EFFECTS OF *CATHA EDULIS* (MIRAA) ON KIDNEY AND LIVER FUNCTION AMONG MIRAA CHEWING ADULTS IN MERU COUNTY, KENYA

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Effects of *Catha Edulis* (Miraa) on Kidney and Liver Function among Miraa Chewing Adults in Meru County, Kenya

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DECLARATION

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

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DEDICATION

This work is dedicated to my husband Newton and my son Gabriel.

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OPERATIONAL DEFINITIONS

Catha edulis / khat: Miraa

Chewer: An individual is considered a chewer even if he/she had chewed only once in his/her lifetime.

Non-chewer: An individual is considered non-chewer if he/she has never chewed *Catha edulis*

Dependence: Addiction

LIST OF ABBREVIATIONS AND ACROMNYMS

ALT	Alanine aminotransferase
ALP	Alkaline phosphatase
ANOVA	Analysis of variance
AST	Aspartate Aminotransferase
BCG	Bromcresol Green
BUN	Blood Urea Nitrogen
Cl^-	Chloride
CKD	Chronic Kidney Disease
Cr	Creatinine
DB	Direct bilirubin
DSA	Diazotized Sulfanilic Acid
IFCC	International Federation of Clinical Chemistry
IQC	Internal Quality Control
KNH/UoN-ERC	Kenyatta National Hospital/University of Nairobi–Ethical Review Committee
K^+	Potassium

Na^+	Sodium
NACADA	National Authority for the Campaign against Alcohol and Drug Abuse
SSA	Sub-Saharan Africa
SPSS	Statistical Package for Social Sciences
TB	Total bilirubin
UK	United Kingdom
Ur	Urea
USA	United States of America
UV	Ultra Violet
WHO	World Health Organization

ABSTRACT

*Catha edulis (*khat) chewing is common in Kenya and it is a daily habit among adults in Meru County. The habit of *Catha edulis* chewing poses a public health problem. Some studies have been done to investigate the effects of khat on kidney and liver in animals. However, only a few of these studies have been conducted in human. This study was conducted to evaluate the possible effects of *Catha edulis* on the levels of various biochemical parameters to assess kidney and liver function and also compare the effects of red and pale green *Catha edulis* varieties on adult human consumers from four constituencies in Meru County. Ethical review was sought from KNH-UoN ERC. A cross-sectional study was conducted from December 2014 to December 2015 involving 198 chewers and 193 non-chewers consenting adults, aged 18-60 years, who met the inclusion criteria were enrolled. Consecutive sampling method was used to recruit participants. A structured questionnaire was used for data collection and blood was drawn from participants. The Humastar 200 automated chemistry analyzer was used to directly determine the values for liver and kidney biochemical parameters while electrolyte i-smart 30 electrolyte analyzer was utilized for electrolytes. The results were computed statistically with SPSS software package version 21. Results were expressed as mean ± SD values, number and percentage values for continuous data. Student t-test was used to compare the different variables between chewers and non-chewers. Comparison between red variety chewers, pale green variety chewers and non-chewers was done using the one way ANOVA test, followed by the post-hoc Tukey's test. Comparison between age groups was done using the one way ANOVA test, followed by the post-hoc Tukey's test. Cross tabulation was done to determine the proportions of the characteristics of the independent variable. The results showed that majority (96.9%) of the participants were male. Alkaline phosphatase activity was significantly increased in the serum of *Catha edulis* consumers than non-consumers. TP, DB and Cr concentration were significantly decreased in the serum of *Catha edulis* consumers than non-consumers. TB, ALA and ALP parameters mean were statistically significantly increased ($p<0.05$) in red variety chewers than pale green variety chewers. Age and gender did not have significant effect on the level of kidney and liver biochemical parameters in *Catha edulis* chewers. In conclusion, liver biochemical parameters were affected in the *Catha edulis* chewing group which was shown by increasing activity of alkaline phosphatase therefore, *Catha edulis* might be responsible for liver damage. *Catha edulis* has no effect on kidney biochemical parameters. Liver biochemical parameters were affected by the red variety *Catha edulis* chewing as compared to the pale green variety. To shed light on *Catha edulis* as a cause of liver problem, retrospective and prospective epidemiological studies of chronic miraa users should be initiated and also studies to determine the concentration of cathinone and cathine in different varieties of *Catha* edulis should be done.

CHAPTER ONE

INTRODUCTION

1.1 Background information

Chronic diseases like kidney and liver diseases constitute a fast increasing burden to society. The World Health Organization (WHO) estimates that 46% of global disease and 59% of mortality is due to chronic diseases. Thirty-five million individuals in the world die each year from chronic diseases and the numbers are increasing steadily (Guilbert, 2003; World Health Organization, 2002). Developing countries are increasingly suffering from high levels of public health problems related to chronic diseases (World Health Organization, 2002). According to the Office for National Statistics in the United Kingdom, after heart disease, stroke, chest disease and cancer, liver disease is the fifth most common cause of death. However, unlike other major causes of mortality, liver disease rates are increasing rather than declining (Leon & Mccambridge, 2006). The magnitude of chronic kidney disease (CKD) in Sub-Saharan Africa (SSA) countries is still poorly characterized, although there are speculations that the incidence rates are 3–4 times higher than those in developed countries (Naicker 2009). It is more difficult to get accurate estimates in the developing countries like Kenya, due to lack of national registries of CKD and limited surveys. However, the risk factors for CKD are known to be just as prevalent in many developing countries as in the developed countries. Therefore, the burden of CKD in those developing countries may be comparable to those of the developed countries. In addition, developing countries exhibit a disproportionate burden of infectious and environmental factors that broaden the spectrum of CKD risk factors and are up to increase CKD burden. Factors such as hypertension, diabetes, cardiovascular diseases have been associated with kidney problem and alcoholism has been associated with liver problem. *Catha edulis* has also been associated with liver and kidney disease. However evidence of effects of *Catha edulis* on liver and kidney is often based on limited numbers of case reports and animal studies. These studies show that *Catha edulis* interferes with normal body functions, which could lead to serious liver and kidney disorders in animals and even in humans (Aden *et al*., 2006; Gebissa 2004; Al-Motarreb *et al*., 2002; Patel, 2000; Kennedy, *et al*. 1983;).

Catha edulis (Khat) belongs to the kingdom Plantae, class Magnoliopsida, order Celastrales, family Celastraceae, genus *Catha* and species *edulis* (Simmons *et al.*, 2008). *Catha edulis* originated in Ethiopia and later spread to Somalia, Kenya, Uganda, Tanzania, Congo, Zambia, Zimbabwe, Afghanistan, Yemen and Madagascar (Fitzgerald, 2009). *Catha edulis* chewing globally ranges from approximately five to ten million people most of which are in the Horn of Africa and Arabian Peninsula (Mateen, 2010). *Catha edulis* (miraa) is cultivated and consumed in Ethiopia as well as in Kenya (Gebissa, 2004).

In Kenya, the plant is extensively cultivated in Meru and Embu counties. Its leaves are widely chewed, especially among men, for their central nervous system-stimulating properties (Luqman, 1976).

According to National Alcohol and Drug Abuse, 2012, there are 1.6 million *Catha edulis* users in Kenya. The prevalence use of *Catha edulis* in Kenya is 5.5%. The use is high among urban as opposed to rural dwellers. In terms of regions, highest use is reported in North Eastern (28%) followed by Nairobi (7.2%), Coast (6.2%) and Eastern (5.4%). Lowest current use is recorded in Western. Chewing of *Catha edulis* (miraa) is a social habit in Kenya (NACADA, 2012). The leaves come from a small evergreen shrub that can grow to tree size (World Health Organization, 2012). Majority of *Catha edulis* chewers are between the age of 25–34 years for both sexes (Al-abed *et al.,* 2013). Cathinone is believed to be the main active ingredient in fresh khat leaves. *Catha edulis* (khat) is categorized as a drug of abuse by WHO that can lead to mild to moderate psychological dependence but to a lesser degree than nicotine and alcohol (Nutt, King & Saulsbury, 2007) and the WHO does not consider *Catha edulis* to be as dangerously addictive as cocaine (Al-Mugahed, 2008). The regular consumption of *Catha edulis* is associated with a variety of health problems affecting the consumers (World Health Organization, 2012). The WHO (2003, 2006) reported that Khat consumption has become a common problem that affects the health aspects of life (kassim & Islam, 2010). Different varieties of *Catha edulis* have different degrees of pharmacological action. Meru farmers recognize two varieties based on the colour of the shoots and growing twigs as "kangeta" predominantly reddish in colour and "giza" pale green in colour. The widespread use and popularity is due to its CNS-stimulating phenylpropylamines, especially to cathinone (α-aminopropiophenone, 'natural amphetamine') and to a much lesser extent to norpseudoephedrine ('cathine') and norephedrine (Kalix, 1988). Currently, *Catha edulis* is illegal in the USA, Canada, and many European countries (Roelandt *et al.*, 2011).

Some studies have been done to investigate the effects of *Catha edulis* on kidney and liver in animals. However, only a few of these studies have been conducted in human and these studies have been carried out in the Middle East countries. This study evaluated the effect of *Catha edulis* on liver and kidney among users in Meru County, Kenya and also compared the effect of red and pale green variety of *Catha edulis* on kidney and liver in human.

1.2 Problem Statement

According to NACADA (2012) drug-use study, there are approximately 1.6 million *Catha edulis* users in Kenya. Problems such as increased blood pressure, decreased sexual function, delayed gastric emptying and gastrointestinal disorders, associated with repeated consumption of *Catha edulis* are becoming evident. *Catha edulis* chewing habit has changed such that what was previously a formalized and strongly regulated social habit presently has features of excessiveness, informality and decoupling from normative control (Yusuf *et al.*, 2012). *Catha edulis* leaves and back of the stem has been chewed for centuries by the people of various countries such as Yemen, Ethiopia, Eriteria, Somalia and Kenya for its stimulant properties (Numan, 2012). However, *Catha edulis* chewing has led to serious public health and socioeconomic problems in these countries (Hussein *et al.*, 2008).

In Kenya, consumption of *Catha edulis* has become part of the youth culture (Neil Carrier, 2005). Most of the people who chew *Catha edulis* are farmers in khat growing areas (Gebissa, 2004). The habit of *Catha edulis* chewing is widespread in Kenya especially in the areas where it is cultivated and as such poses a public health problem. Some studies have suggested that khat consumption leads to induced cytotoxicity in livers and kidneys after oral administration to animals (Fahaid *et al.* 2011). *Catha edulis* induces a fall in average and maximum urine flow rate in healthy men (Hassan *et al.*, 2002). Another report also described a patient with impaired liver function attributed to *Catha edulis* chewing. Serum alkaline phosphatase was increased, there was unusual liver directed autoimmune activity with positive anti smooth muscle antibody titre (1: 40) and liver biopsy findings showed cirrhosis. (Patanwala, Burt & Bassendine, 2011). Severe acute liver injury has been attributed to *Catha edulis* chewing in the United States of America (USA) (Vedula *et al.,* 2010). A case series described jaundice and deranged liver function based on biopsy histology and serum biochemistry in seven United Kingdom (UK) men of Somali origin who were regular *Catha edulis* chewers. All denied any alcohol consumption and no other aetiological factors could be identified (Peevers *et al.,* 2010). Goats fed on *Catha edulis* at the University of Eldoret in Uasin Gishu County showed the plant interferes with normal body functions, which could lead to serious liver and kidney disorders (Ng'wena *et al.*, 2012).

However, few studies have been done to evaluate the effects of *Catha edulis* consumption in human beings. Rania Hussein *et al*., (2013) investigated the biochemical blood constituents changes among khat chewers in Sana'a city, Yemen and concluded that there was increase in serum urea, creatinine and bilirubin in in khat users. Shabbir *et al.*, (2014) studied the effect *Catha edulis* consumption on liver and kidney function in male population of Jazan and concluded that aspartate aminotransferase, alanine aminotransferase and alkaline phosphatase were increased in the serum of *Catha edulis* users. Furthermore these studies already done have been carried out in the Middle East countries such as Yemen. Additionally, different varieties of *Catha edulis* contain different levels of cathinone which may lead to different effects that need to be investigated. Due to high availability and increase use of *Catha edulis* use in Meru County, it is therefore important to determine the effects of *Catha edulis* on liver and kidney functions in adult consumers.

1.3 Justification

The evidence of liver or kidney damage due to *Catha edulis* chewing is often based on limited numbers of case reports and animal studies. Meru County is the largest producer and consumer of *Catha edulis*, a herb associated with development of kidney and liver problem. The effect of *Catha edulis* chewing on the liver and kidney has not been adequately studied in human. There is no data in Meru County on relationship of *Catha edulis* chewing and kidney and liver disease. In order to come up with a remedy to the problem, it is therefore, imperative to carry out further research to investigate the relationship that exists, between *Catha edulis* chewing, renal and liver problems in Meru county where the *Catha edulis* chewing is done when it is very fresh and it is the red variety of *Catha edulis* used, which is considered superior by users.

1.4 Null hypothesis

Catha edulis chewing has no effect on the biochemical parameters of the kidney and liver.

1.5 OBJECTIVES

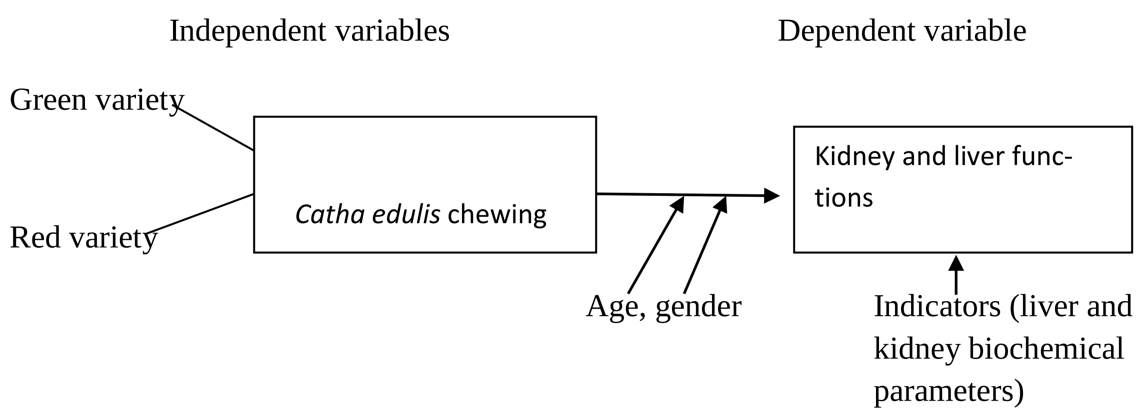
1.5.1 General Objective

To evaluate the effects of *Catha edulis* on kidney and liver function using biochemical parameters.

1.5.2 Specific objective

1. To determine liver and kidney biochemical parameters changes associated with chewing *Catha edulis.*
2. To determine the effect of *Catha edulis* on liver and kidney in relation to age.
3. To determine the effect of *Catha edulis* on liver and kidney in relation to gender.
4. To compare the effects of red and pale green variety of *Catha edulis* on human consumers in relation to kidney and liver biochemical parameters
5. To determine demographic factors and chewing habits related to *Catha edulis* use.

1.6 Conceptual Framework



CHAPTER TWO

LITERATURE REVIEW

2.1 Background information

Catha edulis (miraa) is a large green shrub (figure 2.1) that grows at high altitudes whose leaves are the source of a naturally occurring amphetamine-like substance known as cathinone (Al-Habori & Al-Mamary, 2004). It originated from Ethiopia, specifically in Hararghe with a gradual expansion to different parts of Ethiopia, Yemen and other parts of the world (Huffnagel, 1961). *Catha edulis* use is accepted in Yemen, Ethiopia, Eritrea, Djiboutia, Kenya, Somali and Ugandan cultures (Thomas, 2013; World Health Organization (WHO) Expert Committee on Drug Dependence, 2006) but is prohibited in the United States of America (USA), France, Sweden, and Switzerland. *Catha edulis* use had been tolerated in the Netherlands and in the United Kingdom (UK) for a long time, but in 2012, the recreational use of *Catha edulis* was prohibited in the Netherlands, and the UK followed in 2014 (Klein., 2014). Although the use of *Catha edulis* is widespread, it has until recently remained mostly confined to the regions where the plant is grown since only fresh leaves have the potency (of Cathinone) to produce the desired effects. The fresh stems and leaves are harvested regularly from the trees, and are highly valued for their stimulating properties. Cathinone the major constituent of *Catha edulis* is similar in structure and pharmacological activity to amphetamine (Feyissa *et al.* 2008) which has psychoactive effects; produces an initial euphoria that is later followed by depression, chewers may also experience irritability and insomnia (Lukandu, 2009). Cathinone and cathine are classified as controlled substances according to the 1971 Convention on Psychotropic Substance (Wedegaertner *et al*., 2010). D-amphetamine exerts different forms of hepatotoxicity in-vivo and in-vitro when tested on hepatocytes (Vitcheva *et al*., 2009). *Catha edulis* is expected to cause similar toxic effects on the liver. According to Ogembo, personal communication, 2011, a consultant physician at Cottolengo mission hospital there is increased hepatic and renal complications among *Catha edulis* users in Meru County.

Source: Field data

Figure 2.1: *Catha edulis* plant

2.2 The pharmacology of *Catha edulis*

The leaves and shoots (figure 2.2) of khat contain more than forty complex compounds including the alkaloid stimulants cathinone (figure 2.3) and cathine. Cathinone is the main active ingredient in fresh *Catha edulis* leaves and shoots (Al-Motarreb, Baker & Broadley, 2002). Cathinone has a closer structural similarity with amphetamine (Masoud *et al.,* 2014) and both share common pharmacodynamic features, this led to the conclusion that cathinone is the most important active ingredient of *Catha edulis*, which causes the major pharmacological effects (Al-Habori, 2005; Brenneisen, 1990). Stored *Catha edulis* leaves lose their stimulatory properties rapidly, becoming physiologically inactive after about thirty six hours as the more powerful cathinone decomposes leaving behind the milder, less active, cathine. Chewing *Catha edulis* is an efficient way of extracting cathinone and cathine by the action of enzymes in saliva (Al-Motarreb *et al.,* 2002; Krikorian, 1984). The potential for users to become dependent on *Catha edulis* is limited by two key characteristics. First it takes a long time for the active ingredients to reach maximal levels in the blood. Second the bulky nature of the herbal material limits the maximum dose that can be received. Central stimulation by *Catha edulis* is manifested by euphoria, increased alertness, garrulousness, hyperactivity, excitement, aggressiveness, anxiety, elevated blood pressure, and maniac behavior (Aden *et al.,* 2006). The euphoric effect of *Catha edulis* starts after about one hour of chewing, when plasma concentrations of cathinone start to rise, peak plasma levels are obtained within 1.5 hour to 3.5 hour after the onset of chewing (Cox, 2003). A depressive phase, including insomnia, malaise, and a lack of concentration, almost always follows (Al-Motarreb *et al.,* 2002). Chronic use of *Catha edulis* can lead to behavioural changes and impairment of mental health (Fekaduk *et al.,* 2009).

Source: Field data

Figure 2.2: ***Catha edulis*** **shoot and leaves**

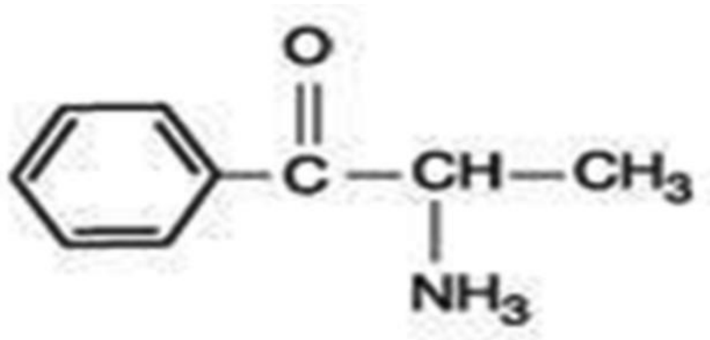


Figure 2.3: Cathinone chemical structure

2.3 Chemical composition of *Catha edulis*

Catha edulis contains many alkaloids, tannis, amino acids, glycosides, minerals and vitamins (Halbach, 1972). Cathedulins and phenylalkylamnes are the major alkaloids. The major active ingredients in *Catha edulis* leaf are phenylalkylamine (-)-alpha aminopropiophenone named as cathinone (psychostimulant component of khat) and cathine (nor-pseudo-ephedrine) (Numan. 2012), these compounds share similarities with amphetamine, with up to 90% being absorbed during chewing, predominantly via the oral mucosa. Cathinone is found mainly in the young leaves and shoots. During maturation, cathinone is metabolized to cathine, and (-)-norephedrine (Kalix, 1985). Other phenylalkylamine alkaloids found in *Catha edulis* leaves are the phenylpentenylamines *merucathinone, pseudomerucathine* and *merucathine.* These seem to contribute less to the stimulant effects of *Catha edulis* (Al-Hebshi, 2005). On average, 100g fresh khat leaves contain 36 - 114 mg cathinone, 83 -120 mg cathine and 8 - 47 mg norephedrine (Toennes & Harder, 2003). Cathinone is labile and is transformed within a few days of harvesting to a dimer (3,6-dimethyl-2,5-diphenylpyrazine) and it is for this reason that *Catha edulis* needs to be consumed while still fresh.

2.4 Environmental health effects of growing *Catha edulis*

Due to the high economic importance of *Catha edulis* as a cash crop, farmers tend to use pesticides and fertilizers heavily on *Catha edulis trees* in order to protect them from pests, to ensure healthy foliage and thus to increase the yield and income (Thabet, 1999). A study by Date *et al* (2004) shows that people who chew khat which is sprayed with pesticides have the highest health risks due to the combination of the pesticides and khat.

2.5 Cultivation and use of *Catha edulis* in Kenya

Catha edulis is predominantly cultivated as a cash crop in parts of Eastern and Central Kenya. However, there are some variants of *Catha edulis* that grow wild in the Rift Valley region of Kenya (NACADA, 2012). The best quality *Catha edulis* comes from Nyambene hills and is highly perishable and consumption of the product must take place within at least thirty six hours after it is picked from the tree. Nyambene is home to two subgroups of the Bantu-speaking Meru: the Tigania and Igembe. Igembe cultivate *Catha edulis* more intensively than the Tigania (Neil Carrier, 2008). *Catha edulis* grows under the same climatic and soil conditions as Arabic Coffee. It grows well on well drained most slopes 1,500 - 300 meters above sea level between latitude 1 8°N - 30°S. Nyambene lies within one degree on either side of the equator and 37°-38° east, longitude. *Catha edulis* plant requires well drained dark red-brown, sandy loam with a low percentage of clay and medium to high amounts of total nitrogen, organic matter, available phosphorus, calcium, potassium and magnesium (Murphy H, 1959). Vegetative propagation is applied in cultivation of *Catha edulis*. Suckers or rooting branches arising from underground near the stem are used. The cultivation is done during the heavy rainy season. Spacing is usually 3-4 meters between plants. The plant takes 4-6 years to yield its first crop which is considered to be of low quantity. Good yield is obtained after 8-10 years. The older the tree the more potent the *Catha edulis* shoots is. The good quality *Catha edulis* shoots are produced by trees over 30 years old. The branches of the *Catha edulis* tree bend under the heavy weight of the foliage. In Meru County the oldest trees are called *mbaine*, and these trees are regarded as producing the finest miraa (Carrier

2005; Goldsmith 1994). *Catha edulis is* often grown in association with different food crops and is highly compatible when intercropped. The young tender shoots that grow directly from the main stems and old branches are harvested by breaking off the young branches from the main branches. This is done about twice a week. *Catha edulis* is harvested year round at any time of the day, but is often harvested in early morning or late afternoon. Generally, harvesting is possible 2-3 times a year from a well-established rain-fed matured *khat* plant depending on the age, management practice and the fertility of the soil (Dechassa Lemessa, 2001).

The harvested shoots are first tied in twos (if they are long) or 4-5 (for very small ones e.g. giza) to form an "apa". Ten apas are tied together to half bandari. Two half bandaris are tied together to form bandari wrapped in fresh green banana leaves and secured with a dry banana fibre. Ten bandaris are finally wrapped together with green banana leaves to form a bunda. The bunda is the major wholesale exchange unit while bandari is the retail exchange procedure, particularly for export market (NACOSTI (National Council for Sience and technology), 1996).

Catha edulis is accepted as fresh for a period of up to 4 days (Leboi type) and 2 days (Kangeta or Giza type) after picking. Miraa is wrapped in green banana leaves to conserve moisture. In the market Leboi type (high moisture content) is considered inferior to Kangeeta or Giza type with low moisture content.

Catha edulis leaves are consumed while fresh to avoid loss of stimulatory potency. For maximum potency, *Catha edulis* must be picked in the morning and chewed that afternoon (Grove *et al.,* 1999). *Catha edulis* leaves losses stimulatory potency after three to four days of harvesting (Halbach, 1972). North Eastern residents and many Somali community in particular are major users of *Catha edulis* in Kenya. *Catha edulis* chewing is a male activity in Eastern Africa communities though presently it has become increasingly popular among women (Aden *et al.,* 2006).

The use of *Catha edulis* starts at a young age and the habit is most likely to develop into a compulsive daily habit lasting lifetime (Patel, 2000). *Catha edulis* induces a significant rise of arterial blood pressure. The peak effect of the arterial pressure is reached three hours after starting to chew followed by a decline, one hour after stopping to chew (Galal *et al.*, 2013). Khat is usually chewed in prolonged sessions, producing mild psychostimulant effects such as increased energy, enhanced mood, reduced appetite and reduced sleep (Douglas, Boyle, & Lintzeris, 2011).

The pleasure derived from *Catha edulis* chewing is attributed to the euphoric actions of its content of 5-cathinone (Al-Motarreb *et al.*, 2002). The effects of *Catha edulis* are similar to, but weaker than those of amphetamine. The consumers get a feeling of well-being and mental alertness with loquacity, excitement and sometimes anxiety (Al-Motarreb *et al.*, 2002). To achieve the climax of such feeling, they continue chewing for four to six hours or even more.

2.6 Mode of consumption

Catha edulis users prefer the leaves from the tips of the branches and those young shoots. The fresh leaves, twigs, and shoots of the *Catha edulis* shrub are taken one by one and thoroughly chewed. *Catha edulis* is chewed differently depending on its type. The type that is grown in Meru, involves the peeling of the bark from the twigs and chewing it (often taking along the bud with). The *Catha edulis* from Embu involves the chewing of the leaves. The juice that comes out of the chewed Catha edulis then swallowed whereas the marcerated material is kept for a while in the cheek and later expectorated (Dhaifalah & Santavy 2004). In the areas where *Catha edulis* use originated, groups of people mainly men gather every day to chew it. These sessions, (known in Ethiopia as barch'a, and in Yemen as majlisalqat (literally "the council of khat"), are informal events.

The ingestion of khat produces sympathetic activation characterized by euphoria, increased intellectual efficiency and alertness, anxiety, high blood pressure, and other effects (Nencini, 1989). These effects are mainly mediated by phenylalkylamines such as cathinone (the major stimulant) and cathine, although several other stimulant compounds are also found in khat (Nencini, 1989). Studies done have shown that many individuals chew *Catha edulis* and drink alcohol concurrently (Beckerleg, 2009; Ihunwo

& Kayanja, 2004). In communities where *Catha edulis* plant is grown, chewing of *Catha edulis* has both social and cultural importance. *Catha edulis* plays a vital role as part of dowry in wedding ceremonies (Kennedy, Teague and Rokaw 1983).

2.7 Social factors leading to the use *Catha edulis*

Catha edulis chewing is a social occasion among addicts, and is typically done as a group activity until late at night (Aden *et al.,* 2006). *Catha edulis*, like coffee, is a stimulant and it is used to improve alertness and performance (Masoud *et al.,* 2014). Night shift workers such as watchmen and long distance truck drivers chew it to stay awake and postpone fatigue (Aden *et al.,* 2006). It *is* also commonly used for social recreation. A significant number of students consume *Catha edulis* to be alert and wakeful at night especially during examination periods (Douglas *et al.,* 2011; Masoud *et al.,* 2014). In Somali *Catha edulis* is used in social settings, in the home, in restaurants or in *Catha edulis* cafes ('mafrishi'), and women and men chew separately. Chewing is done for periods of 4-6 hours and upto 12 hours, and commonly drink sweet drinks while chewing, to counter the bitter taste of khat (Heather Douglas, 2011).

2.8 Varieties of *Catha edulis* based on their pigmentation

In Yemen farmers recognize four varieties based on the colour of shoots and growing twigs as 'Abyadh' predominantly pale green in colour, 'Azraq' purplish, 'Aswad' crimson and 'Ahmar' an intermediate between ;Azraq' and 'Aswad' reddish. In Ethiopia two prominient varieties have been described as dimma (red) and ahde (pale green) (Krikorian, 1984; Al-Motarreb & Baker 2002). In Yemen forty kinds of khat were recognized Ramadan *et al.,* (1981). The alkaloid content of khat varies with the soil, climatic conditions, and cultivation. Khat quality is rated by connoisseurs in a manner similar to tea and coffee. The criteria utilized depend upon the part of the plant that will be used – leaves, buds, or twigs. This is coupled with the degree of maturity, the size of the leaf and the area of plant origin. Larger and older leaves will be tough and not easily chewed. Additionally, these leaves will contain a lower amount of cathinone as compared to younger leaves from the same plant. In addition, the red leaf type known as “dimma” is known to have more cathinone than the pale green leaf type known as “dallota”. Khat varies in strength from region to region. Kenyan khat is considered to be the strongest and, thus, the most popular (Neil, 2007). In Meru farmers recognize different varieties of *Catha edulis* based on their pigmentation. The red type (figure 2.4) known as kangeta and pale green type (figure 2.5) known as giza (Neil, 2017). Scientific investigation of the varieties has not been carried out to determine their taxonomic position.

Source: Field data

Figure 2.4: Red type of *Catha edulis*

Source: Field data

Figure 2.5: Pale green type of *Catha edulis*

2.9 Association of *Catha edulis* chewing with gender and age

Catha edulis consumption was only limited to older men and members of Muslim communities in the past but today consumption is by all societal groups regardless of age and sex (Ayana and Mekonen, 2004). Khat chewing among women and children has increased and has become socially accepted in Yemen (Kandela, 2000). Many studies have demonstrated *Catha edulis* chewing is associated with age and gender (Milaat, Salih & Bani, 2005). In Kenya *Catha edulis* is used by both male and female (NACADA, 2006). A study done in central division of Wajir East Sub County revealed that gender is a demographic determinant of *miraa* chewing. Male are almost two times more likely to chew *miraa* than female. There is greater cultural acceptance of males chewing of *miraa* compared to females (Abdisalan, 2016).

2.10 Liver

The liver is a major organ for metabolism of foreign substances and also functionally interposed between the site of absorption and the systemic circulation. These conditions render the liver not only the most important organ for detoxification of foreign substances but also a major target of their toxicity (Russmann & Kullak-Ublick, 2009). Metabolism is the enzymatic conversion of one chemical compound into another. Most drug metabolism occurs in the liver, although some processes occur in the gut wall, lungs and blood plasma. Metabolism of cathinone the main constituent of *Catha edulis* mainly occurs at the first passage in the liver (Drake, 1988).

An excess of chemicals hinders the production of bile thus leading to the body's inability to flush out the chemicals through waste. Smooth endoplasmic reticulum of the liver is the principal 'metabolic clearing house' for both endogenous chemicals like cholesterol, steroid hormones, fatty acids and proteins, and exogenous substances like drugs and alcohol. The central role played by liver in the clearance and transformation of chemicals exposes it to toxic injury (Saukkonen, Cohn, Jasmer, Schenker, 2006). Production of excess reactive oxygen radicals has been hypothesized as a mediator of *Catha edulis* induced hepatoxicity (Patanwala, Burt & Bassendine, 2011). Intracellular reactive oxygen radicals generated following exposure to *Catha edulis* can provoke a series of signaling cascade that promotes liver cells death (Abid *et al.*, 2013).

2.11 Kidney

The kidney maintains the water, ionic and chemical balance of blood by filtering chemicals from the blood and conserving, or reabsorbing, those chemicals that are needed for adequate metabolism. Because of the ability of the nephron to filter and reabsorb certain chemicals from the blood, the measurement of the concentration of these chemical in the blood and urine serves as a functional evaluation of the kidney. The measurements of the concentrations of blood urea nitrogen, creatinine and electrolytes all serve as functional evaluations of different areas of the kidney (Wendy Arneson, 2007)

2.12 Effects of *Catha edulis* chewing

Notably, an association has been found between khat chewing and acute coronary syndrome, a syndrome that results from coronary artery spasm and produces myocardial infarction (Ali *et al.,* (2010). Other cardiovascular disorders, for example cardiovascular ischemia, thromboembolism, and dilated cardiomyopathy, have also been associated with khat chewing. Additionally increased levels of energy, alertness and self-esteem, enhanced imaginative ability and capacity to associate ideas have been reported by habitual users of *Catha edulis*. Cathinone has a vasoconstrictor activity on isolated perfused guinea-pig heart (Al-Motarreb, 2003). Khat chewing induces a certain state of euphoria and elation with feeling of increased alertness and arousal, which is followed by a stage of vivid discussions, loquacity and an excited mood. Thinking is characterized by a flood of ideas but without the ability to concentrate. However, at the end of khat session the user may experience depressive mood, irritability, anorexia and difficulty of sleeping (Al-Motarreb & Baker, 2002). It is notable that local khat consumers sometimes distinguish between stimulant (*miraa*) and calmant (*hereri)* khat varieties (Balint *et al.*, 1994; summers, 2006). *Catha edulis* produces psychological and physical dependence on long-term use, and chronic use of khat is associated with the development of several systemic and metabolic disorders (Manghi *et al.,* 2009). In young adults, the chronic use of khat is associated with hypertension, as well as stomatitis, esophagitis, gastritis, and constipation (Hassan & Gunaid, 2007).

2.13 Side effects of *Catha edulis*

Chewing *Catha edulis* has shown to affect many organs in the body. *Catha edulis* chewing may have many different side effects since it contains many different compounds. These toxic effects includes general malaise, irritability, impaired sexual potency in men, increased blood pressure, constipation and anorexia (Cox, 2003). Increased blood pressure and heart rate has been reported in khat chewers (Hassan *et al*., 2000). Chronic use of *Catha edulis* may lead to decreased sexual function and impotence *(*Mwenda *et al.,* 2003). Khat chewing affects certain parts of the digestive system and the oral cavity (Hill, 1987). Heymann *et al.,* 1995 reported a delay in gastric emptying after chewing *Catha edulis*. *Catha edulis* prolongs gut motility (Gunaid & El-Khally, 1999). Makonnen Eyassu, 2000 reported that *Catha edulis* produced constipation in mice and antispasmodic action on guinea pig isolated ileum. Nigussie *et al.,* 2013 reported that the prevalence of gastrointestinal disorders was found to be higher among khat chewers. *Catha edulis* use has been associated with metabolic disorders such as diabetes mellitus, and with the development of hepatitis and liver cirrhosis. Biochemically, *Catha edulis* leaves decreased plasma cholesterol, glucose and triglycerides in rabbits (Molham, Al-Habori & Al-Mamary, 2004) and increased plasma alkaline phosphatase and alanine aminotransferase in white rabbits (Molham *et al.,* 2002).

2.14 Catha edulis liver toxicity and renal toxicity

Hepatotoxicity due to *Catha edulis* chewing has been evoked in animals and also human. Toxicological effects of *Catha edulis* leaves have been tested in laboratory animals for a period of six months and the liver biochemical parameters were found to be significantly altered with a profound impact on the alkaline phosphatase (ALP) and alanine aminotransferase (ALT) plasma levels throughout the treatment period (Molham Al-Habori *et al.,* 2002). Some workers have demonstrated that the administration of crude extract of *Catha edulis* to New Zealander white rabbits for three months resulted into hepatocellular jaundice and histopathological abnormalities in the livers of these experimental animals (Molham *et al.,* 2002). Findings have demonstrated that the administration of hydro-ethanol extract of *Catha edulis* into rats resulted in significant increase in the serum level of urea, bilirubin and phosphorus ions, accompanied by significant decreases in serum total protein and albumin levels (Al-Hashem *et al.,* 2011). A study on livers and kidneys of male and female Sprague-dawley rats results showed that in khat-fed rats there was hepatic enlargement, increased serum aspartate aminotransferase (AST), and increased serum alkaline phosphatase (ALP) of male and female SD-rats and decreased serum albumin (A) and increased serum creatinine (Cr) of female as compared to controls (Alsalahi *et al.,* 2012). Some studies have reported an adverse effect of *Catha edulis* on the liver and kidney of humans (Shabbir & Ahmad, 2014). *Catha edulis* induces a fall in average and maximum urine flow rate in healthy men (Hassan *et al.,* 2002). Another report also described a patient with impaired liver function attributed to *Catha edulis* chewing. Serum alkaline phosphatase was increased, there was unusual liver directed autoimmune activity with positive anti smooth muscle antibody titre (1: 40) and liver biopsy findings showed cirrhosis. (Patanwala, Burt & Bassendine, 2011). More recently, severe acute liver injury has been attributed to *Catha edulis* chewing in the United States of America (USA) (Vedula *et al.,* 2010). A case series described jaundice and deranged liver function based on biopsy histology and serum biochemistry in seven United Kingdom (UK) men of Somali origin who were regular *Catha edulis* chewers. All denied any alcohol consumption and no other aetiological factors could be identified (Peevers *et al.,* 2010). Goats fed on *Catha edulis* at the University of Eldoret in Uasin Gishu County showed the plant interferes with normal body functions, which could lead to serious liver and kidney disorders (Ng'wena *et al.,* 2012).

2.15 Therapeutic use of *Catha edulis*

Catha edulis leaves and roots are boiled and the boiled water is drunk so that to treat gonorrhea. To treat influenza, cough and asthma fresh stems are chewed and the juice is swallowed (Al-Hebshi, 2005; Balint & Falkay, 2009). Endogenous people of East Africa and the Meru people of Kenya chew *Catha edulis* leaves and the juice is swallowed to treat malaria and also reduce pain associated with arthritis (Al-Hebshi, 2005; Balint, Falkay, 2009; Graeme, 2010). People in Yemen use *Catha edulis* for treatment of obesity, suppression of appetite and to alleviate their headaches by inhaling the fumes of burning khat leaves (Christian, 1998). In Ethiopia processed (by boiling) *Catha edulis* leaves and roots are used to treat influenza, cough, gonorrhea, asthma and other chest problems whereby people with these infections drink the boiled water.. The roots are used for stomachache and infusions made from them are taken to treat boils (Lemessa, 2001).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Introduction

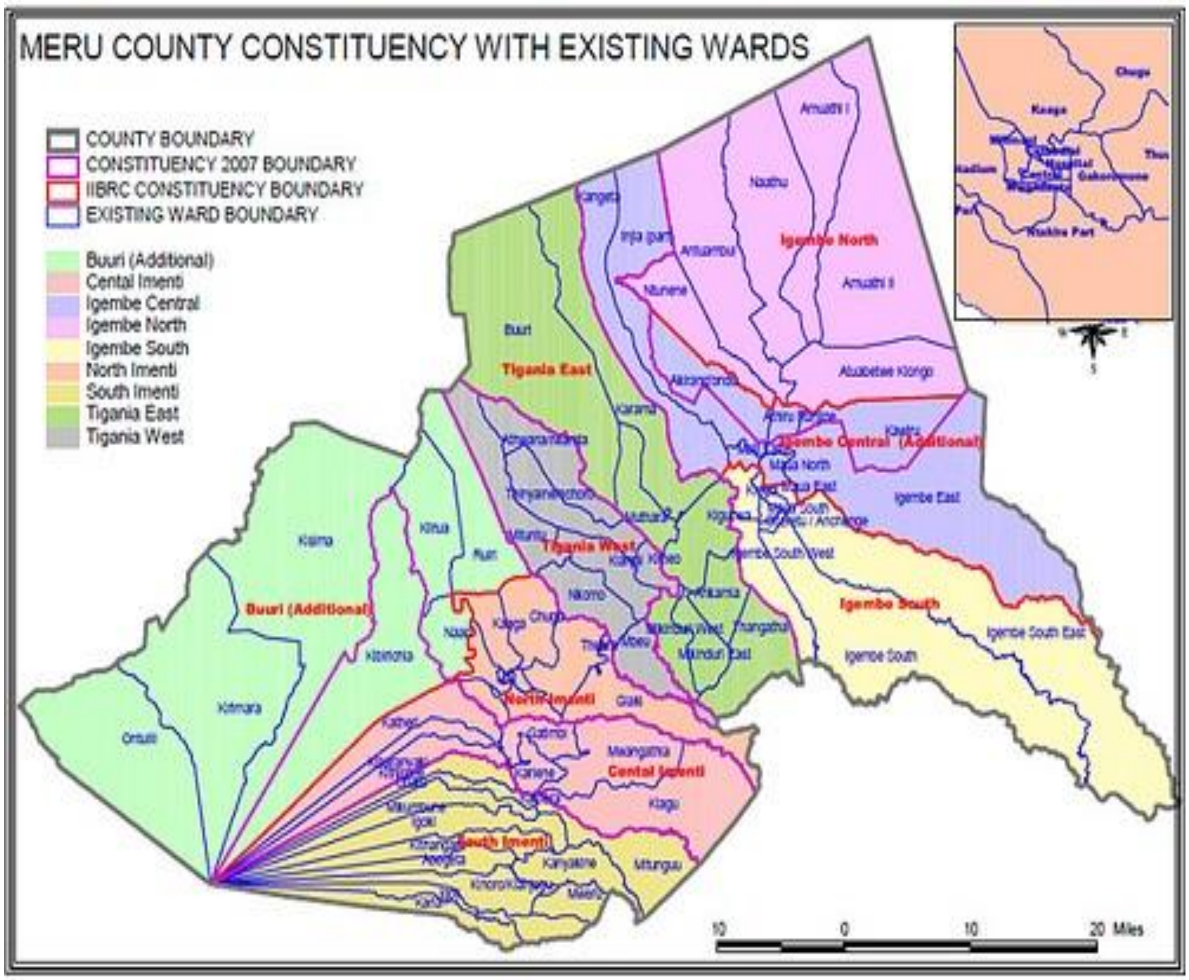
This chapter provides the methodology of the study. It presents a description of research design, location of the study, and population of the study, sample size and sampling procedures, instruments, data collection and data analysis procedures. Ethical issues regarding the study are highlighted.

3.2 Study area

The study was carried out in Meru County, (Figure 3.1). Meru County is found in Eastern region of Kenya, approximately 225 km northeast of the capital, Nairobi. It covers a geographical area of 6,936 sq.kms with a population of 1,365,301 according to 2009 population census of Kenya (KNBS, 2015). Agriculture is the main economic activity in Meru County with a wide scale growing of *Catha edulis* (miraa), a lucrative cash crop for the locals harvested throughout the whole year. *Catha edulis* is mostly grown in Maua, Igembe and Tigania and fetches millions of shillings in the export market for its farmers (Nyongesa & Onyango, 2010).

The study area consisted of four constituencies of Meru County namely, Igembe North and Central**,** Central Imenti and North Imenti. However Igembe North and Central constituencies are the largest *Catha edulis* growing and consumption regions in Kenya**.** This is because of the area's rich loamy soil which is particularly good for cultivation of *Catha edulis* (miraa). Central Imenti and North Imenti are *Catha edulis* consumption areas due to easy access of the *Catha edulis* from the major production areas. Also in these areas *Catha edulis* is produced but in low quantity. Central Imenti constituency areas included: Gatimbi, Kariene, Katheri, Kiagu, Kibaranyaki, Kibirichia and Mwangathia. North imenti constituency areas included; Kaaga, Mwendantu, Gakoromone, Stadium, Ntakira, Ruiri, Thuura, Kiirua and Chugu. Igembe North constituency areas included:

Akirang'ondu, Antuabetwekiongo, Antuambui, Athiru, Runjine, Kabachi, Naathu and Ntunenee. Igembe south constituency areas included: Kangeta, Athi, Maua and Kiengululuma.



Source: Kenya National Bureau of Statistics (2013)

Figure 3.1: Meru County map

3.3: Study design

This was a community-based cross-sectional study to determine the effects of *Catha edulis* on kidney and liver function among chewing adults in four constituencies in Meru

County, between 1st December 2014 and 31st December 2015. Consecutive sampling was used to randomly recruit all accessible and consenting *Catha edulis* chewers and non-chewers. The study employed a mixed research methodology that combined elements of qualitative and quantitative aspects. Mixed methods enable the researcher to converge quantitative and qualitative data to provide a comprehensive analysis of the research problem. Studies that combine elements of the two approaches have been proven to be more comprehensive than those that employ one type of methodology (Creswell, 2003). Adopting a mixed research method was informed by the nature of the research problem under investigation, which required the researcher to collect and analyse data, and make inferences. To evaluate the effects of *Catha edulis* on kidney and liver called for a detailed analysis of the biochemical parameters using quantitative approach. In addition, to be able to describe the participants *Catha edulis* chewing habit and alcohol drinking habit qualitative approach was used.

This study employed a cross-sectional descriptive design to collect data at one point in time from 391 participants, where the effect of *Catha edulis* chewing on the liver and kidney biochemical parameters were evaluated. The design is faster and inexpensive compared to case and cohort studies. It provides self-reported facts about respondents, their feelings, attitudes, opinions and habits (Kombo, Kothari., & Tromp, 2008). It is an excellent vehicle for collecting original data for purposes of studying large populations. With this design, a large population can be studied with only a portion of it being used to provide the required data (Kothari, 2008).

3.4 Assumption of the Study

The respondents provided truthful and honest responses to the items in the questionnaire.

3.5 Study variables

Dependent variables were liver and kidney while independent variables were biochemical parameter changes, variety of *Catha edulis*, gender and age.

3.6 Target population.

The target population in this study constituted all Individuals aged between 18 and 60 years who chewed *Catha edulis and* also non-chewers in Meru County were targeted in the study.

3.7 Inclusion and Exclusion Criteria

3.7.1 Inclusion criteria

The following inclusion criteria were utilized in identifying study participants:

Respondents between 18-60 years of age. This is the age which the liver and kidney bio-chemical parameters like ALP are not affected by age.

Catha edulis chewers and non-chewers who were willing to participate in the study

Both chewers and non-chewers were from the same environment.

For both chewers and non-chewers recruitment a screening checklist was used to help determine the appropriate candidate for the study. The non-chewers were recruited alongside the chewers.

3.7.2 Exclusion criteria

The following exclusion criteria were utilized by use of a checklist whereby respondents answered the questions in the checklist.

Respondents below 18 years and above 60 years of age were excluded from the study. Respondents below 18 years were excluded because of growth spurts in adolescents which may lead to elevated results of ALP. Age above 60 years was excluded because abnormal liver tests occur commonly in elderly people.

Respondents 18 years and above whom did not give informed consent to participate.

Persons with diabetes, liver problems, cardiovascular diseases, renal problem, hepatitis, hypertension, glomerulonephritis and HIV positive because they are risk factor for renal and liver disease.

Pregnant women because of placental ALP elevation.

People under medication were excluded from the study including herbal drugs. This is because drug induced liver injury can occur following the use of prescription and the counter medications, herbal preparations and recreational drugs.

3.8 Sample Size Determination

The sample size (n) was determined using Daniel’s formula (Daniel, 1999).

$$\mathbf{n = \frac{Z^2\, P\, (1-P)}{d^2}}$$

Where, n is required sample size

Z is the standard normal distribution value corresponding to the 95% confidence level;

z = 1.96.

P is the expected prevalence or proportion who chew *Catha edulis*.

d is the precision (acceptable degree of error, d = 0.05).

The proportion of *Catha edulis* chewers among Meru county people was estimated to be 50% because the prevalence is not known. Thus, the sample size was calculated by using the prevalence rate of 50%, 95% level of significance and marginal error of 5%.

$$\mathbf{n = \frac{1.96^2 \times 0.5(1-0.5)}{0.05^2}}$$

$$\mathbf{= 384}$$

The calculated sample size was 384 but to increase the precision and representativeness the sample size was increased to 391.

3.8 Sampling method

The method of this study was cross-sectional community based whereby purposive sampling was used to select four out of nine constituencies in the Meru County, namely Igembe North, Igembe Central, central Imenti and North Imenti. Consecutive sampling technique was used to randomly recruit all accessible and consenting *Catha edulis* chewers and non-chewers from within these four constituencies. Also "snowball sampling technique" (getting *Catha edulis* chewers to refer those they know, these individuals in turn refer those they know and so on) which greatly hastened participants' recruitment, was utilized.

Snowball technique has been applied elsewhere in acquisition of information from hidden populations which are hardly inaccessible by researchers (Gakuubi, 2012; Salganik & Heckathorn, 2004). Purposeful sampling technique ensured that only key respondents with the desired qualities on *Catha edulis* were selected (Russell, 2002). A probability random sampling technique was not applied in this study as not everyone sampled randomly would be *Catha edulis* chewer. Stratified sampling of male and female was not done in this study because male *Catha edulis* chewing is culturally accepted and female *Catha edulis* chewing is culturally restricted. One hundred and ninety eight chewers and one hundred and ninety three non-*c*hewers were recruited. One hundred and ninety three non-*c*hewers persons were recruited alongside study participants.

3.10 Data Collection Instruments and Procedure

3.10.1 Study Instruments

Participants were interviewed face to face and questionnaire was completed. The questionnaire covered a range of socio-demographic characteristics and data on *Catha edulis* chewing. Data was collected from December 2014 to December 2015. Questionnaires were selected on the strength that they are more commonly used in quantitative research as their standardized, highly structured design was compatible with that design. Questionnaires are appropriate for such studies since they collect information that is not directly observable as they inquire about feelings, motivation, attitudes and experiences. The interview method was used because of its high response rate and allowed the researcher to clarify to the respondents what was not clear.

3.11 Sample collection

Participants were interviewed and questionnaire completed to cover the personal history of *Catha edulis* chewing (amount of *Catha edulis* per day, number of days per week and number of years). Five milliliters of blood from each participant was collected from the arm by venipuncture using an evacuated tube collection system. The collected blood (5ml) was put in plain vacutainer tube without anti-coagulant and allowed to clot. The clot samples were centrifuged immediately for 10 minutes at 3000 rpm. After centrifugation, the serum was separated and transferred to clean vials using a sterile pipette. The vials containing serum were transported to Biochemistry laboratory, Nyeri provincial hospital to analyze biochemical parameters.

3.12 Biochemical Analysis

Liver biochemical parameters analyzed included total bilirubin (TB), direct bilirubin (DB), alkaline phosphatase (ALP), albumin (A), alanine aminotransferase (ALT) and aspartate aminotransferase (AST). Kidney biochemical parameters analyzed included urea (Ur), and creatinine (Cr), sodium (Na^+), potassium (K^+) and chloride (Cl^-).

Serum was analyzed using standard methods as follows:

Alanine Aminotransferase (ALT)

Method

UV-assay according to International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) without pyridoxal phosphate activation

Reaction Principle

$$\alpha\text{-oxoglutarate} + \text{L-alanine} \overset{ALT}{\rightleftharpoons} \text{L-glutamate} + \text{pyruvate}$$

LDH

$$\text{Pyruvate} + \text{NADH} + \text{H}^+ \rightleftharpoons \text{L-lactate} + \text{NAD}^+$$

Alanine aminotransferase catalyzes the reversible transamination of L-alanine and α-oxoglutarate to pyruvate and L-glutamate. The pyruvate is then reduced to lactate in the presence of lactate dehydrogenase (LDH) with the concurrent oxidation of reduced β-nicotinamide adenine dinucleotide (NADH) to β-nicotinamide adenine dinucleotide (NAD). This change in absorbance is directly proportional to the activity of ALT in the sample.

Aspartate Aminotransferase (AST)

Method

UV-assay according to International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) without pyridoxal phosphate activation

Reaction Principle

$$\text{L-aspartate} + \alpha\text{-oxoglutarate} \overset{\text{AST}}{\rightleftharpoons} \text{oxaloacetate} + \text{L-glutamate}$$

$$\text{Oxaloacetate} + \text{NADH} + \text{H}^+ \overset{\text{MDH}}{\rightleftharpoons} \text{L-malate} + \text{NAD}^+$$

In the assay reaction, the AST catalyzes the reversible transamination of L-aspartate and α-oxoglutarate to oxaloacetate and L-glutamate. The oxaloacetate is then reduced to malate in the presence of malate dehydrogenase with NADH being oxidized to NAD^+. The rate of the photometrically determined NADH decrease is directly proportional to the rate of formation of oxaloacetate and thus the AST activity.

Alkaline Phosphatase (ALP)

Method

International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) modified method

Reaction Principle

$$p\text{-Nitrophenyl phosphate} + H_2O \underset{Mg^{2+}}{\overset{ALP}{\rightleftharpoons}} p\text{-Nitrophenol} + Pi$$

By the action of ALP and magnesium ions, *p*-Nitrophenyl phosphate is catalysed to p-Nitrophenol, and the absorbency increase is directly proportional to the activity of ALP.

Albumin (ALB)

Method

Bromcresol green（BCG）method

Reaction Principle

$$\text{BCG} + \text{Albumin} \underset{p=4.2}{\overset{\text{Nonionic surfactant}}{\rightleftharpoons}} \text{Glaucous complex}$$

At a slightly acid pH (pH=4.2), serum albumin combines with bromcresol green to produce a glaucous complex. The absorbency increase is directly proportional to the concentration of albumin.

Total Protein (TP)

Method

Biuret method

Reaction Principle

$$Cu^{2+} + \text{Protein} \overset{pH}{\rightleftharpoons} \text{Copper-protein complex (blue-violet colour)}$$

At an alkaline solution (pH>12) copper ions combine with protein to produce a blue-violet colour complex. The absorbency increase is directly proportional to the concentration of protein.

Total bilirubin (TB)

Method

Diazotized Sulfanilic Acid (DSA)

Reaction Principle

$$\text{Bilirubin + Diazo salt} \xrightleftharpoons{H^{+}\text{, Surfactant}} \text{Azobilirubin}$$

The reaction uses a special surfactant to accelerate the solubility of conglutinated bilirubin, total bilirubin with diazo salt at an acid condition to form a red product of azobilirubin. The absorbency increase is directly proportional to the concentration of bilibrubin.

Creatinine (Cr)

Method

Sarcosine Oxidase Method

Reaction Principle

$$\text{Creatinine} + H_2O \xrightleftharpoons{\text{Creatininas}} \text{Creatine}$$

$$\text{Creatine} + H_2O \xrightleftharpoons{\text{CRTase}} \text{Sarcosine}$$

$$\text{Sarcosine} + O_2 + H_2O \xrightleftharpoons{\text{Sarcosine}} \text{Glycin + HCHO} + H_2O$$

$$2H_2O_2 + \text{4-aminoantipyrine + ESPMT} \xrightleftharpoons{\text{Catalase}} \text{Quinonimine} + 4H_2O$$

The absorbency increase at 546 nm of the product Quinonimine is directly proportional to the concentration of creatinine.

Blood Urea Nitrogen (BUN)

Method

Urease-glutamate Dehydrogenase, UV method

Reaction Principle

$$\text{Urea} + 2H_2O \xrightleftharpoons{\text{Urease}} 2NH4^{+} + CO_3^{2-}$$

$$\alpha\text{-Oxoglutarate} + NH4^{+} + NADH \xrightleftharpoons{\text{GLDH}} \text{L-Glutamate} + NAD^{+} + H_2O$$

Urea is hydrolyzed by urease, and one of the products, ammonia, helps to turn NADH to NAD^{+} with the catalysis of GLDH. The absorbency decrease is directly proportional to the concentration of urea.

Liver and renal parameters estimation was done using automated chemistry analyzer (Human Star 200, Human Diagnostic Worldwide, GmbH, Germany) and i-smart 30 electrolyte analyzer for electrolytes.

Serum remaining after testing, used needles and syringes and vacutainers were destroyed through incineration.

3.14 Quality control and calibration of the analytical work

Internal quality control (IQC) was undertaken by the inclusion of particular reference materials (control materials) into the analytical sequence and by duplicate analysis. Three levels of commercial control serum were run for each test series, namely pathological low patient range, pathological high patient range and normal patient range control. The control material was the representative of the test materials under consideration in respect of matrix composition, the state of physical preparation and the concentration range of the analyte. The control materials were analyzed alongside the test material and were treated in exactly the same way as the test material. The interpretation of the test data was based on documented, objective criteria and on statistical principles. Compliance with statistical control was monitored graphically with Levey-Jennings charts. The quality control materials used were traceable to a national/international certified reference material. The control samples were measured every six hours. An active calibration curve was used for all tests. Calibration was done every twenty eight days or whenever new lot numbers of reagents were placed into use.

3.15 Data categorization

Data was grouped according to age (ages 18-30 years, 31-40 years, and 41-60 years) and gender.

3.16 Data analysis

The acquired data was both quantitative and qualitative. Data was analyzed using descriptive and inferential statistics. Descriptive statistics was used to summarize variables into thematic areas and to convey the characteristics of the key variables. Inferential statistics was used to in drawing conclusions. Statistical Package for Social Sciences (SPSS) version 21 was employed in the entry and analysis of data. According to Borg (1996) SPSS is the commonly used set of computer programme in social science research. Before processing the responses, the completed questionnaires were edited for completeness and consistency while the incomplete ones were entirely eliminated. The data was then coded to enable the responses to be grouped into various categories. Results were expressed as mean ± SD values, as number and percentage values for categorical data. Student independent sample t-test was used to compare the different variables between the *Catha edulis* chewers and non-chewers. Mugenda and Mugenda, (1999) notes that t-test is an ideal statistical tool when comparing the means of two groups. Comparison between red variety chewers, pale green variety chewers and non-chewers was done using the one way ANOVA test, followed by the post-hoc Tukey test. Comparison between age groups was done using the one way ANOVA test, followed by the post-hoc Tukey test. Cross tabulation was done to determine the proportions of the characteristics of the independent variable. The significance level was set at $\alpha=0.05$.

3.17 Ethical Considerations

Ethical approval was sought from Kenyatta National Hospital/University of Nairobi – Ethical Review committee (KNH/UoN-ERC) through the ministry of health. Details of the participants of the study were confidential and anonymous in accordance with 2004 guidelines for ethical conduct of biomedical research involving human subjects in Kenya. Informed consent was sought from all the study participants, which was culminated in signing of consent form. Prior to commencement of the study, permission for informants' participation was first sought from the local administrative offices of Meru county of Kenya

CHAPTER FOUR

RESULTS

4.1 Introduction

This section presents the findings from the study, followed by discussion of the findings based on the objectives and hypotheses. Participant's demographic information is presented first followed by other findings. The data qualified for parametric analysis whereby Student's independent *t*-test and analysis of variance (ANOVA) were used because the data are on a quantitative scale, with a normal distribution of the underlying population.

4.2 Demographic characteristics of the study participants

A cross tabulation as shown in Table 4.1 was done to determine the proportions of the characteristics of the independent variable. A total of 391 participants were interviewed and questionnaires were completed and blood sample drawn from them making a response rate of 101.8%. Among the study participants 50.6% were *Catha edulis* chewers and 49.4% were non-chewers. 39.4% are in age group 18-30 years, 33.2% are in age group 31-40 years and 27.4% are in age group 41-60 years. Among the chewers participants 97% were males and 3% were female. Majority of the chewers (97%) were males, 47% were in age group 18-30 years. Among this age group 43.7% chewed the red type and 56.3% chewed the pale green type of *Catha edulis*. Majority (56%) of chewers who chewed the red variety of *Catha edulis* are in age group 31-40, followed by chewers in age group 18-30 (55.6%) who chewed the pale green variety of *Catha edulis*.

Table 4.1 Demographic characteristics of the study participants

Variable	population		Chewers (n=198) Total		Red type		Pale green type		Non-chewers (n=193) Total	
	n	(%)	n	(%	n	(%	n	(%)	n	(**%)**
Gender										
Male	379	(96.9)	192	(**97**)	103	(53.6)	89	(46.4)	187	(**96.9**)
Female	12	(3.1)	6	(**3**)	5	(41.7)	1	(8.3)	6	(**3.1**)
Age group										
18-30	154	(39.4)	96	(**47**)	42	(43.7)	54	(56.3)	62	(**32**)
31-40	130	(33.2)	62	(**31**)	35	(56)	27	(44)	68	(**35**)
41-60	107	(27.4)	44	(**22**)	31	(29.0)	13	(30)	63	(**33**)

N=sample size, %= percentage

4.3 Distribution of the study participants (chewers of both red and pale green varieties, red variety chewers and pale green variety chewers) by *Catha edulis* chewing habit

Majority 138 (69.7%),of chewers chewed *Catha edulis* for more than three days in a week. For the red type majority (60.1%) chewed *Catha edulis* for more than three days in a week and for the pale green type majority (58.3%) chewed for less than three days in a week. In regard to bundles, majority (46.97%) of the chewers chewed one bundle per day. For the red type of *C. edulis* majority (69.6%) of the chewers chewed two bundles per day and for the pale green type majority (58.1%) chewed one bundle per day. Majority (33.3%) of the chewers have chewed *Catha edulis* for more than ten years and for the red type majority (78.9%) of the chewers have chewed *Catha edulis* for less than one year and for the pale green type majority (59.2%) have chewed for five to ten years. *Catha edulis* chewing habit is more frequent among red variety *Catha edulis* chewers group than pale green variety chewers group (54.5% vs 45.5%). *Catha edulis* chewing habits of the chewers are shown in Table 4.2.

Table 4.2 Distribution of the chewers by *Catha edulis* chewing habit

***Catha edulis* Chewing habits**	**Participants by varieties of *C. edulis***					
	Red		**Pale green**		**Total**	
	No	**%**	**No**	**%**	**No**	**%**
Bundles of *Catha edulis* chewed per day						
1	39	41.9	54	58.1	93	46.97
2	48	69.6	21	30.4	69	34.85
3	19	57.6	14	42.4	33	16.67
>5	2	66.7	1	33.3	3	1.51
Frequency of *Catha edulis* chewing						
>3 days per week	83	60.1	55	39.9	138	69.70
<3 days per week	25	41.7	35	58.3	60	30.30
Years of *Catha edulis* chewing						
< 1 year	15	78.9	4	21.1	19	9.60
1-2 years	24	77.4	7	22.6	31	15.70
3-5 years	19	57.6	14	42.4	33	16.70
5-10 years	20	40.8	29	59.2	49	24.70
>10 years	30	45.5	36	54.5	66	33.30

N=sample size, %= percentage

4.4 Participants *Catha edulis* chewing habit in relation to age.

Majority 92 (46.5%) of the chewers were in age group 18-30 years. In terms of bundles chewed per week, majority 92(46.5%) of the participant chewed one bundle per week. With reference to frequency majority 140(70.7%) chewed *Catha edulis* for more than three days in a week and age group 18-30 years had the majority 63 (31.8%) of the participants chewing *Catha edulis* for more than three days in a week. Referring to the duration majority 67 (33.8%) of the participants have chewed for more than ten years and age group 31- 40 years had the majority 29 (14.6%) of the participants who have chewed

Catha edulis for more than ten years as in Table 4.3. Figure 4.1 shows distribution of the study participants by age and variety of *Catha edulis* chewed.

Table 4.3 Participants *Catha edulis* chewing habit in relation to age (n = 198)
N=sample size, %= percentage

		Age group of participants n & % count			
No. of bundles of *C. edulis* chewed per day		**18-30 years**	**31- 40 years**	**41 years & above**	**Total**
	1	45(22.7%)	31(15.7%)	16(8.1%)	92(46.5%)
	2	32(16.2%)	20(10.1%)	18(9.1%)	70(35.4%)
	3	14(7.1%)	9(4.5%)	10(5.1%)	33(16.6%)
	>5	1(0.5%)	1(0.5%)	1(0.5%)	3(1.5%)
Total		92(46.5%)	61(30.8)	45(22.7%)	198(100%)
Frequency of *C. edulis* chewing	>3days	63(31.8%)	42(21.2%)	35(17.7%)	140(70.7%)
	< 3 days	29(14.6%)	19(9.6%)	10(5.1%)	58(29.3)
Duration of *C. edulis* chewing	< 1 year	6(3.0%)	8(4.0%)	5(2.5%)	19(9.6%)
	1-2 years	16(8.1%)	6(3.0%)	7(3.5%)	29(14.6%)
	3-5 years	21(10.6%)	6(3.0%)	6(3.0%)	33(16.7%)
	5-10years	30(15.2%)	12(6.1%)	8(4.0%)	50(25.3%)
	>10 years	19(9.6%)	29(14.6%)	19(9.6%)	67(33.3%)

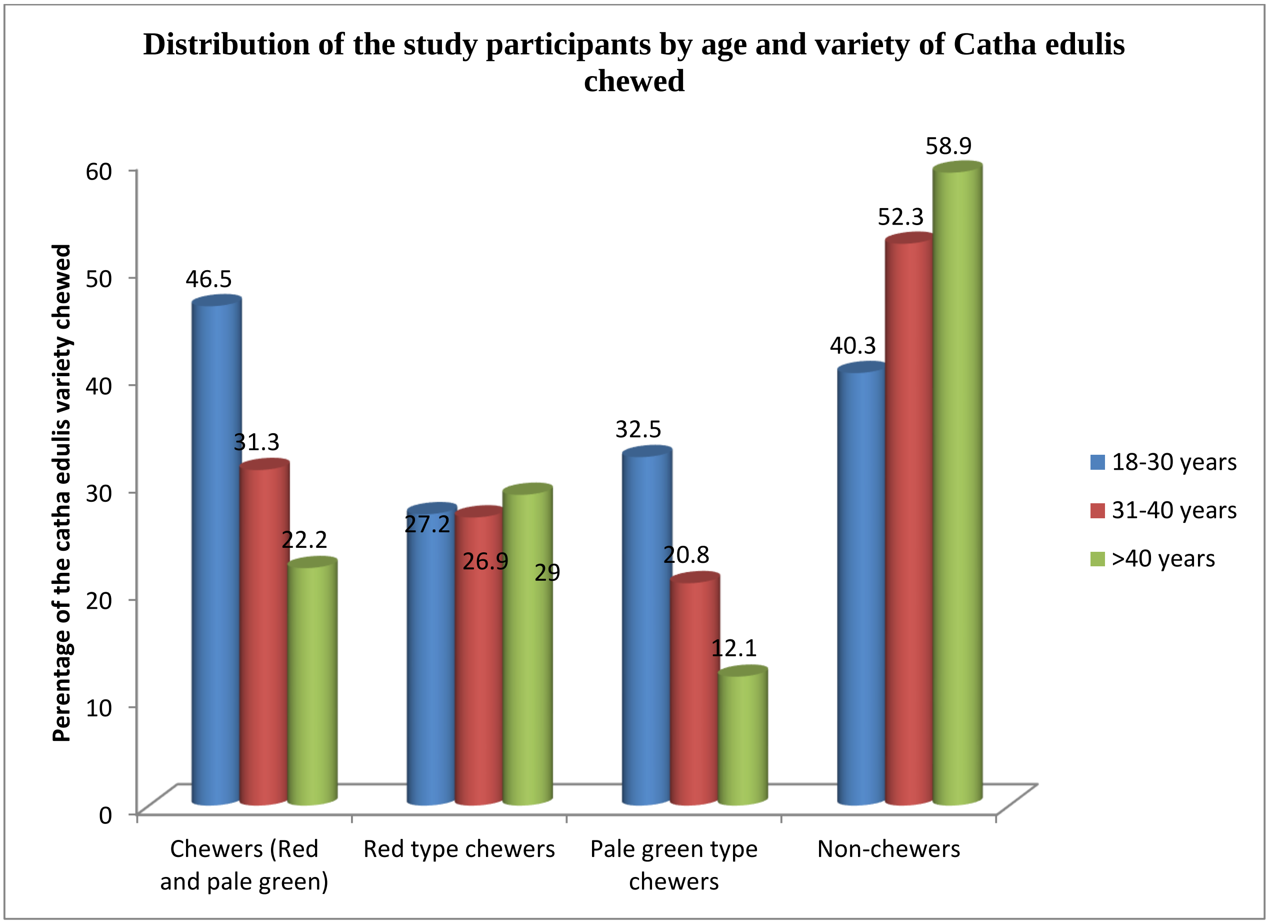


Figure 4.1: Distribution of the study participants by age and variety of *Catha edulis* chewed

4.5 Distribution of the *Catha edulis* participants by alcohol drinking habit

Eighty seven (43.9%) of the participants reported consuming alcohol and 111 (56.1%) not consuming alcohol. 39.1% of the participants who chewed the red type of *Catha edulis* reported consuming alcohol and 66.7% not consuming and for the participants who chewed the pale green type, 60.9% reported consuming and 33.3% not consuming. Majority 59 (67.8%) who consumed alcohol, consumed less than 5 bottles per day. With reference to the red type of *Catha edulis* majority (45.0%) of the participants consumed five to ten bottles per day and for the pale green type majority (75.0%) consumed less than five bottles per day. Bottled alcohol (58.6%) was the most consumed with traditional brew being the most consumed (58.8%) for the participants who chewed the red type of *Catha edulis* and bottled alcohol was the most consumed (56.4%) for the participants who chewed the pale green type of *Catha edulis*. Majority (42.5%) of the participants who consumed alcohol have consumed it for less than five years. Majority (59.5%) of the participants who chewed the red type of *Catha edulis* have consumed alcohol for less than five years while majority (78.3%) of the participants who chewed the pale green type have consumed alcohol for more than ten years as in Table 4.4.

Table 4.4 Alcohol drinking habit of *Catha edulis* chewing participants

Drinking habits	**Participants by *C.edulis* varieties**					
	Red		**pale green**		**Total**	
	No	**%**	**No**	**%**	**No**	**%**
Drink alcohol						
Yes	34	39.1	53	60.9	87	43.9
No	74	66.7	37	33.3	111	56.1
Alcohol type						
Bottled	27	52.9	24	56.4	51	58.6
Traditional	10	58.8	7	41.2	17	19.5
Both bottled and traditional	9	42.8	10	47.6	19	24.1
Don't drink alcohol but chew	74	66.1	37	33.9	111	56.1
Quantity of alcohol drunk/ day						
<5 bottles	24	40.7	35	59.3	59	67.8
5-10 bottles	9	45.0	11	55.0	20	23.0
>10 bottles	2	25.0	6	75.0	8	9.2
Duration of alcohol consumption in years						
<5 years	22	59.5	15	40.5	37	42.5
5-10 years	10	37.0	17	63.0	27	31.03
>10 years	5	21.7	18	78.3	23	26.4

4.6 Changes in Liver and kidney biochemical parameters in chewers and non-chewers

To test the hypothesis that the *Catha edulis* chewing has no effect on the biochemical parameters of the kidney and liver, an independent t-test was performed. The mean of direct bilirubin, creatinine and total protein was statistically significantly ($p<0.05$) decreased in *Catha edulis* chewers as compared to non-chewers. The mean of alkaline phosphatase was statistically significantly ($p<0.05$) increased in *Catha edulis* chewers as compared to non-chewers as in Table 4.5

Table 4.5 Changes in Liver and kidney biochemical parameters in chewers and non- chewers

	Normal range	Chewers		Non-chewers		
Variable		**Mean**	**SD**	**Mean**	**SD**	**P**
Liver biochemical parameters						
TB (μmo/l)	2-21	10.03	5.64	11.97	8.89	.011
DB (μmo/l)	0-3.42	2.67	2.78	2.89	1.91	.355
TP (g/l)	66-88	69.12	13.76	70.73	20.62	.012
ALB (g/l)	35-52	51.05	11.69	49.78	11.43	.277
ALP (U/l)	80-306	237.71	199.72	197.65	102.19	.001
ALA (U/l)	0-42	24.64	44.92	23.37	13.87	.705
ASA (U/l)	0-37	26.44	18.15	27.99	13.56	.339
Renal biochemical parameters						
Ur (mmol/l)	1.7-8.3	4.17	1.33	4.47	2.31	.116
Cr (μmo/l)	53-97	83.07	28.94	94.69	25.17	.000
Na^+(mmo/l)	135-155	139.69	130.35	141.15	5.00	.144
K^+(mmol/l)	3.5-5.5	4.25	.48	4.31	1.28	.548
Cl^-(mmol/l)	97-111	103.07	8.00	103.34	3.11	.660

$p<0.05$ TB-total bilirubin, DB-direct bilirubin, TP-total protein, ALB-albumin, ALP-alkaline phosphatase, ALA-alanine aminotransferase, ASA-aspartate aminotransfer-ase,cret-creatinine,Na^+-sodium,K^+-potassium,Cl^-chloride

4.7 ANOVA for significant difference among chewers age groups with respect to kidney and liver biochemical parameter levels

As shown in Table 4.6 the serum levels of liver and kidney biochemical parameters were not significantly (ANOVA) different between age groups (p>0.05).

Table 4.6 ANOVA for significant difference among chewers age groups with respect to kidney and liver biochemical parameter levels

Variable		Normal range	M	SD	P
Liver biochemical parameters	**Age group in years**				
TB (µmo/l)	18-30	2-21	11.03	7.61	.618
	31-40		11.39	7.84	
	41-60		10.44	6.86	
DB (µmo/l)	18-30	0-3.42	2.70	1.49	.788
	31-40		2.96	3.20	
	41-60		3.03	2.18	
TP (g/l)	18-30	66-88	71.13	20.72	.963
	31-40		70.77	19.40	
	41-60		71.42	12.66	
ALB (g/l)	18-30	35-52	49.16	12.45	.141
	31-40		50.61	12.20	
	41-60		52.02	9.06	
ALP (U/l)	18-30	80-306	209.86	192.99	.914
	31-40		215.43	112.45	
	41-60		217.03	96.41	
ALA (U/l)	18-30	0-42	25.68	20.00	.753
	31-40		27.95	16.74	
	41-60		26.40	16.30	
ASA (U/l)	18-30	0-37	25.50	18.53	.239
	31-40		28.25	15.09	
	41-60		28.39	12.95	
Renal biochemical parameters					
Ur(mmol/l)	18-30	1.7-8.3	4.19	1.40	.447
	31-40		4.29	1.28	
	41-60		3.96	1.27	
Cr (µmo/l)	18-30	53-97	87.23	33.46	.429
	31-40		81.83	28.06	
	41-60		94.50	25.93	
Na^+(mmo/l)	18-30	135-155	140.29	9.919	.830
	31-40		140.25	12.92	
	41-60		140.91	4.185	
K^+(mmol/l)		3.5-5.5	4.39	0.57	.558
			4.46	0.59	
			4.45	0.55	
Cl^-(mmol/l)	18-30	97-111	103.70	3.70	.326
	31-40		101.79	13.26	
	41-60		103.51	8.00	

4.8 Mean ± standard deviation and test of significance of mean values of male and female *Catha edulis* chewers

There is no statistically significant difference ($p>0.05$) in kidney and liver biochemical parameters mean between male and female *Catha edulis* chewers as seen in Table 4.7

Table 4.7 Mean ± standard deviation and test of significance of mean values of male and female *Catha edulis* chewers

	Normal range	Male chewers		Female chewers		
Variable		**Mean**	**SD**	**Mean**	**SD**	**P**
Liver biochemical parameters						
TB (μmo/l)	2-21	10.11	5.70	7.50	2.24	0.266
DB (μmo/l)	0-3.42	2.69	2.82	2.00	0.69	0.552
TP (g/l)	66-88	73.54	19.00	68.87	23.31	0.557
ALB (g/l)	35-52	51.32	11.52	42.35	14.84	0.640
ALP (U/l)	80-306	188.14	93.01	159.33	64.31	0.453
ALA (U/l)	0-42	24.69	45.60	23.00	8.98	0.928
ASA (U/l)	0-37	26.63	18.28	20.33	12.70	0.404
Renal biochemical parameters						
Ur (mmol/l)	1.7-8.3	4.18	1.33	3.81	1.41	0.506
Cr (μmo/l)	53-97	83.29	29.29	75.90	12.22	0.539
Na^+(mmo/l)	135-155	139.79	13.04	136.50	13.64	0.545
K^+(mmol/l)	3.5-5.5	4.26	0.48	3.90	0.34	0.161
Cl^-(mmol/l)	97-111	103.04	8.11	104.40	3.34	0.773

N, sample size; M, mean; SD, standard deviation; TB, total bilirubin; DB, direct bilirubin; ALB, albumin; ALP, alkaline Phosphatase; ALA, alanine aminotransferase; ASA, aspartate aminotransferase; Ur, urea; Cr, creatinine; Na^+, sodium; K^+, potassium; Cl^- ,chloride.

4.9 Mean ± standard deviation and test of significance of mean values of chewers who have chewed *Catha edulis* for more than ten years and those who have chewed for less or equal to ten years

There is no statistically significance difference in all liver and kidney biochemical parameters mean of chewers who have chewed for less or equal to ten years and those who have chewed for more than ten years (p>*0.05*) except for the mean of total protein which was statistically significantly decreased in chewers who have chewed for more than ten years as in Table 4.8.

Table 4.8 Mean ± standard deviation and test of significance of mean values of chewers who have chewed *Catha edulis* for more than ten years and those who have chewed for less or equal to ten years

		***Catha edulis* Chewers**				
	Normal range	**Chewers who have chewed for ≤10 years**		**Chewers who have chewed for >10 years**		
Variable		**Mean**	**SD**	**Mean**	**SD**	**P**
Liver biochemical parameters						
TB (μmo/l)	2-21	9.92	5.60	10.05	5.59	0.879
DB (μmo/l)	0-3.42	2.61	2.69	2.71	2.91	0.822
TP (g/l)	66-88	75.46	21.31	69.11	12.67	0.029
ALB (g/l)	35-52	50.77	11.90	51.49	11.35	0.690
ALP (U/l)	80-306	181.7	83.48	197.0	108.09	0.277
ALA (U/l)	0-42	25.25	53.19	23.23	19.42	0.769
ASA (U/l)	0-37	26.73	18.62	25.56	17.25	0.672
Renal biochemical parameters						
Ur (mmol/l)	1.7-8.3	4.24	1.36	4.05	1.27	0.352
Cr (μmo/l)	53-97	80.66	26.96	87.97	32.52	0.098
Na^+(mmo/l)	135-155	138.83	156.79	1.41	3.38	0.190
K^+(mmol/l)	3.5-5.5	4.24	0.48	4.27	0.47	0.724
Cl^-(mmol/l)	97-111	102.76	95.03	10.37	3.24	0.435

N, sample size; M, mean; SD, standard deviation; TB, total bilirubin; DB, direct bilirubin; ALB, albumin; ALP, alkaline Phosphatase; ALA, alanine aminotransferase; ASA, aspartate aminotransferase; Ur, urea; Cr, creatinine; Na^+, sodium; K^+, potassium; Cl^-,chloride.

4.10 ANOVA results for significance difference between red variety chewers, pale green variety chewers and non-chewers.

A one-way between groups analysis of variance was conducted to compare the effects of red and pale green variety of *Catha edulis* on human consumers. Participants were divided into three groups (red variety chewers, pale green variety chewers and non-chewers).

Serum activity of alanine aminotransferase (ALA), alkaline phosphatase (ALP) and total bilirubin (TB) was statistically significantly ($p<0.05$) different between groups. Post hoc Turkey test indicated the mean of red variety chewers was significantly increased than that of pale green variety chewers.

Serum activity of urea (Ur), and creatinine (Cr) was statistically significantly ($p<0.05$) different between groups. Post hoc Turkey test indicated the mean of red variety chewers was significantly decreased than that of non-chewers.

Serum activity of total protein seemed statistically significantly different between groups ($p<0.05$), however post hoc Turkey test indicated a non-significant different ($p>0.05$).The values of ALP, ALA, TB, Cr and Ur are within the normal reference range (Table 4.9) and (Table 4.10)

Table 4.9 ANOVA results for significance difference between *Catha edulis* red variety chewers, pale green variety chewers and non-chewers.

		Range	M	SD	P
Liver biochemical parameters					
TB (μmo/l)	Red *Catha edulis*	2-21	11.01	6.01	.005
	Pale green *Catha edulis*		8.86	4.96	
	Non chewers		11.97	8.90	
DB (μmo/l)	Red *Catha edulis*	0-3.42	2.74	2.87	.601
	Pale green *Catha edulis*		2.60	2.69	
	Non chewers		2.89	1.91	
TP (g/l)	Red *Catha edulis*	66-88	73.45	15.07	.041
	Pale green Catha edulis		73.36	23.11	
	Non chewers		68.73	17.28	
ALB (g/l)	Red *Catha edulis*	35-52	52.47	9.96	.093
	Pale green *Catha edulis*		49.35	13.34	
	Non chewers		49.78	11.43	
ALP (U/l)	Red *Catha edulis*	80-306	200.88	78.63	.005
	Pale green *Catha edulis*		176.92	110.34	
	Non chewers		169.5	169.5	
ALA (U/l)	Red *Catha edulis*	0-42	29.87	59.22	.050
	Pale green *Catha edulis*		18.37	13.36	
	Non chewers		23.37	13.87	
ASA (U/l)	Red *Catha edulis*	0-37	28.58	19.89	.077
	Pale green *Catha edulis*		23.88	15.54	
	Non chewers		27.99	13.56	
Renal biochemical parameters					
Ur(mmol/l)	Red *Catha edulis*	1.7-8.3	4.04	1.25	.003
	Pale green *Catha edulis*		4.34	1.43	
	Non chewers		4.73	2.03	
Cr (μmo/l)	Red *Catha edulis*	53-97	139.33	16.37	.000
	Pale green *Catha edulis*		140.11	7.33	
	Non chewers		141.15	5.00	
Na^+(mmo/l)	Red *Catha edulis*	135-155	103.28	4.86	.801
	Pale green *Catha edulis*		102.80	10.65	
	Non chewers		103.34	3.11	
K^+(mmol/l)	Red *Catha edulis*	97-111	4.25	.485	.790
	Pale green *Catha edulis*		4.27	.479	
	Non chewers		4.60	.608	
Cl^-(mmol/l)	Red *Catha edulis*				
	Pale green *Catha edulis*				
	Non chewers				

N, sample size; M, mean; SD, standard deviation; TB, total bilirubin; DB, direct bilirubin; ALB, albumin; ALP, alkaline Phosphatase; ALA, alanine aminotransferase; ASA, aspartate aminotransferase; Ur, urea; Cr, creatinine; Na^+, sodium; K^+, potassium; Cl^-,chloride.

Table 4.10: Tukey's Post Hoc Multiple Comparisons red variety chewers, pale green variety chewers and non-chewers in relation to kidney and liver biochemical parameters

Dependent Variable	(I) variety of *Catha edulis*	(J) variety of *Catha edulis*	
TB	Red	Pale green	.043
		Non-chewers	.001
	Pale green	Non-chewers	.107
TP	Red	Pale green	.999
		Non-chewers	.081
	Pale green	Non-chewers	.117
ALP	Red	Pale green	.001
		Non-chewers	.236
	Pale green	Non-chewers	.036
ALA	Red	Pale green	.042
		Non-chewers	.236
	Pale green	Non-chewers	.466
Ur	Red	Non-chewers	.002
	Pale green	Red	.425
		Non-chewers	.168
Cr	Red	Pale green	.631
		Non-chewers	.007
	Pale green	Red	.631
		Non-chewers	.000

N, sample size; M, mean; SD, standard deviation; TB, total bilirubin; DB, direct bilirubin; ALB, albumin; ALP, alkaline Phosphatase; ALA, alanine aminotransferase; ASA, aspartate aminotransferase; Ur, urea; Cr, creatinine; Na^+, sodium; K^+, potassium; Cl^- ,chloride.

4.11 Mean ± standard deviation and test of significance of mean values of chewers who consume alcohol for >5 years and >5bottles/day and chewers who don't consume alcohol

To find out if alcohol was a confounding factor in this study, test of significance of liver biochemical parameters of chewers who don't consume alcohol and chewers who consumed alcohol (consumed more than five bottles per day for more than five years) was done.

There was no statistically significance difference ($p>0.05$) between chewers who don't consume alcohol and those who consumed alcohol (more than five bottles of alcohol per day for more than five years) as shown in Table 4.11.

Table 4.11 mean ± standard deviation and test of significance of mean values of chewers who consume alcohol for >5 years and >5bottles/day and chewers who don't consume alcohol

	Chewers who consume alcohol for >5 years and >5bottles/day			**Chewers who don't consume alcohol**		
Variable	**Normal range**	**M**	**SD**	**M**	**SD**	P
Liver bio-chemical parameters						
TB (μmo/l)	2-21	9.71	5.65	10.31	5.65	.518
DB (μmo/l)	0-3.42	2.43	2.15	2.78	2.93	.311
TP (g/l)	66-88	73.90	20.42	72.93	15.23	.763
ALB (g/l)	35-52	51.38	12.17	51.74	11.56	.860
ALP (U/l)	80-306	187.86	79.90	192.07	192.07	.741
ALA (U/l)	0-42	21.85	16.45	28.56	57.96	.233
ASA (U/l)	0-37	26.04	15.95	27.14	18.20	.563

M, mean; SD, standard deviation; TB, total bilirubin; DB, direct bilirubin; ALB, albumin; ALP, alkaline Phosphatase; ALA, alanine aminotransferase; ASA, aspartate aminotransferase

CHAPTER FIVE

DISCUSSION AND CONCLUSIONS

5.1 Discussion

The aim of this study was to evaluate the effects of *Catha edulis* on kidney and liver using biochemical parameters among miraa chewing adults in Meru County, to compare the effects of red and pale green variety of *Catha edulis* on human consumers and to determine the effect of *Catha edulis* on liver and kidney in relation to age and gender. One hundred and ninety eight (198) *Catha edulis* chewers and one hundred and ninety three (193) *Catha edulis* non-chewers were studied. Majority (97%) of the *Catha edulis* chewers were male. Female *Catha edulis* chewers were at 3%. These findings concurs with previous Kikuvi and Karanja 2013 who found out that 89.1% of chewers were male and 10.9% were female in a study carried out in Embu, Nyeri, Mombasa and Nyeri counties. Hughes (1973); Hijazi (1981) and Ogada *et al,*. (2014) also found out women rarely chewed *Catha edulis*. This might be due to social and cultural influence on females. Female *Catha edulis* chewing is culturally restricted than males (Andualem, 2002). In 1972, the WHO-sponsored Mission to Yemen estimated that approximately 80% of adult men in the major cities and 90% of adult men in the villages of regions in which khat is produced are regular khat chewers. From this study the mean age of chewers was 29 years and that of non-chewers was 28 years. No significant difference was found between chewers and non-chewers in terms of age distribution. This agrees with Jabr, (2013) who found that there was no significant difference between chewers and non-chewers in terms of age distribution.

This study reveals that *Catha edulis* chewing habit in Meru County is common in the younger generation with majority of chewers being in age group 18-30 years. This compares with a study done in Meru by Ogada *et al.,* 2014 where majority of the chewers were in age group 21-30 years and the mean age was 28.73 years. Michuki & Kivuva, (2013) in their study found that age category 18-24 years has the highest lifetime use of

Catha edulis. Ease of availability of *Catha edulis* in this region and approval as compared to other drugs could be the reason as to why the youth are the majority users. A study by Ng'ethe, (2012) in Igembe South district showed that among the factors contributing to khat consumption by the youth include, the ease of availability with majority of the respondents indicating that it is easily available and highest approval rate as compared to other drugs

This study reveals that age did not have a significant effect on the level of kidney and liver biochemical parameters in *Catha edulis* chewers. This agrees with Ramzy *et al.,* 2013 who found out that age has no significant effect on liver enzyme levels.

The study also reveals that there is no statistically significant difference in the mean between male and female *Catha edulis* chewers.

In reference to frequency majority of the participants chewed *Catha edulis* for more than three days in a week. Ease of availability of *Catha edulis* could be the reason for frequent chewing. This is in line with a study done in England where the average frequency of khat use was three days a week (Patel, Alex, & Wright, 2005).

Among chewers there was no difference (in liver and kidney biochemical parameters) between those who chewed for less than ten years compared to those who chewed for more than ten years. This is in agreement with Ramzy *et al.,* 2013 study on effect of chronic khat chewing on liver enzyme levels in Yemen.

In line with a study done in Meru by Ogada *et al.,* 2014 majority of the chewers have chewed *Catha edulis* for more than ten years. This is because the current study was done in *Catha edulis* production and consumption areas. The duration of *Catha edulis* chewing did not have effect on kidney and liver biochemical parameters. This could be due to the majority of the chewers chewed one bundle per day and also majority chewed for less than three days in a week.

43.9% of the participants who chewed *Catha edulis* reported consuming alcohol but there was no significance difference found between alcohol consumption to kidney and liver biochemical parameters. This could be as a result of majority of the participants consuming bottled type of alcohol, one bottle per day for less than five years. This is in agreement with Ogada *et al.,* 2014 who found that half of the population in the study consumed alcohol. Khat chewers drink alcohol to break the stimulating effect of khat after long hours of stimulation by the central inhibitory effect of alcohol (Gelaw & Haile-Amlak, 2004).

Alkaline phosphatase activity was significantly increased in the serum of *Catha edulis* consumers than non-consumers. This indicates leakage of alkaline phosphatase (ALP) enzyme into extracellular fluid. This might be an indication of possible hepatotoxicity due to liver cells being destroyed by *Catha edulis* hence damaged liver fails to excrete ALP made in bones, intestines and liver (Daniel,1999).

Since ALP is not liver specific its increased activity may raise suspicion of other source other than the liver cause. This is in agreement with studies done in animals where ALP was increased in khat extract administered animals (Al-Habori *et al.,* 2002; Al-Hashem *et al.,* 2011). The results are also in agreement with Shabbir *et al.,* 2014 where ALP was significantly increased in male population of Jazan region of Saudi Arabia.

Total protein, direct bilirubin and creatinine concentration were significantly decreased in the serum of *Catha edulis* consumers than non-consumers. Decrease in serum total protein indicate decreased protein synthesis either due to liver cells damage or reduced absorption of amino acids or secondary to diminished protein intake. Decreased protein synthesis either due to liver cells damage may indicate decrease in liver function (Chawla, 1999). This is in agreement with Rania Hussein *et al.*, 2013 who reported a significant decrease in serum total protein among adults in Sana'a city. Also Al-Hashem *et al.*, 2011 who reported decreased total protein in khat extract administered animals. The findings are similar to a study by Rania Hussein *et al.*, 2013 where serum total protein was significantly decreased in khat chewers among adults in Sana'a city Yemen and

Masoud *et al*., 2014 where total protein level was significantly decreased in female khat chewers.

The normal serum activity of aspartate aminotransferase (ASA) and alanine aminotransferase (ALT) could be a good indicator of normal hepatic function because ALT is a more specific marker of hepatocytes integrity, and aspartate aminotransferase is distributed in various organs but it is more concentrated in the hepatocytes (Ozer *et al.,* 2008). This contrasts to Shabbir *et al.,* 2014 where ALT and AST was significantly increased in khat users as compared to non-users. The difference in ALT and AST levels in this study and Shabbir *et al.,* 2014 would be due to shabbir *et al* study was hospital based whereby patients with symptoms of liver problem and had history of *Catha edulis* chewing were recruited for the study. This could have led to increased ALT and AST in shabbir *et al* study whereas in this was a community based study whereby healthy participants without symptoms of liver problem were recruited.

Serum activity of creatinine (Cr) was statistically significantly decreased in the serum of *Catha edulis* users than non-chewers. Having a low level of serum creatinine, normal level of serum urea and a balance of sodium, potassium and chloride in the blood of *Catha edulis* chewers indicates efficient and effective pair of kidneys. This contrasts to Al-Hashem *et al*., 2011 where serum urea was significantly increased in serum of hydro-ethanol extract khat administered rats. Also Rania Hussein *et al.,* 2013 where serum urea and creatinine were significantly increased in khat chewers group more than the control. The difference would be due to Al-Hashem *et al*., 2011study involved administering khat to rats while in this study people chewing *Catha edulis* were involved. Animal study could be a poor predictor of human reactions.

Total bilirubin, alanine aminotransferase and alkaline phosphatase parameters mean were statistically significantly increased in red variety chewers than pale green variety chewers. Important chemical reactions in the body are triggered by several enzymes produced in the liver. These enzymes are normally found within the cells of the liver. If the liver is damaged or injured, the enzymes spill into the blood causing elevated liver enzyme levels. Increased activity of alanine aminotransferase (ALT) in the serum suggests leakage of the enzyme into circulation from ruptured cell membranes of hepatocytes upon exposing to injury thus elevating serum ALT level in blood ((Edith *et al*., 2010; (Kim *et al.,* 2008); Rini *et al.,* 1981). This indicates hepatotoxicity due to red variety *Catha edulis* (hepatotoxicant) because this enzyme is more specific for detecting liver abnormalities since it is primarily found in the cytosol of the hepatocytes and its concentration in the liver far exceeds that in any other organ. The findings were consistent with those reported by Shabbir *et al.,* 2014 where ALT was significantly increased in khat users as compared to non-users and Al-Habori *et al.,* 2002 who declared that long term feeding of Khat leaves to New Zealand white rabbits elevated liver enzyme activities and lead to toxic hepatocellular jaundice.

Increased serum activity of alkaline phosphatase and total bilirubin in red variety *Catha edulis* chewers indicates a cholestatic pattern of liver function test disturbance. Increased serum activity of ALP in red variety chewers than that of pale green variety chewers and non-chewers, suggests leakage of the enzyme into circulation from damaged liver cells. Since total bilirubin and alanine aminotransferase were also increased, it means the increased ALP was of hepatic origin rather than bone origin.

Increased total bilirubin suggests a direct toxic effect of the red variety *Catha edulis* on liver cells leading to decreased uptake and conjugation of bilirubin and reduced secretion into bile ducts (decreased hepatic clearance) causing a build-up of bilirubin in the blood (Al-Hashem *et al.,* 2011).

Total protein mean was statistically significantly increased in pale green variety chewers than non-chewers accompanied by statistically significantly decrease in total bilirubin, alkaline phosphatase, creatinine, and potassium levels in pale green variety chewers than non-chewers

These results are in contrast with those reported in a study conducted by Ramzy *et al.*, 2013 in their study, protein and albumin levels were not statistically significant different between the khat users and non-users.

There was a statistically significantly decrease in creatinine level in the serum of red variety chewers than non-chewers. A good balance of electrolytes, normal serum urea and decreased serum creatinine in the serum of red variety *Catha edulis* chewers is an indicator of well-functioning kidneys.

Chewers using red variety *Catha edulis*, majority (60.1%) have chewed for more than three days in a week and pale green variety chewers majority (58.3%) have chewed for less than three days per week. This shows that increased in amount of *Catha edulis* chewed may cause change in serum level of ALT, total bilirubin and ALP in *Catha edulis* chewers. This is in line with Gemechi *et al.,* 2015 who demonstrated that there is a positive relationship between the increases in dose of crude extract of khat and the change in serum level of ASA, ALT, ALP, total bilirubin and direct bilirubin in rats treated with crude extract of khat.

Despite the fact that 43.9 % of the chewers consumed alcohol none had liver problem because even if ALP and direct bilirubin was increased and total protein decreased in chewers as compared to non-chewers, they were all within the normal range. There was no statistically significantly difference between chewers who drink alcohol and chewers who don’t drink alcohol.

All the kidney and liver biochemical parameters evaluated in this study were all within the normal reference range

5.2 Conclusions

1. Liver biochemical parameters were affected in the *Catha edulis* chewing group which was clear by increasing serum activity of alkaline phosphatase and decreased serum activity of total protein, therefore, constituents of *Catha edulis* with amphetamine like effect might be responsible for the damage in liver of the users.
2. Liver biochemical parameters were affected in the red variety *Catha edulis* chewing group which was clear by increasing serum activity of alkaline phosphatase, alanine aminotransferase, and total bilirubin and decreased serum activity of total protein. This indicates that red variety, *Catha edulis* is more potent than pale green variety.
3. Kidney biochemical parameters were not affected in the *Catha edulis* chewing group and also in the red variety *Catha edulis* chewing group which was clear by normal serum activity of urea, electrolytes and decreased creatinine activity.
4. Age did not have a significant effect on the level of kidney and liver biochemical parameters in *Catha edulis* chewers.
5. Gender did not have a significant effect on level of kidney and liver biochemical parameters in *Catha edulis* chewers, but due to the small sample size of female, effects on gender were not well established. Therefore, a further study on the same should be carried out.
6. Forty three point nine percent (43.9%) of the chewers consumed alcohol, which is a known long term risk factor for liver problem, due to prolonged periods of consumption.

5.3 Recommendations

1. Health professionals should educate users about potential harms (increased liver enzymes) arising from *Catha edulis* use, and promoting responsible use (moderate use) of *Catha edulis* or avoid use in order to minimize negative health effects.
2. Health education talks should be held by health professionals where chewers are advised to chew more of green variety *Catha edulis* and less of red variety.

5.4 Recommendations for further research

1. To shed light on *Catha edulis* as a cause of liver problem, retrospective and prospective epidemiological studies of chronic *Catha edulis* users should be initiated.
2. The concentration of cathinone and cathine in different varieties of *Catha edulis* in Meru County should be evaluated.
3. Research should be undertaken on the quantity (moderate/responsible use) of *Catha edulis* that should be consumed.

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APPENDICES

Appendix 1: Questionnaires

Please respond to all the questions in the questionnaire appropriately and honestly. The information you give will be treated with strict confidence and will only be used for research purposes

1. Age: □
2. Gender: Male □ Female □
3. How many bundles of miraa do you chew in a day

   1 □ 2 □ 3□ >5□

4. Which is the preferred miraa do you chew?

   Red type □ Pale green type □ whatever is available □

5. How often do you chew miraa in a week?

   > 3 days □ <3 days□

6. How long have you chewed miraa

   < 1year □ 1-2 years □ 3- 5 years □ 5 -10 years □ >10 years □

7. Do you drink alcohol?

   Yes □ No □

   If yes, bottled □ traditional □

8. How many bottles of alcohol do you drink per day?

   <5 bottles □ 5-10 bottles □ >10 □bottles

9. For how long have you been drinking alcohol

   <5 years □ 5-10 years □ >10 years □

Appendix II: Informed consent form

Part I: Information sheet

Topic: Effect of *Catha edulis* (miraa) on kidney and liver function among *Catha edulis* chewing adults in Meru County, Kenya.

Consent explanation: My name is Catherine Makandi Mworia, a Masters student at the Department of Medical Laboratory Sciences, Jomo Kenyatta University of Agriculture and Technology. PO Box 62000 Nairobi; Phone: +254-067-52124. I am carrying out an academic research to find out what is the effect of *Catha edulis* (miraa) chewing on your kidney and liver function. I am going to give you information and invite you to be part of this research. You do not have to decide today whether or not you will participate in the research. Before you decide, you can talk to anyone you feel comfortable with about the research.

This consent form may contain words that you do not understand. Please ask me to stop as we go through the information and I will take time to explain. If you have any questions you may ask them now or later, even after the study has started.

Description: Liver and kidney are vital organs of the body. Their primary role is to expel toxins that result from the body's metabolism of food and drink. Previous studies in other regions have shown that people who chew miraa are likely to develop liver and kidney problem.

Purpose: This study is interested in finding out if the biochemical parameters of the liver and kidney are altered among those who chew miraa.

Benefits: You will benefit from free screening for liver and kidney function. In case of any abnormal findings you will be informed in confidentiality and referred to where you will receive treatment.

Risks: One potential risk is the loss of privacy. However, we will do our best to make sure that the personal information gathered during this study is kept private. You will feel a little pain at the time we will be drawing blood.

Procedure: If you accept to participate in this study, we will ask you to attend the hospital laboratory near your area where you will meet study consultant. You will undergo face to face interviews using language you are comfortable with to answer a few questions concerning your demographic parameters as well as collect blood using needle and syringe. 5 mls of blood will be drawn and transported to Nyeri Provincial hospital biochemistry laboratory where the researcher will carry out the analysis.

Voluntarism: Your participation in this research is entirely voluntary. You may change your mind later and stop participating even if you agreed earlier.

Follow up: Participants found with abnormal liver and kidney findings will be contacted for review and referral to a management facility.

Confidentiality: This research will maintain high levels of confidentiality. You may refuse to answer any question you are uncomfortable with. To maintain confidentiality, you will be assigned a code number so that your name will not appear on any of your questionnaires. In addition, all information will be kept in a locked filing cabinet and no identifying information will be used in any written report of the study. Only Catherine Makandi and the research team members involved in this study will have access to the data. Your participation in this study will be kept completely confidential.

Subject's right: If you have any questions or desire further information with respect to this study, you may contact the primary researcher, Catherine Makandi, at 0725 353 341, the research supervisors at the Jomo Kenyatta University of Agriculture, Dr. Waithaka Kinge and Dr. Michael Kahato,416-946-8608 or Dr. Joseph Mwamisi at Kenya Methodist University, phone: 254-064-30301 Ext 8622.If you have questions about your rights as a study participant, or dissatisfied at any time with any aspect of this study, you may contact KNH/UoN ERC (Chairperson of the Scientific Steering Committee, P.O Box 20732 Nairobi, Kenya; Phone: 02-7263000 Ext 44102.

Appreciation: We would appreciate you taking the time to consider participating in this study. If you are eligible and agree to participate, you will be asked to complete a questionnaire so that we may learn more about the participants in the study. A summary of research findings will be made available to you, if requested, at the end of the study.

Part II: Certificate of consent

I have read the foregoing information, or it has been read to me. I have had the opportunity to ask questions about it and any questions I have asked have been answered to my satisfaction. I consent voluntarily to be a participant in this study.

________________	______________			
Name of participant	Signature	or		Date ………
			Thumbprint	

________________	______________	Date ……..
Study Staff witness	Signature	

(Medical laboratory technologist)

Appendix III: Screening checklist for recruitment procedure

This screening checklist is to help determine if you are appropriate candidate for the study. It consists of a few questions. Tick appropriately.

Questions	Yes	No	Don't know
Are you sick today?	☐	☐	☐
Are you on any medication at the moment?	☐	☐	☐
Have you ever been told you have high blood pressure?	☐	☐	☐
Do you suffer any cardiovascular related problem?	☐	☐	☐
Do you suffer kidney problem e.g nephritis, renal failure?	☐	☐	☐
Do you have a liver disease (hepatitis, liver cirrhosis) or jaundice?	☐	☐	☐
Have you ever been told you have diabetes (high blood sugar in your blood)?	☐	☐	☐
Female: Are you pregnant?	☐	☐	☐
Are you on any medication at the moment?	☐	☐	☐

Appendix IV: Ethical Clearance

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

KNH/UON-ERC
Email: uonknh_erc@uonbi.ac.ke
Website: www.uonbi.ac.ke

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

Ref: KNH-ERC/A/370 **Link:www.uonbi.ac.ke/activities/KNHUoN** 13th November 2014

KENYATTA NATIONAL HOSPITAL APPROVED 13 NOV 2014 KNH/UoN-ERC P.O.Box 20723-00202 NRB.

Catherine Makandi Mworia
Reg. No.TM300-1265/2013
Dept. of Medical Laboratory Sciences
JKUAT

Dear Catherine

Research proposal – Effects of Catha Edulis(Miraa) on kidney and liver function among Miraa chewing adults in Meru county, Kenya (P476/08/2014)

This is to inform you that the KNH/UoN-Ethics & Research Committee (KNH/UoN-ERC) has reviewed and **approved** your above proposal. The approval periods are 13th November 2014 to 12th November 2015.

This approval is subject to compliance with the following requirements:

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

For more details consult the KNH/UoN ERC website **www.uonbi.ac.ke/activities/KNHUoN.**

Protect to discover

Yours sincerely

PROF. M. L. CHINDIA
SECRETARY, KNH/UON-ERC

c.c. The Principal, College of Health Sciences, UoN
The Deputy Director CS, KNH
The Assistant Director, Health Information, KNH
The Chairperson, KNH/UON-ERC
Supervisors: Dr.Waithaka Kinge, Dr. Michael Kahato, Dr. Joseph Mwamisi

Appendix V: Meru county research authorization letter

EXECUTIVE OFFICE OF THE PRESIDENCY
MINISTRY OF INTERIOR AND CO-ORDINATION OF NATIONAL GOVERNMENT

Telegrams:
Telephone: .
Email: ccmeru@yahoo.com
Fax:

OFFICE OF THE
COUNTY COMMISSIONER
P.O. BOX 703-60200
MERU.

When replying please quote
Ref: *ED.12/3/69*
and Date

12[h] January 2015

To Whom It May Concern:

RE: RESEARCH AUTHORIZATION

This is to inform you that Catherine Makandi Mworia accompanied by Nicholas Kiriinya ruuri (ID 23906656) has reported to this office as directed by the **Kenyatta National Hospital,** she will be carrying out a research on "*Effects of catha edulis (Miraa) on kidney and liver function among miraa chewing adults in Meru County, Kenya*".

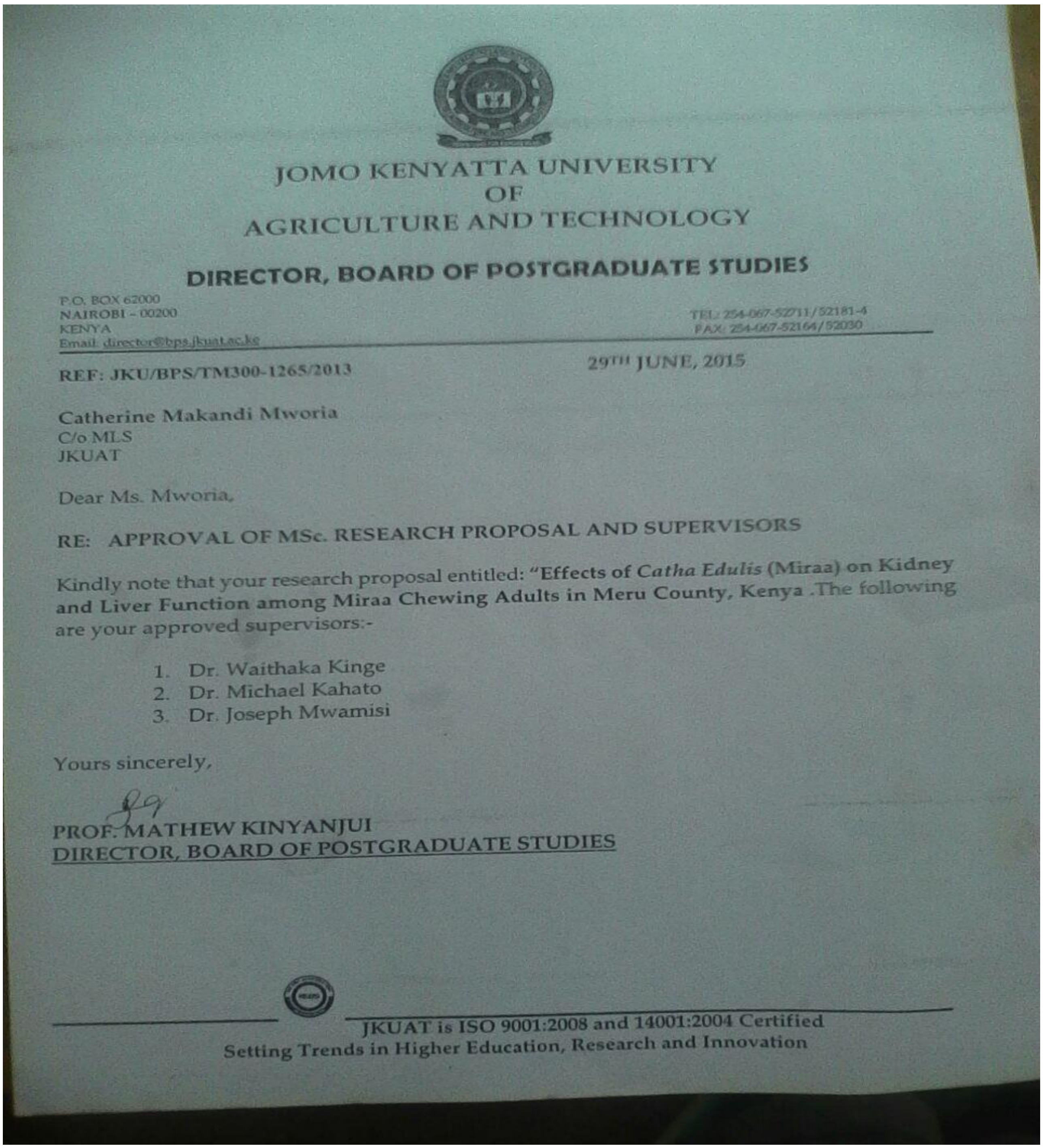
Since authority has been granted by the said Hospital, and the above named has reported to this office, she can embark on her research projects.

Please accord her any necessary support she may require.

COUNTY COMMISSIONER
MERU
P.O. BOX 703 MERU

MAINA GEORGE
FOR: COUNTY COMMISSIONER
MERU

Appendix VI: JKUAT Graduate School research authorization letter



JOMO KENYATTA UNIVERSITY
OF
AGRICULTURE AND TECHNOLOGY

DIRECTOR, BOARD OF POSTGRADUATE STUDIES

P.O. BOX 62000
NAIROBI – 00200
KENYA
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TEL: 254-067-52711/52181-4
FAX: 254-067-52164/52030

REF: JKU/BPS/TM300-1265/2013

29TH JUNE, 2015

Catherine Makandi Mworia
C/o MLS
JKUAT

Dear Ms. Mworia,

RE: APPROVAL OF MSc. RESEARCH PROPOSAL AND SUPERVISORS

Kindly note that your research proposal entitled: **"Effects of *Catha Edulis* (Miraa) on Kidney and Liver Function among Miraa Chewing Adults in Meru County, Kenya**. The following are your approved supervisors:-

1. Dr. Waithaka Kinge
2. Dr. Michael Kahato
3. Dr. Joseph Mwamisi

Yours sincerely,

PROF. MATHEW KINYANJUI
DIRECTOR, BOARD OF POSTGRADUATE STUDIES

JKUAT is ISO 9001:2008 and 14001:2004 Certified
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Appendix VII: East Africa Medial Journal publication cover page

The East African Medical Journal

Established in 1923 ISSN 0012-835X

July 2016 Volume 93 No. 7

CONTENT

www.eamj.org

July 2016 cover.indd 1 05/10/2016 2:25 PM

Appendix VIII: International journal of advanced multidisciplinary research publication certificate

INTERNATIONAL JOURNAL OF ADVANCED MULTIDISCIPLINARY RESEARCH (IJAMR)

ISSN:2393-8870

www.ijarm.com

IMPACT FACTOR: 3.565, ICV:67.87

DARSHAN PUBLISHERS

Editorial Board Member of

International Journal of Advanced Multidisciplinary Research (IJAMR)

is hereby awarding this certificate to

[1].**Catherine Makandi. Mworia Bsc MLS, MMLS**, Clinical Chemistry, Department of Medical Laboratory Sciences, Kenya Methodist University, P.O Box 267-60200 Meru, Kenya.

[2]. **Waithaka Kinge, Msc, PhD,** Clinical Chemistry, Department of Medical Laboratory Sciences, Mount Kenya University, P.O Box 342-01000 ,Thika,Kenya.

[3].**Michael Kahato, Msc, PhD,** Medical Entomology, Department of Medical Laboratory Sciences, Jomo Kenyatta University of Agriculture and Technology, P.O. Box 62000- 00200 , Nairobi, Kenya.

[4].**Joseph Mwamisi, Msc, PhD,** Medical Education, Department of Medical Laboratory Sciences, Kenya Methodist University, P.O Box 267-60200 Meru, Kenya.

Corresponding Author: *kiriinyanicholas@gmail.com*

In recognition of the publication of the paper entitled **"Effects of red and pale green variety of *Catha edulis* on liver and kidney of human consumers in Meru county, Kenya"** published in IJAMR Journal, Volume: 4, Issue: 2, Year: 2017.

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