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DEPARTMENT OF MEDICAL LABORATORY SCIENCES

Assessment of Renal Function Tests and Lipid Profiles among Apparently Healthy Adult Male Khat-Chewers and Non-Khat Chewers in Dilla Town, Southern Ethiopia. A Comparative Cross-sectional Study

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Department of Medical laboratory sciences

This is to certify that the thesis prepared by Abush Getaneh, entitled: **Assessment of Renal Function Tests and Lipid Profiles among Apparently Healthy Adult Male Khat-Chewers and Non-Khat Chewers in Dilla Town, Southern Ethiopia** and submitted in partial fulfilment of the requirements for Master of Science degree in Clinical Laboratory Sciences (Clinical chemistry) complies with the regulations of the University and meets the accepted standards with respect to originality and quality.

Signed by the Examining Committee:

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Advisor ____________________________ Signature ___________ Date ____________

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Abbreviations

- BMI Body mass index
- C-G Cock croft – Gault equation
- CKD Chronic Kidney Disease
- CKD-EPI Chronic Kidney Disease Epidemiology Collaboration
- CVD Cardiovascular disease
- eGFR Estimated glomerulus filtration rate
- GSH Glutathione
- HDL-C High density lipoprotein cholesterol
- IQR Inter quartile range
- KDIGO Kidney disease improving global outcome
- LDL-C Low density lipoprotein cholesterol
- LPL Lipoprotein lipase
- MDRD Modification of Diet in Renal Disease
- ROS Reactive oxygen species
- SOP Standard operational procedure
- SPSS Statistical Package for Social Sciences
- TC Total cholesterol
- TG Triglyceride
- VLDL Very low-density lipoprotein

Abstract

Background: Chewing khat, '*Catha edulis'* is becoming more common in our society with increased harmful health effects, such as lipid metabolism disorder and impaired renal function that are highly associated with acute myocardial infarction. It was categorized as a substance of abuse by the WHO. There is a scarcity of data and contradictory findings on this topic.

Objective: To assess renal function tests and lipid profiles among apparently healthy adult male khat-chewers and non-khat chewers in Dilla Town, Southern Ethiopia.

Methods: A comparative cross-sectional study was undertaken from June to September 2023 in Dilla Town, southern Ethiopia. The study employed convenient sampling techniques. The study included 200 apparently healthy adult male khat-chewers and non-khat chewers. Socio demographic data were collected by using standardized questionnaire. Blood sample collection was done through standardized techniques. Automated Siemens Dimension EXL 200 integrated system were used to analyze the samples. Kruskal-Wallis, Mann-Whitney, and Spearman correlation statistical methods were applied using SPSS Version 26. A P-value of less than 0.05 was considered to indicate statistical significance.

Result: Among khat chewers, the levels of HDL-C (median ± IQR [interquartile range]) were significantly lower (34.0 ± 17 mg/dl) in comparison to non-khat chewers (39.5 ± 25 mg/dl) ($P = 0.007$). Additionally, khat chewers displayed significantly higher levels of TC/HDL ratio (3.81 ± 2.05 vs. 3.17 ± 1.29, $P<0.001$) and TG (95.5 ± 56 mg/dl vs. 80.5 ± 45 mg/dl, $P = 0.005$) than non-khat chewers ($p<0.05$). A significant decrease in eGFR (stage 1) and increased creatinine levels were observed among khat chewers who chewed for more than 10 years in comparison to those who chewed for < 5 and 5–10 years.

Conclusion: Khat chewing has a deleterious effect on HDL, triglyceride, and TC/HDL ratio levels. It may be associated with duration of khat chewing, frequency of khat chewing, bundle of khat used, and absence of physical activity. Thus, awareness creation should be implemented by the concerned bodies to minimize the habit of chewing khat.

Key word: Khat, *Catha edulis*, Lipid profiles, Renal function tests.

1. Introduction

1.1. Background

Substance usage has been prevalent since ancient times [1, 2]. Khat, scientifically referred to as *Catha edulis*, is a small plant with mildly psychoactive properties. It is cultivated extensively in Yemen and East Africa [3]. When the leaves of this plant are chewed, they generate a feeling of exhilaration and stimulation, akin to the effects of amphetamines [4].

There is a wide range of chemical compounds present in khat, including approximately forty alkaloids, glycosides, amino acids, vitamins, tannins, and minerals [5]. Among these compounds, the major alkaloids are phenylalkylamines and cathedulins, which bear structural resemblance to amphetamines and exhibit notable pharmacological properties. Recent research has identified a total of 62 distinct cathedulins in fresh khat leaves [6].

The major pharmacological and biological effects connected to khat are mainly attributed to the cathinone substance [7]. Studies have shown that fresh khat is reported to have an average content of 36 to 343 mg of cathinone, 83 to 120 mg of cathine, and 8 to 47 mg of nor-ephedrine per 100 g of leaves [5, 8]. Cathinone elicits the activation of alpha-1 adrenergic receptors via an indirect sympathomimetic mechanism, thereby enhancing the release of norepinephrine. As a result, this induces contractions in the smooth muscles located in the bladder neck, urethra, and prostate, ultimately causing obstruction in the bladder outlet, reduced urine flow, and a decline in glomerular filtration rate [9, 10].

The use of khat has been shown to cause nephrotoxicity and renal impairment by inducing oxidative stress. The oxidizing agents in khat stimulate the generation of free radicals and inhibit the activity and synthesis of enzymes that metabolize free radicals, such as superoxide dismutase (SOD), catalase, and glutathione (GSH). Consequently, this results in increased production of reactive oxygen species as a result of higher reactive oxygen species (ROS) formation, leading to membrane damage [11-13].

The activation of adenylyl cyclase is initiated by cathinone through the stimulation of β-adrenergic receptors, leading to an increased conversion of ATP to c-AMP [14, 15]. Moreover, the administration of *Catha edulis* extract to rabbits has been found to have a stimulatory effect on adrenocorticotrophic hormone (ACTH) [16]. The activation of adenylyl cyclase is also thought to play a role in mediating ACTH. Increased levels of c-AMP result in a hindering effect on cholesterol production [14]. A significant number of studies have explored and reported on the chemical makeup and characteristics of khat, yet there is a notable lack of literature addressing its effect on lipid profiles and renal function tests. As a result, the current research was initiated to investigate the effects of khat on lipid profiles and renal function test levels in the designated study area.

1.2. Statement of the problem

In Ethiopia, the habit of chewing khat is increasingly becoming the norm, with a significant surge observed among the younger segment of the population [17]. The national STEP-wise survey data showed that the current prevalence of khat chewing is 15.8% [18]. Similarly, findings from the 2016 Ethiopia Demographic and Health Survey revealed that 12% of women and 27% of men acknowledged having experience with chat chewing [19]. In Dilla town, the current prevalence rate of khat chewing is documented at 27.71% [20].

As per the World Health Organization, khat is categorized as a drug of abuse [21]. Different studies have shown that chronic chewing of khat has numerous adverse effects, including physical, physiological, public health, and social implications [22-24].

Dyslipidaemia is one of the main complications of chewing khat regularly, which leads to cardiovascular diseases. A study done in Yemen found that heavy Khat chewers had a 39-fold higher chance of developing an acute myocardial infarction due to dyslipidaemia compared to non-chewers and that 72.2% of patients with acute myocardial infarction were regular chewers [25]. Similarly, in Yemen, it was found that 89.8% of khat chewers and 80.5% of non-khat chewers exhibited dyslipidaemia, indicating a possible association between khat consumption and the development of dyslipidaemia [26].

An investigation carried out in Jimma, Ethiopia, highlighted that regular khat chewing could potentially lead to the development of dyslipidaemia. The proportion of individuals developing dyslipidaemia was notably higher among khat chewers than non-chewers (72.1% versus 23.7%), indicating a possible link between habitual khat consumption and the risk of dyslipidaemia [27].

Nephrotoxicity damage can be observed in white rabbits subjected to the prolonged administration of Khat [28]. Histopathological analysis of kidney tissue sections exhibited a heightened extent of the lesion, which correlated with an increase in the dosage of khat leaves. Notable findings encompassed the presence of fat droplets in the upper cortical tubules, acute cellular swelling, hyaline tubules, and acute tubular nephrosis of the kidneys [24].

Higher doses of khat administration (400 mg/kg) lead to histopathological and biochemical changes, including mild to moderate renal infiltrative inflammation, hypertrophied glomerular capillaries, tubular degeneration, a foamy appearance in the tubular epithelial cells, and a significant rise in serum creatinine and BUN levels [29].

Despite the extensive literature on the pharmacology and chemistry of khat, there is a lack of comprehensive understanding regarding the effect of khat chewing on lipid profiles and renal function test levels. The existing data is limited and inconclusive, with conflicting results arising from a small number of human and animal studies.

In Dilla, located in southern Ethiopia, the practice of chewing khat is common among the residents, despite the potential health risks associated with it. The existing literature suggests that there was a gap in published studies addressing these issues within the study area. This study aimed to investigate the levels of lipid profile and renal function tests among apparently healthy adult male khat chewers and non-chewers residing in Dilla town, located in southern Ethiopia.

1.3. Significance of the study

There is a scarcity of studies, and their findings were inconclusive and contradictory, thus indicating the need for further investigations. To date, no investigations have been conducted, whether human or animal studies, in the study area to show the effect of khat on renal and lipid profile test levels.

The study endeavored to investigate the effect of khat on lipid profiles and renal function test levels. It seeks to provide healthcare professionals with valuable insights into the potential effects of khat chewing on lipid and renal function test levels.

This study may be used as a tool for creating awareness and public health interventions about the effects of khat chewing on renal and lipid profile test levels to encourage people to quit chewing khat in the community. Policymakers can use this data to implement khat chewing discourage policy for the betterment of society. Finally, the study will be used as preliminary data for other broad and nationwide research that will be undertaken in this area.

2. Literature review

2.1. Effect of Khat Chewing on the Levels of Lipid Profile Tests

There are a few different studies conducted on both animal models and human studies to assess the effects of khat chewing on lipid profiles and renal function test levels, but the reports are still conflicting.

A study conducted in Yemen in 2003, involving 107 Yemeni male subjects, indicated that the levels of TC and LDL-C were lowered non-significantly in khat chewers compared to non-khat chewers. The fasting TC and LDL-C levels were recorded at 132.60 ± 43.40 mg/dl and 68.70 ± 39.79 mg/dl in khat chewers, respectively, while non-khat chewers had TC levels of 146.58 ± 37.53 mg/dl and LDL-C levels of 90.00 ± 37.84 mg/dl. Interestingly, HDL-C and triglyceride levels were higher in the khat chewers compared to the non-khat chewers [11].

A cross-sectional study was done in the Jazan region of Saudi Arabia in 2014. The study included 100 participants, with 50 male individuals who were khat chewers and 50 male individuals who were non-khat chewers. The findings of the study revealed that khat chewers had a significantly higher levels of TG and lower levels of HDL-C compared to non-khat chewers. The levels of TG and HDL-C were measured as (186.64 ± 2.58 mg/dl, 53.21 ± 1.10 mg/dl) in khat chewers and (95.24 ± 3.31 mg/dl, 57.99 ± 1.22 mg/dl) in non-khat chewers, respectively. These differences were statistically significant ($P< 0.005$). Interestingly, the levels of TC and LDL-C were found to decrease among khat chewers compared to non-khat chewers, although this decrease was not statistically significant [30].

In 2015, a cross-sectional study was carried out in Ethiopia, encompassing nine regions and two city administrations, with a community-based approach. The study specifically targeted non-communicable diseases. The survey, which involved 9800 respondents, indicated that individuals who chewed khat had lower median total cholesterol, HDL, and LDL values compared to non-chewers. The levels of TC, HDL-C, and LDL-C among khat chewers were 130.0±45 mg/dl, 37.0±17 mg/dl, and 91.3±43.9 mg/dl, while non-chewers had 132.0±48 mg/dl, 40.0±17 mg/dl, and 96.1±47.5 mg/dl, respectively, $P<0.05$ [31].

A cross-sectional study carried out in Dire Dawa, Ethiopia, in 2018 involved 100 volunteers, including 50 khat chewers and 50 non-khat chewers, to investigate the effect of khat leaves on the levels of HgA1c and lipid profiles. The results revealed that khat chewers had higher mean levels of TG and TC compared to non-khat chewers, with statistically significant differences in TG ($P = 0.047$) and TC ($P = 0.045$), while no significant difference were found in their LDL and HDL levels [32].

A case-control investigation conducted in Jimma, Ethiopia, in 2017, which focused on individuals using a facility-based approach, revealed that Khat chewers exhibited higher levels of serum TG in comparison to non-chewers ($P < 0.001$). The study found that 72.1% of Khat chewers had dyslipidaemia, indicated by a TC/HDL ratio greater than 5, compared to 23.7% of non-Khat chewers. Consequently, this literature suggests that Khat chewers are at high risk of developing dyslipidaemia compared to non-Khat chewers [27].

2.2. Effect of Khat Chewing on the Levels of Renal Function Tests

According to a cross-sectional study done in Saudi Arabia in 2018 on 100 male study participants, consisting of 50 khat chewers and 50 non-khat chewers, Regular khat chewers had significantly higher levels of serum creatinine and urea than non-khat chewers [30]. Similarly, a study from Yemen done in 2012 on randomly selected 50 male individuals showed that khat chewing significantly increased serum creatinine and urea levels compared to non-khat chewers, with a value of serum creatinine (2.3 ± 0.675 mg/dl and 0.83 ± 0.32 mg/dl) and urea (43.643 ± 6.93 mg/dl and 32.3 ± 1.92 mg/dl) in khat chewers and non-khat chewers, respectively [33].

A cross-sectional study was carried out in Meru, Kenya, spanning from December 2014 to December 2015. The study involved 198 individuals who were classified as khat chewers and 193 individuals who were categorized as non-chewers. The study findings revealed that the serum creatinine concentrations were significantly lower in khat chewers (83.07 ± 28.94 mMol/l) compared to non-khat chewers (94.69 ± 25.17 mMol/l) ($P<0.001$). Moreover, the levels of serum urea in khat chewers were found to be significantly lowered ($P = 0.16$) [34].

As per the literature reviewed, there have been no published studies investigated in the study area relating to the effect of khat chewing on lipid profile and renal function levels. The literature emphasizes the necessity for additional research on this topic. Given the prevalence of khat chewing in the community, such investigations will provide scientific support for reducing organ damage and metabolic changes caused by khat for the improvement of individuals' health.

2.3. Conceptual frame work

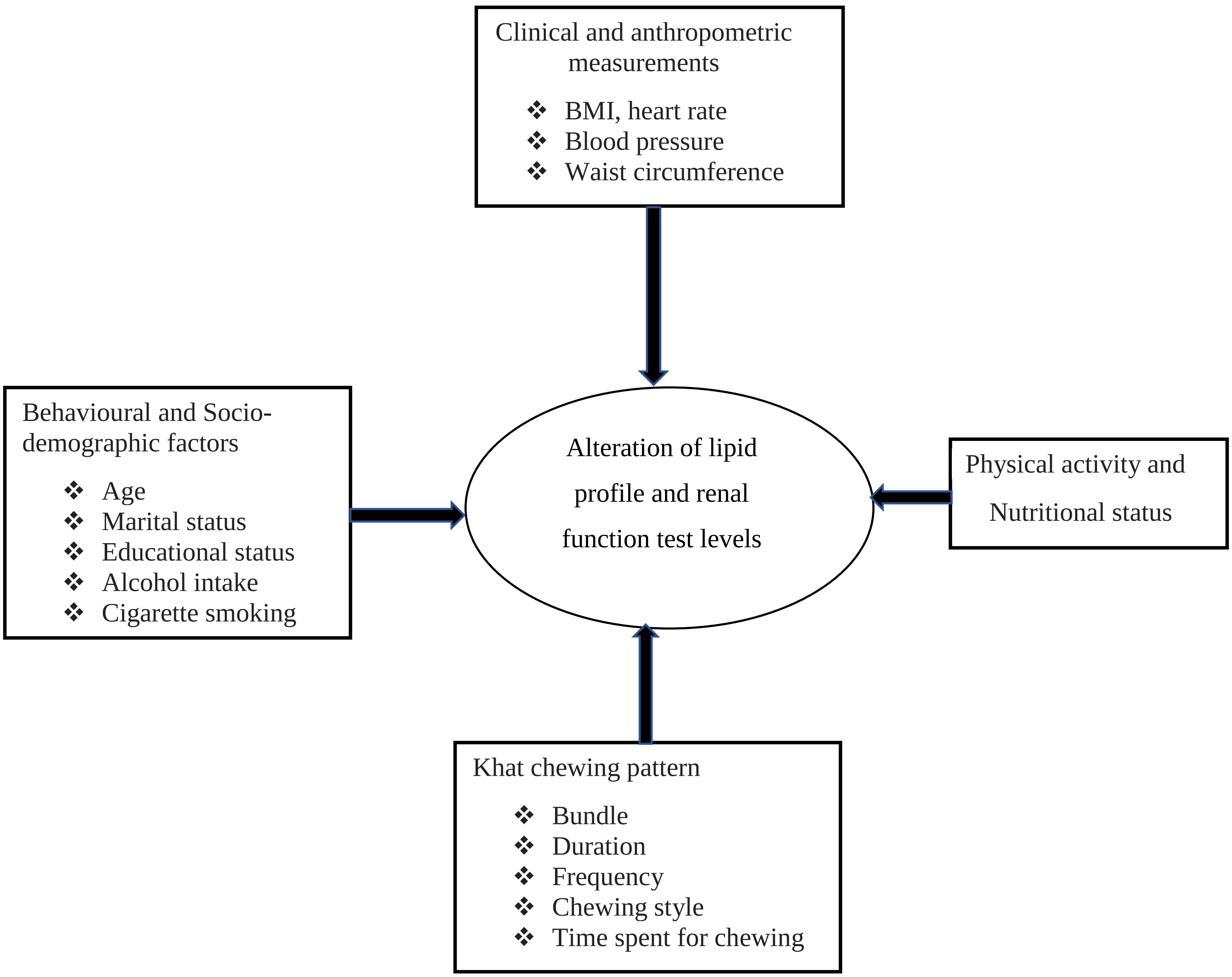

Figure 1: Conceptual frame work

3. Objective

3.1. General Objective

Assessment of renal function tests and lipid profiles among apparently healthy adult male khat-chewers and non-khat chewers in Dilla Town, Southern Ethiopia.

3.2. Specific Objective

- ❖ To compare renal function tests (serum creatinine and urea, eGFR) and lipid profile (TG, HDL-C, LDL-C, TC/HDL ratio, and TC) levels between apparently healthy adult male khat chewers and non-khat chewers.
- ❖ To identify associated factors with the level of renal function tests and lipid profile tests among apparently healthy adult male khat chewers.

4. Hypothesis

4.1. The null hypothesis HO:

HO: The levels of serum lipid profiles and renal function tests among apparently healthy adult male khat chewers and non-khat chewers are the same.

5. Methods and Materials

5.1. Study area

The investigation took place in Dilla town, the administrative center of the Gedeo Zone, which is located on the main road from Addis Ababa to Nairobi, 360 km away from the Ethiopian capital city. The town has a longitude and latitude of 6°24′30″N and 38°18′30″E, with a height of 1570 meters above sea level. Dilla town is administratively divided into five kebeles as follows: Horo-resa, Haro-welabo, Sessa, Odaayya, and Chuchu. As per the Central Statistical Agency of Ethiopia (CSA) projections for July 2023, the total population of Dilla town is projected to be 166,067 individuals, comprising 83,716 men and 82,351 women [35]. In Dilla, the primary source of khat is the town of Chuko, located in close proximity.

The map of the study area is presented in Figure 2.

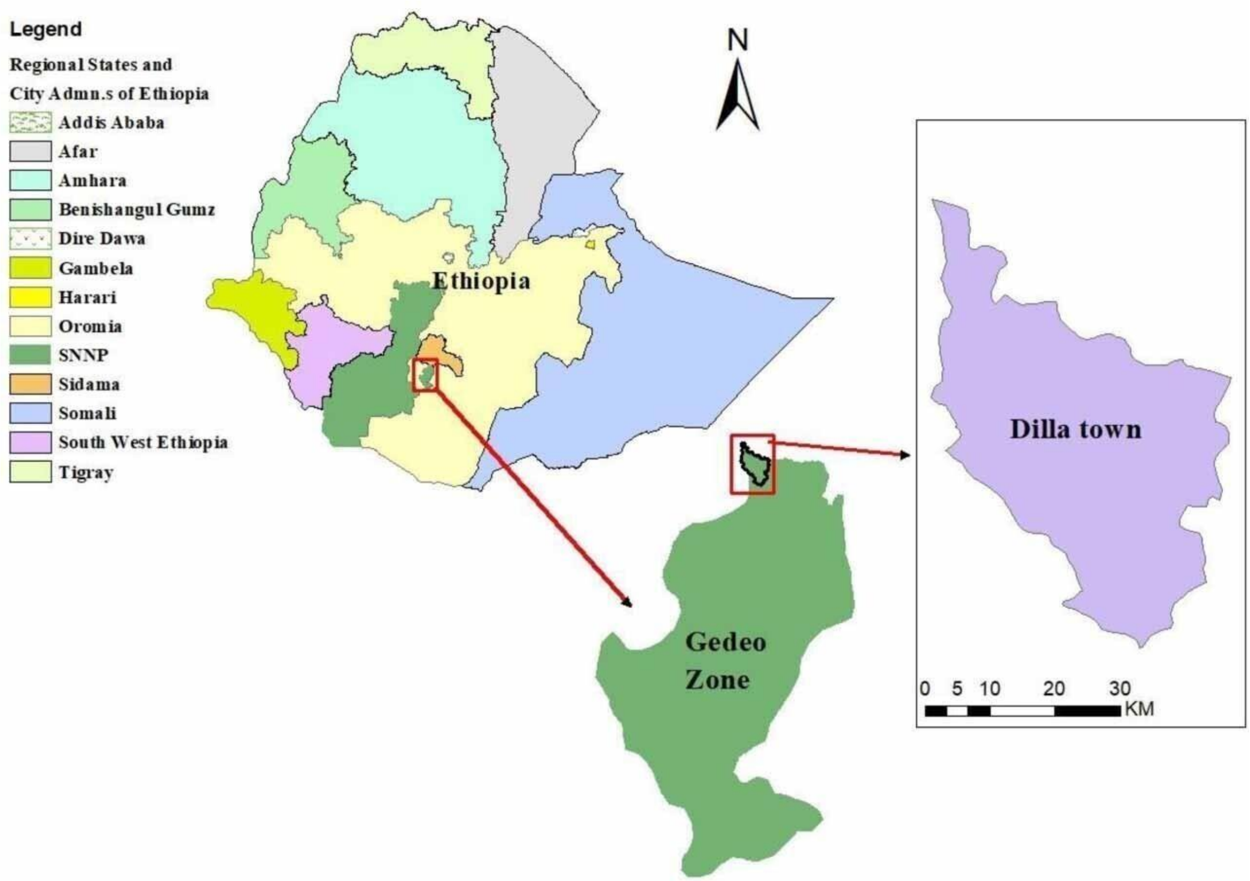

Figure 2: Map of Dilla Town (study area)

5.2. Study design and period

A community-based cross-sectional study was investigated from June to September 2023.

5.3. Population

5.3.1. Source population

All adult male khat chewers and non-khat chewers who lived in Dilla Town were considered sources of population.

5.3.2. Study population

All consented apparently healthy adult male khat chewers and non-khat chewers who met the specified criteria for inclusion.

5.4. Eligibility criteria

5.4.1. Inclusion criteria

Khat chewer group

- Apparently healthy adult male khat-chewers who's aged between 18-60 years old.
- Individuals who lived in Dilla town for at least 6 months and above.
- The study included those who voluntarily volunteered.

Non –khat chewer group

- Apparently healthy adult males who have never chewed khat in their lives and are aged between 18 and 60 years old.
- Individuals who lived in Dilla town for at least 6 months and above.

5.4.2. Exclusion criteria

Exclusion criteria for khat chewer and non-khat chewer groups

- Individuals on a course of medication.
- A person who is not practicing fasting in the morning
- Individuals with acute and chronic diseases; like lipid metabolic disorder, chronic kidney diseases, liver diseases.

5.5. Study variables

5.5.1. Dependent Variable

The levels of:

- Renal function tests (creatinine, urea, and eGFR)
- Lipid profiles (total cholesterol, triglycerides, high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), and TC/HDL ratio)

5.5.2. Independent variable

- Socio-demographic factors
- Anthropometric parameters
- Heart rate, diastolic and systolic blood pressure
- Alcohol consumption and cigarette smoking
- Khat chewing patterns
- Nutrition status
- Physical activity

5.6. Sample size determination and sampling technique

5.6.1. Sample size determination

According to a study conducted in Dire Dawa, the mean value of total cholesterol tests was used to calculate the sample size [32].

$$N = \frac{S1^2 + S2^2\,(Z\beta + Z\alpha/_2)^2}{d^2} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots.. \;[36]$$

Where **S1**= standard deviation of Khat chewer group =18.14, **S2**= standard deviation of non-khat chewer group =13.16, **d** = Expected mean difference between two groups = 6.5, **Zα/2**=1.96, **Zβ** = power = 0.84

$$N= \frac{(18.14^2 + 13.16^2)x(0.84+1.96)^2}{6.5^2} \qquad = \frac{(329.06+173.19)\ x\ 7.84}{42.25} = 93, \text{ make it } 100$$

N.B.: For this study, a sample size of 200 participants was determined, comprising 100 apparently healthy khat chewers and 100 apparently healthy non-khat chewers.

5.6.2. Sampling method

Convenient sampling techniques were applied to recruit study participants. We recruited 100 apparently healthy khat chewers from 3 kebeles, namely Horo-resa, Sessa, and Odaayya, where khat chewing shops and khat chewers exist. At the same time, the control groups (non-khat chewers) were selected around khat chewing shops, and the rest were from the Dilla University Referral Hospital staff.

5.7. Measurement and Data collection

5.7.1. Demographic and clinical data

A clear elucidation of the study's objectives was given to the participants, who then provided written consent for their involvement. A pretest of the questionnaire was carried out on a subset representing 5% of the study participants.

The collection of data or information about the participant involved the use of structured questionnaires as well as the measurement of anthropometric parameters such as height, weight, waist circumference and blood pressure were carried out by trained personnel with a standardized measurement scale. Weight was measured in kilogram, the participants' height was measured in centimeter. BMI (kg/m^2) was determined by dividing the weight of an individual by the square of their height. Following a standard procedure, blood pressure was assessed using a Riester RBP-100 instrument [37]. Prior to obtaining a blood sample, volunteers were instructed to refrain from eating for a duration of 8 to 12 hours overnight.

5.7.2. Blood sample collection

Complying with antiseptic techniques, a lab technologist collected 5 ml of venous blood in a BD serum separator tube, which contained a clot activator and gel for serum separation, from the antecubital vein of a voluntary study participant who had fasted overnight. The blood sample collection were carried out at a nearby clinic and hospital. Fasting specimens are necessary for tests such as triglycerides and LDL-C, as postprandial triglycerides can remain elevated for hours, while total cholesterol and HDL cholesterol levels do not differ significantly between fasting and non-fasting samples. The blood sample was then placed in a serum separator tube labeled with the participant's ID number.

5.7.3. Blood sample processing and analysis

Following a 30-minute blood sample collection, the serum was separated using centrifugation at 3000 RPM for 5 minutes. Subsequently, the serum samples were promptly transported to the clinical chemistry laboratory at Dilla University Referral Hospital. To maintain their integrity, the samples were securely enclosed within an icebox during transit. All standard transportation procedures were rigorously implemented. Subsequent to successfully completing all phases of the standard procedure outlined in the standard operating procedure manual, renal function tests and lipid panel analyses were conducted utilizing the Siemens EXL 200 integrated system at Dilla University Referral Hospital. Following this, the results of all tests were meticulously recorded on the data collection sheet. The samples were stored under -86 ^{o}C deep freezer until the analysis of the tests was done.

5.7.4. Laboratory Analysis

Clinical chemistry analyzer: Siemens EXL 200 integrated system (ELSMED Healthier Diagnostics PLC, a German company).

5.7.4.1. High Density Lipoprotein-Cholesterol (HDL-C)

Chylomicrons, VLDL, and LDL form complexes with dextran sulfate and magnesium sulfate, resistant to PEG-modified cholesterol esterase and cholesterol oxidase. HDL cholesterol is oxidized to Δ4-cholestenone and hydrogen peroxide, forming a colored dye with intensity proportional to serum HDL-C concentration.

5.7.4.2. Low Density Lipoprotein-Cholesterol (LDL-C)

The method involves two reagents: detergent 1 solubilizes non-LDL particles, while detergent 2 solubilizes the remaining LDL particles. Soluble LDL-C is consumed by esterase and oxidase, forming cholestenone and hydrogen peroxide. The color produced is directly proportional to the amount of LDL-C in the sample.

5.7.4.3. Triglyceride (TG)

Triglycerides convert into glycerol and fatty acids when incubated with lipoprotein lipase, transforming. Glycerol kinase phosphorylates glycerol, while peroxidase forms quinoneimine from H2O2, aminoantipyrine, and 4-chlorophenol. The absorbance change is measured using a bichromatic endpoint technique.

5.7.4.4. Total Cholesterol (TC)

Cholesterol esterase hydrolyzes cholesterol esters to produce free cholesterol, which is oxidized by cholesterol oxidase to form cholest-4-ene-3-one and hydrogen peroxide. Horseradish peroxidase uses hydrogen peroxide to oxidize N, N-diethylanilineHCl/4-aminoantipyrine (DEA-HCl/AAP) to produce a chromophore that absorbs at 540 nm. The absorbance is directly proportional to the total cholesterol concentration in the sample.

5.7.4.5. Urea

Urea is hydrolyzed by urease to form ammonium and carbonate. Ammonium reacts with 2-oxoglutarate in the presence of glutamate dehydrogenase (GLDH) and the coenzyme NADH to produce L-glutamate. The reaction involves oxidizing NADH to NAD+, with the decrease in NADH concentration directly proportional to the urea concentration in the sample.

5.7.4.6. Creatinine

The test principle is based on the modified Jaffe reaction technique, where picrate reacts with creatinine to form a red chromophore. The rate of increasing absorbance is measured using a bichromatic (510, 600 nm) rate technique, directly proportional to the creatinine concentration in the sample.

N.B.: In accordance with the manufacturer's guidelines, all biochemical analysis for this study was performed.

5.7.4.7. Determination of eGFR (CKD-EPI Creatinine Equation)

The CKD-EPI equation has emerged as the preferred method for estimating glomerular filtration rate (GFR) due to its superior performance and fewer limitations when compared to the MDRD and C-G formulas. The KDIGO 2012 guidelines advocate for the utilization of the CKD-EPI formula in estimating GFR for the purpose of screening and diagnosing chronic kidney disease (CKD) [38].

Calculation: $GFR = 141 \times \min(SCr/k, 1)^{\mathbf{a}} \times \max(SCr/k, 1)^{-1.209} \times 0.993^{Age} \times 1.018$ [if female] $\times 1.159$ [if black]

Where SCr is serum creatinine in mg/dL, k is 0.7 for females and 0.9 for males, **a**-is -0.329 for females and -0.411 for males, min indicates the minimum of SCr /k or 1, and max indicates the maximum of SCr /k or 1 [39].

5.8. Data Quality Assurance

The questioner was translated from English version into Amharic and back again. Throughout the data collection process, a questionnaire was developed with a unique ID number that aligned with the specimen ID number. After the interview had been completed, thorough cross-checking was conducted on a daily basis to ensure the entirety of the collected information.

In order to guarantee the quality of laboratory findings, all protocols were strictly adhered to, following Standard Operating Procedures (SOP) and Internal Quality Control (IQC) guidelines. Prior to analyzing participant samples, quality control materials, including both normal and pathological samples, were routinely tested on a daily basis. The outcomes of the control tests were assessed based on the Westgard rule.

The precision and accuracy of the test results were guaranteed through the implementation of pre-analytical, analytical, and post-analytical safety measures, which were carefully considered.

5.8.1 Pre-analytical phase: Proper sample collection, transportation, and processing procedures were applied based on standard operating procedures.

5.8.2 Analytical phase: Quality control materials were run before analysing the sample, and the sample was run if the control test results were passed.

5.8.3 Post-analytical: The findings were documented clearly in legible handwriting on the data collection sheet. The results were interpreted according to the reference range.

5.9. Data interpretation and Analysis

Data entry and coding were performed with Epi-data version 4.6, while analysis was conducted using Statistical Package for Social Sciences (SPSS) version 26.0. In order to investigate the normal distribution of the biochemical parameters among khat chewers and non-khat chewers, the Kormogorov-Smorvo test and the Shapiro-Wilk normality test were utilized. Consequently, nonparametric statistical tests were conducted, and the data were presented as Median±IQR.

Nonparametric tests, including the Kruskal-Wallis test (K-independent sample test) and Mann-Whitney test (2-independent sample test), were used in this study to compare the renal function tests and lipid profile levels between and within groups of khat chewers and non-khat chewers, respectively. Furthermore, a nonparametric Spearman correlation was used to evaluate the association between the level of the lipid profile, renal function tests, and associated factors. A 95% confidence interval with a P-value < 0.05 was applied to evaluate statistical significance. Textual summaries, figures and tabular forms were used to display the results.

5.10. Ethical consideration

The study received ethical approval from the Department Research and Ethical Review Committee (DRERC) of the Department of Medical Laboratory Sciences, College of Health Sciences, Addis Ababa University, under the ethical approval protocol number DRERC/718/23/MLS.

Following this, an official letter was sent to the Dilla Town Health office and local administrative offices in order to request authorization to conduct the research. Additionally, official requests were made to the clinic and Dilla University Referral Hospital to obtain permission for the collection and analysis of samples. The study participants were provided with a detailed explanation of the study's goals and the confidentiality measures in place. Prior to gathering data, each participant gave written consent. All participants in the study willingly agreed to and signed the consent form.

5.11. Dissemination of Result

The outcomes of the study will be conveyed to the Department of Medical Laboratory Sciences, College of Health Sciences, Addis Ababa University. Furthermore, dissemination to the Dilla Town Health office, local health institutions, and relevant stakeholders is planned. The research were sent for publication in reputable journals. Afterward, the results will be shared at national and international conferences.

5.12. Operational definitions

- **Dyslipidaemia:** defined if one or more of the following situations are noted: Hypertriglyceridemia (> 150 mg/dl), hypercholesterolemia (> 200 mg/dl), high LDL cholesterol (>130 mg/dl), and low HDL cholesterol (< 40 mg/dl) According to the National Cholesterol Education Program-Adult Treatment Panel III criteria (NCEP-ATP III).
- **Khat-chewers**: Those chewing two or more times per week for the last year and more.
- **Non-khat chewers:** are those who have never chewed khat in their lifetime.

6. Work flows

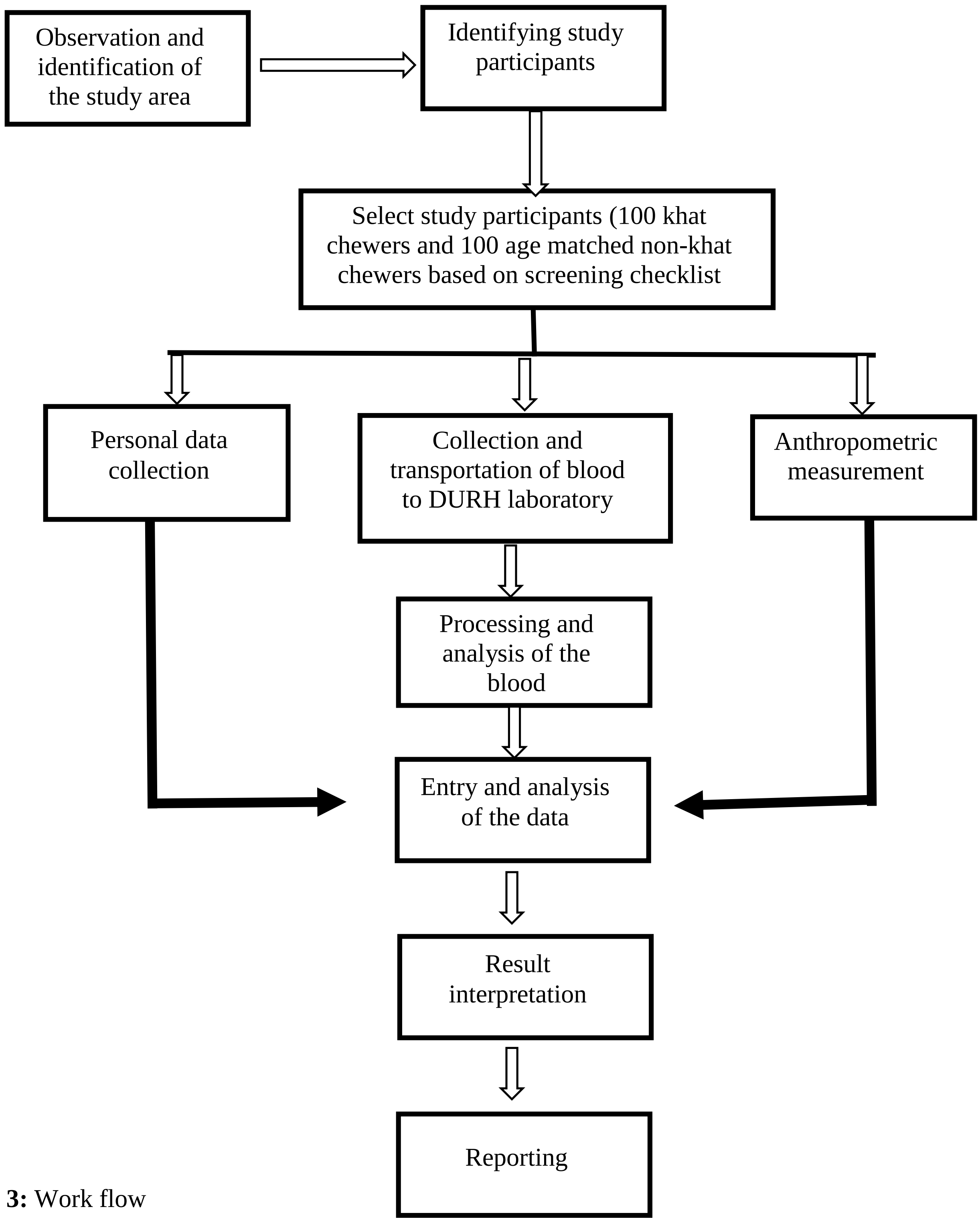

Figure 3: Work flow

7. Result

7.1 Socio-Demographic Characteristics of Study Participants

A total of 200 study participants, consisting of 100 khat chewers and 100 non-khat chewers, apparently healthy individuals, were included. The median ± IQR age of khat chewers and non-khat chewers was 36 ± 12 and 35 ± 14 years, respectively (Table 1).

Table 1: Socio-demographic characteristics of study participants Using Chi-square and Mann-Whitney U-test at Dilla Town, Southern Ethiopia, from June to September 2023 (n =200)

Parameters	Categories	KC	NKC	Total	P value
Age (years)	18-30	22 (22%)	35 (35%)	57 (28.5%)	0.169 [a]
	31-40	46 (46%)	34 (34%)	80 (40%)	
	≥41	32 (32%)	31 (31%)	63 (31.5%)	
Age (Median ± IQR)		36 ± 12	35 ± 14		
Marital status	Single	25 (25%)	22 (22%)	47 (23.5%)	0.639
	Married	70 (75%)	75 (75%)	145 (72.5%)	
	Divorced	5 (5%)	3 (3%)	8 (4%)	
Educational status	No formal education	11 (11%)	12 (12%)	23 (11.5%)	0.375
	Primary school	68 (68%)	59 (59%)	127 (63.5%)	
	Secondary and above	21 (21%)	29 (29%)	50 (25%)	
Intensity of physical activity	Sedentary	63 (63%)	47 (47%)	110 (55%)	0.071
	Low-level intensity	12 (12%)	23 (23%)	34 (17.5%)	
	Vigorous- intensity	13 (13%)	12 (12%)	25 (12.5%)	
	Moderate -intensity	12 (12%)	18 (18 %)	30(15%)	
Frequency of consuming fatty diet	< 3 times per week	72 (72%)	62 (62%)	134 (67%)	0.176
	≥ 3 times per week	28 (28%)	38 (38%)	66 (33%)	
Frequency of Fruit and vegetable consumption	> 3 days per week	39 (39%)	40 (40%)	79 (39.5%)	0.885
	1- 3 days per week	61 (61%)	60 (60%)	121 (60.5)	
History of alcohol consumption	Yes	37 (37%)	29(29%)	66(33%)	0.292
	No	63 (63%)	71 (71%)	134 (67%)	
History of cigarette smoking	Yes	18 (18%)	11 (11%)	29 (14.5%	0.228
	No	82 (82%)	89 (89%)	171 (85.5%)	

Note: Mann-Whitney U-test ([a]).

Abbreviations: KC, khat chewers; NKC, non-khat chewers.

7.2 Anthropometric Characteristics of Study Participants

Among KC and NKC, (Median±IQR, mmHg) SBP was 114±11, and 107.5±13, respectively, and DBP was 75±8, and 72±7, respectively. Both SBP and DBP were significantly higher among khat-chewers (Mann-Whitney U-test, P <0.05).

Table 2: Anthropometric characteristics of study participants Using Mann-Whitney U-test at Dilla Town, Southern Ethiopia, from June to September 2023 (n =200)

Parameters	**Khat-chewers (Median ± IQR)**	**Non-khat chewers (Median ± IQR)**	**P-value**
Diastolic blood pressure (mmHg)	75 ±8	72 ±7	**0.001**
Systolic blood pressure (mmHg)	114 ± 11	107.5 ± 13	**< 0.001**
Body mass index (Kg/m^2)	22.3 ± 2.98	22 ± 2.25	0.350
Waist circumstance (cm)	82 ± 7	81 ± 5	0.108
Heart rate (beats/ minute)	75 ± 7	74 ± 8	0.087

Note: The P value is based on the Mann-Whitney U-test.
Abbreviations: mmHg, millimetre mercury; cm, centimetre; Kg/m^2, kilogram per metre square; IQR, interquartile range.

7.3 The Distribution of Khat Chewing Pattern among Khat-Chewers

Most of the study subjects, 55/100 (55%) among khat chewers, chewed khat for more than 10 years, and 46/100 (46%) of them spent ≥ 6 hours per single day for khat chewing ceremonies. Surprisingly, most of them chew on a daily basis (48%). A large proportion (70%) of khat-chewers chew ≤ 0.5 bundles of khat per single day.

Table 3: Pattern of khat chewing among khat chewers at Dilla Town, Southern Ethiopia, from June to September 2023 (n = 100)

Parameters	Categories	Number (percent)
Chewing frequency per week	≤ 3 days	8 (8%)
	> 3 days	44 (44%)
	Daily	48 (48%)
Chewing duration (years)	< 5	6 (6%)
	5-10	39 (39%)
	> 10	55 (55%)
Bundles of khat chewed per day (zurba)	≤ 0.5 zurba	70 (70%)
	> 0.5 zurba	30 (30%)
Time spent for chewing per single day (hours)	≤ 3	11 (11%)
	4-5	43 (43%)
	≥ 6	46 (46%)
Type of things used along with khat chewing	Water	59 (59%)
	Soft drinks	13(13%)
	Peanuts	17 (17%)
	Others	11 (11%)

7.4 Comparison of Lipid Profiles and Renal Function Test Levels

The levels of some of the lipid profiles was significantly higher among khat chewers than non-khat chewers. The Median ± IQR value of triglyceride for khat chewers and non-khat chewers were 95.5 ± 56 mg/dl and 80.5 ± 45 mg/dl, (P = 0.005); and TC/HDL ratio were 3.81 ± 2.05 and 3.17 ± 1.29 respectively, P<0.001. However, the levels of HDL-C was significantly lower among khat chewers (34.0 ± 17 mg/dl) compared with non-khat chewers (39.5 ± 25 mg/dl), P = 0.007.

Table 4: Comparison of lipid profile and renal function test levels among khat-chewers and non-khat chewers Using Mann-Whitney U-test at Dilla Town, Southern Ethiopia, from June to September 2023 (n =200)

Parameters	Kit reference range	Groups	Median ± IQR	Number (%) subjects with abnormal value	P-value
HDL-C (mg/dl)	≥ 40 (mg/dl)	KC NKC	34. 00 ± 17 39.5 ± 25	68 (68%) 50 (50%)	**0.007**
TG (mg/dl)	< 150 (mg/dl)	KC NKC	95.5 ± 56 80.5 ± 45	16 (16%) 3 (3%)	**0.005**
TC/HDL ratio	< 5	KC NKC	3.81 ± 2.05 3.17 ± 1.29	25 (25%) 11 (11%)	**< 0.001**
LDL-C (mg/dl)	< 100 (mg/dl)	KC NKC	71.0 ± 38 66.5 ± 29	12 (12%) 9 (9%)	0.148
TC (mg/dl)	< 200 (mg/dl)	KC NKC	126.5 ± 46 128.5 ± 43	5 (5%) 2 (2%)	0.423
Urea (mg/dl)	16.6-48.5 (mg/dl)	KC NKC	22.0 ± 5.57 23.5 ± 3.98	0 (0%) 0 (0%)	0.206
Creatinine (mg/dl)	0.70 - 1.30 (mg/dl)	KC NKC	0.52 ± 0.22 0.51 ± 0.16	0 (0%) 0 (0%)	0.476
eGFR (ml/min)	≥ 90 (ml/min)	KC NKC	132.5 ± 23 135.0 ± 15	1 (1%) 0 (0%)	0.239

Note: The P value is based on the Mann-Whitney U-test.
Abbreviations: HDL-C, high-density lipoprotein cholesterol; TG, triglyceride; LDL-C, low-density lipoprotein cholesterol; TC**,** total cholesterol; eGFR, estimated glomerulus filtration rate; ml/min**,** millilitre per minute; mg/dl, milligram per decilitre; IQR**,** interquartile range; KC, khat chewers; NKC**,** non-khat chewers.

7.5 The Association of Lipid and Renal Function Test Levels with Khat Chewing Pattern

In comparison to individuals who chewed khat three times a week or less, as well as those who chewed it four to six times a week, daily khat chewers exhibited decreased levels of TC, HDL-C, eGFR, and an elevated TC/HDL ratio.

Khat chewers who consumed more than half a bundle of khat and chewed for a minimum of six hours per day demonstrated significantly lower levels of HDL-C, eGFR, and higher TC/HDL ratio and TG levels when compared to individuals who consumed half or less than half a bundle of khat and chewed khat for less than six hours per day.

Individuals who chewed khat for a period exceeding 10 years had significantly reduced levels of HDL-C, TC, and eGFR, as well as elevated levels of TC/HDL ratio and creatinine when compared to those who chewed khat for less than 10 years (see Table 5).

Table 5: Association of khat chewing pattern with lipid and renal function test levels of khat chewers at Dilla Town, Southern Ethiopia, from June to September 2023 (n =100)

Parameters	**Frequency of khat chewing** [a]	**Duration of khat chewing** [a]	**Bundle of khat** [b]	**Time spent for chewing of khat** [a]	**Type of drink used** [a]
	P value	**P value**	**P value**	**P value**	**P value**
HDL-C (mg/dl)	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	0.898
LDL-C (mg/dl)	0.165	0.562	0.191	0.803	0.084
TG (mg/dl)	0.505	0.172	**0.001**	**0.026**	0.326
TC (mg/dl)	**0.009**	**0.042**	0.818	0.122	0.260
TC/HDL ratio	**0.006**	**< 0.001**	**<0.001**	**< 0.001**	0.151
Urea (mg/dl)	0.972	0.362	0.625	0.588	0.479
Creatinine (mg/dl)	0.151	**0.008**	0.111	0.141	**0.003**
eGFR (ml/min)	**< 0.001**	**< 0.001**	**< 0.001**	**0.001**	**0.034**

Note: Kruskal-Wallis rank test **([a])**, and the Mann-Whitney two sample rank sum test **([b]).**

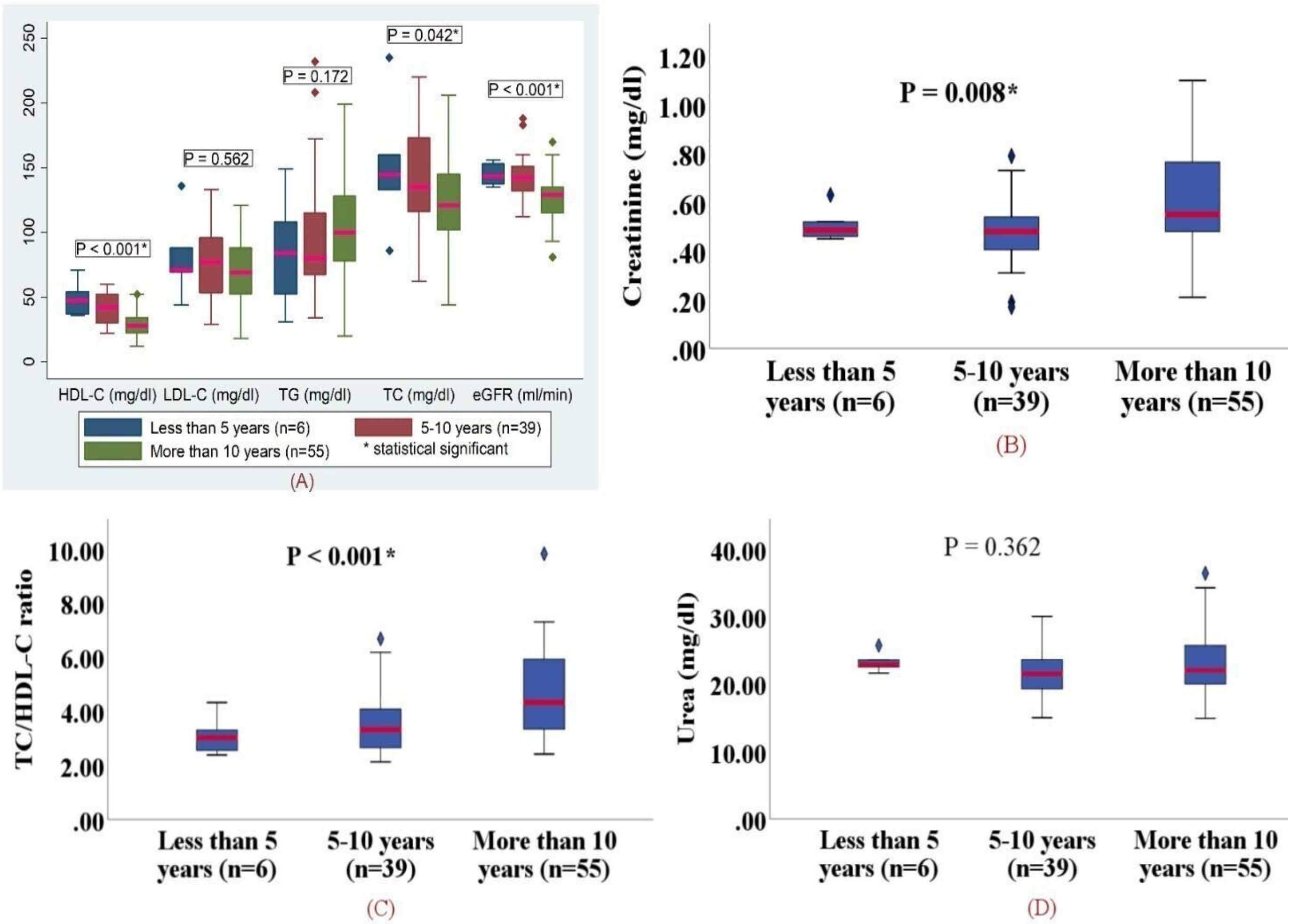

Figure 4: Box-whisker of lipid profiles (HDL-C, LDL-C, TG, TC, and TC/HDL-C ratio) and renal function tests (urea, creatinine and eGFR) levels in relation to duration of khat chewing among khat chewers based on IQR.

7.6 The Correlation of Associated Factors with Lipid Profile and Renal Function Test Levels

The study found that both khat-chewers and non-khat chewers without physical exercise had lower HDL-C levels and higher TC/HDL ratios compared to those who exercised. Khat chewers without exercise also had higher levels of TG compared to those who exercised.

In the khat chewer group, both SBP and DBP were negatively correlated with HDL-C (r = -0.334 and r = -0.318, P = 0.001) and positively with TC/HDL ratio (r =0.366, P<0.001, and r =0.325, P = 0.001), and TG (r =0.359, P< 0.001 and r =0.197, P = 0.050), respectively. The correlation of age with eGFR shows the khat chewer group had a significantly higher negative correlation (r = -0.682 vs. -0.456) compared with non-khat chewers (Table 6).

Table 6: Correlation between lipid and renal function test levels with intensity of physical activity, SBP, DBP, and age in khat chewers and non-khat chewers Using Spearman's rank correlation at Dilla Town, Southern Ethiopia, from June to September 2023 (n =200)

Parameters	Groups	Intensity of physical activity [a]	SBP [c]		DBP [c]		Age [c]	
		P value	r	P value	r	P value	r	P value
HDL-C (mg/dl)	KC	**0.001**	-0.334	**0.001**	-0.318	**0.001**	-0.549	< **0.001**
	NKC	**0.014**	-0.235	**0.018**	0.084	0.407	0.034	0.740
LDL-C (mg/dl)	KC	0.724	0.113	0.263	0.066	0.515	0.091	0.370
	NKC	0.180	-0.079	0.436	0.012	0.906	0.196	0.051
TG (mg/dl)	KC	**0.029**	0.359	< **0.001**	0.197	**0.050**	0.311	**0.002**
	NKC	0.083	0.151	0.134	0.071	0.481	-0.016	0.874
TC (mg/dl)	KC	0.814	0.055	0.583	-0.013	0.900	-0.063	0.533
	NKC	0.067	-0.144	0.153	0.082	0.418	0.086	0.394
TC/HDL Ratio	KC	**0.004**	0.366	< **0.001**	0.325	**0.001**	0.514	< **0.001**
	NKC	**0.002**	0.232	**0.020**	0.003	0.980	0.074	0.464
Urea (mg/dl)	KC	0.293	0.021	0.839	0.173	0.085	0.148	0.142
	NKC	0.241	-0.061	0.546	-0.055	0.589	-0.165	0.085
Creatinine (mg/dl)	KC	0.510	0.055	0.588	-0.016	0.874	0.327	**0.001**
	NKC	**0.016**	-0.040	0.696	-0.177	0.079	0.200	**0.046**
eGFR (ml/min)	KC	0.635	-0.234	**0.019**	-0.121	0.230	-0.682	< **0.001**
	NKC	0.145	0.073	0.471	0.131	0.193	-0.456	< **0.001**

Note: Kruskal-Wallis rank test **([a]),** Spearman's rank correlation ([c] **)**
Abbreviations: SBP, systolic blood pressure; DBP, diastolic blood pressure; KC, khat chewers; NKC, non-khat chewers.

8. Discussion

The study examined the effect of khat chewing on lipid profile and renal function test levels among khat chewers in Dilla town, Ethiopia, and identified risk factors. In our investigation, we have selected 100 khat-chewer individuals and 100 non-khat-chewer individuals from Dilla town. Most khat chewers have been engaging in this habit for more than 10 years, with the average person dedicating 6 or more hours daily to khat chewing.

This study found significantly higher levels of triglyceride (TG) and TC/HDL ratio in khat chewers compared to non-khat chewers, similar to previous cross-sectional studies in Yemen, where both studies found a significantly higher levels of triglyceride among khat chewers [40, 41]. This may be linked to the catecholamine-induced lipolysis, where stored fat is broken down, increasing free fatty acids and subsequent re-esterification into triglycerides in the liver [7]. However, this study contradicts a study conducted in Yemen where a significantly lower levels of TG in khat chewers was reported [26].

This study found that khat chewers have significantly lower HDL-C levels than non-khat chewers. This finding is consistent with previous cross-sectional studies in Yemen and Ethiopia, where HDL-C levels were found to be significantly decreased in khat chewers [31, 40, 41]. However, another cross-sectional study done in Yemen demonstrated increased levels of HDL-C by 15% in khat chewers [11]. This could be a result of impaired cholesterol transport and clearance from the bloodstream [7].

In our study, khat chewers showed non-significantly decreased levels of TC compared to non-khat chewers. Our finding is supported by cross-sectional studies carried out in Saudi Arabia and Yemen [11, 30]. Contrary to our study, a cross-sectional study conducted in Yemen found that the levels of TC increased non-significantly among khat chewers [42]. Similarly, a large cohort study in six Arabic countries found a significant increase TC levels in acute coronary syndrome (ACS) khat-chewers compared to ACS non-khat chewers [43].

The study also demonstrated that there was a non-significantly higher levels of LDL-C among khat chewers compared to non-khat chewers. This is in line with a cross-sectional study done in Yemen, but the degree of increment was statistically significant [26]. In contrast to this, according to a cross-sectional study conducted in Saudi Arabia, a non-significantly lower levels of LDL-C in khat chewers was reported [30]. Our result is also not in line with another cross-sectional study reported from Ethiopia, where significantly lower LDL-C levels were observed [31].

Furthermore, this study suggests that khat chewers demonstrate a non-significantly lower levels of urea. Interestingly, this finding contradicts the results of a cross-sectional study conducted in Saudi Arabia [30] and in Nairobi [44]. Both of which reported a higher levels of urea among khat chewers.

The study reveals that khat chewers have a non-significantly higher levels of creatinine and lower eGFR compared to non-khat chewers. This finding is similar to a study done in Kenya [45] and a study carried out in Yemen on healthy adult males. [33] But the degree of increment in creatinine levels was statistically significant. Our study contradicts a cross-sectional study conducted in Kenya, where the levels of creatinine was significantly lower in khat chewers [34].

The variation observed could be explained by the differences in the types and quantities of khat used, variations in sample sizes, variations in individual health statuses, gender differences, genetic differences, variations in study designs, and differences in nutritional statuses.

The study found that individuals who chewed khat for over 10 years had lower HDL-C and TC levels but higher TC/HDL ratios and creatinine levels in comparison to those who had been chewing for less than 10 years. Supporting this, a study done in Yemen found a significant decrease in total cholesterol levels over a six-month treatment period with all *C. edulis* concentrations (10%, 20%, and 30%) compared to levels at 2 and 4 months [46]. It is also similar to another cross-sectional study conducted in Yemen, which found a significant association between long-term khat chewing (5–30 years) and decreased HDL-C levels as the duration of khat use increased [41]. Furthermore, a cross-sectional study undertaken in Saudi Arabia indicated increased serum creatinine levels and reduced HDL-C levels in those who chewed khat for a prolonged period compared to those who chewed it for a shorter period [30].

Most interestingly, our study has confirmed that frequency of khat chewing and bundle of khat used have an association with higher levels of triglyceride and lower levels of HDL-C and TC. This was similar to a study done on khat chewers receiving different doses orally each day (0, 500, 1000, and 2000 mg/kg body weight) for a continuous 6-week period. The outcomes exhibited the same findings in the two high-dose treatment groups (1000 and 2000 mg/kg) compared to the low-dose treatment group [47].

The study found that the age-adjusted correlation of eGFR with khat had significantly higher negative correlation (r = -0.682 vs. -0.456) among khat chewers compared with non-khat chewers. Furthermore, a longer duration of chewing is associated with a decline in eGFR and an increase in creatinine levels compared to shorter chewing durations.

In line with these findings, a cross-sectional study from Yemen found that chewing khat for over 4 years, for 4–8 hours daily, leads to oxidative stress and renal impairment compared to shorter chewing periods [33]. Similarly, a study conducted in Yemen indicated that prolonged intragastric exposure to khat at therapeutic (20 mg/kg/day) and sub-toxic doses (40 mg/kg/day) caused a marked increase in creatinine levels and renal impairment [48]. Additionally, another study done with an administration dose of 2000 mg/kg/day for a duration of four weeks exhibited renal impairment [45].

The study reveals a significant association between TG, TC/HDL-C ratio, and HDL-C levels and physical activity intensity and time spent chewing. Khat chewers tend to have low physical activity levels. Physical activity increases HDL-C by increasing lipoprotein lipase (LPL) concentration and activity in skeletal muscles, accelerates lipid transfer, decomposition, and excretion, and reduces TG levels [49-51]. Similar findings were reported from a cross-sectional Studies conducted in India and Indonesia also show those who experience moderate and high physical activity (≥ 150 minutes per week) have higher HDL-C levels and lower triglyceride levels compared to those who are physically inactive [52, 53].

The study also demonstrated a positive correlation between systolic and diastolic blood pressure and the levels of TG and TC/HDL-C ratios, while a negative correlation was found with HDL-C levels. This could be due to dyslipidaemia impairing endothelial function, reducing nitric oxide production, and reducing baroreflex sensitivity [54-57]. Another possible explanation may be due to dyslipidaemia may potentially reduce the distensibility of large elastic arteries [58].

Elevated systolic and diastolic blood pressure levels were noted among individuals who chew khat in a community-based cross-sectional study conducted in Gurage Zone and Butajira, located in southern Ethiopia [59, 60]. This finding is consistent with a cross-sectional study carried out in Bangladesh and Poland, where higher levels of TG and lower levels of HDL were reported in hypertensive individuals compared to normotensives [61, 62].

These findings provide valuable insights into the effects of khat chewing on the levels of lipid profile and renal function tests. In light of prospective future research, this study may raise community awareness and motivate people to quit the habit of chewing khat, which would promote a healthier community.

9. Strength and limitation of the study

9.1. Strength of the study

- There is a scarcity of study on the effect of khat on lipid profile and renal function test levels, so it might serve as a reference for other more researches coming ahead.

9.2. Limitation of the study

- The financial and time limitations we encountered restricted our ability to conduct an extensive follow-up with the study participants and evaluate the long-term biochemical progression.
- The study design may not lend itself to establishing a strong causal relationship.
- Unable to perform screening tests.

10. Conclusion and recommendation

10.1. Conclusion

In conclusion, khat chewers have higher triglycerides, a higher TC/HDL ratio, and lower HDL-C levels compared to non-khat chewers. Associate factors like long-term chewing, frequency of chewing, bundle of khat, and time spent chewing are associated with lipid profile and renal function test levels. Long-term chewing (more than 10 years) has an association with higher creatinine and declining eGFR levels compared to shorter durations of chewing (less than 10 years); this shows long-term chewing may lead to renal impairment.

Early detection of biochemical changes in khat-chewers can reduce complications, morbidity, and mortality rates associated with khat consumption. The study can assist in delivering public health interventions and policy decisions regarding khat consumption.

10.2. Recommendation

- ❖ The study only involved male participants, suggesting the need for research involving both genders.
- ❖ To overcome the limitations of cross-sectional studies, it is recommended to conduct a comprehensive case-control study in order to investigate cause-and-effect relationships.
- ❖ We encourage regular medical check-ups to identify potential health issues early.
- ❖ Further research is required to conduct a comprehensive chemical analysis in order to identify the active components of khat that are responsible for this.
- ❖ We strongly recommend performing screening tests.

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Annex

Annex I: - Participants' information sheet (English version):

Principal Investigator: Abush Getaneh (BSc, MSc candidate)

Name of organization: Addis Ababa University, Department of MLS. **Phone:** 011 -275-51-70

Project Title: - Assessment of renal function tests and lipid profiles among apparently healthy adult male khat-chewers and non-khat chewers in Dilla Town, Southern Ethiopia.

Introduction-: My name is Abush Getaneh, an MSc student at Addis Ababa University. I invite you to participate in research as a study subject. Please read or listen to the information sheet.

Purpose of the study: - The purpose of this study is to assess renal function and lipid profiles among apparently healthy khat chewers and non-chewers.

Procedures and the expected participation: - Participation is entirely voluntary, and if you are willing to participate, you need to understand the purpose of the study and give your consent. A blood sample will be taken, and there will be a face-to-face interview for additional questions.

Potential risks and Discomforts: Taking venous blood may pose minimal risk and discomfort, but experienced lab technologists will take the necessary precautions and provide appropriate medical care in case of any issues.

Confidentiality: This study upholds privacy and confidentiality,

Potential benefits to subjects or to the society: You will have the chance to know the health of your kidney and your metabolic status. Hence, you are indirectly benefiting society in this respect.

If you have additional questions about the study, you can contact:

Abush Getaneh cell phone-0919970606 E-mail: abushgetaneh44@gmail.com

Samuel Kinde 0975379824 Samuel.kinde@aau.edu.et

Thank you for your cooperation. If you are willing to participate in the study, I kindly request that you provide your response to the questionnaire on the next page.

Annex II: Informed consent sheet in the Amharic version

የተሳታፊዎች ፈቃድና መተማመኛ ቅፅ (የአማርኛ ቅጂ)

ዋና ተመራማሪ፡ አቡሽ ጌታነህ (BSc፣ MSc እጩ)

የድርጅት ስም፡-አዲስ አበባ ዩኒቨርሲቲ፣ ሕክምና ላቦራቶሪ ሳይንስ ት/ክፍል፣ **ስልክ፡** 011 -275-51-70

የፕሮጀክት ርዕስ፡ - በጫት ቃሚዎች እና በማይቅሙ ጤነኛ ጎልማሳ ወንዶች መካከል ያለውን የኩላሊት ተግባር ምርመራ እና ቅባት ፕሮፋይል ግምገማ፣ ዲላ ከተማ ደቡብ ኢትዮጵያ ።

መግቢያ- አቡሽ ጌታነህ እባላለሁ በአዲስ አበባ ዩኒቨርሲቲ የኤምኤስሲ ተማሪ ነኝ። በምርምር ውስጥ የጥናቱ አካል ሆነው እንዲሳተፉ ተጋብዘዋል። እባክዎ የመረጃ ወረቀቱን ያንብቡ ወይም ያዳምጡ።

የጥናት ፕሮጀክቱ ዓላማ፡- የዚህ ጥናት ዓላማ በጫት ቃሚዎች እና በማይቅሙ ጤነኛ ጎልማሳ ወንዶች መካከል ያለውን የኩላሊት ተግባር ምርመራ እና ቅባት ፕሮፋይል መገምገም ነው።

የጥናቱ አካሄድ እና የሚጠበቁ ተሳትፎዎች፥- በዚህ ጥናት ላይ መሳተፍ በፈቃደኝነት ላይ የተመሰረተ ነው። ለመሳተፍ ፍቃደኛ ከሆኑ የጥናቱን አላማ መረዳት እና ፈቃድዎን መሰጠት ይኖርቦታል። ከዛም የደም ናሙና ከእርሶ ይዎሰዳል በተጨማሪም የገፅ ለ ገፅ ቃለ መጠይቅ ይደረግሎታል።

ጥናቱ የሚያስከትለው ችግርና አለመመቸት፥-የደም ናሙና በሚወሰድበት ግዜ አነስተኛ አለመመቸት ሊያስከትል ይችላል ነገርግን ልምድ ያላቸው የላቦራቶሪ ቴክኖሎጂ ባለሙያዎች በማናቸውም ጉዳዮች ላይ አስፈላጊውን ጥንቃቄ ያደርጋሉ እና ተገቢውን የህክምና አገልግሎት ይደረግሎታል።።።

ሚስጢር ስለመጠበቅ፥- ይህ ጥናት የእርሶን ግላዊ ሚስጥር ይጠብቃል።

ለማህበረሰቡ ወይም ለግለሰብ የሚኖረው ጥቅም ፥- በዚህ ጥናት በመሳተፍዎ የእርሶን የኩላሊት እና ቅባት ፕሮፋይል ጤንነት የማወቅ እድል ይኖርዎታል.

ስለ ጥናቱ ተጨማሪ ጥያቄዎች ካሉዎት የሚከተለውን አድራሻ ይጠቀሙ፥-

ዋና ተመርማሪ፡ አቡሽ ጌታነህ ሞባይል ስልክ-0919970606 ኢሜል፡ abushgetaneh44@gmail.com

ሳሙኤል ክንዴ 0975379824 Samuel.kinde@aau.edu.et

ለትብብርዎ እናመሰግናለን. በጥናቱ ለመሳተፍ ፈቃደኛ ከሆናችሁ በሚቀጥለው ገጽ ላይ ለመጠይቁ ምላሽ እንድትሰጡ በትህትና እጠይቃለሁ።

Annex III: - Informed consent form English version

Code …………………

I had been informed that the objective of this study. The purpose of this study is to assess renal function and lipid profiles among apparently healthy adult male khat chewers and non-chewers in Dilla town. I had been informed about the confidentiality of this study. I have understood that participation in this study is entirely voluntary. I understood that Abush Getaneh is the contact person if I have any questions about the study or about my rights as a study participant.

I hereby give my consent to providing the requested information and specimens.

Signature: ____________ Date_________________

Annex IV. Informed consent form in Amharic version

የተሳታፊዎች ስምምነት ማረጋገጫ (የአማርኛ ቅጂ)

የኮድ ቁጥር …………

የዚህ ጥናት ዓላማ የጥነቱ አላማ በደምብ ተብራርቶልኛል።የዚህ ጥናት ዓላማ በጫት ቃሚዎች እና በማይቅሙ ጤነኛ ጎልማሳ ወንዶች መካከል ያለውን የኩላሊት ተግባር ምርመራ እና ቅባት ፕሮፋይል መገምገም ነው። ስለ ጥናቱ ሚስጢር ጠባቂነት ተነግሮኛል።በዚህ ጥናት ውስጥ መሳተፍ ሙሉ በሙሉ በፈቃደኝነት ላይ የተመሰረተ እንደሆነ ተረድቻለሁ። ስለ ጥናቱ ወይም የጥናት ተሳታፊ የመሆኔን መብት በተመለከተ ጥያቄ ካለኝ አቡሽ ጌታነህ ተጠሪ እንደሆነ ተረድቻለሁ።

እኔ በዚህ የተጠየቀውን መረጃ እና ናሙና ለመስጠት ፈቃደኛነቴን አረጋግጣለሁ።

ፊርማ፡- ____________ ቀን: - ____________

Annex V. Questionnaire English version   Code --------

PART I: Questions on Socio- demographic characteristics of the respondent

No.	Questions	Response categories	Remarks
1	Identification Number	ID NO ______________	
2	Age of respondent	In competed years	
3	Marital Status	1) Single 2) Married 3) Widowed 4) Divorced	
4	Educational status of the respondent	1) Can't write and read 2) Can write and read 3) Primary educational level (1-8) 4) Secondary and above	
Part II: Khat chewing characteristics			
5	Have you ever chewed khat? If no escape to question number 12	1) Yes 2) No	
6	How often do you chew khat per week?	Days/week	
7	How long have you chewed khat?	Year/s	
8	The type of khat chewed	Type	
9	Bundle of khat chews per day?	Bundle	
10	Time spent on chewing per day?	Hours	
11	Things mostly used during khat chewing?	1) Water 2) Soft drinks 3) Peanuts 4) Others (tea, coffee, milk and others) 5) None	
Part III: Physical activity			

12	Do you do any physical activities?	1) Yes 2) No	
13	If yes, the intensity of physical activity?	1) Vigorous intensity 2) Moderate intensity 3) Low level intensity 4) Sedentary	

Part IV: Nutrition status

14	Do you use foods like meat, egg and dairy products?	2) Yes 2) No	
15	If yes, how often do you take?	Day/s per week	
16	Do you take any fruit and vegetable?	1) Yes 2) No	
17	If yes, how often do you take?	Day/s per week	

Part V: Behavioral measurements

Cigarette uses practice			
18	Do you smoke Cigarette?	1) Yes 2) No	
Alcohol uses practice			
19	Do you take alcohol?	1) Yes 2) No	

Part VI: Biochemical and anthropometric measurements

20	Blood pressure (mm/Hg)		26	High density Lipoprotein (mg/dl)	
21	Height (meter)		27	Low density Lipoprotein (mg/dl)	
22	Weight (Kg)		28	Triglycerides (mg/dl)	
23	BMI (Kg/m^2)		29	Total Cholesterol (mg/dl)	
24	Waist circumstance (cm)		30	Urea (mg/dl)	
25	Heart rate (Beats per Minute).		31	Creatinine (mg/dl)	

THANK YOU FOR YOUR PARTICIPATION!!!

Annex VI. Screening checklist English version

Screening checklist for recruitment procedure (tick appropriately in box as ☑)

S. No	Questions	Yes	No	Don't know
1	Are you sick today?			
2	Are you on medication for the last 6 months?			
3	Have you ever been told you have hypertension, DM, liver disease, kidney disease, or other chronic diseases from doctors?			
4	Do you have a family history of any chronic disease like liver disease, kidney disease, diabetes mellitus (DM), hypertension, or other diseases?			

Annex VII. Screening checklist Amharic version

የጥናቱ ተሳታፊዎችን ለመመልመል የሚጠቅም ቅፅ (አባክዎን ሳጥን ዉስጥ ያለውን የራይትምልክት ☑ በማድረግ ጥያቄዎችን ይመልሱ)

ተቁ	ጥያቄዎች	አዎ	አይ	አላዉቅም
1	አሁን ላይ የህመም ስሜት ይሰማዎታል?			
2	ባለፉት 6 ወራት መድሃኒት ወስደው ነበር?			
3	ከዘህ በፊት እንደ ደም ግፊት፣ ስኳር፣ የጉበት በሽታ፣ የኩላሊት በሽታ ወይም ሌሎች ሥር የሰደዱ በሽታዎች እንዳለብዎት ከሃኪም ተነግሮዎት ያውቃል?			
4	ከዘህ በፊት በቤተሰብዎ አባላት ላይ እንደ ጉበት በሽታ፣ ኩላሊት በሽታ፣ ስኳር፣ ደም ግፊት ወይም ሌሎች ቆየት ያሉ በሽታዎች ያለበት ሰው አለ?			

Annex VIII. Questionnaire Amharic version

ኮድ__________

ክፍል I: በተጠያቂዉ ማህበራዊ እና ስነ-ህዝብ ባህሪዎችን በተመለከተ

ተ.ቁ	ጥያቄዎች	የምላሽ ምድቦች	አስተያየት
1	የመለያ ቁጥር		
2	ዕድሜ	አመት	
3	የጋብቻ ሁኔታ	1) ያላገባ 3) መበለት 2) ያገባ 4) የተፋታ	
4	የትምህርት ደረጃ	1) ማንበብ እና መጻፍ የማይችል 2) ማንበብ እና መጻፍ የሚችል 3) የመጀመሪያ ደረጃ ትምህርት ያለው (1-8) 4) ሁለተኛ እና ከዚያ በላይ የትምህርት ደረጃ ያለው	

ክፍል II፥ ጫት መቃምን በተመለከተ

5	ጫት ይቅማሉ? መልሰ አይ አልቅምም ከሆነ አባከዎ ወደ ጥያቄ ቁጥር 12 ይለፉ	1) አዎ 2) አይ	
6	በሳምንት ለስንት ቀናት ጫት ይቅማሉ?	ቀናት	
7	ለምን ያህል ዓመታት ጫት ቅመዋል?	አመት	
8	የሚቅሙት የጫት አይነት?	አይነት	
9	በቀን ዉስጥ የሚቅሙት የጫት ጥቅል ብዛት?	የጥቅል ብዛት	
10	በቀን ዉስጥ ጫት እየቃሙ ለምን ያህል ሰአታት ይቆያሉ?	ሰአት	
11	በአብዛኛው ጫት በሚቅሙበት ጊዜ የሚጠቀሙት ነገር?	1) ዉሃ 2) ለስላሳ መጠጥ 3) ለዉዝ 4) ሌላ (ሻይ፣ቡና፣ ወተት ....) 5) የለም	
ክፍል III: አካላዊ እንቅስቃሴን በተመለከተ			

12	ማንኛውንም የአካል ብቃት እንቅስቃሴዎችን ያደርጋሉ?	1) አዎ 2) አይ	
13	መልሶ አዎ ከሆነ፣ የሚያደርጉት የአካል ብቃት እንቅስቃሴ መጠን?	1) ኃይለኛ ደረጃ ጥንካሬ ያለው 2) መካከለኛ ደረጃ ጥንካሬ ያለው 3) ዝቅተኛ ደረጃ ጥንካሬ ያለው 4) እንቅስቃሴ የማያደርጉ	
ክፍል IV: - የአመጋገብ ልምድን በተመለከተ			
14	የወተት ተዋጽኦዎችን፣ስጋን እና እንቁላልን በምግብነት ይጠቀማሉ?	1) አዎ 2) አይ	
15	መልሶ አዎ ከሆነ፣ በሳምንት ለሰንት ቀናት ይመገባሉ ?	ቀናት/በሳምንት	
16	አትክልትና ፍራፍሬን በምግብነት ይጠቀማሉ??	1) አዎ 2) አይ	
17	መልሶ አዎ ከሆነ፣ በሳምንት ለስንት ቀናት ይጠቀማሉ?	ቀናት/በሳምንት	
ክፍል V: የባህሪ መለኪያዎችን በተመለከተ የትምባሆ አጠቃቀምን በተመለከተ			
18	ሲጋራ ያጨሳሉ?	1) አዎ 2) አይ	
የአልኮል አጠቃቀምን በተመለከተ			
19	አልኮል ይጠጣሉ?	1) አዎ 2) አይ	

ክፍል VI፥ ባዮኬሚካል እና አንትሮፖሜትሪክ መለኪያዎች

20	Blood pressure (mm/Hg)		26	High density Lipoprotein (mg/dl)	
21	Height (meter)		27	Low density Lipoprotein (mg/dl)	
22	Weight (Kg)		28	Triglycerides (mg/dl)	
23	BMI (Kg/m^2)		29	Total Cholesterol (mg/dl)	
24	Waist circumstance (cm)		30	Urea (mg/dl)	
25	Heart rate (Beats per Minute).		31	Creatinine (mg/dl)	

ስለተሳትፎ እናመሰግናለን!!!

Annex IX: Principle of each test (Siemens Dimestion EXL 200 system)

1. Low-density lipoprotein cholesterol (LDL-C)

Principles of Procedure:

The LDL cholesterol assay is a homogeneous method for directly measuring LDL-C levels in human serum or plasma without the need for any off-line pretreatment or centrifugation steps. The color formed is measured using a dichromatic (540, 700 nm) endpoint technique. The color produced is directly proportional to the amount of LDL-C present in the sample.

$$\text{Non-soluble LDL-C, VLDL-C, HDL-C, Chylomi} \xrightarrow[\text{DSBmT+Peroxidase}]{\text{Detergent 1}} \text{Soluble Non-LDL-C}$$

$$\text{Soluble Non-LDL-C} \xrightarrow[\text{Cholesterol oxidase}]{\text{Cholesterol esterase}} \text{Non-color forming}$$

$$\text{Non-Soluble LDL-C} \xrightarrow{\text{Detergent 2}} \text{Soluble LDL –C}$$

$$\text{Soluble LDL-C + O2} \xrightarrow[\text{Cholesterol oxidase}]{\text{Cholesterol esterase}} \text{Cholestenone} + H_2O_2$$

$$H_2O_2 + \text{DSBmT + 4-AA} \xrightarrow{\text{Peroxidase}} \text{Color Development sample.}$$

Assay range 5 – 300 mg/dL [0.13 – 7.8 mmol/L]

Quality Control:

At least once each day of use, analyze two levels of a Quality Control (QC) material with known low-density lipoprotein concentrations. The National Cholesterol Education Program recommends controls that span the medical decision points and are traceable to the National Reference System (NRS/CHOL) reference materials and methods.

Result Interpretation:

The National Cholesterol Education Program Adult Treatment Panel III (NCEP- ATP III) 3 provides the following classifications of LDL-C concentrations:

Optimal (100mg/dl), near optimal/above optima (100-129mg/dl), Borderline High (130-159mg/dl), High (160-189mg/dl) and Very high (≥190 mg/dl).

2. High-Density Lipoprotein cholesterol (HDL-C)

Principles of Procedure:

The color formed (colored dye) is measured using a dichromatic (600/700 nm) endpoint technique. The color intensity of the dye is directly proportional to the serum HDL-C concentration.

HDL, LDL, VLDL, Chylomicrons $\xrightarrow[\text{Magnesium sulfate}]{\text{Dextran Sulfate}}$ Non-reactive LDL, VLDL,

Chylomicrons + HDL cholesterol esters

HDL cholesterol esters + H_20 $\xrightarrow{\text{PEG-Cholesterol esterase}}$ HDL cholesterol + RCOOH

HDL cholesterol + O_2 $\xrightarrow{\text{PEG-Cholesterol Oxidase}}$ Δ4 Cholestenone + H_2O_2

2 H2O2 + 4-aminoantipyrine + $\xrightarrow{\text{Peroxidase}}$ Color development + 5 H20 HSDA + H+ + H20

Quality Control:

Two levels of a Quality Control (QC) material with known HDL-C concentrations were run in day before testing sample. The National Cholesterol Education Program (NCEP) Lipid Standardization Panel (LSP) recommends two levels of controls: one in the normal range (40 – 60 mg/dL [1.04 – 1.55mmol/L]) and one near the concentrations for decision-making (<40 mg/dL [<1.04 mmol/L]).

Result Reporting:

The National Cholesterol Education Program Adult Treatment Panel III (NCEP-ATP III) provides the following classifications of HDL-C concentrations:

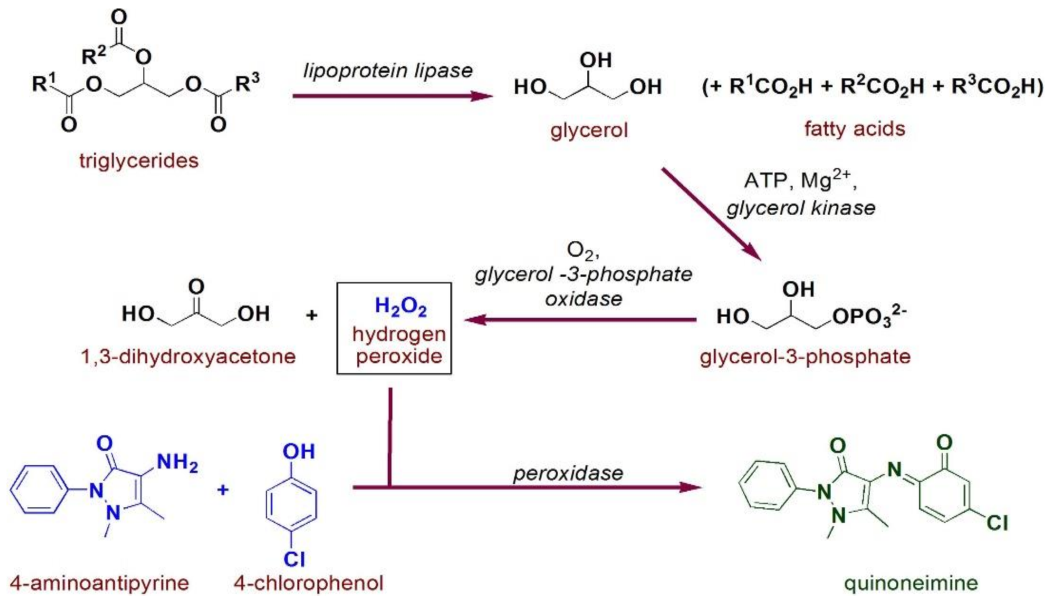
HDL < 40 mg/dL [1.04mmol/L] Low HDL Cholesterol

HDL ≥ 60 mg/dL [1.55mmol/L] High HDL Cholesterol

3. Triglyceride (TGL)

Principles of Procedure:

The change in absorbance of quinoneimine using a bichromatic (510, 700 nm) endpoint technique is directly proportional to the total amount of glycerol, which directly shows the amount of triglyceride in the sample measured.



Quality Control: At least once each day of use, two levels of a quality control material with known triglycerides concentrations were analyzed.

Result Interpretation:

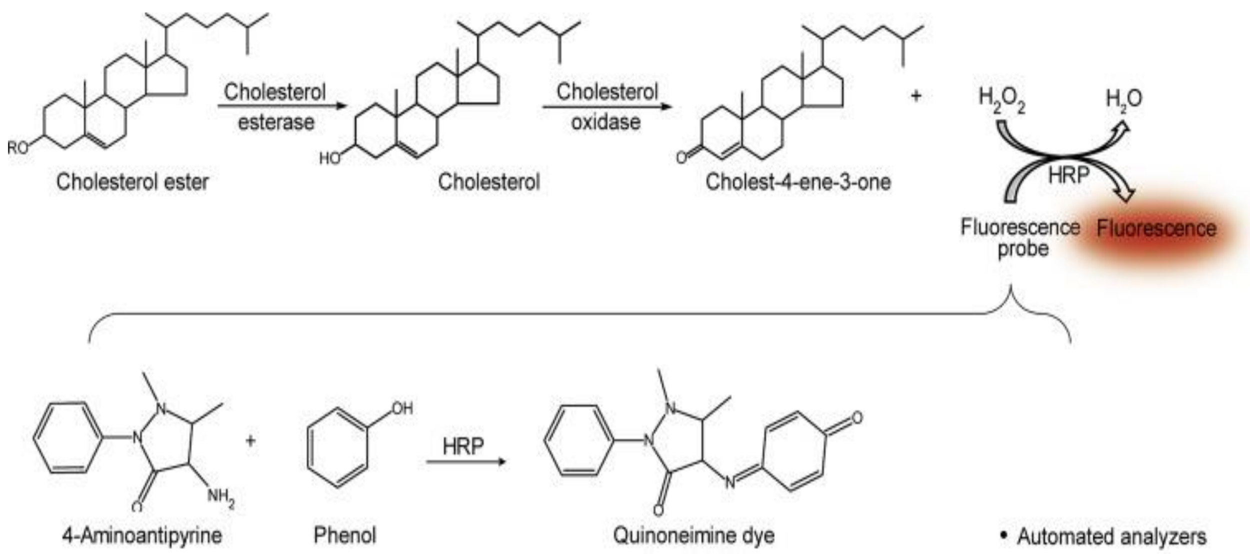
The National Cholesterol Education Program Adult Treatment Panel III (NCEP- ATP III) provides the following categories of triglycerides concentrations:

Normal (< 150 mg/dl), Borderline High (150-199 mg/dl), High (200-499 mg/dl) and Very High (≥ 500 mg/dl).

4. Total Cholesterol (TC)

Principles of Procedure:

The absorbance due to oxidized DEA-HCl/AAP is directly proportional to the total cholesterol concentration and is measured using a polychromatic (452, 540, 700 nm) endpoint technique.



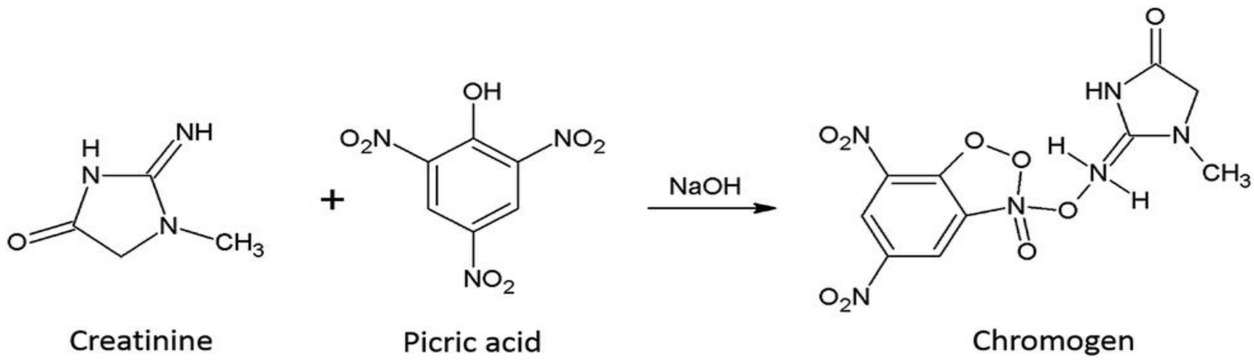
Quality Control: At least once each day of use, two levels of a Quality Control (QC) material with known cholesterol concentrations were analyzed.

Result Interpretation:

Desirable < 200 mg/dL, Borderline High 200 – 239 mg/dL and High ≥ 240 mg/dL.

5. Creatinine (CRE2)

Principles of Procedure: The CRE2 method uses a modified kinetic Jaffe technique. The rate of increasing absorbance at 510 nm due to the formation of this chromophore is directly proportional to the creatinine concentration in the sample and is measured using a bichromatic (510, 600 nm) rate technique. Bilirubin is oxidized by potassium ferricyanide4 to prevent interference.



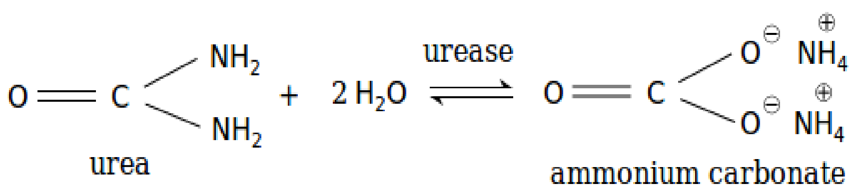
Quality Control: At least once each day of use, two levels of a Quality Control (QC) material with known creatinine concentrations were analyzed.

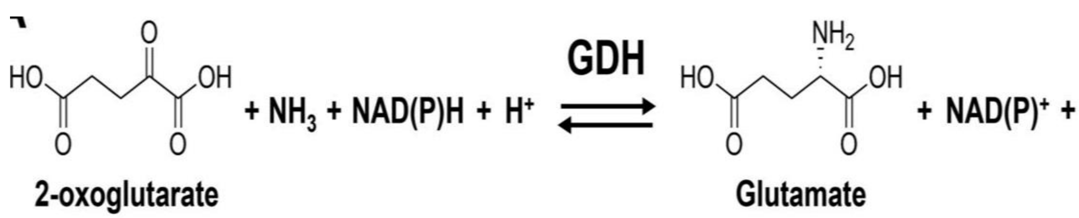
Result Interpretation (Males): 0.70 – 1.30 mg/dL [62 – 115 µmol/L]

6. UREA

Test Principle: based on the principle of Kinetic test with urease and glutamate dehydrogenase

In this reaction two moles of NADH are oxidized to NAD+ for each mole of urea hydrolyzed. The rate of decrease in the NADH concentration is directly proportional to the urea concentration in the specimen and is measured photometrically at 700/340nm.





Quality Control: At least once each day of use, two levels of a Quality Control (QC) material with known urea concentration were analyzed.

Expected values (Adults): 2.76-8.07 mmol/L (16.6-48.5 mg/dL)

Annex X: DECLARATION

I, the undersigned, declare that this M.Sc. thesis is my original work, has not been presented for a degree in this or any other university and that all sources of materials used for the thesis have been duly acknowledged.

M.Sc. candidate: Abush Getaneh (B.Sc.)

Signature: ______________________

Date: _____________/24________

This thesis has been submitted with our approval as advisors.

Advisor: Samuel Kinde (MSc, Assistant professor, PhD fellow)

Signature: ______________________

Date: _____________/24________

Place: Addis Ababa, Ethiopia.

Advisor: Mekdes Alem (MSc)

Signature: ______________________

Date: _____________/24________

Place: Addis Ababa, Ethiopia.