In vivo acute effects of orally administered hydro-ethanol extract of *Catha edulis* on blood glucose levels in normal, glucose-fed hyperglycemic, and alloxan-induced diabetic rats

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ABSTRACT

الأهداف: دراسة تقييم ما إذا كانت الخلاصة المائية الكحولية لنبات القات (كاثا ايدوليس) تخفض مستويات السكر في الجرذان الطبيعية، والجرذان المحدث لها فرط سكر الدم بتناول السكر، وكذلك المصابة بداء السكري واستخدمت مادة الوكسان.

الطريقة: استخدمت 3 طرق تجريبية في هذه الدراسة. في الطريقتين الأولى، والثانية من هذه الدراسة 3 مجموعات من الجرذان تحتوي كل مجموعة على 6 جرذ، واشتملت المجموعات على مجموعة تخدم كشاهد عولجت بالمحلول الملحي الفسيولوجي (NS)، ومجموعة عولجت بخلاصة القات (كاثا ايدوليس)، والمجموعة الأخيرة عولجت بدواء معياري خافض للسكر (جليبنكلامايد). أجريت الدراسة في المختبر الفيسولوجي، كلية الطب، جامعة الملك خالد، أبها، المملكة العربية السعودية. كانت الجرذان المستخدمة في المجموعة الأولى طبيعية، وكانت جرذان المجموعة الثانية مصابة بالسكري. قيست مستويات سكر الدم في 3 مجموعات بعد جرعة حقن واحدة من المحلول الملحي، أو خلاصة القات (كاثا ايدوليس)، أو بالجليبنكلامايد. في المجموعة التجريبية الثالثة تم اختبار فحص تحمل الجلوكوز الفموي (OGTT) بإعطاء الجرذان جرعة جلوكوز تعادل 1.5 غم/كغم من وزن الفئران على 6 مجموعات من الجرذان كل مجموعة تحتوي على 6 جرذ كما في المجموعتين الأولى، والثانية.

النتائج: أظهرت النتائج أن استخدام الخلاصة المائية الكحولية عن طريق الفم لنبات القات (كاثا ايدوليس) لم يغير مستوى سكر الدم في الجرذان الطبيعية سواء أعطيت أم لم تعطى جرعة تحمل السكر، بينما كانت هناك زيادة غير مهمة في سكر الدم عند الجرذان المصابة بالسكري، والتي استخدمت خلاصة نبات القات (كاثا ايدوليس) سواء أعطيت أم لم تعطى جرعة تحمل السكر مقارنة بالمجموعات الشاهدة والمجموعات التي استخدمت دواء خافض للسكر.

خاتمة: أن استخدام الخلاصة المائية الكحولية لنبات القات عن طريق الفم (كاثا ايدوليس) لم يحدث تأثير لخفض السكر الدموي سواء في الجرذان الطبيعية، أو التي استخدمت سكر الجلوكوز، أو المصابة بداء السكري.

Objectives: To investigate the blood glucose lowering effect of khat (*Catha edulis*) extract in normal, glucose-loaded, and alloxan diabetic rats.

Methods: Three experimental protocols were used in this study. In each of the first 2 protocols, 3 groups of rats (6 rats per group) were used as control group (NS), *Catha edulis* (CE) treated, and glibenclamide treated groups. This study was carried out at the Physiological Laboratory of the Medical School of King Khalid University, Abha, Saudi Arabia between October and November, 2009. Normal rats were used in the first protocol while alloxan diabetic rats were used in the second protocol. Blood glucose levels were measured in all 3 groups after single dose injections of saline, CE or glibenclamide. In the third protocol, another 6 groups of rats (6 rats per group) were prepared as in the first 2 protocols and oral glucose tolerance test (OGTT) was performed on each rat after oral administration of glucose (1.5g/kg).

Results: Oral administration of a hydro-ethanol extract of CE caused no statistically significant change in blood glucose levels in normal rats with or without glucose loading. There were slight, non significant increases in blood glucose levels of extract-treated diabetic rats, with and without glucose loading, as compared to the corresponding untreated rats.

Conclusion: Oral administration of CE extract does not exert a hypoglycemic effect in normal, glucose-loaded, and diabetic rats.

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Diabetes mellitus is an endocrine disorder characterized by hyperglycemia, which affects many people, worldwide. It is projected that a total of approximately 239 million people worldwide, will be affected by the disorder by the year 2010.[1] At the present time, there are no adequately effective cure for diabetes mellitus.[2] Currently available therapeutic options, including dietary modification, oral hypoglycemics and insulin, have their limitations.[3,4] Therefore, the search for more efficacious and safer hypoglycemic agents has continued to be an area of active research. One area of great interest lies in safe, natural antidiabetic agents to replace synthetic drugs in diabetes management. The plant kingdom offers a promising field in which to look for natural oral hypoglycemics. More than 400 species have been reported to display hypoglycemic effects, but very few of them have been investigated for their safe use.[5] Some plant products are consumed by the general population for their socializing benefits and by patients for their alleged therapeutic effects, but in many cases no scientific proof exists in support of its therapeutic efficacy; indeed some of these products may have harmful effects on body function. There is, therefore, a need to investigate these plant products to establish their true effects.[5] Khat is one of those plants widely used throughout the world, especially in the Arab world.[6] In 1975, it was estimated that between 5 and 10 million people worldwide used khat (*Catha edulis*) daily and since then the number is likely to have increased.[6] The leaves of khat (*Catha edulis*) are chewed for their stimulating and sympathomimetic effects. Khat juice is swallowed to induce a stimulatory and euphorogenic effect in the user. The most important active ingredient of khat is cathinone, which is reported to produce the major clinical effects of khat.[7] These include anorexia, anxiety, irritability, insomnia, hallucinations and panic attacks. Chronic abusers are at risk of developing personality disorders as well as myocardial infarction.[8] The market value of khat leaves correlates with their cathinone content.[9] Other constituent of khat leaves include tannins, amino acids and a significant amount of ascorbic acid, magnesium, and beta-carotene.[7,9] Many people chew khat leaves simply for its pleasurable and stimulating effects. However, anecdotal evidence also points to a widely-held belief among khat chewers in the Arab region that khat juice helps to lower their blood glucose and maintain it within normal levels.[10-12] This study was carried out to investigate this claim. A search of the local literature turned up only a few articles on the effect of khat on blood glucose, and the reported results were controversial.[10-15] Therefore, we considered it important to revisit earlier claims and establish whether there is indeed any scientific basis for the widespread use of this plant in Arabian countries as a hypoglycemic agent to treat diabetes mellitus.

Methods. ***Preparation of Catha edulis shrub extracts and dose selection.*** This study was carried out at the Physiological Laboratory of the Medical School of King Khalid University, Abha, Saudi Arabia between October and November, 2009. Fresh shrubs of *Catha edulis* (stem tips and leaves) were obtained from the Drug Enforcement Administration (DEA) of Aseer Region of southwestern Saudi Arabia. The Saudi DEA is the only legitimate source of khat in Saudi Arabia, as both the peddling and consumption of khat are prohibited in the country. The plant material was washed, dried, and extracted with 500 ml of water/ethanol mixture (70/30%, v/v) at room temperature overnight, and subsequently filtered. The filtrate was evaporated in a vacuum at 40^{0}C to remove all traces of ethanol. The hydro-ethanol extract obtained (20 gram), constituted 10.7% of the original dry material. It was then dissolved in freshly prepared normal saline to a final concentration of 200 mg/ml and 0.5 ml of this was administered orally to the animal. The dose of the extract used was 100 mg/rat (equivalent to 500 mg/kg as dry plant). This dose selection was based on the average amount of khat leaves estimated to be chewed daily by khat chewers.[9,16]

Experimental animals. Seventy-two white albino rats aged between 14 and 16 weeks were used in this study. Each rat weighing approximately 200 g, was obtained from the Animal House of College of Medicine, King Khalid University, Saudi Arabia. The rats were divided into 12 groups and housed in 12 plastic cages (6 rats per cage) in a climatically controlled room temperature (22°C) and humidity, 55% with 12 hour light/12 hour dark cycles. Water and standard pellet diet were available ad libitum throughout the experimental period. The rats were acclimatized for 10 days prior to the experiment. All studies were conducted in accordance with the National Institute of Health's Guide for the Care and Use of Laboratory Animals.[17] The design of the experiments was approved by the Physiology Department Research Committee of King Khalid University.

Preparation of normal and diabetic rats. In order to achieve steady state levels of serum glucose, normal rats were fasted for 18 hours, and diabetic rats for 6 hours prior to the administration of the extract. Alloxan monohydrate (Sigma chemical Co., Louis, MO, USA) dissolved in distilled water (pH <6.0) was administered intraperitoneal to 18 hour-fasted rats at a dose of 150 mg/kg. The alloxan protocol was executed 48 hours before the onset of the experimental procedure and was followed by the measurement of serum glucose levels. Diabetic rats were defined as rats having a serum glucose of ≥13.9 mmol/L (≥250 mg/dL), while normal, non-

diabetic rats were defined as those having serum glucose in the range of 6.0-7.6 mmol/L (108-137 mg/dL).[18]

Experimental procedure. In all experimental procedures, all treatments were given single oral doses using special stainless-steel cavage needles. Hypoglycemic effect of *Catha edulis* extract on normal fasted rats (**Figure 1a**): Eighteen fasted normal rats were selected at random and divided into 3 groups of 6 rats each (n=6). Group 1, normal rats, received vehicle (normal saline) and served as normal controls. Group 2 was given 0.5 ml *Catha edulis* hydro-ethanol extract at a final concentration of 0.5 mg/kg and served as extract-treated normal rats. Group 3 received glibenclamide (600 mg/kg/day) and served as glibenclamide-treated normal rats.

Antihyperglycemic effect of *Catha edulis* extract on diabetic fasted rats (**Figure 1b**): Eighteen fasted diabetic rats were selected at random and divided into 3 groups of 6 rats each (n=6). Group 1, diabetic rats, was given a vehicle and served as diabetic control. Group 2, diabetic rats, received 0.5 ml Catha edulis hydro-ethanol extract at a final concentration of 0.5 mg/kg and served as extract-treated diabetic rats. Group 3, diabetic rats, was given glibenclamide (600 mg/kg/day) and served as glibenclamide-treated diabetic rats.

Rats in the experimental groups were identified with color coded tail marks and put into different sections of the cage, according to their group. The time point at which the extract was given was defined as 0.0 minute (min). Blood samples were collected from the tail vein of each rat for the measurement of blood glucose at 0, 30, 60, 90, 120, 180 min intervals in normal rats and at 0, 1, 2, 3, 4 hours in diabetic rats.

Oral glucose tolerance tests (Figure 1c). To study the effect of Catha edulis hydro-ethanol extract on oral glucose tolerance test (OGTT) in normal rats and diabetic rats, the animals were divided into 6 groups each of 6 rats (n=6). Group 1, normal rats, was given vehicle (normal saline) and served as normal controls. Groups 2, normal rats, were given 0.5 ml *Catha edulis* hydro-ethanol extract at a final concentration of 0.5 mg/kg and served as normal extract-treated rats. Group 3, normal rats received glibenclamide (600 mg/kg/day). Group 4, diabetic rats, were given a vehicle and served as diabetic controls. Group 5, diabetic rats, received 0.5 ml *Catha edulis* hydro-ethanol extract at a final concentration of 0.5 mg/kg and served as diabetic extract-treated rats. Groups 6, diabetic rats, were given glibenclamide (600 mg/kg/day). A single oral dose of glucose (1.5 g/kg) was given to each animal 10 min after extract dosing in extract-treated groups. Blood was collected from tail veins for glucose estimation before administration of the extract (0 min) and at 30, 60, 90, and 120 min after glucose loading.

Biochemical assays. Blood glucose was estimated using a commercial diagnostic kit (Sigma chemical Co., Louis, MO, USA).

Results were expressed as mean values ± SEM. Statistical differences between groups were assessed by Student's t test. $P<0.05$ were considered significantly different (95% Confidence interval).

Results. ***Hypoglycemic effect of Catha edulis extract on normal fasted rats.*** Fasting blood glucose levels of normal fasted rats are shown in **Table 1**. There was no significant difference in fasting blood glucose levels between *Catha edulis* extract-treated rats and control rats. Compared to control rats, rats given glibenclamide showed a reduction of blood glucose level ($p<0.001$) of 21.6% at 30 min, 31.6% at 60 min , 40.8% at 90 min, 23.2% at 120 min, and 25.8% at 180 min with a maximum reduction observed at 90 min after glibenclamide treatment.

Antihyperglycemic effect of Catha edulis extract on diabetic fasted rats. **Table 2** shows the fasting blood glucose levels of hyperglycemic alloxan-induced diabetic rats. In comparison to diabetic control rats administered the vehicle only, oral administration of the hydro-ethanol extract to diabetic rats resulted in a slight (statistically non significant) increase in blood glucose levels during the first 4 hours after treatment. Diabetic rats administered glibenclamide showed decreased blood glucose levels ($p<0.001$) of 18% at one hour, 35% at 2 hours, 37.1% at 3 hours, and 40% at 4 hours following the treatment. So, the maximum reduction in blood glucose levels in glibenclamide treated diabetic fasted rats was observed at 4 hours after treatment.

Oral glucose tolerance tests. **Table 3** shows the effect of orally administered hydro-ethanol extract of *Catha edulis* on glucose tolerance test in normal and diabetic rats. The experimental induction of hyperglycemia by intragastric ingestion of glucose resulted in a peak increase in serum glucose levels of 30% and 46.2% in normal and diabetic rats. This maximum increase in blood glucose level in both groups was reached at 60 min after glucose loading. Blood glucose levels of normal rats loaded with glucose progressively decreased until the end of the experimental period. The blood glucose level of the diabetic rat group receiving glibenclamide and loaded with glucose increased up to a maximum of 23.2% at the 60 min and progressively decreased until the end of the experimental period. In OGTT, oral administration of *Catha edulis* hydro-ethanol extract caused a non-significant decrease in blood glucose levels in glucose loaded normal rats at 60, 90 and 120 min when compared to the corresponding control rats. Compared to diabetic control rats, oral administration of the extract in glucose loaded diabetic rats, resulted in

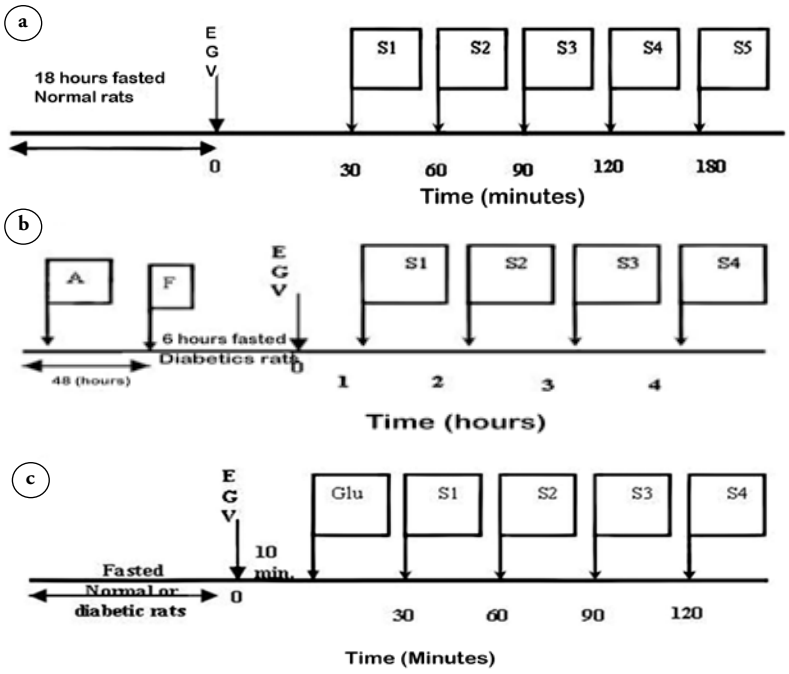

Figure 1 - Experimental protocols of the study. **a)** Experimental procedure in normal non diabetic rats. **b)** Experimental procedure in alloxan induced diabetic rats. **c)** Oral glucose tolerance test experimental procedure in normal and diabetic rats. E - extract, G - glibenclamide, V - vehicle, S - sample, A - alloxan, F - fasting, Glu - glucose

Table 1 - Effect of Khat (*Catha edulis*) hydro-ethanol extract on blood glucose levels in normal fasted rats.

Group	0.0 minute	30 minutes	60 minutes	90 minutes	120 minutes	180 minutes
Normal control	96.00 ± 3.38	95.20 ± 5.02	96.80 ± 1.64	97.80 ± 1.48	98.00 ± 0.71	98.40 ± 1.67
Normal + CE extract (500 mg/kg)	97.80 ± 2.39	96.00 ± 2.50	96.40 ± 2.07	97.00 ± 2.45	98.00 ± 1.58	98.00 ± 0.70
Normal + Glibenclamide	99.40 ± 3.65	74.60 ± 3.6*	66.20 ± 2.05*	57.80 ± 4.32*	75.20 ± 3.35*	73.00 ± 3.24*

Values are given as mean ± SEM for groups of 6 rats each. Values are statistically significant at *p<0.05. Normal rats given the extract (*Catha edulis*) or glibenclamide were compared to normal rats received the vehicle. CE - *Catha edulis*.

Table 2 - Effect of Khat (*Catha edulis*) hydro-ethanol extract on blood glucose levels in Alloxan induced diabetic rats.

Group	0.0 hour	One hour	2 hours	3 hours	4 hours
Diabetic control	264.40 ± 6.31	262.60 ± 5.68	258.60 ± 6.19	259.00 ± 3.39	257.60 ± 5.13
Diabetic + CE (500 mg/kg)	267.40 ± 5.41	265.20 ± 3.63	259.80 ± 2.78	260.20 ± 3.42	260.40 ± 3.91
Diabetic + glibenclamide	262.20 ± 8.26*	215.60 ± 5.32*	168.00 ± 4.12*	163.00 ± 5.79*	155.00 ± 14.61*

Values are given as mean ± SEM for groups of six rats each. Values are statistically significant at *p<0.05. Diabetic rats given the extract (*Catha edulis*) or glibenclamide were compared to diabetic rats which received only vehicle. CE - *Catha edulis*.

Table 3 - Oral glucose tolerance test (OGTT) in normal and alloxan induced diabetic rats.

Group	0.0 minute	30 minutes	60 minutes	90 minutes	120 minutes
Normal control (vehicle)	94.00±3.80	125±3.87	134.8±3.83	125.6±1.81	110.6±2.70
Normal + CE extract (500mg/kg)	97.4±3.05	128±2.55	130.6±1.52	122.4±2.60	106.8±1.72
Normal+ Glibenclamide	94.00±3.00	87.40±6.35*	96.60±7.43*	96.00±4.00*	102.80±5.07*
Diabetic control	265.80±8.67	429.40±6.88	493.40±4.16	436.00±4.85	430.00±1.05
Diabetic + CE extract (500 mg/kg)	269.80±3.63	428.60±2.88	486.20±4.97	438.60+2.30	434.80±2.68
Diabetic + Glibenclamide	264.00±15.89*	420.20±14.13*	344.20±9.65*	280.00±10.36*	274.40±6.1*

Values are given as mean ± SEM for groups of 6 rats each. Values are statistically significant at *p<0.05. All groups were loaded with 1.5 g/kg glucose orally. Normal rats given the extract (*Catha edulis [CE]*) or glibenclamide were compared to normal rats received the vehicle, Diabetic rats given the extract or glibenclamide were compared to diabetic rats received the.

decreased glucose level at 60 min followed by increased levels at 90 and 120 min. These changes were not statistically significant.

Discussion. The health implications of the social habit of khat chewing, prevalent especially among Arabs and East Africans, are not fully known. Even less is known about claims by many diabetic khat devotees that khat exerts hypoglycemic effects which help to lower their blood sugar and maintain it within the normal range. The present study attempted to demonstrate any such effects of khat in normoglycemic, glucose loaded and alloxan-induced hyperglycemic rats. The study also compared any possible antidiabetic effects of khat to those of the well known antidiabetic agent, glibenclamide.

Animal models of diabetes mellitus differ significantly from one another and none reproduces faithfully the essentials of human diabetes mellitus.[19,20] Nevertheless, they have proved increasingly useful for pathophysiologic and pharmacologic studies of the disease because they allow investigations of tightly defined experimental conditions as well as more detailed studies of the biologic effects of the agent under investigation.[21] Alloxan-induced hyperglycemia in rodents is considered a good experimental model since it is less toxic than other chemical diabetes-inducing agents. Oral glucose tolerance test (OGTT) measures the body's ability to use glucose as its main source of energy. Assuming that the plant extract under investigation has some hypoglycemic effects in normal and/or diabetic rats, this effect may be masked because it is well known that a serum glucose level of ≤60 mg/dL induces physiological responses including the release of cortisol, epinephrine, growth hormone and/or glucagons which collectively cooperate to correct serum glucose levels by stimulating glycogenolysis, gluconeogenesis, lypolysis and protein breakdown. To circumvent this interference, we decided to assess the effect of the extract following the administration of glucose loads in both normal and diabetic rats. In this study, the possible antidiabetic effects of *Catha edulis* were compared to those of glibenclamide. The main mechanism of action of glibenclamide is the stimulation of insulin release.[19] It is well known that oral administration of hypoglycemic agents produces hypoglycemia in both normal and alloxan-induced diabetic rats.[22] It has also been reported that glibenclamide is effective in the moderate diabetic state but ineffective in severe diabetic animals where pancreatic β-cells are almost completely destroyed.[19,23] Previous studies have suggested that certain plant extracts cause antihyperglycemic effects by promoting regeneration of β-cells, by protecting pancreatic cell destruction, by restricting glucose load or by promoting unrestricted endogenous insulin action. They may also prompt β-cells to release insulin or activate insulin receptors. Some plants are reported to exhibit properties similar to the well known sulfonylurea drug, gliclazide to reduce blood glucose in normoglycemic animals.[24] Others exert antihyperglycemic effects like biguanides (metformin) but have no effect on blood glucose level in the normal state.[24]

In this study, acute administration of a hydro-ethanol extract of *Catha edulis* to normal rats had no effect on blood glucose levels in three hours following administration, but caused a non significant decrease in blood glucose levels in glucose loaded hyperglycemic rats at 90 and 120 min after glucose loading. Although animal and human studies are not strictly comparable, our results are in agreement with those of Saif et al,[10] showing that blood glucose levels of healthy khat chewers were not affected during khat sessions. They suggested that this was due to the effect of a sympathetically mediated, khat-induced release of insulin on the rising blood glucose. Indeed similar results have previously been reproted by other workers in both animal[11] and human[12] studies. However, our data differ from those reported by Ahmed[13] and Ramadan et al.[14] who found statistically significant decreases in serum glucose of khat chewers, and from that of Karunanayake et al[15] who reported that khat exerts a hypoglycemic effect, either by enhancing peripheral glucose uptake or by interacting directly with ß-cells of the pancreas.

The absence of any marked hypoglycemic effect in the current study cannot be attributed to our experimental animal model since a clear antidiabetic effect was routinely obtained in our laboratory on the same animal model with conventional doses of a classical hypoglycemic drug, glibenclamide.[25,26] Rather, the difference between our results and those of some other studies[13-15] showing antidiabetic properties of *Catha edulis* could be attributed to the fact that these other investigators studied glucose levels in humans after khat session, not in diabetic animal models, or to differences in methods of preparation and extraction of khat.[25,26] The slight, non significant increase in blood glucose levels of normal rats in our study could be attributed to the fact that khat might increase norepinephrine release which has only about one tenth of the potency of epinephrine on blood glucose level in normal individuals.[7,20] The resulting slight increase in blood glucose would be opposed by enhanced responsiveness of pancreatic ß-cells which secrete more insulin to rapidly correct serum glucose by a fall in hepatic glucose output with no stimulation of peripheral glucose utilization.[10] Our results for normal rats (with or without glucose loading)

treated with *Catha edulis* hydro-ethanol extract, followed the same trend as above. In glucose loaded diabetic rats, the oral administration of the extract resulted in a non significant increase in glucose levels at 60 min followed by a non significant decrease at 90 and 120 min after glucose loading. In diabetic rats without glucose loading, the administration of the hydro-ethanol extract resulted in a non significant increase in blood glucose levels throughout the 4 hour treatment period. It could be suggested that diabetic rats exhibit increased glycemic responsiveness to catecholamines due to their decreased ability to secrete insulin as the serum glucose concentration rises.[10] So, the sympathomimetic action of khat may increase glucose production by activating glycogenolysis especially in muscles. This would increase blood glucose by an indirect mechanism through increased glycolysis, and subsequent release of lactate. Also, it has been reported that an increase of peripheral norepinephrine stimulates hormone sensitive lipases which hydrolyze tissue triacylglycerol into free fatty acids and glycerol which are released into the circulation.[26] These fatty acids are then transported to the liver where glycerol acts as a gluconeogenic substrate and fatty acids as energy sources for glucose synthesis by the liver. Cathinone, a major active constituent of khat alkaloids, has been shown to significantly increase free fatty acid levels in rats[27] and rabbits.[13]

It is unlikely that destruction of the active hypoglycemic constituents of khat during extraction could account for the absence of khat induced hypoglycemia in our study. In our study, the plant material was extracted routinely with a water/ethanol mixture (70/30 %, v/v) at room temperature over night, so our extraction procedure is unlikely to have caused damage to the active ingredients. It is also unlikely that the concentration of extract used in this study was sub-therapeutic. Several previous studies used similar[29] or even lower doses[30] of khat extract.

In conclusion, in our study, oral administration of a hydro-ethanol extract of khat (*Catha edulis*) did not exert a hypoglycemic activity on both normal and alloxan-induced diabetic rats. Further studies aimed at determining the effects of khat extract on insulin secretion, glucose transport systems, and on gluconeogenetic and glycolytic enzymes may throw more light on whether khat extract influences blood glucose one way or another.

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Ethical Consent

All manuscripts reporting the results of experimental investigations involving human subjects should include a statement confirming that informed consent was obtained from each subject or subject's guardian, after receiving approval of the experimental protocol by a local human ethics committee, or institutional review board. When reporting experiments on animals, authors should indicate whether the institutional and national guide for the care and use of laboratory animals was followed.