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Effect of *Catha edulis* on Insulin, Resistin and Cortisol Levels in Type-2 Diabetics and Non-Diabetics

Mohamed Ibrahem Kotb El-Sayed and Hatem Al-Kordy Amin

Department of Biochemistry and Molecular Biology,
Faculty of Pharmacy, Helwan University, Helwan, P.O. Box 11790, Cairo, Egypt

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ABSTRACT

In this study, the biochemical effects of *Catha edulis* leaves chewing (as psycho stimulant and aphrodisiac) on the serum concentration of resistin, insulin, cortisol, zinc, calcium, copper and blood glucose in both healthy individuals and type 2 diabetic patients were examined. 80 male subjects aged 35-55 years were recruited in this study, 40 of them were previously diagnosed as type 2 diabetics and the other 40 were healthy non-diabetics. The above two groups were subdivided into two subgroups (n = 20) in accordance on whether they were regular and chronic khat chewers or none into NNK; healthy non-khat chewers, NK; healthy khat chewers, DNK; type 2 diabetic non-khat chewers and DK; type 2 diabetic khat chewers. Khat chewing resulted in elevated resistin, cortisol, FBG, PBG levels and HOMA-IR in either diabetics or healthy khat chewers than those of non-khat chewers and generally in diabetics than healthy. In addition, khat chewing resulted in a significant increase in calcium and copper serum levels. In contrast, serum zinc and insulin levels in diabetic chewers were significantly lower than those of diabetic's non-chewers. *Catha edulis* Forsk chewing adds additional toxic effects to type 2 diabetics by increasing cortisol and resistin levels while decreasing insulin secretion and sensitivity.

Keywords: *Catha Edulis* Forsk, Diabetes Mellitus Type 2, Resistin, Cortisol, Insulin

1. INTRODUCTION

The stimulant alkaloid-containing psycho stimulant leaf *Catha edulis* Forsk popularly known as "khat" comes from a tree which grows in countries bordering the Red Sea, along the east coast of Africa and in west Asia (Cox and Rampes, 2003). Commonly used in East Africa and in the Arabian Peninsula (Warfa *et al*., 2007). khat is chewed habitually by users for its euphoric effects; its chewing session may last 3-7 h (Banjaw and Schmidt, 2006). Being chewed slowly and intermittently to release its active components then swallowed with saliva (Cox and Rampes, 2003).

Khat contains a number of pharmacologically active compounds in leaves and young shoots such as the phenylpentenylamines, cathedulines and the most important major and natural alkaloid of d-(+)-amphetamine like action and structure; cathinone (Graziani *et al*., 2008). Cathinone is relatively unstable and rapidly metabolized to norpseudoephedrine (cathine) and norephedrine (Al-Motarreb *et al*., 2002). Cathinone is released within 15-45 min during chewing and peak plasma levels of cathinone are obtained 1.5-3.5 h after the onset of chewing khat. Cathinone is detectable in plasma for up to 24 h after khat consumption and eliciting euphoric, psycho stimulant effects and Khat-induced analgesia (Graziani *et al*., 2008). The common adverse effects of khat include insomnia, anorexia, hyperthermia, mydriasis, endocrinological disturbances and acute autonomic responses, such as elevated blood pressure and tachycardia (Hassan *et al*., 2000).

Corresponding Author: Mohamed Ibrahem Kotb El-Sayed, Department of Biochemistry and Molecular Biology, Faculty of Pharmacy, Helwan University, Helwan, P.O. Box 11790, Cairo, Egypt

Diabetes mellitus is a metabolism disorder with abnormally high blood glucose levels "hyperglycaemia" (Panda, 2002). Diabetes mellitus Type 2 is a complex heterogeneous group of metabolic conditions characterized by increased levels of blood glucose due to impairment in insulin action and/or insulin secretion (Das and Elbein, 2006). Several facts could link khat chewing with diabetes mellitus due to khat chewing decreases body weight via lipolysis and gastric emptying delay (Heymann *et al*., 1995), this forces the general belief that khat chewing helps in lowering their blood glucose.

Resistin also known as Adipose tissue-Specific Secretory Factor (ADSF) is a cysteine-rich protein consists of 108 amino acid residues in human and encoded by the RETN gene (Wang *et al*., 2002), it has been shown to impair insulin signaling and/or glucose metabolism in many organs (Steppan *et al*., 2005). In addition, an increased cortisol levels are also associated with insulin resistance (Misra *et al*., 2008). Several micronutrients have beneficial effects in healthy subjects and in diabetes. The disturbed metabolism of some micronutrients has been reported and it was postulated that certain metals/metalloids might have specific roles in the pathogenesis and progress of diabetes mellitus (Zheng *et al*., 2008; Tanaka *et al*., 2009).

Limited data were available regarding khat chewing long-term health consequences; particularly in diabetes mellitus type 2 patients. Several studies showed controversial effect of khat on carbohydrate metabolism, insulin and cortisol secretion (Saif-Ali *et al*., 2003; Mwenda *et al*., 2006), while there is no any reported literature regarding effect of khat chewing on resistin, copper, zinc and calcium levels in khat chewing diabetic patients. This study will assess the effect of regular and chronic khat chewing by diabetes mellitus type 2 patients considering resistin, cortisol, insulin, copper, zinc and calcium in fasting and postprandial status.

2. MATERIALS AND METHODS

2.1. Plant Collection

Bundles of the Herari khat (leaves and small branches) were purchased fresh at a local market of Hada; a common harvest area in Southern Sana'a, in its natural habitat, season of collection was in December 2010. The fresh leaves were collected and transported using an ice box. The plant was identified by a taxonomist and a voucher specimen (PD-22122010) was deposited at the Pharmacognosy Department, Faculty of pharmacy, Sana'a University.

2.2. Equipments

Centrifuge 6000 rpm (Hitachi, Germany), Automatic micro-well reader (Lab system multiskan RC, Finland) and ELISA reader (Humareader Human Company 2106/1682). Syringes, vacutainer tubes, tourniquet, epindroff, micropipettes, plastic tubes, UV/Visible spectrophotometer (Shimadzu) and cylinders. Flame Atomic Absorption Spectrometry (FAAS) was on Perkin-Elmer Atomic Absorption Spectrometer (AAS) model 5000. Software HG Graphics II (Perkin-Elmer, Norwalk, CT, USA) were employed for spectrum acquisition and data processing.

2.3. Study Protocol

This study involved the recruitment of 80 Yemeni male subjects (aged 35-55 years, their body mass index average of 18-22 kg m^{-2} and non-smoker), were divided into two groups each consisted of 40 subjects, the first group were previously diagnosed as diabetes type 2 (non-insulin dependent) patients while, the other group were healthy without any history of familial diabetes. Each of the above two groups were subdivided into either regular chronic khat chewers group, (those who chewed khat daily for continued 5 h during the past five years) or non-khat chewers group, (those who never chewed khat). Each group subdivided into NNK; healthy non-khat chewers, NK; healthy khat chewers, DNK; type 2 diabetic non-khat chewers and DK; type 2 diabetic khat chewers. Diabetics received a 500 mg twice/day of metformin.

All subjects presented themselves at the research center of faculty of pharmacy, Sana'a University at 9 am. They arrive fasting for 12 h, overnight fasting samples were taken using vacutainer tubes. There were not given any instructions about their activities for the previous day except for khat chewing for 5 h just 1 h after lunch. Thereafter, they received a standard lunch meal to avoid any difference among them. One hour after lunch, equal amount of the khat (~250 g of fresh Herari khat leaves) were given to each subject of NK and DK Groups to start 5 h of continued chewing. All subjects were instructed not to eat (except for starting khat chewing 1 h after lunch for NK and DK) or drinks (except water for all) during 3 h following the lunch, just till the blood sample withdrawal. The experimental protocol was approved by an institutional review committee for the use of human subjects.

2.4. Blood Samples

Venous blood samples were collected from each subject at 3 h after the lunch (i.e., 2 h after starting continued khat chewing in the case of NK and DK groups)

using vacutainer tubes (test tubes specifically designed for venipuncture). Blood samples were centrifuged immediately at 3500 rpm for 10 min and the serum was separated and divided into five separated aliquot in eppenddrof (micro centrifuge) tubes for postprandial or fasting tests. Blood glucose test was measured immediately after the collection and the rest aliquots were stored at-70°C for later analysis of resistin, copper, zinc and calcium. Fasting samples were used for FBG, insulin and cortisol estimations. All stored samples were used for the various measurements within 2 weeks except; insulin, cortisol and resistin were detected after collection. All samples were coded and analyzed by a technician who blinded to a protocol design.

2.5. Biochemical Estimations/Assays

Serum insulin, cortisol and resistin were measured by sandwich Enzyme Linked Immuno Sorbent Assay (ELISA) kit supplied by DRG Diagnostics Company (Tietz, 1986; Flier *et al*., 1979; Gerber *et al*., 2005). Blood glucose was measured enzymatically by glucose oxidase method, a colorimetric kit supplied by Roche (Bondar and Mead, 1974). Serum Cu and Zn were determined by Flame Atomic Absorption Spectrometry (FAAS) in an air-acetylene flame at 324.8 and 248.3 nm, respectively. A background correction was performed using a deuterium lamp. The operating conditions were based on those suggested by the manufacturer (Beauty and Kerber, 1993) and were presented by (Skrbic and Cupic, 2004), standards used in the calibration procedure were prepared in the same acids as samples andthe analysis was performed in two parallel determinations. Calcium was measured by O-cresolphthalein direct method, colorimetric kit supplied by QCA company ref.99-59-36 (Kessler and Wolfman, 1964). Homeostasis Assessment model was used to assess Insulin Resistance (HOMA.IR) using fasting insulin and glucose concentrations by the formula; HOMA.IR% = fasting serum glucose (mg/dL) X fasting insulin (µIU/mL)/405 (Matthews *et al*., 1985).

2.6. Statistical Analysis

The results were analyzed by the SPSS computer program (version 15), expressed as means ± S.E.M. (Standard Error Mean) and the significance was analyzed by paired sample t-test between groups. Significant differences were indicated when $P < 0.05$.

3. RESULTS

In the current study, Khat chewing resulted in a significant increase in both resistin and cortisol serum levels (NK Vs. NNK or DK Vs. DNK). Moreover, the serum resistin and cortisol levels of DK were significantly higher when compared with those of NK. In addition, the serum resistin, insulin and cortisol levels of DNK were significantly higher when compared with those NNK. Non-significant changes in serum insulin levels were observed either between NK and NNK or between DK and NK. In contrast, serum insulin levels in DK were significantly lower than those of DNK (**Table 1**).

Table 1. Effect of khat chewing on serum resistin (ng/mL), insulin (µIU/mL) and cortisol (ng/mL) levels in normal and type 2 diabetic individuals

	Normal		Type 2 diabetics	
Parameter	Non-khat chewers (NNK) (n = 20)	khat chewers (DK) (n = 20)	khat chewers (NK) (n =20)	Non-khat chewers (DNK) (n =20)
Resistin (ng/mL)	2.38 ± 0.10	4.70 ± 0.16^{aaa}	7.07 ±0.26^{ccc}	8.54 ± 0.31$^{bbb, ddd}$
Insulin (µIU/mL)	10.24 ± 0.13	10.23 ± 0.25	11.25 ± 0.32^{c}	10.02 ± 0.25^{bbb}
Cortisol (ng/mL)	149.35 ± 2.46	155.3 ± 3.18^{a}	156.5 ± 3.07^{cc}	166.6 ± 2.47$^{bbb, ddd}$

Data expressed as mean ± S.E.M. $^{a, c}$ = P < 0.05; ^{cc} = P < 0.01; $^{aaa, bbb, ccc, ddd}$ = P < 0.001. a = NNK vs NK; b = DNK vs DK; c = NNK vs DNK; d = NK vs DK. NNK = normal non- khat chewer; NK = normal khat chewer; DNK = diabetics non- khat chewer; DK = diabetics khat chewer

Table 2. Effect of khat chewing on FBG (mg/dL), PBG (mg/dL) levels, HOMA-IR % of normal and type 2 diabetic individuals

	Normal		Type 2 diabetics	
Parameter	Non-khat chewers (NNK) (n = 20)	khat chewers (NK) (n =20)	Non-khat chewers (DNK) (n =20)	khat chewers (DK) (n = 20)
FBG (mg/dL)	72.0 ± 1.25	87.40 ± 1.47^{aaa}	199.40 ± 3.74^{ccc}	241.80 ± 4.82$^{bbb, ddd}$
PBG (mg/dL)	95.40 ± 1.64	103.65 ± 2.59^{a}	280.35 ± 6.37^{ccc}	373.85 ± 8.27$^{bbb, ddd}$
HOMA-IR %	1.82 ± 0.04	2.20 ± 0.06^{aaa}	5.54 ± 0.20^{ccc}	5.99 ± 0.20$^{b, ddd}$

Data expressed as mean ± S.E.M. FBG; Fasting blood glucose, PBG; Post-prandial blood glucose, HOMA-IR = Homeostasis assessment model of insulin resistance. $^{a, b}$ = P < 0.05; $^{aaa, bbb, ccc, ddd}$ = P < 0.001. a = NNK vs NK; b = DNK vs DK; c = NNK vs DNK; d = NK vs DK. NNK = normal non- khat chewer; NK = normal khat chewer; DNK = diabetics non- khat chewer; DK = diabetics khat chewer

Table 3. Effect of khat chewing on Copper (µg/L), Zinc (µg/L) and Calcium (mg/dL) levels of normal and type 2 diabetic individuals

	Normal		Type 2 diabetics	
Parameter	Non-khat chewers (NNK) (n = 20)	khat chewers (NK) (n =20)	Non-khat chewers (DNK) (n =20)	khat chewers (DK) (n = 20)
Copper (µg/L)	837 ± 30.8	890.5 ± 35.6	1053.5 ± 42.06^{ccc}	1233.5 ± 35.7$^{bbb, ddd}$
Zinc (µg/L)	930.5 ± 16.97	774 ± 46.08^{aa}	729.40 ± 22.63^{ccc}	624.7 ± 19.6$^{bb, d}$
Calcium (mg/dL)	10.95 ± 0.29	13.33 ± 0.22^{aaa}	13.26 ± 0.29^{ccc}	15.16 ± 0.17$^{bbb, ddd}$

Data expressed as mean ± S.E.M. ^{d} = P < 0.05; $^{aa, bb}$ = P < 0.01; $^{aaa, bbb, ccc, ddd}$ = P < 0.001. a = NNK vs NK; b = DNK vs DK; c = NNK vs DNK; d = NK vs DK. NNK = normal non- khat chewer; NK = normal khat chewer; DNK = diabetics non- khat chewer; DK = diabetics khat chewer

Khat chewing has an important effect on carbohydrate metabolism. In the present work both FBG levels and HOMA-IR show a significant increase by khat chewing in both NK and DK groups when compared with those of NNK and DNK groups respectively. The same results were observed for both DNK and DK when compared with NNK and NK respectively. In the same line, PBG levels showed the same results (**Table 2**).

Khat chewing resulted in a significant decrease in zinc serum levels (NK Vs. NNK; DK Vs. DNK). The same results were observed for zinc levels when diabetics compared with healthy individuals (DNK Vs. NNK; DK Vs. NK). In contrast, Khat chewing resulted in a significant increase in calcium serum levels (NK Vs. NNK; DK Vs. DNK). The same results were observed for calcium levels when diabetics compared with healthy individuals (DNK Vs. NNK; DK Vs. NK). In the same line of calcium, copper showed the same results except when NK compared with NNK (non-significant) (**Table 3**).

4. DISCUSSION

Current study had demonstrated Khat chewing resulted in significant increase of resistin and cortisol levels in both diabetics and healthy subjects, while it is significantly decreases serum insulin levels in diabetics' khat chewers than those of diabetics' non-chewers. This finding is in agreement with Gharibeh *et al*. (2010) whom reported that serum resistin level increases in association with diabetes type 2 and insulin resistance, while Beckers *et al*. (2008) failed to identify such relation.

Khat main constituent cathinone is structurally similar to nor-ephedrine and has a similar mechanism of action like amphetamine, through releasing catecholamine from pre-synaptic storage sites; its blood peak is reached up to 2 h after commencement of khat chewing. It increases ACTH hormone levels through stimulation of β-adrenergic receptors by its amphetamine-like effect and inhibits insulin release due to induced nor-adrenaline and activation of pancreatic α2-adrenoceptors by the released catecholamine (Nencini and Fraioli, 1994; Halket *et al*., 1995; Broadley, 1996; Roberts and Wink, 1998; Al-Motarreb *et al*., 2010). In addition, Saif-Ali *et al*. (2003) conclude that, the indirect sympathetic effect of khat may act as insulin antagonist.

In contrast, Mwenda *et al*. (2006), reported khat chewing decreases cortisol levels in the baboon, this might indicate that khat effect on cortisol is a species dependent.

Resistin has a role in mediating hepatic or skeletal muscle insulin resistance, impair insulin signalling and/or glucose metabolism; its expression is regulated by a variety of hormones including, tumor necrosis factor and epinephrine and its level up-regulated by nor-epinephrine release resulted from khat chewing (Shojima *et al*., 2002; Muse *et al*., 2004; Steppan *et al*., 2005; Peschke *et al*., 2011).

Khat chewing has an important effect on carbohydrate metabolism through an increased cortisol levels were leading to a reduced insulin secretion and insulin resistance through induced up-regulation of resistin expression (Shojima *et al*., 2002; Misra *et al*., 2008) and cathinone-induced catecholamines secretion; which would increase blood glucose levels by activation of glycogenolysis in skeletal muscles and the liver; a β2-adrenoceptor-mediated response (Al-Motarreb *et al*., 2010).

In the present work FBG, PBG levels and HOMA-IR % showed a significant increase by khat chewing in either healthy or diabetic khat chewers as compared with those of non-khat chewers. This is in agreement with Al-Habori and Al-Mamary (2004) on rabbits who reported serum glucose was significantly higher after 2 h of khat chewing. In contrast, Ahmed (1984) who reported a significant decreases in serum glucose by khat chewing. However, El Hadrani and Al Hoot (2000) showed that there is an association between chronic Khat chewing and the development of type 2 diabetes mellitus, while Saif-Ali *et al*. (2003), concluded that a chronic khat

chewing does not affect serum glucose and C-peptide in healthy individuals while it increases glucose and C-peptide levels during the khat session in diabetic individuals especially those having serum glucose between 200 and 450 mg dL^{-1} at 2 h post-meal.

Copper, zinc, selenium, iron and manganese are essential components of metalloenzymes, all of them are important in intra- and extra-cellular antioxidant defense (Zheng *et al*., 2008). Zinc acts as an antioxidant by protecting the sulfhydryl groups of proteins and enzymes against free radical attack in the body (DiSilvestro, 2000) and is necessary for the synthesis of insulin hexamer (Meyer and Spence, 2009).

Khat chewing resulted in a significant decrease in zinc serum levels in either healthy or diabetic's khat chewers when compared with non-chewers, which in accordance with Aguilar *et al*. (2007) whom reported that type 2 diabetes is usually associated with decreased plasma serum zinc concentration. An increased urinary excretion of Zn due to hyperglycemia and osmotic diuresis might explain lower levels of zinc in diabetic groups (Marreiro *et al*., 2007). Hyperglycemia and khat chewing increases levels of free radicals and decreases the efficiency of antioxidant defence systems including zinc therefore khat chewing has a role in consuming zinc (Ahmed, 1984). Lower zinc levels for DNK and DK groups might give the explanation for insulin resistance particularly in DNK group, because hexamer conformation of insulin will not formed resulting in immature and/or inactive insulin. Substances such as calcium, fiber and other chelating agents interfere with intestinal absorption of zinc and subsequently lead to an increase of intestinal zinc excretion (Overbeck *et al*., 2008), therefore khat content of fibers, tannins and calcium retards zinc absorption after lunch.

Khat chewing resulted in a significant increase in calcium serum levels among healthy and diabetic chewers. These findings might be refers to that concentration levels of calcium were the higher essential metals in Ethiopian khat (*Catha edulis* Forsk or Herari khat) as reported by Atlabachew *et al*. (2010) and other possibility that khat chewing might has an effect on calcitonin and parathyroid hormones but further studies are required to establish this relation. An increased serum calcium level led to an increased cytosolic calcium level (Aoki and Miyagawa, 1990), which diminish cellular sensitivity to insulin and might participate in the pathogenesis of insulin resistance in type 2 diabetes. calcium was shown to be significantly correlated with resistin; it has been shown that resistin increases cytosolic calcium concentration in hepatic stellate cells (Bertolani *et al*., 2006). In addition, Yamaguchi *et al*. (2011) who stated that serum calcium is positively correlated with fasting plasma glucose and insulin resistance, independent of parathyroid hormone, in male patients with type 2 diabetes mellitus.

Copper showed the same results of calcium, except when NK compared with NNK (non-significant). Copper acts as a prooxidant and may participate in metal-catalyzed formation of free radicals. Copper (Cu) and Zinc (Zn) play a pivotal role in the oxidant/antioxidant mechanism, imbalance leads to increased susceptibility to oxidative damage of tissues, resulted in pathogenesis of diabetes mellitus or diabetic complications (Soinio *et al*., 2007). Some investigators have reported the hypothesis that glycated proteins bind transition metals such as copper and iron, playing an important role in the etiology of peripheral vascular dysfunction and peripheral neuropathies in diabetes mellitus (Zheng *et al*., 2008).

5. CONCLUSION

We conclude that khat chewing resulting in additional adverse effects to type 2 diabetes patients by increasing insulin resistance due to increased resistin levels and its cathinone-induced catecholamine secretion, leading to increased FBG, PBG, HOMA-IR, cortisol, copper, calcium and decreased zinc and insulin levels.

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6.1. Conflict of Interest

The researchers declare no conflicting interests related to the study.

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