A PRELIMINARY STUDY ON THE EFFECTS OF KHAT (*Catha edulis*) ON LIVER AND KIDNEY OF MICE

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DECLARATION

I declare that this thesis is my own original work and has not been presented for award of a degree in any other University

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DEDICATION

This piece of work is whole heartedly dedicated to all great men and women of great minds and souls who know what they have achieved yet they are not confused with what they can still do. Great men and women who will never settle for great work achieved but will forever be determined to settle for nothing less than the full accomplishment of their dreams.

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TABLE OF CONTENT

LIST OF TABLES

LIST OF FIGURES

ABBREVIATIONS AND ACRONYMS

ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
ALW	Absolute liver weight
API	American Proficiency Institute
AST	Aspartate transferase
BUN	Blood urea nitrogen
BW	Body weight
CK	Creatine kinase
Cr	Creatinine
CYPs	Cytochromes
DMSO	Dimethyl sulfoxide
DPX	Distrene plasticizer xylene
GDP	Gross domestic product
GFR	Glomerular filtration rate
GSH	Glutathione stimulating hormone
HC*l*	Hydrochloric acid
HPLC	High pressure liquid chromatography
IT	Information technology
N	Normality
NaOH	Sodium hydroxide
pH	Potential hydrogen
RLW	Relative liver weight
SD	Standard deviation

SOPs	Standard operating procedures
SPSS	Statistical program for social scientists
TLC	Thin layer chromatography
UAE	United Arab Emirates
QA	Quality assurance
QC	Quality control
WHO	World health organization

ABSTRACT

Chewing of Khat, which typically consists of the young leaves and shoots of the *Catha edulis* plant for its stimulant effects, is rampart in East, Central and Southern Africa, UAE, Saudi Arabia, Yemen, Oman, Iraq, Iran Afghanistan, Pakistan and Bangladesh. Khat contains more than 40 alkaloids, glycosides, tannins, amino acids, vitamins and minerals. Most of the effects of chewing Khat are thought to come from two phenylalkylanines-cathinone and cathine which are structurally related to amphetamine. Khat abuse is associated with neurosis, ulcers, increased diastolic pressure, and vasoconstriction of coronary vasculature, gastritis and hemorrhoids. The potential for hepatotoxicity and nephrotoxicity of Khat chewing is however unknown. This study was conducted to evaluate the biochemical, hematological and histocytological effects of Khat extract on liver and kidney mice when administered orally with different non-alcoholic substance consumed alongside by Khat users. The non alcoholic substances used were water, coke, milk, patico sweet, tea, coffee and groundnut in different groups respectively. Forty five male albino mice were used in the study. The mice were grouped into nine. The Khat extracts was administered orally at a dosage of 2000mg/kg/day together with non-alcoholic substances for 30 days. Physical parameters of namely body weight and morphology of the liver and kidney were collected. The liver enzymes (aspartate aminotransferase, alkaline phosphatase and total bilirubin were evaluated. The kidney function enzymes like creatinine and blood urea nitrogen were evaluated. Enzymes were analysed using Cobas Integra® 400 plus automatic Chemistry Analyzer (Roche Diagnostic, Mannheim, Germany). The histocytological analyses for liver and kidney tissues were done using hematoxylin and eosin staining technique. The collected biochemical, hematological and cytohistological data were statistically analysed using paired t-test. Results indicated that there were hepatic enlargement, abnormal elevation in serum aspartate transferase (AST), alkaline phosphatase (ALP), serum bilirubin, blood urea nitrogen (BUN) and serum creatinine (Cr). The cytological and histological findings also indicated cytopathological and histopathological abnormalities in the liver and the kidney. Khat administration in the albino mice orally was associated with hepatic hypertrophy, hepatotoxicity and nephrotoxicity irrespective of the non-alcoholic substance it was administered with. Use of paired t test between a negative control and the test groups indicated that there was a significant difference in relative liver weight at $p < 0.05$. Khat extraction on albino mice leads to increase liver and renal enzymes used as the biomarkers of renal and liver injury. The histological and cytological studies indicated abnormality in cells and tissues of male albino mice. The results of the study provide information on the possible adverse effects on the liver and kidney. The information generated by the study can be used by the public health officer and the clinician to inform the public on the possibilities of Khat to cause liver and kidney problems. Study of the oral effects of Khat on liver and kidney of albino mice need to be carried out using a replication test at logarithmically spaced dose levels to clearly demonstrate the dose-time effects of the Khat extract and corresponding tissue responses at different doses to justify in need the Khat extract have biological effects on the liver and kidney and also to be carried out in large animals and primates to show effects of Khat on human liver and kidney. The study can be used by policy makers on the decision to take concerning consumption of Khat.

CHAPTER ONE

INTRODUCTION

1.1 Background

Khat is an evergreen flowering tree or shrub. Khat grows well in high altitudes of the horn of Africa and the Arabian Peninsula. More than 10 million people worldwide are using Khat for euphorizing and psychostimulating effect (Andualem, 2002; Bongard *et al*., 2011). In many countries Khat is chewed for social and psychological reasons and its use is expanding gradually worldwide (Yeshigeta and Abraham, 2004; Ezekiel, 2005; Wabel, 2011). In the past Khat consumption was only limited to older men and members of Muslim communities. However recent trends indicate consumption by all societal groups regardless of age, sex, affluence, class, education and occupation (Ayana and Mekonen, 2004). With introduction of modern means of transport, Khat production and consumption has expanded rapidly throughout the world faster (Gesesse, 2013).

Khat contains two alkaloids; cathinone and cathine which are responsible for central nervous system stimulation. Chewing of the Khat leaves is the most effective way of extracting cathinone; the chemical constituent of the Khat that produces an amphetamine-like stimulatory effect (Widler *et al*., 1994; Toennes *et al*., 2003).

Khat is a grown in most countries by small scale farmers and is a commodity of foreign currency source, agent of socio-economic and biophysical change (Kalix, 1990; Basunaid and Dongen 2008; Access 2010; Gebissa, 2010). However studies indicate that Khat consumption in large quantities has become a pastime activity and far reached across

large members of the population worldwide resulting in serious consequences on their health and socio-economic conditions (Ashbury, 2005; Dawit *et al*., 2005). With these realities Khat is now a controversial crop in the world in general, it contributes positively to the national economy of both importing and exporting countries. But on the other hand its psychoactive action has largely hampered the social economic and health status of the society (Aden *et al*., 2006; Bongard *et al*., 2011). Khat dependence has been associated with high morbidity, societal and economical costs (Marghi *et al*., 2009). The controversy is also found among international communities where some countries are classifying Khat as an addictive drug. Other countries ban Khat consumption with enforced law such as the Saudi Arabia, UK, Netherlands and USA. The controversy is also in the producing countries where the government neither encourages nor takes any action against Khat cultivation, trade or use (Rawlins, 2005).

A variety of views emerges from different literature sources. Hirst (1997) has labeled Khat as an adaptogene, with an ability to increase body's ability to adapt to stress and changing situations. This has given Khat three favourable traits: it is nontoxic, benefits the body as a whole and helps in the restoration of natural homeostasis processes. Khat is used across the world as a scapegoat for wide range of social and economic ills (Beckerleg, 2008). Khat despite its low addictive potential, chronic use is associated with hypertension, heart rhythm disorders, insomnia and loss of appetite (Pennings *et al*., 2008). Other studies have classified Khat as least harmful compared to popular drugs such as alcohol and tobacco (Nutt *et al*., 2007). Recently, Khat is the most controversial plants in all ramifications. Khat use has a deep root in both religion and socio-cultural

tradition. The legality of Khat use or trade varies from one country to the other. The ethical and morality of chewing Khat is ambiguous, equivocal and unclear. Literature supports the negative effects of chronic use of Khat on human health, though the longitudinal and quantitative data on health issues seem scanty. Since the public debate concerning Khat use and abuse are influenced by genuine concern, personal belief, religious affiliation and political motivation. It is through this background that this study attempts to investigate effects of Khat extract on liver and kidney of mice.

1.2 Problem statement

Khat consumption is associated with many health problems affecting the gastrointestinal system, reproductive system, cardiovascular system and other body systems (Szendrei, 1980; Al-Meshal, 1988; Al-Dubai *et al*., 2006; Brostoff *et al*., 2006; Hassan *et al.*, 2007). Khat abuse has become a real national problem in most East, Central and South African countries and also in Arabian Peninsula. Many people and in particular physicians believe that Khat is associated with health, social and financial problems (Khalil, 1997; Manghi *et al*., 2009). There is a growing concern of Khat health effects worldwide (Manghi *et al*., 2009). Kidney and hepatic pathological conditions have attained epidemic proportions worldwide today. The use *Catha edulis,* for its stimulant effects may lead to serious hepatotoxicity and nephrotoxicity complications and even death. Due to availability and increase in use of *Catha edulis* day after day it is important to study its hepatotoxicity and nephrotoxicity in human.

1.3 Research questions

i. What are the effects of Khat extracts on the total body and organ weight of mice when administered orally?

ii. What are the effects of Khat extracts on the liver and kidney analytes in albino mice when administered orally?

iii. What are the effects of Khat extracts on hepatic and renal tissues in albino mice histology, cytology and morphology after oral administration?

iv. What are the effects of Khat extracts on hematological tests in albino mice after oral administration?

1.4 Hypotheses

Ho_1: *Catha edulis* grown in parts of Kenya and used as stimulant drug has no hepatotoxic and nephrotoxic effects in albino mice when administered orally.

1.5 Objectives

1.5.1 Main objective

To evaluate toxicological effects of *Catha edulis* in mice.

1.5.2 Specific objectives

i. To determine changes in total body and organ weight in mice after oral administration of Khat extract.

ii. To determine changes liver function tests analytes (Alkaline phosphatase, Aspartate aminotransferase and bilirubin) and renal function tests analytes (Blood

urea nitrogen concentration and creatinine) in mice after oral administration of Khat extract.

iii. To determine the histopathological, cytopathological and morphological effects of Khat extract on hepatic and renal tissues and cells in albino after oral administration.

iv. To determine Khat extract effect on hematological (clotting time) test in albino mice after oral administration.

1.6 Justification of the study

There are a number of reviews of the effects of Khat chewing (Halbach, 1972; Al-Meshal *et al*., 1985; Pantelis *et al*., 1989; Al-Motarreb *et al*., 2002; Cox and Rampes, 2003; Al-Habori, 2005; Hassan *et al*., 2007) and this study supplements those earlier reviews to concentrate the adverse effects of Khat use. The study analyzed the effects of Khat on the liver and kidney enzymes, hematological changes and the histopathological changes of liver and kidney of albino mice. Khat abusers, consume Khat in accompaniment of different non-alcoholic substances, this was the reason behind testing the Khat toxicity in combination with different non-alcoholic substances like: - tea, coke, milk, coffee and water. These combinations assisted understand whether Khat toxicity is enhanced by different non-alcoholic substances consumed in accompaniment with Khat. The finding of this study provides evidence on the possible adverse effect of Khat on the kidney and liver of human. The finding of this study provides information to clinician and public health workers to sensitize ‘miraa’ abusers on the adverse effect on liver and kidney Khat may predispose them too.

1.7 Scope of the study

The study investigated hepatic and nephrotic effects of *Catha edulis* in albino mice. Khat extract was administered with different non-alcoholic drinks like coffee, milk, tea, coke, ‘patico’ sweet and groundnuts. This is because Khat abusers chew with these different on-alcoholic substances as the accompaniments. Non-alcoholic substances containing methylxanthines have synergetic effect to Khat (Kalix and Braenden, 1985).

CHAPTER TWO

LITERATURE REVIEW

2.1 Introduction

Khat scientifically known as *Catha edulis* (Vahl.) Endal., Catha edulis Forsk belongs to the family Celastraceae (moonseed)(Krinikonian, 1984 and Baasher, 1987). Celastraceae is a slow growing tree or shrub inhabiting the tropical regions of Arabian Peninsula and East Africa. Khat is found in countries along the coast of East Africa and those of red sea (Luqman and Danowski, 1976). Countries that mainly grown Khat in Africa are:- Ethiopia, Kenya, Madagascar, Somalia, south Africa and Sudan while in Asia it is mainly in Afghanistan, Pakistan and Yemen (Lugman and Danowski, 1976). The botanist Peter Forskal was the first to give scientific report concerning Khat in the eighteenth century. (Baasher, 1980; Al-Hebshi and Skang, 2005). Khat chewing is common in East Africa and Arabian Peninsula countries. The plant has several names according to different communities or countries like:- 'Chat' and 'Qat' in Yemen, 'Qaad' and 'Jaad' in Somalia, 'Miraa' and 'Muguka' in Kenya, 'Chat' in Ethiopia and'Jimma' in the Oromo language. In most western countries it is known as Khat (Luqman and Danowski, 1976).

Khat is evergreen and is cultivated as a small tree (figure 2.1). It reaches heights of 1.5 meters to 20 meters depending on the amount of rainfall and soil conditions. It grows in arid environments, and once established thrives in full sun, with environmental temperature ranging from 5-35°C. It will not usually tolerate frosts, and overwatering will cause it to drop leaves and die. In certain areas it is often grown with coffee plants

and in irrigated terraces (Lugman and Danowski, 1976). The leaves must be chewed while fresh for maximum effects. The plant contains the alkaloid called cathinone which is an amphetamine-like in structural and psychostimulation (Nencini *et al.,* 1989).

The tree leaves are slightly sweet with astringent taste and have aromatic odour. The taste of the khat leaves varies from one kind to another and depends on the tannic acid content. The young leaves are slightly sweet (WHO, 2006). Khat plant do not bear fruits hence it is seedless. Khat plant is able to grow in different climatic region and variety of soils. The plant is pruned to attain a desired shape, for easy harvesting and to encourage growth of shoots (figure 2.2) (Luqman and Danowski, 1976). In the past Khat consumption was localized within the region where 'miraa' is grown. Recently with improved means of transport Khat consumption is widely distributed all over the world. Khat is mainly harvested in the morning hours and immediately transported to the market in term of road and air transport to avoid loss of quality. The Khat stalks are arranged to form a bundle before they are taken to the market (figure2.3). In the market Khat is wrapped properly with banana leaves to preserve their freshness (Lugman and Danowski, 1976).

Khat leaves are consumed while fresh to avoid loss of stimulant potency. Khat leaves losses stimulatory potency after 3-4 days of harvesting. Due to improved means of transportation, Khat nowadays reaches far destinations where they are needed by customers including America and European countries (Halbach, 1972). In 1980, world health organization classified Khat as one drug of abuse which is capable of inducing to mild or moderate psychological dependence. Khat is however legal for production and

sale in many countries whereas some countries consider it to be an illegal or controlled substance.

Mild euphoria with excitement similar to those associated with strong coffee is induced by Khat consumption (Dewick, 2001). Oral consumption of cathinone leads to rapid onset of action. The action is faster than that of amphetamine pills when administered orally. Both Khat and amphetamine consumption leads to manic behaviors and hyperactivity. Some effects of Khat consumption are: - mydriasis (dilated pupils), constipation, increased rate of the heart and raised blood pressure. Some of the withdrawal system of Khat use include: - lethargy, irritability and mild or moderate depression. Upon long time abuse of Khat the withdrawal symptoms may range from lethargy, depression, nightmares and slight tremor. Khat causes an anorexic effect (appetite loss). Use of Khat for a long period of time lead to darkening of teeth or permanent tinge which is greenish, ulcers and low libido (Dewick, 2001). There is an increased renal and hepatic liver complication amongst ‘Miraa’ abusers in Meru according to Ogembo, personal communication, 2011, a consultant physician at Cottolengo mission hospital in Meru County.

Figure 2.1: A young *Catha edulis* plant

Figure 2.2: A well pruned *Catha edulis* plant in a field with shoots which are harvested

Figure 2.3: A bundle of harvested *Catha edulis* stalks ready for market

2.2 Historical background and botanical taxonomy classification

Khat: kingdom, Plantae; class, Magnoliopsida; order, Celastrales; family, Celastraceae; genus, *Catha*, species, *edulis*. Khat is believed to have originated from Ethiopia from where it spread to East Africa and Yemen (Peters, 1952; Radt, 1969; Cox, 2003). There is also a controversial belief that khat originated from Yemen before it spread to Ethiopia and nearby countries (Al-Motarreb *et al*., 2002; ACMD, 2005; NDIC, 2007). Khat from Ethiopia and Yemen is thought to have spread to Kenya, Somalia, Malawi, Uganda, Tanzania, Arabia, Congo, Madagascar, Zimbabwe, Zambia, South Africa, Afghanistan and Turkistan (Luqman, 1976).

Khat has been grown for centuries in the horn of Africa and Arabian Peninsula for use as a stimulant. Khat chewing predates the use of coffee. Khat plant is known by a variety of names such as qat in Yemen, chat in Ethiopia, jaad in Somalia and miraa in Kenya and Tanzania (Kennedy, 1987), it is also known as marungi, Catha, cat, cot, qat, qaad, African salad, tohai, bushmans tea, flower of paradise, four of paradise, Abyssinian tea, African tea, Arabian tea, chafta, ciat, crafta, djimma, ikwa, ischott, iubulu, kaad, kafta, Kat, la salade, liss, liruti, mariongi, mandoma, maonj, marongi, mbugula, mabwe, mdimamadzi, meongi, mfeike, mhulu, mira, mirungi, miungi, mlonge, m'mke, msabukinja, msuruti, msuvuti, msekera, muholo, muhulu, muirungi, mulungi, muraa, musitate, mutsawari, mutawhari, mutsawhri, mwandama, mzengo, nangungwe, ol meraa, ol nerra, qat, quat, salahin, seri, Somali tea, tohai, tohat, tsad, tschad, tschat, tshut, tumayot, waifo, warfi, Kus-es-Salahin, warfo in most western literature it is referred as Khat (Luqman, 1976; Cox and Rampes, 2003).

The khat shrub has slender trunk with thin and smooth bark. The leaves are lancet shaped with a length ranging from 0.5 to 10cm and a width ranging from 0.5 to 5 cm. Young leaves are reddish green but later turn into yellow greenish colour. In frost areas the shrub grows to height higher than 1.5 meters. The shrub is able to grow to height of 20 meters in places with more rainfall. (Kennedy, 1987; Al-Motarreb *et al*., 2002). Khat is a perennial crop able to survive in drought areas where other crops have failed. Khat is propagated through grafting and plant does well in altitude of 1500-2000 meters. Khat leaves have faint aroma with astringent and slight sweet taste. The khat tree takes 3-4 year before they mature. A health khat plant can stay productive for more than 50 years. There is no known disease that affects khat. Khat has a tap root that grows to a depth of 3meters or more (Al-Motarreb *et al*., 2002; NDIC, 2007).

2.3 Ingestion of Khat

Most of the people who use Khat do so through chewing. Small population take it as a drink of dried Khat leaves and some also as a smoke. Chewing Khat results into a mouth full of leave and stalk resulting to release of juice which contains active components swallowed with saliva (Nencini *et al*., 1986). Chewing of Khat plant materials results into a ball that is kept for a while in the cheek, causing a characteristic bulge (Nencini *et al*., 1984). Khat is consumed in social gathering referred to as ‘Khat sessions’. During these sessions, the young tender stalks with leaves are chewed slowly and intermittently release the active components for several hours (Al-Habori, 2006).

2.4 Reasons for chewing Khat

Chewing Khat has both cultural and social importance in communities where it is grown. Khat chewing facilitates social interaction. In some communities Khat plays a vital role as part of dowry in wedding ceremonies (Kennedy *et al.,* 1983). Muslims in Yemen are avid chewers and some have a belief that chewing Khat especially when praying enhances contact with their God (Allah) (Kennedy *et al.,* 1983). Christians and Jews in Israel chew Khat, and is used by many people to increase work performance and alertness (Kalix, 1996). People who work at night mostly use Khat to remain awake and for reducing fatigue. There are also incidences of Khat use amongst students before exams with an aim of improving mental performance. Khat has also been used for medicinal values especially as a pain killer and also in management of arthritis, fever and depression (Kennedy *et al*., 1983). Most of the Khat users prefer using Khat in a social place and few takes its while alone. Khat chewing in a social setting can last for several hours (Kennedy *et al.,* 1983).

In South-West of Arabian Peninsula and East Africa khat is used to ease exhaustion, increase alertness and self esteem, decrease hunger and induce euphoria and feeling of well being (Brenneisen *et al*., 1990; Kalix 1996). Medically Khat is used as an appetite suppressant and an anti-ulcer agent (Carrier, 2008). Khat is also used medically for its aphrodisiac effects and also to manage premature ejaculation (Krinikonian, 1984).

2.5 Active constituents of Khat

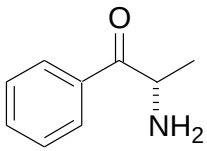
Leaves of a fresh Khat contains a naturally occurring active amphetamine-like stimulants cathinone (S-(-)-a-aminopropiophenone) and cathine (S, S-(-)-norpseudoephedrine) (Houghton and Raman, 1998; Cox, 2003; Corkey *et al*., 2011). Khat contains many different compounds which include:- alkaloids, amino acids, flavonoids, glycosides, terpenoids, minerals, sterols, tannins and vitamins (Halbach, 1972: Kalix and Braenden, 1985; Cox and Rampes, 2003). The chemical profile of the Khat leaves depend with the environment and climatic condition where the plant is found (Geisshusler and Brenneisen, 1987; Al-Motarreb *et al*., 2002). The active ingredients of Khat are cathine (norpseudoephedrine) (figure 2.5) and cathinone (benzoylethanamine) (Figure 2.4). These alkaloids have similar structure and pharmacological activity to amphetamines (Wagner *et al*., 1982). Khat effects come from phenylalkylanines-cathinone and cathine (Nencini *et al*., 1984). The major Khat alkaloids are the phenylalkylamines and the cathedulins. Cathedulins are basically polyester of euonyminol and are based on a polyhydroxylated sesquiterpene skeleton. There are 62 different types of cathedulins characterized from fresh Khat leaves (Kite *et al*., 2003). Khat does not contain amphetamine or caffeine. Other chemical substances in Khat include cathidine, eduline and ephedrine (Giannini *et al*., 1986).

Cathinone and cathine stimulate the central nervous system and suppress appetite hence are referred to as the natural amphetamine. Cathinone is more rapid in stimulation of central nervous system with a half life of about 4 hour shorter than that of amphetamine. Central nervous stimulation is caused by increased levels of dopamine (DA) and

norepinerphrine in the brain. Dopamine and norepinerphrine acts on the catecholaminergic synapses delaying the reuptake and/or enhancing the release of these neurotransmitters (Wagner *et al*., 1982; Patel, 2000).

2.5.1 Cathinone

Cathinone (figure 2.4) is the primary constituent of Khat. (UNDCP, 1996; WHO, 2006). Cathinone is found mainly in the young leaves and shoots. Cathinone is metabolised to cathine [(+) – norpseudoephedrine] during maturation in a ratio approximately 4:1 (UNDCP, 1996; WHO, 2006). Cathinone is the Khat most central nervous system (CNS) stimulant active ingredient. Isolate and synthesized cathinone have shown similar effects to amphetamine though it has lowered potent. Fresh khat leaves contains cathinone amount ranging from 78-343mg/100g. The level of cathinone decreases with storage time. When hat leaves are dried, the psychoactive substance cathinone decomposes to less active psychoactive chemical cathine. Scientific studies confirm than cathinone effects are significantly if not completely diminished if the plant material is dried slowly Cathinone is more potent to cathine by 7-10 times when it comes to central nervous system stimulation. Cathinone is difficult to synthesize hence not suitable for marketing in form of a pure substance (Nencini *et al*., 1989).



Cathinone

Figure 2.4: Chemical structure of cathinone

Young fresh leaves contain 0.1-0.3% (-) cathinone as the principal central nervous system stimulant (Dewick, 2008). Cathinone is relatively unstable, decomposing to (+) norpseudoephedrine (cathine) and norphedrine after harvesting or as the leaves are dried which are related to dexamphetamine structurally (Nencini *et al*., 1984). Decomposition leads to a ‘’dimmer’’ (3, 6-dimethyl – 2, 5 – diphenylpyrazine) and possibly to small fragments. Both the dimmer and phenyl- propanedione have been isolated from khat extracts (WHO, 1980). Presumably, cathinone is the main psychoactive component of Khat, this explain why khat is wrapped up in banana leaves to preserve freshness (WHO, 2006). Cathinone, in central nervous system (CNS) leads to releases of serotonin and dopamine in central nervous system (CNS). Dopamine release in the dopamine terminals offers dopaminergic pathway increased activities (Kalix and Braenden, 1985). Cathinone enhances release of noradrenaline from their storage sites, this facilitates noradrenaline transmission. Noradrenaline uptake can be inhibited by cathine and cathinone (Drake, 1988). The pleasure derive from Khat chewing is attributed to the euphoric actions of cathinone, a sympathomimetic amine with properties similar to amphetamine (Kalix, 1992). The euphoric effect of chewing Khat starts after one hour. Within one hour the levels of cathinone levels start rise and peak plasma levels are obtained within 1.5-3.5 hours after the onset of chewing (Halket *et al*., 1995).

2.5.1.1 Pharmacology

The psychotropic effects of Khat are caused by cathinone and cathine which are amphetamine-like substances. Amphetamine and cathinone acts on different parts of the

brain though they share common effects like interaction with the dopaminergic pathways (Pehek *et al*., 1990). The stimulation of the central nervous system by Khat is manifested by increased alertness, hyperactivity, aggressiveness, elevated blood pressure, euphoria, garrulousness, excitement, manic behaviour and anxiety. Khat stimulation lasts for 3 hour (Kalix, 1996) and is followed by depressive phase which include malaise, insomnia and lack of concentration (Al-Motarreb *et al*., 2002; Hassan *et al*., 2002). The Khat dose is self-limiting because it does not permit the active compounds to rise high enough for toxic psychosis. However, paranoid delusions, usually persecutory, have been seen (Jager and Sireling, 1994).

Catha edulis induced psychotic conditions are described by several case studies (Giannini and Castellani, 1982; Critchlow and Seifert, 1987; McLaren, 1987; Yousef *et al*., 1995). In Eastern Ethiopia where Khat is commonly grown, Khat induced psychotic behaviour is commonly designated as ‘Jezba’ (Kalix, 1988). Khat related psychoses in many instances disappear with few days when consumption of Khat is stopped or if anti-psychotic agents are initiated. However, in return of psychotic symptoms, recidivism occurs. There are little population based studies to associate Khat use and psychiatric problems. Khat use can exacerbate psychotic symptoms in individuals with pre-existing conditions, and precipitate psychiatric disorders in vulnerable subjects (Yousef *et al*., 1995).

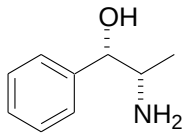
2.5.1.2 Pharmacokinetics of cathinone

During Khat chewing most of the alkaloids are extracted in to the saliva. After chewing, absorption takes place very first starting from the mucosa of the mouth. The stomach and/or the small intestine receive the swallowed juice and are the second phase of absorption. Chewing and masticating the Khat materials effectively liberates the alkaloids from the leaves allowing rapid absorption in the systemic circulation. (Toennes *et al*., 2003). Cathinone effects starts within a period of between 15-30 minutes (Kalix and Braenden, 1985). Cathinone is metabolised rapidly. Metabolism of cathinone mainly occurs at the first passage in the liver (Drake, 1988). Only 2% which is a small fraction of the absorbed cathinone will appear in urine while unchanged. Cathinone is mostly metabolized and excreted in form of norephedrine (Drake, 1988; Niverberg, 2002). The inactivation and absorption rate of cathinone are almost the same hence this offers limitation in attaining blood cathinone levels through chewing (Kalix and Braenden, 1985). When taking Khat, drinks especially non-alcoholics are consumed in large amount. Drinks which contain substances such as methylxanthines offer a synergetic effect to Khat (Kalix and Braenden, 1985).

2.5.2 Cathine

Cathine (figure 2.5) has a minimal function in the stimulatory effect of Khat. Cathine is associated with unwanted harmful effects on the body. Cathinone which is known to cause central nervous system (CNS) stimulation is higher in fresh Khat leaves than cathine (Nencini *et al.,* 1989). Drying of Khat leaves leads to breakdown of cathinone to cathine. Khat users prefer fresh Khat leaves because they offer more stimulation effects

than dry ones. This is as result of higher amounts of cathinone. Khat abusers are advised to use fresh Khat leaves because they do offer little systemic effects (Nencini *et al*., 1989).



Cathine

Figure 2.5: Chemical structure of cathine

Cathine cause inhibition of noradrenaline uptake (Drake, 1988). Cathine has a half life of 3 hours and takes longer to induce stimulatory effects to central nervous system than the cathinone (Nencini *et al*., 1989).

2.6 The liver

The liver is an organ of major importance containing a variety of cell types that are specialized for performing particular functions and are co-ordinated in a complex three dimensional arrangement (Elias, 1955; Elias, 1989; Elias, 1995). Liver is built of parenchymal cells (hepatocytes) and endothelial cells that line the blood vessels, bile ductular cells, connective tissue cells, nerve cells, Kupffer cells and the pit cells that may play a humoral role (Wisse *et al*., 1976). Generally, in normal liver the hepatocytes are the major cell type in terms of number (Abercrombie and Harkness, 1991), and they form a large volume of the liver substance.

2.6.1 Drugs and the liver

Hepatotoxicity of drugs can be principally metabolism-dependent, parent drug-dependent, or a combination of both. Metabolism takes place largely in the liver, which accounts for its susceptibility to drug induced injury (Kaplowitz, 1996). Liver metabolises majority of exogenous substances within the body before they are taken to systemic circulation (Elbers *et al*., 1980; Macfarlane *et al*., 2000). The metabolites may be electrophilic chemicals or free radicals that deplete glutathione stimulating hormone (GSH), covalently bind to proteins, lipids, or nucleic acids or induced lipid peroxidation. The consequences include hepatocellular necrosis, apoptosis or sensitization to cytokines or inflammatory mediators produced by nonparenchymal cells. Alternatively, the reactive metabolites may covalently bind to or alter liver proteins such as cytochrome p450, (CYPs) leading to sensitization and immune-mediated injury (Pessayre, 1995). Liver injury occurs generally over a range period ranging from approximately 15 minutes up to many days after exposure to a toxic agent. Biochemical studies of liver injury *in vivo* tend to fall into the same general time ranges with most analyses of acute injuries being done over a range 5 minutes to 24 hours and chronic lesion being studied over intervals up to several months after toxic injury commenced (Miller, 1970).

2.6.2 Assessment of hepatotoxicity

Liver disease caused by drugs or toxins remain a challenge of modern hepatology. This is not only because the pathogenesis and susceptibility factors for idiosyncratic toxic liver damage are still poorly understood, but also because of the lack of reliable and standardized markers for toxic liver damage (Renner, 1995; Tredger and Sherwood,

1997; Bissel *et al*., 2001). The diagnosis of drug hepatotoxicity relies upon circumstantial evidence of exposure to a potential hepatotoxin as well as the exclusion of other causes of liver injury (Bissel *et al*., 2001).

2.7 The kidney

The morphology and physiology of animal kidney varies depending on the species (Moffat, 1979). Kidney microsomes have a number of enzymatic and cytochrome metabolic mediated activities. The capacity of kidney to transform xenobiotic is approximately 3-50% of the liver (Litterst *et al*., 1975; Navran and Louis-Ferdinand 1975; Fry *et al*., 1978). Under some circumstances it is higher than of the liver (Anders, 1980; Nuyts *et al*., 1989).

2.7.1 Assessment of nephrotoxicity

Earlier methods of assessing damage of the kidney and prediction of their healthy status due to chemical injury are not able to provide relevant information due to differences in various chemical injuries (Bach *et al*., 1989). This is attributed to the fact that kidney has a functional reserve hence it may undergo some injury without any significant clinical indication. Lack of substantial clinical changes masks until renal degeneration reaches up to a substantial amount (Friedlander *et al*., 1989). In the past glomerular filtration rate (GFR) was the most common test used to monitor renal damage. The test was done by use of single blood sample unto which the serum creatinine was determined. The reciprocal of the serum creatinine was then adjusted to the constant surface area of the body (Siersback-Nielson *et al*., 1971). Abnormal functioning of the renal tubules is

positively correlated with the histopathological changes (Buzio *et al*., 1989). Renal function damages can be reversed if the substances leading to injury are withdrawn but this depends with the repair mechanism of the body. In management of renal tissue injury there is a great need of repeated monitoring (Thornley *et al*., 1985).

2.8 Khat adverse effects

Khat is consumed for its psychostimulatory effect (Al-Motarreb *et al*., 2010). Khat chewing is a serious public health problem in areas where Khat is grown and its spreading rapidly worldwide (Al-Motarreb *et al*., 2010). The chewing of Khat leaves involved adult males but it has extended to women too (Kasim *et al*., 2010). The WHO (2003, 2006) reported common health problems associated with Khat consumption. Consumption of Khat for a long period could result in psychoneurological disturbances such as neurosis (Hoffman and Al'Absi, 2010). Other health related problems include: - increased diastolic pressure (Getahun *et al*., 2010); vasoconstriction of coronary vasculature (Ali *et al*., 2010); gastritis (Nencini and Ahmed, 1989); hemorrhoids and duodenal ulcers (Manghi *et al*., 2009). Luqman and Danowski reported that liver cirrhosis among Yemeni Khat chewers might be associated with Khat consumption (Luqman and Danowski, 1976). The hepatotoxicity associated with Khat chewing is still debated in humans (Ardouin *et al*., 1979; Chapman *et al*., 2010; Coton *et al*., 2011). Administration of crude Khat extract to animals like New Zealander white rabbits for three months is linked with toxic hepatocellular jaundice and histopathological abnormalities in liver (Al-Mamary *et al*., 2002). Moreover a companion study on the same species of animals with the same dose of crude extract for six months supported the

former three-month findings; however, after six months histopathological evidence from liver sections suggested periportal fibrosis as an initial indicator of liver cirrhosis without apparent damage to kidneys (Al-Habori *et al*., 2002).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Materials

3.1.1 Plant collection, identification and processing

Freshly picked *Catha edulis* young shoots consisting young leaves from a farm were harvested in early morning hours and presented as a small bundle of twigs. The plant samples were collected from 30 different Khat plants from Meru County, Kenya. The plants were sampled from 5 different farms. Approximately 20 Kilograms were collected. The bundles were wrapped with fresh banana leaves before and during transportation in order to preserve their freshness. The *Catha edulis* from which the samples were collected had not been sprayed with any pesticide or fertilizer for more than six months to avoid chemical residues. In Kenyatta University Biochemistry laboratories, the plant sample were divided into smaller sample portions and were stored at temperature of approximately 4^0C before extraction of cathinone and cathine.

3.1.2 Plant extraction

Protocol by Adugna and Dagne (2009); was followed when extracting alkaloids. The *Catha edulis* plant sample specifically the fresh soft stem barks of the shoots and leaves were collected in the month of September from a domestically grown plants and stored at 4ºC in laboratory refrigerator. The plant samples were weighed and washed three times in clean distilled water. The plant samples were chopped, and ground into a fine in a mortar and pestle, weighed and then placed in an Erlenmeyer flask (conical flask). Approximately 200g of the plant sample paste was suspended in 300mL of 0.1N

hydrochloric acid (0.1N, HC*l*) in conical flask. Thirty conical flasks were used in the experiment. All the conical flasks were covered with aluminium foil to shield the extract from sunlight destruction. The mixture was sonicated for approximately 30 minutes, shook by use of a shaker for 10 minutes (120shakes/minute). The mixture was filtered by use of a suction pump. The plant material that remained in the conical flasks was again washed by 140 ml of 0.1 N HC*l*. Diluted hydrolochloric acid was used to obtain a crude extract of alkaloids since they exist in plant as salts forms of organic acid. The filtrate was extracted two times with 100mL of diethyl ether. The diethyl ether portion was separated by use of 1000mL separatory funnel. The aqueous acidic portion was again extracted two times with chloroform and again the acidified portion separated by use of 1000mL separatory funnel. This procedure was used to remove the fat, oils and chlorophyll in the plant material. Drops of saturated sodium hydroxide (10%, NaOH) were added to acidic aqueous portion until a pH of 10 was attained. The pH monitoring was done by use of a universal indicator pH 1-14. This was done to convert the alkaloids to free base forms from their salts.

The basic mixture was extracted two times by use of 100mL diethyl ether. The diethyl ether was separated by use of separatory funnel. Oxalic acid (1%) in diethyl ether was added to the diethyl ether portion until white precipitate was observed. The mixture with precipitates was left to stand for 20 hours in refrigerator at a temperature of approximately 6^{o}C and then filtered in a No 1 Whatmann filter paper. The precipitate was left to dry at room temperature for 48 hours. One gram (1g) of Khat extract was dissolved in 5mL DMSO and aliquots (each) 200µ*l* were immediately stored in a laboratory freezer

at a temperature of -10ºC. Different extraction batches were routinely used every day. Extract was verified using TLC plates by use of already documented ratio factor values. The eluent mixture of ethyl acetate-methanol-ammonia, 85:10:5 was used. The spots of the cathinone and cathine were viewed in an ultraviolet cabinet at a wavelength of 254 and 366nm and sprayed later with ninhydrin solution.

3.1.3 Reconstitution of plant extract for animal experimentation

The resultant Khat extract weighed 1g in a volume of 5ml; this was used to prepare a working concentration of 200mg/ml. the Miller and Tainter protocol (1970); was used to determine the dosage for Khat extract to be administered. To determine the volume of the extract to be administered for example to a mouse weighing 26.5g at 2000mg/Kg body weight, the weight of the mouse was multiplied by 2000mg/Kg then divided by 1000g and 200mg/ml. Therefore, a mouse weighing 26.5g was administered with 265µl of Khat extract. The volume of Khat extract for all the animals used in the experiment was calculated based on weight and formula above. The accurate measurements of the extract were made possible by use of micropipettes. The dose of the extract was adjusted to the final volume of 0.5ml using normal saline and administered orally.

3.1.4 Experimental animals

Male albino mice with an average age of 6 weeks weighing 20-25g were used. The mice were bred in the Department of Zoological Sciences, Kenyatta University.

The albino mice were housed in group of five in metal wire meshed cages approximately 30 × 30 × 30cm and then placed on a 0.75m raised surface in the animal house. Nine

groups of the study animal were housed in separate cages. Each cage was labeled with cage number (one to nine). Wood shavings beddings were replaced daily. The albino mice had access to standard rodent pellets. The temperature in the animal room was approximately 25^0C. Before the study commenced, all albino mice were clinically observed daily for mortality, morbidity and any physical abnormality.

3.2 Toxicity testing

The experimental animals were treated as indicated in the table 3.1 for *Catha edulis* toxicity.

Table 3.1 Treatment of experimental animals with *Catha edulis* extract and different accompaniments as used by *Catha edulis* users.

Group number (n=5)	*Catha edulis* extract dosage per day	Type of accompaniment	Amount/dosage of accompaniment in a day	Duration of treatment
1	-	water	2ml	30 days
2	-	indomethacin	0.3mg/Kg	30 days
3	2000mg/Kg	water	2ml	30 days
4	2000mg/Kg	coke	2ml	30 days
5	2000mg/Kg	coffee	2ml	30 days
6	2000mg/Kg	milk	2ml	30 days
7	2000mg/Kg	tea	2ml	30 days
8	2000mg/Kg	Patico sweet	2g	30 days
9	2000mg/Kg	groundnut	2g	30 days

3.2.1 Test parameters

Different samples were collected to tests for the *Catha edulis* toxicity in mice. Some of the tests which were carried out involved the biochemical tests, physical tests, hematological test and histocytological tests.

3.2.2 Physical parameters

The body weights in grams of the albino mice were taken at the beginning and at the end of the study. That is on the first day of Khat extract treatment and the last day before they were sacrificed. The absolute liver and the kidney weight for each of the albino mice were taken after washing the liver in normal saline and the blotting with filter paper. Relative liver weights were obtained for each of the albino mice by dividing the liver weight by body weight.

3.2.3 Hematological tests

The hematological testing which was carried out was only the blood clotting time test. The blood samples for hematological test were collected in a container without an anticoagulant. The blood samples were collected from the tail.

3.2.4 Gross pathology examinations

Liver and kidney weight were collected after sacrificing the experimental mice. The organ weights were used to determine the relative weight of the organs. The organs were also observed for any other abnormalities.

3.2.5 Histocytological tests

The experimental animals were sacrificed on the 31st day to obtain kidney and liver for physical, morphological, histological and cytological processing and examination to determine liver and kidney damage.

Histological and cytological examinations were carried out using haematoxylin and eosin staining technique and observed under light microscope for any histopathological and cytopathological changes (Baker *et al*., 1989).

3.2.5.1 Specimen collection for cytological and histological analysis

The histological specimens were collected by the removal of both kidneys and the liver from the sacrificed mice. Immediately after the kidney and liver tissue were collected they were fixed in 10% formal saline. The jars containing the tissues were correctly labeled with the animals' number and also the group number.

3.2.5.2 Histological specimen transportation, processing and storage

The fixed tissues were directly transported in the cytology and histology laboratory while in 10% formal saline. The tissues were trimmed into 2mm sections which were taken straight to 70% ethanol to commence dehydration process. The dehydration process started with 70% ethanol for 1 hour, then to 80% ethanol for 1 hour, 90% ethanol for 2 hours and then to absolute ethanol I for 1 hour, absolute ethanol II for 2 hours and absolute ethanol III for 2 hour.

After dehydration process, the tissues were put into the clearing process. The reason for this process was to replace the dehydrating fluid with substance that is miscible with the embedding medium to be employed. The clearing agent which was used in the process was xylene. To start the clearing process the tissues were placed in chloroform for 1 hour, then to chloroform II for $1^1/_2$ hours, chloroform III for $2^1/_2$ hour and lastly in chloroform IV for 3 hours.

The tissues were impregnated in paraffin wax to completely saturate the tissue with the medium to be used for embedding. The tissues were impregnated by subjecting them to molten wax at approximate temperature of 40ºC for 2 hours in paraffin wax I and for 3 hours in paraffin wax II. Immediately after impregnation of the tissue, the tissues were embedded using paraffin wax by positioning the specimen in a paper boat at the middle with an aid of an applicator stick. The paper boat was filled to the brim with paraffin wax. The paper boat was placed in a dessicator for complete hardening. Hardening was complete when a finger could not make impression on the surface.

The paraffin wax blocks were stored in a cool place. The tissue surfaces were protected from dust by covering with a layer of molten wax. The tissues were later sectioned using Cambridge rocker microtome with biconcave knive. During microtomy process thin ribbon were produced which were spread in water bath at temperatures of 37^0C. The tissue sections were fished from the water bath using slide. The tissue sections were fixed on slides using egg albumin.

The slides were stained in Harri's haematoxylin for 4 minutes and then rinsed in tap water. 1% acid alcohol was used to differentiate the cells by dipping 10 times. The slides were rinsed in tap water and then blued in Scott' s tap water by dipping 10 times before rinsing in 95% ethanol in 10 dips. The slides were stained with orange G 6 solution for 2 minutes and then rinsed in 95% ethanol by dipping 10 times. The slides were stained with Eosin in Azur 50 stain for 5 minutes and then rinsed in 95% ethanol 10 times. The specimen were dehydrated in changes of absolute ethanol by dipping 10 times and then cleared in 3 changes of xylene by dipping 10 times. The slides were mounted using D.P.X. The slides were observed under microscope first at ×40 then at ×100.the nuclei of the cells were blue, red blood cells were orange to pink while the rest of the tissue was in the shade of red and pink.

3.2.6 Biochemical tests

Liver functions were assessed by measuring the concentration of substances produced by the hepatocytes, measuring the serum content of substances that are changed by hepatocytes damage, evaluating the serum concentration of substances of injury, assessing the ability of the liver to perform a metabolic task such as conjugation or detoxification or by measuring enzyme activity and substrate content of the cell and its organelles. The substances tested were the alkaline phosphatase and the aspartate aminotransferase. Renal function tests were assessed by evaluation of glomerular and tubular function. The substances evaluated to assess the function of the glomerular and tubular functions were the levels of bilirubin, creatinine and blood urea nitrogen.

3.2.6.1 Specimen collection for biochemical tests

Blood for biochemistry analysis were collected from the veins in the tail and also from the liver and the heart. The blood samples were collected in red tipped vacuatainers without an anticoagulant.

3.2.6.2 Specimen transportation, processing and storage for biochemical tests

Specimens after collection were transported from the laboratory animal house dissection centre to the processing laboratory in ice packed cool boxes within one hour. Once clotted, the blood specimens were centrifuged at 3000 revolutions per minute for two minutes and serum separated immediately into well labeled cryovials. Serum specimens were then stored in laboratory refrigerator at 4^0C awaiting laboratory analysis at clinical laboratory at Kenyatta national hospital.

3.2.6.3 Biochemical laboratory analysis

Three liver function and two renal functions tests were determined on the sera specimen. The liver function tests determined were alkaline phosphatase, aspartate aminotransferase and total bilirubin. The renal function tests which were determined include: creatinine levels and blood urea nitrogen concentration. All the assays were performed based on the standard operating procedures (SOPs) written and maintained in the Kenyatta national hospital laboratory using Cobas Integra® 400 plus automatic Chemistry Analyzer (Roche Diagnostics, Mannheim, Germany).

3.2.6.4 Calibration of biochemical tests

To calibrate the biochemistry analyzer system, a Calibrator for automated systems was used. The calibration was automatically performed by the system.

3.2.6.5 Quality assurance (QA)/ Quality control (QC)

All the pre-analytical and post analytical precautions were put into consideration to ensure accuracy and precision of the test results. Internal QC materials from Roche diagnostics were the internal QC materials which were run daily while the external QC material for the process were from American Proficiency Institute (API). The American Proficiency Institute external QC was used at Kenyatta national hospital to monitor the performance of the institution laboratories. The American proficiency institute external QC were run twice daily according to manufacturers' instruction and QC protocol. The quality control was automatically performed by the system as per the specification of the test definition (Ohmann, 1997).

3.2.6.6 Biochemical data collection

The data was collected after carrying out laboratory experiments and analyzing serum for liver and renal function tests and also after observing morphological, cytological and histological changes of liver and kidney from experimental albino male mice.

3.3 Data analysis

For statistical analysis SPSS software (version 17 SPSS Inc. Chicago. Illinois) was employed for arithmetic means and standard deviation of biochemical, histocytological,

physical and hematological tests were calculated. The p-values were examined by paired students t-tests. The results were considered significant by a p-value <0.05. All end points were analyzed using 2-tailed test. The average values of biochemical, histocytological, physical and hematological tests were presented using tables and bar graphs.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Changes in total body and organ weights in mice

4.1.1 Body weight

The average body weight of the negative control group (29.92 ± 1.83g) was significantly different with that of the positive control group (26.92 ± 1.80g) at $p < 0.05$. The negative control group average body weight was higher compared to other test groups (Table 4.1). There was no significant difference between the body weight of the negative control and the test groups. There was a clear difference in average body weight between the negative control and other test groups (Figure 4.1).

Table 4.1: Mean body weight of male albino mice in grammes after treatment with *Catha edulis* extract for 30 days at a dosage of 2000mg/Kg/day and different accompaniments as used by *Catha edulis* users

Group	Treatment	Body weight in grams
1	No Khat extract (-ve control)	29.92±1.83
2	Indomethacin (+ve control)	26.92±1.80
3	Extract + water	28.22±1.07
4	Extract + coffee	28.28±1.06
5	Extract + milk	28.59±0.85
6	Extract + tea	28.14±0.77
7	Extract + patico sweet	28.09±1.01
8	Extract + groundnut	28.78±1.83
9	Extract + coke	28.92±1.83

The difference in the body weight of the test animal group and the negative control could be attributed to anorexigenic effect of Khat (Halbach, 1972; Sireeratawong *et al.*, 2008). This could also be due to Khat effects on intestinal absorption due to astringent effect of

Khat (Aziz *et al.*, 2011). Administration of Khat to animals is associated with significant decrease in leptin levels which leads to decrease in body weight (Admassie and Engidawork, 2011). Presence of compounds such as tannins and polyphenols in Khat can

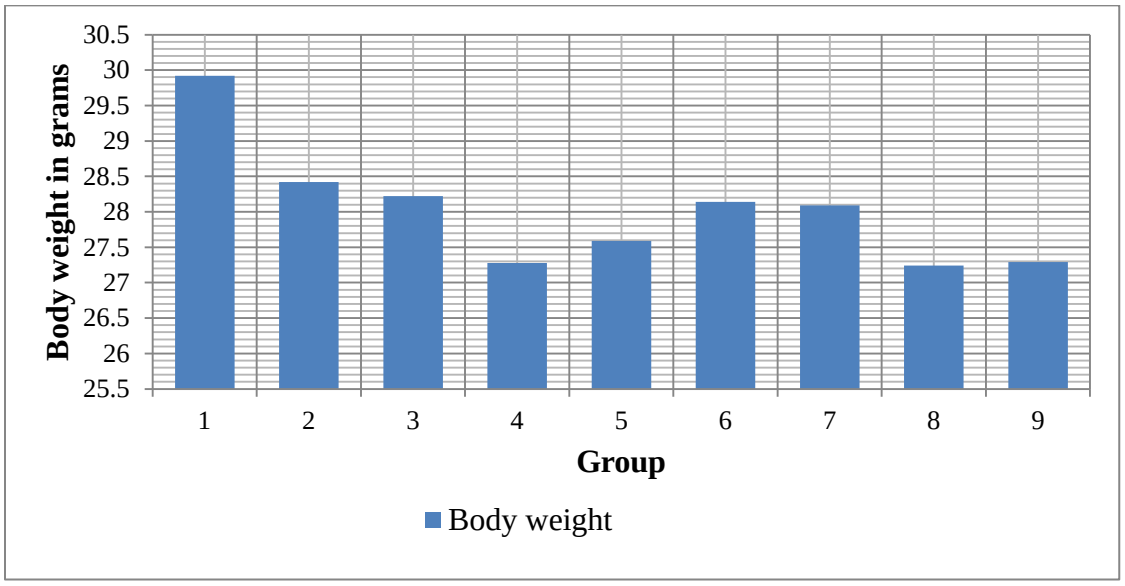


Figure 4.1: The mean body weight of male albino mice in grams after treatment with *Catha edulis* extract for 30 days at a dosage of 2000mg/Kg/day and different accompaniments as used by *Catha edulis* users

be attributed to retarded growth which leads to weight loss because of their inhibition activity to digestive enzymes leading to impaired nutrients absorption (Al-Mamary *et al.*, 2001). According to the study in relation to human, Khat chewers may suffer from loses of weight due to decrease in levels of leptin, malabsortion and loss of appetite.

4.1.2 Changes in liver and kidney weight

The average liver weight amongst the groups was not significantly different at $p < 0.05$. The absolute liver weight of the test groups, negative control and the positive control was not significantly different between the groups at $p < 0.05$. The liver weight increase was

observed among the groups unto which indomethacin and Khat extract was administered (Table 4.2). The absolute liver weight of the positive control was higher than that of the test groups into which Khat extract was administered though that of the tests groups was higher than that of the negative control (Figure 4.2).

Table 4.2: Absolute liver weight (ALW), relative liver weight (RLW) and absolute kidney weight (AKW) in grams of male albino mice after treatment with *Catha edulis* extract for 30 days at a dosage of 2000mg/Kg/day and different accompaniments as used by *Catha edulis* users

Group	Treatment	(ALW) in grams	RLW (W/W %)	AKW in grams
1	No Khat extract (-ve control)	0.95±0.28	3.53±1.26	0.25±0.04
2	Indomethacin (+ve control)	1.55±0.17	5.18±0.08	0.31±0.04
3	Extract + water	1.01±0.29	3.58±1.16	0.26±0.06
4	Extract + coffee	1.44±0.27	5.09±1.20	0.29±0.04
5	Extract + milk	1.37±0.41	4.79±1.64	0.31±0.09
6	Extract + tea	1.25±0.27	4.44±1.08	0.31±0.03
7	Extract + patico sweet	1.34±0.31	4.77±1.19	0.35±0.09
8	Extract + groundnut	1.13±0.33	3.93±1.33	0.28±0.07
9	Extract + coke	1.18±0.27	4.08±1.16	0.27±0.03

ALW: Absolute liver weight, RLW: Relative liver weight, AKW: Absolute kidney weight

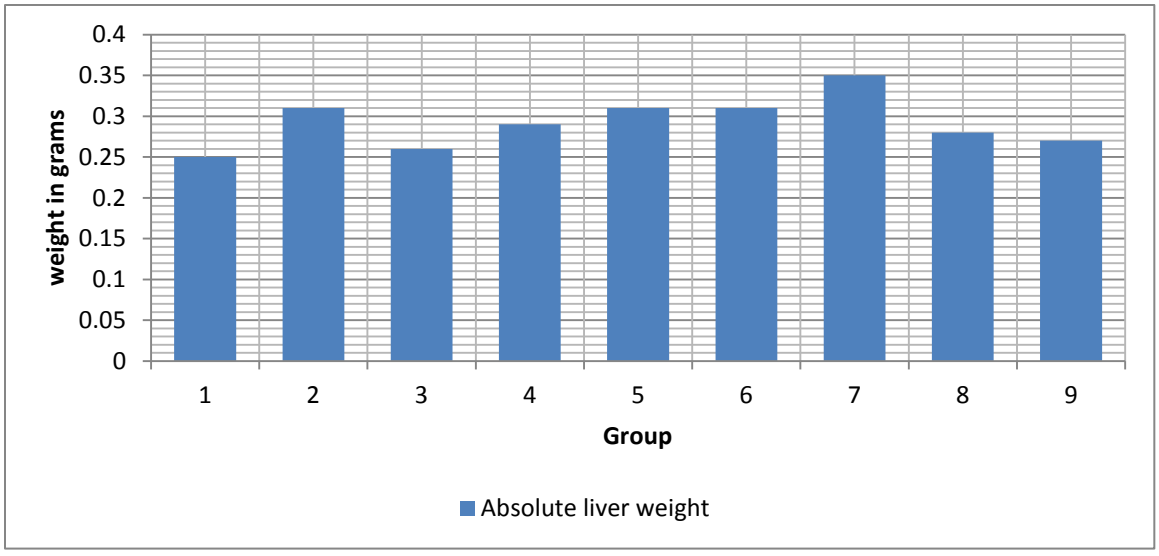


Figure 4.2: The mean absolute liver weight in grams of different groups of male albino mice administered with Khat extract in different combinations of non-alcoholic substances for 30 days at a dosage of 2000mg/Kg/day.

The relative liver weight of the positive control (5.18±0.08g) and the negative control (3.53±1.26g) were significantly different at $p < 0.05$. There was no significant different between relative weight of the positive control and the test groups at $p < 0.05$. The relative liver weight for the albino mice in which Khat extract was administered was higher than that of the negative control (Table 4.2). The relative liver weight of the positive control group was higher than of the negative control and the test groups (Figure 4.3).

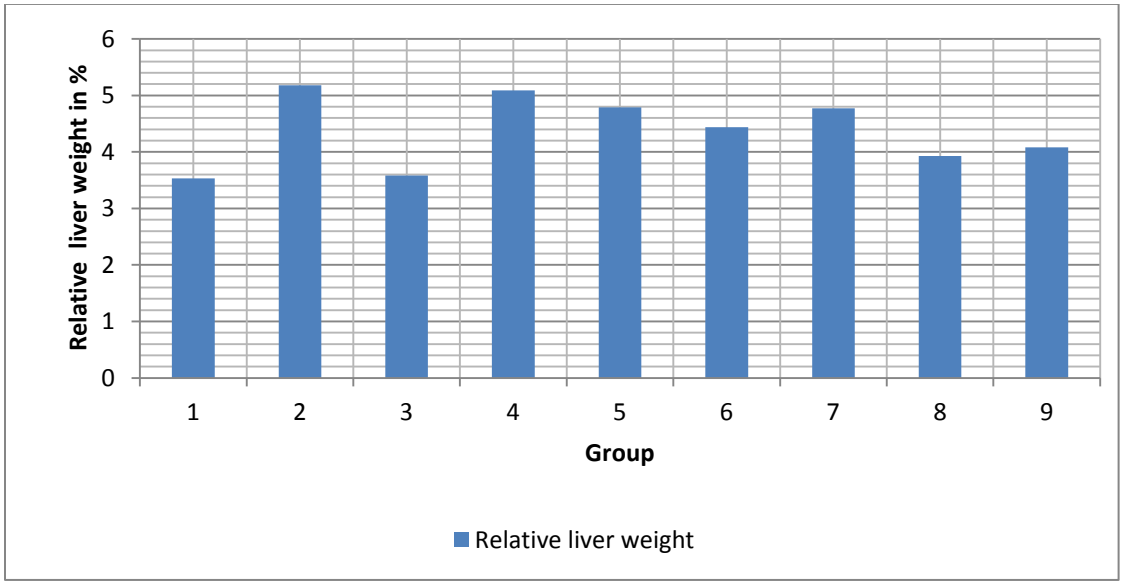


Figure 4.3: The mean relative liver weight in grams of different groups of male albino mice administered with Khat extract in different combinations of non-alcoholic substances for 30 days at a dosage of 2000mg/Kg/day.

The mean absolute kidney weight of the positive control group is slightly higher than that of the test groups in which Khat extract is administered (Table 4.2). The group in which Khat extract and patico sweet was administered had the highest kidney weight

(0.35±0.09g). The absolute kidney weight is well demonstrated in figure 4.4. There was no significant different between the mean absolute weight of the positive control group, negative control group and the test groups unto which Khat extract was administered.

The increase of the liver and kidney weight observed can be associated with hepatic and renal enlargement. Hypertrophied cells of the liver and kidney leads to enlargement and increase in organ weight than normal (Michalopoulos, 2007).

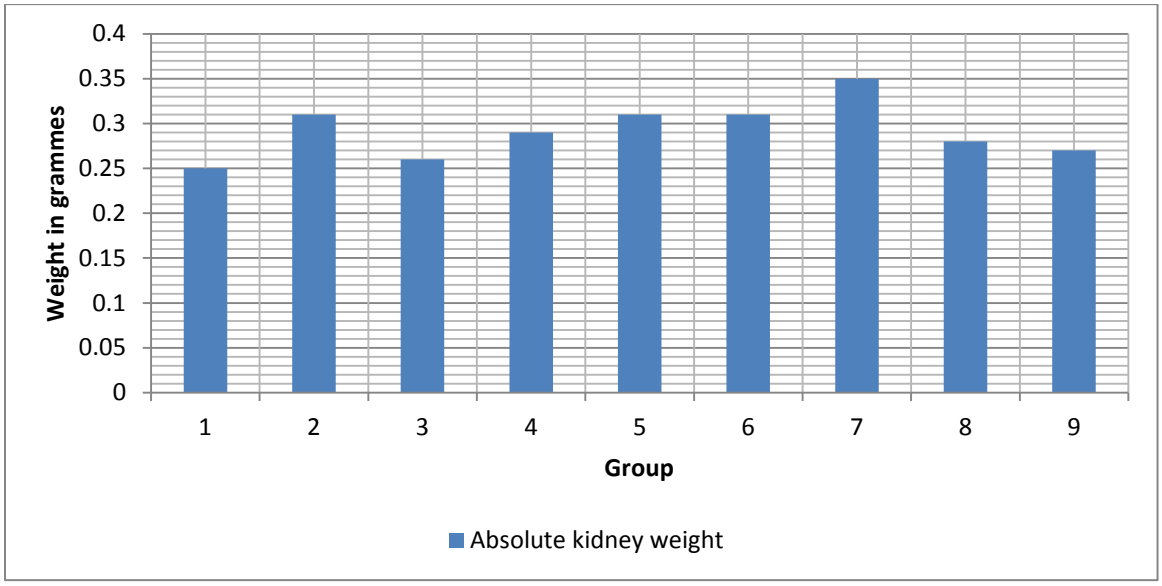


Figure 4.4: The mean absolute kidney weight in grams of different groups of male albino mice administered with Khat extract with different combinations of non-alcoholic substances for 30 days at a dosage of 2000mg/Kg/day.

Hepatic and renal enlargement is a clear feature that represents liver and renal damage due to regeneration process. (Michalopoulos, 2007). A lot of the xenobiotics which are associated with liver and kidney damage results into enlargement of these organs and increase in their weight (Ashafa *et al.*, 2009). Liver enlargement is due to the effect of

the xenobiotic effects to size of the hepatocytes. Liver enlargement is also associated with inflammatory response of the hepatocytes (Ashafa *et al.,* 2009)**.**

4.2 Changes in liver and renal function tests analytes

4.2.1 Liver function tests analytes

The activity of the alkaline phosphatase (ALP) was significantly different between the negative (treated only with rodent pellets and water) control and the test groups at $p < 0.05$ (Table 4.4). According to the study, the average level of the alkaline phosphatase (U/L) was increased in the positive control group (187.82±6.06U/L) as compared to negative (treated only with rodent pellets and water) control group (146.12±18.65U/L) (Table 4.3). There was no significance difference between the positive (treated with indomethacin) control group and the group to which Khat extract was administered with combination of milk, water, and coke, tea, coffee, groundnut and patico sweets at $p < 0.05$ (Table 4.4).

Table 4.3: Biochemical assayed levels of serum alkaline phosphatase units per litre (U/L) in male albino mice after treatment with *Catha edulis* extract for 30 days at a dosage of 2000mg/Kg/day.

Group	**Treatment**	**Alkaline phosphatase (U/L) Mean±SD**	**P < 0.05**
1	No Khat extract (-ve control)	146.12±18.65	-
2	Indomethacin (+ve control)	187.82±6.06	0.017*
3	Extract + water	178.38±10.76	0.035*
4	Extract + coffee	178.10±11.04	0.042*
5	Extract + milk	182.86±3.96	0.009*
6	Extract + tea	176.24±6.16	0.011*
7	Extract + patico sweet	182.92±2.85	0.011*
8	Extract + groundnut	181.24±11.55	0.019*
9	Extract + coke	179.24±5.78	0.009*

*Significance difference using paired t-test as compared with normal control at $p < 0.05$, SD =standard deviation, n= number.

Table 4.4: Serum alkaline phosphatase significance difference at $p < 0.05$ using paired t-test in different groups of male albino mice after treatment with *Catha edulis* extract for 30 days at a dosage of 2000mg/Kg/day.

Treatment	NC	PC	EW	EC	EM	ET	EP	EG	ECk
NC	-	0.017*	0.035*	0.042*	0.009*	0.011*	0.011*	0.019*	0.009*
PC	0.017*	-	0.200	0.175	0.570	0.082	0.225	0.313	0.158
EW	0.035*	0.200	-	0.935	0.395	0.750	0.452	0.781	0.878
EC	0.042*	0.175	0.935	-	0.337	0.802	0.424	0.745	0.867
EM	0.009*	0.257	0.395	0.337	-	0.143	0.982	0.804	0.353
ET	0.011*	0.082	0.750	0.802	0.143	-	0.033	0.327	0.079
EP	0.011*	0.225	0.452	0.424	0.982	0.033	-	0.710	0.198
EG	0.019*	0.313	0.781	0.745	0.804	0.327	0.710	-	0.740
ECk	0.009*	0.158	0.878	0.867	0.353	0.079	0.198	0.740	-

*indicates a significant difference at $p < 0.05$
ALP =Alkaline phosphatase, NC =Normal control, PC=Positive control, EW=Extract and water, EC=Extract and milk, ET=Extract and tea, EP=Extract and patico sweet, EG= Extract and groundnut, ECk=Extract and coke

There was a significant difference in the levels of alkaline phosphatase between the negative control group (the group of mice to which Khat extract was not administered) with the positive (treated with indomethacin) control and the groups in which Khat extract were being administered at $p < 0.05$ (Table 4.4).

The levels of the alkaline phosphatase in the serum of albino male mice were higher in positive (treated with indomethacin) control group as compared to negative (taking only

rodent pellet and water only) control group (Figure 4.5). The levels of alkaline phosphatase were lower in negative control group than the test groups in which Khat extract was administered (Figure 4.5).

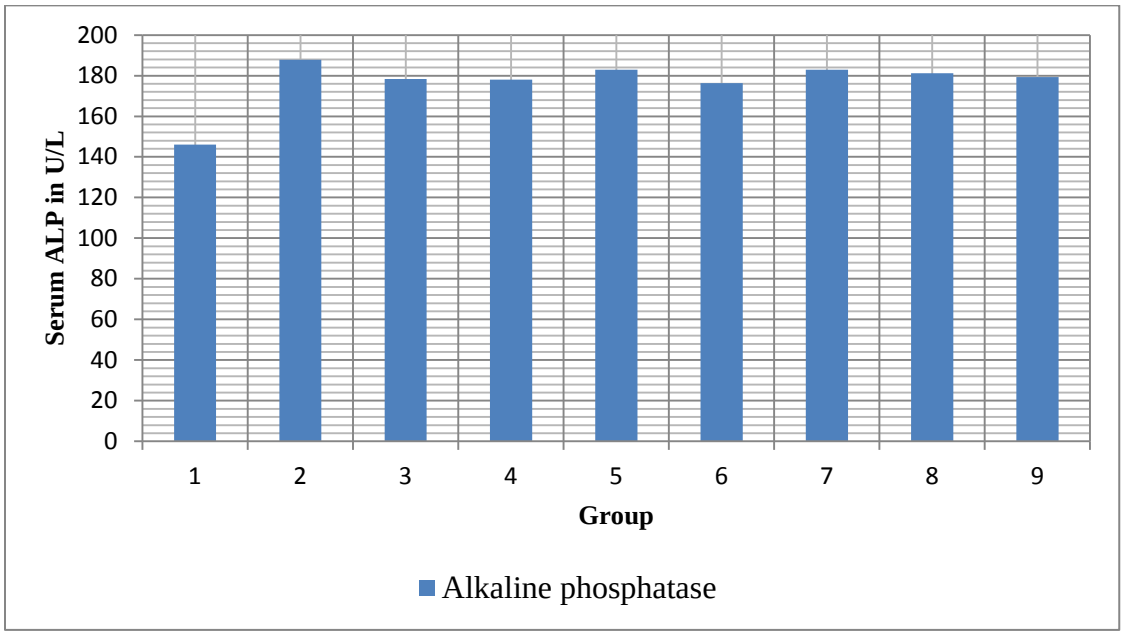


Figure 4.5: Biochemical assayed levels of serum alkaline phosphatase units per litre (U/L) in male albino mice after treatment with *Catha edulis* extract for 30 days at a dosage of 2000mg/Kg/day.

The data presented here clearly indicates Khat extracts (cathinone and cathine) altered the levels alkaline phosphatase in the serum of the albino mice to which it was administered. There was no significance difference at $p < 0.05$ in the alteration of the alkaline phosphatase irrespective of the non-alcoholic substance in which the Khat extract is administered with.

Table 4.5: Biochemical assayed levels of serum aspartate aminotransferase in units per litre (U/L) in male albino mice after treatment with *Catha edulis* extract for 30 days at a dosage of 2000mg/Kg/day.

Treatment	**Group**	**Aspartate aminotransferase (U/L) Mean±SD**	**P<0.05**
No Khat extract (-ve control)	1	153.10±7.07	-
Indomethacin(+ve control)	2	213.86±7.08	0.006*
Extract + water	3	210.38±5.76	0.007*
Extract + coffee	4	206.80±7.43	0.008*
Extract + milk	5	208.86±9.46	0.004*
Extract + tea	6	210.72±5.80	0.008*
Extract + patico sweet	7	208.74±5.95	0.011*
Extract + groundnut	8	209.80±8.28	0.006*
Extract + coke	9	205.38±9.53	0.005*

*Significance difference as compared with normal control at p<0.05 using paired t test.
SD = standard deviation, n =number.

According to the study, the average level of the aspartate aminotransferase (U/L) was higher in the positive control (treated with indomethacin) group (213.86±7.08U/L) as compared to negative control (not treated with Khat extract) group (153.10±7.07U/L) (Table 4.5, figure 4.6). The concentration of Aspartate aminotransferase was significantly different between the negative control and the test groups (Table 4.6). There was no significance difference at $p < 0.05$ between the levels of aspartate aminotransferase levels in the positive control (treated with indomethacin) group and the group to which Khat extract was administered with combination of milk, water, coke, tea, coffee, groundnut and patico sweets (Table 4.6).

Table 4.6: Serum aspartate aminotransferase significance difference at $p < 0.05$ in different groups of male albino mice after treatment with *Catha edulis* extract for 30 days at a dosage of 2000mg/Kg/day.

Treatment	**NC**	**PC**	**EW**	**EC**	**EM**	**ET**	**EP**	**EG**	**ECk**
NC	-	0.006*	0.007*	0.008*	0.004*	0.008*	0.011*	0.006*	0.005*
PC	0.006*	-	0.370	0.140	0.488	0.141	0.202	0.529	0.277
EW	0.007*	0.370	-	0.280	0.827	0.874	0.238	0.912	0.388
EC	*	0.140	0.571	-	0.739	0.340	0.604	0.424	0.746
EM	0.008*	0.488	0.827	0.739	-	0.795	0.987	0.787	0.391
ET	*	0.141	0.874	0.340	0.795	-	0.344	0.881	0.446
EP	0.004*	0.202	0.238	0.604	0.987	0.344	-	0.851	0.594
EG	*	0.529	0.912	0.424	0.787	0.881	0.851	-	0.076
ECk	0.008*	0.277	0.388	0.746	0.391	0.446	0.594	0.076	-
	0.011*								
	0.006*								
	0.005*								

*indicates a significant difference at $p < 0.05$

NC =Normal control, PC=Positive control, EW=Extract and water, EC=Extract and milk, ET=Extract and tea, EP=Extract and patico sweet, EG= Extract and groundnut, ECk=Extract and coke

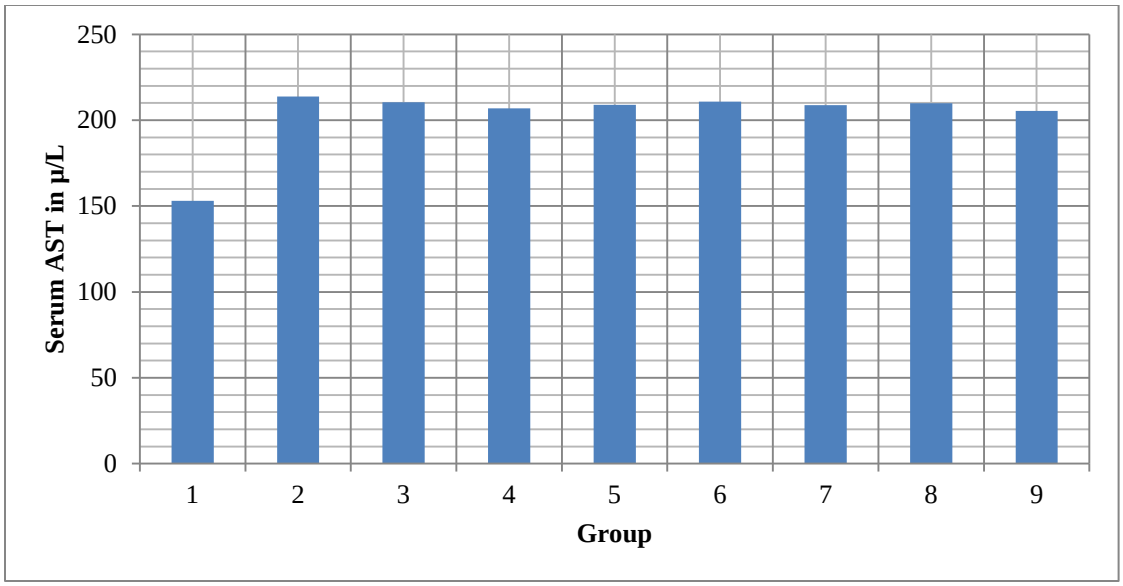


Figure 4.6: The mean serum biochemical assayed levels of aspartate aminotransferase units per litre (U/L) in male albino mice after treatment with *Catha edulis* extract for 30 days at a dosage of 2000mg/Kg/day.

Khat extracts altered the levels aspartate aminotransferase in the serum of the albino mice to which it was administered (figure 4.6).

According to the study, the average level of the total bilirubin (mM/L) was higher in the positive control (treated with indomethacin) group (24.24±3.63mM/L) as compared to negative control (not administered with Khat extract) group (12.44±1.18mM/L) (Table 4.7). There was no significance difference in level of total bilirubin between the positive control group and the groups in which Khat extract was administered in combination of milk, water, coke, tea, coffee, groundnut and patico sweets $p < 0.05$. There was a significance difference in the levels of total bilirubin between the negative control group (the group of mice to which Khat extract was not administered) with the positive control and the groups to which Khat extract were being administered $p < 0.05$. There was no signifince difference in Khat extracts the levels total bilirubin in groups in which Khat

extracts was administered $p < 0.05$(Table 4.8). The levels of total bilirubin were higher in mice which indomethacin and Khat extract was administered (Figure 4.7).

Table 4.7: The mean serum biochemical assayed levels of total bilirubin mMol/L in male albino mice after treatment with *Catha edulis* extract for 30 days at a dosage of 2000mg/Kg/day.

Treatment	**Group**	**Total bilirubin (mMol/L) Mean±SD**	**P<0.05**
No Khat extract (-ve control)	1	12.44±1.18	-
Indomethacin (+ve control)	2	24.24±3.63	0.004*
Extract + water	3	20.60±3.87	0.009*
Extract + coffee	4	22.08±2.86	0.000*
Extract + milk	5	17.80±2.80	0.011*
Extract + tea	6	18.66±4.06	0.017*
Extract + patico sweet	7	21.16±6.17	0.024*
Extract + groundnut	8	19.12±5.29	0.035*
Extract + coke	9	20.02±2.75	0.003*

*Significance difference as compared with normal control at $p<0.05$,
SD = standard deviation, n =number.

Table 4.8: Serum total bilirubin significance difference at $p < 0.05$ in different groups of male albino mice after treatment with *Catha edulis* extract for 30 days at a dosage of 2000mg/Kg/day.

Treatment	**NC**	**PC**	**EW**	**EC**	**EM**	**ET**	**EP**	**EG**	**ECk**
NC	-	0.004*	0.009*	0.000*	0.011*	0.017*	0.024*	0.035*	0.003*
PC	0.004*	-	0.165	0.381	0.073	0.071	0.504	0.166	0.061
EW	0.009*	0.165	-	0.571	0.209	0.140	0.884	0.689	0.816
EC	0.000*	0.381	0.571	-	0.100	0.137	0.705	0.212	0.233
EM	0.011*	0.073	0.209	0.100	-	0.714	0.284	0.613	0.282
ET	0.017*	0.071	0.140	0.137	0.714	-	0.471	0.892	0.585
EP	0.024*	0.504	0.884	0.705	0.284	0.471	-	0.584	0.756
EG	0.035*	0.166	0.689	0.212	0.613	0.892	0.584	-	0.567
ECk	0.003*	0.061	0.816	0.233	0.282	0.585	0.756	0.567	-

	*
	0.035
	*
	0.003
	*

*indicates a significant difference at p <0.05
NC =Normal control, PC=Positive control, EW=Extract and water, EC=Extract and milk, ET=Extract and tea, EP=Extract and patico sweet, EG= Extract and groundnut, ECk=Extract and coke

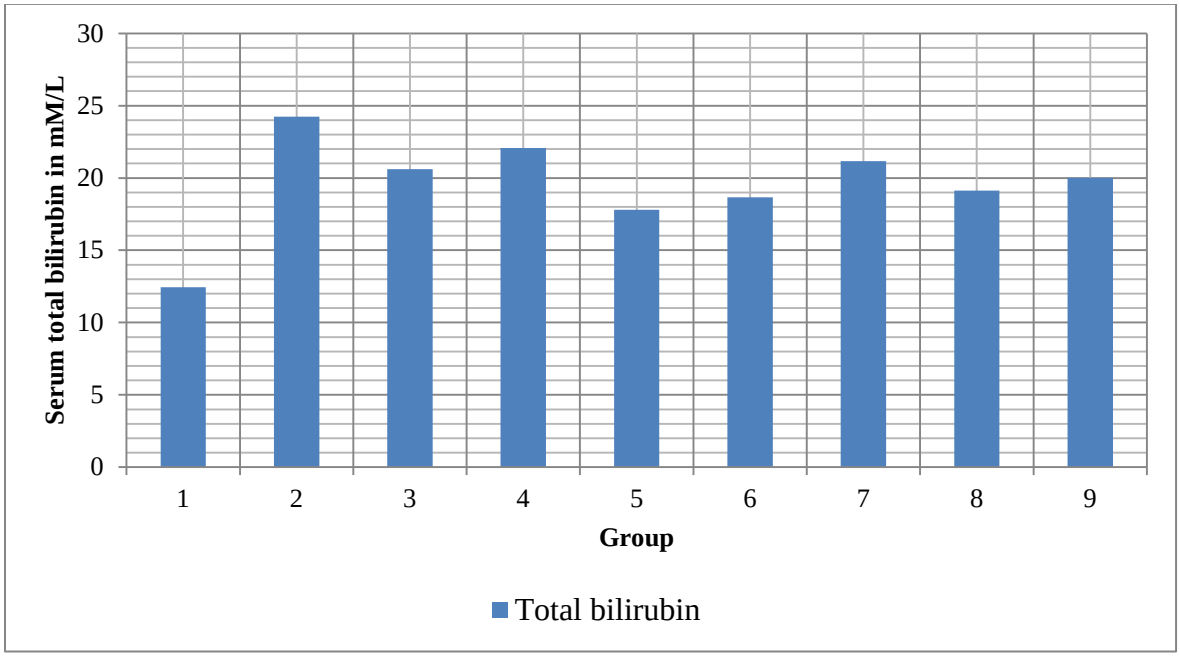


Figure 4.7: The mean serum biochemical assayed levels of total bilirubin mMol/L in male albino mice after treatment with *Catha edulis* extract for 30 days at a dosage of 2000mg/Kg/day

The elevation ALP in serum is an indicator of possibility of hepatotoxicity (Burke, 2002). Aspartate aminotransferase and alkaline phosphatase lack specificity (Pratt and Kaplan, 2000). Alalanine aminotransferase is a good indicator of normal functioning of the liver because it's a more specific marker of hepatocytes integrity (Limdi and Hyde, 2003). The elevation of serum Aspartate aminotransferase (AST) results from a leakage into circulation due to rupturing of the cell membrane of the hepatocytes due to organ injury

(Waner and Nyska, 1991). Aspartate transferase is distributed in various organs, but it is more concentrated in the hepatocytes (Ozer *et al.,* 2008). Aspartate transferase (AST) can be elevated in cases of striated muscles which is associated with increased levels of creatine kinase (CK) (Pratt and Kaplan, 2000). Fragmentation of the haem from haemoglobin of the red blood cells which are aged or damaged lead to formation of the bilirubin. Bilirubin is conjugated in the liver hence liver damage leads to accumulation of bilirubin in the serum leading to jaundice (WHO, 2000).

4.3 Renal function tests

The blood urea nitrogen (BUN) concentration was significantly different between the negative control (no Khat extract administration) and the test groups (administered with Khat extract) (paired t test, $P<0.05$) (Table 4.8). There was no significant difference between the positive control (treated with indomethacin) and the test groups (treated with Khat extract) in which Khat extract was administered (paired t test, $P<0.05$). The blood urea nitrogen (BUN) concentration for the positive control (treated with indomethacin) group was higher than that of the negative control (not treated with Khat extract) group and other test groups which Khat extract was administered (Figure 4.9). The average level of the blood urea nitrogen (mM/L) was higher in the positive control group (treated with indomethacin) (15.64±1.42mMol/L) as compared to negative control (not administered with Khat extract) group (8.46±1.29mMol/L). There was no significant difference between the positive control (treated with indomethacin) group and the group to which Khat extract was administered in combination with:- milk, water, coke, tea,

coffee, groundnut and patico sweets (Table 4:10). The significance differences of serum blood urea nitrogen between test groups were calculated (Table 4.9).

Table 4.9: The mean serum biochemical assayed levels of blood urea nitrogen (BUN) mMol/L in male albino mice after treatment with *Catha edulis* extract for 30 days at a dosage of 2000mg/Kg/day.

Treatment	Group	BUN in mMol/L Mean±SD	P<0.05
No Khat extract (-ve control)	1	8.46±1.29	-
Indomethacin (+ve control)	2	15.64±1.42	0.000*
Extract + water	3	13.54±0.97	0.050*
Extract + coffee	4	13.82±1.00	0.060*
Extract + milk	5	13.86±2.85	0.037*
Extract + tea	6	13.16±1.64	0.008*
Extract + patico sweet	7	12.78±1.40	0.021*
Extract + groundnut	8	13.66±1.39	0.009*
Extract + coke	9	14.30±0.60	0.000*

*Significance difference as compared with normal control at p<0.05,
SD = standard deviation, n =number.

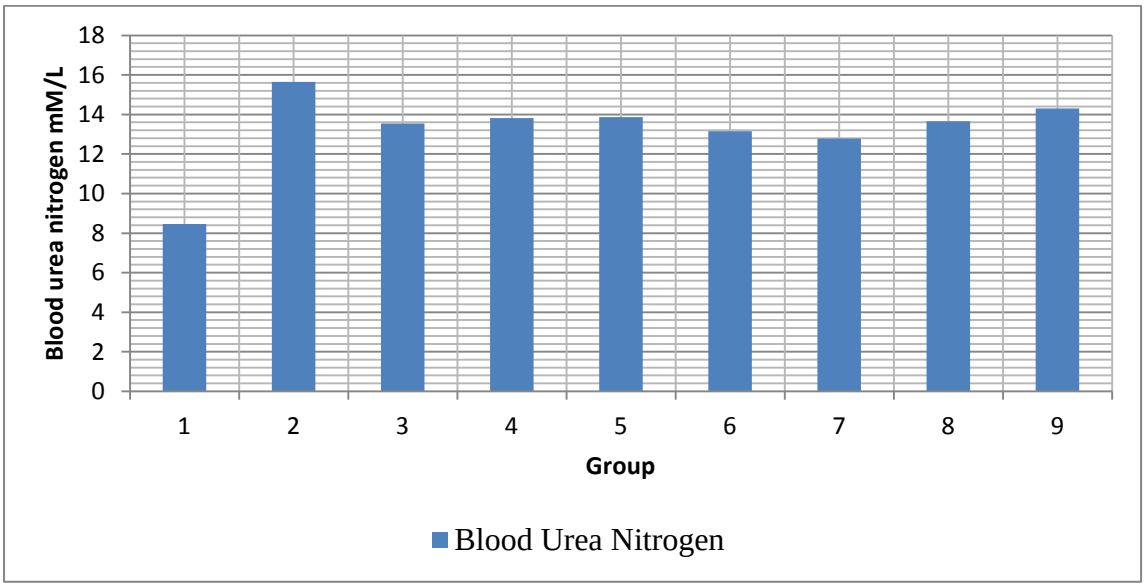


Figure 4.8: The mean serum biochemical assayed levels of blood urea nitrogen (BUN) mMol/L in male albino mice after treatment with *Catha edulis* extract for 30 days at a dosage of 2000mg/Kg/day.

Table 4.10: Serum blood urea nitrogen (BUN) significance difference at $p < 0.05$ in different groups of male albino mice after treatment with *Catha edulis* extract for 30 days at a dosage of 2000mg/Kg/day.

Treatment	**NC**	**PC**	**EW**	**EC**	**EM**	**ET**	**EP**	**EG**	**ECk**
NC	-	0.000*	0.050*	0.060*	0.037*	0.008*	0.021*	0.009*	0.000*
PC	0.000*	-	0.070	0.156	0.386	0.077	0.072	0.139	0.075
EW	0.050*	0.070	-	0.609	0.832	0.685	0.789	0.267	
EC	0.060*	0.156	0.609	-	0.967	0.409	0.326	0.798	0.523
EM	0.037*	0.386	0.832	0.967	-	0.608	0.011*	0.889	0.778
ET	0.008*	0.077	0.685	0.409	0.608	-	0.697	0.251	
EP	0.021*	0.072	0.326	0.011*	0.242	0.668	0.242	0.270	0.159
EG	0.009*	0.139	0.789	0.789	0.889	0.697	0.668	-	0.450
ECk	0.000*	0.075	0.267	0.523	0.778	0.259	-	0.450	-
							0.270		
							0.159		

*indicates a significant difference at p value < 0.05
BUN =Blood urea nitrogen, NC =Normal control, PC=Positive control, EW=Extract and water, EC=Extract and milk, ET=Extract and tea, EP=Extract and patico sweet, EG= Extract and groundnut, ECk=Extract and coke.

Khat extracts oral administration resulted to altered levels blood urea nitrogen (BUN) in the serum of the albino mice to which it was administered.

The blood creatinine concentration was significantly different between the negative control group and the tests groups (paired t test, $P<0.05$) (Table 4.11). There was no significant different between the positive control and the test groups unto which Khat extract was administered (paired t test, $P<0.05$). The blood creatinine concentration for the positive control group was higher than that of the negative control group and other test groups which Khat extract was administered (Figure 4.9). According to the study, the

average level of the blood creatinine concentration (µM/L) was higher in the positive control group (14.62±1.48µM/L) as compared to negative control group (10.64±0.83µM/L). There was no significance difference between the levels blood creatinine concentration in the positive control (treated with indomethacin) group and the group to which Khat extract was administered with combination of milk, water, coke, tea, coffee, groundnut and patico sweets(Table 4:12).

Table 4.11: The mean serum biochemical assayed levels of blood creatinine concentration (µM/L) in male albino mice after treatment with *Catha edulis* extract for 30 days at a dosage of 2000mg/Kg/day.

Treatment	**Group**	**Creatinine (µM/L) Mean±SD**	**P<0.05**
No Khat extract (-ve control)	1	10.64±0.83	-
Indomethacin (+ve control)	2	14.62±1.48	0.003*
Extract + water	3	13.58±1.19	0.024*
Extract + coffee	4	13.36±0.93	0.012*
Extract + milk	5	13.70±1.47	0.036*
Extract + tea	6	13.70±1.34	0.008*
Extract + patico sweet	7	12.34±0.90	0.016*
Extract + groundnut	8	13.48±2.19	0.011*
Extract + coke	9	13.58±2.02	0.044*

*Significance difference as compared with normal control at p<0.05,
SD = standard deviation,=number

Table 4.12: Serum creatinine concentration significance difference at p < 0.05 in different groups of male albino mice after treatment with *Catha edulis* extract for 30 days at a dosage of 2000mg/Kg/day.

	NC	**PC**	**EW**	**EC**	**EM**	**ET**	**EP**	**EG**	**ECk**
NC	-	0.003*	0.024*	0.012*	0.036*	0.008*	0.016*	0.011*	0.044*
PC	0.003*	-	0.245	0.116	0.445	0.222	0.015	0.293	0.463
EW	0.024*	0.245	-	0.742	0.795	0.856	0.087	0.944	1.000
EC	0.012*	0.116	0.742	-	0.661	0.646	0.177	0.924	0.865

EM	0.036*	0.445	0.795	0.661	-	1.000	0.139	0.889	0.887
ET	0.008*	0.222	0.856	0.646	1.000	-	0.007*	0.814	0.887
EP	0.016*	0.015*	0.087	0.177	0.139	0.007*	-	0.225	0.175
EG	0.011*	0.293	0.944	0.924	0.889	0.814	0.225	-	0.939
ECk	0.044*	0.463	1.000	0.865	0.887	0.887	0.175	0.939	-

*indicates a significant difference at $p<0.05$
NC =Normal control, PC=Positive control, EW=Extract and water, EC=Extract and milk, ET=Extract and tea, EP=Extract and patico sweet, EG= Extract and groundnut, ECk=Extract and coke

Creatine phosphate, a storage compound with high energy in the muscle produces creatinine. Creatine phosphate in the muscles is spontaneously degraded to cyclic anhydride creatinine. In a normal individual the levels of serum creatinine remains fairly constant hence it's a good indicator, of assessing the functionality of the kidney (WHO, 2000).

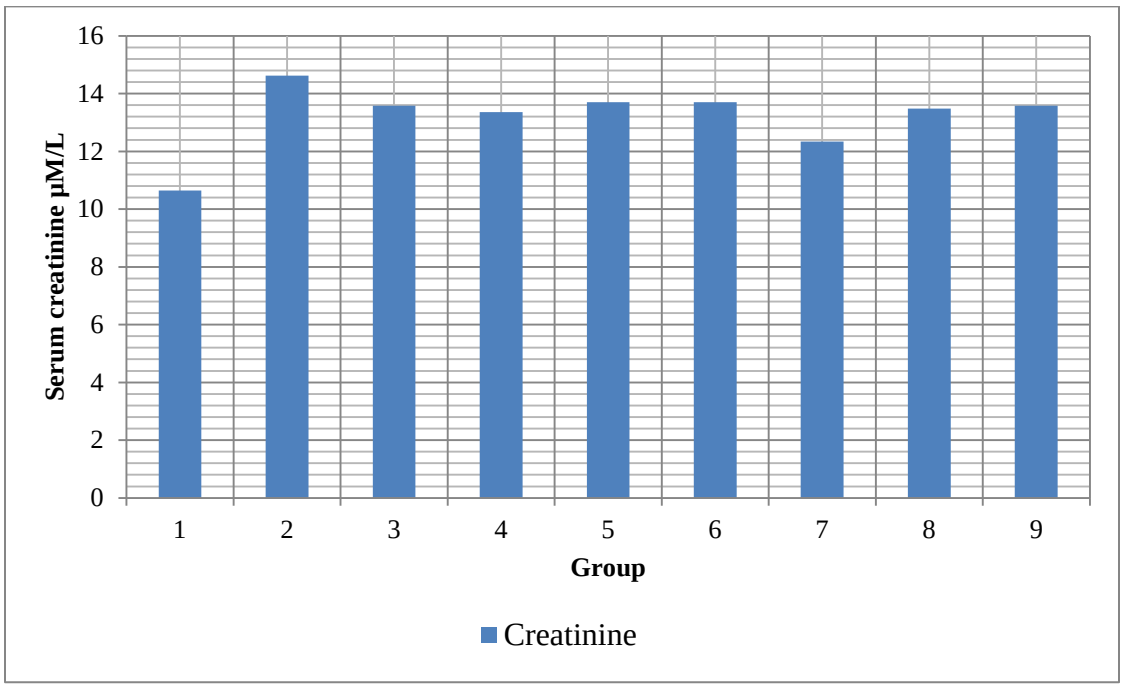


Figure 4.9: The mean serum biochemical assayed levels of serum creatinine concentration µM/L in male albino mice after treatment with *Catha edulis* extract for 30 days at a dosage of 2000mg/Kg/day.

About 45% of the body's non-protein nitrogen is urea. Urea in human is the major catabolic end product. The liver synthesizes the urea after that it is excreted through the kidneys. Assessment of blood urea nitrogen is a vital parameter for assessment of renal and hepatic integrity (WHO, 2000). Creatinine (Cr) and blood urea nitrogen (BUN) elevation indicates xenobiotics effects on the structure and the functions of the kidney (Ashafa *et al,* 2009; and Woodman, 1996). The effect on the levels of creatinine and urea levels in the blood indicates impairement of kidney function or deshydration (dehydration). The physical, hematological and biochemical examinations cannot rule out the effects of khat hepato-and nephrotoxicity without carrying out histological examinations (Chapman *et al.,* 2010*;* Peevers *et al.,* 2010; Coton *et al*., 2011; Stuyt *et al.,* 2011).

4.4 Hematological tests

The clotting time for the positive control group had a significant difference with that of the negative control. The blood clotting time between all the test groups (treated with Khat extract) didn't show any significant difference at $p < 0.05$ (Table 4.13). The clotting time for the positive control group was higher than that of negative control group and other tests groups (Figure 4.10). The average clotting time for other groups was almost the same.

Table 4:13: : The mean blood clotting time in minutes in male albino mice after treatment with *Catha edulis* extract for 30 days at a dosage of 2000mg/Kg/day.

Group	Treatment	Clotting time in minutes Mean±SD	P<0.05
1	No Khat extract (-ve control)	5.22±0.37	-
2		6.60±0.23	0.004*
3	Indomethacin (+ve control)	5.32±0.16	0.486
4	Extract +water	5.24±0.52	0.959
5	Extract+coffee	5.58±0.49	0.149
6	Extract+milk	4.94±0.99	0.590
7	Extract+tea	5.26±0.36	0.897
8	Extract+patico sweet	5.02±0.18	0.249
9	Extract +groundnut Extract+coke	5.02±0.31	0.473

* significance difference as compared with normal control at p<0.05,
SD - standard deviation, n – number, n=5.

Bleeding time indicates the time between the making of a small incision and the moment when the bleeding stops. Tissue factors leads to coagulation in arteries or in veins after tissue damage in the extrinsic coagulation pathway (Caldwell *et al.*, 2006). Apart from factor VIII, all coagulation factors are synthesized in the liver. Vitamin K is required for the synthesis of hepatic factors VII, IX, X and prothrombin (Caldwell *et al.*, 2006). The liver makes six blood clotting factors: I (fibrinogen), II (Prothrombin), IV, V, VI, and VII. Whenever there is an abnormality in any of them bleeding time will be prolonged.

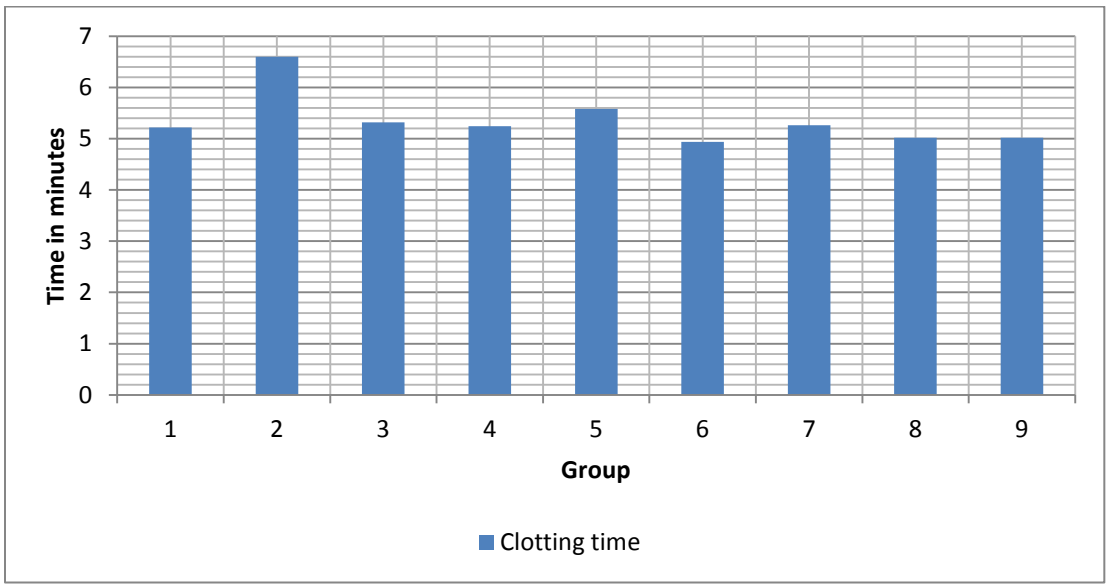


Figure 4.10: The mean blood clotting time in minutes of male albino mice after treatment with *Catha edulis* extract for 30 days at a dosage of 2000mg/Kg/day and different non-alcoholic accompaniments.

Hepatic dysfunction results in the reduced production of functional coagulation factors. The coagulation disorders which are encountered in severe liver disease leads to enhanced fibrinolysis resulting to consumption of clotting factors (Lisman and Porte, 2010). Fibrinolysis triggers breakdown of fibrin leading to fibrin degradation which impair hemostasis by inhibiting fibrin polymerization. Liver diseases are commonly associated with blood clotting/bleeding dysfunction. Liver problems are associated with disturbed balance in the pro-coagulant and anti-coagulant factors which leads to deviation from the normal coagulation cascade (Lisman and Porte, 2010).

4.5 Histopathological examination

4.5.1 Liver histological results

Liver sections of the negative control group (not treated with Khat extract) of the albino mice under microscopic examination showed intact cytoplasm, uncongested central vein, uniform hepatocytes and prominent nuclei of the cells. The sections also did not show any sign of necrosis, inflammation or changes in fatty acids. The sections also composed of hexagonal or pentagonal lobules whereby the central veins and the peripheral hepatic triads or tetrads were embedded in the connective. The hepatocytes were arranged in

trabecules running from the central vein radiantly. There were spaces in between the cell cords which were converging towards the central veins through the Kupffer cells. The hepatocytes of the negative control group were regular and contained nucleus which were large and spherical in shape.

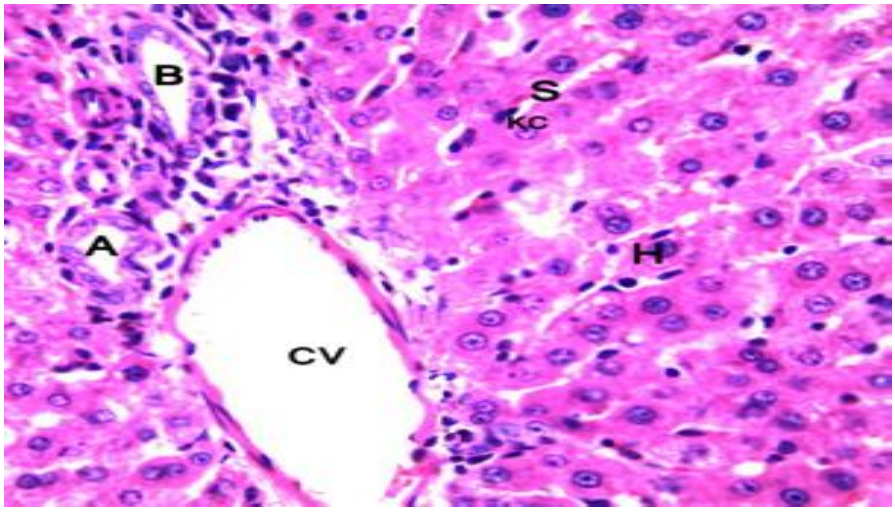


Figure 4:11 Light photomicrography of liver of negative control mice: **(CV):** central vein, **(H):** hepatocytes, **(S):** sinusoids, **(A):** hepatic artery, **(B):** bile duct, **(KC):** Kupffer cell. **H&E. ×400**

The nucleus contained nucleoli which were clearly marked with peripheral chromatin distribution. Some of the cells from the normal control group contained two nuclei (Figure 4.11).

In the positive control (treated with indomethacin) group and the test groups in which Khat extract was administered to male albino mice, there were signs of degeneration of hepatocytes. The hepatocytes were enlarged and had light and foamy cytoplasms filled with numerous vacuoles. The blood sinusoids walls were dilated and had large number of Kupffer cells. There were also presence of necrosis, pycnosis and condensed chromatin in

livers of all test groups. The hepatic areas of the test groups and the positive control also had presence of mononuclear cell infiltrates (Figure 4:12 and 4:13). The frequency of the abnormalities observed in the liver are demonstrated in figure 4.14 below.

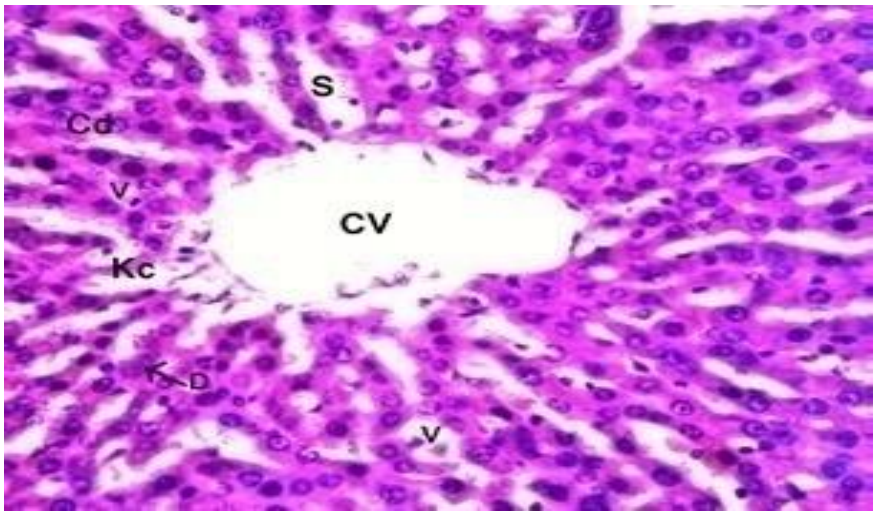


Figure 4.12: Liver injury of albino mice. **(V):** light, foamy and vacuole filled cytoplasm, **(Cd):** enlarged cells with condensed nuclear chromatin, **(D arrow):** single necrotic and pycnotic hepatocytes with contracted nuclei and condensed chromatin, **(S):** strongly acidophilic cytoplasm with accumulation of mononuclear cells in sinusoids, **(KC):** Kupffer cells around sinusoidal walls. **H&E. ×400**

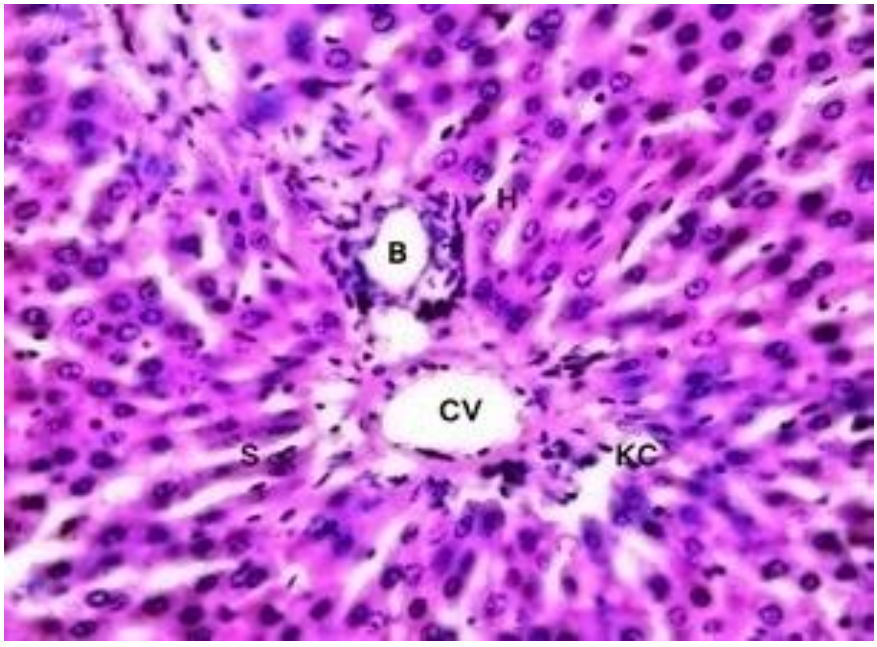


Figure 4.13: Liver injury of albino mice. **(S):** decreased widening of blood sinusoid **(CV):** less fragmentation, lighting of cytoplasm and infiltration of mononuclear cells around the portal system of central vein, **(B):** bile duct. **H&E, ×400.**

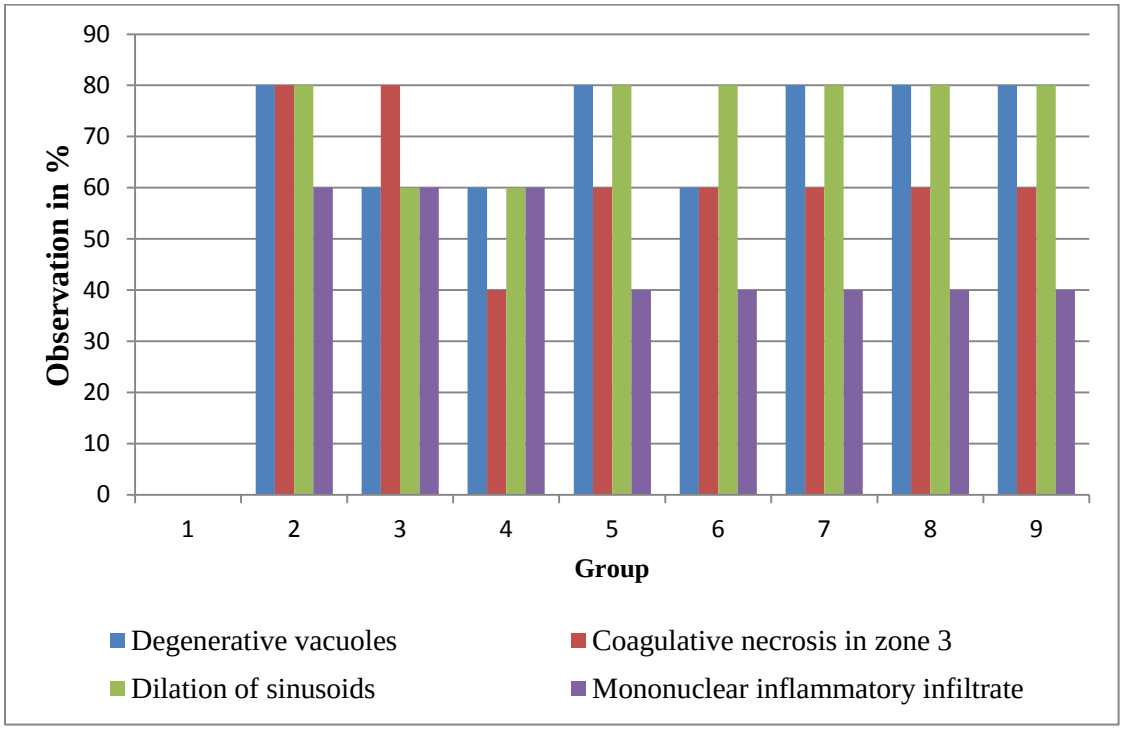


Figure 4.14: Histological observation of albino mice liver tissues.

Observed enlargement of the liver could be as a result of Khat extract exposure. Enlargement of the liver is a clear indicator of regeneration of the liver normally experienced clinically after the damage of the liver. Most xenobiotics are associated with enlargement of the liver because they have direct effect on the size of the hepatocytes (Michalopoulos, 2007) or on the inflammatory responses (Ashafa *et al.*, 2009), this results in the histopathological and cytological changes on the hepatocytes of the mice. The mostly observed changes on the cytological and histological features of the liver were:-vacuolar and coagulative necrosis, dilatation of the sinusoids, mononuclear infiltrates, oedema, congestion and hemorrhage degeneration in parenchymal

hepatocytes. The oedema may have occurred because of energy reduction which is required for the cells ion concentration regulation which leads to water retention in the hepatocytes (Yukiko *et al*., 1977). Liver damages leads to impaired liver functioning hence interfering with plasma proteins secretion (Lacroix *et al*., 2004; Lapeyre-Mestre *et al*., 2006).

Decreased secretion of plasma proteins leads to decrease in osmotic blood pressure resulting to decreased drainage of the tissue fluids hence oedema. The vacuolation of the hepatic lobule in the study predominantly started in the hepatocytes of the peripheral sites and the extended towards the center of the lobules. This can be associated with direction of blood supply in the liver lobular (Amer *et al*., 1998). The vacuolation of the cytoplasm of the hepatocytes is mainly caused by disturbance of the lipid inclusions and the metabolism of the lipid due to pathological changes (Zhang and Wang, 1984). The vacuolar degeneration is an alteration which is produced in order to collect the injurious substances which are found in the hepatocytes (Durhan *et al*., 1990).

4.5.2 Kidney histological results

The microscopic examination of the sections of the kidney obtained from the normal group (group 1) indicated normal tubules, gromerular capillaries, Malpighian corpuscles and Bowman's capsule (Figure 4.15). The kidneys of the albino mice treated with Khat extract and the positive control group indicated many areas of tubular damages ranging from mild to severe. Some of the histopathological observations were:-signs of amorphous Malpighian corpuscles, invasive infiltrative inflammatory cells signs,

hypertrophied glomerular capillaries especially at Malpighian corpuscles of kidney and atypical tubules. In some groups hyperaemia of kidney vessels were also observed (Figure 4.16). The frequencies of abnormalities observed are as indicated in figure 4.17.

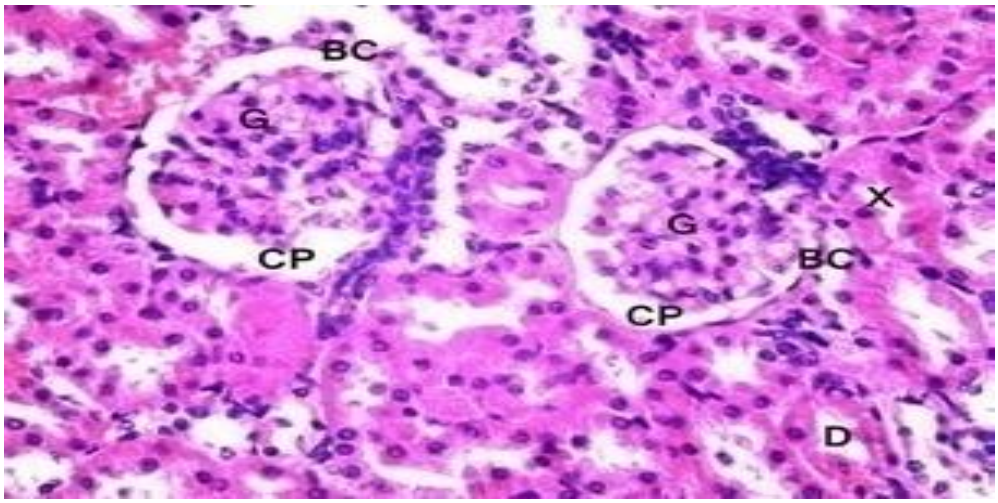


Figure 4.15: Light photomicrography of kidney of negative control. **(G):** normal glomeruli showing normal structure, **(X):** proximal lined with typically thick cubic epithelium, **(D):** distal convulated tubules lined with relatively low simple epithelium, **(CP):** normal capsular space, **(BC):** glomerular capsule lined with flat epithelium. **H&E, ×400**

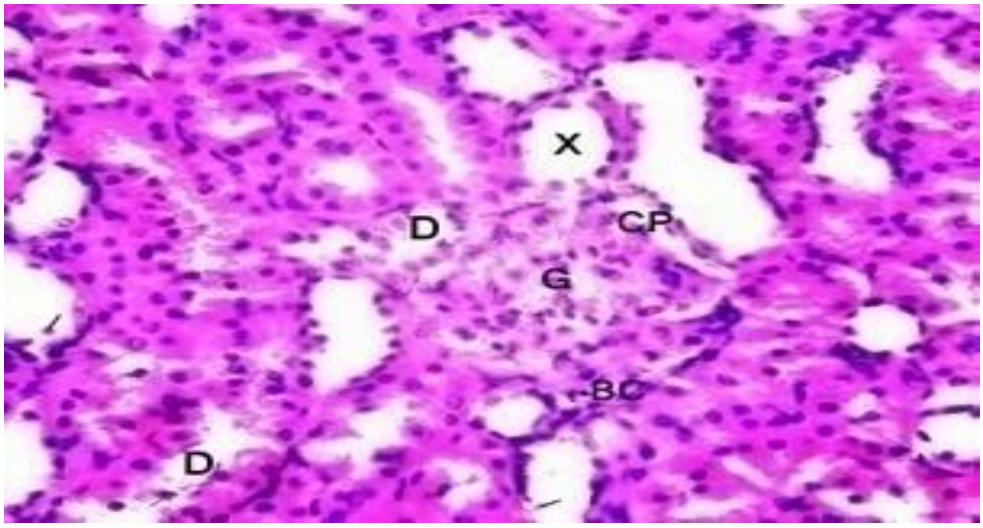


Figure 4.16: Moderate kidney injury photomicrography **(G):** enlarged vascular glomeruli, **(CP):** tightly filled glomerular capsular space, **(BC):** Bowman's capsule with flat epithelial lining, **(X):** proximal tubule epithelium with features of oedema, **(D):** distal convulated tubule with

features of oedema. **(Black lines)** Capillaries filled with blood cells with some tubules containing single desquamated cells. **H&E, ×400**

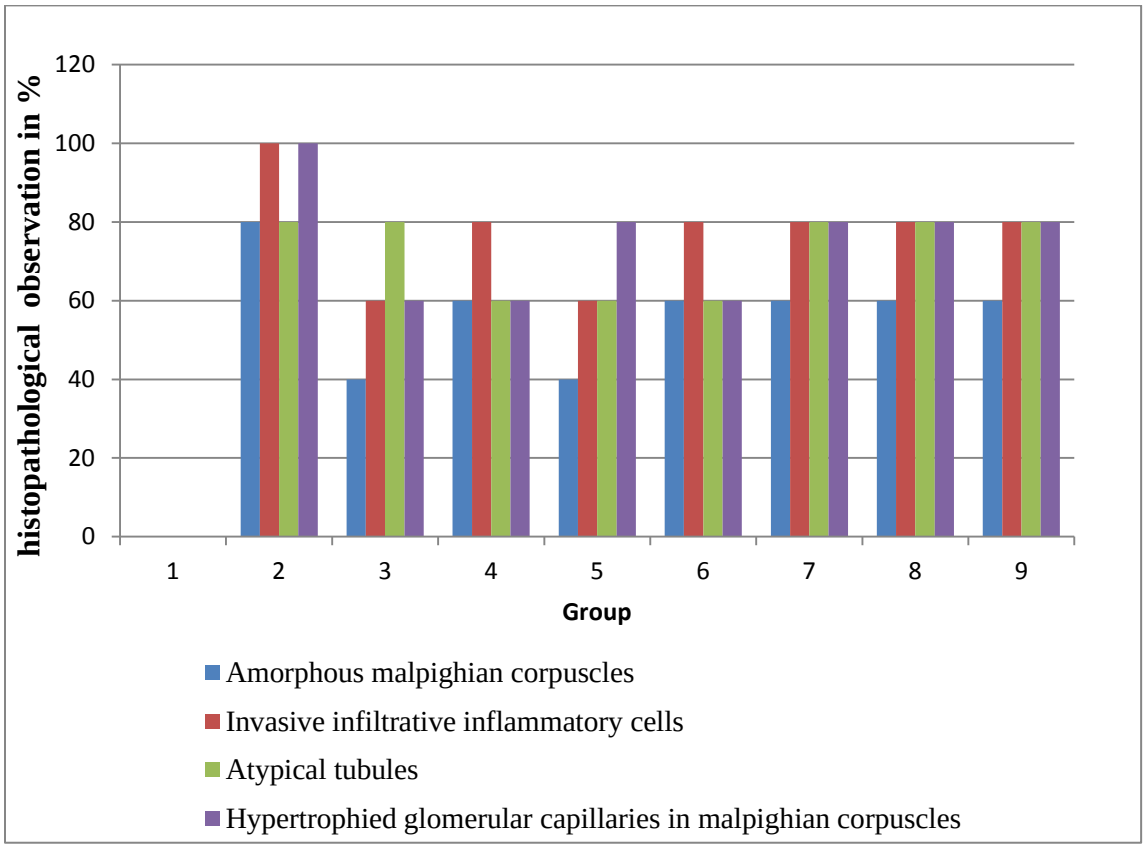


Figure 4.17: Histological observations of albino mice kidney tissues.

Concentration of the drugs in the body affects constriction of the renal capillaries in order to decrease glomerular filtration which minimizes the effects of the drug and protects the tubular cells (Stevens and Lowe, 1997). Capillaries constriction leads to glomeruli shrinkage and atrophy. This may also lead to retraction and contraction of the mesangial cells processes filaments due to angiotensin II stimulation in the cells. After 4 weeks glomerular hypertrophy is due to mesangial cells proliferation because of the secretion of more matrixes. The lesion of tubules with invasion of the inflammatory cells into the intertubular tissues is to minimize the renal tissue injury (El Banhawy *et al*., 1994). The proximal convulated tubules of the renal cells became edematous because of distention

and retraction of microvilli and also destruction of some cells. Edematous of the renal cells is as a result of decreased rate of reabsorption of glomerular filtrate to counteract the toxicity effects of the drug (Jackson and Lawrence, 1978).

CHAPTER FIVE

CONCLUSIONS AND RECOMMENDATION

5.1 Conclusions

Oral administration of Khat extract on albino mice resulted into elevated levels of liver enzymes such as alkaline phosphatase, aspartate aminotransferase and total bilirubin. Alkaline phosphatase (ALP), aspartate transferase (AST) and total bilirubin levels were significantly different between the negative control and the test group (paired t test $P<0.05$) and indication that there is increased levels due to Khat extract administration. An elevated level of liver enzymes is a sign of liver injury.

The study results indicated that there is no significant difference in the levels of alkaline phosphatase, aspartate aminotransferase and total bilirubin among different test groups, this indicated that the non alcoholic substances used, water, coffee, groundnut, tea, milk, coke, and patico sweet did not have any adverse effects on the liver.

The level of kidney function tests:- creatinine and blood urea nitrogen (BUN) concentration were higher in the test groups (treated with Khat extract) as compared to the negative control (taking rodent pellet and water only) which indicated that Khat extract had an adverse effect on the renal cells and tissues. Lack of significant difference in levels of both liver and renal function tests at $p < 0.05$ between the test groups (treated with Khat extract) indicated that different non alcoholic substances like water, coffee, groundnut, tea, milk, coke and patico sweet did not have any adverse effect on the kidney. Administration of Khat extract to albino mice led to decrease in body weight gain as compared to the negative control group.

The histological observation on liver tissue of test groups administered with Khat extract had degenerated vacuoles, coagulated necrotic cells in zone 3, dilated sinusoids and infiltration of mononucleated inflamed cells. The negative control group had normal liver tissue architecture. The different histological observation between tests groups in which the Khat extract was administered and the negative control group indicated that there was adverse effect of Khat extract on the liver tissue.

The negative control group (treated with Khat extract), kidney tissue histological observation showed normal architecture. The histological observation in the kidney tissues like amorphous malphigian corpuscles, invasion and infiltration of inflammed cells, atypical proximal and distal tubules and the hypertrophied glomerular capillaries in malpighian corpuscles indicated adverse effect on tissue due to Khat extract.

Clotting time in minutes of the various test groups (treated with Khat extract) indicated that there was no significant difference at $p < 0.5$ between negative group (treated with rodent pellet and water only) and the tests groups (treated with Khat extract). Clotting time result indicated that the liver damage was acute and not severe. Public health authorities should discourage the use of Khat because it may lead to liver injuries in humans since it has shown signs of liver damage in mice.

The study of both physical, hematological, biochemical and histocytological tests indicates that the hypothesis of the study is to be rejected hence according to the study *Catha edulis* grown in parts of Kenya and used as stimulant drug has hepatotoxic and nephrotoxic effects in albino mice when administered orally

5.2 Recommendations

i. The clinicians need to use the already gathered information to advice the Khat abusers who are suffering from liver and kidney problem to stop abusing the substance.

ii. There is a need to carry out further study on large animals especially the primates so that clear information can be obtained concerning Khat effects on human health.

iii. No study has ever been carried to clarify whether toxic effect of Khat extract on hepatocytes and renal cells and tissues of albino mice is due to the content of the Khat leaves or the pesticides used to improve the harvest. There is a great need to carry out a study to find out the relationship of the toxic effect on two groups of albino mice using plants extract from which the plants have never been subjected to pesticides and the ones which pesticides are being used to improve the harvest. There is a great need to find out whether the level of damage between those two groups is significantly different. The information obtained from such study will be of great use to determine a better method of minimizing the toxic effect of Khat especially on kidney and liver.

iv. There is a great need to carry out more research on effect of Khat on liver and kidney using replication test at logarithmically spaced dose levels to clearly demonstrate the dose-time effects of the Khat extract and corresponding tissue responses at different doses to justify if in need the extract have biological effects on the liver and kidney of albino mice.

v. Same study need to be carried out for a longer period of time to clearly investigate whether there will be any difference between the investigated parameter in different groups of test animals administered with difference non-alcoholic substances.

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APPENDIX 1: Biochemical assayed levels of liver and kidney enzymes concentration µM/L in male albino mice after administration with *Catha edulis* extract for 30 days at a dosage of 2000mg/Kg/day

Animal group and number	BUN (mMol/L)	Alkaline phosphatase (U/L)	Total bilirubin (mMol/L)	Creatinine (µMol/L)	Aspartate aminotransferase (U/L)
1a	6.4	190	2.68	10.70	150.60
1b	6.6	192	2.65	11.50	170.80
1c	6.3	189	2.63	10.60	180.10
1d	6.1	190	2.70	11.10	145.10
1e	6.5	193	2.71	9.30	118.90
2a	7.9	276	10.2	15.25	213.30
2b	8.0	265	10.6	14.90	204.50
2c	7.7	270	10.4	16.70	217.50
2d	7.9	268	10.0	13.50	223.30
2e	6.5	269	10.2	12.90	210.70
3a	16.9	187	3.25	12.70	200.70
3b	17.3	188	3.98	13.10	210.50
3c	17.3	188	4.00	14.90	211.50
3d	17.3	188	3.23	12.40	215.60

3e	17.3	188	2.99	14.80	213.60
4a	7.5	176	2.78	14.70	200.70
4b	7.4	175	2.79	13.10	211.50
4c	7.5	176	2.88	13.50	202.70
4d	7.6	177	3.01	12.10	217.60
4e	7.5	176	3.00	13.40	201.50
5a	7.1	257	4.27	12.90	220.00
5b	7.1	256	4.25	13.70	218.00
5c	7.2	257	4.23	13.60	205.00
5d	7.1	258	4.24	12.20	201.30
5e	7.0	254	4.27	16.10	200.00
6a	5.5	256	8.89	12.90	205.60
6b	5.7	266	8.81	15.50	203.80
6c	5.4	264	8.84	14.50	214.30
6d	5.8	256	8.89	12.10	217.40
6e	5.6	276	8.90	13.50	212.50
7a	5.5	314	3.25	11.50	200.90
7b	5.5	316	3.20	13.50	205.80
7c	5.7	317	3.30	13.10	207.70
7d	5.4	313	3.24	11.70	214.10
7e	5.6	314	3.21	11.90	215.20
8a	7.5	276	4.00	12.90	211.80
8b	7.0	271	4.50	16.50	221.70
8c	8.0	276	3.78	13.50	200.60
8d	7.4	278	4.02	14.10	211.50
8e	6.9	274	4.62	10.40	203.40
9a	7.7	286	3.53	10.30	203.40
9b	7.8	287	3.54	15.40	222.00
9c	7.9	284	3.50	13.50	200.00
9d	7.7	285	3.51	13.60	203.10
9e	7.8	286	3.54	15.10	198.40

APPENDIX 2: Physical parameters data

Negative control group body weight gain (GROUP 1)

Week	**First week**	**Second week**	**Third week**	**Fourth week**
Date	1/9/2012	10/9/2012	20/9/2012	30/9/2012
Animal A	15.064g	17.600g	19.736g	22.187g
Animal B	14.777g	17.409g	19.721g	22.374g
Animal C	15.345g	17.966g	20.348g	22.693g
Animal D	16.109g	18.763g	21.295g	23.949g
Animal E	14.995g	17.460g	19.992g	22.361g

Positive control group body weight gain (GROUP 2**)**

Week	**First week**	**Second week**	**Third week**	**Fourth week**
Date	1/9/2012	10/9/2012	20/9/2012	30/9/2012

Animal A	14.069g	16.081g	17.204g	18.894g
Animal B	15.023g	17.126g	18.649g	20.681g
Animal C	14.982g	16.991g	18.992g	20.615g
Animal D	16.001g	18.316g	20.420g	21.980g
Animal E	15.145g	17.376g	19.241g	20.894g

Animals administered to Khat extract (cathinone and cathine) at 2000mg/Kg/day and 2mls of water body weight gain (GROUP 3)

Week	**First week**	**Second week**	**Third week**	**Fourth week**
Date	1/9/2012	10/9/2012	20/9/2012	30/9/2012
Animal A	16.003g	18.539g	21.162g	23.794g
Animal B	15.235g	17.724g	20.536g	23.072g
Animal C	14.235g	16.595g	18.990g	20.993g
Animal D	15.454g	18.205g	20.957g	23.338g
Animal E	15.983g	18.637g	21.180g	23.778g

Animals administered to Khat extract cathinone and cathine) at 2000mg/Kg/day and 2mls of coffee body weight gain (GROUP 4)

Week	**First week**	**Second week**	**Third week**	**Fourth week**
Date	1/9/2012	10/9/2012	20/9/2012	30/9/2012
Animal A	15.398g	18.051g	20.513g	22.829g
Animal B	15.245g	17.834g	20.347g	23.036g
Animal C	18.349g	19.047g	21.682g	24.321g
Animal D	15.345g	18.168g	20.880g	23.745g
Animal E	14.388g	17.061g	19.417g	22.338g

Animals administered to Khat extract (cathinone and cathine) at 2000mg/Kg/day and 2mls of milk body weight gain (GROUP 5)

Week	**First week**	**Second week**	**Third week**	**Fourth week**
Date	1/9/2012	10/9/2012	20/9/2012	30/9/2012
Animal A	14.591g	17.260g	19.895g	22.579g
Animal B	15.697g	18.394g	21.215g	23.899g
Animal C	16.313g	19.025g	21.684g	24.640g
Animal D	14.341g	16.746g	19.435g	22.247g
Animal E	15.449g	17.805g	20.341g	22.995g

Animals administered to Khat extract (cathinone and cathine) at 2000mg/Kg/day and 2mls of tea body weight gain (GROUP 6)

Week	**First week**	**Second week**	**Third week**	**Fourth week**

Date	1/9/2012	10/9/2012	20/9/2012	30/9/2012
Animal A	15.381g	17.693g	20.278g	22.673g
Animal B	15.457g	17.913g	20.625g	23.319g
Animal C	15.556g	18.021g	20.702g	23.593g
Animal D	14.959g	17.612g	20.103g	22.792g
Animal E	16.006g	18.532g	20.930g	23.886g

Animals administered to Khat extract (cathinone and cathine) at 2000mg/Kg/day and 2g of patico sweet body weight gain (GROUP 7)

Week	**First week**	**Second week**	**Third week**	**Fourth week**
Date	1/9/2012	10/9/2012	20/9/2012	30/9/2012
Animal A	15.381g	17.817g	20.173g	22.854g
Animal B	14.371g	16.884g	19.539g	22.228g
Animal C	15.567g	18.098g	21.061g	24.014g
Animal D	16.023g	18.446g	21.235g	24.035g
Animal E	15.229g	17.793g	20.416g	23.987g

Animals administered to Khat extract (cathinone and cathine) at 2000mg/Kg/day and 2g of groundnut body weight gain (GROUP 8)

Week	**First week**	**Second week**	**Third week**	**Fourth week**
Date	1/9/2012	10/9/2012	20/9/2012	30/9/2012
Animal A	15.281g	17.804g	20.367g	23.351g
Animal B	14.888g	17.353g	19.718g	22.316g
Animal C	15.012g	17.544g	20.057g	23.019g
Animal D	16.002g	18.578g	21.176g	23.825g
Animal E	15.781g	18.146g	20.727g	23.385g

Animals administered to Khat extract (cathinone and cathine) at 2000mg/Kg/day and 2mls of coke body weight gain (GROUP 9)

Week	**First week**	**Second week**	**Third week**	**Fourth week**
Date	1/9/2012	10/9/2012	20/9/2012	30/9/2012
Animal A	15.338g	17.678g	20.216g	22.865g
Animal B	15.481g	17.807g	20.479g	23.128g
Animal C	15.333g	17.846g	20.488g	22.886g
Animal D	15.487g	17.799g	20.711g	23.257g
Animal E	14.987g	17.455g	19.591g	22.152g

APPENDIX 3: Organ weight

Negative control group body weight gain (GROUP 1)

GROUP 1	**Liver weight in g**	**Kidney left weight in g**	**Kidney right weight in g**
Animal A	1.038	0.158	0.136
Animal B	0.736	0.102	0.107
Animal C	1.225	0.159	0.159
Animal D	1.018	0.112	0.117
Animal E	1.001	0.109	0.105

Positive control group body weight gain (GROUP 2)

GROUP 2	**Liver weight in g**	**Kidney left weight in g**	**Kidney right weight in**

			g
Animal A	1.550	0.183	0.148
Animal B	1.671	0.168	0.144
Animal C	1.871	0.201	0.195
Animal D	1.518	0.132	0.147
Animal E	1.121	0.139	0.135

Animals administered to Khat extract (cathinone and cathine) at 2000mg/Kg/day and 2mls of water body weight gain (GROUP 3)

GROUP 3	**Liver weight in g**	**Kidney left weight in g**	**Kidney right weight in g**
Animal A	0.489	0.078	0.084
Animal B	1.252	0.164	0.145
Animal C	0.896	0.117	0.119
Animal D	1.034	0.162	0.166
Animal E	1.101	0.149	0.146

Animals administered to Khat extract cathinone and cathine) at 2000mg/Kg/day and 2mls of coffee body weight gain (GROUP 4)

GROUP 4	**Liver weight in g**	**Kidney left weight in g**	**Kidney right weight in g**
Animal A	1.543	0.163	0.153
Animal B	1.633	0.145	0.162
Animal C	1.130	0.132	0.139
Animal D	1.165	0.119	0.105
Animal E	1.729	0.166	0.173

Animals administered to Khat extract (cathinone and cathine) at 2000mg/Kg/day and 2mls of milk body weight gain (GROUP 5)

GROUP 5	**Liver weight in g**	**Kidney left weight in g**	**Kidney right weight in g**
Animal A	0.804	0.086	0.085
Animal B	1.447	0.163	0.155
Animal C	1.940	0.232	0.214
Animal D	1.426	0.176	0.176
Animal E	1.247	0.156	0.154

Animals administered to Khat extract (cathinone and cathine) at 2000mg/Kg/day and 2mls of tea body weight gain (GROUP 6)

GROUP 6	Liver weight in g	Kidney left weight in g	Kidney right weight in g
Animal A	1.295	0.153	0.148
Animal B	1.070	0.164	0.173
Animal C	1.492	0.143	0.143
Animal D	0.875	0.143	0.145
Animal E	1.502	0.181	0.191

Animals administered to Khat extract (cathinone and cathine) at 2000mg/Kg/day and 2g of patico sweet body weight gain (GROUP 7)

GROUP 7	Liver weight in g	Kidney left weight in g	Kidney right weight in g
Animal A	1.217	0.155	0.176
Animal B	1.481	0.196	0.200
Animal C	1.688	0.231	0.243
Animal D	1.461	0.188	0.178
Animal E	0.868	0.109	0.110

Animals administered to Khat extract (cathinone and cathine) at 2000mg/Kg/day and 2g of groundnut body weight gain (GROUP 8)

GROUP 8	Liver weight in g	Kidney left weight in g	Kidney right weight in g
Animal A	0.822	0.127	0.121
Animal B	1.588	0.204	0.207
Animal C	1.175	0.125	0.152
Animal D	1.229	0.149	0.138
Animal E	0.807	0.102	0.109

Animals administered to Khat extract (cathinone and cathine) at 2000mg/Kg/day and 2mls of coke body weight gain (GROUP 9)

GROUP 9	Liver weight in g	Kidney left weight in g	Kidney right weight in g
Animal A	0.921	0.116	0.117
Animal B	1.642	0.153	0.163
Animal C	1.174	0.145	0.167
Animal D	1.134	0.117	0.123
Animal E	1.037	0.132	0.130

APPENDIX 4: Liver and renal biochemical analytes

(a) Minimum, maximum, mean and standard deviation of blood urea nitrogen in albino mice

GROUP	N	Minimum (mMol/L)	Maximum (mMol/L)	Mean (mMol/L)	SD
1	5	7.00	10.10	8.46	1.29
2	5	13.40	17.30	15.64	1.42
3	5	12.10	14.70	13.54	0.97
4	5	12.90	15.20	13.82	0.99
5	5	10.70	18.50	13.86	2.85
6	5	10.30	14.50	13.16	1.64
7	5	11.10	14.50	12.78	1.40
8	5	11.50	15.10	13.66	1.39
9	5	13.50	15.00	14.30	0.59
VALID N	5				

(b) Minimum, maximum, mean and standard deviation of serum creatinine in albino mice

GROUP	N	Minimum (µMol/L)	Maximum (µMol/L)	Mean (µMol/L)	SD
1	5	9.30	11.50	10.64	0.83
2	5	12.90	16.70	14.62	1.48
3	5	12.40	14.90	13.58	1.19
4	5	12.10	14.70	13.36	0.93
5	5	12.20	16.10	13.70	1.47
6	5	12.10	15.50	13.70	1.33
7	5	11.50	13.50	12.34	0.89
8	5	10.40	16.50	13.48	2.19
9	5	10.30	15.40	13.58	2.02
VALID N	5				

(c) Minimum, maximum, mean and standard deviation of total bilirubin in albino mice

GROUP	N	Minimum (mMol/L)	Maximum (mMol/L)	Mean (mMol/L)	SD
1	5	18.40	28.30	24.24	3.63
2	5	10.50	13.40	12.44	1.18
3	5	17.50	26.70	20.60	3.87
4	5	19.50	25.30	22.08	2.85
5	5	14.50	21.00	17.80	2.79
6	5	14.50	23.40	18.66	4.06
7	5	14.50	27.80	21.16	6.17
8	5	15.40	28.40	19.12	5.28
9	5	17.50	24.50	20.02	2.75
VALID N	5				

(d) Minimum, maximum, mean and standard deviation of serum aspartate aminotransferase in albino mice

GROUP	N	Minimum (U/L)	Maximum (U/L)	Mean (U/L)	SD
1	5	204.50	223.30	213.86	7.07
2	5	118.90	180.10	153.10	23.88
3	5	200.70	215.60	210.38	5.75
4	5	200.70	217.60	206.80	7.43
5	5	200.00	220.00	208.86	9.46
6	5	203.80	217.40	210.72	5.80
7	5	200.90	215.20	208.74	5.95
8	5	200.60	221.70	209.80	8.27
9	5	198.40	222.00	205.38	9.52
VALID N	5				

(e) Minimum, maximum, mean and standard deviation of serum alkaline phosphatase in albino mice

GROUP	N	Minimum (U/L)	Maximum (U/L)	Mean (U/L)	SD
1	5	180.90	195.60	187.82	6.06
2	5	119.40	170.50	146.12	18.65
3	5	159.90	186.50	178.38	10.76
4	5	160.50	188.90	178.10	11.04
5	5	179.50	189.50	182.86	3.96
6	5	166.40	181.50	176.24	6.16
7	5	179.90	186.50	182.92	2.85
8	5	168.40	199.10	181.24	11.55
9	5	169.50	184.50	179.24	5.78
VALID N	5				