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Liver Function Tests and Fasting Blood Glucose level among Apparently Healthy Adult Male Khat Chewers in Dilla Town, Southern Ethiopia: A Comparative Study

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This is to certify that the thesis prepared by Abera Abreham, entitled**: Liver Function Tests and Fasting Blood Glucose levels among Apparently Healthy Adult Male Khat Chewers in Dilla Town, Southern Ethiopia: A Comparative Study** 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 concerning originality and quality.

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Table of Contents

List of Table

List of Figures

List of abbreviations/acronyms

A

Adrenocorticotropic hormone
ACTH, 2
Alanine transaminase
ALT, 1
Alkaline phosphatase
ALP, 1
Aspartate transaminase
AST, 1

B

Body mass index
BMI, 14

D

Diastolic blood pressure
DBP, 14
Direct bilirubin
DBI, 1

F

Fasting blood glucose
FBG, 15

K

Khat chewers
KC, 19

L

Liver function tests
LFTs, 1

N

Non-khat chewers
NKC, 19

S

Standard operating procedures
SOPs, 15
Statistical Package for Social Science
SPSS, 16
Systolic blood pressure
SBP, 14

T

Total bilirubin
TBI, 1
Total protein
TP, 1

W

Waist circumference
WC, 14
World Health Organization
WHO, 3

Abstract

Background: Khat is a natural psychostimulant herbal drug. Khat chewing is associated with adverse health problems such as diabetic mellitus and hepatotoxicity. The practice of khat chewing has risen among adults in Ethiopia over time, despite its potential health issues.

Objective: To determine liver function tests and fasting blood glucose level among apparently healthy adult male khat chewers in Dilla town, Southern Ethiopia, 2024

Methods: A community-based comparative cross-sectional study was carried out from June to September 2023 in Dilla town, Southern Ethiopia. Two hundred apparently healthy adult male participants (100 khat chewers and 100 non-khat chewers) were selected with a convenient sampling technique. Structured, pretested and translated questionnaires were used to collect study participants' data. Five millilitres of blood specimen was collected with a serum separator tube through an aseptic technique and centrifuged at 3000 rpm for 5 minutes to obtain the serum sample. Levels of liver function tests and fasting blood glucose were analysed using Siemen dimension EXL200 automated clinical chemistry analyser. The data were coded and entered into Epi-data version 4.6 and then exported to SPSS version 27 for analysis. Mann-Whitney test, Kruskal Wallis test and Spearman's correlation were used for statistical analysis. A p-value of less than 0.05 was considered statistically significant.

Results: Khat chewers showed a statistically significant increase in the levels of transaminase enzymes (ALT and AST) and FBG than non-khat chewers ($P < 0.001$, $P = 0.007$, and $P = 0.002$), respectively. However, there was no statistically significant difference in the levels of ALP ($P = 0.098$), TP ($P = 0.113$), DBI ($P = 0.139$) and TBI ($p = 0.095$) among khat chewers compared to non-khat chewers. Duration and frequency of khat chewing showed a significant and positive association with ALT, AST, and FBG. Additionally, there was a significant and positive correlation of systolic blood pressure with AST and FBG. Age, diastolic blood pressure and bundle of khat had a significant and positive association with ALT.

Conclusion: The present finding concluded that higher LFTs and FBG are associated with khat chewing depending on the duration, frequency and amount of bundle used.

Keywords*:* *Liver Function Tests, Khat Chewers***,** *Fasting Blood Glucose*

1. Introduction

1.1. Background

Kha*t (Catha edulis)* is a natural psychostimulant herbal drug comprising leaves and shoots. It consists of several active substances, of which cathinone is the principal and the predominant psychoactive ingredient found in khat's fresh leaves and twigs as confirmed by phytochemical chemical analysis [1, 2]. Khat is a green shrub mostly grown in Southern Arabia and East Africa. Ethiopia is a prominent khat producer country in Africa and globally due to its high khat cultivation, export and consumption rates [3].

The liver is a major organ responsible for the metabolism, detoxification and removal of substances from the body. It is a resilient organ that can repair or regenerate damaged cells. However, repeated and sustained damage to the liver results in irreversible changes and malfunction. The status of the liver is assessed and evaluated by liver function tests (LFTs) Total protein (TP), direct bilirubin (DBI) total bilirubin (TBI) and liver enzymes such as Aspartate transaminase (AST), Alanine transaminase (ALT) and Alkaline phosphatase (ALP) are the most requested liver function tests. Liver enzymes give information about the primary origin of the disease (hepatic or cholestatic). Total bilirubin (TBI), direct bilirubin (DBI) and Total protein (TP) are used to assess the functional capacity of the liver [4].

Exogenous substances have an impact on the principal "metabolic clearing house," the smooth endoplasmic reticulum of the liver, as they are washed away. The liver may be susceptible to the harmful effects of khat during the processes of metabolism, clearance and detoxification [5].

Regular and prolonged khat chewing inhibits the activities of cytochrome P450 enzymes, leading to a significant rise in reactive oxygen species. The reactive oxygen species induced by khat can harm liver cell membranes through lipid peroxidation, causing membrane permeability damage and leakage of cellular components into the bloodstream [6, 7].

The impact of khat on blood glucose levels remains inconclusive, with some studies indicating a hyperglycaemic effect and others suggesting a hypoglycaemic effect. Additionally, certain research has found that khat does not have a significant impact on blood glucose [8].

Cathinone elevates the levels of catecholamines in the plasma. As a result of its adrenergic effect, it hinders the action of insulin, leading to the secretion of glucagon and the activation of glycogenolysis in the liver (a response mediated by β-2 adrenoreceptors). It also stimulates the secretion of adrenocorticotropic hormone (ACTH) and suppresses the release of insulin (a response mediated by α-2 adrenoreceptors), ultimately increasing blood glucose levels [9, 10].

In our investigation of different search engines, we found no published studies on liver function tests and fasting blood glucose levels among khat chewers in the study area, Dilla town. However, published articles, including animal studies, have highlighted the significant influence of khat on blood glucose levels, ranging from hyperglycaemic to hypoglycaemic effects and its hepatotoxic effect. Hence, this study aims to shed light on the levels of liver function tests and blood glucose and associated factors among khat chewers in our study area.

1.2 Statement of the Problem

The World Health Organization (WHO) expert committee on drug dependence classified khat as an abuse drug in 2006, and those who abuse it may experience a variety of health problems [11].

Khat chewers have varying opinions on the health implications of khat consumption. While some believe it can lower and regulate blood sugar levels, others view it as harmless. Conversely, certain individuals perceive khat to have negative health consequences. The perception of khat as medically significant among khat users may exacerbate the health problems associated with khat chewing [12, 13].

Approximately 20 million individuals, primarily residing in the Horn of Africa and the Arabian Peninsula, engage in regular khat chewing [14]. Over time, the prevalence of khat chewing has increased in Ethiopia, and it has now become a widespread practice, which needs great attention, among adults in the country [15].

Scientific case reports have provided evidence that chronic khat chewing leads to chronic liver diseases. The following observations were observed that indicated the effects of khat among cases who chronically chew khat. Firstly, when individuals ceased chewing khat, their liver condition improved, but upon resuming khat consumption, further liver damage occurred. Secondly, an elevated level of cathinone, a key component of khat, was found in the blood of khat chewers, with no other identifiable cause for their liver condition [16-19].

Cathinone, the primary component of khat, has been shown to induce liver injury. Research carried out by Maria Joao Valente *et al.*, 2016 revealed that cathinone leads to hepatic damage through mitochondrial dysfunction and oxidative stress [20].

Research conducted in Yemen to evaluate the harmful effects of khat on the liver revealed that khat leads to liver damage. The researchers indicated that hepatotoxicity was prevalent in individuals who chew khat, with 67% of chronic khat chewers experiencing liver injury. The findings suggest that khat consumption may be a contributing factor to the development of liver injury among khat chewers [21]. The study conducted by Shahzad Riyaz et al. also stated the hepatotoxic of effect khat [18].

A study from Ethiopia in 2018 indicated the existence of a strong association and causal relationship between khat chewing and the development of liver diseases. According to the study, khat chewers had a 2.64-fold greater risk of developing liver diseases than non-khat chewers [22].

A study conducted in the Jazan region of Saudi Arabia indicated a significant association of khat chewing with diabetes development. The research found that khat chewers had more than a three-fold greater risk of developing diabetes than non-khat chewers [23]. Another study conducted in Yemen also considered khat as a risk factor for the development of diabetes mellitus [24]. Moreover, a study conducted in the Jazan region of Saudi Arabia demonstrated the aggravation of hyperglycaemia among habitual khat chewers [25].

WHO and Ethiopian Public Health Institute report on non-communicable disease risk factors and the prevalence of specific non-communicable diseases stated that khat chewing is the risk factor for hyperglycaemia [26].

1.3. Significance of the Study

Limited studies and contradictory findings of liver function tests and serum blood glucose levels among khat chewers indicate the necessity for further investigation.

The findings of the present study will assist clinicians in the clinical management of liver function and blood glucose among khat chewers.

The potential health problems of khat are less emphasized in Ethiopia and this study will give more awareness on the health impacts of khat on the liver and blood glucose. Furthermore, the associated factors found in the current study will have an advantage in how khat chewing education is designed, targeted, and implemented to prevent health issues related to khat chewing.

In the scarce data situation on khat's health impacts in Ethiopia, this study provides helpful information about the health impacts of khat.

Moreover, the results of this study will be evidence for further studies.

2. Literature Review

2.1. Khat Chewing and Level of Liver Function Tests

An animal model study conducted on 36 cases and 12 controls of Sprague Dawley rats revealed that khat-fed rats showed significantly increased serum AST and ALP of male rats at 2000mg/kg dose of khat compared to that of the control. According to the study as the dose of khat rises, so does the level of toxicity [27].

AST and ALT activities were considerably elevated in an experimental investigation performed on forty Swiss albino mice that were three weeks old and treated for ninety days. The albino mice fed khat also showed higher total bilirubin concentrations. The research concluded that compounds present in khat such as cathine and cathinone were responsible for the observed abnormalities in the liver function tests [28].

An experimental study conducted on a twenty-four white rabbit over six months to assess the toxicological effect of khat showed a significant increase in plasma levels of ALP, ALT, and AST with all levels of khat leaves tested and throughout the treatment period. TBI was also observed to be affected by all levels of khat leaves in a dose-dependent manner [29]. The study conducted for three months also showed a significant increase in levels of ALP and ALT with all levels of khat leaves tested and throughout the treatment period. In contrast to what was seen in the short-term effect conducted by the same authors for three months, the ALT and AST Enzymes were significantly more noticeable in the long-term effect [30].

An animal study on 35 adult Wistar rats for four weeks showed that crude execrates of khat affect liver function tests. The results revealed that Serum levels of AST, ALT, and ALP were significantly elevated in khat-fed rats while the TP was significantly decreased in khat-fed rats. The concentration of TBI was also significantly increased in the serum of rats treated with khat compared to the control group. The study indicated that the dose is strongly and linearly associated with the liver function tests [31].

A comparative cross-sectional study was conducted in the Jazan region of the Kingdom of Saudi Arabia in 2020 among 38 khat chewers and 20 non-khat chewers to assess the effects of Khat Chewing on liver function tests. According to the study, there was a significant increase in the levels of ALT and ALP among khat chewers when compared to non-khat chewers. The DBI and

TBI had no significant variation among the two groups. However, the level of TP was significantly lower among khat chewers than non-khat chewers [32].

A cross-sectional study done in Kenya among 391 subjects (198 khat chewers and 193 non-khat chewers) in 2017 to determine whether khat has an impact on the levels of liver function showed a significantly higher level of ALP among khat chewers than non-chewers. Significantly lower serum levels of TP and DBI were observed among khat chewers compared to non-khat chewers. There was no significant difference in the levels of AST and ALT among khat chewers compared to non-khat chewers. The study also indicated that ALT increases among red variety khat chewers than pale green type[33, 34].

A case-control study done in Yemen among 30 khat chewers and 20 non-khat chewers in 2013 indicated that the duration of khat chewing has a direct association with the severity of the hepatic problem. According to the study, there was no pronounced difference between khat chewers and non-khat chewers' levels of AST, ALT and TP. However, chewers who had been chewing khat for more than ten years had higher ALT. This change in the ALT enzyme's activity may indicate that liver damage increases with continued khat chewing for a long period [35].

In 2014 a cross-sectional study was conducted among 50 khat chewers and 50 non-khat chewers male population of the Jazan region of the Kingdom of Saudi Arabia and showed that khat has an impact on the level of liver function tests. The findings of the study showed that the activities of ALT and AST Enzymes were statistically significantly increased in the serum of Khat chewers compared to non-khat users. The study revealed no significant difference in the levels of TP and TBI among khat chewers compared to non-khat chewers [36].

2.2. Khat Chewing and Fasting Blood Glucose Level

Twenty non-khat chewers and fifty khat chewers in Yemen participated in a cross-sectional study in 2012 to determine their blood glucose levels. As compared to non-khat chewers, the study found that khat chewers had a statistically significant lower level of fasting blood glucose [37].

A cross-sectional study was conducted in Yemen in 2022 among 22 apparently healthy khat chewers and 16 non-khat chewers. The study showed statistically significantly higher mean fasting blood glucose among khat chewers as compared to non-khat chewers [38].

A study conducted among 20 apparently healthy khat chewers and 20 apparently healthy non-khat chewers in Egypt indicated significantly higher median of fasting blood glucose among khat chewers when compared to that of non-khat chewers. The study concluded that khat chewing raises insulin resistance because of elevated levels of resistin and its cathinone-induced catecholamine secretion, which raises fasting blood glucose level and lowers insulin level [39].

A hospital-based comparative cross-sectional study was conducted among 100 khat chewers (50 apparently healthy and 50 diabetics) and 100 non-khat chewers (50 apparently healthy and 50 diabetics) in Ethiopia in 2020. The study compared fasting blood glucose levels among khat chewers and non-khat chewers and found that khat chewing has a hypoglycaemic effect, khat chewing appeared to be associated with a statistically significant reduction in fasting blood glucose levels as compared to non-chewing khat users [40].

In 2015, a community-based cross-sectional study was conducted in two city administrations and nine regions of Ethiopia to assess the impact of khat chewing on the level of clinical biomarkers in the blood. According to the authors, those who chew khat have a median fasting blood glucose level that is significantly greater than that of non-chewers. They concluded that khat chewing raised blood glucose levels [41].

A cross-sectional study conducted in Yemen in 2003 among 16 apparently healthy male non khat chewers and 20 apparently healthy khat chewers showed non significance difference in fasting blood glucose levels among khat chewers compared to non khat chewers [42].

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2.3. Conceptual Framework

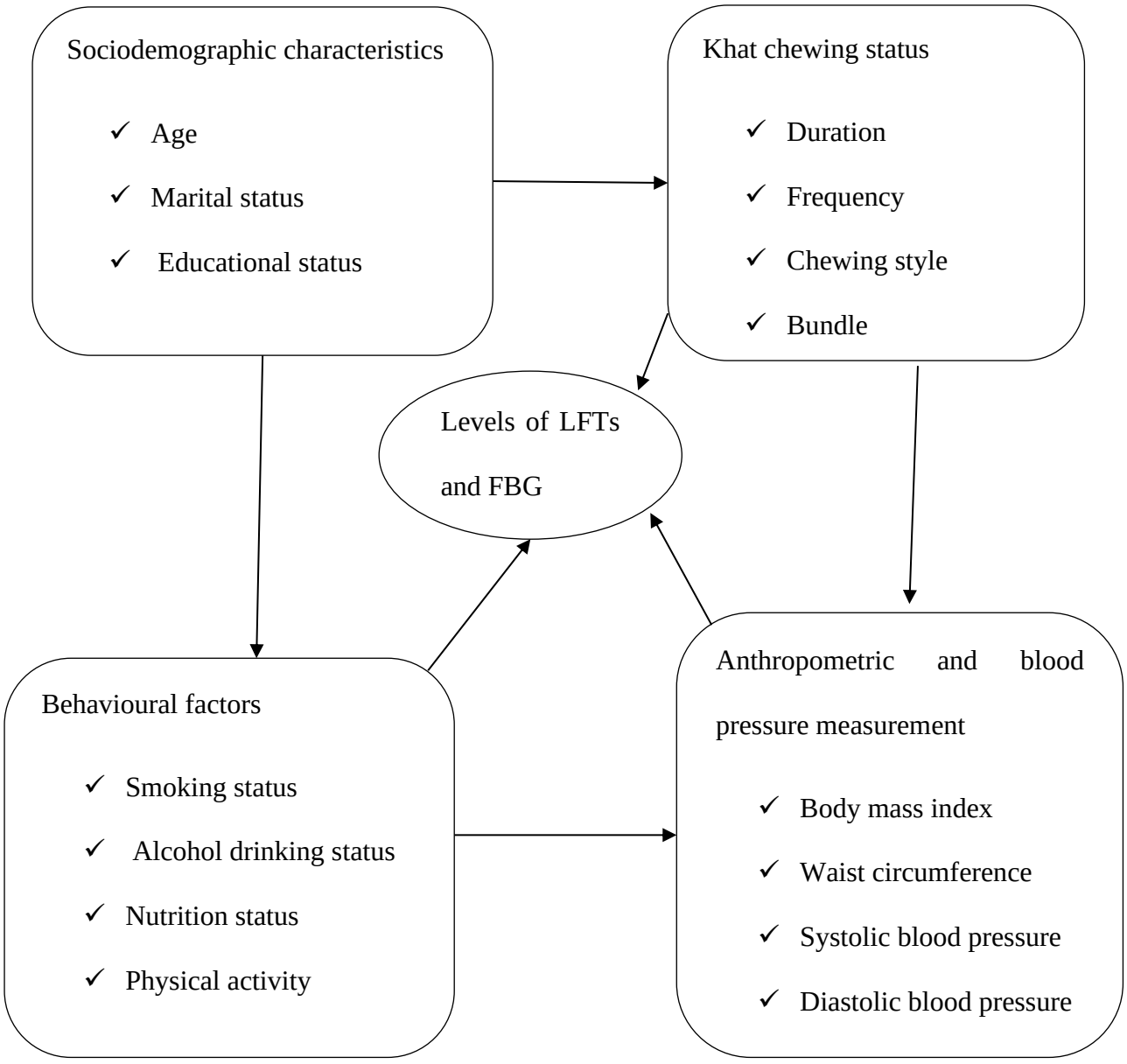

Figure 1: Conceptual framework

3. Objectives

3.1. General Objective

- ✓ To determine liver function tests and fasting blood glucose levels among apparently healthy adult male khat chewers in Dilla town, Southern Ethiopia from June to September 2023

3.2. Specific Objectives

- ✓ To compare liver function tests and fasting blood glucose levels among apparently healthy adult male khat chewers and non-khat chewers in Dilla town, Southern Ethiopia from June to September 2023
- ✓ To assess associated factors with liver function tests and fasting blood glucose levels among apparently healthy adult male khat chewers in Dilla town, Southern Ethiopia from June to September 2023

4. Hypothesis

4.1. HO -There is no statistically significance difference in the liver function tests and fasting glucose levels among apparently healthy adult male khat chewers and non-khat chewers.

5. Methods and Materials

5.1. Study Area

Dilla town, the administrative centre of the Gedeo zone, was our study area. It is located at an elevation of 1570 metres above sea level and approximately 360 kilometres south of Addis Ababa, the capital of Ethiopia. The town's geographical locations are 38° 18' 30"E latitude and 6° 24' 30" N longitude. It is crossed by the main road that links Ethiopia to its southern neighbour, Kenya. Based on the 2023 Central Statistical Agency of Ethiopia, the total population of the town is 166,067 (83,716 male and 82,351 female) [43].

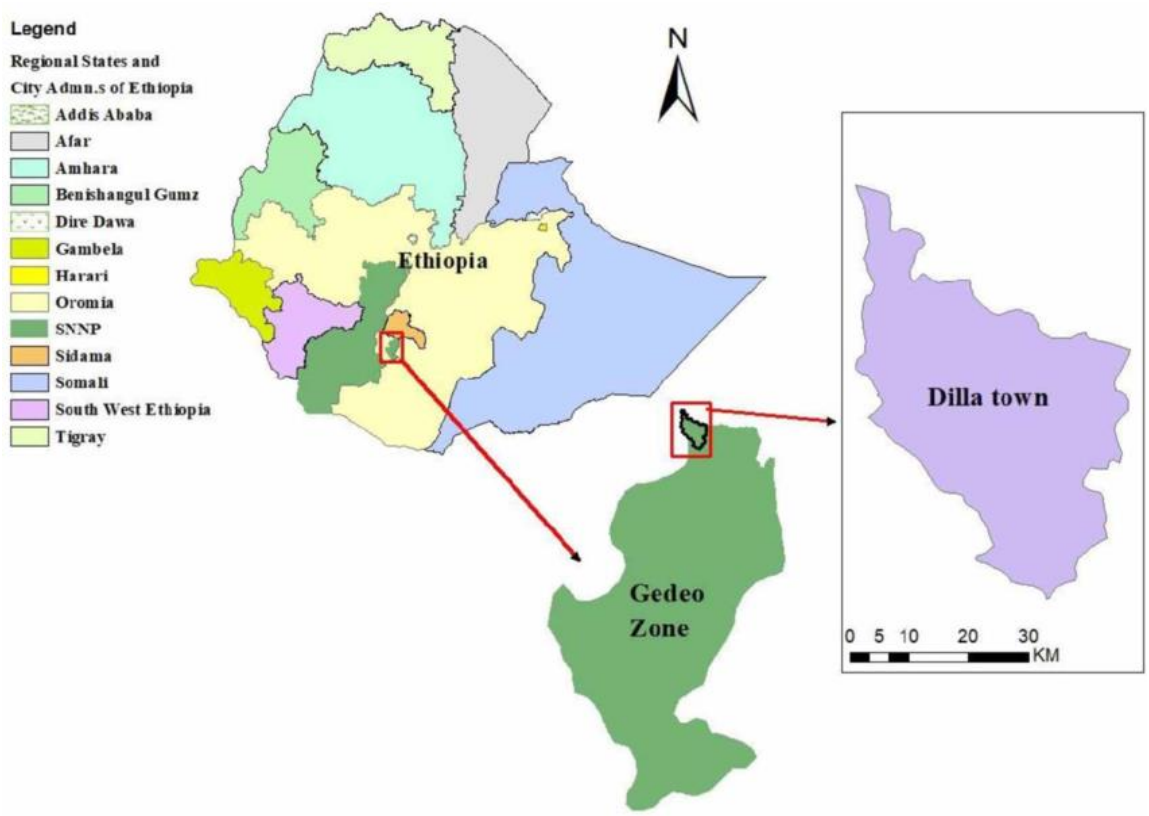

Figure 2: Location of Dilla town

5.2. Study Design and Period

A community-based comparative cross-sectional study was conducted in Dilla town from June to September 2023.

5 .3. Population

5.3.1. Source Population

All apparently healthy adult male khat chewers and non-khat chewers residing in Dilla town.

5.3.2. Study Population

All apparently healthy adult male khat chewers and non-khat chewers residing in Dilla town and fulfilled the inclusion criteria.

5.4. Inclusion and Exclusion Criteria

5.4.1. Inclusion Criteria

- ✓ Adult male khat chewers and non-khat chewers who are apparently healthy
- ✓ Subjects who were willing to voluntarily participate in the study
- ✓ Aged 18 -60 years old
- ✓ Living in Dilla town at least for six months

5.4.2 Exclusion Criteria

- ✓ Those who were under medication
- ✓ Individuals who were not fasting in the morning
- ✓ Individuals who had a history of diabetes mellitus, hypertension, renal and liver health problems

5.5. Study Variables

5.5.1. Dependent Variables

- ✓ Levels of liver function tests and fasting blood glucose

5.5.2. Independent Variables

- ✓ Khat chewing characteristics
- ✓ Socio-demographic characteristics
- ✓ Diastolic and systolic blood pressure
- ✓ Anthropometric measurements
- ✓ Nutrition status
- ✓ Physical activity
- ✓ Alcohol consumption status
- ✓ Cigarette smoking status

5.6. Sample Size Determination and Sampling Technique

5.6.1. Sample Size Determination

The sample size was calculated using two population sample size calculation formula [44]

$n=\frac{r+1}{r}.\frac{sd^2(Z\alpha/2+Z\beta)}{d^2}$ for equal ratio- $\frac{2\ sd^2(Z\alpha/2+Z\beta)}{d^2}$

Where: Sd = Standard deviation

d = expected difference of mean of the two groups

r = Ratio of control to cases

$Z\beta$ = Standard normal variate for power - for 80% power it is 0.84

Za/2 = 1.96

Depending on the study conducted in Yemen [35]

$n=\frac{1+1}{1}\frac{(13.306)^2(1.962+0{,}84)}{(6.2)^2}=73$

A total of 81 sample size was obtained after adding a 10% non-response rate. Since a larger sample size increases the power to identify differences, 100 khat chewers and 100 non-khat chewers were chosen.

5.6.2. Sampling Method

A convenient sampling method was applied to select study participants. From three kebeles of Dilla town, where khat shopping houses and khat chewers were available, one hundred apparently healthy adult male khat chewers participated after providing detailed information about the study and confirming their voluntarism. Additionally, one hundred apparently healthy adult male non-khat chewers were selected from the surroundings of khat shopping houses and Dilla University general hospital staff.

5.7. Measurement and Data Collection

5.7.1. Demographic and Clinical Data Collection

The study participants' data were collected with a structured, pretested, and translated questionnaire. The study participants were directly interviewed by data collectors to complete the questionnaire. The height, weight, waist circumference (WC) and blood pressure were measured with the standard procedures by experienced personnel. Body mass index (BMI) was calculated by dividing weight by height squared. The systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured with the Riester RBP-100 machine.

5.7.2. Blood Specimen Collection

After fasting for 8-12 hours, about 5mL of blood sample was collected by a laboratory technologist with a serum separator tube from each participant through an aseptic/sterile technique. Blood samples were collected at a nearby clinic to Khat shopping houses and at Dilla University general hospital.

5.7.3. Blood Sample Processing and Analysis

The serum was separated from the collected sample by centrifuging at 3000 rpm for five minutes after 30 minutes of blood sample collection. The serum sample was transported to Dilla University general Hospital with an ice pack and stored at -86℃ (deep freezer) until analysis. The serum levels of liver function tests and fasting blood glucose were analysed using Siemen EXL200 chemistry analysers at Dilla University general hospital.

5.7.4. Laboratory Analysis

5.7.4.1. Clinical Chemistry Analyzer-

Siemens EXL200 integrated system (ELSMED Healthier Diagnostics PLC, A German company)

5.7.4.2. Liver Function Tests Analysis

Liver function test includes measurement of the level of AST, ALT, ALP, DBI and TBI in the serum sample. The analysis was done by the principle of spectrophotometry for measuring the absorption spectrum of the analyte at each wavelength.

5.7.4.3. Fasting Blood Glucose (FBG) Level Analysis

Fasting blood glucose level analysis is a blood test that serves as an initial screening tool for abnormalities in blood glucose. It is an in vitro diagnostic test intended for the quantitative determination of glucose in human serum. The hexokinase method is the generally accepted reference method for measuring glucose.

5.8. Data Quality Assurance

The principal investigator was under close contact and supervision throughout the data collection process and conducted the laboratory analysis. The data were cleaned and entered into Epidata version 4.6 and checked for completeness. As part of laboratory quality assurance, pre-analytical, analytical, and post-analytical procedures were maintained.

5.8.1. Pre-Analytical

To ensure that the participants could comprehend the questions during the interview, The questionnaire was translated from English into Amharic and back again. A pretest of a questionnaire was conducted on 5% of study participants before the actual data collection. Samples of blood were collected, transported, and stored according to standard operating procedures (SOPs). The questionnaire was designed with a unique identification number.

5.8.2. Analytical

The participant's samples were analysed after the normal and pathologic controls were conducted and approved (passed),

5.8.3. Post-Analytical

The results that were acquired were accurately recorded and documented on the data collection sheet.

5.9. Data Analysis and Interpretation

The data were coded and entered into Epi-Data version 4.6 and then exported and analysed using Statistical Package for Social Science (SPSS) version 27. The frequency of both khat chewers and khat chewers' data was analysed using descriptive statistics. Mann-Whitney test was used to compare the levels of liver function tests and fasting blood glucose among khat chewers and non-khat chewers. Kruskal-Walis test was used to compare the levels of liver function tests and fasting blood glucose levels among variables that have three or more categories. The correlation of liver function tests and fasting blood glucose level with continuous independent variables was assessed using Spearman's correlation. The difference in categorical variables between khat chewers and non-khat chewers was analysed using Fisher's exact test. The results of the analysis were presented with tables and figures, where appropriate. At a 95% confidence level, a p-value of less than 0.05 was considered statistically significant.

5.10. Ethical Considerations

The study was commenced after ethical clearance for the study was obtained from the Department of Medical Laboratory Science Research and Ethical Review Committee (DRERC), College of Health Science, Addis Ababa University. Permission for informants' participation was first obtained from the Dilla town health and local administrative offices. We obtained permission from the clinic and Dilla University general hospital to collect and analyse the sample. Informed consent was obtained from all the study participants, which was confirmed by the signing of the consent form. There was assurance of the data's confidentiality

5.11. Dissemination of Result

The results of the study were submitted and presented to the Department of Medical Laboratory Science at Addis Ababa University. The outcome of the study will be used by the Dilla town health office. Furthermore, the findings will be published in reputable journals and will be presented at different research conferences.

5.12. Operational Definitions

Adult: an individual whose age is from 18-60 years

Apparently Healthy: those who do not have a history of any systemic disease

Fasting blood glucose level: the level of glucose used to measure blood glucose concentration after fasting for 8-12hrs

Khat Chewer: An Individual who chews khat at least for a year and 2 or more times a week

Liver Function Tests: are groups of blood tests that help diagnose and treat people with liver impairment (AST, ALT, ALP, TP, DBI and TBI).

Low Fruit Consumption:1-3 days per week

Non-Khat Chewers: those who never chew khat in their lifetime.

Sufficient Fruit Consumption: 4-7 days per week

6.Work Flow

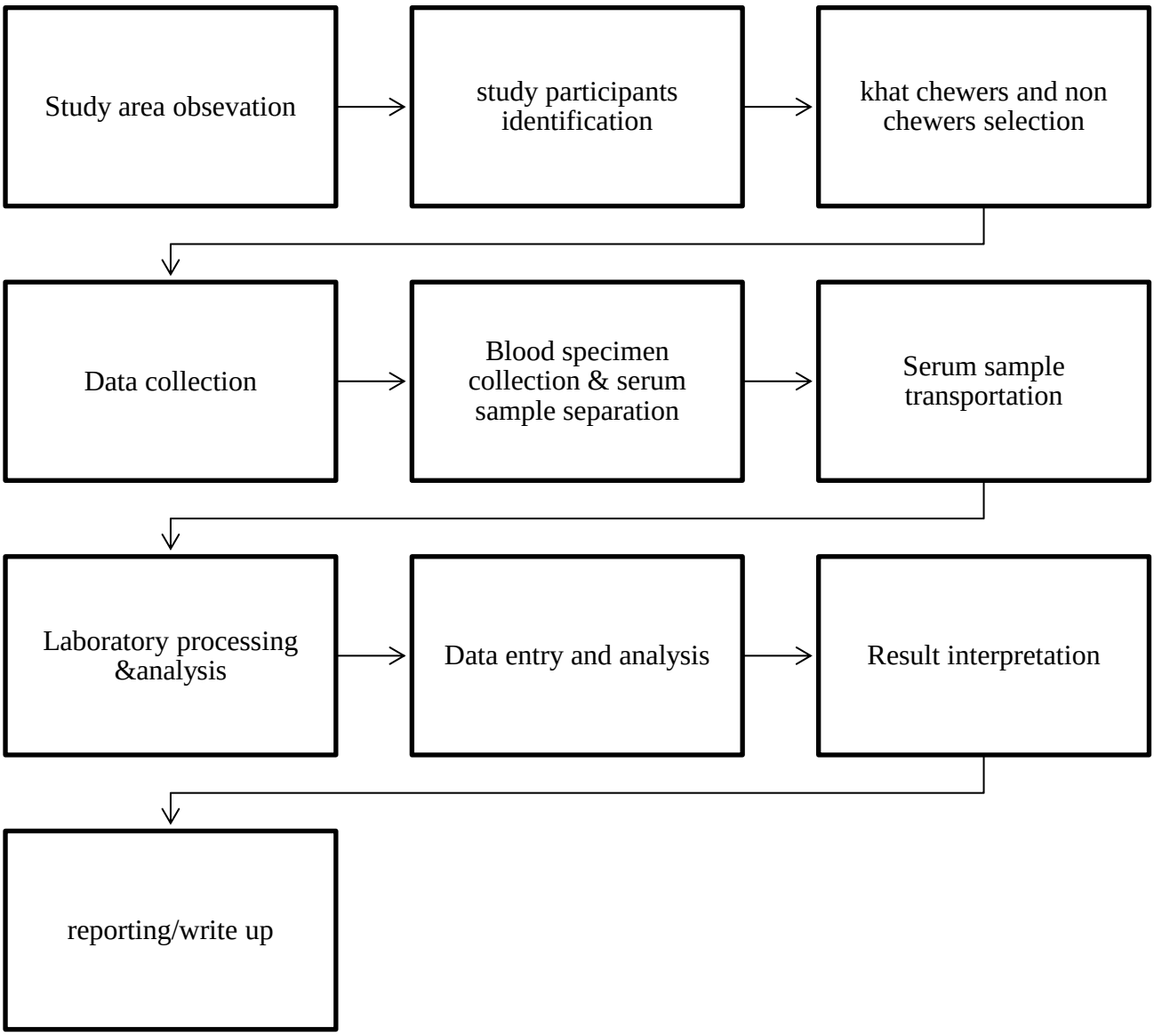

Figure 3: Workflow

7. Results

7.1. General Characteristics of Study Participants

This study involved two hundred (200) apparently healthy adult male study participants (100 khat chewers and 100 non-khat chewers). The median (±IQR) age of khat chewers and non-khat chewers were 36±12 years and 34±14 years, respectively (Table 1)**.**

Table 1: Socio-demographic and behavioural characteristics of study participants in Dilla town, Southern Ethiopia, 2024 (N=200).

Variables	Categories	KC (n = 100)	NKC (n = 100)	Total (%)	P-value*
Age	18–30	22 (22%)	35(35%)	57 (28.5)	
	31–43	56 (56%)	46 (46%)	102 (51)	0.13
	≥ 44	22 (22%)	19 (19%)	41(20.5)	
Educational status	No formal ed.	11 (11%)	12 (12%)	23 (11.5)	0.38
	Primary school	68 (68)	59 (59%)	127(63.5)	
	≥Secondary school	21 (21%)	29 (29%)	50 (25)	
Marital status	Single	25 (25%)	22 (22%)	47 (23.5)	
	Married	70(70%)	75 (75%)	145(72.5)	0.64
	divorced	5 (5%)	3 (3%)	8 (4)	
Smoking status	Yes	18 (18%)	11 (11%)	29 (14.5)	0.23
	No	82 (82%)	89 (89%)	171(85.5)	
Alcohol consumption	Yes	37 (37%)	29 (29%)	66 (33)	0.29
	No	63 (63%)	71 (71%)	134 (67)	
Physical Activity	Vigorous	13 (13%)	12 (12%)	25 (12.5)	
	Moderate	12 (12%)	18 (18%)	30 (15)	0. 07
	Less active	12 (12%)	23 (23%)	25 (12.5)	
	Sedentary	63 (63%)	47 (47%)	110 (55)	
Dairy product consumption	< 3times / week	72 (72%)	62 (62%)	134 (67)	
	≥ 3times / week	28 (38%)	38 (38%)	66 (33)	0.18
Fruit/vegetable consumption	Low	61 (61%)	60(60%)	121(60.5)	
	Sufficient	39 (39%)	40(40%)	79 (39.5)	0.89

KC: - khat chewers, NKC: -Non-khat chewers, *: -fisher's exact test

7.2. Anthropometric and Blood Pressure Measurements Among Study Participants

Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were significantly increased among khat chewers than non-khat chewers.

Table 2: Anthropometric and blood pressure measurements among study participants in Dilla town, Southern Ethiopia, 2024 (N=200)

Parameters	Khat chewers	Non-khat chewers	p-value
	median ± IQR	Median ± IQR	
SBP (mmHg)	114 ±11 mmHg	107 ±13mmHg	< 0.001
DBP (mmHg)	75±8 mmHg	72±7 mmHg	0.001
BMI (kg/m$^{2)}$	22.3 ±2.98 kg/m^2	22 ±2.25 kg/m^2	0.350
WC (cm)	82 ±7 cm	81 ±5cm	0.108

BMI: -body mass index, DBP: -diastolic blood pressure, IQR; - interquartile range, SBP: -systolic blood pressure, WC: waist circumference

7.3. Khat Chewing characteristics among Khat Chewers

Forty-four (44%) of khat chewers chewed for 8-14 years, while Forty-eight (48%) chewed daily. In addition, 70(70) of the khat chewers chewed less than one bundle per day (Table3).

Table 3: Khat chewing status among khat chewers in Dilla, Southern, Ethiopia, 2024 N (100)

Characteristics	Category	N (%)
Duration (years)	≤7 yrs.	24 (24%)
	8-14yrs.	44 (44%)
	≥ 15yrs.	32(32%)
Frequency (days per week)	≤ 6 days per week	52(52%)
	7 days	48(48%)
Bundle per day	<1 bundle per day	70(70%)
	≥1 bundle per day	30(30%)
Chewing style	Water	59(59%)
	Soft drinks	13(8%)
	Peanut	17(17%)
	Others (milk, coffee, tea)	11(11%)

7.4. Comparison of Liver Function Tests and Fasting Blood Glucose Level among Khat Chewers and Non-Khat Chewers.

There was a statistically significant increase in the levels of transaminase enzymes (AST and ALT) and FBG among khat chewers compared to non-khat chewers. The levels of ALP, TP, DBI, and TBI were not statistically significantly varied among khat chewers compared to non-khat chewers (Table 4).

Table 4: Comparison of LFTs and FBG among khat chewers and non-khat chewers in Dilla town, Southern Ethiopia, 2024 (N=200).

Parameters	Khat chewers	Non-khat chewers	P-value
	median ± IQR	Median ± IQR	
AST (IU/L)	30.50±10	27±10	**0.007**
ALT(IU/L)	30±16	25±10	**<0.001**
AST: ALT	1.06±0.46	1.1±0.5	0.254
ALP (IU/L)	81.5±33	77±24	0.098
TP (g/dl),	7±0.9	7.2±0.9	0.113
DBI (mg/dl),	0.1±0.1	0.1±0.1	0.139
TBI (mg/dl),	0.5±0.3	0.5±0.3	0.095
FBG (mg/dl),	83±11	79.5±10	**0.002**

AST: - aspartate aminotransferase, ALT: - alanine aminotransferase, ALP: - alkaline phosphatase, TP: - total protein, DBI: - direct bilirubin, TBI: - total bilirubin, FBG: - fasting blood Glucose, ref: - reference, IQR: -Inter quartile range, KC: - khat chewers, NKC: - Non-khat chewers. Mann-Whitney test was used for comparison.

7.5. Correlations of Liver Function Tests and Fasting Blood Glucose Level with Anthropometric Measurement, Blood Pressure and Age of Study participants

SBP was weakly and positively correlated with AST, ALT and FBG among khat chewers. Age had a moderate and positive correlation with ALT levels, while DBP had a weak correlation with ALT levels among khat chewers (Table 5).

Table 5: Correlations of LFTs and FBG level with anthropometric measurement, blood pressure and age among study participants in Dilla town, Southern Ethiopia, 2024 (N=200).

Parameters	Study groups	AST (IU/L) rho (p)	ALT (IU/L) rho (p)	ALP (IU/L) rho (p)	TP (g/dl) rho (p)	DBI (mg/dl) rho (p)	TBI (mg/dl) rho (p)	FBG (mg/dl) rho (p)
SBP (mmHg)	KC (n=100)	0.201 **(0.045)**	0.341 **(<0.00)**	0.236 (0.018)	0.96 (0.340)	0.071 (0.482)	0.122 (0.225)	0.262 **(0.008)**
	NKC(n=100)	0.022 (0.825)	-0.035 (0.729)	-0.016 (0.875)	-0.54 (0.594)	-0.060 (0.552)	0.053 (0.599)	0.116 (0.252)
DBP (mmHg)	KC(n=100)	0.139 (0.169)	0.310 **(0.002)**	0.200 (0.046)	0.119 (0.238)	0.005 (0.962)	0.052 (0.604)	0.157 (0.118)
	NKC(n=100)	0.265 (0.008)	-0.138 (0.172)	-0.033 (0.748)	-0.011 (0.912)	-0.050 (0.621)	-0.070 (0.487)	0.135 (0.182)
BMI kg/m^2	KC(n=100)	0.189 (0.05)	0.152 (0.132)	0.005 (0.964)	-0.42 (0.679)	0.077 (0.446)	0.024 (0.811)	0.047 (0.645)
	NKC(n=100)	0.043 (0.668)	-0.056 (0.581)	0.065 (0.519)	0.044 (0.664)	0.041 (0.686)	0.002 (0.987)	-0.042 (0.675)
WC (cm)	KC(n=100)	0.011 (0.914)	0.050 (0.623)	-0.019 (0.850)	-0.116 (0.249)	0.059 (0.561)	-0.036 (0.719)	-0.048 (0.634)
	NKC(n=100)	0.049 (0.631)	-0.110 (0.277)	0.023 (0.821)	0.118 (0.242)	-0.111 (0.274)	-0.012 (0.909)	0.034 (0.734)
Age (years)	KC(n=100)	0.75 (0.460)	**0.403 (<0.001)**	0.40 (0.693)	-0.147 (0.144)	**0.222 (0.026)**	0.165 (0.101)	0.183 (068)
	NKC(n=100)	-0.144 (0.153)	0.113 (0.264)	0.153 (0.130)	0.238 (0.017)	0.105 (0.297)	-0.044 (0.663)	0.145 (0.151)

7.6. Correlations of LFTs and FBG Levels with Duration and Frequency of Khat Chewing and Bundle of Khat

Duration of khat chewing was strongly and positively correlated with ALT and weakly associated with AST and FBG. The frequency of khat chewing had a moderate and positive correlation with ALT and a weak correlation with FBG. Bundle of khat was very weakly and positively associated with ALT (Table 6).

Table 6: Correlations of LFTs and FBG with duration of khat chewing, frequency khat chewing and bundle of khat used among khat chewers in Dilla town, Southern Ethiopia, 2024 (n=100)

parameters	Khat chewing characteristics		
	Duration	Frequency	Bundle
	rho(p-value)	rho(p-value)	rho(p-value)
AST (U/L)	0.249 **(0.012)**	0.156 (0.121)	0.116 (0.250)
ALT(U/L)	0.679 **(<0.001)**	0.488 (<**0.001**)	0.323 **(<0.001)**
ALP(U/L)	0.089 (0.377)	0.044 (0.664)	-0.083(0.411)
TP(g/dl)	-0.11 (0.912)	-0.109 (0.281)	-0.024 (0.811)
DBI (mg/dl)	0.137 (0.173)	0.083 (0.413)	0.118 (0.241)
TBI (mg/dl)	0.111 (0.273)	0.077 (0.448)	0.133 (0.187)
FBG (mg/dl)	0.282 **(0.004)**	0.242 **(0.015)**	0.102 (0.344)

Rho: - spearman correlation coefficients, AST: - aspartate amino transferase, ALT: - alanine amino transferase, ALP: - alkaline phosphatase, TP: - total protein, DBI: - direct bilirubin, TBI: - total bilirubin, FBG: - fasting blood Glucose

7.7. The Association of Khat Chewing Duration with AST, ALT and FBG

The duration of khat chewing had a positive association with transaminase enzymes (AST and ALT) and fasting blood glucose levels. As the duration of khat chewing increased, the levels of transaminase enzymes and FBG also increased (Figure 4).

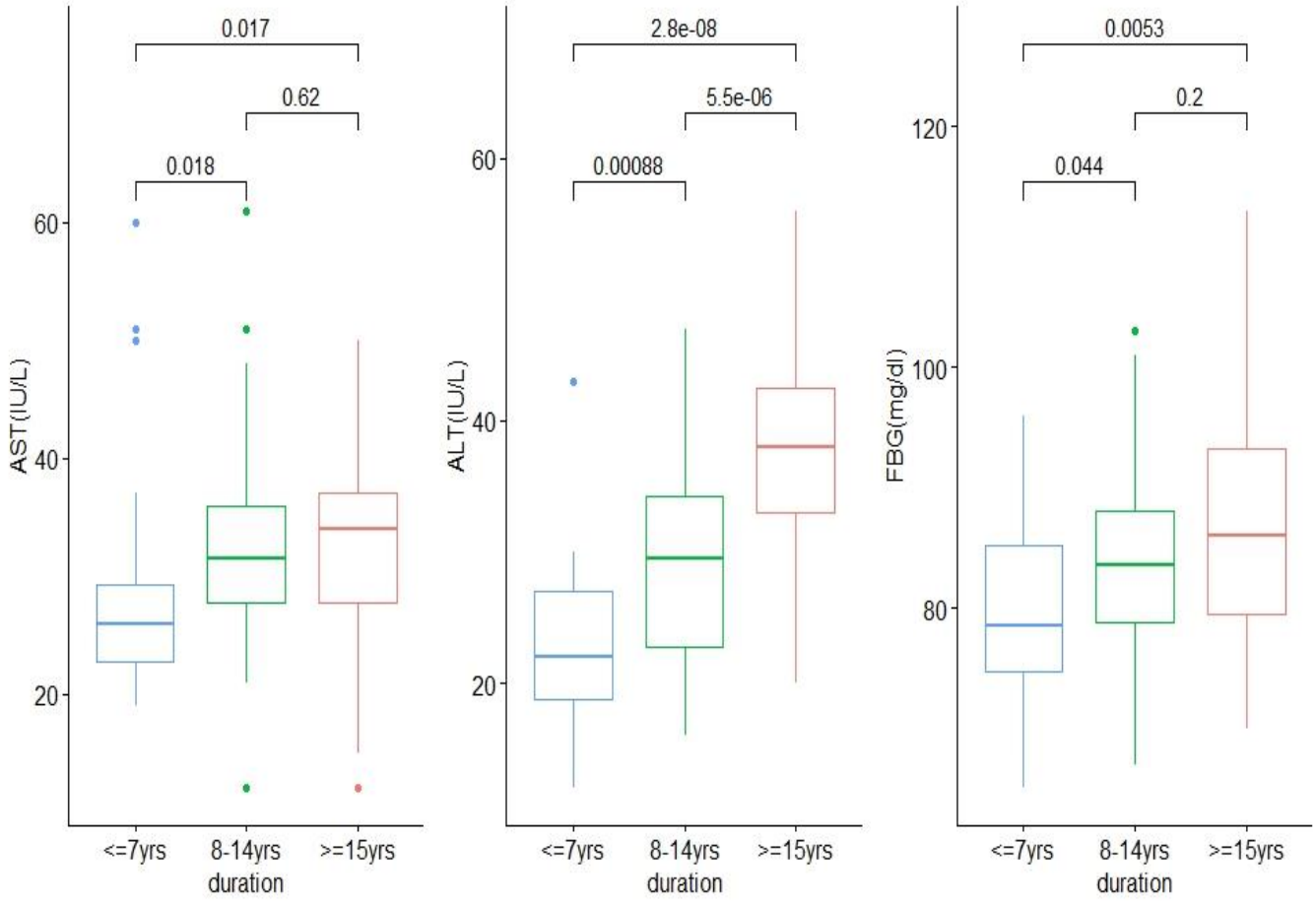

Figure 4: Effect of khat chewing duration on AST, ALT and FBG

*Mann-Whitney test was used to compare AST, ALT and FBG in each group of duration and analysed with R version 4.4.0 software

7.8. The Association of Khat Chewing Duration With ALP, TP, DBI And TBI

There was no statistically significant difference in ALP, TP, DBI and TBI levels among each duration of khat chewing duration (Figure 5).

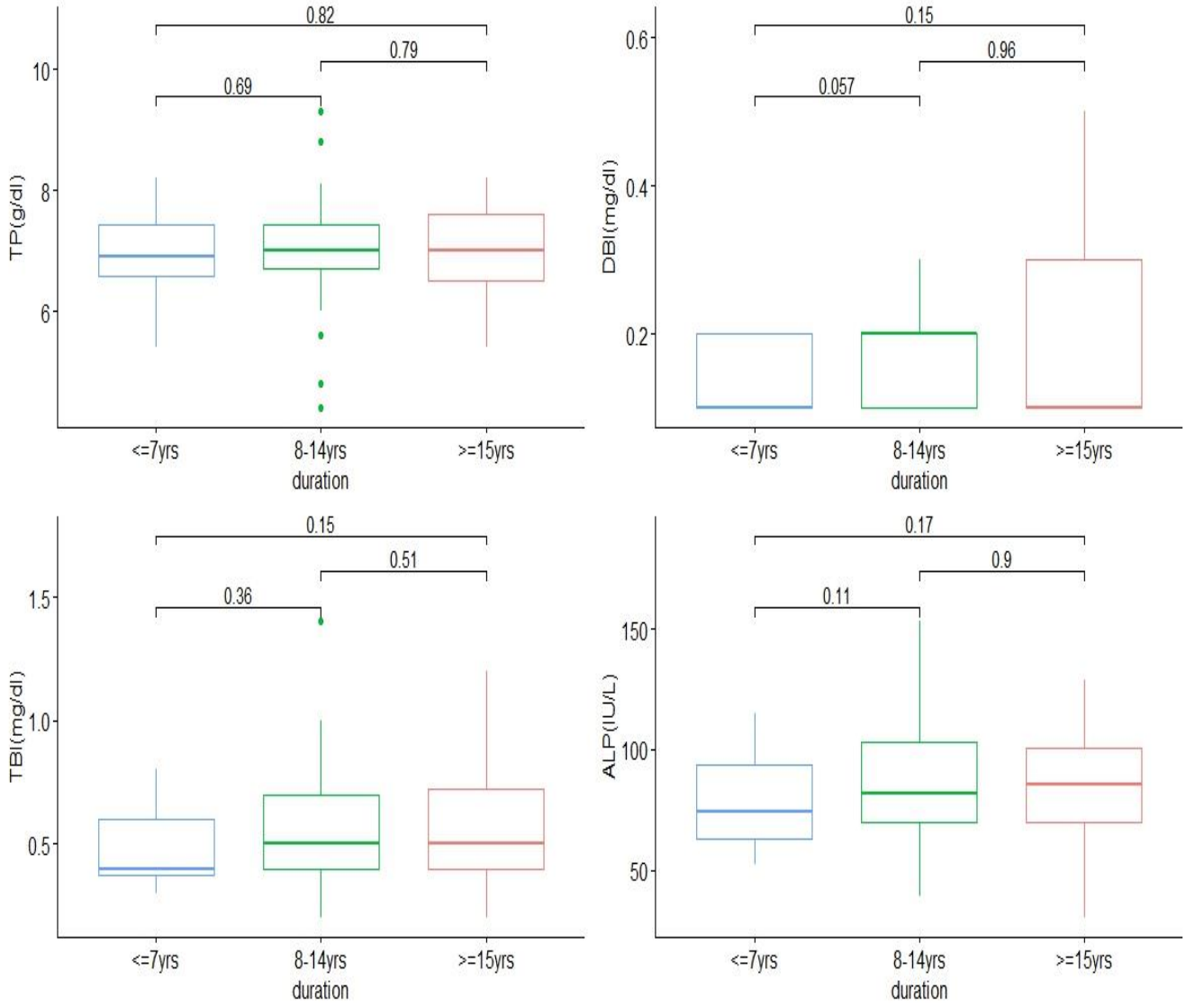

Figure 5: Effect of khat chewing duration on the levels of ALP, TP, DBI and TBI

*Mann-Whitney test was used to compare ALP, TP, DBI and TBI in each group of duration and analysed with R version 4.4.0 software

7.9. Association Of Liver Function Tests and Fasting Blood Glucose Level with Smoking Status Alcohol Consumption, Physical Activity, Dairy Products Consumption, Fruit and Vegetable Consumption and Chewing Style Among Khat Chewers.

There was no statistically significant difference in liver function tests and fasting blood glucose levels among smoking status, alcohol consumption, dairy products, fruit and vegetable consumption, and chewing style among khat chewers. However, a higher FBG level was observed in a sedentary lifestyle (Table 8).

Table 7: Association of LFTs and FBG levels with d/t behavioural characteristics among khat chewers in Dilla town, Southern Ethiopia, 2024 (n=100)

Parameters	Study groups	Factors					
		Smoking* (P-value)	Alcohol* (P-value)	Physical activity# (P-value)	Dairy products* (P-value)	Fruit and vegetable* (P-value)	Chewing style# (P-value)
AST (IU/L)	KC	0.71	0.18	0.32	0.08	0.44	0.75
	NKC	0.40	0.87	0.58	0.33	0.03	NA
ALT (IU/L)	KC	0.81	0.94	0.30	0.66	0.77	0.86
	NKC	0.38	0.01	0.76	0.64	0.35	NA
ALP (IU/L)	KC	0.75	0.54	0.85	0.68	0.54	0.78
	NKC	0.62	0.17	0.31	0.41	0.80	NA
TP (g/dl)	KC	0.22	0.23	0.53	0.26	0.08	0.27
	NKC	0.97	0.73	0.53	0.86	0.80	NA
DBI (mg/dl)	KC	0.63	0.28	0.10	0.46	0.41	0.83
	NKC	0.56	0.67	0.08	0.81	0.62	NA
TBI (mg/dl)	KC	0.69	0.61	0.93	0.73	0.02	0.43
	NKC	0.17	0.41	0.79	0.97	0.94	NA
FBG (mg/dl)	KC	0.41	0.12	**<0.001**	0.03	0.80	0.74
	NKC	0.62	0.41	**<0.001**	0.53	0.69	NA

KC: -khat chewers, NKC: - Non-Khat chewers, *: - Mann-Whitney, #: - Kruskal Walis test, NA: -not applicable

8. Discussion

The current study aimed to assess liver function tests and fasting blood glucose levels in adult male khat chewers (KC) and non-khat chewers (NKC). The results of the study indicated a significant increase in the levels of transaminase enzymes (AST and ALT) and (FBG) among khat chewers when compared to non-khat chewers. However, there was no statistically significant difference in the levels of ALP, TP, DBI and TBI among khat chewers compared to non-khat chewers.

The statistically significant increase in the levels of transaminase enzymes (AST and ALT) among khat chewers compared to non-khat chewers in the current study agreed with studies conducted in the Jazan region of Saudi Arabia [32, 36] and Yemen [45]. The higher levels of transaminase enzymes (AST and ALT) among khat chewers could be due to the components in khat that may have caused the production of reactive oxygen species in khat chewers, which may lead to the disruption of hepatocytes' cytosolic and mitochondrion membrane integrity due to accumulated free radicals generated during metabolic activities of khat [6, 28].

In contrast to the statistically significant increase in the levels of the transaminase enzymes (AST and ALT) in the current study, the study conducted in Kenya [33] and another study conducted in Yemen [35] showed a statistically non-significant difference in transaminase enzymes (AST and ALT) among khat chewers compared to non-khat chewers. However, the study conducted in Kenya indicated that a specific type of khat leads to an increment in the level of ALT [34]. This discrepancy alerts the need for further investigative studies on the impact of types of khat. Another reason for the difference may be the variation in frequency and khat chewing duration.

In the current study, there was no statistically significant difference in the levels of ALP among khat chewers compared to non-khat chewers. Similar results were also reported in Yemen in 2013 [35] and 2023 [45] and Jazan region of Saudi Arabia [36]. However, the present study's finding of ALP contradicted the study done in Kenya [33], which indicated statistically significantly higher ALP levels among khat chewers compared to non-khat chewers. The variation may be due to the type of khat and sex difference (since the study conducted in Kenya involved a female participant, the increased in ALP may be due to placental ALP increment)

The TP levels had no statistically significant difference among khat chewers compared to non-khat chewers in the current study which, was in line with the study conducted in Yemen in 2023

[45] and 2013 [35]. This may be due to serum total protein level used to assess liver synthetic capacity which, will decrease in late-stage liver disease. However, results from Kenya [46] and Jazan [32] were contradictory to the present result. The difference may because of nutritional status.

The present study found no statistically significant difference in the levels of TBI and DBI among khat chewers compared to non-khat chewers. This was supported by the study conducted in Yemen [35] , another study conducted in Yemen [45] and Jazan region of Saud Arabia [32]. Contradicted to our study's findings of DBI , the study conducted in Kenya showed significantly lower DBI levels among khat chewers when compared to non-khat chewers [46]. An animal study conducted on adult male rats showed higher DBI and TBI levels in 250, 350 and 450 mg/kg doses of khat-fed rats compared to the control group but not in 150 mg/kg doses [31]. The variation may be due to doses (bundle used).

The current study found a higher FBG levels among khat chewers than non-khat chewers, Which was supported by the surveillance study conducted in nine regions and two administrative cities of Ethiopia [41] and the study conducted in Egypt [39]. The higher FBG levels among khat chewers compared to non-khat chewers can be explained by the pharmacological action of khat involves elevated sympathomimetic activity through elevated catecholamines, which inhibit insulin action and elevate glucagon secretion. The stimulation of skeletal muscle and liver glycogenolysis results in elevated blood glucose level [47].

Contrary to the present increased fasting blood glucose levels among khat chewers, a study conducted in Yemen in 2003 [42] and 2024 [48] showed a non-significant difference in FBG levels. A study conducted in Ethiopia [40] and Yemen in 2013 [49] also demonstrated a significant decrease of FBG levels among khat chewers than non-khat chewers. This variation may be due to differences in the type of khat, variations in the sample size and lifestyle.

Concerning the association of liver function tests and fasting blood glucose levels with blood pressure, our study indicated that there was a significant increment in the levels of systolic and diastolic blood pressure among khat chewers than non-khat chewers. This was supported by the study conducted in Ethiopia [50]. Furthermore, our study showed a statistically significant positive correlation of blood pressure with transaminase enzymes (ALT and AST). A cross-sectional study conducted in Bangladesh indicated a positive correlation of blood pressure with liver enzymes [51].

This study's correlation of SBP with the FBG was supported by a report from federal ministry of health in Ethiopia which stated that SBP as a risk factor for hyperglycaemia. As the report indicated blood pressure and FBG levels had a positive correlation which is similar to the current study [52].

With regard to the association of khat chewing duration with liver function tests and fasting blood glucose level, this study showed a significant positive association with AST, ALT and FBG. This association may indicate that the longer the duration of khat chewing, the more hepatocellular damage and hyperglycaemia [29, 30]. A study conducted in Yemen also indicated that the duration of khat chewing had a significant effect on the levels of ALT [35].

The levels of ALT and FBG showed a positive correlation with the frequency of khat chewing. Additionally, a statistically significant and positive association was observed between the bundle of khat and ALT levels. The animal model study indicated the linear relationship between the doses of khat and liver function tests[31].

Age was significantly and positively associated with the ALT levels among khat chewers. This was supported by the study conducted in Yemen [35].This may be due to the older the age the longer the duration of khat chewing.

9. Strength and Limitation

9.1. Strength

- ✓ As far as the principal investigator's knowledge this was the first study conducted in the study area
- ✓ The study was a community based addressed a public health issue

9.2. Limitation

- ✓ Due to a cross-sectional study design nature, the present study could not provide adequate evidence of causation
- ✓ The limited human study made the present study difficult to compare with other findings

10. Conclusion and Recommendation

10.1. Conclusion

The current study demonstrated that in comparison to non-khat chewers, transaminase enzymes and fasting blood glucose levels are higher among khat chewers. Therefore, this study concluded that khat may affect liver function tests and blood glucose depending on the duration and frequency of khat chewing and bundle of khat used.

10.2. Recommendation

Even if the current study indicated statistically significantly higher liver function tests and fasting blood glucose levels among khat chewers than non-khat chewers, the findings showed clinically non-significant differences among the two groups. The reason behind this phenomenon may be due to our study participants being apparently healthy and about seventy percent of khat chewers chewed less than one bundle per day as well as the majority (68%) of the khat chewers had chewed for less than fifteen years. Therefore, based on our findings we recommend further studies to be done among chronic and regular khat chewers. Additionally, we recommend a longitudinal study to be done to identify the cause-and-effect relationship between liver function tests and fasting blood glucose levels with khat chewing. The knowledge, attitude and practice (KAP) study participants should be assessed which is beneficial for health promotion.

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Annexes

Annex I: Participants' information sheet

A. English version

Project Title: Liver Function Tests and Fasting Blood Glucose levels among Apparently Healthy Adult Male Khat Chewers in Dilla Town, Southern Ethiopia: A Comparative Cross-Sectional Study

Principal Investigator: Abera Abreham (BSc, MSc candidate)

Institution: Addis Ababa University, College of health Science, Department of Medical Laboratory Science **(Address:011-275-51-70)**

Consent explanation: My name is Abera Abreham, a Master's student in Addis Ababa university college of Health science department of Medical Laboratory Science. You are kindly invited as a study participant in this research.

Purpose: This study is interested in assessing the levels of liver function tests and fasting blood glucose among khat chewers in Dilla town**.**

Benefits: You will benefit from free screening for the liver and fasting blood glucose level. In case of any abnormal findings, you will be informed in confidentiality and referred to where you will receive treatment

Risks: You may feel a little pain at the time of blood drawing. However, during the collection of blood specimens from you, appropriate precautions will be taken and the sample will be collected by an experienced laboratory technologist.

Procedure: If you are volunteer to participate in this research, you will undergo a face-to-face interview using language you are comfortable with to answer questions as well as we will collect blood using a needle and syringe.

Voluntarism: Your participation in this research is entirely voluntary.

Confidentiality: Only Abera and the research team members involved in this study and none one else will access to the data. **Contact information Investigator**: Abera Abreham at 0921726507 or abrihina@gmail.com **Advisor:** Samuel Kinde **0975379824 or samuel.kinde@aau.edu.et**

B.Amharic version

ተሳታፉዎች መረጃ መስጭያ ሰነድ

የፕሮጀክት ርዕስ፡ - በደቡብ ኢትዮጵያ ዲላ ከተማ ውስጥ ጤናማ አዋቂ ወንድ ጫት ቃሚዎች የጉበት አፈፃፀም ምርመራ እና የፆም ግሉኮስ መጠን ምርመራ

ዋና መርማሪ፡ አበራ አብርሃም (BSc, MSc candidate)

የተቋሙ ስም፡ የአዲስ አበባ ዩኒቨርሲቲ የጤና ሳይንስ ኮሌጅ፣ የሕክምና ቤተ ሙከራ ሳይንስ ትምህርት ክፍል

የስምምነት ማብራሪያ፡ ስሜ አበራ አብረሃም ይባላል፣ በአዲስ አበባ ዩኒቨርሲቲ የጤና ሳይንስ ኮሌጅ፣ የሕክምና ላቦራቶሪ ሳይንስ ትምህርት ክፍል የማስተርስ ተማሪ ነኝ። በጫት ቃሚዎች እና ጫት ቃሚ ያልሆኑ ጎልማሶች መካከል የጉበት ተግባርን እና የፆም ግሉኮስን ለመገምገም አካዳሚክ ጥናት እያደረግሁ ነው። በዚህ ጥናት ውስጥ የጥናት ተሳታፊ እንድትሆኑ ተጋብዘዋል።

ዓላማው፡ ይህ ጥናት በዲላ ከተማ በጫት ቃሚዎች እና ጫት በማይቃሙ ጎልማሶች መካከል ያለውን የጉበት ተግባር ምርመራ እና የፆም ደም የግሉኮስ መጠን ደረጃን ለመገምገም ፍላጎት አለው።

ጥቅማ ጥቅሞች፡ ነጻ የጉበት እና የግሉኮስ ምርመራ ተጠቃሚ ይሆናሉ። ያልተለመዱ ግኝቶች በሚኖሩበት ጊዜ በሚስጥራዊነት ይነግሩዎታል እና ህክምና ወደሚያገኙበት ቦታ ይላካሉ

ስጋቶች: ደም በሚወሰድበት ጊዜ ትንሽ ህመም ሊሰማዎት ይችላል. ነገር ግን ከእርስዎ የደም ናሙናዎች በሚሰበሰቡበት ጊዜ ተገቢ ጥንቃቄዎች ይደረጋሉ እና ናሙናው ልምድ ባለው የላቦራቶሪ ቴክኖሎጂ ባለሙያ ይወሰዳል። የሆነ ነገር ከተፈጠረ, ተገቢ የሕክምና እንክብካቤ ይሰጥዎታል።

የአሰራር ሂደት፡ በዚህ ጥናት ላይ ለመሳተፍ ፈቃደኛ ከሆኑ፣ የጥናት አማካሪውን በሚያገኙበት በአቅራቢያዎ ወደሚገኝ የሆስፒታል ላቦራቶሪ እንዲሄዱ ይጠየቃሉ። የእርስዎን የስነ ሕዝብ አወቃቀር መለኪያዎችን የሚመለከቱ ጥቂት ጥያቄዎችን ለመመለስ በሚመችዎ ቋንቋ በመጠቀም ፊት ለፊት ቃለ መጠይቅ ያደርጋሉ።አምስት ሚሊሊሊትር ደም ይሰጣሉ።

በጎ ፈቃደኝነት፡ በዚህ ጥናት ውስጥ ያለዎት ተሳትፎ ሙሉ በሙሉ በፈቃደኝነት ላይ የተመሰረተ ነው።

ሚስጥራዊነት፡ የማይመችዎትን ማንኛውንም ጥያቄ ከመርማሪዎቹ ማብራሪያ የመጠየቅ መብት አልዎት። ምስጢራዊነትን ለመጠበቅ፣ ስምዎ በማንኛውም መጠይቆችዎ ላይ እንዳይታይ ኮድ ቁጥር ይመደብልዎታል። በዚህ ጥናት ላይ የተሳተፉት አበራ እና የምርምር ቡድኑ አባላት ብቻ እንጂ ማንም መረጃውን ማግኘት አይችልም። በዚህ ጥናት ውስጥ ያለዎት ተሳትፎ ሙሉ በሙሉ በሚስጥር ይጠበቃል።

በዚህ ጥናት ሊይ ያልዎትን ጥያቄ በሚከተለት አዴራሻ በማንኛውም መጠቀም ይችላሉ

የኣጥኝው ስም: አበራ አብርሃም ተንቀሳቃሽ ስልክ: +251921726507 Email: abrihina@gmail.com

አማካሪ፡ሳሙኤል ክንዴ ተንቀሳቃሽ ስልክ: +251975379824 **Email** **samuel.kinde@aau.edu.et**

Annex II: Informed consent

A. English version

Information about the study has been explained for me by the investigator. I have understood that the objective of the study is to assess liver function tests and fasting blood glucose level among adult male khat chewers and non-khat chewers in Dilla town. I decide to participate in the study. I agree to participate in the study voluntarily, without any coercion or forceful act by the research team and here I approve my agreement with my signature

ID No._____ Signature _____ Date_____

B. Amharic Version

የፈቃደኝነት ማረጋገጫ ሰነድ

ስለ ጥናቱ መረጃ በተመራማሪው ተብራርቶልኛል። የጥናቱ አላማ በዲላ ከተማ ጫት ቃሚዎች እና ጫት የማይቅሙ አዋቂ ግለሰቦች ላይ የሚደረገውን የጉበት ተግባር እና የግሉኮስ ምርመራ እንደሆነ ተረድቻለሁ። በጥናቱ ውስጥ ለመሳተፍ በሙሉ ፈቃደኝነት ተስማምቻለሁ፣ ይህንንም በፊርማዬ አረጋግጣለሁ።

ልዩ መታወቂያ ቁጥር _____ ፊርማ _____ ቀን_____

Annex III: -Questioner

A. English version of questioner

PART I: Socio- demographic characteristics of the respondent			
No.	**Questions**	**Response categories**	**Remarks**
1	Identification number	ID_____	
2	Age of participant	_____years	
3	Marital status	1.single 3. widowed 2.married 4. divorced	
4	Educational status	1.Can't write and read 2.Can write and read 3. Primary school 4. Secondary school and above	
Part II: *Catha edulis*(khat) Chewing characteristics			
5	Have you ever chewed khat? If no please jump to Q 12	1.Yes 2.No	
6	How often do you chew khat per week?	_____days	
7	How long have you chewed khat?	_____year/s	
8	The type of khat chewed	Type _____	
9	Bundle of khat used per day	----------bundle(s)/day	

10	Chewing style	1. Water 2. Soft drinks 3. peanut 4. others (tea, coffee, milk...) 5. none	
Part III: Physical activity			
11	Physical activity	1. Vigorous intensity 2. Moderate intensity 3. Less active 4. Sedentary	
Part IV: Nutrition status			
12	Do you use foods like meat, egg and dairy products	1.yes 2. No	
13	If yes, how often do you take?	____ day/s per week	
14	Do you take any fruit and vegetable?	1.yes 2. No	
15	If yes, how often do you take?	____ day/s per week	
Part V: Behavioural measurements			
Tobacco use practice			
16	Do you smoke Cigarette?	1. Yes 2. No	
Alcohol drinking practice			
17	Do you drink alcohol?	1. Yes 2. No	

Part VI: Biochemical, clinical and anthropometric measurements

	Clinical and anthropometric measurements	**Result**	**parameters**	**Result**
18	Blood pressure (mm/Hg)		ALT(IU/L)	
19	Height (meter)		AST(IU/L)	
20	Weight (Kg)		ALP(IU/L)	
21	BMI (kg/$m^{2)}$		TP (g/dl)	
22	Waist circumstance (cm)		DBI (mg/dl)	
			TBI (mg/dl)	
			FBG (mg/dl)	

B. Questionnaire Amharic version

ክፊል 1. ማህበራዊ-ስነ-ሕዝብ ባህሪያት			
ተ.ቁ	ጥያቄዎች	ምላሽ	**አስተያየት**
1	የመለያ ቁጥር	_____	
2	ዕድሜ	_____ ዓመት	
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	

		4.ሁለተኛ ደረጃና ከዛ በላይ	
ክፍል 2: *ጫት መቃምን በተመለከተ*			
5	ጫት ይቅማሉ? መልሶ አይ ከሆነ እባክዎ ወደ ጥያቄ ቁጥር 12 ይለፉ	1.አዎ 2. አይ	
6	በሳምንት ለስንት ቀናት ጫት ይቅማሉ?	_____ቀን	
7	ለምን ያህል ዓመታት ጫት ቅመዋል?	_____ዓመት	
8	የሚቅሙት የጫት ዓይነት?	_____	
9	በቀን የሚጠቀሙት የጫት ጥቅል ብዛት?	_____	
10	ጫት በሚቅሙበት ግዜ ከሚከተሉት ውስጥ የትኛውን ብዙን ግዜ ይጠቀማሉ	1. ውሃ 2. ለስላሳ መጠጦች 3. ለውዝ 4. ሌሎች (ቡና ፣ሻይ ወተት....) 5. የለም	
Part III: አካላዊ እንቅስቃሴን በተመለከተ			
11	የአካላዊ እንቅስቃሴ ጥንካሬ	1.ኃይለኛ ደረጃ ጥንካሬ ያለው 2. መካከለኛ ደረጃ ጥንካሬ ያለው 3. ዝቅተኛ ደረጃ ጥንካሬ ያለው 4 እንቅስቃሴ የማያደርጉ	
Part IV: የአመጋገብ ልምድን በተመለከተ			

12	የወተትተዋጽኦችን፣ስጋን እና እንቁላልን በምግብነት ይጠቀማሉ ?	1.አዎ 2. አይ	
13	መልሶ አዎ ከሆነ, በሳምንት ለስንት ቀናት?	_____ ቀን	
14	አትክልት እና ፍራፍሬን በምግብነት ይጠቀማሉ?	1.አዎ 2. አይ	
15	መልሶ አዎ ከሆነ, በሳምንት ለስንት ቀናት?	_____ ቀን	
Part V: የባህሪ መለኪያዎችን በተመለከተ			
የትምባሆ አጠቃቀምን በተመለከተ			
16	ሲጋራ ያጨሳሉ?	1. አዎ 2. አይ	
አልኮል መጠጥን በተመለከተ			
17	አልኮል ይጠጣሉ?	1.አዎ 2. አይ	

Annex IV: Screening checklist English version

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

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

Annex V: laboratory assay principles and interpretations

Alanine Aminotransferase (ALT)

Method

UV-assay according to International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) modified to contain pyridoxal-5-phosphate as an activator.

Reaction Principle

The enzyme Alanine Aminotransferase catalyses the reaction between L alanine and 2 oxoglutarates. The production of pyruvate and its subsequent reduction by NADH to yield L lactate and NAD+ are catalysed by lactate dehydrogenase.

$$\text{L-Alanine + 2-oxoglutarate} \xrightarrow[\text{P5P, Tris, PH 7.4}]{\text{ALT}} \text{pyruvate + L-glutamate}$$

$$\text{Pyruvate + NADH + H+ LDH} \xrightarrow{\text{LDH}} \text{L-lactate + NAD+}$$

The rate of NADH oxidation corresponds directly with the catalytic activity of the enzyme alanine transaminase. The wavelength is 340 nm /700 nm.

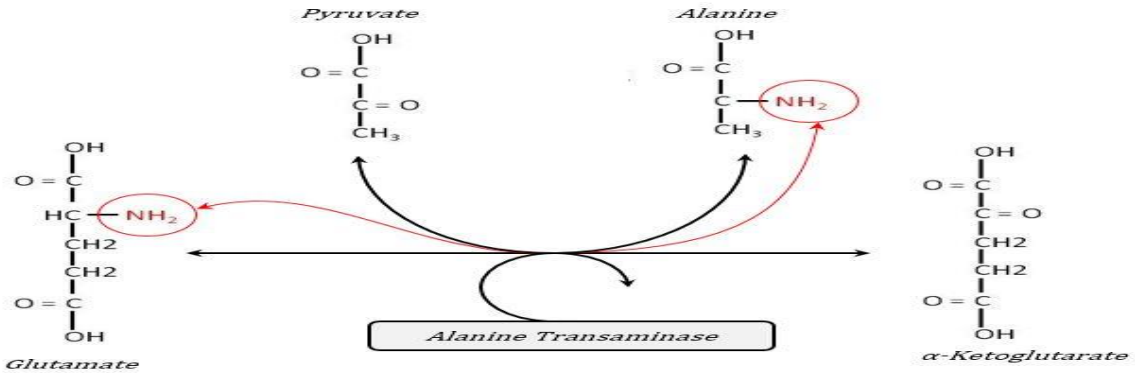

Figure 6:The catalytic activity of Alanine Transaminase

Measuring limits : 6 – 1000 U/L **Expected Values :** 16 – 63 U/L

Aspartate Aminotransferase (AST)

Method

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

Reaction Principle

The sample's aspartate aminotransferase catalyses the transfer of an amino group from L aspartate to 2 oxoglutarates, resulting in the formation of L glutamate and oxaloacetate. Then, in the presence of malate dehydrogenase (MDH), the oxaloacetate combines with NADH to generate NAD+.L glutamate MDH + 2 oxoglutarate + L aspartate + oxaloacetate H+ L malate + NAD + oxaloacetate + NADH

$$\text{L-Aspartate + 2-oxoglutarate} \xrightarrow{AST} \text{oxaloacetate + L-glutamate}$$

$$\text{MDH Oxaloacetate + NADH + H+} \xrightarrow{MDH} \text{L-malate + NAD}$$

The catalytic activity of aspartate aminotransferase directly relates to the rate of NADH oxidation. The activity can be determined by a decrease in absorbance.

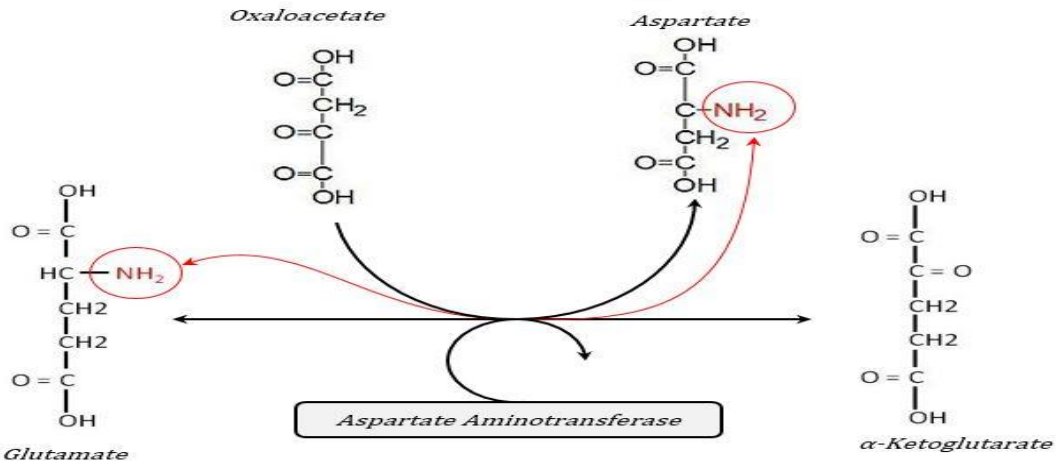

Figure 7:The activity of Aspartate Aminotransferase

Measuring range: 0-700 U/L **Expected Values**:15 – 37 U/L

Alkaline Phosphatase (ALP)

Method

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

Test principle

phosphatases cleave nitrophenyl phosphate to produce phosphate and p- nitrophenol. The following process uses magnesium and zinc ions as cofactors.

$$\text{P-NPP + AMP} \xrightarrow[Mg^{2+}]{ALP} \text{p-NP + AMP + PO4}$$

The amount of p nitrophenol emitted is directly related with ALP's catalytic activity. measured at a 480 nm wave length.

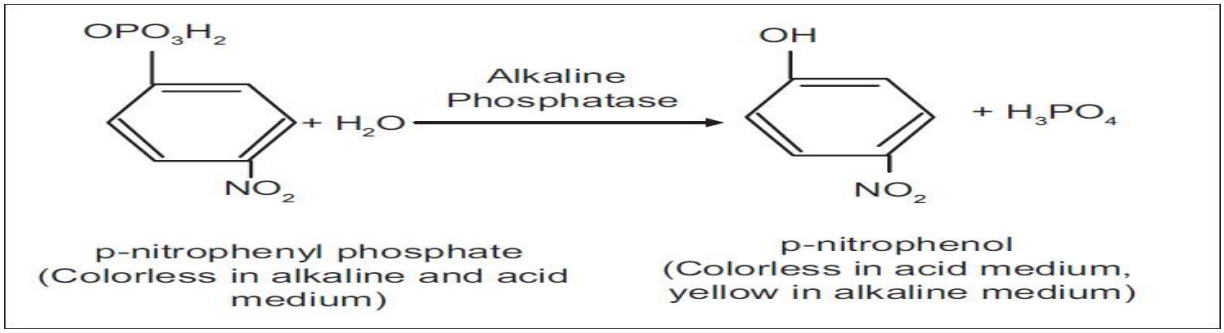

Figure 8:The activity of Alkaline Phosphatase

Expected values :46 – 116 U/ **Measuring range :**10-1200 U/L

Total Bilirubin (TBI)

Method

Diazotized Sulfanilic Acid (DSA)

In a strong acidic media, 3,5 dichlorophenyl diazonium is combined with total bilirubin and an appropriate solubilizing agent.

Solubilized bilirubin + Diazotized sulfanilic acid $\xrightarrow{ACID}$ Red chromophore. The red azo dye's colour intensity, which is measured photometrically at 540 nm, is directly related to the total amount of bilirubin.

Expected values :0.2 – 1.0 mg/dL **Measuring range:** 0.1– 25.0 mg/Dl

Direct bilirubin (DBI)

Principles of Procedure:

Conjugated bilirubin + Diazotized sulfanilic acid $\longrightarrow$ Red chromophore (absorbs at 540 nm)

Expected Values: 0.1– 0.2 mg/dL **Measuring range** :0.05 – 16.0 mg/dL

Total protein (TP)

Method

Biuret method

Test principle

Divalent copper reacts with in proteins peptide bonds in the alkaline solution to generate the distinctive purple biuret complex. Potassium iodide inhibits copper auto-reduction, while sodium potassium tartrate hinders the precipitation of copper hydroxide.

Principles of Procedure:

Cupric ion (Cu++) reacts with the peptide linkages (-C-NH-CH-C-NH-) of protein in a basic solution. The resulting blue copper (II) protein complex is proportionate to the sample's total protein concentration and is quantified using a bichromatic endpoint approach technique (540, 700 nm).

Cu++ + Protein $\longrightarrow$ Complex

(Absorbs at 540 nm)

Expected value :6.4 – 8.2 g/dL **Measuring range** :2.0 – 12.0 g/dL

Fasting blood glucose (FBG)

Method

Hexokinase

Principle:

When glucose is phosphorylated by hexokinase (HK) in the presence of magnesium ions (ADP) and adenosine triphosphate (ATP), glucose-6-phosphate (G-6-P) and adenosine diphosphate are produced. More specifically, nicotinamide adenine dinucleotide (NAD+) is reduced to nicotinamide adenine dinucleotide by glucose-6-phosphate dehydrogenase (G6P-DH), which also lowers G-6-P to 6-phosphogluconate (NADH). The amount of glucose in the sample is reflected in the change of absorbance at 340/380 nm.

$$\text{Glucose + ATP} \xrightarrow{\mathbf{HK,}} \text{Glucose-6-phosphate + ADP}$$

$$\text{Glucose-6-phosphate + NAD+} \xrightarrow[\mathbf{G-6-PDH}]{\mathbf{MG++}} \text{+ 6-phosphogluconate + NADH + H+}$$

Measuring range :0 – 500 mg/dL **Expected values:** 74 – 106 mg/dL

Declaration

I, the undersigned, declare that this MSc. 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

Abera Abreham (BSc, MSc candidate)

Signature: _____

Date of submission: _____

This thesis has been submitted with our approval as advisors.

Advisor: Samuel Kinde (Assistant professor, PhD candidate)

Signature: _____

Date: _____

Place: Addis Ababa, Ethiopia.

Advisor: Gobena Dedefo (BSc, MSc)

Signature: _____

Date: _____

Place: Addis Ababa, Ethiopia.