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Original Research Article

Effect of Crude Extract of Khat (*Catha Edulis)* On the Plasma Glucose Level of Normoglycemic and STZ Induced Type 2 Diabetic Rats

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ABSTRACT

Khat is chewed traditionally in Ethiopia, Somalia, Yemen and Kenya. In Ethiopia, khat chewing is now becoming an everyday substance of abuse for the general population. Clearly, knowledge about the effects of cathinone and khat chewing on plasma glucose and insulin levels is very sparse and controlled studies need to be undertaken in Ethiopia. Therefore, the objective of this study was to investigate the effect of crude extract of Catha edulis on blood glucose level of normal and STZ induced type 2 diabetic rats. Randomized experimental study was conducted for four consecutive weeks to determine the effects of crude extract of Catha edulis on plasma glucose level of 16 normal and 24 diabetic male Wistar rats(N=40). One experimental protocol was used in this study. Diabetic rats in group I were treated with 450mg/kg of crude extract of Catha edulis (8rats per group).Diabetic rats in group II were treated with Glibenclamide (5mg/kg) and was used for comparison(8rats per group). Diabetic rats in group III were treated with distilled water which served as controls. Normal rats in group IV were given 450mg/kg of crude extract of Catha edulis (8rats per group) and normal rats in group V were given distilled water as a control. Oral administration of aqueous extract (450mg/kg) of Catha edulis in diabetic rats reduced the blood glucose level from 296.8±42.8 to 96.7±7.4 (67.4%) ($p<0.01$). Administration of aqueous extract (450mg/kg) of Catha edulis in normal rats reduced the blood glucose level from 112.4±4.27 to 61.0±4.3 (45.7%)($p<0.01$). A administration of aqueous extract (450mg/kg) of Catha edulis in diabetic and normal rats caused reduction in body weight from 304.81±14.46 to 237.37±12.28 (22.1%) ($p<0.01$) Results of present study showed that oral administration of crude extract of Catha edulis exert a hypoglycemic effect in normal and STZ induced type 2 diabetic rats. The study found that crude extract of Catha edulis showed a significant reduction in body weight of normoglycemic and STZ induced Type 2 diabetic rats. Further studies to isolate different active components of Catha edulis to elucidate the exact mechanism of action so as to develop it as a potent antidiabetic drug is recommended.

Keywords: Catha edulis, Crude extract, Wistar rats, plasma glucose, weight gain/loss, Diabetic Mellitus

1. INTRODUCTION

The habit of khat (*Catha edulis*) chewing has prevailed for centuries among populations in the horn of Africa and the Arabian Peninsula. Khat is grown in Yemen and many parts of East Africa, especially Ethiopia and Kenya. The plant belongs to the family Celastracea. Fresh leaves of khat are customarily chewed to attain a state of stimulation. [1] Cathinone in khat leaves has a close structural similarity with amphetamine, and that both share common pharmacodynamic features. It had led to the conclusion that cathinone is the most important active ingredient of khat, causing the major pharmacological effects. [2] Khat

user chews the young leaves and the twings of the plant, the residue is stored in the cheek. The saliva is swallowed or spitted out. The chewing sessions usually lasts for 2 to 4 hours. People chew fresh khat leaves daily on a regular basis mainly in the afternoon, although some people start to chew khat in the morning. Moreover, many social gatherings have made khat chewing more popular. At the present time, easy transportation of khat and easing of importation restrictions has helped this habit spread to countries such as the USA and Western Europe where Yemeni, Somali and other East African communities. [3,4]

It had been used for the management of obesity and depression due to its central stimulant effects. [5] Religious scholars used to chew khat to be alert during the night time to study religious science. Many factors played a role in the extension to its social use in society; easy transportation from village to city, khat market, its availability in cheaper price making it affordable for all and late afternoon free time. People also believe that khat helps them to work more effectively, particularly with manual work, due to increased energy and alertness. Khat chewing, however, has been reported to have adverse effects on various human body systems. Recently, khat chewing has become a real national problem in the Yemen and many people, in particular physicians, believe that khat chewing can damage health in the peripheral systems of the body, including the cardiovascular system and gastrointestinal tract. Comparisons are made with amphetamine and ecstasy in particular on the detrimental effects on the cardiovascular system.

Diabetes mellitus is a metabolic disorder initially characterized by a loss of glucose homeostasis with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action, or both. [6] The incidence of diabetes mellitus (DM) has reached an alarming level despite having been a rare disease 50 years ago, attaining a global incidence of 151 million people per year. The number of patients with diabetes is increasing due to population growth, aging, urbanization, and increasing prevalence of obesity and physical inactivity. [7,8]

The general concept among Yemeni people, in particular diabetics, is that khat chewing controls the level of glucose in the blood and khat has a therapeutic role in the management of high blood glucose [9] The sympathomimetic actions of cathinone would be expected to raise plasma catecholamine levels. Cathinone has a similar mechanism of action to that of amphetamine, acting by releasing catecholamines from presynaptic storage sites. [1] Cathinone and amphetamine induce dopamine release from central dopaminergic nerve terminals thus increasing the activity of dopaminergic pathways. These catecholamines would increase blood glucose levels by activation of glycogenolysis in skeletal muscles and the liver; β- adrenoceptor-mediated response. There is also inhibition of insulin release from the pancreatic β -cells via α-adrenoceptor stimulation which would also elevate blood glucose levels. [10] In diabetic subjects, however, serum glucose was significantly higher after 1 and 2h of khat chewing. [11]

The studies conducted in Yemen Specialized Hospital demonstrated that DDT influences carbohydrate metabolism. The exact mechanism by which DDT induce hyperglycaemia is not clear, however, DDT inhibit pancreatic secretary activity by increasing the activity of gluconeogenic enzymes. [12] Promoting hepatic glycogenolysis by activating glycogen phosphorylase. DDT reduce calcium permeability and hence insulin secretion. The results showed that there is an association between chronic khat chewing and the development of non-insulin dependent diabetes mellitus. This association might be attributed to the longterm effects of pesticides residues on chronic khat chewers. [12]

While some studies show that in healthy non-diabetics, khat does not affect

fasting or post-prandial serum glucose levels, [11] others have suggested a decrease in serum glucose. [13] A study was conducted to investigate the traditional antidiabetic use of indigenous or naturalised plants in South African. [14] The organic leaf extract of *C. edulis* (khat) moderately stimulate glucose utilisation in fat and muscle cells. Fresh leaves chewed in many parts of Africa for their stimulant effect and have been claimed to lower blood glucose levels in diabetes. [14] A study has been conducted to investigate the blood glucose lowering effect of khat (*Catha edulis*) extracts in normal, glucose-loaded, and alloxan diabetic rats. Oral administration of single dose of a hydro-ethanol extract of *Catha edulis* for a month caused no statistically significant change in blood glucose levels in normal rats with or without glucose loading.

Similarly oral administration of *Catha edulis* extract does not exert a hypoglycemic effect in normal, glucose-loaded, and diabetic rats. [15] A study conducted to evaluate the effect of khat chewing on the blood glucose level of normal chewers in comparison to the effects of two antidiabetic drugs in diabetic patients showed that the rate of sugar decrease in healthy khat chewers was significantly higher than the effect of the two antidiabetic drugs. [16] Healthy khat chewers had a 61.22% reduction of blood sugar within 4 hours of khat chewing. Insulin had a greater effect than daonil on the decrease in serum glucose levels. The average decrease of serum glucose in oral daonil was 11.99% and in insulin 22.14%. [16]

A study designed to determine the presence and levels of antidiabetic trace elements in *Catha edulis* in Kenya has bioscreened its antidiabetic potential and found to lower blood glucose levels appreciably in alloxan-induced diabetic mice. It is justifiably postulated that its hypoglycemic potential is due to antidiabetic trace elements like Mg (791±1.2), Cr (97.9±1.5), Mn (8.9±0.5), V (<1), Cu(<5), Zn (47.7±3.7), Ni (<5), Fe (46±5.2), Pb (9.4±0.2) and Sr (<1) contained in it. [17]

Interestingly, in their recent experimental study on rabbits, [18] found that Khat significantly reduced the blood glucose in a dose- and time-dependent manner.

The lowering of blood glucose levels in alloxan-induced diabetic mice by the aqueous leaf extract of *Catha edulis* was shown to be dose dependent. The hypoglycemic action of the extract could be attributed to the presence of flavonoids, flavones, flavonols, saponins and trace elements, which have been shown to be hypoglycaemic. [19] The aqueous extract of C*atha edulis* lowered blood glucose to normal and as effectively as insulin ($p<0.05$) in the third and fourth hours.

Furthermore, the extract of C*atha edulis* may have achieved hypoglycaemic activity by decreasing the rate of carbohydrate absorption into the portal hepatic circulation. [19] That the aqueous extract of C*atha edulis* acted in a dose related manner could be explained by passive diffusion of the active principle across the cell membranes in the peritoneal cavity. This is because it started lowering blood sugar levels almost immediately. That the leaf extract of *Catha edulis* at a dose of 150 mg/kg body weight was not as effective as the dose level of 100 mg/kg body weight could be explained by the fact that the high dose took longer to be absorbed across the peritoneum cavity. That the dose level of 50 mg/kg body weight was not as effective as the two higher doses could be explained by the fast metabolism, clearance and inactivation of the lower concentration of the active principle.

Rabbits fed a diet containing different levels of khat leaves showed an increase in plasma glucose levels after 4 months but a significant reduction after 6 months. Furthermore, plasma levels of the cholesterol and triglycerides have also shown to reduced in them. [18] Possibly tannins and the inorganic ions present in khat may contribute to the delayed

absorption of glucose thereby reducing its level. Moreover, the khat-induced delay of gastric emptying. [20] may also play a role in reducing the blood sugar after food intake.

The effect of khat chewing in diabetic patients is unclear till now. Some believe that the overall effect of khat in diabetic patients is deleterious, because the user is less likely to follow dietary advice, and the consumption of sweetened beverages with khat raises blood sugar. [11] Clearly, knowledge about the effects of cathinone and khat chewing on plasma glucose and insulin levels is very sparse and controlled studies need to be undertaken in Ethiopia. It is therefore, the aim of this study is to investigate the effect of fresh juice of *Catha endulis* on the blood glucose level of normoglycemic and STZ induced type -2 diabetic rats.

Effects of khat chewing on appetite may also indirectly influence blood glucose levels and body weight. Recently a study conducted by. [21] Showed chewing khat significantly decrease subjective feelings of hunger and increase the sensation of fullness but had no effect on ghrelin and peptide YY levels. They concluded that the anorexigenic effect of khat may be secondary to central mechanisms mediated via cathinone. Indeed, one of its uses is in the control of obesity, which indirectly would reduce the risk of diabetes. High plasma levels of the anorectic hormone, leptin, have been found 4 h after a heavy khat chewing session (400g). This hormone may then contribute to the decreased appetite and body weight observed in khat chewers. [22]

Another study was conducted in Yemen to evaluate the effect of crude of *Catha edulis* in WOKW rats with metabolic syndrome. The study reported that reduction of the weight in rats fed with khat is an indication that the plant could be involved in the ongoing metabolic process. [23] found and reported that the increase in metabolic rate and oxygen consumption of rats was caused by cathinone. The significance of increased plasma leptin is in explaining the underlying mechanism of the observed effects associated with khat chewing such as loss of appetite, decreased body weight, and hyperthermia. [18] The decreased body weight was evident from the significantly lower body mass index of the khat leaves chewers group as compared to the non–khat leaves chewers group.

The major therapy option in diabetes mellitus is lifestyle management. Besides exercise, weight control and medical nutrition therapy, oral glucose-lowering drugs and insulin injection are the conventional therapies for the disease. These conventional therapies have adverse side effects, are expensive and require expertise. [24] There is a new trend in the world to turn back to natural substances as alternatives to synthetic drugs either due to cost factors or to sum amount the side effects of the former. Less than a quarter of the estimated 250,000 medicinal plant species have been investigated for hypoglycemic activity. [24] It seems that khat chewing by patients with type 2 diabetes in Yemen is likely associated with poor glycemic control, lower BMI, and probably an earlier age at diagnosis of diabetes. Male patients were found to be more frequent Khat chewers and more prone to earlier onset of the disease. Poor glycemic control was independently related to long duration of diabetes, habitual khat chewing, and lower BMI. [25]

There is known relation between khat and diabetic mellitus. [9] The studies clearly show effects of khat on the most important parameters of metabolic syndrome in rats. They need confirmation in human and could be helpful to evaluate the risks and/or potential of khat for human health. Besides it is obvious that there are partly strong differences between the effects of different types of khat leaves (green or light and crimson or dark khat). Our preliminary chromatographic investigations show that the cathinone content in the dark leaves is higher than that in the light leaves (unpublished). Therefore we assume that cathinone contributes to the observed effects. Further phytochemical

investigations are necessary to analyse the content of bioactive compounds (cathinone, cathin, tannins and other) in different khat leaves and preparations. Possibly such differences could explain some inconsistent data of khat effects in the literature. [26] It was difficult to draw a meaningful conclusion from both the systematic and the meta-analysis with respect to the glycemic effect of Catha edulis since the meta-analysis results were insignificant with high heterogeneity among subgroups and are greatly conflicting. The variation is most likely due to unadjusted experimental factors or is related to Catha edulis itself, such as the differences in the phytochemical composition. Therefore, it is highly recommended that further studies of the glycemic effect of the cultivar of Catha edulis being studied should come with the identification and quantification of phytochemical content so that a meaningful assessment can be made with regard to its hypoglycemic properties. In addition, well-controlled clinical studies should be conducted to confirm whether or not chewing Catha edulis is associated with the development of type 2 DM, since this would be a source of concern seeing that the plant is widely consumed in certain populations. [27] There were also histopathology changes confirm the toxic effect of the khat on the liver of the rabbit. Cessation of Khat consumption has been associated with recovery from hypoglycaemic action of khat and histopathology changes of rabbit livers confirm diverse and adverse effects of khat. Meanwhile, incomplete or mild recovery occurred in all serum lipid profiles. Clearly, our knowledge about the effect of khat chewing on serum glucose and lipids is very sparse and controlled studies are needed to be undertaken. They need confirmation in human and could be helpful to evaluate the risks and/or potential of khat for human health. The results showed that feeding with khat leaves reduced total serum cholesterol, HDL-and LDL cholesterol levels and glucose concentration. After khat withdrawal cholesterol and HDL-cholesterol levels were still significantly decreases, whereas serum glucose content was non-significant and LDL- cholesterol concentration was significantly increases compared to control group. [28] Other study showed that different khat extracts or cathinone produces changes in terms of weight, fat mass, appetite, lipid biochemistry and hormonal levels. These changes are more pronounced at higher doses and long durations of intervention. The most suggested mechanism of these changes is the central action that produces changes in the physiology of dopamine and serotonin. Nonetheless, there are a number of variations in the study design, including species, doses and durations of intervention, which makes it difficult to arrive at a final conclusion about khat regarding obesity. insulin level and khat use found a study that measured plasma insulin and reported a decrease of the insulin plasma level associated with a decrease in the plasma glucose in rats. The effect was more pronounced with the dark khat than light khat extracts. [29,30] Sympathomimetic actions of cathinone would be expected to raise plasma catecholamine levels. These catecholamines would increase blood glucose levels by activation of glycogenolysis in skeletal muscles and liver. There is also inhibition of insulin release from the pancreatic β-cells which would also elevate blood glucose level. While some studies showed that in healthy non-diabetics, khat did not affect fasting or post-prandial serum glucose levels, others have suggested a decrease in serum glucose. In diabetic subjects, however, serum glucose was significantly higher after 1 and 2 hour of khat chewing. [31] Therefore, the main aim of the present study was to investigate the effect of crude extract of Catha edulis on the plasma glucose of normoglycemic and streptozocin (STZ) induced type 2 diabetic rats.

2. MATERIALS AND METHODS

2.1. Collections and preparation of plant materials

The fresh khat (stem tips and leaves) weighing 3kg was purchased from Merkato in Addis Ababa in February, 2011. A voucher specimen of *Catha edulis* has been deposited in Arat Killo Herbarium, College of Natural science, Addis Ababa University Then Arat Killo Herbarium authenticated the botanical identity of the plant.

2.2. Procedures for preparation of aqueous extract of *Catha edulis* (AECE)

Three kg of Bahr Dar fresh khat leaves and stem tips was purchased and washed with distilled water and allowed to dry for 5 days in a dark place (away from direct sunlight). The dried 1500gm of khat was crushed with mechanical mortar and soaked (dissolved) in 3 liter of distilled water for 24 hours. Then it was filtered through filter paper (grade 1, Whatman, Kent, UK) and transferred into clean closed containers (rectangular dishes). The water in the filtrate was made to freeze in the refrigerator and then lyophilized by using lyophilizer machine which directly sublimates the filtrate into extracts or yield. Finally all 3kg fresh khat material yielded 150gm of khat extract and the yield was placed in the dissector until use for the experiment.

2.3. Experimental animals

The experimental animals used in this study were 40 male Wistar rats aged between 14-16 weeks and weighing between 200-320 gm. The animals were purchased from Ethiopian Health and Nutrition Research Institute (EHNRI) for the study. They were given one week of acclimatization period before the start of experiment in the animal house, Black Lion Hospital, Faculty of Medicine, AAU. They were fed standard rat pellets and water. The rats were housed in plastic cages at a controlled ambient temperature of 22 ± 2°C and 50 ± 10% relative humidity, with 12 hour light/12 hour dark cycles. The experimental procedures were carried out in strict compliance with Animal Ethics Committee's rules and regulations followed in Addis Ababa University. The proposal was first submitted to research Ethics committee in the department of Physiology and approved. Then further seen by research committee in the College of Health Sciences before the research was carried out.

2.4. Induction of diabetes in rats.

Diabetes was induced by a single intraperitoneal injection of Streptozocin (STZ) (50 mg kg^{-1} b.wt.) which was prepared in 1.47gm of sodium citrate buffered in 0.01 M citric acid (0.96gm) (pH 4.5) in a volume of 1 mL kg^{-1} b.wt [Jasmine R and Daisy P, 2007]. After 2-3 days of STZ administration rats with fasting blood sugar levels of 140 mg dL^{-1} and above were considered as diabetic rats.

2.5. Diabetic animals

Twenty four male wistar rats were randomly grouped into three; each group consists of 8 rats/cages. Group I diabetic rats were treated with single dose of crude extract of *Catha edulis* (4.5ml/kg). Group II diabetic rats were treated with single dose of Glibenclamide (5mg/kg). Group III diabetic rats were treated with vehicle (2 mL distilled water) and were used as control.

In the acute study conducted for two week, the rats in each group was orally administered with single dose of crude extract and glibenclamide. Administration was conducted for each consecutive 14 days using gavage without damaging their trachea by careful handling of rats' neck and tail by crossing their front legs. In the study crude extract of *Catha edulis* was administered to rats with single dose on a daily base for one month.

2.6. Normoglycemic animals

Sixteen normal male wistar rats were randomly grouped into two; each group consists of eight rats/cages. Group I, rats were treated with single dose of crude extract of *Catha edulis* (450mg/kg), and finally group II were treated with vehicle (2mL distilled water) and were used as control. In the study conducted for two week the rats in each group was orally administered with single dose of crude extract. Administration was conducted for 14 consecutive days. In the study the crude extract (450mg/kg) of *Catha edulis* was

administered to rats with single dose on a daily base for one month.

2.7. Blood sugar determination

A drop of blood was obtained from the tails of the rats by snipping (mincing) the tips with sterile scissors, which had first been sterilized by swabbing with 70% ethanol. Bleeding was then enhanced by gently "milking" the tail from the body towards the tip. After taking a drop of blood, the tips of the tail were again sterilized by swabbing with 70% ethanol.

The blood sugar level in the rats was determined by using SensoCard Glucometer (D45-8001-x, Electronika, Kft.Hungary). Blood glucose test strips (77Electronika Kft.H-1116 Budapset, Hungary) compatible with the glucometer were appropriately inserted in the glucometer and blood from the tail of the rats was placed on the glucometer strip. The blood glucose level was read on glucometer screen in mg/dL. In the study conducted for two week, the blood glucose level was first taken before treatment each day. After treatment, the blood was collected after 2 hours and 4 hours. The blood glucose level was determined after every blood collection. During the experiment, the animals continued to fast for 8 hours but were allowed free access to water. The effects of crude extract of *Catha edulis* was conducted by treating the rats with single dose on a daily base for four weeks. The blood glucose level was measured on every 5^{th} day for four weeks.

2.8. Statistical analysis

Blood glucose levels were expressed in mg/dl as mean±SEM. The statistical analysis of data was done using paired sample t test and one way analysis of variance (ANOVA), followed by Dunnett's test and Post hoc comparisons between the experimental and control groups were made using the software" SPSS16 Statistical software package." P value less than 0.05 and 0.01 was considered to be significant.

3. RESULTS

The effects of crude extract of *Catha edulis* on the plasma glucose level of STZ induced type-2 diabetic rats was indicated in table. 1. The administration of aqueous extract of *Catha edulis* (450mg/kg) reduced the blood glucose level significantly from 320.72±62.7 to 165±31 (49.6%) at 4hr ($p<0.01$) when compared with plasma glucose level of the same group before treatment (0hr) and corresponding value of control group. As indicated in table.1, there was significant decrease in plasma glucose level from 358.7±63.8 to 148.3±37.3 (58.7%) ($p<0.01$) in the group treated with glibenclamide (5mg/kg) at 4hr when compared with plasma glucose level of the same group before treatment (0hr) and corresponding value of control group treated with distilled water.

TABLE.1. EFFECTS OF CRUDE EXTRACT OF *CATHA EDULIS* ON THE PLASMA GLUCOSE LEVEL OF STZ INDUCED TYPE-2 DIABETIC RATS.

One week measurement of plasma glucose level in mg/dl			
Treatment and dose	0hr	2hr	4hr
AECE (450mg/kg)	320.72±62.7	241.7±43*a (24.7%)	165±31**aa (49.6%)
Glibenclamide (5mg/kg)	358.7±63.8	174.9±40.9*a (51.2%)	148.3±37.3*aa (58.7%)
Distilled water	432.6±47.9	487.9±17.6 (-13%)	493.5±22.8 (-14%)

AECE- Aqueous extract of Catha edulis .Values are mean± SEM, n=4, * P<0.05, **P<0 when compared with 0hr (before treatment) of the same group.

Numbers in parenthesis represent percentage reduction of blood glucose from 0hr (100%) to 4hr after each treatment. a=P<0.05 and aa =p<0.01 were considered to be significant and very significant when compared with the corresponding value of control group respectively.

The effects of crude extract of *Catha edulis* on the plasma glucose level of STZ induced type-2 diabetic rats was shown in table 2. The administration of aqueous extract of *Catha edulis* (450mg/kg) reduced the blood glucose level significantly from 296.8±42.8 to 96.7±7.4 (67.4%) in the 4^{th} week ($p<0.01$) when compared with plasma glucose level of the same group before treatment (0 day) and corresponding value of control group.

There was significant decrease in the blood glucose level from 354.6±50.5 to 117±10.4 (67%) ($p < 0.01$) in group treated with Glibenclamide (5mg/kg) in the 4th week when compared with day 0 of the same group and control group treated with distilled water (table 2).

TABLE 2. EFFECTS OF CRUDE EXTRACT OF *CATHA EDULIS* ON THE PLASMA GLUCOSE LEVEL OF STZ INDUCED TYPE-2 DIABETIC RATS

Treatment and dose	Plasma glucose level in mg/dl (Mean±SEM)				
	day 0	1st week	2nd week	3rd week	4th week
AECE (450mg/kg)	296.8±42.8	102.3±7*a (67.5%)	98±6.4**aa (67%)	103.5±8.6**aa (65.1%)	96.7±7.4**aa (67.4%)
Glibenclamide (5mg/kg)	354.6±50.5	194.4±6.8**a (45.2%)	184.4±50**aa (48%)	222.8±61.2*aa (37.2%)	117±10.4**aa (67%)
Distilled H_2O	506.8±18.5	509.2±18.1 (-0.48%)	508.1±25 (-0.3%)	516.3±24.5 (-1.9%)	495.7±28.6 (2.2%)

*Values are mean± SEM, n=4, * P<0.05, **P<0.01* when compared with day 0 of the same group.

Numbers in parenthesis represent percentage reduction of blood glucose from day 0 (100%) to 4th week after each treatment. a=P<0.05 and aa =p<0.01 were considered to be significant and very significant when compared with the corresponding value of control group respectively.

The administration of aqueous extract of *Catha edulis* (450mg/kg) reduced the blood glucose level significantly from 100.96±22.9 to 71.7±7.8 (29%) at 4hr (p<0.01) when compared with plasma glucose level of the same group before treatment (0hr) and corresponding value of control group (table 3).

TABLE.3. EFFECTS OF CRUDE EXTRACT OF *CATHA EDULIS* ON THE PLASMA GLUCOSE LEVEL OF NORMOGLYCEMIC RATS.

One week measurement of plasma glucose level in mg/dl			
Treatment and dose	0hr	2hr	4hr
AECE (450mg/kg)	100.96±22.9	85.9±6.2*a (15%)	71.7±7.8**aa (29%)
Distilled water	110.4±3.6	107.2±2.9 (-2.9%)	108.08±3 (-2.1%)

Values are mean± SEM, n=3, * P<0.05, **P<0.01 when compared with 0hr (before treatment) of the same group.

Numbers in parenthesis represent percentage reduction of blood glucose from 0hr (100%) to 4hr after each treatment. a=P<0.05 and aa =p<0.01 were considered to be significant and very significant when compared with the corresponding value of control group respectively.

As demonstrated in table 4 administration of aqueous extract of *Catha edulis* (450mg/kg) significantly reduced the blood glucose level from 112.4±4.27 to 61.0±4.3 (45.7%) in the 4th week (p<0.01) when compared with plasma glucose level of the same group before treatment (day 0) and corresponding value of control group treated with distilled water.

TABLE.4. EFFECTS OF CRUDE EXTRACT OF *CATHA EDULIS* ON PLASMA GLUCOSE LEVEL OF NORMOGLYCEMIC RATS.

Treatment and Dose	Plasma glucose level in mg/dl (Mean±SEM)				
	day 0	1st week	2nd week	3rd week	4th week
AECE (450mg/kg)	112.4±4.27	101.8±6.92* (9.5%)	79.6±8.17**aa (29.2%)	71±6.4**aa (36.9%)	61.0±4.3**aa (45.7%)
Distilled water	107.6±3.82	103.2±5.45 (4%)	118.8±2.13 (-10.4%)	117.4±2.89 (-9%)	115±2.30 (-7%)

Values are mean± SEM, n=3, * P<0.05, **P<0.01 when compared with day 0 of the same group.

Numbers in parenthesis represent percentage reduction of blood glucose from day 0 (100%) to 4th week after each treatment. a=P<0.05 and aa =p<0.01 were considered to be significant and very significant when compared with the corresponding value of control group respectively.

The administration of aqueous extract of *Catha edulis* (450mg/kg) significantly reduced the body weight of rats from 261.64±7.48 to 209.71±8.34 (19.9%) in the

4th week (p<0.01) when compared with the body weight of the same group before treatment (0 day) and corresponding value of control group treated with distilled water (table 5).

TABLE 5.EFFECTS OF CRUDE EXTRACT OF *CATHA EDULIS* ON THE BODY WEIGHT OF STZ INDUCED TYPE-2 DIABETIC RATS.

Treatment and dose	Body weight of rats in gram (Mean± SEM)				
	day 0	1st week	2nd week	3rd week	4th week
AECE (450mg/kg)	261.64±7.48	225.62±8.25*a (13.8%)	218±4.16*aa (16.7%)	213.57±8.15**aa (18.4%)	209.71±8.34**aa (19.9%)
Glibenclamide (5mg/kg)	306.8±25.89	264.2±17.58* (13.9%)	260.1±13.77*a (15.3%)	254.8±18.56**aa (17%)	244.2±25.89*aa (20.4%)
Distilled H_2O	263.42±5.85	266.94±4.32 (-1.3%)	271.25±4.84 (-3%)	276.78±3.90 (-5%)	276.52±3.53 (-4.9%)

Values are mean± SEM, n=4, * P<0.05, **P<0.01 when compared with day 0 of the same group.

Numbers in parenthesis represent percentage reduction of body weight from day 0 (100%) to 4th week after each treatment. a=P<0.05 and aa =p<0.01 were considered to be significant and very significant when compared with the corresponding value of control group respectively.

As it was illustrated in table 6 the administration of aqueous extract of *Catha edulis* (450mg/kg) significantly reduced the body weight of rats from 233±27.5 to 173.8±20.38 (25.4%) in the 4th week (p<0.01) when compared with the body weight of the same group before treatment (day 0) and corresponding value of control group treated with distilled water.

TABLE. 6. EFFECTS OF CRUDE EXTRACT OF *CATHA EDULIS* ON THE BODY WEIGHT OF NORMOGLYCEMIC RATS.

Treatment	Body weight of rats in gram (Mean± SEM)				
	day 0	1st week	2nd week	3rd week	4th week
AE CE (450mg/kg)	233±27.5	212.6±27.2** (8.8%)	204.6±24.6**a (12.3%)	189.4±21.84**aa (18.8%)	173.8±20.38**aa (25.4%)
Distilled water	266.8±7.21	271.6±7.83 (-1.8%)	273.6±6.51 (-2.5%)	281.4±4.23 (-5.4%)	276.7±4.82 (-3.7%)

Values are mean± SEM, n=4, * P<0.05, **P<0.01 when compared with day 0 of the same group.

Numbers in parenthesis represent percentage reduction (%) of body weight from day 0 (100%) to 4th week after each treatment. a=P<0.05 and aa =p<0.01 were considered to be significant and very significant when compared with the corresponding value of control group respectively.

4. DISCUSSION

Diabetes mellitus is a major public health problem in the world. The number of patients with diabetes is increasing due to population growth, aging, urbanization, and increasing prevalence of obesity and physical inactivity. [7,8]

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

There is a new trend in the world to turn back to natural substances as alternatives to synthetic drugs either due to cost factors or to sum amount the side effects of drugs. [24] In the present study effect of crude extract of *Catha edulis* on the plasma glucose level was tested on STZ induced type 2 diabetic rats. The administration of aqueous extract of *Catha edulis* (450mg/kg) reduced the blood glucose level significantly from 320.72±62.7 to 165±31 (49.6%) at 4hr when compared with plasma glucose level of the same group before treatment (0hr) and corresponding value of control group.

The plasma glucose reduction between groups treated with aqueous extract

(450mg/kg) was similar to that of standard drug (glibenclamide 5mg/kg). This is in agreement with previous hypoglycemic study on intraperitoneal injection of aqueous extract (150 mg/kg) of *Catha edulis* in alloxan induced mice where it lowered blood glucose to normal as effectively as insulin in the third and fourth hours. [19] The hypoglycemic action of the extract tested in the present study could be attributed to the presence of phytochemicals like flavonoids, flavones, saponins and tannins in this plant. [19]

The current study contrasts with findings of [10,11] who reported the sympathomimetic actions of cathinone. These catecholamines would increase blood glucose levels by activation of glycogenolysis in skeletal muscles and the liver; β- adrenoceptor-mediated response. In diabetic subjects serum glucose was significantly higher after 1 and 2 h of khat chewing. This may be due to the exclusion of other ingredients of khat in their study.

This study is in contrary to the reports of [12] who demonstrated that chewing DDT sprayed khat influences carbohydrate metabolism. The exact mechanism by which DDT induce hyperglycaemia is not clear, however, DDT inhibit pancreatic secretary activity by increasing the activity of gluconeogenic enzymes. Promoting hepatic glycogenolysis by activating glycogen phosphorylase. DDT reduce calcium permeability and hence insulin secretion. The results showed that there is an association between chronic khat chewing and the development of non-insulin dependent diabetes mellitus. This association might be attributed to the longterm effects of pesticides residues on chronic khat chewers

This study found that effects of crude extract (450mg/kg) of *Catha edulis* in STZ induced type 2 diabetic rats. The administration of aqueous extract of *Catha edulis* (450 mg/kg) reduced the blood glucose level significantly from 296.8±42.8 to 96.7±7.4 (67.4%) in the 4th week when compared with plasma glucose level of the same group before treatment (day 0) and corresponding value of control group. Hypoglycemic activity of the crude extract of *Catha edulis* in the present study may probably be due to the presence of trace elements. This is in accordance with findings of [17] who have bioscreened *Catha edulis* for its antidiabetic potential and found to lower blood glucose levels appreciably in alloxan-induced diabetic mice. It is justifiably postulated that its hypoglycemic potential is due to antidiabetic trace elements like Mg (791±1.2), Cr (97.9±1.5), Mn (8.9±0.5), V (<1), Cu (<5), Zn (47.7±3.7), Ni (<5), Fe (46±5.2), Pb (9.4±0.2) and Sr (<1) contained in it. [17] In contrary to the present study [15] reported that oral administration of single dose of a hydro-ethanol extract of CE for a month caused no statistically significant change in blood glucose levels in normal rats with or without glucose loading. The Oral administration of *Catha edulis* extract does not exert a hypoglycemic effect in normal, glucose-loaded, and diabetic rats. This could be attributed to difference in effective dose administration. The present study showed effects of crude extract (450mg/kg) of *Catha edulis* in normoglycemic rats. The administration of aqueous extract of CE (450 mg/kg) reduced the blood glucose level significantly from 100.96±22.9 to 71.7±7.8 (29%) at 4hr when compared with plasma glucose level of the same group before treatment (0hr) and corresponding value of control group. The effects of aqueous extract of *Catha edulis* in normal rats has not been reported so far.

The administration of aqueous extract of CE (450 mg/kg) significantly reduced the blood glucose level from 112.4±4.27 to 61.0±4.3 (45.7%) in the 4th week when compared with plasma glucose level of the same group before treatment (0 day) and corresponding value of control group treated with distilled water. Studies which indicate the effects of aqueous extract of *Catha edulis* on the plasma glucose level of normal human subjects have not been reported. This hypoglycemic effect of Catha

edulis in normal rats may be attributed to khat induced delay in gastric emptying. This is similar to other findings by [16,20] who reported that khat extracts may have achieved hypoglycemic activity by decreased rate of carbohydrate absorption into the portal hepatic circulation. Possibly tannins and the inorganic ions present in khat may contribute to the delayed absorption of glucose thereby reducing its level. Moreover, the khat-induced delay of gastric emptying may also play a role in reducing the blood sugar after food intake.

This study also illustrates the effects of aqueous extract (450mg/kg) of *Catha edulis* on body weight of STZ induced type 2 diabetic at rats. The administration of aqueous extract of *Catha edulis* (450mg/kg) significantly reduced the body weight of rats from 261.64±7.48 to 209.71±8.34 (19.9%) in the 4th week when compared with the body weight of the same group before treatment (day 0) and corresponding value of control group treated with distilled water.

There was significant decrease in body weight from 306.8±25.89 to 244.2±25.89 (20.4%) in group treated with Glibenclamide (5mg/kg) in the 4th week when compared with day 0 of the same group and control group treated with distilled water. The reduction of body weight is more Significant in aqueous extract when compared with body weight decrease in rats treated with Glibenclamide.

In the present study the effects of aqueous extract (450 mg/kg) of *Catha edulis* significantly reduced the body weight of normoglycemic rats. The administration of aqueous extract of *Catha edulis* (450 mg/kg) significantly reduced the body weight of rats from 233±27.5 to 173.8±20.38 (25.4%) in the 4th week when compared with the body weight of the same group before treatment (day 0) and corresponding value of control group treated with distilled water. The effects of aqueous extract of *Catha edulis* has not been reported in human subjects. The reduction in body weight of both diabetic and normoglycemic rats treated with khat in this study could be attributed to increase in plasma leptin and metabolic rate induced by *Catha edulis.* This is related with findings of [22,21] which indicated high plasma levels of the anorectic hormone, leptin, have been found 4 h after a heavy khat chewing session (400g).This hormone may then contribute to the hyperthermia, decreased appetite and body weight observed in khat chewers. They concluded that the anorexigenic effect of khat may be secondary to central mechanisms mediated via cathinone. Indeed, one of its uses is in the control of obesity, which indirectly would reduce the risk of diabetes. [23] found and reported that the increase in metabolic rate and oxygen consumption of rats was caused by cathinone.

5. CONCLUSIONS AND RECOMMENDATIONS

Based on the results of this work, the following conclusions can be drawn:

The present study showed that acute effects of crude extract of *Catha edulis* possessed significant hypoglycemic properties in normal and STZ induced type 2 diabetic rats which suggest the presence of biologically active components which may be worth further investigation and elucidation.

In the present study crude extract (450 mg/kg) of *Catha edulis* revealed a hypoglycemic effect as significant as glibenclamide (5 mg/kg) in STZ induced type 2 diabetic rats. Therefore, the mechanism of crude extract is probably different from that of glibenclamide, which is an insulin-independent mechanism.

The current study found that effects of crude extract of *Catha edulis* showed a significant reduction in body weight of normoglycemic and STZ induced type 2 diabetic rats.

Therefore, the following activities for future research are recommended:

- Detailed phytochemical screening and isolation of active ingredient is required to identify the exact chemical compounds responsible for the hypoglycemic activity observed in crude extract of *Catha edulis* so as to develop it as a potent antidiabetic drug.

- Another study should be carried out on diabetic khat chewers and non chewers in comparison with normoglycemic chewers to evaluate the effect of *Catha edulis* on their blood glucose level

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Author contributions

Demeke Ashencho Debecho performed all of the experiments, analyzed the data and wrote the paper. Professor Yekoye Abebe conceived and designed the experiments, reviewed manuscript and provided significant input. Dr. Daniel Seifu conceived and designed the experiments, reviewed manuscript and contributed STZ.

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