ANTI-OBEISITY, COGNITIVE ENHANCING, NEUROBEHAVIORAL, ANTIOXIDANT EFFECTS AND PHYTOCHEMICAL PROFILE OF DICHLOROMETHANE LEAF EXTRACT OF GNIDIA GLAUCA (FRESEN)

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(184/37010/2016)

A THESIS SUBMITTED IN FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF DOCTOR OF PHILOSOPHY (MEDICAL BIOCHEMISTRY) IN THE SCHOOL OF PURE AND APPLIED SCIENCES OF KENYATTA UNIVERSITY

NOVEMBER, 2019
DECLARATION

I, Makori Wycliffe Arika, duly declare that this thesis is my original work and has not been presented for a degree in any other university or for any other award.

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DECLARATION BY SUPERVISORS:

We confirm that the work reported in this thesis was carried out by the candidate under our supervision.

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DEDICATION

This thesis is dedicated to three special ladies who have been so dear to my life: my mother, Damaris Moraa Arika Makori, my daughter, Shanne-Skye Moraa Arika, and my godmother, the late Dr. Joan Murugi Njagi Ngugi. You are all my source of inspiration and am deeply indebted to all of you. Also, a special dedication goes to my father Makori Nyang’ute Harrison for laying a solid academic foundation in my life unto which I am still anchored.
ACKNOWLEDGEMENTS

To work under the esteemed guidance of a genius scientist is a matter of pride. Destiny has bestowed upon me this golden opportunity to work under the supervision of Dr. Mathew Piero Ngugi, the pillar and backbone of my research work. His plausible, valuable guidance and motivation urge instilled in me an immense confidence to continue my search right from the stage of identifying a problem to the accomplishment of goals. Thank you very much Doc. I am extremely thankful to Dr. Cromwell Kibiti, whose stimulating criticism, constructive suggestions, keen interest, and inspiration have contributed immensely towards the successful completion of the work. I record my profound sense of gratitude to the late Dr. Joan Murugi Njagi Ngugi for her constant guidance, supervision, unflagging interest, critical comments and valuable suggestion during the course of this work. I pray for her soul to find favor with our heavenly Father, who believably called her to His place. Eternal rest grant unto her Oh Lord, and May Perpetual Light Shine on her. May she rest in peace, Amen.

I wish to express my gratitude and respect to Prof. Eliud Njagi, Dr. Stephen Runo, Dr. Richard Okoth Oduor, Dr. Anthony Ireri (Cewa) and Dr. Mark Wamalwa for their blessings, kind co-operation, and constant unconditional support and guidance. I wish to express my warm and sincere thanks to Dr. George Omwenga whose nimble attitude, moral support and intellectual suggestions helped me a lot in the accomplishment of the project. During this work, I have collaborated with many colleagues for whom I have great regard. I wish to extend my warmest thanks to Shadrack Njagi, Peterson Mucheni, Stephen Gitahi, Dorothy Wavinya, Stephen Mwihia Joshua Mulele, Phillip Ogola, Japheth Wambani, and
James Kimani. It is due to their keen interest, encouragement and fruitful suggestions that I could complete my work. I would like to express my thanks to Daniel Gitonga Mwaniki, Ibrahim Waweru, James Ngunjiri, Joyce and the Chief Technician, Josephine of the Department of Biochemistry, Microbiology and Biotechnology, Kenyatta University for technical support and guidance during my project work. My humble regards are due to Vincent Maoga and Emmah Ogeto for providing me the logistical and moral support. I especially thank Anthony Njuki Maringa, Eunice Meru Njuki, Niceta Wangari, Margaret Wamuyu, Lucia Muthoni, uncle Charles Arika Abaya and aunty Linet Okemwa for providing me the affection, co-operation, loving companionship, and unflagging interest during my project work.

My parents deserve special mention for their inseparable support and prayers. My Father, in the first place, is the person who showed me the joy of intellectual pursuit ever since I was a child. My Mother is the one who sincerely raised me with her caring and gentle love. I would like to express my heartfelt thanks and great debts of gratitude to my siblings Ruth Kerubo, Enock Makori, Edna Kemunto, Neomy Kwamboka, and dearest nephews Ezra, Fidel and Trevordel and nieces Ivy, Beverly, Bevin, Deborah, Trina and Linda for being supportive, loving and caring.

Words elude me to express my appreciation to my daughter Shanne-Skye Moraa Arika and my girlfriend Juliee Nelima Furaha whose dedication, love and persistent confidence in me, has taken the load off my shoulder. She helped me in different ways and with whom I shared my joys and sorrows. Besides, there are several other people who have knowingly
or unknowingly helped me in the successful completion of this project. I thank all those people for every ounce of efforts they contributed, with my sincere apology, if I could not mention anyone. Above all, I bow my head before the Almighty God with whose grace and beatitude, I moved through this venture.

I finish with a final silence of gratitude for my life.
# TABLE OF CONTENTS

DECLARATION ........................................................................................................... ii  
DEDICATION ................................................................................................................ iii  
ACKNOWLEDGEMENTS .............................................................................................. iv  
TABLE OF CONTENTS ............................................................................................... vii  
LIST OF TABLES ......................................................................................................... xiii  
LIST OF FIGURES ....................................................................................................... xv  
LIST OF PLATES ........................................................................................................... xvi  
LIST OF ABBREVIATIONS AND ACRONYMS ....................................................... xvii  
ABSTRACT .................................................................................................................. xxii  

## CHAPTER ONE ........................................................................................................ 1  
INTRODUCTION .......................................................................................................... 1  
  1.1 Background Information ...................................................................................... 1  
  1.2 Statement of the Problem .................................................................................... 9  
  1.3 Justification of the Study .................................................................................... 10  
  1.4 Hypotheses ......................................................................................................... 12  
  1.5 Objectives .......................................................................................................... 13  
    1.5.1 General Objective ......................................................................................... 13  
    1.5.2 Specific Objectives ....................................................................................... 13  

## CHAPTER TWO ........................................................................................................ 14  
LITERATURE REVIEW ................................................................................................ 14  
  2.1 The Biology of Obesity ....................................................................................... 14  
  2.2 Epidemiology and Impact of Obesity ................................................................. 14  
  2.3 Etiology, Pathophysiology and Chronobiology of Obesity ....................... 16  
    2.3.1 Neurochemical Mediators of Food Intake ................................................. 17  
    2.3.2 Genetic Determinants of Obesity ............................................................. 19  
    2.3.3 Chronobiology of Obesity ....................................................................... 20  
  2.4 Classification of Obesity ................................................................................... 22  
    2.4.1 Android Obesity (Apple Shape) ................................................................. 22  
    2.4.2 Gynoid Obesity (Pear Shape) .................................................................... 22  
  2.5 Diagnosis of Obesity ......................................................................................... 23  
  2.6 Obesity and Spontaneous Emitted Behaviors ............................................... 26
2.7 Psychopathology and Obesity .................................................................29
2.8 Obesity and Cognition............................................................................31
2.9 Obesity and Oxidative Stress .................................................................32
2.10 Management of Obesity .......................................................................34
  2.10.1 Dietary Changes and Physical Activity .............................................34
  2.10.2 Behavioral Modification ..................................................................35
  2.10.3 Pharmacological Treatment of Obesity ..........................................36
  2.10.4 Herbal Approaches to Obesity Management ..................................39
CHAPTER THREE .................................................................................42
IN VIVO ANTI-OBEITY EFFECTS OF DICHLOROMETHANE LEAF EXTRACT
OF Gnidia glauca IN HIGH-FAT DIET (HFD)-INDUCED OBESE RATS ..........42
  3.1 Introduction ..........................................................................................42
  3.2 Materials and Methods ........................................................................44
    3.2.1 Collection of the Leaves of Gnidia glauca .......................................44
    3.2.2 Processing and Extraction of the Plant Material ..............................46
    3.2.3 Preparation of Appropriate Doses for Bioassays ............................46
    3.2.4 Experimental Animals ..................................................................47
    3.2.5 Induction of Obesity ......................................................................47
    3.2.6 Experimental Design .....................................................................48
    3.2.7 Determination of Body Weight .......................................................49
    3.2.8 Determination of Anthropometric and Morphological Measures ......49
    3.2.9 Blood Sampling for Fasting Blood Glucose ....................................50
    3.2.10 Determination of Rectal Body Temperature of Rats .....................51
    3.2.11 Preparation of Blood for Hematological and Biochemical Parameters .51
    3.2.12 Determination of Organ Weights and Relative Organ to Body Weight (Organo-Somatic Index) ..........................................................52
    3.2.13 Determination of Adipose Depots ..................................................52
    3.2.14 Determination of Biochemical Parameters ....................................53
    3.2.15 Determination of Hematological Parameters ................................54
    3.2.16 Determination of Feed Intake .......................................................55
    3.2.17 Data Management and Statistical Analysis ...................................55
  3.3 Results ..................................................................................................56
    3.3.1 Effects of DCM Leaf Extract of Gnidia glauca on Body Weights of HFD-Induced Obese Rats .................................................................56
3.3.2 Effect of DCM Leaf Extract of *Gnidia glauca* on Anthropometric Measures of HFD-Induced Obese Experimental Rats .................................59
3.3.3 Effect of DCM Leaf Extract of *Gnidia glauca* on Organ Weights and Relative Organ to Body Weights of HFD-Induced Obese Rats ..........76
3.3.4 The Effect of DCM Leaf Extract of *Gnidia glauca* on Total Fat Content in HFD-Induced Obese Laboratory Rats .................................81
3.3.5 Effect of DCM Leaf Extract of *Gnidia glauca* on Lipid Profiles in HFD-Induced Obese Rats ..................................................................................85
3.3.6 The Effect of DCM Leaf Extract of *Gnidia glauca* on Body Adiposity Index (BAI) and Atherogenic Index (AI) in HFD-Induced Obese Rats ..................................................................................88
3.3.7 Effect of DCM Leaf Extract of *Gnidia glauca* on Fasting Blood Glucose Levels of HFD-Induced Obese Laboratory Rats .......................89
3.3.8 Effect of DCM Leaf Extract of *Gnidia glauca* on ALT, AST, ALP, LDH, GGT, Creatinine and Urea in HFD-Induced Obese Laboratory Rats ..................................................................................91
3.3.9 Effect of DCM Leaf Extract of *Gnidia glauca* on Total Protein, Total Albumin, Direct Bilirubin and Indirect Bilirubin in HFD-Induced Obese Laboratory Rats ..................................................................................93
3.3.10 Effect of DCM Leaf Extract of *Gnidia glauca* on Some End-Point Haematological Parameters in HFD-Induced Obese Laboratory Rats ..................................................................................95
3.3.11 Effect of DCM Leaf Extract of *Gnidia glauca* on Rectal Body Temperature in HFD Induced Obese Laboratory Rats ......................101
3.3.12 Effect of DCM Leaf Extract of *Gnidia glauca* on Feed Intake in HFD-Induced Obese Rats ..................................................................................104

3.4 Discussion .................................................................................................................................................................106

CHAPTER FOUR ....................................................................................................................................................................123

EFFECTS OF DCM LEAF EXTRACT OF *Gnidia glauca* ON HIPPOCAMPAL-DEPENDENT SPATIAL LEARNING AND MEMORY RETENTION IN HFD-INDUCED OBESE RATS ..................................................................................123

4.1 Introduction ....................................................................................................................................................................123
4.2 Materials and Methods .................................................................................................................................................127
4.2.1 Preparation of the Plant Material, Handling of Experimental Rats and Induction of Obesity .........................................................127
4.2.2 Morris Water Maze ......................................................................................................................................................127
4.2.3 Data Management and Statistical Analysis ..................................................................................................................132

4.3 Results ............................................................................................................................................................................133
4.3.1 Effect of DCM Leaf Extract of G. glauca On Hippocampal-Dependent Spatial Learning and Memory Retention in High Fat Diet-Induced Obese Rats .................................................. 133
4.3.2 Latency Period ........................................................................... 134
4.3.3 Navigation Distance ................................................................. 138
4.3.4 Swimming Speed ..................................................................... 142
4.3.5 Spatial Memory Retention ......................................................... 146
4.4 Discussion ...................................................................................... 148

CHAPTER FIVE .................................................................................... 157
EFFECT OF DCM LEAF EXTRACT OF Gnidia glauca ON LOCOMOTOR ACTIVITY, ANXIETY AND EXPLORATION-LIKE BEHAVIORS IN HFD-INDUCED OBESE RATS ................................................................................ 157
5.1 Introduction .................................................................................... 157
5.2 Materials and Methods ................................................................. 161
  5.2.1 Preparation of the Plant Material, Handling of Experimental Rats and Induction of Obesity .......................................................... 161
  5.2.2 Open Field Arena ..................................................................... 161
  5.2.3 Data Management and Statistical Analysis .............................. 164
5.3 Results .......................................................................................... 165
  5.3.1 Effect of DCM Leaf Extract of Gnidia glauca on Locomotor Activities, Anxiety and Exploration-Like Behaviors in HFD-Induced Obese Laboratory Rats ............................................................... 165
5.4 Discussion ...................................................................................... 168

CHAPTER SIX ...................................................................................... 183
IN VITRO ANTIOXIDANT POTENTIAL AND FREE RADICAL SCAVENGING ACTIVITIES OF DICHLOROMETHANOLIC LEAF EXTRACT OF Gnidia glauca .......................................................... 183
6.1 Introduction .................................................................................... 183
6.2 Materials and Methods ................................................................. 187
  6.2.1 Collection and Preparation of the Medicinal Plant .................... 187
  6.2.2 Determination of Ferric Reducing Antioxidant Power (FRAP) .... 187
  6.2.3 Determination of DPPH Free Radical Scavenging Activity ........... 188
  6.2.4 Determination of Nitric Oxide Radical Scavenging Activity .......... 189
  6.2.5 Determination of Superoxide Radical Scavenging Activity ........... 190
  6.2.6 Determination of Hydroxyl Radical (OH) Scavenging Activity .... 191
  6.2.7 Determination of Lipid Peroxidation Activity .............................. 192
  6.2.8 Determination of Hydrogen Peroxide Radical Scavenging Activity .... 193
6.2.9 Iron (Fe²⁺) Chelating Activity Assay .......................................................... 194
6.2.10 Data Management and Statistical Analysis .............................................. 195
6.3 Results ............................................................................................................. 196

6.3.1 *In Vitro* Ferric Reducing Antioxidant Power (FRAP) of DCM Leaf Extract of *G. glauca* .......................................................... 196

6.3.2 *In Vitro* DPPH Radical Scavenging Activity of DCM Leaf Extract of *G. glauca* .......................................................... 197

6.3.3 *In Vitro* Nitric Oxide Radical Scavenging Activity of DCM Leaf Extract of *G. glauca* .......................................................... 199

6.3.4 *In Vitro* Superoxide Radical Scavenging Activity of DCM Leaf Extract of *G. glauca* .......................................................... 201

6.3.5 *In Vitro* Hydroxyl Radical Scavenging Activity of DCM Leaf Extract of *G. glauca* .......................................................... 203

6.3.6 *In Vitro* Lipid Peroxidation Inhibition Activity of DCM Leaf Extract of *G. glauca* .......................................................... 205

6.3.7 *In Vitro* Hydrogen Peroxide Radical Scavenging Activity of DCM Leaf Extract of *G. glauca* .......................................................... 207

6.3.8 *In Vitro* Iron Chelating Activity of DCM Leaf Extract of *G. glauca* .......................................................... 209

6.4 Discussion ....................................................................................................... 211

CHAPTER SEVEN .................................................................................................. 225

PHYTOCHEMICAL SCREENING OF DICHLOROMETHANE LEAF EXTRACT OF *Gnidia glauca* .......................................................... 225

7.1 Introduction ...................................................................................................... 225

7.2 Materials and Methods .................................................................................. 227

7.2.1 Collection and Preparation of the Medicinal Plant, *Gnidia glauca* ...... 227

7.2.2 Sample Preparation for GC-MS Analysis .................................................. 227

7.2.3 Gas Chromatography-Mass Spectrometry (GC-MS) Analysis .............. 227

7.2.4 Data Management and Statistical Analysis .............................................. 228

7.3 Results ............................................................................................................. 229

7.4 Discussion ....................................................................................................... 232

CHAPTER EIGHT .................................................................................................. 242

GENERAL SUMMARY, CONCLUSIONS AND RECOMMENDATIONS ........ 242

8.1 General Summary .......................................................................................... 242

8.2 Conclusions ................................................................................................... 245

8.3 Recommendations ......................................................................................... 245

8.3.1 Recommendations from The Study .......................................................... 245
LIST OF TABLES

Table 3.1: Coordinates of Site of Collection of the Plant Sample .................................................45
Table 3.2: Composition of High Fat Diet ..........................................................................................48
Table 3.3: Experimental Design for In Vivo Anti-obesity Assays .....................................................49
Table 3.4: Effect of DCM Leaf Extract of *Gnidia glauca* On Body Weights of HFD-Induced Obese Laboratory Rats ........................................................................................................58
Table 3.5: Effect of DCM Leaf Extract of *Gnidia glauca* on Obesity Index (OI) of HFD-Induced Obese Laboratory Rats .................................................................61
Table 3.6: Effect of DCM Leaf Extract of *Gnidia glauca* on Abdominal Circumference (AC) of HFD-Induced Obese Laboratory Rats .................................................................63
Table 3.7: Effect of DCM Leaf Extract of *Gnidia glauca* on Thoracic Circumference (TC) of HFD-Induced Obese Laboratory Rats .................................................................66
Table 3.8: Effect of DCM Leaf Extract of *Gnidia glauca* on Abdominal Circumference to Thoracic Circumference Ratio (AC:TC) of HFD-Induced Obese Laboratory Rats .................................................................69
Table 3.9: Effect of DCM Leaf Extract of *Gnidia glauca* on Abdominal Circumference to Height Ratio (ACHtR) of HFD-Induced Obese Laboratory Rats .................................................................72
Table 3.10: Effect of DCM Leaf Extract of *Gnidia glauca* on Thoracic Circumference to Height Ratio (TCHtR) Of HFD-Induced Obese Laboratory Rats .................................................................75
Table 3.11: Effect of DCM Leaf Extract of *Gnidia glauca* on Organ Weights of HFD-Induced Obese Rats .................................................................................................................78
Table 3.12: Effect of DCM Leaf Extract of *Gnidia glauca* on Relative-Organ Weights (Organo-Somatic Index) of HFD-Induced Obese Rats .................................................................80
Table 3.13: The Effect of DCM Leaf Extract of *Gnidia glauca* on Total Fat Content in HFD-Induced Obese Rats .................................................................................................................84
Table 3.14: The Effect of Oral Administration of DCM Leaf Extract of *Gnidia glauca* on Lipid Profiles in HFD-Induced Obese Rats .................................................................................................................87
Table 3.15: The Effect of DCM Leaf Extract of *Gnidia glauca* on Body Adiposity Index (BAI) and Atherogenic Index (AI) in HFD-Induced Obese Rats .................................................................................................................88
Table 3.16: Effect of DCM Leaf Extract of *Gnidia glauca* on Fasting Blood Glucose Levels in HFD-Induced Obese Laboratory Rats .................................................................................................................90
Table 3.17: Effect of DCM Leaf Extract of *Gnidia glauca* on ALT, AST, ALP, LDH, GGT, Urea and Creatinine in HFD-Induced Obese Laboratory Rats .................................................................................................................92
Table 3.18: Effect of DCM Leaf Extract of *Gnidia glauca* on Total Protein, Total Albumin, Direct Bilirubin and Indirect Bilirubin in HFD-Induced Obese Laboratory Rats .................................................................................................................94
Table 3.19: Effect of DCM Leaf Extract of *Gnidia glauca* on Erythrocytes and Related Parameters in HFD-Induced Obese Laboratory Rats .................................................................................................................96
Table 3.20: Effect of DCM Leaf Extract of *Gnidia glauca* on White Blood Cells and Differential Leucocytes Counts in HFD-Induced Obese Laboratory Rats .................................................................................................................98
Table 3.22: Effect of Oral Administration of DCM Leaf Extract of *Gnidia glauca* On Platelets Count and Related Variants in HFD-Induced Obese Laboratory Rats ..............................................100
Table 3.23: Effect of DCM Leaf Extract of *Gnidia glauca* on Rectal Body Temperature in HFD-Induced Obese Laboratory Rats ......................................................103

Table 4.1: Effect of DCM Leaf Extract of Gnidia glauca on Escape Latency in HFD-Induced Obese Rats During Acquisition Training ..............................................135
Table 4.2: Effect of DCM Leaf Extract of *Gnidia glauca* On Escape Latency in HFD-Induced Obese Rats During Reverse Training ..............................................137
Table 4.3: Effect of DCM Leaf Extract of *Gnidia glauca* on Navigation Distance in HFD-Induced Obese Rats During Acquisition Training ..............................................139
Table 4.4: Effect of DCM Leaf Extract of *Gnidia glauca* on Navigation Distance in HFD-Induced Obese Rats During Reverse Training ..............................................141
Table 4.5: Effect of DCM Leaf Extract of *Gnidia glauca* on Swimming Speed in HFD-Induced Obese Rats During Acquisition Training ..............................................143
Table 4.6: Effect of DCM Leaf Extract of *Gnidia glauca* on Swimming Speed in HFD-Induced Obese Rats During Reverse Training ..............................................145

Table 5.1: Effect of DCM Leaf Extract of *Gnidia glauca* on Locomotor Activities, Anxiety and Exploration-Like Behaviors In HFD-Induced Obese Rats .............167

Table 7.1: The Concentrations of Compounds Identified in DCM Leaf Extract of *Gnidia glauca* ..............................................................................................................230
**LIST OF FIGURES**

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.1</td>
<td>Map showing Nthawa Location, Mbeere North Sub-County, Embu County</td>
<td>45</td>
</tr>
<tr>
<td>3.2</td>
<td>Effect of DCM Leaf Extract of <em>Gnidia glauca</em> on Feed Intake in HFD-Induced Obese Rats</td>
<td>105</td>
</tr>
<tr>
<td>4.1</td>
<td>Morris Water Maze/Navigation Task</td>
<td>128</td>
</tr>
<tr>
<td>4.2</td>
<td>An illustration of the four quadrants of the water maze</td>
<td>129</td>
</tr>
<tr>
<td>4.3</td>
<td>Effect of DCM leaf extract of <em>G. glauca</em> on spatial memory retention in HFD-induced obese rats during acquisition training</td>
<td>146</td>
</tr>
<tr>
<td>4.4</td>
<td>Effect of DCM leaf extract of <em>G. glauca</em> on spatial memory retention in HFD-induced obese rats during reverse training</td>
<td>147</td>
</tr>
<tr>
<td>5.1</td>
<td>The Open Field Arena</td>
<td>162</td>
</tr>
<tr>
<td>6.1</td>
<td>In vitro ferric reducing antioxidant power of DCM leaf extract of <em>G. glauca</em></td>
<td>196</td>
</tr>
<tr>
<td>6.2</td>
<td>In vitro DPPH radical scavenging activity of DCM leaf extract of <em>G. glauca</em></td>
<td>198</td>
</tr>
<tr>
<td>6.3</td>
<td>In vitro nitric oxide radical scavenging activity of DCM leaf extract of <em>G. glauca</em></td>
<td>200</td>
</tr>
<tr>
<td>6.4</td>
<td>In vitro superoxide radical scavenging activity of DCM leaf extract of <em>G. glauca</em></td>
<td>202</td>
</tr>
<tr>
<td>6.5</td>
<td>In vitro hydroxyl radical scavenging activity of DCM leaf extract of <em>G. glauca</em></td>
<td>204</td>
</tr>
<tr>
<td>6.6</td>
<td>In vitro lipid peroxidation inhibition activity of DCM leaf extract of <em>G. glauca</em></td>
<td>206</td>
</tr>
<tr>
<td>6.7</td>
<td>In vitro hydrogen peroxide radical scavenging activity of DCM leaf extract of <em>G. glauca</em></td>
<td>208</td>
</tr>
<tr>
<td>6.8</td>
<td>In vitro iron chelating activity of DCM leaf extract of <em>G. glauca</em></td>
<td>210</td>
</tr>
</tbody>
</table>
LIST OF PLATES

Plate 2.1: A photomicrograph of *Gnidia glauca* taken on June, 2016 at Siakago Sub County, Embu County, Kenya.........................................................41
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>AC</td>
<td>Abdominal circumference</td>
</tr>
<tr>
<td>ACHtR</td>
<td>Abdominal circumference to height ratio</td>
</tr>
<tr>
<td>ACTC</td>
<td>Abdominal thoracic to thoracic circumference ratio</td>
</tr>
<tr>
<td>AchE</td>
<td>Acetylcholinesterase</td>
</tr>
<tr>
<td>ASP</td>
<td>Acylation-stimulating protein</td>
</tr>
<tr>
<td>AMPK</td>
<td>Adenosine monophosphate-activated protein kinase</td>
</tr>
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<td>Adrenocorticotropic hormone</td>
</tr>
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<td>Agouti-related protein</td>
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</tr>
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<td>Alkaline phosphatase</td>
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<tr>
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<td>Alpha melanocyte stimulating hormone</td>
</tr>
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<td>Aβ</td>
<td>Amyloid beta proteins</td>
</tr>
<tr>
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<td>Analysis of variance</td>
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<td>Angiotensinogen</td>
</tr>
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<td>Apolipoprotein E</td>
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</tr>
<tr>
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<td>Atherogenic Index</td>
</tr>
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<td>AIP</td>
<td>Atherogenic Index of Plasma</td>
</tr>
<tr>
<td>BAS</td>
<td>Basophils</td>
</tr>
<tr>
<td>BDZs</td>
<td>Benzodiazepines</td>
</tr>
<tr>
<td>BBB</td>
<td>Blood Brain Barrier</td>
</tr>
<tr>
<td>BMI</td>
<td>Body Mass Index</td>
</tr>
<tr>
<td>BMPs</td>
<td>Bone morphogenetic proteins</td>
</tr>
<tr>
<td>BMAL1</td>
<td>Brain- and muscle ANRT-like protein-1</td>
</tr>
<tr>
<td>BDNF</td>
<td>Brain-derived neurotrophic factor</td>
</tr>
<tr>
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<td>Brown adipose tissue</td>
</tr>
<tr>
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<td>Butylated hydroxyanisole</td>
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<tr>
<td>Acronym</td>
<td>Description</td>
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<tr>
<td>---------</td>
<td>-------------</td>
</tr>
<tr>
<td>CREB</td>
<td>Camp-Response-Element-Binding protein</td>
</tr>
<tr>
<td>CVDs</td>
<td>Cardiovascular Diseases</td>
</tr>
<tr>
<td>CNS</td>
<td>Central Nervous System</td>
</tr>
<tr>
<td>CCK</td>
<td>Cholecystokinin</td>
</tr>
<tr>
<td>CLOCK</td>
<td>Circadian Locomotor Output Cycles Kaput</td>
</tr>
<tr>
<td>CoQ10</td>
<td>Co-enzyme Q-10</td>
</tr>
<tr>
<td>CHD</td>
<td>Coronary Heart Disease</td>
</tr>
<tr>
<td>CRH</td>
<td>Corticotropin-releasing hormone</td>
</tr>
<tr>
<td>cAMP</td>
<td>Cyclic adenosine monophosphate</td>
</tr>
<tr>
<td>COX-1 &amp; 2</td>
<td>Cyclooxygenase-1 &amp; 2</td>
</tr>
<tr>
<td>DPPH</td>
<td>2,2-diphenyl-1-picrylhydrazine</td>
</tr>
<tr>
<td>DMS</td>
<td>Degrees Minutes Seconds</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic Acid</td>
</tr>
<tr>
<td>DCM</td>
<td>Dichloromethane</td>
</tr>
<tr>
<td>DEXA</td>
<td>Dual-Energy X-ray Absorptiometry technique</td>
</tr>
<tr>
<td>EOS</td>
<td>Eosinophils</td>
</tr>
<tr>
<td>EDTA</td>
<td>Ethylenediaminetetraacetic acid</td>
</tr>
<tr>
<td>ERV</td>
<td>Expiratory reserve volume</td>
</tr>
<tr>
<td>FRAP</td>
<td>Ferric Reducing Antioxidant Power</td>
</tr>
<tr>
<td>FasR</td>
<td>First apoptosis signal receptor</td>
</tr>
<tr>
<td>FFA</td>
<td>Free fatty acids</td>
</tr>
<tr>
<td>FRC</td>
<td>Functional residual capacity</td>
</tr>
<tr>
<td>GGT</td>
<td>Gamma(γ)-Glutamyl Transferase</td>
</tr>
<tr>
<td>GABA</td>
<td>Gamma-aminobutyric acid</td>
</tr>
<tr>
<td>GC-MS</td>
<td>Gas chromatography linked to mass spectrometry</td>
</tr>
<tr>
<td>GLP-1</td>
<td>Glucagon-like peptide-1</td>
</tr>
<tr>
<td>GPCR</td>
<td>G-Protein Coupled Receptors</td>
</tr>
<tr>
<td>GFR</td>
<td>Growth Factor Receptors (GFR)</td>
</tr>
<tr>
<td>Hb</td>
<td>Haemoglobin</td>
</tr>
<tr>
<td>HDL-c</td>
<td>High-Density Lipoprotein Cholesterol</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Full Form</td>
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</tr>
<tr>
<td>HFD</td>
<td>High-fat diet</td>
</tr>
<tr>
<td>HSL</td>
<td>Hormone-sensitive lipase</td>
</tr>
<tr>
<td>hGH</td>
<td>Human growth hormone</td>
</tr>
<tr>
<td>HPA</td>
<td>hypothalamic-pituitary-adrenal axis</td>
</tr>
<tr>
<td>iNOS</td>
<td>Inducible nitric oxide synthase</td>
</tr>
<tr>
<td>IR</td>
<td>Insulin Receptors</td>
</tr>
<tr>
<td>IDE</td>
<td>Insulin-degrading enzyme</td>
</tr>
<tr>
<td>IL-6</td>
<td>Interleukin 6</td>
</tr>
<tr>
<td>KDHS</td>
<td>Kenya Demographic and Health Survey</td>
</tr>
<tr>
<td>LDH</td>
<td>Lactate dehydrogenase</td>
</tr>
<tr>
<td>LPS</td>
<td>Lipopolysaccharides</td>
</tr>
<tr>
<td>LPL</td>
<td>Lipoprotein lipase</td>
</tr>
<tr>
<td>LTP</td>
<td>Long-term potentiation</td>
</tr>
<tr>
<td>LDL-c</td>
<td>Low-density lipoprotein Cholesterol</td>
</tr>
<tr>
<td>LYM</td>
<td>Lymphocytes</td>
</tr>
<tr>
<td>MDA</td>
<td>Malonyldialdehyde</td>
</tr>
<tr>
<td>MCH</td>
<td>Mean Corpuscular Hemoglobin</td>
</tr>
<tr>
<td>MCHC</td>
<td>Mean corpuscular hemoglobin concentration</td>
</tr>
<tr>
<td>MCV</td>
<td>Mean Corpuscular Volume</td>
</tr>
<tr>
<td>MPV</td>
<td>Mean platelet volume</td>
</tr>
<tr>
<td>MC4-R</td>
<td>Melanocortin 4 receptor</td>
</tr>
<tr>
<td>MetS</td>
<td>Metabolic syndromes</td>
</tr>
<tr>
<td>MAPK</td>
<td>Mitogen-Activated Protein Kinases</td>
</tr>
<tr>
<td>MCP-1</td>
<td>Monocyte chemotactic protein</td>
</tr>
<tr>
<td>MON</td>
<td>Monocytes</td>
</tr>
<tr>
<td>MUFAs</td>
<td>Mono-unsaturated fatty acids</td>
</tr>
<tr>
<td>MWM</td>
<td>Morris water maze</td>
</tr>
<tr>
<td>NACOSTI</td>
<td>National Commission for Science, Technology and Innovation</td>
</tr>
<tr>
<td>NIST</td>
<td>National Institute of Standards and Technology</td>
</tr>
<tr>
<td>NGF</td>
<td>Nerve growth factor</td>
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<tr>
<td>Abbreviation</td>
<td>Full Form</td>
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<tr>
<td>NPY</td>
<td>Neuropeptide Y</td>
</tr>
<tr>
<td>NEU</td>
<td>Neutrophils</td>
</tr>
<tr>
<td>NBT</td>
<td>Nitro blue tetrazolium</td>
</tr>
<tr>
<td>NMDAR</td>
<td>N-methyl-D-aspartate receptor</td>
</tr>
<tr>
<td>NIDDM</td>
<td>Non-Insulin Dependent Diabetes Mellitus</td>
</tr>
<tr>
<td>NFE2L2 or Nrf2</td>
<td>Nuclear factor (erythroid-derived 2)-like 2</td>
</tr>
<tr>
<td>NF-kβ</td>
<td>Nuclear factor kappa-beta</td>
</tr>
<tr>
<td>OSA</td>
<td>Obstructive sleep apnea</td>
</tr>
<tr>
<td>OD</td>
<td>Optical Density</td>
</tr>
<tr>
<td>OSI</td>
<td>Organo-Somatic Index</td>
</tr>
<tr>
<td>OS</td>
<td>Oxidative stress</td>
</tr>
<tr>
<td>PCV</td>
<td>Packed Cell Volume</td>
</tr>
<tr>
<td>PER2</td>
<td>Period Circadian Protein-2</td>
</tr>
<tr>
<td>PRDXs</td>
<td>Peroxiredoxins</td>
</tr>
<tr>
<td>PGC-1α</td>
<td>Peroxisome proliferator-activated receptor γ coactivator-1α</td>
</tr>
<tr>
<td>PI3K</td>
<td>Phosphatidylinositol 3-Kinase</td>
</tr>
<tr>
<td>PAI-1</td>
<td>Plasminogen activating inhibitor</td>
</tr>
<tr>
<td>PDW</td>
<td>Platelet distribution width</td>
</tr>
<tr>
<td>PCOS</td>
<td>Polycystic ovaries syndrome</td>
</tr>
<tr>
<td>PUFAs</td>
<td>Poly-unsaturated fatty acids</td>
</tr>
<tr>
<td>PSD-95</td>
<td>Postsynaptic density protein 95</td>
</tr>
<tr>
<td>POMC</td>
<td>Pro-opiomelanocortin</td>
</tr>
<tr>
<td>PG</td>
<td>Propyl gallate</td>
</tr>
<tr>
<td>PKA</td>
<td>Protein Kinase A</td>
</tr>
<tr>
<td>PKC</td>
<td>Protein Kinase C</td>
</tr>
<tr>
<td>RNS</td>
<td>Reactive nitrogen species</td>
</tr>
<tr>
<td>ROS</td>
<td>Reactive oxygen species</td>
</tr>
<tr>
<td>RBCs</td>
<td>Red blood cells</td>
</tr>
<tr>
<td>RDW</td>
<td>Red cell distribution width</td>
</tr>
<tr>
<td>RNA</td>
<td>Ribonucleic acid</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Full Form</td>
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<tr>
<td>SNP</td>
<td>Sodium nitroprusside</td>
</tr>
<tr>
<td>SD</td>
<td>Standard Deviation</td>
</tr>
<tr>
<td>SREBP1c</td>
<td>Sterol regulatory element-binding transcription factor 1c</td>
</tr>
<tr>
<td>SAP</td>
<td>Stretch attend postures</td>
</tr>
<tr>
<td>SCN</td>
<td>Suprachiasmatic Nucleus</td>
</tr>
<tr>
<td>SNS</td>
<td>Sympathetic Nervous Systems</td>
</tr>
<tr>
<td>SYP</td>
<td>Synapsin I</td>
</tr>
<tr>
<td>TBHQ</td>
<td>Tert-butylhydroxyquinone</td>
</tr>
<tr>
<td>TZDs</td>
<td>Thiazolidinediones</td>
</tr>
<tr>
<td>TBA</td>
<td>Thiobarbituric acid</td>
</tr>
<tr>
<td>TBARS</td>
<td>Thiobarbituric acid reactive substance</td>
</tr>
<tr>
<td>TC</td>
<td>Thoracic circumference</td>
</tr>
<tr>
<td>TCtHR</td>
<td>Thoracic Circumference to Height Ratio</td>
</tr>
<tr>
<td>TLC</td>
<td>Total lung capacity</td>
</tr>
<tr>
<td>TG</td>
<td>Triglycerides</td>
</tr>
<tr>
<td>TNF-α</td>
<td>Tumor necrosis factor-α</td>
</tr>
<tr>
<td>TrkB</td>
<td>Tyrosine kinase receptor</td>
</tr>
<tr>
<td>UCP-1</td>
<td>Uncoupling protein-1</td>
</tr>
<tr>
<td>UTM</td>
<td>Universal Transverse Mercator coordinate system</td>
</tr>
<tr>
<td>VLDL-c</td>
<td>Very Low-Density Lipoprotein Cholesterol</td>
</tr>
<tr>
<td>WC</td>
<td>Waist Circumference</td>
</tr>
<tr>
<td>WtHR</td>
<td>Waist-to-height ratio</td>
</tr>
<tr>
<td>WHR</td>
<td>Waist-to-hip ratio</td>
</tr>
<tr>
<td>WAT</td>
<td>White Adipose Tissue</td>
</tr>
<tr>
<td>WBCs</td>
<td>White Blood Cells</td>
</tr>
<tr>
<td>WHO</td>
<td>World Health Organization</td>
</tr>
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ABSTRACT

Obesity is a chronic metabolic disorder characterized by increased adipose tissue mass due to positive energy balance. The epidemic of obesity is currently on the rise probably due to increasingly sedentary lifestyles combined with easy availability of palatable, high-fat foods. It presents modifiable risk factor for cognitive impairments, anxiety and motor deficits. Globally, its prevalence has shown a startling increase in all age groups and have been associated with increased morbidity and mortality. Prescription of anti-obesity drugs can be useful adjuncts to diet and exercise for obese patients who have failed to achieve weight loss. However, these drugs are ineffective, not readily available, unaffordable and have been associated with adverse effects. Therefore, medicinal plants have drawn a sharp focus in recent times as complementary and alternative medicines owing to their biocompatibility, affordability and are assumed to be safe due to their long-term clinical application. Hereby, determination of therapeutic activities and identification of active principles from herbal prescriptions have become the prime focus in the validation of their folkloric usage and in drug discovery programs. The present study aimed to determine the anti-obesity effects, cognitive enhancing, neurobehavioral, antioxidant effects and phytochemical profile of dichloromethane leaf extract of Gnidia glauca. Obesity was experimentally induced by feeding the rats with prepared high-fat-diet (HFD) and water ad libitum for a period of 6 weeks. The in-vivo anti-obesity effects were determined by oral administration of the extract at dosage levels of 200, 250 and 300mg/kg body weight in HFD-induced obese rats from the 6th to 12th week along with HFD. Cognitive-enhancing effects of the extract in HFD-induced obese rats were determined using the Morris Water Maze experiment. The effects of the extract on neurobehaviors (locomotor activity, anxiety and exploration-like behaviors) in HFD-induced obese rats were tested using the Open Field Test. The in vitro antioxidant properties of the extract were determined using non-enzymatic assays. The phytochemical profile of the extract was determined using GC-MS. The results indicated that the extract exhibited potent anti-obesity effects in HFD-induced obese rats. It significantly reduced the body weight, organ weights, organo-somatic indices, anthropometric indices, the total fat content, adiposity index, atherogenic index as well as the lipid profiles (Triglycerides, Total Cholesterol, Low-Density Lipoproteins, and Very Low-Density Lipoproteins). However, it significantly increased levels of High-Density Lipoproteins. The extract increased levels of white blood cells, differential leukocyte counts, platelet count as well as red blood cells and related parameters. The extract improved hippocampal-dependent spatial learning and memory retention in HFD-induced obese rats. Moreover, it showed anxiolytic effects, increased spontaneous locomotor activity and exploration-like behaviors in HFD-induced obese rats. The extract also indicated in vitro antioxidant effects. The phytochemical analysis revealed the presence of 28 bioactive compounds in the extract. The anti-obesity effects, antioxidants activities, cognitive-enhancing effects and the improved locomotor and exploration-like behaviors could be attributed to the phytochemical compounds present in the plant extract. The present study, therefore, scientifically validated the traditional use of this plant and generated data that can serve as guide in the recruitment of the extract as a potential candidate for the synthesis of a new effective drug against obesity and associated complications. However, there is a need for bioassay-guided fractionation of bioactive compounds in Gnidia glauca. Besides, it is recommended to conduct comprehensive toxicity studies to establish the safety profiles of Gnidia glauca.
CHAPTER ONE

INTRODUCTION

1.1 Background Information

Obesity is a chronic disease characterized by pathophysiological processes that result in increased adipose tissue mass due to positive energy balance (Garber et al., 2013). Obesity involves an interaction of both genetic and environmental factors (such as behavioral, social, cultural, physiological) that can singly or synergistically contribute to its pathogenesis (Kaufer et al., 2001; Vaidya, 2006). Fundamentally, obesity represents a phenotypic consequence of an energy imbalance between calories consumed and calories expended (Halicioglu, 2013). This may be due to increased intake of energy-dense foods that are high in fat combined with decreased physical activity as a result of increasingly sedentary nature of many forms of work, transport and urbanization (WHO, 2013).

Pathological obesity is characterized by hypertrophied and hyperplastic adipocytes (Dulloo(751,511),(797,526)) et al., 2010). The hypertrophic-hyperplastic adipocytes result in an anomalous fat distribution that leads to weight gain equaling to or greater than 20% of the standard weight (Westerterp-Plantenga, 2005). Once considered a high-income country burden, obesity is now on the rise in low- and middle-income countries, particularly in urban settings and has been linked to more deaths worldwide than underweight (WHO, 2012). It presents modifiable risk factors for type II diabetes mellitus, coronary heart disease, hypertension, psychological disorders, musculoskeletal disorders (knee osteoarthritis) and certain cancers (endometrial, breast, and colon) (Luppino et al., 2010; Barouki et al., 2012).
The worldwide prevalence of obesity has more than doubled between 1980 and 2014. In 2014, more than 1.9 billion adults, 18 years and older, were overweight and at least 600 million of them were clinically obese (WHO, 2015). Overall, about 13% of the world’s adult population (11% of men and 15% of women) were obese in 2014 (WHO, 2015). During the same year, 39% of adults aged 18 years and over (38% of men and 40% of women) were overweight (WHO, 2015). In 2013, 42 million children under the age of 5 were overweight or obese and out of this, more than 30% were from developing countries (WHO, 2015). Recent evidence indicates that overweight and obesity are increasing in Sub-Saharan Africa, including Kenya, at a rate of 5% per year on average (Ziraba et al., 2009). Approximately, 17.3% of men and 43.4% of women were diagnosed to be clinically obese in Kenya (Ettarh et al., 2013). Besides, a study conducted by Mkuu et al. (2018) reported that one of every three Kenyan women is either obese or overweight (accounting for about 32.8% of women). On a global scale, obesity has been ranked as the fifth leading risk factor for global deaths and is projected to be the third leading cause of death by 2030 (WHO, 2011; WHO, 2015).

Obesity is associated with increased circulating levels of free fatty acids and systemic pro-inflammatory cytokines, chemokines, prostaglandins, immune cells, reactive oxygen species and reactive nitrogen species which in turn precipitates oxidative stress (Marseglia et al., 2014). Chronic exposure to lipid-rich diets stimulates the production of reactive oxygen species (ROS) through mitochondrial and peroxisomal oxidation of fatty acids (Fernández-Sánchez et al., 2011). High production of ROS is associated with low-grade chronic systemic inflammation in adipose tissue (Marseglia et al., 2014). This condition
activates the innate immune system in the adipose tissue and stimulates the secretion of pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α), interleukin (IL)-1β, and IL-6 (Fonseca-Alaniz et al., 2007). A rise in the concentration of pro-inflammatory cytokines by macrophages and monocytes further increases the generation of ROS and reactive nitrogen species (RNS) (Shoelson et al., 2007).

Under obesogenic states, the compromised redox homeostatic status characterized by attenuated antioxidant defense systems serves as a pre-requisite for the pathogenesis of degenerative disorders such as cognitive impairment (Guidi et al., 2006) and neural disorders that directly or indirectly mediate behaviors (Stachowiak et al., 2013). Ostensibly, this degree of relative adiposity, increased production of proinflammatory cytokines and increased levels of ROS and RNS modulates a wide range of neurobehaviors among which are, spontaneously emitted behaviors (activity patterns, anxiety and exploration), motivated behaviors (feeding, drinking and sexual behaviors) and operant performance, attentional processes, learning and memory (Sas et al., 2007; Sharma and Fulton, 2013).

Peripheral pro-inflammatory cytokines infiltrate the Blood-Brain Barrier (BBB) and initiate an indirect signaling cascade within the CNS that induces reactive astrogliosis in the hypothalamus (astrocytes) exacerbating a further production of cytokines that drives inflammatory responses (Sofroniew and Vinters, 2010). These stimuli also activate the primary mediators of the central nervous system’s immune defense system, the microglia, to release further proinflammatory cytokines, chemokines, nitric oxide, and some
superoxide species (Loane and Byrnes, 2010). Proinflammatory cytokines such as IL-1β, IL-6, and TNF-α play a significant role in cognitive processes such as synaptic plasticity, neurogenesis, neuromodulation, memory consolidation and long-term potentiation (LTP) (Nguyen et al., 2014).

A higher concentration of IL-1 β in the hippocampus under pathophysiological conditions inhibits the induction of LTP and neurogenesis (McAfoose and Baune, 2009). Overexpression of IL-6 impacts cognitive function by dysregulating hippocampal neurogenesis, synaptic plasticity and disruption of neural circuits (Gemma and Bickford, 2007; McAfoose and Baune, 2009). Pathological levels of TNF-α has been postulated to inhibit LTP biphasically in the dentate gyrus of the hippocampus (Stellwagen and Malenka, 2006). Moreover, TNF-α has exhibited its effects on glial–neuronal homeostatic synaptic plasticity and alteration of nerve growth factor (NGF) and neuropeptide Y (NPY) levels resulting in cognitive impairment (Turrigiano, 2006). Therefore, chronic consumption of a high-fat diet significantly aggravates the production of proinflammatory cytokines that drives inflammatory responses within the hypothalamus leading to cognitive impairment (Garcia-Caceres et al., 2013).

The increased circulating levels of ROS and RNS interacts with biomolecules (DNA and RNA), proteins, lipids as well as carbohydrates and exacerbates oxidative damage through carbonylation, peroxidation, nitration and nitrosylation reactions (Halliwell, 2006; Newsholme et al., 2012). In the brain, the resultant molecular modifications contribute to dysregulated neuronal functioning, loss of synaptic plasticity, neurodegeneration and
consequently cognitive impairment (Valko et al., 2007; Reddy et al., 2012; Chen and Zhong, 2013; Butterfield et al., 2014). The compromised redox homeostatic status due to attenuated antioxidant defenses culminates in impaired neurogenesis, synaptic remodeling and reduction in neuronal spine density as well as neuronal apoptosis, deregulated HPA axis, neurodegeneration and brain atrophy (Morrison et al., 2010; Freeman et al., 2013; Pepping et al., 2013; Tucsek et al., 2013).

Chronic exposure to obesogenic diets is often associated with physical inactivity due to altered coordination of motor and reflexive responses (Belczak et al., 2014; Bouchard et al., 2015). Increased adiposity characterized by high levels of proinflammatory cytokines, ROS and RNS alter motor function through enhanced decrements in balance, muscle strength, and coordination (Muramoto et al., 2014). High-fat diets potentiate an oxidative attack on the brain resident cells resulting in activation of the cholinergic motor inhibitory system (Abubakar and Salka, 2010). Besides, chronic exposures to obesogenic diets contribute to striatum damage thereby affecting the dopamine synthesis and release as well as striatal-dopamine receptor function (Kravitz et al., 2016). Deficiencies in dopamine synthesis, striatal dopamine release, as well as defective striatal dopamine receptors contributes to reductions in motor output, often termed “physical inactivity” akin to classical movement disorders such as Parkinson’s disease (Huang et al., 2001; Bouchard et al., 2015; Kravitz et al., 2016).

Anxiety is a state of excessive fear and worry characterized by motor sympathetic hyperactivity, muscular tension, apprehension, restlessness, reduced concentration, fatigue
and vigilance syndromes (Adedayo and Seun, 2018). It often results in feelings of dread over something that is unlikely to occur (Adedayo and Seun, 2018). The positive association between obesity and anxiety disorders such as panic disorder (Barry et al., 2008), social phobias (Mather et al., 2009), obsessive-compulsive disorder (Herpertz et al., 2006), post-traumatic stress disorder and generalized anxiety disorder (Lykouras and Michopoulos, 2011) have been reported. Obesity as a causal factor for anxiety involves several paths such as social discrimination against obese persons (Puhl and Heuer, 2009), low self-esteem in unfriendly social network (Muennig, 2008), distress from illness burden (especially diabetes mellitus, asthma and cardiovascular diseases) and adverse drug effects (Beuther and Sutherland, 2007). Obese subjects interpret that the way others see them is the way they see themselves (Puhl and Brownell, 2003). The image obese subjects have of themselves is one of dissatisfaction and rejection (Puhl and Heuer, 2009). They blame themselves for being fat and try to use various coping strategies to get thinner usually without proper planning and often results in failure (Esmaily et al., 2015).

Weight control, preoccupation and aggressive personality traits contribute to increased food intake favoring hedonic reward of eating and therefore increases positive energy balance which results in excessive worry and anxiety (Hasler et al., 2004; Kroenke et al., 2007). Anxiety disorders as the causal factor for obesity have been correlated with disruption of hypothalamic-pituitary-adrenal (HPA) axis resulting in deregulation of autonomic functions in the hedonic reward circuitry (Kiernan and Bars, 2009; Esmaily et al., 2015). These factors create a continuous vicious cycle (obesity-anxiety cycle) (Esmaily et al., 2015).
Exploration is one of the main domains of behaviors referring to the tendency to investigate a novel environment (Heyser and Chemero, 2012). It is, therefore, curiosity and attraction to novelty (Edagha et al., 2015). Locomotor activity is driven by exploration since its reduced form could possibly reflect reduced exploration (Kravitz et al., 2016). Similarly, studies have indicated that reduction in explorative interest in obese rats is suggestive of symptoms of depressive disorders consistent with those observed in patients suffering from anxiety disorders (Su et al., 2012). Obesity exacerbates psychological distress through low social adjustment, low self-esteem and numerous physical complaints in obese subjects (Glinski et al., 2001). These patients display very little cooperativeness when participating in societal roles due to their persistent failure to visualize themselves as autonomous and integrated (Garaulet et al., 2010b). In response, they are subject to prejudice and discrimination from their colleagues. Social discrimination and low self-esteem exacerbate social avoidance, psychological distress and anxiety scales among obese subjects (Carr and Friedman, 2005; Puhl and Heuer, 2009).

The mainstay of non-pharmacological treatment of obesity includes dietary changes, behavioral modification, and exercise program that have been individualized to the patient’s lifestyle and physical needs (WHO, 2002). For patients who cannot achieve sufficient weight loss through lifestyle and behavioral modification, anti-obesity drug therapy is often useful as an adjunct (Kang and Park, 2012). Despite the urgent need for safe and efficient therapeutics and the potential size of the market for anti-obesity drugs, the current status for the development of such drugs is still unsatisfactory (Shrestha et al., 2007). Besides, the unaffordability, unavailability and the potentially hazardous side-
effects associated with conventional drugs, necessitates a need for an alternative strategy for developing effective and safe anti-obesity drugs from medicinal plants especially in resource poor economies (Bagri et al., 2009).

The postulated mechanisms of activity of herbal medicines in management of obesity is through appetite suppression via central receptors (NPY, AgRP, CB-1 and dopamine receptors), inhibition of triglyceride absorption, increase in lipolysis, improvement of glycemic control, adipose tissue differentiation as well as increase in energy expenditure and thermogenesis (Leung et al., 2003). The main mediators of anti-obesity effects are the bioactive compounds contained in them. These compounds modulate inflammation and protection from oxidative damage (Amirkhizi et al., 2007). The antioxidants principles isolated from herbal medicines inhibit oxidative metabolism in cells through free radical scavenging mechanisms, neutralization of free radicals into less active stable products, blockage of the initiators of free radical attack (Halliwell et al., 1992), regulation of electron transport chain, repair of oxidized proteins, termination of chain reaction effect and salvage of the oxidized antioxidants thereby restoring their functional capacity (Kohen and Nyska, 2002; Ishino et al., 2010). Detoxification of cells and tissue from these highly reactive molecules by means of antioxidants is critical for cell viability, activation, proliferation, and general organ function (Amirkhizi et al., 2007; Mirończuk-Chodakowska et al., 2018).

In traditional African medicine, many herbs have been therapeutically applied against various ailments. One such medicinal plant is *Gnidia glauca*, in the Thymelaeaceae family
(Bhandurge et al., 2013). *Gnidia glauca* is well known for its hypoglycemic activities (Ghosh et al., 2012). It is regarded as a strong vesicant with agrochemical applications such as insecticidal, piscicidal, larvicidal and molluscicidal agents (Rao et al., 2013). Despite the folkloric use of *Gnidia glauca* against obesity and associated complications, it has not been empirically determined to support the traditional practitioners’ claims. The present study, therefore, aimed at assessing the phytochemical profile, anti-obesity, cognitive enhancing, neurobehavioral and antioxidant effects of dichloromethane leaf extract of *Gnidia glauca*. The generated data will act as a source of ‘qualified leads’ in the synthesis of new, effective and affordable anti-obesity drug, alternative antioxidant supplement and/or neurological agent.

1.2 Statement of the Problem

Obesity has attained epidemic proportions worldwide. It presents modifiable risk factors for cognitive impairment, type II diabetes mellitus, coronary heart disease, hypertension, psychological disorders, knee osteoarthritis, and certain cancers. Obesity is ranked as the fifth leading risk factor for global deaths and is projected to be the third leading cause of death by the year, 2030. Obesity has been associated with cognitive decline, poorer cognitive performance and increased risk of depression, anxiety, dementia and Alzheimer’s disease. Moreover, it exacerbates the odds of developing spontaneously emitted behaviors such as activity patterns, anxiety and exploration. It alters the motor functions thereby decreasing locomotor activity and exploration. Although the medical consequences of obesity are of central concern to researchers and clinicians, obesity and the resultant metabolic complications appears to affect adversely an individual's capacity to live a comfortable and active life.
Obesity and associated health problems account for a significant amount of Kenyan health care spending costs. Direct medical costs include preventive, diagnostic and treatment services related to obesity. Indirect costs are related to lost income from decreased productivity, restricted activity, and absenteeism from work, as well as a loss of future income due to premature death.

For patients who cannot achieve sufficient weight loss through lifestyle and behavioral modification, anti-obesity drug therapy is often useful as an adjunct. However, such conventional drugs have been found to be unaffordable, unavailable and often associated with undesirable side effects. These have necessitated the upsurge in utilization of herbal prescriptions due to their affordability, easy accessibility and are firmly embedded within wider belief systems of many people especially in poor resource economies. Therefore, herbal medicines remain a key resource for finding more efficacious and cheaper alternative therapies in the prevention and control of obesity.

1.3 Justification of the Study
The epidemic of obesity is currently on the rise and has been associated with increased morbidity and mortality worldwide. Approximately 600 million adults (18 years and older) and over 42 million children (under the age of 5) were diagnosed to be clinically obese. In Kenya, 17.3% of men and 43.4% of women were diagnosed to be clinically obese. One of every three Kenyan women is either obese or overweight. Moreover, obesity is projected to be the leading risk factor for global deaths by the year 2030. Obesity presents a modifiable risk factor for cognitive impairment, anxiety disorders and spontaneously
emitted behaviors such as locomotor activity and exploration. These conditions significantly contribute to reduced quality of life of the affected obese subjects and their caregivers. Attempts to decrease fat mass via pharmacological reduction of energy intake and fat mobilization have had limited potency and/or intolerable side effects. The development of a chemical agent that would be safe, efficacious and target the complex pathophysiology of obesity has proved to be a challenging task. However, newer insights in traditionally used medicinal plants are indispensable for exploration of their novel bioactive components.

Medicinal plants have drawn a sharp focus in recent times as complementary and alternative medicines owing to their biocompatibility, unlike the chemically synthesized drugs which have been associated with side effects and numerous health hazards. Herbal medicines have been a key bio-resource that meets the health needs of many people, especially in developing nations due to their affordability and easy accessibility. Usually, they have been widely perceived by traditional practitioners as being natural, healthful and free from side effects. The rationale for their utilization has rested largely on long-term clinical experience. However, the continued upsurge in use of herbal medicines has not been accompanied with scientific research to empirically determine their efficacy and safety profiles to support the traditional practitioner’s claims. Besides, herbal medicines have been viewed by the pharmaceutical industry as a source of ‘qualified leads’ in the synthesis of modern drugs. The present study, therefore, aimed at determining the anti-obesity, cognitive enhancing, neurobehavioral and antioxidant effects as well as phytochemical profiles of dichloromethanolic leaf extract of *Gnidia glauca*. The findings
from this study will provide ‘qualified leads’ in the synthesis of new anti-obesity, alternative antioxidant supplement and/or neurological agent.

1.4 Hypotheses

i. The dichloromethane leaf extract of *G. glauca* has no anti-obesity effects in high fat diet-induced obese rats.

ii. The dichloromethane leaf extract of *G. glauca* has no cognitive enhancing effects in high fat diet-induced obese rats.

iii. The dichloromethane leaf extract of *G. glauca* has no effects on locomotor activity, anxiety and exploration-like behaviors in high fat diet-induced obese rats.

iv. The dichloromethane leaf extract of *G. glauca* do not exhibit antioxidant activities.

v. There are no phytochemicals associated with anti-obesity, cognitive enhancing, neurobehavioral and antioxidant effects in dichloromethane leaf extract of *G. glauca*. 
1.5 Objectives

1.5.1 General Objective

To determine the anti-obesity, cognitive enhancing, neurobehavioral, antioxidant effects and phytochemical profile of dichloromethane leaf extract of *Gnidia glauca*.

1.5.2 Specific Objectives

i. To investigate the *in vivo* anti-obesity effects of dichloromethane leaf extract of *G. glauca* in high fat diet-induced obese rats.

ii. To explore the cognitive enhancing effects of dichloromethane leaf extract of *G. glauca* in high fat diet-induced obese rats.

iii. To assess the effects of dichloromethane leaf extract of *G. glauca* on locomotor activity, anxiety and exploration-like behaviors in high fat diet-induced obese rats.

iv. To determine the *in vitro* antioxidant activities of dichloromethane leaf extract of *G. glauca*.

v. To examine the presence of anti-obesity, cognitive enhancing, neurobehavioral and antioxidant phytochemicals in dichloromethane leaf extract of *G. glauca*. 
CHAPTER TWO
LITERATURE REVIEW

2.1 The Biology of Obesity

Obesity is a chronic metabolic disorder that is characterized by increased adipose tissue mass due to positive energy balance (Bray, 2007; De-la-Garza et al., 2011). Integrating factors of genetic, behavioral, social, cultural and physiological are also believed to contribute to its genesis (Mbochi et al., 2012; Hryhorczuk et al., 2013). Pathological obesity is characterized by an anomalous fat distribution that result in weight gain equaling to or greater than 20% of the standard bodyweight (Westerterp-Plantenga, 2005).

2.2 Epidemiology and Impact of Obesity

The epidemic of obesity is currently on the rise due to increasingly sedentary lifestyles combined with easy availability of palatable, high-fat foods (Lykouras and Michopoulos, 2011). Once considered a problem only in high-income countries, obesity is now dramatically on the rise in low- and middle-income countries, particularly in urban settings (WHO, 2015). Globally, its prevalence has shown a startling increase in all age groups and has been associated with morbidity and mortality. In 2014, in excess of 1.9 billion adults, 18 years and older, were overweight and among them, at least 600 million were diagnosed to be clinically obese (WHO, 2015). Overall, about 13% of the world’s adult population (11% of men and 15% of women) were obese in 2014 (WHO, 2015). During the same year, 39% of adults aged 18 years and over (38% of men and 40% of women) were overweight (WHO, 2015). In 2013, 42 million children under the age of 5 were overweight or obese and out of this, more than 30% were from developing countries (WHO, 2015).
The Kenya Demographic and Health Survey (KDHS) of 2009 showed that the national prevalence of overweight and obesity for women (15–49 years old) in Kenya was 23% and 7% for men in the same category (KNBS, 2010). In the year 2013, 17.3% of men and 43.4% of women were diagnosed to be clinically obese in Kenya (Ettarh et al., 2013). Mkuu et al. (2018) also reported that one of every three Kenyan women is either obese or overweight (accounting to about 32.8% of women). The incidence of overweight and obese cases in Kenya was found to occur at a rate of 5% per year on average (Ziraba et al., 2009).

Obesity represents the most significant current health promotion and disease prevention priorities worldwide due to increased risks of associated morbidity and mortality (Garber et al., 2013). It presents modifiable risk factors for cognitive impairment (Guidi et al., 2006), neural disorders (Sas et al., 2007), Alzheimer’s disease (Smith et al., 2000), ageing (Hyun et al., 2006), type II diabetes mellitus (Luppino et al., 2010) and coronary artery disease (Lykouras and Michopoulos, 2011). Although the medical consequences of obesity are of central concern to researchers and clinicians, obesity also appears to affect adversely an individual's capacity to live a full and active life (Karasu, 2012). It exacerbates an individual’s psychological disorders such as poor self-esteem, depression and poor employment prospects which substantially impacts a person's functional capacity and quality of life (Richards et al., 2011).

The escalating public health crisis accounts for a significant amount of healthcare spending across nations (Fontaine et al., 2003). Direct medical costs especially in preventive care, diagnoses and treatment result in greater utilization of services and correspondingly greater
healthcare costs (Oliver and Lee, 2005). Indirect costs relate to lost income from decreased productivity, restricted activity, absenteeism as well as a loss of future income due to shortened lifespan (De-la-Garza et al., 2011). These, therefore, highlights the need for effective therapeutic strategies to prevent obesity and its associated complications (De-la-Garza et al., 2011).

2.3 Etiology, Pathophysiology and Chronobiology of Obesity

Obesity could be conceptualized as a consequence of the interaction of exogenous factors (environmental) and endogenous factors (individual genetic predisposition) (Gurevich-Panigrahi et al., 2009). The precise pathogenesis of obesity is difficult to establish; however, the basic premise of the obese phenotype is the positive energy balance due to an imbalance in energy metabolism such that energy input exceeds energy expenditure (Chugh and Sharma, 2012).

The energy balance is determined by an interplay between caloric intake (ingestion and absorption of calories), energy storage and energy output (energy expended in physical activity, basal metabolic rate, and thermic effect of food) (Ravussin and Bogardus, 2000; York and Bouchard, 2000). Increasingly sedentary lifestyles or comforts of affluence, easy availability of palatable high-fat foods combined with minimized physical activity, reduced metabolic rate and thermogenesis are key determinants of positive energy balance (Hryhorczuk et al., 2013).

The cellular processes involved in the maintenance of the energy balance are tightly regulated through neural and humoral signal transduction between the gastrointestinal tract
and the CNS (Chugh and Sharma, 2012). Failure of fat cells to generate and send an appropriate signal to the central control site (the hypothalamus) for integration and/or infectivity in the relay and response mechanisms contributes to the development of obesity (Gurevich-Panigrahi et al., 2009). A number of chemical mediators and neurochemical pathways are involved in the regulation of feed intake, satiety, energy storage, and energy output and, hence, obesity (Chugh and Sharma, 2012).

2.3.1 Neurochemical Mediators of Food Intake

Leptin, a peptide hormone encoded by the ob gene, is mainly secreted by the white adipose tissue (WAT) which exclusively express leptin mRNA (Carr et al., 2004). The concentration of this hormone in circulation is modulated by feeding behaviors and is proportional to fat deposits and body mass index (BMI) in normal subjects (Gautron and Elmquist, 2011). The production of leptin is potentiated by profound hyperphagia, insulin, glucocorticoids, and estrogens and is decreased by β-adrenergic agonists (Diamond and Eichler, 2002; Bjorkman and Kahn, 2004).

In the hypothalamus, the two clusters of neurons of the arcuate nucleus express insulin and leptin receptors responsive to food intake and levels of fat depots (Schwartz et al., 2000). Neuropeptide Y (NPY) and agouti-related protein (AgRP) are colocalized in the first cluster while the other cluster of neurons contains the neuropeptide pro-opiomelanocortin (POMC) derivatives which act on melanocortin-4 receptors (MC4-R) releasing alpha melanocyte stimulating hormone (α-MSH) (Schwartz et al., 2000).
Food deprivation or a fall in energy stores reduces leptin levels and stimulates the release of the first cohort of neurons (NPY and AgRP) to increase appetite that in turn increases food intake and decrease sympathetic outflow thus lowering energy expenditure (Schwartz et al., 2000). The NPY also activates lipoprotein lipase in adipose tissue and promotes storage and synthesis of fats (Diamond and Eichler, 2002). The AgRP, in turn, inhibits α-MSH from exerting its anorexigenic capacity. High food intake increases leptin levels and stimulates the second group of neurons suppressing appetite and decreasing food intake (Schwartz et al., 2000). Optimal activity of this intricate physiology is essential for the maintenance of energy balance; however, any abnormality may disrupt the normal homeostatic processes resulting in undernutrition or obesity (Schwartz et al., 2000). Other neuropeptides such as orexin-A and B and melanin concentrating hormones (MCH) increases appetite while neuropeptides like corticotropin-releasing hormone (CRH), 5-hydroxy tryptamine (5HT), prolactin-releasing peptide, serotonin and cocaine, and amphetamine-related transcripts contributes to a decrease in food intake (Srivastava et al., 2007).

Serum insulin induces hypoglycemic states by increasing glucose uptake, stimulates leptin release from fat cells and centrally inhibits secretion of NPY. Ghrelin is an orexigenic, somatotrophic and adipogenic hormone synthesized in the stomach from its prohormone pro-ghrelin by posttranslational processing (Hosoda et al., 2002; Hosoda et al., 2006). Ghrelin stimulates appetite and decreases energy expenditure through its activation of NPY and inhibition of pro-opiomelanocortin neurons (Van-der-Lely et al., 2004). Obestatin is another preproghrelin peptide hormone synthesized in the stomach. It is an anorexic
hormone with contrasting biological activities with ghrelin. Obestatin reduces gastric emptying, inhibit feed intake and thus prevent weight gain (Wren et al., 2001). Cholecystokinin (CCK) is secreted by duodenum postprandial and acts on the CCK-A receptor in the gastrointestinal tract to decrease food intake. Cholecystokinin does not cross the blood-brain barrier, however, the centrally synthesized CCK binds with the CCK-B receptors and functions as a satiety factor (Gurevich-Panigrahi et al., 2009).

2.3.2 Genetic Determinants of Obesity
Genetics plays a key role in the pathogenesis of obesity or significantly enhances susceptibility to its development (Banning, 2005; Gurevich-Panigrahi et al., 2009). Single gene mutations in leptin and leptin receptors (Lepr), pro-opiomelanocortin (POMC), melanocortin 4 receptor (MC4-R) and prohormone convertase-1 (PC1) represents the most common causes of monogenic obesity (Karam and McFarlane, 2007; Gurevich-Panigrahi et al., 2009). Multiple allelic variants occurring in the same molecular pathway of determinants of monogenic obesity may interact additively or synergistically to produce a set of associated clinical obese phenotypes (syndromic obesity) due to accelerated adiposity (Chung, 2012). The most common dysmorphic syndromic forms of obesity for which genetics play a crucial role include the Prader-Willi syndrome, the Laurence-Moon-Biedl syndrome, Ahlstrom’s syndrome, Cohen’s syndrome, and Carpenter’s syndrome (O’Rahilly and Farooqi, 2006). Mutations in other factors important in thermogenesis (such as beta-3-adrenergic receptors and uncoupling proteins) and appetite regulation (such as NPY, AgRP and α-MSH are relatively common in severely obese patients (Glowinska et al., 2003).
Presence of endocrinologic disorders such as polycystic ovaries syndrome (PCOS), Cushing’s syndrome, hypothyroidism, growth hormone deficiency, hypogonadism, hypothalamic tumors, trauma and inflammation results in obesogenic states (Karam and McFarlane, 2007).

2.3.3 Chronobiology of Obesity

Maintenance of the homeostatic balance mediates precise timing of the endogenous circadian clock that enables an individual’s capacity to predict, anticipate and temporally adjust the physiological, biochemical and behavioral functions to the daily environmental changes (Garaulet et al., 2010a). However, the current societal habits such as high snacking frequency, a decrease in the time spent sleeping, jet-lag, shift work and increased exposure to contemporary artificial blue lights during the night, induces a breakdown or loss of ‘feeling’ for internal circadian rhythms and the 24-hr environmental cycles (Erren and Reiter, 2009; Garaulet et al., 2010a). This disruption of the internal temporal order or the circadian systems (chronodisruption) by external rhythms leads to obesity with an increased risk of developing cardiovascular diseases, premature aging, some cancers (such as endometrial, breast, melanoma, prostate and colorectal) (Otalora et al., 2008), mood disorders and cognitive impairment (Wirz-Justice, 2006; Froy, 2007).

The main components of the circadian system include central oscillators (such as suprachiasmatic nucleus (SCN), a main central pacemaker, located in the hypothalamus) and the peripheral oscillators (mainly seen in specific organs and tissues including the adipose tissue, heart, liver, lung, intestine and adrenal gland) (Guilding and Piggins, 2007).
Peripheral oscillators are self-sustained oscillators but are sensitive to their own synchronizers such as feeding time, body temperature, retinoic acid, glucocorticoids, adrenaline, noradrenaline, prostaglandins, angiotensin-II and glucose (Zvonic et al., 2006). The conceptualized core machinery of the molecular circadian clock that modulates the adipocyte differentiation and lipogenesis include the Circadian Locomotor Output Cycles Kaput (CLOCK), the Period Circadian Protein-2 (PER2) and the Brain- and muscle ANRT-like protein-1 (BMAL1) (Kohsaka and Bass, 2007).

In our modern 24/7 society, the main mediators of chronodisruption that predisposes an individual to weight gain include suboptimal spectrum or insufficient intensity of the nocturnal artificial blue lights which have a longer wavelength relative to the natural light (Turner and Mainster, 2008) and nocturnal leisure activities such as excess energy intake that disrupt temporal communication between food intake signals through modification of the duration, strength and frequency in their rhythmicities (Gomez-Abella et al., 2010). Other obesity-induced chronodisruption mediators include jet-lag and shift work that alters the rates of synchronization between SCN and peripheral oscillators due to an induced difference in their coupling strengths, as well as the presence of mutations in molecular circadian clock proteins (such as BMAL1, PER2 and CLOCK genes) (Kondratov, 2007).
2.4 Classification of Obesity

Obesity can be classified based on the pattern of fat distribution in the body. In some people, excess fat can be stored primarily in the truncal-abdominal region while others in the gluteal and femoral area giving rise to two patterns of obesity, namely: android obesity and gynoid obesity (Bray, 2007).

2.4.1 Android Obesity (Apple Shape)

This is the type of obesity in which fat is stored primarily in the abdominal area, defined as a waist circumference greater than 102 cm in men and greater than 88 cm in non-pregnant women (Bray, 2007). It is predominantly associated with males. It is a public health concern because of its association with disease states such as hypertension, insulin resistance, cognitive deficits, breast cancer, stroke, diabetes and CVDs (Wildman and Medeiros 2000).

2.4.2 Gynoid Obesity (Pear Shape)

It is also known as gluteo-femoral obesity. Gynoid obesity is the typical female pattern where excess fat stores accumulate in the periphery, specifically hips, thighs, and buttocks. Individuals with a gynoid fat distribution (often called “pear” shape), are at a greater risk of mechanical problems (locomotor activity) (Haslam et al., 2006).
2.5 Diagnosis of Obesity

In clinical settings, the obesity diagnostic algorithm in a crude population incorporates assessment of body fat mass using ethnicity-adjusted anthropometric indices as well as the presence and severity of specific obesity-related complications (Garber et al., 2013). In humans, obesity is widely quantitated in terms of Body Mass Index (BMI) defined as the ratio of the weight (kg) and the square of height (m²) (Heymsfield et al., 2009). Based on BMI, obesity can be categorized into class I obesity, defined by a BMI between 30.0-34.9, class II obesity, defined by BMI of 35.0-39.9, and class III obesity (Morbid obese), defined by BMI≥40.0. The BMI between 25.0 and 29.9 defines people as overweight (pre-obese) while BMI of 18.5-24.9 and ≤18.5 indicates victims as being normal and underweight, β respectively (Goacher et al., 2012).

In animal rodent models, obesity is usually assessed based on the gain of body weight or the Lee obesity index and/or an increase in body fat content (Thibault and Hariri, 2010). The Lee index for assessing obesity in rat models is similar to BMI in humans. It is defined as the cube root of body weight (g) divided by the naso–anal length (cm) and multiplied by 1000. Values above 310 are diagnostic of obesity (Lee, 1929; Thibault and Hariri, 2010). However, across age, gender, animal species as well as ethnicity (in case of humans), BMI is limited by differences in body adiposity due to its inability to differentiate between fat mass and lean mass (Jackson et al., 2002).

Since metabolic complications of obesity are more closely related to visceral adiposity than overall adiposity, other anthropometric measures of adiposity which considers body fat
distribution such as waist circumference (WC), waist-to-hip ratio (WHR) and waist-to-height ratio (WHtR) are more likely to complement than substitutes (WHO, 2003; Cornier et al., 2011). Waist circumference is a useful predictor of the intra-abdominal fat thickness (visceral fat mass) in normal subjects and has been shown to significantly correlate with cardiovascular disease in humans (Han et al., 1995; Roopakala et al., 2009). A waist circumference greater than 40 inches (102 cm) in men or 35 inches (88 cm) in non-pregnant women defines abdominal/central obesity (Bray, 2007). However, WC does not account for differences in height, therefore, potentially over- and under-evaluating risk for tall and short individuals respectively (Browning et al., 2010). To compensate for this perceived deficit, current meta-analyses and systematic reviews have proposed the waist-to-height ratio (WHtR) as an alternative to WC since it offers a better prediction of CVD risk factors (Ashwell et al., 2012; Savva et al., 2013). A WHtR cutoff value of 0.5 in both males and females has been proposed as a better predictor of cardiometabolic risk (Li et al., 2016).

The ratio of waist-to-hip circumferences (WHR) like other anthropometric measurements is inexpensive, quick and easy to perform measures of the degree of peripheral (subcutaneous) and central (visceral, abdominal) adiposity. Visceral fat mass is a major risk for metabolic disorders, whereas peripheral fat mass appears to be benign to metabolic complications (Ferreira et al., 2004). The WHR above 0.84 in females and 0.94 in males suggests an undesirable obesity pattern of abdominal fat accumulation (Gurevich-Panigrahi et al., 2009).
Additional screening procedures that are standard for ‘new’ patient visits in a clinical setup includes the physical examination of the patient, family history, blood pressure, fasting lipid panel (such as total cholesterol, triglycerides, LDL-c, VLDL-c and HDL-c), fasting glucose, hepatic transaminases, creatinine as well as assessment of diet, meal pattern preferences and levels of physical activity (Lin et al., 2012). The manifestation of three or more of the following risk factors confers a high absolute risk: patient age of 45 years or older for men or 55 years or older for women; cigarette smoking; family history of premature coronary heart disease (myocardial infarction or sudden death at or before age 55 years in father or age 65 years in mother); High-density lipoprotein (HDL) cholesterol less than 35 mg/dL; impaired fasting glucose between 110 to 125 mg/dL; hypertension (systolic blood pressure 140 mm Hg or greater or diastolic blood pressure 90 mm Hg or greater); Low-density lipoprotein (LDL) cholesterol of 160 mg/dL or greater (Bray, 2013a).

According to the American Academy of Pediatrics, childhood obesity is defined as a percentile of a BMI range plotted on an appropriate age/gender CDC (Center for Disease Control) growth chart at or above the 95th percentile (Barlow, 2007). The BMI in children can be calculated as ([weight (kg)/height (cm)] ÷ [height (cm) x 10,000]) and plotted using the Centers for Disease (CDC) growth charts (CDC, 2013). A BMI between the 85th and 95th percentiles on an age/gender appropriate growth chart defines overweight while a BMI between the 5th and 85th percentile is considered healthy weight (Fowler and Kahwati, 2004; US Preventive Services Task Force, 2010).
Other techniques which have been applied for assessment of body fat include bioelectrical impedance analysis, air-displacement plethysmography, Computerized Tomography (CT), Dual-energy X-ray Absorptiometry (DXA), Magnetic Resonance Imaging (MRI) and skinfolds measurements using a Vernier caliper (Lobstein et al., 2004). These techniques provide an additional accurate, precise and practical approach for the assessment of body fat mass (Lobstein et al., 2004).

2.6 Obesity and Spontaneous Emitted Behaviors

Obesity is the main component of metabolic syndromes involving distinct etiologies which target different underlying behavioral and biological functions within the brain structures and circuits. An alteration in the neuronal circuitry stemming from abdominal or central adiposity stimulates a cascade of changes in neurochemical signaling that directly or indirectly mediate spontaneously emitted behaviors such as locomotor activity patterns, anxiety, and exploration (Stachowiak et al., 2013; Marrisal-Arvy et al., 2014).

Obesity is associated with decreases in motor output, often termed “physical inactivity” due to altered coordination of motor and reflexive responses (Bouchard et al., 2015). Increased adiposity contributes to physical inactivity which is largely characterized by decrements in balance, muscle strength, and coordination (Muramoto et al., 2014). Studies have demonstrated that deficits in striatal dopaminergic signaling have contributed to the persistent reductions in motor output in obesity (Kravitz et al., 2016).
Chronic exposures to obesogenic diets affect the dopamine synthesis, dopamine release and striatal-dopamine receptor function (Kravitz et al., 2016). The motor circuitry can also be altered by the decreases in expression levels of brain-derived neurotrophic factor (BDNF) and its tyrosine kinase receptor, TrkB, in hypothalamic nuclei which affects the strength of synaptic connections or dendritic spine density (Kravitz et al., 2016). Moreover, inhibition of the acetylcholinesterase (AchE) activity and damage to the peripheral muscle due to necrosis of skeletal muscle fibers enhances reduction of locomotor activity in animal models (Haque et al., 2001). Orexin A stimulates spontaneous physical activity and non-exercise activity thermogenesis (Kotz et al., 2002; Kiwaki et al., 2004). The orexin neurons project to the dopaminergic neurons in the substantia nigra that innervate the striatum and forms a critical component of motor activity (Hara et al., 2001). Therefore, an alteration in the expression of orexin, and/or it’s signaling, could exacerbate spontaneous physical inactivity and contributes to weight gain (Hara et al., 2001).

Locomotor activity is driven by exploration since its reduced form could possibly reflect reduced exploration. While locomotor activity is the function of performance on motor tasks, exploration involves some quality never previously experienced or familiar items arranged in unfamiliar ways (Edagha et al., 2015). Exploration is thus curiosity and attraction to novelty (Edagha et al., 2015).

The cognitive map theory postulates novelty as misrepresentation of an item or place in the cognitive mapping/locale system. The locale system is located within the hippocampus containing mental representations of previously perceived stimuli. Therefore, the
hippocampal system supposedly signals a lack of information about the current environment and exploration becomes a direct response to a mismatch detected (Crusio, 2001). Studies have indicated that reduction in explorative interest in obese rats indicates symptoms of depressive disorders consistent with those observed in patients suffering from anxiety disorders (Su et al., 2012).

The limbic circuitry that includes the amygdala modulates motivational states, such as desire, fear, and anxiety (Hong et al., 2014). Anxiety is a psychological disorder characterized by excessive fear, worry, muscular tension, restlessness, reduced concentration and vigilance syndromes (Adedayo and Seun, 2018).

The mechanisms underpinning obesity as a causal factor for anxiety relate to obesity-induced oxidative stress and inflammation (Popa-Wagner et al., 2013). Chronic exposures to calorically high dense diets have been shown to activate glial cells that forms the hallmark of inflammation in the brain (Orr et al., 2002). Activated microglia secretes neurotoxic inflammatory stress signals, such as tumor necrosis factor-alpha (TNF-α) (Alzoubi et al., 2013), interleukins (1β, IL-2, IL-6, IL-8, and IL-12) (Sahebkar, 2014) and monocyte chemoattractant protein (MCP). These proinflammatory mediators, in turn, precipitate inflammatory signaling cascade by activating other resident cells to produce additional molecules that perpetuate the activation of microglia in a positive feedback loop (Kreutzberg, 1996).
Anxiety disorders as the causal factor for obesity have been correlated with disruption of hypothalamic-pituitary-adrenal (HPA) axis which results in deregulation of autonomic functions in the hedonic reward circuitry (Kiernan and Bar’s, 2009; Esmaily et al., 2015). These factors create a continuous vicious obesity-anxiety cycle (Esmaily et al., 2015).

2.7 Psychopathology and Obesity

Recent studies have indicated an unusual prevalence of psychopathology in morbidly obese patients (Glinski et al., 2001). The most frequent finding showed that morbidly obese patients present with personality traits among which are self-doubt, sensitivity, insecurity, paranoia, poor impulse control, dependence and emotional instability (Van-Hout et al., 2004). A higher score on depression and anxiety disorders were recorded in many patients with morbid obesity. A preponderance of females compared to male obese patients was noted concerning interpersonal sensitivity, depression, somatization, paranoid ideation, obsessive-compulsive behavior, hostility and anxiety (Papageorgiou et al., 2002). Besides, obese female patients display an avoidance wait-and-see and passive response patterns as a coping mechanism towards relationships which are unreliable and relatively non-romantic (Horchner et al., 2002).

Obesity exacerbates psychological distress through low social adjustment, low self-esteem and numerous physical complaints (Glinski et al., 2001). These patients display very little cooperativeness when participating in societal roles due to their persistent failure to visualize themselves as autonomous and integrated (Garaulet et al., 2010a). In response, they are subject to prejudice and discrimination from their colleagues. Obese patients
predominantly record high scores on the compulsive scales through the expression of simplistic, rigid and sometimes moralistic ways of thinking (Glinski et al., 2001; Van-Hout et al., 2004). Additionally, their schizoid and paranoia states are high and correspond with interpersonal sensitivity and difficulties in expressing aggressive feelings (Glinski et al., 2001).

It is hypothesized that social discrimination and low self-esteem among the obese subjects contribute to high scores on the social avoidance, psychological distress and anxiety scales (Carr and Friedman, 2005; Puhl and Heuer, 2009). They interpret that the way others see them is the way they see themselves (Puhl and Brownell, 2003). The image of obese subjects has of themselves is one of dissatisfaction and rejection (Puhl and Heuer, 2009). They blame themselves for being fat and try to use various coping strategies to get thinner usually without proper planning and often results in failure. Weight control, preoccupation and aggressive personality traits contribute to increased food intake favoring hedonic reward of eating and, therefore, increases positive energy balance resulting in excessive worry and anxiety (Hasler et al., 2004; Kroenke et al., 2007). Distress from illness burden associated with obesity (such as cognitive deficits, anxiety, diabetes, hypertension, coronary artery disease, and some cancers) together with pharmacological factors used in their management, further enhances anxiety and/or depression (Muennig et al., 2006). In this way, another vicious circle is born (Ostbye et al., 2007).
2.8 Obesity and Cognition

Chronic exposures to high-fat diets have been linked with neuropathological changes that culminate in obesity-related cognitive dysfunction and brain alterations (Cho et al., 2016; Medic et al., 2016). These alterations in mental functioning are associated with comorbid conditions related to obesity such as diabetes mellitus, hyperlipidemia and cardiovascular diseases (Jurdak and Kanarek, 2011). The cognitive domains are mainly sub-served by the hippocampus and to a smaller extent the prefrontal cortex (Park et al., 2014). These domains play a critical role in learning (Molteni et al., 2002; Murray et al., 2009), memory (Kanoski and Davidson, 2011; Kosari et al., 2012), and the executive functioning (McNeilly et al., 2011). The hippocampus is specifically essential in the consolidation of short-term memories into long-term memories and is particularly important for spatial memory (Henke, 2010). The hippocampus is highly susceptible to any endo-or exogenous insults (Kanoski and Davidson, 2011). This suggests that any slight alteration in its morphology and function leads to deficits in episodic memory, spatial learning and memory retention (Neves et al., 2008; Lucassen et al., 2013).

Obesity and/or chronic consumption of high-fat diet is characterized by increased circulating levels of free fatty acids and systemic pro-inflammatory cytokines, chemokines, immune cells, and prostaglandins (Alzoubi et al., 2013; Miller and Spencer 2014). Peripheral proinflammatory cytokines may infiltrate the BBB and activates brain’s resident immune cells, the microglia, and astrocytes, to further produce inflammatory mediators which exacerbate oxidative damage in the hippocampus (Loane and Byrnes, 2010; Pistell et al., 2010; Popa-Wagner et al., 2013). These events, therefore, culminates in impaired
neurogenesis, synaptic remodeling and reduction in neuronal spine density (Morrison et al., 2010; Freeman et al., 2013). They also contribute to neuronal apoptosis, deregulated HPA axis, neurodegeneration and brain atrophy (Pepping et al., 2013; Tucsek et al., 2013).

The cross-link between insulin and leptin signaling pathways in the hypothalamus has a fundamental role in energy homeostasis and cognition (Taouis, 2011; Kumar et al., 2015). Leptin plays a key role in neurogenesis, synaptogenesis and axon growth and their low plasma levels have resulted in direct cognitive impairment (Boret, 2010; Taouis, 2011). Low insulin levels and insulin resistance in CNS represents a hallmark of cognitive deficits due to the facilitated accumulation of a neurotoxic protein known as beta-amyloid (Aβ) and neurofibrillary tangles of Tau (a microtubule-interacting/binding protein) (Takeda et al., 2010). The levels of Aβ corresponds with the severity of cognitive deficits as it modulates neuronal function, apoptosis-induced neuronal loss, degeneration of synapses, and depletion of neurotransmitters (Yoon and AhnJo, 2012; Castillo and Hernández, 2015).

2.9 Obesity and Oxidative Stress

Obesity, an energy-rich condition associated with overnutrition, impairs systemic metabolic homeostasis and elicits systemic oxidative stress (Furukawa et al., 2004). Oxidative stress represents a consequence of increased production of ROS and RNS as well as the attenuated capacity of antioxidant defenses (Ferry and Roussel, 2011; Sohal and Orr, 2012). The ROS and RNS interact with nucleic acids (DNA and RNA), proteins, lipids, carbohydrates and exacerbates oxidative damage through carbonylation, peroxidation, nitration and nitrosylation reactions (Halliwell, 2006; Newsholme et al., 2012).
Under obesogenic states, the increased production of ROS and RNS occurs through mitochondrial and peroxisomal oxidation of fatty acids (Fernández-Sánchez et al., 2011). Besides, facilitated adiposity causes low-grade inflammation of the adipose tissues (Marseglia et al., 2014). This condition activates the innate immune system in adipose tissue and promotes the secretion of pro-inflammatory cytokines such as TNF-α, interleukin (1β and 6) (Fonseca-Alaniz et al., 2007). A rise in the concentration of pro-inflammatory cytokines further increases the generation of ROS and RNS by macrophages and monocytes. The ROS and RNS also induce expression of adhesion molecules and growth factors (such as connective tissue growth factor, insulin-like growth factor-1 (IGF-1), platelet-derived growth factor, and vascular cell adhesion molecule-1) (Lavrovsky et al., 2000) through redox-sensitive transcription factors, particularly NF-κB and the NADPH oxidase pathway (NOX) (Shoelson et al., 2007).

Oxidative stress due to catalytic action of ROS and/or RNS on serves as a pre-requisite for the pathogenesis of many pathological conditions such as cognitive impairment (Guidi et al., 2006), neurological disorders (Shetty et al., 2008), atherosclerosis (Touyz, 2004) and some cancers (Kinnula and Crapo, 2004).
2.10 Management of Obesity

The general goals for weight management are to reduce body weight, maintain a lower weight over the long term and to prevent further weight gain. Principally, these can only be achieved through the maintenance of the negative energy balance (Chugh and Sharma, 2012). However, rapid weight loss is almost always followed by a gain of the lost weight (Redinger, 2007). A comprehensive approach is essential to losing weight permanently. Initial management involves lifestyle changes such as increased physical activity, behavioral therapy as well as dietary modification (low-calorie diet) (Chugh and Sharma, 2012). Anti-obesity drugs can be useful adjuncts to diet and exercise for obese patients. However, for morbidly obese patients, bariatric surgery (such as gastric banding, gastric bypass, sleeve gastrectomy, and duodenal switch) can be undertaken laparoscopically. Besides, surgical removal of the adipose tissue through liposuction or omentectomy may also be a treatment of choice for morbidly obese patients (Hocking et al., 2013). Here follows a summary of the main approaches to obesity management:

2.10.1 Dietary Changes and Physical Activity

Dietary management and physical activity are fundamental to obesity treatment (Sui et al., 2007). The ability of the patient to adhere to required diets not only depends on diet composition (such as low-carbohydrate diets, low-glycemic-index diets, low-fat diets, and high-protein diets) but also the total calories consumed (Sacks et al., 2009). Meta-analysis indicates that physical activity can produce favorable effects on cardiorespiratory fitness and decreases adverse effects associated with obesity (Church et al., 2007). However, physical activity alone has a limited effect on body weight, but the addition of exercise to
a dietary intervention increases the odds of successful long-term weight loss management (Catenacci et al., 2007; Dansinger et al., 2007).

### 2.10.2 Behavioral Modification

Behavioral modification is often useful as an adjunct to diet and physical activity to obese patients (Bray, 2013b). The goal of behavioral therapy is to enable patients to reduce and manage their weight by monitoring and modifying their food intake, increasing their physical activity level as well as recognizing and controlling those cues that trigger overeating (Bray, 2013b). High-intensity interventions such as self-monitoring, goal setting, and planning to address barriers to maintaining lifestyle changes over time should be practiced if obesity was to be effectively managed (LeBlance et al., 2011).

Maintenance of a constant homeostatic circadian system is an essential strategy for active weight management programs. The regularization of sleep-wake pattern, decrease in exposure to bright light, timed and regular feeding/nutrient intake rhythms and physical exercise are key in the prevention of circadian disruption (Garaulet et al., 2010b). All pharmacological, dietary and occupational habits that improve the circadian system status of an individual are critical in reducing the risk of obesity as well as improving the success of treatment (Garaulet et al., 2010b).
2.10.3 Pharmacological Treatment of Obesity

The successful management of obesity is possible through lifestyle changes in diet and physical activity (Yun, 2010). However, prescription of anti-obesity drugs can be helpful adjuncts to diet and exercise for obese adults who have failed to achieve weight loss through diet and exercise (Sharma et al., 2005). Pharmacotherapy is usually initiated only after 6 months of combined lifestyle therapy when the patient has lost < 0.4 kg/week for patients with a BMI ≥ 27 kg/m² with concomitant obesity-related risk factors or BMI ≥ 30 kg/m² (without concomitant obesity-related risk factors). Monitoring for adverse effects is essential when pharmacotherapy is initiated (Chugh and Sharma, 2012).

2.10.3.1 Centrally Acting Drugs

1. Neuropeptide Y Receptor Antagonists.

Obinepitide is a synthetic analog of a pancreatic polypeptide hormone. Pancreatic polypeptide is normally released upon ingestion of a meal and act as a satiety signal to regulate appetite and food intake. Obinepitide acts selectively on Y2 and Y4 receptors. Subcutaneous administration of this agent significantly inhibited the consumption of food for up to 9 hours in phase I/II clinical trials. It was found to be safe and was well tolerated (Chugh and Sharma, 2012).

2. Agouti-Related Protein Inhibitor

The TTP435 is a potent oral acting selective inhibitor of Agouti-related protein (AgRP). The AgRP is released by neurons in the arcuate nucleus to increase food intake and decrease energy expenditure (Chugh and Sharma, 2012). Studies in animal models of
obesity demonstrated that TTP435 reduced food intake and body weight gain, reduced-fat composition and decreased insulin levels in a dose-dependent manner. This novel compound is being evaluated in phase II clinical trials (Chugh and Sharma, 2012).

### 2.10.3.2 Drugs Affecting Fat Metabolism

#### 1. Methionine Aminopeptidase 2 Inhibitor.

Methionine aminopeptidase 2 inhibitor known as Beloranib (ZGN-433) modulates fat metabolism (Chugh and Sharma, 2012). Preclinical studies in mice and dogs indicated an improvement in cardiovascular risk factors such as lipid profile, leptin, and adiponectin. Moreover, it resulted in the loss of weight with no net increase in blood pressure. Beloranib (ZGN-433) is now under evaluation for its safety and pharmacokinetics (Chugh and Sharma, 2012).

#### 2. Diglyceride Acyltransferase (DGAT) Inhibitor.

Diglyceride acyltransferase is an enzyme that participates in triglyceride synthesis and adipose tissue formation. The AZD7687 is a DGAT-1 inhibitor which limits adipocyte formation and subsequent obesity-related inflammation (Smith et al., 2000). Previous studies have demonstrated that deficiency of DGAT1 confers resistance to obesity and diet-induced hepatic steatosis in mice. Therefore, inhibition of adipogenesis could subsequently decrease levels of inflammatory mediators associated with metabolic abnormalities and obesity (Chugh and Sharma, 2012).
2.10.3.3 Drugs Affecting Fat Digestion

1. Lipase Inhibitors

**Orlistat** is a saturated derivative of lipstatin that inhibits the activity of pancreatic lipase resulting in reduced dietary fat absorption (Viner et al., 2010). It is approved for long-term use (>12 weeks) for weight management in obese patients (Li et al., 2005). The long-term clinical application together with an energy-restricted diet results in weight loss (Neovius et al., 2008). Orlistat increases fecal fat loss up to 30% with minimized systemic absorption (Padwal and Majumdar, 2007). The decrease in intestinal lipid digestion has been associated with the reduction in the intra-abdominal fat content (Rubio et al., 2007). Orlistat is also able to modestly decrease tachycardia, improve oral glucose tolerance and prevent the onset of type II diabetes mellitus (Torgerson et al., 2004). The commonly reported adverse effects associated with its long-term clinical use includes a wide range of gastrointestinal side effects such as bloating, steatorrhea, oily spotting, fecal urgency, and fecal incontinence as well as hepatic adverse effects (Viner et al., 2010).

**Cetilistat** is a highly lipophilic benzoxazinone novel drug that inhibits the activity of pancreatic lipases. Cetilistat has demonstrated a significant reduction in weight loss, improvement in glycemic control with significant reductions in plasma HbA1c and waist circumference, an important risk factor for Metabolic Syndromes. It is currently in phase III trials (Kopelman et al., 2010).
2.10.4 Herbal Approaches to Obesity Management

Globally, the upsurge in the prevalence of obesity and associated morbidity has presented unmet medical needs for safe and effective therapies (Chugh and Sharma, 2012). Attempts to decrease fat mass via pharmacological reduction of energy intake and fat mobilization have had limited potency and/or intolerable side effects (Goodpaster et al., 2005).

The development of a chemical agent that would be safe, efficacious and target the complex pathophysiology of obesity has proved to be a challenging task. However, newer insights in traditionally used medicinal plants are indispensable for exploration of their novel bioactive components (Arika et al., 2015). Medicinal plants have drawn a sharp focus in recent times as complementary and alternative medicines owing to their biocompatibility, unlike the chemically synthesized drugs which have been associated with side effects and numerous health hazards (Piero et al., 2015). Herbal medicines have been a key bi-resource that meets the health needs of many people, especially in developing nations due to their affordability and easy accessibility. Usually, they have been widely perceived by traditional practitioners as being natural, healthful and free from side effects. The rationale for their utilization has rested largely on long-term clinical experience (Arika et al., 2016).

The prospective treatment and/or preventive agents of herbal medicines are attributed to the phytochemicals contained in them (Pengelly et al., 2012). These phytobiotics confers multiple physiological effects that serve to protect from pathogenicity (De-la-Garza et al., 2011). Major phytococonstituents responsible for such activity among others are, polyphenols (curcumin, catechins, flavones, flavanols, monoterpenes, phenolic acids
(rosmarinic acid), tannins, chalcones and quercetin, allicin; and Saponins (triterpenoid, steroid saponins) (Slanc et al., 2009; De-la-Garza et al., 2011).

The pharmacological relevance underlying the mechanistic approach to management of obesity and associated complications by these phytobiotics include appetite suppression via central receptors (NPY, AgRP, CB-1 and dopamine receptors), inhibition of triglyceride absorption, increase in lipolysis, improvement of glycemic control, adipose tissue differentiation as well as increase in energy expenditure and thermogenesis (Leung et al., 2003). Therefore, herbal medicines act as a source of ‘qualified leads’ in the synthesis of anti-obesity drugs.

In traditional African medicine, many herbs have been therapeutically applied against various ailments. One such medicinal plant is *Gnidia glauca*. *Gnidia glauca* is a genus that belongs to the family of Thymelaeaceae (Bhandurge et al., 2013). It is an evergreen shrub arising from a woody base or rhizome with a smooth to rough textured bark with or without lenticels. It is often ericoid with slender branches which are alternately positioned with opposite sessile leaves. *Gnidia glauca* is a very showy plant due to its conspicuous flowers (Bhandurge et al., 2013).

*Gnidia glauca* has been traditionally applied for the treatment of sore throat, abdominal pain, wounds, burns, and snake bites (Amarajeewa et al., 2007). It is well known for its anti-obesity, antidiabetic anticancer activities (Kareru et al., 2007). It is regarded as a strong vesicant with agrochemical applications as a molluscicide, insecticide, piscicide,
and even larvicidal agents (Rao et al., 2013). Even though *Gnidia glauca* has had traditional phytomedicinal and folkloric remedies against obesity and associated metabolic complications, it has not been empirically determined to validate traditional practitioner’s claims. The present study, therefore, aimed to determine the anti-obesity, cognitive enhancing, neurobehavioral and antioxidative effects and phytochemical profile of DCM leaf extract of *Gnidia glauca*. The generated data will form a proper guide for the synthesis of new, effective, affordable and safe anti-obesity drug, alternative antioxidant supplements and/or neurological agents.

Plate 2.1: A photomicrograph of *Gnidia glauca* taken on June, 2016 at Siakago Sub County, Embu County, Kenya.
CHAPTER THREE

IN VIVO ANTI-OBESEITY EFFECTS OF DICHLOROMETHANE LEAF EXTRACT OF Gnidia glauca IN HIGH-FAT DIET (HFD)-INDUCED OBESE RATS

3.1 Introduction

Obesity, a phenotypic manifestation of abdominal adiposity due to increased lipid consumption and sedentary lifestyle, is linked with impairment of systemic metabolic homeostasis that results in reduced life expectancy and/or increased health complications (González-Castejón and Rodriguez-Casado, 2011). Although genetic factors clearly contribute to the propensity of an individual to become obese, overconsumption of a high fat diet, altered regulatory signals of hunger and satiety, reduced physical activity, reduced thermogenesis and resting metabolic rate over a prolonged period of time may promote a positive energy balance leading to overweight and obesity (Swinburn and Egger, 2002). Hyperphagia, energy density and post-ingestive effects of the dietary fat are the main mediators of dietary obesity (Thibault and Hariri, 2010).

The procedures for quali-quantitative measurement of body adiposity ranges from simple and effective anthropometric methods (such as body mass index (BMI), waist circumference (WC), waist-to-hip ratio (WHR) and waist-to-height ratio (WHtR)) to magnetic resonance imaging and computerized technologies such as Dual-energy X-ray Absorptiometry (DEXA) technique (Savva et al., 2013). These techniques are essential for diagnosis, risk assessment and precise tracking of obese patients in terms of quantity and fat mass distribution (Savva et al., 2013). Intra-abdominal fat thickness (visceral fat mass) has been shown to be a significant predictor of metabolic disturbances including
cardiovascular diseases, atherosclerosis, dyslipidemia and hypertension (Parinita et al., 2012; Savva et al., 2013). Any significant change in the levels of lipids, atherogenic index and body adiposity index predisposes individuals to the development of atherosclerotic diseases, endothelial dysfunction as well as some cancers (Parinita et al., 2012).

The mainstay of non-pharmacological treatment of obesity is diet, behavioral modification and exercise program that has been individualized to the patient’s lifestyle and physical needs (WHO, 2002). However, prescription of anti-obesity drugs can be a useful adjunct for obese patients who have failed to achieve weight loss through diet and exercise program (Kang and Park, 2012). Despite the remarkable progress in the management of obesity by synthetic drugs, there has been a renewed interest in medicinal plants owing to their availability, affordability, and the easy biocompatibility, unlike the chemically synthesized drugs which have been associated with adverse effects and numerous health hazards (Kang and Park, 2012). Identification and evaluation of active principles from herbal prescriptions have become the prime focus in the validation of their folklore use and drug discovery programs. The anti-obesity effects of these bioactive compounds are mediated by regulation of various pathways including lipid absorption, feed intake and expenditure of energy, increased lipolysis, decreased lipogenesis as well as differentiation and proliferation of pre-adipocytes (Park and Kim, 2011). In this chapter, the in vivo anti-obesity activity of DCM leaf extract of *G. glauca* was determined in HFD-induced obese rats.
3.2 Materials and Methods

3.2.1 Collection of the Leaves of *Gnidia glauca*

The collection, processing, and extraction of the plant material were conducted as described in Fresh leaves of *Gnidia glauca* were collected from their natural habitat at Makunguru Village, Nthawa Location, Siakago Division, Mbeere North Sub-County, Embu County, Kenya (Figure 3.1; Table 3.1). An acknowledged taxonomist authenticated the botanical identity of the plant and a voucher specimen deposited at the National Museums of Kenya Herbarium for future reference. The specimen was assigned a voucher number as WAM-V1. Coordinates for locations of collection points were taken using a hand-held GPS machine model type Garmin etrex H and recorded as shown in Table 3.1. The study was undertaken in the animal handling and experimental laboratory at the Department of Biochemistry, Microbiology and Biotechnology, Kenyatta University.
Figure 3.1: Map showing Nthawa Location, Mbeere North Sub-County, Embu County

Table 3.1: Coordinates of Site of Collection of the Plant Sample

<table>
<thead>
<tr>
<th>Plant species</th>
<th>UTM Eastings</th>
<th>UTM Northings</th>
<th>Latitude DMS</th>
<th>Longitude DMS</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>G. glauca</em></td>
<td>348,712.48</td>
<td>9,936,131.99</td>
<td>0°34’39.61”S</td>
<td>37°38’25.72”E</td>
</tr>
</tbody>
</table>
3.2.2 Processing and Extraction of the Plant Material

Fresh leaves of *G. glauca* were collected and shade-dried at room temperature for 21 days. The dried leaves were milled into fine powder by use of an electric mill. The powdered plant material was kept at room temperature away from direct sunlight in a dry airtight plastic container ready for extraction.

Five hundred grams of powdered *G. glauca* leaves were soaked in 1litre of dichloromethane for 48hrs. The solution was decanted and then filtered using muslin cloth into a different dry clean conical flask. The filtrate was concentrated under reduced pressure using a rotary evaporator at 40°C to obtain a semi-solid residue (Evans *et al*., 2009). The yield of the plant extract was determined and subsequently refrigerated at -20°C until used for analysis.

3.2.3 Preparation of Appropriate Doses for Bioassays

The appropriate bioassay doses of DCM-leaf extract of *G. glauca* for 5 animals per group were prepared by dissolving 0.23g in 2.5ml of 1% DMSO (200mg/kg body weight), 0.29g in 2.5ml of 1% DMSO (250mg/kg body weight), and 0.35g in 2.5ml of 1% DMSO (300mg/kg body weight). Similarly, the dose of the reference drug, Orlistat, was prepared by dissolving 0.035g in 2.5ml of 1% DMSO (30mg/kg body weight). The 1% DMSO was prepared by mixing 9 ml of PBS with 1ml of 10% DMSO solution. In the entire dosing period, each experimental animal received a daily single-dose oral administration of 0.5ml of treatment solution. All the treatment solutions were stored at -20°C until used for bioassay.
3.2.4 Experimental Animals

Thirty female white albino Wistar rats of about eight to ten weeks and weighing 120±10g were bought from Kenya Medical Research Institute, Nairobi, Kenya and used in the present study. Before initiation of the experiment, the rats were housed in standard polypropylene cages and maintained under controlled room temperature (23±2°C) and humidity (55±5%) with 12hrs light and 12hrs dark cycle for one week for acclimatization (National Research Council, 2010). During this period, the rats were fed on standard laboratory diet, in the form of rodent pellets from Unga Feeds Limited, Nairobi, Kenya, and tap water ad libitum. The experimental protocols were approved by the Institutional Animal Ethics Committee of Kenyatta University and National Commission for Science, Technology and Innovation (NACOSTI).

3.2.5 Induction of Obesity

Obesity was induced by feeding the experimental animals with a prepared High Fat Diet (HFD) and water ad libitum for a period of 12 weeks. The composition of the experimental diet (g/kg diet) was done according to the formula described by Srinivasan (Srinivasan et al., 2005) with some modifications as shown in Table 3.2. Rats with Lee obesity index value of 310 (equivalent to BMI in humans) and above were considered obese (Lee, 1929) and used in the study.
Table 3.2: Composition of High Fat Diet

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Diet (g/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Powdered NPD</td>
<td>375</td>
</tr>
<tr>
<td>Lard</td>
<td>290</td>
</tr>
<tr>
<td>Casein</td>
<td>265</td>
</tr>
<tr>
<td>Corn oil</td>
<td>10</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>10</td>
</tr>
<tr>
<td>Vitamin and mineral mix</td>
<td>60</td>
</tr>
<tr>
<td>DI Methionine</td>
<td>03</td>
</tr>
<tr>
<td>Yeast Powder</td>
<td>01</td>
</tr>
<tr>
<td>Sodium Chloride</td>
<td>01</td>
</tr>
</tbody>
</table>

3.2.6 Experimental Design

In this study, a total of 30 rats were randomly grouped into six different groups of five rats each. Group I (normal control) consisted of normal rats maintained on standard chow diet for 12 weeks. There was no treatment given to these group of rats but for 1% DMSO from the 6th-12th week. Group II (negative control) comprised of rats maintained on high fat diet for 12 weeks to induce obesity. Besides 1% DMSO was administered to this group of rats from the 6th-12th week of study. Group III (positive control) consisted of HFD-induced obese rats treated with the reference drug, Orlistat, from the 6th to 12th week. Group IV-VI (experimental groups) comprised of HFD-induced obese rats that were administered with the DCM leaf extract of *G. glauca* at different doses of 200, 250 and 300 mg/kg body weight from the 6th to 12th week. During the entire dosing period, all the treated rats were maintained on a high fat diet. All the experimental rats received water *ad libitum* throughout the study period. The experimental design is summarized in Table 3.3.
Table 3.3: Experimental Design for *In Vivo* Anti-obesity Assays

<table>
<thead>
<tr>
<th>Groups (5 rats per group)</th>
<th>Treatments</th>
<th>Duration of Obesity Induction (weeks)</th>
<th>Duration of Treatments (weeks)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I (Normal control)</td>
<td>1% DMSO</td>
<td>No induction</td>
<td>No treatment</td>
</tr>
<tr>
<td>II (Negative control)</td>
<td>HFD+DMSO</td>
<td>6</td>
<td>No treatment</td>
</tr>
<tr>
<td>III (Positive control)</td>
<td>Orlistat+HFD</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>IV (Experimental group)</td>
<td><em>G. glauca</em> (200mg/kgbw) + HFD</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>V (Experimental group)</td>
<td><em>G. glauca</em> (250mg/kgbw) + HFD</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>VI (Experimental group)</td>
<td><em>G. glauca</em> (300mg/kgbw) + HFD</td>
<td>6</td>
<td>6</td>
</tr>
</tbody>
</table>

3.2.7 Determination of Body Weight

During the experimental period the body weight of each rat, was assessed in grams after every seven days using a digital Mettler PJ 3000 weighing balance.

3.2.8 Determination of Anthropometric and Morphological Measures

The anthropometric and morphological measures were determined once every week. The obesity index was defined by Lee index. The Lee index was calculated according to the formula described by Lee (1929).

\[
\text{Lee index (\%)} = \frac{3\sqrt[3]{\text{Body Weight (g)}}}{\text{Nose-to-Anus Length (cm)}} \times 1000
\]

Obesity was defined by a Lee index of greater than 310 (Lee, 1929). Following exposure to HFD (except for normal control group) for 6 weeks, all the rats in the negative control, positive control and extract-administered experimental groups attained the target
diagnostic value of obesity, indicating the end to the obesity induction phase. The Naso–Anal Length (NAL) (cm) of rats was measured by a non-extensible thread and readings taken using a ruler with an accuracy of 0.1 cm.

The abdominal circumference (AC) was assessed on the largest zone of the rat abdomen in front of the hindlegs using a non-extensible thread. Thereafter, the thoracic circumference (TC) was similarly measured behind the forelegs. During the entire period, the rats were placed in ventral position. The readings of both abdominal and thoracic circumferences were taken using a ruler with an accuracy of 0.1 cm. The abdominal circumference to thoracic circumference ratio (AC: TC) was calculated using the formula described by Novelli et al. (2007).

\[
AC: TC = \frac{\text{Abdominal Circumference (cm)}}{\text{Thoracic Circumference (cm)}}
\]

The Thoracic Circumference to Height Ratio (TCHtR) was also calculated according to the formula described by Novelli et al. (2007).

\[
TCHtR = \frac{\text{Thoracic Circumference (cm)}}{\text{Nosal–Anal Length (cm)}}
\]

(Novelli et al., 2007).

### 3.2.9 Blood Sampling for Fasting Blood Glucose

Blood sampling for determination of fasting blood glucose levels was carried out by lateral caudal vein tail bleeding with the subjects having been fasted for 8 hours. The tail was sterilized with 70% alcohol and then nipped using a 24G /26G needle. Blood samples were obtained once in every 7 days. Fasting blood glucose was measured each time with a glucose analyzer model (Hypoguard, Woodbridge, England).
3.2.10 Determination of Rectal Body Temperature of Rats

Rat body temperature was measured on the 84th day using Rectal probe thermometry method (inserting a thermometer into rectal cavity of a rat) (Lomax, 1966). Rectal temperatures were recorded at intervals of 30 min for two and half hours after feeding rats with a high fat diet and administration of the treatments.

3.2.11 Preparation of Blood for Hematological and Biochemical Parameters

On the day of sacrifice, all the animals were euthanized using an overdose of isoflurane in a glass vacuum desiccator following an overnight fast. The blood was drawn from the heart of each sacrificed rat through cardiac puncture (laterally or ventrally) using a 5 ml syringe with a 23G1 needle. The blood samples were collected into two carefully labelled vacutainers. The first portion of blood was used for determination of hematological parameters. A drop of blood from this sample was used to determine fasting blood glucose level using a glucose analyzer model (Hypoguard, Woodbridge, England). The other portion of blood in the second vacutainer was allowed to stand for 3 hours to ensure complete clotting. The clotted blood was centrifuged at 3000 rpm for 10 minutes and the resulting supernatant stored at -20°C until required for analysis of lipid profiles and other biochemical parameters.
3.2.12 Determination of Organ Weights and Relative Organ to Body Weight (Organo-Somatic Index)

The liver, kidneys, spleen, heart, lungs and brain were carefully dissected out and weighed using a digital Mettler PJ 3000 weighing balance. These organs were then stored in 10% neutral buffered formalin. Organo-somatic index (relative organ to body weight) was calculated using the formulae described by Vani et al. (2000).

Relative organ to body weight (%) = \( \frac{\text{Actual weight of the organ (g)}}{\text{Body weight of an individual rat on day of sacrifice (g)}} \times 100 \)

3.2.13 Determination of Adipose Depots

A midline laparotomy was performed following the sagittal plane, the intestines were removed, and retroperitoneum was exposed. The parametrial fat pad was cut at the midpoint of the base of the uterus and trimmed away along the length of the horns of both left and right ovaries. The retroperitoneal fat pad was excised as a triangular section extending laterally from the lower pole of the kidneys. While the kidneys were pulled toward the midline, the perirenal fat pad, that is seen embedded with adrenal glands, was dissected out just above the kidneys. Mesenteric fat pad found along the small intestines was dissected (by pulling gently) from the duodenum till the end of the colon. Placing the animal on its side, the flap of skin just below the ribcage was carefully peeled back rostrally exposing the inguinal subcutaneous fat pad which was dissected away from the underlying muscle. Finally, the BAT was dissected away from the interscapular depot in the neck region of the animal.

The four dissected intra-abdominal white fat pads (visceral WAT depots) (the retroperitoneal, perirenal, parametrial and mesenteric) as well as the one excised
subcutaneous white fat pad (inguinal) and BAT interscapular depot were weighed using Mettler PJ 3000 weighing balance. The weights of these tissues were combined to form the *ex-vivo* Fat Mass.

As a measure of adiposity, the Body Adiposity Index (BAI) was calculated by using the formula described by Singh *et al.* (2010).

\[
BAI \, (\%) = \left( \frac{\sum \text{Mesenteric} + \text{Retroperitoneal} + \text{Parameterial} + \text{Perirenal} + \text{Subcutaneous} + \text{BAT}}{\text{Final \ Body \ Weight}} \right) \times 100
\]

### 3.2.14 Determination of Biochemical Parameters

The separated sera were used for estimation of lipid profile, enzyme activities and other blood analytes. Lipid profiles such as the total cholesterol (TC), LDL-cholesterol, HDL-cholesterol and triglycerides (TAG) levels were determined using Olympus 640 chemistry auto analyzer. The activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP), lactate dehydrogenase (LDH), γ-glutamyltransferase (GGT) were also determined using Olympus 640 chemistry auto analyzer. Moreover, the blood levels of total bilirubin, direct and indirect bilirubin, creatinine and urea as well as total proteins were quantified using the same automated instrument. All the assays were performed based on the standard operating procedures (SOPs) written and maintained in the Department of Laboratory Medicine, Kenyatta National Hospital.
The levels of serum Very Low-Density Lipoprotein (VLDL) were calculated in accordance with the formula described by Friedewald and Fredrickson. (1972).

\[ \text{VLDL} = \frac{\text{Triglycerides (TG)}}{5} \]

Atherogenic index of plasma (AIP) was calculated using the formula as described by Nwangha. (2010).

\[ \text{Atherogenic index (AIP)} = \log \left( \frac{\text{Triglycerides}}{\text{HDL} - \text{C}} \right) \]

3.2.15 Determination of Hematological Parameters

Some end point haematological parameters such as red blood cells (RBCs) and related indices (hemoglobin, mean corpuscle hemoglobin concentration, mean corpuscle hemoglobin and packed cell volume/hematocrit), white blood cells (WBCs) as well as platelets and related variants (mean platelet volume and platelet distribution width) were determined using coulter count system Beckman Coulter (Thermo Fisher, UK). Air dried thin blood films stained with giemsa stain were examined microscopically using magnification X200 and X400 for differential leukocyte counts (DLCs) and cell morphologies, respectively. Neutrophil (N), lymphocyte (L), eosinophils (E), monocytes (M) and basophils (B) absolute counts (number of cells x 10^9) per liter were obtained by expressing their percent differential counts against the total WBC absolute counts (Jain, 1986).
3.2.16 Determination of Feed Intake

All the food intake was measured daily for the period of 6 weeks (during the treatment phase) on a particular time on per cage basis and the average food consumed calculated. Each animal was provided with each food component daily and feed intake determined by the food remnants method. The remnant feed was weighed before and after diet consumption. A digital Mettler PJ 3000 weighing balance was used for taking measurements throughout the study period.

3.2.17 Data Management and Statistical Analysis

The quantitative data on the anti-obesity effects of DCM leaf extract of *G. glauca* in HFD-induced obese rats was entered in the Microsoft® Excel spread sheet, where it was organized and then exported to statistical software Minitab for analysis. The data was found to conform with the assumptions of parametric data using box plot. One-way ANOVA was used to test the significance among the normal control group rats, negative control group rats, Orlistat-treated group of rats and extract-treated group of rats. The data was further subjected to Tukey’s post hoc for pairwise separation and comparison of means. The results were expressed as the Mean±Standard Deviation (SD) and presented in tables and graphs. All differences were considered statistically significant if $p \leq 0.01$. The Minitab Software (Version 17.1, NC, USA) was used to perform all the statistical analyses.
3.3 Results

3.3.1 Effects of DCM Leaf Extract of Gnidia glauca on Body Weights of HFD-Induced Obese Rats

As Table 3.4 shows, during the first week of study, the rate of weight gain in the normal control rats significantly differed from the gain in body weight in the second week \((p \leq 0.01)\). However, the rate of weight gain in the same group of rats did not differ significantly in the 3\(^{rd}\), 4\(^{th}\), 5\(^{th}\) and 6\(^{th}\) week of the study period \((p > 0.01; \text{Table 3.4})\). In the negative control group, there was a marked increase in body weight throughout the study period. Indeed, the rate of weight gain ranged from 10.87±2.71 % in the 1\(^{st}\) week to 36.34±4.27 % in the 6\(^{th}\) week (Table 3.4). On the other hand, treatment of rats with the reference drug, Orlistat, and the three extract doses markedly and progressively caused decreases in body weights from the 1\(^{st}\) to the 6\(^{th}\) week of the study period (Table 3.4). The rat models administered with the DCM leaf extract of G. glauca at a dose of 200mg/kg body weight showed negative changes in body weight from -5.45±1.79 % in the 1\(^{st}\) week to -27.32±2.63 % in the 6\(^{th}\) week (Table 3.4).

Analysis of the weekly percentage change of the body weights of rats was also conducted across all the experimental groups (Table 3.4). It was observed that there was a positive change in the rate of weekly body weights of rats in both the negative control and normal control groups (Table 3.4). However, the weekly percentage increase in body weights of rats in the negative control group were significantly higher than those of rats in the normal control group \((p \leq 0.01)\). The extract-treated and reference drug, Orlistat, treated groups of rats showed a negative weekly percentage change in body weights during the study period.
(Table 3.4). However, the extract treated group of rats had a significantly higher rate of decrease in weight per week than Orlistat-treated group of rats ($p \leq 0.01$; Table 3.4).
### Table 3.4: Effect of DCM Leaf Extract of *Gnidia glauca* on Body Weights of HFD-Induced Obese Laboratory Rats

<table>
<thead>
<tr>
<th>TREATMENT (mg/kg bw)</th>
<th>Percentage Change in Weekly Body Weights</th>
<th>% Δ in body weight/Week</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Week 1</td>
<td>Week 2</td>
</tr>
<tr>
<td>Normal Control</td>
<td>6.30±1.03&lt;sup&gt;c&lt;/sup&gt;</td>
<td>10.98±1.93&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Negative Control</td>
<td>10.87±2.71&lt;sup&gt;e&lt;/sup&gt;</td>
<td>14.69±2.87&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Positive Control</td>
<td>-1.14±0.41&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-3.02±0.97&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>HFD+200mg/kg</td>
<td>-5.45±1.79&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-8.54±1.91&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>HFD+250mg/kg</td>
<td>-3.61±1.57&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-6.94±1.36&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>HFD+300mg/kg</td>
<td>-3.40±0.40&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-6.89±0.51&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Means within respective rows followed by superscript of similar lowercase letters are not significantly different (p>0.01); means within respective columns followed by superscript of similar uppercase letters are not significantly different (p>0.01); **Key** -Δ- represents change in.
3.3.2 Effect of DCM Leaf Extract of *Gnidia glauca* on Anthropometric Measures of HFD-Induced Obese Experimental Rats

Generally, changes in anthropometric parameters were observed following treatment of HFD-induced obese rats with DCM leaf extract of *Gnidia glauca* (Tables 3.5-3.10). The results showed an increase in obesity index in the normal and negative control group of rats from the 1st to the 6th week of treatments (Table 3.5). On the other hand, treatment of rats with the reference drug, Orlistat, and the three doses of the plant extract caused a persistent decrease in the obesity index from the first to the last week of the study period (Table 3.5).

In the negative control group, obesity index increased from 5.54±1.946% in the 1st week to 9.54±4.28% in the 6th week of the study period. The normal control group rats also showed an increase of the obesity index from 1.59±0.66% in the 1st week to 5.62±1.88% in the 6th week (Table 3.5). Administration of the extract at doses of 250 and 300mg/kg body weight caused a negative change in obesity index from -1.63±0.77% and -1.56±0.29% in the 1st week to -8.25±1.50% and -10.59±0.68% in the 6th week of study respectively (Table 3.5). Similarly, rats treated with the reference drug, Orlistat, caused a reduction in obesity index from -0.43±0.29% in the 1st week to -5.94±1.73% in the 6th week of treatment (Table 3.5).

Analysis of the weekly rate of change in obesity index indicated a positive change in both the normal control and the negative control group rats (Table 3.5). However, the weekly percentage change in the obesity index was significantly higher in rats in the negative control group than those in the normal control group ($p \leq 0.01$; Table 3.5). Treatment of rats
with the three doses of the plant extract and the reference drug, Orlistat, caused a negative weekly percentage change in obesity index (Table 3.5). However, the extract-treated rats showed significantly decreased weekly rate of change in obesity index as compared to rats treated with the reference drug, Orlistat ($p \leq 0.01$; Table 3.5).
Table 3.5: Effect of DCM Leaf Extract of *Gnidia glauca* on Obesity Index (OI) of HFD-Induced Obese Laboratory Rats

<table>
<thead>
<tr>
<th>TREATMENT (mg/kgbw)</th>
<th>Obesity Index (OI) (%)</th>
<th>% Δ in OI/Week</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Week 1</td>
<td>Week 2</td>
</tr>
<tr>
<td>Normal Control</td>
<td>1.59±0.66&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.70±1.07&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>Negative Control</td>
<td>5.54±1.946&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6.04±1.66&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>Positive Control</td>
<td>-0.43±0.29&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.14±1.07&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>HFD+200mg/kg</td>
<td>-2.71±1.02&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-2.72±1.19&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>HFD+250mg/kg</td>
<td>-1.63±0.77&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-1.42±0.91&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>HFD+ 300mg/kg</td>
<td>-1.56±0.29&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-1.79±0.44&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Means within respective rows followed by superscripts of similar lowercase letters are not significantly different (p>0.01); means within respective columns followed by superscript of similar uppercase letters are not significantly different (p>0.01); **Key** -Δ- represents change in.
It was observed that rats in the normal control and negative control groups recorded a statistically significant positive change in abdominal circumference in the entire study period. Conversely, rats treated with the reference drug, Orlistat, and those treated with three extract doses indicated a statistically significant negative change in abdominal circumference throughout the study period (Table 3.6). In the normal control group, there was a statistically significant marked increase in abdominal circumference from $3.36\pm1.95\%$ in the 1st week to $18.85\pm0.92\%$ in the 6th week of study (Table 3.6). Similarly, in the negative control group, the abdominal circumference of rats statistically increased significantly from $4.88\pm1.47\%$ in the 1st week to $24.22\pm2.04\%$ in the 6th week of study (Table 3.6). The extract-treated groups of rats and Orlistat-treated rats showed significant reductions in abdominal circumference from the first to the last week of treatments ($p\leq0.01$; Table 3.6) which were comparable.

Analysis of the weekly percentage change of abdominal circumference of rats among experimental groups revealed a positive rate of change in abdominal circumference of rats in the normal control and negative control groups (Table 3.6). On the contrary, a negative weekly percentage change in abdominal circumference was indicated in rats treated with the reference drug, Orlistat, and those treated with the three extract doses (Table 3.6). The weekly percentage increase in abdominal circumference was significantly higher in negative control group of rats than normal control group rats ($p\leq0.01$). On the other hand, the weekly percentage decreases in abdominal circumference in the extract-treated rats and Orlistat-treated rats were comparable ($p>0.01$; Table 3.6).
Table 3.6: Effect of DCM Leaf Extract of *Gnidia glauca* on Abdominal Circumference (AC) of HFD-Induced Obese Laboratory Rats

<table>
<thead>
<tr>
<th>TREATMENT (mg/kgbw)</th>
<th>Percentage Change in Abdominal Circumference/Week (%)</th>
<th>% Δ in AC/Week</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Week 1</td>
<td>Week 2</td>
</tr>
<tr>
<td>Normal Control</td>
<td>3.36±1.95&lt;sup&gt;d&lt;/sup&gt;</td>
<td>4.78±2.59&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Negative Control</td>
<td>4.88±1.47&lt;sup&gt;d&lt;/sup&gt;</td>
<td>7.72±1.14&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Positive Control</td>
<td>-6.73±1.54&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-11.00±1.67&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>HFD+200mg/kg</td>
<td>-6.69±1.99&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-11.73±0.72&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>HFD+250mg/kg</td>
<td>-6.94±0.61&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-11.42±0.79&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>HFD+300mg/kg</td>
<td>-6.73±0.39&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-11.20±1.56&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Means within respective rows followed by superscript of similar lowercase letters are not significantly different (p>0.01); means within respective columns followed by superscript of similar uppercase letters are not significantly different (p>0.01); **Key** -Δ- represents change in.
Generally, results indicated that there was a statistically significant increase in the thoracic circumference of rats in the normal and negative control groups from the 1\textsuperscript{st} to the 6\textsuperscript{th} week of study (Table 3.7). On the contrary, the extract-treated rats and Orlistat-treated rats exhibited a statistically significant reduction in the thoracic circumference from the 1\textsuperscript{st} to the 6\textsuperscript{th} week of the study period (Table 3.7).

The normal control group indicated a significant increase in thoracic circumference between the 2\textsuperscript{nd} and the 5\textsuperscript{th} week ($p \leq 0.01$). However, there was no significant increase in thoracic circumference between the 5\textsuperscript{th} and 6\textsuperscript{th} week ($p > 0.01$). In the negative control group, there was a marked increase in thoracic circumference from 5.58$\pm$2.26\% in the 1\textsuperscript{st} week to 17.65$\pm$2.49\% in the 6\textsuperscript{th} week (Table 3.7). Findings also indicated that administration of the three extract doses caused significant reductions in the thoracic circumference of rats from the 1\textsuperscript{st} to the 6\textsuperscript{th} week of treatments ($p \leq 0.01$; Table 3.7). For instance, rats treated with the extract at a dose of 300mg/kg body weight caused a negative change in thoracic circumference from -5.72$\pm$2.04\% in the 1\textsuperscript{st} week to -29.23$\pm$0.53\% in the 6\textsuperscript{th} week of study (Table 3.7). Treatment of rats with the reference drug, Orlistat also caused significant reductions in the thoracic circumference of rats from the 1\textsuperscript{st} to the 6\textsuperscript{th} week of treatment ($p \leq 0.01$; Table 3.7). For example, Orlistat-treated rats indicated a negative change in thoracic circumference from -6.20$\pm$0.77\% in the 1\textsuperscript{st} week to -29.53$\pm$1.45\% in the 6\textsuperscript{th} week (Table 3.7).

Analysis performed on the weekly rate of change in thoracic circumference among the experimental groups indicated a positive change in thoracic circumference of rats in normal
control and negative control groups (Table 3.7). However, the weekly percentage change in the thoracic circumference of rats in the negative control was significantly higher than that of rats in the normal control group ($p \leq 0.01$; Table 3.7). Findings also showed a negative weekly percentage change in thoracic circumference of extract-treated rats and Orlistat-treated rats (Table 3.7). However, the weekly percentage decrease in the thoracic circumference of rats treated with the three extract doses was not statistically significant to that of rats treated with the reference drug, Orlistat ($p > 0.01$; Table 3.7).
Table 3.7: Effect of DCM Leaf Extract of *Gnidia glauca* on Thoracic Circumference (TC) of HFD-Induced Obese Laboratory Rats

<table>
<thead>
<tr>
<th>TREATMENT</th>
<th>Percentage Change in Thoracic Circumference/Week (%)</th>
<th>% Δ in TC/Week</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Week 1</td>
<td>Week 2</td>
</tr>
<tr>
<td>Normal Control</td>
<td>2.67±1.70&lt;sup&gt;d&lt;/sup&gt;</td>
<td>4.74±2.24&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Negative Control</td>
<td>5.58±2.26&lt;sup&gt;d&lt;/sup&gt;</td>
<td>8.84±2.25&lt;sup&gt;cd&lt;/sup&gt;</td>
</tr>
<tr>
<td>Positive Control</td>
<td>-6.20±0.77&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-9.60±1.19&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>HFD+200mg/kg</td>
<td>-6.19±0.86&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-10.31±1.34&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>HFD+250mg/kg</td>
<td>-5.84±2.25&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-9.63±1.61&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>HFD+ 300mg/kg</td>
<td>-5.72±2.04&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-9.50±0.99&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Means within respective rows followed by superscript of similar lowercase letters are not significantly different (*p*>0.01); means within respective columns followed by superscript of similar uppercase letters are not significantly different (*p*>0.01); **Key** -Δ- represents change in.
As Table 3.8 shows, rats in the normal and the negative control groups showed an increase in abdominal circumference to thoracic circumference ratio from the 1\textsuperscript{st} to the 6\textsuperscript{th} week of the study period (Table 3.8). On the contrary, findings indicated a decrease in abdominal circumference to thoracic circumference ratio in extract-treated rats and Orlistat-treated group of rats (Table 3.8).

Results indicated that the abdominal circumference to thoracic circumference ratio of rats in the normal control group increased from 3.07±0.63\% in the 1\textsuperscript{st} week to 11.54±2.72\% in the 6\textsuperscript{th} week (Table 3.8). Similarly, the abdominal circumference to thoracic circumference ratio of the HFD-induced obese untreated rats in the negative control group exponentially increased from 4.32±0.50\% in the 1\textsuperscript{st} week to 16.39±1.76\% in the 6\textsuperscript{th} week (Table 3.8). Treatment of rats with the extract dosages of 200, 250 and 300mg/kg body weight caused a statistical significant decrease in abdominal circumference to thoracic circumference ratio from -1.46±1.23\%, -0.95±1.94 and -1.39±1.03\% in the 1\textsuperscript{st} week to -7.33±1.44\%, -10.35±8.63\% and -15.14±5.91\% in the 6\textsuperscript{th} week respectively (Table 3.8). Administration of the reference drug, Orlistat, caused a statistically significant decrease in abdominal circumference to thoracic circumference ratio from -1.31±1.38\% in the 1\textsuperscript{st} week to -7.44±0.66\% in the 6\textsuperscript{th} week of the study period (Table 3.8).

The Table 3.8 also shows the weekly percentage change in abdominal circumference to thoracic circumference ratio. The results indicated that there was a positive change in the weekly rate of change of abdominal circumference to thoracic circumference ratio of rats in the negative control and normal control groups. However, the weekly percentage change
in abdominal circumference to thoracic circumference ratio of rats in the negative control group was significantly higher than that of rats in the normal control group \((p \leq 0.01; \text{ Table 3.8})\). Findings also revealed that there was a negative weekly percentage change in abdominal circumference to thoracic circumference ratio of rats treated with the three extract doses and those treated with the reference drug, Orlistat, (Table 3.8). The effects of the treatment with the three extract doses on the weekly percentage decrease in abdominal circumference to thoracic circumference ratio of extract-treated rats were not significantly different from those observed in rats treated with the reference drug, Orlistat \((p > 0.01; \text{ Table 3.8})\).
Table 3.8: Effect of DCM Leaf Extract of *Gnidia glauca* on Abdominal Circumference to Thoracic Circumference Ratio (AC:TC) of HFD-Induced Obese Laboratory Rats

<table>
<thead>
<tr>
<th>TREATMENT</th>
<th>Percentage Change in Abdominal Circumference to Thoracic Circumference Ratio/Week (%)</th>
<th>% Δ in AC:TC/Week</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Week 1</td>
<td>Week 2</td>
</tr>
<tr>
<td>Normal Control</td>
<td>3.07±0.63d</td>
<td>4.36±0.53cd</td>
</tr>
<tr>
<td>Negative Control</td>
<td>4.32±0.50e</td>
<td>5.88±0.49d</td>
</tr>
<tr>
<td>Positive Control</td>
<td>-1.31±1.38a</td>
<td>-2.10±1.38a</td>
</tr>
<tr>
<td>HFD+200mg/kg</td>
<td>-1.46±1.23a</td>
<td>-1.93±1.51a</td>
</tr>
<tr>
<td>HFD+250mg/kg</td>
<td>-0.95±1.94a</td>
<td>-1.97±1.60a</td>
</tr>
<tr>
<td>HFD+300mg/kg</td>
<td>-1.39±1.03a</td>
<td>-2.80±0.70ab</td>
</tr>
</tbody>
</table>

Means within respective rows followed by superscript of similar lowercase letters are not significantly different (*p*>0.01); means within respective columns followed by superscript of similar uppercase letters are not significantly different (*p*>0.01); Key -Δ- represents change in.
The results exhibited a statistically significant increase in abdominal circumference to height ratio of rats in the negative control and normal control groups from the 1\textsuperscript{st} to 6\textsuperscript{th} week of study (Table 3.9). On the other hand, there was a statistically significant decrease in abdominal circumference to height ratio of rats in extract-treated groups as well as those treated with the reference drug, Orlistat (Table 3.9). Treatment with the reference drug, Orlistat, caused a statistically significant decline in abdominal circumference to height ratio from -6.46±1.38\% in the 1\textsuperscript{st} week to -22.06±1.89\% in the 6\textsuperscript{th} week of study (Table 3.9). Similarly, treatment with the plant extract at dosage levels of 200 and 250mg/kg body weight caused a decrease in abdominal circumference to height ratio from -6.59±1.65\% and -6.57±0.60\% in the 1\textsuperscript{st} week to -20.13±3.52\% and -23.34±3.04\% in the 6\textsuperscript{th} week, respectively (Table 3.9). In addition, administration of the extract dose of 300mg/kg body weight significantly decreased abdominal circumference to height ratio from -6.45±0.49\% in the 1\textsuperscript{st} week to -25.10±2.22\% in the 6\textsuperscript{th} week of the study (Table 3.9).

Table 3.9 also shows the weekly percentage changes in abdominal circumference to height ratio among the experimental groups. The findings revealed a positive weekly percentage change in abdominal circumference to height ratio of rats in the normal and negative control groups. However, the weekly percentage increase in abdominal circumference to height ratio was significantly high in the negative control group rats relative to that of the normal control group rats \((p\leq0.01;\text{ Table 3.9})\). Further, results indicated a negative weekly percentage change in abdominal circumference to height ratio of rats treated with the three extract doses and those treated with the reference drug, Orlistat (Table 3.9). However, no significant difference in the weekly percentage change in abdominal circumference to
height ratio was indicated in the extract-treated rats and Orlistat-treated rats ($p>0.01$; Table 3.9).
Table 3.9: Effect of DCM Leaf Extract of *Gnidia glauca* on Abdominal Circumference to Height Ratio (ACHtR) of HFD-Induced Obese Laboratory Rats

<table>
<thead>
<tr>
<th>TREATMENT</th>
<th>Percentage Change in Abdominal Circumference to Height Ratio (ACHtR)/Week (%)</th>
<th>% Δ in ACHtR/Week</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Week 1</td>
<td>Week 2</td>
</tr>
<tr>
<td>Normal Control</td>
<td>0.71±1.90b</td>
<td>1.93±3.44ab</td>
</tr>
<tr>
<td>Negative Control</td>
<td>1.48±1.80d</td>
<td>3.67±3.15cd</td>
</tr>
<tr>
<td>Positive Control</td>
<td>-6.46±1.38a</td>
<td>-7.98±1.93a</td>
</tr>
<tr>
<td>HFD+200mg/kg</td>
<td>-6.59±1.65a</td>
<td>-8.65±1.97ab</td>
</tr>
<tr>
<td>HFD+250mg/kg</td>
<td>-6.57±0.60a</td>
<td>-7.46±2.42a</td>
</tr>
<tr>
<td>HFD+300mg/kg</td>
<td>-6.45±0.49a</td>
<td>-8.00±1.62a</td>
</tr>
</tbody>
</table>

Means within respective rows followed by superscript of similar lowercase letters are not significantly different (p>0.01); means within respective columns followed by superscript of similar uppercase letters are not significantly different (p>0.01); **Key** -Δ-represents change in.
The results showed a statistically significant progressive increase in thoracic circumference to height ratio of rats in both the normal control and negative control groups during the entire study period (Table 3.10). However, treatment with DCM leaf extract of *G. glauca* and the standard drug, Orlistat, caused a significant decrease in thoracic circumference to height ratio of rats throughout the treatment period (Table 3.10).

The HFD-induced untreated obese rats indicated an increase in thoracic circumference to height ratio from 5.55±4.21% in the 1st week to 17.74±4.87% in the 6th week of the study (Table 3.10). There was a significant decrease in thoracic circumference to height ratio of rats treated with the reference drug, Orlistat, from -5.91±0.10% in the 1st week to -23.26±1.62% in the 6th week of study (Table 3.10). A progressive decrease in the thoracic circumference to height ratio of rats was observed in the group of rats treated with the extract dose of 300mg/kg body weight from -5.43±1.93% in the 1st week to -21.10±1.69% in the 6th week of study (Table 3.10). However, there was no significant difference in the thoracic circumference to height ratio between the first two weeks of treatment with the same extract dose (*p>*0.01). It was also observed that there was a gradual decrease in the thoracic circumference to height ratio in rats treated with the reference drug, Orlistat, from 5.91±0.10% in the 1st week to -23.26±1.62% in the 6th week of treatment (Table 3.10).

Analysis of the weekly percentage change in the thoracic circumference to height ratio showed a positive weekly percentage change in thoracic circumference to height ratio of rats in both the normal control and negative control groups (Table 3.10). However, the weekly rate of change in the thoracic circumference to height ratio was significantly higher
in negative control group rats than that of rats in the normal control group ($p \leq 0.01$; Table 3.10). Findings also indicated a negative weekly percentage change in the thoracic circumference to height ratio in extract-treated rats and Orlistat-treated group of rats (Table 3.10). The activities of the three extract doses on the weekly rate of change in the thoracic circumference to height ratio was comparable to that of the reference drug, Orlistat ($p > 0.01$) (Table 3.10).
Table 3.10: Effect of DCM Leaf Extract of *Gnidia glauca* on Thoracic Circumference to Height Ratio (TCHtR) Of HFD-Induced Obese Laboratory Rats

<table>
<thead>
<tr>
<th>TREATMENT</th>
<th>Percentage Change in Thoracic Circumference to Height Ratio (TCHtR)/Week (%)</th>
<th>% Δ in TCHtR/Week</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Week 1</td>
<td>Week 2</td>
</tr>
<tr>
<td>Normal Control</td>
<td>1.97±1.11&lt;sup&gt;d&lt;/sup&gt;</td>
<td>3.89±1.34&lt;sup&gt;cd&lt;/sup&gt;</td>
</tr>
<tr>
<td>Negative Control</td>
<td>5.55±4.21&lt;sup&gt;c&lt;/sup&gt;</td>
<td>8.05±2.78&lt;sup&gt;bc&lt;/sup&gt;</td>
</tr>
<tr>
<td>Positive Control</td>
<td>-5.91±0.10&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-6.52±1.97&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>HFD+200mg/kg</td>
<td>-6.08±0.613&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-7.17±2.51&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>HFD+250mg/kg</td>
<td>-5.46±2.38&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-5.58±3.08&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>HFD+ 300mg/kg</td>
<td>-5.43±1.93&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-6.24±0.79&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Means within respective rows followed by superscript of similar lowercase letters are not significantly different (p>0.01); means within respective columns followed by superscript of similar uppercase letters are not significantly different (p>0.01); Key -Δ-represents change in.
3.3.3 Effect of DCM Leaf Extract of *Gnidia glauca* on Organ Weights and Relative Organ to Body Weights of HFD-Induced Obese Rats

The results demonstrated that treatment with the DCM leaf extract of *G. glauca* caused changes in organ weights of HFD-induced obese rats (Tables 3.11). The weights of the liver, kidney, heart, lungs and brain were significantly higher in the negative control group rats than the normal control group rats, Orlistat-treated rats and extract-treated rats (*p*≤0.01; Table 3.11). On the contrary, the weight of the spleen was significantly low in the negative control group rats relative to that of rats in the normal control, positive control and extract-treated groups (*p*≤0.01; Table 3.11).

The extract-treated rats indicated a significantly lower weight of the liver than the normal control group rats (*p*≤0.01). However, the weight of the liver of extract-treated rats was not statistically significant from that of Orlistat-treated rats (*p*>0.01; Table 3.11). Similarly, there was no significant difference in the weights of the kidneys and the brain between extract-treated rats and Orlistat-treated rats (*p*>0.01; Table 3.11). However, the weights of the two organs were significantly higher in the normal control group rats than extract-treated group of rats (*p*≤0.01). The weight of the heart of rats treated at extract dosages of 250 and 300mg/kg body weight was statistically significant compared to that of rats treated with the reference drug, Orlistat (*p*≤0.01; Table 3.11).

There was a significant decrease in weight of lungs in extract-treated rats relative to the negative control group rats (*p*≤0.01). The extract treatment lowered the weight of lungs of rats in all the three extract doses compared to that observed in normal control group rats.
(p≤0.01). However, the weights of lungs of rats administered with the three extract doses was not significantly different from that of Orlistat-treated rats (p>0.01; Table 3.11).
Table 3.11: Effect of DCM Leaf Extract of *Gnidia glauca* on Organ Weights of HFD-Induced Obese Rats

<table>
<thead>
<tr>
<th>TREATMENT</th>
<th>Organ weights (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Liver</td>
</tr>
<tr>
<td>(mg/kgbw)</td>
<td></td>
</tr>
<tr>
<td>Normal Control</td>
<td>10.33±0.44b</td>
</tr>
<tr>
<td>Negative Control</td>
<td>23.13±0.74a</td>
</tr>
<tr>
<td>Positive Control</td>
<td>6.22±0.37c</td>
</tr>
<tr>
<td>HFD+200mg/kg</td>
<td>6.65±0.17c</td>
</tr>
<tr>
<td>HFD+250mg/kg</td>
<td>6.55±0.33c</td>
</tr>
<tr>
<td>HFD+ 300mg/kg</td>
<td>6.07±0.07c</td>
</tr>
</tbody>
</table>

Means followed by similar lower-case letters within respective columns are not statistically different (p>0.01).
The DCM leaf extract of *G. glauca* caused changes on relative-organ weights (organo-somatic index) of HFD-induced obese rats (Tables 3.12). The organo-somatic indices of liver, kidney, heart, lungs and brain of rats in the negative control group were significantly higher than those of rats administered with reference drug, Orlistat, and the three extract doses of *G. glauca* (*p*≤0.01; Table 3.12). However, the organo-somatic indices of the spleen were significantly low in the negative control group rats relative to extract-treated and Orlistat-treated groups of rats (*p*≤0.01; Table 3.12). The effect of the extract was as effective as that of the reference drug, Orlistat, on the organo-somatic indices of the kidneys, spleen and brain (*p*>0.01; Table 3.12). Further, the extract-treated rats showed significantly lower weight of liver than normal control group rats and Orlistat-treated rats (*p*≤0.01; Table 3.12).

Findings also indicated that the organo-somatic indices of heart of rats treated with the extract dose of 200mg/kg body weight were statistically similar to those of rats in the normal control group and Orlistat-treated group of rats (*p*>0.01; Table 3.12). However, the organo-somatic indices of the heart at the extract dosages of 250 and 300mg/kg body weight were significantly lower than those of rats in the normal control group and Orlistat-treated group of rats (*p*≤0.01; Table 3.12). Notably, the organo-somatic indices of lungs of extract-treated rats at the dose of 200mg/kg body weight were statistically similar to those of the normal control group rats and Orlistat-treated rats (*p*>0.01; Table 3.12). However, administration of extract doses of 250 and 300mg/kg body weight significantly lowered the organo-somatic indices of lungs as compared to those of rats in the normal control group (*p*≤0.01; Table 3.12).
Table 3.12: Effect of DCM Leaf Extract of *Gnidia glauca* on Relative-Organ Weights (Organo-Somatic Index) of HFD-Induced Obese Rats

<table>
<thead>
<tr>
<th>TREATMENT (mg/kgbw)</th>
<th>Relative Organ to Body Weights (Organo-Somatic Index) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Liver</td>
</tr>
<tr>
<td>Normal Control</td>
<td>4.45±0.39&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Negative Control</td>
<td>7.10±0.10&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Positive Control</td>
<td>3.16±0.16&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>HFD+200mg/kg</td>
<td>3.90±0.20&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>HFD+250mg/kg</td>
<td>3.62±0.15&lt;sup&gt;cd&lt;/sup&gt;</td>
</tr>
<tr>
<td>HFD+300mg/kg</td>
<td>3.40±0.13&lt;sup&gt;de&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Means followed by similar lower-case letters within respective columns are not statistically different (<i>p</i>&gt;0.01).
3.3.4 The Effect of DCM Leaf Extract of *Gnidia glauca* on Total Fat Content in HFD-Induced Obese Laboratory Rats

The weights of different fat pads such as mesenteric, retroperitoneal, parametrial, perirenal, subcutaneous and total fat content varied differently across the experimental groups (Table 3.13). The weight of mesenteric fat pad was significantly higher in HFD-induced obese untreated rats than all other experimental groups ($p \leq 0.01$; Table 3.13). The weight of mesenteric fat pad was significantly lower in the extract-treated group of rats than that of rats treated with the reference drug, Orlistat ($p \leq 0.01$; Table 3.13). However, there was no significant difference in weight of mesenteric fat pad of rats in the normal control and rats treated with the reference drug, Orlistat ($p > 0.01$; Table 3.13). Similarly, the weight of mesenteric fat pads in the group of rats treated with 200 mg/kg body weight extract dose and the reference drug, Orlistat were comparable ($p > 0.01$; Table 3.13). It was also observed that the weight of retroperitoneal fat pad was significantly increased in the negative control group of rats relative to those of rats in the normal control, positive control and extract-treated groups ($p \leq 0.01$; Table 3.13).

The parametrial fat pad weights were significantly higher in the negative control group rats than rats treated with the three extract doses ($p \leq 0.01$; Table 3.13). Further, all extract treated groups of rats exhibited significantly lower weights of parametrial fat pad than the normal control group rats ($p \leq 0.0$). However, no significant difference in weight of parametrial fat pad was indicated between rats treated with the reference drug, Orlistat, and those treated with the three extract doses ($p > 0.01$; Table 3.13).
The weights of perirenal fat pads were observed to be significantly higher in the negative control rats than those of rats in the normal control, positive control and extract-treated groups \((p\leq0.01;\ \text{Table}\ 3.13)\). Treatment of rats with the extract dose of 300mg/kg body weight led to the lightest perirenal fat pad (Table 3.13). The activities of the reference drug, Orlistat, in reduction of weights of the perirenal fat pad were comparable to those of the extract at a dose of 200 mg/kg body weight \((p>0.01;\ \text{Table}\ 3.13)\).

The weight of subcutaneous fat pads in the HFD-induced untreated obese rats was significantly high as compared to those of rats in the normal control, positive control and extract-treated groups \((p\leq0.01;\ \text{Table}\ 3.13)\). Remarkably, all the concentrations of the plant extract indicated significantly reduced weights of the subcutaneous fat pad than those of rats treated with the reference drug, Orlistat \((p\leq0.01;\ \text{Table}\ 3.13)\). The highest concentration (300 mg/kg body weight) of the extract showed the lightest weight of the subcutaneous fat pad (Table 3.13).

Treatment of rats with the DCM leaf extract of \textit{G. glauca} significantly reduced the weight of brown adipose tissue (BAT) fat pads relative to the negative control obese untreated rats \((p\leq0.01;\ \text{Table}\ 3.13)\). As shown, the highest extract dose recorded the lowest weight of BAT (Table 3.13). However, no significant change in weight of BAT was observed between rats treated with the reference drug, Orlistat, and those treated with the three extract doses \((p>0.01;\ \text{Table}\ 3.13)\).
The results also revealed that rats in the negative control group showed significantly higher weight of the total fats than those of rats in the normal control, positive control and extract-treated groups ($p \leq 0.01$; Table 3.13). The weights of the total fats gradually reduced as the concentration of the plant extracts increased with the highest dose of 300mg/kg body weight recording the lightest weight of total fats (Table 3.13).
Table 3.13: The Effect of DCM Leaf Extract of *Gnidia glauca* on Total Fat Content in HFD-Induced Obese Rats

<table>
<thead>
<tr>
<th>TREATMENT</th>
<th>MESENTERIC</th>
<th>RETROPERITONEAL</th>
<th>PARAMETRIAL</th>
<th>PERIRENAL</th>
<th>SUBCUTANEOUS</th>
<th>BAT</th>
<th>TOTAL FAT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal Control</td>
<td>2.77±0.30&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.30±0.03&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.28±0.06&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.15±0.28&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.61±0.11&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.29±0.10&lt;sup&gt;b&lt;/sup&gt;</td>
<td>9.41±0.27&lt;sup&gt;bc&lt;/sup&gt;</td>
</tr>
<tr>
<td>Negative Control</td>
<td>5.89±0.28&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.14±0.13&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.44±0.11&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.05±0.34&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.72±0.23&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.29±0.09&lt;sup&gt;a&lt;/sup&gt;</td>
<td>22.54±0.38&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Orlistat-30mg/kg</td>
<td>2.58±0.12&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>1.31±0.13&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.16±0.04&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.95±0.19&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.85±0.31&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.17±0.02&lt;sup&gt;c&lt;/sup&gt;</td>
<td>10.01±0.40&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>HFD+200mg/kg</td>
<td>2.15±0.18&lt;sup&gt;cd&lt;/sup&gt;</td>
<td>1.44±0.16&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.15±0.02&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.85±0.13&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>3.24±0.37&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.17±0.03&lt;sup&gt;c&lt;/sup&gt;</td>
<td>9.00±0.74&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>HFD+250mg/kg</td>
<td>1.95±0.17&lt;sup&gt;d&lt;/sup&gt;</td>
<td>1.23±0.05&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.11±0.04&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.47±0.12&lt;sup&gt;cd&lt;/sup&gt;</td>
<td>2.93±0.19&lt;sup&gt;cd&lt;/sup&gt;</td>
<td>0.11±0.02&lt;sup&gt;c&lt;/sup&gt;</td>
<td>7.79±0.32&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>HFD+ 300mg/kg</td>
<td>1.87±0.14&lt;sup&gt;d&lt;/sup&gt;</td>
<td>1.34±0.31&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.09±0.01&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.36±0.06&lt;sup&gt;d&lt;/sup&gt;</td>
<td>2.69±0.20&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.10±0.02&lt;sup&gt;c&lt;/sup&gt;</td>
<td>7.44±0.33&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Means followed by similar lower-case letters within respective columns are not statistically different (*p*>0.01).
3.3.5 Effect of DCM Leaf Extract of *Gnidia glauca* on Lipid Profiles in HFD-Induced Obese Rats

It was observed that administration of DCM leaf extract of *G. glauca* in HFD-induced obese rats altered the levels of serum lipid profiles (Table 3.14). The results showed that there was a significant increase in triglycerides (TG) in the negative control group rats relative to the normal control, positive control and the extract-treated groups rats (*p*≤0.01; Table 3.14). Findings also indicated that the reduction of triglycerides in the positive control group were statistically similar to that of the extract-treated groups (*p*>0.01). Treatment with the extract at dosage levels of 250 and 300mg/kg body weight significantly reduced the triglycerides levels as compared to the normal control group (*p*≤0.01; Table 3.14).

Administration of rats with DCM leaf extract of *G. glauca* caused a significant reduction of the total cholesterol levels compared to the negative group rats (*p*≤0.01). Nonetheless, the amount of total cholesterol in rats administered with 300mg/kg body weight of the extract was not significantly different from that of rats in the normal control group (*p*>0.01; Table 3.14). Treatment of rats with extract doses of 200 and 300mg/kg body weight showed statistically similar levels of total cholesterol as positive control group rats (*p*>0.01; Table 3.14).

A dose-dependent elevation of HDL-C levels was observed in rats treated with the three extract doses (Table 3.14). The results indicated that the levels of HDL-C of rats in the negative control group were significantly low as compared to rats treated with the three extract doses and the reference drug, Orlistat (*p*≤0.01). Further, there was no significant
difference in levels of HDL-C between rats treated with the reference drug, Orlistat, and rats treated with the extract at the concentrations of 200 and 250 mg/kg body weight ($p>0.01$; Table 3.14). On the other hand, HDL-C levels were significantly increased in the group of rats treated with 300mg/kg body weight of the plant extract ($p\leq0.01$; Table 3.14).

The results also demonstrated significantly high levels of LDL-C in the negative control group of rats relative to the extract-treated and Orlistat-treated groups of rats ($p\leq0.01$). Nevertheless, the difference in the levels of LDL-C of rats treated with the three extract doses were not statistically different from rats treated with the reference drug, Orlistat ($p>0.01$; Table 3.14). It is worth noting that the levels of LDL-C of rats in the normal control group and those of rats treated with the plant extract were comparable ($p>0.01$; Table 3.14).

The levels of VLDL decreased as the concentration of the extract increased, however, there was no significant difference in VLDL levels among the administered dosages of the plant extract ($p>0.01$; Table 3.14). Results indicated that the HFD-induced untreated obese rats had significantly increased levels of VLDL as compared to extract-treated and Orlistat-treated rats ($p\leq0.01$; Table 3.14). Treatment of rats with the three extract doses showed comparable levels of the VLDL with Orlistat-treated rats ($p>0.01$; Table 3.14). The extract appeared to normalize levels of VLDL since levels of VLDL of rats in the normal control group were comparable to those of rats treated with the reference drug, Orlistat ($p>0.01$; Table 3.14).
Table 3.14: The Effect of Oral Administration of DCM Leaf Extract of *Gnidia glauca* on Lipid Profiles in HFD-Induced Obese Rats

<table>
<thead>
<tr>
<th>TREATMENT (mg/kgbw)</th>
<th>Lipid Profiles (mmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TG</td>
</tr>
<tr>
<td>Normal Control</td>
<td>1.44±0.11\textsuperscript{b}</td>
</tr>
<tr>
<td>Negative Control</td>
<td>2.74±0.52\textsuperscript{a}</td>
</tr>
<tr>
<td>Positive Control</td>
<td>1.02±0.16\textsuperscript{bc}</td>
</tr>
<tr>
<td>HFD+200mg/kg</td>
<td>0.98±0.13\textsuperscript{bc}</td>
</tr>
<tr>
<td>HFD+250mg/kg</td>
<td>0.96±0.11\textsuperscript{c}</td>
</tr>
<tr>
<td>HFD+ 300mg/kg</td>
<td>0.84±0.15\textsuperscript{c}</td>
</tr>
</tbody>
</table>

Means followed by similar lower-case letters within respective columns are not statistically different ($p>0.01$). TG = triacylglycerols; TC = total cholesterol; HDL-C = high density lipoprotein cholesterol; LDL-C = low density lipoprotein cholesterol; VLDL = very low density lipoprotein.
3.3.6 The Effect of DCM Leaf Extract of *Gnidia glauca* on Body Adiposity Index (BAI) and Atherogenic Index (AI) in HFD-Induced Obese Rats

As depicted in Table 3.15, administration of DCM leaf extract of *G. glauca* in HFD-induced obese rats caused a significant decrease in the atherogenic index and adiposity index as compared to the HFD-induced untreated obese rats in the negative control group \( (p \leq 0.01; \text{Table 3.15}) \). Besides, administration of the extract doses of 250 and 300mg/kg body weight in rats resulted in a significantly low body adiposity index as compared to Orlistat-treated group of rats \( (p \leq 0.01) \). Interestingly, the body adiposity indices of rats in the extract treated groups were not significantly different from those of rats in the normal control group \( (p > 0.01; \text{Table 3.15}) \). Results also revealed that the atherogenic indices of rats treated with the three extract doses were significantly lower than those of rats in the normal control group \( (p \leq 0.01) \). Further, treatment of rats with the extract dose of 300mg/kg body weight attenuated levels of atherogenic than was observed in Orlistat-treated group of rats \( (p \leq 0.01; \text{Table 3.15}) \).

**Table 3.15: The Effect of DCM Leaf Extract of *Gnidia glauca* on Body Adiposity Index (BAI) and Atherogenic Index (AI) in HFD-Induced Obese Rats**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Body Adiposity Index (BAI) (%)</th>
<th>Atherogenic Index (AI) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal Control</td>
<td>4.05±0.16&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.12±0.05&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Negative Control</td>
<td>6.92±0.20&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.64±0.12&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Positive Control</td>
<td>5.09±0.29&lt;sup&gt;b&lt;/sup&gt;</td>
<td>-0.12±0.06&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>HFD+200mg/kg</td>
<td>4.88±0.34&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>-0.14±0.07&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>HFD+250mg/kg</td>
<td>4.30±0.08&lt;sup&gt;c&lt;/sup&gt;</td>
<td>-0.20±0.05&lt;sup&gt;cd&lt;/sup&gt;</td>
</tr>
<tr>
<td>HFD+300mg/kg</td>
<td>4.17±0.23&lt;sup&gt;c&lt;/sup&gt;</td>
<td>-0.33±0.11&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Means followed by similar lower-case letters within respective columns are not statistically different \( (p > 0.01) \).
3.3.7 Effect of DCM Leaf Extract of *Gnidia glauca* on Fasting Blood Glucose Levels of HFD-Induced Obese Laboratory Rats

As results indicate, treatment with the DCM leaf extract of *G. glauca* and reference drug, Orlistat, caused a reduction in levels of fasting blood glucose in HFD-induced obese rats from the 1\textsuperscript{st} to the 6\textsuperscript{th} week of the study period (Table 3.16). However, rats in the normal control and negative control groups indicated a positive change in fasting blood glucose levels throughout the experimental period (Table 3.16). Rat models administered with DCM leaf extract of *G. glauca* at dose levels of 200 and 300mg/kg body weight caused a significant decrease in levels of blood glucose from -5.26±1.54\% and -7.43±1.67\% in the 1\textsuperscript{st} week to -49.37±4.22\% and -57.45±1.62\% in the 6\textsuperscript{th} week (Table 3.16). On the contrary, rats in the negative control group indicated a significant percentage increase in fasting blood glucose levels from 5.85±2.47\% in the 1\textsuperscript{st} week to 58.93±11.00\% in the 6\textsuperscript{th} week of the study period (Table 3.16).

The weekly percentage change in fasting blood glucose levels indicates a positive rate of change in fasting blood glucose levels of rats in the normal control and negative control groups (Table 3.16). Conversely, rats treated with the three extract doses and the reference drug, Orlistat, showed a negative weekly rate of change in fasting blood glucose levels (Table 3.16). The weekly percentage increase in fasting blood glucose levels of rats in negative control group were significantly higher than those of rats in the normal control group (*p*≤0.01). However, no significant differences in the weekly percentage decreases in fasting blood glucose levels were observed between rats treated with the reference drug, Orlistat and those treated with the three extract doses (*p*>0.01) (Table 3.16).
Table 3.16: Effect of DCM Leaf Extract of *Gnidia glauca* on Fasting Blood Glucose Levels in HFD-Induced Obese Laboratory Rats

<table>
<thead>
<tr>
<th>TREATMENT (mg/kgbw)</th>
<th>Percentage Change in Fasting Blood Glucose Levels/Week (%)</th>
<th>% Δ in Fasting Glc levels/Week</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Week 1</td>
<td>Week 2</td>
</tr>
<tr>
<td>Normal Control</td>
<td>5.20±1.10&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.61±1.74&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Negative Control</td>
<td>5.85±2.47&lt;sup&gt;c&lt;/sup&gt;</td>
<td>14.34±4.20&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>Positive Control</td>
<td>-5.94±0.42&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-14.53±2.35&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>HFD+200mg/kg</td>
<td>-5.26±1.54&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-16.27±1.47&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>HFD+250mg/kg</td>
<td>-6.85±2.68&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-16.81±3.48&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>HFD+300mg/kg</td>
<td>-7.43±1.67&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-19.90±3.84&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Means within respective rows followed by superscript of similar lowercase letters are not significantly different (*p* > 0.01); means within respective columns followed by superscript of similar uppercase letters are not significantly different (*p* > 0.01); **Key** -Δ- represents change in.
3.3.8 Effect of DCM Leaf Extract of *Gnidia glauca* on ALT, AST, ALP, LDH, GGT, Creatinine and Urea in HFD-Induced Obese Laboratory Rats

The DCM leaf extract of *G. glauca* had varied effects on AST, ALT, ALP, LDH, GGT, Urea and Creatinine in HFD-Induced obese laboratory rats (Table 3.17). As observed, all the extract-treated groups of rats had significantly lower levels of AST, ALT, LDH, GGT, Urea and Creatinine than rats in the negative control group (*p*≤0.01; Table 3.17). However, levels of AST, ALT, LDH, GGT, Urea and Creatinine in rats treated with the three extract doses were not statistically different from those of rats treated with the reference drug, Orlistat (*p*>0.01; Table 3.17). Interestingly, levels of AST, ALT, LDH, GGT, Urea and Creatinine in rats treated with three extract doses were comparable with levels of these parameters in the normal control group rats (*p*>0.01; Table 3.17). Further, the results indicated that extract-treated rats showed significantly reduced levels of ALP relative to obese untreated negative control group rats (*p*≤0.01). It was apparent that treatment of rats with the extract dose of 300mg/kg body weight caused significantly lower levels of ALP than was observed in rats treated with the reference drug, Orlistat (*p*≤0.01; Table 3.17).
Table 3.17: Effect of DCM Leaf Extract of *Gnidia glauca* on ALT, AST, ALP, LDH, GGT, Urea and Creatinine in HFD-Induced Obese Laboratory Rats

<table>
<thead>
<tr>
<th>TREATMENT (mg/kgbw)</th>
<th>AST (U/L)</th>
<th>ALT (U/L)</th>
<th>ALP (U/L)</th>
<th>LDH (U/L)</th>
<th>GGT (U/L)</th>
<th>UREA</th>
<th>Creatinine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal Control</td>
<td>38.40±9.24^b</td>
<td>41.20±7.79^b</td>
<td>55.40±12.95^bc</td>
<td>22.60±6.95^b</td>
<td>3.80±1.48^b</td>
<td>5.70±2.36^b</td>
<td>58.86±3.57^b</td>
</tr>
<tr>
<td>Negative Control</td>
<td>128.60±4.04^a</td>
<td>139.20±5.81^a</td>
<td>204.60±18.61^a</td>
<td>131.60±14.43^a</td>
<td>11.40±1.14^a</td>
<td>10.25±0.48^a</td>
<td>133.86±7.42^a</td>
</tr>
<tr>
<td>Positive Control</td>
<td>34.20±12.85^b</td>
<td>40.40±5.18^b</td>
<td>58.60±11.06^bc</td>
<td>26.20±5.50^b</td>
<td>3.60±0.55^b</td>
<td>6.74±0.76^b</td>
<td>56.14±2.80^b</td>
</tr>
<tr>
<td>HFD+200mg/kg</td>
<td>35.80±1.93^b</td>
<td>41.20±3.96^b</td>
<td>75.80±14.34^b</td>
<td>24.00±4.47^b</td>
<td>3.00±1.23^b</td>
<td>7.26±1.19^b</td>
<td>60.86±5.94^b</td>
</tr>
<tr>
<td>HFD+250mg/kg</td>
<td>30.60±4.22^b</td>
<td>37.00±3.54^b</td>
<td>55.20±11.56^bc</td>
<td>21.00±2.92^b</td>
<td>2.40±1.34^b</td>
<td>6.87±1.10^b</td>
<td>60.12±7.14^b</td>
</tr>
<tr>
<td>HFD+ 300mg/kg</td>
<td>28.00±3.16^b</td>
<td>36.60±1.67^b</td>
<td>47.80±5.26^c</td>
<td>19.00±3.32^b</td>
<td>1.80±0.84^b</td>
<td>6.54±1.13^b</td>
<td>58.12±3.13^b</td>
</tr>
</tbody>
</table>

Means followed by similar lower-case letters within respective columns are not statistically different (*p* >0.01). ALT = alanine transaminase; AST = aspartate transaminase; ALP = alkaline phosphatase; GGT = γ-glutamyltransferase; LDH = lactate dehydrogenase.
3.3.9 Effect of DCM Leaf Extract of *Gnidia glauca* on Total Protein, Total Albumin, Direct Bilirubin and Indirect Bilirubin in HFD-Induced Obese Laboratory Rats

Treatment with DCM leaf extract of *G. glauca* altered levels of the total proteins, total albumin, direct bilirubin and indirect bilirubin in HFD-Induced obese laboratory rats (Table 3.18). It was noted that levels of all these analytes in rats treated with the three extract doses were comparable with rats treated with the reference drug, Orlistat, and those of normal control group rats (*p* > 0.01; Table 3.18). In contrast, the levels of total proteins, total albumin, direct bilirubin and indirect bilirubin were significantly higher in obese untreated rats in the negative control group than levels observed in rats treated with the three extract doses, Orlistat-treated rats and normal control group rats (*p* ≤ 0.01; Table 3.18).
Table 3.18: Effect of DCM Leaf Extract of *Gnidia glauca* on Total Protein, Total Albumin, Direct Bilirubin and Indirect Bilirubin in HFD-Induced Obese Laboratory Rats

<table>
<thead>
<tr>
<th>TREATMENT (mg/kgbw)</th>
<th>Total Protein (g/L)</th>
<th>Albumin (g/L)</th>
<th>Total Bilirubin (µmol/L)</th>
<th>Direct Bilirubin (µmol/L)</th>
<th>Indirect Bilirubin (µmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal Control</td>
<td>74.24±7.19&lt;sup&gt;b&lt;/sup&gt;</td>
<td>41.00±2.02&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.90±0.78&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.18±1.19&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.72±0.46&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Negative Control</td>
<td>90.20±5.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>63.94±5.28&lt;sup&gt;a&lt;/sup&gt;</td>
<td>15.52±1.92&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8.76±0.82&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.76±1.70&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Positive Control</td>
<td>75.86±1.66&lt;sup&gt;b&lt;/sup&gt;</td>
<td>40.44±4.91&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6.04±1.05&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.46±0.86&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.58±1.56&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>HFD+200mg/kg</td>
<td>71.10±2.92&lt;sup&gt;b&lt;/sup&gt;</td>
<td>39.42±1.58&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6.10±0.81&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.58±0.90&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.52±0.87&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>HFD+250mg/kg</td>
<td>69.58±5.23&lt;sup&gt;b&lt;/sup&gt;</td>
<td>38.76±1.59&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.66±1.83&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.50±0.67&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.16±1.67&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>HFD+ 300mg/kg</td>
<td>67.34±5.41&lt;sup&gt;b&lt;/sup&gt;</td>
<td>37.84±1.29&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.58±0.77&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.40±0.46&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.18±1.03&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Means followed by similar lower-case letters within respective columns are not statistically different (*p*>0.01).
3.3.10 Effect of DCM Leaf Extract of *Gnidia glauca* on Some End-Point Haematological Parameters in HFD-Induced Obese Laboratory Rats

3.3.10.1 Effect of DCM Leaf Extract of *Gnidia glauca* on Erythrocytes and Related Parameters in HFD-Induced Obese Laboratory Rats

The results showed that treatment with DCM leaf extract of *G. glauca* increased the levels of red blood cells (RBCs), haemoglobin (Hb), packed cell volume (PCV), mean cell haemoglobin (MCH), mean cell haemoglobin concentration (MCHC), mean cell volume (MCV) and red cell distribution width (RDW) (Table 3.19). The extract-treated rats showed significantly higher levels of all these parameters than was observed in obese untreated rats in the negative control group (*p*≤0.01). Similarly, administration of the highest dose of the extract (300mg/kg body weight) resulted in significantly high levels of RBCs as compared to those of Orlistat-treated rats and normal control group rats (*p*≤0.01; Table 3.19). Levels of RBCs in rats treated with extract doses of 200, 250mg/kg body weight were, however, not significantly different from those of Orlistat-treated rats and normal control group rats (*p*≤0.01; Table 3.19). The levels of Hb, MCV and RDW were statistically similar in the extract-treated rats and Orlistat-treated rats (*p>*0.01). Similarly, levels of PCV were comparable to extract-treated rats at dosage levels of 200 and 250mg/kg body weight and Orlistat-treated rats (*p>*0.01). Treatment with the highest dose of extract (300mg/kg body weight) showed significantly higher levels of PCV, MCV and MCHC than those observed in Orlistat-treated rats (*p*≤0.01; Table 3.19). Nevertheless, rats treated with low doses of the extract (200 and 250mg/kg body weight) showed no significant difference in levels of MCV and MCHC than Orlistat-treated rats (*p>*0.01; Table 3.19).
Table 3.19: Effect of DCM Leaf Extract of *Gnidia glauca* on Erythrocytes and Related Parameters in HFD-Induced Obese Laboratory Rats

<table>
<thead>
<tr>
<th>TREATMENT</th>
<th>Red Blood Cells and Related Indices</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>RBC (x10^6/µL)</td>
</tr>
<tr>
<td>Normal Control</td>
<td>5.40±0.63^b</td>
</tr>
<tr>
<td>Negative Control</td>
<td>2.90±0.62^c</td>
</tr>
<tr>
<td>Positive Control</td>
<td>5.72±0.63^b</td>
</tr>
<tr>
<td>HFD+200mg/kg</td>
<td>6.07±0.67^b</td>
</tr>
<tr>
<td>HFD+250mg/kg</td>
<td>6.83±1.02^ab</td>
</tr>
<tr>
<td>HFD+300mg/kg</td>
<td>8.33±0.97^a</td>
</tr>
</tbody>
</table>

Means followed by similar lower-case letters within respective columns are not statistically different ($p>0.01$).
3.3.10.2 Effect of DCM Leaf Extract of Gnidia glauca on White Blood Cells and Differential Leucocytes Counts in HFD-Induced Obese Laboratory Rats

The white blood cells (WBC), lymphocytes (LYM), monocytes (MON), neutrophils (NEU), eosinophils (EOS) and basophils (BAS) were observed to vary upon treatment with DCM leaf extract of G. glauca in HFD-Induced obese rats (Table 3.20). Results revealed that extract-treated rats and Orlistat-treated rats had significantly higher levels of WBC, LYM, MON, NEU, EOS and BAS than HFD-induced obese rats in the negative control group (p≤0.01; Table 3.20). However, the levels of LYM, MON, NEU, EOS and BAS in rats treated with the three extract doses and the reference drug, Orlistat, were not significantly different from those of rats in the normal control group (p>0.01; Table 3.20). Further, levels of LYM, MON, NEU, EOS and BAS were comparable between extract-treated rats and Orlistat-treated rats (p>0.01; Table 3.20). Interestingly, the levels of WBC in extract-treated rats were significantly higher than those of rats in the normal control group (p≤0.01; Table 3.20). However, the difference between levels of WBC in the Orlistat-treated rats and extract-treated rats were not statistically significant (p>0.01; Table 3.20).
Table 3.20: Effect of DCM Leaf Extract of *Gnidia glauca* on White Blood Cells and Differential Leucocytes Counts in HFD-Induced Obese Laboratory Rats

<table>
<thead>
<tr>
<th>TREATMENT</th>
<th>WBC (x10^3/µL)</th>
<th>LYM (x10^3/µL)</th>
<th>MON (x10^3/µL)</th>
<th>NEU (x10^3/µL)</th>
<th>EOS (x10^3/µL)</th>
<th>BAS (x10^3/µL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal Control</td>
<td>9.50±0.90&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>4.96±0.67&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.22±0.13&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.26±0.21&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.21±0.10&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.28±0.08&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Negative Control</td>
<td>7.02±0.85&lt;sup&gt;c&lt;/sup&gt;</td>
<td>3.08±0.40&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.76±0.11&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.06±0.50&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.38±0.16&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.14±0.05&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Positive Control</td>
<td>11.32±0.93&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>5.74±0.44&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.40±0.14&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.42±0.13&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.01±0.13&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.28±0.08&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>HFD+200mg/kg</td>
<td>11.68±1.38&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>6.02±0.61&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.30±0.13&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.32±0.13&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.20±0.10&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.28±0.08&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>HFD+250mg/kg</td>
<td>12.24±0.89&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.18±0.26&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.34±0.05&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.48±0.41&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.26±0.05&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.30±0.10&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>HFD+300mg/kg</td>
<td>12.96±2.24&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.34±0.57&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.40±0.07&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.66±0.56&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.30±0.12&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.32±0.13&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Means followed by similar lower-case letters within respective columns are not statistically different (p>0.01).
3.3.10.3 Effect of DCM Leaf Extract of *Gnidia glauca* on Platelets Count and Related Variants in HFD-Induced Obese Laboratory Rats

Results indicated that the levels of platelets (PLT), mean platelet volume (MPV) and platelet distribution width (PDW) were significantly low in the negative control group rats than was observed in extract-treated rats, Orlistat-treated rats and normal control group rats ($p \leq 0.01$; Table 3.21). The extract-treated group of rats at the dosage of 200mg/kg body weight showed comparable levels of PLT with that of the Orlistat-treated rats ($p > 0.01$). However, treatment with the extract at dose levels of 250 and 300mg/kg body weight resulted in significantly higher levels of PLT than rats treated with the reference drug, Orlistat ($p \leq 0.01$) (Table 3.21). Findings further revealed that the levels of MPV of rats treated with the extract at dosage levels of 200 and 250mg/kg body weight were statistically similar to that of the normal control group rats and Orlistat-treated rats ($p > 0.01$). However, treatment with the highest dose of the extract (300mg/kg body weight) showed a greater amount of MPV than was observed in rats treated with the Orlistat and those of normal control group rats ($p \leq 0.01$; Table 3.21). It was worth noting that levels of PDW in extract-treated rats were comparable with those of Orlistat-treated rats and normal control group rats ($p > 0.01$; Table 3.21).
Table 3.21: Effect of Oral Administration of DCM Leaf Extract of *Gnidia glauca* on Platelets Count and Related Variants in HFD-Induced Obese Laboratory Rats

<table>
<thead>
<tr>
<th>TREATMENT (mg/kg bw)</th>
<th>PLT (x10^3/µL)</th>
<th>MPV (fL)</th>
<th>PDW (fL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal Control</td>
<td>392.80±40.50^c</td>
<td>6.68±1.43^ab</td>
<td>18.78±0.74^a</td>
</tr>
<tr>
<td>Negative Control</td>
<td>136.20±14.10^d</td>
<td>3.90±0.53^c</td>
<td>9.64±0.88^b</td>
</tr>
<tr>
<td>Positive Control</td>
<td>547.60±87.90^b</td>
<td>6.52±1.31^b</td>
<td>18.84±2.12^a</td>
</tr>
<tr>
<td>HFD+200mg/kg</td>
<td>634.80±51.66^b</td>
<td>6.30±1.41^b</td>
<td>19.00±0.49^a</td>
</tr>
<tr>
<td>HFD+250mg/kg</td>
<td>754.00±62.90^a</td>
<td>6.36±0.10^b</td>
<td>20.20±1.71^a</td>
</tr>
<tr>
<td>HFD+300mg/kg</td>
<td>842.20±55.30^a</td>
<td>8.86±0.74^a</td>
<td>20.38±0.92^a</td>
</tr>
</tbody>
</table>

Means followed by similar lower-case letters within respective columns are not statistically different (p>0.01).
3.3.11 Effect of DCM Leaf Extract of *Gnidia glauca* on Rectal Body Temperature in HFD Induced Obese Laboratory Rats

As depicted in Table 3.22, administration of the DCM leaf extract of *G. glauca* and reference drug, Orlistat, caused a significant positive percentage changes in the rectal body temperature of rats (Table 3.22). However, rats in a significant negative control groups indicated a significant negative percentage change in the rectal body temperature throughout the study period (Table 3.22).

Treatment with the DCM leaf extract of *G. glauca* at dosages of 250 mg/kg and 300 mg/kg body weight caused a significant percentage increase in rectal body temperature from 0.85±0.45% and 0.84±0.22% in the 30th min to 3.29±1.50% and 3.21±1.52% in the 150th min, respectively (Table 3.22). The rats in the negative control group indicated a significant decrease in the rectal body temperature from the 30th to the 150th minute (p≤0.01; Table 3.22). For instance, at 30th min the percentage decrease in rectal body temperature of rats was -10.25±3.07% while at 150th min it was -36.98±3.70% (Table 3.22). Treatment with a standard drug (Orlistat at 30mg/kg, p.o.) caused a significant percentage increase in rectal body temperature from 0.21±0.42% in the 30th min to 1.19±1.11% in the 150th min. The rats in the normal control group showed a decrease in rectal body temperature from -1.91±1.17% in the 60th to -0.98±1.06% in the 150th minute of study (p>0.01; Table 3.22).

Analysis of the weekly percentage change in rectal body temperature across experimental groups revealed a significant negative rate of change in the normal control rats and negative control group rats (Table 3.22). On the contrary, there was a positive weekly percentage change in rectal body temperature of rats treated with the reference drug, Orlistat, and those
treated with the three extract doses (Table 3.22). The weekly percentage increase in rectal body temperature of rats were significantly lower in the negative control group than rats in the normal control group ($p \leq 0.01$). On the other hand, the weekly percentage increases in rectal body temperature in the extract-treated groups of rats and those of rats treated with the reference drug, Orlistat, were comparable ($p > 0.01$; Table 3.22).
Table 3.22: Effect of DCM Leaf Extract of *Gnidia glauca* on Rectal Body Temperature in HFD-Induced Obese Laboratory Rats

<table>
<thead>
<tr>
<th>TREATMENT</th>
<th>Percentage Change in Rectal Body Temperature/Week (%)</th>
<th>30min</th>
<th>60min</th>
<th>90min</th>
<th>120min</th>
<th>150min</th>
<th>% Δ in Rectal Temp/Week</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Normal Control</td>
<td>-0.87±2.78&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-1.91±1.17&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.26±1.22&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-1.81±1.02&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-0.98±1.06&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-1.06±1.07&lt;sup&gt;B&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Negative Control</td>
<td>-10.25±3.07&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-24.38±4.30&lt;sup&gt;b&lt;/sup&gt;</td>
<td>-31.60±3.28&lt;sup&gt;c&lt;/sup&gt;</td>
<td>-40.77±2.84&lt;sup&gt;d&lt;/sup&gt;</td>
<td>-36.98±3.70&lt;sup&gt;cd&lt;/sup&gt;</td>
<td>-28.80±3.10&lt;sup&gt;C&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Positive Control</td>
<td>0.21±0.42&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.39±0.73&lt;sup&gt;abc&lt;/sup&gt;</td>
<td>1.24±0.81&lt;sup&gt;abc&lt;/sup&gt;</td>
<td>2.39±1.48&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.19±1.11&lt;sup&gt;abc&lt;/sup&gt;</td>
<td>1.29±0.74&lt;sup&gt;A&lt;/sup&gt;</td>
<td></td>
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<tr>
<td>HFD+200mg/kg</td>
<td>0.83±0.50&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.61±0.62&lt;sup&gt;abc&lt;/sup&gt;</td>
<td>1.71±1.32&lt;sup&gt;abc&lt;/sup&gt;</td>
<td>2.70±0.86&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.98±1.02&lt;sup&gt;abc&lt;/sup&gt;</td>
<td>1.76±0.79&lt;sup&gt;A&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>HFD+250mg/kg</td>
<td>0.85±0.45&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.44±0.95&lt;sup&gt;abc&lt;/sup&gt;</td>
<td>1.97±1.24&lt;sup&gt;abc&lt;/sup&gt;</td>
<td>4.03±1.46&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.29±1.50&lt;sup&gt;abc&lt;/sup&gt;</td>
<td>2.31±0.97&lt;sup&gt;A&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>HFD+300mg/kg</td>
<td>0.84±0.22&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.52±0.44&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>2.05±1.19&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>4.21±1.39&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.21±1.52&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>2.37±0.91&lt;sup&gt;A&lt;/sup&gt;</td>
<td></td>
</tr>
</tbody>
</table>

Means within respective rows followed by superscript of similar lowercase letters are not significantly different (*p*>0.01); means within respective columns followed by superscript of similar uppercase letters are not significantly different (*p*>0.01); **Key** -Δ- represents change in.
3.3.12 Effect of DCM Leaf Extract of *Gnidia glauca* on Feed Intake in HFD-Induced Obese Rats

Results showed a significant increase in the mean cumulative food intake in HFD-induced obese untreated rats as compared to that of rats treated with the three extract doses and the reference drug, Orlistat ($p \leq 0.01$; Figure 3.2). Treatment of rats with the extract at dose of 200mg/kg body weight caused a weekly percentage decrease in total feed intake from 72.53% in the 1<sup>st</sup> week to 28.53% in the 6<sup>th</sup> week of study (Figure 3.2). Similarly, treatment of rats with the extract dosage of 300mg/kg body weight caused a weekly percentage decrease in total feed intake from 72.8% in the 1<sup>st</sup> week to 21.1% in the 6<sup>th</sup> week of study period (Figure 3.2). Treatment of rats with the reference drug, Orlistat, caused a weekly percentage decrease in total feed intake from 73.06% in the 1<sup>st</sup> week to 33.1% in the 6<sup>th</sup> week of the study period (Figure 3.2). However, provision of normal chow diet (rodent pellet and water *ad libitum*) to normal rats in the normal control group progressively increased total feed intake of rats from 60.13% in the 1<sup>st</sup> week to 76.4% in the 6<sup>th</sup> week of the experimental period (Figure 3.2).
Figure 3.2: Effect of DCM Leaf Extract of *Gnidia glauca* on Feed Intake in HFD-Induced Obese Rats
3.4 Discussion

The present study tested the effect of administration of therapeutic doses of DCM leaf extract of *G. glauca* in an animal model of dietary obesity. The consumption of calorically dense (high-fat) diets caused a significantly higher weekly body weight gain in the HFD-fed untreated group of rats relative to the normal control group rats throughout the experimental period (*p*≤0.01). The results confirmed a proposition that chronic exposure to high fat diet results in obesogenic states that is coupled with positive impairment of the energy balance equation (Lennox *et al.*, 2015). This is due to a high rate of acylation of saturated fatty acids into triglycerides that is subsequently stored in the adipose tissue hence weight gain (Storlien *et al.*, 2001). In addition, post-ingestive effects of high fat diets contribute to weight gain through reduction of satiety signals (leptin, cholecystokinin and peptide YY) and attenuated oxidation of fatty acids (Little *et al.*, 2007).

The oral administration of DCM leaf extract of *G. glauca* plant extract at graded doses of 200, 250 and 300mg/kg body weight led to a significant reduction in the weekly body weight of obese rats as compared to the HFD-fed untreated rats (*p*≤0.01). However, the effect of the extract was comparable to that of the reference drug, Orlistat, a pancreatic lipase inhibitor (*p*>0.01). Similar studies showed that treatment of high fat-induced obese rats with *Moringa oleifera* at doses of 200 mg and 400 mg/kg body weight for 7 weeks led to a significant decrease in body weight as compared to untreated HFD-induced obese rats (Bais *et al.*, 2014). The potential to decrease the body weight of the experimental rats by the extract could be due to its ability to mediate various pathways among which are stimulation of satiety signals (such as leptin), reduction of triglycerides absorption (by
inhibiting the action of pancreatic lipase), facilitation of energy expenditure and inhibition of differentiation and proliferation of pre-adipocytes (Rayalam et al., 2008).

The results revealed that the obese untreated-rats chronically fed with high fat diets exhibited a weekly percentage increase in various anthropometric measures. There was a significant increase in the rate of weekly change in obesity index of HFD-induced untreated rats as compared to that of extract-treated rats from the 1st to 6th week of treatments ($p \leq 0.01$). Similarly, the abdominal and thoracic circumferences of obese untreated-rats were significantly higher than those of extract-treated rats in the entire study period. The obese untreated-rats indicated positive weekly changes in abdominal and thoracic circumferences as opposed to the negative changes observed in the extract-treated rats. Moreover, the weekly percentage increase in the abdominal circumference to thoracic circumference ratio, abdominal circumference to height ratio and thoracic circumference to height ratio were significantly higher in HFD-induced untreated rats than those of extract-treated rats ($p \leq 0.01$). Consistent with this study, rats in the negative control group fed on an HFD for 12 weeks showed a significant increase in body weight, body mass index (BMI) and waist hip ratio as compared to rats treated with the aqueous and ethanol leaf extracts of Aegle marmelos at 200, 250, 400, and 500 mg/kg doses (Singh and Garg, 2015).

These observations suggest that exposures of rats to calorically dense diets result in facilitated fat accumulation in the abdominal and thoracic regions due to a high effective energy content of high-fat diets (DeLany et al., 2000). In this regard, chronic exposures to a high fat diet mediates a decrease in resting metabolic rate accompanied with reduced diet-
induced thermogenesis resulting in a higher preferential storage of triglycerides in the adipose tissue leading to an overall increase in anthropometrical measures as indicated in the HFD-fed untreated obese rats (Gerbaix et al., 2010). Consistent with this study, previously reported data also revealed that exposure of adult male Wistar rats to high-fat diets (60% of energy) for 8 weeks resulted in greater intra-abdominal and intra-thoracic fat mass (Okere et al., 2006). The observed increase in body weight may also be due to excessive ingestion of high caloric diet with facilitated fat accumulation in the adipose tissue.

The obesity index, abdominal and thoracic circumference have been established to be the best predictors of intra-abdominal fat thickness in rats and, therefore, of central obesity (Gerbaix et al., 2010). In humans, abdominal circumference, waist circumference, waist hip ratio (WHR) and waist to height ratio (WHtR) have been identified to be simple reliable estimates of body fat content and are among the established criteria for diagnosis of human metabolic syndromes (Novelli et al., 2007; Gerbaix et al., 2010).

Findings from this study indicated that treatment of rats with DCM leaf extract of G. glauca resulted in a negative weekly percentage change in the measured anthropometric parameters from the 1st to 6th week of treatments. The observed weekly reduction in these anthropometric measures could be attributed to the effects of the extract’s bioactive compounds in augmentation of satiety, suppression of absorption and digestion of dietary lipids as well as inhibition of pancreatic lipase activity (De-la-Garza et al., 2011; Ahmed et al., 2014). Therefore, the general reduction in bioavailability of circulating lipids results in decrement in the intra-abdominal fat content (Rubio et al., 2007).
The results also indicated that untreated rats chronically fed on a high-fat diet for twelve weeks resulted in a marked increase in the organ weights and organo-somatic index of the liver, kidney, lungs, heart and brain. However, the organ weight and organo-somatic index of the spleen was significantly reduced than those of extract-treated rats \((p \leq 0.01)\). Treatment of rats with DCM leaf extract of *G. glauca* led to a significant \((p \leq 0.01)\) decrease in organ weights and organo-somatic index of liver, kidney, lungs, heart and brain relative to obese untreated-rats. Besides, the organ weight and organo-somatic index of spleen in extract-treated rats was higher than that of rats in the HFD-induced untreated obese rats \((p \leq 0.01)\). These results were in accordance with the results reported from the previous study where treatment with *Moringa oleifera* remarkably decreased the organ weight and relative organ to body weight of rats fed on high-fat diet for 8 weeks (Bais *et al*., 2014).

Reduction in organ weights and organo-somatic indices could be associated with the observed decreases in body weights of extract-treated rats. Regulation of hunger and appetite signals plays a key role in maintenance of homeostatic balance. Activation of satiety signals such as leptin and melanocyte-stimulating hormones promotes weight reduction through suppressed feed intake (Horvath, 2010). Moreover, facilitated release of peptide YY (PYY), cholecystokinin (CCK), and glucagon-like peptide-1 (GLP-1) slows down gastric emptying and intestinal transit thereby generating indirect satiety effects (Diepvens *et al*. 2007).

Findings from the present study also revealed an approximately 2-3-fold significant \((p < 0.01)\) increase in fat pad weights (Mesenteric, Retroperitoneal, parametrial, perirenal,
subcutaneous) in HFD-induced untreated obese rats relative to those of normal control rats. Moreover, treatment of experimental rats with DCM leaf extract of *G. glauca* at different doses of 200, 250 and 300 mg/kg for six weeks significantly reduced the body fat content as compared to HFD-induced untreated obese rats. A similar study on adult male Wistar rats showed that feeding high-fat diets (60% of energy) for 8 weeks resulted in greater intra-thoracic fat mass in animals fed with a saturated fat acids-rich diet (cocoa butter) and greater reduction in intra-abdominal and epididymal fat mass in those fed with PUFA (sunflower-seed oil) (Okere *et al.*, 2006).

Dietary obesity involves either an increase in the number of adipocytes (hyperplasia) or their size (hypertrophy) (Mancini *et al.*, 2001). Chronic ingestion of high caloric/fat diet has been shown to correlate with total body fat (Fenton *et al.*, 2009). The decrease in the relative weights of the total visceral and subcutaneous fat-depot following treatments with DCM leaf extract of *G. glauca* may be due to inhibitory effect in the formation of new adipocytes from precursor cells resulting in reduced adipocyte proliferation and differentiation (Singh and Garg, 2015). The reduction in body weight of extract-treated rats was accompanied by a depletion of body fat stores as evidenced by the reduced total fat mass. In addition, extract-treated rats showed significantly lowered body adiposity index relative to HFD-induced untreated obese rats (*p*<0.01). The body adiposity index is used as a measure of adiposity since the degree of fat tends to increase gradually with obesity (Taylor and Phillips, 1996).
The evaluation of serum lipid profile of experimental rats showed that oral administration of DCM leaf extract of *G. glauca* for six weeks significantly (*p*<0.01) decreased levels of TC, TG, LDL and VLDL with a concomitant increase in HDL as compared to those of HFD-induced untreated obese rats. Previous studies on obese rats treated with the polyherbal formulation of *Anogeissus Latifolia* (Bark), *Trichodesma amplexicaule* (Whole plant) and *Holostemma annularis* (Roots) for 6 weeks resulted in significantly decreased levels of TC and TG as compared to negative control obese rats fed on cafeteria and atherogenic diets (Avanapu and Dachani, 2013). Similarly, treatment of progesterone-induced obese mice with *Lantana camara* at doses of 200 and 400mg/kg body weight led to a reduction in levels of TG, LDL-C, VLD-C and an increase in HDL-C (Gundamaraju *et al.*, 2012).

It is reported that obesity causes an adverse pattern of plasma lipoproteins (Chang *et al.*, 2011). Facilitated visceral adiposity is associated with dyslipidemia which is characterized by elevated TG and reduced HDL-C concentrations (Velasquez and Bhathena, 2007). The TGs are involved in the ectopic accumulation of lipid stores in the liver and adipose tissues and are associated with metabolic syndromes. The elevation of plasma TGs in the untreated group of rats supplied with high fat diets is indicative of increased *de-novo* lipid biosynthesis (Welty, 2013). Hypertrophied and hyperplastic adipocytes facilitate the synthesis and release of free fatty acids which are transported to the liver where they are re-esterified in hepatocytes to form triglycerides, packaged into VLDL and secreted into blood circulation (Welty, 2013). This contributes to lipotoxicity (Welty, 2013).
High dietary intake of simple carbohydrates and fats can also be converted into triacylglycerols in the liver and exported as VLDLs to the adipose tissues (Welty, 2013). The adipocytes take up these fatty acids and reconver them back to triacylglycerols for storage in intracellular lipid droplets. The loss of triacylglycerol converts some VLDL to intermediate density lipoprotein (IDL); further loss of triacylglycerol from VLDL remnants produces low-density lipoprotein (LDL). The LDL are rich in cholesterol and cholesteryl esters and contain apoB-100 as their major apolipoprotein. The LDLs transports cholesterol to extrahepatic tissues that have specific plasma membrane receptors that recognize apoB-100. Intake of foods rich in cholesterol increases levels of LDL since cholesterol enters blood circulation in the form of LDL. The buildup in circulating levels of cholesterol results in hypercholesterolemia which has been associated with plaque formation in the arteries leading to atherosclerosis and stroke (Chang et al., 2011).

The high-density lipoprotein (HDL) originates from the liver and ileum as small protein-rich particles with relatively low cholesterol and no cholesteryl esters. The HDLs predominantly contain apoA-I, apoC-I, apoC-II as well as the enzyme lecithin-cholesterol acyl transferase (LCAT) (Ansell et al., 2003). ApoA-I activates LCAT on the surface of newly synthesized HDL particles which catalyzes conversion of cholesterol and phosphatidylcholine (lecithin) of chylomicron and VLDL remnants into cholesteryl esters. The formation of cholesteryl esters transforms the disk-shaped nascent HDL into a mature spherical HDL particle. The mature HDL particle rich in cholesterol is picked up by the liver through the apoE receptor and delivers cholesterol through the scavenger receptor B1 (SR-B1) (Chang et al., 2011). Some of this cholesterol is converted to bile salts for
subsequent excretion (Chang et al., 2011; Ansell et al., 2003). The HDL particle also transfers the cholesterol to an IDL reforming a normal, unoxidized LDL particle. The observed increase in levels of HDL in extract-treated rats could be responsible for the lowered levels of TG, LDL and VLDL and hence reduced risk for atherosclerotic plaques formation.

The contribution of caloric intake to obesity development in this model elicited a significant increase in atherogenic index in HFD-induced untreated obese rats than was observed in rats treated with DCM leaf extract of G. glauca. Lipid profile and atherogenic index have been shown to be significant predictors for metabolic disturbances including atherosclerosis, cardiovascular diseases, hypertension and dyslipidemia (Parinita, 2012). Any positive change in the levels of lipids make individuals to be more inclined to develop atherosclerotic plaques, cardiovascular diseases and endothelial dysfunction (Ansell et al., 2003; Parinita, 2012).

Findings of the fasted blood glucose revealed a significant weekly percentage increase in blood glucose levels in HFD-induced untreated obese rats relative to the extract-treated group of rats ($p<0.01$). Raised levels of circulating blood glucose is characteristic of hyperglycemia as a results of an absolute insulin deficiency and/or insulin resistance (Arika et al., 2016). Obesity-related diabetes is mainly associated with insulin resistance and/or hyperinsulinemia due to reduced number of insulin receptors, impaired insulin-receptor binding and disruption in post-receptor insulin signal transduction (Laakso, 2004). The probable mechanisms for antidiabetic potential of the extract includes restoration of insulin
sensitivity, facilitation of uptake of blood glucose by peripheral tissues mediated by an insulin dependent glucose transporter, GLUT-IV, potentiation of insulin release from pancreatic beta cells of Islet of Langerhans as well as elevation of the peripheral glucose utilization (Piero et al., 2015). Similarly, *Panax quinquefolius* was shown to confer its hypoglycemic effects by decreasing absorption of carbohydrates into hepatic portal circulation, reduction of carbohydrates breakdown and increase in insulin release and sensitivity (Xie et al., 2005).

Chronic exposure to high fat diet enhances deposition of toxic lipid metabolites (such as fatty acyl CoA, ceramide and diacylglycerol) in pancreatic beta cells, adipocytes, muscle, liver and arterial tissues resulting in insulin resistance, beta cell dysfunction and accelerated atherosclerosis in type 2 diabetes (Kahn and Flier, 2000). Insulin resistance is a fundamental aspect of the etiology of type 2 diabetes and is also linked to a wide array of other pathophysiologic sequelae including hypertension, hyperlipidemia, and atherosclerosis (Kahn and Flier, 2000). Visceral fat mass has been shown to be a significant predictor for metabolic disturbances including cardiovascular diseases, atherosclerosis, dyslipidemia and hypertension (Savva et al., 2013).

Chronic consumption of the HFD for six weeks without any therapeutic intervention resulted in increased levels of biomarkers of hepatocellular injuries (ALP, ALT, AST, GGT, LDH, total bilirubin, direct and indirect bilirubin. Chronic exposures to high fat diets has been shown to be associated with non-alcoholic fatty liver disease that is the prerequisite for hepatocellular injuries (Caldwell et al., 2004). Non-alcoholic fatty liver
disease is an independent risk factor for the development of liver fibrosis/cirrhosis and hepatocellular carcinoma (HCC) (Caldwell et al., 2004). Hepatic steatosis is commonly asymptomatic and its presence is often associated with elevations in serum aminotransferases (Bruno et al., 2008). The observed increased organ weight and organo-somatic index of liver could be attributed to steatosis of the liver (Caldwell et al., 2004). However, administration of therapeutic doses of DCM leaf extract of *G. glauca* exhibited a hepatoprotective effects, indicated by decreased levels of liver biomarkers and reduction of organ weight and organo-somatic index of liver. Similarly, previous studies showed that dietary supplementation of *Camellia sinensis* (Green Tea) for 6 weeks caused a protective effect against the development of hepatic steatosis and injury in obese (*ob/ob*) mice (Bruno et al., 2008).

Results also indicated that HFD-induced untreated rats recorded higher levels of markers of kidney function such as creatinine and urea. The elevated serum creatinine level and plasma urea signifies impaired kidney function or kidney disease (Lameire et al., 2005). Chronic exposure to high fat diets could account for the increased weight of the kidney and retroperitoneal fat depot in HFD-induced untreated rats. Facilitated fat accumulation in the retroperitoneum lead to fat accretion in the renal sinus thereby altering the renal structure (Montani et al., 2004). Increased retroperitoneal fat within the renal sinus is associated with the increased intra-abdominal pressure due to compression of the renal papilla, renal vein and renal lymphatic vessels (Montani et al., 2004). The resultant increase in renal interstitial pressure leads to a net decrease in fractional sodium excretion and consequently arterial hypertension occurs (Montani et al., 2004). Treatment of obese rats with the three
extract doses significantly decreased levels of creatinine and urea. Similarly, oral administration of *Acalypha wilkesiana* at 100, 200 and 300 mg/kg body weight doses in diabetic rats lowered plasma urea and creatinine levels (Ikewuchi *et al.*, 2011). The reduction in weight of the kidneys and retroperitoneal fat pad in extract-treated rats may be associated with amelioration of kidney injury as evidenced by reduced levels of kidney biomarkers.

Oral administration of therapeutic doses of DCM leaf extract of *G. glauca* significantly (*p*≤0.01) normalised levels of some end-point haematological parameters (such as erythrocytes, Mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC), red cell distribution width (RDW), white blood cells, differential leucocytes counts as well as platelets count, mean platelet volume (MPV) and platelet distribution width (PDW)) relative to HFD-induced untreated obese rats. The observed decrease in weight of the spleen might be indicative of the reduced levels of some hematological indices in HFD-induced untreated obese rats.

The MCV defines the size of the red blood cells while the MCH quantifies the amount of hemoglobin per red blood cell. The MCHC indicates the amount of hemoglobin per unit volume while the RDW represents the coefficient of variation of the red blood cell volume distribution (Moreno *et al.*, 2003). These red cell indices are useful in elucidating the etiology of anemias. The reduction in levels of these indices in the HFD-induced untreated obese rats might be indicative of impaired bone marrow function, decreased number of precursor cells, reduced bone marrow infiltration and nutrients deficiency (such as folate
or B12). Therefore, the resultant reduced levels of MCV, MCH and MCHC are suggestive of microcytic hypochromic anemias (Moreno et al., 2003). Thrombopoietin stimulates myeloid stem cells to produce platelets (Arika et al., 2016). Platelets are mediators of coagulation that forms sticky plugs that helps stop the bleeding during physical injuries (Arika et al., 2016). Platelets also contribute to development of atherosclerosis through hardening of the arteries in hyperglycemic states. The decreased levels of platelet count (thrombocytopenia) in HFD-induced obese untreated rats may either be associated with bone marrow injury and/or hemolysis (Barazzoni et al., 2014). The normalization of levels of platelets and related indices in extract-treated rats are indicative of extracts protective potential against hemophilia, hyperglycemia and coronary thrombosis, a pre-requisite of myocardial infarction (Barazzoni et al., 2014).

The reduced levels of white blood cell count imply a reduced ability of the body to respond to infections (Hoורand et al., 2006). Reduced levels of neutrophils and monocytes imply impaired ability to protect the body against bacteria; reduced lymphocyte levels imply impaired antibody production against foreign organisms and protection against viruses; reduced eosinophils imply impaired ability to kill parasites and impaired allergic responses; reduced basophil levels imply impaired allergic responses (Hoורand et al., 2006). The improved white blood cells and related leukocyte counts in extract-treated rats shows the extracts capacity to potentiate immunity. The improved immune function implies that these indices can specifically recognize diverse range of antigens, establish an immunologic memory, amount an attack and destroys all these antigens (Hoורand et al., 2006). The leaves of Acalypha wilkesiana were shown to improve the hemopoietic system of diabetic
rats (Ikewuchi et al., 2011). It increased levels of total white cell, neutrophil count, platelet counts, red cell and mean cell volume (Ikewuchi et al., 2011).

Results also provided insight into reduced thermogenic effect in obese untreated group of rats following chronic exposure to high fat diets relative to extract-treated rats. Similarly, high fat diet-induced obese rats showed a remarkable reduction in rectal body temperature; however, rats treated with Moringa oleifera at doses of 200 mg/kg and 400 mg/kg body weight indicated increase in rectal body temperature (Bais et al., 2014). Overfeeding with high caloric diet results in a positive energy balance accompanied by a decrease in energy expenditure, which substantially lowers fecal energy loss hence decreased rectal body temperatures as recorded in obese untreated rats (Lammert et al., 1998; Richard and Picard, 2011). Brown adipose tissue (BAT) is specialized in adaptive thermogenesis through lipid oxidation-mediated heat generation by the uncoupling protein-1 (UCP-1) during the mitochondrial respiratory chain (Gurevich-Panigrahi et al., 2009). Activation of adrenergic receptors (ARs) on brown adipose tissue increases lipolysis (β-oxidation of fatty acids) resulting in heat production (Bachman et al., 2002). Chronic exposure to high-fat diet mediate under-expression and unresponsiveness of ARs and a general change in BAT, often, leading to a dysfunction of heat production (Kuhn et al., 2012). As observed, the thermogenic competence of the obese rats might have been increased pharmacologically by administration of the graded doses of DCM leaf extract of G. glauca.

The present study also demonstrated that chronic exposure to HFD led to an increase in cumulative food intake in obese untreated group rats relative to extract-treated rats from
the 1st to 6th week of study period. It has been proposed that a high-fat diet stimulates hyperphagia, which is solely responsible for the increased body fat content (West and York, 1998). Eating behavior is modulated by the brain reward systems through mechanisms that involves the homeostatic need to feed as well as the hedonic and cognitive value of ingestion (Little et al., 2007). Overconsumption of high-fat diets has been associated with lesions in the hippocampus that impairs the rats’ ability to distinguish between the state of hunger and that of satiety (Davidson et al., 2009). Chronic exposure to HFD promotes positive energy balance that leads to orexigenic responses due to increased activity of appetite-stimulant neuronal group such a neuropeptide Y (NPY), agouti-related protein (AGRP) and the neurotransmitter chemical called gamma-aminobutyric acid (GABA) (Horvath, 2010). These neuronal groups potently stimulate food intake, reduce energy expenditure and inhibit the activity of proopiomelanocortin (POMC) (Horvath, 2010). The inhabited activity of this precursor, POMC, reduces the subsequent generation and signaling of the key satiety signaling molecule, the melanocyte-stimulating hormone (MSH) (Horvath, 2010). The observed decrease in the quantity of feed intake upon treatment with the plant extract could be attributed to their potential to increase satiety signals that mediates reduction of feed intake, decreases body weight and increases energy expenditure (Horvath, 2010).

The anti-obesity effect of DCM leaf extract of G. glauca might be attributed to a singly, additive and/or synergistic effects of the contained phytochemicals. The presence of condensed tannins, Gallocatechin-catechin flavan in the DCM leaf extract of G. glauca contributes to reduction in feed intake by decreasing palatability (Zhang et al., 2015).
Palatability is reduced because tannins are astringent. Astringency is the sensation caused by the formation of complexes between tannins and salivary glycoproteins. Low palatability depresses feed intake. Digestibility reduction negatively influences intake because of the fullness effect associated with undigested feedstuff (Zhang et al., 2015). Besides, condensed tannins influence fat digestion through inhibition of small-intestine micelle formation and the inhibition of α-glucosidase activity leading to a decrease in triacylglycerol absorption (Kim et al., 2010). The reduction of absorption of triglycerides would ultimately result in reduced body fat mass (Thom, 2007).

The extracts flavanols such as catechin significantly reduces intracellular lipid accumulation by facilitated inhibition of fat absorption and lipogenesis (Kim et al., 2010). Catechins promotes a reduction of body fat by inducing fat oxidation and thermogenesis (Tucci, 2010). Luteolin is the flavone that affect appetite centrally by acting on the hypothalamus to stimulate release of satiety signals. Luteolin enhances sympathomimetic activity leading to a delay in gastric emptying (Zheng et al., 2010). It decreases hunger and increases feeling of fullness associated with prolonged gastric emptying (Murray et al., 2008).

The GC-MS profile of the extract indicated the presence of long-chain polyunsaturated fatty acids such as omega-3 fatty acids (alpha linolenic acid, docosahexaenoic acid and eicosapentanoic acid), and omega-9 fatty acid (oleic acid). Poly- and mono-unsaturated fatty acids (PUFAs and MUFAs) have been shown to increase the release of satiety hormones such as cholecystokinin (CCK) (Pasman et al., 2008). The CCK delays gastric
emptying and produces a subsequent increased feeling of satiety and a decreased appetite (Lawton et al., 2000). The PUFAs have been reported to increase the synthesis and release of HDLs (Guiné et al., 2009). Neryl acetate, an essential oil confers its anti-obesity effects by enhancing the release of peptide YY, CCK and GLP-1 which inhibit upper gut motility (to slow gastric emptying and intestinal transit) generating an indirect satiety effect (Burns et al. 2002; Diepvens et al. 2007).

Terpenoids such as squalene, phytol, α-Amyrin and β-Amyrin promotes weight reduction through suppressed de novo fatty acid synthesis, increased lipid oxidation and reduced food intake (Gugler et al., 2013). Diterpene reduces body weight by increasing the acting on adenylate cyclase that converts ATP to cyclic adenosine monophosphate (cAMP). Cyclic adenosine monophosphate (cAMP) promotes lipolysis, increases the body’s basal metabolic rate, increases use of body fat and protein degradation and/or decreases protein synthesis (Huang et al., 2016). Enhanced satiety may also account for the reported suppression of energy consumption (Gugler et al., 2013). Squalene have also been reported to confer anti-obesity and hypoglycemic activity through enhancement of lipolysis and regeneration of pancreatic islets cells, respectively (Elliot et al. 2000).

Phytosterols such as stigmasterol and γ-sitosterol decrease body weight through a combination of central and peripheral mechanisms. These compounds have been shown to induce lipogenesis in the adipose tissues (Atif et al., 2003). In the central structures, they decrease appetite by amplifying the signaling of the energy sensing function in the
hypothalamus (Kuriyan et al. 2007). The γ-sitosterol exhibit antihyperlipidemic effects through reduction of serum total cholesterol and triglycerides (Balamurugan et al., 2011).

Generally, the observed medicinal value of G. glauca in the management of obesity lies in the contained phytochemicals whose synergistic effects mediate regulation of various pathways including reduction in lipid absorption, decrease in energy intake, increase in energy expenditure, decrease in pre-adipocyte differentiation and proliferation, decrease in lipogenesis and increase lipolysis (Chandrasekaran et al., 2012). They also confer anti-inflammatory effects and free radical scavenging activities (Rayalam et al., 2008). Multiple-phytochemicals combinations may result in synergistic activities that increases their bioavailability and action on multiple molecular targets, thus offering advantages over treatments with single chemicals (González-Castejón and Rodriguez-Casado, 2011).
CHAPTER FOUR

EFFECTS OF DCM LEAF EXTRACT OF *Gnidia glauca* ON HIPPOCAMPAL-DEPENDENT SPATIAL LEARNING AND MEMORY RETENTION IN HFD-INDUCED OBESE RATS

4.1 Introduction

Obesity is a significant modern health concern with widespread implications to society, family, as well as for individual health and well-being (Norris *et al*., 2016). The negative systemic effects on cardiovascular, metabolic physiology and neuropsychological sequelae of obesity has attracted contemporary research in these fields (Sellbom and Gustard, 2012). Chronic exposure to high fat diet results in an obesogenic state that is coupled with positive impairment on the energy balance equation (Lennox *et al*., 2015). In particular, a high fat diet has been linked with neuropathological changes that culminate in obesity-related cognitive dysfunction and brain alterations (Cho *et al*., 2016; Medic *et al*., 2016). The cognitive domains mostly affected by obesogenic diet include learning (Molteni *et al*., 2002; Murray *et al*., 2009), memory performance (Kanoski and Davidson, 2011; Kosari *et al*., 2012), and the executive function (McNeilly *et al*., 2011). These cognitive behaviors are mainly sub-served by the hippocampus and the prefrontal cortex of the brain (Park *et al*., 2014; Kim *et al*., 2016).

The hippocampus is part of the limbic system bilaterally located in the medial temporal lobes of the brain critical for learning and memory processes (Neves *et al*., 2008). It is highly susceptible to any endo-or exogenous insults (Kanoski and Davidson, 2011). This signifies that any slight alteration in its morphology and function have implications for diverse behaviors such as cognitive flexibility, eating behaviors, working memory,
emotional regulation, episodic memory, stress reactivity, spatial learning and memory retention (Neves et al., 2008; Lucassen et al., 2013).

The brain cannot synthesize its own fuel nor reserve it and, therefore, food provides it with a continuous immediate source of energy (Cunnane, 2010). This unique metabolic profile of the brain may consequently influence its structure and function hence need to balance the energy equation for its optimal activity and growth (Molteni et al., 2004).

Numerous preclinical findings have demonstrated that chronic consumption of a high fat diet is associated with cognitive decline, poorer cognitive performance and increased risk of depression, dementia and Alzheimer’s disease (Balistreri et al., 2010; Miller and Spencer, 2014; Cho et al., 2016). Cognitive performance examining short-term information retention and executive function in rats fed on a 45% high fat diet for 3 months was consistently poor in the operant-based delayed matching to position task (McNeilly et al., 2011). Likewise, rats fed on a 25% high fat diet for 3 months demonstrated cognitive deficits in locating a hidden escape platform in an open field water maze test (Alzoubi et al., 2013).

Long-term high fat dietary exposures appear to confer deleterious effect on cognitive performance (Pengelly et al., 2012). Reduced cognitive faculties in the form of short-term memory and executive function deficits are a frequent consequence of obesity (Lott and Dierssen, 2010). The potential avenues underlying obesity and cognitive impairment
involve oxidative stress, systemic and central inflammation, brain atrophy, breakdown of BBB and disruption in cerebrovascular function (Lucke and Partridge, 2013).

The limited number of the available conventional therapies have shown some potential to improve cognition but for their ineffectiveness and inability to change the underlying disease process thereby exacerbating neurocognitive defects (Baskys and Cheng, 2012). As such, current meta-analysis on alternative herbal-based remedies have demonstrated better therapeutic effects than most conventional therapies in enhancing cognitive performance thus appearing to be the supreme promising option (May et al., 2009). For instance, evidence indicates that *Salvia officinalis* and *Melissa officinalis* (Akhondzadeh et al., 2003) as well as *Gingko biloba* (Birks and Grimley, 2004) modulate cholinergic systems, thereby improving the cognitive function.

The prospective treatment or preventive agents attributable to herbal medicines are the phytochemicals contained in them (Pengelly et al., 2012). These phytobiotics confer multiple physiological effects that serve to protect the brain from pathogenesis (De-la-Garza et al., 2011). Major phytocompounds responsible for such activities include polyphenols (curcumin, resveratrol, flavones, flavanols, monoterpenes, phenolic acids, tannins, chalcones, resveratrol, quercetin, and allicin) and saponins (triterpenoid and steroid saponins) (Slanc et al., 2009; De-la-Garza et al., 2011).

The pharmacological relevance underlying the mechanistic approach to the treatment or improvement of cognition by these phytobiotics include acetylcholinesterase inhibition
butyrylcholinesterase inhibition (Orhan et al., 2008; Cho et al., 2012), protection of dopaminergic neurons (Kim et al., 2006), activation of neurotransmitter on neurons (Williams and Spencer, 2012), inhibition of beta amyloid plaques formation (Ono et al., 2008; Wang et al., 2008a) as well as protection from neurotoxicity of amyloid β-peptide (Ansari et al., 2009). Other probable mechanisms of action include sequestration of an insoluble toxic protein aggregates (Poulose et al., 2014), increase in free radical scavenging activity and reduction of cortisol levels (Howes and Houghton, 2003; Slanc et al., 2009; Pengelly et al., 2012), enhancement of insulin and leptin sensitivity, enhancement of cerebral blood flow and reduction of inflammation (May et al., 2010; Poulose et al., 2014).

*Gnidia glauca* is traditionally used for various therapeutic effects in subtropical Africa. It has demonstrated significant superior efficacy in management of obesity. Moreover, it has been a useful adjuvant and a key adjunct to dietary control in diabetic patients (Hasani-Ranjbar and Larijani, 2015). It is postulated that the anti-obesity effect of *G. glauca* are linked with amelioration of cognitive defects in animal models. Therefore, this chapter explores the effects of DCM leaf extract of *G. glauca* on cognitive function in HFD-induced obese rats.
4.2 Materials and Methods

4.2.1 Preparation of the Plant Material, Handling of Experimental Rats and Induction of Obesity

The collection of medicinal plant, processing and extraction of the plant material were conducted as described in chapter 3 (Section 3.2.1-2). The preparation of appropriate doses of DCM-leaf extract of *G. glauca* for bioassays were as described in chapter 3 (Section 3.2.3). Similarly, the age, average weight, acclimatization period and handling of experimental rats were as described in chapter 3 (Section 3.2.4-7). Further, the composition of high fat diet, the induction of obesity and the experimental design used in the present study were as described in chapter 3 (Section 3.2.5).

4.2.2 Morris Water Maze

Spatial learning and memory retention (cognitive function) were determined using the Morris water maze (MWM) experiment (Morris, 1984; Sharma *et al.*, 2010) to ascertain the effect of a six-week oral administration of *G. glauca*.

4.2.2.1 The Water Maze Apparatus

The water maze apparatus consisted of a cylindrical metal barrel drum (pool) (diameter=130 cm, height=35 cm) (Figure 4.1). The pool was filled with water (22°C±2°C) and was made opaque by addition of 1 kg of skim milk powder to ensure camouflage of the escape platform. A Plexiglas cylinder with a stripped top (diameter=9 cm, height=20 cm) was used as the escape platform in the maze (Figure 4.1). The cylindrical escape platform was filled with water to weigh it down in the pool. The level of the water in the pool was adjusted to 1 cm below the surface of the striped top of the platform, thus creating
a visible escape platform, and to 1 cm above the striped top of the platform, thus creating
an invisible escape platform. The pool was divided into four quadrants: Northwest,
Northeast, Southwest and Southeast in the center of which a mark was made to ensure
proper placement of the escape platform (branded 1-4) (Figure 4.2). Boundaries of these
quadrants were marked on the edges of the pool with a masking tape and labeled: North
(N), South (S), East (E) and West (W) (Figure 4.2). Clearly visible cues were mounted on
the walls of the pool for orientation (Figure 4.1). Experimental sessions were captured by
a video camera placed above the maze. The recorded trials were then fed to a detection
system (HVS Image), which allowed tracking the navigation paths and quantification of
several parameters (Figure 4.1).

Figure 4.1: Morris Water Maze/Navigation Task
4.2.2.2 Procedure

The water maze test consisted of an acquisition phase, a reversal phase and a probe trial phase that lasted ten days. A total of four trials were conducted for each replicate in each experimental group. The first day was an acquisition training with a visible platform followed by another acquisition training with an invisible submerged platform for the 3 proceeding consecutive days. On day five, an acquisition probe trial was conducted with no escape platform. On day six, reverse trials were conducted using the visible platform. Days 7–9 were reversal training days, again with an invisible platform. On the tenth day, a reverse probe trial was conducted with no escape platform.

4.2.2.3 Acquisition (Platform in North West quadrant for days 1, 2, 3, 4, 5)

During acquisition training, the water level was adjusted appropriately such that the escape platform was submerged by 1 cm of water (invisible platform). The platform was positioned on the mark in the center of the Northwest quadrant. Each animal received four
trials of 60 seconds (max) per day. The starting positions of the animals were predetermined, which prevented any sequence of trials to be repeated by the same animal during any other day. The selected possible start positions were at the boundaries of the quadrants (such as West, North, East or South). For each trial, the rats were placed in the water, facing the wall of the tank, in one of the four start locations. The rats were then permitted to explore the pool and to search for the hidden escape platform for 60 seconds. If the rats found the platform, the timer was stopped and the animal was permitted to remain on the platform for 15 sec. If the animal couldn’t find the platform during the allotted time, the animal was guided onto the platform and allowed to remain on the platform for 15 sec to visually explore their surroundings. After each training session, the rats were dried with a towel and returned to their holding cage.

4.2.2.4 Reversal (Platform in South East quadrant for days 6, 7, 8, 9, 10)

The invisible escape platform was moved to the opposite quadrant (Southeast quadrant), and rats were again assigned to appropriate start positions. The same procedures adapted in acquisition training was replicated during the entire period of reversal training.

4.2.2.5 Probe Trial (No platform, Day 5 and Day 10)

To assess spatial memory retention ability, the animals were subjected to the 60 sec probe trial following the last training session of each phase (acquisition phase and reversal phase) upon removal of the escape platform from the maze. The frequency of occupancy in the target quadrant was recorded.
4.2.2.6 Visible Platform (Visible platform in Northwest quadrant for day 1 and Southeast quadrant for day 5)

To obtain a visible platform, the water level was adjusted appropriately such that the platform emerged 1 cm above the water surface. The visible platform was stationed in the Northwest quadrant of the pool on day 1 in acquisition phase while on day 5, the visible platform was in Southeast quadrant during the reversal phase. The same procedures as described above in both acquisition and reversal training were followed.

4.2.2.7 Running of Experimental Animals

During the entire experimental period, the animals were held in a cage lined with a paper towel to allow rats to dry. The paper towels were replaced when they become completely wet. Rats were then run sequentially per group with 5 minutes between each trial for each rat.
4.2.3 Data Management and Statistical Analysis

The data on time latency to reach the escape platform, swimming speed, navigation distance and quadrant frequency was computed by the WatermazeBeta, Actimetrics Software configured on an IBM PC-compatible computer. The data for each navigation variable was exported to Microsoft® Excel spreadsheet, where it was organized and later transferred to Minitab software version 17.1 for analysis. The data was subjected to descriptive statistics and expressed as Mean±Standard Deviation (SD). One-way ANOVA was used to test the significance within the normal control group rats, negative control group rats, Orlistat-treated group of rats and extract-treated group of rats at 99% confidence interval. The data was further subjected to Tukey’s post hoc for pairwise comparison and separation of means. The findings were presented in tables and figures.
4.3 Results

4.3.1 Effect of DCM Leaf Extract of G. glauca on Hippocampal-Dependent Spatial Learning and Memory Retention in High Fat Diet-Induced Obese Rats

4.3.1.1 Analysis of the Navigation Behavior

The rats adopted the characteristic adult swimming posture of forepaws tucked under the head and hindlegs used to propel it forward. The head largely remained above water surface except for brief moments. Initially, the rats were thigmotaxic, swimming around the perimeter of the pool near or against the side walls, making occasional efforts to escape by forepaw climbing movements against the side. Later, they swam out into open water, crossing the pool several times during each of the initial habituation sessions. The rats readily climbed onto the escape platforms when they encountered them or were guided to locate the platform when they failed to locate it within the experimental period. Regularly, the rats would then rear and/or turn around for a few seconds before making vigorous “wet-dog” shakes followed by grooming and occasional face-washing. There was no sign of the animals treating the “underwater” platform any different from the “above-water” platform as a refuge from the water.
4.3.2 Latency Period

4.3.2.1 Acquisition Training

During the first day of the visible platform tests, the negative control group rats exhibited significantly longer latency period to escape onto the visible platform relative to the extract-treated rats and Orlistat-treated rats ($p \leq 0.01$; Table 4.1). In the hidden platform tests (2nd - 4th day), the extract-treated rats and Orlistat-treated rats showed a shorter mean escape latency period onto the hidden platform than negative control group rats ($p \leq 0.01$; Table 4.1). From the 1st to the 3rd day, no significant difference in latency period was indicated among the extract-treated rats, normal control group rats and Orlistat-treated rats in both visible and invisible platform tests ($p > 0.01$). However, on the 4th day, the extract-treated rats showed significantly lower latency period than Orlistat-treated rats and normal control group rats ($p \leq 0.01$; Table 4.1).
Table 4.1: Effect of DCM Leaf Extract of *Gnidia glauca* on Escape Latency in HFD-Induced Obese Rats During Acquisition Training

<table>
<thead>
<tr>
<th>TREATMENT</th>
<th>Escape Latency (sec)</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 1</td>
<td>Day 2</td>
<td>Day 3</td>
<td>Day 4</td>
</tr>
<tr>
<td>Normal Control</td>
<td>35.20±0.84&lt;sup&gt;b&lt;/sup&gt;</td>
<td>43.60±2.07&lt;sup&gt;b&lt;/sup&gt;</td>
<td>32.20±1.30&lt;sup&gt;b&lt;/sup&gt;</td>
<td>24.20±1.30&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>High-fat Diet</td>
<td>46.80±1.30&lt;sup&gt;a&lt;/sup&gt;</td>
<td>53.20±0.84&lt;sup&gt;a&lt;/sup&gt;</td>
<td>45.80±1.48&lt;sup&gt;a&lt;/sup&gt;</td>
<td>36.00±1.58&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>HFD+Orlistat (30mg/kg)</td>
<td>32.80±1.92&lt;sup&gt;b&lt;/sup&gt;</td>
<td>43.40±2.79&lt;sup&gt;b&lt;/sup&gt;</td>
<td>32.60±2.07&lt;sup&gt;b&lt;/sup&gt;</td>
<td>22.40±0.89&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>HFD+200mg/kg</td>
<td>33.20±2.17&lt;sup&gt;b&lt;/sup&gt;</td>
<td>41.00±2.00&lt;sup&gt;b&lt;/sup&gt;</td>
<td>30.80±1.64&lt;sup&gt;b&lt;/sup&gt;</td>
<td>20.00±1.00&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>HFD+250mg/kg</td>
<td>33.40±1.52&lt;sup&gt;b&lt;/sup&gt;</td>
<td>40.60±1.14&lt;sup&gt;b&lt;/sup&gt;</td>
<td>29.60±1.52&lt;sup&gt;b&lt;/sup&gt;</td>
<td>17.80±1.30&lt;sup&gt;cd&lt;/sup&gt;</td>
</tr>
<tr>
<td>HFD+300mg/kg</td>
<td>33.80±0.84&lt;sup&gt;b&lt;/sup&gt;</td>
<td>40.20±0.84&lt;sup&gt;b&lt;/sup&gt;</td>
<td>30.00±1.87&lt;sup&gt;b&lt;/sup&gt;</td>
<td>16.00±0.71&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Means followed by similar lower-case letters within columns are not statistically different (*p*>0.01).
4.3.2.2 Reverse Training

When the platform was moved to the opposite quadrant, the extract-treated rats indicated a significant reduction in the latency period onto escape platforms (in both the visible and invisible platforms tests) relative to the negative control group rats \((p \leq 0.01; \text{Table 4.2})\). Further, on the 6th and 7th day, it was observed that there was no significant difference in the latency period between the extract-treated rats and Orlistat-treated rats \((p > 0.01)\). However, in both days, the extract-treated rats at doses of 250 and 300mg/kg body weight recorded lower latency periods compared to those of rats in the normal control group \((p \leq 0.01; \text{Table 4.2})\). On the 8th day, rats in the normal control group took significantly more time to reach the escape platform than the extract-treated rats and Orlistat-treated rats \((p \leq 0.01; \text{Table 4.2})\). On the 9th day, rats treated with the three extract doses showed significantly decreased latency period to reach the escape platform relative to the normal control group rats \((p \leq 0.01)\). However, on the same day, no significant difference in latency period was observed in the normal control group rats and Orlistat-treated rats \((p > 0.01; \text{Table 4.2})\).
Table 4.2: Effect of DCM Leaf Extract of *Gnidia glauca* on Escape Latency in HFD-Induced Obese Rats During Reverse Training

<table>
<thead>
<tr>
<th>TREATMENT (mg/kgbw)</th>
<th>Escape Latency (sec)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 6</td>
<td>Day 7</td>
</tr>
<tr>
<td>Normal Control</td>
<td>40.80±0.84&lt;sup&gt;b&lt;/sup&gt;</td>
<td>48.60±0.55&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>High-fat Diet</td>
<td>50.60±0.55&lt;sup&gt;a&lt;/sup&gt;</td>
<td>57.60±1.14&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>HFD+Orlistat</td>
<td>37.40±1.14&lt;sup&gt;c&lt;/sup&gt;</td>
<td>46.20±1.92&lt;sup&gt;bc&lt;/sup&gt;</td>
</tr>
<tr>
<td>HFD+200mg/kg</td>
<td>37.40±1.52&lt;sup&gt;c&lt;/sup&gt;</td>
<td>45.80±2.59&lt;sup&gt;bc&lt;/sup&gt;</td>
</tr>
<tr>
<td>HFD+250mg/kg</td>
<td>38.20±0.84&lt;sup&gt;c&lt;/sup&gt;</td>
<td>44.80±0.84&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>HFD+300mg/kg</td>
<td>38.60±1.14&lt;sup&gt;c&lt;/sup&gt;</td>
<td>45.00±1.00&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Means followed by similar lower-case letters within columns are not statistically different (*p*>0.01).
4.3.3 Navigation Distance

4.3.3.1 Acquisition Training

Analysis of the navigation distance exhibited differences among the experimental groups (Table 4.3). The results demonstrated that rats treated with the three extract doses as well as those in the normal control and positive control groups covered significantly shorter distance to reach the visible escape platform on the 1st day relative to the negative control group ($p \leq 0.01$; Table 4.3). Similarly, rats in the extract-treated groups, normal control and positive control groups covered significantly shorter distance to reach the invisible escape platform from the 2nd to the 4th day than rats in the negative control group ($p \leq 0.01$). Notably, in each day of the acquisition training, there was no significant difference in swimming path lengths among extract-treated rats, normal control group rats and Orlistat-treated rats ($p > 0.01$; Table 4.3).
Table 4.3: Effect of DCM Leaf Extract of *Gnidia glauca* on Navigation Distance in HFD-Induced Obese Rats During Acquisition Training

<table>
<thead>
<tr>
<th>TREATMENT</th>
<th>Navigation Distance/Path Length (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 1</td>
</tr>
<tr>
<td>Normal Control</td>
<td>230.02±20.04&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>High-fat Diet</td>
<td>390.72±20.07&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>HFD+Orlistat</td>
<td>207.70±23.20&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>HFD+200mg/kg</td>
<td>220.30±25.60&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>HFD+250mg/kg</td>
<td>218.92±18.61&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>HFD+300mg/kg</td>
<td>209.80±16.46&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

 Means followed by similar lower-case letters within columns are not statistically different ($p>0.01$).
4.3.3.2 Reverse Training

The reverse trials yielded a considerable trial-to-trial variability between the rats in the negative control group and rats in other experimental groups (Table 4.4). During the entire period of reverse training, rats in the negative control group covered considerably longer distance to reach both the visible and invisible escape platforms relative to the extract-treated rats, Orlistat-treated rats and rats in the normal control group ($p \leq 0.01$; Table 4.4). On the contrary, rats treated with the three extract doses did not show any statistical difference in the navigation distance as compared to the Orlistat-treated rats and rats in the normal control group ($p > 0.01$; Table 4.4).
Table 4.4: Effect of DCM Leaf Extract of *Gnidia glauca* on Navigation Distance in HFD-Induced Obese Rats During Reverse Training

<table>
<thead>
<tr>
<th>TREATMENT</th>
<th>Navigation Distance/Path Length (cm)</th>
<th>Day 6</th>
<th>Day 7</th>
<th>Day 8</th>
<th>Day 9</th>
</tr>
</thead>
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<tr>
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</tr>
<tr>
<td>Normal Control</td>
<td></td>
<td>253.62±17.44&lt;sup&gt;b&lt;/sup&gt;</td>
<td>342.32±8.59&lt;sup&gt;b&lt;/sup&gt;</td>
<td>271.60±39.6&lt;sup&gt;b&lt;/sup&gt;</td>
<td>235.32±16.85&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>High-fat Diet</td>
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</tr>
<tr>
<td>HFD+Orlistat</td>
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<td>249.74±20.12&lt;sup&gt;b&lt;/sup&gt;</td>
<td>326.40±16.14&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>HFD+300mg/kg</td>
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<td>260.92±21.29&lt;sup&gt;b&lt;/sup&gt;</td>
<td>215.80±21.22&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Means followed by similar lower-case letters within columns are not statistically different (p>0.01).
4.3.4 Swimming Speed

4.3.4.1 Acquisition Training

The analysis of the speed variable indicated significantly higher swimming speed in extract-treated rats than rats in the negative control group ($p \leq 0.01$; Table 4.5). Similarly, Orlistat-treated rats and normal control group rats swam relatively faster than rats in the negative control group ($p \leq 0.01$; Table 4.5). On the 1<sup>st</sup>, 2<sup>nd</sup> and 3<sup>rd</sup> day of the acquisition training, the swimming speed of rats in the normal control and that of Orlistat-treated rats were statistically similar to that of extract-treated rats ($p > 0.01$; Table 4.5). However, on the 4<sup>th</sup> day, the swimming speed of extract-treated rats were significantly higher than that of rats in the normal control group ($p \leq 0.01$; Table 4.5). Further, rats treated with the extract doses of 250 and 300mg/kg body weight showed significantly higher swimming speed than Orlistat-treated rats ($p \leq 0.01$; Table 4.5). However, no significant difference was indicated in the swimming speed between the Orlistat-treated rats and normal control group rats ($p > 0.01$; Table 4.5).
Table 4.5: Effect of DCM Leaf Extract of *Gnidia glauca* on Swimming Speed in HFD-Induced Obese Rats During Acquisition Training

<table>
<thead>
<tr>
<th>TREATMENT</th>
<th>Swimming Speed (cm/s)</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Day 1</td>
<td>Day 2</td>
<td>Day 3</td>
</tr>
<tr>
<td>Normal Control</td>
<td>12.22±0.46&lt;sup&gt;a&lt;/sup&gt;</td>
<td>11.86±1.07&lt;sup&gt;a&lt;/sup&gt;</td>
<td>14.61±0.73&lt;sup&gt;a&lt;/sup&gt;</td>
<td>16.77±1.16&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>High-fat Diet</td>
<td>6.21±0.32&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7.16±0.35&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7.62±0.53&lt;sup&gt;b&lt;/sup&gt;</td>
<td>8.05±0.71&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>HFD+Orlistat</td>
<td>12.47±1.12&lt;sup&gt;a&lt;/sup&gt;</td>
<td>11.69±0.88&lt;sup&gt;a&lt;/sup&gt;</td>
<td>14.09±1.41&lt;sup&gt;a&lt;/sup&gt;</td>
<td>18.26±1.39&lt;sup&gt;cd&lt;/sup&gt;</td>
</tr>
<tr>
<td>HFD+200mg/kg</td>
<td>12.73±1.54&lt;sup&gt;a&lt;/sup&gt;</td>
<td>12.19±0.56&lt;sup&gt;a&lt;/sup&gt;</td>
<td>14.74±1.37&lt;sup&gt;a&lt;/sup&gt;</td>
<td>20.31±1.25&lt;sup&gt;bc&lt;/sup&gt;</td>
</tr>
<tr>
<td>HFD+250mg/kg</td>
<td>12.57±0.84&lt;sup&gt;a&lt;/sup&gt;</td>
<td>12.46±0.42&lt;sup&gt;a&lt;/sup&gt;</td>
<td>15.53±1.34&lt;sup&gt;a&lt;/sup&gt;</td>
<td>22.24±2.74&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>HFD+300mg/kg</td>
<td>12.13±0.61&lt;sup&gt;a&lt;/sup&gt;</td>
<td>12.56±0.24&lt;sup&gt;a&lt;/sup&gt;</td>
<td>14.53±1.39&lt;sup&gt;a&lt;/sup&gt;</td>
<td>23.67±1.57&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Means followed by similar lower-case letters within columns are not statistically different (*p*<0.01).
4.3.4.2 Reverse Training

The analysis of the speed variable in the entire reverse trial indicated that rats treated with the DCM leaf extract of *G. glauca* swam significantly faster than rats in the negative control group (*p*≤0.01; Table 4.6). On the 6th and 7th day of the reverse trials, results indicated that there was no significant difference in the swimming speed among the extract-treated rats, the normal control group rats and Orlistat-treated rats (*p*>0.01; Table 4.6). On the 8th day, the swimming speeds of rats treated with the extract dose of 300mg/kg body weight were statistically different from that of Orlistat-treated rats (*p*≤0.01; Table 4.6). Moreover, at this extract dose, the treated rats swam relatively faster than normal control group rats (*p*≤0.01; Table 4.6). On the 9th day, the extract-treated rats had a significantly higher swimming speed than rats in the normal control group (*p*≤0.01; Table 4.6). Similarly, on the same day, rats treated with the extract dose of 300mg/kg body weight swam faster than Orlistat-treated rats (*p*≤0.01; Table 4.6). However, Orlistat-treated rats did not significantly swim faster than rats in the normal control group (*p*>0.01; Table 4.6).
Table 4.6: Effect of DCM Leaf Extract of *Gnidia glauca* on Swimming Speed in HFD-Induced Obese Rats During Reverse Training

<table>
<thead>
<tr>
<th>TREATMENT</th>
<th>Swimming Speed (cm/s)</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 6</td>
<td>Day 7</td>
<td>Day 8</td>
<td>Day 9</td>
</tr>
<tr>
<td>Normal Control</td>
<td>11.12±0.51&lt;sup&gt;a&lt;/sup&gt;</td>
<td>11.16±0.12&lt;sup&gt;a&lt;/sup&gt;</td>
<td>12.68±0.41&lt;sup&gt;b&lt;/sup&gt;</td>
<td>15.12±0.82&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>High-fat Diet</td>
<td>6.54±0.28&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6.98±0.35&lt;sup&gt;b&lt;/sup&gt;</td>
<td>8.13±1.07&lt;sup&gt;c&lt;/sup&gt;</td>
<td>9.02±0.69&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>HFD+Orlistat</td>
<td>12.03±0.61&lt;sup&gt;a&lt;/sup&gt;</td>
<td>11.41±0.46&lt;sup&gt;a&lt;/sup&gt;</td>
<td>13.35±0.74&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>16.21±1.10&lt;sup&gt;cd&lt;/sup&gt;</td>
</tr>
<tr>
<td>HFD+200mg/kg</td>
<td>12.15±1.06&lt;sup&gt;a&lt;/sup&gt;</td>
<td>11.59±0.51&lt;sup&gt;a&lt;/sup&gt;</td>
<td>13.53±0.64&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>17.81±0.40&lt;sup&gt;bc&lt;/sup&gt;</td>
</tr>
<tr>
<td>HFD+250mg/kg</td>
<td>11.87±0.71&lt;sup&gt;a&lt;/sup&gt;</td>
<td>11.92±0.49&lt;sup&gt;a&lt;/sup&gt;</td>
<td>13.86±1.15&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>19.93±1.91&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>HFD+ 300mg/kg</td>
<td>11.22±0.44&lt;sup&gt;a&lt;/sup&gt;</td>
<td>11.81±0.30&lt;sup&gt;a&lt;/sup&gt;</td>
<td>14.41±0.33&lt;sup&gt;a&lt;/sup&gt;</td>
<td>21.26±1.65&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Means followed by similar lower-case letters within columns are not statistically different (*p*>0.01).
4.3.5 Spatial Memory Retention

4.3.5.1 Acquisition

The analysis performed on the probe test on the 5th day in which the platform was withdrawn from the water indicated that rats treated with the three extract doses exhibited preference for North-West quadrant (which was the correct location of escape platform during the acquisition training) than rats in the negative control group \( (p \leq 0.01; \text{Figure 4.3}) \). Similarly, the extract-treated rats exhibited significantly higher quadrant frequency in the correct quadrant than both the Orlistat-treated rats and normal control group rats \( (p \leq 0.01; \text{Figure 4.3}) \). However, the Orlistat-treated rats showed no significant difference in spatial bias towards the target quadrant than the normal control group rats \( (p \leq 0.01; \text{Figure 4.3}) \).

Figure 4.3: Effect of DCM leaf extract of *G. glauca* on spatial memory retention in HFD-induced obese rats during acquisition training. Values followed by the same superscript across treatments are not significantly different from each other \( (p > 0.01) \).
4.3.5.2 Reverse Training

During the reverse trial tests on the 10th day, it was observed that the Orlistat-treated rats and extract-treated rats had a high number of entries into the correct quadrant (South-East quadrant) relative to the negative control group rats \( (p \leq 0.01; \text{Figure 4.4}) \). Besides, the extract-treated rats concentrated their search in the quadrant where the platform was previously located during the training sessions more than the normal control group rats (Figure 4.4). The rats treated with extract doses of 250 and 300mg/kg body weight recorded significantly higher quadrant frequency than Orlistat-treated rats \( (p \leq 0.01; \text{Figure 4.4}) \). However, there was no significant difference in the number of entries into the target quadrant between rats treated with the reference drug, Orlistat and rats treated with the extract at a dose level of 200mg/kg body weight \( (p>0.01; \text{Figure 4.4}) \).

Figure 4.4: Effect of DCM leaf extract of *G. glauca* on spatial memory retention in HFD-induced obese rats during reverse training. Values followed by the same superscript across treatments are not significantly different from each other \( (p>0.01) \).
4.4 Discussion

The hippocampal formation is critical in cognitive processes such as spatial learning and memory retention (Lucassen et al., 2013). The Morris water maze (MWM) is a versatile behavioral technique employed to test hippocampal-dependent spatial learning and memory retention for rodent models relying on distal/extra-maze cues to navigate around an open swimming arena from identified start positions to locate a submerged invisible escape platform (Sharma, 2009). To determine spatial learning, repeated trials are practiced while reference memory is assessed by probe test or preference for the target quadrant while the escape platform is absent (Vorhees and Williams, 2006).

The HFD-induced obesity has been implicated to increase the risk of development of cognitive impairment in form of short-term memory, executive function deficits, dementia and Alzheimer’s disease (Murray et al., 2009; Woo et al., 2013). Short-term exposure to an obesogenic diet even for only 72 hours is sufficient to impair hippocampal-dependent memory (Beilharz et al., 2014; Hsu and Kanoski, 2014). The hippocampal-dependent spatial learning examines ability of the experimental animal to acquire spatial information by measuring various variables including escape latency, swimming speed and navigation distance while spatial memory retention is determined using the probe test of the MWM (Sharma, 2009).

The current study aimed at evaluating the effect of G. glauca on memory and learning processes in HFD-induced obese rats relying on the integrity of the hippocampus through a vision dependent allocentric based navigation task in the Morris water maze. The results
exhibited that chronic exposures to high fat diets had a significant influence on the rats’ navigation behaviors in the Morris water maze test. The analysis of both acquisition training and transfer/reversal trials confirmed that HFD-fed untreated obese rats exhibited a significant longer latency to escape onto the hidden platform, swam slower and covered longer distances to reach the submerged platform than their treated counterparts in the maze. In the probe test, the HFD-fed untreated obese rats searched the escape platform rather randomly and spent more time in non-target quadrants, indicating their inferior memory of the learning task. Several high-energy feeding studies in rodent models have demonstrated marked impairments of cognitive functions determined by latency to find the hidden platform (Kuang et al., 2014), swimming speed, navigation distance and probe trial of the MWM (Kim et al., 2006; Soares et al., 2013).

The escape/time latency and navigation distance variables reflect the integrity of the hippocampus to locate the hidden escape platform and ought to translate in shorter time latency and path distances (Bélanger et al., 2004). Besides, the speed/velocity variables are influenced by weight and, therefore, reflects the perceptive or motor capacities of the experimental animals (Bélanger et al., 2004). Spatial learning and memory retention as determined by the probe test demonstrate whether navigation behavior towards the escape platform depended on allocentric processing/cues and ought to define the integrity of the hippocampus (Bélanger et al., 2004).

Chronic exposure to HFD is often associated with hyperglycemia due to insulin insensitivity (Shoelson et al., 2007). The consequent diabetic state may result in
retinopathy, which renders poor vision (Piero et al., 2012b). The HFD-fed untreated obese rats performed dismally in the Morris water maze and this could be attributed to poor vision, which renders the rats to inappropriately perceive the surrounding external cues that are necessary to locate the escape platform. These observations corroborate with findings of Bélanger et al. (2004), whose 8-week untreated diabetic ZDF rats developed cataracts, which consequently resulted in poor performance in the maze.

The observation that HFD-fed untreated obese rats had slower swimming speeds clearly indicates impaired motor functions. The altered motor capacities of these obese rats might be contributed by their overweight condition. A study by Frisbee and Stepp (2001) showed that the muscular tissues of ZDF rats deteriorate faster in comparison to normal control rats. Several studies on Rosmarinus officinalis (Rosemary) (Moss et al., 2003), Bacopa monnieri (Calabrese et al., 2008) and Centella asiatica (Wattanathorn et al., 2008) have also demonstrated positive modulation of ambulatory, cognition, mood, anxiety and depression.

The potential mediators underlying the link between obesity and the risk of cognitive decline include brain atrophy, breakdown of BBB, systemic and central inflammation as well as oxidative stress (Shefer et al., 2013). Increased adiposity has shown a positive correlation with reduced hippocampal volume and cognitive decline (Debette et al., 2011). A high dietary fat was shown to induce hippocampal-hypothalamic neuronal apoptosis (Rivera et al., 2015) and a reduction in hippocampal weight (Calvo-Ochoa et al., 2014). Besides, it decreases levels of hippocampal brain-derived neurotrophic factor (BDNF)
which mediates neuronal changes involved in learning, memory, neurogenesis as well as synaptic plasticity (Francis and Stevenson, 2013). The metabolic and dietary consequences of a high fat diet intake influence the brain function by disrupting the integrity of the BBB (Davidson et al., 2012). The pathophysiological mechanism relating obesity to BBB dysfunction, neuronal impairment and memory loss involve plasma accumulation of amyloid proteins (Aβ) that pathologically affects the cerebrovasculature (Jahangiri et al., 2013).

Systemic and central inflammation have been shown to contribute to cognitive decline via cytokine-mediated production (McAfoose and Baune, 2009). Synthesis and release of proinflammatory cytokines such as IL-1β, IL-6 and TNF-α contribute to cognitive impairment by affecting cognitive processes such as synaptic plasticity, neurogenesis, neuromodulation, memory consolidation and long-term potentiation (LTP) (Nguyen et al., 2014). Further, chronic consumption of high fat diet exacerbates oxidative damage in the hippocampus due to attenuated antioxidant defenses and facilitated production of pro-inflammatory cytokines, chemokines and brain’s resident immune cells, the microglia and astrocytes (Loane and Byrnes, 2010; Pistell et al., 2010). These events, therefore, culminates in impaired neurogenesis, synaptic remodeling and reduction in neuronal spine density (Freeman et al., 2013). It also results in neuronal apoptosis, deregulated HPA-axis, neurodegeneration and brain atrophy and, hence, cognitive impairment (Morrison et al., 2010; Freeman et al., 2013).
The analysis of both acquisition training and reverse training indicated that rats treated with the DCM leaf extract of *G. glauca* exhibited a significant shorter latency to escape onto the hidden platform, swam faster and covered shorter distances to reach the submerged platform than HFD-induced obese untreated rats in the maze. In the probe test, the extract-treated rats showed novel behavioral strategy of concentrating their search in the target quadrant where the platform was located in the previous training sessions. This suggests that treatment with the three extract doses might have improved obesity-induced memory loss. Consistent with this study, it was reported that the high-fat and high-fructose diet-induced obese mice treated with *Camellia sinensis* (green tea) showed a significantly lower escape latency and escape distance than the high-fat and high-fructose diet-induced obese untreated mice on each test day. Besides, the treated mice spent a longer time in the target quadrant and had greater number of platform crossings than the untreated obese mice (Liu *et al.*, 2014b).

The probable mechanisms attributed to the positive influences on cognitive functions by *G. glauca* among others include the normalization of antioxidant mechanisms (Altmann *et al.*, 2016), reduction of inflammation (Sim, 2014; Baek, 2016), increment in expression of hippocampal neurotrophic factors (BDNF), and enhancement of neurogenesis which facilitates neural plasticity in the hippocampus (Ma *et al.*, 2017; Seo *et al.*, 2014).

The pharmacological relevance underlying the cognitive modulatory activity of *G. glauca* may be due to its chemical compounds, the phytochemicals, such as polyphenols (luteolin, catechins, Galloatechin-catechin flavan, stilbenes, curcumín, quercetin, naringinen and
flavonoid), alkaloids, long-chain polyunsaturated fatty acids and Vitamin E. These phytocompounds confer multiple physiological effects that not only serve to protect the brain from the pathogenicity but also reverses the underlying disease process (Slanc et al., 2009; De-La Garza et al., 2011).

Luteolin has been shown to ameliorate HFD-induced cognitive impairments (Liu et al., 2014a). Treatment of HFD-induced obese mice with luteolin restored blood adipocytokines level to normal, alleviated neuroinflammation, reduced oxidative stress and neuronal insulin resistance in the mouse brain (Liu et al., 2014a). Luteolin significantly increased the level of brain-derived neurotrophic factor (BDNF), enhanced the action of synapsin I (SYP) and postsynaptic density protein 95 (PSD-95) in the cortex and hippocampus (Liu et al., 2014a).

Catechins such as epigallocatechin-3-gallate has been implicated to have the potential to alleviate high-fat and high-fructose-induced insulin resistance and cognitive impairment in mice (Liu et al., 2014a). Catechins treatment of high-fat and high-fructose-induced mice significantly increased the average time spent in the target quadrant and had greater numbers of platform crossings than their obese untreated counterparts, an indication of improved memory (Liu et al., 2014a). Catechins exhibit their protective effects by preventing Aβ-induced neuronal injury through scavenging of ROS (Choi et al., 2001). Specifically, catechins decreases levels of malonyldialdehyde (MDA) and caspase thereby resulting in decreased ROS (Choi et al., 2001). Catechins inhibits fibrillogenesis of Aβ plaques through their direct binding to the unfolded polypeptides Aβ converting them into
unstructured, nontoxic Aβ-oligomers instead of β-sheet-rich aggregates (Ehrnhoefer et al., 2008).

Stilbenes such as pinosylvin and resveratrol are polyphenolic compounds which have been reported to reduce the accumulation of amyloid plaques (Aβ peptide) in Tg2576 neuron cultures (Vingtdeux et al., 2008). Amyloid plaques trigger microglial activation by interacting with toll-like receptors (TLR) such as TLR-4. Activated microglia induces neuronal inflammation and cell death (Vingtdeux et al., 2008). The anti-inflammatory activities of stilbenes, therefore, protect microglia against Aβ-induced inflammation (Capiralla et al., 2012).

Quercetin is a flavonoid exhibiting antioxidant, antiapoptotic and anti-inflammatory properties (Nijveldt et al., 2001). Quercetin was reported to confer cognitive enhancing effects through reduction of β-amyloid plaque aggregation (Nijveldt et al., 2001). Quercetin treatment of 3xTg-Alzheimer’s-Diseased mice decreased IL-1β/COX-2/iNOS proinflammatory signaling in the hippocampal CA1 region (Vargas-Restrepo et al., 2018).

Curcumin is a natural polyphenol whose role in regulating cognition function has received wide attention in the last 10 years. Curcumin supplementation has been reported to ameliorate HFD-induced cognitive deficits (Yu et al., 2013). Curcumin modulate cognition by improving synaptic plasticity through alterations of N-methyl-D-aspartate receptor (NMDAR) and calcium/ calmodulin-dependent kinase II (CaMKII) (Sun et al., 2013). Curcumin reduces oxidative stress and promotes the synthesis of Docosahexaenoic acid
(DHA) from its precursor, α-linolenic acid, by stimulating the activity of enzymes involved in the synthesis of DHA such as elongase and 2-fatty acid desaturase 2 (FADS2) in the brain (Ataie et al., 2010; Wu et al., 2015).

Growing evidence proposes that condensed tannins such as gallicatechin-catechin flavan and anthocyanins reduces Aβ-induced neurotoxicity by reducing ROS formation upon exposure of Aβ1-40 and Aβ25-35 to neuro-2A cells, perturbation of calcium balance and decrease in apolipoprotein E (ApoE) metabolism (Shih et al., 2011). Condensed tannins promote the formation of non-toxic forms of Aβ aggregates instead of the toxic amyloid fibrils by direct binding to Aβ molecules thereby suppressing amyloid fibril formation (Yamakawa et al., 2016).

Naringenin chalcone neuroprotective effects have been well characterized. It enhances learning and memory ability of mice, reduces senile plaque formation, reverses glucose uptake defects in the brain and ameliorates cognitive deficits (Wang et al., 2012). Naringenin enhances cognition through inhibition of GSK3β activity, increases the activity of CaMKII, mitigates mitochondrial dysfunction mediated oxidative stress and suppresses acetylcholinesterase activity (Wang et al., 2013; Sachdeva et al., 2014). It decreases levels of TNF-α levels (Wang et al., 2013).

Alkaloid isolated from Huperzia serrata was shown to be a potent, reversible and selective inhibitor of acetylcholinesterase (AChE) (Tang and Han, 1999). Alkaloids exhibit
memory-enhancing efficacy due to their ability to penetrate the blood-brain barrier and long inhibitory activity against acetylcholinesterase action (Tang and Han, 1999).

Previous studies have reported that vitamin E is an important component of the body antioxidant systems. Its antioxidant activity and anti-inflammatory properties contribute to its neuroprotective effects (Nishida et al., 2009). Vitamin E inhibits Aβ accumulation in the brain. Moreover, the reduction of oxidative stress by vitamin E protects against the formation of Aβ-induced tau phosphorylation through the inhibition of the activation of p38-MAPK (Giraldo et al., 2014).

Long-chain polyunsaturated fatty acids such as omega-3 fatty acids (such as alpha linolenic acid, docosahexaenoic acid and eicosapentanoic acid), and omega-9 fatty acid (oleic acid) have been targeted in reversing the cognitive deficits. Omega-3 and omega-9 fatty acids are predominantly found in the brain (Youdim et al., 2000). A low dietary ratio of omega-3/omega-6 is linked with cognitive impairments and dementia (Heude et al., 2003; Barberger-Gateau et al., 2007). The dietary omega-3/omega-6 ratio has been shown to be directly correlated with cognitive decline and hippocampal inflammation (Grundy et al., 2014). Omega-3 supplementation increases molecular markers involved in plasticity such as BDNF and tropomyosin receptor kinase B (TrkB) (Gomez-Pinilla, 2011). Mice feeding with a high omega-3/omega-6 ratio diet had low mRNA expression levels of hippocampal inflammatory markers such as TNF-α and IL-1β (Labrousse et al., 2012). Therefore, this contributes to prevention of neuroinflammatory processes (Grundy et al., 2014).
CHAPTER FIVE

EFFECT OF DCM LEAF EXTRACT OF Gnidia glauca ON LOCOMOTOR ACTIVITY, ANXIETY AND EXPLORATION-LIKE BEHAVIORS IN HFD-INDUCED OBESE RATS

5.1 Introduction

Metabolic abnormalities stemming from abdominal or central obesity are increasingly linked to impairments in central nervous system (CNS) function (Hryhorczuk et al., 2013). The hypertrophied and hyperplastic adipose tissue stimulates a cascade of changes in neurochemical signaling that directly or indirectly mediate behaviors (Stachowiak et al., 2013; Marrisal-Arvy et al., 2014). Ostensibly, this degree of relative adiposity is implicated in a wide range of neuro-behaviors among which are, spontaneously emitted behaviors (activity patterns, anxiety and exploration), motivated behaviors (feeding, drinking, sexual behavior) and operant performance, attentional processes, learning and memory (Kelley et al., 1989; Sharma and Fulton, 2013).

The obese phenotype and/or chronic exposures to high-fat diet (HFD) markedly exacerbates the odds of developing spontaneously emitted behaviors (Kanoski and Davidson, 2011). A study indicated that mice fed on HFD for 12 weeks showed reduced locomotor and exploratory behaviors in open field tests and elevated plus maze as well as depressive-like features characterized by reduced ambulatory activity in the forced swim tasks (Sharma and Fulton, 2013).

Exploration is one of the main domains of behaviors referring to the tendency to investigate a novel environment. It is closely related to curiosity (Heyser and Chemero, 2012). The
cognitive map theory postulates novelty as misrepresentation of an item or place in the cognitive mapping/locale system. The locale system is located within the hippocampus containing mental representations of previously perceived stimuli. Therefore, the hippocampal system supposedly signals a lack of information about the current environment and exploration becomes a direct response to the mismatch detected (Crusio, 2001).

Chronic exposure to obesogenic diets is often associated with physical inactivity due to altered coordination of motor and reflexive responses (Belczak et al., 2014; Bouchard et al., 2015). Increased adiposity alters motor function through enhanced decrements in balance, muscle strength, and coordination (Muramoto et al., 2014). Deficits in motor performance might be due to alterations in the striatal dopaminergic signaling in the cerebellum (Joseph et al., 2009; Kravitz et al., 2016). Deficiencies in dopamine synthesis, striatal dopamine release, as well as defective striatal dopamine receptors are associated with impairments in striatal dopamine function (Kenny et al., 2013; Volkow et al., 2013).

Anxiety disorders, being the most prevalent mental disorders, globally contribute to reduced quality of life and predisposes affected individuals to other psychiatric comorbidities (Skilton et al., 2007; Kessler and Wang, 2008). Anxiety, fear, chronic worry, muscle tension, panic attacks and apprehension are the main psychological symptoms while physical symptoms involve chest dysphoria, fatigue and tension (Lykouras and Michopoulos, 2011). Anxiety disorders are majorly categorized into specific and social

Studies have reported a positive association between obesity and anxiety disorders such as panic disorder (Barry et al., 2008), specific phobia (Herpertz et al., 2006) and social phobia (Mather et al., 2009). Obesity as a causal factor for anxiety involves several paths such as social discrimination against obese persons (Puhl and Heuer, 2009), low self-esteem in unfriendly social network (Muennig, 2008), distress from illness burden (especially diabetes mellitus, asthma and cardiovascular diseases) and adverse drug effects (Beuther and Sutherland, 2007). Anxiety disorders as the causal factor for obesity has been correlated with disruption of hypothalamic-pituitary-adrenal (HPA) axis which results in dysregulation of autonomic functions (Kiernan and Bar’s, 2009; Esmaily et al., 2015). These factors create another vicious cycle (obesity-anxiety cycle).

Inflammation and oxidative stress due to increased adiposity plays a pivotal role in the pathogenesis of neuropsychiatric disorders through their effect on the hypothalamus, amygdala and the hippocampus (Su, 2012). Chronic consumption of high fat diet stimulates production of proinflammatory cytokines (TNF-α) (Alzoubi et al., 2013), interleukins (1β, 2, 6, 8 and 12) (Sahebkar, 2014), chemokines, immune cells, prostaglandins and nitric oxide which in turn precipitates hypothalamic-mediated oxidative stress (Miller and Spencer, 2014). The compromised redox homeostatic status characterized by attenuated antioxidant defenses activates brain’s resident immune cells, the microglia and astrocytes,
to further produce inflammatory mediators, which exacerbates an oxidative damage in the hippocampus (Popa-Wagner et al., 2013).

Pharmacological agents available for the treatment of neurologic and psychiatric disorders have had limited potency or intolerable adverse effects (Zhao et al., 2009). Therapeutic herbs and nutrients have, therefore, provided an effective alternative treatment with minimized side effects and capacity to potentiate the effect of prescribed medications (Arika et al., 2015). The present study aimed to determine the modulatory effect of DCM leaf extract of *Gnidia glauca* on locomotor activity, exploration and anxiety-like behaviors in HFD-induced obese rats in an open field arena. The generated data will provide qualified leads in drug design from this plant for the treatment and/or management of neurologic disorders.
5.2 Materials and Methods

5.2.1 Preparation of the Plant Material, Handling of Experimental Rats and Induction of Obesity

The collection of medicinal plant, processing and extraction of the plant material were conducted as described in chapter 3 (Section 3.2.1-2). The preparation of appropriate doses of DCM-leaf extract of *G. glauca* for bioassays were as described in chapter 3 (Section 3.2.3). The age, average weight, acclimatization period and handling of experimental rats were as described in chapter 3 (Section 3.2.4-7). Further, the composition of high fat diet, the induction of obesity and the experimental design used in the present study were as described in chapter 3 (Section 3.2.5).

5.2.2 Open Field Arena

In order to determine whether *G. glauca* modulates gross locomotor activity, anxiety and exploration-like behaviors in HFD-induced obese rats, animals were tested in an open field arena (Brown *et al*., 1999) after 6 weeks of oral administration of therapeutic doses of the plant extract.

5.2.2.1 Apparatus

The open field apparatus consisted of an open top box (72 cm x 72 cm) with 36 cm high walls. Blue visible lines were drawn on the floor using a marker into sixteen 18 x 18 cm squares (Figure 5.1). A center square (18 cm x 18 cm) was drawn in the middle of the arena (within the four inner squares) with a red marker (Figure 5.1). A 60-Watt white light bulb provided lighting. The floor was covered by a sheet of clear Plexiglas which was cleaned using 70% ethyl alcohol in each trial. Animal behavior in the arena was recorded and
tracked by an overhead video camera connected to a PC with Ethovision XT software (Figure 5.1).

![Diagram of the Open Field Arena]

**Figure 5.1: The Open Field Arena**

### 5.2.2.2 Experimental Procedure

The animals were transferred to the testing room in their home cages and allowed to acclimatize to this room prior to testing. Each rat was gently placed in the center of an open field arena and left freely to explore the arena for 5 minutes while recording scores of its behaviors. At the end of the 5-minute test period, animals were returned to their respective
home cages. The Plexiglas was removed, cleaned and disinfected with 70% ethyl alcohol between each trial.

To determine the effect of the DCM leaf extract of *G. glauca* on locomotor activity, exploration and anxiety-like behaviors, the following animal behaviors were assessed: number of line/grid crossing (frequency with which the rats crossed one of the grid lines with all four paws), center square entries (frequency with which the rats crossed one of the red lines with all four paws into the central square), latency period (duration of stay in the central square), rearing (frequency with which the rats stood on their hind legs in the arena), stretch-attend-postures (frequency with which the rats demonstrated forward elongation of the head and shoulders followed by retraction to their original position), grooming (duration of time the animal spent licking or scratching itself while stationary), freezing (duration with which the animal was completely stationary), urination (number of puddles or streaks of urine) and defecation (number of fecal boli produced per animal) (Brown *et al.*, 1999).

Locomotor activity for each animal was assessed by the sum of line/grid crosses and number of rears. The behavioral domains used to test exploration-like behaviors included the frequency of rearing, central square entries and latency in the central square. The animal behavioral domains used to assess anxiety included latency period, fecal boli score, frequency of urination, grooming, stretch attend postures and freezing.
5.2.3 Data Management and Statistical Analysis

To assess the performance in the open field arena, each determinant of the behavioral domain was recorded and tracked by an overhead video camera connected to a PC with Ethovision XT software. The data for each behavioral domain was exported to Microsoft® Excel spread sheet, where it was organized and later transferred to statistical software Minitab (Version 17.1) for analysis. The data was found to conform to the assumptions of parametric data using box plot. One-way ANOVA was used to test the significant differences among the normal control group rats, negative control group rats, Orlistat-treated group of rats and extract-treated group of rats. The data was further subjected to Tukey’s post hoc for pairwise comparison and separation of means. The criterion for significance was set at $p \leq 0.01$. The findings were presented in a table.
5.3 Results

5.3.1 Effect of DCM Leaf Extract of *Gnidia glauca* on Locomotor Activities, Anxiety and Exploration-Like Behaviors in HFD-Induced Obese Laboratory Rats

Treatment of HFD-induced obese rats with DCM leaf extract of *G. glauca* resulted in a significantly higher grid crossing score relative to the negative control group rats (*p*≤0.01; Table 5.1). Further, rats treated with the plant extract showed a higher grid crossing than those in the normal control (*p*≤0.01). Administration of extract dosages of 250 and 300mg/kg body weight resulted in a significant increase in grid crossings than those of rats treated with the reference drug, Orlistat (*p*≤0.01; Table 5.1).

It was further observed that extract-treated rats had a higher number of rearing episodes relative to negative control rats (Table 5.1). Besides, there was no significant variation in the number of rearing episodes observed among extract-treated rats at dosages of 200 and 250mg/kg body weight, normal control group rats and rats in the positive control groups (*p*>0.01). Results also indicated a higher defecation and urinating episodes in rats in the negative control than those of rats in the extract-treated groups (*p*≤0.01). Similarly, the number of stretch attend postures and urinating episodes were significantly high in negative control group rats relative to extract-treated rats (*p*≤0.01). Further, it was observed that the number of visits to the central square after the initial exit were more on extract-treated and Orlistat rats than those rats in the negative control group (Table 5.1). Remarkably, no significant difference in defecation, urination, center square entries and stretch attend postures were observed among rats in the extract-treated, positive control and normal control groups (*p*>0.01; Table 5.1).
The results also showed that negative control group rats had a significantly longer latency period in the central square upon entry into the open field arena than extract-treated rats (\(p \leq 0.01\)). Treatment of rats at a dosage levels of 250 and 300mg/kg body weight of the extract resulted in a shorter latency period in the central square than rats administered with the reference drug, Orlistat (Table 5.1). However, the latency period was statistically similar in rats treated with the reference drug, Orlistat and those in the normal control group (\(p > 0.01\)).

The results also revealed that rats in the negative control group substantially froze longer in the open field arena than rats treated with the three extract doses (Table 5.1). However, the effect was not statistically significant among rats treated with the reference drug, Orlistat and those treated with the plant extract at dosage levels of 200 and 250mg/kg body weight (\(p > 0.01\)). Treatment of rats with the highest extract dose of 300mg/kg body weight significantly reduced immobility time (freezing period) than that of rats treated with the reference drug, Orlistat (\(p \leq 0.01\); Table 5.1).

Results also demonstrated that administration of \(G. \ glauca\) leaf extract significantly reduced the grooming behavior of rats relative to the untreated obese rats in the negative control group (\(p \leq 0.01\)). However, the propensity to groom in the extract-treated groups of rats and those treated with the reference drug, Orlistat were comparable (\(p > 0.01\); Table 5.1).
**Table 5.1: Effect of DCM Leaf Extract of *Gnidia glauca* on Locomotor Activities, Anxiety and Exploration-Like Behaviors In HFD-Induced Obese Rats**

<table>
<thead>
<tr>
<th>Treatments (mg/kg bw)</th>
<th>Grid Crossings</th>
<th>Rearing</th>
<th>Defecation score</th>
<th>Urination Center square entries</th>
<th>Stretch attend postures</th>
<th>Latency period (Sec)</th>
<th>Freezing (Sec)</th>
<th>Grooming (Sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal Control</td>
<td>37.20±1.92c</td>
<td>10.00±1.58b</td>
<td>3.60±0.55b</td>
<td>9.20±1.48a</td>
<td>3.80±0.84b</td>
<td>23.20±2.39b</td>
<td>21.60±2.70b</td>
<td>15.80±2.77b</td>
</tr>
<tr>
<td>Negative Control</td>
<td>21.80±2.39d</td>
<td>3.20±0.84c</td>
<td>9.00±0.71a</td>
<td>5.60±0.55a</td>
<td>11.00±1.58a</td>
<td>38.80±2.59a</td>
<td>46.20±4.15a</td>
<td>29.00±2.45a</td>
</tr>
<tr>
<td>Positive Control</td>
<td>39.00±2.24c</td>
<td>9.40±1.14b</td>
<td>3.60±0.55b</td>
<td>8.00±1.58a</td>
<td>4.60±1.14b</td>
<td>23.40±2.30b</td>
<td>19.40±2.41bc</td>
<td>13.80±1.48bc</td>
</tr>
<tr>
<td>HFD+200</td>
<td>41.60±2.97bc</td>
<td>11.20±1.48ab</td>
<td>3.20±0.84b</td>
<td>9.20±1.79a</td>
<td>4.20±1.30b</td>
<td>20.40±1.14bc</td>
<td>17.80±1.79bcd</td>
<td>12.40±2.07bc</td>
</tr>
<tr>
<td>HFD+250</td>
<td>46.00±4.30ab</td>
<td>11.00±1.58ab</td>
<td>3.40±0.55b</td>
<td>9.40±1.14a</td>
<td>3.80±0.84b</td>
<td>18.80±1.92cd</td>
<td>15.60±1.52cd</td>
<td>11.80±1.30c</td>
</tr>
<tr>
<td>HFD+ 300</td>
<td>50.40±3.36a</td>
<td>12.80±1.30a</td>
<td>2.60±0.55b</td>
<td>10.20±1.30a</td>
<td>3.00±0.71b</td>
<td>15.60±2.30d</td>
<td>14.00±1.58d</td>
<td>10.20±1.30c</td>
</tr>
</tbody>
</table>

Means followed by similar lower-case letters within columns are not statistically different ($p>0.01$).
5.4 Discussion

The open-field test provides simultaneous measures of locomotion (ambulatory activity), exploration and anxiety (emotionality) (Koofreh et al., 2013). It is a model not only useful in the assessment of the behavioral performance of the test animals but also contributes knowledge on the neurobiological mechanisms mediating behaviors (Koofreh et al., 2013).

Locomotor/ambulatory activity is the function of performance on motor tasks while exploration or novelty may involve some quality never previously experienced or familiar items arranged in unfamiliar ways (Edagha et al., 2015). Exploratory behavior is thus curiosity and attraction to novelty (Edagha et al., 2015). Animal behaviors such as frequency of line crosses, frequency of rearing, central square entries and latency in the central square are used as measures of locomotor activity and exploration. A higher frequency of these parameters indicates increased locomotion and exploration and vice versa (Koofreh et al., 2013). The present study indicated a number of clear effects on these behavioral domains.

The frequency of line/grid crosses measures the horizontal exploration or locomotor behavior and represents the horizontal distance covered (Edagha et al., 2015). Line crossing is the frequency with which the rat cross each of the lines with all four paws. In the present study, line crossing was significantly ($p \leq 0.01$) increased in the extract-treated rats as compared to the rats in the negative control group. Similarly, administration of ethanolic extract and acetone extract of Cedrus deodara increased locomotor activity in neonatal rats (Patil et al., 2011). In normal circumstances, rats naturally move in order to
find the location of feeds, gather nesting materials, search for nesting places and sexual partners or flee themselves from enemies (Deacon, 2006). However, the reduced locomotor activity observed in the negative control group rats could be due to their overweight nature, generalized muscle fatigue and/or increased behavioral despair (Edagha et al., 2015). Besides, the diminished ambulatory/locomotor activity could be as a result of the damage in the primary motor area and/or distress from illness burden associated with increased adiposity (Kiernan and Barr’s, 2009; Edagha et al., 2015). The ability of the extract to reduce body weight might be attributed to the observed increased ambulatory activity in extract-treated rats. Moreover, the extract might have resulted in a positive effect on striatal dopaminergic signaling through increased striatal receptor sensitivity and dopamine synthesis thereby improving motor activity (Kravitz et al., 2016).

The frequency of rearing or vertical exploration was significantly decreased in HFD-fed untreated obese rats as compared to their treated counterparts ($p \leq 0.01$). Rearing measures exploratory behavior or otherwise vertical locomotor activity. When rearing, the animal stands upright on its hind limbs often using their tail as support with its forelimbs freely suspended in the air or resting on the wall of the open-field arena. Through rearing, olfactory signals can be taken in from the air, as well as from visual cues (Voigt et al., 2005). Locomotor activity is driven by exploration and its reduced form possibly reflects reduced exploration as it is accompanied by reduced rearing frequency (Voigt et al., 2005). Previous studies demonstrated that oral administration of ethanolic extracts of Nauclea latifolia and Emilia sonchifolia increased locomotion and exploratory activities as evidenced with a high frequency of rearing in mice (Edagha et al., 2015).
The state of being obese is associated with decreases in the motor output, often termed as “physical inactivity” (Bouchard et al., 2015). Chronic exposures to obesogenic diets contribute to striatum damage thereby affecting the dopamine synthesis and release as well as striatal-dopamine receptor function (Kravitz et al., 2016). The striatal-dopamine plays a key role in the proper control of movement and, therefore, its impairment contributes to physical inactivity in obesity akin to classical movement disorders such as Parkinson’s disease (Huang et al., 2001; Kravitz et al., 2016). The motivated locomotor and exploratory behaviors observed in extract-treated rats could be linked to facilitated dopamine synthesis and release as well as restored striatal dopamine receptor function (Johnson and Kenny, 2010; Alsio et al., 2014).

The chronic mobility problems of joints and muscles in obese patients are largely contributed by alteration of motor circuitry in the brain (Kravitz et al., 2016). Besides, the obesity-induced adaptations due to altered motor circuitry could continue to contribute to physical inactivity even after weight loss (Kravitz et al., 2016). The reduction of expression levels of brain-derived neurotrophic factor (BDNF) and its tyrosine kinase receptor, TrkB, in hypothalamic nuclei affects the strength of synaptic connections or dendritic spine density leading to altered satiety signals and locomotor activity (Kernie et al., 2000). High-fat diets potentiate an oxidative attack on the brain resident cells resulting in activation of the cholinergic motor inhibitory system (Abubakar and Salka, 2010). Alteration of the activity of acetylcholinesterase (AchE) and damage to the peripheral muscle due to necrosis of skeletal muscle fibers enhances the reduction of locomotor activity in animal models (Haque et al., 2001).
The increased ambulatory or spontaneous physical activity (SPA) characteristic to extract-treated rats could also be as a result of the action of neuropeptide, orexin-A, independent of feeding behavior (Teske et al., 2006). Orexin A robustly stimulates spontaneous physical activity and non-exercise activity thermogenesis (Kotz et al., 2002; Kiwaki et al., 2004). Central administration of orexin-A (into the hypothalamic paraventricular nucleus) was found to increase rearing frequency and locomotor activity in rats (Kiwaki et al., 2004). The orexin neurons project to the dopaminergic neurons in the substantia nigra that innervate the striatum and forms a critical component of motor activity (Hara et al., 2001). Therefore, an alteration in the expression of orexin, and/or its signaling, could exacerbate spontaneous physical inactivity and contribute to weight gain (Hara et al., 2001).

The test for anxiety is usually based on the conflicting tendencies of rats to explore a novel environment in contrast to the aversive features of a brightly lit open arena or an elevated space (Koofreh et al., 2013). Moreover, in the open field arena, this behavioral domain may be mediated by two key factors namely, agoraphobia and individual testing. Agoraphobia is a function for anxiety based on the size of the test area relative to the size of an animal while individual testing is a function for anxiety based on the separation of an animal from its social group (Bourin and Hascoet, 2003). Animal behaviors such as increased latency period, greater fecal boli score, higher frequency of urination, increased grooming period, fewer rears, higher frequency of stretch attend postures and increased freezing duration are used as measures of anxiety. A higher frequency or an increased duration of these parameters are indicative of increased anxiety (Koofreh et al., 2013).
Analysis of stretch attend postures (SAP) revealed a significantly ($p \leq 0.01$) increased frequency in obese untreated rats relative to extract-treated rats. The SAP is the frequency with which the animal demonstrated forward elongation of head and shoulder followed by retraction to its original position. These are risk assessment behaviors of fear and anxiety which indicates that the animal is hesitant to move from its present position of comfort to a new position. Thus, decreased levels of this behavior are indicative of a low level of anxiety and fear and vice versa (Blanchard and Griebel, 2001). These results were consistent with the finding that mice with HFD-induced obesity demonstrated a high frequency of SAP relative to obese mice treated with the herbal extracts from *Morus alba*, *Melissa officinalis*, and *Artemisia capillaris* (Lee *et al.*, 2008).

Results also showed that the extract-treated rats had an increased frequency of entry to the inner zone of the open field arena relative to HFD-fed untreated obese rats ($p \leq 0.01$). A high frequency of movements into the center of the arena in open field tests is reflective of reduced anxiety, increased locomotor activity and exploration (Koofreh *et al.*, 2013). Rats are generally thigmotactic, they avoid open areas and prefer moving alongside walls where they perceive tactile stimuli via their vibrissae (Voigt *et al.*, 2005). However, when the animal is less anxious their exploratory behavior increases and tend to move all over the holding cage or arena. Consistent with the present study, previous studies observed that treatment of mice with hydroalcoholic extract of *Coriandrum sativum* increased the frequency of entry to the inner zone of the open field arena (Mahendra and Bisht, 2011). The sedative and muscle relaxant effects of *Coriandrum sativum* are indicative of its anxiolytic effects (Mahendra and Bisht, 2011).
The HFD-fed untreated group showed a longer latency period in the central square upon entry into the open field arena, an indicator of higher anxious states due to the anxiogenic effects of chronic exposure to high-fat diets. The quicker the retreat from the center square of the arena in extract-treated rats is indicative of increased automatic and exploratory behaviors due to extracts anxiolytic effects (Zhang et al., 2014). These findings were in agreement with a study that demonstrated that HFD-induced obese rats showed less explorative interest due to lower cross lattice number and reduced percent of time spent in the center of the arena and open arms (Dutheil et al., 2016). The reduction in explorative interest in HFD-induced obese untreated rats appears to be symptoms of depressive disorders consistent with those observed in patients suffering from anxiety disorders (Su et al., 2012). Current studies have demonstrated that chronic intake of HFD has led to depressive- and anxious-like behaviors (Aslani et al., 2015; Dutheil et al., 2016).

Immobility time (freezing duration) was significantly \((p\leq0.01)\) increased in HFD-fed untreated rats compared to extract-treated rats, an indicator of increased anxious state and hypoactivity or impaired locomotor activity (Koofreh et al., 2013). Freezing often occurs in response to a sudden change in the surroundings where the animal usually stands still with its forelegs raised while looking up. Previous studies demonstrated that HFD-induced obese rats exhibited a significantly low frequency of rearing as compared to rats treated with therapeutic doses of aqueous extract of *Ginkgo biloba* (Kuribara et al., 2003). The anxiolytic effects of the *G. glauca* leaf extract might be accompanied by increases in the brain levels of monoamines such as serotonin, norepinephrine, and dopamine.
Serotonin and norepinephrine are neurotransmitters that play a key role in mood regulation (Xu et al., 2007; Kasper, 2009).

Increased fecal boli and urination scores observed in HFD-fed untreated obese rats are suggestive of fear and anxious states. Comparatively, the low scores of these parameters in the extract-treated rats may be attributed to the presence of bioactive chemicals responsible for down-regulation of receptors and connectivity in the amygdala, a key center of fear (Kiernan and Bar’s, 2009; Koofreh et al., 2013). Previous studies have demonstrated that high-fat feeding and obesity increases the production of BDNF and phospho-CREB in the striatum contributing to negative emotional states and depressive-like symptoms (Sharma and Fulton, 2013). This biochemical alterations in the brain reward circuitry could be implicated for the observed increased fecal boli and urination scores in HFD-fed untreated obese rats.

Grooming is a de-arousing self-directed behavior associated with anxiety upon displacement of animals into a novel environment or aversive situations such as an open field arena (Kalueff et al., 2016). Grooming duration is the time the animal spends licking or scratching itself with paws and face washing actions while in a stationary position. This stereotypical behavioral sequence is usually increased in anxious states. Anxiolytic drugs, however, reduces the grooming behavior, whereas anxiogenic drugs facilitate grooming (Voigt et al., 2005). Treatment with therapeutic doses of the plant extract led to a significant ($p \leq 0.01$) decrease in grooming duration relative to HFD-fed untreated obese rats. Consistent with this study, the open-field tests performed to test the neurobehavioral effect
of *Nauclea latifolia* and *Emilia sonchifolia* (Edagha et al., 2015) and *Mammea africana* (Okokon and Davies, 2014) in rodents indicated reduced grooming frequency together with increased spontaneous locomotion and exploratory activities (Shukitt-Hale et al., 2005).

Self-grooming is a highly stereotyped pattern of sequential movements (syntactic chain pattern) that is modulated by circuits that incorporate the basal ganglia such as the striatum, substantia nigra and nucleus accumbens in the forebrain (Kalueff et al., 2007; Kalueff et al., 2016). Striatal circuits majorly sub-serve the basal ganglia and are mainly involved in learning, motivation and motor sequencing (Jin et al., 2014). Lesions of the striatum completely impair sequential syntactic self-grooming chains (Kalueff et al., 2016). The limbic circuitry that includes the amygdala and the hypothalamus also modulates self-grooming behavior in rodents. The amygdala mainly modulates motivational states, such as desire, fear, and anxiety (Hong et al., 2014). Studies showed correlations between reduced dopamine release within the amygdala and increased anxiety-like behaviors in low and high grooming rats respectively (Homberg et al., 2002).

The hypothalamic paraventricular nucleus is another limbic region that has been implicated in the regulation of self-grooming in rodents (Homberg et al., 2002). Besides, the hypothalamic-pituitary stress-related hormones such as corticotropin-releasing hormone (CRH) and adrenocorticotropic hormone (ACTH) also influences self-grooming in rodents (Kalueff et al., 2016). The anxiolytic effects observed in the extract-treated rats as evidenced by reduced self-grooming, increased exploratory and locomotor activities, could be attributed to the effects of the extract on the dopamine release in the nigrostriatal and mesolimbic systems (Nin et al., 2012). Dopamine plays a critical role in locomotor
function, self-grooming and other complex behavioral patterns (Kalueff et al., 2016). The 
G. glauca leaf extract might have also contributed to the reduction of stress-induced self- 
grooming by enhancing the GABAergic tone through attenuating the intensity of the 
perception of anxiogenic stimuli (Leonard et al., 1994; Nin et al., 2012).

The mechanistic basis underlying obesity as a causal factor for anxiety could relate to 
oxidative stress and neuroinflammation (Popa-Wagner et al., 2013). Studies have 
demonstrated that exposures to chronically high energy diets influence the activity of glial 
cells that mediates endogenous immune system within the microenvironment in the CNS 
(Kreutzberg, 1996). The activation of glial cells is the hallmark of inflammation in the 
brain (Orr et al., 2002). Activated microglia produce neurotoxic inflammatory stress 
signals, such as, tumor necrosis factor-alpha (TNF-α) (Alzoubi et al., 2013), interleukins 
(1β, IL-2, IL-6, IL-8 and IL-12) (Sahebkar, 2014), lipoxygenase (Loane and Byrnes, 2010), 
cyclooxygenase-2 (COX-2) (O’Banion, 1999), monocyte chemoattractant protein (MCP) 
(Esmaily et al., 2015), growth factors and complement proteins (Bodles and Barger, 2004). 
These proinflammatory mediators, in turn, precipitate an inflammatory signaling cascade 
by activating other resident cells to produce additional molecules that perpetuate microglia 
activation in a positive feedback loop (Kreutzberg, 1996).

Increased inflammation due to chronic exposure to the high-fat diet increases the 
vulnerability of neurotransmitter receptors to oxidative stress through activation of the 
oxidative stress-sensitive nuclear factor-kappa-β (NF-kβ) (Durany et al., 1999). The 
activated NF-kβ, in turn, upregulates the inflammatory response resulting in a further
increase in ROS such as superoxide species and nitric oxide (NO) as well as increased expression of inducible nitric oxide synthase (iNOS) (Shukitt-Hale et al., 2016). High levels of ROS exacerbate oxidative stress and inflammation, and thus, vulnerability to further stressors (Floyd and Hensley, 2002). Facilitated central adiposity might also precipitate oxidative damage due to compromised redox homeostatic status characterized by attenuated antioxidant defenses thus exacerbating neuropsychiatric damage (Hemmer et al., 2001; Popa-Wagner et al., 2013). The therapeutic effects exhibited by the G. glauca leaf extract could be due to its ability to mitigate inflammation and oxidative stress by down-regulating the activity and release of proinflammatory mediators and restoration of redox homeostatic status through activation of antioxidant defenses (Esmaily et al., 2015). The normalization of NF-κβ levels reduces the expression of the pro-inflammatory cytokines and consequently results in low levels of ROS in the hippocampus (Joseph et al., 2007).

Pharmacological manipulations of anxiety disorder by anxiolytic agents like benzodiazepines (BDZs) and Allopregnanolone enhance GABAergic tone. Binding of the anxiolytic agents to one of the two gamma subunit of the GABA-A receptor causes a structural modification of the receptor and allosterically increases GABA-A receptor activity (Burguiere et al., 2013; Adedayo et al., 2018). This binding also facilitates the opening of GABA activated chloride channels thereby increasing chloride ion conductance and inhibition of the action potential (Vogel and Vogel, 2002). The eventual allosteric binding of GABA to the gamma subunit of the GABA-A receptor decreases the excitability of neurons and augments a calming effect (Vogel and Vogel, 2002; Adedayo et al., 2018). The observed anxiolytic properties of the G. glauca leaf extract may be attributed to
stimulation of the binding of gamma-aminobutyric acid (GABA) to GABA-A receptors that occurs abundantly on the surfaces of neurons in the amygdala and other parts of the limbic system and, therefore, brings a calming effect (Kiernan and Bars, 2009).

In the present study, the observed anxiolytic effects and increased locomotor and exploration-like behaviors in extract-treated rats could be attributed to the presence of a number of bioactive compounds in the DCM leaf extract of G. glauca. The synergistic and/or additive effects of these phytochemical compounds might be implicated in amelioration of symptomatic complications of obesity viz; anxiety, locomotor activity, and exploration.

The phenolic compounds such as catechins and epicatechins were found to confer neuroprotective effects by mitigating oxidative and metabolic insults (Guo et al., 2007). Catechins exhibit neuroprotective activities by activating multiple signaling pathways that exerts cell-survival and anti-inflammatory actions, including altering the expression of pro-apoptotic and anti-apoptotic proteins and upregulating antioxidant defenses (Sutherland et al., 2006). Catechins activate protein kinase C (PKC) and transcription factors that promote the expression of cell-survival genes (Zhang et al., 2007). Several studies have reported that catechins and epicatechins exhibit protective effects of dopaminergic neurons from damage induced by 6-hydroxydopamine in a rat model of Parkinson's disease (Guo et al., 2007). In addition, catechin and epicatechin suppresses neuroinflammation, attenuate activation of microglia and inhibit release of the mediators associated with apoptotic death of neurons (Wu et al., 2009).
Curcumin was shown to ameliorate impaired hippocampal neurogenesis and increase the expression levels of brain-derived neurotrophic factor (BDNF) in severely stressed rats (Xu et al., 2007). Curcumin has been used in the prevention and management of neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease and stroke (Xu et al., 2007). It mitigates oxidative stress and inflammation by down-regulating the activity of lipoxygenase, COX-2 and inhibiting the generation of proinflammatory cytokines such as TNF-α, interleukins 1, 2, 6, 8 and 12 as well as monocyte chemoattractant protein (MCP) (Goel et al., 2008; Sahebkar, 2014). Besides, curcumin deactivate the transcription factor NF-kβ through induction of the expression of antioxidant enzymes and Bcl-2 (Kumar et al., 2015). Curcumin activate multiple signaling pathways through ligand binding to various receptors that include Growth Factor Receptors (GFR), G-Protein Coupled Receptors (GPCR) and Insulin Receptors (IR). These receptors in turn activate the kinase cascades involving Phosphatidylinositol-3-Kinase (PI3K), Mitogen-Activated Protein Kinases (MAPK) and Protein Kinase C (PKC) (Kumar et al., 2015).

Quercetin has been shown to improve brain cell function and signaling by mitigating extra-neuronal parameters of survival—the oxidative stress (Traustadottir et al., 2009). Quercetin reduced oxidative stress and protects cultured hippocampal neurons against nitric-oxide-mediated cell death (Guo et al., 2007). Quercetin ameliorates calcium dysregulation thereby protection from ischemic injury, neuronal cell death and consequently brain damage (Ansari et al., 2009). Quercetin treatment decreased acid-mediated intracellular calcium levels and inhibited spectrin breakdown by inactivation of calcium-dependent protease cabin (Pandey et al., 2011). Quercetin significantly decreased
protein oxidation, Aβ-induced toxicity and apoptosis in primary hippocampal cell cultures (Ansari et al., 2009). This novel antioxidant offers an effective and safe means of bolstering body's defense against free radicals (Alsio et al., 2010).

Stilbenes such as pinosylvin and resveratrol are phytophenols that have been shown to exhibit antioxidant activity (Parker et al., 2005). Pinosylvin and resveratrol protected cultured neurons against oxidative damage by scavenging nitric oxide radicals (Huang et al., 2001). Similarly, in a model relevant to Parkinson's disease, resveratrol protected cultured dopaminergic neurons against oxidative-induced cell death (Huang et al., 2001). Current findings indicate that administration of resveratrol and/or pinosylvin to rats confers protection of neurons in the brain and spinal cord from ischemic injury (Huang et al., 2001). In models relevant to Alzheimer's disease, stilbenes promoted clearance of amyloid β-peptide from cultured cells hence preventing neuronal cell damage (Marambaud et al., 2005).

Flavonoids were reported to modulate neuronal function and prevent neurodegeneration (Kumar and Khanum, 2012). Flavonoids were shown to improve memory and learning through stimulation of neuronal regeneration and enhancement of neuronal function (Kumar et al., 2015). They inhibit TNF-α, IL-1β and nitric oxide in activated microglia cells (Kumar et al., 2015). Flavanone maintain nigro-striatal integrity and functionality and serve as a potential neuroprotective agent against 6-hydroxydopamine (Kumar and Khanum, 2012). Flavonoids activate PI3 kinase-mTOR cascade and ERE-CREB pathway resulting in changes in synaptic plasticity. Flavanones were found to inhibit oxidative-
induced neuronal apoptosis through phosphorylation of signaling proteins essential in pro-survival pathways (Kumar et al., 2015).

Previous studies reported that condensed tannins such as gallocatechin-catechin flavan and anthocyanins have the ability to diffuse through the central nervous system and cross the Blood-Brain Barrier (BBB) (Talav´era et al., 2005). Gallocatechin-catechin flavan and anthocyanins confers neuroprotective function through their antioxidative properties. In human SH-SY5Y neuroblastoma cells, condensed tannins reduced Aβ- induced neurotoxicity by enhancing the formation of Aβ fibril formation thus reciprocally modulating local Aβ clearance (Feng et al., 2009). Gallocatechin-catechin flavan and anthocyanins have been shown to have potent anti-inflammatory activities. They inhibit inflammatory mediators COX-2 (Seeram et al., 2003).

Alkaloids increases gamma aminobutyric acid (GABA) in the synapses of the brain (Halliwell, 2007). They are a highly potent vasodilator agents, enhances cerebral blood flow, facilitate glucose uptake by brain cells and protects from hypoxia and ischemia (Halliwell, 2007).

Ellison and α-amyrin were shown to activate the Transient Receptor Potential (TRP) ion channels in the cell membrane of neurons (Oi et al., 1999). This results in Ca^{2+} influx which in turn activates neuroprotective kinase signaling cascades via Camp-Response-Element-Binding protein (CREB) and Mitogen-Activated Protein Kinases (Oi et al., 1999). The CREB stimulates expression levels of a major neurotrophic factor, the Brain Derived
Neurotrophic Factor (BDNF). The BDNF activates the PI-3K/Akt and MAPK/ERK pathways through binding to its tyrosine kinase TrkB receptor thereby activating downstream molecules which can promote neurogenesis and cell survival (Cheng et al., 1999).

Ferulic acids attenuates the stress-induced behavior in the depression-like model in mice (Feng et al., 2009). Ferulic acids influence the function of ionotropic receptors for GABA in the brain, therefore, enhancing its anxiolytic effects (Kumar et al., 2015).

Terpenoids (such as monoterpenes, triterpenoid and sesquiterpene alkaloid) isolated from the rhizome of Valerian officinalis exhibited a broad range of neuroprotective actions (Ortiz et al., 1999). Terpenoids were shown to confer sedating effects in mice through the activation of GABA-A receptor activity and other pathways upstream of nuclear factor erythroid 2-related factor 2 (Nrf2) (Ortiz et al., 1999).

Polyunsaturated fatty acids (PUFAs) such as oleic acid, linoleic acid and α-linolenic acid maintain the integrity of the structural components of neurons (Crews et al., 2005). The fatty acid composition of the neuronal membrane is necessary for maintenance of appropriate electrical gradients across the membrane and neurotransmission in the synaptic cleft (McCann and Ames, 2005). The PUFAs improve membrane fluidity and, therefore, affects membrane biophysical properties. In neuronal membranes, PUFAs participates in signaling cascades that promote synaptic plasticity, neuronal function and neuroprotection (Yehuda et al., 2002).
CHAPTER SIX

IN VITRO ANTIOXIDANT POTENTIAL AND FREE RADICAL SCAVENGING
ACTIVITIES OF DICHLOROMETHANOLIC LEAF EXTRACT OF
Gnidia glauca

6.1 Introduction

Oxidative stress (OS) is a state of compromised redox equilibria due to an altered pro-
oxidants-antioxidants balance in favor of pro-oxidants attributed to endogenous and/or
exogenous stressor(s) (Bisht and Dada, 2017). Oxidative stress represents a consequence
of increased production of reactive oxygen species (ROS) and reactive nitrogen species
(RNS) as well as attenuated capacity of antioxidant defenses (Ferry and Roussel, 2011;
Sohal and Orr, 2012).

Reactive oxygen species (ROS) and reactive nitrogen species (RNS) interact with
biomolecules (nucleic acids, proteins, lipids, carbohydrates) and exacerbate an oxidative
damage through carbonylation, peroxidation, nitration and nitrosylation reactions
(Newsholme et al., 2012; Weidinger and Kozlov, 2015). These ultimately result in
depletion of endogenous antioxidant capacity, inadvertent enzyme activation and oxidative
damage to cellular systems (Halliwell, 2006; Kaliora et al., 2006; Mirończuk-
Chodakowska et al., 2018). The compromised integrity of cellular systems by ROS and
RNS serves as a pre-requisite for pathogenesis of many degenerative diseases and disorders
such as cardiovascular diseases (Singh and Jialal, 2006), cancers (Kinnula and Crapo,
2004), Alzheimer’s disease (Smith et al., 2000), ageing (Hyun et al., 2006), mild cognitive
impairment (Guidi et al., 2006) and neural disorders (Sas et al., 2007).
Obesity, an energy-rich condition associated with overnutrition, impairs systemic metabolic homeostasis and elicits systemic oxidative stress (Furukawa et al., 2004). Chronic exposure to lipid-rich diets stimulates production of ROS through mitochondrial and peroxisomal oxidation of fatty acids (Fernández-Sánchez et al., 2011). High production of ROS is associated with low-grade chronic inflammation of adipose tissues (Marseglia et al., 2014). This condition activates innate immune system in adipose tissue and promotes secretion of pro-inflammatory cytokines including tumor necrosis factor-alpha (TNF-α), interleukin (IL)-1β, and IL-6 (Fonseca-Alaniz et al., 2007). A rise in concentration of pro-inflammatory cytokines further increases generation of ROS and nitrogen reactive species (RNS) by macrophages and monocytes (Shoelson et al., 2007). Therefore, detoxification of cells and tissue from these highly reactive molecules by means of antioxidants is very critical for cell viability, activation, proliferation, and the general organ function (Amirkhizi et al., 2007; Londhe et al., 2009; Mirończuk-Chodakowska et al., 2018).

To maintain the redox homeostasis and protect the cells and organ systems against ROS and RNS, humans have evolved a complex and highly sophisticated antioxidant system (Umar et al., 2012). Components of antioxidants defense systems are both endogenous and exogenous in origin which function interactively and synergistically to neutralize ROS and RNS (Ishino et al., 2010). These components include dietary antioxidants such as ascorbic acid (vitamin C), β-carotene, α-tocopherol, tocotrienols (vitamin E), glutathione, and uric acid (Naik, 2003). They also include antioxidant enzymes such as superoxide dismutase (SOD), glutathione peroxidase (GPx), glutathione reductase and catalase (CAT) which...
catalyze free radical quenching reactions (Ishino et al., 2010). Antioxidant components also include metal binding proteins (such as ferritin, albumin, ceruloplasmin and lactoferrin) which sequesters Cu\(^+\) and Zn\(^{2+}\) ions in the cytoplasm or Mn\(^{2+}\) in the mitochondrion which acts as catalytic components in redox reactions (Kaliora et al., 2006). Antioxidant phytonutrients from plant sources also constitute antioxidant components (Naik, 2003).

Synthetic antioxidants such as propyl gallate (PG), butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and tert-butyldihydroxyquinone (TBHQ) have been associated with limited potency and some negative health effects such as hepatic damages and malignancies in animal models (Hwang et al., 2001; Gandomi et al., 2014). Currently, there is an upsurge of interest to substitute synthetic antioxidants for naturally occurring antioxidants from plants to act as either antioxidant additives or as nutritional supplements (Salazar et al., 2008; Saxena et al., 2012).

Antioxidant principles from medicinally important plants possess enormous potential in ameliorating oxidative stress related degenerative diseases with minimal cytotoxicity (Ozata et al., 2002; Londhe et al., 2009). The natural biologically active components are idiosyncratic in terms of their structures, biological properties and mechanisms of actions. Therefore, their relative potency is largely proportional to their interactions and synergistic effects with endogenous antioxidants in eradication of free radicals (Willcox et al., 2004; Amari et al., 2014).
*Gnidia glauca* is traditionally used as therapy for various ailments in sub-tropical Africa. This plant has demonstrated significant efficacy in management of fever, inflammation, sore throat, wounds, burns and snake bites (Amarajeewa *et al*., 2007). Moreover, it has been a useful adjuvant and a key adjunct to dietary control in obese and diabetic patients (Hasani-Ranjbar and Larijani, 2015). The hypothesized pharmacological relevance underlying the mechanistic approach to the management of these diseases is through inhibition of oxidative stress in cellular systems (Londhe *et al*., 2009). However, evidence to back up the postulated mechanism of activity of *G. glauca* remains elusive. The aim of this chapter is therefore to determine the *in vitro* antioxidant potential and free radical scavenging activities of DCM leaf extract of *G. glauca* through various non-enzymatic antioxidant assays.
6.2 Materials and Methods

6.2.1 Collection and Preparation of the Medicinal Plant

The collection of medicinal plant, processing and extraction of the plant material were conducted as described in chapter 3 (Section 3.2.1-2).

6.2.2 Determination of Ferric Reducing Antioxidant Power (FRAP)

Principle

This method is based on the conversion of Fe$^{3+}$/ferricyanide complex to its ferrous form to form a violet-coloured solution, whose intensity is proportional to the concentration of sample. A higher absorbance of the reaction mixture is indicative of a greater reducing power of the extract (Gupta et al., 2012).

Procedure

The ferric reducing power of the plant extract was determined by the method described by Athukoralala et al. (2006). A reaction mixture containing 1ml of 2.5ml of phosphate buffer (200 mM, pH 6.6) and 2.5 ml of potassium ferricyanide (30 mM) and the extract at different concentrations (50 to 250 μg/ml), was incubated at 50°C for 20 min. Thereafter, 2.5 ml of trichloroacetic acid (TCA) (600 mM) was added to the reaction mixture and centrifuged at 3000rpm for 10min. Then, a 2.5 ml supernatant was collected and mixed with 2.5 ml of distilled water and 0.5 ml of FeCl$_3$ (6 mM). The absorbance was then determined at a wavelength of 700 nm. The blank contained all the reactants except the extract. Ascorbic acid was used as a standard. All tests were run in triplicates.
6.2.3 Determination of DPPH Free Radical Scavenging Activity

**Principle**

This method is based on an antioxidant compounds’ hydrogen donating or radical scavenging ability to reduce 2, 2-diphenyl-1-picrylhydrazyl radical to 2, 2-diphenyl-1-picrylhydrazine resulting in a pale-yellow solution. The decrease in the absorbance as the colour of the solution fades (from deep violet to light yellow) is monitored at 517 nm (Afolayan et al., 2010).

**Procedure**

The plant extract was prepared at various concentrations ranging from 0.05 to 5 mg/ml in methanol. The reaction mixture consisted of 1 ml of sample, 3 ml of methanol and 0.5 ml of 1 mM methanolic solution of DPPH. The reaction mixture was then vortexed and left to stand for 5 min. The absorbance of the resulting solution was measured at 517 nm. A mixture of methanol and DPPH solution served as a blank while a reaction mixture of methanol, DPPH and standard (Vitamin C) served as positive control. All tests were run in triplicates. The percentage radical scavenging activity was calculated according to the following formula:

\[
\% \text{ DPPH Radical Scavenging activity} = \frac{\text{Control OD} - \text{Sample OD}}{\text{Control OD}} \times 100
\]

Where: \( \text{Control OD} \) = Optical density of the blank

\( \text{Sample OD} \) = Optical density of the extract or standard

The percentage radical scavenging activity was then plotted against various concentrations and the IC\(_{50}\) (Half maximal inhibitory concentration) was determined graphically.
6.2.4 Determination of Nitric Oxide Radical Scavenging Activity

**Principle**

This assay is based on the theory that sodium nitroprusside (SNP) spontaneously generates nitric oxide which interacts with molecular oxygen to form nitrite ions that may be estimated using Griess reagent. Scavengers of nitric oxide in the extract compete with molecular oxygen resulting in reduced production of nitrite ions (Bajpai and Agrawal, 2015).

**Procedure**

Nitric oxide radical scavenging activity of *G. glauca* was determined according to the method described by Farhan *et al.* (2012). The reaction mixture constituting of a solution of sodium nitroprusside (SNP) (5 mmolL\(^{-1}\)) in phosphate buffered saline pH 7.4 and different concentrations of the extract ranging from 250-2500 μg ml\(^{-1}\), prepared in methanol, was incubated for 30 minutes at 25°C. After incubation, an aliquot of the incubated solution (1.5 mL) was diluted with 1.5 mL of Griess reagent (0.1% N-1- naphthyl ethylene diamine dihydrochloride (NED), 1% sulphanilamide and 2% phosphoric acid). Quercetin was used as a standard drug. Diazotization of nitrite ions with sulphanilamide and subsequent coupling with NED generated a pink chromophore, whose absorbance was measured spectrophotometrically at 546 nm against a blank (Farhan *et al.*, 2012). The blank contained all the reactants except the extract. All the tests were performed in triplicate. The percentage radical scavenging activity was computed using the formula below:

\[
\% \text{ Nitric Oxide Radical Scavenging activity} = \left( \frac{A_0 - A_1}{A_0} \right) \times 100
\]
Where $A_0$ is the absorbance of control reaction (blank) and $A_1$ is the absorbance of the extract of quercetin.

### 6.2.5 Determination of Superoxide Radical Scavenging Activity

#### Principle

This assay is based on the ability of the extract to inhibit formazan formation through the reduction of nitro blue tetrazolium (NBT) by scavenging the superoxide radicals generated in Riboflavin–light–NBT system (Beauchamp and Fridovich, 1971).

#### Procedure

A 3 ml reaction mixture was prepared containing 50 mM sodium phosphate buffer (pH 7.6), 20 μg Riboflavin, 12 mM EDTA and 0.1 mg NBT and various concentrations (50-250 μg/ml) of the plant extract or standard compound. The reaction mixture was then illuminated for 90s. The illuminated reaction mixture without the extract served as the negative control. The unilluminated reaction mixture without plant extract served as the blank. Immediately after illumination, the absorbance of the reaction mixture was measured at 562 nm against a blank to determine the quantity of formazan generated. All tests were performed three times each and quercetin served as positive control. The percentage radical scavenging activity was calculated using the following equation:

\[
\% \text{ Superoxide Radical Scavenging activity} = \frac{A_0 - A_1}{A_0} \times 100
\]

Where $A_0$ is the absorbance of control reaction (blank) and $A_1$ is the absorbance of test reaction.
6.2.6 Determination of Hydroxyl Radical (OH) Scavenging Activity

Principle

This assay is based on the ability of the extract to inhibit hydroxyl radical-mediated deoxyribose degradation by the Fenton’s reaction using Fe$^{3+}$-EDTA-ascorbic acid and H$_2$O$_2$ reaction mixture (Harsha and Latha, 2012; Bajpai et al., 2014).

Procedure

A reaction mixture contained 28 mM 2-deoxy-2-ribose (100 μl), 20 mM KH$_2$PO$_4$-KOH buffer (pH 7.4), 200 μM FeCl$_3$ (1:1 v/v), 200 μl EDTA (1.04 mM), 100 μl of H$_2$O$_2$ (1.0 mM), 100 μl ascorbic acid (1.0 mM) and the extract (100-500 μg/ml) to a final volume of 1 ml. The mixture was incubated at 37°C for 1 hour. After incubation, 1.0 ml of 1% thiobarbituric acid (TBA) and 1.0 ml of 2.8% trichloroacetic acid (TCA) were added and further incubated at 100°C for 20 min to develop pink colour. After cooling, the optical density was measured at λ 532 nm. The blank solution contained all the reactants without the extract. Gallic acid was used as a positive control (standard). All experiments were performed in triplicate (Elizabeth and Rao, 1990). The formula below was used to compute for percentage hydroxyl radical scavenging activity:

\[
\text{\% Hydroxyl Radical Scavenging activity} = \frac{A_0 - A_1}{A_0} \times 100
\]

Where:

- \(A_0\) = Control absorbance (blank)
- \(A_1\) = Extract or standard absorbance.
6.2.7 Determination of Lipid Peroxidation Activity

Principle

Malondialdehyde (MDA), an end product of lipid peroxidation (breakdown of polyunsaturated fatty acids) reacts with TBA (thiobarbituric acid) to produce a pink-colored product with an absorption maxima of 532nm (Halliwell and Gutteridge, 1984). Therefore, inhibition of the formation of MDA denotes the extracts antioxidant potential.

Procedure

Assay of Malondialdehyde was measured according to the method described by Wills (1969) with slight modifications. The reaction mixture of final volume of 1.0 ml contained 2.0 ml of the TCA-TBA-HCl reagent (15% (w/v) TCA, 0.375% (w/v) TBA and 0.25N HCl) and the plant extract (50 µg/ml to 250 µg/ml). The reaction mixture was heated on a water bath at 90°C for 10 minutes, cooled and centrifuged at 10,000 rpm for 10 minutes to remove the TCA precipitate forming light pink colored supernatant (MDA). Ascorbic acid was used as a reference drug. All tests were performed three times. The amount of malondialdehyde formed in each of the samples was assessed by measuring the absorbance of clear supernatant at 532 nm against reference blank. All tests were performed in triplicate. The percentage lipid peroxidation inhibition was calculated using the following equation:

% Lipid Peroxidation = \frac{A_0 - A_1}{A_0} \times 100

Where, \( A_0 \) is the absorbance of control (blank) and \( A_1 \) is the absorbance of test sample.
6.2.8 Determination of Hydrogen Peroxide Radical Scavenging Activity

Principle

This method is based on the decrease in absorbance of H$_2$O$_2$ following reduction of H$_2$O$_2$ by the antioxidant compound to two molecules of water (Malik et al., 2011).

Procedure

The hydrogen peroxide scavenging assay was performed according to the modified method of Ruch et al., (1989). A solution of 40 mM hydrogen peroxide (H$_2$O$_2$) was prepared in phosphate buffer pH 7.4. The plant extract (at different concentrations of 0.1-0.5 mg/ml) was added to hydrogen peroxide solution, incubated for 10 minutes and absorbance measured at 230 nm against a blank solution containing phosphate buffer without the hydrogen peroxide. Ascorbic acid was used as a positive control. All tests were run in triplicate and hydrogen peroxide radical scavenging activity was calculated using the following formula:

\[
\text{% Hydrogen Peroxide Radical Scavenging Activity} = \frac{A_0 - A_1}{A_0} \times 100
\]

Where, $A_0$ is the absorbance of control (blank) and $A_1$ is the absorbance of test sample.
6.2.9 Iron (Fe$^{2+}$) Chelating Activity Assay

**Principle**

This test is based on antioxidants capacity to inhibit the transfer of electrons by forming coordinate complex with the metal ions thereby arresting the oxidation reactions and formation of free radicals. The presence of chelating agents competes with ferrozine for the ferrous ions resulting in decolorization of the red coloured iron (II)-ferrozine complex (Soler-Rivas et al., 2000). The chelating activity of ferrous ions can be measured by the decrease in absorbance at 562 nm (Dinis et al., 1994).

**Procedure**

The chelating activity of ferrous ions was determined by a standard spectrophotometric method (Dinis et al., 1994). Briefly, 1ml of different concentrations of plant extract (50-250 µg/ml) was added to a solution of 1 ml of 0.125 mM ferrous sulphate. The reaction was initiated by addition of 1ml of ferrozine (0.3125 mM), vortexed and incubated for 10 min at room temperature and the absorbance measured at 562 nm. The negative control test (blank) was performed without addition of the extract. All tests were run in triplicate and EDTA was used as a positive control. The capacity of sample to chelate the ferrous ion was calculated relative to the control using the following formula:

\[
\% \text{ Iron II Chelating Activity} = \left( 1 - \frac{A_1}{A_0} \right) \times 100
\]

Where $A_0$-Absorbance of control (blank), $A_1$-Absorbance of sample.
6.2.10 Data Management and Statistical Analysis

The data on absorbance measures was entered in the Microsoft® Excel spread sheet one-word programme, where it was organized and then exported to Minitab statistical software for analysis. The data was found to conform with assumptions of parametric data using box plot and expressed as Means ± Standard Deviations (SD). Inferential statistics were performed using one way-ANOVA followed by Tukey’s post hoc tests for pairwise separation and comparison of means at 99% confidence interval. Unpaired Student t-test was used to compare the percentage free radical scavenging activity between the standard compounds and the plant extract at different concentrations. All statistical analyses were performed using Minitab (Minitab 17.1 Version, NC, USA). The analyzed data was presented in tables and graphs.
6.3 Results

6.3.1 *In Vitro* Ferric Reducing Antioxidant Power (FRAP) of DCM Leaf Extract of *G. glauca*

The five tested concentrations of the DCM leaf extract of *G. glauca* demonstrated a concentration-related increase in ferric reductive activity. All the *G. glauca* leaf extract concentrations were statistically similar to the concentration of ascorbic acid in terms of ferric reducing capacity (*p*≥0.01) (Figure 6.1; Appendix 7.1). However, the ferric reducing activity of the tested extract concentrations was significantly different from each other with the highest extract concentration being the most effective (*p*≤0.01) (Figure 6.1; Appendix 7.1).

*Figure 6.1:* In vitro ferric reducing antioxidant power of DCM leaf extract of *G. glauca*. Bar graphs with different letters across the tested concentrations are statistically significant (*p*≤0.01). Bar graphs with asterisks (*) within the same concentration are not significantly different (*p*>0.01).
6.3.2 *In Vitro* DPPH Radical Scavenging Activity of DCM Leaf Extract of *G. glauca*

The DCM leaf extract of *G. glauca* demonstrated a concentration-dependent increase in DPPH radical scavenging activity (Figure 6.2; Appendix 7.2). As figure 6.2 shows, in all the tested concentrations, the DPPH radical scavenging activity of *G. glauca* was statistically similar to that of the standard compound, vitamin C (*p*>0.01) (Figure 6.2; Appendix 7.2). Further, the DPPH radical scavenging activity was significantly different among all the tested extract concentrations (*p*≤0.01). The lowest extract concentration showed the least DPPH radical scavenging activity while the highest extract concentration exhibited the highest activity (Figure 6.2; Appendix 7.2).

The results also revealed that the concentration of DCM leaf extract of *G. glauca* required to scavenge the initial DPPH radical concentration by 50% (IC$_{50}$ value) was 1.33±0.03 mg/ml, whereas the IC$_{50}$ value of the standard compound, Vitamin C, was 1.39±0.06 mg/ml (Appendix 7.2).
Figure 6.2: *In vitro* DPPH radical scavenging activity of DCM leaf extract of *G. glauca*. Bar graphs with different letters across the tested concentrations are statistically significant (*p* ≤0.01). Bar graphs with asterisks (*) within the same concentration are not significantly different (*p* >0.01).
6.3.3 *In Vitro* Nitric Oxide Radical Scavenging Activity of DCM Leaf Extract of *G. glauca*

The study showed that the DCM leaf extract of *G. glauca* caused a concentration-dependent increase in nitric oxide radical scavenging activity (Figure 6.3; Appendix 7.3). The nitric oxide radical scavenging activity of all the *G. glauca* leaf extract concentrations was not significantly different from that of the standard compound, Quercetin (*p* > 0.01) (Figure 6.3; Appendix 7.3). Across all the tested concentrations, the nitric oxide radical scavenging activity of the *G. glauca* was significantly different from each other whereby the highest concentration showed significantly higher activity than those of lower extract concentrations (*p* ≤ 0.01) (Figure 6.3; Appendix 7.3).

The DCM leaf extract of *G. glauca* also showed a lower IC$_{50}$ value of 665.76±334.12 µg/ml than the standard, Quercetin, which had an IC$_{50}$ value of 748.00±145.38 µg/ml (Appendix 7.3).
Figure 6.3: *In vitro* nitric oxide radical scavenging activity of DCM leaf extract of *G. glauca*. Bar graphs with different letters across the tested concentrations are statistically significant (*p* ≤ 0.01). Bar graphs with asterisks (*) within the same concentration are not significantly different (*p* > 0.01).
6.3.4 *In Vitro* Superoxide Radical Scavenging Activity of DCM Leaf Extract of *G. glauca*

As figure 6.4 indicates, the DCM leaf extract of *G. glauca* caused an exponential increase in superoxide radical scavenging activity from the lowest extract concentration to the highest extract concentration (Figure 6.4; Appendix 7.4). The superoxide radical scavenging activities of *G. glauca* and the standard compound, Quercetin were comparable (*p*>0.01) (Figure 6.4; Appendix 7.4). The effectiveness of the extract in scavenging the superoxide radicals was higher in the highest extract concentration than was observed in lower extract concentrations (Figure 6.4; Appendix 7.4).

The concentration of the DCM leaf extract of *G. glauca* required to inhibit superoxide radical formation by 50% was 119.73±0.20 µg/ml, whereas the standard, Quercetin, showed a higher IC$_{50}$ value of 121.16±8.64 µg/ml (Appendix 7.4).
Figure 6.4: *In vitro* superoxide radical scavenging activity of DCM leaf extract of *G. glauca*. Bar graphs with different letters across the tested concentrations are statistically significant (p≤0.01). Bar graphs with asterisks (*) within the same concentration are not significantly different (p>0.01).
6.3.5 *In Vitro* Hydroxyl Radical Scavenging Activity of DCM Leaf Extract of *G. glauca*

The DCM leaf extract of *G. glauca* displayed potent efficacy of hydroxyl radical scavenging activity across all the extract concentrations (Figure 6.5; Appendix 7.5). The ability of *G. glauca* leaf extract to scavenge hydroxyl radicals occurred in a concentration-dependent manner (Figure 6.5; Appendix 7.5). As figure 7.5 shows, the hydroxyl radical scavenging activity of the standard compound, Gallic acid, was statistically similar to that of *G. glauca* leaf extract in all the tested concentrations (*p* > 0.01). Among all the *G. glauca* leaf extract concentrations, the hydroxyl radical scavenging activity was significantly different from each other (Figure 6.5; Appendix 7.5). As the concentrations increased, the capacity of the extract to scavenge hydroxyl radicals also increased significantly with the highest extract-concentration exhibiting the highest activity (*p* ≤ 0.01) (Figure 6.5, Appendix 7.5).

Findings of the present study also revealed that the DCM leaf extract of *G. glauca* had a lesser IC₅₀ value of 204.34±10.64 µg/ml than the standard compound, Gallic acid, whose IC₅₀ value was 210.05±8.80 µg/ml (Appendix 7.5).
Figure 6.5: *In vitro* hydroxyl radical scavenging activity of DCM leaf extract of *G. glauca*. Bar graphs with different letters across the tested concentrations are statistically significant (*p* ≤ 0.01). Bar graphs with asterisks (*) within the same concentration are not significantly different (*p* > 0.01).
6.3.6 In Vitro Lipid Peroxidation Inhibition Activity of DCM Leaf Extract of *G. glauca*

The DCM leaf extract of *G. glauca* exhibited a concentration-dependent increase in lipid peroxidation inhibition (Figure 6.6; Appendix 7.6). The lipid peroxidation (MDA) inhibitory effect of the *G. glauca* leaf extract was significantly different among the extract-concentrations (*p*≤0.01) (Figure 6.6; Appendix 7.6). Further, the highest extract concentration showed significantly greater inhibition (86.86%) than the lowest extract concentration which inhibited 19.71% of malonaldehyde (MDA). The inhibition of lipid peroxidation by *G. glauca* at different concentrations was statistically similar to that of the standard compound, ascorbic acid (*p*>0.01) (Figure 6.6; Appendix 7.6).

The DCM leaf extract of *G. glauca* showed a lower IC\textsubscript{50} value of 120.56±2.51 µg/ml than the standard, ascorbic acid, which had an IC\textsubscript{50} value of 128.53±5.99 µg/ml (Appendix 7.6).
Figure 6.6: *In vitro* lipid peroxidation inhibition activity of DCM leaf extract of *G. glauca*. Bar graphs with different letters across the tested concentrations are statistically significant (*p* ≤ 0.01). Bar graphs with asterisks (*) within the same concentration are not significantly different (*p* > 0.01).
6.3.7 In Vitro Hydrogen Peroxide Radical Scavenging Activity of DCM Leaf Extract of G. glauca

As observed in Figure 6.7, the activity of DCM leaf extract of G. glauca in scavenging hydrogen peroxide radical occurred in a concentration dependent manner (Figure 6.7; Appendix 7.7). All the tested concentrations of the G. glauca leaf extract showed statistically similar hydrogen peroxide radical scavenging activity to that of the standard compound, ascorbic acid ($p>0.01$) (Figure 6.7; Appendix 7.7). The hydrogen peroxide radical scavenging activities among all the tested concentrations of the G. glauca leaf extract were statistically significant from each other ($p \leq 0.01$). The highest extract concentration was more effective than those of lower extract concentrations (Figure 6.7; Appendix 7.7).

The concentration of the DCM leaf extract of G. glauca required to inhibit hydrogen peroxide radical formation by 50% (IC$_{50}$ value) was 0.24±0.01 mg/ml, whereas the IC$_{50}$ value for the standard, ascorbic acid, was 0.25±0.01 µg/ml (Appendix 7.7).
Figure 6.7: *In vitro* hydrogen peroxide radical scavenging activity of DCM leaf extract of *G. glauca*. Bar graphs with different letters across the tested concentrations are statistically significant (*p* ≤ 0.01). Bar graphs with asterisks (*) within the same concentration are not significantly different (*p* > 0.01).
6.3.8 *In Vitro* Iron Chelating Activity of DCM Leaf Extract of *G. glauca*

The results also revealed that there was a concentration-related increase in iron chelating activity of the DCM leaf extract of *G. glauca* (Figure 6.8; Appendix 7.8). The potential to inhibit the formation of iron (II)-ferrozine complex was significantly different amongst all the concentrations ($p\leq 0.01$) (Figure 6.8; Appendix 7.8). The highest extract concentration showed significantly higher activity than those of the lower extract concentrations ($p\leq 0.01$). The iron chelating activities exhibited among the five extract concentrations were statistically comparable to that of the standard compound, EDTA ($p>0.01$) (Figure 6.8; Appendix 7.8).

Further, it was observed that the IC$_{50}$ value of the DCM leaf extract of *G. glauca* was 114.91±1.72 µg/ml, whereas the IC$_{50}$ value for the standard, EDTA, was 119.22±1.76 µg/ml (Appendix 7.8).
Figure 6.8: *In vitro* iron chelating activity of DCM leaf extract of *G. glauca*. Bar graphs with different letters across the tested concentrations are statistically significant (*p* ≤ 0.01). Bar graphs with asterisks (*) within the same concentration are not significantly different (*p* > 0.01)
6.4 Discussion

A redox imbalance in favor of pro-oxidants results in overproduction of ROS, which constitutes primary catalysts that initiate bimolecular oxidation that causes oxidative stress (Rackova et al., 2007; Hazra et al., 2008). Antioxidants reconcile the upshot of free radicals by directly reacting, neutralizing or competing for substrates whose terminal electron acceptor is molecular oxygen (O$_2$) (Lalianrawn, 2013). Molecular oxygen acts as a thermodynamic sink (Andre et al., 2010). Several synthetic antioxidant agents commercially available are reported to be toxic and carcinogenic, offering natural antioxidants from medicinal plants as better alternatives against oxidative deterioration (Wojdylo et al., 2007). Herbal medicines offer useful therapeutic agents in management and prevention of oxidative stress-related degenerative diseases (Afolayan et al., 2010). In this study, the dichloromethanolic leaf extract of G. glauca demonstrated significant *in vitro* antioxidant and free radical scavenging activities.

The reducing power ability of a chemical compound is based on its reductive capacity in a Fe$^{3+}$-Fe$^{2+}$ system (Halliwell, 1991; Oktay et al., 2003). Usually, biologically active compounds with ferric reducing power capacity are electron donors and are able to reduce the oxidized intermediates such as those of lipid peroxidation processes (Yen and Chen, 1995; Wojdylo et al., 2007). The results obtained in this study indicated that the ferric reducing capacity of G. glauca at various concentrations (50-250 μg/ml) conformed to Beer’s Law at 700 nm (Bajpai et al., 2014). The reduction of Fe$^{3+}$ to Fe$^{2+}$ is an indicator of the extract’s electron donating ability (Farhan et al., 2012). The amount of Fe$^{2+}$ complex [Perl’s Prussian blue ferric ferrocyanide, (Fe$_4$[Fe (CN)$_6$]$_3$)] formed was directly
proportional to the measured absorbance at 700 nm and is indicative of an increase in reductive ability of the extract. Therefore, an increase in optical density indicates higher reductive ability (Naskar et al., 2011). Previous studies on ethanolic seed extracts of *Trachyspermum ammi* also demonstrated a concentration-dependent increase in reducing power potential (Bajpai and Agrawal, 2015). Besides, the reducing capabilities of the root extract of *Biophytum sensitivum* was found to be dose dependent and comparable to the reference compound, Quercetin (Kalita et al., 2013).

The *in vitro* DPPH radical inhibitory assay is based on an antioxidant’s hydrogen donating ability to reduce DPPH radical in methanol to form the non-radical DPPH-H (Kedare and Singh, 2011). In the present study, the *G. glauca* extract demonstrated a remarkable concentration-dependent DPPH radical scavenging activity. The interaction between the extract and DPPH might have occurred through transfer of electrons and hydrogen ions to DPPH to form a stable DPPH molecule (Matthaus, 2002). The DPPH radical usually has a strong absorbance at the wavelength of 517 nm. However, upon acceptance of an electron or hydrogen atom from an antioxidant compound, it becomes a stable diamagnetic molecule with decreased absorbance at 517 nm (Aliev et al., 2009). The resulting color change from purple to pale yellow determines the anti-radical power of an antioxidant (Aliev et al., 2009). A stable diamagnetic free radical, DPPH, has been widely applied as a sensitive and rapid tool for estimation of free radical scavenging activities of both lipophilic and hydrophilic antioxidants (Matthaus, 2002). The IC<sub>50</sub> value of *G. glauca* was less than that of the standard, Vitamin C. Lower IC<sub>50</sub> value is an indication of a high DPPH free radical scavenging activity at low extracts concentrations (Malik et al., 2011).
Previous researches have also demonstrated DPPH radical scavenging activities of various plant extracts. Methanolic whole plant extract of *Biophytum sensitivum* exhibited anti-radical activity in scavenging DPPH radical with a maximum inhibition of about 43.96% (Kalita *et al.*, 2013). The leaf, flower and stem extracts of *Thymelaea hirsuta* also demonstrated a concentration dependent scavenging activity on DPPH radicals (Amari *et al.*, 2014).

Sodium nitroprusside spontaneously generates nitric oxide in aqueous solution at physiological pH, which interacts with molecular oxygen forming nitrite ions that may be estimated using Griess reagent (Bajpai and Agrawal, 2015). In the present study, *G. glauca* demonstrated a concentration-dependent increase in nitric oxide radical scavenging activity. The half maximal activity (IC$_{50}$) of the DCM leaf extract was also lower than that of the standard. Consistent with this study, *Strychnos henningsii* extract was found to cause a moderate concentration-dependent scavenging of nitric oxide with an IC$_{50}$ of 0.49 mg/ml (Afolayan *et al.*, 2010). The antioxidant potency of scavenging nitric oxide by methanolic leaf extracts of *Phyllanthus freternus*, leaves, barks and roots of *Triumfetta rhomboidae* and barks of *Casuarina littorea* resulted in linear time-dependent decrease in nitrite production (Parul *et al.*, 2013). *Newbouldia laevis* was also found to inhibit nitrite formation by direct competition with molecular oxygen (Habu and Ibeh, 2015).

Nitric oxide is a cell signaling molecule generated by specific nitric oxide synthase through which arginine is metabolized to citrulline with the formation of NO via a five-electron oxidative reaction (Parul *et al.*, 2013). Nitric oxide plays a vital physiological role in
respiratory, immune, and neuromuscular systems (Dadashpour et al., 2011). It affects the release of neurotransmitters, neuronal excitability, enhances neurotoxin-induced cellular damage and neuronal cell death (observed in Parkinson’s disease and Alzheimer’s disease). It modulates spatial learning and memory retention processes (cognitive impairment) (Dadashpour et al., 2011). Nitric oxide is also associated with inflammatory bowel syndrome, juvenile diabetes, sepsis, arthritis, carcinomas, dementia, multiple sclerosis, stroke as well as ulcerative colitis (Hazra et al., 2008).

The riboflavin–light–NBT system generates superoxide anions that reduce the yellow dye (NBT\(^{2+}\)) to produce the blue formazan monitored spectrophotometrically at 562 nm. Antioxidants inhibit formazan formation by scavenging the superoxide radicals in the reaction mixture (Beauchamp and Fridovich, 1971; Farhan et al., 2012). The observed decrease in absorbance caused by the G. glauca extract at 562 nm is indicative of the ability of the extract to quench the superoxide radicals in the reaction mixture (Farhan et al., 2012). The lower IC\(_{50}\) value of the extract than that of the standard exhibits stronger free radical scavenging activity (Farhan et al., 2012). Superoxide radical scavenging activities of the Newbouldia laevis plant extract increased markedly with increasing concentrations (Habu and Ibeh, 2015). Similarly, previous studies also demonstrated the abilities of fruit extracts of Terminalia chebula, Terminalia belerica and Emblica officinalis to quench superoxide radicals from the reaction mixture in a concentration dependent manner (Hazra et al., 2010).
The superoxide anion is an oxygen-centered and relatively weak oxidant with a selective reactivity generated by numerous biological and metabolic reactions in the human body (Hazra et al., 2010). Although superoxide radicals exhibit only limited chemical reactivity in biological systems, they act as potential precursors of highly ROS such as hydroxyl radical, hydrogen peroxide and singlet oxygen, which result in lipid peroxidation exacerbating oxidative stress (Meyer and Isaksen, 1995; Hazra et al., 2010). Therefore, superoxide radical scavenging capacity is a first line defense mechanism against oxidative damage (Hazra et al., 2010).

In this study, the ability of the *G. glauca* leaf extract to inhibit hydroxyl radical-mediated deoxyribose damage was evaluated by the Fenton’s reaction using iron (II)-dependent DNA damage assay (Harsha and Latha, 2012). The hydroxyl radicals generated by the Fenton’s reaction degrade DNA deoxyribose sugar, using Fe$^{2+}$ salts as a catalytic component (Balu et al., 2005). The *G. glauca* exhibited ability to quench hydroxyl radicals from the sugar, halting the reaction and thereby forming a fading pink chromophore with the concomitant increase in plant extract concentrations. Similarly, *Trachyspermum ammi* seeds showed hydroxyl radical scavenging activities in a concentration-dependent manner (Bajpai and Agrawal, 2015). The leaf, stem and root extracts of *Clerodendrum viscosum* also exhibited the ability to inhibit hydroxyl radical-mediated deoxyribose degradation in a Fe$^{3+}$-EDTA-ascorbic acid and H$_2$O$_2$ reaction mixture (Dey et al., 2012).

Hydroxyl radical is the most potent ROS in free radical pathology of biological systems capable of damaging exclusively all cellular components (Valko et al., 2007). Hydroxyl
radicals are usually formed from superoxide anion and hydrogen peroxide in the presence of metal cations such as Fe$^{2+}$ and Cu$^+$. These highly reactive radicals cause an oxidative damage to DNA, lipids and proteins (Balu et al., 2005). Hydroxyl radical oxidizes polyunsaturated fatty acid moieties of the cell membrane phospholipids and initiates lipid peroxidation (Valko et al., 2007). The hydroxyl radical also damages nucleic acids by causing polynucleotide strand breakage and alteration of the structure of DNA bases thereby contributing to cytotoxicity, mutagenicity and carcinogenicity (Balu et al., 2005).

Lipid peroxidation is a free radical mediated glycation and protein-modifying reactions in cellular components (Sharma et al., 2012). In this study, the inhibition of hydroxyl ion-induced lipid peroxidation by the G. glauca extract resulted in a concentration dependent decrease in malondialdehyde (MDA) production estimated by thiobarbituric acid (TBA) reaction with an absorption maximum at 532 nm. This capacity might be due to the extracts’ ability to scavenge the hydroxyl radicals generated during decomposition of hydrogen peroxide (Gulcin et al., 2010). Consistent with this study, reports have indicated that essential oils derived from Eryngium creticum exhibited an anti-lipid peroxidation effect in a similar manner (Farhan et al., 2012).

In biological systems, lipid peroxidation is initiated by generation of hydroxyl and superoxide radicals, which accelerates the decomposition of lipid hydroperoxides into peroxyl and alkoxyl radicals that eventually propagate the chain reaction in lipids (Bajpai et al., 2015). Several aldehyde products are eventually produced, among which malondialdehyde is the most important derivative (Baratta et al., 1998). Production of
excess amount of highly reactive oxygen species in the biological systems forms the hallmark of the modifications in cellular membrane function and structure through reduction of membrane lipid fluidity and increase in membrane permeability (Geetha and Vasudevan, 2004).

Malondialdehyde is an important biomarker of lipid peroxidation, which have been associated with the pathogenesis of various disorders among which are inflammation, cancer (Cirak et al., 2003), atherosclerosis (Pari and Amarnath, 2004), diabetes mellitus (Kesavulu et al., 2001), Alzheimer’s disease (Bourdel-Marchasson et al., 2001) as well as degradation of lysosomes and mitochondrial swellings and disintegration (Harsha and Latha, 2012).

Hydrogen peroxide is an important ROS with the ability to directly inactivate enzymes by oxidation of essential thiol (-SH) groups (Sharma et al., 2012). It rapidly penetrates biological membranes and once inside the cell, it interacts with redox-active transitional elements such as Fe$^{2+}$ and possibly Cu$^{+}$ ions via the Harber–Weiss reaction to generate the highly reactive hydroxyl radicals initiating an oxidative attack (Kushad et al., 1998; Gulcin et al., 2003). In this study, the DCM leaf extract G. glauca exhibited the ability to inhibit hydrogen peroxide radical in a concentration related manner. This activity may be attributed to the presence of phytocompounds in the plant extract that can donate electron to H$_2$O$_2$ and thus neutralizing it to water (Mathew and Abraham, 2006). In addition, this could be due to extracts ability to catalyze peroxidases to decompose hydrogen peroxide to water and oxygen (Sharma et al., 2012). Consistent with this study, the leaves, flowers
and fruits of *Crataegus monogyna* extracts demonstrated the ability to scavenge hydrogen peroxide in an amount dependent manner (Keser *et al*., 2012). Another study demonstrated that methanolic extract of *Trichodesma zeylanicum* caused a strong dose-dependent inhibition of hydrogen peroxide (Ngonda, 2013).

Ferrozine can quantitatively react with Fe$^{2+}$ to form a red colored complex (Sharma *et al*., 2012). The presence of a chelating agent in the reaction mixture limits the formation of ferrozine-Fe$^{2+}$ complex and results in the decrease in the intensity of the red color formed with an increase in the concentration of the chelating agent (Sharma *et al*., 2012). As demonstrated in this study, the plant extract caused a concentration-dependent reduction in color change, due to its competition with ferrozine for the ferrous ions, thereby inhibiting the formation of ferrozine-Fe$^{2+}$ complex (Soler-Rivas *et al*., 2000). A similar study demonstrated a dose-dependent inhibition of the formation of ferrozine-Fe$^{2+}$ complex by the action of *Clerodendrum viscosum* (Dey *et al*., 2012). *Mellilotus arvensis* extract inhibited the formation of ferrous and ferrozine complex, signifying that it has chelating activity and captures ferrous ions before ferrozine (Ebrahimzadeh *et al*., 2008).

The dual oxidation state characteristic of iron enables it to accept or donate electrons through redox reactions (Sharma *et al*., 2012). The capacity of iron to interact with superoxide anion (O$_2^-$) and hydrogen peroxide results in the formation of reactive hydroxyl radical (OH·) through Haber-Weiss reaction, which exacerbates damage to cell membrane, proteins and nucleic acids (Sharma *et al*., 2012). Moreover, the dual oxidation state of iron enables acceleration of lipid peroxidation through decomposition of lipid hydroperoxides.
into peroxyl and alkoxyl radicals responsible for perpetuation of the oxidative-chain reactions (Mohan et al., 2012).

Obesity is associated with increased circulating levels of free fatty acids and systemic pro-inflammatory cytokines, prostaglandins and nitric oxide, which in turn precipitates oxidative stress (Marseglia et al., 2014). High production of ROS is associated with low-grade chronic systemic inflammation of adipose tissues (Marseglia et al., 2014). This condition activates innate immune system in adipose tissue and promotes secretion of pro-inflammatory cytokines including tumor necrosis factor-alpha (TNF-α), interleukin (IL)-1β, and IL-6 (Fonseca-Alaniz et al., 2007). A rise in concentration of pro-inflammatory cytokines further increases generation of ROS and RNS by macrophages and monocytes (Shoelson et al., 2007). However, antioxidants inhibit oxidative metabolism in living cells through free radical scavenging mechanisms, neutralization of free radicals into less active stable products, blockage of the initiators of free radical attack (Ishino et al., 2010), regulation of electron transport chain, repair of oxidized proteins, termination of chain reaction effect and salvage of the oxidized antioxidants thereby restoring the cells’ functional capacity (Kohen and Nyska, 2002; Ishino et al., 2010).

The antioxidant activity exhibited by DCM leaf extract of G. glauca can be attributed to the presence of various phytochemicals that are thought to function interactively and synergistically to neutralize ROS and RNS (Ishino et al., 2010). The GC-MS analysis of G. glauca revealed the presence of bioactive compounds among which are phenolic compounds (flavonoids, stilbenes, chalcones, tannins), lipids (fatty acid esters and
phytosterols), terpenoids (monoterpane, diterpenes and triterpenes) as well as vitamin E (Chapter 3). These bioactive compounds have been shown to maintain the redox homeostasis through multiple step processes of antioxidant reactions which involves initiation, propagation, branching and termination of free radicals (Ishino et al., 2010).

Flavonoids are bioactive phenols with anti-oxidant, anti-inflammatory, anti-obesity and anti-diabetic properties (Batra and Sharma, 2013). Flavonoids exerts their anti-oxidant activities through quenching or scavenging of free radicals, chelating of metal ions, donation of an electron and hydrogen ion, and inhibition of enzymatic systems responsible for generation of free radicals (Firuzi et al., 2005). Flavonoids bind with transition metal elements such as iron and copper and plays a key role in inhibiting metal-catalyzed free radical formation (Treml and Smejkal, 2016). The chelation effects of flavonoids inhibit lipid peroxidation, Fe^{2+} catalyzed oxidation of glutamine synthase and oxidation of linoleic acid through removal of metal ions from catalytic sites and scavenging of free radicals (Andjelković et al., 2006). The reducing power (ability to donate electron and hydrogen ions) of flavonoids contributes to the termination of lipid peroxidation chain reaction (Prakash and Gupta, 2009). Flavonoids interact with various signaling pathways that regulate cell cycle, differentiation and apoptosis (Gee and Johnson, 2001).

Stilbenes such as pinosylvin and resveratrol have been implicated in scavenging of most oxidizing molecules such as singlet oxygen, and other free radicals (Agati et al., 2012). They suppress the formation of reactive oxygen species, scavenge reactive species, chelate
metal ions involved in the production of free-radicals as well as protects and up-regulate antioxidant defenses (Agati et al., 2012).

Quercetin exhibits its antioxidant activities through its ability to scavenge superoxide radicals, hydroxyl radicals and lipid peroxyl radicals (Dong et al., 2014). Previous studies in mice showed that quercetin supplementation normalized the concentration of nitric oxide, glutathione and glutathione peroxidase thereby protecting the liver from oxidative damage (Stevenson and Hurst 2007). Another study reported that quercetin conferred neuroprotection against neurotoxicity of amyloid β-peptide via its acetylcholinesterase inhibitory property and free radical scavenging effects (Ansari et al., 2009). Quercetin modulates its anti-inflammatory effects via anti-oxidant responsive elements (ARE), NF-κB and xenobiotic responsive elements (Moskaug et al. 2004). The anti-adipogenesis effects of quercetin are mediated by the adenosine monophosphate-activated protein kinase (AMPK) in preadipocytes and mitogen-activated protein kinases signaling pathways (MAPK) in mature adipocytes (Ahn et al., 2008).

Studies have shown that vitamin E (tocopherols and tocotrienols) results in significant reduction in lipid peroxides, nitrogen dioxide, singlet oxygen, and superoxide anion in plasma (Rietjens et al., 2002). The mechanism of their enhanced efficiency of scavenging hydroxyl, peroxyl and alkoxy radicals is based on the ease with which the hydrogen on the hydroxyl group of their chroman ring can be donated to neutralize the free radicals. This, in turn, creates a more stable tocopheroxyl radical (Holubková et al., 2012). Vitamin E increases the levels of serum glutathione and stimulate the catalytic activity of
glutathione peroxidase and catalase (Borek, 2004). Tocotrienols are also reported to inhibit low-density lipoprotein (LDL) oxidation (Tiwari, 2001). Oxidized LDL is a potent chemokine that induces an influx and adhesion of monocytes as well as facilitates the activation and recruitment of macrophages (Devaraj et al., 2008). The presence of monocytes and activated macrophages stimulates the production of proinflammatory cytokines such TNF-α and IL-6 (Holubková et al., 2012). The ability of vitamin E to inhibit the oxidation of LDL prevents buildup of plaques in arteries thereby conferring protection against cardiovascular diseases such as atherosclerosis, heart attack and stroke (Devaraj et al., 2008). Besides, it signifies its anti-inflammatory effects (Holubková et al., 2012).

Terpenoids and Sterols quench free radicals, acts as reducing agents and are involved in termination of the free radical chain reaction (Ragasa et al., 2009). Sterol esters reduces the concentration of LDL cholesterol in plasma (Ragasa et al., 2009). The α-Amyrin acetate isolated from the fruits of Ficus racemose showed hypoglycemic effects in streptozotocin-induced diabetic rat model and prevented oxidation of low-density lipoprotein (LDL) (Ragasa et al., 2009). Squalene is implicated in protection from obesity-mediated inflammation through its antioxidant activity (Zhang et al., 2015). Squalene inhibits gene expression of proinflammatory mediators by enhancing histone deacetylase activity and activates the transcription factors that antagonize chronic inflammation (Chuang and McIntosh, 2011).

Naringenin chalcone showed neuroprotective activity by reducing inflammatory load and prevention from oxidative damage thereby increasing neurogenesis and intraneuronal
signaling (Poulose et al., 2014). It also showed its anticancer activity by downregulating the gene expression of cyclooxygenase 2 (COX-2) (Kumar et al., 2012).

Curcumin has been shown to inhibit the generation of ROS in macrophages and red blood cells (Daniel et al., 2004). Curcumin effectively scavenges different classes of free radicals such as superoxide and hydroxyl anions. The metal-chelating activity of curcumin and the metal complexes of curcumin are reported to be effective radical scavengers (Baum and Ng, 2004). In addition, curcumin inhibit nitric oxide synthase enzymes resulting in decreased levels of nitric oxide (Amin and Bano, 2012). Curcumin was found to upregulate the expression levels and the catalytic activity of different antioxidant enzymes such as catalase, superoxide dismutase, activated protein-1, heme oxygenase-1 and glutathione peroxidase (Panahi et al., 2012). Studies demonstrated that treatment with curcumin efficiently mitigated lipid peroxidation (Sahebkar et al., 2013). Curcumin regulate the antioxidant response by inhibiting the phosphorylation of Akt and ERK (Chun et al., 2003). Moreover, curcumin modulates cell death by reducing the expression levels of TNF-α and endogenous Bcl-xL and Bcl-2 (Deeb et al., 2003).

Catechins exert their antioxidant and anti-inflammatory effects through enhancing the activity of antioxidant enzymes that are regulated by nuclear factor erythroid 2p45 (NF-E2)-related factor 2 (Nrf2) bound to the AREs (antioxidant response elements) (Tan et al., 2011; Su et al., 2012). Catechins reduce inflammation via inhibition of prostaglandin production and nuclear factor-κB activity (Hutchins-Wolfbrandt and Mistry, 2011; Costa et al., 2013). Studies have demonstrated that catechins decreases uptake of LDL by
macrophages, inhibit LDL oxidation and lowers LDL aggregation (Sahebkar et al., 2013). Galloctechin-catechin flavan reduces the levels of inflammatory cytokines such as TNF-α a and IL-6 in plasma (Hwang et al., 2005; Su et al., 2012).

Gallocatechin-catechin flavan reduces the levels of inflammatory cytokines such as TNF-α a and IL-6 in plasma (Hwang et al., 2005; Su et al., 2012).

Gallic acid is a strong antioxidant compound that has been found to inhibit lipid peroxidation through reduction in levels of oxidized plasma malondialdehyde (MDA) (Da-Silva’Porto et al., 2003). Gallic acid hinders the oxidation of low-density lipoprotein (LDL) and reduces levels of plasma thiobarbituric acid reactive substance (TBARS) (Jang et al., 2001). Another compound, Neryl acetate, increases the bioavailability of reduced glutathione and stimulates synthesis and catalytic activity of glutathione peroxidase (Valko et al., 2007). Ferulic acids reduces levels of cytokines and C-reactive proteins in plasma (Al-Lahham et al., 2010). The increased circulating levels of pro-inflammatory cytokines and C-reactive proteins are definitive of low-grade systemic inflammation, a hallmark of metabolic syndromes. Ferulic acids inhibits transcription factor Nfκ-β and increases the threshold for an inflammatory response by enhancing the binding of short chain fatty acids to G-protein coupled receptors (Belobrajdic and Bird, 2013).

Luteolin reduces levels of lipopolysaccharides (LPS) in obesity-related inflammatory liver diseases (Cani et al., 2009). Diet supplementation of luteolin decreased markers of inflammation in adipose tissue by restricting the translocation of LPS from the large bowel in high fat diet fed mice models (Belobrajdic and Bird, 2013).
CHAPTER SEVEN
PHYTOCHEMICAL SCREENING OF DICHLOROMETHANE LEAF
EXTRACT OF *Gnidia glauca*

7.1 Introduction

The contribution of medicinal plants to the therapeutic arsenal in the fight against diverse ailments is evidence of man’s ingenuity since time immemorial (Charles *et al.*, 2011). The rationale behind the vast usage and greater dependence on herbal medicines as preferred prescription agents rest upon their long-term clinical experience (Piero *et al.*, 2012a). Medicinal plants provide a major reservoir of effective chemotherapeutics essential for the maintenance of human health (Garza *et al.*, 2011). These bioactive compounds are assumed to be non-phytotoxic, easily biodegradable and are cost-effective alternatives to synthetic drugs (Janakiraman *et al.*, 2012).

The pharmacological efficacy of the herbal medicines may be due to the synergistic effects of various phytocompounds contained in them (Zwenger and Basu, 2008). Authentication of these chemical constituents is not only essential for the discovery of new therapeutic agents but also discloses new sources of economic phytocompounds as well as the appreciation of the significance of folkloric remedies (Mojab *et al.*, 2010).

Owing to biocompatibility and overlapping mechanism of action associated with phytochemicals, a thorough validation of the active principles of the herbal medicines is inevitable. Mass spectrometry coupled with chromatographic separations such as gas chromatography linked to mass spectrometry (GC-MS) is one of the most popular technique employed for identification and characterization of components existing in
natural products (Cheong et al., 2016). The GC-MS has proved to be a valuable technique that is increasingly applied for the analysis of non-polar compounds and volatile essential oils, fatty acids, lipids and alkaloids (Cheong et al., 2016). Therefore, this study was carried out to profile major phytochemicals present in DCM leaf extract of *G. glauca* using GC-MS. It is postulated that GC-MS could yield good detection and identification of phytocompounds with associated anti-obesity activity, cognitive enhancing and neurobehavioral effects as well as antioxidant properties.
7.2 Materials and Methods

7.2.1 Collection and Preparation of the Medicinal Plant, Gnidia glauca
The collection of medicinal plant, processing and extraction of the plant material were conducted as described in chapter 3 (Section 3.2.1-2).

7.2.2 Sample Preparation for GC-MS Analysis
A 1 mg of the dried crude dichloromethane leaf extract of G. glauca was dissolved in 1mL dichloromethane (sigma Aldrich gc-grade). The sample was vortexed for 30s and sonicated in an ultra-bath for 15 min before being centrifuged at 14,000 rpm for 5 min. The supernatant was passed through anhydrous Na₂SO₄ to remove moisture. The resultant stock solution (1mg/mL) was used to prepare an experimental sample whose final concentration was 100ng/µL. The samples were prepared in triplicate.

7.2.3 Gas Chromatography-Mass Spectrometry (GC-MS) Analysis
Analysis of the sample was carried out using GC-MS (7890/5975 Agilent Technologies, Inc., Beijing, China) consisting of a gas-chromatograph interfaced to a mass spectrometer instrument. The GC-MS was equipped with an HP-5 MS (5% phenyl methyl siloxane) low bleed capillary column of 30m length, 0.25mm diameter, and 0.25µm film thickness. For GC-MS detection, an electron ionization system with ionization energy of 70Ev was used. The carrier gas used was helium (99.99%) at a constant flow rate of 1.25 ml/min in split mode. The injector and mass transfer line temperature were set at 250°C and 200°C respectively, and an injection volume of 1 µl was employed. The oven temperature was
programmed from 35°C for 5 minutes, with an increase of 10°C/min to 280°C for 10.5
minutes, then 50°C/min to 285°C for 29.9 minutes with a run time of 70 minutes.

The mass spectrometry operating parameters were as follows: ionization energy, 70eV; ion
source temperature, 230°C; solvent cut time, 3.3 min; relative detector gain mode; scan
speed, 1666 µ/sec; scan range of 40-550 m/z and the interface temperature of 250°C.

7.2.4 Data Management and Statistical Analysis

Compound identities were proposed based on their general fragmentation pattern and using
reference spectra published by the library–MS databases (National Institute of Standards
and Technology (NIST) 05, 08). The retention indices were determined using C5-C32
hydrocarbons range. The identity of the spectra above 60% of the library match was
required for the identification of phytocompounds. The compound name, molecular
weight, chemical class and structure of the components of the plant extract were
ascertained. The relative concentrations of each component were expressed as mg/kg with
peak-area normalization. The three replicate measures were subjected to descriptive
statistics using analysis of variance (ANOVA) and results expressed as Mean ± SD.
7.3 Results

The GC-MS analysis of DCM leaf extract of *G. glauca* revealed the presence of 28 compounds (Table 7.1). Based on analysis results, Oleic acid (21.05±2.34 mg/kg) had the highest concentration followed by γ-Sitosterol (18.84±1.0 mg/kg), Curcumin (16.91±2.30 mg/kg), Quercetin (15.74±1.01 mg/kg), 3,5-dihydroxy-trans-stilbene (13.39±4.06 mg/kg) and Vitamin E (12.25±1.67 mg/kg), Phenol, 2,4-bis (1,1-dimethyl ethyl)- (11.20±1.38 mg/kg), Phytol (11.04±1.18 mg/kg), Octadecanoic acid (Stearic Acid) (10.73±1.55 mg/kg), Phytol acetate (10.65±1.31 mg/kg), D-Allose (10.58±0.99 mg/kg), Gallocatechin-catechin flavan (10.40±1.00 mg/kg), Gallic acid (10.24±1.02 mg/kg), Ferulic acid (10.18±1.14 mg/kg), Flavonols (10.15±1.58 mg/kg) and others (Table 7.1). The molecular formula, molecular weight, concentration (mg/kg), retention time, and a chemical class of identified compounds in *Gnidia glauca* leaf extract were presented in Table 7.1.
### Table 7.1: The Concentrations of Compounds Identified in DCM Leaf Extract of *Gnidia glauca*

<table>
<thead>
<tr>
<th>RT</th>
<th>Compound Name</th>
<th>Molecular Formula</th>
<th>Chemical Class</th>
<th>M+H (g/mol)</th>
<th>Concentration (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>11.51</td>
<td>3,4,5-trihydroxybenzoic acid (Gallic acid)</td>
<td>C₇H₆O₅</td>
<td>Phenolic acid</td>
<td>170.12</td>
<td>10.24±1.02</td>
</tr>
<tr>
<td>13.36</td>
<td>D-Allose</td>
<td>C₈H₁₂O₆</td>
<td>Aldohexose sugar</td>
<td>180.1559</td>
<td>10.58±0.99</td>
</tr>
<tr>
<td>15.12</td>
<td>3,7-dimethyl-2,6-octadiene-1-ol acetate (Neryl acetate)</td>
<td>C₁₂H₂₀O₂</td>
<td>Essential oil</td>
<td>196.29</td>
<td>9.77±1.81</td>
</tr>
<tr>
<td>15.19</td>
<td>Ferulic acid</td>
<td>C₁₀H₁₀O₄</td>
<td>Phenolic acid</td>
<td>194.18</td>
<td>10.18±1.14</td>
</tr>
<tr>
<td>18.20</td>
<td>Phenol, 2,4-bis (1,1-dimethylethyl)-</td>
<td>C₁₄H₂₂O</td>
<td>Polyphenol</td>
<td>206.329</td>
<td>11.20±1.38</td>
</tr>
<tr>
<td>21.53</td>
<td>Flavonols</td>
<td>C₁₅H₁₀O₂</td>
<td>Flavonoid</td>
<td>222.243</td>
<td>10.15±1.58</td>
</tr>
<tr>
<td>23.06</td>
<td>Oleic acid</td>
<td>C₁₈H₃₆O₂</td>
<td>Fatty acid</td>
<td>282.47</td>
<td>21.05±2.34</td>
</tr>
<tr>
<td>24.73</td>
<td>3,5-dihydroxy-trans-stilbene (pinsylvin)</td>
<td>C₁₄H₁₂O₂</td>
<td>Polyphenol</td>
<td>212.24</td>
<td>13.39±4.06</td>
</tr>
<tr>
<td>24.92</td>
<td>Catechins</td>
<td>C₁₅H₁₆O₆</td>
<td>Tannins</td>
<td>290.26</td>
<td>9.27±2.05</td>
</tr>
<tr>
<td>25.44</td>
<td>Octadecanoic acid (Stearic Acid)</td>
<td>CH₃(CH₂)₁₆COOH</td>
<td>Fatty acid</td>
<td>328.488</td>
<td>10.73±1.55</td>
</tr>
<tr>
<td>25.96</td>
<td>Naringenin chalcone</td>
<td>C₁₅H₁₂O₅</td>
<td>Polyphenol</td>
<td>272.256</td>
<td>7.71±1.63</td>
</tr>
<tr>
<td>26.35</td>
<td>9,12,15-Octadecatrienoic acid, (Z,Z,Z)-(α-Linolenic acid)</td>
<td>C₁₈H₃₀O₂</td>
<td>Fatty Acid</td>
<td>278.43</td>
<td>9.74±2.85</td>
</tr>
<tr>
<td>26.98</td>
<td>Luteolin</td>
<td>C₁₅H₁₀O₆</td>
<td>Flavonoid</td>
<td>286.24</td>
<td>9.77±2.62</td>
</tr>
<tr>
<td>27.90</td>
<td>Eicosapentaenoic acid</td>
<td>C₂₀H₃₀O₂</td>
<td>Fatty acid</td>
<td>302.451</td>
<td>7.62±0.89</td>
</tr>
<tr>
<td>28.48</td>
<td>Docosahexaenoic acid</td>
<td>C₂₂H₃₂O₂</td>
<td>Fatty acid</td>
<td>278.524</td>
<td>7.94±0.44</td>
</tr>
<tr>
<td>29.22</td>
<td>Curcumin</td>
<td>C₂₁H₂₀O₆</td>
<td>Polyphenol</td>
<td>368.38</td>
<td>16.91±2.30</td>
</tr>
<tr>
<td>30.07</td>
<td>Phytol</td>
<td>C₂₀H₄₀O</td>
<td>Acyclic diterpene</td>
<td>296.539</td>
<td>11.04±1.18</td>
</tr>
<tr>
<td>30.24</td>
<td>Quercetin</td>
<td>C₁₅H₁₀O₇</td>
<td>Flavonoid</td>
<td>302.236</td>
<td>15.74±1.01</td>
</tr>
<tr>
<td>30.79</td>
<td>γ-Sitosterol</td>
<td>C₂₀H₃₀O</td>
<td>Phytosterol</td>
<td>414.718</td>
<td>18.84±1.04</td>
</tr>
<tr>
<td>32.23</td>
<td>Gallocatechin-catechin flavan</td>
<td>C₁₅H₁₄O₆</td>
<td>Tannins</td>
<td>306.27</td>
<td>10.40±1.00</td>
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<tr>
<td>32.97</td>
<td>Phenol, 2,4-bis (1-methyl-1-phenylethyl)-</td>
<td>C₂₂H₂₅O</td>
<td>Polyphenol</td>
<td>302.417</td>
<td>9.85±0.49</td>
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<tr>
<td>34.33</td>
<td>Phytol acetate&lt;E-&gt;</td>
<td>C₂₂H₄₂O₂</td>
<td>Acyclic diterpenoid</td>
<td>338.576</td>
<td>10.65±1.31</td>
</tr>
<tr>
<td>RT</td>
<td>Compound</td>
<td>Chemical Formula</td>
<td>Class</td>
<td>MW</td>
<td>Mean ± SD</td>
</tr>
<tr>
<td>-----</td>
<td>--------------------------</td>
<td>------------------</td>
<td>----------------</td>
<td>------</td>
<td>-----------</td>
</tr>
<tr>
<td>34.92</td>
<td>Cholecalciferol (vitamin D)</td>
<td>C$<em>{27}$H$</em>{44}$O</td>
<td>Vitamin</td>
<td>384.64</td>
<td>9.95±1.42</td>
</tr>
<tr>
<td>35.41</td>
<td>Vitamin E</td>
<td>C$<em>{29}$H$</em>{50}$O$_2$</td>
<td>Vitamin</td>
<td>430.717</td>
<td>12.25±1.67</td>
</tr>
<tr>
<td>35.48</td>
<td>Stigmasterol</td>
<td>C$<em>{29}$H$</em>{46}$O</td>
<td>Phytosterol</td>
<td>412.702</td>
<td>7.75±2.23</td>
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<tr>
<td>36.29</td>
<td>Squalene</td>
<td>C$<em>{30}$H$</em>{50}$</td>
<td>Triterpene</td>
<td>410.718</td>
<td>7.48±0.52</td>
</tr>
<tr>
<td>36.82</td>
<td>α-Amyrin</td>
<td>C$<em>{30}$H$</em>{50}$O</td>
<td>Triterpene</td>
<td>426.729</td>
<td>5.25±0.78</td>
</tr>
<tr>
<td>37.85</td>
<td>β-Amyrin</td>
<td>C$<em>{32}$H$</em>{52}$O$_2$</td>
<td>Triterpene</td>
<td>468.766</td>
<td>6.98±2.47</td>
</tr>
</tbody>
</table>

Concentrations of compounds identified in *Gnidia glauca* leaf extract (mg/kg). Results are expressed as Means ± SD for replicate measurement n=3. RT is the retention time and [M+H] is the Relative molecular mass.
7.4 Discussion

In the present study, phytochemical profiles of dichloromethane leaf extract of *G. glauca* were identified and characterized using GC-MS. The GC-MS analysis identified 28 known bioactive compounds among which are phenolic compounds (flavonoids, stilbenes (pinosylvin), chalcones and tannins), lipids (fatty acid esters and phytosterols), terpenoids (monoterpene, diterpenes, and triterpenes) as well as vitamins (vitamin D and E). Oleic acid, γ-Sitosterol, curcumin, quercetin, and stilbenes were the most abundant phytocompounds in DCM leaf extract of *G. glauca*.

The gas chromatogram revealed concentrations of various phytocompounds eluted at different retention times. Analysis of the nature of the eluted compounds at different retention times were determined using Mass Spectrophotometer. The fragmentation pattern indicates disintegration of large fragments into smaller compounds with peaks at different m/z ratios. The mass spectra provided a blueprint of phytocompounds of DCM leaf extract of *G. glauca* which were identified from the data library of the National Institute of Standards and Technology (NIST).

The results of the GC-MS analysis of *G. glauca* revealed the presence of phenolic compounds such as phenolic acids, stilbenes, curcuminoids (curcumin), chalcones, lignans (such as flavones and flavonoids and tannins). Phenolic compounds are bioactive secondary metabolites containing benzene rings with one or more hydroxyl substituents (Velderrain-Rodríguez et al., 2014). These phytocompounds are synthesized via the shikimic acid pathway, pentose phosphate pathway and metabolization of phenylpropanoid in plants (Randhir et al., 2004).
Phenolic acids are the simplest bioactive phytochemicals that occur as esters or glycosides conjugated with other natural compounds such as alcohols, hydroxy fatty acids, flavonoids, glucosides and sterols (Castillo and Hernández, 2015). They contain a hydroxyl group bonded to a carbon atom that is part of an aromatic ring (Castillo and Hernández, 2015). The GC-MS analysis of *G. glauca* indicated phenolic acids such as ferulic acid and gallic acids. These phenolic acids confer protective roles against oxidative damage by acting as free radical acceptors and/or chain breakers (Robbins, 2003). Gallic acid plays a critical role in oxidative stress-mediated diseases such as coronary heart disease, diabetes mellitus, stroke, and some cancers (Robbins, 2003). Similarly, isolated gallic acid from the leaves of *Stewartia pseudocamellia* indicated antioxidant, antidiabetic and anticarcinogenic effects. Ferulic acid contains hypolipidemic properties, reduces serum cholesterol levels and could be effective in lowering the risk of high fat diet-induced obesity (Son et al., 2010). Further, ferulic acids have been found to be a potent inhibitor of tumorigenesis (Srinivasan et al., 2007). Ferulic acids isolated from the aqueous poplar bud extract of *Populus nigra* exhibited anti-inflammatory, antidiabetic and antioxidants activities (Dudonne et al., 2011).

Phytochemical analysis of the DCM leaf extract of *G. glauca* also revealed the presence of stilbenes. Stilbenes such as pinosylvin and resveratrol are phenolic compounds that have been shown to exhibit anti-obesity and hypoglycemic effects (Rayalam et al., 2008). Stilbenes increases lipolysis, induces apoptosis and decreases adipogenesis by downregulating adipocyte-specific genes (such as SREBP-1c, PPAR, FAS, C/EBP, LPL, and HSL) and adipocyte-specific transcription factors (Rayalam et al., 2008; Baile et al., 2007).
2011). Besides, stilbenes decrease the expression levels of inflammatory mediators such as IL-6, TNF-α IL-8, COX-2, MCP-1 and PAI-1 in mature adipocytes (Olholm et al., 2010). It inhibits TNF-α activated NF-β signaling thereby hindering TNF-α induced secretion (Baile et al., 2011). Stilbenes such as resveratrol increases mRNA expression of IL-6 and adiponectin (Kang et al., 2010). In adipocytes, resveratrol improves insulin sensitivity through phosphorylation of Ser/Thr residues of the insulin receptor substrate-1, and downstream AKT (Kang et al., 2010). Therefore, it increases insulin-stimulated glucose uptake in the adipocytes, but, simultaneously, inhibits lipolysis (Fischer-Posovszky et al., 2010). Stilbenes isolated from Polygonum cuspidatum were found to have strong antioxidant activity, reduce the oxidation of LDL cholesterol, total cholesterol and the risk of cardiovascular diseases (Ghanim et al., 2010).

Curcumin is a turmeric-derived polyphenol, which was also identified in the DCM leaf extract of G. glauca. Curcumin is therapeutically applied in the management of oxidative stress-induced diseases such as obesity, diabetes mellitus and some cancers (Tang and Chen, 2010). Curcumin modulates energy metabolism and intracellular lipids levels (Ejaz et al., 2009; Alappat and Awad, 2010). It suppresses angiogenesis in adipose tissues hindering tissue growth (Alappat and Awad, 2010). Curcumin improves obesity-induced inflammation and associated comorbidities such as hyperlipidemia, hypercholesterolemia, insulin resistance, and hyperglycemia, (Aggarwal, 2010). Previous studies have indicated that treatment with curcumin increases adipose tissue adiponectin secretion, reduces macrophage infiltration into white adipose tissue and reduces the activity of hepatic NF-β and markers of hepatic inflammation (Weisberg et al., 2008). Cumulatively, curcumin
contributes to lower body fat and body weight gain (Ejaz et al., 2009). Curcumin isolated from *Curcuma longa* caused a decrease in triglycerides accumulation and an improvement in inflammatory and metabolic derangements (Weisberg et al., 2008; Lee et al., 2009).

Naringenin chalcone is another polyphenolic compound identified in *G. glauca* leaf extract. Naringenin chalcones have been reported to modulate lipid and carbohydrate metabolism (Hirai et al., 2007). It is useful for ameliorating the inflammatory changes in adipose tissue, alleviate oxidative stress, improve β-cell function, stimulate insulin secretion as well as attenuates hyperglycemia, dyslipidemia and insulin resistance (Yu et al., 2009). Naringenin and other phenolic compounds found in the leaf extracts of *Lawsonia inermis* and *Kaempferia galanga* were attributed to their anti-obesity, antidiabetic and free radical scavenging activities (Mustafa et al., 2010).

Analysis of the DCM leaf extract of *G. glauca* also revealed the presence of flavonoids such as flavanols (catechin, epicatechin), flavonols (quercetin) and flavones (luteolin). Flavonoids are phenolic structures containing one carbonyl group (Shahidi and Ambigaipalan, 2015). Observational and intervention studies have demonstrated that flavonols such as quercetin are associated with anti-inflammatory, anti-obesity, anti-proliferative effects, free radical scavenging activity as well as inhibition of the hydrolytic and oxidative enzyme (Atanassova and Georgieva, 2012). Quercetin exerts its therapeutic effects against Type II diabetes mellitus through modulation of α-glycosidase activity, glucose cotransporters and aldose reductase activity (Romano et al., 2013). Quercetin plays a critical role in obesity management through inhibition of adipogenesis by activating the
AMPK signaling pathway in preadipocytes (Ahn et al., 2008). It reduces the expression levels of adipogenesis-related factors and induces apoptosis in adipocytes by modulation of the ERK and JNK pathways (Hu et al., 2006; Ahn et al., 2008). *Punica granatum* (Pomegranate) rich in flavonols, exhibited antidiabetic and anti-obesity activities through enhancement of the activities of antioxidant enzymes (such as catalase, superoxide dismutase, glutathione reductase, and peroxidases) as well as induction of metal chelating activity (Banihani et al., 2013).

The GC-MS analysis also revealed the presence of a flavanol called catechins in the DCM leaf extracts of *G. glauca*. Catechins have been demonstrated both in cell culture and animal models of obesity to decrease fat mass and body weight through reduction of adipocyte differentiation and proliferation (Wolfram et al., 2006) They also increases β-oxidation of fatty acids and thermogenesis (Richard et al., 2009). Catechins significantly reduce intracellular lipid accumulation by facilitated inhibition of fat absorption and lipogenesis (Kim et al., 2010). A case study in human subjects recorded a 4.6% and 4.48% decrease in body weight and waist circumference, respectively, upon treatment with 25% catechins for three months (Hara, 2011). The postulated mechanism of activity of catechins could be through the inhibition of gastric lipases and increase in thermogenesis (Boschmann and Thielecke, 2007; Hara, 2011). Catechins isolated from *Camellia sinensis* demonstrated anti-obesity, anti-tumorigenic, hypoglycemic and antioxidant activities (Vuong et al., 2010). Administration of Epigallocatechin gallate isolated from the leaf extract of *Camellia sinensis* caused a decrease in body weight gain in obese Zucker rats (Wolfram et al., 2006; Moon et al., 2007).
Luteolin is the flavone identified in DCM leaf extract of *G. glauca* and has been reported to exhibit anti-obesity effects through inhibition of gene expression of cell adhesion molecules (CAM) (Zheng *et al*., 2010). Cell adhesion molecules are highly expressed in obese states and therefore, luteolin inhibits their expression by blunting the activation of NF-β (Zheng *et al*., 2010). Further, luteolin has been shown to enhance insulin sensitivity via activation of PPAR transcriptional activity in adipocytes (Zheng *et al*., 2010). Luteolin isolated from the *Genista tenera* exhibited an antihyperglycemic effect in streptozotocin–induced diabetic rats (Rauter *et al*., 2010).

The GC-MS analysis of DCM leaf extract of *G. glauca* also revealed the presence of tannins. Tannins are polymeric phenolic substances that exist as either hydrolyzable tannins or condensed tannins (Zhang *et al*., 2015). The GC-MS analysis indicated the presence of gallocatechin-catechin flavan. Gallocatechin-catechin flavan also termed as condensed tannins are dimers, oligomers, and polymers of catechinods (Banihani *et al*., 2013). Condensed tannins isolated from the leaf extract of *Punica granatum* were reported to attenuate hyperglycemia, dyslipidemia and insulin resistance; modulate carbohydrate and lipid metabolism; improve adipose tissue metabolism, alleviate oxidative stress and inflammatory processes (Castillo and Hernández, 2015). The postulated mechanisms of activity of tannins involve the induction of phosphatidylinositol 3-kinase (PI3K) to increase glucose uptake, they enhance metal chelating activity as well as modulates the activities of transcriptional factors, such as PPAR-γ and nuclear factor-kappa-β (Kumar *et al*., 2010; Banihani *et al*., 2013). Condensed tannins confer anti-obesity effects through stimulation of long-term lipolysis by increasing PKA and cAMP in 3T3-L1 adipocytes.
(Kumar et al., 2010). Administration of therapeutic dose of condensed tannins such as proanthocyanidin isolated from Cassia mimosoides in HFD-induced obese rats for 8 weeks, resulted in a decrease in body weight gain due to the inhibitory activity of pancreatic lipase (Yamamoto et al., 2000). Tannins have also been shown to prevent the development of long-term diabetes complications, including cardiovascular disease, neuropathy, nephropathy and retinopathy (Derong et al., 2016).

The GC-MS analysis of the DCM leaf extract of G. glauca revealed the presence of lipids such as fatty acids (fats and oils) and steroids (phytosterols such as sterols and stanols). The GC-MS profile of the extract indicated the presence of long-chain polyunsaturated fatty acids (essential fatty acids) that includes omega-3 fatty acids (such as alpha-linolenic acid, docosahexaenoic acid, and eicosapentanoic acid), and an omega-9 fatty acid (oleic acid). The omega-3 fatty acids are derived from linolenic acid while the omega-9 fatty acids from oleic acid (Guiné et al., 2009; Pereira et al., 2011). The alpha-linolenic acid is usually converted into docosahexaenoic acid and eicosapentanoic acid which are more usable forms of essential fatty acids (Guiné et al., 2009; Pereira et al., 2011). Studies have suggested that omega 3 and 9 fatty acids contain free radical-scavenging activity, decreases triglycerides and "bad" cholesterol (LDL) levels, increases serum HDL-cholesterol levels, improves cognition, improves glucose intolerance, improves immune function as well as prevents from cardiovascular diseases (Chan, 2008).

The DCM leaf extract of G. glauca also contained an essential oil called neryl acetate. Essential oils are mixtures of phenylpropane derivatives and terpenes whose chemical and
structural differences are insignificant (Oprean et al., 2001; Castillo and Hernández, 2015). Neryl acetate has been shown to exhibit antidiabetic, antioxidant and anti-obesity activities (Boukhris et al., 2012). Similarly, essential oil isolated from the leaf extract of Meriandra dianthera exhibited anti-diabetic, antioxidant and anti-obesity activities (Sium et al., 2017).

Based on the GC-MS analysis, the main components of phytosterols identified in the DCM leaf extract of G. glauca include stigmasterol and γ-sitosterol. Similar studies on Strobilanthes crispus also indicated the presence of stigmasterol (10.93%) and gamma-sitosterol (6.70%) (Muslim et al., 2010). Sitosterol and stigmasterol are the most abundant plant sterol that structurally resembles cholesterol (Ghosh et al., 2011). These phytosterols form the main structural components of plant cell membranes (Bouic, 2001). Besides, they have been reported to possess antidiabetic, anti-inflammatory and cholesterol-lowering activities (Bouic, 2001). Oral administration of γ-sitosterol in STZ-induced diabetic rats for 21 days caused a significant decrease in blood glucose (Balamurugan et al., 2011). The γ-sitosterol exhibited antihyperlipidemic effect through reduction of serum total cholesterol and triglycerides coupled with an increase in high-density lipoproteins (Balamurugan et al., 2011). Plant sterols also contain anti-tumor effects against different types of cancers including prostate (Bennani et al., 2007), breast (Atif et al., 2003), stomach (de Stefani, 2000a), esophagus (de Stefani, 2000b), ovary (McCann et al., 2003) endometrial (McCann et al., 2000) and lung (Schabath et al., 2005).
The GC-MS analysis of the DCM leaf extract of *G. glauca* also revealed the presence of terpenoids such as squalene, phytol, α-Amyrin, and β-Amyrin. Terpenoids known as isoprenoids are oxygen-rich hydrocarbons that include aldehydes, ketones or alcohols (Gugler *et al*., 2013; Castillo and Hernández, 2015). Squalene is a significant precursor for the synthesis of phytosterols such as stigmasterols, sitosterols, and campesterols (Maguire *et al*., 2004). It is also an important precursor for the synthesis of triterpenoids such as amyrin, betulin, and lupeol (Maguire *et al*., 2004). Studies have demonstrated that squalene is a chemopreventive agent against inflammation, diabetes and oxidative stress (Maguire *et al*., 2004).

The curative potential of the identified diterpenes (phytol and phytol acetate) and triterpenes (α-Amyrin and β-Amyrin) is multidirectional (Jager *et al*. 2009). Terpenes have exhibited important bioactivity in ameliorating hyperlipidemia in obese patients (Cui *et al*., 2013), inflammation (Leite *et al*., 2014; Huang *et al*., 2016) and type II diabetes (Nazaruk and Borzym-Kluczyk, 2015; Li *et al*., 2016). These compounds manifest their anti-diabetic activities by enhancing the regeneration of pancreatic islets cells (Oh, 2015), inhibiting enzymes involved in glucose metabolism (such as α-amylase, α-glucosidase, aldose reductase, glycogen phosphorylase and protein tyrosine phosphatases) (Ansley and Wang, 2013) as well as improving glucose tolerance (Tan *et al*., 2008; Mosa *et al*., 2015). Terpenes inhibit the formation of advanced glycation end products that modulates the pathogenesis of diabetic complications such as diabetic nephropathy, diabetic neuropathy, diabetic embryopathy and impaired wound healing (Liu *et al*., 2014a; Nazaruk and Borzym-Kluczyk, 2015). The dichloromethane leaves extract of *Ficus pseudopalma* and
*Ficus ulmifolia* also indicated the presence of α-Amyrin and β-amyrin, phytol as well as squalene which were attributed to their hypoglycemic effect, triglyceride, and cholesterol-lowering effects as well as antioxidant properties (Ragasa *et al.*, 2009).

The GC-MS analysis of DCM leaf extract of *G. glauca* revealed the presence of vitamins such as vitamin E and cholecalciferol (vitamin D). Similar studies on *Strobilanthes crispus* also indicated the presence of vitamin E (9.75%) (Muslim *et al.*, 2010). Vitamins are organic compounds supplied in small quantities and are essential for metabolic processes. Vitamin D plays a significant role in bone metabolism by enhancing calcium and phosphorus absorption (Bang *et al.*, 2012). Vitamin E has been found to enhance the immune function and prevent hemolytic anemia among premature infants (Fishman *et al.*, 2000). The therapeutic efficacy of vitamins chiefly dwelt on their capacity to scavenge free radicals (Fishman *et al.*, 2000). Their antioxidant activities have been associated with protection from DNA damage, malignant transformations, inflammation as well as increased metabolism of proteins, carbohydrates, and lipids (Rahal *et al.*, 2014). The biological efficacy of the vitamins is also evidenced with their antitumorigenic, antidiabetic, antileukemic, anti-aging, antiulcerogenic and antidermatitic activities (Rahal *et al.*, 2014).
CHAPTER EIGHT

GENERAL SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

8.1 General Summary

The present study focused on determination of phytochemical profiles of the DCM leaf extract of *G. glauca*. It also focused on determination of *in vivo* anti-obesity, cognitive enhancing and neurobehavioral effects of DCM leaf extract of *G. glauca* in HFD-induced obese rats. Further, the study sought to determine the *in vitro* antioxidant activities of the DCM leaf extract of *G. glauca*.

The GC-MS analysis revealed the presence of various secondary metabolites (phytochemicals) at different concentrations in the DCM leaf extract of *G. glauca*. These phytocompounds include Oleic acid, γ-Sitosterol, Curcumin, Quercetin, Pinosylvins, α-Linolenic acid, Vitamins D and E, Phenol, Neryl acetate, Naringenin chalcone, Octadecanoic acid (Stearic Acid), Phytol acetate, D-Allose, Flavonols, Gallocatechin catechin flavan, Catechins, Gallic acid, Ferulic acid, Luteolin, Stigmasterol, Squalene, Phytol, 2,4-bis (1,1-dimethylethyl), Eicosapentanoic acid, Docosahexaenoic acid, α-Amyrin and β-Amyrin. These bioactive phytocompounds are thought to ameliorate obesity through appetite suppression, inhibition of pancreatic lipase activity, stimulation of lipolysis, reduction of lipogenesis, increment of energy expenditure, enhancement of apoptosis of fat cells as well as inhibition of precursor cell proliferation and differentiation. They are also thought to function interactively and synergistically to neutralize ROS and RNS. They contribute to the restoration and maintenance of the redox homeostasis status.
through multiple step processes of antioxidant reactions which involves initiation, propagation, branching and termination of free radicals.

The DCM leaf extract of *G. glauca* exhibited its anti-obesity effects through reduction in body weight, obesity index (OI), abdominal circumference (AC), thoracic circumference (TC), abdominal circumference to thoracic circumference ratio (ACHtR), abdominal circumference to height ratio, thoracic circumference to height ratio (TCHtR), organ weights, organo-somatic index, total fat content, atherogenic index (AI), body adiposity index (BAI), and feed intake. Besides, a decrease in levels of serum glucose, triglycerides, total cholesterol, LDL-C, VLDL and an increase in HDL-C and rectal body temperature are indicative of the *G. glauca* leaf extract anti-obesity properties. The observed anti-obesity effects might be attributed to the presence of various bioactive phytocompounds in the DCM leaf extract of *G. glauca*.

It was also established that the DCM leaf extract of *G. glauca* ameliorates learning and cognitive deficits in HFD-induced obese rats. The improvement in cognition could relate to extracts ability to confer protection against obesity-induced oxidative damage, restoration of redox homeostatic status, reduction of central inflammation, enhancement of the gene expression of hippocampal neurotrophic factor (BDNF) and increment of neurogenesis which facilitates neuronal plasticity in the hippocampus. The learning and cognitive enhancing effects of *G. glauca* leaf extract might have a positive implication in the management of dementia and Alzheimer’s disease.
The study also established that DCM leaf extract of *G. glauca* showed an effect on spontaneous emitted behaviors (such as locomotor activity, exploration and anxiety) which were tested on an open field arena. The DCM leaf extract of *G. glauca* showed anxiolytic effects, increased locomotor activity and exploration-like behaviors in HFD-induced obese rats. The therapeutic effects observed could relate to *G. glauca* leaf extract ability to mitigate inflammation and oxidative stress by down-regulating the activity and release of proinflammatory mediators and restoration of redox homeostatic status through activation of antioxidant defenses.

The different *in vitro* antioxidant assays conducted in this study revealed that DCM leaf extract of *G. glauca* possess potent antioxidant activities which might be attributed to its anti-obesity, cognitive enhancing, anxiolytic, exploration and locomotor activities. The presence of phytochemicals in *G. glauca* leaf extract might be responsible for its antioxidant and free radical-scavenging activities. The synergistic and additive effects of these bioactive compounds increase their bioavailability and action on multiple molecular targets thereby correcting imbalance-mediated oxidative stress.

As the result of the present study indicate, all the observed effects of DCM leaf extract of *G. glauca* are attributed to secondary metabolites in the extract. This study therefore, provides a platform on which to further study the DCM leaf extract of *G. glauca* as a plant-derived source of a drug against obesity, oxidative stress-related disorders and treatment of symptomatic complications of obesity viz; anxiety, memory loss and locomotor activity.
8.2 Conclusions

In conclusions, the present study established that:

i. The DCM leaf extract of *G. glauca* has potent *in vivo* anti-obesity effects in HFD-induced obese rat models.

ii. The DCM leaf extract of *G. glauca* caused improvement in hippocampal dependent spatial learning and memory retention in HFD-induced obese rat models.

iii. The DCM leaf extract of *G. glauca* showed anxiolytic effects, increased spontaneous locomotor activity and exploration-like behaviors in HFD-induced obese rat models.

iv. The DCM leaf extract of *G. glauca* exhibited *in vitro* antioxidant effects.

v. The DCM leaf extract of *G. glauca* contains phytochemical compounds associated with anti-obesity and antioxidants effects. These phytochemicals are also associated with beneficial effects against symptomatic complications of obesity viz; anxiety, memory loss and locomotor activity.

Therefore, the null hypotheses formulated in this study were rejected.

8.3 Recommendations

8.3.1 Recommendations from The Study

i. The extract has phytochemicals that can be harnessed for management of obesity, oxidative stress and symptomatic complications of obesity.

ii. Upon establishment of safety profiles, this plant could be used as a potential alternative anti-obesity agent.
iii. In addition to its use in the management of obesity, *G. glauca* can also be used as an alternative therapeutic agent against symptomatic complications of obesity viz; anxiety, memory loss and locomotor activity.

iv. Upon establishment of safety profiles, this plant could be used as an antioxidant agent due to its antioxidant activities.

### 8.3.2 Recommendations for Further Studies

i. Conduct bioassay-guided fractionation of active compounds in *G. glauca*.

ii. Perform comprehensive toxicity studies to establish the safety profile of *G. glauca*.

iii. Determine the effect of *G. glauca* on molecular markers of obesity.

iv. Use of solvents with different polarities other than DCM and comparison of results among extracts.
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APPENDICES

Appendix 1: The Plant Species Voucher Information

<table>
<thead>
<tr>
<th>Species (Botanical identity)</th>
<th>Collection Location</th>
<th>Collection Date</th>
<th>Voucher number</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Gnidia glauca</em></td>
<td>Mbeere North Sub-County, Embu County, Kenya</td>
<td>July, 2013</td>
<td>WA-V1: Wycliffe Arika-V1</td>
</tr>
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</table>

Appendix 2: Method of Drug Administration (Oral Administration)

<table>
<thead>
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<th>Restrainer</th>
<th>Cannula</th>
<th>Max. administration volume</th>
<th>Frequency</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>No</td>
<td>Irrigation cannula or gavage</td>
<td>10 ml/kg</td>
<td>1 per day</td>
<td>Maximum 5 ml; with viscous substances (such as oils) max. 4 ml.</td>
</tr>
</tbody>
</table>

A rat being orally administered with the DCM leaf extract of *Gnidia glauca*. 
Appendix 3: Method of Blood Sampling

Appendix 3.1 Cardiac Puncture

<table>
<thead>
<tr>
<th>Puncture site</th>
<th>Sampling frequency</th>
<th>Sampling volume</th>
<th>Anesthesia</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Right or left ventricle</td>
<td>Once (only of the sacrificial day)</td>
<td>Suitable for exsanguination</td>
<td>Yes</td>
<td>Results in cardiac arrest</td>
</tr>
</tbody>
</table>

Puncturing of the ventricle can lead to substantial injury (bleeding into the pericardium which consequently results in cardiac arrest). This method was applied on the day of sacrifice.

The two approaches used to draw blood from the heart are shown below:

Appendix 3.2 Caudal Tail Bleeding

<table>
<thead>
<tr>
<th>Puncture of vessel</th>
<th>Sampling frequency</th>
<th>Sampling volume</th>
<th>Anesthesia</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>24 G - 26 G needle</td>
<td>Once per week</td>
<td>Small quantities of blood</td>
<td>No, Restrainer</td>
<td>Stress can lead to vasoconstriction; warm the vessel if necessary, to dilate it. Alternate the injection site cases of repeated sampling.</td>
</tr>
</tbody>
</table>

Blood samples were obtained from the lateral veins without anesthesia. For this, the animals were placed in a restraining device.
Position of Caudal Vessels

The figure illustrates the relevant vessels through which blood was collected from:

a ventral caudal artery  
b/f lateral caudal veins  
d dorsal caudal vein  
e caudal vertebra  
e skin  
g muscle

Appendix 4: Some of The Sampled Adipose Tissues

A Visceral adipose tissue compartments

Mesenteric  Omental  Retroperitoneal
Appendix 5: Normal Biochemical and Hematology Reference Values

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Reference Values</th>
<th>Parameters</th>
<th>Reference Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood volume (ml/100 g)</td>
<td>5.4 - 7.0</td>
<td>MCH (pg)</td>
<td>14.1-19.3</td>
</tr>
<tr>
<td>Blood pressure (mmHg)</td>
<td>84 - 184/58 - 145</td>
<td>MCHC (g/dL)</td>
<td>30.2 – 34.2</td>
</tr>
<tr>
<td>Serum protein (g/dl)</td>
<td>5.6 - 7.6</td>
<td>MCV (fL)</td>
<td>45.4 – 60.3</td>
</tr>
<tr>
<td>Albumin (g/dl)</td>
<td>2.8 - 5.3</td>
<td>WBC (x 103/L)</td>
<td>1.8-10.7</td>
</tr>
<tr>
<td>Globulin (g/dl)</td>
<td>1.8 - 3.0</td>
<td>Neutrophil (%)</td>
<td>6.6-38.9</td>
</tr>
<tr>
<td>Cholesterol (mg/dl)</td>
<td>40 - 130</td>
<td>Lymphocytes (%)</td>
<td>55.8-91.6</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>0 – 1.7 mmo/L</td>
<td>Monocytes (%)</td>
<td>0.0-7.5</td>
</tr>
<tr>
<td>HDL - cholesterol</td>
<td>1.2 – 3.0 mmol/L</td>
<td>Glucose (mg/dl)</td>
<td>50 - 135</td>
</tr>
<tr>
<td>Calculated LDL cholesterol</td>
<td>1.0 – 3.0 mmol/L</td>
<td>Urea-Nitrogen (BUN, mg/dl)</td>
<td>15 - 23</td>
</tr>
<tr>
<td>Calculated Non-HDL - cholesterol</td>
<td>&lt;2.5 mmol/L</td>
<td>Creatinine (mg/dl)</td>
<td>0.2 - 0.8</td>
</tr>
<tr>
<td>LDH (S)</td>
<td>240 -480 U/L</td>
<td>Total bilirubin (mg/dl)</td>
<td>0.2 - 0.6</td>
</tr>
<tr>
<td>Total Protein (S)</td>
<td>60 – 80g/L</td>
<td>Bilirubin (S)</td>
<td>0 – 21 μmol/L</td>
</tr>
<tr>
<td>PCV (%) / Hematocrit (Vol.-%)</td>
<td>35.1-45.4</td>
<td>Alanine Transaminase (ALT)</td>
<td>Adult F 10 - 35 U/L M 10 – 50 U/L</td>
</tr>
<tr>
<td>Hb (g/dL)</td>
<td>11.0-15.1</td>
<td>Alkaline Phosphatase (S)</td>
<td>30 - 130 U/L</td>
</tr>
<tr>
<td>RBC (x 106/μL)</td>
<td>6.36-9.42</td>
<td>Aspartate transaminase (AST)</td>
<td>F 0 – 32 M 0 – 40 IU/L</td>
</tr>
</tbody>
</table>

(Sharp and LaRegina, 1998; Wolfensohn and Lloyd, 2003; Weiss et al., 2014; Gabrisch and Zwart, 2015).
Appendix 6: Anesthesia, Analgesia and Method of Killing the Rats

General anesthesia was induced by injecting a solution of ketamine (80mg/kg body weight) and xylazine (5mg/kg body weight). This solution of drugs was administered intraperitoneally. The duration of anesthesia was 20 minutes. Carprofen, a non-steroidal anti-inflammatory drug was used as an analgesic drug. It was administered subcutaneously at a dosage level of 5 mg/kg body weight per day. The euthanasia method applied was an overdose of isoflurane in a glass vacuum desiccator (Henke and Erhardt, 2001; Henke et al., 2004).
Appendix 7: Antioxidant Assay Results

Appendix 7.1: *In vitro* Ferric Reducing Antioxidant Power (FRAP) of DCM Leaf Extract of *G. glauca*

<table>
<thead>
<tr>
<th>Concentrations</th>
<th>50µg/ml</th>
<th>100µg/ml</th>
<th>150µg/ml</th>
<th>200µg/ml</th>
<th>250µg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>G. glauca</em></td>
<td>0.12±0.01\textsuperscript{e*}</td>
<td>0.20±0.01\textsuperscript{d*}</td>
<td>0.27±0.01\textsuperscript{c*}</td>
<td>0.35±0.02\textsuperscript{b*}</td>
<td>0.42±0.02\textsuperscript{a*}</td>
</tr>
<tr>
<td>Ascorbic acid</td>
<td>0.13±0.01\textsuperscript{e*}</td>
<td>0.20±0.01\textsuperscript{d*}</td>
<td>0.27±0.01\textsuperscript{c*}</td>
<td>0.36±0.01\textsuperscript{b*}</td>
<td>0.43±0.02\textsuperscript{a*}</td>
</tr>
</tbody>
</table>

Means with different letters across the concentrations are statistically significant (*p*≤0.01). Means with asterisks (*) within the same concentration are not significantly different (*p*>0.01).

Appendix 7.2: *In Vitro* DPPH Radical Scavenging Activity of DCM Leaf Extract of *G. glauca*

<table>
<thead>
<tr>
<th>Concentrations</th>
<th>0.5mg/ml</th>
<th>1mg/ml</th>
<th>1.5mg/ml</th>
<th>2.0mg/ml</th>
<th>2.5mg/ml</th>
<th>3.0mg/ml</th>
<th>IC$_{50}$</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>G. glauca</em></td>
<td>0.35±0.01\textsuperscript{a*}</td>
<td>0.29±0.01\textsuperscript{b*}</td>
<td>0.23±0.01\textsuperscript{c*}</td>
<td>0.15±0.01\textsuperscript{d*}</td>
<td>0.08±0.01\textsuperscript{e*}</td>
<td>0.03±0.02\textsuperscript{f*}</td>
<td>1.33±0.02</td>
</tr>
<tr>
<td>Vitamin C</td>
<td>0.35±0.01\textsuperscript{a*}</td>
<td>0.28±0.01\textsuperscript{b*}</td>
<td>0.22±0.01\textsuperscript{c*}</td>
<td>0.13±0.01\textsuperscript{d*}</td>
<td>0.08±0.01\textsuperscript{e*}</td>
<td>0.02±0.01\textsuperscript{f*}</td>
<td>1.39±0.06</td>
</tr>
</tbody>
</table>

Means with different letters across the concentrations are statistically significant (*p*≤0.01). Means with asterisks (*) within the same concentration are not significantly different (*p*>0.01). IC$_{50}$ is the half maximal inhibitory concentration.

Appendix 7.3: *In Vitro* Nitric Oxide Radical Scavenging Activity of DCM Leaf Extract of *G. glauca*

<table>
<thead>
<tr>
<th>Concentrations</th>
<th>250µg/ml</th>
<th>500µg/ml</th>
<th>1000µg/ml</th>
<th>2000µg/ml</th>
<th>2500µg/ml</th>
<th>IC$_{50}$</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>G. glauca</em></td>
<td>0.04±0.01\textsuperscript{a*}</td>
<td>0.03±0.01\textsuperscript{b*}</td>
<td>0.03±0.01\textsuperscript{c*}</td>
<td>0.02±0.01\textsuperscript{d*}</td>
<td>0.01±0.01\textsuperscript{e*}</td>
<td>665.76±334.11</td>
</tr>
<tr>
<td>Quercetin</td>
<td>0.05±0.01\textsuperscript{a*}</td>
<td>0.04±0.01\textsuperscript{b*}</td>
<td>0.03±0.01\textsuperscript{c*}</td>
<td>0.02±0.01\textsuperscript{d*}</td>
<td>0.02±0.01\textsuperscript{e*}</td>
<td>748.00±145.38</td>
</tr>
</tbody>
</table>

Means with different letters across the concentrations are statistically significant (*p*≤0.01). Means with asterisks (*) within the same concentration are not significantly different (*p*>0.01). IC$_{50}$ is the half maximal inhibitory concentration.
### Appendix 7.4: *In Vitro* Superoxide Radical Scavenging Activity of DCM Leaf Extract of *G. glauca*

<table>
<thead>
<tr>
<th>Concentrations</th>
<th>50µg/ml</th>
<th>100µg/ml</th>
<th>150µg/ml</th>
<th>200µg/ml</th>
<th>250µg/ml</th>
<th>IC&lt;sub&gt;50&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>G. glauca</em></td>
<td>0.31±0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.27±0.01&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.23±0.01&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.17±0.01&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.10±0.02&lt;sup&gt;e&lt;/sup&gt;</td>
<td>119.73±0.20</td>
</tr>
<tr>
<td>Quercetin</td>
<td>0.33±0.02&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.27±0.02&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.21±0.01&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.12±0.01&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.08±0.01&lt;sup&gt;e&lt;/sup&gt;</td>
<td>121.16±8.64</td>
</tr>
</tbody>
</table>

Means with different letters across the concentrations are statistically significant (*p*≤0.01). Means with asterisks (*) within the same concentration are not significantly different (*p*>0.01). IC<sub>50</sub> is the half maximal inhibitory concentration.

### Appendix 7.5: *In Vitro* Hydroxyl Radical Scavenging Activity of DCM Leaf Extract of *G. glauca*

<table>
<thead>
<tr>
<th>Concentrations</th>
<th>100µg/ml</th>
<th>200 µg/ml</th>
<th>300 µg/ml</th>
<th>400 µg/ml</th>
<th>500 µg/ml</th>
<th>IC&lt;sub&gt;50&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>G. glauca</em></td>
<td>0.27±0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.21±0.01&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.17±0.01&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.11±0.01&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.04±0.01&lt;sup&gt;e&lt;/sup&gt;</td>
<td>204.34±10.64</td>
</tr>
<tr>
<td>Gallic Acid</td>
<td>0.28±0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.21±0.01&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.18±0.01&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.11±0.01&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.05±0.01&lt;sup&gt;e&lt;/sup&gt;</td>
<td>210.05±8.80</td>
</tr>
</tbody>
</table>

Means with different letters across the concentrations are statistically significant (*p*≤0.01). Means with asterisks (*) within the same concentration are not significantly different (*p*>0.01). IC<sub>50</sub> is the half maximal inhibitory concentration.

### Appendix 7.6: *In Vitro* Lipid Peroxidation Inhibition Activity of DCM Leaf Extract of *G. glauca*

<table>
<thead>
<tr>
<th>Concentrations</th>
<th>50µg/ml</th>
<th>100µg/ml</th>
<th>150µg/ml</th>
<th>200µg/ml</th>
<th>250µg/ml</th>
<th>IC&lt;sub&gt;50&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>G. glauca</em></td>
<td>0.44±0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.36±0.01&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.25±0.01&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.14±0.01&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.07±0.01&lt;sup&gt;e&lt;/sup&gt;</td>
<td>120.56±2.51</td>
</tr>
<tr>
<td>Ascorbic acid</td>
<td>0.46±0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.37±0.01&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.26±0.01&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.16±0.01&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.08±0.01&lt;sup&gt;e&lt;/sup&gt;</td>
<td>128.53±5.99</td>
</tr>
</tbody>
</table>

Means with different letters across the concentrations are statistically significant (*p*≤0.01). Means with asterisks (*) within the same concentration are not significantly different (*p*>0.01). IC<sub>50</sub> is the half maximal inhibitory concentration.
Appendix 7.7: *In Vitro* Hydrogen Peroxide Radical Scavenging Activity of DCM Leaf Extract of *G. glauca*

<table>
<thead>
<tr>
<th>Concentrations</th>
<th>0.1mg/ml</th>
<th>0.2 mg/ml</th>
<th>0.3 mg/ml</th>
<th>0.4 mg/ml</th>
<th>0.5 mg/ml</th>
<th>IC₅₀</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>G. glauca</em></td>
<td>0.39±0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.30±0.01&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.22±0.01&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.16±0.02&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.08±0.01&lt;sup&gt;e&lt;/sup&gt;</td>
<td>0.24±0.01</td>
</tr>
<tr>
<td>Ascorbic Acid</td>
<td>0.40±0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.32±0.01&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.23±0.01&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.16±0.02&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.08±0.01&lt;sup&gt;e&lt;/sup&gt;</td>
<td>0.25±0.01</td>
</tr>
</tbody>
</table>

Means with different letters across the concentrations are statistically significant (p ≤ 0.01). Means with asterisks (*) within the same concentration are not significantly different (p > 0.01). IC₅₀ is the half maximal inhibitory concentration.

Appendix 7.8: *In Vitro* Iron Chelating Activity of DCM Leaf Extract of *G. glauca*

<table>
<thead>
<tr>
<th>Concentrations</th>
<th>50µg/ml</th>
<th>100µg/ml</th>
<th>150µg/ml</th>
<th>200µg/ml</th>
<th>250µg/ml</th>
<th>IC₅₀</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>G. glauca</em></td>
<td>0.35±0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.28±0.01&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.20±0.01&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.14±0.01&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.08±0.01&lt;sup&gt;e&lt;/sup&gt;</td>
<td>114.91±1.72</td>
</tr>
<tr>
<td>EDTA</td>
<td>0.36±0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.29±0.01&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.21±0.01&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.14±0.01&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.07±0.01&lt;sup&gt;e&lt;/sup&gt;</td>
<td>119.22±1.76</td>
</tr>
</tbody>
</table>

Means with different letters across the concentrations are statistically significant (p ≤ 0.01). Means with asterisks (*) within the same concentration are not significantly different (p > 0.01). IC₅₀ is the half maximal inhibitory concentration.
Appendix 8: Summary of the Project

Obesity Induction with HFD (6-weeks)

- Normal Rat
- HFD-induced Obese Rat

No Treatment

Collection of Gnidia glauca

Collection of Gnidia glauca

Extraction with DCM

Treatment with Gnidia glauca (6 Weeks)

GC-MS Analysis

In vitro antioxidant Assays

Effects attributed to Phytochemicals in G. glauca

- ↑Body Weight
- ↑Blood glucose levels
- ↑Feed Intake
- ↑Meta-inflammation of Adipose tissues
- Hyperlipidemia
- Hypercholesterolemia
- ↑ Obesity-induced Oxidative attack
- Cognitive impairment
- ↑Anxiety
- ↓ Locomotor activity
- ↓ Exploratory Behaviors
- ↓ Energy Expenditure

- ↓Body Weight
- ↓Blood glucose levels
- ↓Feed Intake
- ↓Meta-inflammation of Adipose tissues
- ↓Serum Lipid levels
- ↓Cholesterol levels
- ↓ Obesity-induced Oxidative attack
- Improved Cognition
- ↓Anxiety
- ↑Locomotor activity
- ↑Exploratory Behaviors
- ↑ Energy Expenditure
Appendix 9: Publication 1

Research Article

Modulation of Cognition: The Role of Gnidia glauca on Spatial Learning and Memory Retention in High-Fat Diet-Induced Obese Rats

Wycliffe Makori Arika,1 Cromwell Mwiti Kibiti,2 Joan Murugi Njagi,3 and Mathew Piero Ngugi1

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Received 27 April 2019; Revised 22 July 2019; Accepted 13 August 2019; Published 3 September 2019

Guest Editor: Ahammed Ally

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Chronic exposures to high-fat diets are linked to neuropathological changes that culminate in obesity-related cognitive dysfunction and brain alteration. Learning, memory performance, and executive function are the main domains affected by an obesogenic diet. There are limited effective therapies for addressing cognitive deficits. Thus, it is important to identify additional and alternative therapies. In African traditional medicine, Gnidia glauca has putative efficacy in the management of obesity and associated complications. The use of Gnidia glauca is largely based on its long-term traditional use. Its therapeutic application has not been accompanied by sufficient scientific evaluation to validate its use. Therefore, the current study sought to explore the modulatory effects of dichloromethane leaf extracts of Gnidia glauca on cognitive function in the high-fat diet- (HFD-) induced obese rats. Obesity was induced by feeding the rats with prepared HFD and water ad libitum for 6 weeks. The in vivo antiobesity effects were determined by oral administration of G. glauca at dosage levels of 200, 250, and 300 mg/kg body weight in HFD-induced obese rats from the 6th to the 12th weeks. The Lee obesity index was used as a diagnostic criterion of obesity. The Morris water maze was employed to test spatial learning and memory retention in rats. The results indicated that Gnidia glauca showed potent antiobesity effects as indicated in the reduction of body weight and obesity index in extract-treated rats. Moreover, Gnidia glauca exhibited cognitive-enhancing effects in obese rats. The positive influences on cognitive functions might be attributed to the extracts’ phytochemicals that have been suggested to confer protection against obesity-induced oxidative damage, reduction of central inflammation, and increased neurogenesis. The therapeutic effects observed suggest that Gnidia glauca might be an alternative to current medications for the symptomatic complications of obesity, such as learning and memory loss. Further studies are therefore needed to establish its toxicity profiles.
Appendix 10: Publication 2

In Vitro Antioxidant Properties of Dichloromethanolic Leaf Extract of Gnidia glauca (Fresen) as a Promising Antiobesity Drug

Arika Wycliffe, PhD1, Kibiti Cromwell Mwiti, PhD2, Murugi Joan Njagi, PhD1, and Piero Mathew Ngugi, PhD1

Abstract
The acquisition of ethnobotanical information from traditional practitioners remains an empirical aspect of understanding the ethnopharmacology research. However, integration of information on chemical composition of plant extracts and their pharmacological activities forms a key resource for synthesis of new and effective therapeutics. In traditional Afr-can medicine, Gnidia glauca has folkloric remedies against obesity and its associated oxidative stress-mediated complications. However, the upsurge in its use has not been accompanied with scientific validations to support these claims. The present study aimed to determine the antioxidant potential of G glauca as a promising antiobesity agent. The antioxidant effects of the extract were assessed against 1,1-diphenyl-2-picrylhydrazyl, hydroxyl, hydrogen peroxide, nitric oxide, and superoxide radicals as well as lipid peroxidation, iron-chelating effect, and ferric-reducing power. Phytochemical analysis was conducted using gas chromatography linked to mass spectrophotometry. The results revealed that G glauca exhibited scavenging activities against all radicals formed. Besides, the extract showed iron chelation and ferric reducing abilities. The extract indicated a lower half maximal inhibitory concentration value than the standards used. For instance, the extract inhibited 50% of the formation of 2,2-diphenyl-1-picyrylhydrazine at the concentration of 1.33 ± 0.03 mg/mL relative to 1.39 ± 0.06 mg/mL of the standard, vitamin C at 1% confidence limit. Similarly, the extract scavenged 50% of hydroxyl radical at 204.34 ± 10.64 μg/mL relative to 210.05 ± 8.80 μg/mL of gallic acid. The extract also contained various phytochemicals that have been associated with antiobesity effects. The synergistic effects of these phytochemicals increase their bioavailability and action on multiple molecular targets thereby correcting obesity-induced oxidative stress.

Keywords
obesity, oxidative stress, antioxidants, Gnidia glauca, reactive oxygen species, reactive nitrogen species, free radicals, pH

Received August 5, 2019. Accepted for publication September 14, 2019.

The contribution of medicinal plants to the therapeutic arsenal in the fight against diverse ailments is evidence of human ingenuity since time immemorial. The rationale behind the vast usage and greater dependence on herbal medicines as preferred prescription agents rests upon their long-term clinical experience. Medicinal plants provide a major reservoir of effective chemotherapeutics essential for the maintenance of human health. These phytochemicals are idiosyncratic in terms of their mechanism of action, biological properties, and chemical structures. They possess an enormous potential in ameliorating many diseases among which are anemia, diabetes mellitus, obesity, liver and kidney disorders, wounds, and steatosis. These bioactive compounds have been associated with minimal cytotoxicity, are biodegradable, easily available, and affordable to many people especially those in poor resource economies unlike the chemically synthesized drugs.

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Appendix 10: Preprint 1

Effects of DCM leaf Extract of *Gnidia glauca* (Fresen) on Locomotor Activity, Anxiety and Exploration-Like Behaviors in High Fat Diet-Induced Obese Rats

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Appendix 11: Manuscript Accepted by Heliyon Journal (Elsevier) Awaiting Publication

Anti-Obesity Effects of Dichloromethane Leaf Extract of Gnidia glauca in High Fat Diet-Induced Obese Rats

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