ANTIDIABETIC PROPERTIES AND SAFETY OF AQUEOUS EXTRACTS OF *TRITICUM AESTIVUM, HORDEUM VULGARE, URTICA MASSAICA* AND *CYNANCHUM VIMINALE* IN A RAT MODEL

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MAY 2021

DECLARATION

I 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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This thesis is dedicated to my spouse Alice Gathoni and our children Kimani Kennedy Njoroge, Kimani Wilson Nginyangi and Kimani Christine Njoki towards the Global efforts in prevention and management of noncommunicable diseases.

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF PLATES

ACRONYMS AND ABBREVIATIONS

ADA	American Diabetic Association
AMY	Amylase
CK	Creatinine Kinase
EDTA	Ethylene Diamine Tetra-acetic Acid
ESRD	End Stage Renal Disease
HBA1C	Glycated Hemoglobin
IDDM	Insulin Dependent Diabetes Mellitus
IDF	International Diabetic Federation
KNH	Kenyatta National Hospital
LDH	Lactate Dehydrogenase
LFTs	Liver Function Tests
NIDDM	Non Insulin Diabetes Mellitus
OGTT	Oral Glucose Tolerance Test
RFTs	Renal Function Tests
ROS	Reactive Oxygen Species
CAM	Complementary and Alternative Medicine
TXRF	Total X-ray Fluorescence
UK	United Kingdom
WBC	White Blood Cell
WHO	World Health Organization
GDM	Gestational Diabetes Mellitus
STZ	Streptozotocin
IGT	Impared Glucose Tolerance
IFG	Impared Fasting Glycemia
MOPHS	Ministry of Public Health and Sanitation
ROK	Republic of Kenya
NENT	Norwegian National Committee for Research Ethics in Science and Technology

ABSTRACT

The primary goal in the management of diabetes mellitus is to realize normoglycemia as much as possible. In conventional medicine, oral insulin secretagogues and parenteral insulin are used as hypoglycemic agents. However, they have known side effects such as insidious hypoglycemia, lactic acidosis, liver injury and neurological deficit. On the other hand, numerous phytotherapeutics that have been used in traditional, complimentary or alternative medicine for the treatment of diabetes have not been evaluated for their effectiveness and or their toxicity effects. The objective of this study was to evaluate the *in vivo* glycemic and toxicity effects of aqueous extracts of *Triticum aestivum, Hordeum vulgare, Urtica massaica* and *Cynanchum viminale.* Diabetes was induced by intraperitoneally administering 10% alloxan monohydrate into Wistar rats. Rats with fasting blood glucose levels above 200 mg/L 72 hr post alloxan administration were used in this study to evaluate efficacy of doses of aqueous plants extracts against glibenclamide and insulin as reference drugs. Toxicity studies were done by oral or intraperitoneal administration of doses of aqueous plant extracts for 28 days. The rats were euthenized and blood taken for hematological and biochemical studies. Gross and relative organ weights were taken. Presence of minerals, phytonutrients and phytochemicals in the plants extracts was also established. A $p < 0.05$ was consindered statistically significant. The results obtained in this study indicates that both orally and intraperitoneally administered aqueous plants extracts used in this study exhibited varying degrees of blood glucose lowering effects. When orally administered, all the plants extracts did not have significant different effect on cardiac and pancreatic systems, liver and kidneys relative to the control. Significant variations in some gross organ and relative organ weights between the extracts administered and control rats though observed were not supported by haematological or biochemical changes hence not pathologically associated. However, statistically significant effects were recorded when some of the plants extracts were intraperitoneally administered compared to the controls. Flavonoids, alkaloids, saponins, steroids, cardiac glycosides, minerals and vitamins were found to be present in the aqueous plants extracts. In conclusion, the aqueous plants extracts evaluated in this study have demonstrated blood glucose reduction properties and validates their use in control and mitigation of diabetes mellitus. Further, results obtained on phytonutrients and bioactive secondary metaboloites assay indicate the presence of various functional groups. The synergistic activities of the various compounds present in the aqueous plants extracts in this study are attributable to the plant's observed blood glucose lowering effects. When orally taken, none of the logarithmic doses of either of the plants extracts demonstrated any toxicity effects while some of the plants extracts exhibited toxicity when intraperitoneally administered. Thus, based on the findings of this study, continued use of the plants extracts and following proper monitoring and evaluation protocol in management of diabetes mellitus is recommended. However, caution should be observed in intraperitoneal administration of some of the extracts. Further isolation and structural characterization of the active constituents of phytochemicals of the plants and ellucidation of the actual mode of action is recommended. Translational clinical reseach is equally recommended on the background of the data obtained in this study to facilitate advances and the integration of the studied alternative botanical therapies for treating diabetes into modern medicine.

CHAPTER ONE

INTRODUCTION

1.1 Background Information

Diabetes mellitus is a chronic, debilitating and costly disease associated with major complications that pose severe health risks to the individual and the society. It is a clinical syndrome characterized by elevated blood glucose, which could be attributed to lack or relative deficiency of insulin, the hormone regulating the utilization of glucose in the body (MOPHS, 2010; WHO, 2016a). Hyperglycemia causes macrovascular and microvascular alterations that lead to numerous complications in the body such as neuropathy, nephropathy, cardiovascular disease (heart attack and stroke), loss of limbs and vision (Giugliano *et al*., 2008; Piero *et al*., 2014).

Despite great development of therapeutics and other measures for mitigating diabetes, there has been a steady global rise in its prevalence. Data on prevalence estimates indicated 285 million people aged between 20-79 years to have been living with diabetes in 2010 of whom 70% of the diabetic population were living in developing countries. The prevalence was expected to reach 438 million people by the year 2030 (Piero *et al*., 2014; Nada *et al*., 2015). According to the World Health Organization (WHO), 108 million people were living with diabetes in 1980, but the prevalence quadrupled to 422 million in 2014. In the adult population, global prevalence almost doubled to 8.7% from 4.7% in 1980 (WHO, 2016a).

Increase in diabetic cases has been more in the low and middle income countries. According to the Kenya National Diabetes Strategy 2010-2015, the prevalence of diabetes is projected to rise to 4.5% by the year 2025 (MOPHS, 2010). It is estimated

that 5% of the global deaths per year are attributed to diabetes (WHO, 2016a; WHO, 2016b). In 2012, diabetes mellitus directly caused 1.5 million deaths and an additional 2.2 million lost their lives due to higher than normal blood glucose that increases the risks of heart diseases and other diabetes related complications. Deaths due to diabetes mellitus are likely to increase unless urgent action is taken (Demoz *et al*., 2015). Diabetes and its related complications cause huge economic loses in individuals, families and nations which is attributed to loss of work and wages, disability, high health budget and high costs of treatment (Piero *et. al*., 2014; WHO, 2016a).

The major classes of diabetes mellitus are Type I and Type II. Type I diabetes mellitus, also known as Juvenile onset diabetes and insulin-dependent diabetes mellitus (IDDM), occurs due to absence or destruction of beta cells of the islets of langerhans in the pancreas which is attributable to an autoimmune process. Type I diabetes mellitus individuals are prone to ketoacidosis and are dependent on insulin (Piero *et. al*., 2014). Type II diabetes mellitus, the maturity or adult onset diabetes also referred to as non-insulin dependent diabetes mellitus (NIDDM) occurs as a result of reduced insulin secretion or relative insulin deficiency due to resistance to its action. Majority of people living with diabetes mellitus have Type II diabetes (Demoz *et al*., 2015). The development of Type II diabetes mellitus is predisposed by being overweight or obese, consumption of unhealthy diets with high intake of total fats and inadequate fiber, physical inactivity and genetics predisposition (MOPHS, 2010). Other conditions described as diabetes include gestational diabetes mellitus (GDM) a disorder in glucose metabolism that occurs during pregnancy, impaired glucose tolerance (IGT) and impaired fasting blood glucose (IFG). Diabetes may also result

due to pathological conditions such as pancreatic diseases (pancreatitis, cystic fibrosis, and hemachromatosis), endocrine system disorders (acromegaly, cushing's syndrome) and administration of steroids and thiazides diuretics (MOPHS, 2010: WHO, 2016a).

The diagnosis of diabetes mellitus involves a clinical laboratory demonstration of high levels of glucose in the blood and in urine of individuals who present with a history of glycosuria, polyuria, polydipsia, polyphagia and increased loss of weight (Piero *et. al*., 2014). An individual is said to be diabetic if the glycemic levels are above the normal reference level of fasting plasma glucose of above or equal to 7.0 mmol/L (126 mg/dl) or a 2 hour post prandial glucose level equal to or above 11.1 mmol/L (200 mg/dl). If hyperglycemia is established in more than one occasion, a definitive diagnosis of diabetes mellitus Type II is made on ruling out stress hyperglycemia, hyperglycemia due to drug therapy and factitious hyperglycemia. However, the condition may be asymptomatic where glycosuria and hyperglycemia may only be detected in a routine checkup and an oral glucose tolerance test (OGTT) is necessary in inconclusive blood sugar results. The determination of glycated-hemoglobin (HBA_{1c}) has been used to assess glycemic control in people with diabetes as well as glucose tolerance in people without diagnosed diabetes mellitus (Bing *et. al*., 2016).

Upon diagnosis, the primary goal in the management of diabetes mellitus is to realize optimal glycemic control and maintain normoglycemia as much as possible. When diet therapy and body weight reduction fail to lower glycemic levels, oral hypoglycemic agents or insulin injections are used. Antidiabetic or antihyperglycemic

drugs include oral insulin secretagogues such as sulfonylureas, meglitinides, thiazolidinedione and alpha glucosidase inhibitors as well as insulin for parenteral use (Hui *et al*., 2009; Alam *et al*., 2013). Despite their antidiabetic properties, these drugs have serious side effects such as insidious hypoglycemia, lactic acidosis, hepatic failure, low libido and drug loss of activity (Neustadt, 2008). Furthermore, the treatments are not universally accessible due to international or national barriers and cost (Alam *et al*., 2013; WHO, 2016a). Due to the inactivity and side effects experienced with conventional diabetic therapy, the use of complementary and alternative medication has been on the rise. Equally, where conventional medicine is not available, societies have had their traditional antidiabetic therapy which includes use of herbs (WHO, 2016a).

Phytotherapy has been used in traditional medicine for control and management of diabetes mellitus. Other than that, plants have been used alongside conventional medicaments due to their perceived better clinical outcome in therapy and reduction in diabetes related complications. Hyperglycemia exercibates oxidative stress with consequent microvascular and macrovascular alterations that results in diabetes related complications. Other than the antihyperglycemic properties, plants based therapies are also known to have antioxidant activities that scavenge free radicals hence reducing oxidative stress (Bisht S and Sisodia SS, 2010). The young plants of cereals *Triticum aestivum* and *Hordeum vulgare* as well as shrubs *Urtica massaica and Cynanchum viminale* are used both in developed and developing nations for control and treatment of diabetes mellitus. Despite widespread use, empirical data is not available and their safety has not been evaluated (Yogesha, 2013; Khalid, 2014). It is against this background that this study has been designed.

1.2 Statement of the problem and justification of the study

Inspite of great strides in development of both oral and parenteral antihyperglycemic agents, diabetes mellitus continues to pose a major risk to human health and economic development. Conventional diabetes therapy has been compromised due to loss of therapeutic effects and numerous side effects associated with the drugs. As a result, search of new antidiabetic drugs which are more active and considered safer has been enhanced. Plants, being a source of numerous drugs in conventional medicine, are equally a target in development of antidiabetic treatments. As people become more proactive towards their health, they are seeking other forms of antidiabetic treatments either as alternatives or complimentary to current therapy. This is in addition to traditional utilization of numerous plants as therapies for management and control of diabetes mellitus and hence the great increase in the use of crude plants as antidiabetic treatments in the twentieth century. However, there is limited scientific data on the antidiabetic effects and safety concerns of the plants. In the absence of empirical data on the efficacy and safety of phytotherapy, continued use of the non-validated plant preparations may exacerbate the diabetic epidemic, related complications and increase in mortality. Likewise, full benefits of the use of botanicals may not be realized and individual or institutional resistance to their use may arise even in situations where they could be beneficial.

Randomized controlled studies have been used to demonstrate *in vivo* antihyperglycaemic activities as well as safety of the plants. The use of experimental animals is a primary requirement in development of drugs and conducting this study preliminary information key to development of additional antidiabetic therapies, which are equally considered to be safer than synthetic medicines. Data generated in

this study will be used to push the case for prioritized translational research in botanicals with potential therapeutic properties. The choice of the plants in this study was informed by their increased use both in developing and developed countries in mitigation of diabetes mellitus and its complications.

Therefore, it's justified that *Triticum aestivum, Hordeum vulgare, Urtica massaica* and *Cynanchum viminale* need to be scientifically evaluated for their efficacy and safety.

1.3 Research questions

i) What are the effects on blood glucose by logarithmic doses of aqueous extracts of *Triticum aestivum, Hordeum vulgare, Urtica massaica* and *Cynanchum viminale* in alloxan induced diabetic rat?

ii) What is the *in vivo* safety of logarithmic doses of aqueous extracts of *Triticum aestivum, Hordeum vulgare, Urtica massaica* and *Cynanchum viminale* in experimental rat?

iii) Does *Triticum aestivum, Hordeum vulgare, Urtica massaica* and *Cynanchum viminale* have any phytochemical and micronutrient components that could be associated with blood glucose lowering properties?

1.4 Objectives

1.4.1 Broad objective

To evaluate the *in vivo* effects on blood glucose and safety of aqueous extracts of *Triticum aestivum, Hordeum vulgare, Urtica massaica* and *Cynanchum viminale* in white Winstar rats.

1.4.2 Specific objectives

i) To determine *in vivo* hypoglycemic effects of logarithmic doses of aqueous extracts of *Triticum aestivum, Hordeum vulgare, Urtica massaica* and *Cynanchum viminale* in diabetic rats.

ii) To determine *in vivo* toxicity of aqueous extracts of *Triticum aestivum, Hordeum vulgare, Urtica massaica* and *Cynanchum viminale* in normal rats.

iii) To determine phytochemical and micronutrient composition of *Triticum aestivum, Hordeum vulgare, Urtica massaica* and *Cynanchum viminale* aqueous plants extracts.

1.5 Significance of the study and anticipated output

This study endeavors to evaluate the antidiabetic effects and safety of *Triticum aestivum, Hordeum vulgare, Urtica massaica* and *Cynanchum viminale* commonly used by various communities in Kenya for management and control of diabetes mellitus. Efficacy studies will generate data to elucidate antihyperglycemic activity of the studied plants. The data collected will be used as a guide to the use and safety of the complementary or alternative therapies for management and control of diabetes mellitus. This study will add additional plants to the inventory of plant species used as traditional therapies for diabetes mellitus in Kenyan. The generated data will equally be utilized to push the case for prioritized translational research in plant species with proven therapeutic benefits in diabetes therapy. The data will also be useful while teaching alternative and complementary medicine in medical schools and commercialization of developed products.

CHAPTER TWO

LITERATURE REVIEW

2.1 Diabetes Mellitus

Diabetes mellitus is a metabolic disorder characterized by chronic hyperglycemia with derangements of carbohydrate, fat and protein metabolism resulting from impaired insulin secretion, insulin action, or both. The body fails to regulate the utilization of glucose in the body leading to hyperglycemia and glycosuria (Piero *et al*., 2015). Current estimates on global burden of diabetes mellitus indicates that 9.3% (483 million) people aged between 20-79 years are living with diabetes. Baesd on the 2019 estimates, It's projected that the prevalence of diabetes mellitus will increase to 578 and 700 million people by the year 2030 and 2045 respectively. In Kenya, out of 25, 588 000 people aged 20-79 years, 522, 000 (2%) are diabetic. Diabetes and its complications related global mortality was 4.2 million people in the year 2019. Economically, global health expenditure on diabetes is expected to rise to USD 825 billion by 2030 and USD 845 billion by 2045 from current expenditure of USD 760 billion (IDF, 2019; Tran *et al*, 2020).

2.1.1 Metabolism and utilization of carbohydrates

Carbohydrates are consumed by man as the principal source of energy. Carbohydrate metabolic endproducts are also utilized as constituents of mucopolysaccharides, lipids and conjugated proteins, promoters or catalysts, therapeutic substances such as antibiotics and cardiac glycosides, synthesis of fatty acids, cholesterol and amino acids or conjugation of toxins, drugs, hormones and bilirubin (Chatterjea and Shinde, 2012a). Upon absorption, glucose is transported to the liver which withdraws it from the hepatic portal vein and releases it to the systemic circulation. Withdrawal involves

the conversion of hexoses such as galactose and fructose to glucose, glycogenesis (conversion of glucose to glycogen), glycolysis for energy production and synthesis of fatty acids and amino acids. Release to the blood involves formation of glucose from hexoses and release to the blood circulation, glycogenolysis (conversion of glycogen to glucose) and formation of glucose from amino acids, pyruvates, lactates, glycerol and propionyl CoA (gluconeogenesis). Circulating glucose is also removed by storage in the liver and skeletal muscles as glycogen (glycogenesis) and triacyl glycerol in fat depots (lipogenesis), conversion to other carbohydrates and non-essential amino acids. The tissue uptake and regulation of glucose metabolism is regulated by level of glucose, endproducts and hormones. Insulin enhances the uptake of glucose in skeletal muscles, adipose tissue, mammary glands, cardiac muscles etc but is not required for the liver's glucose uptake (Voet and Voet, 2011; Chatterjea and Shinde, 2012a)

2.1.2 Oxidation of glucose

The main oxidation of glucose occurs via the Embden-meyerhof glycolytic pathway where glucose is degraded to pyruvate which in turn is oxidized to carbon dioxide and water in the presence of oxygen (Glycolysis) for the generation of energy. Glycolysis is stimulated and regulated by insulin and glucagon hormones. Other lesser pathways of oxidation of glucose are the hexose monophosphate (HMP) shunt pathway which provides nicotinamide adenine diphosphate (NADPH) for reductive synthesis and pentoses for nucleic acid synthesis and the Uronic acid pathway which provides D-glucuronic acid which is utilized for mucopolysaccharides synthesis and conjugation of bilirubin, hormones and drugs (Chatterjea and Shinde, 2012a).

2.2 Insulin Hormone Biosynthesis and Biosignaling

Insulin is a hormone produced by the beta cells of islets of Langerhans of the pancrease. The precursor of insulin processed in the Golgi apparatus is proinsulin which is produced through a DNA or RNA directed synthesis. Proinsulin is a long strand single chain protein molecule which on hydrolysis yields the two chain 51 amino acids insulin and C peptide having 31 amino acids. The first chain of insulin is made up of 30 amino acids while the second chain has 21. The two chains of insulin are joined together by disulphide bonds and has a molecular weight of 5808 Daltons. Insulin is stored in the beta cells granules as crystals of zinc and insulin. Insulin is secreted by the pancrease upon stimulation by sugars (glucose and mannose), amino acids (leucine and arginine) and activation by the vagas nerve. An increase in intracellular calcium triggers the release of insulin (Kleine and Rossmanith, 2016; Vecchio *et al.*, 2018). Insulin has dual action of being excitatory by stimulation of glucose uptake and lipid synthesis as well as being inhibitory by suppression of glycogenolysis, gluconeogenesis, lipolysis, ketogenesis and proteolysis (Qaid and Abdelrahman, 2016)

2.3 Classification of Diabetes

Depending on the aetiology, diabetes can be classified as primary or secondary. The majority of diabetic cases are primary and clinically categorized as Type I and Type II diabetes. Secondary diabetes are of low incidence and may results as a consequence of an identifiable pathological process in diseases such as pancreatitis, haemachromatosis and carcinoma which cause destruction of the pancreas, abnormal concentration of hormones antagonistic to insulin, gestational diabetes or liver disease (Voet and Voet, 2011; IDF, 2019).

2.3.1 Type I diabetes

Type I diabetes encompasses cases due to absence or destruction of beta cells of the pancreas, attributable to an autoimmune process, prone to ketoacidosis and dependent on insulin. Based on treatment, type I diabetes is also known as insulin-dependent diabetes mellitus [IDDM]). It has a genetic predisposition and occurs early in life thus also referred to as juvenile diabetes. Demonstration of lymphocytic and plasma cells infiltration and detection of auto-antibodies to pancreatic islet cells with concomitant destruction of the beta cells has been a strong indication that autoimmunity to the pancreatic cells is a major cause of type I diabetes. Autoimmune response to viral (e.g Coxsackivirus B1 and B4) infections has been associated with the occurrence of type I diabetes. Insulin injections are necessary to maintain glucose homeostasis (Chatterjea and Shinde, 2012a; IDF, 2019).

2.3.2 Type II diabetes

Type II diabetes also referred to as maturity onset diabetes is non-insulin dependent (NIDDM) and occurs as a result of reduced insulin secretion or relative insulin deficiency due to resistance to its action, has an onset in adulthood and occurs in the majority of diabetic patients in the world. A reduction in the number of insulin receptors and the occurrence of antibodies to the receptor sites has directly been associated with diabetes Type II. Hyperglycemia in Type II diabetes is related to hyperinsulinemia and Type II patient may respond to high level doses of insulin with a slight reduction in blood glucose levels. The disease is precipitated by obesity, diet, over indulgence and low physical activity. The disease is hereditary in nature and has familial tendencies (WHO, 2016; IDF, 2019).

2.3.3 Gestational diabetes

Hyperglycemia in pregnancy may occur when the body is not able to make and use all the insulin it needs for pregnancy. Gestational diabetes should not be confused with diabetes in pregnancy. A distinction of the two is established based on fasting and 2 hour post glucose load blood glucose. If a fasting blood glucose of 5.1-6.9 mmol/l (92 -125 mg/dl) and an OGTT 2 hour glucose level of 8.5-11.0 mmol/l (153 -199 mg/dl) is obtained, a diagnosis of gestational diabetes is made. In comparison, diabetes in pregnancy is diagnosed on getting a fasting plasma glucose above or equal to 7.0 mmol/l (126 mg/ dl), a 2 hour post glucose load blood glucose of 11.1 mmol/l (200 mg/dl) or above and random blood sugar equal to or above 11.1 mmol/l (200 mg/ dl) and manifestations of diabetes (WHO, 2016, IDF, 2019).

2.3.4 Intermediate hyperglycemia

Impaired glucose tolerance (IGT) and impaired fasting glycaemia (IFG) are conditions where the 2 hour post glucose load and fasting blood glucose is above the expected normal values < 7.8 mmo/l and ≤ 6.1 mmol/l respectively but not high enough to be categorized as diabetic. The conditions presents a risk of developing diabetes mellitus especially Type II (WHO, 2016).

2.3.5 Pituitary diabetes

Alterations in glucose metabolism resulting to IGT and IFG are a consequence in many individuals with chronic pituitary hypersecretion of growth hormone (GH) and concomitant increased levels of circulating insulin-like growth factor 1 (IGF-1). On the other hand, 16 to 56% of patients with acromegaly has been diagnosed with diabetes mellitus. Hypersecretion of adrenocorticotrophic hormone (ACTH) in

Cushing's disease results into secondary diabetes (Barbot *et al*, 2018; Ferrau *et al*., 2018). With multiple hormones secreted by the pituitary gland exerting regulatory functions in other endocrine organs (adrenal cortex, thyroid and gonads), hypophyseal health is key in glucose homeostasis in health and disease. There is a great relationship between the pituitary gland and diabetes mellitus, and the oral glucose tolerance test (OGTT) is imperative in individuals with acromegaly and Cushings's disease (Barbot *et al.*, 2018; Ferrau *et al*., 2018; Bhattacharya *et al*., 2020).

2.4 Diagnosis of Diabetes

The diagnosis of diabetes lies in a clinical laboratory demonstration of high levels of glucose in the blood and in urine of individuals who present with a history of glycosuria, polyuria, polydipsia, polyphagia and increased loss of weight. An individual is said to be diabetic if blood glucose is above the normal reference level. Fasting plasma glucose of above or equal to 7.0 mmol/L (126 mg/dl) or a 2 hour post prandial glucose level equal to or above 11.1 mmol/L (200 mg/dl) is an indication of diabetes. In a definitive diagnosis, hyperglycemia due to stress, drug therapy and factitious hyperglycemia must be ruled out. However, the condition may be asymptomatic where glycosuria and hyperglycemia may only be detected in a routine checkup. An oral glucose tolerance test (OGTT) is necessary in inconclusive blood sugar results. The determination of glycated-hemoglobin (HBA1c) has been used to assess glycemic control in people with diabetes as well as glucose tolerance in people without diagnosed diabetes (WHO, 2016; IDF, 2019). Post prandial blood glucose is elevated depending on the level of exogenous glucose in the circulation, glucose content of a meal and rate of absorption and should be twice the post absorptive glucose production.

2.5 Complications of Diabetes Mellitus

Complications of diabetes mellitus occur as a manifestation of vascular disease. Hyperglycemia exacerbates the risk of microvascular (abnormality of small blood vessels) which causes retinopathy, nephropathy and neuropathy. Damage of the large blood vessels leads to macrovascular complications which include ischemic heart disease, stroke and peripheral vascular disease. There is diminished quality of life, reduced life expectancy and significant morbidity (WHO, 2016). Oxidative stress is high in diabetic patients due to the enhanced generation of reactive oxygen species (ROS) that are involved in the development and progression of complications attributed to diabetes (Shradha, 2010). Mortality, mostly due to coronary heart disease (CHD) is two to four times higher in a diabetic population than in the non-diabetic population while people with diabetes have an increased risk of stroke (WHO, 2016, IDF 2019).

Diabetes is the leading cause of end stage renal disease (ESRD) both in developed and developing countries. Amputations of the lower limbs is ten times more in people with diabetes than in non-diabetic individuals in developed countries, and more than half of all non-traumatic lower limb amputations are due to diabetes. In developed countries, diabetes is one of the leading causes of visual impairment and blindness. People with diabetes require two to three times the health care resources of people who do not have diabetes, and diabetes care accounts for up to 15% of national healthcare budgets. The American Diabetes Association (ADA) estimated the national costs of diabetes in the USA for 2002 to be $US 132 billion, increasing to $US 192 billion in 2020 (WHO, 2016, IDF 2019).

2.6 Management and Control of Diabetes

2.6.1 Conventional diabetes therapy

2.6.1.1 Oral antidiabetic or antihyperglycemic drugs

Oral antidiabetic drugs used in conventional medicine are grouped according to their target or mode of action. Mechanisms of hypoglycemic drugs are:-

i. Stimulation of pancreatic insulin secretion (insulinotropics). The drugs include Insulin secretagogues and sulfonylureas. The common drugs are glibenclamide, glipizide and rapaglininde (such as tobultamide, tolazamide, chloropropamide, etc.).
ii. Improve the action of insulin. In this class are the thiazolidinediones (e.g. rosiglitazone, pioglitazone, troglitazone).
iii. Reduce hepatic glucose production. This are biguanides and meglitinides (repaglinide, metformin, pheformin and buformin).
iv. Delay digestion and absorption of intestinal carbohydrates through inhibition of alpha glucosidase. Examples are acarbose and miglitol.

2.6.1.2 Parenteral antidiabetic therapy

The other line of antidiabetic drugs is insulin for intramuscular or intravenous use. Four types of insulin formulations are available based on their activities as ultra- short acting, short acting, intermediate acting and Long acting. The commercial insulin preparations differ in the animal species, purity, concentration and solubility, time of onset and duration of biological action. Type I diabetics solely depend on insulin injections while type II diabetics may have a combination of both oral and injectable antidiabetic therapy. Despite their antidiabetic properties, the drugs have serious side

effects such as insidious hypoglycemia, lactic acidosis, hepatic failure, low libido and drug loss of activity (Neustadt, 2008).

2.6.2 Alternative and complementary diabetes therapies

The use of complementary and alternative medicine (CAM) is widespread and has increased dramatically in the 20th century. In developing countries, about 80% of the population use CAM for their primary health care needs. In the developed Nations, up to 65% use CAM and an estimated 15 million adults take herbal remedies or high-dose vitamins along with prescription drugs. World Health Assembly passed a resolution on traditional medicine in 2003 urging member state of the WHO to provide reliable information on CAM to consumer and to promote proper use. WHO provided guidelines on developing consumer information on proper use of complementary and alternative medicine as well as the traditional medicine strategy 2002-2005 (WHO, 2013).

An increase to use of CAM has been occasioned by the increasing cases of chronic diseases. This has been driven by high cost, ineffectiveness and unavailability of conventional therapies. CAM have been said to cause lesser adverse events than conventional medicine. People are also taking a more proactive approach to their own health and are seeking different forms of self-care. In addition, CAMs are generally available, have a wide diversity and flexible. They are affordable and low level of technology is required hence their wide spread use in low and middle income countries.

The body of evidence on the therapeutic potential of botanicals is quite huge. Numerous chemical substances have been derived from plants and are used worldwide as remedies for both acute and chronic illness and are safer and less toxic than synthetic drugs. The use of botanicals for therapeutic purposes is not only in CAM, but conventional medicine also utilizes drugs derived from plants. For instance, the latest antimalarial drug artemisinin, has been developed from a widely used herbal plant *Artemisia annua* (Joy, 2013).

Use of botanicals and nutritional supplements in their natural and unprocessed forms has been practiced as alternative therapy by man (Mariangela, 2020). They are used for both preventive and therapeutic purposes. Herbal (plant based) therapies are the most used in CAM health practices and some of them have been proven to be beneficial to human health (Neelash *et al*., 2010; Fantini, 2010; Yogesha, 2013; Khalid, 2014; Sheikh, 2016). Herbal therapies utilizes herbal materials that include herb (crude plant material such as leaves, flowers, fruits, seed, stem, bark, roots, rhizomes or other part of the plant used whole, fragmented or powdered), fresh juices collected in forests and administered by traditional herbalists while others are common foodstuff known as functional foods or food supplements. The consumption of a food in a different manner than what is usual is akin to taking a herbal material. For instance taking juices of carrots, cabbages and other green vegetables rather than cooking them. CAM are found for almost all health challenges afflicting man. Examples of various CAM are shown in Table 2.1(Joy Kurian and John Perumal, 2013). The overall biological effects of plants based therapies may vary depending on the geographical region, climate and different parts of the plant being used (Pradeep *et al*., 2014).

Table 2.1: Commonly used herbal therapies in CAM

Clinical indications	**Plants used**	
	Common Names	**Scientific Names**
Cancer	Madagascar periwinkle	*Catharanthus roseus*
	African stink wood	*Prunus Africanus*
	Wheatgrass	*Triticum aestivum*
	Asparagus spears	*Asparagus officinalis*
Prostate cancer	Pumpkin.	*Cucurbita maxima*
	Watermelon	*Citrullus vulgaris*
	Saw palmetto	*Serenoa repens*
Diabetes	Onion	*Allium sepa.*
	Java plum	*Syzygium cuminii.*
	Wheatgrass	*Triticum aestivum*
Arthritis	Drumstick	*Moringa oliefera*
	Stinging nettle	*Urtica massaica*
	Cilantus (Ndania)	*Coriandrum sativum*
	African greenheart	*Warbugia ugandensis*
Artherosclerosis	Sunflower	*Helianthus anuus*
	Chilli	*Capsicum frutenscens*
	Garlic	*Allium saitvum*
	Garden spinach	*Spinacea oleracea*

Source; Joy Kurian and John Perumal, 2013.

2.7 Plants Used in the Study

The plants used in this study are consumed in Kenya as anti-diabetes therapy or complimentary to conventional antidiabetic therapy.

2.7.1 *Triticum aestivum* (wheatgrass)

Triticum aestivum is the scientific name of the common wheat, a major nutritional source since the history of man. It belongs to the Kingdom plantae, family *Poaceae (Gramineae)* and genus *Triticum L*. Numerous health benefits have been attributed to the young plant of wheat (Singh *et al*., 2012). Therapeutic potential of some wheat based products has been documented (Mancinelli *et. al*., 2009; Swati *et al*., 2010).

In vitro and *in vivo* studies has demonstrated that wheatgrass has anticancer properties (Khan *et al*., 2015; Bhulabhai, 2016). It has been demonstrated that wheatgrass can be

used as a monotherapy for treatment of distal ulcerative colitis, reduce the need of blood transfusion in thalassemia (Swati *et al*., 2010), has antidiabetic and antioxidant properties (Yogesha, 2013), has antimicrobial activity (Sundaresan, 2015) and has been reported to have potent growth inhibitory activity on human breast and cervical cancer (Hussai *et al*., 2014). Phytochemical and nutritional analysis on wheatgrass have indicated its potential of being a valuable source of the bioactive compounds (Chauhan, 2014; Polshettiwar, 2016). Seven day old wheatgrass was found to have better activity than fourteen or twenty one day's old wheatgrass (Sundaresan, 2015). No study has been carried out to determine antidiabetic activity and safety of the aqueous extract of *Triticum aestivum* (wheatgrass).

Plate 2.1: *Triticum aestivum* (Photo taken on 26th November 2014 in Ondiri village, Kikuyu division, Kiambu County)

2.7.2 *Hordeum vulgare* (barleygrass)

Hordeum vulgare just like *Triticum aestivum* is a cereal producing plant and commonly referred to as barley. It belongs to the Kingdom plantae, family Poaceae (Gramineae) and genus Hordeum (Lahouar *et al*., 2015). Barley has been considered to be weed competitive with allelopathic proprieties against pathogens and pests. The

presence of various secondary metabolites such as phenolic compounds, cyanoglucosides, alkaloids and polyamines contributes to the allelopathic activity of *Hordeum vulgare*. Several species of *Hordeum vulgare* studied have demonstrated differences in their phytochemical contents and allelopathic properties (Bouhaouel *et al*., 2019). The species differences identified in barley has eqully been associated with the NAC (NAM, ATAF, CUC) genes regulatory roles of transcription factor such as plant development and response to hormone (Christiansen *et al*., 2011). Barley bran has been demonstrated to have hepatoprotective and hypocholesterolemic properties (Abulnaja and El Rabey, 2015). Barley grass is the young grass of the barley plant whose consumtion has been attributed to its therapeutic properties. The health benefits of barley grass emanates from its rich contents of functional ingredients such as minerals, vitamins, diertary fiber, fat, enzymes and polyphenols. It exerts therapeutic effects as anticancer, anti-inflammatory, antioxidant, antidiabetes, hypolipidemic, and antidepressant among others. It's used as a powder or low temperature squeezed juice (Sinha *et al*., 2012; Sheikh, 2016; Woo *et al*., 2017; Zeng *et al*., 2018; Benicasa *et al*., 2019).

Plate 2.2: *Hordeum vulgare* (Photo taken on 26th November 2014 in Ondiri village, Kikuyu division, Kiambu County)

2.7.3 *Urtica massaica*

Urtica massaica is a species of flowering rhizomatous perennial herb plant growing up to 2 meters tall. It belongs to the kingdom plantae, family urticaceae (nettle) and genus *Urtica*. Though still in consideration, there are over thirty species of the genus *Urtica* spread all over the wild. Sharing the common name "stinging nettle" *Urtica massaica* also refered to as forest nettle is largely distributed in Africa and mostly in East Africa. The plant has heart-shaped leaves covered in stinging hairs and serrated edges. Its normal habitation include forests near mountains, and around cattle in human surroundings (Hussain, 2019). As other species of the genus *Urtica*, the medicinal properties of *Urtica massaica* has attracted a lot of attention in the recent past (Said *et al*., 2015; Hussain, 2019). This plant is used for food and medicine in several African nations. In Kenya, it has been used for numerous purposes such as a tonic, treatment of stomach ache, diabetes, fractures, skin and venereal diseases among others (Alphonse *et al*., 2008; Joseph *et al*., 2015; Yvonne *et al*., 2018). The methanolic extract of the plant has been demonstrated to have antimicrobial activity agaist *Escherichia coli* (Allan *et al*., 2019).

Plate 2.3: *Urtica massaica* (Photo taken on 10th December 2014 in Arkroad village, Aboni sublocation, Nyeri County)

2.7.4 *Cynanchum viminale*

Cynanchum viminale also referred to as *Sarcostemma viminale* or *Sarcostema acidum* is a leafless succulent plant with cylindrical stems. It belongs to the Kingdom Plantae, Family Apocynaceae and Genus cynanchum (sarcostemma). The taxonomic dissolution of *Sarcostemma* into *Cynanchum* was effected due to results of recent molecular studies (Meve and Liende-Schumann, 2012).

The plant grows throughout much of Africa, the Middle East and the Far East as a shrub or a vine. The stems produce copious amounts of milky exudates when broken. This exudate is caustic in nature and can cause burns if it contacts human skin. The environmental range of the species is very broad, extending from rainforest margins, monsoon forests to arid deserts and dry rocky areas. The plant exhibits various therapeutic properties including anticancer, tocolytic and hepatoprotective abilities. It has traditionally been used to treat a range of conditions such as sores and wounds, smallpox, eye infections, diarrhea, intestinal and skin parasites and insufficient milk production. The plant should be handled with care as the sap may be poisonous and caustic in nature although the toxicity varies from place to place (Bagyanathan and Thoppil, 2015; Safari *et al*., 2016). In Kenya the plant grows in arid areas such as Makueni County where it’s commonly known as Mungendya thenge and used for the treatment of hypertension and diabetes.

Plate 2.4: *Cynanchum viminale* (Photo taken on 15th October 2014 in Linga village, Kisau division, Makueni County)

As we strive to harness and embrace knowledge in CAM, efficacy and safety of botanic therapeutic products should be established. Hence the objective of determining *in vivo* hypoglycemic activity and safety of *Triticum aestivum, Hordeum vulgare, Urtica massaica* and *Cynanchum viminale*.

2.8 Experimental Models of Diabetes

Drug bioscreening is an important and inescapable aspect in drug developments for human use where living organisms mostly animals are used in identification of useful drugs as comprehensively and rapidly possible with minimal expenses. Bioscreening includes determination of efficacy and toxicity effects of a product. The use of laboratory animals in bioscreening is based on the fact that drugs may act in similar ways in animals and human beings hence making animals suitable for use as model analogs for man. Human diseases are emulated or duplicated in animals during the initial evaluation of activity and development of potential therapeutic substances from

natural products and depends largely on the animal models used. The findings of an *in vivo* study using animal models are used in extrapolation for human diseases (Clarke, 2009; Kehinde, 2013; Wojcikowski, 2014; Andrade *et. al*., 2016).

2.8.1 Chemical induction diabetes model

Various chemicals have been used to induce diabetes in laboratory animals. They include alloxan monohydrate, streptozotocin, ditizona, antiinsulin serum and ferric nitrilotriacetate. Parenterally (intravenously, intraperitoneally or subcutaneously) administered Streptozotocin (STZ) and alloxan monohydrate are the most frequently used and their diabetogenic effects depends on the dose administered, the animal species, route of administration and nutritional status (Etuk *et al*., 2010; Kumar *et al*., 2012: Mostafavinia *et al*., 2016).

2.8.1.1 Streptozotocin

Streptozotocin (2-Deoxy-2[(methylnitrosoamino)-carbonyl] amino]- D-glucopyranose), a synthetic nitrosoureido glucopyranose derivative is a fermentation product of *Streptomyces achromogenes.* Streptozotocin is used in cancer chemotherapy and is also referred to as streptozocin, zanosar or izostazin. It's used for the induction of diabetes models in laboratory animals. In mice, a dose of 150 milligram per kilogram body weight or in rats at 80 milligram per kilogram body weight in citrate buffer (pH 4.5 0.1M) administered intraperitoneally is used. Streptozotocin acts by preventing the binding of the substrate to DNA or inhibiting enzymatic systems in synthesis of DNA resulting to cell death (Etuk *et al*., 2010; Mostafavinia *et al*., 2016).

Streptozotocin can also be used to determine the mechanism of action of the substance under study due to its delayed diabetogenic action. Animals with a blood glucose level between 180-500 mg/dL are considered diabetic after four or seven days for mice and rats respectively post STZ administration. Streptozotocin is limited to its use for chronic experiments due to its oncogenic action which leads to formation of kidney and liver tumours or an insulinoma which contribute to hypoglycemic activity (Etuk *et al*., 2010; Mostafavinia *et al*., 2016).

2.8.1.2 Alloxan monohydrate

Alloxan (2, 4, 5, 6 tetraoxypyrimidine; 5-6-dioxyuracil) is a urea derivative used to induce diabetes in laboratory animals. Alloxan diabetogenic action is due to selective necrosis of the pancreatic islet β-cells which is similar to type I diabetes but it's equally used to induce Type II diabetes in animals. The severity of diabetes in the animal depends with the laboratory animal used, response of the animal, dose administered and route of administration. Intravenous injection requires a lower dose than intraperitoneal and subcutaneous administration and determination of the optimal dose of alloxan for use is paramount for each batch of alloxan (Etuk *et al*., 2010; Mostafavinia *et al*., 2016).

Animals with a fasting blood glucose post alloxanization ranging from 180 to 250 milligram per deciliter are classified as having moderate diabetes while those with above 250 milligram per deciliter are said to have severe diabetes. Animals with moderate diabetes are the most suitable for use as those with severe diabetes are similar to diabetic animals due to pancreatectomy and compounds that act by stimulating production of insulin from β cells such as sulphonylureas (e.g.

tolbutamide) will have negligible hypoglycemic effect. Plant extracts that produce hypoglycemic effects in severe alloxan induced diabetes will be doing so in a different mode of action other than insulinotropics activity. Animals are fasted for up to eighteen hours prior to alloxan administration and allowed access to food and water ad libitum post alloxanization (Etuk *et al*., 2010; Mostafavinia *et al*., 2016).

Alloxan and its reduction product dialuric acid establish a redox cycle with the formation of superoxide radicals. These radicals undergo dismutation to hydrogen peroxide. Thereafter, highly reactive hydroxyl radicals are formed by fenton reaction. The action of reactive oxygen species with a simultaneous massive increase in cytosolic calcium concentration causes rapid destruction of *beta* cells (Szkudelski, 2001). One of the targets of the reactive oxygen species is DNA of pancreatic islets. Its fragmentation takes place in *beta* cells exposed to alloxan. Alloxan administration in experimental animals has been reported to produce pancreatic lesion which is proportional to the dose of the drug administered and the size of the lesion also correlates with the pancreatic insulin content. This perhaps explains why the drug at a low or medium dose does not produce absolute but insufficient insulin deficiency in experimental animals. Therefore the experimental dose of the drug must be carefully selected in order to avoid excessive pancreatic tissue damage (Etuk *et al*., 2010; Mostafavinia *et al*., 2016).

2.8.1.3 Ferric nitrilotriacetate diabetes model

Diabetes induction is achieved through parenteral administration of large dose of ferric nitrilotriacetate (Fe-NTA) in rats and rabbits for up to 60 days for hyperglycemia to occur. Fe-NTA induces oxidative damage to DNA, lipids and

protein through decrease of antioxidant enzymes resulting in an increased production of lipid peroxides and hydrogen peroxide (Rimbachi *et al*., 2000; El-desouky *et al*., 2019).

2.8.2 Total pancreatectomy

This is the total removal of the animal pancreas through surgery. It has been used to induce diabetes in rats, pigs, dogs and primates. However, its use is limited due to requirement of high level of technical expertise, facilities and post-surgical management of the animals (Etuk, 2010; Kumar *et al.*, 2012).

2.8.3 Genetic models of diabetes

The models available are the spontaneously developed diabetic rats and the genetically engineered diabetic mice. Spontaneously developed diabetic models are obtained by breeding glucose-intolerant nondiabetic animals over several generations and are genetically modelled to type 11 diabetes. An example of the spontaneously developed diabetic models is the Goto-Kakizaki rat. Genetical engineering to develop diabetes models target proteins that are involved in glucose metabolism where the rodents will either over express (transgenic) or under express (knockout) the proteins. Type 1 diabetes mellitus model is obtained by inserting into the rodent a viral protein which expresses itself in the pancreatic islets and generate an autoimmune mediated destruction of beta cells of the islets of Langerhans. The genetic models of diabetes enable bioscreening of potential therapeutic products devoid of side effects that arise in use of alloxan and streptozotocin. However, they are highly technical and costly hence rarely used (Frode, 2008; Etuk, 2010; Wojcikowski, 2014).

2.8.4 Other experimental models for diabetes

2.8.4.1 Normal healthy animal models

This employs the use of healthy animals with normal blood glucose and is used together with diabetic animals. It facilitates testing effect of the potential antidiabetic agent in animals with normal pancreatic activity which may provide important information on mode of action (Frode, 2008: Etuk, 2010: Shama *et al*., 2013).

2.8.4.2 Glucose loading animal model

In this model, rabbits or male rat's animals are fasted overnight and a glucose load of 1 to 2.5 milligram per kilogram body weight is orally administered and the blood glucose levels are monitored with time. The procedure is similar to the oral glucose tolerance test (OGTT) which is used in clinical setups and is appropriately referred to as physiological induction of diabetes. However, the hyperglycemia achieved is not stable hence not a suitable experimental model for diabetes (Kumar *et al*., 2012: Sharma *et al*., 2013).

2.9 Toxicology

Adverse effects, the undesirable, abnormal or harmful change due to exposure to potential toxicants in phytotherapy are said to be minimal. However, there is limited data on the safety of numerous plants despite their wide spread use (Neelash *et al*., 2010). It is imperative that medicinal plants be submitted to efficacy and safety test just as synthetics drugs. Defining tolerable levels as well as pharmacologic effects of natural products designed for clinical trials entails extensive preclinical safety data integral in support of the safety of such studies. In that respect, toxicological profiles of plants used for therapeutic interventions in human beings are being documented

(Mounanga *et al*., 2015). Toxicity effects are categorized as acute, sub acute or chronic. Toxicities may ultimately lead to death in severe cases. However, less severe toxicities are exhibited in alterations in food consumption, changes in body and organ weights, pathological alteration in organ toxicity biomarkers and histological changes (George, 2011; Fatima and Nayeem, 2016).

2.9.1 Total body and organ weight

Body weight is an early indicator of adverse effects of administered toxicants. It provides information on food and water intake and by extension, the physiological state of the experimental animal. More than 10% reduction in initial body weight indicates adverse effects on the rat model (Chanda *et al*., 2015). Effects on body weight attributed to medicinal plants may vary between species (Bussmann *et al*., 2011). Toxic effects of a plant depend on the type of specific phytochemical present as well as their concentration. (Karau *et al*., 2014; Ameni *et al.,* 2015; Mutungi *et al*., 2017).

Organ weight is a sensitive predictor of toxicological effects of xenobiotics and change in organ weights occur before morphological changes are evident. Comparing the organ weight of the control and treated groups indicates changes to organs that results in atrophy, hypertrophy or organomegally and swelling (Michael *et al.*, 2007; Sellers *et al*., 2007). Evaluated organs include the heart, liver, kidneys, lungs, spleen and brain as they are major organ affected by toxicants (Piao *et al.,* 2013; Chanda *et al*., 2015).

2.9.2 Haematology

The study of blood (complete blood count-CBC) in both human beings and rats is used as an evaluation and diagnostic tool in various conditions. It is a powerful tool in establishing the presence of a disease, assess the severity of the disease and response to treatment. The CBC is also useful as a tool to differentiate various illnesses that manifest in deranged blood parameters. The hematological parameters are equally used in determining the safety of various drugs developed to treat, control and prevent human diseases (Chanda *et al*., 2015: OECD, 2018).

A total red blood cell count (TRBC), hemoglobin (HB), hematocrit/packed cell volume (HCT/PCV), mean cell volume (MCV), mean cell hemoglobin (MCH) and mean cell hemoglobin concentration (MCHC) are useful in a complete evaluation of the red blood cells (erythrocytes). TRBC and HB provide information on the number of circulating red blood cells and the oxygen carrying capacity of the blood. An increased number of RBCs with concomitant increase in hemoglobin levels can indicate polycythemia in a well hydrated individual or if decreased, indicate anemia. HCT which is a measure of the content of red blood cells in milliliter of whole blood centrifuged in a micro-hematocrit tube. HCT can also be calculated by using RBC and MCV values in an automated hematology analyzer. It's reported in percentage and is about three times the HB level. The HCT serves as an indicator of anemia, polycythemia and when interpreted with total protein as an indication of dehydration and inflammation.

The morphology and staining of RBCS evaluated by the microscopical study of a thin or peripheral blood film is also useful in providing more information on size

(normocytic, microcytic and macrocytic) and HB level (mostly hypochromia) of the RBCs. However, auto determination of the MCV is an indicator of the overall size of the RBC while the MCH and MCHC denotes hemoglobin concentration. MCH and MCHC can be increased in cases of in vivo or in vitro hemolysis, lipemia or the presence of Heinz bodies. Increased MCH is also an indicator of intravascular hemolysis. The values of the red blood cell indices (TRBC, HB, HCT, MCV, MCH and MCHC) provides a differential diagnosis for anemia (iron deficiency and megaloblastic anemia), polycythemia, dehydration and indication of bone marrow toxicity (Chanda *et al*., 2015: Sharif *et al*., 2015; Arika *et al*., 2016).

The parameters in evaluation of the leucocytes are the total white blood cell (leucocyte) counts (TLC), differential leucocyte counts (DLC) and morphology. Significantly elevated or decreased WBC count referred to as leukocytosis and leucopenia respectively are abnormalities that can be encountered in WBC derangements. TLC measures the cellular immunity of phagocytic cells. A differential count for neutrophils, eosinophils, lymphocytes, monocytes and basophils is important in detailing the cause of the abnormalities either infection, allergy/hypersensitivity, inflammation, neoplasia, stress or excitement. Increased levels of WBC and differential leucocte counts indicate direct effect on bone marrow and leucocytes blast formation and hence an immunostimulatory effect indicator (Sharif *et al*., 2015; Arika *et al*., 2016).

2.9.3 Biochemistry

In evaluation of the cardiac system, creatine kinase (CK) and lactate dehydrogenase (LD) enzymes are used as indicators of myocarditis and myocardial infarction. The

enzymes are released from the tissues upon damage and CK levels are elevated twice the upper reference limit in cardiac involvement. However, an elevated CK can arise due to skeletal muscle disease and other non-cardiac diseases but a differential diagnosis can be made by assay of CK isoenzymes CK-MM (mainly cardiac origin) and CK-MB (Skeletal origin). Lactate dehydrogenase, though also released from other tissues like the skeletal muscles, kidney, liver and blood cells complement the interpretation of creatine kinase levels as elevated values of both CK and LDH indicate cardiac source (Kim *et al*., 2016; Aydin *et al*., 2019). In evaluation of pancreatic system, amylase and lipase which are both digestive enzymes were used. Their values increase in cases of pancreatitis (Usborne *et al*., 2014; Meher *et al*., 2015).

The liver is the main organ for metabolism of drugs or bioactive compounds (Kifayatulah *et al*., 2015). The diagnostic parameters for liver damage commonly used are alanine aminotransferase (AST), aspartate aminotransferase (ALT), alkaline phosphatase (ALP), γ-Glutamyltransferase (GGT), total protein and albumin. The transaminases (AST and ALT) are both present in the hepatocytes and are released during hepatocellular damage. The most specific of the enzymes is ALT as AST is also found in cardiac and skeletal muscles and erythrocytes. In liver disease, ALT levels may rise 100 times above normal. While both enzymes are found in the hepatocytes cytosol, AST is also found in the mitochondria. Thus, in cases of severe damage to the liver cells, AST levels are higher than those of ALT (Olaniyan *et al*., 2016). ALP is found in the sinusoidal surface of the hepatocytes as well as in the bile. Enzyme induction in cholestasis raises ALP levels. The ALP levels can be elevated because of bone disease and other non-hepatic diseases involving the intestines and

placenta. However, high levels are mostly seen in hepatic and bone disease. A differential diagnosis is made in reference to other parameters of AST and ALT in which case if all are elevated, then it indicates a liver source (Martha and Rolando, 2016; OECD, 2018).

Elevated serum levels of the transaminases (AST and ALT) may not be directly as a result of liver injury. However, increased levels may occur as a result of inflammation, cellular leakage and damage of cell membrane to cells in the liver. Gamma (γ)-glutamyltransferase (GGT) is widely distributed in the body and found in the liver, kidneys, pancreas and prostate. Above normal levels of GGT indicates hepatic origin. High levels of GGT does not always indicate a histologically demonstrable liver pathology. Drugs and alcohol induce higher levels through proliferation of the endoplasmic reticulum. High levels of GGT attributed to a diagnosis of liver disease (cholestatic liver disease, hepatocellular damage) will be in relation to elevated levels of AST, ALT and ALP (Martha and Rolando, 2016; Olaniyani *et al*., 2016).

Kidney injury may result as a consequence of various clinical conditions such as hypertention, diabetes and therapeutic drugs interventions. However, the progression of the disease maybe asymptomatic. Evaluation of renal function or diagnosis of kidney disease is done by use of biomarkers such as blood urea nitrogen (BUN), creatinine (CREAT), sodium (Na+) and potassium (K^+) (Urbschat *et al*., 2011; Dhondup *et al*., 2017; Rysz *et al*., 2017).

2.10 Macro and Micronutrients

The maintenance of good health and effective treatment of diseases requires provision of different nutrients in adequate quantities. The biological importance of amino acids and their importance in sustaining the health of the human body cannot be over emphasized. They are the building blocks of proteins vital for gene expression and facilitation of messenger RNA (mRNA) through adjustment of protein function (Wu, 2010; Chatterjea and Shinde, 2012b; Zhang *et al*., 2018).

Other than the macromolecules (carbohydrates, proteins, lipids and fiber), micronutrients are essential nutrients which are required in small quantities. Micronutrients deficiency is a major challenge in public health especially in developing countries. Micronutrients of interest include trace elements such as sodium, chloride, potassium, iron, calcium, copper, cobalt, phosphorus, manganese, magnesium, iodine, zinc fluoride, molybedum, Sulphur, selenium and chromium (Soetan *et al*., 2010).

Trace elements deficiencies has been associated with several diseases. Replacement of a particular element that results to recovery from an illness is an indicator of deficiency of such element. Enzyme systems require trace elements for ultimate function and their deficiencies results in serious derangement in metabolism and tissue integrity. The numerous minerals plays different and interrelating roles in the body making them vital in optimal metabolic processes (Soetan *et al*., 2010).

Specific elements are vital in optimal glucose homeostasis. Such elements include sodium and potassium which are the principal cations in extracellular and intracellular fluids respectively. They both regulate body fluids homeostasis and acid-base balance,

maintain proper cell membrane functions, conduction of nerve impulses, muscle contraction (K^+ particulary in cardiac muscles) and normal Na+/K+-ATPase activity. Absorption of pyrimidines, bile salts, amino acids and monosaccharides requires the presence of sodium. Within the epithelial calls of the intestines, choroid plexus and renal tubules, sodium plays a key role in glucose absorption from a low concentration outside the cell to a high concentration inside the cell-concentration gradient. The secondary active transport of glucose is facilitated by the sodium dependent glucose transporter 1 (SGLUT-1) in a co-trasport of sodium from high concentration in the lument to low concentration in the cell is done by the Na^+/K^+-ATPase system (Voet and Voet, 2011: Harada and Inagaki, 2012). Potassium plays numerous roles in enzymatic reactions maily gluconeogenesis and transfer of ATP to pyruvic acid (Biswajit *et al*., 2017). Elevated plasma sodium (hypernatremia) occurs due to dehydration for instance in excessive sweating, therapeutic sodium replacement, high cortisol (Cushing's syndrome), diabetes insipidus, administration of adrenocorticotropic hormone (ACTH) and sex hormones (Soetan *et al*., 2010; Biswajit *et al*., 2017).

Magnesium is key in activation of enzymes where thymine pyrophosphate is a cofactor such as myokinase (phosphate-transferring enzymes), pyruvic acid carboxylase, pyruvic acid oxidase, diphophopyridine nucleotide kinase, creatine kinase, and condensing enzyme in the citric acid cycle (Soetan *et al*., 2010; Piero *et al.*, 2012). Manganese is a vital element as a cofactor in enzymatic systems involved in metabolism of carbohydrates, amino acids and cholesterol. Optimal metabolism of manganese is required for normal synthesis of insulin and its secretion. It is involved in regulation of blood glucose, cellular energy, immune and antioxidants system,

glycoprotein and proteoglycan synthesis (Biswajit *et al*., 2017). Altered carbohydrate and lipid metabolism, glucose intolerance, impaired insulin secretion, and skeletal abnormalities are associated with manganese deficiency (Piero *et al*., 2012; Koh *et al*., 2014; Sium *et al*., 2016; Olafisoye *et al*., 2017).

Zinc functions as a cofactor and is a constituent of many enzymes that are involved in nucleic and amino acid metabolism, cell replication and gene expression (Rajan *et al*., 2014; Olafisoye *et al*., 2017). Zinc is an integral constituent of insulin and is vital in its storage and release, growth. It's vital in synthesis of DNA and RNA, stability of cell membranes and utilization of vitamin A from the liver. Depleted levels of zinc in serum results in insulin resistance in type II diabetes mellitus (Sium *et al*., 2016; Biswait *et al.*, 2017).

Plants have been realized to accord a lot of benefits to human health and there is a global shift in consumption of plants based fruits, vegetables and medicines. The activity of medicinal plants has equally been associated with their micronutrients contents. Hence, it's imperative to determine the trace element content of plants used as therapeutic interventions.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Area

The study was carried out at Kenyatta University Department of Biochemistry, Microbiology and Biotechnology, School of Pure and Applied Sciences. Kenyatta University is off Thika road super highway and 23 kms from Nairobi central business district.

3.2 Collection and Preparation of Plant Materials

The plant materials for this study were obtained as follows: sprouts of *Triticum aestivum* (wheatgrass) and *Hordeum vulgare* (barley grass) were grown indoors in soil put in wooden trays in a purposely built building at Ondiri Village, Kikuyu Location, Kikuyu Division, Kiambu County of Kenya using the procedure described by Springs Healthcare (K) limited (Appendix 1). *Urtica Massaica* leaf powder was obtained from processors of *Urtica massaica* harvested from Aberdare forest at Arkroad Village, Aboni sublocation in Nyeri County of Kenya.

Fresh stems of *Cynanchum viminale* were collected from Linga Village, Waia Location, Kako sublocation, Kisau Division - Mbooni East, Makueni County of Kenya under the direction of a local herbalist. The plant were authenticated by a botanist at the national museum of kenya East African Herbarium ref:NMK/BOT/CTX/1/2 (Appendix 2). The plants were dried indoors for three weeks, cut into small pieces and ground using an electric mill.

3.3 Aqueous Extraction

Aqueous extraction and lyophylization was done at the Department of Pharmacy laboratory of Kenyatta University. Two hundred gram weight of each of the fine ground plant material was weighed and put separately into 2000 mL conical flasks. Then, 1750 mL of distilled water was added and thoroughly mixed. The conical flasks containing the sample/water mixture were placed in a shaker water bath and left to run for 12hrs at 60^0 C. The mixture was decantend and the crude extract filtered using a muslin cloth at negative pressure. Filtered extracts were then transferred into 200mls freeze-drying plates and put in a deep-freezer at -20^0 C. for 12hrs. The frozen material were transferred to a lyophylizer (FDL-10N-50-BA/mrc laboratory equipment ltd) set at at -50^0 C. and 1.0 Pascal's pressure for 24hrs. The plant materials were obtained as powders, weighed and refrigerated at 4^0 centigrade.

3.4 Determinatinon of *in vivo* hypoglycemic effects

3.4.1 Experimental animals

The study used 8 weeks old Wistar rats with a mean weight of 119 grams. The rats were bred in the Animal house at the Department of Biochemistry, Microbiology and Biotechnology of Kenyatta University. They were housed at room temperature and fed on rodent pellets and water *ad libitum*. Experimental rats were radomly picked from the breeding cages, weighed, placed in experimetal rooms and acclimatized for 72 hours. The care and handling of the rats were handled as per the National Research Council, USA guidelines (USA-NRC, 2011; NENT, 2018).

3.4.2 Experimental design

This was a randomized controlled study design (RCD). The experimental rats were randomly grouped into seven groups of five animals each. The experiment protocol is presented in Table 3.1.

Table 3.1: Grouping and treatment of experimental animals for efficacy evaluation

Animal Group	Treatment
Group I (baseline)	Normal/physiological saline
Group II (Negative control)	Normal/physiological saline
Group III (positive control)	Glibenclamide (oral) or insulin (IP)
Aqueous plants extract doses (mg/kg bwt) orally or intraperitoneally adminstered	
Group IV	50
Group V	91
Group VI	165
Group VII	300

Group II to VII consisted of alloxan induced diabetic rats

3.4.3 Induction of diabetes mellitus in experimental animals

Diabetes was induced in white Wistar rats to obtain suitable diabetic animal models (Etuk, 2010) by use of alloxan monohydrate (Soltesova, 2011; Karau *et. al*., 2012). The Wistar rats were left to fast overnight for 8-12 hours. Hyperglycemia was induced by a single intraperitoneal administration of 186.9 mg/kg body weight of freshly prepared 10% alloxan monohydrate (2,4,5,6 tetraoxypyrimidine; 5-6-dioxyuracil) obtained from Sigma (Steinhein, Switzerland). The animals were given feeds and water *ad libitum* and evaluated for fasting blood glucose levels 72 hours after alloxan administration using Accu Check perfoma glucometer. Rats with blood glucose levels above 200 mg/L (11.1 mmol/L) were considered diabetic and used in this study (Soltesova, 2011; Karau *et. al*., 2012).

The blood glucose meter utilizes electrical biosensors to provide blood glucose levels in whole blood within a short time. When a drop of blood (1-2 µL) is placed on the tip of the testing strip, the blood is sucked into the strip by capillary action. The blood comes into contact with immobilized glucose oxidase which reacts with the glucose in the blood to form gluconic acid. Gluconic acid then reacts with potassium ferricyanide to form ferrocyanide. A current that is directly proportional to the blood glucose concentration is generated by oxidation of the ferrocyanide (Khandur 2019; Cho *et al*., 2020).

3.4.4 Preparation of logarithmic doses of the plants extracts

A dose level of 50 mg/kg body weight was taken as the smallest dose and 300 mg/kg body weight was taken as the highest dose for efficacy evaluation. Two other doses were established as 91 mg/kg body weight and 165mg/kg body weight using the formula;

$F = \sqrt[r]{I}$

Where; (F = incremental factor, r = N (number of doses in the log series)-1, I = large dose /small dose) (Thomson 1985). The concentration of the logarithmic doses of freeze-dried plant extracts in consideration of the mean rat's body weight were prepared as in Appendix 3.1.

3.4.5 Blood glucose, rate constant and half life determination

Blood sampling for blood glucose determination was done by sterilizing the tail with 10% alcohol and nipping the tail of the rats at the start of the experiment (0 hr) and repeated at 1, 2, 3, 4, 6, 12 and 24 hours. The blood glucose levels were determined

using a glucose point of care analyzer Accu-Chek® perfoma model and Accu-Chek® perfoma strips.

The order of reaction was established by plotting the natural logarithim of blood glucose concentration (Ln [$C_6H_{12}O_6$]) against time (t). On ploting, a straight line was obtained which was negative to the first order rate constant (-k). The first order rate constant was hence derived using the formular described by voet and voet (2011);

$$v = \frac{d[A]}{dt} = k[A]hour^{-1}$$

The time required to reduce the initial blood glucose (ln $[A]_0$ by half (half life; $t_{1/2}$) was computed using the formula:

$$t_{1/2} = \ln\frac{2}{k} = \frac{0.693}{k}$$

3.5 Evaluation of *In Vivo* Safety

Logarithmic doses were prepared based on the mean body weight of animals used for respective plants. The concentrations of the plants extracts for use were calculated using the formula:

$F=\sqrt[r]{I}$.

Where

F = incremental factor

r = N (number of doses in the log series)-1

I = large dose (Ld)/Small dose (Sd)

The doses to be administered were prepared as outlined in Appendix 3.2.

3.5.1 Experimental animals

Normal 8 weeks old Wistar rats with a mean weight of 117 grams were used as the experimental animals. The rats were bred and housed in the Animal house at the Department of Biochemistry, Microbiology and Biotechnology of Kenyatta University. Acclimatization of the rats in experimetal rooms prior to commencement of treatments was done for 72 hours. The care and handling of the rats was conducted as per the National Research Council, USA guidelines (USA-NRC, 2011).

3.5.2 Experimental design

This was a randomized controlled study. The rats were randomly placed into five groups of five rats each for safety evaluation. Group I consisted of normal rats and were administered 0.5 mL physiological saline. Group II to V were normal rats administered 0.5 ml of the aqueous plants extracts reconstituted in physiological saline. Treatments were administered daily for 28 days and the experiment was done separately for oral or intraperitoneal administration. During the experimental period, the rats were allowed free access to mice pellet and water and observed for any manifestation of illness, change in behavior and mortality. The body weight of each rat was determined at the start of the experiment and after every seven days during the dosing period up to and including the 28^{th} day. A summary of the experimental design for safety evaluation is presented in Table 3.2.

Table 3.2: Grouping of animals for safety evaluation

Treatment group	Treatment (oral or intraperitoneal administration)
Group I. Control	0.5 mL normal saline
Aqueous extracts administration (mg/kg body weight)	
Group II	300
Group III.	448
Group IV	669
Group V.	1000

3.5.3 Collection and preparation of pathological samples

On the 28th, the rats were euthanized by placement in a chloform chamber. Blood samples were obtained from the rats by cardiac puncture. The blood obtained was put into test tube containing no anticoagulant or with ethylene diamine tetra acetic acid (EDTA) as an anticoagulant for biochemical and hematological parameters respectively. Blood samples collected in plain blood containers were allowed to clot at room temperature. The clotted blood samples were centrifuged at 3000 rpm for 10 min and serum samples were aspirated off, put in cryovials, stored at -20 centigrade until assayed or delivered immediately for biochemical investigations. Whole blood samples in EDTA tubes were immediately dispatched for hematological investigations. The pathological samples were analysed following the standard operating procedures for analytes measurement at Kenyatta National Hospital, Nairobi, Kenya.

After collecting the blood, all the vital organs such as liver, kidney, heart, spleen, brain and lungs were separated and individual organs were weighed using an electronic balance and stored in 10% formalin.

3.5.2.1 Determination of hematological parameters

Red blood cells counts (RBC), hemoglobin, mean cell hemoglobin, mean cell hemoglobin concentration, mean cell volume, White blood cells counts (WBC), WBC differentials counts for neutrophils, lymphocytes, eosinophils, basophils, monocytes and platelets were determined using the Coulter Counter System (Beckman Coulter®, ThermoFisher, UK). Air-dried thin blood films stained with giemsa stain were examined microscopically using magnification 400 and 1000 for

cell morphologies. The blood sample collected in K_3-EDTA tubes was used to estimate hematological parameters.

3.5.2.2 Determination of Biochemical Parameters

Biochemical investigations were carried out using an automated clinical chemistry analyzer to determine alanine aminotransferase (AST), aspartate aminotransferase (ALT), alkaline phosphatase (ALP), γ-Glutamyltransferase (GGT), total protein, albumin, blood urea nitrogen (BUN), creatinine (CREAT), sodium (Na^+), potassium (K+), amylase (AMY), creatinine kinase (CK) and lactate dehydrogenase (LDH). The analytes were determined photometrically based on the Beer-Lambert law that materials selectively absorb light and the amount of light absorbed correlates with the concentration of the material. The general principles described on the chemistry parameters part of the operator's manual is (CS 300 B autoanalyzer, Version No: REV. 06, 2014):

When the monochromatic light with a specific wavelength goes through a cuvette with a sample, the monochromatic light absorbency is directly proportional to the sample concentration and the distance that the light goes through the sample.

$$A = \frac{\log(I)}{T} = \log\left(\frac{Io}{It}\right) = \varepsilon bc$$

where,

A = Absorbancy of the light when passing the solution.

T = Transmitted intensity and incident intensity ratio: transmittance It/I0.

I_0 = Incident intensity.

I_t = Transmitted intensity.

ε = Molar absorption coefficient of solution ($mL \times mmol^{-1} \times cm^{-1}$).

c = Mol concentration of the solution (mmol/mL).

b = Solution layer thickness (cm).

Solution layer thickness (b): Optical path known and fixed by the analyzer. Molar absorption coefficient (ε) is the correlation coefficient of the wavelength, solution and solution temperature. Individual analytes are determined by specific reagent principles as per the general principle.

3.6 Determination of Phytochemicals

3.6.1 Qualitative phytochemicals determination

The determination of phytochemicals was done to establish qualitatively presence or absence of tannins, flavonoids, terpenoids, saponins, steroids, phlobatannins, antharaquinones, cardiac glycosides and alkaloids using methods described by Iqbal *et.al* (2011), James (2012) and Pradeep *et.al.* (2014) where the results were reported as present (+) or absent (-).

3.6.1.1 Test for alkaloids

The alkaloids were determined by adding 3ml of aqueous plant's extract to 3ml of 10% hydrochloric acid on a steam bath. Mayer and Hager's reagent was then added drop by drop to the extract. Turbidity of the resulting precipitate and yellow coloration was taken as an evidence for the presence of alkaloid.

3.6.1.2 Test for tannins

The tannins were determined using ferric chloride test. 2ml of the aqueous extract was stirred with 3ml of distilled water and few drops of ferrous chloride solution were added. The formation of green color precipitate indicates presence of tannins.

3.6.1.3 Test for saponins

The saponins were determined by foam test. 3ml of aqueous extract was shaken vigorously with equal volume of distilled water in a test tube and warmed. The formation of stable foam persisting for about 5-10 minutes was taken as an indication of the presence of saponins.

3.6.1.4 Test for phlobatannins

The phlobatannins were determined by adding 3ml of aqueous plant's extract to 2ml of 10% hydrochloric and the mixture boiled. Deposition of a red precipitate was taken as an evidence for the presence of phylobatannins.

3.6.1.5 Test for flavonoids

The flavonoids were determined by lead acetate test. 3ml of aqueous plant's extract were added to 1ml of 10% lead acetate solution. The formation of a yellow precipitate was taken as a positive result for flavonoids.

3.6.1.6 Test for terpenoids

The presence of terpenoids in the plants extracts was determined by dissolving 3 ml of the plant extract in 2ml of chloroform and evaporated to dryness. 2 mL of

concentrated sulphuric acid was then added and heated for about 2 min. Development of greyish color indicated the presence of terpenoids.

3.6.1.7 Test for steroids

Salkowski test was done to determine the presence of steroids. The crude extract was mixed with 2 mL of chloroform and concentrated sulphuric acid ($H_2SO_{4)}$ added. A red color produced in the lower chloroform layer indicated the presence of steroids. Additional test for steroid was performed by mixing the crude extract with 2 mL of chloroform. Then 2 mL of concentrated H_2SO_4 and 2 mL of acetic acid were poured into the mixture. The development of a greenish coloration indicated the presence of steroids.

3.6.1.8 Test for reducing sugars

Reducing sugars were determined by Fehlings test. 2 mL of Fehling reagents was added to the crude plant extract and gently boiled. A brick red precipitate appearing at the bottom of the test tube indicated the presence of reducing sugars.

3.6.1.9 Test for tannins

Phenols and tannins were determined by addition of 2 mL of 2% solution of ferrous chloride to the plant extract. Occurrence of blue-green or black coloration indicated the presence of phenols and tannins.

3.6.1.10 Test for cardiac glycosides

Cardiac glycosides were determined by Keller-kilani test. The crude extract was mixed with 2 mL of glacial acetic acid in a test tube. 1-2 drops of 2% solution of

ferrous chloride was added then 2mL of concentrated H_2SO_4. A brown ring at the interphase indicated the presence of cardiac glycosides.

3.6.2 Spectrophotometric determination of compounds present in aqueous extracts of *T. aestivum, H. vulgare, U. massaica* and *C. viminalle*

The lyophilized aqueous extracts of the plants in this study were prepared for analysis with UPLC-MS/MS (ACQUITY UPLC I-class system -Waters Corp., Milford, MA). One gram of the plant extract was dissolved in 1mL solution of methanol and double distilled water at a ratio of 90:10 (MeOH: ddH_2O). The mixture was vortexed for 10 seconds, sonicated for 30 min and centrifuged at 14000 rpm for 10 min at 4°C. The supernatant was decanted and analyzed using the UPLC-MS/MS. 0.1µL of the sample was injected for chromatographic separation with an ACQUITY UPLC BEH C18 column (2.1 mm X 150 mm, 1.7 µm particle size; Waters Corp., Wexford, Ireland, oven temp 45°C). The autosampler tray was cooled to 5°C. The mobile phase comprised of (A) water and (B) methanol (solvent B) both acidified with 0.01% formic acid. The gradient system used was 0-2 min, 5% B, 2-4 min, 40% B, 4-7 min, 40% B, 7-8.5 min 60% B, 8.5-10 min 60% B, 10-15 min, 80% B, 15-19 80% B, 19-20.5 min, 100% B, 20.5-23 min, 100% B, 23-24 min 95% B, 24-26 min, 95% B. The flow rate was held constant at 0.2 mL/min.

The UPLC was interfaced with an electrospray ionization (ESI) Waters Xevo TQ-S operated in full scan MS in positive ionization mode. Data were acquired over the m/z range 40-2000 with a capillary voltage of 0.5 kV, sampling cone voltage of 30 V, source temperature 150°C and desolvation temperature of 120°C. The nitrogen desolvation flow rate was 800 L/h. Data was acquired using MassLynx version 4.1

SCN 712 (Waters). Potential assignments of compounds were determined after the generation of the mass spectrum for each peak, establishing the molecular ion peaks using adducts, common fragments and literature online databases (METLIN, ChemSpider).

3.7 Determination of Macro and Micronutrients composition

3.7.1 Determination of total carbohydrates

Carbohydrates contents in the aqueous plants extracts were determined by anthrone method (Raunkjaer, 1994).

3.7.1.1 Principle

The carbohydrate (polysaccharides) content of the plants extracts are hydrolyzed by hydrochloric acid to monosaccharides. The resultant monosaccharides (glucose) is dehydrated to hydroxymethyl furfural. Hydroxymethyl furfural reacts with anthrone to yield a green colored product with an absorption maximum at 630nm.

3.7.1.2 Procedure

100 mg of the aqueous plant extract was weighed into a boiling tube. The extract was hydrolyze by addition of 5 ml of 2.5N hydrochloric acid and placing the mixture in boiling water bath for 3 hours with 5mL of 2.5 N-HCl. Solid sodium carbonate was added to the cooled mixture until effervescence stopped. The volume was made up to 100 ml using distilled water and centrifuged. Aliquots of the supernatant (0.5 to 1 ml) were used for analysis.

Glucose serial standards were prepared by taking 0, 0.2, 0.4, 0.6, 0.8 and 1 ml of the working standard. '0' served as the blank. The volume in all the tubes was filled to 1 ml using distilled water. 4 ml of anthrone reagent was added. Heating of the tubes using a boiling water bath was done for eight minutes. The preparation was cooled rapidly and absorbance of the green to dark green solution read at 630nm. A standard graph plotting concentration of the standard on the X-axis and absorbance on the Y-axis was drawn.

The computation of the carbohydrate content in a 100mg of the sample was done using the formular;

$$\frac{mg\ of\ glucose}{Volume\ of\ test\ sample} x\ 100$$

The concentration of the carbohydrate content was reported in mg/l. Figure 3.1 presents the calibration curve of carbohydrate concentration.

3.7.2 Determination of protein

Protein was determined using the semi micro Kjeldal method. 2 g of the plant aqueous extract was weighed into a digestion flask. 5 g potassium sulphate (catalyst), 0.5 g of copper sulphate and 15 mL of sulphuric acid were added to the flask. The mixture was heated in a fume hood till the color of the mixture turned blue. The blue color signified the end of the digestion process.

The digest was cooled, transferred to 100 ml volumetric flask and topped up to the mark with deionized water. A blank digest with the catalyst was also made. 10 mL of diluted digest was transferred into a distilling flask and washed with distilled water. 15 mL of 40% NaOH was added and washed with distilled water. Distillation was

done to a volume of about 60 ml distillate. The distillate was titrated using 0.02 N HCL to an orange color of the mixed indicator, which signified the end point.

$$\%\ Nitrogen = (V_1 - V_2)\ x\ N\ x\ F\ x\ \frac{100}{(V\ x\ \frac{100}{S})}$$

Where V_1 is the titre for sample in ml, V_2 is titre for blank in ml, N is normality of standard HCL (0.02), f is factor of standard HCL solution, V is volume of diluted digest taken for distillation (10 ml) and S is weight of sample taken for distillation (1 g). The protein content was reported as % protein.

$$\%\ Protein = \%\ Nitrogen\ x\ Protein\ factor\ (625)$$

3.7.3 Determination of amino acids and mineral contents

Amino acids and mineral contents of the aqueous plants extracts were determined using Shimadzu LC/MS at the Department of Chemistry of Kenyatta University. Two mobile phase were applied. A 10 mL sample loop was used for injection of the sample and the flow rate of the mobile phase was maintained at 0.7 mL/min and the column at 25°C. Spectrum of the Amino acids were used to correlate with known molecular weights. Calibration curves (Appendix 13) using standards of the minerals were generated and the samples mineral contents were calculated using the absorbance of the standard and the unknown as well as the concentration of the standard and the concentration of minerals reported in grams/kilogram (g/kg)

3.8 Approval and Ethical Considerations

This study was primarily approved by the Kenyatta University Graduate School Board vide Ref: I84/27460/14 (Appendix 5). Further approval was accorded by National Commision for Science, Technology and Innovation (NACOSTI/P/15/7291/6442;

Appendix 6). In addition, the Kenya Ministry of Interior and Co-ordination of National Government County Commisioner, Kiambu, approved the research through Ref; ED.12/1/VOL.II/204 (Appendix 7). Approval sought from the Kenyatta National Hospital and University of Nairobi Ethical Research Committee (KNH-UON ERC) was granted (Ref: KNH- ERC/A/401: Appendix 8). The research was partly funded by the National Commision for Science, Technology and Innovation in Kenya (NACOSTI/RCD/ST&I/7TH CALL/PHD/008; Appendix 9). The experimental animals were handled following protocols on ethical handling and use of laboratory animals by the USA-National Research Council and the Norwegian National Committee for Research Ethics in Science and Technology (USA-NRC, 2011; NENT, 2018).

3.9 Data Management and Statistical Analysis

Parametric data obtained for evaluation of effect on blood glucose and safety was entered into excel spread sheet. The data was cleaned and exported to Minitab version 17 for statistical analysis. The data was analyzed for descriptive statistics (Mean± standard deviation). Comparison of the blood glucose level means between untreated control groups, standard antidiabetic treatments and plants extracts treated groups of rats was done using one way analysis of variance (ANOVA) followed by Tukey's post hoc test where ANOVA statistics was significant. Student's t test was used to compare blood glucose lowering activity of similar doses of same aqueous plants extracts when administered orally and intraperitoneally. Mean values for biomarkers of safety data of control rats and plants extracts treated rats were analyzed using ANOVA followed by Tukey's post hoc test. Values of $p < 0.05$ were considered significantly different. Data for effect on blood glucose and safety evaluation are expressed in form of either tables or graphs.

CHAPTER FOUR

RESULTS

4.1 Lyophilized Yields of Aqueous Extractions

On cryodesiccation, yields of lyophilized aqueous plants extracts of *T. aestivum, H. vulgare, U. massaica* and *C. viminale* were as indicated in Table 4.1.

Table 4. 1: Yields of aqueous extracts of *T. aestivum, H. vulgare, U. massaica* and *C. viminale*

Extract	Yield (mg/g)	Color
T. aestivum	227	Red-brown
H. vulgare	244.5	Brown
U.massaica	220	Black
C. viminale	69.5	Dark grey

4.2 *In vivo* hypoglycemic effects

4.2.1 Effects of aqueous extract of *T. aestivum* in alloxan induced diabetic rat model

Orally administered logarithmic doses of aqueous extracts of *T. aestivum* at 50, 91, 165 and 300 mg caused reduction in blood glucose in the rat's model. The four doses of *T. aestivum* reduced the blood glucose levels in a statistically insignificant version relative to the reference drug - glibenclamide ($p > 0.05$; Table 4.2).

The four doses of *T. aestivum* when administered intraperitoneally, reduced the blood glucose of the alloxan induced diabetic rats in a significantly different ($p < 0.05$) manner relative to insulin the standard intraperitoneal drug from zero to the third hour (Table 4.2). However, on the third hour, the blood glucose lowering effect of the plant extract at 50 mg/kg body weight was not significantly different ($p > 0.05$) compared to that of insulin. From the fourth to the 24^{th} hour, reduction of blood glucose resulting from the intraperitoneally administered four doses of *T. aestivum* on of the

rat's model was not significantly different ($p > 0.05$) to that of insulin in the same period. The reduction in blood glucose of the diabetic rats treated by the plant extract was not dose dependent throughout the experiment (Table 4.2).

In comparison, at the dose level of 50 mg/kg body weight *T. aestivum*, the lowering effect on blood glucose was significantly different ($p < 0.05$) between orally and intraperitoneally administered plant extract on the 12^{th} and 24^{th} hour. The blood glucose lowering effect of the plant extract was however not significantly different ($p > 0.05$) when either orally or intraperitoneally administered at dose levels from 91 to 300 mg/kg body weight during the entire period of the experiment (Table 4.2).

Table 4. 2: *E*ffects of aqueous extracts of *T. aestivum* logarithmic doses on blood glucose levels in alloxan induced diabetic rats

Treatment group		Blood glucose levels at varying times (mmol/l)							
		0hr	1hr	2hr	3hr	4hr	6hr	12hr	24hr
Physiological saline	Oral	5.8 ± 0.9^{B}	6.0 ± 0.6^{B}	5.9 ± 0.2^{B}	5.8 ± 0.3^{B}	5.6 ± 0.5^{C}	5.3 ± 0.5^{B}	$4.6\pm0.3^{BC*}$	$4.5\pm0.7^{B*}$
	IP	6.1 ± 0.2^{b}	7.0 ± 1.0^{c}	7.1 ± 1.0^{c}	6.6 ± 0.5^{c}	6.98 ± 0.5^{b}	5.6 ± 0.3^{b}	4.7 ± 0.2^{b}	4.0 ± 0.4^{b}
Diabetic/Physiological saline	Oral	18.04 ± 2.5^{A}	18.6 ± 2.9^{A}	19.1 ± 2.6^{A}	19.9 ± 2.6^{A}	20.2 ± 2.0^{A}	19.4 ± 1.6^{A}	15.3 ± 1.3^{A}	9.6 ± 3.6^{A}
	IP	21.8 ± 4.3^{a}	23.2 ± 4.6^{a}	22.1 ± 4.4^{a}	20.9 ± 4.6^{a}	21.1 ± 6.5^{a}	18.7 ± 5.9^{a}	16.8 ± 6.8^{a}	13.2 ± 4.95^{a}
Diabetic/reference drugs	Gliben	18.8 ± 2.8^{A}	17.9 ± 1.9^{A}	15.2 ± 1.7^{A}	14.4 ± 2.0^{A}	12.6 ± 2.2^{ABC}	7.4 ± 1.5^{B}	4.3 ± 1.4^{BC}	4.6 ± 0.9^{B}
	Insulin	23.3 ± 6.6^{a}	4.4 ± 2.3^{c}	4.9 ± 3.4^{c}	6.9 ± 5.1^{c}	8.4 ± 5.4^{b}	10.5 ± 5.7^{b}	8.7 ± 5.8^{b}	4.5 ± 1.2^{b}
Therapeutic aqueous extract doses (mg/kg body weight) of *T. aestivum*									
50	Oral	20.1 ± 6.2^{A}	18.6 ± 4.5^{A}	16.9 ± 4.6^{A}	16.3 ± 5.0^{A}	13.9 ± 4.8^{AB}	7.4 ± 3.1^{B}	3.2 ± 0.9^{C}	3.3 ± 0.7^{B}
	IP	17.98 ± 2.8^{a}	16.2 ± 4.3^{b}	13.6 ± 2.8^{b}	10.4 ± 2.0^{bc}	7.8 ± 2.0^{b}	5.1 ± 1.2^{b}	4.3 ± 0.8^{b}	4.4 ± 0.6^{b}
91	Oral	18.8 ± 5.8^{A}	17.1 ± 6.2^{A}	15.4 ± 5.2^{A}	12.7 ± 3.7^{AB}	11.1 ± 2.9^{BC}	6.6 ± 2.5^{B}	3.8 ± 1.5^{C}	3.5 ± 1.1^{B}
	IP	18.4 ± 3.2^{a}	17.7 ± 2.7^{ab}	16.1 ± 2.9^{ab}	16.0 ± 4.5^{ab}	13.5 ± 4.7^{ab}	9.3 ± 5.1^{b}	5.4 ± 1.2^{b}	5.8 ± 2.4^{b}
165	Oral	21.3 ± 6.0^{A}	18.0 ± 6.5^{A}	16.9 ± 6.6^{A}	15.2 ± 6.8^{A}	13.4 ± 7.2^{ABC}	8.2 ± 4.7^{B}	6.6 ± 1.3^{B}	5.9 ± 2.4^{AB}
	IP	18.1 ± 4.9^{a}	17.6 ± 3.8^{ab}	16.6 ± 4.1^{ab}	15.4 ± 5.0^{ab}	13.6 ± 4.4^{ab}	8.5 ± 3.4^{b}	5.5 ± 2.6^{b}	5.7 ± 0.8^{b}
300	Oral	19.7 ± 3.2^{A}	15.4 ± 3.6^{A}	13.6 ± 3.4^{B}	12.4 ± 4.1^{AB}	10.2 ± 4.0^{BC}	7.3 ± 2.0^{B}	4.0 ± 1.3^{C}	4.8 ± 1.7^{B}
	IP	21.7 ± 4.4^{a}	18.7 ± 3.2^{ab}	16.0 ± 2.0^{ab}	15.2 ± 3.4^{ab}	14.2 ± 3.5^{ab}	8.1 ± 2.1^{b}	4.9 ± 2.7^{b}	4.1 ± 1.5^{b}

Values are expressed as Mean ±Standard Deviation (SD) blood glucose of five rats per group. Means in a column that share a superscripted capital or small letter indicate no significant difference. Gliben = glibenclamide. Means in a column followed by a superscripted asterix indicates significant difference ($p < 0.05$) between the blood glucose lowering effects of orally and intraperitoneally administered extract at specific doses.

The trend in reduction of blood glucose resulting from the four orally administered *T. aestivum* extract doses was equally not significantly different from the effect caused by glibenclamide (Figure 4.1). *T. aestivum* extract reduced the blood glucose in a dose depedent manner in the first hour post treatments administration. The highest reduction in blood glucose was caused by the 300 mg/kg body weight dose of *T. aestivum* by 22.2% while the 50, 90 and 165 mg/kg body weight doses reduced the blood glucose by 5.2%, 10.2% and 16% respectively in the first hour. However, the lowering effect on blood glucose by the four doses of *T. aestivum* extract was not significantly different from that of the standard oral drug which reduced blood glucose by 2.2% in the same hour. Similar trend in reduction of blood glucose by glibenclamide and *T. aestivum* doses occurred up to the fourth hour. Though not significantly different ($p > 0.05$), the respective extract doses reduced baseline blood glucose to 70.4%, 61.4%, 59.4% and 50.6% respectively while glibenclamide reduced the blood glucose to 68.6% on the fourth hour. From the 12^{th} to the 24^{th} hour, reduction of blood glucose was not dependent on the treatment administered. During this period, a statistically insignificant increase in blood glucose occurred in the 50 and 300 mg/kg body weight *T. aestivum* as well as glibenclamide treated rats. At dose levels of 91 and 165 mg/kg body weight *T.aestivum*, insignificant decrease in blood glucose of the diabetic rats occurred from the 12^{th} to the 24^{th} hour (Figure 4.1).

Figure 4.2 presents the trend in reduction in blood glucose caused by intraperitoneally administered therapeutic doses of *T. aestivum*. An hour post administration of the treatments, the extract reduced the blood glucose in a dose independent manner by between 1.2% and 13.1 % which was significantly different ($p < 0.05$) relative to insulin which reduced blood glucose by 62.5% in the same hour. Further, the rate of

reduction in blood glucose of the rats continued at significantly different ($p < 0.05$) rate between the four extract doses and insulin treated rats up to the fourth hour. On the fourth hour, the plants extracts reduced the blood glucose to 44.5, 74, 75.1 and 65% at dose level of 50, 91, 165 and 300 mg/kg body weight, respectively, which was significantly different ($p < 0.05$) to insulin which reduced the blood glucose to 18%. On the sixth hour, *T. aestivum* extract reduced the blood glucose in a dose independent rate. At 50 and 300 mg/kg body weight dose, *T. aestivum* reduced blood glucose of the rat's model to 28.6% and 38.6%, respectively, an effect which was not significantly different ($p < 0.05$) to that of the intraperitoneal standard drug which reduced the blood glucose to 19.6% in the same hour (Figure 4.2).

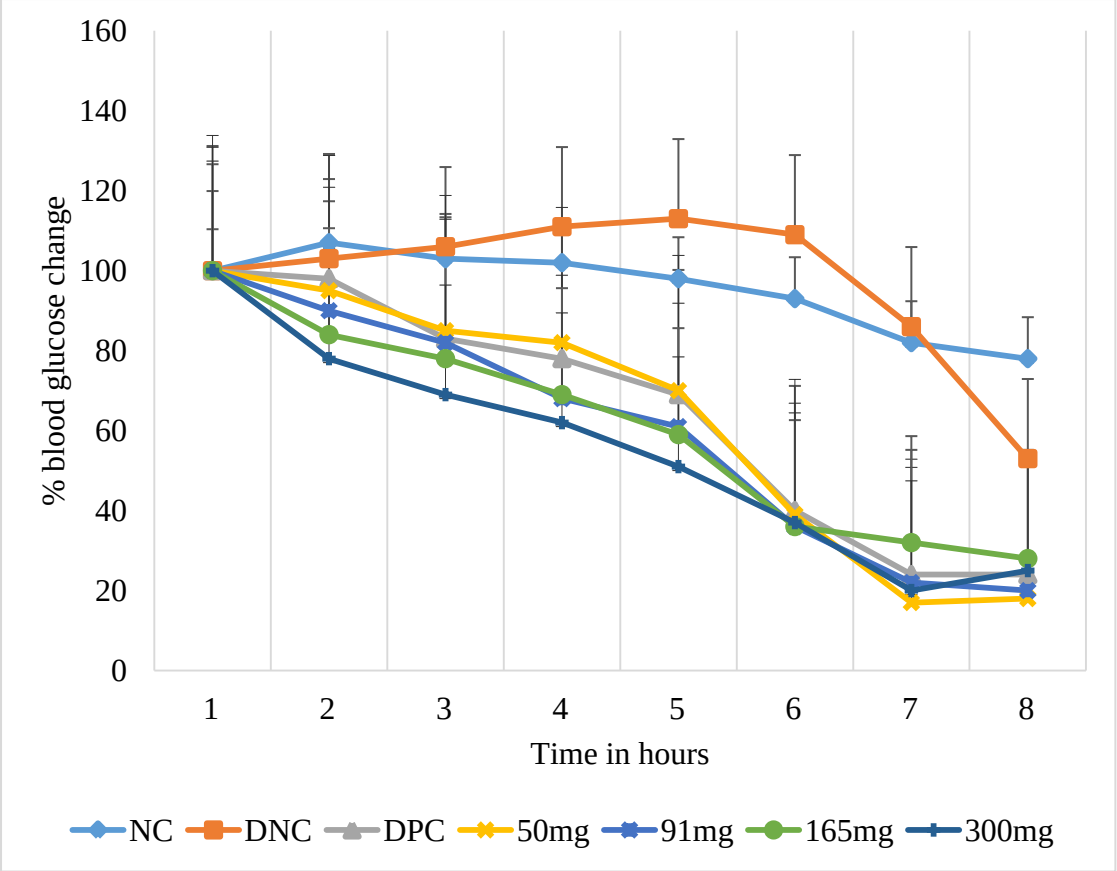

Figure 4. 1: Change in blood glucose levels post oral administration of logarithmic doses of aqueous extracts of T. aestivum in diabetic rats. NC = Normal control, DNC = Diabetic negative control, DPC = Diabetic positive control. 50mg, 91mg, 165mg and 300mg represents doses (mg/kg body weight) of T. aestivum administered.

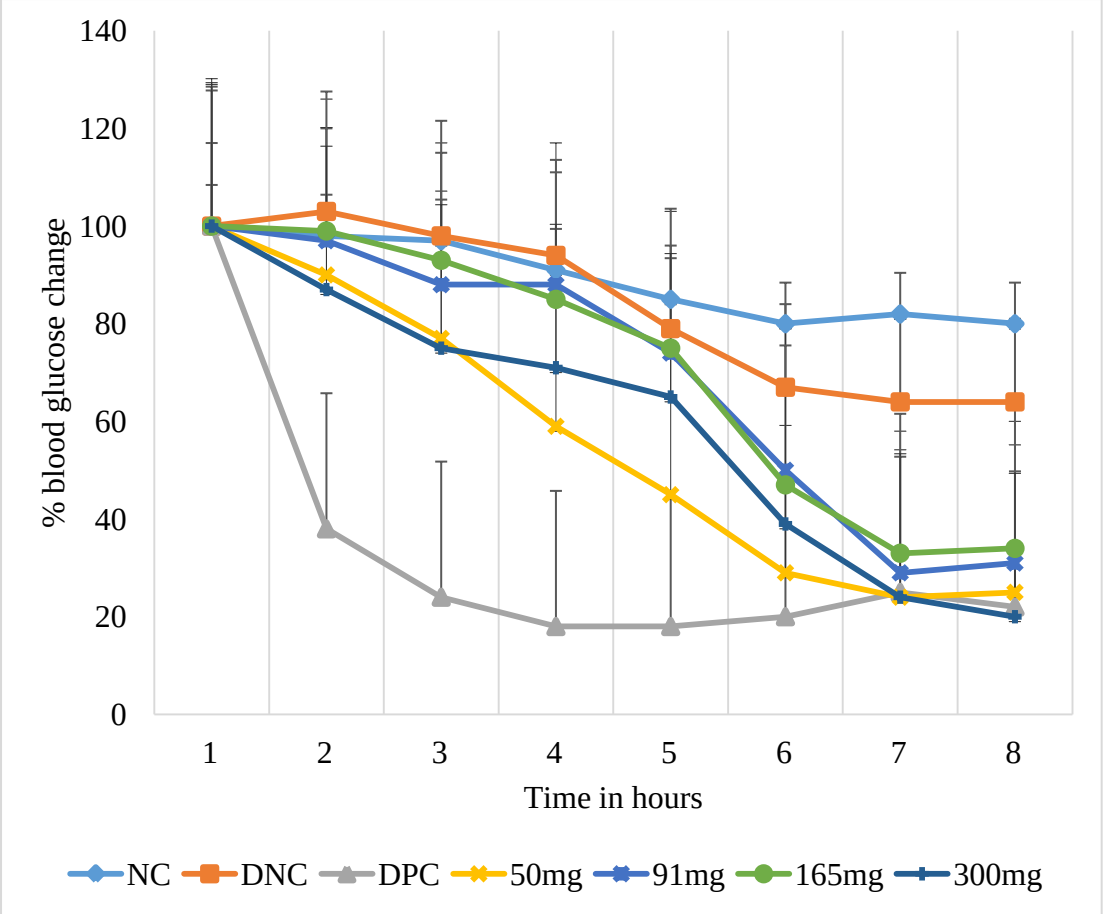

Figure 4.2: Change in blood glucose levels post intraperitoneal administration of aqueous extracts of *T. aestivum* doses in induced diabetic rats. NC = Normal control, DNC = Diabetic negative control, DPC = Diabetic positive control. 50mg, 91mg, 165mg and 300mg represents doses of *T. aestivum* administered.

Table 4.3 presents the pharmacokinetics of orally and intraperitoneally administered extracts of *T. aestivum*. The first order rate constant of insulin and glibenclamide were 0.48 and 0.34 while the half life was 1.44 and 2.04, respectively. *T. aestivum* orally administered at dose levels of 50, 91, 165 and 300 mg/kg body weight had a half life of 1.82, 1.54, 2.31 and 1.82, respectively. The half life of the orally administered 165 mg *T. aestivum* extract was higher while the rate constant was lower relative to that of glibenclamide. *T. aestivum* orally administered at 50, 91 and 300 mg/kg body weight had lower half lifes but higher rate constants than the standard oral drug. The half life of *T. aestivum* intraperitoneally administered dose of 50 mg/kg body weight (0.92 hrs) was lower than that of insulin (1.44 hrs). Further, half life of 1.61, 2.48 and 1.98 hours for intraperitoneally administered *T. aestivum* at 91, 165 and 300 mg/kg body weight respectively were higher than that of insulin. However, the first order rate constants of intraperitoneally administered *T. aestivum* at doses of 91, 165 and 300 mg/kg body weight were lower than the rate constant of insulin.

Table 4.3: Four hours pharmacokinetics of *in vivo* hypoglycemic activity of orally and intraperitoneally administered aqueous extracts of *T. aestivum* in alloxan induced diabetic Wistar rats

Drug (dose)	Route	Rate constant ($hour^{-1}$)	Half-life (hours)
Insulin	IP	0.48	1.44
Glibenclamide	Oral	0.34	2.04
Extract (mg/kg body weight)			
50	IP	0.75	0.92
	Oral	0.38	1.82
91	IP	0.43	1.61
	Oral	0.45	1.54
165	IP	0.28	2.48
	Oral	0.3	2.31
300	IP	0.35	1.98
	Oral	0.38	1.82

Results are expressed as Means of five rats for each time point. $T_{50} = 0.693/k$

4.2.2 Effects of aqueous extract of *H. vulgare* in alloxan induced diabetic rat model

Doses of aqueous extract of *H. vulgare* when orally administered at 50, 91, 165 and 300 mg/kg body weight reduced the blood glucose of the alloxan induced diabetic rat in statistically insignificant manner ($p > 0.05$) compared to glibenclamide the reference drug (Table 4.4).

Upon intraperitoneal administration, the four doses of aqueous extracts of *H. vulgare* reduced the blood glucose of the alloxan induced diabetic rats in a significantly different ($p < 0.05$) manner from that of insulin up to the 4^{th} hour. From the 6^{th} hour, the hypoglycemic effect of all the four administered extract doses was not significantly different ($p > 0.05$) to the control drug (Table 4.4).

Table 4.4: Effects of aqueous extract of *H. vulgare* logarithmic doses on blood glucose levels of alloxan induced diabetic rat

Treatment group		Blood glucose levels at varying times (mmol/l)							
		0hr	1hr	2hr	3hr	4hr	6hr	12hr	24hr
Physiological saline	Oral	5.8 ± 1.1^{B}	5.9 ± 1.3^{B}	5.8 ± 1.3^{B}	5.8 ± 1.0^{B}	5.5 ± 0.5^{B}	4.8 ± 0.5^{B}	3.5 ± 1.1^{B}	3.0 ± 0.7^{B}
	IP	4.8 ± 0.2^{b}	5.2 ± 0.4^{b}	4.5 ± 0.3^{b}	5.2 ± 0.5^{bc}	5.3 ± 0.6^{bc}	4.7 ± 0.3^{c}	4.1 ± 0.2^{a}	4.2 ± 0.3^{b}
Diabetic/Physiological saline	Oral	24.9 ± 4.7^{A}	24.0 ± 3.9^{A}	23.7 ± 4.7^{A}	24.5 ± 7.0^{A}	22.5 ± 7.8^{A}	20.4 ± 9.5^{A}	15.9 ± 7.6^{A}	11.4 ± 5.0^{A}
	IP	22.3 ± 5.3^{a}	19.98 ± 4.5^{a}	18.9 ± 4.4^{a}	18.2 ± 3.9^{a}	18.6 ± 4.8^{a}	16.2 ± 5.0^{a}	11.8 ± 1.8^{a}	10.2 ± 0.6^{a}
Diabetic/reference drug	Gliben	24.7 ± 7.7^{A}	22.5 ± 7.8^{A}	19.1 ± 9.3^{A}	17.5 ± 8.3^{AB}	15.0 ± 8.4^{AB}	10.9 ± 4.7^{AB}	8.6 ± 3.7^{AB}	5.2 ± 1.9^{B}
	Insulin	22.9 ± 5.5^{a}	5.1 ± 1.0^{b}	2.8 ± 0.5^{b}	2.8 ± 0.7^{c}	3.8 ± 0.4^{c}	6.1 ± 0.9^{bc}	5.4 ± 1.5^{a}	4.2 ± 1.0^{b}
Therapeutic aqueous extract doses (mg/kg body weight) of *H. vulgare*									
50	Oral	18.9 ± 3.5^{A}	18.3 ± 3.0^{A}	16.5 ± 4.8^{AB}	15.3 ± 4.3^{AB}	14.8 ± 5.4^{AB}	11.7 ± 4.5^{AB}	5.96 ± 4.0^{B}	8.3 ± 3.9^{B}
	IP	23.7 ± 2.7^{a}	23.8 ± 2.7^{a}	22.96 ± 6.5^{a}	19.1 ± 8.2^{a}	16.0 ± 6.4^{a}	12.7 ± 4.95^{abc}	8.4 ± 4.8^{a}	5.1 ± 2.2^{b}
90.9	Oral	24.7 ± 3.1^{A}	22.4 ± 2.9^{A}	18.2 ± 4.9^{AB}	14.9 ± 6.4^{AB}	13.4 ± 7.3^{AB}	11.5 ± 7.2^{AB}	4.6 ± 2.6^{B}	3.4 ± 0.6^{B}
	IP	18.1 ± 3.6^{a}	17.9 ± 2.9^{a}	15.1 ± 1.3^{a}	13.5 ± 2.0^{ab}	12.9 ± 1.9^{ab}	10.5 ± 1.0^{abc}	6.2 ± 1.2^{a}	4.4 ± 0.6^{b}
165.1	Oral	17.5 ± 3.0^{A}	15.3 ± 5.6^{AB}	12.6 ± 4.0^{AB}	9.98 ± 4.7^{B}	8.1 ± 4.2^{B}	5.8 ± 1.7^{B}	4.3 ± 1.0^{B}	4.1 ± 0.7^{B}
	IP	22.6 ± 9.7^{a}	20.8 ± 6.6^{a}	19.2 ± 7.2^{a}	17.2 ± 6.8^{a}	16.9 ± 7.4^{a}	14.5 ± 7.6^{ab}	7.8 ± 7.3^{a}	4.1 ± 1.1^{b}
300	Oral	20.4 ± 6.3^{A}	21.2 ± 7.6^{A}	20.2 ± 9.95^{A}	15.6 ± 7.1^{AB}	11.9 ± 4.6^{AB}	8.2 ± 4.7^{B}	2.6 ± 0.7^{B}	3.4 ± 0.9^{B}
	IP	22.5 ± 2.5^{a}	20.6 ± 3.8^{a}	20.2 ± 3.6^{a}	19.2 ± 3.7^{a}	17.8 ± 3.7^{a}	14.8 ± 5.3^{ab}	10.4 ± 7.0^{a}	7.0 ± 4.4^{a}

Values are expressed as Mean ±Standard Deviation (SD) blood glucose of five rats per group. Means that share a superscripted capital or small letter in a column are not significantly different. Means in a column do not have a superscripted asterix down the column indicating no significant difference ($p > 0.05$) between effects on blood glucose by orally and intraperitoneally administered extract.

The four orally administered doses of aqueous extract of *H. vulgare* decreased the blood glucose in a trend not significantly different ($p > 0.05$) from that of glibenclamide (Figure 4.3). An hour after administration of the treatments, *H. vulgare* extract at the dose level of 300 mg/kg body weight caused an increase in blood glucose by 4.5% though the effect was insignificant ($p > 0.05$) relative to the standard drug which reduced blood glucose by 10% in the same hour. At the same hour, the dose level of 50, 91 and 165 mg/kg body weight decreased the blood glucose in an insignificantly different ($p > 0.05$) dose dependent trend by 2.4%, 9.5% and 14.1% respectively. In the second hour, in exclusion of the 300 mg/kg body weight plant extract dose which reduced blood glucose by 6.2% to 93.8%, *H.vulgare* reduced the blood glucose in a dose depedent rate by 14.1%, 27.2% and 29.1% at the dose level of 50, 91 and 165 mg/kg body weight respectively, which was not significantly different ($p > 0.05$) from the standard oral drug that reduced the blood glucose by 27.2% in the same hour.

From the second to the fourth hour, the extract at dose level of 300 mg/kg body weight reduced blood glucose at a higher rate to 57.1% though not significantly different ($p > 0.05$) from the other extract doses (50, 91 and 165 mg/kg body weight) that lowered the blood glucose to 76.1%, 52.2% and 44.3% respectively as well as the standard oral drug which reduced blood glucose to 55.7% in the 4^{th} hour. By the sixth hour, the plant extract at 50, 91, 165 and 300 mg/kg body weight doses had lowered the blood glucose in a dose depedent manner to 60.2%, 44.5%, 33.2% and 38% respectively compared to glibenclamide which had lowered blood glucose to 41.9% in the same hour. The change in blood glucose levels from the 12^{th} to the 24^{th} hour as a

result of the four extract doses did not significantly vary ($p > 0.05$) from the standard oral drug (Figure 4.3).

An hour after administration of the treatments, the extract doses decreased the blood glucose depending on the dose by between 0% and 8.4% while insulin reduced blood glucose levels in a significantly different ($p < 0.05$) rate from the four extract doses by 77.3% in the same hour. By the 6^{th} hour, *H. vulgare* extract at doses of 50 and 91 mg/kg body weight weight decreased the blood glucose in a statistically insignificant rate ($p > 0.05$) to 55.3% and 60.4% respectively relative to insulin that reduced blood glucose to 28.3% in the same hour. However, at dose level of 165 and 300 mg/kg body weight, the rate of reduction in blood glucose was lower (62% and 66% respectively; $p > 0.05$) compared to that of insulin by the 6^{th} hour. The rate in blood glucose reduction caused by the four doses of the plant extract was not significantly different ($p < 0.05$) from insulin from the 12^{th} to the 24^{th} hour (Figure 4.4).

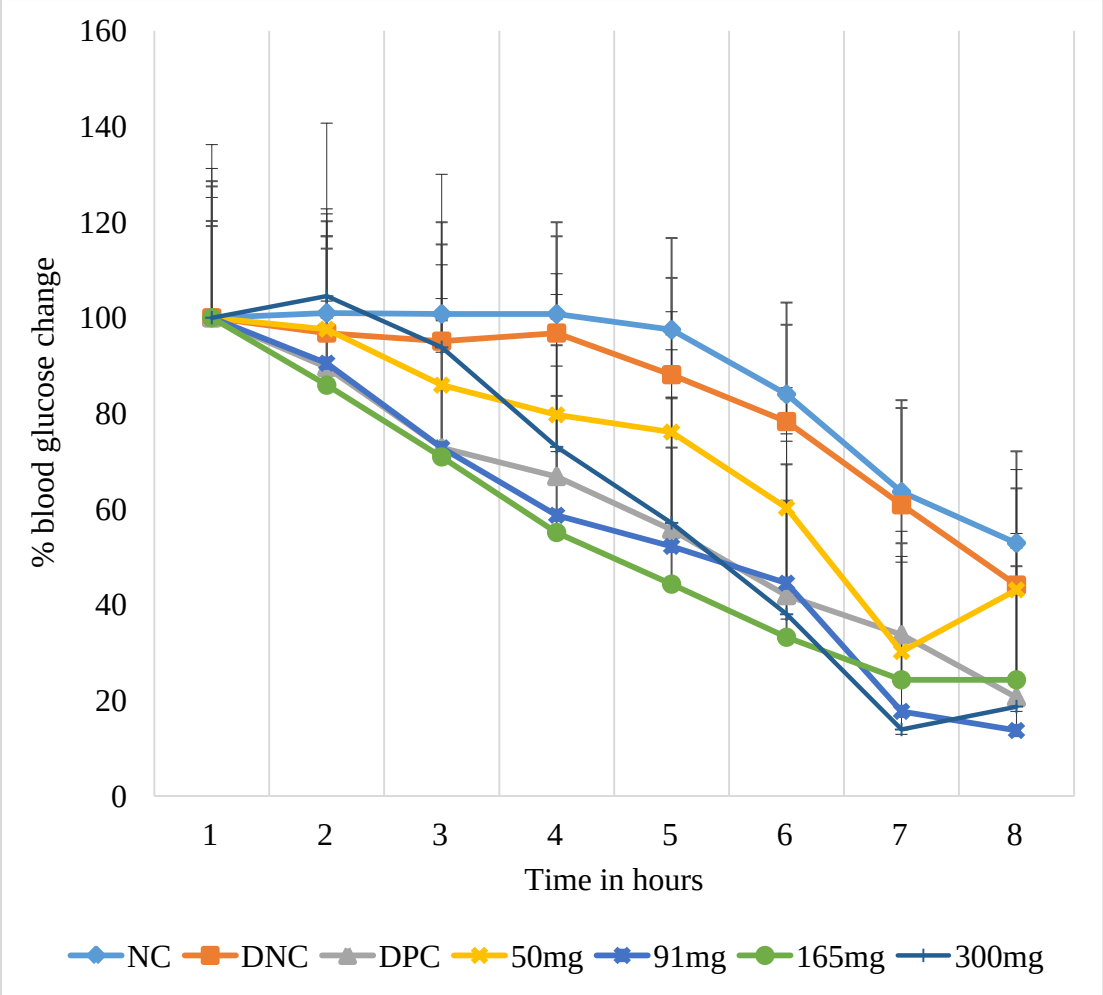

Figure 4.3: Mean percentage change in blood glucose levels post oral administration of aqueous extracts of *H. vulgare* doses in diabetic rats. NC = Normal control, DNC = Diabetic negative control, DPC = Diabetic positive control, 50, 91, 165 and 300 represents doses of *H. vulgare* (mg/kg body weght) administered.

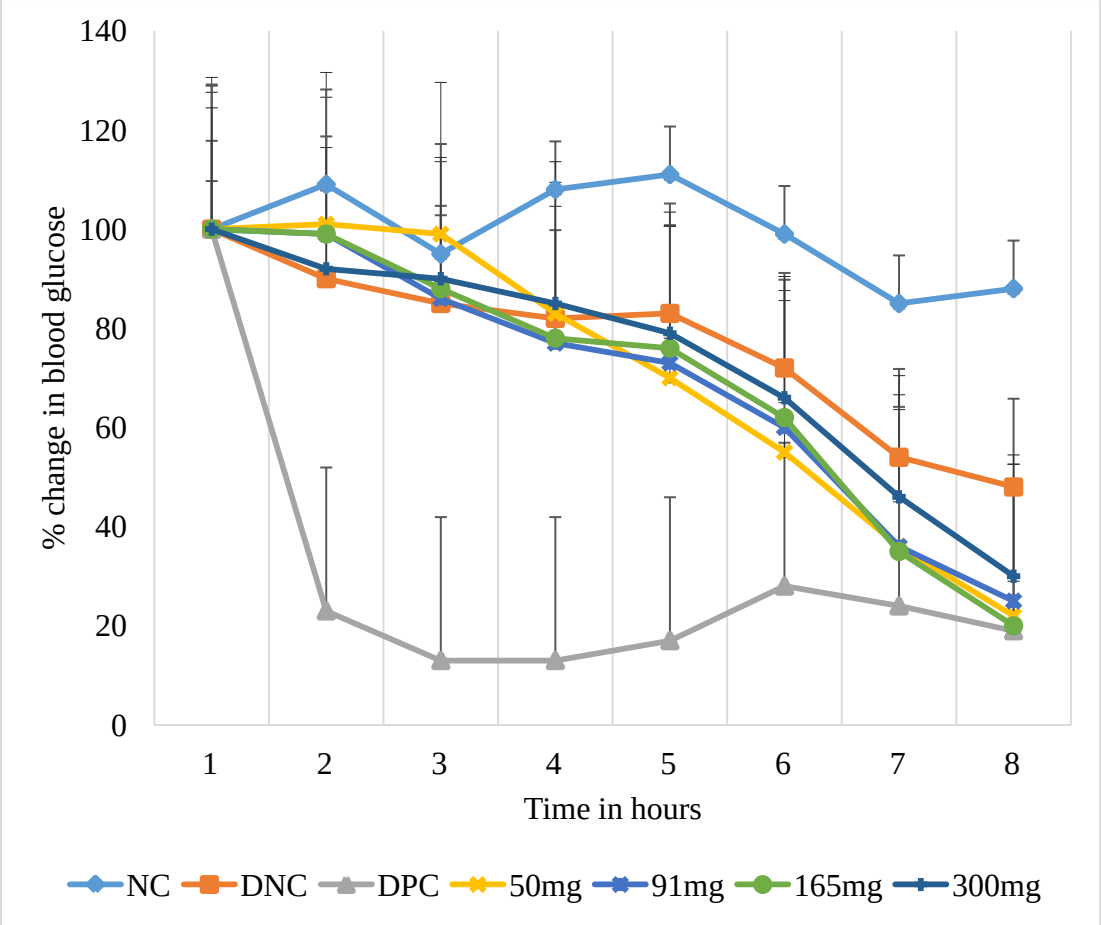

Figure 4.4: Change in blood glucose levels following intraperitoneal administration of aqueous extracts of *H. vulgare* in diabetic rats. NC = Normal control, DNC = Diabetic negative control, DPC = Diabetic positive control. 50mg, 91mg, 169mg and 300mg represents doses (mb/kg bwt) of *H. vulgare* administered.

The pharmacokinetics of *in vivo* blood glucose lowering effect of *H. vulgare* are presented in Table 4.5. While the rate constant of insulin and glibenclamide were 0.29 and 0.48, respectively, the rate constant of intraperitoneally and orally administered four doses of *H. vulgare* ranged from 0.31 to 0.49 and 0.49 to 0.66, respectively. The first order rate constants of *H. vulgare* either intraperitoneally or orally administered were higher than that of the standard drugs. The half life of 50, 91, 165 and 300 mg/kg body weight of *H. vulgare* intraperitoneally administered were 1.41, 2.17, 1.69 and 2.24 hours, respectively, and lower than that of insulin of 2.39 hours. Orally administered *H. vulgare* half life of 1.41, 1.05, 1.1 and 1.05 at specified doses respectively was lower than that of glibenclamide of 1.44 hours (Table 4.5).

Table 4.5: Four hours pharmacokinetics of *in vivo* hypoglycemic activity of orally and intraperitoneally administered aqueous extracts of *H. vulgare* in alloxan induced diabetic Wistar rats

Drug (dose)	Route	Rate constant ($hour^{-1}$)	Half-life (hours)
Insulin	IP	0.29	2.39
Glibenclamide	Oral	0.48	1.44
Extract (mg/kg body weight)			
50	IP	0.49	1.41
	Oral	0.49	1.41
91	IP	0.32	2.17
	Oral	0.66	1.05
165	IP	0.41	1.69
	Oral	0.63	1.1
300	IP	0.31	2.24
	Oral	0.66	1.05

Results are expressed as Means of rats for each time point. $T_{50} = 0.693/k$

4.2.3 Effects of aqueous extracts of *U. massaica* in alloxan induced diabetic rat model

Table 4.6 shows effect on blood glucose resulting from orally administered aqueous extract of *U. massaica* at doses of 50, 91, 165 and 300 mg/kg body weight. The four doses of *U. massaica* when orally administered caused a blood glucose lowering effect in the diabetic rat model which was not significantly different ($p > 0.05$) to that of glibenclamide throughout the experimental period. The blood glucose lowering effect of the plant extract was not dose dependant ($p > 0.05$).

Upon intraperitoneally administering the four doses of the plant extract, the blood glucose was lowered by the extract in a significantly different manner ($p < 0.05$) from that of insulin up to the 2^{nd} hour post treatments (Table 4.6). However, by the third hour, the blood glucose loweing effect by the plant extract at dose levels of 50, 165 and 300 mg/kg body weight was insignificantly different ($p > 0.05$) relative to insulin. At 91 mg/kg body weight dose of *U. massaica*, reduction in blood glucose was significantly lower ($p < 0.05$) compared to insulin from the 3^{rd} to 12^{th} hour (Table 4.6).

Table 4.6: Effects of aqueous extract of *U. massaica* logarithmic doses on blood glucose levels in diabetic rats

Treatment group		Blood glucose levels at varying times (mmol/l)							
		0hr	1hr	2hr	3hr	4hr	6hr	12hr	24hr
Physiological saline	Oral	5.8 ± 0.4^{B}	5.8 ± 0.2^{B}	5.3 ± 0.7^{B}	5.4 ± 0.7^{B}	5.2 ± 0.9^{C}	4.6 ± 0.5^{B}	4.5 ± 0.5^{B}	4.3 ± 0.8^{B}
	IP	5.2 ± 0.5^{a}	5.8 ± 0.2^{b}	5.5 ± 0.3^{bc}	5.8 ± 0.6^{c}	5.3 ± 0.3^{c}	6.1 ± 0.6^{bc}	6.0 ± 1.0^{bc}	5.1 ± 0.5^{b}
D/physiological saline	Oral	23.6 ± 3.4^{A}	23.7 ± 3.6^{A}	22.5 ± 4.5^{A}	22.3 ± 5.0^{A}	20.9 ± 4.8^{A}	16.1 ± 3.7^{A}	12.6 ± 2.4^{A}	11.1 ± 1.9^{A}
	IP	21.2 ± 6.6^{b}	20.4 ± 4.5^{a}	18.7 ± 4.8^{a}	17.0 ± 4.6^{a}	16.2 ± 3.6^{a}	15.3 ± 4.2^{a}	13.7 ± 3.4^{a}	11.6 ± 1.7^{a}
D/reference drug	Gliben	27.5 ± 4.1^{A}	23.7 ± 2.3^{A}	19.9 ± 3.8^{A}	17.1 ± 2.7^{A}	13.8 ± 4.5^{AB}	9.2 ± 5.6^{AB}	5.0 ± 1.8^{B}	4.8 ± 1.5^{B}
	Insulin	21.4 ± 4.2^{a}	3.8 ± 2.0^{b}	3.1 ± 1.3^{c}	4.1 ± 0.9^{c}	4.5 ± 1.6^{c}	4.5 ± 1.2^{c}	4.0 ± 1.1^{bc}	4.2 ± 1.5^{b}
Therapeutic aqueous extract doses (mg/kg body weight) of *U. massaica*									
50	Oral	22.5 ± 1.4^{A}	19.8 ± 2.6^{A}	17.8 ± 2.6^{A}	16.1 ± 3.6^{A}	12.4 ± 3.3^{ABC}	5.5 ± 1.2^{B}	4.1 ± 0.5^{B}	5.0 ± 0.9^{B}
	IP	16.4 ± 1.8^{a}	16.4 ± 3.4^{a}	14.3 ± 4.3^{a}	7.9 ± 3.2^{bc}	7.5 ± 2.9^{bc}	6.1 ± 1.2^{bc}	3.9 ± 1.3^{bc}	4.1 ± 1.0^{b}
90.9	Oral	26.1 ± 4.6^{A}	23.3 ± 3.8^{A}	22.1 ± 4.3^{A}	20.0 ± 3.5^{A}	16.6 ± 3.6^{AB}	11.1 ± 4.9^{AB}	5.3 ± 1.6^{B}	6.4 ± 4.4^{B}
	IP	22.8 ± 7.4^{a}	22.2 ± 6.1^{a}	16.6 ± 6.7^{a}	16.4 ± 6.2^{ab}	14.7 ± 5.2^{ab}	11.3 ± 4.4^{ab}	7.1 ± 2.3^{b}	4.6 ± 0.9^{b}
165.1	Oral	25.7 ± 5.6^{A}	21.0 ± 4.7^{A}	20.0 ± 4.5^{A}	18.1 ± 5.7^{A}	14.2 ± 5.8^{AB}	8.2 ± 3.3^{B}	4.2 ± 2.1^{B}	4.4 ± 2.6^{B}
	IP	20.5 ± 3.4^{a}	20.4 ± 5.3^{a}	17.5 ± 5.4^{a}	7.8 ± 2.3^{bc}	5.8 ± 1.6^{c}	3.6 ± 0.8^{c}	2.9 ± 0.7^{c}	4.3 ± 0.9^{b}
300	Oral	22.3 ± 3.0^{A}	19.5 ± 4.1^{A}	17.98 ± 4.4^{A}	15.8 ± 5.3^{A}	10.7 ± 5.1^{BC}	5.7 ± 5.0^{B}	4.0 ± 2.8^{B}	3.5 ± 2.0^{B}
	IP	21.0 ± 5.9^{a}	16.3 ± 1.6^{a}	13.3 ± 3.9^{ab}	9.98 ± 7.5^{abc}	8.1 ± 6.8^{bc}	6.2 ± 5.7^{bc}	2.8 ± 1.4^{c}	4.3 ± 1.6^{b}

Values are expressed as Mean ±Standard Deviation (SD) blood glucose of five rats per group. Means that share a superscripted capital or small letter in a column are not significantly different. D = diabetic, gliben = glibenclamide. Means in a column are not followed by a superscripted asterix indicating no significant difference ($p < 0.05$) between effects on blood glucose by orally and intraperitoneally administered *U. massaica*.

The blood glucose lowering rate by the plant extract was not significantly different ($p > 0.05$) from glibenclamide (Figure 4.5). An hour post treatments administration, the extract reduced blood glucose to 87.6%, 89.3%, 83.1% and 87% at dose levels of 50, 91, 165 and 300 mg/kg body weight respectively while glibenclamide reduced the blood glucose to 87.3% in the same hour. The insignificantly different ($p > 0.05$) pattern in blood glucose reduction between the extract doses and the standard oral drug persisted throughout the 24 hr experimental period (Figure 4.5).

Figure 4.6 present the rate at which blood glucose of the alloxan induced diabetic rats was reduced by the intraperitoneally administered four doses of *U. massaica* aqueous extract. Effect of the plant extract on blood glucose was delayed in the 1st hour after administration of 50, 91 and 165 mg/kg body weight doses of the extract while an immediate onset of lowering blood glucose resulted upon intraperitoneally administering 300 mg/kg body weight dose. The effect on blood glucose by the extract was dose dependent on the 1st hour though not statisticaly significant ($p > 0.05$) between the doses. At the extract dose level of 165 and 300 mg/kg body weight, blood glucose of the rats was lowered by 1.8% and 18.4% respectively while at 50 and 91 mg/kg body weight, blood glucose increased by 0.8% and 0.1 % respectively in the 1st hour. Comparatively, the rate of blood glucose reduction caused by the standard intraperitoneal drug (reduced the blood glucose to 17.6% by 1st hour) was significantly different from the the four doses of the plant's extract (Figure 4.6). The dose of 300 mg/kg body weight *U. massaica* caused a consistent decrease in blood glucose up to the 12th hour. However, at the dose levels of 50, 91 and 165 mg/kg body weight, the blood glucose lowering effect occured in phases. By the third and 4th hour, except at 91 mg ($p < 0.05$), the other doses of the extract reduced blood glucose of the

rats in an insignificantly different ($p > 0.05$) manner relative to the reference drug. From the sixth to the 24^{th} hour, the blood glucose lowering effect by all the doses of the extract was not statistically significant ($p > 0.05$) from that of insulin.

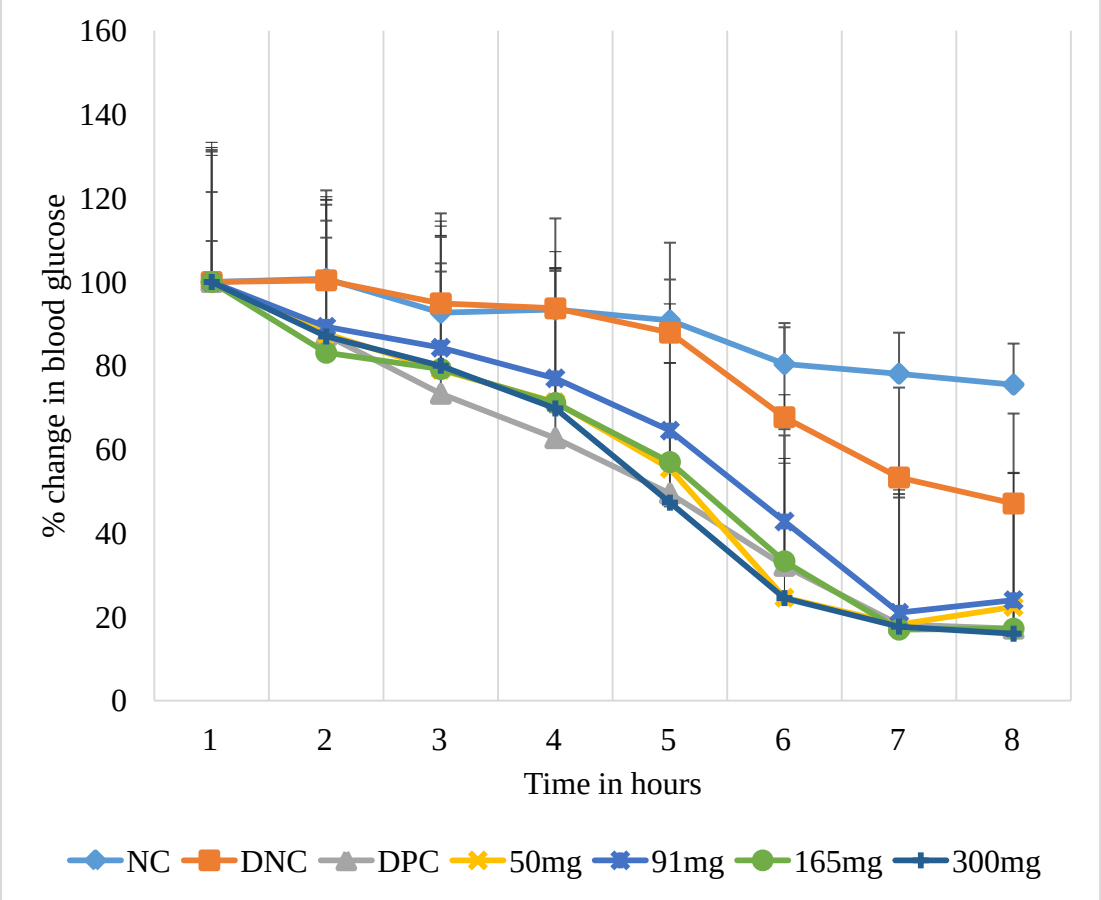

Figure 4.5: Change in blood glucose levels post oral administration of aqueous extracts of *U. massaica* doses in diabetic rats. NC = Normal control, DNC = Diabetic negative control, DPC = Diabetic positive control. 50, 91, 165 and 300 mg represents doses per kg body weight administered.

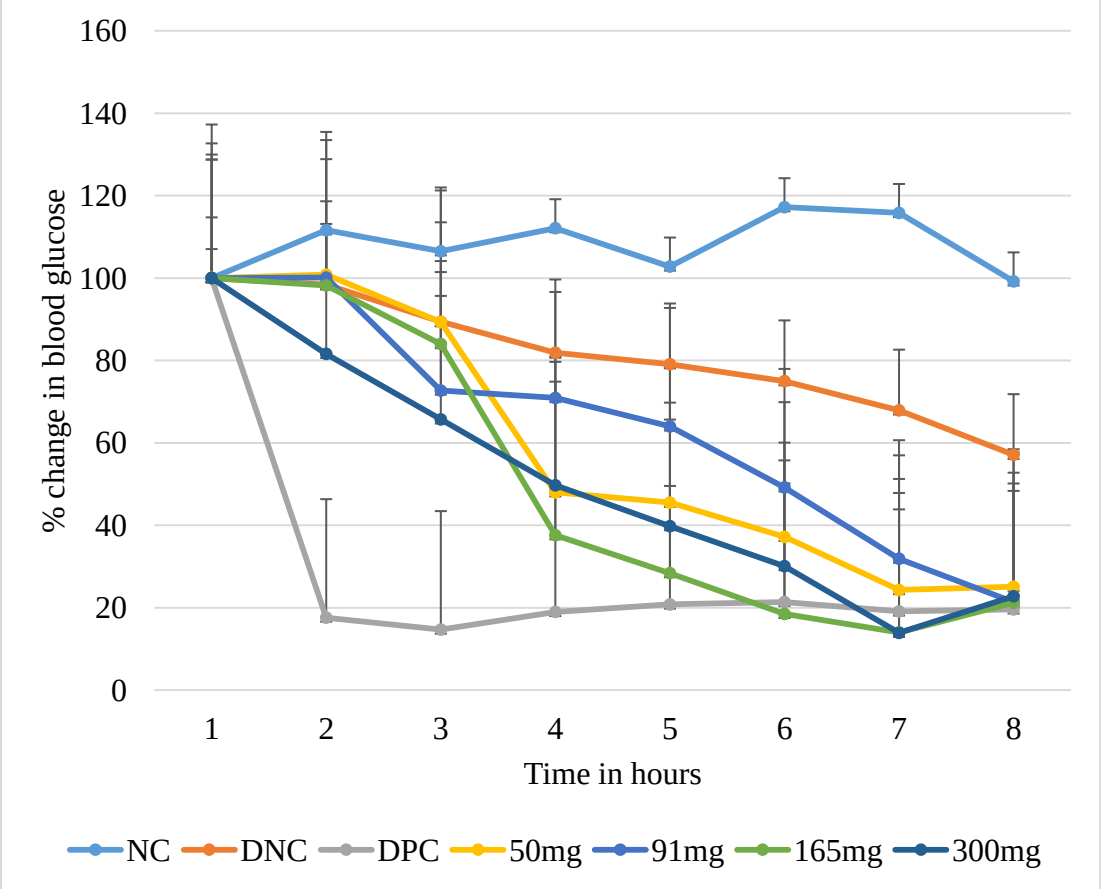

Figure 4.6: Change in blood glucose levels post intraperitoneal administration of aqueous extracts of *U. massaica* doses in diabetic rats. NC = Normal control, DNC = Diabetic negative control, DPC = Diabetic positive control. 50, 91, 165 and 300mg represents doses per kg body weight administered.

Table 4.7 presents the blood glucose pharmacokinetics of orally and intraperitoneally administered *U. massaica*. The first order rate constant of the four doses of *U. massaica* intraperitoneally administered were higher compared to that of insulin. The rate constant of 50 and 91 mg/kg body weight were both at 0.61 while 165 and 300 mg/kg body weight *U. massaica* rate constants were 0.85 and 0.99, respectively. Insulin and glibenclamide had a first order rate constant of 0.33 and 0.65, respectively. Orally administered *U. massaica* at 300 mg/kg body weight had a higher rate constant than glibenclamide while the lower doses of *U. massaica* had lower rate constant compared to glibenclamide. The half life of intraperitoneally administered *U. massaica* 50. 91, 165 and 300 mg/kg body weight were lower than that of insulin. Other than 300 mg/kg body weight *U. massaica* dose, the other doses had a higher first order rate constant.

Table 4.7: Four hours pharmacokinetics of *in vivo* hypoglycemic activity of orally and intraperitoneally administered aqueous extracts of *U. massaica* in alloxan induced diabetic Wistar rats

Drug (dose)	Route	Rate constant ($hour^{-1}$)	Half-life (hours)
Insulin	IP	0.33	2.1
Glibenclamide	Oral	0.65	0.11
Extract (mg/kg body weight)			
50	IP	0.61	1.14
	Oral	0.47	0.15
91	IP	0.61	1.14
	Oral	0.36	0.19
165	IP	0.85	0.82
	Oral	0.41	0.17
300	IP	0.99	0.7
	Oral	0.68	0.10

Results are expressed as Means of five rats for each time point. $T_{50} = 0.693/k$

4.2.4 *In vivo* hypoglycemic effects of aqueous extracts of *C. viminale* in alloxan induced diabetic rat model

Orally administered aqueous extracts of *C.viminale* at doses of 50, 91, 165 and 300 mg/kg body weight decreased the blood glucose of the rats in a statistically insignificant manner relative to the reference drug ($p > 0.05$; Table 4.8). The decline in blood glucose was not dose depedent. Blood glucose of diabetic rats treated with the four extract doses increased though not significantly ($p > 0.05$) from the 12^{th} to 24^{th} hour (Table 4.8; Figure 4.7).

Upon intraperitoneal administration, the four doses of the extract decreased the blood glucose in a significantly lower version compared to insulin up to the third hour ($p < 0.05$: Table 4.8). By the fourth hour, the blood glucose lowering effect of the extract at doses level of 50, 91 and 300 mg/kg body weight was not significantly different ($p > 0.05$) from insulin except at 165 mg/kg body weight dose of the extract. From the sixth hour, the extract reduced the blood glucose in a statistically insignificant ($p > 0.05$) manner compared to the reference drug (Table 4.8).

Table 4.8: Effects of aqueous extracts of *C. viminale* logarithmic doses on blood glucose levels in diabetic rats

Treatment group		Blood glucose levels at varying times (mmol/l)							
		0hr	1hr	2hr	3hr	4hr	6hr	12hr	24hr
Physiological saline	Oral	5.8 ± 0.4^{C}	5.8 ± 0.2^{B}	5.3 ± 0.7^{B}	5.4 ± 0.7^{C}	5.2 ± 0.9^{C}	4.6 ± 0.5^{C}	4.5 ± 0.5^{B}	4.3 ± 0.8^{B}
	IP	4.8 ± 0.2^{b}	5.2 ± 0.4^{b}	4.5 ± 0.3^{b}	5.2 ± 0.5^{bc}	5.3 ± 0.6^{b}	4.7 ± 0.3^{b}	4.1 ± 0.2^{b}	4.2 ± 0.3^{bc}
D/physiological saline	Oral	23.6 ± 3.4^{AB}	23.7 ± 3.6^{A}	22.5 ± 4.5^{A}	22.3 ± 5.0^{A}	20.9 ± 4.8^{A}	16.1 ± 3.6^{A}	12.6 ± 2.4^{A}	11.1 ± 1.9^{A}
	IP	22.3 ± 5.3^{a}	19.98 ± 4.5^{a}	18.9 ± 4.4^{a}	18.2 ± 3.9^{a}	18.6 ± 4.8^{a}	16.2 ± 5.0^{a}	11.8 ± 1.8^{a}	10.2 ± 0.6^{a}
D/reference drug	Gliben	27.5 ± 4.1^{A}	23.7 ± 2.3^{A}	19.9 ± 3.8^{A}	17.1 ± 2.7^{AB}	13.8 ± 4.5^{ABC}	9.2 ± 5.6^{ABC}	5.0 ± 1.8^{B}	4.8 ± 1.5^{B}
	Insulin	22.9 ± 5.5^{a}	5.1 ± 1.0^{b}	2.8 ± 0.5^{b}	2.8 ± 0.7^{c}	3.8 ± 0.4^{b}	6.1 ± 0.9^{ab}	5.4 ± 1.5^{b}	4.2 ± 0.9^{bc}
Therapeutic aqueous extract doses (mg/kg body weight) of *C. viminalle*									
50	Oral	26.5 ± 2.5^{AB}	22.8 ± 2.6^{A}	20.8 ± 3.3^{A}	19.6 ± 3.8^{AB}	18.1 ± 3.7^{AB}	14.5 ± 3.9^{A}	4.8 ± 1.4^{B}	6.4 ± 1.3^{B}
	IP	20.8 ± 3.9^{a}	18.3 ± 3.0^{a}	15.5 ± 4.4^{a}	14.0 ± 4.3^{ab}	12.3 ± 4.5^{ab}	10.0 ± 5.4^{ab}	5.3 ± 1.6^{b}	4.1 ± 1.0^{bc}
90.9	Oral	23.9 ± 4.3^{AB}	19.4 ± 3.6^{A}	16.8 ± 1.96^{A}	14.5 ± 2.9^{B}	11.5 ± 6.1^{BC}	6.3 ± 2.9^{BC}	3.3 ± 0.7^{B}	5.4 ± 1.2^{B}
	IP	18.4 ± 6.3^{a}	17.5 ± 7.9^{a}	16.9 ± 7.7^{a}	14.0 ± 6.1^{ab}	10.4 ± 6.5^{ab}	8.8 ± 6.4^{ab}	5.1 ± 2.2^{b}	3.8 ± 0.8^{bc}
165.1	Oral	20.0 ± 4.7^{B}	21.4 ± 4.9^{A}	20.7 ± 6.8^{A}	17.3 ± 4.7^{AB}	16.0 ± 5.4^{AB}	12.4 ± 4.8^{AB}	5.0 ± 1.4^{B}	4.8 ± 0.9^{B}
	IP	17.5 ± 5.1^{a}	22.1 ± 6.1^{a}	20.0 ± 5.1^{a}	17.2 ± 5.2^{a}	16.1 ± 5.5^{a}	12.3 ± 7.5^{ab}	8.1 ± 3.1^{ab}	5.9 ± 2.1^{b}
300	Oral	21.5 ± 2.4^{AB}	20.2 ± 2.5^{A}	18.0 ± 2.1^{A}	17.6 ± 3.1^{AB}	15.4 ± 3.9^{AB}	8.7 ± 3.3^{ABC}	5.1 ± 0.8^{B}	5.2 ± 1.5^{B}
	IP	18.3 ± 5.8^{a}	15.7 ± 5.8^{a}	14.6 ± 6.7^{a}	12.5 ± 6.6^{ab}	12.0 ± 8.5^{ab}	8.8 ± 5.6^{ab}	5.5 ± 2.8^{b}	3.2 ± 0.9^{c}

Values are expressed as Mean ±Standard Deviation (SD) blood glucose of five rats per group. Means that share a superscripted capital or small letter in a column are not significantly different. D = diabetic, Gliben = glibenclamide. Means in a column are not followed by a superscripted asterix indicating no significant difference ($p < 0.05$) between effects on blood glucose by orally and intraperitoneally administered extract.

Up to the third hour post treatment administration, *C. viminalle* at dose level of 165 mg decreased the blood glucose at a lower rate ($p < 0.05$) than the reference drug. However, the trend in reduction of blood glucose by the other extract's three doses (50, 91 and 300 mg/kg body weight) was not significantly different ($p > 0.05$) from the standard oral drug during the same period (Figure 4.7). By the third hour, the extract dose of 165 mg/kg body weight lowered blood glucose to 86% ($p < 0.05$) compared to glibenclamide which decreased the blood glucose to 62.7%. At the same time, *C. viminalle* doses of 50, 91 and 300 mg/kg body weight reduced the blood glucose to 73.8%, 61.8% and 81.7% respectively which was not significantly different ($p > 0.05$) relative to the oral standard drug. On the fourth hour, at the dose level of 50, 91, 165 and 300 mg/kg body weight, the extract had reduced the blood glucose to 68.1%, 47.8%, 78.2% and 71.3% respectively ($p > 0.05$) compared to glibenclamide which reduced blood glucose to 49.5% in the same hour. The blood glucose lowering effect by all the four doses of the extract was statistically insignificant ($p > 0.05$) relative to the reference drug from the 4^{th} to the 24^{th} hour (Figure 4.7).

As shown in Figure 4.8, the blood glucose lowering rate of the extract an hour after intraperitoneally administering the treatments was significantly different ($p < 0.05$) to that of insulin. While at the extract's dose level of 165 mg/kg body weight an increase in blood glucose to 129% occured, a reduction in blood glucose to 88.5%, 92% and 88% resulted from extract administered at the dose level of 50, 91 and 300 mg/kg body weight respectively and the reference drug decreased the blood glucose to 22.7% in the same hour significantly different ($p < 0.05$) from the extract treated diabetic rats. In the 6^{th} hour, the extract had reduced the blood glucose as effectively as insulin except at the dose level of 165 mg/kg body weight. In this hour, the extract

reduced the blood glucose to 46.9%, 44.0% and 46.4% respectively at the dose levels of 50, 91 and 300 mg/kg body weight which was not significantly different ($p < 0.05$) from the reference drug that reduced the blood glucose to 28.3% in the same hour. During this time, the extract administered at 165 mg/kg body weight dose lowered the blood glucose to 70.2% which was a significantly lower rate ($p > 0.05$) compared to the standard intraperitoneal drug. The trend in the rate of blood glucose reduction by the extract recorded in the 6^{th} hour relative to the reference drug remained similar up to the 24^{th} hour (Figure 4.8).

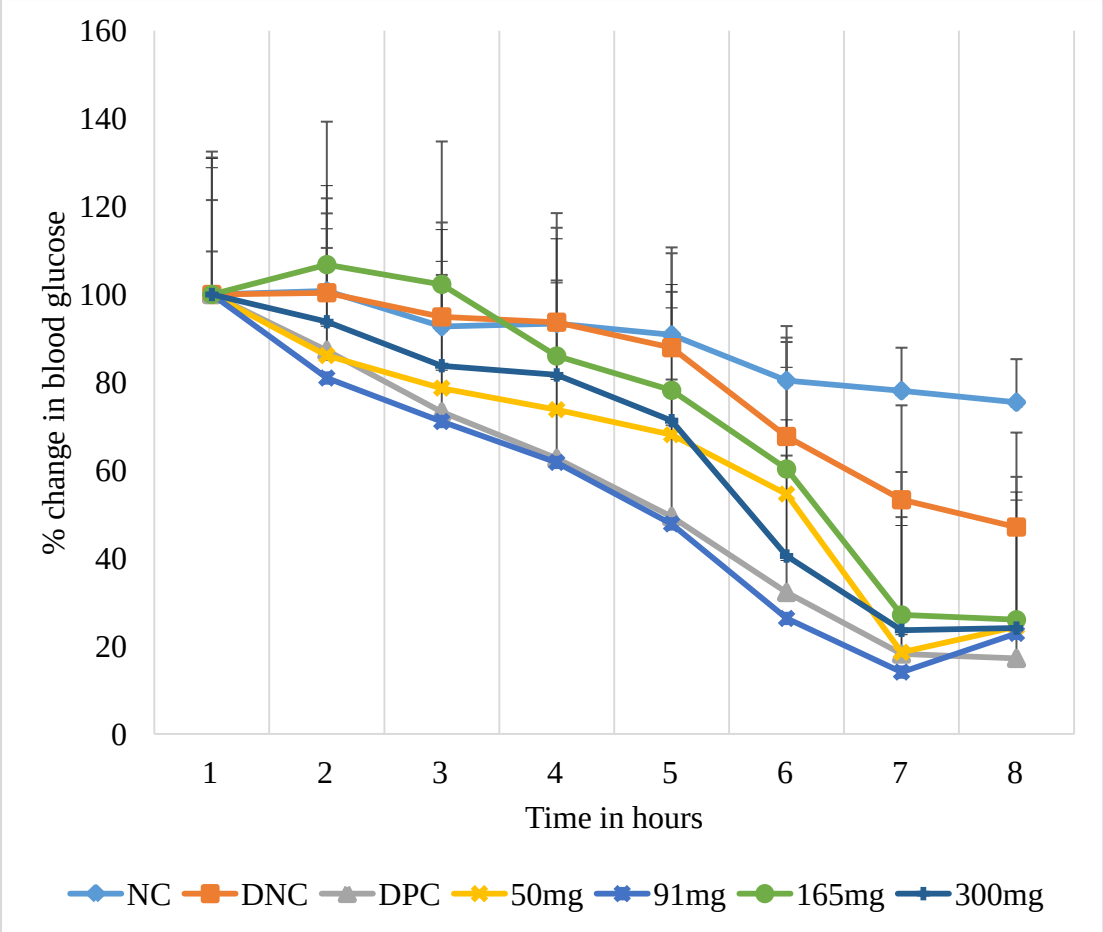

Figure 4.7: Change in blood glucose levels following oral administration of aqueous extracts of *C. viminale* doses in diabetic rats. NC = Normal control, DNC = Diabetic negative control, DPC = Diabetic positive control. 50, 91, 165 and 300mg represents doses of extract per kg body weight administered.

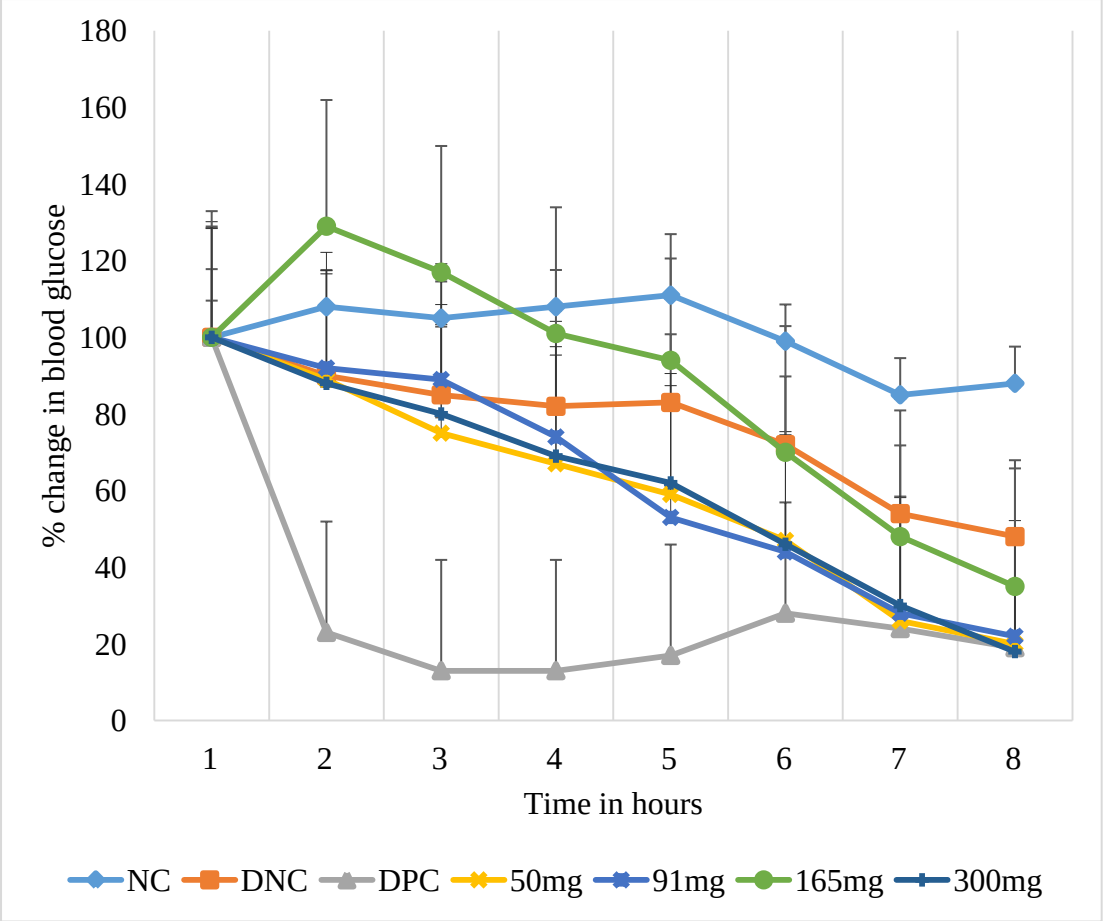

Figure 4.8: Change in blood glucose levels following intraperitoneal administration of aqueous extracts of *C. viminale* in diabetic rats. NC = Normal control, DNC = Diabetic negative control, DPC = Diabetic positive control. 50mg, 91mg, 165mg and 300 mg represents doses per kg bwt administered.

Pharmacokinetics for orally and intraperitoneally administered *C. viminale* indicates a first order rate constant of 0.31 and 0.6 for insulin and glibenclamide respectively. The rate constant for intraperitoneally administered *C. viminale* doses of 50, 91, 165 and 300 mg/kg body weight was higher compared to insulin. The orally administered four specified doses of *C. viminale* extract had lower rate constants than the standard oral drug glibenclamide. While the half life of intraperitoneally administered *C. viminale* was lower for all the four doses compared to insulin, it was higher for the orally administered extract compared to glibenclamide (Table 4.9).

Table 4.9: Four hours pharmacokinetics of *in vivo* hypoglycemic activity of orally and intraperitoneally administered aqueous extracts of *C. viminale* in alloxan induced diabetic Wistar rats

Drug (dose)	Route	Rate constant ($hour^{-1}$)	Half-life (hours)
Insulin	IP	0.31	2.24
Glibenclamide	Oral	0.6	1.16
50	IP	0.45	1.54
	Oral	0.35	1.98
91	IP	0.59	1.18
	Oral	0.54	1.28
165	IP	0.39	1.78
	Oral	0.39	1.78
300	IP	0.44	1.58
	Oral	0.43	1.61

Results are expressed as Means of five rats for each time point. $T_{50} = 0.693/k$

4.3 Evaluation of uptake safety of aqueous extracts of *T. aestivum*

4.3.1 Effects of orally and intraperitoneally administered aqueous extract of *T. aestivum* on the relative organ weight of Wistar rats

Orally administered aqueous extracts of *T. aestivum* at 300, 448, 669 and 1000 mg/kg body weight did not have significant different ($p > 0.05$) effect on the general body weight of the rats compared to the control. Likewise, the average weekly change in body weight resulting from the four administered extract doses did not have any significant variation ($p > 0.05$) from the control (Table 4.10). Orally administered four doses (300, 448, 669, 1000 mg/kg body weight) of aqueous extract of *T. aestivum* in normal rats did not affect the organ and percentage organ to body weight of the heart, kidney, lungs, spleen and brain of the rats differently ($p > 0.05$) to the physiologic saline administered in control rats (Table 4.10). However, the four doses of the extract resulted to a dose independent significantly lower ($p < 0.05$) liver weights compared to the control. Orally administered dose levels of 669 and 1000 mg/kg body weight of the extract caused a dose dependent significantly lower ($p < 0.05$) percentage organ to body weight of the liver compared to that of the control. The percentage organ to body weight of the liver was not significantly affected ($p > 0.05$) by orally administered *T. aestivum* at doses of 300 and 448 mg/kg body weight relative to the control (Table 4.10).

Table 4.11 shows the effect on the relative weight of the rats upon intraperitoneal administration of the four non therapeutic doses of aqueous extract of *T.* aestivum. There was consistent increase in the general body weight as a consequence of the extract administration. The increase in weight was not significantly different ($p > 0.05$) between the four doses of *T. aestivum* administered and control rats. Further, the

average week gain was not significantly different ($p > 0.05$) between the control rats and *T. aestivum* treated rats (Table 4.11). Intraperitoneally administered non therapeutic doses of aqueous extract of *T. aestivum* used in this study did not affect the organ and percentage organ weight of the heart, lungs, spleen and brain of the rats differently ($p > 0.05$) to the normal control (Table 4.11). Though the percentage organ to body weight of the extract treated rat's kidney was not significantly different ($p > 0.05$) to the control, the organ's weight was significantly higher ($p < 0.05$) for all the four extract doses relative to the control. Intraperitoneally administered *T. aestivum* did not have a significant effect ($p > 0.05$) on the actual liver weight compared to physiological saline administration. However, the percentage organ to body weight of the liver was significantly higher ($p < 0.05$) than the control rats at the dose level of 669 and 1000 mg/kg body weight *T. aestivum* extract.

Table 4.10: Effects of orally administered logarithmic doses of aqueous extract of *T. aestivum* on organ and percentage organ to body weight

Treatment	Δ weight change (g/week)	Organ (g) and percent organ to body weight (g/100g)					
		Heart	Kidney	Lungs	Spleen	Liver	Brain
Control	11.8± 11.2[a]	0.6±0.1[a] (0.4±0.0)[a]	1.5±0.3[a] (0.9±0.1)[a]	1.6±0.5[a] (0.9±0.2)[a]	1.2±0.3[a] (0.7±0.2)[a]	9.1±1.4[a] (5.5±0.5)[a]	1.2±0.2[a] (0.8±0.2)[a]
Extract dose (mg/kg body weight)							
300	8.8±7.4[a]	0.5±0.1[a] (0.4±0.0)[a]	1.2±0.2[a] (0.8±0.1)[a]	1.5±0.6[a] (1.0±0.6)[a]	0.9±0.3[a] (0.6±0.2)[a]	6.2±0.9[b] (4.2±0.4)[ab]	1.2±0.2[a] (0.8±0.2)[a]
448	8.2±7.9[a]	0.5±0.1[a] (0.4±0.1)[a]	1.2±0.2[a] (0.9±0.2)[a]	1.3±0.2[a] (0.9±0.1)[a]	0.9±0.2[a] (0.6±0.2)[a]	6.4±1.5[b] (4.3±1.2)[ab]	1.3±0.2[a] (0.9±0.2)[a]
669	9.4±7.5[a]	0.6±0.1[a] (0.4±0.0)[a]	1.3±0.2[a] (0.8±0.1)[a]	1.3±0.2[a] (0.8±0.0)[a]	1.0±0.3[a] (0.7±0.2)[a]	6.4±1.6[b] (4.0±0.5)[b]	1.3±0.1[a] (0.9±0.2)[a]
1000	8.7±8.1[a]	0.6±0.1[a] (0.4±0.0)[a]	1.1±0.1[a] 0.8±(0.1)[a]	1.2±0.3[a] (0.8±0.3)[a]	0.7±0.2[a] (0.5±0.1)[a]	5.3±1.0[b] (3.5±0.5)[b]	1.4±0.1[a] (0.9±0.2)[a]

Results are expressed as Mean ± Standard deviation (SD) for five rats per group. Percentage organ to body weight means are in parentheses. Values that share a superscripted letter down the columns are not significantly different.

Table 4.11: Effects of intraperitoneally administered logarithmic doses of aqueous extract of *T. aestivum* on the organ and percentage organ to body weight of rats

Treatment	Δ weight change (g)	Organ (g) and percent organ to body weight (g/100g)					
		Heart	Kidney	Lungs	Spleen	Liver	Brain
Control	15±15[a]	0.8±0.4[a] (0.4±0.2)[a]	0.9±0.3[b] (0.5±0.2)[a]	1.6±0.2[a] (0.9±0.1)[a]	1.1±0.3[a] (0.6±0.2)[a]	7.0±0.9[ab] (3.8±0.5)[b]	1.0±0.1[a] (0.6±0.1)[a]
Extract dose (mg/kg body weight)							
300	12±9[a]	0.5±0.1[a] (0.3±0.0)[a]	1.4±0.2[a] (0.8±0.05)[a]	1.5±0.2[a] (0.9±0.3)[a]	0.8±0.2[a] (0.5±0.1)[a]	6.4±1.3[b] (3.8±0.6)[b]	1.2±0.1[a] (0.7±0.2)[a]
448	15±12[a]	0.6±0.1[a] (0.3±0.1)[a]	1.4±0.1[a] (0.8±0.1)[a]	1.4±0.3[a] (0.8±0.1)[a]	0.9±0.3[a] (0.5±0.2)[a]	7.8±1.0[ab] (4.4±0.8)[ab]	1.1±0.1[a] (0.6±0.1)[a]
669	10±7[a]	0.6±0.1[a] (0.3±0.0)[a]	1.3±0.2[a] (0.8±0.1)[a]	1.3±0.2[a] (0.8±0.1)[a]	1.1±0.4[a] (0.7±0.2)[a]	8.6±0.6[a] (5.3±0.7)[a]	1.0±0.2[a] (0.6±0.1)[a]
1000	9±6[a]	0.5±0.04[a] (0.4±0.1)[a]	1.3±0.2[a] (0.8±0.1)[a]	1.5±0.1[a] (0.98±0.1)[a]	0.7±0.1[a] (0.5±0.1)[a]	7.8±1.0[ab] (5.3±1.0)[a]	1.0±0.1[a] (0.7±0.1)[a]

Results are expressed as Mean ± Standard deviation (SD) for five rats per group. Percentage organ to body weight means are in parentheses. Values that share a superscripted letter down the columns are not significantly different.

4.3.2 Effects of orally and intraperitoneally administered aqueous extracts of *T. aestivum* on hematological parameters of Wistar rats

Hematological parameters for red blood cells and platelets post oral and intraperitoneal administration of *T. aestivum* at doses of 300, 448, 669 and 1000 mg/kg body weight are presented in Table 4. 12 and 4.13, respectively.

Orally administered four non-therapeutic doses of aqueous extract of *T. aestivum* did not affect the red blood cells significantly ($p > 0.05$) relative to normal control. The extract administered doses caused dose independent higher haemonglobin level than the control though the levels were not significantly different ($p > 0.05$) except at dose level of 448 mg/kg body weight. The packed cell volume (PCV), mean cell volume (MCV), Mean cell hemoglobin (MCH) and mean cell hemoglobin concentration (MCHC) were not affected by orally administered *T. aestivum* doses used in this study. Likewise, there was no effect on platelets that resulted due to orally administered non-therapeutic doses of *T. aestivum* different from the control (Table 4.12). The results indicate no effect on the total white blood cell (WBC) and differential counts that resulted from administration of 300, 448, 669 and 1000 mg/kg body weight *T. aestivum* significantly different ($p > 0.05$) to the control by either route of administration.

Intraperitoneally administered non therapeutic doses of *T. aestivum* did not affect the RBC count and the rest of the erythrocytes indices and platelets differently ($p > 0.05$) compared to the control except decreased levels ($p < 0.05$) of MCH at doses of 300 and 1000 mg/kg body weight of the aqueous extract of *T. aestivum*.

Table 4.12: Effects of orally administered logarithmic doses of aqueous extract of *T. aestivum* on rat's haematological parameters

Haematological parameter		Normal	Doses of *T. aestivum* administered (mg/kg body weight)			
			300	448	665	1000
RBC ($\times 10^6/\mu L$)	Oral	6.1 ± 1.1^a	7.1 ± 0.4^a	7.4 ± 0.5^a	6.7 ± 0.2^a	6.7 ± 1.0^a
Hb (g/dL)	Oral	12.2 ± 0.8^b	13.5 ± 0.4^{ab}	14.3 ± 1.2^a	13.2 ± 1.0^{ab}	12.9 ± 1.3^{ab}
PCV (%)	Oral	41.5 ± 9.0^a	44.9 ± 5.9^a	48.7 ± 2.8^a	44.0 ± 3.4^a	42.9 ± 5.6^a
MCV (fL)	Oral	67.7 ± 3.5^a	63.4 ± 8.4^a	65.8 ± 7.2^a	65.6 ± 5.1^a	64.8 ± 7.5^a
MCH (pg)	Oral	20.5 ± 4.5^a	19.0 ± 1.0^a	19.2 ± 1.0^a	19.7 ± 1.5^a	19.5 ± 2.6^a
MCHC (g/dL)	Oral	30.4 ± 7.1^a	30.4 ± 4.3^a	29.6 ± 3.7^a	30.3 ± 3.8^a	30.3 ± 4.8^a
PLT ($\times 10^3/\mu L$)	Oral	632 ± 217^a	569 ± 295^a	789 ± 158^a	746 ± 113^a	752 ± 194^a
WBC ($x10^9/L$)	Oral	3.8 ± 1.3^a	5.4 ± 4.2^a	4.7 ± 2.3^a	5.1 ± 2.9^a	4.8 ± 1.9^a
Neutrophils	Oral	0.9 ± 0.7^a	0.6 ± 0.4^a	0.9 ± 0.7^a	1.6 ± 1.3^a	0.8 ± 0.3^a
Lymphocytes	Oral	1.9 ± 1.1^a	3.8 ± 3.6^a	2.4 ± 2.1^a	2.8 ± 2.6^a	2.3 ± 1.0^a
Monocytes	Oral	0.7 ± 0.6^a	0.7 ± 0.7^a	0.5 ± 0.3^a	0.7 ± 0.5^a	0.9 ± 0.8^a
Eosinophils	Oral	0.1 ± 0.1^a	0.1 ± 0.1^a	0.3 ± 0.0^a	0.1 ± 0.1^a	0.1 ± 0.1^a
Basophils	Oral	0.1 ± 0.1^a	0.0 ± 0.0^a	0.1 ± 0.0^a	0.1 ± 0.0^a	0.1 ± 0.1^a

Results are expressed as Mean ± Standard Deviation (SD) for five rats per group. Values that share a superscripted letter along the row are not significantly different at $p<0.05$. RBC = red blood cell count, Hb = hemoglobin, PCV = packed red cell volume, MCV = mean cell volume, MCH = mean corpuscular hemoglobin, MCHC = mean corpuscular hemoglobin concentration, PLT= platelets. WBC = white blood cells count.

Table 4.13: Effects of intraperitoneally administered logarithmic doses of aqueous extract of *T. aestivum* on rat's haematological parameters

Haematological parameter		Normal	Doses of *T. aestivum* administered (mg/kg body weight)			
			300	448	665	1000
RBC ($\times 10^6/\mu L$)	IP	6.4±0.5[a]	7.5±1.1[a]	8.1±1.0[a]	6.7±0.9[a]	6.5±3.1[a]
Hb (g/dL)	IP	13.2±0.7[a]	13.7±1.4[a]	15.2±1.5[a]	12.8±1.5[a]	13.4±1.6[a]
PCV (%)	IP	47.1±1.5[a]	48.0±5.9[a]	51.7±3.4[a]	47.4±6.3[a]	47.7±6.6[a]
MCV (fL)	IP	73.9±4.9[a]	64.2±3.3[a]	64.8±5.2[a]	71.9±11.8[a]	65.7±6.2[a]
MCH (pg)	IP	20.7±1.1[a]	18.3±0.9[b]	19.0±0.6[ab]	19.3±1.0[ab]	18.1±1.5[b]
MCHC (g/dL)	IP	28.0±1.1[a]	28.6±1.5[a]	29.4±1.8[a]	27.2±3.1[a]	28.4±1.1[a]
PLT ($\times 10^3/\mu L$)	IP	799±163[a]	690±160[a]	888±183[a]	940±296[a]	849±191[a]
WBC ($x10^9/L$)	IP	7.7±6.0[a]	6.5±1.3[a]	6.9±3.2[a]	11.5±8.1[a]	6.4±3.2[a]
Neutrophils	IP	1.0±0.5[a]	1.0±0.5[a]	1.0±0.5[a]	1.3±1.0[a]	1.4±1.1[a]
Lymphocytes	IP	5.8±5.7[a]	4.5±1.1[a]	5.1±3.4[a]	9.5±7.0[a]	4.2±2.6[a]
Monocytes	IP	0.5±0.4[a]	0.6±0.6[a]	0.5±0.5[a]	0.5±0.5[a]	0.5±0.5[a]
Eosinophils	IP	0.1±0.1[a]	0.1±0.1[a]	0.1±0.1[a]	0.1±0.1[a]	0.0±0.0[a]
Basophils	IP	0.2±0.2[a]	0.3±0.2[a]	0.1±0.1[a]	0.1±0.1[a]	0.2±0.1[a]

Results are expressed as Mean ± Standard Deviation (SD) for five rats per group. Values that share a superscripted letter along the row are not significantly different at $p<0.05$. RBC = red blood cell count, Hb = hemoglobin, PCV = packed red cell volume, MCV = mean cell volume, MCH = mean corpuscular hemoglobin, MCHC = mean corpuscular hemoglobin concentration, PLT= platelets. WBC = white blood cells count.

4.3.3 Effects of orally and intraperitoneally administered aqueous extracts of *T. aestivum* on biochemical parameters of Wistar rats

In vivo effects of aqueous extracts of *T. aestivum* on cardiac and pancreatic organs of normal rats are presented in Table 4.14 and Table 4.15. Orally administered aqueous extract at dose levels of 300, 448, 669 and 1000 mg/kg body weight did not affect cardiac and pancreatic functions significantly different ($p > 0.05$) from the control (Table 4.14). Further, the results indicate that the orally administered plant extract at the four stated doses did not have a significantly different ($p > 0.05$) effect on the function of the liver relative to the control (Table 4.14). As presented in Table 4.14, control rats had significantly increased potassium levels compared to normal rats orally administered the four doses of *T. aestivum*.

Further, the four doses of the plant extract when intraperitoneally administered did not affect the biomarkers for cardiac and pancreatic functions significantly different ($p > 0.05$) from the control (Table 4.15). Intraperitoneal administration of 1000mg *T. aestivum* increased the alkaline phosphatase (ALP) levels considerably compared with the control rats. However, the effect at the *T. aestivum* highest dose was not different from other *T. aestivum* doses intraperitoneally administered. Administration of *T. aestivum* at 300, 448 and 669 mg/kg body weight did not affect the ALP levels relative to the control rats (Table 4.15). Intraperitoneally administered *T. aestivum* at doses of 300, 669 and 1000 mg/kg body weight doses increased the urea levels of the rats compared to the control rats. The effect in urea levels was not different between the control rats and 448 mg/kg body weight *T. aestivum* treated rats. Creatinine, sodium and potassium levels were not affected differently by intraperitoneal administration of the four doses of *T. aestivum* compared to control rats (Table 4.15).

Table 4.14: Effects of orally administered logarithmic doses of aqueous extract of *T. aestivum* on cardiac and pancreatic function tests of rats

Biochemistry parameter	Normal	Doses of *T. aestivum* administered (mg/kgbwt)			
		300	448	665	1000
LDH (IU/L)	1517±814[a]	1990±621[a]	1344±223[a]	1478±580[a]	1674±230[a]
CK (UI/L)	909±447[a]	1335±1103[a]	2587±1103[a]	2928±1333[a]	1923±1510[a]
AMYLASE (IU/L)	1346±409[a]	1318±511[a]	1268±304[a]	1228±410[a]	901±206[a]
LIPASE (IU/L)	16±1.0[a]	15±0.8[a]	16±2.6[a]	14±0.9[a]	15±0.5[a]
AST (IU/L)	415±191[a]	639±394[a]	449±211[a]	546±179[a]	759±197[a]
ALT (IU/L)	185±44[a]	228±159[a]	113±29[a]	224±181[a]	160±56[a]
ALP (IU/L)	283±142[a]	249±127[a]	227±130[a]	234±158[a]	162±81[a]
GGT (IU/L)	3.5±2.1[a]	4.4±2.4[a]	2.6±1.8[a]	8.3±3.7[a]	7.6±4.0[a]
TP (g/L)	70±16[a]	76±7[a]	70±3[a]	69±3[a]	70±5[a]
ALB(g/L)	34±8[a]	30±5[a]	30±9[a]	28±3[a]	25±3[a]
UREA (mmol/L)	11±2[a]	12±1[a]	11±3[a]	11±1[a]	10±2[a]
CREA (µmol/L)	106±19[a]	86±10[a]	79±19[a]	85±24[a]	74±12[a]
Na^+(mmol/L)	156±1[a]	158±5[a]	153±3[a]	154±6[a]	150±3[a]
K^+(mmol/L)	22±8[a]	11±3[b]	9±1[b]	10±3[b]	9±1[b]

Results are expressed as Mean ± Standard Deviation (SD) for five rats per group. Values that share a superscripted letter along the row are not significantly different at $p < 0.05$. LDH = lactate dehydrogenase, CK = creatine phosphokinase, AST = aspartate aminotransferase, ALT = alanine aminotransferase, ALP = alkaline phosphatase, GGT = gamma glutamyl transferase, TP = total protein and ALB = albumin.

Table 4.15: Effects of intraperitoneally administered logarithmic doses of aqueous extract of *T. aestivum* on biochemical parameters of rats

Biochemistry parameter	Normal	Doses of *T. aestivum* administered (mg/kg body weight)			
		300	448	669	1000
LDH (IU/L)	605±154[a]	609±238[a]	438±273[a]	473±278[a]	476±302[a]
CK (UI/L)	2557±1180[a]	2700±1530[a]	1268±1217[a]	2402±1575[a]	1555±1171[a]
AMYLASE (IU/L)	1039±157[a]	1126±75[a]	1118±180[a]	1036±78[a]	1146±42[a]
LIPASE (IU/L)	30±0.5[a]	33±5.2[a]	29±3.0[a]	31±4.1[a]	32±3.7[a]
AST (IU/L)	284±98[a]	239±146[a]	156±81[a]	224±82[a]	302±129[a]
ALT (IU/L)	121±14[a]	219±181[a]	152±81[a]	175±67[a]	154±34[a]
ALP (IU/L)	209±38[b]	227±33[ab]	270±109[ab]	361±165[ab]	459±211[a]
GGT (IU/L)	3.4±1.1[a]	5.6±3.7[a]	4.8±2.1[a]	5.0±3.7[a]	4.4±1.7[a]
TP (g/L)	62±2.9[a]	64±2.6[a]	66±4.3[a]	63±7.2[a]	68±3.9[a]
ALB(g/L)	32±2.7[ab]	30±1.6[ab]	33±1.1[a]	28±2.9[b]	29±2.2[ab]
UREA (mmol/L)	9.0±0.8[b]	11.7±2.1[a]	10.3±1.5[ab]	11.7±0.7[a]	11.5±0.8[a]
CREATININE (µmol/L)	57.8±3.4[a]	57.4±4.6[a]	54.8±5.9[a]	56.2±5.6[a]	55.8±3.8[a]
SODIUM (mmol/L)	149±2.5[a]	152±2.2[a]	150±3.5[a]	152±3.7[a]	152±5.9[a]
POTASSIUM (mmol/L)	6.6±0.9[a]	8.7±1.5[a]	8.1±3.2[a]	10.96±4.2[a]	8.3±3.4[a]

Results are expressed as Mean ± Standard Deviation (SD) for five rats per group. Values that share a superscripted letter along the row are not significantly different at $p < 0.05$. LDH = lactate dehydrogenase, CK = creatine phosphokinase, AST = aspartate aminotransferase, ALT = alanine aminotransferase, ALP = alkaline phosphatase, GGT = gamma glutamyl transferase, TP = total protein and ALB = albumin.

4.4 Evaluation of *in vivo* safety of aqueous extracts of *U. massaica*

4.4.1 Effects of orally and intraperitoneally administered aqueous extract of *U. massaica* on the relative organ weights of Wistar rats

Orally administered extract of *U. massaica* at dose levels of 300, 448, 669 and 1000 mg/kg body weight did not affect the general body weight of the rats differently ($p > 0.05$) from the control. Consequently, the extract did not affect the weight of the heart, kidney, lungs, spleen and brain differently from the control. However, 300 mg/kg body weight *U. massaica* reduced the weight of the liver compared to the control. The percentage organ to body weight of the spleen and liver was reduced by orally administered 300 mg/kg body weight *U. massaica* extract relative to the control. *U. massaica* at the dose level of 448, 669 and 1000 mg/kg body weight did not affect the percentage organ to body weight different from that of the control (Table 4.16).

Intraperitoneally administered *U. massaica* aqueous extract at the four doses resulted into the death of the rats within the 1st week of administration. By the end of the seven days, 20%, 60%, 100% and 80% mortality was recorded for 300, 448, 669 and 1000 mg/kg body weight respectively resulting to exit of the experiment at this doses. Intraperitoneally administered *U. massaica* at 300 mg/kg body weight increased the weight of the rats though not significantly different to control rats. Further, the extract dose caused an increase in the weight of the liver relative to control rats ($p < 0.05$). However, there was no significant difference ($p > 0.05$) between the control and *U. massaica* treated rat's percentage organ to body weight of the liver. The effects of intraperitoneally administered *Urtica massaica* at 300 mg/kg body weight on the heart, kidney, lungs, spleen and brain was not significantly different to the physiological saline administered control rats ($p >0.05$; Table 4.17).

Table 4.16: Effects of orally administered logarithmic doses of aqueous extract of *U. massaica* on the organ and percentage organ to body weights of rats

Treatment	Δ w**ee**kly weight change (g)	Organ (g) and percent organ to body weight (g/100g)					
		Heart	Kidney	Lungs	Spleen	Liver	Brain
Control	11.8±11.2[a]	0.6±0.1[a] (0.4±0.0)[a]	1.5±0.3[a] (0.9±0.1)[a]	1.6±0.5[a] (0.9±0.2)[a]	1.2±0.3[a] (0.7±0.2)[a]	9.1±1.4[a] (5.5±0.5)[a]	1.2±0.2[a] (0.8±0.2)[a]
Extract dose (mg/kgbwt)							
300	12.8±9.3[a]	0.6±0.1[a] (0.3±0.0)[a]	1.4±0.1[a] (0.8±0.0)[a]	1.7±0.1[a] (1.0±0.1)[a]	0.7±0.1[a] (0.4±0.1)[b]	6.1±0.9[b] (3.6±0.4)[c]	1.3±0.2[a] (0.8±0.1)[a]
448	10.8±10.0[a]	0.6±0.1[a] (0.4±0.1)[a]	1.3±0.2[a] (0.8±0.1)[a]	1.6±0.6[a] (1.0±0.1)[a]	1.1±0.4[a] (0.7±0.2)[a]	7.3±1.6[ab] (4.6±0.6)[ab]	1.3±0.2[a] (0.9±0.3)[a]
669	13.4±13.4[a]	0.6±0.1[a] (0.4±0.1)[a]	1.4±0.3[a] (0.8±0.0)[a]	1.7±0.1[a] (1.0±0.2)[a]	0.9±0.2[a] (0.5±0.1)[ab]	7.4±1.6[ab] (4.3±0.4)[bc]	1.5±0.1[a] (0.9±0.1)[a]
1000	10.95±9.8[a]	0.8±0.2[a] (0.5±0.1)[a]	1.6±0.3[a] (1.0±0.3)[a]	2.2±0.9[a] (1.3±0.6)[a]	0.9±0.2[a] (0.5±0.1)[ab]	6.7±1.3[ab] (4.0±0.6)[bc]	1.6±0.1[a] (1.0±0.2)[a]

Results are expressed as Mean ± Standard Deviation (SD) for five rats per group. Values that share a superscripted letter down the column are not significantly different at $p < 0.05$.

Table 4.17: Effects of intraperitoneally administered logarithmic doses of aqueous extract of *U. massaica* on rat's organ and percentage organ to body weights

Treatment	Δ weekly weight change (g)	Organ (g) and percent organ to body weight (g/100g)					
		Heart	Kidney	Lungs	Spleen	Liver	Brain
Control	14±11.9[a]	0.6±0.1[a] (0.3±0.1)[a]	1.4±0.1[a] (0.8±0.1)[a]	1.7±0.3[a] (1.0±0.1)[a]	0.8±0.2[a] (0.5±0.1)[a]	9.0±0.2[b] (5.0±0.6)[a]	1.5±0.2[a] (0.8±0.2)[a]
300 mg/kgbwt	9.96±8.6[a]	0.5±0.0[a] (0.3±0.0)[a]	1.4 ±0.1[a] (0.8±0.1)[a]	1.4±0.3[a] (0.8±0.1)[a]	1.1±0.6[a] (0.6±0.3)[a]	10±0.8[a] (5.7±0.5)[a]	1.5±0.1[a] (0.8±0.1)[a]

Results are expressed as Mean ± Standard Deviation (SD) for five rats per group. Values that share a superscripted letter down the column are not significantly different at $p < 0.05$. UM = *U. massaica.*

4.4.2 Effect of orally and intraperitoneally administered aqueous extracts of *U. massaica* on hematological parameters of Wistar rats

The effect of orally and intraperitoneally administered non therapeutic doses of *U. massaica* on hematological parameters are presented in Table 4.18 and Table 4.19 respectively. Except the mean corpuscular volume (MCV), orally administered *U. massaica* did not affect the red blood cells indices and platelets of the treated rats relative to the control ($p < 0.05$). Orally administered dose level of 300 mg of the plant resulted to decreased ($p < 0.05$) MCV compared to the control. Orally administered *U. massaica* at the specified doses, did not have significant different ($p > 0.05$) effects on the total white blood cells count (WBC), differential counts of neutrophils, lymphocytes, monocytes, eosinophils and basophils compared to the controls (Table 4.18).

The extract at 300 mg/kg body weight dose while intraperitoneally administered did not ($p > 0.05$) affect erythrocytes (RBC), hemoglobin (Hb), packed red cell volume (PCV), mean cell volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) and platelets (PLT) differently from the controls. Likewise, intraperitoneally administered dose level of 300 mg/kg body weight *U. massaica* did not alter WBC and differential leucocytes counts differently from the controls (Table 4.19).

Table 4.18: Effects of orally administered logarithmic doses of aqueous extract of *U. massaica* on the red blood cells indices and platelets of rats

Haematological parameter	Normal	Doses of *U. massaica* administered (mg/kgbwt)			
		300	448	665	1000
RBC ($\times 10^6/\mu L$)	6.1 ± 1.1^a	7.3 ± 0.3^a	7.3 ± 0.6^a	7.1 ± 0.3^a	7.2 ± 0.9^a
HB (g/dL)	12.2 ± 0.8^a	13.5 ± 0.8^a	13.3 ± 1.8^a	13.8 ± 0.7^a	14.1 ± 1.3^a
PCV (%)	41.5 ± 9.0^a	39.98 ± 2.4^a	49.1 ± 2.8^a	42.8 ± 4.6^a	42.0 ± 4.3^a
MCV (fL)	67.7 ± 3.5^a	54.6 ± 2.1^b	67.2 ± 4.8^a	60.6 ± 5.8^{ab}	60.1 ± 3.5^{ab}
MCH (pg)	20.5 ± 4.5^a	18.5 ± 0.5^a	18.4 ± 3.1^a	19.6 ± 1.6^a	19.7 ± 1.8^a
MCHC (g/dL)	30.4 ± 7.1^a	33.9 ± 0.6^a	27.1 ± 4.6^a	32.5 ± 3.3^a	33.5 ± 1.3^a
PLT ($\times 10^3/\mu L$)	632 ± 217^a	615 ± 240^a	770 ± 244^a	759 ± 167^a	647 ± 170^a
WBC ($x10^9/L$)	3.3 ± 1.3^a	4.3 ± 0.6^a	3.4 ± 0.5^a	4.2 ± 1.8^a	4.1 ± 1.8^a
Neutrophils	0.9 ± 0.7^a	1.5 ± 0.7^a	1.3 ± 0.4^a	1.2 ± 0.7^a	1.1 ± 0.9^a
Lymphocytes	1.9 ± 1.1^a	2.5 ± 1.5^a	1.4 ± 0.9^a	2.5 ± 1.4^a	2.5 ± 1.2^a
Monocytes	0.3 ± 0.2^a	0.2 ± 0.1^a	0.2 ± 0.2^a	0.2 ± 0.1^a	0.3 ± 0.2^a
Eosinophils	0.3 ± 0.2^a	0.4 ± 0.4^a	0.4 ± 0.4^a	0.3 ± 0.2^a	0.2 ± 0.2^a
Basophils	0.1 ± 0.0^a	0.0 ± 0.0^a	0.1 ± 0.0^a	0.0 ± 0.0^a	0.1 ± 0.0^a

Results are expressed as Mean ± Standard Deviation (SD) for five rats per group. Values that share a superscripted letter in a row are not significantly different at $p < 0.05$. RBC = red blood cell count, Hb = hemoglobin, PCV = packed red cell volume, MCV = mean cell volume, MCH = mean corpuscular hemoglobin, MCHC = mean corpuscular hemoglobin concentration, PLT= platelets, WB = white blood cells.

Table 4.19: Effects of intraperitoneally administered 300mg/kgbwt dose of aqueous extract of *U. massaica* on rat's red blood cells parameters and platelets

Haematological parameters	Normal control	*U. massaica*
RBC ($\times 10^6/\mu L$)	7.7 ± 0.6^a	7.1 ± 0.7^a
Hb (g/dL)	13.6 ± 0.7^a	13.0 ± 0.6^a
PCV (%)	47.0 ± 2.8^a	47.7 ± 2.4^a
MCV (fL)	60.9 ± 1.4^a	67.1 ± 9.0^a
MCH (pg)	17.7 ± 0.5^a	18.2 ± 1.2^a
MCHC (g/dL)	29.0 ± 0.6^a	27.3 ± 2.0^a
PLT ($\times 10^3/\mu L$)	613 ± 80^a	955 ± 326^a
WBC ($x10^9/L$)	8.8 ± 2.7^a	8.2 ± 1.8^a
Neutrophils	0.9 ± 0.6^a	0.3 ± 0.1^a
Lymphocytes	6.8 ± 2.1^a	6.3 ± 2.0^a
Monocytes	1.0 ± 0.7^a	1.6 ± 1.0^a
Eosinophils	0.1 ± 0.1^a	0.0 ± 0.0^a
Basophils	0.0 ± 0.0^a	0.0 ± 0.0^a

Results are expressed as Mean ± Standard Deviation (SD) for five rats per group. Values that share a superscripted letter in a row are not significantly different at $p< 0.05$. RBC = red blood cell count, Hb = hemoglobin, PCV = packed red cell volume, MCV = mean cell volume, MCH = mean corpuscular hemoglobin, MCHC = mean corpuscular hemoglobin concentration, PLT= platelets, WBC = white blood cells.

4.4.3 Effect of orally and intraperitoneally administered aqueous extracts of *U. massaica* on biochemical parameters of Wistar rats

Orally administered *U. massaica* at doses of 300, 448, 669 and 1000 mg/kg body weight did not affect lactate dehydrogenase (LDH) and amylase differently ($p > 0.05$) compared to the control. However, orally administered dose level of 1000 mg of the plant extract increased ($p < 0.05$) creatine phosphokinase (CK) and decreased ($p < 0.05$) lipase levels relative to control. The effect on CK and lipase by orally administered 300, 448, 669 and 1000 mg/kg body weight doses of *U. massaica* was not significantly different ($p > 0.05$) in comparison to the control. All the four doses of *U. massaica* did not affect aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), Gamma glutamyl transferase (GGT) and total protein (TP) significantly different ($p > 0.05$) from the control. Albumin levels were decreased ($p < 0.05$) by orally administered dose level of 300 mg/kg body weight of the plant extract compared to the control but effect was similar ($p > 0.05$) to the other doses of *U. massaica* administered (Table 4.20). *In vivo* effect of orally administered *U. massaica* extract on blood urea nitrogen (BUN), creatinine (CREAT) sodium (Na^+) and potassium (K^+) is presented in Table 4.20. The results indicate that orally administered extract of *U. massaica* at 300, 448, 669.5 and 1000 mg/kg body weight doses did not affect urea and sodium levels of the treated rats significantly different ($p > 0.05$) compared to control rats. The four doses reduced creatinine and potassium levels compared to control.

Intraperitoneally administered aqueous extract of *U. massaica* at the dose level of 300 mg did not have a significantly different ($p > 0.05$) effect on LDH, CK, and amylase relative to the control. However, lipase was increased ($p < 0.05$) by intraperitoneally

administered *U. massaica* at this dose compared to the control (Table 4.21). Intraperitoneally administered 300 mg/kg body weight dose of *U. massaica* extract increased ($p < 0.05$) the levels of total protein compared to the control. However, the other parameters for evaluation of liver function were not affected (Table 4.21). The effect on all parameters for renal evaluation by intraperitoneally administered 300 mg/kg body weight dose of *U. massaica* was not significantly different ($p > 0.05$) from the control (Table 4.21).

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Table 4.20: Effects of orally administered logarithmic doses of aqueous extract of *U. massaica* on biochemistry of rats

.Biochemistry parameter	Normal	Doses of *U. massaica* administered (mg/kg body weight)			
		300	448	665	1000
LDH (IU/L)	1517±814[a]	1249±313[a]	1665±798[a]	1626±432[a]	932±305[a]
CK (UI/L)	909±447[b]	2145±1089[ab]	2730±1525[ab]	2028±1573[ab]	3585±350[a]
AMYL (IU/L)	1346±409[a]	1648±804[a]	1639±321[a]	1423±229[a]	1344±366[a]
LIPASE (IU/L)	15.5±1.0[a]	13.8±0.8[b]	13.8±0.5[b]	14.2±0.8[ab]	13.5±0.6[b]
AST (IU/L)	415±191[a]	506±294[a]	639±271[a]	717±260[a]	371±91[a]
ALT (IU/L)	185±44[a]	160±71[a]	180±65[a]	172±75[a]	141±32[a]
ALP (IU/L)	282.5±142[a]	153±49[a]	180±25[a]	240±63[a]	173±73[a]
GGT (IU/L)	3.5±2[a]	3.6±2[a]	6.2±3[a]	6.2±3[a]	4.0±2[a]
TP (g/L)	69.7±16.3[a]	65±2.0[a]	69.8±3.4[a]	68.2±4.6[a]	67.3±2.1[a]
ALB (g/L)	34±7.5[a]	23.2±2.8[b]	25.2±3.9[ab]	25.4±3.7[ab]	24.8±2.9[ab]
UREA (mmol/L)	11.1±2.1[a]	12.0±0.7[a]	13.1±0.6[a]	10.8±1.4[a]	11.0±1.7[a]
CREAT (µmol/L)	105.8±18.6[a]	65.4±5.9[b]	74.4±11.3[b]	64.2±6.9[b]	66.0±6.2[b]
Na^+(mmol/L)	155.5±1.3[a]	148.1±0.6[a]	154.6±7.7[a]	150.8±0.9[a]	150.3±1.5[a]
K^+(mmol/L)	22.0±7.5[a]	8.1±1.0[b]	10.3±2.7[b]	11.1±1.9[b]	10.7±0.6[b]

Results are expressed as Mean ± Standard Deviation (SD) for five rats per group. Values that share a superscripted letter down the column are not statistically different at $p < 0.05$. LDH = lactate dehydrogenase and CK = creatine phosphokinase, AST = aspartate aminotransferase, ALT = alanine aminotransferase, ALP = alkaline phosphatase, GGT = gamma glutamyl transferase, TP = total protein and ALB = albumin.

Table 4.21: Effects of intraperitoneally adminisered logarithmic doses of aqueous extract of *U. massaica* on biochemistry of rats

Biochemistry parameter	Normal	Doses of *U. massaica* administered 300mg/kg body weight
LDH (IU/L)	703±444[a]	466±241[a]
CK (UI/L)	1386±636[a]	624±106[a]
AMYLASE (IU/L)	869±164[a]	1032±141[a]
LIPASE (IU/L)	31±2.5[b]	35±2.5[a]
AST (IU/L)	328±125[a]	242±151[a]
ALT (IU/L)	131±42[a]	183±107[a]
ALP (IU/L)	172±53[a]	206±71[a]
GGT (IU/L)	1.8±1.1[a]	2.3±1.0[a]
TP (g/L)	60±1.5[b]	63±2.6[a]
ALB (g/L)	30±1.1[a]	32±2.8[a]
UREA (mmol/L)	7.8±1.2[a]	8.2±1.0[a]
CREA (µmol/L)	61.8±4.8[a]	59.8±6.2[a]
Na^+ (mmol/L)	134±5.4[b]	145±3.7[a]
K^+ (mmol/L)	11.2±1.5[a]	14.9±7.0[a]

Results are expressed as Mean ± Standard deviation (SD) for five rats per group. Values that share a superscripted letter in a row are not significantly different at $p < 0.05$. LDH = lactate dehydrogenase and CK = creatine phosphokinase, AST = aspartate aminotransferase, ALT = alanine aminotransferase, ALP = alkaline phosphatase, GGT = gamma glutamyl transferase, TP = total protein and ALB = albumin.

4.5 Evaluation of *in vivo* safety of aqueous extracts of *H. vulgare*

4.5.1 Effects of orally and intraperitoneally administered aqueous extract of *H. vulgare* on the relative organ weights of Wistar rats

Orally administered *H. vulgare* at dose levels of 300, 448, 669 and 1000 mg/kg body weight increased the body weight of the rat's insignificantly different ($p < 0.05$) to the control. The heart, kidney, lungs, spleen, liver and brain whole organ and percentage organ to body weights were not affected by orally administered *H. vulgare* at the specified doses relative to the control ($p > 0.05$; Table 4.22).

Intraperitoneally administered *H. vulgare* at the dose levels of 669 and 1000 mg/kg body weight resulted into death of the rats that was dose dependent. By the fourth day post treatment administration, 20% (n = 1) and 100% (n = 5) of rats administered 669 and 1000 mg dose of *H. vulgare* respectively had died. However, no further deaths were recorded at the dose level of 669 mg of *H. vulgare* thereafter. Intraperitoneally administered *H. vulgare* extract caused an increase in weight of the rats at the dose level of 300 and 448 mg/kg body weight. Likewise, at the dose level of 669 mg/kg body weight, the weight of the remaining rats (n =4) increased. However, the weight increase as a result of the administered doses of the plant extract was not significantly different ($p > 0.05$) from the control (Table 4.23).

Intraperitoneally administered 300 mg dose of *H. vulgare* extract did not affect the gross weight of the heart, kidney, lungs, spleen, liver and brain significantly different ($p > 0.05$) relative to the control. However, the percentage organ to body weight of the liver was significantly higher ($p < 0.05$) compared to the control. Intraperitoneally administered *H. vulgare* at the dose level of 448 mg/kg body weight increased the

gross weight of the kidney and the liver significantly higher ($p \leq 0.05$) than the control. However, the percentage weight of the kidney of rats administered the plant extract at this dose was not significantly different ($p > 0.05$) from the control. At the dose level of 669 mg/kg body weight, *H. vulgare* extract increased the gross organ and percentage organ to body weight of the kidney ($p < 0.05$) relative to the control. In addition, the 669 mg/kg body weight *H. vulgare* dose raised the percentage organ to body weight of the kidney, liver and brain significantly ($p < 0.05$) compared to the controls (Table 4.23).

Table 4.22: Effects of orally administered logarithmic doses of aqueous extract of *H. vulgare* on the gross organ and percentage organ to body weight of rats

Treatment	Δweekly weight change (g)	Organ (g) and percent organ to body weight (g/100g)					
		Heart	Kidney	Lungs	Spleen	Liver	Brain
Control	19.3±15.4[a]	0.6±0.1[a] (0.3±0.0)[a]	1.7±0.3[a] (0.8±0.1[a]	2.4±0.3[a] (1.1±0.3)[a]	1.7±0.6[a] (0.8±0.3)[a]	10.5±2.0[a] (4.9±0.6)[a]	1.5±0.1[a] (0.7±0.1)[a]
Extract dose (mg/kg body weight)							
300	16.5±13.3[a]	0.7±0.0[a] (0.3±0.0)[a]	1.8±0.2[a] (0.8±0.0)[a]	1.9±0.2[a] (0.9±0.1)[a]	1.4±0.2[a] (0.6±0.1)[a]	10.2±0.7[a] (4.6±0.3)[a]	1.5±0.2[a] (0.7±0.1)[a]
448	16.7±12.5[a]	0.7±0.1[a] (0.3±0.1)[a]	1.6±0.2[a] (0.8±0.1)[a]	1.9±0.6[a] (0.9±0.2)[a]	1.5±0.1[a] (0.7±0.1)[a]	9.9±1.2[a] (4.7±0.4)[a]	1.5±0.1[a] (0.7±0.1)[a]
669	17.3±9.9[a]	0.7±0.0[a] (0.3±0.0)[a]	1.7±0.3[a] (0.8±0.0)[a]	2.1±0.2[a] (1.0±0.2)[a]	1.2±0.5[a] (0.6±0.2)[a]	9.7±1.0[a] (4.8±0.4)[a]	1.5±0.1[a] (0.8±0.1)[a]
1000	12.0±9.7[a]	0.7±0.1[a] (0.3±0.1[a]	1.5±0.2[a] (0.8±0.1)[a]	1.7±0.5[a] (0.9±0.2)[a]	1.2±0.3[a] (0.6±0.1)[a]	8.7±1.5[a] (4.6±0.4)[a]	1.5±0.1[a] (0.8±0.1)[a]

Results are expressed as Mean ± Standard Deviation (SD) for five rats per group. Mean percentage organ to body weight is in parentheses. Values that share a superscripted letter down the column are not statistically different at $p < 0.05$.

Table 4.23: Effects of intraperitoneally administered doses of aqueous extract of *H. vulgare* on gross organ and percentage organ to body weight of rats

Treatment	Δ weekly weight change (g)	Organ (g) and percent organ to body weight (g/100g)					
		Heart	Kidney	Lungs	Spleen	Liver	Brain
Control	15±15[a]	0.8±0.4[a] (0.4±0.2)[a]	0.9±0.3[b] (0.5±0.2)[b]	1.6±0.2[a] (0.9±0.1)[a]	1.1±0.3[a] (0.6±0.2)[a]	7.0±0.9[b] (3.7±0.4)[c]	1.0±0.1[a] (0.5±0.1)[b]
Extract dose (mg/kg body weight)							
300	13.8±9[a]	0.6±0.1[a] (0.4±0.1)[a]	1.3±0.0[ab] (0.7±0.1)[ab]	1.4±0.5[a] (0.8±0.3)[a]	0.8±0.2[a] (0.4±0.1)[a]	8.9±1.5[ab] (5.0±0.8)[b]	1.1±0.1[a] (0.6±0.1)[ab]
448	17.1±10[a]	0.6±0.1[a] (0.3±0.0)[a]	1.5±0.3[a] (0.7±0.1)[ab]	1.6±0.6[a] (0.8±0.2)[a]	0.9±0.2[a] (0.5±0.1)[a]	10.9±2.8[a] (5.4±0.8)[ab]	1.1±0.2[a] (0.6±0.1)[b]
669*	9±5[a]	0.6 ± 0.1[a] (0.4±0.1)[a]	1.5± 0.2[a] (1.0±0.2)[a]	1.3± 0.1[a] (0.8±0.1)[a]	1.2± 0.5[a] (0.8±0.4)[a]	10.7± 1.5[a] (6.7±0.6)[a]	1.3± 0.3[a] (0.9±0.3)[a]

Results are expressed as Mean ± Standard Deviation (SD) for five rats per group (* n = 4). Mean percentage organ to body weight is in parentheses. Values that share a superscripted letter down the column are not statistically different at $p < 0.05$.

4.5.2 Effect of orally administered aqueous extracts of *H. vulgare* on hematological parameters of Wistar rats

Table 4.24 presents the effect on haematological parameters as a result of orally administered logarithmic doses of *H. vulgare* extract in rats. At the dose level of 300 mg/kg body weight, *H. vulgare* decreased the mean corpuscular haemoglobin (MCH) significantly ($p < 0.05$) than the control. However, orally administered specified doses of *H. vulgare* did not affect the red blood cells, other erythrocyte indices (except MCH) and platelets significantly different ($p > 0.05$) from the controls. Equally, the results indicate that orally administered *H. vulgare* at the specified doses did not have significantly different ($p > 0.05$) effect on the total and differential white cells counts compared to the controls.

Intraperitoneally administered 300 mg/kg body weight *H. vulgare* increased ($p < 0.05$) the red blood cells counts (RBC) but decreased ($p < 0.05$) the mean corpuscular volume (MCV) significantly compared to the controls. At the dose levels of 448 and 669 mg/kg body weight, *H. vulgare*, did not significantly alter ($p > 0.05$) the RBCs, red blood cells indices and platelets differently from the controls (Table 4.25). Intraperitoneally administered *H.vulgare* at doses of 300. 448 and 669 mg/kg body weight had an insignificant different ($p > 0.05$) effect on the white blood cells relative to the control (Table 4.25).

Table 4.24: Effects of orally logarithmic doses of aqueous extract of *H. vulgare* on haematological indices of rats

Haematological parameter	Normal	Doses of *H. vulgare* administered (mg/kg body weight)			
		300	448	665	1000
RBC ($\times10^{12}$/L)	6.4±0.6^{a}	6.9±1.3^{a}	6.4±0.8^{a}	6.7±0.4^{a}	5.1±2.4^{a}
Hb (g/dL)	13.9±0.9^{a}	12.8±2.1^{a}	13.9±1.4^{a}	13.9±0.5^{a}	13.0±1.1^{a}
PCV (%)	45.7±3.1^{a}	43.3±4.8^{a}	44.5±4.4^{a}	44.1±1.0^{a}	41.7±2.5^{a}
MCV (fL)	72.2±3.5^{a}	64.2±7.7^{a}	70.4±4.4^{a}	65.7±4.7^{a}	64.0±7.4^{a}
MCH (pg)	21.9±1.0^{a}	18.8±1.9^{b}	22.0±1.3^{a}	20.7±1.4^{ab}	19.9±1.9^{ab}
MCHC (g/dL)	30.3±0.2^{ab}	29.5±1.9^{b}	31.2±0.2^{ab}	31.5±0.6^{a}	31.1±0.98^{ab}
PLT ($\times10^{9}$/L)	569±142^{a}	657±74^{a}	616±103^{a}	642±95^{a}	474±69^{a}
WBC (x10^{9}/L)	13.7±4.0^{a}	13.2±6.6^{a}	10.7±2.1^{a}	10.1±4.5^{a}	12.9±5.2^{a}
Neutrophils	1.6±1.3^{a}	2.4±2.0^{a}	1.6±0.7^{a}	1.4±0.9^{a}	1.2±0.8^{a}
Lymphocytes	11.4±3.5^{a}	10.2±5.7^{a}	8.4±1.3^{a}	7.8±4.3^{a}	10.7±4.2^{a}
Monocytes	0.1±0.1^{a}	0.1±0.1^{a}	0.3±0.2^{a}	0.6±0.5^{a}	0.5±0.5^{a}
Eosinophils	0.5±0.5^{a}	0.4±0.4^{a}	0.3±0.2^{a}	0.1±0.1^{a}	0.1±0.0^{a}
Basophils	0.2±0.0^{a}	0.2±0.1^{a}	0.2±0.2^{a}	0.2±0.1^{a}	0.2±0.1^{a}

Results are expressed as Mean ± Standard Deviation (SD) for five rats per group. RBC = red blood cell count, Hb = hemoglobin, PCV = packed red cell volume, MCV = mean cell volume, MCH = mean corpuscular hemoglobin, MCHC = mean corpuscular hemoglobin concentration, PLT= platelets, WBC = white blood cells count.Values that share a superscripted letter along the row are not significantly different at $p<0.05$.

Table 4.25: Effects of intraperitoneally administered logarithmic doses of aqueous extract of *H. vulgare* on haematological indices of rats

Haematological parameter	Normal	Doses of *H. vulgare* administered (mg/kg body weight)		
		300	448	665
RBC ($\times 10^{12}$/L)	6.4±0.5^{b}	7.9±0.5^{a}	7.2±0.7^{ab}	6.5±0.4^{b}
Hb (g/dL)	13.2±0.7^{a}	14.8±1.5^{a}	14.1±1.7^{a}	13.3±1.1^{a}
PCV (%)	47.1±1.5^{a}	46.4±1.1^{a}	45.6±7.9^{a}	48.6±7.5^{a}
MCV (fL)	73.9±4.9^{a}	58.6±3.4^{b}	63.6±6.3^{ab}	75.1±15.7^{a}
MCH (pg)	20.7±1.1^{a}	18.7±2.2^{a}	19.7±1.2^{a}	20.4±1.7^{a}
MCHC (g/dL)	28.0±1.1^{a}	31.9±3.2^{a}	31.1±1.8^{a}	27.8±4.2^{a}
PLT ($\times 10^{9}$/L)	799±163^{a}	1148±140^{a}	787±275^{a}	863±335^{a}
WBC (x10^{9}/L)	8.0±5.7^{a}	10.5±0.5^{a}	7.4±1.5^{a}	8.2±2.1^{a}
Neutrophils	1.0±0.5^{a}	1.4±1.3^{a}	1.2±0.8^{a}	0.7±0.6^{a}
Lymphocytes	5.8±5.7^{a}	7.6±1.3^{a}	4.5±1.6^{a}	5.7±2.5^{a}
Monocytes	0.7±0.6^{a}	1.1±1.0^{a}	0.5±0.4^{a}	0.5±0.5^{a}
Eosinophils	0.1±0.1^{a}	0.1±0.1^{a}	1.1±1.1^{a}	0.7±0.6^{a}
Basophils	0.2±0.2^{a}	0.3±0.3^{a}	0.1±0.1^{a}	0.1±0.0^{a}

Results are expressed as Mean ± Standard Deviation (SD) for five rats per group. RBC = red blood cell count, Hb = hemoglobin, PCV = packed red cell volume, MCV = mean cell volume, MCH = mean corpuscular hemoglobin, MCHC = mean corpuscular hemoglobin concentration, PLT= platelets, WBC = white blood cells count.Values that share a superscripted letter along the row are not significantly different at $p< 0.05$.

4.5.3 Effect of orally administered aqueous extracts of *H. vulgare* on biochemistry parameters of Wistar rats

Biochemistry values obtained for lactate dehydrogenase (LDH), creatine phosphokinase (CK), amylase and lipase indicates no significantly different ($p > 0.05$) effect on the cardiac and pancreatic systems caused by orally administered *H. vulgare* in comparison to the control (Table 4.26). In evaluation of liver function, orally administered *H. vulgare* extract at the specified four doses did not ($p > 0.05$) affect alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), gamma glutamyl transferase (GGT), total protein (TP) and albumin (ALB) relative to the control (Table 4.26). In addition, the results indicates that no effects on the kidney that occurred as a result of orally administered *H. vulgare* at 300, 448, 669 and 1000 mg/kg body weight doses resulted to alterations of urea, creatinine and sodium differently ($p > 0.05$) relative to the control. However, the results also shows that *H. vulgare* administered at the specified doses lowered potassium levels significantly ($p < 0.05$) at the dose level of 669 mk/kg body weight compared to the control (Table 4.26).

As indicated in Table 4.27, intraperitoneally administered *H. vulgare* at the dose level of 300 and 448 mg reduced lipase levels significantly lower ($p < 0.05$) than the control. The other biomarkers of cardiac and pancreatic functions were not affected by intraperitoneally administered 300, 448 and 669 mg/kg body weight doses of *H. vulgare*. Intraperitoneally administered *H. vulgare* at the dose level of 300 and 448 mg had an insignificant different ($p > 0.05$) effect in all liver function biomarkers used in this study compared with the control. However, as the dose of *H. vulgare* increased, the levels of ALT equally were raised. Eventually, at the dose level of 669

mg/kg body weight, the ALT levels were significantly elevated ($p < 0.05$) compared to that of the controls. The effect on aspartate aminotransferase (AST), alkaline phosphatase (ALP), gamma glutamyl transferase (GGT), total protein (TP) and albumin (ALB) by intraperitoneally administered *H. vulgare* at 669 mg/kg body weight was not significantly different ($p > 0.05$) from the controls. Intraperitoneally administered *H. vulgare* at the dose levels of 300, 448 and 669 mg/kg body weight affected kidney function insignificantly different ($p > 0.05$) from the controls (Table 4.27).

Table 4.26: Effects of orally administered logarithmic doses of aqueous extract of *H. vulgare* on biochemistry parameters of rats

Biochemistry parameter	Normal	Doses of *T. aestivum* administered (mg/kg body weight)			
		300	448	665	1000
LDH (IU/L)	585±307[a]	323±79[a]	345±218[a]	441±161[a]	339±111[a]
CK (UI/L)	948±552[a]	476±116[a]	505±174[a]	541±98[a]	559±207[a]
AMYLASE (IU/L)	2243±512[a]	2060±293[a]	2256±753[a]	1857±323[a]	1823±371[a]
LIPASE (IU/L)	360±28[a]	391±34[a]	363±15[a]	392±56[a]	374±38[a]
AST (IU/L)	305±121[a]	198±29[a]	222±27[a]	210±38[a]	236±29[a]
ALT (IU/L)	189±31[a]	166±32[a]	150±39[a]	154±21[a]	144±21[a]
ALP (IU/L)	252±32[a]	196±76[a]	183±55[a]	184±69[a]	160±57[a]
GGT (IU/L)	7.4±1.5[a]	7.2±1.3[a]	7.0±1.4[a]	9.2±3.5[a]	5.6±3.7[a]
TP (g/L)	68±1.3[a]	68±4.9[a]	75±4.2[a]	75±4.9[a]	70±4.1[a]
ALB(g/L)	33±1.8[a]	34±2.1[a]	35±1.5[a]	34±2.3[a]	32±1.8[a]
UREA (mmol/L)	11.2±1.[a]	12.8±2.4[a]	11.2±1.2[a]	10.9±1.0[a]	10.6±1.2[a]
CREA (µmol/L)	82±4.2[a]	87±8.4[a]	87±4.7[a]	90±3.7[a]	91±9.0[a]
Na^+(mmol/L)	133±3.7[a]	133.0±2.5[a]	132.0±1.9[a]	133.2±1.9[a]	132.0±0.7[a]
K^+(mmol/L)	7.8±2.4[a]	5.6±1.0[ab]	5.7±0.8[ab]	4.5±0.5[b]	6.6±1.8[ab]

Results are expressed as Mean ± Standard deviation (SD) for five rats per group. Values that share a superscripted letter in a row are not significantly different at $p < 0.05$. LDH = lactate dehydrogenase and CK = creatine phosphokinase, AST = aspartate aminotransferase, ALT = alanine aminotransferase, ALP = alkaline phosphatase, GGT = gamma glutamyl transferase, TP = total protein and ALB = albumin.

Table 4.27: Effects of intraperitoneally administered logarithmic doses of aqueous extract of *H. vulgare* on biochemistry parameters of rats

Biochemistry parameter	Normal	Doses of *T. aestivum* administered (mg/kg body weight) 300	448	669
LDH (IU/L)	540±291[a]	560±238[a]	465±145[a]	495±431[a]
CK (UI/L)	1748±869[a]	1632±1228[a]	1024±663[a]	1022±860[a]
AMYLASE (IU/L)	1039±157[a]	1095±153[a]	1181±68[a]	1203±72[a]
LIPASE (IU/L)	30±0.5[a]	27±1.7[bc]	26±2.0[c]	30±2.6[ab]
AST (IU/L)	284±98[a]	260±111[a]	247±32[a]	252±119[a]
ALT (IU/L)	121±14[b]	115±23[b]	133±29[ab]	181±35[a]
ALP (IU/L)	209±38[a]	231±61[a]	268±72[a]	279±107[a]
GGT (IU/L)	3.4±1.1[a]	3.2±1.3[a]	3.0±0.7[a]	2.8±1.0[a]
TP (g/L)	62±2.9[a]	61±3.3[a]	64±2.6[a]	68±5.7[a]
ALB(g/L)	32±2.7[a]	32±2.4[a]	35±1.2[a]	34±1.6[a]
UREA (mmol/L)	9.0±0.8[a]	7.7±1.7[a]	7.8±0.8[a]	9.1±0.7[a]
CREA (µmol/L)	57.8±3.4[a]	59.4±4.6[a]	62.8±7.5[a]	56.8±6.2[a]
Na^+(mmol/L)	149±2.5[a]	147±3.5[a]	147±6.1[a]	144±4.7[a]
K^+(mmol/L)	6.6±0.9[a]	6.0±0.8[a]	6.9±2.7[a]	8.7±1.9[a]

Results are expressed as Mean ± Standard Deviation (SD) for five rats per group. Values that share a superscripted letter down the column are not significantly different at $p< 0.05$. AST = aspartate aminotransferase, ALT = alanine aminotransferase, ALP = alkaline phosphatase, GGT = gamma glutamyl transferase, TP = total protein and ALB = albumin.

4.6 Evaluation of *in vivo* safety of aqueous extracts of *C. viminale*

4.6.1 Effects of orally and intraperitoneally administered aqueous extract of *C. viminale* on the relative organ weight of Wistar rats

Orally administered *C. viminale* four doses used in this study effect on the relative organ weight of normal rats is presented in Table 4.28. As the results indicate, though *C. viminalle* consistently increased the body weight of the experimental rats, the increase in the general body weight was not significantly different ($p > 0.05$) from the control (Table 4.28). Orally administered 300, 448, 669 and 1000 mg/kg body weight did not affect the organ and percentage organ to body weight of the heart, kidney, lungs, liver and brain significantly different ($p > 0.05$) from the controls. However, at the dose level of 1000 mg/kg body weight, *C. viminale* increased the weight of the spleen significantly ($p < 0.05$) relative to the control. The percentage organ to body weight of the spleen was however not significantly different ($p > 0.05$) from the control (Table 4.28).

Intraperitoneally administered *C. viminale* nontherapeutic doses specified in this study resulted into the deaths of the rats in a dose dependent manner. By the 7th day of extract administration, 20% (n = 1) of rats administered *C. viminale* doses of 300 and 669 and 60% (n = 3) of those administered 1000 mg/kg body weight dose of *C. viminale* had died. On the 1st week of intraperitoneal administration of the extract, *C. viminale* decreased the body weight of the rats at doses of 300, 448 and 669 mg/kg body weight. However, the extract's effect on the body weight in the 1st week was not significantly different ($p > 0.05$) from the controls. By the 14th day, no further mortality had been attributed to the dose of 300 mg/kg body weight *C. viminale* administered. However, mortality was recorded at the dose level of 448 at 60%, and at

80% at the dose levels of 669 and 1000 mg/kg body weight *C. viminale* intraperitoneally administered. By the 21st day, intraperitoneally administered dose of 300 mg *C. viminale* had resulted to 60% (n = 3) mortality of the rats. Following the deaths of the animals, evaluation of the safety of intraperitoneally administered *C. viminalle* was exited in the third week of the experiment.

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Table 4.28: Effects of orally administered logarithmic doses of aqueous extract of *C. viminale* on rat's organ and percentage organ to body weights

Treatment	(Δ) weekly weight change (g)	Organ (g) and percent organ to body weight (g/100g)					
		Heart	Kidney	Lungs	Spleen	Liver	Brain
Control	13.9±9.5[a]	0.6±0.1[a] (0.3±0.1)[a]	1.4±0.3[a] (0.8±0.2)[a]	2.1±0.4[a] (1.2±0.3)[a]	0.8±0.2[b] (0.4±0.2)[a]	8.0±1.2[a] (4.4±1.0)[a]	1.4±0.3[a] (0.8±0.2)[a]
Extract dose (mg/kg body weight)							
300	8.6±7.7[a]	0.6±0.1[a] (0.4±0.1)[a]	1.5±0.1[a] (1.0±0.1)[a]	2.3±0.9[a] (1.5±0.9)[a]	0.8±0.1[ab] (0.5±0.2)[a]	8.0±0.6[a] (4.9±0.4)[a]	1.5±0.2[a] (0.9±0.1)[a]
448	12.1±9.2[a]	0.6±0.1[a] (0.3±0.0)[a]	1.6±0.2[a] (0.9±0.0)[a]	2.1±0.5[a] (1.2±0.3)[a]	0.7±0.1[b] (0.4±0.1)[a]	8.5±0.9[a] (4.7±0.22)[a]	1.4±0.3[a] (0.8±0.1)[a]
669	10.1±7.1[a]	0.6±0.1[a] (0.3±0.0)[a]	1.8±0.3[a] (1.0±0.2)[a]	1.8±0.3[a] (1.0±0.1)[a]	0.9±0.1[ab] (0.5±0.1)[a]	8.4±1.4[a] (4.6±0.2)[a]	1.5±0.3[a] (0.8±0.2)[a]
1000	11.2±8. 6[a]	0.6±0.0[a] (0.3±0.0)[a]	1.7±0.2[a] (0.9±0.1)[a]	1.7±0.3[a] (1.0±0.2)[a]	1.1±0.3[a] (0.6±0.2)[a]	9.1±0.7[a] (5.1±0.6)[a]	1.6±0.1[a] (0.8±0.1)[a]

Results are expressed as Mean organ weights± Standard Deviation (SD) for five rats per group. Percentage organ to body weight means are in parenthesis. Values that share a superscripted letter along the column are not significantly different at $p < 0.05$.

4.6.2 Effect of orally and intraperitoneally administered aqueous extracts of *C. viminale* on hematological indices of Wistar rats

Presented in Table 4.29 are the results obtained for erythrocyte indices and platelets post administration of aqueous extract of *C. viminale*. The results indicates that orally administered *C. viminale* at the dose levels of 300, 448, 669 and 1000 mg/kg body weight did not affect erythrocyte and platelets significantly different ($p > 0.05$) from the controls (Table 4.29). Orally administered *C. viminale* extract at doses of 300, 448, 669 and 1000 mg/kg body weight affected the white blood cells total count insignificantly different ($p > 0.05$) from the control. However, at the dose level of 448 and 1000 mg/kg body weight, orally administered *C. viminale* decreased the level of basophils ($p < 0.05$) relative to the control.

Table 4.29: Effects of orally administered logarithmic doses of aqueous extract of *C. viminale* on haematological parameters of rats

Haematological parameter	Normal	Doses of *T. aestivum* administered (mg/kg body weight)			
		300	448	665	1000
RBC ($\times 10^6/\mu L$)	7.3 ± 0.5^{ab}	7.3 ± 0.5^{ab}	7.7 ± 0.3^{a}	7.6 ± 0.3^{ab}	6.6 ± 0.6^{b}
Hb (g/dL)	14.6 ± 1.3^{a}	14.6 ± 1.3^{a}	15.3 ± 1.2^{a}	15.8 ± 1.2^{a}	13.9 ± 1.5^{a}
PCV (%)	46.4 ± 4.1^{ab}	49.2 ± 2.4^{a}	49.2 ± 2.9^{a}	47.7 ± 2.9^{ab}	42.3 ± 3.9^{b}
MCV (fL)	64.0 ± 3.0^{a}	67.8 ± 5.4^{a}	63.8 ± 1.9^{a}	62.6 ± 1.7^{a}	64.5 ± 1.0^{a}
MCH (pg)	20.1 ± 1.5^{a}	20.1 ± 1.9^{a}	20.0 ± 1.3^{a}	20.6 ± 0.7^{a}	21.2 ± 0.7^{a}
MCHC (g/dL)	31.6 ± 3.4^{a}	29.8 ± 3.8^{a}	31.2 ± 3.2^{a}	33.0 ± 0.6^{a}	32.9 ± 0.7^{a}
PLT ($\times 10^3/\mu L$)	717 ± 81^{a}	798 ± 84^{a}	887 ± 161^{a}	798 ± 116^{a}	698 ± 85^{a}
WBC ($x10^9/L$)	10.7 ± 7.3^{a}	10.8 ± 2.3^{a}	6.3 ± 3.8^{a}	8.3 ± 5.3^{a}	7.2 ± 3.0^{a}
Neutrophils	1.4 ± 1.0^{a}	1.5 ± 0.7^{a}	0.6 ± 0.4^{a}	0.7 ± 0.6^{a}	1.0 ± 0.6^{a}
Lymphocytes	8.6 ± 6.7^{a}	9.0 ± 2.7^{a}	5.5 ± 3.4^{a}	6.9 ± 4.6^{a}	5.6 ± 2.7^{a}
Monocytes	0.3 ± 0.2^{a}	0.2 ± 0.1^{a}	0.2 ± 0.1^{a}	0.3 ± 0.2^{a}	0.2 ± 0.2^{a}
Eosinophils	0.2 ± 0.1^{a}	0.1 ± 0.1^{a}	0.1 ± 0.0^{a}	0.2 ± 0.1^{a}	0.2 ± 0.1^{a}
Basophils	0.3 ± 0.2^{a}	0.2 ± 0.0^{ab}	0.1 ± 0.0^{b}	0.2 ± 0.1^{ab}	0.1 ± 0.0^{b}

Results are expressed as Mean ± Standard Deviation (SD) for five rats per group. RBC = red blood cell count, Hb = hemoglobin, PCV = packed red cell volume, MCV = mean cell volume, MCH = mean corpuscular hemoglobin, MCHC = mean corpuscular hemoglobin concentration, PLT= platelets, WBC = white blood cells count. Values that share a superscripted letter along the row are not significantly different at $p<0.05$.

4.6.3 Effect of orally and intraperitoneally administered aqueous extracts of *C. viminale* on biochemical parameters of Wistar rats

Presented in Table 4.30 are the results on biochemistry biomarkers used in this study. The results shows that the levels for lactate dehydrogenase (LDH), creatine kinase (CK), amylase and lipase were not affected by the four orally administered doses of *C. viminale* significantly different ($p > 0.05$) from the controls. Orally administered doses of 300, 448, 669 and 1000 mg/kg body weight *C. viminale* did not result to any effect on aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), gamma glutamyltransferase (GGT), total protein and albumin that significantly varied ($p > 0.05$) from the controls. Further, the effect of orally administered 300, 448, 669 and 1000 mg/kg body weight doses of *C. viminale* did not adversely affect the renal system. There was no significant difference ($p > 0.05$) in the levels of urea, creatinine, sodium and potassium between the extract treated rats and the control (Table 4.30).

Table 4.30: Effects of orally administered logarithmic doses of aqueous extract of *C. viminale* on biochemical parameters of rats

Biochemistry parameter	Normal	Doses of *T. aestivum* administered (mg/kg body weight)			
		300	448	665	1000
LDH (IU/L)	291±129[a]	477±178[a]	478±228[a]	478±168[a]	374±136[a]
CK (UI/L)	475±215[a]	660±270[a]	773±389[a]	499±125[a]	504±176[a]
AMYLASE (IU/L)	1740±817[a]	1529±286[a]	1962±582[a]	1774±772[a]	2192±346[a]
LIPASE (IU/L)	13.8±3.2[a]	12.4±1.82[a]	12.4±2.07[a]	12.7±2.08[a]	11.5±1.00[a]
AST (IU/L)	150±35[a]	196±41[a]	204±64[a]	163±27[a]	173±24[a]
ALT (IU/L)	83.6±7[a]	111± 46[a]	108±13[a]	115±8.5[a]	102±4[a]
ALP (IU/L)	239± 63[a]	323±267[a]	253±46[a]	291±35[a]	223±69[a]
GGT (IU/L)	2.2±1.3[a]	2.0±1.6[a]	1.8±0.8[a]	1.7±1.5[a]	1.8±1.3[a]
TP (g/L)	64.0±2.5[a]	65.8±3.0[a]	68.4±2.1[a]	66.0±2.0[a]	72.5±8.8[a]
ALB(g/L)	35.4±3.7[a]	36.2±4.3[a]	38.2±4.1[a]	34.7±4.9[a]	39.5±1.3[a]
UREA (mmol/L)	7.2±1.6[a]	6.7±1.2[a]	6.4±0.7[a]	5.9±0.4[a]	6.4±1.1[a]
CREA (µmol/L)	61±9.9[a]	62±13.3[a]	58±12.7[a]	60±15.1[a]	52±2.06[a]
Na^+(mmol/L)	139±2.1[a]	139±2.2[a]	140±4.6[a]	140±3.5[a]	140±3.5[a]
K^+(mmol/L)	5.7±1.1[a]	6.6±1.5[a]	6.2±0.9[a]	5.9±0.9[a]	6.3±0.3[a]

Results are expressed as Mean ± Standard Deviation (SD) for five rats per group. Values along the row that share a superscripted are not significantly different at $p<0.05$. LDH = lactate dehydrogenase and CK = creatine phosphokinase, AST = aspartate aminotransferase, ALT = alanine aminotransferase, ALP = alkaline phosphatase, GGT = gamma glutamyl transferase, TP = total protein and ALB = albumin

4.7 Phytochemical, Macro and Micronutrients Composition of *T. aestivum, H. vulgare, U. massaica* and *C. viminale*.

4.7.1 Phytochemical composition of *T. aestivum, H. vulgare, U. massaica* and *C. viminale*

A phytochemical assay done to determine qualitatively the phytochemical composition of the plants extracts yielded the following results for phenolic compounds (Table 4.31). Tannins were present in *U. massaica, C. viminale, T. aestivum* and *H. vulgare*. Flavonoids were also present in all the four plant's extracts. Terpenoids were present in *T. aestivum* and *H. vulgare* but absent in *U. massaica* and *C. viminale.* Saponins, cardiac glycosides and steroids were present in *T. aestivum, H. vulgare, C. viminale* and *U. massaica.* Phylobatannins were absent in *C. viminale* but present in *U. massaica, T. aestivum* and *H. vulgare.* Alkaloids were not detected in all of the plants extracts by both Mayer's and Hager's test (Table 4.31).

Table 4.31: Qualitative phytochemical constituents of *T. aestivum, H. vulgare, U. massaica* and *C. viminale* aqueous extracts

Phytochemical	*T. aestivum*	*H. vulgare*	*U. massaica*	*C. viminale*
Tannins	+	+	+	+
Flavanoids	+	+	+	+
Terpenoids	+	+	-	-
Saponins	+	+	+	+
Steroids	+	+	+	+
Cardiac glycosides	+	+	+	+
Reducing sugars	+	+	-	-
Phylobatannins	+	+	+	-
Alkaloids	M - H -	M - H -	M - H -	M - H -

Key: + Present, - absent, M= Mayers, H = Hagers

4.7.2 Compounds present in *T. aestivum, H. vulgare, U. massaica and C. viminale*

The LCMS analysis of the four plants aqueous extracts profiled possible number of compounds in each of the extracts. *H. vulgare* had the highest number of possible compounds at 174, while *U. massaica* had the lowest at 74. *C. viminalle* and *T.aestivum* had 151 and 109 possible compounds, respectively.

T. aestivum and *H. vulgare* both in the family of apocanceae had similar compounds (except three not present in *H. vulgare*) though at different concentrations. Among the various class of secondary metabolites identified, all plants extracts in this study had flavonoids, phenylpropanoids, alkaloids and steroids. The concentration of flavonoids was highest in *C. viminalle* followed by *T. aestivum* while *U. massaica* had the lowest. Terpenoids were not present in *H. vulgare* and *U. massaica* while gamma amino acids were not present in *U. massaica* and *C. viminalle*. Glycoside (apigenin) was only identified in *T. aestivum* (Figure 4.9). Numerous compounds in individual plants extracts were not identified (that is they were unknown). The compounds positively identified in each of the aqueous extracts of the plants in this study are presented in Table 4.32, 4.33, 4.34 and 4.35 for *T. aestivum*, *H. vulgare*, *U. massaica* and *C. viminale,* respectively. The LCMS total ion current spectra is in Appendix 9. Data on possiple fragments, peak area and concentrations of the compounds identified are presented in Appendix 10. The calibration curve is presented in Appendix 11.

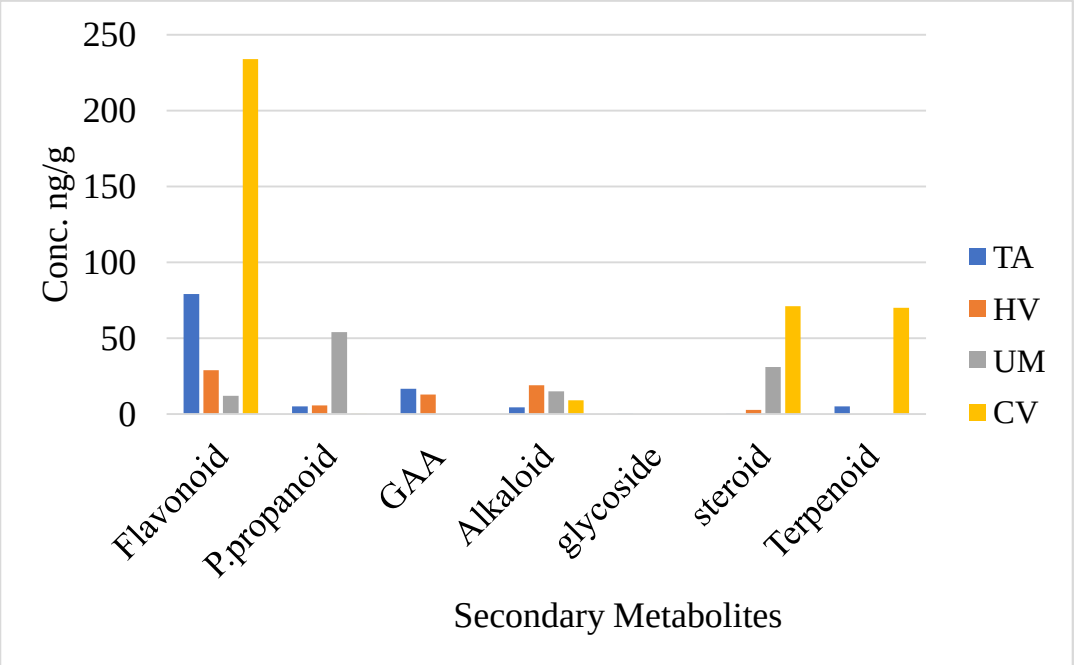
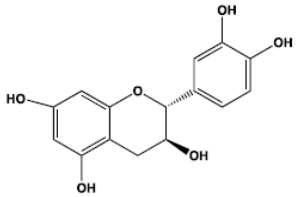
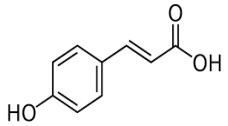
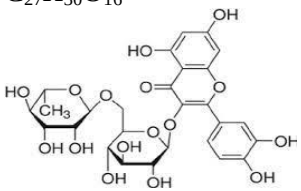
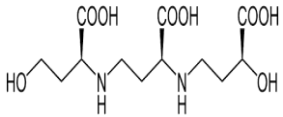
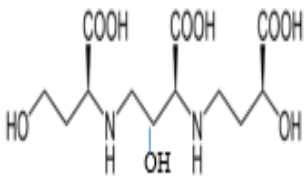
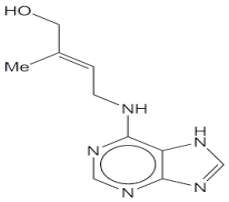
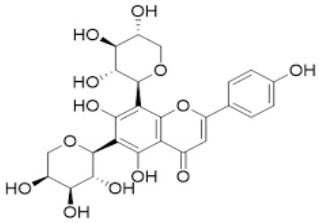
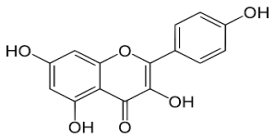
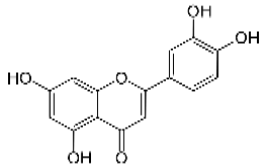
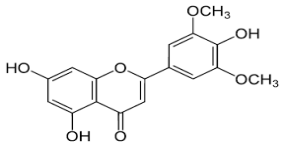
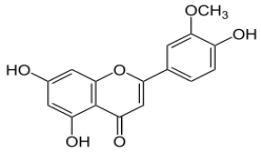
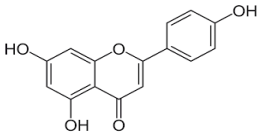


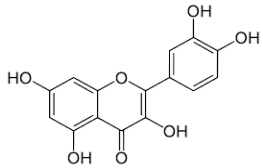
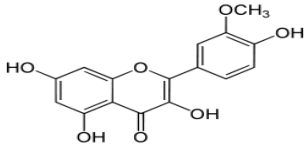
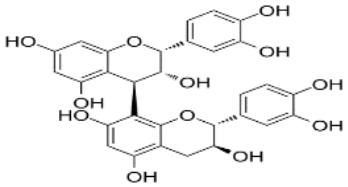
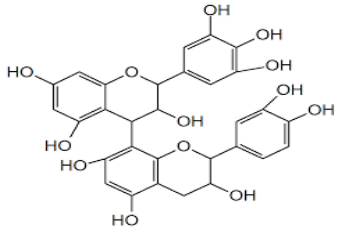
Figure 4.9: Quantitative concentration of secondary metabolites in aqueous extracts of TA (*T aestivum*), HV (*H.vulgare*), UM (*U. massaica*) and CV (*C. viminalle*). P. propanoid = phenylpropanoid, GAA = gamma amino acid, ng/g = nanogram/gram.

Table 4.32 Compounds present in aqueous extract of *T. aestivum*

Peak#	RT	Compound name	Formula and structure	Phytochemical class	Concentration (ng/g)	Ethnobotanical/biological use
1	1.47	Catechin (MW 290.3 g/mol)	$C_{15}H_{14}O_6$ 	Flavanoid	6.27	Antimicrobial. Antioxidant. Prevention of cancer, obesity, diabetes and neurodegenerative diseases. (Gaikwad, Mohan and Rani, 2014; Aba and Asuzu, 2017; Bharti *et al*., 2018; Isemura *et al*., 2019)
2	2.747	ρ-Coumaric acid (MW 164.1 g/mol)	$C_9H_8O_3$ 	Phenylpropanoid	4.95	Antioxidant, anti-cancer, antimicrobial, antivirus, anti-inflammatory, antiplatelet aggregation, anxiolytic, antipyretic, analgesic, and anti-arthritis activities (Zhu *et al*., 2018; Boo, 2019)
3	4.871	Rutin (quercetin-3-O-rutinoside and sophorin) (MW 610.5 g/mol)	$C_{27}H_{30}O_{16}$ 	Glycoside flavonol	7.75	Antioxidant. Promote utilization of vitamin C and formation of collagen. (Aba and Asuzu, 2017: Bharti *et al*., 2018, Enogieru *et al*., 2018).
4	4.977	Avenic acid (MW 322.3 g/mol)	$C_{12}H_{22}N_2O_8$ 	Gamma (γ) amino acid	15.33	Phytosiderophore (iron chelator). (Tsednee *et al*., 2012)

5	5.389	Hydroxyavenic acid	$C_{12}H_{23}N_2O_9$ 	Gamma (γ) amino acid	1.34	Phytosiderophore (iron chelator). (Tsednee *et al*., 2012; Michel *et al*., 2019)
6	5.688	Zeatin (MW 219.3 g/mol)	$C_{10}H_{13}N_5O$ 	Alkaloid	4.37	Antiageing. Protects brain from proteinaceous deposits and prevent formation of amyloid plaques, antidiabetic. (Agrawal, Sethiya and Mishra, 2013; Bharti *et al*., 2018)
7	8.582	Apigenin 6-C-glucoside (MW 564.5g/mol)	$C_{26}H_{28}O_{14}$ 	Glycoside	0.09	Antioxidant, antidiabetic, anticancinogenic and antimutagenic (Bharti *et al*., 2018)
8	8.943	Kaempferol (MW 286.23g/mol)	$C_{15}H_{10}O_6$ 	Flavonoid	8.64	Antiinflamatory, antioxidant, anti-inch, antiatherogenic. (Bharti *et al*., 2018; Ren *et al*., 2019)

9	9.022	Luteolin (MW 286.23g/mol)	$C_{15}H_{10}O_6$ 	Flavonoid	11.49	Antioxidant, anti-inflammatory, immune system modulator, anticancers. (Saxena *et al*., 2013; Aba and Asuzu, 2017; Bharti *et al*., 2018; Isemura *et al*., 2019)
10	9.134	Tricin (MW 330.29g/mol)	$C_{17}H_{14}O_7$ 	Flavonoid	6.32	Antibacterial, antioxidant, anticancer. Eye and ear treatment. (Li et al., 2016)
11	9.175	Chrysoeriol (MW 300.26g/mol)	$C_{16}H_{12}O_6$ 	Flavonoid (3'-O- methyl derivative of Luteolin)	9.77	Antiinflamatory, antineoplastic, antioxidant, antibacterial (Bashyal *et al*., 2018; Vestena *et al*., 2019)
12	9.236	Apigenin (MW 270.05g/mol)	$C_{15}H_{10}O_5$ 	Flavonoid	11.38	Induces autophagy in leukaemia cells, antineoplastic, muscle relaxation, sedation, antiinflamatory (Aba and Asuzu, 2017; Bharti *et al*., 2018)
13	9.638	Quercetin (MW 302.24g/mol)	$C_{15}H_{10}O_7$	Flavonoid	0.40	Antiviral, antioxidant, antiinflamatory, anticancer (Aba and Asuzu, 2017; Bharti *et al*., 2018; Missoun *et al*., 2018)

						
14	9.706	Isorhamnetin (MW 316.26g/mol)	$C_{16}H_{12}O_7$ 	Flavonoid	5.03	Antiviral, antioxidant, antiinflamatory, anticancer (Antunes-Ricardo *et al*., 2014; Aba and Asuzu, 2017; Bharti *et al*.,2018)
15	9.794	Procyanidin B1 (MW 578.5g/mol)	$C_{30}H_{26}O_{12}$ 	Biflavonoid	4.33	Antioxidant (Bharti et al., 2018)
16	9.842	Prodelphinidin B3 (MW 594.52g/mol)	$C_{30}H_{26}O_{13}$ 	Flavonoid	8.02	Antiprostate cancer
17	10.795	Campesterol (MW	$C_{28}H_{48}O$	Steroid	0.01	Cholesterol lowering, anticancer, treatment of cardiovascular diseases

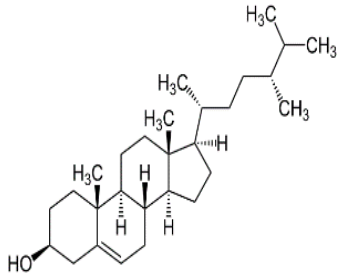
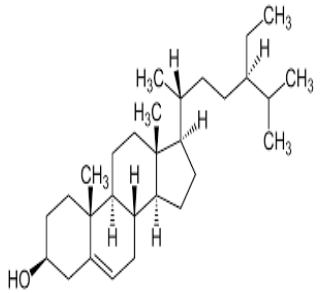
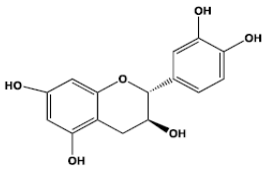
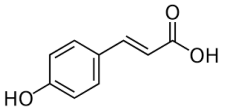
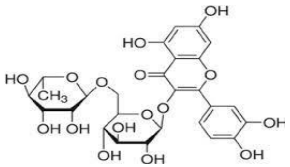
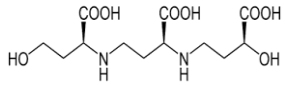
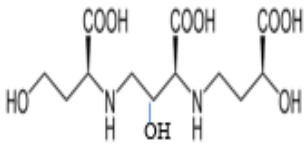
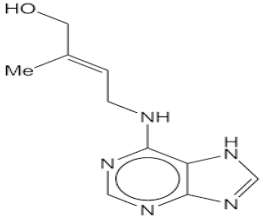
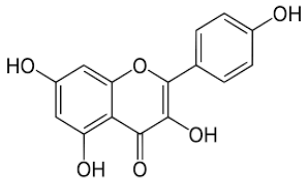
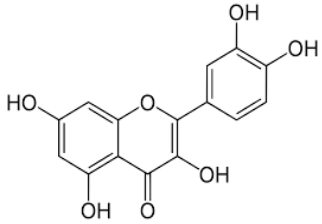
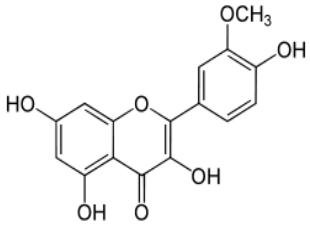
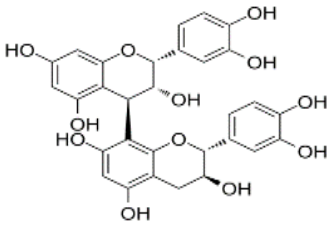
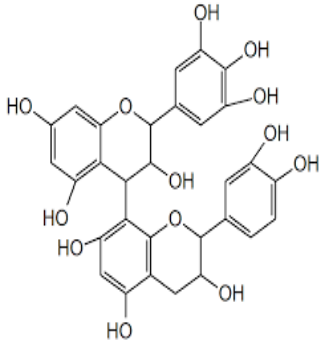
		400.68g/mol)				
18	10.925	Beta-Sitosterol (MW 414.71g/mol)	$C_{29}H_{50}O$ 	Steroid	0.24	Erectile dysfunction, anti benign prostatic hyperplasia (BPH), lower cholesterol, immune modulator (Gaikwad, Mohan and Rani, 2014)

Table 4.33 Compounds present in aqueous extract of *H. vulgare*

Peak#	RT	Compound name	Formula and structure	Phytochemical class	Concentration (ng/g)	Ethnobotanical/biological use
1	1.49	Catechin (MW 290.3 g/mol)	$C_{15}H_{14}O_6$ 	Flavanoid	2.87	Antimicrobial. Antioxidant. Prevention of cancer, obesity, diabetes and neurodegenerative diseases. (Gaikwad, Mohan and Rani, 2014; Aba and Asuzu, 2017; Bharti *et al*., 2018; Isemura *et al*., 2019)
2	2.74	ρ-Coumaric acid (MW 164.1 g/mol)	$C_9H_8O_3$ 	Phenylpropanoid	5.68	Antioxidant, anti-cancer, antimicrobial, antivirus, anti-inflammatory, antiplatelet aggregation, anxiolytic, antipyretic, analgesic, and anti-arthritis activities (Zhu *et al*., 2018; Boo, 2019)
3	4.72	Rutin (quercetin-3-O-rutinoside and sophorin) (MW 610.5 g/mol)	$C_{27}H_{30}O_{16}$ 	Glycoside flavonoid	2.78	Antioxidant. Promote utilization of vitamin C and formation of collagen. Antioxidant. Promote utilization of vitamin C and formation of collagen. (Aba and Asuzu, 2017: Bharti *et al*., 2018, Enogieru *et al*., 2018).
4	4.99	Avenic acid (MW 322.3 g/mol)	$C_{12}H_{22}N_2O_8$ 	Gamma (γ) amino acid	11.22	Phytosiderophore (iron chelator). Phytosiderophore (iron chelator). (Tsednee *et al*., 2012)

5	5.38	Hydroxyavenic acid (MW 339.14g/mol)	$C_{12}H_{23}N_2O_9$ 	Gamma (γ) amino acid	1.66	Phytosiderophore (iron chelator). (Tsednee *et al*., 2012; Michel *et al*., 2019)
6	5.67	Zeatin (MW 219.3 g/mol)	$C_{10}H_{13}N_5O$ 	Cytokinin	18.88	Antiageing. Protects brain from proteinaceous deposits and prevent formation of amyloid plaques, antidiabetic. (Agrawal, Sethiya and Mishra, 2013; Bharti *et al*., 2018)
7	8.90	Kaempferol (MW 286.23g/mol)	$C_{15}H_{10}O_6$ 	Flavonoid	3.08	Antiinflamatory, antioxidant, anti-inch, antiatherogenic. (Bharti *et al*., 2018; Ren *et al*., 2019)
8	9.64	Quercetin (MW 302.24g/mol)	$C_{15}H_{10}O_7$ 	Flavonoid	0.01	Antiviral, antioxidant, antiinflamatory, anticancer (Aba and Asuzu, 2017; Bharti *et al*., 2018; Missoun *et al*., 2018)

9	9.70	Isorhamnetin (MW 316.26g/mol)	$C_{16}H_{12}O_7$ 	Flavonoid	8.13	Antiviral, antioxidant, antiinflamatory, anticancer (Antunes-Ricardo *et al*., 2014; Aba and Asuzu, 2017; Bharti *et al*.,2018)
10	9.79	Procyanidin B1 (MW 578.5g/mol)	$C_{30}H_{26}O_{12}$ 	Flavonoid	5.26	Antioxidant (Bharti et al., 2018)
11	9.84	Prodelphinidin B3 (MW 594.52g/mol)	$C_{30}H_{26}O_{13}$ 	Flavonoid	7.59	Antiprostate cancer

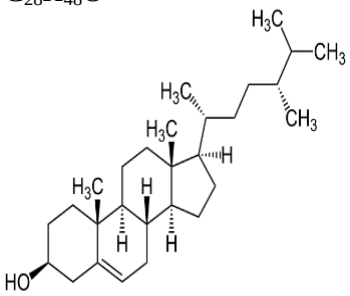
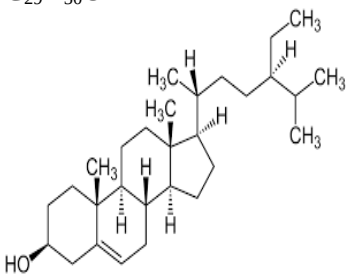
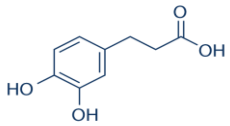
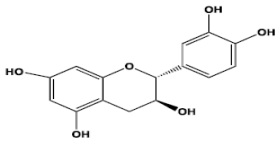
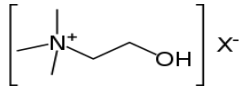
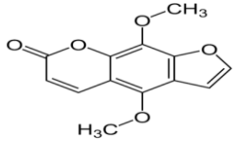
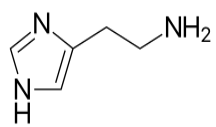
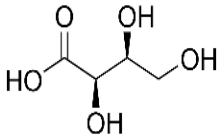
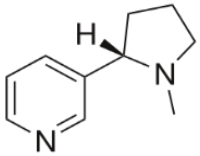
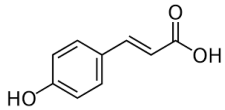
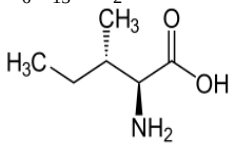
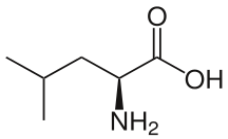
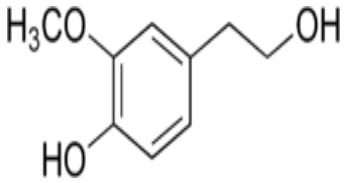
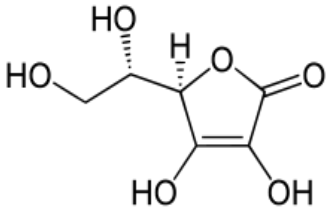
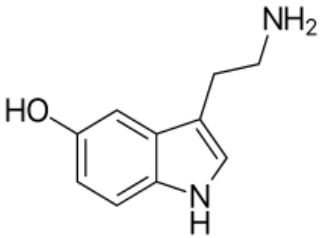
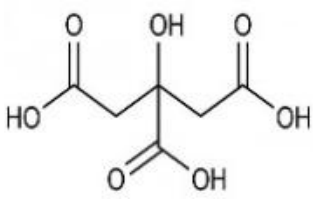
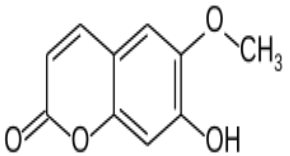
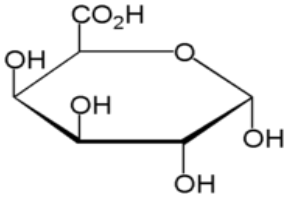
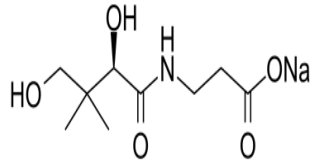
12	10.79	Campesterol (MW 400.68g/mol)	$C_{28}H_{48}O$ 	Steroid	0.62	Cholesterol lowering, anticancer, treatment of cardiovascular diseases
13	10.92	Beta-Sitosterol (MW 414.71g/mol)	$C_{29}H_{50}O$ 	Steroid	2.07	Erectile dysfunction, anti benign prostatic hyperplasia (BPH), lower cholesterol, immune modulator (Gaikwad, Mohan and Rani, 2014)

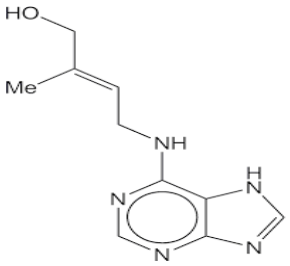
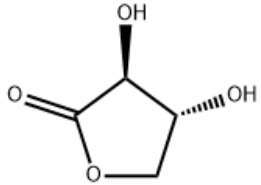
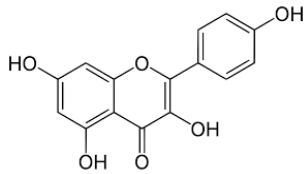
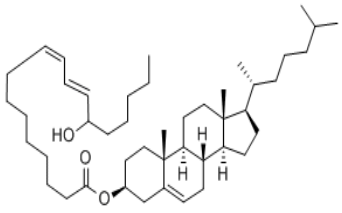
Table 4.34 Compounds present in aqueous extract of *U. massaica*

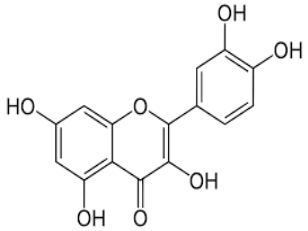
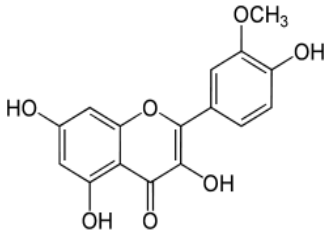
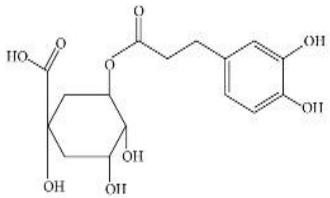
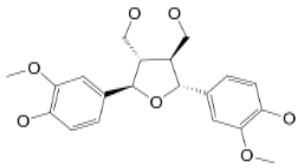
Peak#	RT	Compound name	Formula and structure	Phytochemical class	Concentration (ng/g)	Ethnobotanical/biological use
1	1.32	3,4-Dihydroxyhydrocinnamic acid (MW 182.17g/mol)	$C_9H_{10}O_4$ 	Phenylpropanoid	16.74	Antioxidant
2	1.47	Catechin (MW 290.3 g/mol)	$C_{15}H_{14}O_6$ 	Flavanoid	8.80	Antimicrobial. Antioxidant. Prevention of cancer, obesity, diabetes and neurodegenerative diseases. (Gaikwad, Mohan and Rani, 2014; Aba and Asuzu, 2017; Bharti *et al*., 2018; Isemura *et al*., 2019)
3	1.52	Choline (MW 104.17g/mol)	$C_5H_{14}NO$ 	Alkaloid	6.53	Synthesize components of cell membranes (phospholipids);-phosphatidylcholine and sphingomyelin. Production of neurotransmitter acetylcholine. Modulating gene expression, cell membrane signaling, lipid transport and metabolism, and early brain development.
4	1.56	Isopimpinellin (MW 246.21g/mol)	$C_{13}H_{10}O_5$ 	Phenylpropanoid lactone	10.76	Lipid metabolism (proote lipolysis and suppress antilipolytic hormones. Anticoagulant anticonvulsant

5	1.60	Histamine (MW 111.15g/mol)	$C_5H_9N_3$ 	Alkaloid	1.21	Immune response, neurotrasmitter
6	2.53	Threonic acid (MW 136.10g/mol)	$C_4H_8O_5$ 	Sugar	15.66	Treatment of androgenic allopecia
7	2.68	Nicotine (MW 162.24g/mol)	$C_{10}H_{14}N_2$ 	Alkaloid	4.08	Recreational stimulant/anxiolytic. Suppreses symptoms of tobacco smoking withdrawal
8	2.70	ρ-Coumaric acid (MW 164.1g/mol)	$C_9H_8O_3$ 	Phenylpropanoid	3.47	Antioxidant, anti-cancer, antimicrobial, antivirus, anti-inflammatory, antiplatelet aggregation, anxiolytic, antipyretic, analgesic, and anti-arthritis activities (Zhu *et al.*, 2018; Boo, 2019)
9	2.88	Isoleucine (MW 131.17g/mol)	$C_6H_{13}NO_2$ 	Aliphatic amino acid	2.10	Vitamins useful in wound healing, detoxification, stimulating immune function, promoting secretion of several hormones. Haemoglobin synthesis, glucose metabolism

10	3.14	Leucine (MW 131.17g/mol)	$C_6H_{13}NO_2$ 	Nonpolar aliphatic amino acid	6.93	Growth and repair of muscle, skin and bone.
11	3.54	Homovanillyl alcohol (MW 168.19g/mol)	$C_9H_{12}O_3$ 	Phenylethanoid	0.75	Preventive and mitigation factor in cardiovascular disease.
12	3.79	Ascorbic acid	$C_6H_8O_6$ 	Vitamin	5.63	Tissue repare, neurotransmitters production in enzymatic systems, antioxidant, anticancer
13	3.80	5-Hydroxy Tryptamine (MW 176.22g/mol)	$C_{10}H_{12}N_2O$ 	Derivative of tryptophan	8.82	Intermediate in synthesis of serotonin neurotrasmitter

14	4.82	Citric acid	$C_6H_8O_7$ 	Carboxylic acid	1.18	Energy metabolism, prevent formation of kidney stones
15	5.27	Scopoletin (MW 194.14g/mol)	$C_{10}H_8O_4$ 	Phenylpropanoid	12.64	Antifungal
16	5.39	Galacturonic acid (MW 194.14g/mol)	$C_6H_{10}O_7$ 	Sugar acid	5.20	Acidifying agent
17	5.67	Pantothenic acid (MW 219.23g/mol)	$C_9H_{17}NO_5$ 	Alkaloid	2.12	Precursor of coenzyme A

18	5.73	Zeatin (MW 219.13g/mol)	$C_{10}H_{13}N_5O$ 	Nicotinimic alkaloid	0.86	Antiageing. Protects brain from proteinaceous deposits and prevent formation of amyloid plaques, antidiabetic. (Agrawal, Sethiya and Mishra, 2013; Bharti *et al*., 2018)
19	8.40	Threono-1,4-lactone (MW 118.09g/mol)	$C_{10}H_{22}O_4Si_2$ 	Modified sugar	9.33	Antiathritic. Promote bone mineralization
20	8.91	Kaempferol (MW 286.23g/mol)	$C_{15}H_{10}O_6$ 	Flavonoid	0.35	Antiinflamatory, antioxidant, anti-inch, antiatherogenic, antiinflamatory, antioxidant, anti-inch, antiatherogenic. (Bharti *et al*., 2018; Ren *et al*., 2019)
21	9.46	13-hydroxy-9,11-octadecadienoic acid (MW 296.4g/mol)	$C_{18}H_{32}O_3$ 	Steroidal fatty acid derivative	2.37	Anticancer, immunomodulation, antiatherosclerotic

22	9.68	Quercetin (MW 302.24g/mol)	$C_{15}H_{10}O_7$ 	Flavonoid	2.02	Antiviral, antioxidant, antiinflamatory, anticancer (Aba and Asuzu, 2017; Bharti *et al*., 2018; Missoun *et al*., 2018)
23	9.77	Isorhamnetin (MW 316.26g/mol)	$C_{16}H_{12}O_7$ 	Flavonoid	1.04	Antiviral, antioxidant, antiinflamatory, anticancer (Antunes-Ricardo *et al*., 2014; Aba and Asuzu, 2017; Bharti *et al*.,2018)
24	9.89	Chlorogenic acid (MW 354.31g/mol)	$C_{16}H_{18}O_9$ 	Glycoside phenylpropanoid	1.53	Antibacterial, antioxidant, anticarcinogenic, antidiabetic, antihyperlipidaemia.
25	10.04	Neoolivil (MW 376.4g/mol)	$C_{20}H_{24}O_7$ 	Phenylpropanoid derivative	4.94	Antiproliferative

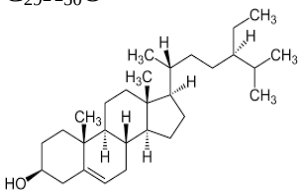
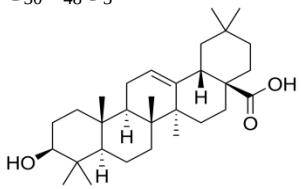
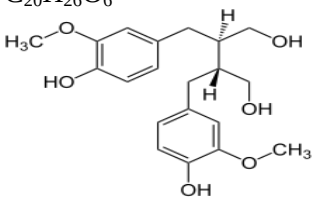
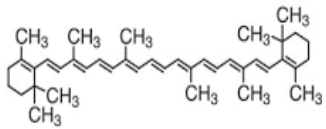
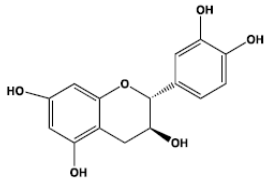
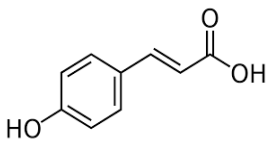
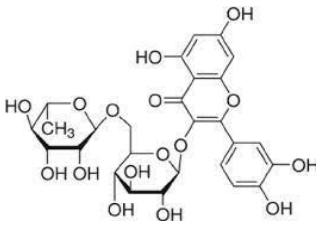
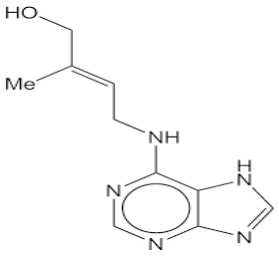
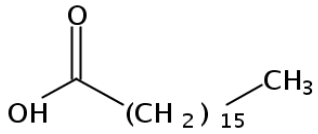
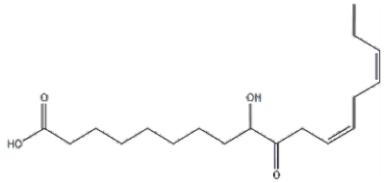
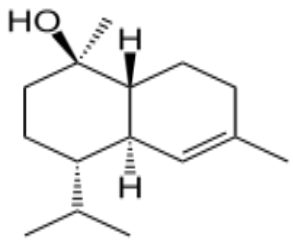
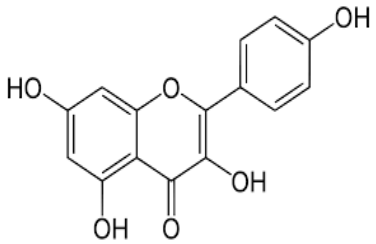
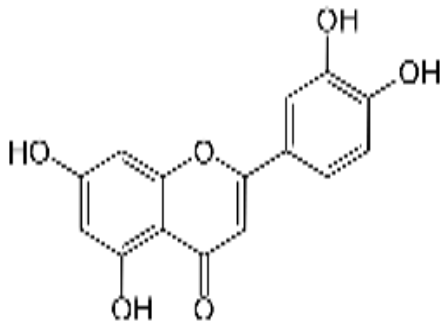
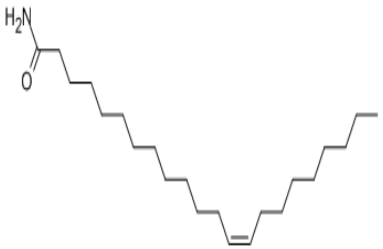
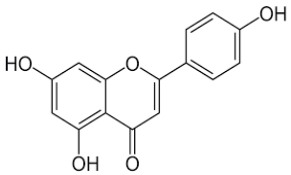
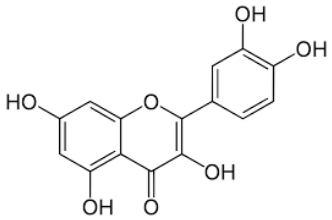
26	10.09	Beta-Sitosterol (MW 414.71g/mol)	$C_{29}H_{50}O$ 	Steroid	0.70	Erectile dysfunction, anti benign prostatic hyperplasia (BPH), lower cholesterol, immune modulator (Gaikwad, Mohan and Rani, 2014)
27	10.24	Oleanolic acid (MW 456.7g/mol)	$C_{30}H_{48}O_3$ 	Pentacyclic triterpenoid (PCCT)	3.51	Antioxidant, anti-inflammatory, antiviral, anti-diabetic.
29	10.44	Secoisolariciresinol glucoside (MW 362.42g/mol)	$C_{20}H_{26}O_6$ 	Phenylpropanoid derivative	2.96	Antiproliferative, antioxidant, antiestrogenic, antiangiogenic.
30	10.56	Beta-carotene (MW 536.87g/mol)	$C_{40}H_{56}$ 	Tetraterpenoid	1.40	Provitamin A carotenoid, antioxidant, immune modulator, antineoplastic, chemopreventive.

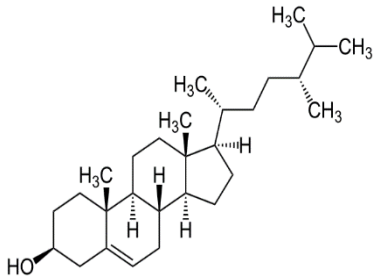
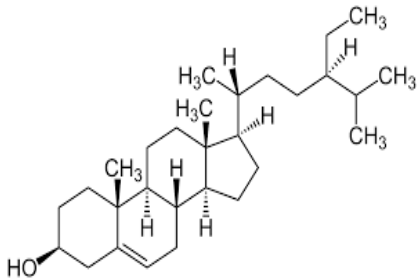
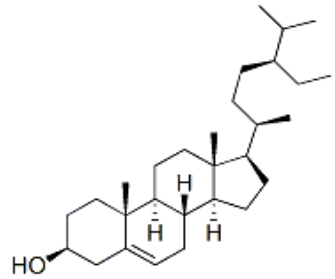
Table 4.35 Compounds present in aqueous extract of *C. viminale*

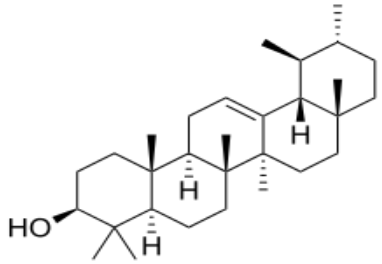
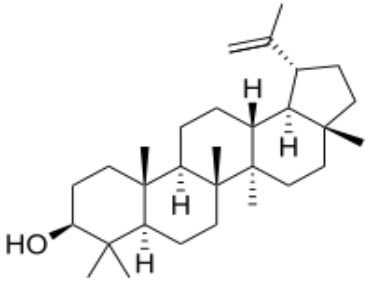
Peak#	RT	Compound name	Formula and structure	Phytochemical class	Concentration (ng/g)	Ethnobotanical/biological use
1	1.48	Catechin (MW 290.3 g/mol)	$C_{15}H_{14}O_6$ 	Flavanoid	70.0	Antimicrobial. Antioxidant. Prevention of cancer, obesity, diabetes and neurodegenerative diseases. (Gaikwad, Mohan and Rani, 2014; Aba and Asuzu, 2017; Bharti *et al*., 2018; Isemura *et al*., 2019)
2	2.726	ρ-Coumaric acid (MW 164.1 g/mol)	$C_9H_8O_3$ 	Phenylpropanoid	0.1	Antioxidant, anti-cancer, antimicrobial, antivirus, anti-inflammatory, antiplatelet aggregation, anxiolytic, antipyretic, analgesic, and anti-arthritis activities (Zhu *et al*., 2018; Boo, 2019)
3	4.759	Rutin (quercetin-3-O-rutinoside and sophorin) (MW 610.5 g/mol)	$C_{27}H_{30}O_{16}$ 	Glycoside flavonoid	2.8	Antioxidant. Promote utilization of vitamin C and formation of collagen. (Aba and Asuzu, 2017: Bharti *et al*., 2018, Enogieru *et al*., 2018).

4	5.719	Zeatin (MW 219.3g/mol)	$C_{10}H_{13}N_5O$ 	Alkaloid	9.0	Antiageing. Protects brain from proteinaceous deposits and prevent formation of amyloid plaques, antidiabetic. (Agrawal, Sethiya and Mishra, 2013; Bharti *et al*., 2018)
5	7.183	Hexadecanoic acid, methyl ester (MW 270.45g/mol)	$C_{18}H_{36}O_2$ 	Fatty acid ester	8.1	Antifungal
6	7.247	Heptadecanoic acid (MW 270.45g/mol)	$C_{17}H_{34}O_2$ 	Fatty acid	5.5	Anti-cancer (lung)
7	7.309	9, 12-Octadecadienoic acid (Z, Z)- (MW 280.4g/mol)	$C_{18}H_{32}O_2$ 	Fatty acid	10.2	

8	7.421	Alpha-cadinol (MW 222.37g/mol)	$C_{15}H_{26}O$ 	Sesquiterpenoid	17.7	Antifungal
9	9.011	Kaempferol (MW 286.23g/mol)	$C_{15}H_{10}O_6$ 	Flavonoid	52.7	Antiinflamatory, antioxidant, anti-inch, antiatherogenic. (Bharti *et al*., 2018; Ren *et al*., 2019)
10	9.076	Luteolin (MW 286.23g/mol)	$C_{15}H_{10}O_6$ 	Flavonoid	5.0	Antioxidant, anti-inflammatory, immune system modulator, anticancers. (Saxena *et al*., 2013; Aba and Asuzu, 2017; Bharti *et al*., 2018; Isemura *et al*., 2019)

11	9.161	13-Docosenamide, (Z)- (MW 337.58g/mol)	$C_{22}H_{43}NO$ 	Fatty acid derivative	27.5	
12	9.215	Apigenin (MW 270.05g/mol)	$C_{15}H_{10}O_5$ 	Flavonoid	57.3	Induces autophagy in leukaemia cells, antineoplastic, muscle relaxation, sedation, antiinflamatory (Aba and Asuzu, 2017; Bharti *et al*., 2018)
13	9.613	Quercetin (MW 302.24g/mol)	$C_{15}H_{10}O_7$ 	Flavonoid	45.7	Antiviral, antioxidant, antiinflamatory, anticancer (Aba and Asuzu, 2017; Bharti *et al*., 2018; Missoun *et al*., 2018)

14	10.771	Campesterol (MW 400.68g/mol)	$C_{28}H_{48}O$ 	Steroid	15.7	Cholesterol lowering, anticancer, treatment of cardiovascular diseases
15	10.856	Beta-Sitosterol (MW 414.71g/mol)	$C_{29}H_{50}O$ 	Steroid	8.8	Erectile dysfunction, anti benign prostatic hyperplasia (BPH), lower cholesterol, immune modulator (Gaikwad, Mohan and Rani, 2014)
16	10.907	Stigmast-5-en-3-ol, (3.beta.)- (MW 414.71g/mol)	$C_{29}H_{50}O$ 	Steroid	46.7	Antidiabetic

17	11.02	Alpha-amyrin (MW 426.7g/mol)	$C_{30}H_{50}O$ 	Pentacyclic triterpenoid (PCCT)	4.1	Antinociceptive, anti-inflammatory
18	11.084	Lupeol (MW 426.72g/mol)	$C_{30}H_{50}O$ 	Pentacyclic triterpenoid (PCCT)	48.4	Anticancer, antiinflamatory, antioxidant, antiarthritic, wound healing (Saleem M., 2009)

4.7.3 Macronutrients and minerals composition of *T. aestivum, H. vulgare, U. massaica* and *C. viminale*

Table 4.36 presents the composition of macronutrients and minerals established in the aqueous extracts of *T. aestivum, H. vulgare, U. massaica* and *C. viminale*. Carbohydrates contents was highest in *U. massaica*. *T. aestivum* and *H. vulgare* had the equal proteins contents compared to the other extracts. Calcium, copper, iron, potassium, sodium, magnesium, manganese, zinc and phosphates were present in all the plants extracts in varying concentrations. Notably, *U. massaica* had highest levels of calcium and magnesium. *T. aestivum* was highest in levels of potassium, manganese and phosphates while *H. vulgare* was highest in the levels of zinc. Calcium and zinc levels were notably higher in *C. viminalle* than *T. aestivum* while zinc contents of *C. viminalle* were higher than in *T. aestivum* and *U. massaica*. The levels of calcium, iron and sodium were not distinctively different between the plants extracts.

Table 4.36: Macronutrients and minerals composition of aqueus extracts of *T. aestivum, H. vulgare, U. massaica* and *C. viminale*

Substance	Units of reporting	*T. aestivum*	*H. vulgare*	*U. massaica*	*C. viminale*
Carbohydrates	mg/L	1614	1350	1950	856
Proteins	%	39.4	39.4	35.0	8.8
Calcium	g/kg	3.0	2.0	42.4	37.0
Copper	g/kg	1.04	1.20	0.88	1.10
Iron	g/kg	0.22	0.12	0.24	0.19
Potassium	g/kg	68.9	50.2	46.6	51.3
Sodium	g/kg	3.48	4.00	3.2	3.68
Magnesium	g/kg	1.82	1.88	12.79	2.2
Manganese	g/kg	0.98	0.34	0.11	0.05
Zinc	g/kg	2.91	13.96	0.22	9.34
Phosphates	g/kg	3.15	2.74	0.83	1.1

4.7.4 Amino acids composition of *T. aestivum, H. vulgare, U. massaica* and *C. viminale*

Table 4.37 presents the amino acids contents in the plant under study. A total of seventeen amino acids were listed for determination. From the LC/MS spectrum generated, the mass of individual amino acid was used in deriving the amino acids present in the plants extracts. Threonine, glutamine, valine, isoleucine and lysine were present in all plants extracts. Amino acids not detected in either of the plants extracts included serine, glycine, methionine, leucine, tyrosine, phenylalanine and histidine. Tryptophan and alanine were found in *T. aestivum* and *U. massaica,* respectively. Asparagine was not detected in *T. aestivum* while arginine was not detected in *C. viminale*. Proline was found in *T. aestivum* and *U. massaica* but was not present in *C. viminale* and *H. vulgare*. Respective spectrophotometric spectrums are presented in Appendix 12.

Table 4.37: Amino acids composition of aqueous extracts of *T. aestivum, H. vulgare, U. massaica* and *C. viminale* by LC/MS

No.	AMINO ACID	Mol. Wt (g/mol	*H. vulgare*	*T. aestivum*	*U. massaica*	*C. viminale*
1	Asparagine	134.1	+	ND	+	+
2	Threonine	120.1	+	+	+	+
3	Serine	106.1	ND	ND	ND	ND
4	Tryptophan	205.2	ND	+	ND	ND
5	Glutamine	147.1	+	+	+	+
6	Glycine	76.1	ND	ND	ND	ND
7	Alaninie	90.1	ND	ND	+	ND
8	Valine	118.1	+	+	+	+
9	Methionine	150.2	ND	ND	ND	ND
10	Isoleucine	132.2	+	+	+	+
11	Leucine	132.2	NSTD	NSTD	NSTD	NSTD
12	Tyrosine	182.2	ND	ND	ND	ND
13	Phenylalanine	166.2	ND	ND	ND	ND
14	Histidine	156.2	ND	ND	ND	ND
15	Lysine	147.2	+	+	+	+
16	Argenine	175.2	+	+	+	ND
17	Proline	116.1	ND	+	+	ND

Key: ND = not determined, NSTD = standard not available, + = present.

CHAPTER FIVE

DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

5.1 Discussion

This study was carried out to determine the *in vivo* hypoglycemic effects of orally and intraperitoneally administered aqueous extracts of *T. aestivum*, *H. vulgare*, *U. massaica* and *C. viminale* in rat models. The study also endevoured to evaluate the safety of the plants extracts upon intake.

Intraperitoneally administered alloxan monohydrate at a dose of 186.9 mg per kilogram body weight increased the blood glucose of the Wistar rats. Alloxan monohydrate is a common glucose analogue that act by selectively causing necrosis of the pancreatic β cells of the rats leading to hyperglycemia (Hashemi *et al*., 2009). Rats with blood glucose level of 11.1 mmol/L (200mg/dl) and above were considered diabetic. Alloxan induced diabetes has been used extensively as a suitable experimental model of type II diabetes (Karau *et al*., 2012). The blood glucose of the alloxan treated rats in the current study was two to five times higher compared to that in control rats. Thus, the results obtained in this study indicate that alloxan monohydrate at the dose level used in this study is diabetogenic in Wister rats.

The results obtained in this study showed that orally or intraperitoneally administered aqueous extracts of *T. aestivum*, *H. vulgare*, *U. massaica* and *C. viminale* at dose levels of 50, 91, 165 and 300 mg/kg body weight decreased the blood glucose of alloxan induced diabetic rats. The findings concur with a study by Yogesha *et al*. (2013), who reported significant blood glucose lowering ability by orally administered ethanolic extract of *T. aestivum* in streptozotocin induced diabetic

Winstar rats. Similary, blood glucose lowering potential of methanolic extracts of *Aloe camperi, Meriandra deathra* and a mixture of *Lepidium sativum, Brassica nigra and nigella sativa* was demonstrated in alloxan induced diabetic Winstar rats (Demoz *et al*., 2015). Further, aqueous and ethanolic extracts of *Moringa oliefera* leaves were reported to have blood glucose reducing potential in streptozotocin induced diabetic rats (Nada *et al*., 2015).

The *in vivo* blood glucose lowering abilities of the plant extracts used in this study could be linked to their constituent secondary metabolites. As reported in the current study, the studied plants had varying phytochemical composition. Qualitatively, all the four aqueous plant extracts in the present study were found to contain tannins, flavonoids, saponins, steroids and cardiac glycosides. Terpenoids and reducing sugars were not present in *U. massaica* and *C. viminale* extracts. Phylobatanins were not present in *C. viminale* extract. Alkaloids were not detected in all the studied plants extracts (Table 4.31)

Further elucidation of the phytochemical composition of the plants extracts by liquid chromatography/mass spectrophotometric (LCMS) analysis described the compounds present in various classes of secondary metabolites (Tables 32, 33, 34, 35). The LCMS assay indicated the major classes' of phytochemicals being flavonoids, phenylpropanoids, alkaloids and steroids whose concentrations varied between the extracts (Figure 4.9). Other than the major phytoconstituemts of the plants presented, the extracts equally contained sugar, derived sugars and amino acids. Several other compounds were not identified (that is, they were unknown).

The phytoconstituents of plants have been of great interest and the results obtained in the current study concurs with others that have reported varying phytochemicals in medicinal plants. A study by Olanipekun *et al*. (2013) identified different classes of phytochemicals in aqueous extracts of *Ocimum bascilicum, Ocimum gratisimum, Chromola enaodorata, Zingiber officinale, Cissalmpelou sowariensis, Aframomum meleguata, Parquetin anigrensis, Tithonia diversifolia, Venonia amygdalina and. Pergulari adaemia.* Likewise, different composition of phytochemicals were demonstrated in acetone, methanolic, benzene and aqueous extracts of *Lantana camara, Annona squamosal, Carica papaya and Acalypha indica by* Pradeep *et al.* (2014). In assessment of secondary metabolites in plants, ethanolic extracts of *Bacopa monnisa* (L), *Pennel grandis* and *Coccinia grandis* (L) were reported by Alamgir *et al*. (2014) to have varying composition of phytochemicals. Other researchers have equally demonstrated varying presence of secondary metabolites in different plants extracts (Karau *et al*., 2012; Wadood *et al*., 2013; Madumelu *et al*., 2013; Mahalakshmi *et al*., 2016).

The pharmacological properties of medicinal plants have been attributed to their phytochemical compositions and ethnobotany continues to document plants with observed medicinal properties. The secondary metabolites biosynthesized in plants synergistically exhibits an array of biological properties such as their antioxidant, anti-inflammatory, antimicrobial, antinoniceptive, antiallergic, anticancer and antidiabetic activities (Saxena *et al*., 2013). In a review of antidiabetic potential of medicinal plants, phytochemicals such as flavonoids, terpenoids, glycosides, alkaloids, carotenoids were reportedly associated with demonstrated hypoglycemic effects of numerous medicinal plants (Malviya *et al*., 2010). Further, Malviya *et al.*

(2010) reported the antihyperglycaemic properties of plants to be attributed to their ability to rejuvenate pancreatic tissues function resulting to increased insulin output or inhibition of the intestinal absorption of glucose, or to facilitate metabolites involved in insulin dependent processes.

The antidiabetic potential of medicinal plants is multimodal and is derived through antioxidant properties, reduction of cholesterol, increased production and utilization of insulin, regulation of glucose (carbohydrate) metabolism and improvement of microcirculation (Bharti *et al.*, 2018). Specifically, the flavonoids exerts their antidiabetic activity by modulation of cell signaling enhancing hepatic hexokinases and glucose-6-phosphate dehydrogenases activities, reduced glycosylation of haemoglobin, increasing levels of insulin and ameriolation of oxidative stress by increasing activities of superoxide dismutase (SOD), catalase and glutathione peroxidase. Alkaloids act by control of cholesterol and triglycerides, reductions in phosphoenol pyruvate carboxykinase and aldose reductase, improvement in the activity of GLUT 4, glucokinase and peroxisome PPARɣ, increased hepatic glycogen content and reduction of glucose-6-phosphatase activity. Terpenoids antidiabetic activity is through suppression of pro-inflammatory (TNFα) and increase anti-inflammatory agent (adiponectin), activation of the AMPK pathway as well as translocation of Glut 4. The glycosides act by decreasing the activities of serum α-amylase and lactate dehydrogenase antioxidant activity (Aba and Asuzu, 2018).

As reported in this study, all the plants extracts reduced the blood glucose of alloxan induced diabetic Winstar rats. Various classes of phytochemicals and individual compounds present in the aqueous extracts of the plants as documented in the current

study explains the plant hypoglycemic capability. For instance, the flavanoids identified in the plants extracts in this study include the flavon-3-ol (Catechin), the flavonols (isorhamnetin, kaempferol, rutin and quercetin) and the flavones (apeginin and luteolin). Isorhamnetin reduces oxidative stress, improves lipid metabolism and prevents accumulation of sorbitol. Kaempferol inhibits apoptosis, enhances cAMP signalling and insulin synthesis and secretion, and reduces caspase-3 activity in beta cells, oxidative stress, IL-1ß, TNFa, lipid peroxidation and glycosylated haemoglobin. Rutin restores the status of the liver, improves insulin secretion and glycogenenolysis, ameriolate oxidative stress and inhibits advanced glycation end products (AGEs) formation. Quercetin actions are exerted by increasing antioxidant enzymes, reduction in lipid peroxidation and inhibition of GLUT 2 thus reducing intestinal glucose absorption. Quercetin also blocks tyrosine kinase activity, mediates through AMPK pathway and promote proliferation and recovery of pancreatic cells. Apigenin acts by enhancement of translocation of glucose transporter 4 (GLUT 4) and preservation of the pancreatic beta cells while Luteolin improves insulin secretion. Beta-sitosterol normalize insulin and blood sugar levels by inducing the secretion of insulin even in the absence of any stimulatory glucose concentration (Gaikwood *et al*., 2014; Aba and Asuzu, 2018).

The findings in this study concurs with similar studies on other antidiabetic plants. While reporting on the hypoglycemic potential of ethanolic extract of *T. aestivum* in winstar albino rats, Yogesha *et al*. (2013) equally attributed the hypoglycemic effect to improvement of insulin sensitivity, enhancement of glucose dependent insulin secretion, inhibiting glucose absorption and activity of intestinal disacharidase enzymes and increasing peripheral glucose utilization as possible mechanisms of the

phytocompounds present in the plant (Yogesha *et al*., 2013). A study on *Terminalia arjuna* antidiabetic effect on alloxan induced diabetic rats linked tannins, flavonoids, saponins and others to stimulation of insulin production by the pancreatic β cells, which, inturn enhance tissue uptake of glucose, increase activity of glucokinase, phosphofructokinase and hexose. This leads to increased hepatic glycolysis while decreasing glucose-6-phosphatase and fructose-1, 6-diphosphatase, thus suppressing gluconeogenesis (Ragavan and Krishnakumari, 2006). The findings in the current study further support the report of Hui *et al*. (2009) in a review of hypoglycemic herbs and their mode of action. Probable hypoglycemic mechanism of medicinal plants were described to be promotion of insulinotropic activity of the pancrease, increasing glucose uptake by extrahepatic tissue or suppressing intestinal glucose absorption and gluconeogenesis (Hui *et al*., 2009). The reduction of blood glucose exhibited by the plants in the current study equally concurs with findings of locally conducted studies on antidiabetic plants by Karau *et al*. (2012) on *Pappea capensis* (L), Demoz *et al*. (2015) on *Aloe camperi* and *Meriandra diathera* as well as Mukundi *et al*. (2015) on *Acacia nilotica*.

The hypoglycemic potential of the aqueous extracts of the plants in this study can further be attributed to or enhanced by their mineral composition. Results obtained in this study indicate the aqueous extracts of *T. aestivum, H. vulgare, U. massaica* and *C. viminalle* to contain considerable amounts of calcium, copper, iron, potassium, sodium, magnesium, manganeses, zinc and phosphates (Table 4.32). The importance of trace elements and their presence in antidiabetic plant has been supported by earlier studies. Soetan *et al*. (2010) review the importance of mineral elements for humans, domestic animals and plants and gives details on biochemical functions of various

trace elements (Soetan *et al*., 2010). The variability in micro elements composition in medicinal plants has been highlighted (Silva *et al.,* 2016). A study on the trace elements contents in selected Kenyan antidiabetic plants, reported appreciable contents of Fe, Zn, Pb, Mg, Mn, Mo, Cr, and Nitrates in *Bidens pilosa, Erythrina abyssinica, Aspilia pluriseta, Strychnos henningsii* and *Catha edulis* whose antihyperglycaemic activities had been proven (Piero *et al.,* 2012). Further, Biswajit and Bhabesh, (2017) reported the presence of microelements that play vital roles in blood glucose reduction in *Aegle marmelos, Musa paradisiac* and *Garcinia peduncula* in a study on antidiabetic medicinal plants grown in North East India.

That the aqueous extracts of the plants in this study reduced the blood glucose when orally administered in a similar trend to glibenclamide points out to a probable mode of action of the plants extracts similar to that of glibenclamide (Njoroge *et al*., 2017). Glibenclamide (glyburide), an oral hypoglycemic second-generation sulfonylurea, is insulinotropic. It acts by increasing insulin secretion from pancreatic beta cells, which reduces blood glucose. In addition, glibenclamide also acts through reduction of serum glucagon levels in type I diabetes and an increase of insulin action on target tissues (Rambiritch, 2014).

For intraperitoneally administered treatments, insulin at 1 IU/kg body weight was used as the positive control. An hour post treatment administration, blood glucose levels of insulin treated rats was significantly lower than in all the rats treated with the plants extracts. From the 6^{th} to the 24^{th} hour, levels of blood glucose between the insulin and plants extracts treated diabetic rats were not statistically different. The trends in blood glucose decline was similarly recorded. The results obtained in this

study indicate hypoglycemic properties of the plants extracts intraperitoneally administered. However, the activity is delayed and prolonged compared to that of insulin. Insulin acts by promoting the synthesis and storage of glycogen, proteins and triglycerides by liver, muscle and adipose tissue. Effect on blood glucose observed post intraperitoneal administration of the plants extracts can be attributed to mode of action similar to that for orally administered extracts.

The current study evaluated the safety of uptake of *T. aestivum*, *H. vulgare*, *U. massaica* and *C. viminale.* The *in vivo* study focused on effects on body weight, absolute organ and percentage (relative) organ to body weight, hematopoietic system, cardiotoxicity, pancreatoxicity, hepatotoxicity and nephrotoxicity as a result of either orally or intraperitoneally administered plants extracts (Chanda *et al*., 2015; OECD, 2018). Orally administered aqueous extracts of the four plants in this study at the dose levels of 300, 448, 669 and 1000 mg per kilogram body weight did not affect the body weight of the Wistar rats. However, intraperitoneally administered same doses of the different plant extracts resulted in varying effects on the body weight. While intraperitoneally administered specified four doses of aqueous extracts of *T. aestivum* did not adversely affect the rats, *C. viminale* caused acute toxic effects from the dose level of 300 mg/kg body weight, *U. massaica* caused death of the rats from doses level equal to or above 448 mg/kg body weight while intraperitoneally administered *H. vulgare* was acutely toxic at 1000 mg/kg body weight. A study by Ameni *et al.* (2015) reported that oral administration of *Casearia sylvetris* did not alter the body weight of the rats despite the presence of rutin, quercetin, luteolin and chlorogenic flavonoids. In contrast, the presence of alkaloids (ρ-octopamine and synephrines), tannins and flavonoids (chlorogenic acid) were asscocated with reduction in the

general body weight (Karau *et al*., 2014; Mutungi *et al*., 2017). Cardenolide glycosides are known to be highly toxic. In this study, all the aqueous extracts were found to have cardiac glycosides. Cardiac glycosides inhibit myocardial Na^+-K^+ ATP-ase thereby increasing heart contraction which result in cardiac arrest. Cardenolide cardiac glycosides present in *Digitalis purpurea*, *Nerium oleander*, *Thevetia peruviana* and *Asclepias curassavica* have been linked to fatal toxicosis (Morsy, 2017). That different effects on the rats resulting from intraperitoneally administered non therapeutic doses of the plant extracts are recorded in this study, determination of the specific type and quantities of cardiac glycosides as well as other phytochemicals present will ellaborate more on the observed effects.

The results obtained in this study, indicated low absolute and relative weight of the liver as a result of orally administered *T. aestivum* (Table 4.10) and *U. massaica* (Table 4.16). The resultant decrease in the weight of the liver would point to a possibility of organ atrophy. However, evaluation of the serum enzyme biomarkers aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and gammaglutanyl transferase (GGT) have no indication of liver injury (Martha and Rolando, 2016). Decreased weight of the liver could also occur as a result of fasting *prior* to sacrificing of the rats, enzyme induction and age (Piao *et al*., 2013). Results obtained in this study compare to that on *Casearia slvestris* chronic toxicity as reported by Ameni *et al*. (2015). Likewise, there was no adverse effects observed as a result of high dose administration of ethanolic extracts of *Pericamplylus glaucus* (Kifayatullah *et al*., 2015).

The results obtained in this study further indicate that orally administered aqueous extracts of *T. aestivum, H. vulgare, U. massaica* and *C. viminale* at the specified doses did not have any adverse effect on the haematopoietic system. Absence of toxicologically significant differences between the extract treated rats and control in red blood cells, hemoglobin, and other red blood cells related indices, platelets, white blood total and differential counts for neutrophils, lymphocytes, monocytes, eosinophils and basophils indicate the plants have no adverse effects on the bone marrow. Furthermore, the increase in hemoglobin in the treated groups in relation to the normal values of the other RBC indices does not indicate any pathological contribution and is a positive indicator as a useful therapy for anemia. In addition, the results indicate no adverse effect on white blood cells as a result of orally administered aqueous extract of *T. aestivum, H. vulgare, U. massaica* and *C. viminale* at 300, 448, 669 and 1000 mg/kg body weight doses. Intraperitoneally administered, *T. aestivum* is safe up to the dose level of 1000mg/kg body weight, *U. massaica* at the dose level of 300 mg/kg body weight and *H.vulgare* is safe up to the dose of 669 mg/kg body weight. The results of the present study agree with others that reported no toxicological alteration to the haematopoietic system as a result of the administered plants extracts (Ameni *et al*., 2015; Kifayatullah *et al*., 2015).

Results in this study indicate no significant changes attributed to oral administration of plant extracts at the specified doses on biomarkers for cardiac, pancreatic, liver and kidney. Fluctuations in some biochemical parameters can be attributed to non pathological causes such as hemoloysis of cells leading to hyperkalaemia. Lower levels of creatinine recorded for *U. massaica* treated rats compared to the control indicate a creatinine lowering potential of the plant and this aspect should be

evaluated further. As such, orally administered aqueous extracts of *T. aestivum, H. vulgare, U. massaica and C. viminale* do not have *in vivo* toxic effects at doses specified in this study, further justifying their use.

A comparison of the CK and LDH values of normal and treated rats in this study indicate no significance variation between the levels obtained. Hence, no cardiac damage could be linked to orally administered aqueous extracts of *T. aestivum, H. vulgare, U. massaica* and *C. viminale* plants. The results in evaluation of pancreatic system function biomarkers (amylase and lipase) indicate no injurious effect on the pancreatic system arising from orally administered aqueous extracts of the plants in this study. Further, though there were variations in some enzyme biomarkers of liver function, the findings were not corroborated by other biochemical findings. In addition, the results obtained for total protein and albumin were not significantly different between the normal group and *T. aestivum, H. vulgare, U. massaica* and *C. viminale* treated rats. In general, the contribution of all the result obtained for liver function tests does not point to hepatotoxicity as a result of orally administered aqueous extracts of the plants and hence are not toxicologically significant. Other than the significantly high level of potassium noted for the normal rats which is most probably attributed to cell lysis, all the other parameters in evaluation of the renal function did not have any significant variation. As a result, no toxicity to the renal system resulted from orally administered aqueous extracts of the plants in this study was recorded.

The results obtained in this study corroborates the findings by Kifayatullah *et al*. (2015) who reported that 28 days administration of ethanolic extracts of

Pericamplylus glaucus at 600 and 1000 mg/kg body weight did no have deleterious effects on rats. Organ weights, haematological and biochemical results were not toxicologically different between *P. glaucus* administered rats and control (Kifayatullah *et al*., 2015). Likewise, subchronic toxicity evaluation of hydroethanolic extracts of *Casearia sylvestris* at 60, 120 and 240 mg/kg body weight indicated safe use of the plant for medicinal purposes. There was was no alteration in body weight, relative organ weight and hematological parameters. However, there were variations in some biochemical parameters whose toxicological insignificance was attributed to lack of support by other parameters or signs of toxicity to growth and normal development (Ameni *et al*., 2015).

Oxidative stress is a consequence of prolonged hyperglycemia. This results to the micro and macrovascular complications experienced in diabetes such as cardiovascular diseases, neuropathy, nephropathy, etc (Sarma, 2010; Shradha, 2010). With a diminished endogenous antioxidant systems, exogenous antioxidants plays a big role in scavenging free radicals and neutralizing their cytotoxic effects. Flavonoids are known potent antioxidants. Some of the flavonoids with antioxidant effects include Flavanones, Flavanols, Flavan-3-ol, Flavones, Flavonols and Isoflavonoids. The antioxidant properties of phytotherapies has been reported which is attributed to the presence of flavonoids amongst other antioxidants. The aqueous extracts of *T. aestivum* and *H. vulgare* have been reported in this study to have flavonoids and hence have antioxidant activities. Coupled with their hypoglycemic effects, the plants has a great advantage while used as hypoglycemic agents than the conventional antidiabetic drugs (Oboh *et al*., 2013; Yogesha *et al*., 2014; Chauhan, 2014). Flavonoids were not detected in *C. viminale* aqueous extracts.

Amino acids analysis of the plants in this study indicated the presence of ten amino acids though in varied contents between the plants. Five of the ten amino acids were essential amino acids. All the four plant extracts had isoleucine, lysine, threonine and valine but *T. aestivum* had tryptophan in addition of the rest. The method of amino acids determination used in this study is similar to what was used in analysis of amino acids in barley (Dahl-lassen *et al*., 2018)). The findings in this study concurs with Zhang on variations on profiles of amino acids in duck weed *(Spirodela polyhiza, Landoltia punctate, lemna aequinoctialis*). The variations were attributed to species pattern, growth environment and growth pattern (Zhang *et al*., 2018). Similary, the amino acids contents have been described in other medicinal plants (Hwang *et al*., 2013). Though not all amino acids targeted were detected in the plants extracts in the current study, the results obtained serve to indicate their importance as a source of valuable amino acids. However, application of a different analysis method may provide additional information on amino acids present in the plants evaluated in the current study.

5.2 Conclusions

In conlusion,

i) The aqueous plant extracts of *T. aestivum, H. vulgare, U. massaica* and *C. viminalle* has significant *in vivo* blood glucose lowering abilities. The four plant extracts reduced the blood glucose of the alloxan induced diabetic rats at the dose levels of 50, 91, 165 and 300 mg/kg body weight whether orally or intraperitoneally administered. The hypoglycemic effects of the four plant extracts when orally administered correlates with that of glibenclamide. Further, though response to hypoglycemic effect of intraperitoneally administered plant extracts was not

immediate, the plant extracts had prolonged glycemic effects in some instances which was better than insulin. Hence, the aqueous plants extracts are suitable in control of blood glucose in diabetes mellitus and their use in complementary or supportive therapy valid. However, determination of a specific mode of action of the observed glycemic effects of the plants was not exhausted.

ii) It is further concluded that the four aqueous plant extracts in this study have phytochemicals of pharmaceutical importance. The phytochemicals has previously been linked to antihyperglycaemic effects and thus it is equally concluded that they contribute to the hypoglycemic effect of the plants as demonstrated in this study. Further, the phytochemicals are potential free radical scavenging components which are paramount in preventing diabetic associated complications. In addition, the mineral composition of the four plants further indicate them as potential source of trace elements which are vital in blood glucose regulation and potentiating the observed hypoglycemic properties of the plants. The four plant extracts in this study contain carbohydrates, proteins and amino acids which are vital to human health.

iii) The results obtained in this sudy further concludes that orally administered doses of the plant extracts at 300, 448, 669 and 1000 mg/kg body weight are not toxic and hence safe for use. Likewise, intraperitoneally administered *T. aestivum* at the specified non therapeutic doses are safe for intake. However, all doses of *C. viminalle* intraperitoneally administered has acute toxicity effects, intraperitoneally administered *H. vulgare* is toxic at levels above 669 mg/kg body

weight and intraperitoneally administered *U. massaica* at doses above 300 mg/kg body weight is toxic.

5.3 Recommendations

5.3.1 Recommendations from the study

As per the findings of this study, it is recommended that:

i) Orally or intraperitoneally administered aqueous extracts of *T. aestivum, H. vulgare, U. massaica* and *C. viminale* can be used for mitigation of diabetes and its complications. However, guided consumption is adviced to achieve and evaluate desired hypoglycemic objetives.

ii) The plants evaluated in this study are a good source of phytochemicals that can be used in development of medicines for treatment of diabetes mellitus. Equally, the plants can be used as a source of nutrients and as functional foods.

iii) Orally administered aqueous plant extracts of *T. aestivum, H. vulgare, U. massaica* and *C. viminale,* having exhibited no toxicity on the rats, can reasonably be predicted safe for human beings thus recommended for use. Equally, intraperitoneally administered *T. aestivum* is considered safe and thus its use through this route is recommended. Nevertheless, caution should be observed when consindering intraperitoneal administration of *H. vulgare* and *U. massaica.* Lastly, it is recommended that *C. viminale* should not be considered for intraperitoneal administration.

5.3.2 Recommendations for further studies

The results obtained on the plants in the current sudy opens the door for further studies. Consequently, it is recommended that.

i) There is need for further isolation and structural characterization of the specific active constituents of phytochemicals present in the plants.

ii) The possible hypoglycemic activity of the plants in this study by blocking the sodium glucoseco-transporters 2 (SGLT-2) in the kidneys thus facilitating glucose excretion in urine should be evaluated.

iii) As most chronic illneses are associated with disruption of the human DNA by oxidative stress, genetic studies should be done to establish which genes are suppressed or expressed by use of the plants in various ailments.

iv) Pathological alterations as a consequence of intraperitoneally administered plant extracts should be evaluated through histopathological studies of various organs.

v) Post pre-clinical translational clinical studies on Human beings should be conducted on specific plant extracts to authoritatively propagate their use in medical practice. This equally opens the door for pharmaceutical formulations of convenient consumption forms of the plant extracts.

The antihyperglycemic potential of *T. aestivum, H. vulgare, U. massaica and C. viminalle* supports the tranditional or complimentary use of the plants in management and mitigation of diabetes. Likewise, elemental and micronutrients analysis of the plants under this study indicate that the plants can be considered as suitable sources of different elements and amino acids which are key in maintenance of good health. Thus, the plants are recommended for prevention and control of non communicable diseases.

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APPENDICES

Appendix 1: Growing and primary processing of *T. aestivum* and *H. vulgare*

I. Seeds of *T. aestivum* and *H. vulgare* were obtained from farmers, thoroughly washed and left soaked in water overnight (8-12hrs).

II. The water was completely drained and the seeds put in a sisal sack and left to sprout in a warm environment for 24 hrs.

III. Red soil enriched with manure from field fed sheep was put in trays, properly leveled and adequate water added to the soil.

IV. The sprouted seeds were placed on the wet soil closely together, covered with a thin layer of soil and low quantities of water sprinkled twice daily until the plants were three inches high and the plants left to grow and harvested on the eighth day from the day of placement on the soil as single blades.

V. The plants were initially dried under shade and UV protection for six hours.

VI. Total drying of the plants were done by placing them in a hot air electrical drying system at temperatures up to 60 degrees centigrade. Total drying was confirmed by a crispy feeling by hand.

VII. The dried *T. aestivum* and *H. vulgare* plant were size reduced to a fine powder by grinding using an electric mill.

Appendix 2: Authentication of *Cynanchum viminalle*

NATIONAL MUSEUMS OF KENYA

WHERE HERITAGE LIVES ON

The East African Herbarium
P.O. Box 45166 00100 Nairobi, Kenya
Telephone: 3743513, 3742131/4 ext 2274
Fax: 3741424
E-Mail: botany@museums.or.ke

4th December, 2020

REF: NMK/BOT/CTX/1/2

Kimani Njoroge
Kenyatta National Hospital

Dear Kimani,

PLANT IDENTIFICATION

The plant specimen that you deposited at the East African Herbarium for identification was determined as follows:

Cynanchum viminale (L.) L. (Family: Apocynaceae)

Thank you for visiting the EA Herbarium.

Yours Faithfully,

Kennedy Matheka

1910-2010
CELEBRATING A CENTURY OF HERITAGE MANAGEMENT

Appendix 3: Preparation of logarithmic doses of aqueous extracts of *T. aestivum, H. vulgare, U. massaica* and *C. viminale.*

Appendix 3.1: Preparation of aqueous plants extract doses for evaluation of the plants hypoglycemic effects

Four logarithmic doses were prepared for administration. The lowest was 50 milligram per kilogram body weight (mg/kg bdw) while the highest was 300mg/kg bdw. The two doses between the high and low were derived as follows:-

$F = \sqrt[r]{I}$

Where

F = incremental factor

r = N (number of doses in the log series) - 1

$$I = \frac{\text{Large dose (LD)}}{\text{Small dose (SD)}}$$

in substitution;

N = 4. Therefore r = N-1 = 3

Large dose = 300 while small dose = 50. Thus I = 300/50 = 6

Therefore $F = \sqrt[3]{6}$

F = 1.817120593 (incremental factor)

Hence, the two doses derived are:-

I. 50 x 1.817120593 = 90.856 (91)

II. 91 x 1.817120593 = 165

The four logarithmic doses for efficacy evaluation were thus:-

Treatment group	Dosage (mg/kg body weight)
IV	50
V	91
VI	165
VII	300

Appendix 3.2: Preparation of aqueous plants extract doses for toxicity evaluation

The logarithmic doses required for toxicity studies were prepared as follows. The highest dose used in efficacy evaluation (300mg/kg bdw) was taken as the lowest dose while the highest was 1000mg/kg bdw. Four doses were used. The middle range doses were prepared as follows:-

$F = \sqrt[r]{I}$

Where

F = incremental factor

r = N (number of doses in the log series) - 1

$$I = \frac{\text{Large dose (LD)}}{\text{Small dose (SD)}}$$

in substitution;

N = 4. Therefore r = N-1 = 3

Large dose 1000 while small dose 300. Thus I = 1000/300 = 3.333

Therefore $F = \sqrt[3]{3.333}$

F = 1.493801582 (incremental factor)

The two doses are thus:-

i. 300 x 1.493801582 = 448

ii. 448 x 1.493801582 = 669

The four logarithmic doses are:-

Treatment group	Dose (mg/kg body weight)
II	300
III	448
IV	669
V	1000

Appendix 4: Graduate school authority

KENYATTA UNIVERSITY
GRADUATE SCHOOL

E-mail: dean-graduate@ku.ac.ke
Website: www.ku.ac.ke

P.O. Box 43844, 00100
NAIROBI, KENYA
Tel. 810901 Ext. 57530

Internal Memo

FROM: Dean, Graduate School

DATE: 16th May, 2015

TO: **Mr. Njoroge Gideon Kimani**
C/o Biochemistry & Biotechnology
Kenyatta University

REF: I84/27460/14

SUBJECT: APPROVAL OF RESEARCH PROPOSAL

This is to inform you that Graduate School Board at its meeting of **15th April, 2015** approved your Research Proposal for the Ph.D. Degree. **Entitled "In Vivo Hypoglycaemic activity and safety of *Triticum Aestivum, Hordeum Vulgare, Urtica Massaica and Cynanchum Viminale.*".**

You may now proceed with data collection, subject to clearance with the Permanent Secretary, Ministry of Higher Education, Science and Technology.

As you embark on your data collection, please note that you will be required to submit to Graduate School completed Supervision Tracking forms per semester. The form has been developed to replace the progress report forms. The supervision Tracking Forms are available at the University's website under Graduate School webpage downloads.

By copy of this letter, the registrar (Academic) is hereby requested to grant you Substantive registration for your Ph.D studies.

Thank you.

DAVID NJOROGE
FOR: DEAN, GRADUATE SCHOOL

c.c. Chairman, Biochemistry & Biotechnology Department.

Registrar (Academic)

Supervisors:

1. Prof. Eliud N.M Njagi
   Department of Biochemistry & Biotechnology
   Kenyatta University
2. Prof. Nicholas Gikonyo
   C/o Biochemistry & Biotechnology
   Kenyatta University
3. Dr. Mathew Piero Ngugi
   C/o Biochemistry & Biotechnology
   Kenyatta University

Appendix 5: NACOSTI authority

NATIONAL COMMISSION FOR SCIENCE, TECHNOLOGY AND INNOVATION

Telephone: +254-20-2213471,
2241349, 310571, 2219420
Fax: +254-20-318245, 318249
Email: secretary@nacosti.go.ke
Website: www.nacosti.go.ke
When replying please quote

9th Floor, Utalii House
Uhuru Highway
P.O. Box 30623-00100
NAIROBI-KENYA

Ref: No.

Date:
20th July, 2015

NACOSTI/P/15/7291/6442

Gideon Kimani Njoroge
Kenyatta University
P.O. Box 43844-00100
NAIROBI.

RE: RESEARCH AUTHORIZATION

Following your application for authority to carry out research on ***"Invivo hypoglycaemic activity and safety of triticum aestivum, hordeum vulgare, urtica massaica and cyanachum viminale,"*** I am pleased to inform you that you have been authorized to undertake research in **Kiambu County** for a period ending **31st May, 2016.**

You are advised to report to **the County Commissioner and the County Director of Education, Kiambu County** before embarking on the research project.

On completion of the research, you are expected to submit **two hard copies and one soft copy in pdf** of the research report/thesis to our office.

DR. S. K. LANGAT, OGW
FOR: DIRECTOR-GENERAL/CEO

Copy to:

The County Commissioner
Kiambu County.

The County Director of Education
Kiambu County.

Appendix 6: Ministry of interior authority

MINISTRY OF INTERIOR AND CO-ORDINATION OF NATIONAL GOVERNMENT
COUNTY COMMISSIONER, KIAMBU

Telephone: 066-2022709
Fax: 066-2022644
E-mail: countycommissionerkiambu@kenya.go.ke
When replying please quote

County Commissioner
Kiambu County
P.O. Box 32-00900
KIAMBU

ED.12/1/VOL.II/204

22nd July, 2015

Gideon Kimani Njoroge
Kenyatta University
P.O. Box 43844-00100
NAIROBI

RE: RESEARCH AUTHORIZATION

Reference is made to National Commission for Science, Technology and Innovation letter Ref. No. NACOSTI/P/15/7291/6442 dated 20th July, 2015.

You have been authorized to conduct research on ***"Invivo hypoglycaemic activity and safety of triticum aestivum, hordeum vulgare, urtica massaica and cyanachum viminale, in Kiambu County" for*** a period ending ***31st may, 2016.***

You are requested to share your findings with the County Director of Education upon completion of your research.

ESTHER MAINA
COUNTY COMMISSIONER
KIAMBU COUNTY

Cc County Director of Education
KIAMBU COUNTY

National Commission for Science, Technology and Innovation
P.O. Box 30623-00100
NAIROBI

Appendix 7: KNH-ERC authority

KENYATTA NATIONAL HOSPITAL APPROVED 12 OCT 2016 KNH/UoN-ERC P.O. Box 20723-00202 NRB.

UNIVERSITY OF NAIROBI
COLLEGE OF HEALTH SCIENCES
P O BOX 19676 Code 00202
Telegrams: varsity
Tel:(254-020) 2726300 Ext 44355

KNH-UON ERC
Email: uonknh_erc@uonbi.ac.ke
Website: http://www.erc.uonbi.ac.ke
Facebook: https://www.facebook.com/uonknh.erc
Twitter: @UONKNH_ERC https://twitter.com/UONKNH_ERC

KENYATTA NATIONAL HOSPITAL
P O BOX 20723 Code 00202
Tel: 726300-9
Fax: 725272
Telegrams: MEDSUP, Nairobi

Ref: KNH-ERC/A/401

12th October 2016

Njoroge Gideon Kimani
Reg. No.184/27460/2014
Department of Biochemistry and Biotechnology
School of Pure and Applied Sciences
Kenyatta University

Dear Gideon

Revised research proposal: *Invivo* Hypoglycaemic Activity and Safety of *Triticum eastivum, Hordeum vulgare, Urtical massaica* and *Cynanchum viminale* **(P470/06/2016)**

This is to inform you that the KNH- UoN Ethics & Research Committee (KNH- UoN ERC) has reviewed and **approved** your above revised proposal. The approval period is from 12th October 2016 – 11th October 2017.

This approval is subject to compliance with the following requirements:

a) Only approved documents (informed consents, study instruments, advertising materials etc) will be used.
b) All changes (amendments, deviations, violations etc) are submitted for review and approval by KNH-UoN ERC before implementation.
c) Death and life threatening problems and serious adverse events (SAEs) or unexpected adverse events whether related or unrelated to the study must be reported to the KNH-UoN ERC within 72 hours of notification.
d) Any changes, anticipated or otherwise that may increase the risks or affect safety or welfare of study participants and others or affect the integrity of the research must be reported to KNH- UoN ERC within 72 hours.
e) Submission of a request for renewal of approval at least 60 days prior to expiry of the approval period. (*Attach a comprehensive progress report to support the renewal*).
f) Clearance for export of biological specimens must be obtained from KNH- UoN ERC for each batch of shipment.
g) Submission of an *executive summary* report within 90 days upon completion of the study. This information will form part of the data base that will be consulted in future when processing related research studies so as to minimize chances of study duplication and/ or plagiarism.

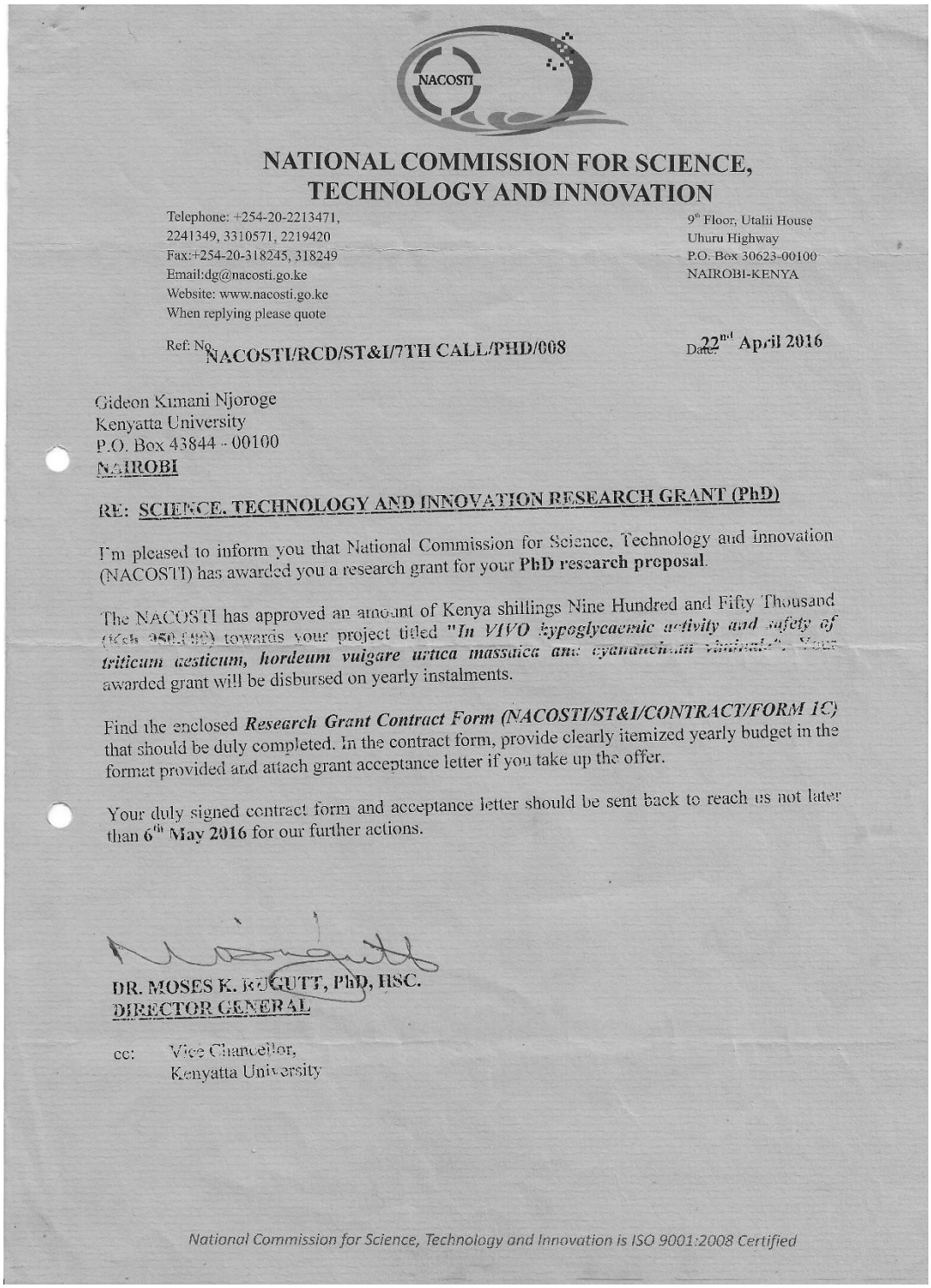
For more details consult the KNH- UoN ERC website **http://www.erc.uonbi.ac.ke**

Yours sincerely,

PROF M. L. CHINDIA
SECRETARY, KNH-UoN ERC

c.c. The Principal, College of Health Sciences, UoN
The Deputy Director, CS, KNH
The Assistant Director, Health Information, KNH
The Chairperson, KNH- UoN ERC
Supervisors: Dr. Andrew Nyerere, Dr. Mourine Kangogo

Appendix 8: NACOSTI funding



NACOSTI

NATIONAL COMMISSION FOR SCIENCE, TECHNOLOGY AND INNOVATION

Telephone: +254-20-2213471,
2241349, 3310571, 2219420
Fax:+254-20-318245, 318249
Email:dg@nacosti.go.ke
Website: www.nacosti.go.ke
When replying please quote

9th Floor, Utalii House
Uhuru Highway
P.O. Box 30623-00100
NAIROBI-KENYA

Ref: No. NACOSTI/RCD/ST&I/7TH CALL/PHD/008

Date: 22nd April 2016

Gideon Kimani Njoroge
Kenyatta University
P.O. Box 43844 - 00100
NAIROBI

RE: SCIENCE, TECHNOLOGY AND INNOVATION RESEARCH GRANT (PhD)

I'm pleased to inform you that National Commission for Science, Technology and Innovation (NACOSTI) has awarded you a research grant for your **PhD research proposal**.

The NACOSTI has approved an amount of Kenya shillings Nine Hundred and Fifty Thousand (Kshs 950,000) towards your project titled ***"In VIVO hypoglycaemic activity and safety of triticum aesticum, hordeum vulgare urtica massaica and cyanarchum virinale"***. Your awarded grant will be disbursed on yearly instalments.

Find the enclosed ***Research Grant Contract Form (NACOSTI/ST&I/CONTRACT/FORM 1C)*** that should be duly completed. In the contract form, provide clearly itemized yearly budget in the format provided and attach grant acceptance letter if you take up the offer.

Your duly signed contract form and acceptance letter should be sent back to reach us not later than **6th May 2016** for our further actions.

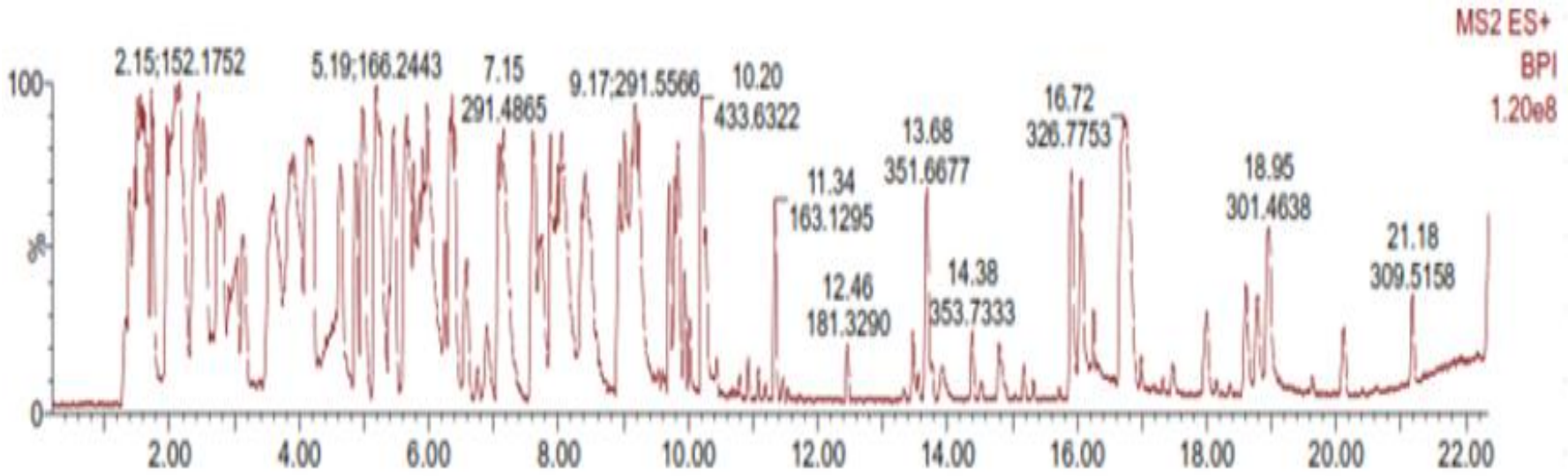
DR. MOSES K. KUGUTT, PhD, HSC.
DIRECTOR GENERAL

cc: Vice Chancellor,
Kenyatta University

National Commission for Science, Technology and Innovation is ISO 9001:2008 Certified

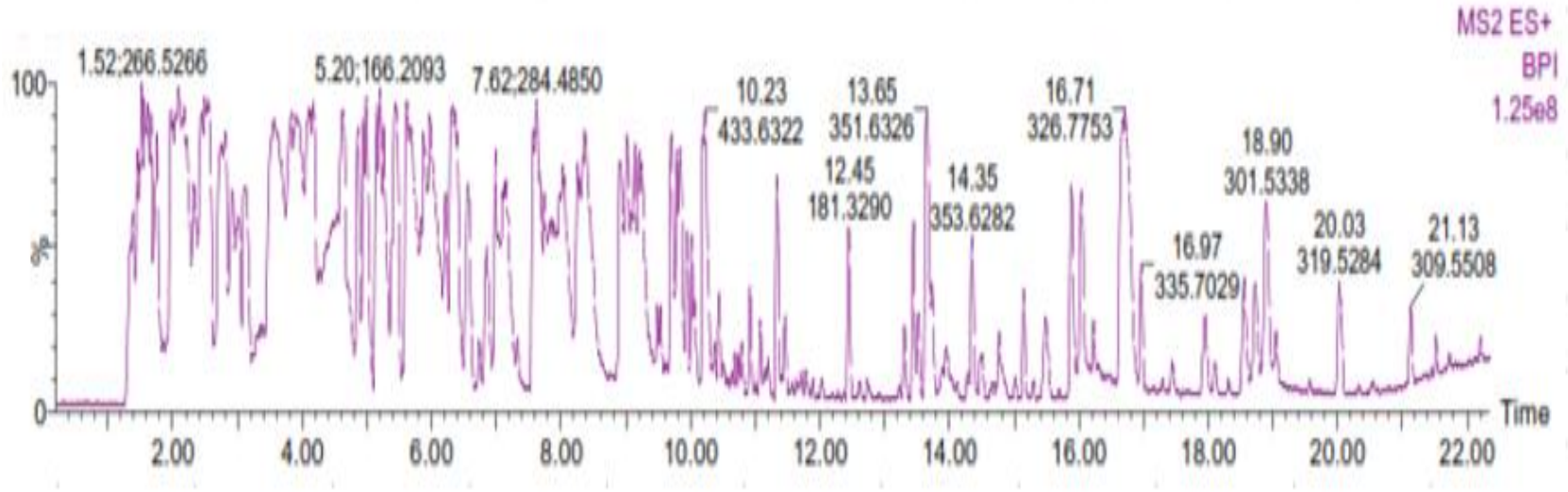
Appendix 9. Ionization patterns of compounds present in aqueous extracts of *T. aestivum*, *H. vulgare*, *U. massaica* and *C. viminalle*.

Appendix 9.1. LC-MS Total ion current (TIC) spectrum for aqueous extract of *T. aestivum.*



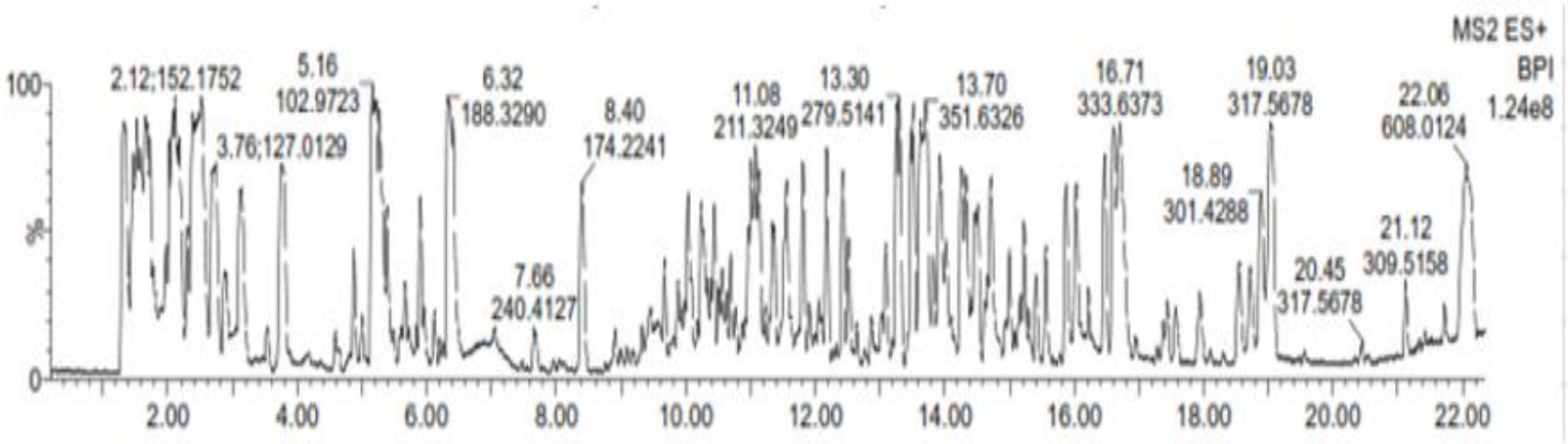
Retention Time (Min)

Appendix 9.2. LC-MS Total ion current (TIC) spectrum for aqueous extract of *H.vulgare*



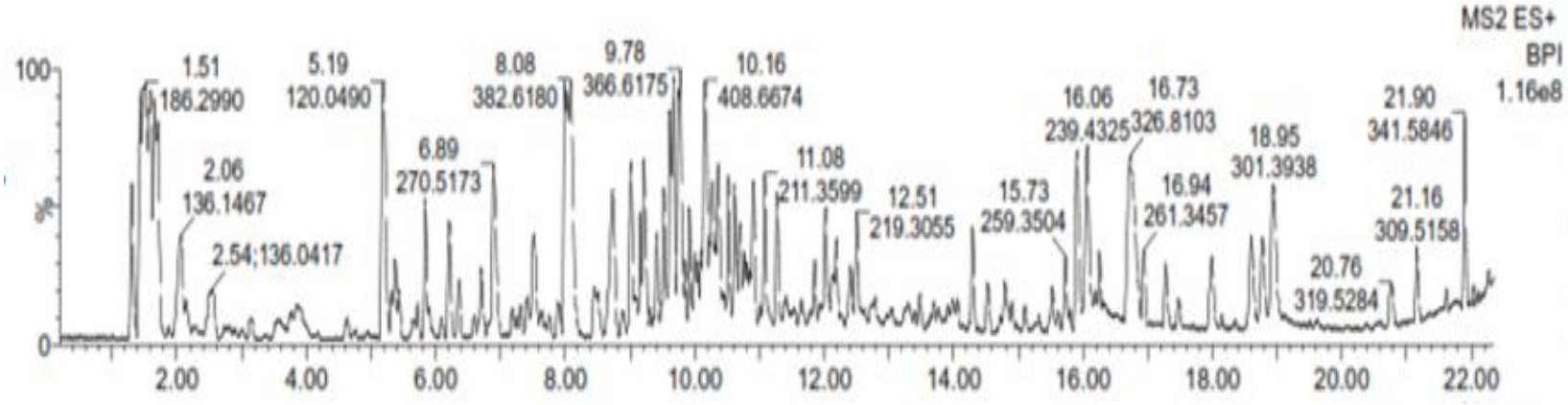
Retention Time (Min)

Appendix 9.3. LC-MS Total ion current (TIC) spectrum for aqueous extract of *U. massaica*



Retention Time (Min)

Appendix 9.4. LC-MS Total ion current (TIC) spectrum for aqueous extract of *C. viminalle*



Retention Time (Min)

Appendix 10. Compounds present in aqueous extract of *T.aestivum, H. Vulgare, U.massaica and C.viminalle*

Appendix 10.1. Compounds present in aqueous extract of *T.aestivum*

RT (min)	Major Fragments	Compound Name	Peak Area	Conc (pg/10µl)	Conc (pg/1000µl)	Conc (pg/g)	Conc (ng/g)
1.47	103.7421, 104.337, 122.8486, 207.0546, 291.2065 (M+H), 304.5446 [M+H+Na]	Catechin	4257413	62.71	6271.35	6271.35	6.27
2.747	147.2300, 165.2410 [M+H), 187.2311 [M+H+Na], 203.1102	P-coumaric	3443767.3	49.51	4950.92	4950.92	4.95
4.871	449.3352, 611.0025 [M+H], 633.1154, 649.2210	Rutin	5165663.5	77.45	7745.30	7745.30	7.75
4.977	269.2211, 211.2260,305.6699, 323.2200[M+H], 345.2258 [M+H+Na], 361.2254	Avenic acid	9840837	153.32	15332.41	15332.40	15.33
5.389	114.2200, 100.2200, 321.5560, 339.1442[M+H], 361.2200 [M+H+Na], 377.1522	Hydroxyavenic acid	1217171	13.38	1337.49	1337.49	1.34
5.688	202.2124, 220.3063[M+H], 242.1122 [M+H+Na], 258.2004	Zeatin	3088085.3	43.74	4373.70	4373.70	4.37
8.582	379.0022, 349.3366, 397.2254, 337,313, 283415.1225, 433.1120[M+H], 455.1230 [M+H+Na], 471.2063	Apigenin 6-C-glucoside	451094.63	0.94	94.26	94.26	0.09
8.943	268.1233, 287.1102[M+H], 309.1140 [M+H+Na], 325.0014	Kaempferol	5714795	86.37	8636.46	8636.4	8.64
9.022	268.1122, 287.0522[M+H], 309.0100 [M+H+Na], 325.2256	Luteolin	7476219	114.95	11494.99	11494.99	11.49
9.134	258.6321, 270.6323, 287.0052, 315.2011, 331.0910[M+H], 353.2201[M+H+Na], 369.2586	Tricin	4285924	63.18	6317.62	6317.62	6.32
9.175	201.4512, 258.6360, 286.3369,301.0706[M+H], 323.2201[M+H+Na], 361.3211	Chrysoeriol	6410564.5	97.66	9765.59	9765.59	9.77
9.236	197.5230,243.2260,271.0590[M+H], 293.6320, 309.1214	Apigenin	7402966	113.76	11376.11	11376.11	11.38
9.638	285.0014, 303.2036[M+H], 325.0012 [M+H+Na], 341.2220	Quercetin	147331.11	-3.99	-398.70	-398.70	-0.40

9.706	299.0023, 317.2256[M+H],339.5522 [M+H+Na], 355.0021	Isorhamnetin	3495245.3	50.35	5034.46	5034.46	5.03
9.794	289.1123, 579.0012[M+H], 601.3352 [M+H+Na],617.0021	Procyanidin B1	3058218.8	43.25	4325.24	4325.24	4.33
9.842	303.1225, 439.6196577.3325,595.8964(M+H), 617.8327 [M+H+Na], 633.1124	Prodelphinidin B3	5336437.5	80.22	8022.44	8022.44	8.02
10.795	383.2259, 401.6223[M+H], 423.1152 [M+H+Na],439.2201	Campesterol	399185.83	0.10	10.02	10.02	0.01
10.925	397.0122, 415.2652[M+H], 437.1122 [M+H+Na], 453.0055	Beta-Sitosterol	542656.38	2.43	242.86	242.86	0.24

Appendix 10.2. Compounds present in aqueous extract of *H. vulgare*

RT (min)	Major Fragments	Compound Name	Peak Area	Conc (pg/10µl)	Conc (pg/1000µl)	Conc (pg/g)	Conc (ng/g)
1.49	103.7421, 104.337, 122.8486, 207.0546, 291.2065 (M+H), 304.5446 [M+H+Na]	Catechin	2161655	28.70	2870.25	2870.25	2.87
2.74	147.2300, 165.2410 [M+H), 187.2311 [M+H+Na], 203.1102	P-coumaric	3895098	56.83	5683.36	5683.36	5.68
4.72	449.3352, 611.0025[M+H],633.1154, 649.2210	Rutin	2107074	27.82	2781.67	2781.67	2.78
4.99	269.2211, 211.2260, 305.6699, 323.2200 [M+H], 345.2258 [M+H+Na], 361.2254	Avenic acid	7308660	112.23	11223.06	11223.06	11.22
5.38	114.2200, 100.2200, 321.5560, 339.1442[M+H], 361.2200[M+H+Na], 377.1522	Hydroxyavenic acid	1412892	16.55	1655.12	1655.12	1.66
5.67	202.2124, 220.3063 [M+H], 242.1122 [M+H+Na], 258.2004	Zeatin	12027922	188.82	18881.72	18881.72	18.88
8.90	268.1233, 287.1102 [M+H], 309.1140 [M+H+Na], 325.0014	Kaempferol	2292126	30.82	3081.98	3081.98	3.08
9.64	285.0014, 303.2036 [M+H], 325.0012 [M+H+Na], 341.2220	Quercetin	397705.7	0.08	7.62	7.62	0.01
9.70	299.0023, 317.2256 [M+H], 339.5522 [M+H+Na], 355.0021	Isorhamnetin	5402472	81.30	8129.60	8129.60	8.13
9.79	289.1123, 579.0012 [M+H], 601.3352 [M+H+Na], 617.0021	Procyanidin B1	3632312	52.57	5256.90	5256.90	5.26
9.84	303.1225, 439.6196577.3325, 595.8964 (M+H), 617.8327 [M+H+Na], 633.1124	Prodelphinidin B3	5068833	75.88	7588.16	7588.16	7.59
10.79	383.2259, 401.6223 [M+H], 423.1152 [M+H+Na], 439.2201	Campesterol	774551.3	6.19	619.19	619.19	0.62
10.92	397.0122, 415.2652 [M+H], 437.1122 [M+H+Na], 453.0055	Beta-Sitosterol	1671174	20.74	2074.27	2074.27	2.07

Appendix 10.3. Compounds present in aqueous extract of *U. massaica*

RT (min)	Major Fragments	Compound Name	Peak Area	Conc (pg/10µl)	Conc (pg/1000µl)	Conc (pg/g)	Conc (ng/g)
1.32	109.9708, 183.149 (M+H), 205.1995 (M+H+Na)	3,4-Dihydroxyhydrocinnamic acid	10706930	167.38	16737.94	16737.94	16.74
1.42	47.1236(M+H), 69.0236 (M+H+Na), 94.0365, 116.2456	Formic acid	1231425	13.61	1360.62	1360.62	1.36
1.47	103.7421, 104.337, 122.8486, 207.0546, 291.2065 (M+H), 304.5446 [M+H+Na]	Catechin	5813215	87.96	8796.18	8796.18	8.80
1.50	43.0236, 61.2415 (M+H), 83.0263(M+H+Na), 122.1230, 144.0036	Acetic acid	11045108	172.87	17286.76	17286.76	17.29
1.52	105.022 [M+H], 127.0575 (M+H+Na), 162.2545,	Choline	4415943	65.27	6528.62	6528.62	6.53
1.56	104.057, 119.0692, 130.0575, 148.1855, 159.1747, 184.234, 235.407, 247.3085[M+H]	Isopimpinellin	7023261	107.60	10759.90	10759.90	10.76
1.58	71.0369, 89.0212(M+H), 111.2256 (M+H+Na), 178.2365	Butyric acid	1125630	11.89	1188.93	1188.93	1.19
1.65	104.057, 116.0247 [M+H], 138.0365 [M+H+Na]	Maleamic acid	5055807	75.67	7567.02	7567.02	7.57
1.60	94.0369, 112.0342[M+H), 134.4066 [M+H+Na], 224.1402	Histamine	1136982	12.07	1207.36	1207.36	1.21
1.77	115.0799, 130.0575 [M+H), 152.2369 [M+H+Na]	N,N-dimethylvaleramide	3162612	44.95	4494.65	4494.65	4.49
2.08	101.2036, 119.0342 [M+H), 141.4066 [M+H+Na], 238.1402	Succinic acid	6268356	95.35	9534.80	9534.80	9.53
2.53	102.9023, 116.0597, 122.0087,137.0916 [M+H], 158.1247 [M+H+Na]	Threonic acids	10045058	156.64	15663.83	15663.83	15.66
2.68	145.0022, 163.1152 [M+H), 185.2036 [M+H+Na],	Nicotine	2905378	40.77	4077.20	4077.20	4.08

	201.0235						
2.70	147.2300, 165.2410 [M+H), 187.2311 [M+H+Na], 203.1102	P-coumaric	2530110	34.68	3468.19	3468.19	3.47
2.88	112.0354, 132.0173 [M+H], 154.1002 [M+H+Na], 170.1120	Isoleucine	1688601	21.03	2102.55	2102.55	2.10
3.14	112.0354, 132.0173 [M+H], 154.1001 [M+H+Na], 170.1120	Leucine	4660261	69.25	6925.11	6925.11	6.93
3.54	151.1051, 169.9292 [M+H], 191.3016 [M+H+Na], 207.4659	Homovanillyl alcohol	856071	7.52	751.48	751.48	0.75
3.79	159.0011, 177.0011 [M+H],199.2311 [M+H+Na], 215.1100	Ascorbic acid	3864181	56.33	5633.19	5633.19	5.63
3.80	159.1011, 177.2011 [M+H],199.0311 [M+H+Na], 215.2100	5-hydroxy tryptamine	5828123	88.20	8820.37	8820.37	8.82
4.82	175.1102, 193.0012 [M+H], 215.0011 [M+H+Na], 231.2322	Citric	1117586	11.76	1175.88	1175.88	1.18
5.27	175.0063, 193.1123 [M+H],215.2011 [M+H+Na], 231.0322	Scopoletin	8183130	126.42	12642.19	12642.19	12.64
5.39	177.0025, 195.1145 [M+H], 217.4001 [M+H+Na], 233.0052	Galacturonic acid	3598798	52.03	5202.51	5202.51	5.20
5.67	202.0120, 220.0060 [M+H], 242.0122 [M+H+Na], 258.0006	Pantothenic acid	1697249	21.17	2116.59	2116.59	2.12
5.73	202.2124, 220.3063 [M+H], 242.1122 [M+H+Na], 258.2004	Zeatin	924601	8.63	862.69	862.69	0.86
8.40	245.0012, 263.1125 [M+H], 285.0012 [M+H+Na], 301.0021	Threono-1,4-lactone	6140863	93.28	9327.90	9327.90	9.33
8.91	268.1233, 287.1102 [M+H], 309.1140 [M+H+Na], 325.0014	Kaempferol	610616	3.53	353.14	353.14	0.35
9.46	279.0211, 297.0056 [M+H], 319.1120 [M+H+Na], 335.2200	13-hydroxy-9,11-octadecadienoic acid	1855446	23.73	2373.32	2373.32	2.37

9.68	285.0014, 303.2036 [M+H], 325.0012 [M+H+Na], 341.2220	Quercetin	1637684	20.20	2019.92	2019.92	2.02
9.77	299.0023, 317.2256 [M+H],339.5522 [M+H+Na], 355.0021	Isorhamnetin	1033968	10.40	1040.18	1040.18	1.04
9.89	337.0023, 355.2253 [M+H],377.0022 [M+H+Na], 393.2650	Chlorogenic acid	1332762	15.25	1525.08	1525.08	1.53
10.04	359.0021, 377.2205 [M+H],399.0055 [M+H+Na], 415.0022	Neoolivil	3439330	49.44	4943.72	4943.72	4.94
10.09	397.0122, 415.2652 [M+H], 437.1122 [M+H+Na], 453.0055	Beta-Sitosterol	825046	7.01	701.13	701.13	0.70
10.24	439.2235, 457.2057 [M+H],479.2250 [M+H+Na], 495.2200	Oleanolic acid	2552869	35.05	3505.13	3505.13	3.51
10.44	363.0012, 507.1200, 525.0012 [M+H], 547.2230, 563.1140	Secoisolariciresinol glucoside	2214843	29.57	2956.56	2956.56	2.96
10.56	519.1203, 537.0011 [M+H], 559.2241 [M+H+Na], 575.1100	Beta-carotene	1252650	13.95	1395.07	1395.07	1.40

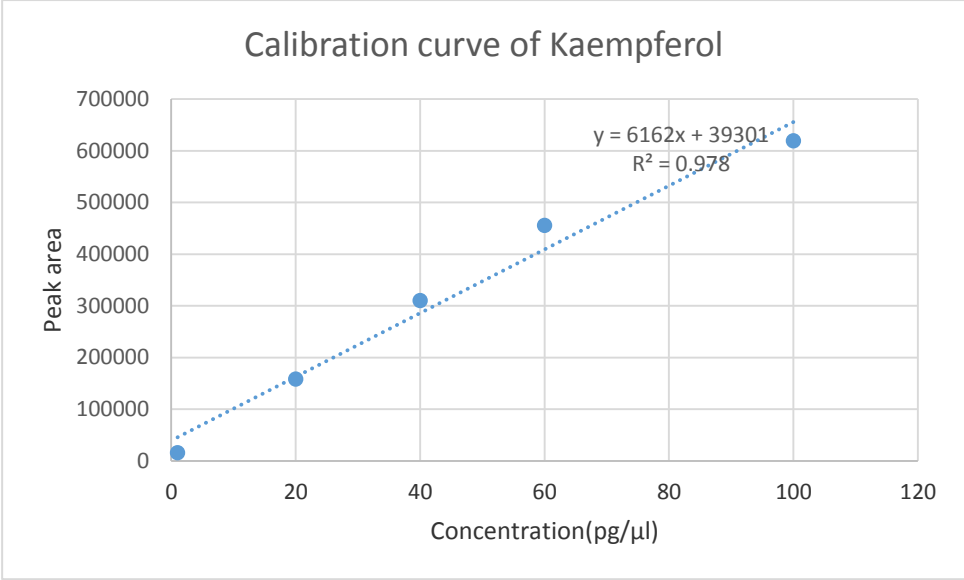
Appendix 10.4. Compounds present in aqueous extract of *C.viminalle*

RT (min)	Major Fragments	Compound Name	Peak Area	Conc (pg/10µl)	Conc (pg/1000µl)	Conc (pg/g)	Conc (ng/g)
1.48	103.7421, 104.337, 122.8486, 207.0546, 291.2065 (M+H), 304.5446 [M+H+Na]	Catechin	43502675	699.61	69960.51	69960.51	70.0
2.726	147.2300, 165.2410 [M+H), 187.2311 [M+H+Na], 203.1102	P-coumaric	468207.7	1.22	122.04	122.04	0.1
4.759	449.3352, 611.0025 [M+H], 633.1154, 649.2210	Rutin	2136194	28.29	2828.93	2828.93	2.8
5.719	202.2124, 220.3063 [M+H], 242.1122 [M+H+Na], 258.2004	Zeatin	5914759	89.61	8960.97	8960.97	9.0
7.183	240.2100, 271.0253 [M+H],293.1326 [M+H+Na], 253.0071	Hexadecanoic acid, methyl ester	5386853	81.04	8104.26	8104.26	8.1
7.247	253.1203, 271.1203 [M+H], 293.6320 [M+H+Na], 253.6321	Heptadecanoic acid	3756917	54.59	5459.12	5459.12	5.5
7.309	263.2541, 281.2541 [M+H], 303.2510 [M+H+Na] 319.2011	9, 12-octadecadienoic acid (Z, Z)-	6707297	102.47	10247.14	10247.14	10.2
7.421	205.1214, 223.0251 [M+H], 245.3696 [M+H+Na], 261.1470	Alpha-cadinol	11312969	177.22	17721.45	17721.45	17.7
9.011	268.1233, 287.1102 [M+H], 309.1140 [M+H+Na], 325.0014	Kaempferol	32880500	527.22	52722.32	52722.32	52.7
9.076	268.1122, 287.0522 [M+H], 309.0100 [M+H+Na], 325.2256	Luteolin	3462734	49.82	4981.70	4981.70	5.0
9.161	322.0123, 338.2013 [M+H], 360.2125 [M+H+Na],376.2545	13-docosenamide, (Z)-	17364123	275.42	27541.57	27541.57	27.5
9.215	197.5230, 243.2260, 271.0590 [M+H], 293.6320, 309.1214	Apigenin	35715750	573.24	57323.5	57323.5	57.3
9.613	285.0014, 303.2036 [M+H], 325.0012 [M+H+Na], 341.2220	Quercetin	28542863	456.83	45682.98	45682.98	45.7

10.771	383.2259, 401.6223 [M+H+Na],439.2201	[M+H],	423.1152	Campesterol	10050057	156.72	15671.94	15671.94	15.7
10.856	397.0122, 415.2652 [M+H+Na], 453.0055	[M+H],	437.1122	Beta-Sitosterol	5793176	87.64	8763.66	8763.66	8.8
10.907	396.2210, 415.3620 [M+H+Na], 452.1123	[M+H],	436.2010	Stigmast-5-en-3-ol, (3.beta.)-	29183120	467.22	46722.02	46722.02	46.7
11.02	408.2211427.1200 [M+H], 464.1200		448.2201[M+H+Na],	Alpha-amyrin	2914948	40.93	4092.73	4092.73	4.1
11.084	409.2121, 427.2215 [M+H+Na], 465.3302	[M+H],	449.2301	Lupeol	30230465	484.22	48421.71	48421.71	48.4

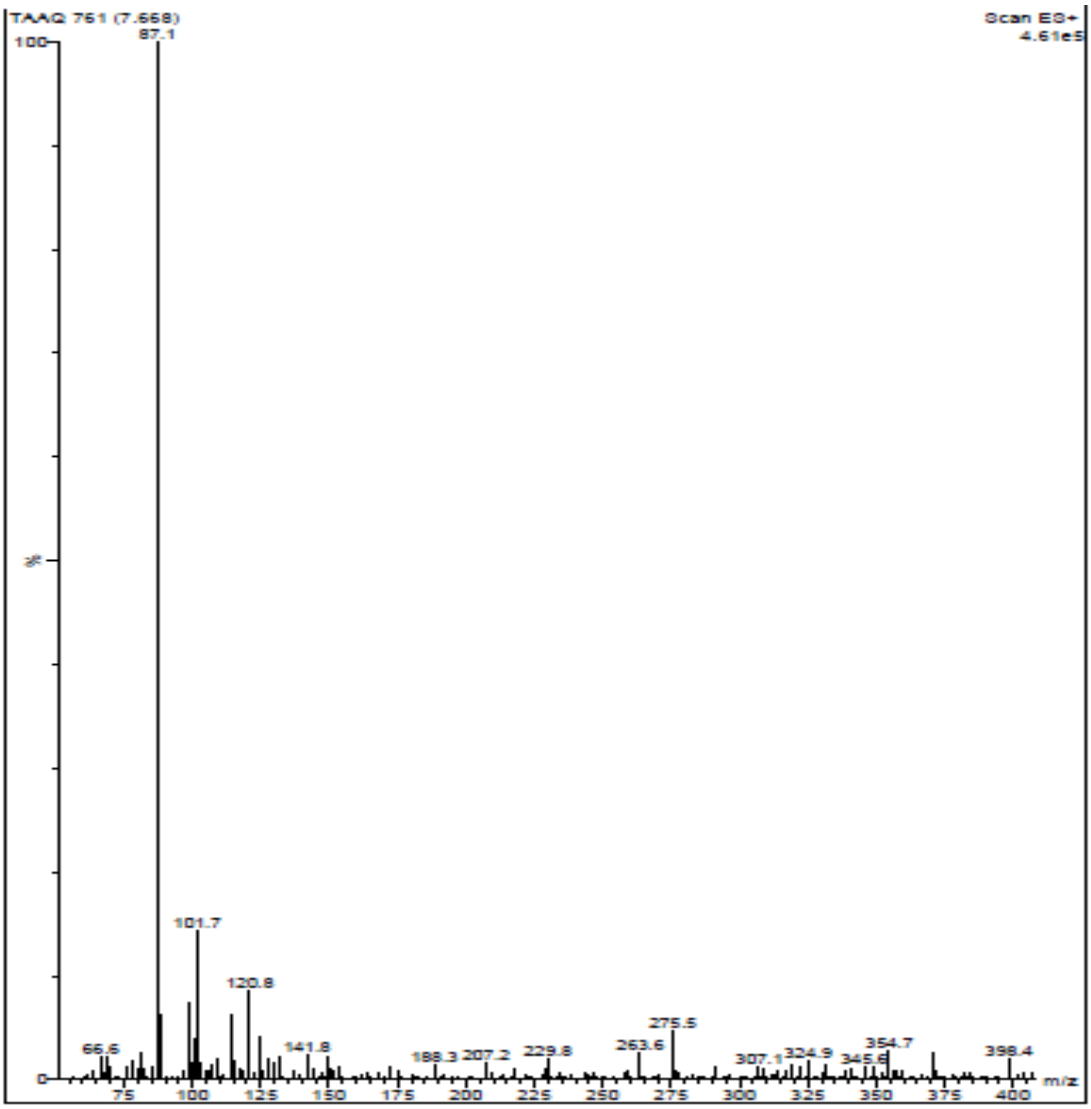
Appendix 11. LCMS calibration curve for the determination of compounds present in aqueous extracts of *T.aestivum, H. vulgare, U.massaica* and *C.viminalle*

Conc (pg/µl)	area
1	15584.26
20	158108.7
40	310036
60	455459.2
100	619127.7

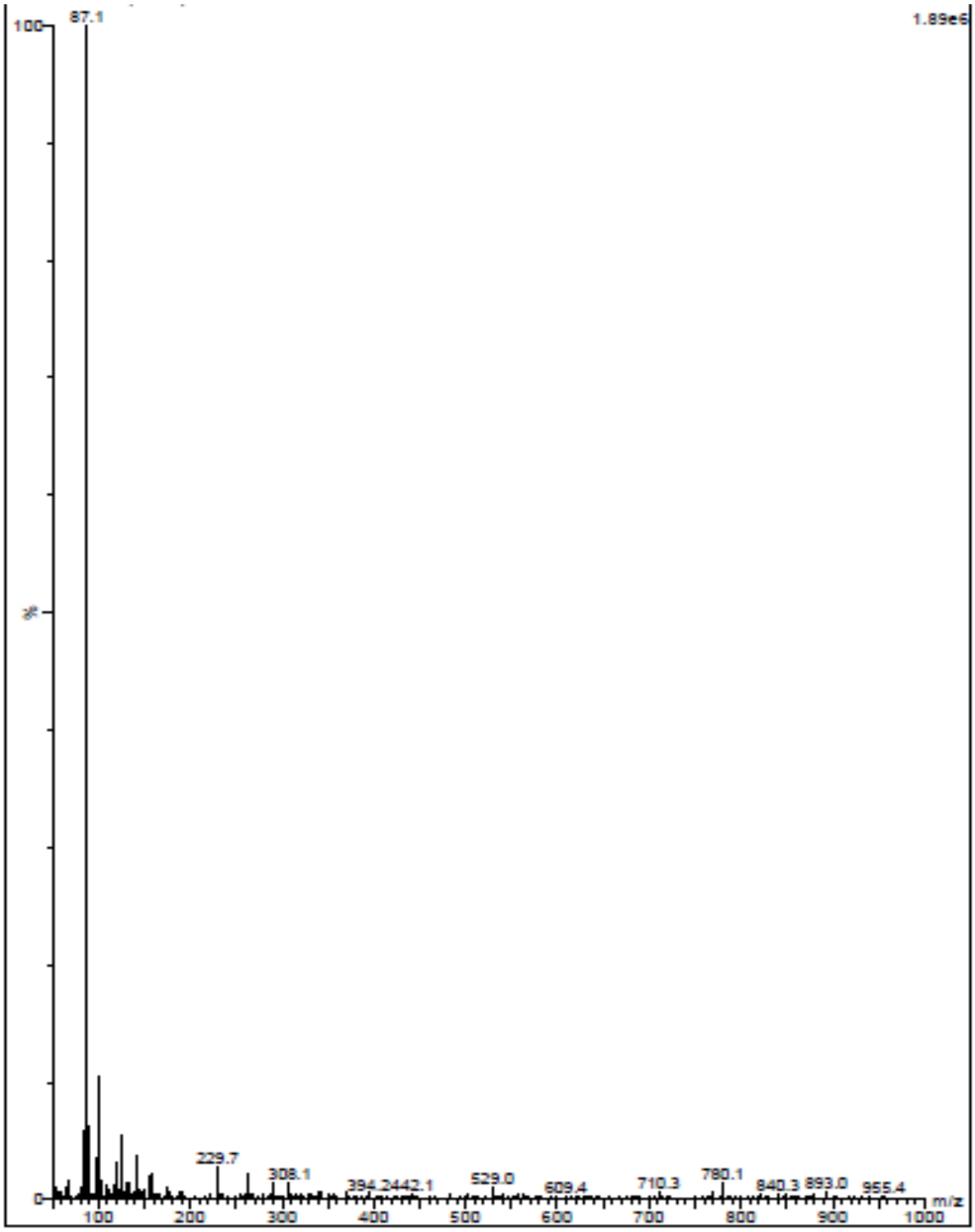


Appendix 13. Amino acids LCMS spectrums of *T. aestivum, H. vulgare, U.massaica* and *C viminalle*

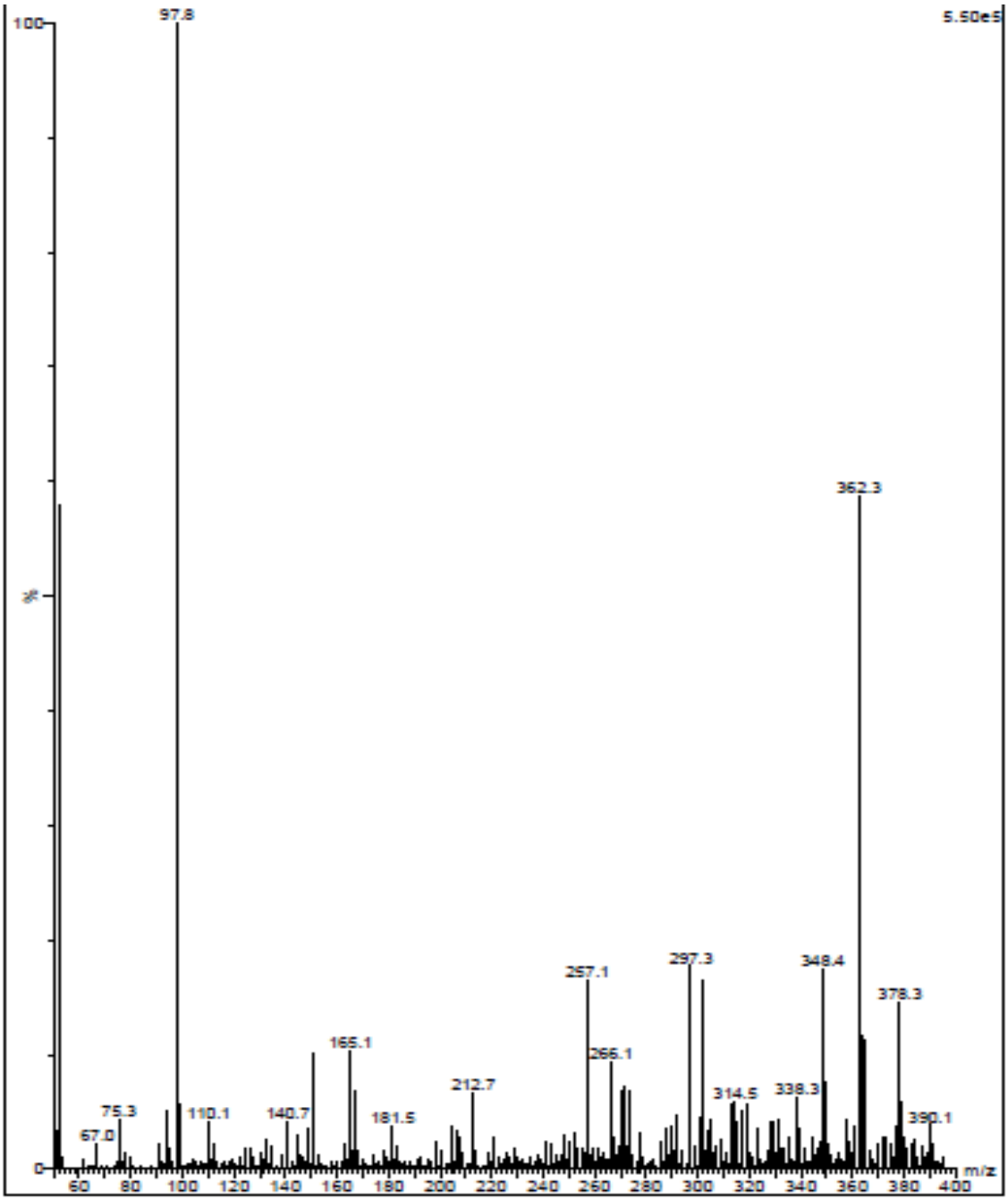
Appendix 13.1 Amino acids LCMS spectrums of *T. aestivum*



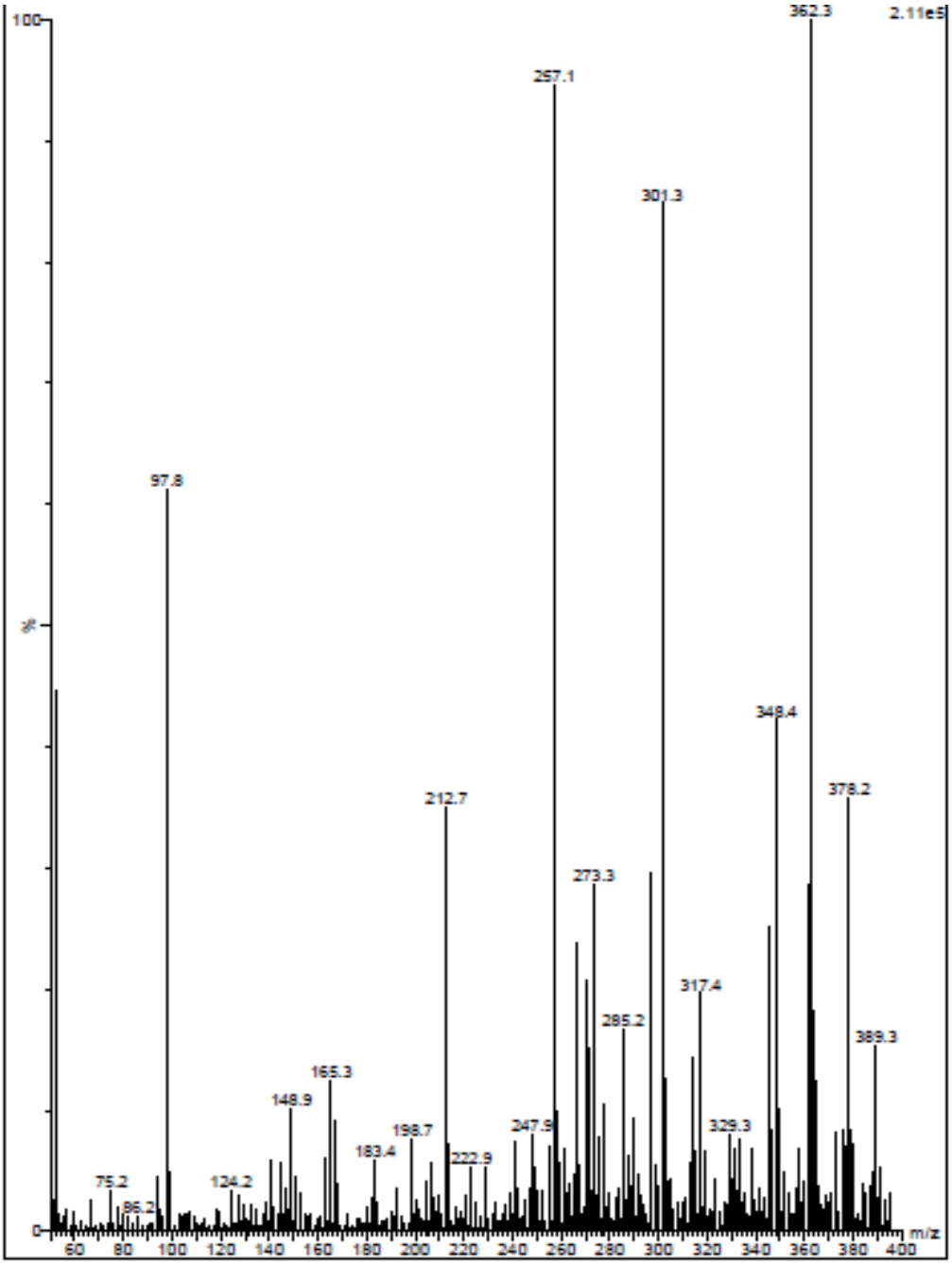
Appendix 13.2 Amino acids LCMS spectrums of *H.vulgare*



Appendix 13.3: Amino acids LCMS spectrums of *U. massaica*



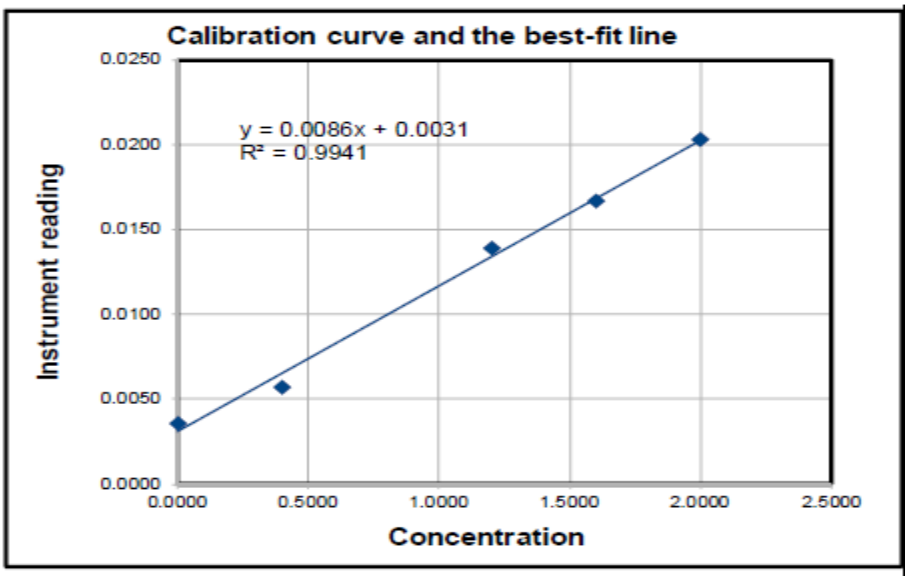
Appendix 13.4: Amino acids LCMS spectrums of *C. viminalle*

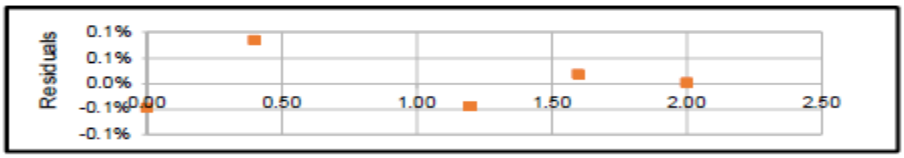


Appendix 14: Determination of calcium (representative for all) contents in aqueous extract of *T. aestivum*, *H. vulgare*, *U. massaica* and *C. viminalle*.

Calibration data

Concentration of standards	Instrument readings
0.0000	0.0036
0.4000	0.0057
1.2000	0.0139
1.6000	0.0167
2.0000	0.0203





Standard deviation of the residuals = 10.4%

	value	Error (σ)	% Error
Slope	0.0086	0.0004	4.45%
Intercept	0.0031	0.0005	15.81%

Readings of the unknowns	Calculated concentration	Calculated error (σ)	% Calculated error	sample id
0.0760	8.4748	0.381	4.50%	UM
0.0206	2.0351	0.107	5.25%	HV
0.0292	3.0348	0.146	4.83%	TAA
0.0269	2.7674	0.136	4.90%	TAC
0.0221	2.2094	0.114	5.14%	BAC
0.0667	7.3938	0.334	4.51%	CV

Appendix 15. Publications from the study

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•Journal Publications •Research Consultancy

Original Article

IN VIVO ANTIDIABETIC POTENTIAL AND SAFETY OF AQUEOUS EXTRACT OF *TRITICUM AESTIVUM* (WHEATGRASS)

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ABSTRACT

Primary goal in management of diabetes mellitus is to realize normoglycaemia. Like many other plants, Triticum aestivum has been used widely in complementary and alternative medicine but minimal data is available on its effectiveness and toxicity effects. This study was done to evaluate in vivo glycemic and toxicity effects of Triticum aestivum. 10% alloxan monohydrate was administered intraperitoneally in Swiss White Albino rats to induce diabetes in determination of efficacy. Rats with blood glucose levels above 200 mg/L were orally administered with aqueous extracts of Triticum aestivum plant at 50, 90.9, 165.1 and 300mg/kg body weight. Glibenclamide was used as the positive control. Toxicity studies were done post oral administration of either 300, 448.14, 669.4 or 1000mg per kilogram body weight of plant extracts for 28 days. The weights of the rats organs, haematological and biochemical parameters were used for toxicity studies. $p \leq 0.05$ was considered statistically significant. The results obtained in this study indicated that the young plant of Triticum aestivum has therapeutic benefits in treatment of diabetes mellitus. No chronic toxicity effects were established. Use of Triticum aestivum as mono therapy for diabetes should be recommended on carrying out clinical studies in humans.

KEYWORDS : Normoglycemia, Triticum aestivum, Glibenclamide, In vivo, Alloxan, Efficacy & Toxicity

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INTRODUCTION

Despite great development of therapeutic and other measures for mitigating diabetes, there has been a steady global rise in its prevalence. In 1980, 108 million people were living with diabetes but the prevalence quadrupled to 422 million in 2014. The increase in diabetic cases has been more in the low and middle income countries over the last decade (WHO, 2016a). In Kenya, a low middle income economy, diabetes has been on a steady rise and the prevalence is currently at 4% in a population of 46 million people (WHO, 2016b). Diabetes and its related complications cause huge economic loses in individuals, families and national economies which is attributed to loss of work and wages, disability, high health budget and high costs of treatment. An individual is said to be diabetic if his or her glycaemic levels are above the normal reference level of fasting plasma glucose of above or equal to 7.0 mmol/l (126 mg/dl) or a 2 hour post prandial glucose level equal to or above 11.1 mmol/L (200 mg/dl) or a deranged oral glucose tolerance test (OGTT) (WHO, 2016a; Aronson and Rayfield, 2002; Piero et al., 2014).

Upon diagnosis, the primary goal in the management of diabetes mellitus is to realize optimal glycaemic control and maintain normoglycaemia as much as possible. When diet therapy fails in normalizing glycemia, oral

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Original Article

INVIVO GLYCEMIC AND TOXICITY EFFECTS OF AQUEOUS EXTRACTS OF URTICA MASSAICA

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ABSTRACT

Phytotherapy as traditional, alternative or complementary medicine is the most popular therapeutic intervention for noncommunicable diseases.Urtica massaica has been used for various ailments; one among them is diabetes mellitus. However, concerns over the blood glucose lowering potential as well as its safety for human use has not been scientifically demonstrated. The objective of this study was to evaluate the invivo glycemic and toxicity effects of aqueous extracts of Urtica massaica. In evaluation of efficacy, aqueous extract ofUrtica massaica at dose levels of 50, 90.9, 165.1 and 300mg/kg body weight was orally administered into Alloxan induced diabetic rats with a fasting blood glucose equal to or above 200 mg/L. Glycemia was determined hourly and the results obtained after a 24 hour experimental period were compared to normal (non diabetic) and glibenclamide treated rats. Safety aspects were evaluated by orally administering either dose of 300, 448.14, 669.4 or 1000mg/kg body weight of the plant'saqueous extracts for 28 days. Mean blood glucose values and results on the effect on body weight, blood, liver, pancrease, heart and renal system relative to the control. We determined presence of minerals, phytonutrients and phytochemicals in the plants extracts, which was established using standard methods. Urtica massaica aqueous extracts have considerable antihyperglycaemic activity and is a promising antidiabetic monotherapy. Flavonoids, alkaloids, saponins, steroids, cardiac glycosides, minerals and amino acids were present in the aqueous extracts. The current study validates the use of Urtica massaicaas as it has been utilized for mitigation of diabetes and its continued use is recommended. However, its ethno botanical use can be enhanced by bioassay guided fractionation of bioactive compounds and translational clinical research is recommended.

KEYWORDS: *Invivo, Urtica Massaica, Glycaemia, Ethnobotanical & Bioactive Compounds*

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INTRODUCTION

Urtica massaica is a species of flowering rhizomatous perennial herb plant that grows up to 2 meters tall. It belongs to the kingdom plantae, family *Urticaceae* (nettle) and genus *Urtica*. It is commonly known as stinging nettle or forest nettle and largely spread across East Africa. The plant has heart-shaped leaves covered with stinging hairs and serrated edges. This plant is used for food and medicine in several African nations (PROTA, 2004; Alphonse, 2008). In Kenya, it is used as a tonic, to treat stomach ache, diabetes or fractures and venereal diseases.The current study evaluates the invivo effectiveness of *Urtica massaica* as an antihyperglycaemic agent and safety concerns related to its chronic consumption.

METHOD

Plant Material