ADDIS ABABA UNIVERSITY

COLLEGE OF HEALTH SCIENCES

DEPARTMENT OF MEDICAL LABORATORY SCIENCES

Comparison of Fasting blood glucose level among khat chewers and non-khat chewers in diabetics and healthy individuals at Hiwot Fana Specialized University Harar Eastern Ethiopia.

By: Yordanos Mengistu

Advisors: Mr. Samuel Kinde (PhD Candidate)

Mr. Gobena Dedefo (BSc, MSc**)**

A research thesis submitted to the Department of Medical Laboratory Sciences, College of Health Science, Addis Ababa University, in partial fulfillment of Master of Science Degree in Clinical Laboratory Sciences (Clinical chemistry).

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This is to certify that the thesis prepared by YORDANOS MENGISTU, entitled: **Comparison of blood glucose level among khat chewer and non khat chewer diabetics and healthy individuals in Harar eastern Ethiopia, 2020** and submitted in partial fulfillment 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.

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Abbreviations

- ACTH adreno corticotropic hormone
- ADSF adipose tissue specific secretary factor
- BMI body mass index
- BGL blood glucose level
- BMI body mass index
- DM diabetes mellitus
- FBS fasting blood sugar
- GOD glucose oxidase
- GHK glucose hexokinase
- HgbA1C hemoglobin A1C
- IQR Inter quartile range

Operational definition

- Fasting blood sugar: - blood glucose measured after at least 8 hours of overnight fasting.
- Diabetic khat chewer: Confirmed diabetic patient who started khat chewing before diagnosis of diabetes, had been chewing khat at least for a year on daily bases.
- Healthy khat chewer: Individual who chew khat at least for a year on daily bases.
- Non-chewer: -Individuals who never chewed khat, chew khat previously, occasional khat chewer.
- Apparently healthy individuals: Normoglycemic individuals, who is not a known a case of diabetes and do not have history any systemic disease and also having normal BMI and blood glucose level at the time of data collection.
- Family history of Diabetes: - Having either of the two parents diagnosed with diabetes.
- Body mass index (Kg/m^2): - classified as Normal: - 18.5 - 24.9 Kg/m^2, Over weight: - 25.0 - 29.9Kg/m^2, Obese: >30 Kg/m^2.
- Blood pressure (mm/Hg): - Classified as (systolic/diastolic) normotensive 120-129/80-84, prehypertensive 130-139/85-89, hypertensive >140/>90 according to British hypertension association.
- Waist circumference Men: -(Inches): -Low risk<37, Intermediate risk 38-39.9, High risk≥40
- Waist circumference Women (Inches): - Low risk<31.9, Intermediate risk 32-34.9, High risk≥35.

Abstract

Background: -Khat chewing is a widely heled practice in our society. Moreover, studies about khat chewing effect are contradictory. There is limited data about khat effect on blood glucose specially in our setting. Therefore, this study aims to investigate this issue.

Objective: In this study, we compared FBS among khat chewer and khat chewer diabetic and healthy subjects.

Method: A cross-sectional study include 200 confirmed diabetic and healthy subjects grouped in to four groups (diabetic khat chewers n=50, diabetic non khat chewer n=50, healthy khat chewer n=50 and healthy non-khat chewer n=50). FBS was determined by enzymatic method (GOD&G HK) using Biosystem A25 and COBAS 6000 chemistry analyzers at Hiwot Fana and St. Peter hospital clinical chemistry laboratory respectively. Non-parametric tests Mann-Whitney test (2-independent sample test) and Kruskal-Wals test (K-independents sample test) were used to compare fasting blood glucose among khat chewer and non-khat chewer with in the groups and between groups respectively. $P<0.05$ is taken to be statistically significant.

Result: - Of the total respondents 83(41.5%) and 117(58.5%) were male and female respectively. Without age and gender adjustment the overall Median±IQR FBS difference among khat chewers and non-khat chewers were (159.5±83) mg/dl and (202±79) mg/dl respectively in diabetic patients using GOD. Similarly, when we compare between healthy non-khat chewers and khat chewers, khat chewers have lower (Median±IQR) fasting blood glucose level (82±18) mg/dl than that of the non-khat chewers (94±13) mg/dl when tested by GOD. Generally, there was significantly lower blood glucose level measured by both GOD and GHK methods in both diabetics and healthy groups of khat chewers. There was also percentage difference of median FBS with in the same group when measured by GOD and GHK methods.

Conclusion and recommendation: -There was significant effect of khat on blood glucose level among khat chewer and non-khat chewer in diabetic and healthy individuals. Based on the present study finding, further studies have to be conducted to identify khat constituent associated with hypoglycemic effect and further elucidate khat effect on systemic metabolism.

Key word: Khat chewing, serum fasting glucose level

1. Introduction

1.1. Background

The plant leaves Catha edulis Forsk is called khat(1). Khat constituents have been studied by narcotic laboratories and it is mainly consisted of Cathinone , Cathine and Norephedrine (2). Ascorbic acid also found in khat leaves. Approximately, 150mg ascorbic acid found per 100gm of khat(3).Cathinone which is responsible for major pharmacological effects is the most active constituent (4, 5). It accounts 77.7-342 mg/100 gm of khat(6).

Cathinone raise catecholamines level in plasma; due to its adrenergic effect it counteracts insulin action resulted in glucagon secretion, activation of glycogenolysis in liver, (β-2 adrenoreceptor m ediated response), adrenocorticotropic hormone (ACTH) secretion, suppress insulin release (α-2 adrenoreceptor mediated response) which generally leads to increased blood sugar level.(7). In adipocytes cathinone upregulates expression of resistin which has impact on metabolism of carbohydrate through increased cortisol level. This decrease insulin production and induce insulin resistance(8).

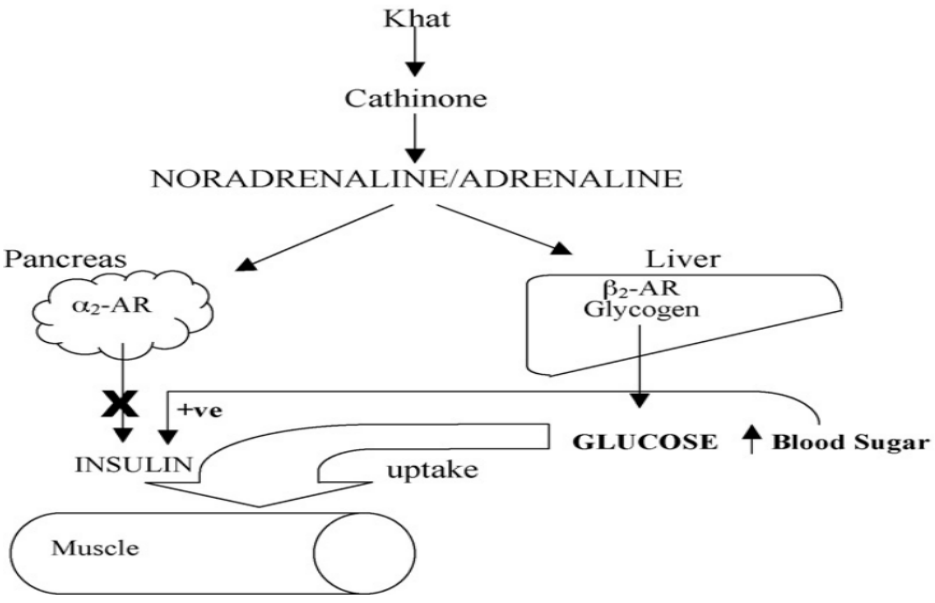

Figure 1:- Effect of khat on blood glucose level through $\alpha_2$and β_2 adrenoreceptors **(7).**

In experimental study conducted on khat fed rats cathinone decrease glutathione peroxidase activity , free radical scavenging enzyme, and hence heightening oxidative stress which attributes to development of diabetes and diabetes complications(9, 10).

Much is not clearly known about the mechanism by which vitamin C lowers blood sugar level but it was postulated that it an antioxidant vitamin, plays an important role in protecting free radical-induced damage(11). Vitamin C is structurally similar to glucose and can replace it in many chemical reactions and thus is effective for prevention of nonenzymatic glycosylation of protein(12)

Reports about khat effect on blood glucose are contradictory. Ranges from no significant effect(13), significant reduction of blood glucose level(14) and highly significant increase of blood glucose levels in diabetic human(15) had been reported. Therefore, further studies should be conducted to draw objective conclusion. Many studies had been conducted and published about chemical composition and its chemistry but very few are available about its effect on glucose level. Therefore, the present study was conducted to study the effect of khat on glucose level in the study area. Moreover, there is interference of Vitamin C in in biochemical assay of glucose using enzymatic glucose oxidase method. In the present study FBS was determined by both glucose oxidase (GOD) and glucose hexokinase (GHK) methods.

1.2. Statement of the problem

Khat chewing habit is highly practiced in majority of the population in Ethiopia specially in Harar for many generations. From 2015 national non-communicable disease survey the prevalence of khat chewing in Harar town is 5.8%(16). Study conducted in Harar indicated there is negative impact of khat on the economy of consumer households by influencing their income as well us time management. Households with khat chewing habit have significant additional burden with regard to their time spending 112.5 hr monthly only on khat related activity and expend 1800ETB (30%) of their monthly income for khat ceremony. Khat also negatively impact on work culture of chewers(17).

Khat has a harmful effect in diabetic khat chewer because they may not follow dietary advice and use sweetened materials (peanut, soft drink, sugar) in khat chewing that could worsen pre-existing hyperglycemia(18).They have enhanced glycemic responsiveness to catecholamines (epinephrine, nor-epinephrine) released as a result of cathinone, which result in reduced insulin secretion and sensitivity(19). Another possible explanation for this could be the effect of cortisol and resistin or adipocyte secretary factor (ADSF) which increase following khat chewing as result of cathinone release from khat in both diabetics and healthy individuals. In diabetes khat chewers it significantly decrease serum insulin level (20).

Resistin is exclusively produced by adipocytes and its expression is upregulated by nor-adrenalin which is released as a result of cathinone. It impairs insulin signaling, mediate hepatic and skeletal muscle insulin resistance (21). Khat increase blood glucose level through indirect mechanism by increasing the rate of glycolysis accompanied by lactate release which is transported to liver and serve as input for gluconeogenesis(22).There is also stimulation of lipase by nor-epinephrine. It hydrolyzes triglycerides in to fatty acids and glycerol. Glycerol serve as substrate in gluconeogenesis(23).

Despite the fact that khat is used simply for its stimulatory effect and entertainment, evidently there is common belief among khat chewers in Ethiopia and other part of the world that khat lowers blood sugar and keep it with in normal level(3). In fact, khat consumption in diabetic patients as well as healthy individual is deleterious as it could cause further damage to β-cells because of insecticides used on khat trees which are not highly selective and may cause toxicity (24).

There is discrepancy among reports about khat effect in both diabetic patients and healthy individuals. In Yemen experimental study in diabetic subjects showed non-significant change in blood glucose level has been reported(25). Similarly another experimental study revealed khat increase blood glucose level in diabetic patients with serum glucose between 200 and 450 mg/dl at 2h post prandial (26). Another study in Somalia in non-diabetic male subjects khat didn't significantly affect blood glucose level(27).

Khat also might be risk factor or contribute to the development of non-insulin dependent diabetes in healthy individuals(24). Prospective study in Yemen showed that [65 (31.7%) out of 205] chronic khat chewers were diagnosed with diabetes compared to [20 (10.7%) out of 187] non-chewer khat chewer subjects and khat increase the risk of having diabetes by 3.8 times in this population(28). Ascorbic acid which also found in khat leaves has effect on blood glucose level among diabetic patients(29), it lowers FBS by 25% and HgbA1C by 12% compared to level recorded before it is given(30).

In Harar, Eastern part of Ethiopia, Khat chewing is widely held practice by the population and it is the major source of income for majority of population and it accounts majority of trade in the Eastern Ethiopia. In addition to this, the inconsistent body of evidence in the literature calls for more investigations to be made on the effects of the habit of chewing khat in the setting of diabetes. According to literature, as to our knowledge, in the study setting there were no published literature regarding the effect of khat on blood glucose level among diabetics and healthy individuals. It is therefore, the interest of this study was to investigate the effect of khat chewing on blood glucose in diabetics (both type 1 and type 2) and normoglycemic individuals.

1.3. Significance of the study

According to prospective study conducted in Yemen in 2018,high prevalence of diabetes has been reported in khat chewers (31.7%) compared to non khat chewers(10.7%)(28).Despite the fact that khat is used for entertainment or other purpose, it has extreme adverse health effect including effect on plasma glucose level. Reports are contradictory about the effect of khat on blood glucose level in diabetics as well as healthy individuals. In the study area, no previous human study had been conducted so far to show the effect of khat on blood glucose level.

This study tried to address the effect of khat chewing on blood glucose level among diabetic patients and healthy individuals by using two methods as there is negative interreference by Vitamin C, (which is found in khat) in glucose assay using enzymatic oxidase method/peroxidase base reaction. This may contribute scientific justification for the misconception of khat chewing has hypoglycemic effect in some localities. May also contribute scientific evidence for further investigations for the use of traditional medicine for better treatment of diabetes. Clinicians, policy makers, moreover; diabetic patients will get benefit from this in such a way that khat's elements which have anti-hyperglycemic effect may be used to make potent antidiabetic drug or supplement for better diabetes management in combination with modern medicine.

2. Literature review

Different animal and human studies had been conducted so far and reported different findings. Includes no effect on blood glucose level, significant reduction of blood glucose level and highly significant increase of blood glucose levels in diabetic human has been reported. This shows khat effect on blood glucose is conflicting.

In a case-control study 260 type two diabetic patients in Yemen were enrolled in the study to investigated the effect of Khat chewing on the level of blood glucose determined by FBS in 2010. Grouped in to 130 non-Khat chewers and 130 Khat chewer. There was no significant change in fasting blood sugar before and after khat chewing. The mean fasting blood glucose level in mg/dl in diabetic non-khat chewer was (149.5±42), in diabetic khat chewer (157.7±74.6) (p value 0.443)(31).

In 21012, 80 male subjects 40 with diabetes and 40 without diabetes included a cross-sectional study conducted in Yemen. FBS was measured enzymatically by glucose oxidase method. Both normal and diabetic khat chewer groups have higher FBS compared to non khat chewers. The mean FBS (mg/dl) were found to be as follows, normal non Khat chewer (72±1.25), normal khat chewer (87.4±1.47),Diabetic non-Khat chewer (199.4±3.7), Diabetic khat chewer (241±9.82 (20).

In 2010, experimental study involving 30 male subjects 20 with diabetes and 10 non diabetics conducted in Yemen FBS and post prandial glucose was measured enzymatically before and after khat chewing. Khat decrease blood glucose level in diabetics and healthy khat chewers participants during the experimental period(22).

In experimental study conducted in Pensuila, rabbits were used as experimental and control group on exposure to cathedulins (khat). FBS was determined the mean FBS (mg/dl) for control group were found to be, (167.8±0.14) and (165.6±0.13) for khat fed rats. This study showed that khat decrease blood glucose level significantly in khat fed rats (P value=0.005) (32).

In another experimental study conducted in Saudi Arabia, 72 diabetic and non-diabetic rats were used as experimental group who were feeding khat and control group without khat. They underwent FBS measurement and post prandial glucose measurement. Mean Fasting blood glucose of non-diabetic control were (96±3.38), non-diabetic exposed group (97.8±2.39), in diabetic control group (264.4±6.31) and (267.9±5.41) for exposed group. This study showed that khat increase blood glucose in both diabetic and non-diabetics(33).

In experimental study on non-diabetic and diabetic rats, in Ethiopia, Addis Ababa 40 normal and diabetic rats were used. Cathedulins were given to the experimental group and glucose was measured at the end. Oral administration of a fresh juice of khat in diabetic and normal rats reduced the fasting blood glucose level significantly ($P<0.001$)from (223.7 ± 27.6) and (115.4 ± 2.48) mg/dl to (106 ± 18.2)and (79.6 ± 3.41)mg/dl, respectively at the end of study(34).

As far as my literature goes there is no published study conducted in the study area regarding the effect of khat chewing on blood glucose level in human beings. The literatures reviewed above shows the need for further investigations on this issue. Since diabetes is continued to be a leading cause of morbidity and mortality with its high prevalence both in developed and developing countries, studies have to be conducted on this issue for better patient care (restrain disease progression and limit organ damage caused by diabetes).

3. Objective

3.1. General Objective

To compare blood glucose level among khat chewer and non khat chewer diabetic and healthy individuals at Hiwot Fana specialized university hospital, Harar eastern Ethiopia January-March 2020.

3.2. Specific Objective

- To compare mean blood glucose level among diabetic khat chewers and non khat chewers.
- To compare mean blood glucose level among apparently healthy khat chewers and non khat chewers.

4. Hypothesis

4.1. The Alternative hypothesis HA:

There is significant difference in median blood glucose level among khat chewer and non-khat chewer in diabetic and apparently healthy individuals.

5. Methods and materials

5.1. Study area

This study was conducted in Harar town Hiwot Fana specialized university hospital. Harar is the capital city of the Harari Regional State, and it is located in the eastern extension of the Ethiopian Highlands about 526km East of Addis Ababa at an elevation of 1885 m. Central Statistical Agency in 2012, reported the estimated population of Harar was 203,000 (35). In the town, there are four governmental hospitals, Hiwot Fana Specialized University Hospital (HFSUH), Jugel Hospital (JH), Federal Harar Police Hospital (FHPH) and South-East command III Hospital (SECIIIH) and two private hospitals, and eight health centers. The study was conducted in Hiwot Fana specialized university hospital which is administered by Haramaya University. It has the largest client load with an average bed occupancy rate of 83%. The hospital consists of six major wards: Medical, Surgical, Obstetrics, Gynecology, Malnutrition, and Pediatric wards. Also serves outpatient services like diabetic clinic, hypertension clinic, TB clinic, ART clinic etc. The hospital has 402 healthcare workers (Harari health bureau).

5.2. Study design and period

A hospital based comparative cross-sectional study was conducted from January-March 2020.

5.3. Population

5.3.1. Source population

The source population for khat chewer and non-khat chewer group were type 1 and type 2 diabetic patients coming to diabetic clinic at Hiwot Fana specialized university hospital and normoglycemic individuals.

5.3.2. Study population

The study population were all consented khat chewer and non khat chewer diabetic patients and healthy individuals from Haramaya University and Hiwot Fana specialized university hospital who fulfilled the inclusion criteria.

5.4. Eligibility criteria

5.4.1. Inclusion criteria

- Inclusion criteria for khat chewer groups
  - Individual of any sex whose age 18-65.
  - Diabetic patient (type-1 and type-2) who chew khat (Harar khat leaves) before diagnosis of diabetes.
  - Diabetic patient who is on follow-up at least for 6 months
  - Healthy khat chewer, (Harar khat leaves) without previous history of systemic disease.
- Inclusion criteria for non-khat chewer group
  - Individual of any sex whose age is18-65.
  - Healthy non khat chewer without previous history of systemic disease
  - Diabetic non khat chewer who is on follow-up at least for 6 months

5.4.2. Exclusion criteria

- Exclusion criteria for khat chewer and non-khat chewer groups
  - Pregnant women
  - Individual who is not in fasting state in the morning
  - Not willing to participate or were critically ill.

5.5. Study variables

5.5.1. Dependent Variable

- Fasting blood sugar level

5.5.2. Independent variable

- Age
- Sex
- Occupation
- Resident
- Khat chewing
- BMI

- Waist circumference
- Blood pressure

5.4. Sample size determination and sampling technique

5.4.1. Sample size determination

Based on the rule of thumb suggested by Van Voorhis and Morgan, where 30 participants per group are required to detect deference which lead to 80% power(36). Thus, the total sample size determined for this study was 200. Each of the above participants were categorized as healthy(n=100) and diabetic(n=100). Then subjects were subdivided in to groups as khat chewer and non-khat chewer consisting 50 individuals in each category.

5.4.2. Sampling method

Convenient sampling method was applied to collect data from study participants.

5.5. Measurement and Data collection

5.5.1. Data collection procedure

The objective of the study was clearly explained to study participants. Questioner was pretested on 5% of study participants. Data on sociodemographic and behavioral characteristics, was collected using structured questioner from volunteers. Detailed medical history of diabetic patients was reviewed and recorded by nurse at diabetic clinic regarding Blood pressure, history of hypertension, type of anti-diabetic drug and last 2-months FBS result. Anthropometric measurements (height, weight, waist circumference) were measured using standard height and weight measurement scale. BMI calculated as weight divided by height squared and expressed as kg/m2. Waist circumference were measured by placing meter between lower ribs and hip and reported in cm and converted to inches.

5.5.1.1. Blood sample collection

About 5 ml of fasting blood sample from forearm antecubital vein of consented study subject following antiseptic technique was collected by laboratory technologist in serum separator tube which is labeled with participant serial number that match with the questioner. Blood sample was

collected from 2-4am after overnight fasting and before morning insulin injection or oral antidiabetic therapies.

5.5.1.2. Blood sample processing and analysis

Blood specimen was centrifuged at 2500 rpm for 3 minutes to separate serum within 20 minutes after collection. Biosystem(R) A25 clinical chemistry analyzer at Hiwot Fana specialized universit y clinical chemistry laboratory was used for glucose determination using enzymatic glucose oxidase method. Left over specimen were poured to 2ml Nunc tube and stored under -20^{o}c and transported to St. Peter hospital for hexokinase glucose assay using COBAS(R) 6000 chemistry analyzer. Laboratory results were recorded on data collection sheet.

5.5.1.3. Laboratory principle and interpretation

Fasting blood glucose of the study participants was determined by colorimetric enzymatic reaction in glucose oxidase and glucose hexokinase method described as follows.

- **Glucose oxidase method**

Test principle

Glucose oxidase oxidizes glucose to gluconic acid and hydrogen peroxide. The formed H_2O_2 reacts under the catalysis of peroxidase with phenol and 4-aminoantipyrine to give a red violet quinoneimine dye as indicator (Trinder's reaction). Absorbance of the chromogen measured spectrophotometrically at 500nm which is proportional to concentration of glucose in the sample

Reaction principle

Glucose + H_2O_2 +O_2 **Glucose oxidase** Gluconic acid + H_2O_2

$2H_2O_2$+ 4-aminoantipyrine+phenol **Peroxidase** $4H_2O$ + quinoneimine

(Color-less) (Brown/ red violet)

Reagent and calibrator:

Mono reagent :Glucose oxidase ≥10kat U/L; peroxidase≥2Kat U/l; Phosphate buffer100mmol/l; PH 7.5;Phenole 5mmol/L;4-aminoantipyrine 0.5mmol/L. DiaSys TruCal U calibrator expiration date 2020-10 used to calibrate the instrument.

Reagent stablity

The reagent is stable up to the given expiry date when stored at 2-8^{o}c and it is in ready to use and inserted in test kit Lot# 28225, expiration date 2021-01,production date 2019-03 ;clonatest Spain.5 µl of serum and 500µL reagent mixed and analyzed. Biosystem A25-China authomethod clinical chemistry analyzer used for assay.

Quality control

Both pathological and normal commercially available quality control materials were run to monitor the analytical phase.

Pathological control: Range 228-309mg/dl, Lot# 23482, Expiration date 2021-01.

Normal control**:** Range 92-112 mg/dl, Lot#23486, expiration date 2021-08.

Linearty range

The test is linear up to glucose concentration within a measuring range from 1-400mg/dl (0.06 - 22.2 mmol/L).

Limit of detection

The lowest limmit of ditection or sensetivity of the test is 1 mg/dL (0,06 mmol/L).

- **Glucose hexokinase method**

Test principle

Glucose in clinical specimen determined by end point assay.Phosphorylation of glucose to glucose-6-phosphate by ATP is catalyzed by hexokinase as showen below. The enzyeme hexokinase is not specific for glucose and other hexose may react.

Glucose + ATP $\xrightarrow{\text{Glucose hexokinase}}$ Glucose-6-Phosphate+ ADP

Another enzyme, Glucose 6-phosphate dehydrogenase which is specific to glucose, oxidizes glucose-6-phosphate to 6-phosphogluconate in the presence of NADP. The rate of NADPH formation is directly glucose concentration in the sample.Absorbance is measured photometrically at 340nm.

Glucose -6-Phosphate + NADP $\xrightarrow{\text{Glucose\_6 phosphate dehydrogenase}}$ 6-phosphogluconate+ NADPH + H^+

Reagent and calibrator

R1: -TRIS buffer: 100 mmol/L, pH 7.8; Mg2+: 4 mmol/L; ATP: ≥ 1.7 mmol/L; NADP: ≥ 1.0 mmol/L; preservative

R2:-HEPES buffer: 30 mmol/L, pH 7; Mg2+: 4 mmol/L; HK (yeast):≥ 130 µkat/L; G-6-PDH (E. coli): ≥ 250 µkat/L; preservative.150µl R1 ,30µl R2 and mixed with 2µl sample to react in the assay procedure. And water used as blank solution.

Reagent stability

The reagent is stable up to the given expiry date when stored at 2-8^{o}c and it is in ready to use and inserted in test kit Lot# 43230301, expiration date 2020-12-31, Roche diagnostic company-Germany. COBAS 6000 c501Roche diagnostic company Germany clinical chemistry analyzer used for assay. S-2 C.f.a.s multi calibrator, expiration date 2021-09 used to calibrate the instrument.

Quality control materials

Pathological control: Range 207-255mg/dl, Lot# 32434500, Expiration date 2020-08.

Normal control: Range 92-112 mg/dl, Lot#324220900, expiration date 2021-06.

Linearity range

The test is linear up to glucose concentration of 0.11-41.6 mmol/L or 2-750 mg/dL**.**

Limitt of ditection

The lowest limmit of ditection or sensetivity of the test is 0.11 mmol/L (2 mg/dL)**.**

Interpretation of result:-A fasting blood sugar level less than 100 mg/dL (5.6 mmol/L) is normal. A fasting blood sugar level from 100 to 125 mg/dL (5.6 to 6.9 mmol/L) is considered prediabetes. If it's 126 mg/dL (7mmol/L) or higher on two separate tests, the individual has diabetes.

5.6. Data quality assurance

During data collection for each study participants questioner was designated with unique serial number that match with specimen collection container. Prior to actual data collection questioner was pretested on 5% of study subjects. Completeness and consistency of each collected data were checked at the end of each day. Incomplete and mismatched questioners were discarded. For reliability and representativeness of the study only complete and consistent data were incorporated. In order to maintain the quality of laboratory result every laboratory procedure following SOP and IQC were performed to check the performance of chemistry analyzer by running quality control materials (both normal and pathological) daily before analysis of the samples and analysis of specimen has been carried out and control result were governed by Westgard rule. Pre-analytical, analytical and post-analytical activities were also implemented as part of laboratory quality assurance. Quality of anthropometric measurements were also maintained as follows. Weight by standard weight measuring instrument put to zero prior to measurement and subjects wear least tiny close. Height were also measured using standard height measuring meter standing upright position. Waist circumference of subjects wearing tiny close were measured using meter put between lower ribs and hips. All anthropometric measurements were reported to the nearest 0.5cm.

5.6.1. Pre-analytical phase

Prior to sample collection each of the study participants were assigned with unique serial number that matches with questioner and serum separator test tube. Proper patient preparation (tourniquet application, sitting) were considered before and during specimen is collected. Above all GLP (good laboratory practice) through following SOP were applied for specimen collection as well as processing. 5ml whole blood specimen was collected in serum separator test

tube cleaning the fore arm of participants with 70% alcohol. The specimen was centrifuged with in 20minutes of collection. Left over specimen were stored under -20^{o}c. Hemolyzed specimen were rejected.

5.6.2. Analytical phase

Before analysis of specimen commercially prepared quality control materials run. Calibration of the chemistry analyzer were performed as recommended by manufacturer in the reagent package kit insert and the specimen was mixed with the required volume of reagent. S-2 C.f.a.s a multi calibrators were used for the case of hexokinase method using COBAS 6000cS chemistry analyzer and DiaSys TruCal U calibrator used for the case of glucose oxidase using Biosystem A25 -China clinical chemistry analyzer used.

5.6.3. Post-analytical

The laboratory analysis result for fasting blood sugar were recorded with legible hand writing on data collection sheet which was labeled with the participant identification number. The result of laboratory test for fasting blood sugar interpreted according to the reference value recommended by American diabetes association.

5.7. Data analysis and Interpretation

Data that has been checked for completeness and consistency entered to SPSS version 21 software (IBM corporation USA) and analyzed. Kormogorov-smorvo test and histogram used to examine data distribution. Data were reported as frequency and percentages for categorical variable, (Mean±SD) for normally distributed continuous variable, median with interquartile range (Median±IQR) for continuous variable with skewed distribution. Non-parametric tests Mann-Whitney test (2-independent sample test) and Kruskal-Wals test (K-independents sample test) were used to compare fasting blood glucose among khat chewer and non-khat chewer with in the groups and between groups respectively. Data were presented as frequency and percentage for categorical variables. P-value of less than 0.05 considered as statistically significant. The results are presented in table.

5.8. Ethical consideration

The study conducted after written ethical approval is from Addis Ababa University College of health science medical laboratory department ethics committee. Support letter has been submitted to Hiwot Fana Specialized University Hospital. The study participants had been provided with detailed explanation about the aim of this research, the risk, benefit, and their right to get their result for free and it' confidentiality as well as written informed consent was obtained from each participant before actual data collection. Participants who agreed and signed the consent form were included in the study. There was strict confidentiality for laboratory result as well as information obtained from participants throughout the study.

5.9. Dissemination of result

The study finding will be disseminated to Addis Ababa university college of health science, Harar Health Bureau, Hiwot Fana specialized university hospital Harar, other local health institut ions and concerned bodies. More importantly the study will also be submitted to reputable journals for publication. Local studies may also use the study finding for further study.

6. Result

6.1 General characteristics of study participants

In the study a total of 100 diabetic patients who had visited diabetic clinic during the study period and 100 healthy normoglycemic individuals were included. Of all the respondents 83(41.5%) and 117(58.5%) were male and female respectively. The Mean±SD age of diabetic patients and healthy individuals were 44.2±8 and 42 ±7.4 years respectively.

Table 1:- Socio- demographic and behavioral characteristics of study participants with diabetes and healthy groups in Harar eastern Ethiopia January-March 2020. (N=200)

Variable		Study groups				Total	%
		Diabetics		Healthy			
		Khat chewer (n=50)	Non-khat chewer(n=50)	Khat chewer (n=50)	Non-khat chewer(n=50)		
Age	25-34	9(18%)	4(8%)	9(18%)	7(14%)	29	14
	35-44	14(28%)	21(42%)	21(42%)	23(46%)	79	39
	45-54	22(44%)	16(32%)	16(32%)	17(34%)	71	35
	≥55	5(10%)	9(18%)	4(8%)	3(6%)	21	10
Sex	Male	21(42%)	18(36%)	26(52%)	18(36%)	83	41
	Female	29(58%)	32(64%)	24(48%)	32(64%)	117	58.5
Marital status	Single	2(4%)	4(8%)	9(18%)	12(24%)	27	13
	Married	34(68%)	34(68%)	26(52%)	25(50%)	119	60
	Divorced	3(6%)	5(10%)	8(16%)	10(20%)	26	13
	widowed	1122(%)	7(14%)	7(14%)	3(6%)	28	14
Occupation	Government	9(18%)	12(24%)	50(100%)	50(100%)	121	60.5
	Private	7(14%)	9(18%)	-	-	16	8
	Farmer	7(14%)	1(2%)	-	-	8	4
	Marchant	12(24%)	10(20%)	-	-	22	11
	unemployed	15(30%)	18(36%)	-		33	16.5
Address	Urban	30(60%)	40(80%)	50(100%)	50(100%)	170	85
	Rural	20(40%)	10(20%)	-	-	30	15
Alcohol consumption	Yes	13(26%)	27(54%)	31(62%)	28(56%)	99	49.5
	No	37(74%)	23(46%)	19(38%)	22(44%)	101	50.5
Cigarette smoking	Yes	15(30%)	4(8%)	9(18%)	3(6%)	31	15.5
	No	35(70%)	46(92%)	41(82%)	47(94%)	169	84.55

6.2 Clinical characteristics of study participants

Most of the study subjects (51%) were diagnosed as diabetics for more than 7 years ago. Median SBP (Median±IQR; mmHg) were 119.5±25 in diabetics and 108±10 in healthy subjects Median± IQR; mmHg) were 88±20 for diabetics and 79±4 for healthy subjects. The (Mean±SD, kg/m^2) body mass index was 26.1±3.6 and 23±1.5 in diabetics and healthy individuals respectively. BMI and FBS showed and FBS showed significant correlation in diabetic subjects and healthy subjects. The (Mean±SD, inches) waist circumference was 35.3±4.1in diabetics and 33.3±2.9 in healthy subjects.

Table 2: - Clinical data and anthropometric measurement of study participants at Hiwot Fana specialized university hospital Harar eastern Ethiopia. (N=200).

Variable		Study groups				Total	%
		Diabetics		Healthy			
		Khat chewer (n=50)	Non-khat chewer(n=50)	Khat chewer (n=50)	Non-khat chewer(n=50)		
Family history of DM	Yes	11(22%)	24(48%)	16(32%)	10(20%)	61	30.5
	No	39(78%)	26(52%)	34(68%)	40(80%)	139	69.5
History of hypertension	Yes	15(30%)	17(34%)	-	-	32	16
	No	35(70%)	33(66%)	50(100%)	50(100%)	168	84
Type of anti-diabetic drug	Insulin	23(46%)	21(42%)	-	-	44	44
	Metformin	15(30%)	11(22%)	-	-	26	26
	Insulin+metformin	12(24%)	18(36%)	-	-	30	30
Diabetes duration	≤7 years	33.(%)	30(60%)	-	-	63	63
	>7 years	17(34%)	20(40%)	-	-	37	37
BMI (kg/m^2)	Normal	23(46%)	16(32%)	50(100%)	50(100%)	126	63
	Overweight	16(32%)	26(52%)	-	-	53	26.5
	Obese	11(22%)	8(16%)	-	-	21	10.5
Waist circumference (inches)	Low risk	24(48%)	13(26%)	48(96%)	42(84%)	128	64
	Intermediate risk	17(34%)	31(62%)	2(4%)	8(16%)	55	27.5
	High risk	9(18%)	6(12%)	-	-	17	8.5
Blood pressure (mm/Hg)	Normotensive	35(70%)	34(68%)	50(100%)	50(100%)	169	84.5
	Pre-hypertensive	7(14%)	4(8%)	-	-	11	5.5
	hypertensive	8(16%)	12(24%)	-	-	20	0.1

6.3 Khat and fasting blood glucose level among diabetics and healthy individuals

There was hypoglycemic effect of khat chewing on serum glucose level in both diabetics and healthy subject of khat chewers compared to non-khat chewer groups using Mann-Whitney test. Diabetic khat chewers had significantly lower (Median±IQR) blood glucose level (159.5±83) than non khat chewers with diabetes (202±79) when tested by GOD (p=0.002). Similarly, when measured by enzymatic hexokinase method, diabetic khat chewers had significantly lower (Median±IQR) FBS (141±80) compared to non khat chewers (188±79) (p<0.001).

Percentage difference of FBS between khat chewers and non-khat chewers when measured by GOD was 21%. This implies that fasting sugar level of diabetic khat chewers was 21% lower than that of diabetic non-khat chewers (p=0.002). Similarly, in enzymatic hexokinase method diabetic khat chewers fasting blood glucose level was 25% lower when compared to diabetic non khat chewers (p<0.001) **table 3.**

Table 3 - Comparison of serum glucose level using Mann-Whitney test among khat chewer and no khat chewer diabetic patients and healthy individuals.

Fasting blood glucose	**Diabetics**		**Healthy individuals**	
	khat chewer (n=50	**non-khat chewer (n=50)**	**khat chewer (n=50)**	**non-khat chewer (n=50)**
GOD (Median±IQR)	159±83	202±79	82±18	94±13
P value	P=0.002		P<0.001	
GHK (Median±IQR)	141±80	188±79	71±12	81±10
P value	P<0.001		P<0.001	
Percentage difference measured by GOD	21%		12.7%	
Percentage difference measured by GHK	25%		12.3%	
% difference between the same groups measured by two methods.	11.3%	6.9%	13.4%	13.8%

Similarly, when we compare between healthy non-khat chewers and khat chewers, khat chewers have lower (Median±IQR) fasting blood glucose level (82±18) than that of the non khat chewers (94±13) when tested by GOD (p<001). (Median±IQR) fasting blood glucose level when measured by enzymatic hexokinase (GHK) method, healthy khat chewers have significantly lower fasting glucose level (71±12) than that of non khat chewers (94±13).

In healthy khat chewers and non khat chewers there was 12.7% percentage difference when measured by GOD. This means that fasting sugar level of healthy khat chewers was 12.7% lower than that of non-khat chewers (p<0.001). In enzymatic hexokinase method there was 12.3% percentage difference between healthy khat chewers and non-khat chewers. Which implies fasting blood sugar was 12.3% lower in healthy khat chewers when compared to non khat chewers.

Generally, there was significantly lower blood glucose level measured by both GOD and GHK methods in both diabetics and healthy groups of khat chewers. There was also percentage difference of median FBS with in the same group when measured by GOD and GHK methods.

6. Discussion

The major therapy option in diabetes mellitus is lifestyle management. Besides exercise, weight control and medical nutrition therapy, oral glucose-lowering agents and insulin injection are the conventional therapies for diabetes though they have adverse side effects, are expensive and require expertise.

In the current study the effect of khat chewing on fasting sugar level were examined and fasting plasma glucose level in khat chewer was significantly lower compared to non khat chewers in both diabetics and healthy groups. In khat chewer groups the hypoglycemic effect of khat showed by the present study might be due to the presence of detectable amount of Mg, Zn, Fe, Pb, Cu, in khat leaves in which their presence at desirable physiological concentration is very important for glucose hemostasis (Mg) and also increase the number of insulin receptors as well as affinity. (Zn) also play role in insulin synthesis and release (37) .

Another possible explanation for the present study could be due to the presence of ascorbic acid which present on khat leaves(7) which has an anti-oxidant function and combats the harmful effects of free radicals in diabetic patients(38).Study revealed that it lowers fasting blood glucose level in type-2 diabetics although the exact mechanism by which Vitamin C brought this change is not known.(29). Though in the present study we couldn't measure serum vitamin C level of study subjects.

The present study is similar with finding in (Kenya,2011). Hypoglycemic activity by Kenyan plants including C.edulins that are traditionally used to manage diabetes have been reported (39). Aqueous extract of this plant lower blood glucose level effectively like that of insulin. This plant may achieve hypoglycemic effect through different mechanisms like stimulating glucose catabolizing enzymes (serving as cofactor), decrease absorption of carbohydrates to portal circulation.

The present study is in line with study conducted in (Yemen,2009) involving 20 confirmed diabetic patients and 10 Healthy subjects to study the effect of khat on blood glucose level. The result showed that there was significant decrease in blood glucose among khat chewer diabetic as well as healthy individuals who underwent khat chewing session. There was reduction of blood glucose

by 61.2% in healthy khat chewers within 4hr of consumption(22). And again, in line with similar finding in (Yemen,2013) in which they found significantly decreased plasma glucose level in diabetics and healthy subjects (40). This hypoglycemic effect of khat in both diabetics and non-diabetics can be explained the presence minerals, tannins (7–14% in dried material), vitamins (Vitamin c), flavonoids, saponin(41).

Contrary to our study increased blood glucose level in diabetic khat chewers while no effect in healthy khat chewers during khat chewing experimental period were reported in (Yemen,2002)(26).This can explained by khat's sympathomimetic effect by releasing nor-adrenalin (counteract insulin action) increase blood sugar level in healthy as well as diabetic subjects(7). In non-diabetic subjects this can be overcome by pancreatic beta cells compensatory action by release of insulin to suppress hepatic glucose output and keep blood glucose level normal. There is 60-100% increment of peripheral insulin level for 10–15 mg/dl rise in plasma glucose level in healthy non diabetic subjects(42).

The present study contradicts with finding in (Yemen,2012) reported that khat significantly increase blood glucose level in khat chewers with diabetes as well as healthy compared to non-khat chewers (20). This could be due to enhanced glycemic response by subjects with diabetes toward catecholamines (adrenaline, nor adrenaline) released as a result of cathinone owing to underlying defect in insulin action and secretion(19) or could be due to the effect of cortisol and resistin (ADSF) which increase following khat chewing as result of cathinone release from khat in both diabetics and healthy individuals. In diabetes khat chewers it significantly decrease insulin level(8). Furthermore, sweetened beverage used along with khat aggravates the existing hyperglycemia in diabetic patients.

This study is also in contrary to the reports in (Yemen, 2000) where chronic khat chewing leads to the development of diabetes among chronic khat chewers(28). Though the exact mechanism by which DDT induce hyperglycemia is not clear, however, DDT inhibit pancreatic secretary activity by increasing the activity of gluconeogenic enzymes. Promoting hepatic glycogenolysis by activating glycogen phosphorylase(43). This association might be attributed to the long-term effects of pesticides residues on chronic khat chewers

7. Conclusion and recommendation

Conclusion: - Based on the present finding, Khat significantly lower fasting blood glucose level in khat chewer groups of both diabetics and healthy individuals. This study did not address overall effect of khat on system metabolic activities. This warrants the need to assess overall metabolic effect of fractionalized khat.

Recommendation: -Based on the present study we recommend,

- Further investigation or phytochemical analysis is needed to identify khat's active ingredient which is associated with hypoglycemia so that in combination with modern medicine more potent antidiabetic drug can be made.
- More importantly objective nutritional advice should be given to individuals for misconception that khat lower blood glucose as it causes several adverse health effects.
- More studies should be conducted in different geographical and socioeconomic settings, as well as study design that could strongly show the casual relationship to further elucidate the cause-effect of khat on systemic metabolism.

8. Strength and limitation of the study

Strength

- Khat chewing is widely held practice in the eastern Ethiopia the current study tried to assess the effect of khat on body metabolite (blood glucose) using two methods to possibly assess the interference of khat constituent on the assay.

Limitation

- Similar number of khat chewing session/hr and amount of khat consumed were not taken in to consideration.
- The use of FBS to compare khat effect may not be reliable as it is affected by several factors.
- There might be recall bias for some response and selection bias with convenient sampling method.
- The casual relationship may not be strong with this study design.

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10. Annexes

Annex I: - Participant information sheet

Information sheet English version

Project Title: - Comparison of blood glucose level among khat chewer and non khat chewer in diabetic and healthy individuals at Hiwot Fana specialized hospital Harar Eastern Ethiopia.

Principal Investigator: Yordanos Mengistu (BSc, MSc candidate)

Name of the Organization: - Hiwot Fana Specialized University Hospital Harar Eastern Ethiopia.

Introduction-: My name is Yordanos Mengistu. I am MSc student in Addis Ababa university college of Health science department of Medical Laboratory Technology. You are invited to parti cipate as a study subject in a research to compare blood glucose level among khat chewer and non khat chewer diabetic and apparently healthy individuals. Please take as much time as you need to read or listen in the information sheet.

Purpose of the Research Project: - The purpose of this study is to Compare blood glucose level among khat chewer and non khat chewer in diabetic and apparently healthy individuals at Hiwot Fana specialized hospital Harar Eastern Ethiopia.

Procedures and the expected participation: - If you are willing to participate, you need to understand the purpose of the study and give your consent. Not only this but also the required volume of fasting blood specimen will be collected by experienced laboratory technologist in clinical chemistry department. Then, you are requested to give your consent to the sample collector. After consent, a sample will be taken from your arm by lumbar puncture. Moreover, there will be a face-to-face interview for additional questions.

Potential risks and Discomforts

There might be some minimal risk and discomfort when we take venous blood. However, during collection of blood specimens from you, appropriate precaution will be taken and sample will be collected by experienced laboratory technologist. If anything happened, appropriate medical care will be provided to you.

Confidentiality

We respect your privacy and confidentiality in this study. Any information that identifies you will not be shared with anyone else outside the study team. The information we will collect from you as part of the study will be kept in a locked file cabinet, or be protected by a password on the computer only accessible to personnel involved in the study. Results will be coded by unique number. Without your permission your personal information will not be disclosed to anybody.

Potential benefits or incentives to subjects and/or to the society

You will not receive any payment for your participation in this research study as compensation. However, the result of the study will be used by clinicians an is beneficial in the management of diabetes and maintain blood glucose in normal level. Hence, you are indirectly benefiting other patients and the society in this respect.

Participation and Withdrawal from the Study

The participation is voluntary and you have the right not to participate in this study. You may withdraw at any time and place without consequences of any kind. You may also reject to give any sample. You can ask any questions regarding to this study and you have a right to get a laboratory diagnosis result free.

Contact information

If you have any questions about this study you can contact the following research team for further information.

Yordanos Mengistu phone # 0940880200 Email: - yordimengistu8@gmail.com

Annex II:-Information sheet Amharic version

የተሳታፊዎች ፈቃድና መተማመኛ ቅፅ

የጥናቱ ራእስ፥- በሂወት ፋና ስፔሻላይዝድ ዩኒቨርሲቲ ሆስፒታል: ቻት በሚቅሙ እና በማይቅሙ የስኳር ታማሚዎች እና ጤናማ ሰዎች መካከል የ ስኳር መጠንን ማወዳደር ፡

የጥናቱ ተመራማሪ፥- ዮረዳኖስ መንግስቱ

የተቋሙ ስም፥- ሂወት ፋና ስፔሻላይዝድ ዩኒቨርሲቲ ሆስፒታል

መግቢያ፥- ዮረዳኖስ መንግስቱ እባላለሁ።በ አ አ ዩኒቨርሲቲ ጤናና ሳይንስ ኮሌጅ የ ክሊኒካል ኼሚስትሪ ማስተርስ ተማሪ ነኝ። ጭት መቃም በሰውነታችን የስኳር መጠን ላይ የሚኖረውን ግንኙነት በሚጠናው ጥናት ላይ አንደ ጥናቱ አካል በመሆን አንዲሳተፉ ተጋብዘዋል። አባከዎን ግዘ ወስደው የመተማመኛ ቅፅ ያንቢቡት።

የፕሮጀክቱ አላማ፥- የጥናቱ አላማ ቻት በሚቅሙ እና በማይቅሙ የስኳር ታማሚዎች እና ጤናማ ሰዎች መካከል የ ስኳር መጠንን ማወዳደር ነው፡ ጥናቱ የሚካሄደውም በሂወት ፋና ስፔሻላይዝድ ዩኒቨርሲቲ ሆስፒታል ታካሚዎች ላይ ነው።

የጥናቱ አካሄድ እና የሚተበቁ ተሳትፎዎች፥- እርሶ በዚህ ጥናት ላይ ተሳታፊ ከሆኑ የጥናቱን አላማ መረዳት እና ፈቃደኛ መሆን ይኖርቦታል።ይህም ብቻ ሳይሆን ልምድ ያለው የላቦራቶሪ ባለሞያ ለጥናቱ የሚያስፈልገውን የደም ናሙና ከእርሶ ይወስዳል። ስለዚህም ናሙና ለሚሰበስበው ሰው ፈቃደኝነቶን መረጋገጥ የኖርቦታል።ከዚያ ቡሃላ ከክንድ የደም ስር ይወሰዳል።በተቸማሪም የገስፅ ለ ገፅ ቃለ መተይቅ የደረግሎታል።

ጥናቱ የሚያስከትለው ችግርና አለመመቸት፥-የደም ናሙና በሚወሰድበት ግዘ መጠነሳኛ የሆነ መጎዳትና አለመመቸት ሊኖር የችላል።ቢሆንም ግን ናሙና ሲወሰድ ተገቢውን ጥንቃቄ ይወሰዳል።በተጨማሪም ናሙናውን የሚሰበስበው ላምድ ያለው የላቦራቶርሪ ባለሙያ ነው።ምንም አይነት ችግር ቢፈጥር ተገቢውን የህክምና አንክብካቤ ይደረግሎታል።

ሚስጢር ስለመጠበቅ፥- በዚህ ጥናት ሚስጥሮ የጠበቀ ነው።ከዚህ የጥናት ቡድን ውጪ ለ ሌላ ለማነኛውም ሰው ማንነቶን የሚገልፅ መረጃ አይሰጥም።ለ ጥናቱ የሚሰጡንን መረጃ በተቆለፈ ካቢኔት ወይም በኮምፒተር ፓስዋርድ ይቀመጣል። ቁልፉም ለ ጥናት ቡድኑ አባላት ብቻ የሚሰጥ ይሆናል።ፋይቹ ለጥናቱ ተሳታፊዎች ብቻ የተፈቀዱ ይሆናሉ።ውጤቶም በሚስጥራዊ ቁጥር የተሰየሙ የሆናሉ።ያለ አርስዎ ፈቃድ ውጤቶ ለማንም ተላልፎ አይሰጥም።

ለማህበረሰቡ ወይም ለግለሰብ የሚኖረው ጠቀመታ ወይም ጥቅማጥቅም፥- በዚህ ጥናት ስለተሳተፉ አንደ ማካካሻ ክፍያ ወይም አበል አይኖርም።ነገር ግን የጥናቱ ውጤት ለህክምና ባለሞያዎች የስኳር ህመምተኞችን የስኳር መጠን ለመቆጣጠር አንዲረዱ በ ግበትነት ሊጠቀሙበት ይችላሉ። ስለዚህ በዚ መልኩ ማህበረሰቡንና ለሎች ታካሚዎችን ይጠቅማሉ።

በጥናቱ ስለመሳተፍና አቋርጦ ስለመውጣት፥-በዚህ ጥናት ላይ መሳተፍ በ ፍቃደኝነት ላይ የተመሰረተ ሲሆን ያለመሳተፍ መብት አንደተጠበቀ ነው።ምንም የሚደርስበት ነገር ሳይኖር ከ ጥናቱ አቋርጦ መውጣትም ይችላል።የላቦራቶሪ ውጤቶን የለምንም ከፍያ ሳይጠየቁ መውሰድ ይችላሉ።

ጥያቄ ካሎት ለማነጋገር፥- ስለጥናቱ ንም አይነት ጥያቄ ቢኖሮት የሚከተሊትን ኣድራሻ ይጠቀሙ።

ዮርዳኖስ መንግስቱ ስ።ቁ 0940880200 ኢሜል yordimengistu8@gmail.com

Annex III: - Information sheet Afan Oromo version

Guca Waliigaltee Fi Heyyamamummaa Hirmaattootaa

Mata Duree Qorannichaa:- Hospitaala Ispeeshaalaayizidii Hiwoot Faanaatti, Garaagarummaa hanga Shukkaaraa Dhukkubsattoota dhibee Shukkaara kan Jimaa fayyadamanii fi hin fayyadamnee fi akkasumas, Namoota dhukkuba sukkaara hin qabne jidduu jiru.

Qaama Qorannicha Gaggeesse: **Yordaanoos Mangistuu**

Maqaa Dhaabbatichaa:- "Hiwot Faanaa Ispeeshaalaayizid Yunivaristii Hospitaal"

Seensa:- Maqaan koo Yordaanoos Mangistuu Jedhama. Yuunivaristii Finfinnee Kollejjii Saayinsii Fayyaa Keessatti barattuu Digrii Lammaffaa Kilinikaal Keemistiriitti. Qaama mata- duree qorannoo "Jimaa qama'uun walitti dhufeenya inni hanga shukkaara qaama keenyaa waliin qabu" jedhu ta'uun akka hirmaatan afeeramtanii jirtu. Kanaafuu guca waliigaltee kana of eeggannoon dubbisaa.

Kaayyoo Piroojaktichaa:- Qorannoon kun Yaalamtoota Hospitaala Ispeeshaalaayizidii Hiwoot Faanaa irratti kan gaggeefamu yoo ta'u, kaayyoon qorannichaas garaagarummaa hanga shukkaaraa dhukkubsattoota dhibee shukkaara kan jimaa fayyadamanii fi hin fayyadamnee fi akkasumas, namoota dhukkuba sukkaara hin qabne jidduu jiru adda baasuu dha.

Haala Adeemsaa fi Hirmaanaa Qorannichaa:- Qaama qorannoo kanaa ta'uun yemmuu hirmaatan, kaayyoo qoranichaa hubachuunii fi fedhinnaa qabachuun isin irraa eegama. Akksumas ogeessa laabraatoorii muuxannoo fi gahuumsa qabuun dhiigni bicuu qorannoof gargaaru hidda dhigaa harka keessan irraa kan fudhatamu waan ta'eef, fedhinaa qabaachuu keessan mirkaneessuun barbaachisaa dha. Dabalataanis af-gaafiin kan isiniif godhamu ta'a.

Rakkoo fi Dhiibbaa Walqabataa Qorannicha:- Yeroo iddattoon dhiigaa fudhatamu qaama keenya irratti hanga tokko nutti dhagahamuu danda'a. Haa ta'u malee of eggannoon barbaachisaa ta'e nu godhama. Iddattoon dhigaatis kan fudhatamu ogeessaa Laabiraatoorii gahumsaa fi muuxannoo qabun ta'a. Rakkoon kan uumamu yoo ta'e, kunuunsi fayyaa ni taasifama.

Iciitii Eeguu:- Qorannoo kana irratti iciitiin keessan gutuumaan guututti kan eegame ta'a. Garee qorannoo kan gaggeessuun alatti odeeffannoon eenyummaan keessan ibsu qaama biraatiif dabarsamee hin kennamu. Odeeffannoon isin irraa sassaabame akka sanduqa keessatti kan itti cufamee ta'uu fi kan kompitaraan barraahe immoo 'paaswordiin hidhamee akka taa'u godhama. Furtuun fi faayila dhuunfaa keessan kan qabatu fi kan fayyadamu garee qorannoo kana irratti hirmaatan qofa ta'a. Bu'aan qorannoo keessanis Lakkoofsi icitii kan kennamuuf ta'a. Fedhii fi beekamtii keessan malee bu'aan qorannoo keessan qaama birootiif dabarfamee hin kennamu.

Bu'aa fi Faayidaa Qoronnichi Akka Dhuunfaa fi Hawaasatti Qabu:- Bu'aan fi faayidaan qorannoo kana irratti hirmaachuun argamuu fi kennamu hin jiru. Haa ta'u malee, ogeessootni fayyaa bu'aa qoranichaa hanga shukkaraa dhukkubsattoota shukkaraa ittin to'achuuf akka galteetti akka itti dhimmi bahan gochuun ni danda'ama. Kanaafuu, bu'aan qoranichaa bifa Kanaan hawaasaa fi dhukkubsataa dhuunfaaf faayidaa kan kennu ta'a jechuudha.

Mirga Qoranicha irratti hirmaachuu fi Addaan kutuu:- Hirmaannaan qoranichaa fedhii irratti kan hundaa'u waan ta'eef mirgi hirmaachuu fi hirmaachuu dhabuu keessan kan eegame ta'a. Rakkon tokkoolle oso hin gahiin qoranicha addaan kutanii bahuun ni danda'ama. Bu'aa qorannoo Laabraatorii keessan kaffaltii tokko malee fudhachuun ni danda'ama.

Odeeffannoo Dabalataaf:- Qoranicha ilaalchisee odeeffannoo dabalataa fi gaafii yoo qabaatan teessoo armaan gadiin kan na argatan ta'a:-

Maqaa:- **Yordaanoos Mangistuu**

Lakk. Bilbilaa:- 0940880200 E-mail:-yordimengistu8@gmail.com

Annex IV: - Informed consent form English version

code ……

I had been informed that the objective of this study. The purpose of this study is to compare blood glucose level among khat chewer and non khat chewer diabetic and non- diabetic healthy

individuals in Harar town. I had been informed about the confidentiality of this study. The principal investigator requested me to participate in the study that would require my willingness to provide the required data and blood sample, and filling questionnaire. Therefore, with full understanding of the importance of the study, I agreed voluntarily to provide the requested samples and my benefit will be only from the free laboratory investigation result/s.

I_____ hereby give my consent for providing the requested information and specimens.

Signature: _____ Date_____

Annexe V: -Informed consent form Amharic version

የተሳታፊዎች ስምምነት ማረጋገጫ

የኮድ ቁጥር ……

የጥነቱ አላማ በደምብ ተብራርቶልኛል።የጥናቱ አላማ ጫት በሚቅሙ እና በማይቅሙ የስኳር ታማሚዎች እና ጤናማ ሰዎች መካከል የ ስኳር መጠንን ማወዳደር ነው።ስለ ጥናቱ ሚስጢር ጠባቂነት ተነግሮኛል።የጥናቱ ዋና ተመራማሪ በጥናቱ ላይ ተሳታፊ ሆኜ የደም ናሙና እና ቃለ መጠይቅ በመስጠት አንድሳተፍ ፍላጎተን ጠይቆኛል። በመሆኑም የጥናቱ ጥቅም ሙሉ በ ሙሉ በመረዳት ለመሳተፍ ፍቃደኛ መሆነን አረጋግጣለሁ።

አኔ……

የሚጠበቅብኝን መረጃና ናሙና ለመስጠት ፈቃደኛነቴን አረጋግጣለሁ።

ፊርማ…… ቀን……

Annex VI: - Informed Consent form Afan Oromo Version

Guca Mirkaneessa Waliigaltee Hirmaattoota Qorannoo

Koodii:_____

Kaayyoon qorannichaa sirritti naaf ibsamee hubadheen jira. kaayyoon qorannichaas garaagarummaa hanga shukkaaraa dhukkubsattoota dhibee shukkaara kan jimaa fayyadamanii fi hin fayyadamnee fi akkasumas, namoota dhukkuba sukkaara hin qabne jidduu jiru adda baasuu dha. Iciitiin qorannoo dhunfaa kiyya akka naaf eegamu natti himamee jira. Gaggeessaa dursaan Qoranichaa dhiiga qorannichaaf ta'u fedhii kiyyaan akka kennuu fi af-gaafiif hayyamamaa ta'uu kiyya na gaafatee jira.Kanaafuu faayidaa qorannichi gutuumaan gutuutti hubachuun qoranicha irratti hirmaachuuf hayyamamaa ta'uu koo nan mirkaneessa.

Ani Obbo/Adde _____

Kan jedhamu odeeffannoo narraa barbaadamuu fi iddattoo/naamunaa dhigaa kennuuf hayyamamaa ta'uu koo nan mirkaneessa.

Mallattoo: _____

Guyyaa: _____

Annex VII:-Questioner

English version of questioner

Code --------

S. No	Part 1: - Sociodemographic and clinical characteristics

	Study Variables	**Response**	
1.	Age in years		
2.	Sex	Male	1
		Female	2
3.	Female (age 20-49) use of contraceptive currently at least for three months?	Yes No	1 2
4.	Marital status	Single	1
		Married	2
		Divorced	3
		Widowed	4
5.	Are you currently Pregnant?	Yes	1
		No	2
6.	Work status	Government	1
		Private	2
		Farmer	3
		Marchant	4
		Others	5
7.	Address	Urban	1
		Rural	2
8.	History of any chronic disease like Liver, kidney disease, cancer etc for the last 1 year?	Yes	1
		No	2
9.	Any medication you take for illness?	Yes	1
		No	2
10.	Family history of diabetes?	Yes	1
		No	2
11.	Have you been diagnosed with diabetes?	Yes	1
		No	2
12.	If yes what type of drug you use and for how long?	Drug -------- Duration -------- in years	
	Part 2:- Behavioral characteristics		
13.	Do you live with khat chewers?	Yes	1
		No	2
14.	Have you ever chewed khat? If no skip to 20	Yes	1
		No	2
15.	How often do you chew khat?	Daily	1
		Occasionally	2
		Every weekend	3
16.	How long have you been chewing khat / years and amount in gm per day?	Years ---------- Amount in gm ---------	
17.	Have you been chewing khat before dx of diabetes?	Yes	1
		No	2
18.	Khat chewing status	Current	1
		Previous	2
		Current+ previous	3
19.	Which of the following you use most during khat chewing?	Water	1
		Sugar	2

		Peanut	3
		Soft drink	4
20.	How often do you smoke cigarettes? If you don't, skip this part?	Daily	1
		Occasionally	2
		Never	4
21.	Smoking status	Current	1
		Previous	2
		Current +previous	3
22.	How often do u drink alcohol? If you don't drink skip this part	Daily	1
		Every weekend	2
		occasionally	3

Part 5: -Biochemical and anthropometric measurements

FBS	**GOD**	
	GHK	
Blood pressure (mm/Hg)		
Heart rate		
Weight (Kg)		
Height (meter)		
Calculated BMI		
Waist circumference (cm)		

Annex VI: - laboratory SOP

Glucose determination procedure

- Collect fasting whole blood of 5ml from for arm and transfer gently to the pale serum separator test tube from the syringe.
- Allow to clot and centrifuge the whole blood at 2500 rpm to 2-3 minute.
- Separate the serum to white sample container cup (nunc tube), if delay is necessary to store the specimen at the right temperature (-18oc) for the right time until analysis.
- Turn on the clinical chemistry analyzer machine
- Check the expire date of glucose reagents
- Check the daily, weekly, monthly, quarterly and yearly controls, standards and calibration results of the analyzer
- Analyze the specimen based on the leaflet procedure for each clinical chemistry parameter tests.
- Read the absorbance measured and record carefully.

Declaration

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: Yordanos Mengistu (B.Sc.)

Signature: _____

Date of submission: 4 / 8/ 2020

This thesis has been submitted with our approval as advisors.

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

Signature: _____

Date:- 4 / 8/ 2020

Advisor: Gobena Dedefo (MSc)

Signature: _____

Date: - 4 / 8/ 2020