EFFECTS OF MAALOX PLUS® ANTACID AND PURECAL® CALCIUM SUPPLEMENTS ON LIVER AND KIDNEY FUNCTION FOLLOWING HEAVY ALCOHOL INTAKE IN RATS

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156/CTY/PT/24940/2013

A Thesis Submitted in Partial Fulfillment of the Requirements for the Award of the Degree of Master of Science (Medical Biochemistry) in the School of Pure and Applied Sciences of Kenyatta University

OCTOBER 2017
DECLARATION

This thesis is my original work and has not been presented for a degree in any other university or any other award.

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DEDICATION

To my loving mum Rebecca Onyango.

Thus far the lord has brought us.
ACKNOWLEDGEMENT

It is with much gratitude that I would like to appreciate the invaluable assistance in practice and in kind of individuals who went out of their way to ensure that this work was exemplary. The time and resources they spent did not go unnoticed and will forever be indelible in my memory.

I would first like to thank my supervisors Dr. David Mburu and Dr. Mathew Piero Ngugi of the Department of Biochemistry and Biotechnology at Kenyatta University. They were both full of words of encouragement, were not hesitant to guide me through grey areas and gave me room for this to be my own work.

I would also like to thank technicians at the Department of Biochemistry and Biotechnology laboratories and animal house who were full of assistance during my bench work: James Ngunjiri, Wycliffe Wenwa and James Adino. Without their passionate assistance my bench work would not have been as smooth.

My list would not be complete without mentioning my fellow colleagues: Kelvin Juma, James Kimani, Peter Nthiga and Francis Wanyama. They were always full of valuable and timely advice while also imparting precious skills that were helpful in navigating my way through the study. To all those who participated directly and indirectly towards making this work a success, am much obliged.
# TABLE OF CONTENTS

DEDICATION ........................................................................................................................ iii

ACKNOWLEDGEMENT ........................................................................................................ iv

LIST OF TABLES .................................................................................................................. viii

LIST OF FIGURES ................................................................................................................ ix

LIST OF APPENDICES .......................................................................................................... x

ABBREVIATIONS AND ACRONYMS .................................................................................. xi

ABSTRACT .......................................................................................................................... xii

CHAPTER ONE ..................................................................................................................... 1

INTRODUCTION ................................................................................................................... 1

1.1 Background ................................................................................................................... 1

1.2 Problem statement ......................................................................................................... 5

1.3 Justification of the study ............................................................................................... 5

1.4 Research questions ........................................................................................................ 6

1.5 Null Hypothesis ............................................................................................................. 6

1.6 Objectives ...................................................................................................................... 6

1.6.1 General objective ....................................................................................................... 6

1.6.2 Specific objectives ..................................................................................................... 7

CHAPTER TWO ................................................................................................................... 8

LITERATURE REVIEW ......................................................................................................... 8

2.1 Alcohol and its effects .................................................................................................. 8

2.1.1 Overview .................................................................................................................. 8

2.1.2 Physical and chemical properties ............................................................................. 9

2.1.3 Absorption of alcohol ............................................................................................. 10

2.1.4 Tissue distribution of alcohol ................................................................................ 11

2.1.5 Metabolism of alcohol ............................................................................................ 12
<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.1.6 Excretion of alcohol</td>
<td>14</td>
</tr>
<tr>
<td>2.1.7 Mode of action of alcohol</td>
<td>15</td>
</tr>
<tr>
<td>2.1.8 Toxicity of alcohol</td>
<td>16</td>
</tr>
<tr>
<td>2.1.9 Health effects of alcohol</td>
<td>17</td>
</tr>
<tr>
<td>2.1.10 Management of alcohol intoxication</td>
<td>20</td>
</tr>
<tr>
<td>2.2 Magnesium homeostasis and alcohol toxicity</td>
<td>21</td>
</tr>
<tr>
<td>2.3 Calcium homeostasis and alcohol toxicity</td>
<td>23</td>
</tr>
<tr>
<td>CHAPTER THREE</td>
<td>26</td>
</tr>
<tr>
<td>MATERIALS AND METHODS</td>
<td>26</td>
</tr>
<tr>
<td>3.1 Materials</td>
<td>26</td>
</tr>
<tr>
<td>3.2 Experimental animals</td>
<td>26</td>
</tr>
<tr>
<td>3.3 Experimental design</td>
<td>27</td>
</tr>
<tr>
<td>3.4 Physical parameters</td>
<td>27</td>
</tr>
<tr>
<td>3.5 Sampling</td>
<td>28</td>
</tr>
<tr>
<td>3.6 Hematological analysis</td>
<td>29</td>
</tr>
<tr>
<td>3.7 Biochemical analysis</td>
<td>29</td>
</tr>
<tr>
<td>3.8 Histopathological examination</td>
<td>31</td>
</tr>
<tr>
<td>3.9 Tissue analysis</td>
<td>31</td>
</tr>
<tr>
<td>3.10 Data management and statistical analysis</td>
<td>32</td>
</tr>
<tr>
<td>CHAPTER FOUR</td>
<td>34</td>
</tr>
<tr>
<td>RESULTS</td>
<td>34</td>
</tr>
<tr>
<td>4.1 Effects of alcohol on behavioural characteristics of rats</td>
<td>34</td>
</tr>
<tr>
<td>4.2 Effects of treatments on body weight</td>
<td>35</td>
</tr>
<tr>
<td>4.3 Effects of alcohol, Maalox plus® and PureCal® on hematological parameters</td>
<td>38</td>
</tr>
<tr>
<td>4.4 Effects of alcohol, Maalox plus® and PureCal® on biochemical profiles</td>
<td>41</td>
</tr>
<tr>
<td>4.5 Calcium and magnesium levels in muscle and femur of rats</td>
<td>45</td>
</tr>
</tbody>
</table>
4.6 Effects of alcohol, Maalox plus® and PureCal® on histopathology of the liver tissues ..... 47
4.7 Effects of alcohol, Maalox plus® and PureCal® on histopathology of the kidney tissues . 51

CHAPTER FIVE .................................................................................................................................................. 55

DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS .............................................................................. 55

5.1 Discussion ..................................................................................................................................................... 55

5.2 Conclusions .................................................................................................................................................. 64

5.3 Recommendations ...................................................................................................................................... 65

5.3.1 Recommendations from the study .......................................................................................................... 66

5.3.2 Recommendations for further research .................................................................................................... 66

REFERENCES ................................................................................................................................................... 67

APPENDICES ..................................................................................................................................................... 83
LIST OF TABLES

Table 3.1: Treatment regimens...........................................................................................................28

Table 4.1: Comparison of mean weight for rats over a four week period.................................37

Table 4.2: Comparison of hematological parameters.................................................................40

Table 4.3: Comparison of biochemical parameters.................................................................43

Table 4.4: Aspartate aminotransferase to alanine aminotransferase ratio................................44

Table 4.5: Comparison of magnesium and calcium levels in tissues .........................................46

Table 4.6: Histological changes of liver tissue..............................................................................48

Table 4.7: Histological changes of kidney tissue........................................................................52
LIST OF FIGURES

**Figure 3.1**: Selectra chemical analyzer system ................................................................. 30

**Figure 3.2**: Bresser LCD Micro light microscope ................................................................. 32

**Figure 4.1**: Behavioral characteristics of rats ................................................................. 34

**Figure 4.2**: Histopathology of the liver of rats ................................................................. 49

**Figure 4.3**: Histopathology of the kidney ................................................................. 53
LIST OF APPENDICES

Appendix I: Haematological and biochemical assessment charts..............................83

Appendix II: NACOSTI research authorization..........................................................96
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADH</td>
<td>Alcohol dehydrogenase</td>
</tr>
<tr>
<td>ALT</td>
<td>Alanine amino transaminases</td>
</tr>
<tr>
<td>AST</td>
<td>Aspartate amino transaminases</td>
</tr>
<tr>
<td>BUN</td>
<td>Blood Urea Nitrogen</td>
</tr>
<tr>
<td>Ca</td>
<td>Calcium</td>
</tr>
<tr>
<td>GGT</td>
<td>Gamma glutamyl transferase</td>
</tr>
<tr>
<td>GSH</td>
<td>Glutathione</td>
</tr>
<tr>
<td>HDL</td>
<td>High Density Lipoprotein</td>
</tr>
<tr>
<td>MDA</td>
<td>Malondialdehyde</td>
</tr>
<tr>
<td>MEOS</td>
<td>Microsomal Ethanol Oxidizing System</td>
</tr>
<tr>
<td>Mg</td>
<td>Magnesium</td>
</tr>
<tr>
<td>ROS</td>
<td>Reactive oxygen species</td>
</tr>
<tr>
<td>TBA</td>
<td>Thiobarbituric acid</td>
</tr>
<tr>
<td>T-CHOL</td>
<td>Total Cholesterol</td>
</tr>
<tr>
<td>ALDH</td>
<td>Acetaldehyde dehydrogenase</td>
</tr>
<tr>
<td>NAD</td>
<td>Nicotinamide adenine dinucleotide</td>
</tr>
<tr>
<td>CNS</td>
<td>Central nervous system</td>
</tr>
<tr>
<td>VTA</td>
<td>Ventral tegmental area</td>
</tr>
<tr>
<td>PFC</td>
<td>Prefrontal cortex</td>
</tr>
<tr>
<td>GABA</td>
<td>Gamma aminobutyric acid</td>
</tr>
<tr>
<td>WHO</td>
<td>World health organization</td>
</tr>
<tr>
<td>CDC</td>
<td>Centre for disease control</td>
</tr>
</tbody>
</table>
Habitual intake of alcohol is associated with various health complications including depletion of the body’s stores of magnesium and calcium. In chronic alcoholism, treatment with Mg and Ca has been shown to normalize elevated enzyme activities and some clinically relevant parameters. Currently there is no information on the effects of mineral supplements on adverse effects associated with acute alcohol intoxication. This study therefore examined the effects of Maalox plus® antacid and PureCal® calcium which are rich in Mg and Ca, respectively, on the haematological and biochemical profiles of rats exposed to acute alcohol intoxication. The experimental design included alcohol being administered orally at 5 g/kg for five days and supplements for two days of the week for 28 days. Haematological and biochemical profiles were determined using automated analyzers. Statistical comparison was done using one way ANOVA followed by Tukey’s test. Alcohol caused neutrophilia, eosinophilia and basopenia ($p < 0.05$). Maalox plus® and PureCal® were ineffective against neutrophilia but effective in normalizing eosinophilia. Basopenia was normalized by 4.25 mg/kg Mg and Ca at 4.25 mg/kg and 8.5 mg/kg. Macrocytic anaemia was another alcohol disorder that was reversed by Maalox plus® and PureCal® except in animals given 8.5 mg/kg Mg and those co-treated with Ca and Mg. The alcohol induced thrombocytopenia ($p < 0.05$) was unaffected by the drugs. Alcohol did not significantly affect liver enzymes, lipids, creatinine and BUN levels ($p > 0.05$). However, the drugs lowered blood urea nitrogen levels in animals given 8.5 mg/kg Mg, 4.25 mg/kg Ca and those co-treated with Ca and Mg ($p < 0.05$). Alcohol did not influence serum levels of Mg$^{2+}$ ($p > 0.05$) but significantly increased Ca$^{2+}$ and lowered K$^+$ ($p<0.05$). Maalox plus® and PureCal® normalized the hypercalcemia in a dose-dependent manner. Alcohol induced hypokalemia was normalized by 4.25 mg/kg Mg and 17mg mg/kg Ca. Hypomagnesemia in bone was also reversed by both Maalox plus® and PureCal®. Liver histology showed that alcohol caused cellular infiltration and cytoplasmic vacuolization. Cellular infiltration and widening of tubules was observed in the kidney. Visible improvement of the liver histology architecture was discernible in rats co-treated with Ca and Mg at a ratio of 1:1 as well as 2:1. These results showed that alcohol altered tissue architecture and the renal cation exchange mechanism as shown by the variation in serum Ca$^{2+}$ and K$^+$ levels. Maalox plus® and PureCal® alleviated the alcohol induced eosinophilia, basopenia, macrocytic anaemia, hypercalcemia, hypokalemia, bone hypomagnesemia, liver and kidney pathology. These findings suggest that the drugs are useful agents that could have applications in the management of adverse effects associated with acute alcohol intoxication.
CHAPTER ONE

INTRODUCTION

1.1 Background

Alcohol is an addictive drug that has proved to be socially accepted over many centuries (Guo and Ren, 2010). The earliest evidence of alcohol use by man dates back to 4000 BC. The dangers of alcohol were recognized early and surviving literature of the Greeks shows that around 1700 BC there was warning against excessive consumption of alcohol (George and Figueredo, 2010).

The global figures for alcohol consumption are high and they average 6.2 litres per person aged 15 years or older, which translates into 13.5 grams of pure alcohol per day (WHO, 2014). In Kenya alcohol consumption is also high and is estimated at 4.3 liters per person (WHO, 2014). According to a countrywide survey by NACADA (2007) about 13% of the Kenyan population does regularly consume alcohol.

Alcohol consumption starts early during adolescence and this has been associated with poor outcomes in adulthood (Hingson, 2006). This led Odgers et al. (2008) to suggest that health strategies to reduce or delay early substance exposure should focus on adolescents. Factors that contribute to excessive alcohol consumption are diverse. In Kenya unemployment compounded by corruption and high prevalence of ‘second generation’ alcoholic drinks and traditional illicit
Alcohol consumption at a young age is very likely to lead to dependence, social harm and deterioration of health in adulthood (McCambridge et al., 2011).

Alcohol consumption in moderation has been reported to lower the risk of contracting coronary heart disease which is a major contributor of mortality in developed countries (George and Figueredo, 2010). Kloner and Rezkalla (2007) attributed the beneficial effect of alcohol on the cardiovascular system to alcohol elevating high density lipoprotein, fibrinolysis and endothelial function while on the other hand reducing plasma viscosity, fibrinogen concentration, platelet aggregation and coagulation.

On the contrary excessive alcohol consumption could lead to serious health consequences. Rhem et al. (2009) ranked alcohol as the third largest causative agent for disease that contributes 4% to the global disease burden. It is estimated that 2.5 million deaths are attributed directly to alcohol yearly (WHO, 2011). Harmful effects of alcohol include damage to the liver (Cederbaum et al., 2009), brain (Welch et al., 2013) and the gastrointestinal tract (Rocco et al., 2014). Abuse of alcohol is also known to cause fetal abnormalities, cardiovascular disease and inhibition of CNS activity (Gohlke et al., 2008). According to Yeligar et al., (2016), alcohol use increases susceptibility to lung and respiratory infections. Excessive consumption is also associated with malnutrition which includes deficiencies of proteins, vitamins and minerals (Rossi et al., 2015).
Many toxic effects of alcohol appear to be mediated through electrolyte imbalances especially hypomagnesemia and hypocalcemia. Magnesium deficiency is a common episode among alcoholics and this is because the body excretes 260% more magnesium within minutes of drinking alcohol (Kulkarani and Ravindra, 2015). Other factors contributing to hypomagnesemia include diarrhoea, vomiting, ketosis and starvation (Mouw et al., 2005). Magnesium deficiency can cause a wide variety of features including hypocalcemia, hypokalemia, cardiac and neurological manifestations (Naderi and Reilly, 2008). Animal studies have shown that magnesium deficiency aggravates the hepatic damage that is caused by alcohol (Long and Romani, 2014).

The resultant alteration of electrolyte balance together with acid-base disorders as a consequence of alcohol intoxication have a hand in morbidity and mortality observed in alcoholics (Elisaf and Kalaitzidis, 2015). Oral magnesium supplementation is necessary for restoration of magnesium balance and concomitant correction of hypocalcemia (Rude and Singer, 1981; Pham et al., 2014). Studies suggest that magnesium treatment in alcoholics may help normalize elevated enzyme activities such as aspartate aminotransferase (AST), alanine aminotransferase (ALT) and gamma glutamyl transferase (GGT) and some clinically relevant parameters (Poikolainen and Alho, 2008).

Hypocalcemia is another common feature in alcoholics with Elisaf et al., (2001) estimating the prevalence to be 5-15%. Hypocalcemia is mostly associated with
magnesium depletion. Heavy alcohol intake affects calcium levels in several ways: failure to produce parathyroid hormone to convert vitamin D to its active form; inability to absorb calcium; increase in bone loss; and low androgen production in males (Hanson, 2016). Hypocalcemia is characterized by serum calcium levels < 2.12 mmol/L and is mostly as a result of low levels of vitamin D or hypoparathyroidism. Symptoms associated with hypocalcemia include muscle spasms, seizures, cramps, neuromuscular irritability, cognitive impairment and heart failure (Shoback, 2008). Cardiovascular disease and ischemic heart disease can lead to mortality and studies have hinted at their inverse relationship with calcium supplementation (Kaluza et al., 2010).

Disturbances in serum concentration of calcium and magnesium are associated with disturbances of physiologic functions manifested by numerous clinical symptoms and signs. An earlier study by Dai et al. (2013) reported that balancing calcium and magnesium intake ratios has an alleviatory effect on coronary vascular disease. The current study uses magnesium and calcium singly and in combination to come up with a prevention strategy towards management of acute alcohol toxicity.
1.2 Problem statement

Calcium and magnesium have roles in cell signaling, blood clotting, muscle contraction and are co-factors to enzymes with magnesium being associated with over 300 enzymes. Alcohol consumption causes diuresis of both calcium and magnesium consequently causing deficiency. Alcohol induced mineral imbalance causes a wide variety of features including liver damage, acid-base disorders, neurological manifestations and cardiovascular disease. Therefore, investigation on the role of formulations rich in calcium and magnesium in amelioration of adverse effects associated with alcohol toxicity is worthwhile.

1.3 Justification of the study

Prior studies have proved that alcohol has negative effects on many organs as well as electrolyte imbalances (Kulkarani and Ravindra, 2015). Some of the electrolytes imbalances experienced by alcoholics include hypomagnesemia and hypocalcemia. Therapeutic values of magnesium supplementation towards alcohol toxicity have been reported (Markiewicz-Górka et al., 2011) but similar studies on calcium supplementation are missing. The current study therefore examines the effects of the brand names Maalox plus® and Purecal®, which are rich in magnesium and calcium, respectively, on modulation of the effects of liver and kidney function following acute intake of alcohol in rats. The findings of this study could have applications in the management of adverse effects associated with acute alcohol intoxication.
1.4 Research questions

i) What is the effect of acute intake of alcohol on hematological parameters, biochemical profile and tissue magnesium and calcium in rats?

ii) What is the effects of Maalox plus® antacid supplementation on the liver and kidney function after acute intake of alcohol in rats?

iii) What is the effect of Purecal® calcium supplementation on the liver and kidney functions after acute intake of alcohol in rats?

1.5 Null Hypothesis

Magnesium and calcium when supplemented after chronic alcohol administration have no attenuating effect on the liver and kidney function.

1.6 Objectives

1.6.1 General objective

To determine the effects of the brands Maalox plus® antacid and Purecal® calcium supplements on the liver and kidney function following alcohol intake in rats.
1.6.2 Specific objectives

i. To determine the effect of alcohol on hematological parameters, biochemical profile and tissue magnesium and calcium in a rat model.

ii. To determine the effects of Maalox plus® antacid on the hematological and biochemical profile of rats subjected to alcohol intoxication.

iii. To determine the effects of Purecal® calcium supplementation on hematological and biochemical profile of rats subjected to alcohol intoxication.
2.1 Alcohol and it’s effects

2.1.1 Overview

Alcohol is a popular legal drug that is easily accessible and affordable. On average the worldwide consumption of alcohol is 6.2 litres per person aged 15 years or older, which translates into 13.5 grams of pure alcohol per day. In Kenya alcohol consumption is estimated at 4.3 litres per person (WHO, 2014). The pleasurable effects of alcohol is due to the release of endorphins and that is why it is enjoyed by a large population.

Excessive alcohol consumption has both short term and long term detrimental effects on the consumer’s health (Mongan et al., 2007; Chweya and Auya, 2014). Some of the short term health risks associated with alcohol include: falls, burns, motor vehicle crashes, suicide, homicide, miscarriage, fetal alcohol syndrome and alcohol poisoning. In the long term, excessive alcohol intake is known to lead to chronic diseases such as liver diseases, hypertension, cardiac diseases, stroke, cancer and depression (D’Souza El-Guindy et al., 2010).

Mwithaga (2013) identified alcohol abuse as constituting the largest proportion of substance abuse in Kenya, with the socioeconomic impact being enormous. The socioeconomic effects take the form of loss of productivity at the workplace, unemployment and family problems (Kaithuru and Asatsa, 2015). As a
developing economy the prevalence of alcohol use in Kenya is set to continue increasing and so are the negative effects on health. Investment of time and resources in therapeutic measures to alleviate the negative effects of alcohol on health is thus worthwhile.

2.1.2 Physical and chemical properties

Alcohol belongs to a class of compounds that comprise of carbon and a functional hydroxyl group. The products of their combustion in the presence of oxygen include carbon dioxide and water. Based on the number of carbon atoms, alcohol is categorized into primary, secondary and tertiary groups. Compounds with three or less carbon chain are completely soluble in water but after the seven carbon chain they, are considered insoluble. Examples of miscible alcohol include methanol, ethanol and propanol, while decanol is insoluble (Desai, 2014).

Ethanol, also known as ethyl alcohol is a clear, colorless liquid, with a boiling point of 78.3°C, melting point of -114°C, specific gravity of 0.79 and pH of 7.33 (Spedding, 2015). It belongs to the primary group of alcohols and is the only type of alcohol that can be safely consumed if it has not been denatured or does not contain toxic impurities. Ethanol is present in alcoholic beverages that are the end products of fermentation process involving action of yeast on sugars in the absence of oxygen (CDC, 2010). The ethanol content in alcoholic beverages
range between 40-50 g/L in normal strength beer; in wine it is 40-120 g/L; and for spirits it is 400-500 g/L (Lachenmeier et al., 2010).

2.1.3 Absorption of alcohol

Absorption of alcohol into the blood stream occurs by diffusion through the gastrointestinal tract (Abramson and Singh, 2000; Zaman et al., 2002; Barceloux et al., 2002) with peak concentration being achieved after 30 to 60 minutes. Since alcohol does not require digestion, it essentially passes through the stomach where 20% is absorbed and up to 80% being absorbed in the upper part of small intestines due to the large surface area to volume ratio and the rich blood supply (Manzo-Avalos, 2010). The absorption rate of alcohol is slow when it is consumed with food particularly those rich in protein (Lieber, 2000). Actually, D’Souza El-Guindy et al. (2010) have suggested that peak concentration of alcohol can be achieved faster if it is administered through oral gavage on an empty stomach. On their part, Korsten et al. (1975) noted that malnourished alcoholics ingesting a given amount of alcohol exhibit a higher blood alcohol level which is sustained longer than in normally nourished individuals. The finding was supported by the observations made by Baraona et al. (2001) and Lapham (2010).

In the gastrointestinal tract, alcohol causes damage to the cells lining the stomach and intestines thereby impairing nutrient absorption (Bode and Bode 1997; Puorohit et al., 2008; Fisher et al., 2008). Irritation of the mucous membrane by
alcohol becomes severe depending on how high the ethanol content of the beverage being consumed is and this tends to slow down the absorption rate. In order to relieve the irritation effects of alcohol the body employs the reflex action of vomiting (Pan et al., 2008).

2.1.4 Tissue distribution of alcohol

The temporary presence of alcohol in various body tissues is what is referred to as distribution. Upon absorption, alcohol attains a volume of distribution similar to that of body water and this is largely due to its solubility with water and lipids. Ethanol has an approximate volume of distribution of 0.5 L/kg with a half–life of 2 to 6 hours (Kraut and Kurtz, 2008) with whole blood ethanol concentration being 1.2 times higher than in plasma. A higher blood concentration of alcohol yields a longer half-life due to saturation of the enzyme alcohol dehydrogenase (Matsumoto and Fukui, 2002; Gemma et al., 2006). Blood alcohol concentration is indicative of the amount of alcohol in one’s body and is determined by the type and quantity of alcoholic beverage, weight, sex, rate of elimination and food after ingestion of alcohol (Toffolo et al., 2012; Caderbaum, 2012). Women have a faster rate of metabolizing alcohol by 17% compared to men but at the same time have a 10% lower volume of distribution (Baselt, 2000). As alcohol enters the bloodstream it dilates the blood vessels leading to greater flow of blood to the skin and increased heat loss.
2.1.5 Metabolism of alcohol

Elimination of alcohol from the blood stream is through a combination of metabolism, excretion and evaporation. The liver metabolizes 95% of alcohol with the rest being excreted through the kidneys and evaporation through breath. Ethanol is primarily metabolized in the liver via alcohol dehydrogenase (ADH) pathway in the cytosol or the soluble fraction of the hepatocytes and the microsomal ethanol oxidizing system (MEOS) located in the endoplasmic reticulum (Haseba and Ohno, 2010). Haber et al. (2003) pointed out that ethanol has the potential to displace other substrates in the liver as it is the preferred fuel. This explains the association between heavy alcohol consumption and liver disease that was recognized more than 200 years ago (Smart and Mann, 1992).

Alcohol dehydrogenase which has broad substrate specificity catalyzes the first step in oxidation of alcohol in the liver, a step that is critical in its biotransformation (Kraut and Kurtz, 2008). The MEOS oxidizes alcohol to acetaldehyde by means of Cytochrome P450 2E1 or Cytochrome 2E1 which is located in the endoplasmic reticulum of hepatocytes. Cytochrome 2E1 normally functions at a low level but presence of alcohol stimulates it to a higher level (Maher, 1997; Cederbaum, 2012). The microsomal ethanol oxidizing system becomes increasingly important as alcohol consumption becomes heavier and chronic. Induction of the activity of MEOS is associated with the synergistic activity of other enzymes like Cytochrome P550 reductase, Cytochrome P540 and gamma glutamyl transferase leading to changes that are responsible for alcoholics
being resistant to the effects of common anesthetics and barbiturates (Caro and Cederbaum, 2004; Lau et al., 2009);

1. \[ \text{CH}_3\text{CH}_2\text{OH}_+ + \text{NAD}^+ \leftrightarrow \text{CH}_3\text{CHO} + \text{NADH} + \text{H}^+ \]

2. \[ \text{CH}_3\text{CHO} + \text{NAD} \rightarrow \text{CH}_3\text{COOH} + \text{NADH} + \text{H}^+ \]

The ADH and MEOS pathways display increased conversion of NAD to NADH leading to redox changes that are responsible for oxidative stress and liver injury (Markiewicz-Górka et al., 2011). Genetic differences which can be brought about by differences in race could lead to differences in hepatic enzymes that are involved in metabolism of alcohol, most notably ADH, and this contributes to predisposition to development of alcoholic liver disease. Enzymes like acetaldehyde dehydrogenase (ALDH) have the potential of undergoing polymorphism that is genetically determined as observed in Caucasians leading to low enzyme activity hence accumulation of acetaldehyde (Haber et al., 2003; Cederbaum, 2012).

The ALDH2 enzyme deficiency is discernible in people of Japanese descent making them susceptible to liver disease (Enomoto et al., 1991). McCullough and O’connor (1998) pointed out that, women are quicker at developing alcoholic liver disease and do so at a lower dose compared to men. While comparing the gender differences in alcohol metabolism Kwo et al. (1998) and Norberg et al. (2003) expounded that despite women having a smaller body weight, their alcohol clearance and volume of liver is similar to that of men.
2.1.6 Excretion of alcohol

According to Kraut and Kurtz (2008) routes of elimination of ethanol include the liver, lungs and kidney at 95%, 2% and 3%, respectively. Barceloux et al. (2002) reported that the kidneys excrete 2% to 5% of ethanol with the rest being excreted through sweat and the lungs. In one study, the amount of ethanol excreted unchanged was estimated to be 5% to 8% (Dahl et al., 2002) while in another it was put at a modest value of 2% to 5% (Manzo-Avalos and Saavedra-Molina, 2010). In the estimates put forth by the latter, urine accounts for 0.3% of ethanol excreted while unaltered with diuresis induced by ethanol being severe for the first 1 to 2 hours after drinking.

When taken in copious amounts ethanol causes to deleterious effects on the kidney. Increase in frequency of urination experienced when taking alcohol is due to ethanol leading to inhibition of the pituitary gland thus a reduction in the release of antidiuretic hormone that is responsible for facilitating reabsorption of water at the kidney tubules (Adewale and Ifudu, 2014). This explains episodes of dehydration that accompany indulgence in alcohol. Xenobiotics also tend to be made more water soluble through conjugation, with ethanol being conjugated with uridine 5’-diphosphoglucuronic acid thus facilitating elimination through urine as ethyl glucuronide (Dahl et al., 2002).
2.1.7 Mode of action of alcohol

Alcohol appears to act through modification of cell membranes. Since ethanol is uncharged it easily dissolves in the lipid bi-layer of the cell membrane thus increasing their fluidity. The pleasurable feeling and possibility of addiction that comes with alcohol is due to its ability to stimulate the dopaminergic reward pathway (Chitel, 2011). A pathway that consists of ventral tegmental area (VTA) that extends axons to areas in the prefrontal cortex (PFC) and nucleus accumbens both believed to have a role in motivation. The feeling of pleasure emanates from a rewarding stimulus being present thus triggering the VTA to increase its release of dopamine, a neurotransmitter in the extended axons (Kandel et al., 2012).

Alcohol inhibits the central nervous system and as such is classified as a sedative and a hypnotic drug (CDC, 2010). Inhibition of the central nervous system by ethanol has been traced to its interaction with the gamma aminobutyric acid, an inhibitory neurotransmitter (Davies, 2003). Aryal et al. (2009) was able to demonstrate the ability of ethanol in opening ionic channels known as G protein gated inwardly-rectifying potassium (GIRK) leading to brain cells eliminating potassium resulting in a reduction of their activity thus slowing down brain function with the drinker experiencing a relaxing sensation. Craving, tolerance, dependence, withdrawal and relapse are some experiences that are associated with alcohol abuse and can be traced back to alcohol targeting the N-methyl-D-aspartate glutamate receptor (NMDAR) (Krystal et al., 2003). In a study
conducted by Parsons et al. (1999) a well-tolerated NMDAR antagonist known as memantine was shown to reduce alcohol craving.

### 2.1.8 Toxicity of alcohol

The oxidative properties of alcohol make the liver and the kidney prone to damage since they play a vital role in its metabolism. Ethanol induced lipid peroxidation of cellular membranes plays a crucial role in hepatotoxic and nephrotoxic action of ethanol. A high malondialdehyde level is a marker of lipid peroxidation which is a fallout of oxidative damage (Macdonald et al., 2010). Subir (2008) reported that ethanol treatment raises thiobarbituric acid reactive substances, suggesting association between increased lipid peroxidation and prolonged ethanol consumption.

The activity of antioxidant enzymes superoxide dismutase and catalase is enhanced in the liver and kidney exposed to ethanol (Jurczuk et al., 2004). Glutathione which is synthesized in the liver plays a crucial role in the cell defense system yet it has been reported that ethanol exposure results in a reduction in glutathione concentration in the liver and kidney in a rat model (Jurczuk et al., 2006). In summary, antioxidant depletion, inhibition of glutathione synthesis by alcohol, inadequate intake of antioxidants and pro-oxidant formation are the causes of oxidative stress seen in alcoholic liver disease (Tome and Lucey, 2004).
Alcohol related liver damage can be divided into 3 categories; fatty liver, alcoholic hepatitis and alcoholic cirrhosis. A daily intake of more than 60 g of alcohol in men and 20 g in women significantly increases the risk of liver cirrhosis (Grant, 1988). The kidneys are mostly spared where chronic alcoholism has not resulted in stages of advanced alcoholic liver disease or hepato-renal syndrome. In their study Heidland et al. (1985) pointed out that consumption of alcohol regularly raises blood pressure which is a risk factor for renal damage.

2.1.9 Health effects of alcohol

Alcohol intoxication has detrimental effect on many organs in the body with promotion of organ damage being attributed to aldehyde forming protein and DNA adducts (Thiele et al., 2008; Seitz and Stickel, 2010). The consequences of alcohol consumption include a direct toxic effect on the bone marrow, blood cell precursors and mature white blood cells thus compromising the immune system as reported by Szabo and Mandrekar (2009).

Alcohol metabolism also tends to cause derangement in metabolism of carbohydrates, protein and lipids, while at the same time affecting cellular signaling (Baroana and Lieber, 1979; Sabesin, 1981; Tuma et al., 1991; Den Boer et al., 1997; Wilkie et al., 2007). The redox alterations brought about by alcohol have been shown to drive pro atrophy signaling pathways that result in muscle atrophy (Jeffery et al., 2007).
When large amounts of alcohol are taken followed shortly by impairment of attention, lack of coordination and loss of consciousness it is known as acute intoxication (Vonghia et al., 2008). After this episode, side effects experienced include hangover that is characterized by headache, nausea and vomiting. These symptoms appear as a result of the dehydrating effect of alcohol.

The heart is also affected by heavy intake of alcohol with diseases like atrial fibrillation, congestive heart failure, heart attack and stroke as a result of damage of heart muscles. Though atrial fibrillation may not be serious, if it tends to last for long it may lead to heart failure or stroke. On the contrary, moderate alcohol consumption has a protective effect on the development of cardiac heart disease (Moore and Pearson, 1986) with the increase in high density lipoprotein cholesterol being advanced as the possible mechanism.

Alcohol damages and narrows the esophagus thus making the swallowing of food difficult and thus increasing likelihood of developing gastro-esophageal reflux disease. This disease is characterized by stomach acid splushing into the esophagus hence causing burning pain and increasing the likelihood of developing cancer of the esophagus. Ailments caused by alcohol on the gastrointestinal tract include intestinal hyperpermeability, endotoxaemia and cancer (Benedetti et al., 2009; Keshavarzian et al., 2009).
Heavy alcohol consumption can also result in acute inflammation known as pancreatitis which is reversible while chronic pancreatitis on the other hand is life threatening. Hanck and Whitcomb (2004) named pancreatitis and cancer as some of the diseases that affect the pancreas as a result of alcohol ingestion.

Previous studies have pointed to chronic alcohol consumption leading to acute respiratory distress syndrome which has a 40% to 50% death rate. The respiratory tract is afflicted by airway diseases and lung infections (Sisson, 2007; Boé et al., 2009). Incidences of pneumonia arising from an unconscious drank person vomiting and then inhaling the vomited material which cause the disease have been reported (Kuhadja et al., 2015).

Alcohol intake has extensive effects on the brain and it impairs the ability to think, learn, understand and solve problems. The drug is also known to induce amnesia and dementia while also causing brain shrinkage and atrophy accompanied by memory and cognitive loses (Mukherjee, 2013). Birth defects commonly referred to as fetal alcohol syndrome are a consequence of alcohol consumption by pregnant mothers (Vall et al., 2015).

Alcoholic liver disease is the single most important health scare associated with alcohol abuse. It is usually as a result of metabolites formed during alcohol breakdown and it involves the conditions of fatty liver, steatosis, steatohepatitis, fibrosis and hepatocellular carcinoma (O’shea et al., 2009; Seitz and Stickel,
When alcohol is taken concurrently with some drugs there is bound to be interaction. Ethanol is known to potentiate the destructive effect of paracetamol on the liver (Gonzalez, 2005), cause irritation to the stomach when taken with aspirin and compound the sedative effects of sleeping pills as well as relief resulting from use of opiates. Cases of alcohol increasing the breakdown of prescription medicine by activating the liver enzymes involved in the breakdown thus sabotaging the therapeutic effect have also been reported (Zakhari, 2006).

### 2.1.10 Management of alcohol intoxication

Abstinence remains the most important approach in management of alcohol toxicity as articulated by Teli et al. (1995) who associated abstinence with reversing of alcoholic steatosis. Pharmacotherapy can also be used to achieve abstinence (Jarvis et al., 1995; De Sousa et al., 2010; Hagedorn et al., 2016). Action of alcohol on the dopaminergic reward pathway can be countered by naltrexone, an orally active opioid antagonist which is however hepatotoxic at higher doses (Sax et al., 1994; Anton, 2008; Zindel and Kranzler, 2014). Inhibition of the central nervous system N-methyl-D-aspartate receptors and gamma-aminobutyric (GABA) transmission can be achieved by administration of acamprosate which is not metabolized in the liver (Saivin et al., 1998). Acetaldehyde dehydrogenase (ALDH) is inhibited by disulfiram thus acetaldehyde accumulates upon alcohol consumption hence causing aversive
reaction with nausea and vomiting. Disulfiram is also hepatotoxic and inhibits CYP2E1 and could potentially lead to drug interactions (Karla et al., 2014).

Excessive consumption of alcohol is usually accompanied by poor food intake, decreased intestinal assimilation and hepatic storage of nutrients (Tome and Lucey, 2004). Long term alcohol intake is also associated with alcoholic ketoacidosis syndrome and vomiting hence the metabolic alkalosis in alcoholic patient (Elisaf et al., 1994). Nutritional therapy has thus been employed in management of various toxic effects of alcohol. Alcoholic ketoacidosis is treated using intravenous fluids and carbohydrates. Alcoholic patients are usually deficient in thiamine which has the potential of resulting in Wernicke’s encephalopathy which can be reversed with thiamine supplementation. Supplementation with magnesium (Poikilanen and Alho, 2008) and folate is also used in treatment of alcoholics.

2.2 Magnesium homeostasis and alcohol toxicity

Magnesium is the fourth most abundant cation and it is estimated that over 300 enzymes are dependent on magnesium. In the body, its balance is maintained by renal regulation of reabsorption. An earlier study by Elin (1987) pointed the major store for magnesium to be in the bone at 53%, followed by the soft tissues at 19% with erythrocytes and serum having 0.5% and 0.3% respectively. The bone, kidneys and small bowels have an interaction that maintains magnesium
homeostasis. Its deficiency and hypomagnesaemia can be as a result of gastrointestinal and renal loses (Swaminathan, 2003).

Many toxic effects of alcohol appear to be mediated through imbalances in micronutrients. In the gastrointestinal tract alcohol causes damage to the cells lining the stomach and intestines thereby impairing nutrient absorption (Bode and Bode, 1997). Actually, magnesium deficiency is a common episode among alcoholics and this is because the body excretes about 260% more magnesium within minutes of drinking alcohol (Sarai et al., 2013). Magnesium deficiency can cause a wide variety of features including hypocalcemia, hypokalemia and cardiac and neurological manifestations (Naderi and Reilly, 2008).

Poikolainen and Alho. (2008) allude to magnesium treatment ameliorating elevated activities of liver transaminases and some clinically relevant parameters. Magnesium supplementation reduces blood pressure since when it’s done for 3 to 24 weeks a decrease in systolic blood pressure by 3-4 mmHg and diastolic blood pressure by 2-3 mmHg can be observed (Bullarbo et al., 2013). In their study in rats Altura et al. (1984) noted that development of hypertension is associated with dietary magnesium deficiency. Whelton et al. (1989) went a step further and reported that magnesium alleviates hypertension. Higher magnesium intake might reduce the risk of diabetes possibly because of the important role of magnesium in glucose metabolism (Rude, 2012).
Animal studies have shown that magnesium deficiency aggravates the hepatic damage that is caused by alcohol (Long and Romani, 2014). In a related study Markiewicz-Gorka et al. (2011) showed that alcohol was responsible for causing liver lesions and that magnesium supplementation alleviated the histopathological changes by diminution of cytoplasmic vacuolation, infiltration of inflammatory cells and disappearance of steatosis and focal hepatocellular necrosis.

2.3 Calcium homeostasis and alcohol toxicity

There is more calcium in the body than any other mineral and it has several important functions that include: helping to build strong bones and teeth; regulating muscle contraction including heart beat; ensuring blood clots normally; transmitting nervous system messages and enzyme functioning (Lakshmi, 2014). Other biochemical roles of calcium include regulating enzyme activity, permeability of ion channels, and activity of ion pumps and as a component of cytoskeleton (Koolman, 2005). On the other hand, healthy kidneys turn vitamin D into Calcitriol which is an active hormone that helps increase calcium absorption from the intestines into blood.

Holick and Garabedian (2006) pointed out that the body is able to maintain serum calcium levels at physiological levels through the action of vitamin Upon activation through hydroxylation in the liver and kidney, the resultant 1, 25-
dihydroxyvitamin D interacts with receptors localized in the small intestines thus increasing absorption of dietary calcium (Bouillon, 2001).

The level of serum calcium is involved in the tight regulation of hydroxylation of vitamin D. Calbindin D 9K which is a calcium binding protein and Ca ATPase that are involved in facilitation of calcium absorption are induced by vitamin D. If a condition arises where there is inadequate dietary calcium to meet the body’s demand then vitamin D is involved in mobilization of bone pre-osteoclasts which become mature osteoclasts. The mature osteoclasts contribute to the dissolution of bone mineral and matrix by releasing proteolytic enzymes and hydrochloric acid is involved. This process results in calcium being made available for use by the body (Kholsa, 2001; Holick, 2004).

Heavy alcohol intake affects calcium levels in several ways. Alcohol blocks antidiuretic hormone leading to diuresis which has been reported to be the leading loss of calcium together with the urine (Lieber et al., 2004; Subir et al., 2008). In addition, the liver plays a critical central role in vitamin D metabolism and a disturbance in this metabolism in alcoholic liver disease is associated with disturbances in calcium homeostasis.

Calcium and magnesium compete for absorption into the bloodstream and if there is an excess of the calcium cation it can effectively prevent the magnesium cation from entering the cell, or acting to elicit biochemical reactions by binding to its
cognate molecule. In their study Whiting and Wood (1997) reported that magnesium absorption is significantly depressed when calcium is increased in the diet. On the other hand studies have also reported scenarios where an increase of magnesium in the diet significantly reduces fecal calcium in humans (Heaton and Pyrah, 1962; Clark, 1969).

Higher calcium to magnesium ratio encourages magnesium deficiency. Disturbances in serum concentration of these minerals are associated with disturbances of physiologic functions manifested by numerous clinical symptoms and signs. High amounts of calcium cause constipation a situation opposite to that of magnesium that is a laxative. In addition the process of absorption of both calcium and magnesium is also similar. The various interactions between the two elements have informed the drug therapy used in the study herein to combat the toxic effects of acute alcohol intoxication.
3.1 Materials

Alcohol (Smirnoff Vodka 37.5%, UDV Kenya Ltd, Nairobi, Kenya) was purchased from the Kenya Breweries staff shop. Maalox plus® antacid (Winthrop Pharmaceuticals Ltd, Guilford, UK) suspension containing 200 mg magnesium hydroxide per 5 ml was used as the source of magnesium. Calcium supplementation was achieved through administration of PureCal® calcium (Fountil Life Sciences Ltd, Mumbai, India) that contained 160 mg calcium per 5 ml. Both the antacid and calcium supplement were procured from local chemists.

3.2 Experimental animals

A total of 50 male wistar rats aged between 7-8 weeks were used in this study. The animals were bred and housed at the Department of Biochemistry and Biotechnology Animal House. They were housed in cages at conventional conditions at a temperature of 22-25 °C and a 12 hour light and dark cycle. The animals were fed on commercially available standard chow diet and water ad libitum. Animal studies were done following the ethical guidelines of the Guide for the Care and Use of Laboratory Animals (Institute of Laboratory Animal Resources, 1996).
3.3 Experimental design

The animals were allocated randomly to ten groups of five animals each. The negative controls received water only while the positive control group was treated with alcohol. The other groups were first treated with alcohol followed by either Maalox plus® antacid, or PureCal® calcium or a combination of both. Details of the treatment regimens are as presented in table 3.1.

Alcohol was administered orally at a dose of 5 g/kg body weight for five days in a week. The drugs were administered for the subsequent two days after alcohol ingestion as was practiced by Markiewicz-Gorka et al. (2011). Maalox plus was diluted 1:40, 1:20 and 1:10 to achieve dosages of 4.25 mg/kg, 8.5 mg/kg and 17 mg/kg of magnesium, respectively. On the other hand PureCal® was diluted 1:32, 1:16 and 1:8 to come up with dosages of 4.25 mg/kg, 8.5 mg/kg and 17 mg/kg of calcium, respectively. All the solutions were administered through the oral gavage (Nadro et al., 2006) using a cannula and treatment was continued for 28 consecutive days.

3.4 Physical parameters

Behavioral characteristics, food and water intake and body condition were studied during the treatment period. Weekly body weight for each animal was also recorded.
Table 3.1 Treatment regimen of experimental animals

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Water (negative control)</td>
</tr>
<tr>
<td>B</td>
<td>5 g/kg alcohol (positive control)</td>
</tr>
<tr>
<td>C</td>
<td>5 g/kg alcohol + 4.25 mg/kg magnesium</td>
</tr>
<tr>
<td>D</td>
<td>5 g/kg alcohol + 8.5 mg/kg magnesium</td>
</tr>
<tr>
<td>E</td>
<td>5 g/kg alcohol + 17 mg/kg magnesium</td>
</tr>
<tr>
<td>F</td>
<td>5 g/kg alcohol + 4.25 mg/kg calcium</td>
</tr>
<tr>
<td>G</td>
<td>5 g/kg alcohol + 8.5 mg/kg calcium</td>
</tr>
<tr>
<td>H</td>
<td>5 g/kg alcohol + 17 mg/kg calcium</td>
</tr>
<tr>
<td>I</td>
<td>5 g/kg alcohol + 4.25 mg/kg magnesium + 4.25 mg/kg calcium</td>
</tr>
<tr>
<td>J</td>
<td>5 g/kg alcohol + 4.25 mg/kg magnesium + 8.5 mg/kg calcium</td>
</tr>
</tbody>
</table>

3.5 Sampling

Forty eight hours after the final day of alcohol administration venous blood was collected from the tail of the rats and used for hematological analysis. The animals were euthanized using ether and blood drawn through cardiac puncture. Serum was processed and used for biochemical analysis. Liver and kidney tissues were harvested for histological analysis while bone and muscle tissues were collected for tissue mineral analysis.
3.6 Hematological analysis

The first drop of venous blood was wiped off and the following five drops collected in EDTA vials. A full haemogram was conducted using a haematological analyzer (Mindray Beckman Coulter 6800, Shenzhen Mindray Bio-Medical Electronica Co Ltd. Shenzhen, China) using the protocol described by Grillone et al. (2014). The parameters determined were total white blood cells counts (WBC), neutrophils (NEUT), eosinophils (EOSI), basophils (BASO), lymphocytes (LYMP), red blood cells (RBC), platelets (PLT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC).

3.7 Biochemical analysis

Blood was obtained with the aid of a syringe through cardiac puncture and transferred to a plain tube. The blood was allowed to clot at room temperature and then centrifuged at 1500 x g for 10 minutes. The supernatant was then transferred into a polypropylene tube. Biochemical parameters were analyzed using a chemical analyzer (Selectra ProS, ELITechGroup, Milsbeek Netherlands) (Figure 3.1) as per the manufacturer’s recommendation. The parameters analyzed were alanine amino transferase (ALT), aspartate amino transferase (AST), gamma glutamyl transferase (γ-GT), creatinine (CREAT), total cholesterol (T-CHOL), high density lipoproteins (HDL), blood urea nitrogen (BUN), magnesium (Mg^{2+}), calcium (Ca^{2+}) and potassium (K^{+}).
The reagents ALT/GPT 4 + 1 SL, AST/GOT 4 +1 SL, GAMMA GT SL were used to assay ALT, AST and γ-GT, respectively. Total cholesterol, HDL, BUN and CREAT were determined using CHOLESTEROL PAP SL, CHOLESTEROL HDL SL 2G, UREA UV SL and CREATININE PAP SL stable liquid reagents, respectively. Magnesium, calcium and potassium were determined by the use of ion selective electrodes. All reagents and the standards were supplied by ELITech Clinical Systems, Netherlands.

Figure 3.1: Selectra chemical analyzer system.
3.8 Histopathological examination

Liver and kidney were excised and fixed in 10% buffered formalin. The liver was washed in 70% alcohol while the kidney was washed in tap water. The tissues were then dehydrated in ascending grades of alcohol (35%, 50%, 70%, 95%, and 100%) (Alarami, 2015). They were then cleared in xylene and embedded in paraffin wax. The specimens were cut into sections of 5µm using a rotary microtome and samples transferred to a microscope slide. Specimens were stained with haematoxylin and eosin (Sangeeta, 2014). Histological analysis was performed under Bresser LCD Micro light microscope at X 10 (North Rhine-Westphalia, Germany)(Figure 3.2). The liver sections were examined for cytoplasmic vacuolization, inflammatory vacuolization, inflammatory infiltration, steatosis, hepatocellular necrosis and nuclear disintegration as outlined by Markiewicz-Gorka et al. (2011). The kidney sections were examined for cellular infiltration, tubular lumen widening, hemorrhage, tubular cast, tubular degeneration, glomerular shrinkage and glomerular degeneration as described by Alarami (2015).

3.9 Tissue analysis

The hind limb was disarticulated and femur cleaned of soft tissues using hydrogen peroxide (DePaula et al., 2005). Thigh muscle was also obtained from the hind limb. The tissues were then ashed overnight in a kiln at 500°C and dissolved in 10% nitric acid (Rayssigueir and Larvor, 1978). Calcium and magnesium levels in
the solution derived from the bone and muscle tissues were determined using flame atomic absorption spectrometry (Prescha et al., 2014). Standards for calcium and magnesium were prepared from calcium nitrate and magnesium nitrate hexahydrate, respectively, and a calibration curve was generated in each case.

![Bresser LCD Micro light microscope](image.jpg)

Figure 3.2: Bresser LCD Micro light microscope.

### 3.10 Data management and statistical analysis

Experimental data on body weight, haematological and biochemical parameters was obtained and results presented as means±standard error of mean. A one way analysis of variance (ANOVA) was performed to determine whether there were statistically significant differences among the ten experimental groups. This was
followed by Tukey’s *post hoc* test for multiple comparisons to compare individual groups and to determine which means differed significantly \( p < 0.05 \). All statistical analysis was performed using WINKS statistical data analysis (SDA) and graphs software (Elliot, 2013). Data was presented as text, tables and figures.
CHAPTER FOUR

RESULTS

4.1 Effects of alcohol on behavioural characteristics of rats

During the first week of the study the alcohol treated rats were less active; had difficulty in walking and went to sleep usually huddled together in one corner of the cage. The animals also had poor appetite and had a rough coat. From the second week onwards they began exhibiting less sluggishness and as time progressed they were seen to be more active after alcohol intake and could be seen feeding. The negative control rats on the other hand were alert and active, had a smooth coat and a good appetite throughout the study. Figure 4.1 shows the behavioral characteristics of the rats during treatment.

Figure 4.1: Behavioral characteristics of rats. A = Control rats (alert), B = Rats are subdued and huddled together after administration of alcohol.
4.2 Effects of treatments on body weight

For the entire period of the study animals were given standard feed and water *ad libitum*. The negative controls received no other treatment; positive controls were treated with alcohol for five days of the week while the rest received alcohol for five days plus different doses of Maalox plus® antacid and PureCal® calcium for the remaining two days of the week. The mean weight of the animals is presented in table 4.1 and it shows an increase of 109.6% for the negative control and 70.5% for the positive control. Rats that were treated with 4.25, 8.5 and 17 mg/kg magnesium had 75.4%, 89.3% and 84.5% increase in weight respectively. Those that were administered with doses of 4.25, 8.5 and 17 mg/kg calcium exhibited 55%, 45.4% and 51.5% increase of their body weight. Cotreatment with both magnesium and calcium at a ratio of 1:1 and 1:2 had an increase of 73% and 77.4% respectively.

Tukey’s post hoc test for multiple comparisons was carried out to determine whether there were significant differences in weight over the four week treatment period. For the negative control there was significant (*p* < 0.05) weight gain in week 1 but for alcohol treated group the weight change was insignificant (*p* > 0.05). From week 2 to 4 both groups recorded significant (*p* < 0.05) weight gain.

When the alcohol-fed rats were treated with Maalox plus®, which is rich in magnesium, or PureCal®, which is rich in calcium, there was insignificant (*p* > 0.05) weight change in the first two weeks. The group that received 8.5 mg/Kg
magnesium recorded significant \((p < 0.05)\) weight increase in week 4. When the dose was doubled significant \((p < 0.05)\) weight increase was recorded in both week 3 and 4. For calcium, there was no significant weight change over the 4 weeks period. When treatment was offered as a combined dose of both drugs there was a significant weight gain in week 4.
Table 4.1: Comparison of mean weight for rats over a four week period

<table>
<thead>
<tr>
<th></th>
<th>NC</th>
<th>PC</th>
<th>4.25 Mg</th>
<th>8.5 Mg</th>
<th>17 Mg</th>
<th>4.25 Ca</th>
<th>8.5 Ca</th>
<th>17 Ca</th>
<th>4.25 Mg + 4.25 Ca</th>
<th>4.25 Mg + 8.5 Ca</th>
</tr>
</thead>
<tbody>
<tr>
<td>Week 0</td>
<td>93.80±11.10</td>
<td>106.40±8.62*</td>
<td>57.51±69.14</td>
<td>84.00±21.87</td>
<td>111.40±20.06</td>
<td>114.20±43.97</td>
<td>120.80±40.62</td>
<td>118.40±44.84</td>
<td>112.80±30.87</td>
<td>110.40±33.20</td>
</tr>
<tr>
<td>Week 1</td>
<td>124.00±8.00</td>
<td>129.40±16.65</td>
<td>73.03±79.72</td>
<td>100.20±24.90</td>
<td>136.00±26.66</td>
<td>131.00±42.60</td>
<td>129.60±45.07</td>
<td>127.40±51.95</td>
<td>129.40±32.04</td>
<td>129.60±37.33</td>
</tr>
<tr>
<td>Week 2</td>
<td>150.80±12.81</td>
<td>145.80±16.72</td>
<td>81.26±91.27</td>
<td>107.60±27.37</td>
<td>146.40±35.22</td>
<td>146.00±35.92</td>
<td>143.00±51.11</td>
<td>138.00±52.98</td>
<td>144.20±40.44</td>
<td>151.80±39.24</td>
</tr>
<tr>
<td>Week 3</td>
<td>175.20±16.86*</td>
<td>162.00±19.97</td>
<td>90.99±100.43</td>
<td>132.60±25.74</td>
<td>176.00±30.85</td>
<td>158.60±31.41</td>
<td>152.80±45.87</td>
<td>141.00±48.23</td>
<td>170.60±45.00</td>
<td>174.60±39.36</td>
</tr>
<tr>
<td>Week 4</td>
<td>196.60±17.60*</td>
<td>181.40±20.38*</td>
<td>100.89±113.86*</td>
<td>159.00±29.44*</td>
<td>206.00±27.50</td>
<td>177.00±29.66</td>
<td>175.60±51.37</td>
<td>179.40±54.84</td>
<td>195.20±46.69</td>
<td>195.80±30.10</td>
</tr>
</tbody>
</table>

The p values for multiple comparison between week 1 and the subsequent three weeks together with week 0 (initial weight) *p < 0.05. NC=Negative Control; PC=Positive Control; 4.25 Mg=4.25 mg/Kg Magnesium; 8.5 Mg=8.5 mg/Kg Magnesium; 17 Mg=17 mg/Kg Magnesium; 4.25 Ca=4.25 mg/Kg Calcium; 8.5 Ca=8.5 mg/Kg Calcium; 17 Ca=17 mg/Kg Calcium; 4.25 Mg + 4.25 Ca=4.25 mg/Kg Magnesium + 4.25 mg/Kg Calcium; 4.25 Mg + 8.5 Ca=4.25 mg/Kg Magnesium + 8.5 mg/Kg Calcium
4.3 Effects of alcohol, Maalox plus® and PureCal® on hematological parameters

Alcohol at a dose of 5 g/kg body weight resulted in increased proliferation (p < 0.05) of neutrophils and eosinophils and the suppression (p < 0.05) of basophils (Table 4.2). Treatment with Maalox Plus® antacid and PureCal® calcium was ineffective in reversing the alcohol induced neutrophilia. On the contrary Maalox Plus® and PureCal®, either singly or in combination, were effective in normalizing the alcohol induced eosinophilia. Magnesium at a dose of 4.25 mg/kg and calcium at 4.25 mg/kg and 8.5 mg/kg also normalized the basophil levels. All other treatments had no influence on the alcohol induced basopenia.

On the other hand alcohol had no significant effects on the lymphocyte and monocyte levels (p > 0.05). Surprisingly, administration of Maalox Plus® and PureCal®, either individually or in combination, was found to cause lymphopenia (p < 0.05). For Maalox Plus® this effect was dose dependent but that for PureCal® it was dose independent.

Alcohol did not have significant effect on the mean corpuscular hemoglobin and the mean corpuscular hemoglobin concentration (p > 0.05). However, treatment with 8.5 mg/kg Mg and a combined dose of Mg and Ca in a ratio of 1:2 did significantly (p < 0.05) lower the mean corpuscular hemoglobin values. Alcohol was also found to suppress the platelet levels (p < 0.001) and treatments with
Maalox plus® and PureCal® did not influence the alcohol induced thrombocytopenia (Table 4.3).
Table 4.2: Comparison of hematological parameters

<table>
<thead>
<tr>
<th>Groups</th>
<th>WBC (10^3/µL)</th>
<th>NEUT (%)</th>
<th>LYMP (%)</th>
<th>MONO (%)</th>
<th>EOSI (%)</th>
<th>BASO (%)</th>
<th>RBC (10^3/µL)</th>
<th>MCV (fL)</th>
<th>MCH (pg)</th>
<th>MCHC (g/dL)</th>
<th>PLT (10^3/µL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative control</td>
<td>11.26 ± 4.07</td>
<td>13.28 ± 1.82</td>
<td>66.76 ± 2.98</td>
<td>5.46 ± 1.61</td>
<td>1.66 ± 0.68</td>
<td>12.84 ± 2.63</td>
<td>7.00 ± 1.13</td>
<td>68.62 ± 4.90</td>
<td>20.38 ± 1.54</td>
<td>29.72 ± 0.70</td>
<td>951.80 ± 91.97</td>
</tr>
<tr>
<td>Positive control</td>
<td>6.78 ± 1.56</td>
<td>37.14 ± 14.48*</td>
<td>49.72 ± 6.45</td>
<td>3.94 ± 4.26</td>
<td>3.80 ± 0.99*</td>
<td>5.40 ± 5.04*</td>
<td>3.87 ± 1.32*</td>
<td>78.86 ± 9.06*</td>
<td>20.58 ± 0.93</td>
<td>93.63 ± 3.63</td>
<td>610.40 ± 93.91*</td>
</tr>
<tr>
<td>Mg 4.25 mg/kg</td>
<td>7.04 ± 0.57</td>
<td>44.78 ± 1.60*</td>
<td>40.12 ± 2.40*</td>
<td>5.06 ± 2.06</td>
<td>2.32 ± 1.07</td>
<td>7.72 ± 1.22</td>
<td>5.87 ± 1.03</td>
<td>60.68 ± 3.58</td>
<td>18.14 ± 0.80</td>
<td>26.36 ± 0.79</td>
<td>721.40 ± 26.46*</td>
</tr>
<tr>
<td>Mg 8.5 mg/kg</td>
<td>12.88 ± 4.18</td>
<td>53.12 ± 15.91*</td>
<td>37.14 ± 13.40*</td>
<td>1.26 ± 0.86</td>
<td>2.40 ± 1.26</td>
<td>6.08 ± 4.81*</td>
<td>4.20 ± 1.11*</td>
<td>66.78 ± 2.81</td>
<td>17.50 ± 1.47*</td>
<td>26.16 ± 1.03</td>
<td>481.40 ± 94.37*</td>
</tr>
<tr>
<td>Mg 17 mg/kg</td>
<td>12.26 ± 2.84</td>
<td>68.18 ± 9.03*</td>
<td>23.18 ± 8.30*</td>
<td>1.50 ± 0.96</td>
<td>2.34 ± 1.23</td>
<td>4.80 ± 2.91*</td>
<td>4.95 ± 0.78</td>
<td>65.12 ± 1.87</td>
<td>18.52 ± 0.45</td>
<td>28.42 ± 1.14</td>
<td>535.40 ± 48.90*</td>
</tr>
<tr>
<td>Ca 4.25 mg/kg</td>
<td>8.46 ± 1.53</td>
<td>47.40 ± 11.61*</td>
<td>35.76 ± 7.57*</td>
<td>5.92 ± 3.28</td>
<td>3.18 ± 0.62</td>
<td>7.74 ± 2.52</td>
<td>5.15 ± 1.50</td>
<td>63.28 ± 4.94</td>
<td>18.56 ± 0.44</td>
<td>29.24 ± 2.38</td>
<td>719.00 ± 70.66*</td>
</tr>
<tr>
<td>Ca 8.5 mg/kg</td>
<td>8.74 ± 1.30</td>
<td>43.76 ± 7.36*</td>
<td>43.76 ± 7.36*</td>
<td>6.72 ± 3.93</td>
<td>3.18 ± 0.88</td>
<td>9.50 ± 2.03</td>
<td>6.20 ± 1.10</td>
<td>62.70 ± 3.60</td>
<td>18.70 ± 1.04</td>
<td>29.66 ± 1.07</td>
<td>762.20 ± 67.09*</td>
</tr>
<tr>
<td>Ca 17 mg/kg</td>
<td>8.46 ± 0.83</td>
<td>51.26 ± 9.21*</td>
<td>35.72 ± 10.86*</td>
<td>4.36 ± 3.21</td>
<td>2.80 ± 0.25</td>
<td>5.86 ± 1.71*</td>
<td>4.79 ± 0.48</td>
<td>63.66 ± 4.20</td>
<td>18.60 ± 1.63</td>
<td>28.94 ± 0.93</td>
<td>658.80 ± 52.97*</td>
</tr>
<tr>
<td>Mg + Ca (4.25)</td>
<td>12.64 ± 4.57</td>
<td>53.82 ± 9.60*</td>
<td>38.94 ± 7.48*</td>
<td>1.56 ± 1.15</td>
<td>1.88 ± 0.39</td>
<td>3.80 ± 1.28*</td>
<td>4.49 ± 1.14*</td>
<td>67.32 ± 2.96</td>
<td>18.28 ± 1.98</td>
<td>16.07 ± 2.08</td>
<td>467.60 ± 67.08*</td>
</tr>
<tr>
<td>Mg 4.25 + Ca 8.5</td>
<td>11.20 ± 2.84</td>
<td>56.02 ± 5.99*</td>
<td>34.08 ± 4.12*</td>
<td>1.14 ± 0.64</td>
<td>3.46 ± 1.07</td>
<td>5.30 ± 3.34*</td>
<td>5.95 ± 1.11</td>
<td>61.28 ± 1.03</td>
<td>17.30 ± 0.52*</td>
<td>28.44 ± 1.11</td>
<td>501.00 ± 83.11*</td>
</tr>
</tbody>
</table>

The values are expressed as mean ±SD for five animals per group. The statistical comparison was between the negative control and the other groups; *p < 0.05.

WBC=White Blood Cells; NEUT=Neutrophils; LYMP=Lymphocytes; MONO=Monocytes; EOSI=Eosinophils; BASO=Basophils; RBC=Red Blood Cells; MCV=Mean Corpuscular Volume; MCH=Mean Corpuscular Hemoglobin; MCHC=Mean Corpuscular Hemoglobin Concentration; PLT=Platelets; NC=Negative Control; PC=Positive Control; Mg = Magnesium; Ca = Calcium.
4.4 Effects of alcohol, Maalox plus® and PureCal® on biochemical profiles

The biochemical profiles of the experimental animals are presented in Table 4.3. Although alcohol treatment showed an elevation in the liver enzymes these changes were statistically insignificant ($p > 0.05$). When the AST to ALT ratios were computed the negative controls had a value of 1.47 and this increased to 1.60 in the alcohol treated group (Table 4.4). Maalox plus® and PureCal® treatments were found to lower these ratios and the lowest value of 1.11 was recorded in the group treated with 8.5 mg/kg of calcium.

Likewise animals that were treated with alcohol recorded an increase in the total cholesterol, high density lipoprotein and creatinine but the elevation was insignificant ($p > 0.05$) (Table 4.3). It was also observed that animals that were given alcohol followed by treatment with Maalox plus® and PureCal® had significantly low levels of total cholesterol (1.11-1.27 vs 1.92, $p < 0.050$) and high density lipoprotein (0.29-0.86 vs 1.77, $p < 0.050$) than those that were treated with alcohol alone. Alcohol did not significantly alter the blood urea nitrogen levels ($p > 0.05$). Interestingly, treatment with 8.5 mg/kg, 17 mg/kg magnesium, 4.25 mg/kg Ca and combined doses of Ca and Mg recorded significant reduction in serum urea levels ($p < 0.05$).

Alcohol treatment also did not influence serum levels of $\text{Mg}^{2+}$ ($p < 0.05$) but it significantly increased $\text{Ca}^{2+}$ and lowered $\text{K}^+$ levels ($p < 0.05$). Treatment with Maalox plus® and PureCal®, either singly or in combination, normalized the
alcohol induced hypercalcemia in a dose-dependent manner. On the other hand, Magnesium at 4.25 mg/kg and calcium at 17mg mg/kg normalized the alcohol induced hypokalemia but all other doses did not.

When serum Ca:Mg and Ca:K ratios were computed the untreated group had values of 1.56 and 0.03, respectively (Table 4.5). Alcohol treatment significantly ($p < 0.05$) raised the ratio threefold to 4.69 and 0.10, respectively. Maalox plus® and PureCal® were able to normalize the calcium to magnesium ratio. Maalox plus® and PureCal® also to normalized the calcium to potassium ratio except for the group that was co-treated with the two drugs in a ratio of 1:2.
Table 4.3: Comparison of biochemical parameters

<table>
<thead>
<tr>
<th>Groups</th>
<th>ALT (U/L)</th>
<th>AST (U/L)</th>
<th>GGT (U/L)</th>
<th>T CHOL (mmol/L)</th>
<th>HDL (mmol/L)</th>
<th>BUN (mmol/L)</th>
<th>CREAT (µmol/L)</th>
<th>Mg²⁺ (mmol/L)</th>
<th>Ca²⁺ (mmol/L)</th>
<th>K⁺ (mmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative control</td>
<td>115.00 ± 16.08</td>
<td>170.20 ± 52.02</td>
<td>4.49 ± 1.32</td>
<td>1.60 ± 0.30</td>
<td>1.02 ± 0.15</td>
<td>4.93 ± 0.69</td>
<td>55.28 ± 10.30</td>
<td>0.19 ± 0.06</td>
<td>0.26 ± 0.08</td>
<td>9.34 ± 2.04</td>
</tr>
<tr>
<td>Positive control</td>
<td>126.60 ± 22.74</td>
<td>200.40 ± 49.34</td>
<td>4.80 ± 0.65</td>
<td>1.92 ± 0.05</td>
<td>1.77 ± 0.02</td>
<td>4.63 ± 0.19</td>
<td>59.34 ± 5.69</td>
<td>0.13 ± 0.01</td>
<td>0.62±0.19*</td>
<td>6.70 ± 1.77*</td>
</tr>
<tr>
<td>4.25 mg/Kg Mg</td>
<td>117.40 ± 15.32</td>
<td>139.20 ± 7.33</td>
<td>2.54 ± 0.71</td>
<td>1.11 ± 0.21</td>
<td>0.54 ± 0.36</td>
<td>4.76 ± 0.66</td>
<td>56.15 ± 9.56</td>
<td>0.41 ± 0.58</td>
<td>0.24 ± 0.05</td>
<td>7.02 ± 0.43</td>
</tr>
<tr>
<td>8.5 mg/Kg Mg</td>
<td>129.40 ± 20.53</td>
<td>166.00 ± 13.08</td>
<td>5.00 ± 1.04</td>
<td>1.08 ± 0.33</td>
<td>0.57 ± 0.27</td>
<td>3.63 ± 0.36*</td>
<td>47.10 ± 4.89</td>
<td>0.28 ± 0.28</td>
<td>0.29 ± 0.05</td>
<td>6.20 ± 0.89*</td>
</tr>
<tr>
<td>17 mg/Kg Mg</td>
<td>115.80 ± 19.32</td>
<td>164.60 ± 22.10</td>
<td>3.58 ± 1.22</td>
<td>1.27 ± 0.22</td>
<td>0.84 ± 0.46</td>
<td>3.42±0.27*</td>
<td>54.88 ± 4.73</td>
<td>0.14 ± 0.04</td>
<td>0.34 ± 0.09</td>
<td>6.72 ± 1.08*</td>
</tr>
<tr>
<td>4.25 mg/Kg Ca</td>
<td>137.40 ± 15.96</td>
<td>174.60 ± 20.57</td>
<td>6.63 ± 1.47</td>
<td>1.19 ± 0.34</td>
<td>0.66 ± 0.47</td>
<td>3.02±0.39*</td>
<td>52.34 ± 5.13</td>
<td>0.16 ± 0.04</td>
<td>0.29 ± 0.02</td>
<td>6.82 ± 0.96*</td>
</tr>
<tr>
<td>8.5 mg/Kg Ca</td>
<td>146.80 ± 5.31</td>
<td>162.20 ± 3.90</td>
<td>5.52 ± 1.43</td>
<td>1.13 ± 0.33</td>
<td>0.53 ± 0.52</td>
<td>4.27 ± 0.27</td>
<td>52.84 ± 8.17</td>
<td>0.16 ± 0.04</td>
<td>0.28 ± 0.04</td>
<td>6.40 ± 0.23*</td>
</tr>
<tr>
<td>17 mg/Kg Ca</td>
<td>136.00 ± 16.63</td>
<td>167.00 ± 23.92</td>
<td>6.06 ± 4.06</td>
<td>1.17 ± 0.13</td>
<td>0.29 ± 0.25</td>
<td>5.25 ± 0.79</td>
<td>47.91 ± 10.31</td>
<td>0.20 ± 0.03</td>
<td>0.28 ± 0.06</td>
<td>7.78 ± 0.98</td>
</tr>
<tr>
<td>4.25 Mg + 4.25 Ca</td>
<td>112.40 ± 19.39</td>
<td>149.40 ± 14.54</td>
<td>3.52 ± 1.03</td>
<td>1.14 ± 0.22</td>
<td>0.86 ± 0.41</td>
<td>3.12±0.04*</td>
<td>54.28 ± 3.08</td>
<td>0.27 ± 0.04</td>
<td>0.29 ± 0.04</td>
<td>5.76±0.65*</td>
</tr>
<tr>
<td>4.25 Mg + 8.5 Ca</td>
<td>107.60 ± 11.19</td>
<td>138.60 ± 5.68</td>
<td>3.58 ± 1.24</td>
<td>1.49 ± 0.29</td>
<td>1.18 ± 0.42</td>
<td>3.51±0.27*</td>
<td>56.70 ± 4.68</td>
<td>0.17 ± 0.02</td>
<td>0.42 ± 0.14</td>
<td>5.88±0.88*</td>
</tr>
</tbody>
</table>

The values are expressed as mean ±SD for five animals per group. The statistical comparison was between the negative control and the other groups; *p < 0.05.
ALT = Alanine aminotransferase; AST = Aspartate aminotransferase; GGT = Gamma glutamyl transferase; T CHOL = Total cholesterol; HDL = High density lipoproteins
BUN = Blood Urea Nitrogen; CREAT = Creatinine; Mg²⁺ = Magnesium; Ca²⁺ = Calcium; K⁺ = Potassium; NC = Negative control; PC = Positive control; Mg = Magnesium; Ca = Calcium.
Table 4.4: Aspartate amino transferase to alanine amino transferase ratio

<table>
<thead>
<tr>
<th>Groups</th>
<th>AST: ALT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative control</td>
<td>1.47±0.35</td>
</tr>
<tr>
<td>Positive control</td>
<td>1.60±0.39</td>
</tr>
<tr>
<td>4.25 mg/Kg Mg</td>
<td>1.20±0.18</td>
</tr>
<tr>
<td>8.5 mg/Kg Mg</td>
<td>1.30±0.15</td>
</tr>
<tr>
<td>17 mg/Kg Mg</td>
<td>1.44±0.27</td>
</tr>
<tr>
<td>4.25 mg/Kg Ca</td>
<td>1.30±0.30</td>
</tr>
<tr>
<td>8.5 mg/Kg Ca</td>
<td>1.11±0.07</td>
</tr>
<tr>
<td>17 mg/Kg Ca</td>
<td>1.23±0.16</td>
</tr>
<tr>
<td>4.25 mg/Kg Mg + 4.25 mg/Kg Ca</td>
<td>1.34±0.13</td>
</tr>
<tr>
<td>4.25 mg/Kg Mg + 8.5 mg/Kg Ca</td>
<td>1.30±0.10</td>
</tr>
</tbody>
</table>

ALT = Alanine aminotransferase; AST = Aspartate aminotransferase
Mg = Magnesium; Ca = Calcium.
4.5 Calcium and magnesium levels in muscle and femur of rats

As expected magnesium and calcium levels were higher in bone than muscle. In the untreated group the calcium levels were 17 times higher while magnesium was 1.6 times higher in bone compared to the muscle tissue (Table 4.6). Following alcohol treatment calcium was 200 times higher in bone than muscle while magnesium was 1.6 times.

Administration of alcohol in rats was found to lower the level of minerals in tissues but this was only significant ($p < 0.05$) for magnesium in bone (Table 4.6). Treatment of the rats with Maalox plus® or PureCal® reversed the alcohol induced hypomagnesaemia in bone.
Table 4.5: Comparison of magnesium and calcium levels in tissues

<table>
<thead>
<tr>
<th></th>
<th>Muscles</th>
<th>Bones</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Magnesium (ppm)</td>
<td>Calcium (ppm)</td>
</tr>
<tr>
<td>Negative control</td>
<td>5.70±1.35</td>
<td>8.88±4.65</td>
</tr>
<tr>
<td>Positive control</td>
<td>4.10±1.41</td>
<td>1.13±5.56</td>
</tr>
<tr>
<td>4.25 mg/kg Mg</td>
<td>5.46±0.53</td>
<td>13.49±3.48</td>
</tr>
<tr>
<td>8.5 mg/Kg Mg</td>
<td>5.04±1.17</td>
<td>13.46±7.58</td>
</tr>
<tr>
<td>17 mg/Kg Mg</td>
<td>5.85±0.60</td>
<td>13.29±3.99</td>
</tr>
<tr>
<td>4.25 mg/Kg Ca</td>
<td>4.37±1.76</td>
<td>9.29±4.96</td>
</tr>
<tr>
<td>8.5 mg/Kg Ca</td>
<td>6.03±0.91</td>
<td>10.46±5.30</td>
</tr>
<tr>
<td>17 mg/Kg Ca</td>
<td>5.05±1.32</td>
<td>10.79±4.89</td>
</tr>
<tr>
<td>4.25 Mg+4.25 Ca</td>
<td>5.41±0.76</td>
<td>14.21±5.34</td>
</tr>
<tr>
<td>4.25 Mg+8.5 Ca</td>
<td>7.13±0.33</td>
<td>15.29±4.09</td>
</tr>
</tbody>
</table>

The statistical comparison was between the negative control and the other groups; *p < 0.05.
Mg = Magnesium; Ca = Calcium.
4.6 Effects of alcohol, Maalox plus® and PureCal® on histopathology of the liver tissues

The architecture of the liver tissue of untreated rats is shown in Figure 4.2. Alcohol administration resulted in only mild tissue pathology. The pathology displayed by the animals exclusively treated with alcohol was the infiltration of inflammatory mononuclear and single neutrophils cells; and cytoplasmic vacuolization (Figure 4.2; Table 4.7). Liver tissues of the experimental group that received magnesium and calcium in a 1:2 ratio were free of this pathology. Those that received magnesium at 8.5 mg/kg did not show cytoplasmic vacuolization but had infiltration of inflammatory cells. While those that received calcium at 4.25 mg/kg and 17 mg/kg and those that were given combined doses of magnesium and calcium in a 1:1 ratio were devoid of cellular infiltration.

The liver tissues of the animals that were fed alcohol did not show any signs of fat infiltration in the liver cells. However, the animals treated with 4.25 and 8.5 mg/kg of calcium had mild to moderate steatosis with evidence of cellular infiltration.
Table 4.6: Histological changes of liver tissue

<table>
<thead>
<tr>
<th>Groups</th>
<th>Cytoplasmic vacuolization</th>
<th>Infiltration of Inflammatory cells</th>
<th>Mild to moderate steatosis</th>
<th>Focal hepatocellular necrosis (sporadic)</th>
<th>Nuclear disintegration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative control</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Positive control</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>4.25 Mg</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>8.5 Mg</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>17 Mg</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>4.25 Ca</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>8.5 Ca</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>17 Ca</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>4.25Mg+4.25Ca</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>4.25Mg+8.5Ca</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

4.25 Mg = 4.25 mg/Kg Magnesium; 8.5 Mg = 8.5 mg/Kg Magnesium; 17 Mg = 17 mg/Kg Magnesium; 4.25 Ca = 4.25 mg/Kg Calcium; 8.5 Ca = 8.5 mg/Kg Calcium; 17 Ca = 17 mg/Kg Calcium; 4.25 Mg + Ca = 4.25 mg/Kg Magnesium + 4.25 mg/Kg Calcium; 4.25 Mg + 8.5 Ca = 4.25 mg/Kg Magnesium + 8.5 mg/Kg Calcium.
continued next page
Figure 4.2: Histopathology of the liver of rats: A = Control; B = Positive Control, C = 4.25 mg/Kg Magnesium; D = 8.5 mg/kg Magnesium; E = 17 mg/Kg Magnesium; F = 4.25 mg/Kg Calcium; G = 8.5 mg/Kg Calcium; H = 17 mg/Kg Calcium; I = 4.25 mg/Kg Magnesium + 4.25 mg/Kg Calcium; J = 4.25 mg/Kg Magnesium + 8.5 mg/Kg Calcium. CV = Cytoplasmic vacuolization; INF = Infiltration; S = Steatosis. Magnification ×40.
4.7 Effects of alcohol, Maalox plus® and PureCal® on histopathology of the kidney tissues

The kidney tubules of the negative control exhibited normal histological features. While the alcohol treated group showed mild tissue pathology. The pathology displayed by the animals treated with alcohol only was cellular infiltration and widening of the tubular lumen (Figure 4.3).

Treatment with Maalox plus® and PureCal® was found to have no influence on cellular infiltration or the widening of the lumen of the kidney tubules. Magnesium treatment was associated with hemorrhage and so was calcium at 8.5 mg/kg and 17 mg/kg. Calcium at 17 mg/kg was also associated with tubular cast formation and tubular degeneration. No pathology was associated with the glomerulus.
Table 4.7: Histological changes of kidney tissue

<table>
<thead>
<tr>
<th></th>
<th>Cellular infiltration</th>
<th>Tubular widening lumen</th>
<th>Hemorrhage</th>
<th>Tubular cast</th>
<th>Tubular degeneration</th>
<th>Glomerular shrinkage</th>
<th>Glomerular degeneration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative control</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Positive control</td>
<td>+</td>
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<td>–</td>
<td>–</td>
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</tr>
<tr>
<td>4.25 Mg</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
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<td>–</td>
</tr>
<tr>
<td>8.5 Mg</td>
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<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>17 Mg</td>
<td>+</td>
<td>+</td>
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<td>–</td>
<td>–</td>
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<td>–</td>
</tr>
<tr>
<td>4.25 Ca</td>
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<td>+</td>
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<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>8.5 Ca</td>
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<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
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<tr>
<td>17 Ca</td>
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<td>+</td>
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<td>–</td>
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<td>–</td>
</tr>
<tr>
<td>4.25 Mg + 8.5 Ca</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

4.25 Mg = 4.25 mg/Kg Magnesium; 8.5 Mg = 8.5 mg/Kg Magnesium; 17 Mg = 17 mg/Kg Magnesium; 4.25 Ca = 4.25 mg/Kg Calcium; 8.5 Ca = 8.5 mg/Kg Calcium; 17 Ca = 17 mg/Kg Calcium; 4.25 Mg + Ca = 4.25 mg/Kg Magnesium + 4.25 mg/Kg Calcium; 4.25 Mg + 8.5 Ca = 4.25 mg/Kg Magnesium + 8.5 mg/Kg Calcium.
Figure 4.3: Histopathology of the kidney of rats. A = Control; B = Positive Control; C = 4.25 mg/Kg Magnesium; D = 8.5 mg/Kg Magnesium; E = 17 mg/Kg Magnesium; F = 4.25 mg/Kg Calcium; G = 8.5 mg/Kg Calcium; H = 17 mg/Kg Calcium; I = 4.25 mg/Kg Magnesium + 4.25 mg/Kg Calcium; J = 4.25 mg/Kg Magnesium + 8.5 mg/Kg Calcium; TWL = Tubular widening lumen; INF = Infiltration; HE = Hemorrhage; TD = Tubular degeneration; TC = Tubular cast. Magnification (×40).
5.1 Discussion

In the first week of the study animals that were treated with alcohol were lethargic, had a rough coat, poor appetite and decreased weight gain compared with the negative control group. However, from the second week onwards they were more active, had good appetite and recorded significant weight gain. Studies on the effect of alcohol on body weight from previous researches showed mixed results. In their study, Macdonald et al. (2010) attributed the significant decrease in body weight gain in rats to alcohol altering the storage, excretion and inhibiting breakdown of nutrients. Ning et al. (2012) observed that excessive alcohol intake led to rats loosing appetite, being apathetic and having slow responses. Other studies opine to alcohol resulting in positive energy balance and cause weight gain (Arif and Rohrer, 2005; Aguiar et al., 2009; Wakabayashi, 2009; Yeomans, 2010). Traversy and Chaput (2015) linked the contradictory evidence on alcohol and body weight to a myriad of factors including gender, genetics, physical activity level, medication use, psychological problems, type, frequency and amount of alcohol intake.

In this study the contradictory effects of alcohol on body weight of rats was demonstrated whereby in week one there was a decrease in weight and this was most likely due to the reduced feed intake and the resultant malnutrition, while
from week two onwards the animals developed tolerance to alcohol and the consequent weight gain was attributed to the calorific effects of ethanol.

Animals that were treated with PureCal® calcium had insignificant ($p > 0.05$) weight change over four weeks period. These results are consistent with those of Zhang and Tordoff (2004) who reported that calcium supplementation had no notable effect on body weight and similar findings have been reported by Reid et al. (2015). On the contrary Zemel et al. (2000) reported a dose dependent diminutive effect of calcium on body weight in mice; this was attributed to a decline in the adipocyte fatty acid synthase activity and an elevation in lipolysis. Calcium supplementation in this study did not seem to have any effect on the synthesis or lysis of adipose tissue hence the insignificant change in body weight of the rats.

The effect of Maalox plus® on body weight was inconclusive. Previous studies have associated magnesium deficiency with reduction in body weight (Orden et al., 2006; Ige et al., 2016). This is because of the central role magnesium plays in growth and development (Shenkin, 2006) especially in the process of cell proliferation and protein synthesis (Bertinato et al., 2016).

Alcohol treatment resulted in increased proliferation ($p < 0.05$) of neutrophils and eosinophils and suppression ($p < 0.05$) of basophils. Neutrophilia is associated with alcoholic hepatitis and it occurs as a result of inappropriate activation and
homing of neutrophils to the microvasculature (Okubo et al., 2006; Xu et al., 2014). In this study the biomarkers of liver function were normal so liver disease was ruled out as the cause of neutrophilia. It is however known that low magnesium levels have been associated with inflammatory response that leads to leukocytosis especially eosinophilia and neutrophilia (Malpuech-Brugere et al., 2000; Tam et al., 2003).

Maalox Plus® antacid and PureCal® had no effect on neutrophilia but were able to normalize eosinophilia while Maalox Plus® normalized basopenia. Eosinophilia plays an important role in destroying invading parasitic pathogens. However, a marked increase is undesirable as it is implicated with various disorders such as allergies and asthma (Wardlaw et al., 2000). This data suggests that the two drugs could be used to treat alcohol induced eosinophilia. In addition they could be used for managing idiopathic eosinophilia that is currently treated using corticosteroids which are known to cause multiple side effects including habituation (Minakuchi et al., 2003). On the other hand Maalox Plus® could have applications in managing basopenia especially one that is associated with acute hypersensitivity reactions.

Alcohol was found to significantly increase the mean corpuscular volume and decrease the total number of red blood cells. This observation is in agreement with several studies that have linked common alcohol use with macrocytosis which may or may not be associated with anaemia (Tefferi et al., 2005; Kaferle
and Strzoda, 2009; Stouten et al., 2016). Macrocytosis and anaemia occurs as a result of the direct damage of alcohol to erythroid precursors in the bone marrow (Das and Vasudevan, 2005). The present findings show that MCV is a sensitive marker for detecting excessive alcohol consumption thus supporting its use as part of the screening procedure for detecting alcohol abuse (Kaferle and Strzoda, 2009; Das and Arya, 2014).

Maalox Plus® antacid and PureCal® were generally effective in reversing the alcohol induced macrocytic anaemia. A review of the literature does not offer direct evidence on the role of calcium and magnesium in alleviation of the condition. However, both micronutrients are known to play a key regulatory role in various cellular processes. Soetan et al. (2010) noted that one of the biochemical functions of calcium in human and animals is its requirement for membrane permeability. Rinehart et al. (2010) made similar observations but also implicated low intracellular magnesium in the regulation of potassium chloride cotransport. It is thus evident that both magnesium and calcium play a role in the integrity of the RBC membrane and this could explain their therapeutic effect towards the macrocytic anaemia encountered in this study.

Treatment with Maalox Plus® and PureCal®, either individually or in combination, was found to cause lymphopenia in rats and this was considered a side effect. This finding concurs with the observation of Orden (2006) who found that supplementation with magnesium alleviated lymphocytosis in rats. The same
study also noted the importance of distinguishing the proportion or percentage of the varied subsets of lymphocytes which include T and B cells. Son et al. (2007) were also of the opinion that magnesium has the potential of suppressing B cell proliferation and consequently a reduction in antibody production. The lymphopenia observed in this study suggests that the synthesis of a particular subset might have been decreased or catabolism decreased. It is impossible to discern which among the subsets was affected since the level of lymphocytes is reported as a whole.

Alcohol given at 5 g/kg for 5 days a week for 4 weeks did not significantly affect the levels of ALT, AST, GGT, T-CHOL, HDL, BUN creatinine and magnesium. There are many reports showing that alcohol has significant toxic effects on blood biochemistry and liver morphology (Minemura et al., 2009; Jang et al., 2012; Borole et al., 2012; Saalu et al., 2012; Deshpande et al., 2014). Equally there are numerous studies that have reported contrary views (Aguiar et al., 2009; Broulik et al., 2010). There are various factors that can contribute to these contrasting differences including duration of alcohol consumption and dosage (Jang et al., 2012; Borole et al., 2012; Deshpande et al., 2014; Hassan et al., 2015).

The adverse effects of short term (binge and sporadic drinking, also termed as acute intoxication) and long-term (chronic abuse) are well documented in the review by D’ Souza (2010). Both acute and chronic excessive alcohol consumption deliver unique pathological consequences with biochemical markers
being more pronounced following chronic abuse. This is in agreement with the current study where biochemical markers were not elevated in this acute model that involved giving 5 g/kg of alcohol for 5 days followed by 2 days of abstinence over a four week period. Although there is no universal definition as to what constitutes acute or chronic treatment in laboratory models the findings of the present study seem to support the conclusion by D’ Souza et al. (2010) that acute treatment would constitute feeding of alcohol for a period of four weeks or less.

Ethanol is known to increase the activity of acetaldehyde dehydrogenase and increases concentration of nicotinamide adenine dinucleotide thus affecting fatty acid β oxidation and the tricarboxylic acid cycle consequently elevating synthesis of triacylglycerol and ultimately liver steatosis (Lieber, 2005). This explains the observation by Chen et al. (2012) of 10 g/day alcohol consumption leading to high triglyceride levels. They also noted that a dosage greater than 50 g/day minimizes development of low level of HDL while maximizing attainment of high levels of cholesterol. Just like in our findings, Borole et al. (2012) did not observe any significant change in serum cholesterol though there was significant depreciation in high density lipoprotein levels in ethanol fed groups. The findings of the present study in conjunction with the observations by Chen et al. (2012) and Borole et al. (2012) suggest that alcohol doses above 5 g/kg are required to observe an effect on triglyceride levels.
On the other hand alcohol caused significant \( p < 0.05 \) increase in serum calcium and a decrease in potassium levels. Published reports show diverse findings on the effect of alcohol on serum calcium and potassium levels with results showing increase, decrease and no effect (Ljunghall et al., 1985; Shaper et al., 1985; Altura et al., 1987; Money et al., 1990; Oduola et al., 2005; Kwano, 2010). The fate of potassium and calcium in the body is controlled by magnesium (Sacks et al., 1995). In the present study alcohol treated rats had lower magnesium than the negative control. Rude et al. (2003) cite magnesium deficiency as a causative factor of increased serum calcium levels due to increased intestinal absorption. Elisaf and Kalaitzidis (2015) attribute the hypokalemia seen in alcoholics to the increase in excretion of non reabsorbable bicarbonates that induces high potassium excretion through urine.

Maalox Plus\textsuperscript{®} and PureCal\textsuperscript{®} were effective in normalizing calcium levels but were ineffective in correcting the alcohol induced hypokalemia. Dørup et al. (1993) suggested that oral supplementation with magnesium for a period of at least 6 months is required for complete normalization of potassium levels. This length of time could explain the reason why the oral magnesium supplementation did not yield a correction of the potassium levels.

Blood urea nitrogen is a kidney degeneration biomarker that is indicative of oxidative stress leading to kidney injury (Kadkhodaee et al., 2012). Maalox Plus\textsuperscript{®} and PureCal\textsuperscript{®} were found to significantly reduce the BUN levels and similar
findings have been reported by Parvizi et al. (2014). It was hypothesized that micronutrients are able to counteract the effect of the free radicals in a dose dependent manner by significantly elevating the superoxide dismutase levels (Ali et al., 2002). Magnesium and calcium therefore have the capacity to alleviate oxidative stress hence the observed reduction of BUN upon supplementation.

Alcohol caused significant reduction in the magnesium levels in bones but it had no effects on magnesium levels in muscle and calcium levels in both tissues. Approximately half of total body magnesium is located in soft tissues intracellularly, with the rest found in bones as surface bound divalent cations that are exchangeable or in the hydroxyapatite. The exchangeable divalent surface bound magnesium cations on the bones act as reservoir for maintenance of extracellular magnesium levels (Dimai et al., 1998). Magnesium deficiency is a feature found mostly in alcoholics and even in cases where the serum magnesium is normal marked intracellular deficiency is possible (Poikolainen et al., 2008). In the current study the serum magnesium levels are not affected in the alcohol ingesting subjects. The significant reduction in the bone magnesium is thus due to the loss of the surface bound magnesium cations due to use in maintenance of serum magnesium levels. The loss of magnesium in bone was effectively reversed through supplementation.

Alcohol is a potent liver toxicant and the severity of the disorder is highly influenced by the concentration of ethanol consumed (Hassan et al., 2015). In the
present study alcohol leads to cellular infiltration and cytoplasmic vacuolization in the liver. Ramaiah and Jaeschke (2007) have attributed infiltration of neutrophils to the apoptosis of hepatocytes, inflammatory mediators, chemokines, cytokines and adhesion molecules. In a study conducted by Markiewicz-Górka et al. (2011) similar results were reported with antioxidant deficiency as a result of alcohol induced malnutrition being reported to be the cause of damage.

Treatment with Maalox plus® and PureCal® in a ratio of 1:2 was found to protect the animals from liver pathology. Potential therapeutic effect of magnesium on the liver damaged due to alcohol toxicity has been documented (Poikolainen and Alho, 2008). Markiewicz-Górka et al. (2011) points out that magnesium enhances the oxidative defense by increasing the activity of antioxidant enzymes thereby strengthening the host’s defense by intensifying the anti-oxidative process. Eshraghi et al. (2015) is in agreement with these findings when they deduced that MgSO₄ combats inflammatory responses and oxidative damage that are experienced when suffering from cholestasis liver injury. While using rabbit subjects Liang et al. (2012) was able to give an insight to the ameliorative properties of calcium on hepatotoxicity induced by fluoride. Just like with magnesium, in their study calcium was able to increase the activity of superoxide dismutase, glutathione peroxidase and brought about ultrastructural repair of the liver. It is thus evident that both elements have a curative effect on liver pathophysiology and that it was the 1:2 ratio of Mg:Ca that turned out to be more effective in repairing the liver architecture.
Alcohol was responsible for mild pathology on the kidney in form of cellular infiltration and widening of the tubular lumen. This corroborates the assertion by Das et al. (2008) that the essence of functional disturbances of the kidney after ethanol exposure is down to the ultrastructural abnormalities experienced. Hassan et al. (2015) attribute the kidney degeneration to alcohol leading to a surge in protein oxidation and acetaldehyde oxidation, causing an increase in reactive oxygen species which are responsible for oxidative stress. Alcohol is known to cause oxidative stress that triggers an inflammatory response that causes cellular infiltration as was observed in the kidney tissue. Treatment with Maalox plus® and PureCal® was found to have no influence on the cellular infiltration nor the widening of the lumen of the kidney tubules.

5.2 Conclusions

i) Alcohol caused neutrophilia, eosinophilia, macrocytosis, anaemia, basopenia and thrombocytopenia. Maalox plus® and PureCal® were able to normalize the eosinophilia and macrocytic anaemia that was experienced by the alcoholic subjects.

ii) Alcohol was found not to affect most of the biochemical parameters but it disrupted the electrolyte balance by significantly increasing serum calcium levels and reducing the serum potassium. Oral supplementation with both Maalox plus® and PureCal® normalized the serum calcium with
the former drug at a dose of 4.25 mg/kg being able to normalize the serum potassium levels.

iii) Findings from tissue analysis showed that alcohol did significantly reduce the bone magnesium levels but it did not affect the bone calcium and the muscle magnesium and calcium levels. Both regimens of Maalox plus® and PureCal® exemplified their therapeutic effect by normalizing the levels of bone magnesium.

iv) Liver tissues from the alcoholic subjects where characterized with cellular infiltration and vacuolization. On the other hand the kidney tissues from the same subjects also displayed cellular infiltration accompanied by widening of the tubular lumen. Though Maalox plus® and PureCal® were not able to alleviate the conditions observed in the kidneys, when administered in a ratio of 1:2 they proved to be hepatoprotective. Administration of PureCal® singly was detrimental as it resulted in steatosis.

5.3 Recommendations

5.3.1 Recommendations from the study

i) Alcohol at 5 g/kg given for 5 days a week for 28 days is an appropriate model for studying acute alcohol intoxication in rats.
ii) The consequent adverse effects caused by the acute alcohol intoxication can be managed through supplementation with both Maalox plus® and PureCal® given at a ratio of 1:2.

5.3.2 Recommendations for further research

i) Determine the appropriate dosage of calcium that does not have adverse effects on the liver and kidney and would improve the level of magnesium in bones.

ii) To discern which subsets of lymphocytes are affected by the calcium and magnesium micronutrients during the Maalox plus® antacid and PureCal® calcium induced lymphopenia.
REFERENCES


Parsons C.G. Danysz W. and Quack G. (1999). Memantine is a clinically well tolerated N-methyl-D-aspartate (NMDA) receptor antagonist—a review of preclinical data. *Neuropharmacology* 38:735.


APPENDICES

Appendix I: Haematological and biochemical charts

Note: n=5, p<0.05, a = Negative Control, b = Positive Control, c = Magnesium 4.25 mg/Kg, d = Magnesium 8.5 mg/Kg, e = Magnesium 17mg/Kg, f = Calcium 4.25 mg/Kg, g = Calcium 8.5 mg/Kg, h = Calcium 17mg/Kg, i = Magnesium 4.25 mg/Kg + Calcium 4.25 mg/Kg, j = Magnesium 4.25 mg/Kg + Calcium 8.5 mg/Kg.
BUN (mmol/L)

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CREAT (μmol/L)

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Appendix II: NACOSTI research authorization

NATIONAL COMMISSION FOR SCIENCE, TECHNOLOGY AND INNOVATION

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Ref. No.

NACOSTI/P/16/67994/12574

9th February, 2017

Tonny Omondi Onyango
Kenyatta University
P.O. Box 43844-00100
NAIROBI.

RE: RESEARCH AUTHORIZATION

Following your application for authority to carry out research on “Effects of malaolx plus antacid and purecal calcium supplements on liver and kidney function following heavy intake of alcohol in rats,” I am pleased to inform you that you have been authorized to undertake research in Nairobi County for the period ending 26th August, 2017.

You are advised to report to the County Commissioner and the County Director of Education, Nairobi 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.

BONIFACE WANYAMA
FOR: DIRECTOR-GENERAL/CEO

Copy to:

The County Commissioner
Nairobi County.

The County Directors of Education
Nairobi County.