EFFECTS OF PARACETAMOL ON THE LIVER AND KIDNEY FUNCTIONS OF A RAT MODEL FOLLOWING PROLONGED ALCOHOL ADMINISTRATION

OLOO QUENTER PENDO (BSc. Biochemistry)
I56/CTY/PT/28573/2013

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

APRIL, 2021

DECLARATION

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

Oloo Quenter Pendo (BSc Biochemistry)
I56/CTY/PT/28573/2013

Department of Biochemistry, Microbiology and Biotechnology

Signature..Date...

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

Dr. David Mburu

Department of Biochemistry, Microbiology and Biotechnology
Kenyatta University

Signature..Date...

Dr. Mathew Ngugi

Department of Biochemistry, Microbiology and Biotechnology,
Kenyatta University

Signature..Date...

DEDICATION

I dedicate this work to my family and friends who supported me.

ACKNOWLEDGMENTS

I would like to acknowledge my supervisors Dr. David Mburu and Dr. Mathew Ngugi for their consistent guidance, advice and the support they offered me throughout the project.

My special thanks to Mr. Kelvin Juma and Mr. Eliakim Mbaka, Department of Biochemistry, Microbiology and Biotechnology, Kenyatta University, for their moral and technical support.

I also thank Mr. Francis Wanyama, Department of Human pathology, University of Nairobi; Mr. Joseph Kihara, Department of Human Anatomy, Kenyatta University: Mr. Daniel Gitonga, and Mr. James Ngunjiri, Department of Biochemistry, Microbiology and Biotechnology, Kenyatta University; for their continued technical support.

Lastly, I would like to thank Kenyatta University for the opportunity to pursue my course in the Department of Biochemistry, Microbiology and Biotechnology, School of Pure and Applied Sciences,

TABLE OF CONTENTS

DECLARATION .... **II**

DEDICATION .... **III**

ACKNOWLEDGMENT .... **IV**

TABLE OF CONTENTS .... **V**

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 information 1

1.2 Problem of statement and justification 3

1.3 Research questions 4

1.4 Hypothesis 4

1.5 Objectives 4

1.5.1 General objective 4

1.5.2 Specific objectives 5

CHAPTER TWO **6**

LITERATURE REVIEW 6

2.1 Alcohol 6

2.1.1 Overview 6

2.1.2 Physical and chemical properties 6

2.1.3 Pharmacokinetic properties 7

2.1.3.1 Absorption and distribution 7

2.1.3.2 Alcohol metabolism and elimination 8

2.1.4 Pharmacological properties 9

2.1.5 Mechanisms of action 10

2.1.6 Adverse effects 10

2.1.6.1 Overall effects 10

2.1.6.2 Hepatotoxicity 11

2.1.6.3 Nephrotoxicity 12
2.1.6.4 Haematotoxicity 13
2.1.6.5 Effects on blood glucose levels 14
2.1.6.6 Effects on body weight 14
2.1.6.7 Effects of alcohol on fertility and fetal development…………..15
2.1.7 Treatment of alcohol toxicity 16
2.2 Paracetamol...17
2.2.1 Overview 17
2.2.2 Physical and chemical properties...18
2.2.3 Pharmacokinetic properties 18
2.2.3.1 Absorption 18
2.2.3.2 Distribution 18
2.2.3.3 Metabolism 19
2.2.3.4 Elimination..19
2.2.4 Pharmacological properties...20
2.2.5 Mechanism of action...20
2.2.6 Indications 20
2.2.7 Adverse effects 21
2.2.7.1 Overall effects 21
2.2.7.2 Hepatotoxicity 21
2.2.7.3 Nephrotoxicity 24
2.2.7.4 Haematotoxicity 26
2.2.7.5 Effect on blood sugar...26
2.2.7.6 Effect on body weight 27
2.2.8 Treatment of toxicity...27
2.3 Combination of alcohol and paracetamol on liver and kidney damage 28

CHAPTER THREE 30

MATERIALS AND METHODS 30
3.1 Laboratory animals...30
3.2 Chemicals..30
3.3 Preparation of working concentrations of alcohol 31
3.4 Preparation of working concentrations of paracetamol..................31
3.5 Experimental design 31
3.6 Physical parameters 33
3.7 Blood collection and analysis...33
3.7.1 Hematological analysis...33

3.7.2 Blood glucose levels 34
3.7.3 Biochemical analysis 34
3.8 Histopathological analysis 35
3.9 Statistical analysis 35

CHAPTER FOUR 36

RESULTS 36
4.1 Morphological characteristics of rats subjected to alcohol and paracetamol treatment 36
4.2 Effects of alcohol and paracetamol on body weight of rats 37
4.3 Effect of alcohol and paracetamol on hematological parameters of rats 38
4.4 Effects of alcohol and paracetamol on biochemical parameters 43
4.4.1 Liver function 43
4.4.2 Kidney function 47
4.4.3 Blood sugar levels 48
4.5 Effects of paracetamol and alcohol on rat tissues 49
4.5.1 Liver histopathology 49
4.5.2 Kidney histopathology 49

CHAPTER FIVE 56

DISCUSSION, CONCLUSIONS, RECOMMENDATIONS AND FUTURE RESEARCH 56
5.1 Discussion 56
5.2 Conclusions 62
5.3 Recommendation 62
5.4 Future Research 63

REFERENCES 64

APPENDIX I: APPROVAL OF RESEARCH PROPOSAL 86
APPENDIX II: RESEARCH AUTHORIZATION 87

LIST OF TABLES

Table 3.1: Details of the treatment regimens……………………………….…32

Table 4.1: Effects of alcohol and paracetamol on the leukocyte profiles of rats ………………………………………………………..40

Table 4.2: Effects of alcohol and paracetamol on the erythrocytic parameters of rats……………………………………………..41

Table 4.3: Platelet parameters of rats following alcohol and paracetamol Administration……………………………………………….....42

Table 4.4: Liver function tests of rats following administration of alcohol and paracetamol………………………………………………...45

Table 4.5: Effects of alcohol and paracetamol on urea and creatinine levels in rats……………………………………………….....47

Table 4.6: Histological changes on liver of rats following alcohol and paracetamol administration………………………………………50

Table 4.7: Histological changes on kidney following alcohol and paracetamol administration…………………………………....53

LIST OF FIGURES

Figure 4.1: Morphological characteristics of rats subjected to alcohol and paracetamol administration...36

Figure 4.2: Changes in body weight of rats following administration of alcohol and paracetamol..39

Figure 4.3: Effects of alcohol and paracetamol on the ratio of aspartate amino transferase to alanine amino transferase......................46

Figure 4.4: Glucose level in rats subjected to alcohol and paracetamol administration...48

Figure 4.5: Liver photomicrographs showing the morphological changes induced by alcohol and paracetamol........................51

Figure 4.6: Kidney photomicrographs for the morphological changes induced by alcohol and paracetamol..................................54

LIST OF APPENDICES

Appendix I: Kenyatta University Research Approval letter…………………86

Appendix II: National Commission for Science, Technology and Innovation (NACOSTI) Research authorization Letter…………………..….87

ABBREVIATIONS AND ACRONYMS

ALD	Alcoholic liver disease
ALF	Acute liver failure
ALT	Alanine aminotransferase
ANOVA	Analysis of variance
AST	Aspartate aminotransferase
BUN	Blood urea nitrogen
EDTA	Ethylene diamine tetra-acetic acid
FDA	Food and drug administration
GABA	Gamma-aminobutyric acid
GGT	Gamma glutamyltransferase
Hb	Hemoglobin
Hct	Hematocrit
MCH	Mean corpuscular hemoglobin
MCHC	Mean corpuscular hemoglobin concentration
MCV	Mean corpuscular volume
MPV	Mean platelet volume
NAC	N-acetylcysteine
NAPQI	N-acetyl-p-benzoquinoneimine
NSAID	Non-steroidal anti-inflammatory drug
PDW	Platelet distribution width
RBC	Red blood cells
RDW	Red cell distribution width volume
ROS	Reactive oxygen species
TWBC	Total white blood cells

ABSTRACT

Alcohol is a widely used psychoactive drug that is safe when consumed moderately. However, overindulgence results in hangover and multiple organ injury. On the other hand paracetamol is a non-steroidal anti-inflammatory drug that is widely used for management of pain and fever. At therapeutic doses, the drug is well tolerated, however, overuse is associated with hepatotoxicity. Due to its safety and efficacy, paracetamol is widely misused in management of hangover among regular consumers of alcohol. In spite of the potential health risks of the combined use of the two drugs, there is limited scientific data on their interaction. This study was therefore conceived with the aim to provide clarity on the effects of the interaction of the two drugs on the biological systems in a rat model. The animals were divided into twelve groups. The negative and positive controls received distilled water and alcohol, respectively. Alcohol was administered at 2.5, 3.5 and 4.5 g/kg orally for 4 weeks. While paracetamol was given at doses of 40 and 400 mg/kg. Half of the groups received combined doses of the two drugs. Hematological and blood chemistry were determined using auto-analyzers while histostructure was scored under light microscopy. The output data was analyzed using Minitab software. Comparison of weight, hematological and biochemistry values were done using one way ANOVA followed by Tukey's test as the *post hoc* test. Alcohol and paracetamol caused changes in the physical characteristics of rats but the effect was non additive. In the case body weight, alcohol induced a dose and time dependent gain but for the combined drugs the effect was mixed. Conversely, alcohol and paracetamol did not affect ($p > 0.05$) the hematological profiles of the experimental animals and by extension had no influence on the bone marrow and immune system activity. In analyses of blood chemistry, the drugs resulted in a dose dependent elevation of liver enzymes, bilirubin, urea, reduced albumin levels and various degrees of liver and renal pathology. Thus, in moderate doses, paracetamol is safe, but high doses of the drug and chronic use of alcohol is hepatotoxic. It is as well inferred that AST/ALT index is a more predictive tool for alcohol exposure and liver injury since all alcohol treated groups had ratios above the normal value. Individually alcohol and paracetamol had a low risk of renal damage but when used together the risk is increased. In conclusion, heavy use of alcohol and regular use of paracetamol in management of alcohol induced hangover are discouraged as this can increase the risk of liver and kidney diseases.

CHAPTER ONE

INTRODUCTION

1.1 Background information

Alcohol is one of the most popular psychoactive drug that is legally available. The drug is often consumed to produce pleasurable effects such as loss of inhibition, euphoria, sedation and mild anaesthesia (Paton, 2005; Vengelien *et al*., 2008). Moderate consumption of alcohol has beneficial effects such as lowered risk of developing diabetes, liver and heart diseases (Ruhl and Everhart, 2003; Ioannou *et al.,* 2006; George and Figueredo, 2010). However, excessive usage has serious negative health, social and economic consequences (Hingson *et al.,* 2006; Silva *et al.,* 2015).

The most important clinical manifestation of chronic alcohol abuse is alcoholic liver disease (ALD). The condition encompasses a range of disorders including alcoholic fatty liver (steatosis), alcoholic hepatitis and alcoholic cirrhosis (Seitz and Becker, 2007). Alcoholic fatty liver occurs as a result of increased $NADH/NAD^+$ ratio which promotes free fatty acids and triglyceride synthesis while inhibiting beta-oxidation of free fatty acids (Maher, 2002; Albano, 2006). Alcohol also causes induction of cytochrome P450 (CYP2E1) and subsequent production of reactive oxygen species (Saetre *et al*., 2007). The reactive oxygen species reduce antioxidant glutathione and stimulates hepatic stellate cells that are

responsible for the fibrosis and hence cirrhosis (Lieber, 2004; Friedman, 2008). Other mediators of alcoholic hepatitis and cirrhosis include acetaldehyde adducts, inflammatory cytokines and endotoxins (Jampana and Khan, 2011).

Chronic abuse of alcohol has also been implicated in kidney damage (Massey *et al.,* 2015). In rats, alcohol has been reported to produce significant renal dysfunction and abnormalities in morphological structure (Van Thiel *et al.*, 1977; Onyango *et al.,* 2017). The kidney is susceptible to alcohol-induced oxidative damage because it has high content of long-chain polyunsaturated fatty acids which promote lipid peroxidation (Das and Vasudevan, 2007; Das *et al.,* 2008).

Paracetamol is a non-steroidal anti-inflammatory drug (NSAID) that is widely used to treat pain and fever. At therapeutic doses, paracetamol is well tolerated and has lower incidences of adverse effects compared to other NSAID such as aspirin (Hyllested *et al.*, 2002). However, over dosage of the drug has been reported to cause liver and kidney diseases and even death (Ganey *et al.,* 2007; Abdel-Zaher *et al.*, 2007; Daly *et al.*, 2008). Due to its safety paracetamol is widely misused for the relief of alcohol induced hangover symptoms.

Studies on the combined effects of alcohol and paracetamol are controversial. Some reports suggest that paracetamol at therapeutic doses is safe and effective even in chronic alcoholics (Kuffner *et al.,* 2001; Graham *et al.*, 2005). However,

other reports suggest that the drug can cause liver and kidney diseases in patients who are glutathione depleted or who take drugs such as alcohol that stimulate the cytochrome P450 enzymes (Blakely and McDonald, 1995; Dart *et al*., 2000). A major gap in these studies is that they have focused on the individual effects, but overlooked the combined effects of alcohol and paracetamol on the liver, and kidney function. In addition, there is limited information on the effects of these drugs on other biological systems. Lastly, the dose relationship between alcohol and paracetamol is also unclear. There is, therefore, need to conduct a well controlled study that examines the relationship between paracetamol and alcohol interaction on the physical parameters, hematological profile and the functions of the liver and kidney in a rat model.

1.2 Problem statement and justification

At therapeutic doses paracetamol has lower incidences of adverse effects. However, in patients with compromised liver function, even therapeutic doses of paracetamol can be toxic. Chronic and excessive usage of alcohol is known to cause multiple organ injury that increases the risk of developing hypertension, diabetes, cancer and heart, kidney and liver diseases (Santolaria *et al.,* 2003; Turati *et al.,* 2012; Reynolds, 2012). Most habitual consumers of alcohol do regularly use paracetamol to relieve the hang over symptoms and this is likely to place them at greater risks of developing more health complications.

There is limited information on the effects of combined use of alcohol and paracetamol on the biological systems. Therefore, this study aims to investigate that relationship with the hope of elucidating the interaction between the two drugs. The data generated will allow inference to be made about the safety of chronic use of paracetamol in habitual alcohol drinkers.

1.3 Research questions

i) What are the effects of individual and combined use of alcohol and paracetamol on blood cells?

ii) What are the effects of individual and combined use of alcohol and paracetamol on the structure and function of the liver?

iii) What are the effects of individual and combined use of alcohol and paracetamol on the structure and function of the kidney?

1.4 Hypothesis

When given separately alcohol and paracetamol are less toxic than when used together.

1.5 Objectives

1.5.1 General objective

To determine the systemic effects of alcohol and paracetamol on an animal model.

1.5.2 Specific objectives

i) To compare the effects of individual and combined treatments of alcohol and paracetamol on the hematological profile in a rat model.

ii) To determine the impact of alcohol and paracetamol on the histological structure and function of the rat liver.

iii) To determine the effects of alcohol and paracetamol on the histological structure and function of the rat kidney.

CHAPTER TWO

LITERATURE REVIEW

2.1 Alcohol

2.1.1 Overview

Ethanol or ethyl alcohol, is a popular recreational drug. Actually, besides nicotine, it is the most widely used psychoactive drug in our society (Guo and Ren, 2010). The widespread use of alcohol is because it is very addictive and people drink it to socialize, celebrate and relax (Mitchell *et al.,* 2014).

In small doses, alcohol has good medicinal values and it has been reported to exert protective effects against development of diabetes, liver and heart diseases (Ruhl and Everhart, 2003; Ioannou *et al.,* 2006). However, chronic and excessive usage is known to cause multiple organ injury that increases the risk of developing hypertension, diabetes, cancer, heart, kidney and liver diseases (Santolaria *et al.,* 2003; Baliunas *et al*., 2009; White *et al.,* 2009; Turati *et al.,* 2012; Reynolds, 2012).

2.1.2 Physical and chemical properties of alcohol

Alcohol is a colorless, volatile liquid with a molecular weight of 46.07, boiling point of 78.29^{o}C, melting point of $^{-}$114.1^{o}C and density of 0.789 g/ml (Lieber, 1997; Lide, 2005). The chemical formulae of alcohol is C_2H_5OH. In the presence of an oxidizing agent, alcohol is oxidized to aldehyde. Upon further oxidation, the

aldehyde is converted to acetic acid acid (Lide, 2005). Dehydration of alcohol results in the formation of ethane which later decomposes to form ethylene (Erdőhelyi *et al.,* 2006).

2.1.3 Pharmacokinetic properties of alcohol

2.1.3.1 Absorption and distribution

Alcohol is readily absorbed through the skin and gastrointestinal tract (Gentry, 2000). After oral ingestion, 20% of alcohol is absorbed from the stomach while 80% is absorbed from the upper small intestine (Paton, 2005). The rate of alcohol absorption is affected by various factors including gastric emptying, intestinal transit time and portal blood flow (Sadler, 2009; Mukherjee, 2014). Blood alcohol concentration is influenced by both the rate of absorption and the amount consumed (Ramchandani *et al.,* 2001). Following absorption, alcohol diffuses into the circulation and is evenly dispersed throughout the body (Mcdonough, 2003). Due to its low molecular weight it is readily distributed in all body fluids and tissues including the central nervous system (Mukherjee, 2014). Diffusion of alcohol is continuous until an equilibrium is achieved between the blood and tissue concentration ((Sadler, 2009; Kent, 2012). This equilibrium is usually achieved within 1-2 hours (Ferreira and Willoughby, 2007). Gastric emptying, intestinal transit time and portal blood flow are some of the factors that influence the rate of alcohol distribution in the body which in turn affects blood alcohol concentration (Sadler, 2009).

2.1.3.2 Alcohol metabolism and elimination

Alcohol is mainly metabolized in the liver through alcohol dehydrogenase in the cytosol, aldehyde dehydrogenase in the mitochondria and microsomal ethanol-oxidizing system in the endoplasmic reticulum (Zakhari, 2006). About 90% of absorbed alcohol is metabolized via hepatic alcohol dehydrogenase (Vonghia *et al.,* 2008). While the remaining 10% is oxidized via microsomal cytochrome P450 system (Fraser 1997). Alcohol dehydrogenase oxidizes alcohol to acetaldehyde (a reactive and toxic molecule) which is rapidly oxidized by aldehyde dehydrogenase to acetate which is further oxidized to carbon dioxide and water (Lieber, 2005). According to Lieber (1997), the rate of alcohol metabolism is influenced by blood alcohol concentration and individual genetic composition. The rate of alcohol metabolism is also influenced by amount and duration of alcohol consumption which influence induction of enzymes involved in alcohol metabolism (Zakhari, 2006). Most of the alcohol consumed is metabolized and excreted in urine as acetate and water (Zakhari, 2006). While only a small portion (2%-5%) is excreted unchanged in urine, sweat or breathe (Vonghia *et al.,* 2008). The rate of alcohol elimination is determined mainly by the activity of hepatic alcohol dehydrogenase which is the primary catabolic enzyme (Mitchell *et al.,* 2014).

Ramchandani *et al*. (2001) observed that food does increase the rate of alcohol elimination. Likewise the rate of alcohol elimination has also been demonstrated

to be higher in females than in males (Mumenthaler, 1999) and this has been attributed to the abundance of reproductive hormones dihydrotestosterone in males which inhibit alcohol dehydrogenase activity (Mezey *et al.*, 2001) and oestrogen in females which enhance hepatic alcohol dehydrogenase activity (Paton, 2005).

2.1.4 Pharmacological properties of alcohol

Alcohol is a central nervous system depressant that causes various biological and behavioral changes (Costardi *et al.,* 2015). Some of the effects of alcohol include sedation, impaired inhibition of activities and loss of relaxation (Oscar-Berman *et al.,* 1997). Due to these properties of alcohol, it is often consumed to produce pleasurable effects such as inhibition impairment, euphoria, sedation and mild anaesthesia (Paton, 2005; Vengelien *et al*., 2008). This therefore makes it a popular beverage in a variety of situations including social gatherings (Mitchell *et al.,* 2014). However, consumption of high levels of alcohol causes attention deficits, blackout and impaired sleep and wake regulation (Castro and Baltieri, 2004)

2.1.5 Mechanisms of action of alcohol

Alcohol exerts its effects by acting on gamma-aminobutyric acid (GABA) receptors potentiating the action of gamma-aminobutyric acid (the main inhibitory neurotransmitter of central nervous system) responsible for impaired inhibition (Charlton *et al.,* 1997; Davies, 2003). In addition, alcohol acts on glutamate receptors causing a reduction in excitatory neurotransmission and an increase in intracellular calcium responsible for reduced attention, blackout and impaired sleep and wake regulation (Hartley *et al.,* 1993; Castro and Baltieri, 2004). It has also been reported that alcohol acts on opioid and doperminergic systems to produce feeling of pleasure and increased chances of alcohol addiction (Aminoff, 2007; Gilpin and Koob, 2008). Alcohol also acts on serotonin receptor which results to symptoms of well-being and mood elevations ((Vengelien *et al*., 2008).

2.1.6 Adverse effects of alcohol

2.1.6.1 Overall effects

Alcohol abuse has the potential of causing multiple organ injury that results in various disease conditions of which alcoholic liver disease is the most prominent. The major mechanism for alcohol-induced organ injury is oxidative stress (Bondy, 1992; Nordman *et al.,* 1992; Tsukamoto and Lu, 2001). Which is defined as an imbalance between pro-oxidants and antioxidants due to enhanced generation of reactive oxygen species and depletion of antioxidant defense system (Schlorff *et al.,* 1999).

2.1.6.2 Hepatotoxicity

Chronic excessive consumption of alcohol results to alcoholic liver disease, a syndrome that encompasses a spectrum of liver alterations ranging from simple steatosis to more advanced stages such as alcoholic steatohepatitis, cirrhosis and hepatocellular carcinoma (Marsano *et al.*, 2003; Zakhari and Li, 2007; O'shea *et al.,* 2010; Neuman *et al.,* 2015).

Alcohol induced hepatotoxicity results from interactions between the direct toxic effects of alcohol and its metabolites such as acetaldehyde on various cell types in the liver as well as induction of reactive oxygen species (ROS) and up-regulation of inflammatory cascade in the liver (Neuman *et al.,* 2001). Reactive oxygen species such as hydrogen peroxide, superoxide and hydroxyl radicle (Nordmann *et al.*, 1992) enhance lipid peroxidation of hepatocyte membrane hence liver damage (Wu and Cederbaum, 2003). In the case of the inflammatory response, the cytochrome P450 (CYP2E1) has also been suggested to play a role in liver injury (Xu *et al.,* 2017).

Due to cellular damage, alcohol is known to affect liver enzymes. It has been reported that alcohol consumption results in the elevation of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) (Clark *et al.,* 2003; Song *et al.,* 2006; Hritz *et al.,* 2008; Kim *et al.,* 2008). While ALT is only found

in the liver, AST can be found in the liver, cardiac muscle, skeletal muscle, kidney, brain, pancreas, lungs, leucocytes and erythrocytes (Lazo *et al.,* 2008)`

Histopathological features associated with alcohol toxicity include cellular infiltration, cytoplasmic vacuolization, steatosis, inflammation, fibrosis and necrosis (Koop *et al.,* 1997; Kono *et al.,* 2000; Onyango *et al.,* 2017).

2.1.6.3 Nephrotoxicity

Chronic alcohol consumption has been associated with chronic kidney disease (Shankar *et al*., 2006) and there is evidence associating moderate to heavy alcohol consumption with increased urinary albumin excretion. In addition, alcohol consumption has been experimentally proven to have a direct and acute nephrotoxic effect on the kidney (Van Thiel *et al.,* 1977). Alcohol exhibit oxidative properties that make the kidney prone to its induced lipid peroxidation since the kidney is the major organ involved in alcohol elimination (Das *et al.,* 2008).

Some studies have reported indirect relationship between chronic alcohol use in humans with increased risk of chronic kidney disease as a result of alcohol-induced hypertension (Corrao *et al.,* 2000; Parekh and Klag 2001). In contrast, other studies have reported an inverse relationship between alcohol consumption and risk of developing chronic kidney disease (Reynolds *et al.,* 2008; Schaeffner

et al., 2005). Chronic alcoholism may also contribute to defects in renal tubular function and abnormalities of serum electrolytes (De Marchi *et al.,* 1993).

There have been reports suggesting decreased serum creatinine with chronic alcohol consumption (Chung *et al.,* 2005; Kronborg *et al.,* 2008). However, other studies have reported no significant changes in creatinine and blood urea nitrogen levels following alcohol intake (Onyango *et al.,* 2017). Chronic administration of alcohol produces significant renal dysfunction and abnormalities in kidney morphological structure in rats which is characterized by cellular infiltration and widening of tubular lumen (Van Thiel *et al*., 1977; Onyango *et al.,* 2017). Alcohol dependence has also been associated with renal papillary necrosis and acute renal failure (Pablo *et al*., 1986; Muthukumar *et al.,* 1999).

2.1.6.4 **Haematotoxicity**

Alcohol has also been reported to have detrimental effects on the bone marrow and blood cell precursors (Szabo and Mandrekar, 2009). It directly damages erythroid precursors. Chronic alcoholism causes hemolytic anaemia due to alterations in the erythrocyte membrane lipids (Nordmann and Rouach, 1996).

In a study by Onyango *et al.* (2017) reported that in a rat model, alcohol at a dose of 5g/kg did not significantly affect mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration. Chronic alcohol consumption has been

shown to cause a rise in the leucocytes particularly the lymphocytes, and also a fall in platelet levels (Myrhed *et al*., 1977; Onyango *et al.,* 2017).

2.1.6.5 Effects of alcohol on blood glucose levels

Low to moderate doses of alcohol may have a protective effect against the risk of developing diabetes and other cardiovascular diseases (Liu *et al.,* 2008). However, heavy drinking has been associated with higher plasma glucose levels increasing the risk of developing diabetes (Athyros *et al.,* 2007). Alcohol affect the release of insulin and glucagon which are the hormones involved in glucose counter-regulation (Kolaczynski *et al*., 1988) and acute alcohol intoxication can rapidly elevate blood glucose levels by inhibiting the release of insulin (Vonghia *et al.,* 2008).

2.1.6.6 Effects of alcohol on body weight

The relationship between alcohol intake and body weight changes has been studied and it shows mixed results. On one hand regular alcohol intake of more than 30 g/day contributes directly to increased body weight (Wannamethee and Shaper, 2003). This can be explained by increased hepatic production and release of acetate into the plasma which consequently inhibits lipolysis in peripheral tissues (Suter and Tremblay, 2005). Acetate also activates expression of lipogenic genes (Gao *et al.,* 2016) hence development of a positive energy balance and weight gain (Wang *et al.,* 2010).

In contrast, heavy drinking has been reported to significantly lower body weight and body mass index (Das and Vasudevan, 2005) and this is attributed to reduced adipose tissue as a result of inadequate nutritional intake (Addolorato *et al.*, 2000). Consumption of high amounts of alcohol also alters taste receptors reducing sensory pleasure in food (Neto *et al.,* 2011). In addition, alcohol interferes with absorption and metabolism of nutrients causing significant decrease in body weight gain (Heckmann and Silveira, 2009; Macdonald *et al.,* 2010).

The loss of weight as a consequent of over indulgence has also been attributed to loss of appetite and malnutrition (Santolaria *et al*., 2003). However, other studies reported that alcohol does not significantly affect the mean body weight of rats (Geidam *et al.,* 2004). In a study using a rat model it was shown that alcohol does not significantly affect body weight in the first week. However, significant weight gain was observed in the subsequent weeks (Onyango *et al.,* 2017).

2.1.6.7 Effects of alcohol on fertility and fetal development

Chronic consumption of alcohol has been shown to negatively affect fertility by causing changes in menstrual cycle regulation and ovulation (Eggert *et al.,* 2004). Heavy drinking can also lead to diminished ovarian reserve as well as decreasing the hormones that lead to testosterone and sperm production (Gude, 2012). Maternal consumption of alcohol during pregnancy can result to varying

embryonic developmental abnormalities depending on the amount and duration of alcohol exposure (O'Neil, 2012).

2.1.7 Treatment of alcohol toxicity

Various compounds have been studied on their effects to manage alcohol related toxicity. The leading compounds include silymarin, pentoxyfylline and corticosteroids and findings are of mixed results.

Silymarin has been used for many years to treat and as a prophylaxis against alcohol-induced injuries (Farghali *et al.,* 2000; Lieber *et al.,* 2003). It is a flavonoid extract which is abundant in the milk thistle *Silibum marianum* (Luper, 1998) and functions as an anti-inflammatory compound modulating cytokine production as well as an antioxidant scavenging free radicals (Song *et al.,* 2006). Sylimarin protects red blood cells membrane against lipid peroxidation caused by alcohol intoxication (Kvasnička *et al.,* 2003). The flavonoid extract is considered very safe with few and rare adverse effects such as gastrointestinal disturbances and allergic skin rashes (Jacobs *et al.,* 2002).

Pentoxyfylline is an inexpensive and widely used dimethylxanthine derivative that attenuates release and action of pro-inflammatory cytokines, exerts antifibrotic action and protects against hepatorenal syndrome (Girolami *et al.,* 1999; Akriviadis *et al.,* 2000). Pentixyfylline, which is a phosphodiesterase inhibitor has a fairly good safety profile (Arteel, 2003). However, associated side

effects including epigastric pain, vomiting and dyspepsia have been reported (Haber *et al.,* 2003).

Corticosteroids such as prednisolone have also been reported to improve survival in patients suffering from acute and severe alcohol intoxication although there have been concerns over their use because of high mortality rate especially in patients with renal impairment and concomitant infections (Mathurin *et al*., 2002). They inhibit pro-inflammatory cytokine production, decrease immune response by inhibiting leukocyte migration and have antifibrotic effects (Elenkov *et al.,* 1996; Arteel, 2003; Tamura *et al.,* 2005). The major side effects associated with the use of corticosteroids include gastrointestinal bleeding and increased susceptibility to infections (Tilg and Day, 2007).

2.2 Paracetamol

2.2.1 Overview

Paracetamol, also known as acetaminophen, is one of the most commonly used non-prescription drug for management of pain and fever (Oyedeji *et al.,* 2013).The drug was discovered in Germany at the end of 19th century. However, it was not widely used until midway through the 20th century (Sheen *et al*., 2002). To date, paracetamol is one of the most widely used over the counter drug for management of pain and fever. Incidentally, the drug is highly abused in the management of alcohol induced hangover.

2.2.2 Physical and chemical properties

Paracetamol, is a white odorless crystalline solid with a molecular weight of 151.165 g/mol and a melting point of 168ºC (Lide, 2005). It is slightly soluble in cold water but very soluble in boiling water and alcohol (O'Neil, 2013). The chemical structure of paracetamol consists of a phenol ring and an acyl group (Oyedeji *et al.*, 2013) and is formed via interaction of *p*-aminophenol and an aqueous solution of acetic anhydride at a pH of between 5.5 to 6.5 (Foye, 1995). Paracetamol is oxidized in the presence of an oxidizing agent such as chlorine to N-acetyl-para benzoquinone imine (Pinkston and Sadlak, 2004) which in aqueous solution is readily hydrolyzed to 1,4- benzoquinone (Andreozzi *et al.,* 2003).

2.2.3 Pharmacokinetic properties

2.2.3.1 Absorption

Following oral administration, paracetamol is readily absorbed from the gastrointestinal tract (Hodgman and Gerrard, 2012). The extent of absorption is not affected by the presence or absence of food in the stomach (FDA, 2011).

2.2.3.2 Distribution

After ingestion, paracetamol is rapidly distributed with peak plasma concentrations achieved within 90 minutes (FDA, 2011). At therapeutic concentrations, paracetamol is minimally bound to protein with a volume of distribution of 0.9 L/kg and a half-life of 2 to 2.5 hours (Hodgman and Gerrard, 2012). Paracetamol readily diffuses across the blood brain barrier with rapid and

high levels in the cerebrospinal fluid (Singla *et al.*, 2012), and is present in breast milk as it crosses the placenta (Van der marel, 2003).

2.2.3.3 Metabolism

Paracetamol is metabolized via glucuronidation and sulfation in the liver (Gu *et al.*, 2005). Glucuronidation accounts for 55% to 66% of paracetamol metabolism. While sulfation accounts for 20% to 30% of paracetamol metabolism in adults (Brayfield, 2014). In children up to 12 years of age, sulfation predominates (Prescott, 2000). A small, yet significant amount of less than 15% of paracetamol is metabolized via cytochrome P-450 enzyme producing N-acetyl-para-benzoquinoneimine (NAPQI) which at non-toxic doses is efficiently conjugated by glutathione into urinary excreted non-toxic metabolites (Botting and Ayoub, 2005).

2.2.3.4 Elimination

Paracetamol is excreted through the kidney in the form of glucuronide and sulfate which can be detected in urine of patients after paracetamol administration ((Gu *et al.*, 2005; Gelotte *et al.,* 2007). About 47-62% of paracetamol absorbed is excreted as paracetamol glucuronide while 25-36% is excreted as paracetamol sulfate (Prescott, 2000). However, only 1-4% of paracetamol is excreted in urine unchanged (Oscier and Milner, 2009). N-acetyl-para-benzoquinone imine resulting from paracetamol metabolism is conjugated with glutathione and excreted in urine as cysteine and mercapturate metabolites (Gelotte *et al.,* 2007).

2.2.4 Pharmacological properties

Paracetamol (*N*-acetyl-*p*-aminophenol) is a non- prescription drug commonly used as antipyretic and analgesic agent (Anderson *et al*., 1998). The drug has lower incidences of adverse effects and is a preferred NSAIDS especially in high risk patients (Hyllested *et al*., 2002). Paracetamol has minimal but significant anti-inflammatory effects (Bjornsson *et al.,* 2003).

2.2.5 Mechanism of action

Paracetamol acts on the central nervous system and various mechanisms have been suggested to be responsible for the analgesic and antipyretic effects (Mallet *et al*., 2010). It has been reported that paracetamol acts as a cannabinoid receptor agonist (Ottani *et al.,* 2006) reinforcing the descending inhibitory serotonergic pain pathways (Pickering *et al.,* 2008) and inhibits the L- arginine nitric oxide pathway (Bujalska, 2004) to exert analgesic properties. The antipyretic effect of paracetamol is as a result of inhibition of prostaglandin synthesis through the cyclooxygenase pathway (Graham and Scott, 2005; Anderson, 2008).

2.2.6 Indications

Paracetamol is a safe, effective and widely used antipyretic and analgesic agent (Wang *et al.,* 2014) that can be prescribed for general pain management in sickle cell patients undergoing mild pains (Adzu *et al.,* 2001), post-operative period such as post-ceasarean delivery (McNicol *et al*., 2011) or post orthopedic procedures (Lachiewicz, 2013).

Many international guidelines such as the Department of Health, Queensland Government have recommended paracetamol as a drug of first choice for relieving mild to moderate pain (Klotz, 2012) and for management of acute and chronic pain in patients with impaired kidney function (Murphy, 2005). Paracetamol is the preferred drug for relieving pain in osteoarthritis and low back pains (Nikles *et al.*, 2005) and is used in combination with other drugs to manage pain in cancer patients (Stockler *et al.*, 2004). Paracetamol is arguably the most widely abused drug used for management of headache and other adverse effects associated with alcohol hangover, especially in binge drinking (Evers and Marziniak, 2010).

2.2.7 Adverse effects

2.2.7.1 Overall effects

Paracetamol toxicity has been recognized since 1966 (James *et al.,* 1975). Adverse effects associated with paracetamol include nausea, vomiting, allergic reactions such as rashes and malaise (Hodgman and Gerrard 2012). On the other hand overdose of the drug has been reported to cause damages to multiple organs especially the liver and kidney (Lee, 2004).

2.2.7.2 Hepatotoxicity

The most remarkable feature of paracetamol overdose is hepatotoxicity (Rømsing *et al.,* 2002) which accounts for a very high percentage of cases annually (Litovitz *et al.,* 2002). In 2007, paracetamol over-dosage was reported to be the leading

cause of acute liver failure in the United States (Bower *et al*., 2007). Poisoning and hepatotoxicity of paracetamol occurs at doses between 10 and 15 grams per day, however, doses of between 20 and 25 grams per day can be fatal (Korpi-Steiner *et al.,* 2008).

Some studies have reported that hepatic injury results from the binding of N-acetyl-para-benzoquinoneimine (NAPQI) to cellular proteins and initiate lipid peroxidation (Mitchell *et al.,* 1973; Hart *et al.,* 1994). In large quantities, hepatic cytochrome P450 enzyme system metabolizes paracetamol to a reactive metabolite, N-acetyl-para-benzoquinoneimine (Manyike *et al*., 2000).

Large amounts of N-acetyl-para-benzoquinoneimine depletes glutathione and directly damages and kills hepatocytes (Lee, 2004). Glutathione is a free radical scavenger and depletion by excess N-acetyl-para-benzoquinoneimine exposes hepatocytes to oxidative stress damage (Smilkstein *et al.,* 1988). The reactive metabolite can as well cause liver damage by binding the liver microsomal protein (Ruepp *et al*., 2002). In addition, NAPQI is a strong oxidant and promotes lipid peroxidation and damage to macromolecules (Song *et al.,* 2004). Furthermore, the metabolite can simultaneously enhance formation of reactive oxygen species such as superoxide anion, hydroxide radicle and hydrogen peroxide as well as reactive nitrogen radicals such as nitric oxide (Hinson *et al.,* 2010).

Paracetamol toxicity results in elevated levels of serum alanine aminotransferase, aspartate aminotransferase and bilirubin concentrations (Ganey *et al*., 2007). In addition, the drug toxicity results to elevation in serum alkaline phosphatase enzyme (Green *et al.,* 2010). Paracetamol at a dose of 500 mg/kg causes significant elevations in serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and bilirubin (Parmer *et al.,* 2010). In addition, paracetamol overdose results to a reduction in albumin levels and insignificant changes in gamma glutamyl transferase (Juma *et al.,* 2015).

In rats paracetamol toxicity is also manifested by abnormal histological changes in the liver which are dose related (Hewawasam *et al.,* 2004; Juma *et al.,* 2015). Over dosage of paracetamol has been reported to cause vacuolization, hemorrhage and necrosis of liver cells (Blazka *et al*., 1996; Bhanwra *et al*., 2000; Gulnaz *et al*., 2010; Hinson *et al.,* 2010).

Liver sections of animals treated with paracetamol have been reported to exhibit severe and intense congestion, degeneration and occasional necrosis (Kanchana and Sadiq, 2011). In addition, it has been observed that paracetamol over dosage results to centrilobular vacuolization, necrosis and inflammation with peak destruction at 9-hour post administration (Kofman *et al.,* 2005). Necrosis results from binding of paracetamol active metabolite (N-acetyl-para-benzoquinone

imine) to sulfhydryl groups of proteins due to depletion of glutathione (Kanchana and Sadiq, 2011).

2.2.7.3 Nephrotoxicity

Paracetamol can cause acute renal failure as the primary manifestation of toxicity with an onset within two to five days and peak damage observed between three to sixteen days after over dosage or may occur in combination with hepatic damage (Blakely and McDonald, 1995; Eguia and Materson, 1997). Nephrotoxicity occurs in approximately 1-2% of patients with paracetamol over dosage and habitual paracetamol exposure can increase the chances of renal insufficiency which eventually lead to end-stage renal disease and death (Boutis and Shannon, 2001; Sarumathy, 2011).

Several studies have suggested various mechanisms responsible for paracetamol-induced renal damage. The damage may be due to formation of N-acetyl-p-benzoquinone imine -glutathione conjugate that cause depletion of glutathione hence inhibiting the detoxification of the reactive metabolite (McMurtry *et al*., 1978; Abdel-Zaher *et al*., 2007). Nephrotoxicity is due the accumulation of the reactive metabolite in the renal papillary (Sheen *et al.,* 2002). Renal damage can also be due to prostaglandin endoperoxidase synthetase enzyme that enhances the activation of paracetamol into NAPQI in the medulla of kidney (Mugford and Tarloff, 1997).

Toxic doses of paracetamol resulted to marked increase in serum creatinine and urea compared to control group (Palani *et al.,* 2010). Increased levels of serum creatinine and urea are considered as indices of assessing nephrotoxicity (Ali *et al.,* 2001). However, other studies have reported that paracetamol causes no significant differences in blood urea nitrogen and creatinine compared with the control (Payasi *et al.,* 2010).

Studies have reported histological changes in the kidney manifested by shrunken glomeruli, vascular congestion and tubular necrosis as a result of paracetamol toxicity (Abraham, 2005; Ucheya and Igweh, 2006). A dose of 500 mg/kg of paracetamol resulted to loss of renal tubular architecture with rearrangement of renal tubules and glomerulus. The renal tubules showed cellular swelling and narrowing of lumen (Pathan *et al.,* 2013). The renal damage may be due to oxidation of paracetamol in the kidney by microsomal mixed function oxidases to the reactive metabolite (N-acetyl-para-benzoquinoneimine) which covalently binds to tissue nucleophiles (Thomsen *et al.*, 1995). The damage may be as a result of increased lipid peroxidation caused by oxidative stress (Li *et al.,* 2003) or due to deacetylation of paracetamol in the kidney to form *p*-aminophenol (Mugford and Tarloff, 1997).

2.2.7.4 Haematotoxicity

Paracetamol poisoning causes no significant differences in the hematological profile of Wistar rats (Payasi *et al*., 2010). However, some studies have reported that rats treated with paracetamol show significant decrease in red blood cells count and hemoglobin (Adedapo *et al.,* 2007; Ikpi and Nku**,** 2008; Juma *et al.,* 2015). Similarly, paracetamol has been reported to cause significant decrease in mean values of haemoglobin, haematocrit and total erythrocyte count (Nwodo *et al.,* 2010).

Haematopoetic system is susceptible to xenobiotic attack since blood is involved in transportation of substances (Adeniyi *et al.,* 2010). Treatment of rats with paracetamol causes non-significant changes in total white blood cells, neutrophil, eosinophil, monocyte and lymphocyte counts as well as platelet count (Oyedeji *et al.,* 2013). However, another study reported that paracetamol overdose results to significant increase in mean corpuscular hemoglobin and a rise in lymphocytes (Juma *et al.,* 2015).

2.2.7.5 Effect on blood sugar

It was noted that paracetamol may have beneficial effect on blood glucose levels and is cardio protective (Kendig *et al.,* 2008; Blough and Wu, 2011) and that the drug can normalize increased blood glucose levels (Shertzer *et al.,* 2008; Jambulingappa *et al.,* 2012). This is due to the ability of paracetamol to function

as an antioxidant (Merrill and Goldberg, 2001). However, the drug toxicity has been associated with extra-hepatic manifestations such as hyperglycemia (Yang *et al.,* 2001). This can be as a result of oxidative stress which plays a major role in pathogenesis of diabetes mellitus (Maritim *et al.,* 2003, Rains and Jain, 2011). In addition, insulin resistance has been linked to oxidative damage of essential macromolecules in insulin sensitive tissues (Styskal *et al*., 2012).

2.2.7.6 Effect on body weight

Some studies have demonstrated that paracetamol causes no significant changes in body weight of rats (Oyedeji *et al.,* 2013; Di pierro and Rossoni, 2013). However, other studies have showed that paracetamol results to a significant decrease in net body weight of Wistar rats (Mossa *et al.,* 2012; Juma *et al.,* 2015). Reduction in weight could be due to compromised nutritional status of rats as a consequent of gastrointestinal tract derangement caused by paracetamol (Ucheya and Igweh, 2006).

2.2.8 Treatment of toxicity

N-acetyl cysteine (NAC) has been reported to be an effective antidote for paracetamol poisoning (Buckley *et al.,* 1999; Prescott, 2005; Dodd *et al.,* 2008). N-acetyl cysteine is a sulfhydryl donor that provides cysteine for the replenishment of depleted glutathione (Woo *et al.,* 2000) as well as enhancing metabolism and elimination of paracetamol via sulfation pathway (Saito *et al.,*

2010). The antidote can be administered orally or intravenously based on the patient body weight and condition (Yarema *et al.,* 2009).

2.3 Combination of alcohol and paracetamol on liver and kidney damage

Chronic alcohol abuse activates paracetamol metabolism through cytochrome P-450 pathway thus potentiates paracetamol-induced hepatotoxicity (Schiodt *et al.,* 2002). It has been reported that chronic alcoholism depletes glutathione thus reducing the margin of safety of paracetamol in patients (Blakely and McDonald, 1995). Other studies have demonstrated that alcohol and paracetamol interact to increase the production of highly reactive and toxic metabolite of paracetamol, NAPQI (Łukasik-Głebocka and Klimaszyk, 2001).

Alanine aminotransferase (ALT), aspartate aminotransferase (AST), total bilirubin and direct bilirubin are significantly elevated after chronic alcohol abuse and paracetamol poisoning (Korpi-Steiner *et al.,* 2008). Elevation in the plasma transaminases levels is attributed to the damaged structural integrity of the liver because ALT and AST are cytoplasmic enzymes in nature and are released into the circulation after cellular damage (Bilgin *et al.,* 2011). Chronic alcohol abuse and paracetamol poisoning results to hemolytic anemia evidenced by low hemoglobin (Dhaliwal *et al.,* 2004) and the hemolysis may be due to oxidative damage to hemoglobin (Beuttler, 1994).

Studies have shown that paracetamol at therapeutic doses is safe and effective even in chronic alcoholic patients (Kuffner *et al.,* 2001). However, other reports suggest that the drug can cause liver and kidney diseases in patients who are glutathione depleted or who take drugs such as alcohol that stimulate the cytochrome P450 enzymes (Blakely and McDonald, 1995; Dart *et al*., 2000).

This study therefore attempts to address the unresolved controversy of the toxic effects of paracetamol on subjects intoxicated with alcohol. This is an important issue of public health concern due to the widespread misuse of paracetamol in managing alcohol related hangover. The significance of the study resonates with the use a controlled animal model using high and low dosages of paracetamol under standard conditions.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Laboratory animals

Adult Wistar rats of 3-4 weeks, weighing between 120 and 200 grams were used in this study. The weight range was selected based on previous protocols (Garba *et al.,* 2009; Bekheet, 2010; Ghoneim *et al.,* 2014). The rats were bred and housed in plastic cages under standard laboratory conditions in the animal house of the Department of Biochemistry and Biotechnology, Kenyatta University. The animals were fed on commercially available pelleted feed and water *ad libitium.* The experiments were carried out in accordance with the 1996 guidelines for the care and use of laboratory animals (Clark *et al.,* 1997).

3.2 Chemicals

Alcohol (Smirnoff Vodka®, 37.5 %) produced by East Africa Breweries Limited (Nairobi, Kenya) was bought from a local vendor (Nakumatt Supermarket, Nairobi, Kenya). Cipladon®1000 (Cipla Ltd., Mumbai, India) containing 1000 mg paracetamol was sourced from Rangechem Pharmaceuticals (Nairobi, Kenya).

3.3 Preparation of working concentrations of alcohol

In the case of rats that received alcohol at doses of 2.5 g/kg and 3.5 g/kg body weight, the working concentration of the administered solution was 0.17 g/ml alcohol, which was prepared by mixing 10 ml of neat vodka (37.5%) with 12 ml of distilled water. Those that received alcohol at a dose of 4.5 g/kg bodyweight, the working concentration was 0.22 g/ml of alcohol, which was prepared by mixing 10 ml of neat vodka with 7 ml of distilled water.

3.4 Preparation of working concentrations of paracetamol

The animals that received paracetamol at a dose of 40 mg/kg bodyweight, the working concentration was 7.4 mg/ml and this was prepared by dissolving one tablet of Cipladon®1000 in 135 ml of distilled water. Those that were treated with 400 mg/kg bodyweight, the working concentration was 74 mg/ml, which was prepared by dissolving four tablets of Cipladon®1000 in 54 ml of distilled water.

3.5 Experimental design

The rats were separated into twelve groups of five animals each. The negative control group was treated with distilled water. While the rest were given either alcohol or paracetamol or a combination of both. The rats treated with alcohol at a dose of 2.5 g/kg body weight were administered with 1 ml of the prepared working solution. Those treated with alcohol at doses of 3.5 g/kg and 4.5 g/kg body weight received 1.4 ml of the respective working solutions. Alcohol was

administered twice daily with half of the dose given in the morning and the other in the evening for 28 days (Iimuro *et al.,* 1997, Kono *et al*., 2000). Paracetamol was given on day 29. Details of the treatment regimens are presented in Table 3.1. The treatments were done orally using a cannula (Nadro *et al*., 2006).

Table 3.1: Treatment regimens

Group	**Treatment**
A	Distilled water (negative control)
B	2.5 g/kg alcohol
C	3.5 g/kg alcohol
D	4.5 g/kg alcohol
E	40 mg/kg paracetamol
F	400 mg/kg paracetamol
G	2.5 g/kg alcohol + 40 mg/kg paracetamol
H	2.5 g/kg alcohol + 400 mg/kg paracetamol
I	3.5 g/kg alcohol + 40 mg/kg paracetamol
J	3.5 g/kg alcohol + 400 mg/kg paracetamol
K	4.5 g/kg alcohol + 40 mg/kg paracetamol
L	4.5g/kg alcohol + 400mg/kg paracetamol

3.6 Determination of Physical parameters

Individual body weights of rats were determined using a weighing balance shortly before the start of the experiment and weekly thereafter until the end of the study. The general physical appearance and behavior of rats was also monitored throughout the experiment period.

3.7 Blood collection and analysis

At the end of the experiment blood was collected from the rats for hematological, blood sugar and biochemical analysis as described below.

3.7.1 Hematological analysis

The tail of rat was sterilized with 10% alcohol and the tip cut with sterilized scissors. Blood was then collected by gently massaging the tail. The first drop of blood was wiped off and the subsequent drops were collected in ethylene diaminetetra-acetic acid (EDTA) containing vials.

Haematological parameters were assayed using Mindray BC-5300 auto hematological analyzer (Shenzen Mindray Bio-Medical Electronics Co., Ltd, Shenzhen, China) as per the manufacturer's recommendations (Lu and Gu, 2012). The analyzer was subjected to daily quality control according to the standard operating procedures. The parameters analyzed were total white blood cells count, lymphocytes, monocytes, granulocytes, red blood cells, mean corpuscular volume

(MCV), hematocrit, mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red cell distribution width volume (RDW), hemoglobin, thrombocytes, mean platelet volume (MPV), pleteletocrit and platelet distribution width (PDW).

3.7.2 Blood glucose levels

Blood was collected from the tip of the tail as described in section 3.7.1. A drop of blood was then placed at one end of the glucometer strip and reading taken after 10 seconds using On Call Plus glucometer (ACON laboratories, San Diego, USA).

3.7.3 Biochemical analysis

The rats were euthanized using diethyl ether. Blood was drawn using sterile needle and syringe by cardiac puncture. The blood was collected in plain vacutubes and was allowed to stand and clot at room temperature for one hour then centrifuged using 24900xg centrifuge at 3000 rpm for 10 minutes. Serum was transferred into polypropylene tubes using sterile pasture pipette (Parmar *et al.,* 2010).

Biochemical analysis were performed using Selectra ProS clinical chemistry analyzer (ELITech group clinical systems, Milsbeek, Netherlands) following the manufacturer’s instructions. The parameters analysed were albumin, total bilirubin (TBI), direct bilirubin (DBIL), gamma glutamyltransferase (GGT),

aspartate aminotransferase (AST), alanine aminotransferase (ALT), urea and creatinine. The ratio of AST/ALT was calculated manually by dividing the AST values by the ALT values.

3.8 Histopathological analysis

The liver and kidney tissues were removed and weighed. The tissues were fixed in 10% formalin. The fixed tissues were rinsed in water and embedded in liquid paraffin. The embedded specimens were sectioned into thin slices using a microtome and stained with hematoxylin and eosin (Gujral *et al.,* 2001; Song *et al.,* 2006). Liver and kidney sections were then examined microscopically for presence or absence of pathology.

3.9 Data management

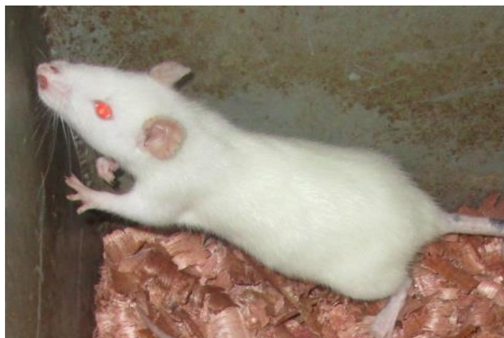
Descriptive data were summarized in Excel (Microsoft Corporation, Redmond, USA) and reported as mean ± standard deviation. Statistical analysis was done using a statistical package Minitab (version 17, Minitab Inc., State college, PA, USA), which generated all statistical calculations. Comparisons of weights, biochemical and hematological parameters were analyzed using one-way analysis of variance (ANOVA). Tests for multiple comparisons were done using Tukey's post hoc test and statistical significance was reported at $p \leq 0.05$. Data were presented as text, tables and figures.

CHAPTER FOUR

RESULTS

4.1 Morphological characteristics of rats subjected to alcohol and paracetamol treatment

Control group rats and those treated with 2.5 g/kg alcohol, 40 mg/kg of paracetamol and 2.5 g/kg of alcohol plus 40 mg/kg of paracetamol were active, had glossy coat and had good appetite throughout the treatment period (Figure 4.1A). Those that received 3.5 g/kg or 4.5 g/kg of alcohol were initially active and had good appetite. However, from days sixteen and nine respectively, they had poor appetite. The remaining five groups of rats, which were either treated with 400 mg/kg of paracetamol or co-treated with alcohol and paracetamol, had rough hair coat, poor appetite, were inactive and they huddled in a corner of the cage (Figure 4.1B).



A **B**

Figure 4.1: Morphological characteristics of rats subjected to alcohol and paracetamol treatment. (A) Control rat (alert and glossy skin) and (B) Rat co-treated with alcohol and paracetamol (dormant, sickly and rough skin).

4.2 Effects of alcohol and paracetamol on body weight of rats

The control group recorded the highest weight gain of 31.8±8.79 g in the first week but it also displayed the lowest weight gain of only 7±1.87 g in the fourth week (Figure 4.2). In the first week of study, rats that were independently treated with alcohol or paracetamol did not have a significant ($p > 0.05$) change on body weight as compared to control. However, those that were co-treated with alcohol and paracetamol had significantly ($p < 0.05$) low weight gain. In the second week, there were no significant ($p > 0.05$) change in weight in all groups.

Likewise, in the third week, all treatment groups showed insignificant ($p > 0.05$) change in weight except for the group that was treated with a combination of 4.5 g/kg of alcohol plus 400 mg/kg of paracetamol that recorded a significant ($p < 0.05$) weight gain.

In week four, the groups treated with 3.5 g/kg and 4.5 g/kg of alcohol recorded significant ($p < 0.05$) weight gain. A similar finding was observed in rats treated with 40 mg/kg paracetamol. There was also significant weight increase in all the groups that were given the two drugs together except for the one that received 3.5 g/kg alcohol plus 400 mg/kg paracetamol where weight change was non-significant ($p > 0.05$) (Figure 4.2).

4.3 Effect of alcohol and paracetamol on hematological parameters of rats

Treatment with alcohol or paracetamol, either individually or combined, did not have a significant ($p > 0.05$) effect on the total white blood cells, lymphocytes, monocytes and granulocytes counts (Table 4.1). A similar pattern ($p > 0.05$) was also observed with the erythrocytic parameters of RBC counts, mean corpuscular volume, hematocrit, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration and hemoglobin (Table 4.2). Likewise alcohol and paracetamol did not have significant ($p > 0.05$) effects on the platelet counts and the related parameters of mean platelet volume, plateletocrit and platelet distribution width (Table 4.3).

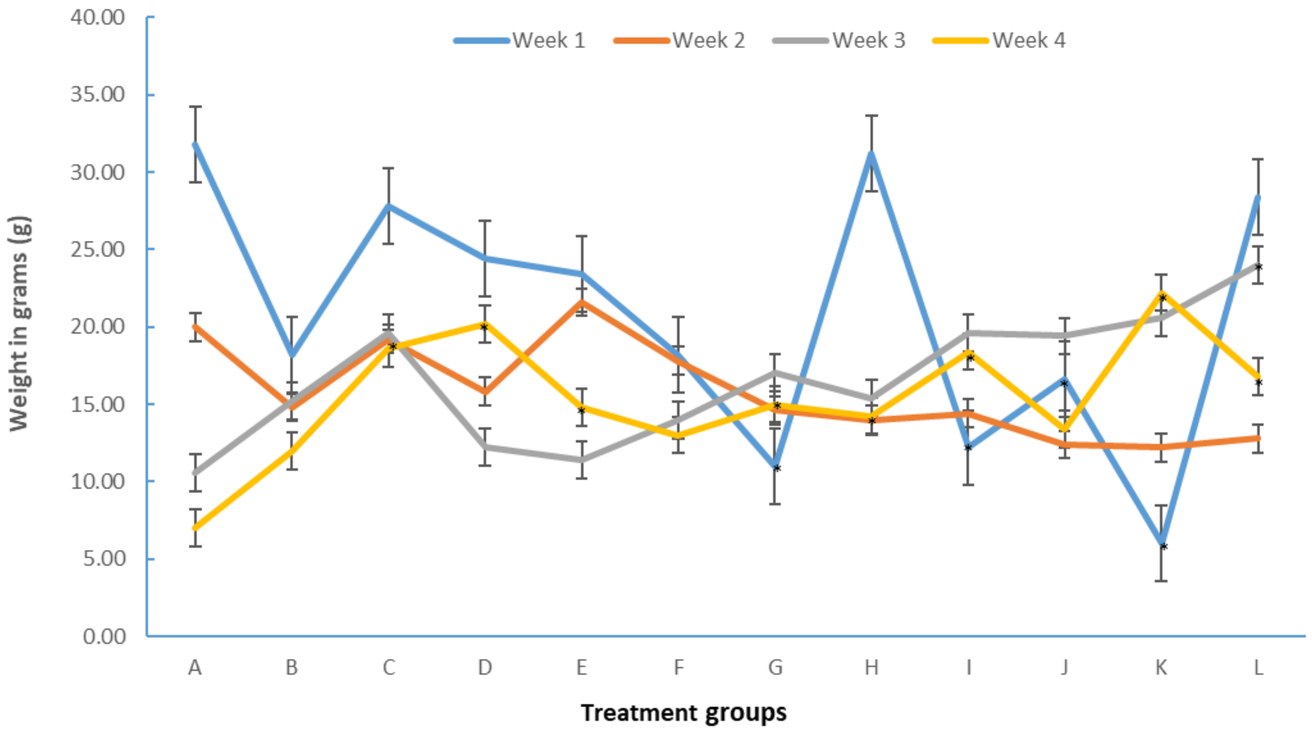


Figure 4.2: Change in body weight of rats following treatment with alcohol and paracetamol.

* Indicates $P < 0.05$ when compared to the corresponding control.

A = Control
B = Alcohol 2.5 g/kg
C = Alcohol 3.5 g/kg
D = Alcohol 4.5 g/kg
E = Paracetamol 40 mg/kg
F = Paracetamol 400 mg/kg
G = Alcohol 2.5 g/kg + paracetamol 40 mg/kg
H = Alcohol 2.5 g/kg + paracetamol 400 mg/kg
I = Alcohol 3.5 g/kg + paracetamol 40 mg/kg
J = Alcohol 3.5 g/kg + paracetamol 400 mg/kg
K = Alcohol 4.5 g/kg +paracetamol 40 mg/kg
L = Alcohol 4.5 g/kg +paracetamol 400 mg/kg

Table 4.1: Comparison of the effects of alcohol and paracetamol on the leukocyte profiles of rats.

Groups	TWBC (m/mm^3)	Lymphocytes (%)	Monocytes (%)	Granulocytes (%)
Control	9.06±1.50	85.54±3.22	5.86±1.76	8.60±1.97
Alc 2.5g/kg	12.59±6.78	92.58±3.36	3.20±1.72	4.22±2.07
Alc 3.5g/kg	11.93±4.37	89.65±3.29	5.35±2.74	5.00±1.31
Alc 4.5g/kg	10.38±3.62	79.90±10.74	9.68±5.36	10.42±5.65
Para 40mg/kg	10.69±5.25	85.46±5.37	7.88±3.31	6.66±2.76
Parac 400mg/kg	9.44±3.30	80.96±5.96	7.78±2.92	11.26±4.03
Alc 2.5g/kg + Para 40mg/kg	12.48±4.52	89.50±2.46	4.88±1.21	5.62±1.95
Alc 2.5g/kg + Para 400mg/kg	13.65±5.89	86.44±5.01	7.82±4.96	5.74±1.72
Alc 3.5g/kg + Para 40mg/kg	12.90±6.22	86.67±5.45	6.93±3.38	6.40±2.71
Alc 3.5g/kg + Para 400mg/kg	12.17±1.84	81.38±8.85	9.06±3.69	9.56±5.38
Alc 4.5g/kg + Para 40mg/kg	7.49±4.48	87.74±3.14	6.32±2.79	5.94±3.58
Alc 4.5g/kg + Para 400mg/kg	10.01±5.24	84.98±3.75	6.70±3.01	8.32±2.72

TWBC=Total white blood cells, Alc= Alcohol and Para= Paracetamol. The control and other treatments were non-significantly ($p > 0.05$) different.

Table 4.2: Effects of alcohol and paracetamol on the erythrocytic parameters of rats.

Groups	RBC (M/mm^3)	MCV (fl)	Hct (%)	MCH (pg)	MCHC (g/dl)	RDW	Hb (g/dl)
Control	7.20±0.82	59.80±1.30	42.96±4.15	20.88±0.49	35.04±0.89	9.42±0.52	15.08±1.61
Alcohol 2.5g/kg	7.29±0.91	57.06±1.67	41.50±4.66	19.78±0.86	34.74±1.18	9.62±0.40	14.42±1.49
Alc 3.5g/kg	8.46±1.24	57.33±0.53	48.45±6.89	19.73±0.46	34.52±0.66	9.73±0.29	16.717±2.15
Alc 4.5g/kg	8.37±0.99	57.40±1.50	47.86±4.56	19.38±1.85	33.80±2.55	9.68±0.96	16.12±0.91
Paracetamol 40mg/kg	7.46±1.11	56.28±1.83	41.98±6.87	19.52±0.52	34.76±0.46	10.02±0.30	14.62±2.42
Paracetamol 400mg/kg	7.75±0.78	57.20±2.40	44.16±3.39	20.48±1.22	35.90±1.45	9.94±0.76	15.84±0.83
Alc 2.5g/kg + Para 40mg/kg	7.89±0.59	58.70±2.34	46.22±3.02	20.38±0.83	34.84±0.93	9.40±0.74	16.10±0.74
Alc 2.5g/kg + Para 400mg/kg	7.40±1.60	60.92±5.53	44.44±6.99	20.56±0.91	33.94±1.71	9.52±0.59	15.14±2.73
Alc 3.5g/kg + Para 40mg/kg	8.23±1.11	56.23±2.36	46.35±7.91	19.75±0.87	35.23±0.59	10.32±0.62	16.33±2.63
Alc 3.5g/kg + Para 400mg/kg	7.69±0.66	58.90±0.66	45.24±3.85	20.80±1.03	35.40±1.54	9.96±0.82	16.02±1.13
Alc 4.5g/kg + Para 40mg/kg	7.39±1.58	56.72±2.31	41.74±8.37	20.20±1.66	35.72±2.13	9.20±0.84	14.84±2.54
Alc 4.5g/kg + Para 400mg/kg	7.40±1.49	58.14±1.74	43.04±9.13	20.38±0.66	35.12±0.58	9.44±0.59	15.14±3.17

RBC= Red blood cells, MCV= Mean corpuscular volume, Hct= Hematocrit, MCH= Mean corpuscular hemoglobin, MCHC = Mean corpuscular hemoglobin concentration, RDW= Red cell distribution width, Hb= Hemoglobin, Alc= Alcohol and Para= Paracetamol. No significant ($p > 0.05$) variations between control and treatments.

Table 4.3: Platelet parameters of rats subjected to alcohol and paracetamol treatment.

Groups	Thromboctes (m/mm3)	MPV (fl)	Plateletocrit (%)	PDW
Control	1498±410.47	6.50±0.12	0.97±0.27	7.34±0.38
Alcohol 2.5g/kg	1633±792.67	6.42±0.16	1.05±0.52	7.30±0.10
Alc 3.5g/kg	1961±731.41	6.40±0.24	1.26±0.47	6.88±0.28
Alc 4.5g/kg	2700±527.37	6.56±0.15	1.78±0.36	7.44±0.37
Paracetamol 40mg/kg	2074±469.26	6.52±0.15	1.35±0.30	7.42±0.25
Paracetamol 400mg/kg	2711±1152.42	6.46±0.27	1.76±0.76	7.38±0.16
Alc 2.5g/kg + Para 40mg/kg	2571±625.46	6.54±0.32	1.67±0.34	7.66±0.32
Alc 2.5g/kg + Para 400mg/kg	2572±1636.75	6.82±0.30	1.91±1.24	7.56±0.59
Alc 3.5g/kg + Para 40mg/kg	2268±552.27	6.50±0.13	2.078±0.36	7.62±0.13
Alc 3.5g/kg + Para 400mg/kg	2482±602.32	6.60±0.29	1.64±0.42	7.50±0.51
Alc 4.5g/kg + Para 40mg/kg	1079±542.64	6.68±0.44	0.74±0.43	7.60±0.47
Alc 4.5g/kg + Para 400mg/kg	1073±237.69	6.40±0.12	0.69±0.16	7.38±0.05

MPV= Mean platelet volume, PDW= Platelet distribution width, Alc= Alcohol and Para= Paracetamol. There were non-significant ($p > 0.05$) variations between control and other treatments,

4.4 Effects of alcohol and paracetamol on biochemical parameters

4.4.1 Liver functions

Most of the treatments with alcohol or paracetamol were found not to affect the serum albumin levels. However, rats given 4.5 g/kg of alcohol, 400 mg/kg paracetamol, and 2.5 g/kg plus 400 mg/kg paracetamol were found to have significantly ($p < 0.05$) low levels of serum albumin (Table 4.4).

The total and direct serum bilirubin levels were significantly ($p < 0.05$) elevated in rats treated with 4.5 g/kg alcohol, 3.5 g/kg of alcohol plus 400 mg/kg of paracetamol, 4.5 g/kg of alcohol plus 40 mg/kg and 4.5 g/kg of alcohol plus 400 mg/kg of paracetamol. The rats treated with 400 mg/kg of paracetamol had direct serum bilirubin levels significantly ($p < 0.05$) elevated.

Gamma glutamyltransferase was significantly ($p < 0.05$) elevated in the groups treated with paracetamol 400 mg/kg, 3.5 g/kg of alcohol plus 400 mg/kg of paracetamol, 4.5 g/kg of alcohol plus 40 mg/kg of paracetamol and 4.5 g/kg of alcohol plus 400 mg/kg of paracetamol.

Serum profiles of enzyme aspartate aminotransferase (AST) was significantly ($p < 0.05$) elevated in rats treated with alcohol 3.5 g/kg and 4.5 g/kg, paracetamol 400 mg/kg, alcohol 2.5 g/kg plus paracetamol 400 mg/kg, 4.5 g/kg of alcohol plus 40 mg/kg paracetamol and 4.5 g/kg of alcohol plus 400 mg/kg (Table 4.4). However, for ALT, it was only elevated in rats treated with alcohol 4.5 g/kg.

The critical AST/ALT ratio was determined and it was observed that the control group had a value of 1.93 which was within the normal range of ≤ 2 (Figure 4.3). The group that was that treated with 40 mg/kg of paracetamol had normal ratio. All the other groups' ratios were above the normal values with the 4.5 g/kg of alcohol plus 400 mg/kg of paracetamol treated group recording a very high ratio of 3.23.

Table 4.4: Comparison of liver function tests of rats following treatment with alcohol and paracetamol.

Groups	ALB (mg/ml)	TBIL (µmol/L)	DBIL (µmol/L)	GGT (U/L)	AST (U/L)	ALT (U/L)
Control	41.8±3.90	2.49±0.60	0.73±0.34	0.94±0.18	193.6±21.65	104±26.08
Alc 2.5 g/kg	37.07±1.92	2.77±0.66	1.28±0.61	1.86±0.68	319.60±37.67	109.80±14.36
Alc 3.5 g/kg	37.05±2.24	3.19±0.71	1.79±0.40	2.14±0.66	337.80±34.97*	118.60±12.31
Alc 4.5 g/kg	31.77±3.26*	4.48±1.40*	3.06±0.91*	2.34±0.80	451.20±85.02*	160.20±29.02*
Para 40mg/kg	40.13±3.42	2.59±0.55	0.67±0.14	1.76±0.89	190.80±40.80	111.00±17.56
Para 400mg/kg	32.64±1.65*	3.23±0.65	2.21±0.51*	4.28±1.76*	357.20±33.69*	132.80±33.66
Alc 2.5 g/kg + Para 40mg/kg	35.70±2.23	2.47±0.17	096±0.46	2.06±1.02	249.40±42.32	104.60±11.41
Alc 2.5 g/kg + Para 400mg/kg	33.21±7.25*	3.25±0.35	1.35±0.19	2.30±1.11	332.80±75.80*	137.00±31.21
Alc 3.5 g/kg + Para 40mg/kg	40.32±6.73	3.25±0.25	1.58±0.19	3.32±1.73	324.60±29.75	125.60±12.69
Alc 3.5 g/kg + Para 400mg/kg	34.56±160	4.62±0.70*	2.00±0.68*	4.84±1.50*	321.40±106.70	136.60±33.89
Alc 4.5 g/kg + Para 40mg/kg	34.48±1.83	4.66±1.37*	2.77±0.74*	4.00±1.71*	378.00±52.92*	136.60±20.38
Alc 4.5 g/kg + Para 400mg/kg	34.08±3.75	4.76±1.02*	3.10±0.50*	4.44±1.21*	484.20±93.05*	149.20±15.32

There were significant variations between control and treatment groups ($p < 0.05$). ALB= Albumin, TBIL= Total bilirubin, DBIL= Direct, AST=Aspartate aminotransferase, ALT=Alanine aminotransferase, GGT=Gamma glutamyl transferase, Alc= Alcohol and Para = Paracetamol.

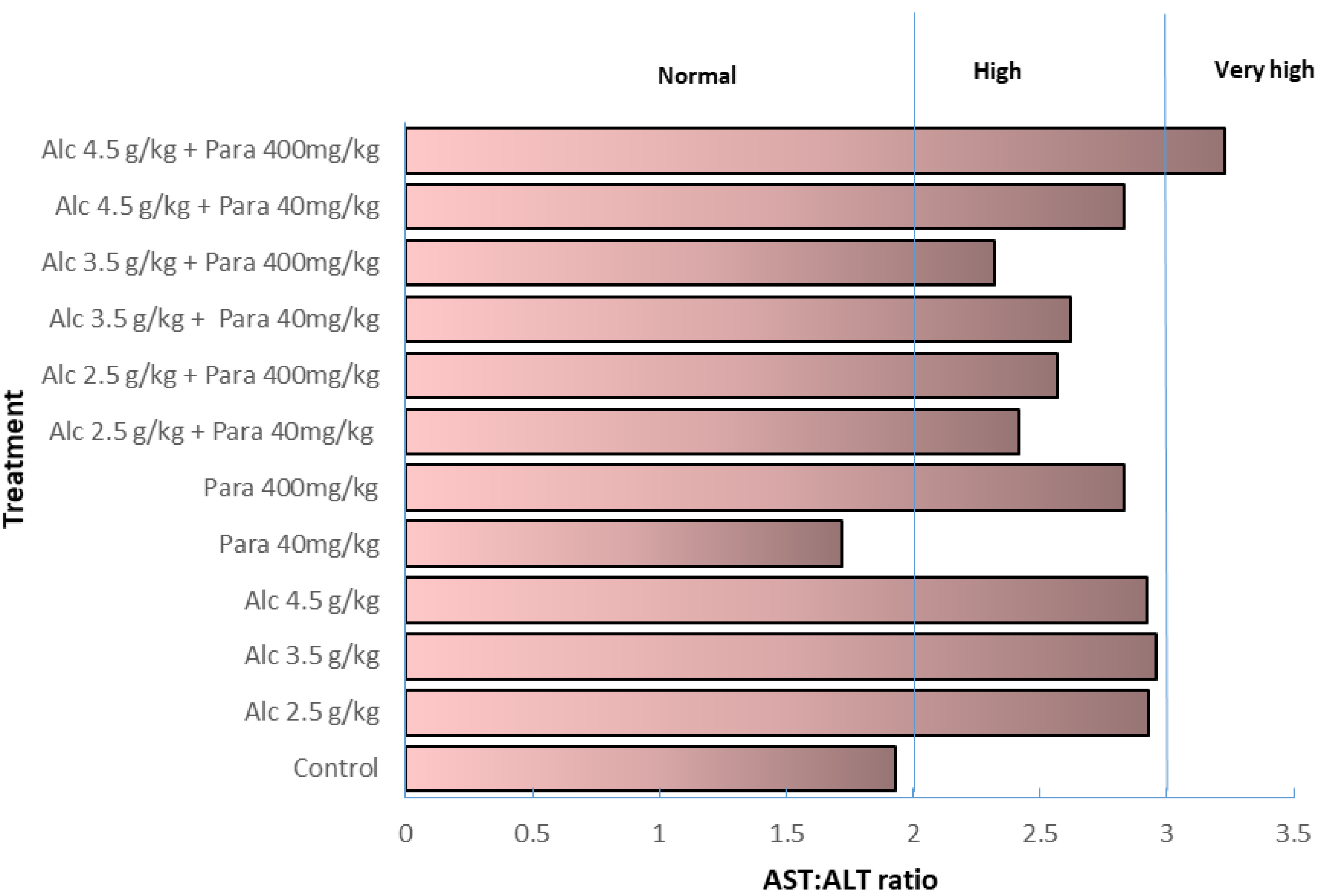


Figure 4.3: Effects of alcohol and paracetamol on the ratio of aspartate aminotransferase (AST) to alanine aminotransferase (ALT). Alc = alcohol; Para = paracetamol.

4.4.2 Kidney functions

Urea and creatinine levels in the control group were 8.71±1.54 mmol/L and 44.53±7.98 µmol/L respectively (Table 4.5). In the treatment groups, the levels of urea and creatinine were stable except in the group that was treated with 4.5 g/kg of alcohol where urea levels were significantly ($p < 0.05$) elevated to 11.25±0.80 mmol/L and in the groups treated with 4.5 g/kg of alcohol plus 400 mg/kg of paracetamol where urea and creatinine levels were significantly ($p < 0.05$) elevated to 11.56±1.12 mmol/L and 73.20±15.67 µmol/L respectively (Table 4.5).

Table 4.5: Effects of alcohol and paracetamol on urea and creatinine levels in a rat model.

Groups	Urea (mmol/L)	Creatinine (µmol/L)
Control	8.71±1.54	44.53±7.98
Alc 2.5 g/kg	10.47±1.06	46.94±12.50
Alc 3.5 g/kg	10.69±1.50	46.55±3.21
Alc 4.5 g/kg	11.25±0.80*	59.61±11.19
Para 40mg/kg	8.94±1.18	57.66±7.97
Para 400mg/kg	9.40±0.48	56.61±4.23
Alc 2.5 g/kg + Para 40mg/kg	9.24±1.00	54.06±11.19
Alc 2.5 g/kg + Para 400mg/kg	9.45±1.40	54.28±6.37
Alc 3.5 g/kg + Para 40mg/kg	9.87±1.63	48.17±9.34
Alc 3.5 g/kg + Para400mg/kg	11.06±0.73	49.28±6.45
Alc 4.5 g/kg + Para 40mg/kg	10.71±0.99	55.45±8.59
Alc 4.5 g/kg + Para 400mg/kg	11.56±1.12*	73.20±15.67*

There was significant difference ($p < 0.05$) between control and other treatments.
Alc= Alcohol, Para= Paracetamol.

4.4.3 Blood sugar levels

The glucose levels in rats treated with 3.5g/kg alcohol, 4.5 g/kg alcohol, 3.5 g/kg alcohol plus 40 mg/kg paracetamol and 4.5 g/kg of alcohol plus 40 mg/kg of paracetamol were significantly ($p < 0.05$) lower than those of the control group. In contrast, glucose levels were significantly ($p < 0.05$) elevated in groups treated with 2.5 g/kg of alcohol plus 400 mg/kg of paracetamol and 4.5 g/kg of alcohol plus 400 mg/kg of paracetamol (Figure 4.4).

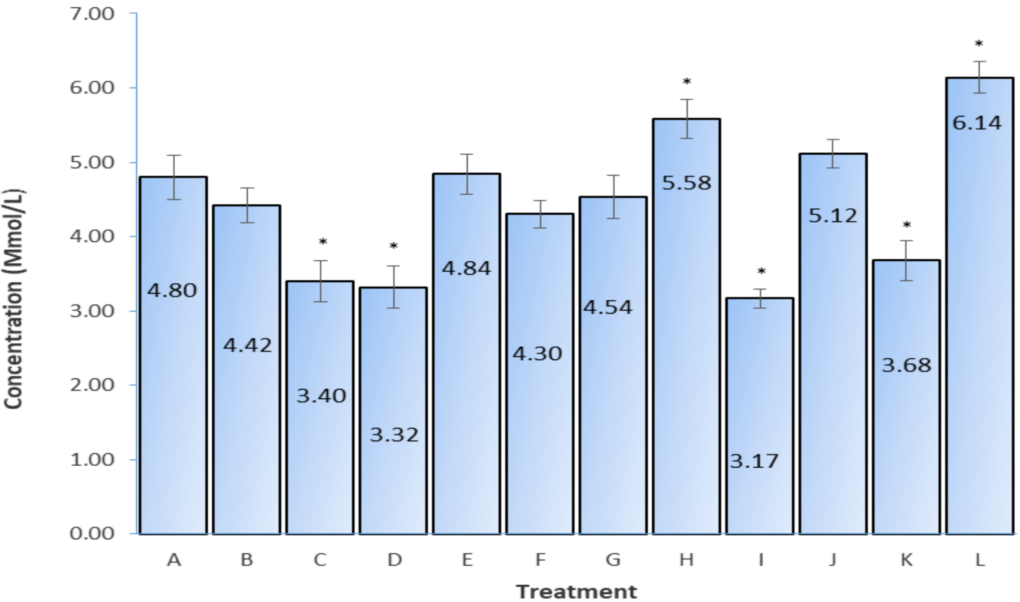


Figure 4.4: Glucose level in rats subjected to alcohol and paracetamol treatment.

The *p* value is for multiple comparisons between control and other treatments: * $p < 0.05$. Para = Paracetamol and Alc= Alcohol.

A = Control	G = Alcohol 2.5 g/kg + paracetamol 40 mg/kg
B = Alcohol 2.5 g/kg	H = Alcohol 2.5 g/kg + paracetamol 400 mg/kg
C = Alcohol 3.5 g/kg	I = Alcohol 3.5 g/kg + paracetamol 40 mg/kg
D = Alcohol 4.5 g/kg	J = Alcohol 3.5 g/kg + paracetamol 400 mg/kg
E = Paracetamol 40 mg/kg	K = Alcohol 4.5 g/kg +paracetamol 40 mg/kg
F = Paracetamol 400 mg/kg	L = Alcohol 4.5 g/kg +paracetamol 400 mg/kg

*

4.5 Effects of paracetamol and alcohol on rat tissues

4.5.1 Liver histopathology

Control group and those treated with 40 mg/kg paracetamol did not show tissue pathology. However, all other treatments showed cytoplasmic vacuolization and infiltration by inflammatory cells (Table 4.6). Steatosis and hepatocellular necrosis was observed in most treatment groups except those that received alcohol at doses of 2.5 and 3.5 g/kg (Table 4.6; Figure 4.5).

4.5.2 Kidney histopathology

Histological features of the control group indicated normal tissue devoid of pathology (Table 4.7; Figure 4.6). All treatment groups showed cellular infiltration and cytoplasmic vacuolization, but the latter was absent in rats that received 40 mg/kg of paracetamol. Rats that were treated with 400 mg/kg of paracetamol and combinations of alcohol and paracetamol had patches of cellular necrosis. While those treated with 400 mg/kg paracetamol, 2.5 g/kg alcohol plus 400 mg/kg paracetamol, 3.5 g/kg alcohol plus 400 mg/kg paracetamol and 4.5 g/kg alcohol plus 400 mg/kg paracetamol in addition had sinusoid formation (Table 4.7; Figure 4.6).

Table 4.6: Histological changes on liver of rats subjected to different treatment regimens of alcohol and paracetamol.

Groups	Cytoplasmic vacuolization	Infiltration of inflammatory cells	Steatosis	Hepatocellular necrosis
Control	-	-	-	-
Alc 2.5 g/kg	+	+	-	-
Alc 3.5 g/kg	+	+	-	-
Alc 4.5 g/kg	+	+	+	+
Para 40 mg/kg	-	-	-	-
Para 400 mg/kg	+	+	+	+
Alc 2.5 g/kg + Para 40 mg/kg	+	+	+	+
Alc 2.5 g/kg + Para 400 mg/kg	+	+	+	+
Alc 3.5 g/kg + Para 40 mg/kg	+	+	+	+
Alc 3.5 g/kg + Para 400 mg/kg	+	+	+	+
Alc 4.5 g/kg + Para 40 mg/kg	+	+	+	+
Alc 4.5 g/kg + Para 400 mg/kg	+	+	+	+

Alc= Alcohol and Para= Paracetamol

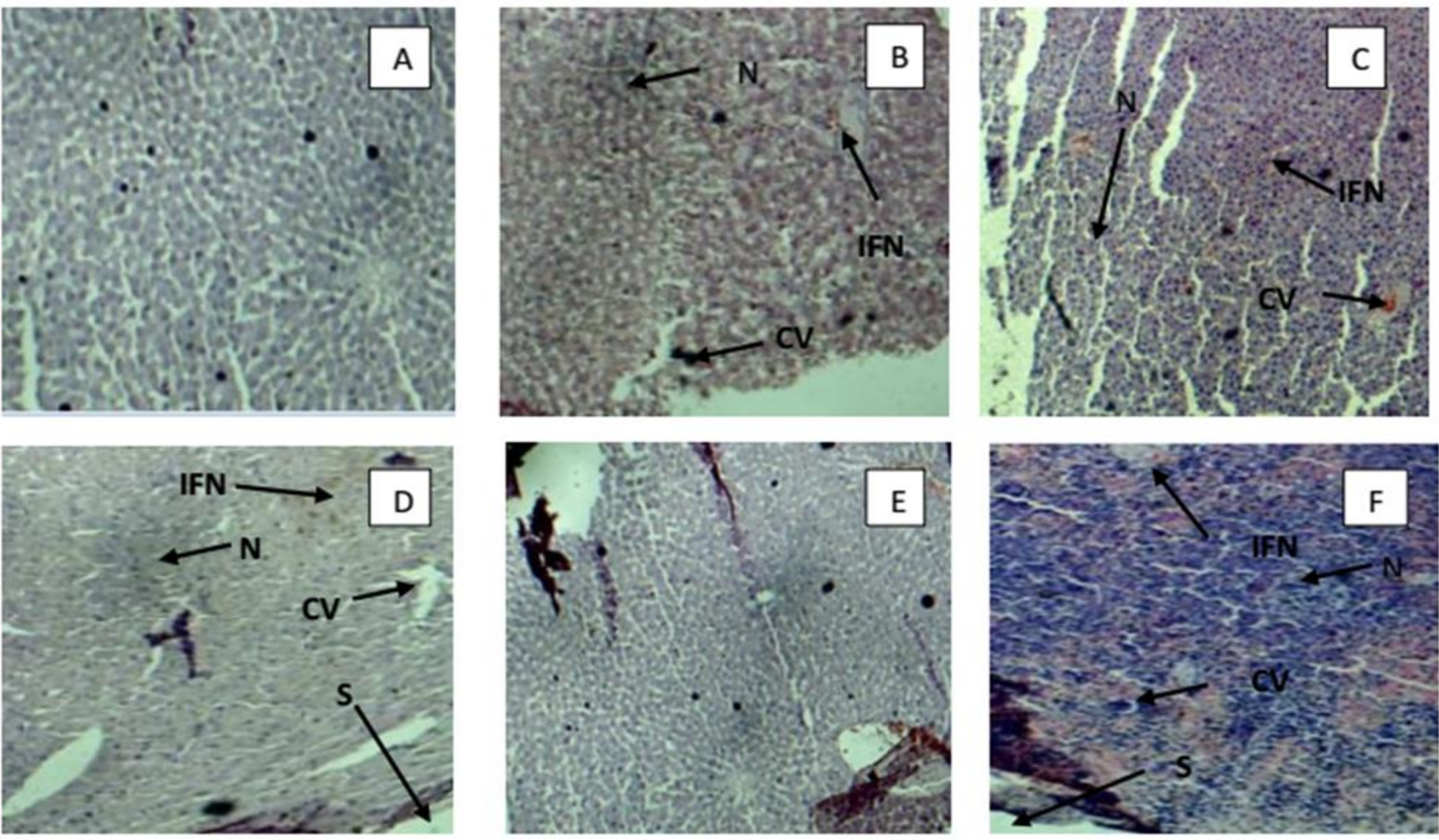


Figure 4.5: Liver photomicrographs showing the morphological changes induced by alcohol and paracetamol: (A) Control, (B) Alcohol 2.5 g/kg, (C) Alcohol 3.5 g/kg, (D) Alcohol 4.5 g/kg, (E) Paracetamol 40 mg/kg, (F) Paracetamol 400 mg/kg. Labelled regions indicate changed morphology as marked (CV; cell vacuolization, S; steatosis, IFN; infiltration, N; necrosis). Magnification X400.

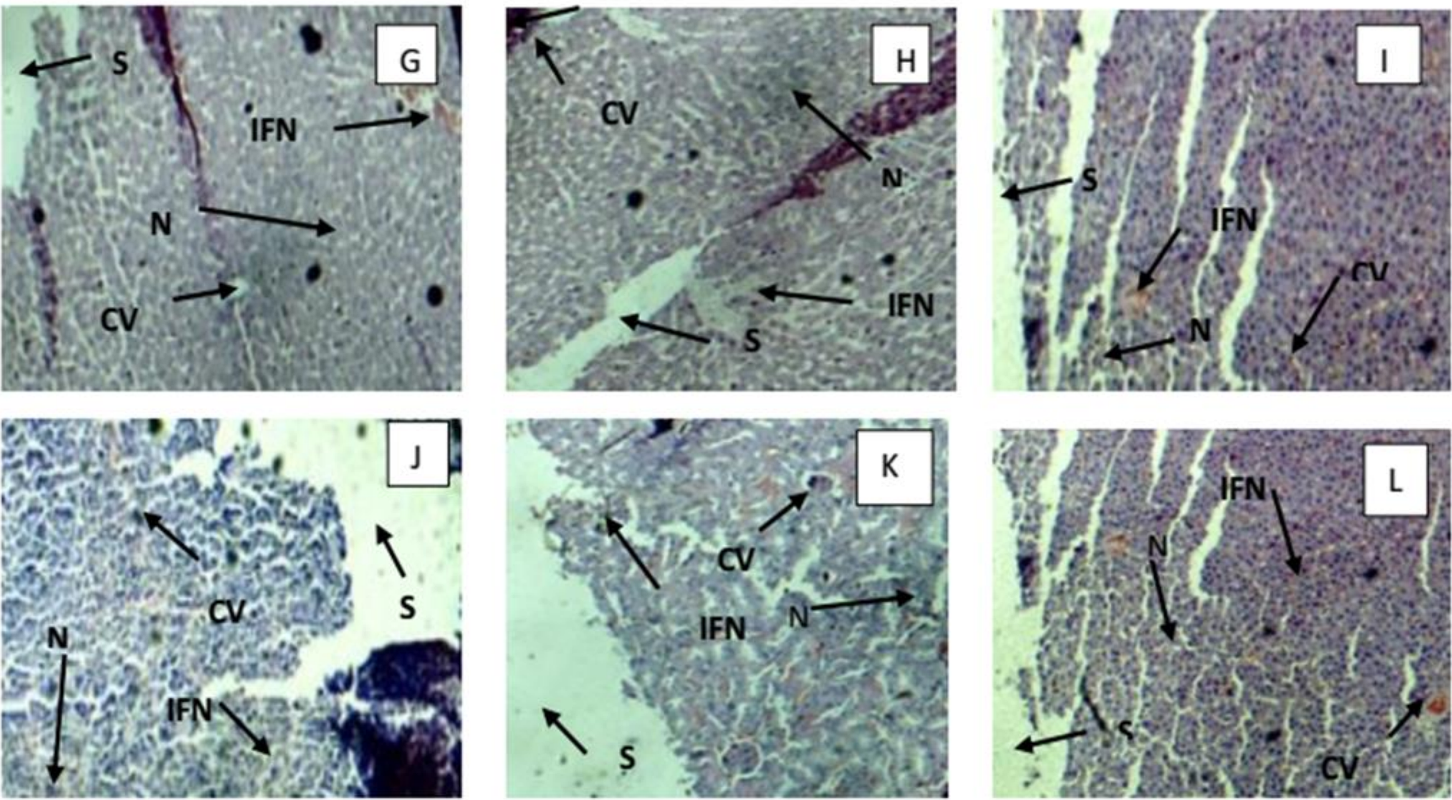


Figure 4.5: Liver photomicrographs showing the morphological changes induced by alcohol and paracetamol: (G) Alcohol 2.5 g/kg + paracetamol 40 mg/kg, (H) Alcohol 2.5 g/kg + paracetamol 400 mg/kg, (I) Alcohol 3.5 g/kg + paracetamol 40 mg/kg, (J) Alcohol 3.5 g/kg + paracetamol 400 mg/kg, (K) Alcohol 4.5 g/kg + paracetamol 40 mg/kg, (L) Alcohol 4.5 g/kg + paracetamol 400 mg/kg. Labelled regions indicate changed morphology as marked (CV; cell vacuolization, S; steatosis, IFN; infiltration, N; necrosis). Magnification X400

Table 4.7: Histological changes on kidney of rats subjected to different treatment regimens of alcohol and paracetamol.

Groups	Cytoplasmic vacuolization	Infiltration of inflammatory cells	Sinusoid formation	Necrosis
Control	-	-	-	-
Alc 2.5 g/kg	+	+	-	-
Alc 3.5 g/kg	+	+	-	-
Alc 4.5 g/kg	+	+	-	-
Para 40 mg/kg	-	+	-	-
Para 400 mg/kg	+	+	+	+
Alc 2.5 g/kg + Para 40mg/kg	+	+	-	+
Alc 2.5 g/kg + Para 400mg/kg	+	+	+	+
Alc 3.5 g/kg + Para 40mg/kg	+	+	-	+
Alc 3.5 g/kg + Para 400mg/kg	+	+	+	+
Alc 4.5 g/kg + Para 40 mg/kg	+	+	-	+
Alc 4.5 g/kg + Para 400 mg/kg	+	+	+	+

Alc= Alcohol, Para= Paracetamol

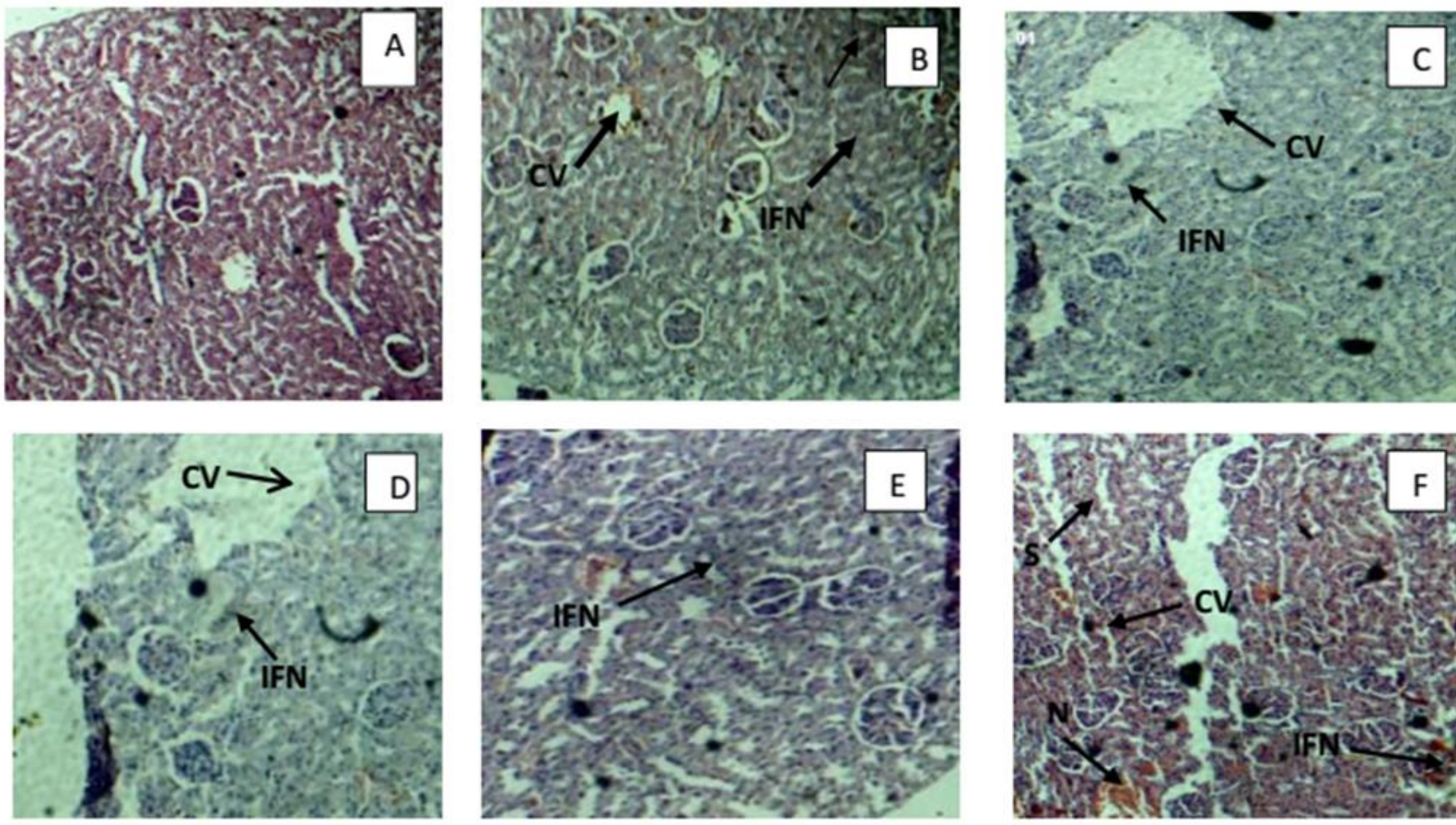


Figure 4.6: Kidney photomicrographs the morphological changes induced by different treatments: (A) Control, (B) Alcohol 2.5 g/kg, (C) Alcohol 3.5 g/kg, (D) Alcohol 4.5 g/kg, (E) Paracetamol 40 mg/kg, (F) Paracetamol 400 mg/kg. Marked regions indicate regions with changed morphology (S; sinusoids, CV; cell vacuolization, IFN; infiltration and N; necrosis). Magnification X400.

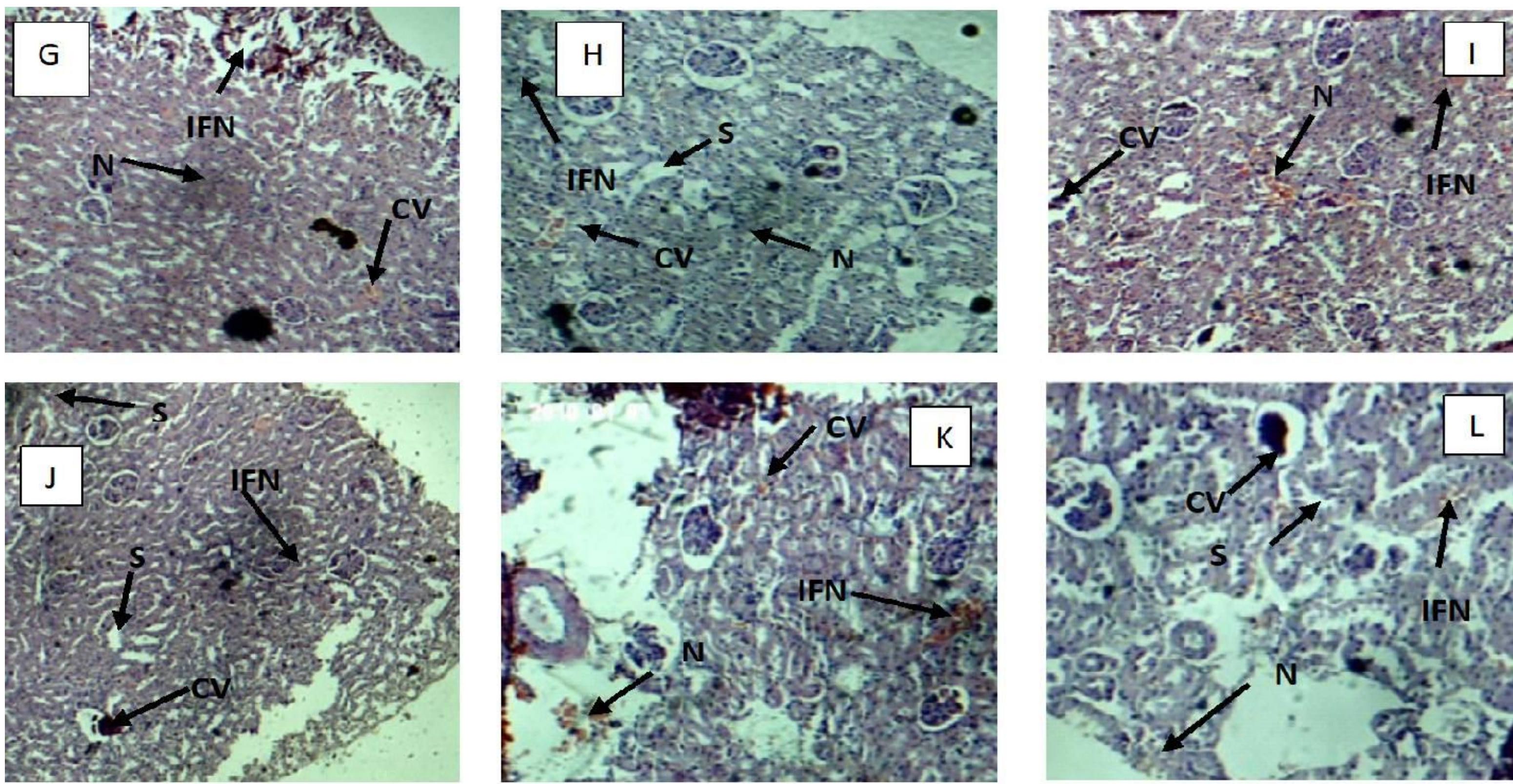


Figure 4.6: Kidney photomicrographs the morphological changes induced by different treatments: (G) Alcohol 2.5 g/kg + paracetamol 40 mg/kg, (H) Alcohol 2.5 g/kg + paracetamol 400 mg/kg, (I) Alcohol 3.5 g/kg + paracetamol 40 mg/kg, (J) Alcohol 3.5 g/kg + paracetamol 400 mg/kg, (K) Alcohol 4.5 g/kg + paracetamol 40 mg/kg, (L) Alcohol 4.5 g/kg + paracetamol 400 mg/kg. Marked regions indicate regions with changed morphology (S; sinusoids, CV; cell vacuolization, IFN; infiltration and N; necrosis). Magnification X400.

CHAPTER FIVE

DISCUSSION, CONCLUSIONS, RECOMMENDATIONS AND FUTURE RESEARCH

5.1 Discussion

This study compared the effects of individual and combined treatments of alcohol and paracetamol on the biological systems of a rat model. Individually, alcohol (≥3.5g/kg) and paracetamol (400 mg/kg) caused listlessness, rough hair coat and poor appetite in rats. These results are consistent with those of Ning *et al*. (2012) and Juma *et al.* (2015). Review of literature shows no information on the effects of combined use of alcohol and paracetamol on physical features of animal model. When the two drugs were used together in this study, they had similar effects on morphological features as the individual treatments. Factors that affect morphological characteristics of rodents include climate, sickness, distress and pain (Evans and Maltby, 1989; Lee *et al*., 2003; Mayer, 2007; Feng and Himsworth, 2014). Here, the subtle changes in the physical features of the study animals is likely due the effects of the drugs.

Multiple comparisons of the control and treated groups showed that initially alcohol had insignificant effect on body weight but on the fourth week there was significant weight gain. Previous studies on the impact of alcohol on bodyweight are contentious with some reports showing a positive correlation (Arif *et al.*,

2005; Aguiar *et al.,* 2009) and others an inverse association (French *et al.,* 2010; Macdonald *et al*., 2010). The weight gain reported here is indicative of positive energy balance due to the calorific effect of ethanol (Wang *et al.,* 2010). The mechanism through which alcohol may modify energy balance and subsequently body weight, include impact on nutrient digestion and absorption, interference with lipid oxidation, thermogenesis, and enhanced adenosine triphosphate breakdown (Lieber, 1991; Suter and Tremblay, 2005; Wang *et al.,* 2010).

Paracetamol also had an insignificant effect on the body weight except for the 40 mg/kg group that recorded weight gain in week four. This is a new finding for the drug and further research is required to establish whether the weight gain is drug related or an isolated event. When administered together alcohol and paracetamol showed initial weight loss that was reversed to weight gain in week four. This implies that simultaneous use of the drugs can result either an energy surplus or deficit, and the former is due to the effects of alcohol.

In the current study, alcohol and paracetamol had no significant effect on the hematological profile. Previous studies on the same show inconsistent findings with alcohol reporting increase (Geidam *et al.*, 2004) who used 1ml/100g body weight of 30% alcohol, decrease (Das and Vasudevan, 2005; Onyango *et al.*, 2017) who used 1.14g/kg and 5g/kg body weight respectively or no effect (Stanly *et al.*, 2003; Oyedeji *et al.*, 2013) who used 2.4g/kg and 10mg/kg body weight of

alcohol respectively. Similar mixed results have also been observed with paracetamol (Payasi *et al*., 2010; Senthilkumar *et al*., 2014; Dwivedi *et al.,* 2015; Samuel *et al*., 2015; Guiloski *et al*., 2017) who used 16.6, 33.6 and 66.6 mg/kg, 2000mg/kg, 900mg/kg body weight, 300mg and 250mg of paracetamol respectively. Disagreement in results between studies could be attributed to factors such as the amount and duration of alcohol treatment and the experimental model employed. Changes in hematological profiles is an indication of interference with the bone marrow and the immune system. It is therefore concluded that alcohol and paracetamol may not have influenced the activity of the two systems.

The most important clinical manifestation of chronic alcohol abuse is alcoholic liver disease. The condition is characterized by steatosis, hepatitis and cirrhosis (Seitz and Bucker, 2007). Due to cellular damage, elevated liver enzymes is a common scenario among patients suffering from liver disease (Marsano *et al.,* 2003; Hyder *et al.,* 2013; Torruellas *et al.,* 2014). In this study, AST was high in animals treated with 3.5 g/kg alcohol while those given 4.5 g/kg had elevated AST and ALT. The elevated enzymes, reduced albumin, and increased bilirubin is an indication of liver injury, a phenomenon that was supported by histopathological data. These results reaffirms that alcohol is hepatotoxic and the effect was dose dependent. Interestingly, AST/ALT ratio was higher than the cut off value of 2 in all alcohol treatments. This implies that the index is a more

predictive biomarker tool for alcohol exposure and hepatic injury. A result also consistent with the histopathology results of the liver sections.

At 400 mg/kg dose, paracetamol caused elevation of GGT and AST. The fact that GGT was only elevated with the high dose of paracetamol and not alcohol is an indication that the former was more hepatotoxic. This could be due to differences in the LD_{50} and mechanisms of toxicity of the drugs. For alcohol, the LD_{50} in rat is 7060 mg/kg (LHS, 2004) and hepatocytes injury is caused by oxidative stress due to enhanced generation of reactive oxygen species and depletion of antioxidant defense system (Schlorff *et al.,* 1999; Neuman *et al.,* 2015). For paracetamol the LD_{50} is >4000 mg/kg (Huynh, 2009) and cellular damage is due to lipid peroxidation induced by the reactive metabolite NAPQI (Song *et al.,* 2004).

When alcohol and paracetamol were administered separately at 4.5 g/kg and 40 mg/kg, respectively, the GGT levels were normal, but the enzyme levels were elevated when the drugs were administered together. Besides, that 4.5 g/kg of alcohol and 400 mg/kg of paracetamol, had AST/ALT ratio that was >3, a value that is indicative of advanced liver injury (Nyblom *et al.,* 2004). These results show that combined use of paracetamol and alcohol exhibited more extensive liver damage and this was supported by histopathological analysis findings. Toxicity due to the combined use of the drugs can be attributed to the fact that

alcohol reduces glutathione content thus reducing the margin of safety of paracetamol (Weathermon and Crabb, 1999). This means that chronic and excess use of paracetamol in management of hangover among heavy users of alcohol can increase the risk of liver disease. On the other hand moderate dosages of the drugs are well tolerated and they therefore exhibit a lower risk of liver disease.

Assessment of renal function showed that paracetamol did not affect serum urea and creatinine but 4.5 g/kg alcohol caused uraemia. High blood urea is a consequence of several conditions such as kidney disease, blocked urinary tract, high protein diets, congestive heart failure and dehydration (Gotsman *et al.,* 2010; Gowda *et al.,* 2010; Schwingshackl and Hoffmann, 2014; Lopez-Giacoman and Madero, 2015). In this experimental model, it is reasonable to conclude that the uraemia is pathologically linked to the alcohol induced renal injury.

Studies on concurrent use of alcohol and paracetamol are controversial. Some reports suggest that paracetamol at therapeutic doses is safe and effective even in chronic alcoholics (Kuffner *et al.,* 2001, Graham *et al*., 2005). However, other indicate that the drug can cause kidney diseases in patients with alcohol dependency (Blakely and McDonald, 1995; Dart *et al*., 2000). In the current work simultaneous use of the two alcohol and paracetamol resulted in significant elevation of urea and creatinine in animals treated with 4.5 g/kg of alcohol and 400 mg/kg of paracetamol. Histopathological results showed more renal

pathology when both drugs were used together than when used individually. Increased toxicity can be explained from the observation that interaction of alcohol and paracetamol result in the increased production of NAPQI, the highly toxic metabolite of paracetamol (Łukasik-Głebocka and Klimaszyk, 2001).These result therefore suggest that use of high dosages of paracetamol (400mg/kg) among chronic users of alcohol increases the risk of kidney disease.

Experimental animals treated with paracetamol had normal levels of blood glucose, while those given 3.5 g/kg and 4.5 g/kg of alcohol displayed low sugar levels. Depending on the circumstance alcohol can cause hypoglycaemia (Liu *et al.,* 2008) or hyperglycaemia (Athyros *et al.,* 2008) and this is because of its influence over insulin and glucagon, both of which are the hormones involved in glucose counter-regulation (Kolaczynski *et al*., 1988).

When alcohol and paracetamol were given together there was no clear pattern on their effect on blood sugar as levels were either normal, low or high. Although information on the combined effect of the drugs on blood sugars is limited, reports on individual drugs indicate contrasting results. Liu *et al.* (2008) reported that low to moderate doses of alcohol have a protective effect against diabetes, while Vonghia *et al.* (2008) found that acute alcohol intoxication can elevate blood glucose. For paracetamol the drug can normalize increased blood glucose (Shertzer *et al.,* 2008; Jambulingappa *et al.,* 2012) but it is also associated with

hyperglycemia (Yang *et al.,* 2001). This study and others shows that the effects of alcohol or paracetamol on blood sugar is influenced by factors such as dosage and experimental design. This work further suggests that neither of the drugs has a predominant effect over the other in modulating blood glucose.

5.2 Conclusions

i) Alcohol and paracetamol individually or combined did not affect the hematological profile of rats.

ii) In moderate doses paracetamol is safe, but high doses of the drug and chronic use of alcohol is hepatotoxic. In combination, the drugs increased the risk of liver injury.

iii) Individually, alcohol and paracetamol have a low risk of renal damage but when used together the risk is increased.

5.3 Recommendations

i) Paracetamol at a dose of 40 mg/kg is safe. However, a dose of 400 mg/kg is toxic and can cause liver and kidney damage.

ii) Caution to be taken against routine use of paracetamol among heavy users of alcohol as there is increased risk of hepatic and renal diseases.

5.4 Future Research

i) To perform dose response experiments to clarify whether combined use of alcohol and paracetamol causes positive or negative energy balance.

ii) To conduct experiments to establish the sensitivity range of the AST/ALT index as a biomarker tool for hepatic injury in heavy alcohol users.

REFERENCES

Abdel-Zaher, A. O., Abdel-Rahman, M. M., Hafez, M. M. and Omran, F. M. (2007). Role of nitric oxide and reduced glutathione in the protective effects of aminoguanidine, gadolinium chloride and oleanolic acid against acetaminophen-induced hepatic and renal damage. *Toxicology*, 234: 124-134.

Abraham, P. (2005). Oxidative stress in paracetamol-induced pathogenesis: (I) Renal damage.

Addolorato, G., Capristo, E., Leggio, L., Ferrulli, A., Abenavoli, L., Malandrino, N. and Mirijello, A. (2006). Relationship between ghrelin levels, alcohol craving, and nutritional status in current alcoholic patients. *Alcoholism: Clinical and Experimental Research*, 30: 1933-1937.

Adedapo, A. A., Abatan, M. O. and Olorunsogo, O. O. (2007). Effects of some plants of the spurge family on haematological and biochemical parameters in rats. *Veterinarski arhiv 77*: 29-38.

Adeniyi, S. A., Orjiekwe, C. L., Ehiagbonare, J. E. and Arimah, B. D. (2010). Preliminary phytochemical analysis and insecticidal activity of ethanolic extracts of four tropical plants (Vernonia amygdalina, Sida acuta, Ocimum gratissimum and Telfaria occidentalis) against beans weevil (Acanthscelides obtectus). *International Journal of Physical Sciences*, 5: 753-762.

Adzu, B., Garba, M., Haruna, A., Maman, M., Wambebe, C. and Gamaniel, K. (2001). Effect of Niprisan® on single oral dose pharmacokinetics of paracetamol in rats. *European journal of drug metabolism and pharmacokinetics*, 26: 201-204.

Aguiar A.S.D. Boaventura G. Abrahao R.F. Freitas T.L. Takiya C.M. Filho P.J.S. and SilvaV.A.D. (2009). Ethanol in low chronic dosage levels attenuates major organic effects in malnourished rats. *Biological Research Journal,* 42: 31-40.

Akriviadis, E., Botla, R., Briggs, W., Han, S., Reynolds, T. and Shakil, O. (2000). Pentoxifylline improves short-term survival in severe acute alcoholic hepatitis: a double-blind, placebo-controlled trial. *Gastroenterology*, 119: 1637-1648.

Albano, E. (2006). Alcohol, oxidative stress and free radical damage. *Proceedings of the nutrition society*, 65: 278-290.

Ali, B. H., Ismail, T. B. and Bashir, A. A. (2001). Sex difference in the susceptibility of rats to gentamicin nephrotoxicity: Influence of gonadectomy and hormonal replacement therapy. *Indian Journal of Pharmacology*, 33: 369-373.

Aminoff, M. J. (2007). Neurology and General Medicine. 4th. Ed.*: Churchill Livingstone*.

Anderson, B.J., Hollford, N.H.G. and Woolard, G.A. (1998). Paracetamol plasma and cerebrospinal fluid pharmacokinetic in children. *British Journal of Clinical Pharmacology,* 46: 237-243.

Anderson, B. J. (2008). Paracetamol (Acetaminophen): Mechanisms of action. *Pediatric Anesthesia*, 18: 915-921.

Andreozzi, R., Caprio, V., Marotta, R. and Vogna, D. (2003). Paracetamol oxidation from aqueous solutions by means of ozonation and H_2O_2/UV system. *Water research*, 37: 993-1004.

Arif, A.A. and Rohrer, J.E. (2005). Patterns of alcohol drinking and its association with obesity: data from the Third National Health and Nutrition Examination Survey, 1988-1994. *BMC Public Health*, 5:126.

Arteel, G. E. (2003). Oxidants and antioxidants in alcohol-induced liver disease. *Gastroenterology*, 124: 778-790.

Athyros, V. G., Liberopoulos, E. N., Mikhailidis, D. P., Papageorgiou, A. A., Ganotakis, E. S., Tziomalos, K. and Elisaf, M. (2008). Association of drinking pattern and alcohol beverage type with the prevalence of metabolic syndrome, diabetes, coronary heart disease, stroke, and peripheral arterial disease in a Mediterranean cohort. *Angiology*, 58: 689-697.

Baliunas D.O., Taylor B.J. and Irving H. (2009). Alcohol as a risk factor for type 2 diabetes a systematic review and meta-analysis. *Diabetes Care,* 32: 2123–32.

Bekheet, S. H. (2010). Morphine sulphate induced histopathological and histochemical changes in the rat liver. *Tissue and Cell*, 42: 266-272.

Beutler, E. (1994). Glucose-6-phosphate dehydrogenase deficiency. *Blood*, 84: 3613-3636.

Bhanwra, S., Singh, J. and Khosla, P. (2000). Effect of Azadirachta Ondica (Neem) leaf aqueous extract on paracetamol-induced liver damage in rats. *Indian journal of physiology and pharmacology*, 44: 64-68.

Bilgin, H. M., Atmaca, M., Obay, B. D., Özekinci, S., Taşdemir, E. and Ketani, A. (2011). Protective effects of coumarin and coumarin derivatives against carbon tetrachloride-induced acute hepatotoxicity in rats. *Experimental and toxicologic pathology*, 63: 325-330.

Bjornsson, T. D., Callaghan, J. T., Einolf, H. J., Fischer, V., Gan, L., Grimm, S. and Kumar, G. (2003). The conduct of in vitro and in vivo drug-drug interaction studies: a Pharmaceutical Research and Manufacturers of America (PhRMA) perspective. *Drug Metabolism and Disposition*, 31: 815-832.

Blakely, P., and McDonald, B. R. (1995). Acute renal failure due to acetaminophen ingestion: a case report and review of the literature. *Journal of the American Society of Nephrology*, 6: 48-53.

Blazka, M. E., Elwell, M. R., Holladay, S.D., Willson, R. E. and Luster, M. I. (1996). Histopathology of acetaminophen-induced liver changes: role of interleukin 1α and tumor necrosis factor α. *Toxicologic Pathology,* 24: 181-189.

Blough, E. and Wu, M. (2011). Acetaminophen: beyond pain and fever-relieving. *Frontiers in pharmacology*, 2: 72.

Bondy, S.C. (1992). Ethanol toxicity and oxidative stress. *Toxicology Letter,* 63: 231–242.

Botting, R. and Ayoub, S. S. (2005). COX-3 and the mechanism of action of paracetamol/acetaminophen. *Prostaglandins, leukotrienes and essential fatty acids*, 72: 85-87.

Boutis, K. and Shannon, M. (2001). Nephrotoxicity after acute severe acetaminophen poisoning in adolescents. *Journal of Toxicology: Clinical Toxicology*, 39: 441-445.

Bower, W. A., Johns, M., Margolis, H. S., Williams, I. T. and Bell, B. P. (2007). Population-based surveillance for acute liver failure. *The American Journal of Gastroenterology,* 102: 2459-2463.

Brayfield, A. (2014). Paracetamol. Martindale*: The Complete Drug Reference*. London, UK: Pharmaceutical Press.

Buckley, N. A., Buckley, N., Whyte, I. M., O'Connell, D. L. and Dawson, A. H. (1999). Oral or intravenous N-acetylcysteine: which is the treatment of choice for acetaminophen (paracetamol) poisoning? *Journal of Toxicology: Clinical Toxicology*, 37: 759-767.

Bujalska, M. (2004). Effect of nitric oxide synthase inhibition on antinociceptive action of different doses of acetaminophen. *Pharmacological Reports*, 56: 605-610.

Castro, L. A. and Baltieri, D. A. (2004). The pharmacologic treatment of the alcohol dependence. *Revista Brasileira de Psiquiatria*, *26*: 43-46.

Charlton, M. E., Sweetnam, P. M., Fitzgerald, L. W., Terwilliger, R. Z., Nestler, E. J. and Duman, R. S. (1997). Chronic ethanol administration regulates the expression of GABAA receptor α1 and α5 subunits in the ventral tegmental area and hippocampus. *Journal of neurochemistry*, 68: 121-127.

Chung, F. M., Yang, Y. H., Shieh, T. Y., Shin, S. J., Tsai, J. C. R., and Lee, Y. J. (2005). Effect of alcohol consumption on estimated glomerular filtration rate and creatinine clearance rate. *Nephrology Dialysis Transplantation*, 20: 1610-1616.

Clark, J. D., Gebhart, G. F., Gonder, J. C., Keeling, M. E. and Kohn, D. F. (1997). The 1996 guide for the care and use of laboratory animals. *ILAR journal*, 38: 41-48.

Clark, J. M., Brancati, F. L. and Diehl, A. M. (2003). The prevalence and etiology of elevated aminotransferase levels in the United States. *The American Journal of Gastroenterology*, 98: 960-967.

Corrao, G., Rubbiati, L., Bagnardi, V., Zambon, A. and Poikolainen, K. (2000). Alcohol and coronary heart disease: a meta-analysis. *Addiction,* 95: 1505-1523.

Costardi, J. V. V., Nampo, R. A. T., Silva, G. L., Ribeiro, M. A. F., Stella, H. J., Stella, M. B. and Malheiros, S. V. P. (2015). A review on alcohol: from the central action mechanism to chemical dependency. *Revista da associação médica brasileira*, *61*(4), 381-387.

Daly, F.S., Fountain, J.S., Murray, L., Graudins, A. and Buckley, N.A. (2008). Guidelines for the management of paracetamol poisoning in Australia and New Zealand-explanation and elaboration. *Medical Journal of Australia,* 188(5).

Dart, R. C., Kuffner, E. K., and Rumack, B. H. (2000). Treatment of pain or fever with paracetamol (acetaminophen) in the alcoholic patient: a systematic review. *American Journal of Therapeutics*, 7: 123-134.

Das, S.K. and Vasudevan, D.M. (2005). Biochemical diagnosis of alcoholism. *Indian Journal of Clinical Biochemistry*, 81: 177–187.

Das, S.K. and Vasudevan, D.M. (2007). Alcohol-induced oxidative stress. *Life Sciences*, 20: 35–42.

Das, S. K., Varadhan, S., Dhanya, L., Mukherjee, S., and Vasudevan, D. M. (2008). Effects of chronic ethanol exposure on renal function tests and oxidative stress in kidney. *Indian Journal of Clinical Biochemistry*, 23: 341-344.

Davies, M. (2003). The role of GABAA receptors in mediating the effects of alcohol in the central nervous system. *Journal of Psychiatry and Neuroscience*.

De Marchi, S., Cecchin, E., Basile, A., Bertotti, A., Nardini, R. and Bartoli, E. (1993). Renal tubular dysfunction in chronic alcohol abuse--effects of abstinence. *New England Journal of Medicine*, 329: 1927-1934.

Dhaliwal, G., Cornett, P. A. and Tierney Jr, L. M. (2004). Hemolytic anemia. *American Family Physician,* 69: 2599-2606

Di Pierro, F.and Rossoni, G. (2013). An amino acids mixture improves the hepatotoxicity induced by acetaminophen in mice. *Journal of Amino Acids*, *2013*.

Dodd, S., Dean, O., Copolov, D. L., Malhi, G. S. and Berk, M. (2008). N-acetylcysteine for antioxidant therapy: pharmacology and clinical utility. *Expert opinion on biological therapy*, 8: 1955-1962.

Dwivedi, V., Mishra, J. and Shrivastava, A. (2015). Efficacy study of livartho against paracetamol induced hepatotoxicity in adult Sprague Dawley rats. *Journal of Drug Metabolism and Toxicology*, 5: 175-181.

Eggert, J., Theobald, H. and Engfeldt, P. (2004). Effects of alcohol consumption on female fertility during an 18-year period. *Fertility and sterility*, 81: 379-383.

Eguia, L., and Materson, B. J. (1997). Acetaminophen-Related Acute Renal Failure without Fulminant Liver Failure. *Pharmacotherapy: The Journal of Human Pharmacology and Drug Therapy*, 17: 363-370.

Elenkov, I. J., Papanicolaou, D. A., Wilder, R. L. and Chrousos, G. P. (1996). Modulatory effects of glucocorticoids and catecholamines on human interleukin-12 and interleukin-10 production: clinical implications. *Proceedings of the Association of American Physicians*, 108: 374.

Erdőhelyi, A., Raskó, J., Kecskés, T., Tóth, M., Dömök, M., & Baán, K. (2006). Hydrogen formation in ethanol reforming on supported noble metal catalysts. *Catalysis Today*, 116: 367-376.

Evans, E. I. and Maltby, C. J. (Eds.). (1989). Technical laboratory animal management. MTM Associates.

Evers, S. and Marziniak, M. (2010). Clinical features, pathophysiology, and treatment of medication-overuse headache. *The Lancet Neurology*, 9: 391-401.

Farghali, H., Kmoníčková, E., Lotkova, H. and Martinek, J. (2000). Hepatoprotective Agents in Oxidative Stress Injury of Perfused Hepatocytes. *Physiological Research*, 49: 261-268.

Feng, A. Y. T. and Himsworth, C. G. (2014). The secret life of the city rat: a review of the ecology of urban Norway and black rats (Rattus norvegicus and Rattus rattus). *Urban Ecosystem,* 17:149–162.

Ferreira, M. P. and Willoughby, D. (2007). Alcohol consumption: the good, the bad, and the indifferent. *Applied Physiology, Nutrition, and Metabolism*, 33: 12-20.

Food and Drug Administration. (2011). Prescription drug products containing acetaminophen; actions to reduce liver injury from unintentional overdose. *Federal Register*, 76: 2691-2697.

Foye, W. O. (1995). Cancer chemotherapeutic agents. *American Chemical Society.*

Fraser, A. G. (1997). Pharmacokinetic interactions between alcohol and other drugs. *Clinical Pharmacokinetics*, 33: 79-90.

French, M. T., Norton, E. C., Fang, H. and Maclean, J. C. (2010). Alcohol consumption and body weight. *Health Economics*, 19: 814-832.

Friedman, S. L. (2008). Mechanisms of hepatic fibrogenesis. *Gastroenterology*, 134: 1655-1669.

Ganey, P.E., Luyendyk, J.P., Newport, S.W., Eagle, T.M., Madox, J.F., Mackmay, N. and Roth, R.A. (2007). Role of the coagulation system in acetaminophen-induced hepatotoxicity in mice. *Hepatology*, 46: 1177-1186.

Gao, Y., Chu, S. F., Xia, C. Y., Zhang, Z., Zhang, S. and Chen, N. H. (2016). Rg1 attenuates alcoholic hepatic damage through regulating AMP-activated protein kinase and nuclear factor erythroid 2-related factor 2 signal pathways. *Journal of Asian Natural Products Research*, 18: 765-778.

Garba, S. H., Sambo, N. and Bala, U. (2009). The effect of the aqueous extract of Kohautia grandiflora on paracetamol induced liver damage in albino rats. *Nigerian Journal of Physiological Sciences*, 24.

Geidam, M. A., Pakmam, I. and Laminu, H. (2004). Effects of aqueous stem bark extract of Momordica balsamina Linn on serum electrolytes and some hematological parameters in normal and alcohol fed rats. *Pakistan Journal of Biological Sciences 7*: 1430-1432.

Gelotte, C. K., Auiler, J. F., Lynch, J. M., Temple, A. R. and Slattery, J. T. (2007). Disposition of acetaminophen at 4, 6 and 8g/ day for 3 days in healthy young adults. *Clinical Pharmacology and Therapeutics,* 81: 840-848.

Gentry, R. T. (2000). Effect of food on the pharmacokinetics of alcohol absorption. *Alcoholism: Clinical and Experimental Research*, 24: 403-404.

George, A. and Figueredo, V. M. (2010). Alcohol and arrhythmias: a comprehensive review. *Cardiology Faculty Papers,* 11: 221-228.

Ghoneim, F. M., Khalaf, H. A., Elsamanoudy, A. Z. and Helaly, A. N. (2014). Effect of chronic usage of tramadol on motor cerebral cortex and testicular tissues of adult male albino rats and the effect of its withdrawal: histological, immunohistochemical and biochemical study. *International Journal of Clinical and Experimental Pathology*, 7: 7323.

Gilpin, N. W. and Koob, G. F. (2008). Neurobiology of alcohol dependence: focus on motivational mechanisms. *Alcohol Research and Health*, 31, 185.

Girolami, B., Bernardi, E., Prins, M. H., ten Cate, J. W., Hettiarachchi, R., Prandoni, P. and Büller, H. R. (1999). Treatment of intermittent claudication with physical training, smoking cessation, pentoxifylline, or nafronyl: a meta-analysis. *Archives of Internal Medicine*, 159: 337-345.

Gotsman, I., Zwas D, Planer D, Admon D, Lotan C, Keren A (2010). The significance of serum urea and renal function in patients with heart failure. *Medicine (Baltimore)*, 89: 197-203.

Gowda, S., Desai, P. B., Kulkarni, S. S., Hull, V. V., Math, A. A., & Vernekar, S. N. (2010). Markers of renal function tests. *North American Journal of Medical Sciences*, *2*: 170-3.

Graham, G. G. and Scott, K. F. (2005). Mechanism of action of paracetamol. *American Journal of Therapeutics*, 12: 46-55.

Graham, G. G., Scott, K. F. and Day, R. O. (2005). Tolerability of paracetamol. *Drug Safety*, 28: 227-240.

Green, T. J., Sivilotti, M. L., Langmann, C., Yarema, M., Juurlink, D., Burns, M. J. and Johnson, D. W. (2010). When do the aminotransferases rise after acute acetaminophen overdose? *Clinical Toxicology*, 48: 787-792.

Gu, J., Cui, H., Behr, M., Zhang, L., Zhang, Q. Y., Yang, W. and Ding, X. (2005). In vivo mechanisms of tissue-selective drug toxicity: effects of liver-specific knockout of the NADPH-cytochrome P450 reductase gene on acetaminophen toxicity in kidney, lung, and nasal mucosa. *Molecular Pharmacology*, 67: 623-630.

Gude, D. (2012). Alcohol and fertility. *Journal of Human Reproductive Sciences*, 5: 226.

Guiloski, I. C., Ribas, J. L. C., Piancini, L. D. S., Dagostim, A. C., Cirio, S. M., Fávaro, L. F. and de Assis, H. C. S. (2017). Paracetamol causes endocrine disruption and hepatotoxicity in male fish Rhamdia quelen after subchronic exposure. *Environmental Toxicology and Pharmacology*, 53: 111-120.

Gujral, J. S., Bucci, T. J., Farhood, A. and Jaeschke, H. (2001). Mechanism of cell death during warm hepatic ischemia-reperfusion in rats: apoptosis or necrosis? *Hepatology*, 33*:* 397-405.

Gulnaz, H., Tahir, M., Munir, B. and Sami, W. (2010). Protective effects of garlic oil on acetaminophen induced nephrotoxicity in male albino rats. *Biomedica*, 26: 9-15.

Guo, R. and Ren, J. (2010). Alcohol and acetaldehyde in public health: from marvel to menace. *International Journal of Environmental Research and Public Health*, 7:1285-1301.

Haber, P. S., Warner, R., Seth, D., Gorrell, M. D. and Mccaughan, G. W. (2003). Pathogenesis and management of alcoholic hepatitis. *Journal of Gastroenterology and Hepatology*, 18: 1332-1344.

Hart, S. E., Beierschmitt, W. P., Wyand, D. S., Khairallah, E. A. and Cohen, S. D. (1994). Acetaminophen nephrotoxicity in CD-1 mice: I. Evidence of a role for in situ activation in selective covalent binding and toxicity. *Toxicology and Applied Pharmacology*, *1*26: 267-275.

Hartley, D. M., Kurth, M. C., Bjerkness, L., Weiss, J. H. and Choi, D. W. (1993). Glutamate receptor-induced 45Ca2+ accumulation in cortical cell culture correlates with subsequent neuronal degeneration. *Journal of Neuroscience*, *13*.

Heckmann, W. and Silveira, C. M. (2009). Alcohol dependence: Clinical aspects and diagnosis.

Hewawasam, R. P., Jayatilaka, K. A. P. W., Pathirana, C. and Mudduwa, L. K. B. (2004). Hepatoprotective effect of Epaltes divaricata extract on carbon tetrachloride induced hepatotoxicity in mice. *Indian Journal of Medical Research*, 120: 30-34.

Hingson, R. W., Heeren, T. and Winter, M. R. (2006). Age at Drinking Onset and Alcohol Dependence. *Archives of Pediatrics and Adolescent Medicine,* 160:739-746.

Hinson, J. A., Roberts, D. W. and James, L. P. (2010). Mechanisms of acetaminophen-induced liver necrosis. *In Adverse Drug Reactions,* 369-405. Springer Berlin Heidelbrg.

Hodgman, M. J., and Garrard, A. R. (2012). A review of acetaminophen poisoning. *Critical Care Clinics*, 28: 499-516.

Hritz, I., Mandrekar, P., Velayudham, A., Catalano, D., Dolganiuc, A., Kodys, K. and Szabo, G. (2008). The critical role of toll-like receptor (TLR) 4 in alcoholic liver disease is dependent of the common TLR adapter MyD88. *Hepatology,* 48: 1224-1231.

Huynh (2009). Pharmacological reviews: Acetaminophen. Centre for drug evaluation and research. Food and Drug Administration, USA. *https://www.accessdata.fda.gov/drugsatfda_docs/nda/2010/022450Orig1s000PharmR.pdf*

Hyder, M.A., Hasan, M., & Mohieldein, A.H. (2013). Comparative Levels of ALT, AST, ALP and GGT in Liver associated Diseases. *European Journal of Experimental Biology,* 3(2):280-284.

Hyllested, M., Jones, S., Pedersen, J. L. and Kehlet, H. (2002). Comparative effect of paracetamol, NSAIDs or their combination in postoperative pain management: a qualitative review. *British Journal of Anaesthesia*, 88: 199-214.

Iimuro, Y., Gallucci, R. M., Luster, M. I., Kono, H. and Thurman, R. G. (1997). Antibodies to tumor necrosis factor alfa attenuate hepatic necrosis and inflammation caused by chronic exposure to ethanol in the rat. *Hepatology*, 26: 1530-1537.

Ikpi, D. and Nku, C. (2008). Effect of ethanolic extract of Dennettia tripetala fruit on haematological parameters in albino Wistar rats. *Nigerian Journal of physiological sciences*, 23:1-2.

Ioannou, G. N., Weiss, N. S., Boyko, E. J., Mozaffarian, D. and Lee, S. P. (2006). Elevated serum alanine aminotransferase activity and calculated risk of coronary heart disease in the United States. *Hepatology*, 43: 1145-1151.

Jacobs, B. P., Dennehy, C., Ramirez, G., Sapp, J. and Lawrence, V. A. (2002). Milk thistle for the treatment of liver disease: a systematic review and meta-analysis. *The American Journal of Medicine*, 113: 506-515.

Jambulingappa, K. L., Somashekar, D., Acharya, A., Santhosh Ramakrishna, D., Gokul, D., & Adake, P. (2012). Effect of Tramadol and paracetamol combination on blood glucose level in type 2 diabetes patients with osteoarthritis: An observational study. *Journal of Pharmacy Research*, 5: 891-893.

James, O., Roberts, S. H., Douglas, A., Lesna, M., Pulman, L., Smith, P. A., & Watson, A. J. (1975). liver damage after paracetamol overdose comparison of liver-function tests, fasting serum bile acids, and liver histology. *The Lancet*, 306: 579-581.

Jampana, S. C. and Khan, R. (2011). Pathogenesis of alcoholic hepatitis: Role of inflammatory signaling and oxidative stress. *World journal of hepatology*, 3: 114.

Juma, K. K., Maina, S. G., Muriithi, J. N., Mwangi, B. M., Mworia, K. J., Mwonjoria, M. J., Ngeranwa, J. N. and Mburu, N. D. (2015). Protective effects of *Urtica dioica* and cimetidine® on liver function following acetaminophen induced hepatotoxicity in mice. *Journal of Developing Drugs,* 4: 130.

Kanchana, N. and Sadiq, A. M. (2011). Hepatoprotective effect of Plumbago zeylanica on paracetamol induced liver toxicity in rats. *International Journal of Pharmacy and Pharmaceutical Sciences,* 3: 151-154.

Kendig, E. L., Schneider, S. N., Clegg, D. J., Genter, M. B. and Shertzer, H. G. (2008). Over-the-counter analgesics normalize blood glucose and body composition in mice fed a high fat diet. *Biochemical Pharmacology*, 76: 216-224.

Kent, W. (2012). The pharmacokinetics of alcohol in healthy adults.

Kim, W. R., Flamm, S. L., Di Bisceglie, A. M. and Bodenheimer, H. C. (2008). Serum activity of alanine aminotransferase (ALT) as an indicator of health and disease. *Hepatology*, 47: 1363-1370.

Klotz, U. (2012). Paracetamol (acetaminophen) a popular and widely used non-opioid analgesic. *Arzneimittel-Forschung.* 62:355-359.

Kofman, A. V., Morgan, G., Kirschenbaum, A., Osbeck, J., Hussain, M., Swenson, S. and Theise, N. D. (2005). Dose-and time-dependent oval cell reaction in acetaminophen-induced murine liver injury. *Hepatology*, 41: 1252-1261.

kolaczynski, J. W., Ylikahri, R., HÄrkonen, M. and Koivisto, V. A. (1988). The acute effect of ethanol on counterregulatory response and recovery from insulin-induced hypoglycemia. *The Journal of Clinical Endocrinology & Metabolism*, 67: 384-388.

Kono, H., Rusyn, I., Yin, M., Gäbele, E., Yamashina, S., Dikalova, A. and Bradford, B. U. (2000). NADPH oxidase–derived free radicals are key oxidants in alcohol-induced liver disease. *The Journal of Clinical Investigation*, 106: 867-872.

Koop, D. R., Klopfenstein, B., Iimuro, Y. and Thurman, R. G. (1997). Gadolinium chloride blocks alcohol-dependent liver toxicity in rats treated chronically with intragastric alcohol despite the induction of CYP2E1. *Molecular Pharmacology*, 51: 944-950.

Korpi-Steiner, N. L., Hoyne, J. B., Hoyer, J. D. and Saenger, A. K. (2008). Hemolytic anemia following attempted suicide. *Clinical Chemistry,* 54: 2071-2074.

Kronborg, J., Solbu, M., Njølstad, I., Toft, I., Eriksen, B. O., and Jenssen, T. (2008). Predictors of change in estimated GFR: a population-based 7-year follow-up from the Tromsø study. *Nephrology Dialysis Transplantation*, 23: 2818-2826.

Kuffner, E. K., Dart, R. C., Bogdan, G. M., Hill, R. E., Casper, E. and Darton, L. (2001). Effect of maximal daily doses of acetaminophen on the liver of alcoholic patients: a randomized, double-blind, placebo-controlled trial. *Archives of Internal Medicine*, 161: 2247-2252.

Kvasnička, F., Bıba, B., Ševčík, R., Voldřich, M. and Kratka, J. (2003). Analysis of the active components of silymarin. *Journal of Chromatography A*, 990: 239-245.

Lachiewicz, P. F. (2013). The role of intravenous acetaminophen in multimodal pain protocols for perioperative orthopedic patients. *Orthopedics*, *36*: 15-19.

Lazo, M., Selvin, E. and Clark, J. M. (2008). Brief communication: clinical implications of short-term variability in liver function test results. *Annals of Internal Medicine*, 148: 348-352.

Lee, J. W., Paape, M. J., & Zhao, X. (2003). Recombinant bovine soluble CD14 reduces severity of experimental Escherichia coli mastitis in mice. *Veterinary Research*, 34: 307-316.

Lee, W. M. (2004). Acetaminophen and the US Acute Liver Failure Study Group: lowering the risks of hepatic failure. *Hepatology,* 40: 6-9.

LHS (2004). Living by Chemistry - Toxins© UC Regents, Lethal dose table. http://whs.rocklinusd.org/documents/Science/Lethal_Dose_Table.pdf

Li, C., Liu, J., Saavedra, J. E., Keefer, L. K. and Waalkes, M. P. (2003). The nitric oxide donor, V-PYRRO/NO, protects against acetaminophen-induced nephrotoxicity in mice. *Toxicology*, 189: 173-180.

Lide, D. R. (2005). Physical Constant of Inorganic Compound. *Handbook of Chemistry and Physics*, *474*.

Lieber C. S. (1991). Perspectives: do alcohol calories count? *American Journal of Clinical Nutrition*, 54:976–82.

Lieber, C. S. (1997). Cytochrome P-4502E1: its physiological and pathological role. *Physiological Reviews*, 77: 517-544.

Lieber, C. S., Leo, M. A., Cao, Q., Ren, C. and DeCarli, L. M. (2003). Silymarin retards the progression of alcohol-induced hepatic fibrosis in baboons. *Journal of Clinical Gastroenterology*, 37: 336-339

Lieber, C. S. (2004). Alcoholic fatty liver: its pathogenesis and mechanism of progression to inflammation and fibrosis. *Alcohol*, 34: 9-19.

Lieber, C. S. (2005). Metabolism of alcohol. *Clinics in Liver Disease*, 9: 1-35.

Litovitz, T. L., Klein-Schwartz, W., Rodgers, G. C., Cobaugh, D. J., Youniss, J., Omslaer, J. C. and Benson, B. E. (2002). 2001 Annual report of the American Association of Poison Control Centers toxic exposure surveillance system. *The American Journal of Emergency Medicine,* 20: 391-452.

Liu, L., Wang, Y., Lam, K. S. and Xu, A. (2008). Moderate wine consumption in the prevention of metabolic syndrome and its related medical complications. *Endocrine, Metabolic and Immune Disorders-Drug Targets (Formerly Current Drug Targets-Immune, Endocrine and Metabolic Disorders)*, 8: 89-98.

Lopez-Giacoman, S. and Madero, M. (2015). Biomarkers in chronic kidney disease, from kidney function to kidney damage. *World Journal of Nephrology*, 4: 57-73.

Lu, X. J. and Gu, J. X. (2012). Performance evaluation of mindray BC-5300 hematology analyzer. *Chinese Medical Equipment Journal*, 2: 47.

Łukasik-Głebocka, M. and Klimaszyk, D. (2001). [Alcohol-paracetamol syndrome: a case report]. *Przeglad Lekarski*, 59: 381-383.

Luper, S. (1998). A review of plants used in the treatment of liver disease: part 1. *Alternative Medicine Review: A Journal of Clinical Therapeutic*, 3: 410-421.

Macdonald, I. O., Olusola, O. J. and Osaigbovo, U. A. (2010). Effects of chronic alcohol administration on body weight, reduced glutathione, malondialdehyde levels and glutathione-s-transferase activity in rats. *New York Science Journal,* 3: 39–47.

Maher, J. J. (2002). Alcoholic steatosis and steatohepatitis. In *Seminars in gastrointestinal disease*, 13: 31-39.

Mallet, C., Barrière, D. A., Ermund, A., Jönsson, B. A., Eschalier, A., Zygmunt, P. M. and Högestätt, E. D. (2010). TRPV1 in brain is involved in acetaminophen-induced antinociception. *PLoS one*, 5: e12748.

Manyike, P. T., Kharasch, E. D., Kalhorn, T. F. and Slattery, J. T. (2000). Contribution of CYP2E1 and CYP3A to acetaminophen reactive metabolite formation. *Clinical Pharmacology and Therapeutics*, 67: 275-282.

Maritim, A. C., Sanders, R. A. and Watkins, J. B. (2003). Diabetes, oxidative stress, and antioxidants: a review. *Journal of Biochemical and Molecular Toxicology,* 17: 24–38.

Marsano L. S., Mendez C., Hill D., Barve S. and McClain C.J. (2003). Diagnosis and treatment of alcoholic liver disease and its complications. *Alcohol Research and Health*, 27(3): 247–256.

Massey, V.L., Beier J.I., Itzenthaler, J.D., Roman, J., and Arteel, G.E. (2015). Potential role of the gut/liver/lung axis in alcohol-induced tissue pathology. *Biomolecules*, 5: 2477-2503.

Mathurin, P., Mendenhall, C. L., Carithers Jr, R. L., Ramond, M. J., Maddrey, W. C., Garstide, P.and Poynard, T. (2002). Corticosteroids improve short-term survival in patients with severe alcoholic hepatitis (AH): individual data analysis of the last three randomized placebo controlled double blind trials of corticosteroids in severe AH. *Journal of Hepatology*, 36: 480-487.

Mayer, J. (2007). Use of behavior analysis to recognize pain in small mammals. *Laboratory animal*, *36*: 43.

McDonough, K. H. (2003). Antioxidant nutrients and alcohol. *Toxicology*, 189: 89-97.

McMurtry, R. J., Snodgrass, W. R. and Mitchell, J. R. (1978). Renal necrosis, glutathione depletion, and covalent binding after acetaminophen. *Toxicology and Applied Pharmacology*, 46: 87-100.

McNicol, E. D., Tzortzopoulou, A., Cepeda, M. S., Francia, M. B. D., Farhat, T. and Schumann, R. (2011). Single-dose intravenous paracetamol or propacetamol for prevention or treatment of postoperative pain: a systematic review and meta-analysis. *British Journal of Anaesthesia*, 106: 764-775.

Merrill, G. F. and Goldberg, E. (2001). Antioxidant properties of acetaminophen and cardioprotection. *Basic Research in Cardiology*, 96: 423-430.

Mezey, E., Rennie-Tankersley, L. and Potter, J. J. (2001). Liver alcohol dehydrogenase is degraded by the ubiquitin–proteasome pathway. *Biochemical and Biophysical Research Communications*, 285: 644-648.

Mitchell, J. R., Jollow, D. J., Potter, W. Z., Davis, D. C., Gillette, J. R. and Brodie, B. B. (1973). Acetaminophen-induced hepatic necrosis. I. Role of drug metabolism. *Journal of Pharmacology and Experimental Therapeutics*, 187: 185-194.

Mitchell, M. C., Teigen, E. L., and Ramchandani, V. A. (2014). Absorption and peak blood alcohol concentration after drinking beer, wine, or spirits. *Alcoholism: Clinical and Experimental Research*, 38: 1200-1204.

Mossa, A. T. H., Heikal, T. M. and Omara, E. A. A. (2012). Physiological and histopathological changes in the liver of male rats exposed to paracetamol and diazinon. *Asian Pacific Journal of Tropical Biomedicine*, 2: S1683-S1690.

Mugford, C. A. and Tarloff, J. B. (1997). The contribution of oxidation and deacetylation to acetaminophen nephrotoxicity in female Sprague-Dawley rats. *Toxicology Letters*, 93: 15-22.

Mukherjee, S. (2014). Alcohol metabolism and generation of free radicals: a deep insight. *OA Alcohol*, 2: 10.

Mumenthaler, M. S., Taylor, J. L., O'Hara, R. and Yesavage, J. A. (1999). Gender differences in moderate drinking effects. *Alcohol Research and Health*, 23: 55-64.

Murphy, E. J. (2005). Acute pain management pharmacology for the patient with concurrent renal or hepatic disease. *Anaesthesia and Intensive Care*, 33: 311-322.

Muthukumar, T. V., Wanchoo, A., Bambery, P. and Sakhuja, V. (1999). Acute renal failure due to nontraumatic rhabdomyolysis following binge drinking. *Renal failure*, 21: 545-549.

Myrhed, M., Berglund, L. and Bottiger, L.E. (1977). Alcohol consumption and hematology. *Acta Medica Scandinavica*, 202: 11-15.

Nadro, M. S., Arungbemi, R. M., & Dahiru, D. (2006). Evaluation of Moringa oleifera leaf extract on alcohol-induced hepatotoxicity. *Tropical Journal of Pharmaceutical Research*, 5: 539-544.

Neto, F. X. P., Targino, M. N., Peixoto, V. S., Alcântara, F. B., de Jesus, C. C., de Araújo, D. C., and de Lacerda Marçal Filho, E. F. (2011). Sensorial abnormalities: Smell and taste. *Arquivos Internacionais de Otorrinolaringologia*, 15: 350-358.

Neuman, M. G., Brenner, D. A., Rehermann, B., Taieb, J., Chollet-Martin, S., Cohard, M. and Shear, N. H. (2001). Mechanisms of alcoholic liver disease: cytokines. *Alcoholism: Clinical and Experimental Research*, 25: 251S-253S.

Neuman, M. G., Maor, Y., Nanau, R. M., Melzer, E., Mell, H., Opris, M. and Malnick, S. (2015). Alcoholic Liver Disease: Role of Cytokines. *Biomolecules*, 5: 2023-2034.

Nikles, C. J., Yelland, M., Del Mar, C. and Wilkinson, D. (2005). The role of paracetamol in chronic pain: an evidence-based approach. *American Journal of Therapeutics*, 12: 80-91.

Ning, J., Lin, G., Ji, F., Xu, J. and Sharify, **N. (2012).** Preventive effects of geranylgeranylacetone on rat ethanol-induced gastritis. *World Journal of Gastroenterology,* 18(18): 2262-2269.

Nordmann, R., & Rouach, H. (1996). Alcohol and free radicals: from basic research to clinical prospects. In *Annales de Gastroentérologie et d'hépatologie* , 32: 128-33.

Nordmann, R., Ribière, C. and Rouach, H. (1992). Implication of free radical mechanisms in ethanol-induced cellular injury. *Free Radical Biology and Medicine*, 12: 219-240.

Nwodo, O. F. C., Joshua, P. E., Ozoemena, N. F. and Onwuzu, N. J. (2010). Haematological evaluation of the effect of coconut (Cocos nucifera) water on Paracetamol-intoxication in laboratory rats. *Journal of Pharmacy Research,* 3: 1831-1834.

Nyblom, H., Berggren, U., Balldin, J. and Olsson, R. (2004). High AST/ ALT ratio may indicate advanced alcoholic liver disease rather than heavy drinking. *Alcohol and Alcoholism,* 39: 336-339.

O'Neil, E. (2012). Developmental Timeline of Alcohol-Induced Birth Defects. *Embryo Project Encyclopedia*.

O'Neil, M. J. (Ed.). (2013). The Merck index: an encyclopedia of chemicals, drugs, and biologicals. RSC Publishing.

Onyango, T.O., Mburu, D. N., Ngugi, M. P., Kamau, J. K. and Juma, K. K. (2017). Effects of Maalox Plus® Antacid and PureCal® Calcium Supplementson Haematological and Biochemical Parameters of Rats Subjected to Alcohol Intoxication. *Journal of Alcohol and Drug Dependence* 5(3): 2329-6488.

Oscar-Berman, M., Shagrin, B., Evert, D. L., & Epstein, C. (1997). Impairments of brain and behavior. *Alcohol Health and Research World*, 21: 65-75.

Oscier, C. D. and Milner, Q. J. W. (2009). Peri-operative use of paracetamol. *Anaesthesia*, 64: 65-72.

O'Shea, R. S., Dasarathy, S. and McCullough, A. J. (2010). Alcoholic liver disease. *Hepatology*, 51: 307-328.

Ottani, A., Leone, S., Sandrini, M., Ferrari, A. and Bertolini, A. (2006). The analgesic activity of paracetamol is prevented by the blockade of cannabinoid CB1 receptors. *European Journal of Pharmacology*, 531: 280-281.

Oyedeji, K. O., Bolarinwa, A. F. and Ojeniran, S. S. (2013). Effect of paracetamol (acetaminophen) on haematological and reproductive parameters in male albino rats. *IOSR Journal of Pharmacy and Biological Sciences (IOSR-JPBS)*, 4: 1-6.

Oyedeji, K. O., Bolarinwa, A. F. and Fashina, A. M. (2013). Effect of alcohol consumption on haematological and reproductive parameters in female Albino rats. *Journal of Dental and Medical Sciences*, 3: 76-79.

Pablo, N. C., Churg, J., Needle, M. A. and Ganesharajah, M. (1986). Renal papillary necrosis: relapsing form associated with alcoholism. *American Journal of Kidney Diseases*, 7: 88-94.

Palani, S., Raja, S., Naresh, R. and Senthil Kumar, B. (2010). Evaluation of nephroprotective, diuretic, and antioxidant activities of plectranthus amboinicus

on acetaminophen-induced nephrotoxic rats. *Toxicology Mechanisms and Methods*, 20: 213-221.

Parekh, R. S. and Klag, M. J. (2001). Alcohol: role in the development of hypertension and end-stage renal disease. *Current Opinion in Nephrology and Hypertension,* 10: 385-390.

Parmar, S. R., Vashrambhai, P. H. and Kalia, K. (2010). Hepatoprotective activity of some plants extract against paracetamol induced hepatotoxicity in rats. *Journal of Herbal Medicine Research*, 4: 101-106.

Pathan, M. M., Khan, M. A., Moregaonkar, S. D., Somkuwar, A. P. and Gaikwad, N. Z. (2013). Amelioration of paracetamol induced nephrotoxicity by Maytenus emarginata in male Wistar rats. *International Journal of Pharmacy and Pharmaceutical Sciences*, *5*:471-474.

Paton, A. (2005). Alcohol in the body. *British Medical Journal,* 330.

Payasi A. Chaudhary m, singhBM, Cupta A and sehgal R (2010). Sub-Acute Toxicity Studies of Paracetamol Infusion in Albino wister rats. *International Journal of Pharmaceutical Sciences and Drug Research,* 2: 142 -145.

Pickering, G., Esteve, V., Loriot, M. A., Eschalier, A. and Dubray, C. (2008). Acetaminophen reinforces descending inhibitory pain pathways. *Clinical Pharmacology and Therapeutics*, 84: 47-51.

Pinkston, K. E. and Sedlak, D. L. (2004). Transformation of aromatic ether-and amine-containing pharmaceuticals during chlorine disinfection. *Environmental Science and Technology*, 38: 4019-4025.

Prescott, L. (2005). Oral or intravenous N-acetylcysteine for acetaminophen poisoning? *Annals of Emergency Medicine*, 45: 409-413.

Prescott, L. F. (2000). Paracetamol: past, present, and future. *American Journal of Therapeutics*, 7: 143-147.

Prescott, L.F. (2000). Paracetamol, alcohol and the liver. *British Journal of Clinical Pharmacology,* 49: 291-301.

Rains, J. L. and Jain, S. K. (2011). Oxidative stress, insulin signaling, and diabetes. *Free Radical Biology and Medicine*, 50: 567-575.

Ramchandani, V. A., Bosron, W. F. and Li, T. K. (2001). Research advances in ethanol metabolism. *Pathologie Biologie*, 49: 676-682.

Reynolds, K., Gu, D., Chen, J., Tang, X., Yau, C. L., Yu, L. and He, J. (2008). Alcohol consumption and the risk of end-stage renal disease among Chinese men. *Kidney International,* 73: 870-876.

Reynolds, P. (2012). Smoking and breast cancer. *Journal of Mammary Gland Biology and Neoplasia*, 18: 15-23.

Rømsing, J., Møiniche, S. and Dahl, J. B. (2002). Rectal and parenteral paracetamol, and paracetamol in combination with NSAIDs, for postoperative analgesia. *British Journal of Anaesthesia*, 88: 215-226.

Ruepp, S. U., Tonge, R.P., Shaw, J., Wallis, N. and Pognan, F. (2002). Genomics and proteomics analysis of acetaminophen toxicity in mouse liver. *Toxicology Science*, 65: 135–150.

Ruhl, C. E. and Everhart, J. E. (2003). Determinants of the association of overweight with elevated serum alanine aminotransferase activity in the United States. *Gastroenterology*, 124: 71-79.

Sadler D. (2009). Alcohol and alcoholism. http://www.dundee.ac.uk/forensicmedicine/notes/notes.html.

Saetre, P., Emilsson, L., Axelsson, E., Kreuger, J., Lindholm, E. and Jazin, E. (2007). Inflammation-related genes up-regulated in schizophrenia brains. *Bmc Psychiatry*, 7: 46.

Saito, C., Zwingmann, C. and Jaeschke, H. (2010). Novel mechanisms of protection against acetaminophen hepatotoxicity in mice by glutathione and N-acetylcysteine. *Hepatology*, 51: 246-254.

Samuel, S. A., Francis, A. O., Ayomide, O. and Onyinyechi, U. O. (2015). Effects of paracetamol-induced liver damage on some hematological parameters: red blood cell (RBC) count, white blood cell (WBC) count, and packed cell volume (PCV) in wistar rats of either sex. *Indo American Journal of Pharmaceutical Research, 5*: 2593-2599.

Santolaria, F., Pérez-Cejas, A., Alemán, M. R., González-Reimers, E., Milena, A., De La Vega, M. J. and Gómez-Rodríguez, M. A. (2003). Low serum leptin levels and malnutrition in chronic alcohol misusers hospitalized by somatic complications. *Alcohol and Alcoholism*, 38: 60-66.

Sarumathy, K., Rajan, M. D., Vijay, T. and Jayakanthi, J. (2011). Evaluation of phytoconstituents, nephro-protective and antioxidant activities of Clitoria ternatea. *Journal of Applied Pharmaceutical Science*, 1:164-172.

Schaeffner, E. S., Kurth, T., de Jong, P. E., Glynn, R. J., Buring, J. E. and Gaziano, J. M. (2005). Alcohol consumption and the risk of renal dysfunction in apparently healthy men. *Archives of Internal Medicine,* 165: 1048-1053.

Schiodt, F. V., Lee, W. M., Bobdesen, S., Ott, P. and Christensen, E. (2002). Influence of acute and chronic alcohol intake on the clinical course and outcome in acetaminophen overdose. *Alimentary Pharmacology and Therapeutics,* 16: 707-715.

Schlorff, E. C., Husain, K. and Somani, S. M. (1999). Dose-and time-dependent effects of ethanol on plasma antioxidant system in rat. *Alcohol*, 17: 97-105.

Schwingshackl, L. and Hoffmann, G. (2014). Comparison of High vs. Normal/Low Protein Diets on Renal Function in Subjects without Chronic Kidney Disease: A Systematic Review and Meta-Analysis. *PLoS ONE*, 9: e97656.

Seitz, H. K. and Becker, P. (2007). Alcohol metabolism and cancer risk. *Alcohol Research and Health*, 30: 38.

Senthilkumar, R., Chandran, R. and Parimelazhagan, T. (2014). Hepatoprotective effect of Rhodiola imbricata rhizome against paracetamol-induced liver toxicity in rats. *Saudi Journal of Biological Sciences*, 21: 409-416.

Shankar A., Klein R. and Klein B. E. (2006). The association among smoking, heavy drinking, and chronic kidney disease. *American Journal of Epidemiology,* 164: 263-271.

Sheen, C. L., Dillon, J. F., Bateman, D. N., Simpson, K. J. and Macdonald, T.M. (2002). Paracetamol toxicity: epidemiology, prevention and costs to healthcare system. *Quartely Journal of Medicine*, 95: 609-619.

Shertzer, H. G., Schneider, S. N., Kendig, E. L., Clegg, D. J., D'Alessio, D. A. and Genter, M. B. (2008). Acetaminophen normalizes glucose homeostasis in mouse models for diabetes. *Biochemical Pharmacology*, 75: 1402-1410.

Silva, C. S., Dias, V. R., Almeida, J. A., Brazil J. M., Santos, R.A. and Milagres, M. P. (2015) Effect of Heavy Consumption of Alcoholic Beverages on the Perception of Sweet and Salty Taste. *Alcohol and Alcoholism*, 51(3): 302–306.

Singla, N. K., Parulan, C., Samson, R., Hutchinson, J., Bushnell, R., Beja, E. G. and Royal, M. A. (2012). Plasma and cerebrospinal fluid pharmacokinetic parameters after single-dose administration of intravenous, oral, or rectal acetaminophen. *Pain Practice*, 12: 523-532.

Smilkstein, M. J., Knapp, G. L., Kulig, K. W. and Rumack, B. H. (1988). Efficacy of oral N-acetylcysteine in the treatment of acetaminophen overdose. *New England Journal of Medicine,* 319: 1557-1562.

Song, Z., Zhou, Z., Uriate, S., Wang, L., Kang, Y. J., Chen, T. and McClain, C. J. (2004). S-adenosylhomocysteine sensitizes to TNF-α hepatotoxity in mice and liver cells: A possible etiological factor in alcoholic liver disease. *Hepatology,* 40: 989-997.

Song, Z., Deaciuc, I., Song, M., Lee, D. Y. W., Liu, Y., Ji, X. and McClain, C. (2006). Silymarian Protects Against Acute Ethanol-Induced Hepatotoxicity in Mice. *Alcoholism*: *Clinical and Experimental Research,* 30: 407-413.

Stanly, P. C., Wakwe, V. C. and Ejele, O. A. (2003). Haematological profile in chronic alcohol consumers. *Nigerian Journal of Clinical Practice*, 6: 84-86.

Stockler, M., Vardy, J., Pillai, A. and Warr, D. (2004). Acetaminophen (paracetamol) improves pain and well-being in people with advanced cancer already receiving a strong opioid regimen: a randomized, double-blind, placebo-controlled cross-over trial. *Journal of Clinical Oncology*, *22*: 3389-3394.

Styskal, J., Van Remmen, H., Richardson, A. and Salmon, A. B. (2012). Oxidative stress and diabetes: what can we learn about insulin resistance from antioxidant mutant mouse models? *Free Radical Biology and Medicine*, 52: 46-58.

Suter, P. M. and Tremblay, A. (2005). Is alcohol consumption a risk factor for weight gain and obesity? *Critical Reviews in Clinical Laboratory Sciences*, 42: 197-227.

Szabo, G. and Mandrekar, P. (2009). A recent perspective on alcohol, immunity, and host defense. *Alcoholism: Clinical and Experimental Research*, 33: 220-232.

Tamura, H., Miyamoto, K., Kiryu, J., Miyahara, S., Katsuta, H., Hirose, F. and Yoshimura, N. (2005). Intravitreal injection of corticosteroid attenuates leukostasis and vascular leakage in experimental diabetic retina. *Investigative Ophthalmology and Visual Science*, 46: 1440-1444.

Thomsen, M. S., Loft, S., Roberts, D. W. and Poulsen, H. E. (1995). Cytochrome P4502E1 inhibition by propylene glycol prevents acetaminophen (paracetamol) hepatotoxicity in mice without cytochrome P4501A2 inhibition. *Pharmacology and Toxicology*, 76: 395-399.

Tilg, H. and Day, C. P. (2007). Management strategies in alcoholic liver disease. *Nature Reviews Gastroenterology and Hepatology*, 4: 24.

Torruellas C., French S. W. and Medici V. (2014). Diagnosis of alcoholic liver disease. *World Journal of Gastroenterology*, 20(33): 11684-11699.

Tsukamoto, H. and Lu, S. C. (2001). Current concepts in the pathogenesis of alcoholic liver injury. *The FASEB Journal*, 15: 1335-1349.

Turati, F., Garavello, W., Tramacere, I., Pelucchi, C., Galeone, C., Bagnardi, V., Corrao, G., Islami, F., Fedirko, V., Boffetta, P., La Vecchia, C. and Negri, E. (2012). A meta-analysis of alcohol drinking and oral and pharyngeal cancers: results from subgroup analyses. *Alcohol and Alcoholism* 48:107–118.

Ucheya, R. E. and Igweh, J. C. (2006). Histological changes in kidney structure following a long–term administration of paracetamol (acetaminophen) in pregnant sprague dawley rats. *Nigerian Journal of Physiological Sciences*, 21: 1-2.

Van der Marel, C. D., Anderson, B. J., van Lingen, R. A., Holford, N. H., Pluim, M. A., Jansman, F. G. and Tibboel, D. (2003). Paracetamol and metabolite pharmacokinetics in infants. *European Journal of Clinical Pharmacology*, 59: 243-251.

Van Thiel, D. H., Gavaler, J. S., Little, J. M., and Lester, R. (1977). Alcohol: its effect on the kidney. *Metabolism*, 26: 857-866.

Vengelien, B. A., Molander, A.and Spanagel, R. (2008). Neuropharmacology of alcohol addiction. *British Journal of Pharmacology*, 154: 299-15.

Vonghia, L., Leggio, L., Ferrulli, A., Bertini, M., Gasbarrini, G. and Addolorato, G. (2008). Acute alcohol intoxication. *European Journal of Internal Medicine*, 19: 561–567.

Wang, C., Allegaert, K., Tibboel, D., Danhof, M., van der Marel, C. D., Mathot, R. A. and Knibbe, C. A. (2014). Population pharmacokinetics of paracetamol across the human age-range from (pre) term neonates, infants, children to adults. *The Journal of Clinical Pharmacology*, 54: 619-629.

Wang, L., Lee, I., Manson, J., Buring, J. and Sesso, H. (2010). Alcohol Consumption, Weight Gain, and Risk of Becoming Overweight in Middle-aged and Older Women. *Archives of Internal Medicine*. 170(5): 453–461.

Wannamethee, S. G. and Shaper, A. G. (2003). Alcohol, body weight, and weight gain in middle-aged men. *The American Journal of Clinical Nutrition*, 77: 1312-1317.

Weathermon, R. and Crabb, D. W. (1999). Alcohol and medication interactions. *Alcohol Research*, 23: 40.

White, S. L., Polkinghorne, K. R. Cass, A., Shaw J. E., Atkin R. C. and Chadban S.J. (2009). Alcohol consumption and 5-year onset of chronic kidney disease. The AusDiab study. *Nephrology Dialysis Transplantation,* 24: 2464-2472.

Woo, O. F., Mueller, P. D., Olson, K. R., Anderson, I. B. and Kim, S. Y. (2000). Shorter duration of oral N-acetylcysteine therapy for acute acetaminophen overdose. *Annals of emergency medicine*, 35: 363-368.

Wu, D. and Cederbaum, A. I. (2003). Alcohol, oxidative stress, and free radical damage. *Alcohol Research and Health*: 27: 277.

Xu, D., Chen, L., Chen, X., Wen, Y., Yu, C., Yao, J. and Kong, X. (2017). The triterpenoid CDDO-imidazolide ameliorates mouse liver ischemia-reperfusion injury through activating the Nrf2/HO-1 pathway enhanced autophagy. *Cell death & disease*, 8: e2983-e2983.

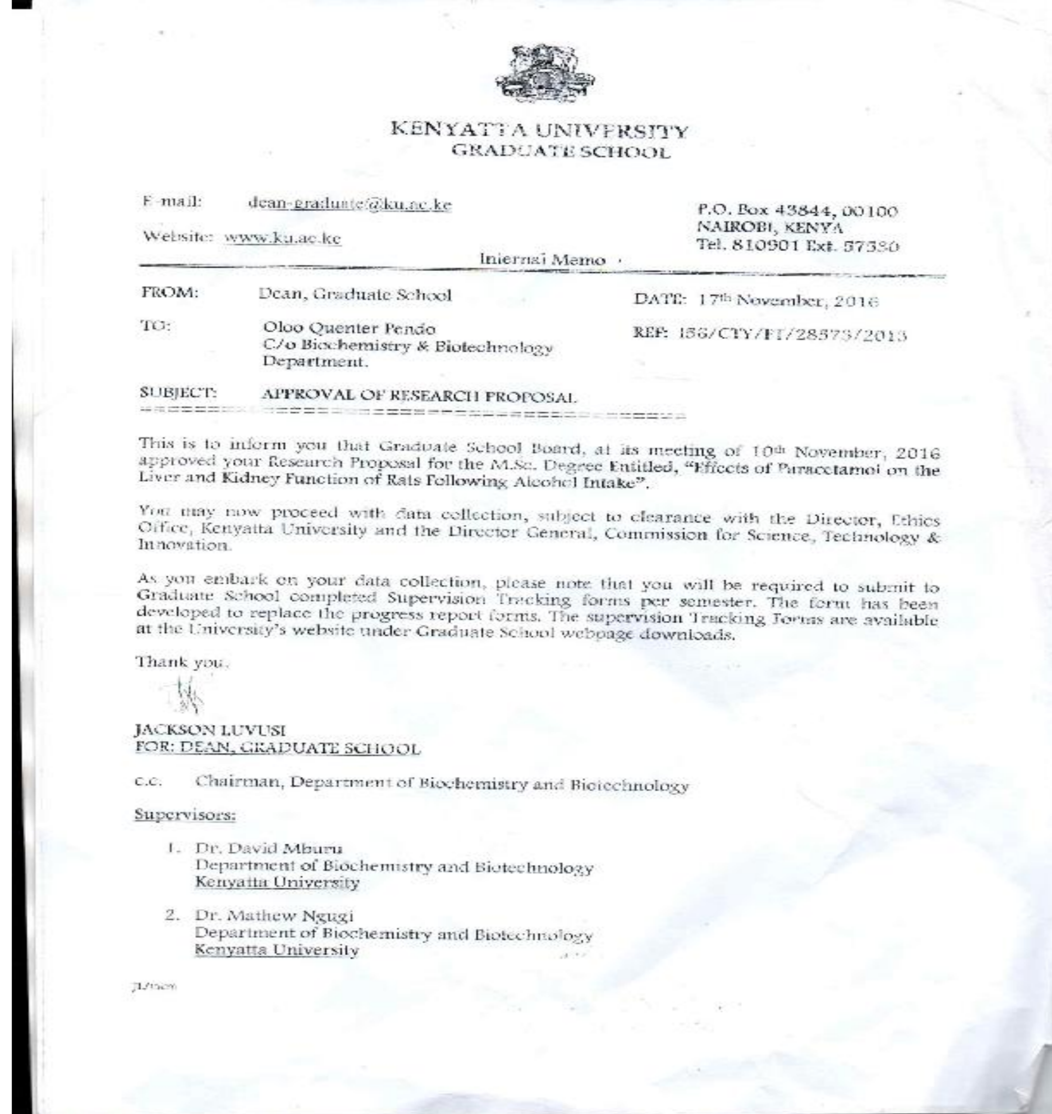
Yang, C. C., Deng, J. F. and Lin, T. J. (2001). Pancytopenia, hyperglycemia, shock, coma, rhabdomyolysis, and pancreatitis associated with acetaminophen poisoning. *Veterinary and Human Toxicology*, 43: 344-348.

Yarema, M. C., Johnson, D. W., Berlin, R. J., Sivilotti, M. L., Nettel-Aguirre, A., Brant, R. F. and Plint, A. C. (2009). Comparison of the 20-hour intravenous and 72-hour oral acetylcysteine protocols for the treatment of acute acetaminophen poisoning. *Annals of Emergency Medicine*, 54: 606-614.

Zakhari, S. (2006). Overview: how is alcohol metabolized by the body? *Alcohol Research and Health*, 29: 245-255.

Zakhari, S. and Li, T. K. (2007). Determinants of alcohol use and abuse: impact of quantity and frequency patterns on liver disease. *Hepatology*: 46: 2032-2039.

Appendix I: Approval of research proposal



KENYATTA UNIVERSITY
GRADUATE SCHOOL

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

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

Internal Memo

FROM: Dean, Graduate School
DATE: 17th November, 2016

TO: Oloo Quenter Pendo
C/o Biochemistry & Biotechnology Department.
REF: I56/CTY/PT/28573/2013

SUBJECT: APPROVAL OF RESEARCH PROPOSAL

This is to inform you that Graduate School Board, at its meeting of 10th November, 2016 approved your Research Proposal for the M.Sc. Degree Entitled, "Effects of Paracetamol on the Liver and Kidney Function of Rats Following Alcohol Intake".

You may now proceed with data collection, subject to clearance with the Director, Ethics Office, Kenyatta University and the Director General, Commission for Science, Technology & Innovation.

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

Thank you.

JACKSON LUVUSI
FOR: DEAN, GRADUATE SCHOOL

c.c. Chairman, Department of Biochemistry and Biotechnology

Supervisors:

1. Dr. David Mburu
   Department of Biochemistry and Biotechnology
   Kenyatta University
2. Dr. Mathew Ngugi
   Department of Biochemistry and Biotechnology
   Kenyatta University

JL/mcm

Kenyatta University Research Approval letter.

Appendix II: National Commission for Science, Technology and Innovation (NACOSTI) Research authorization Letter.

**NATIONAL COMMISSION FOR SCIENCE,
TECHNOLOGY AND INNOVATION**

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

NACOSTI, Upper Kabete
Off Waiyaki Way
P.O. Box 30623-00100
NAIROBI-KENYA

Ref: No. **NACOSTI/P/18/20118/24094**

Date: **1st August, 2018**

Queenter Pendo Oloo
Kenyatta University
P.O Box 43844-00100
NAIROBI

RE: RESEARCH AUTHORIZATION

Following your application for authority to carry out research on ***"Effects of paracetamol on the liver and kidney function of rats following alcohol intake"*** I am pleased to inform you that you have been authorized to undertake research in **Nairobi County** for the period ending **30th July, 2019.**

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

Kindly note that, as an applicant who has been licensed under the Science, Technology and Innovation Act, 2013 to conduct research in Kenya, you shall deposit **a copy** of the final research report to the Commission within **one year** of completion. The soft copy of the same should be submitted through the Online Research Information System.

GODFREY P. KALERWA MSc., MBA, MKIM
FOR: DIRECTOR-GENERAL/CEO

Copy to:

The County Commissioner
Nairobi County.

The County Director of Education
Nairobi County.

National Commission for Science, Technology and Innovation is ISO9001: 2008 Certified