Research Journal of Pharmacology 1 (1): CCCC, 2008
ISSN: 1815-9362

Biochemical Effects of Sub-Chronic Administration of *Catha edulis* (Khat) Crude Extract in Rats

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Abstract: The leaves of khat (*Catha edulis*) are found to have stimulating and pleasurable effects and are chewed habitually by people of East Africa and Arabian Peninsula. Due to the habitual widespread use of khat in many African countries and the Yemen and in order to investigate the effects of khat on various biochemical parameters, the present study was undertaken to evaluate the sub-chronic (13 weeks) administration of crude khat leaves extract in male Sprague-Dawley rats. Three groups were exposed to 0, 1000 and 2000 mg kg^{-1} body weight/day for 13 consecutive weeks. Fasting plasma levels of Malondialdehyde (MDA) were reduced ($p<0.05$) in both treatment groups (27 and 29%, respectively) compared to the control group. In contrast, serum values of total cholesterol and HDL cholesterol were shown to be increased ($p<0.05$) in the 1000 mg kg^{-1} body weight treatment group. The plasma MDA reducing effect of khat crude extract administration in rats may be attributed to the long term effects of polyphenolic contents of khat leaves, which have been reported to have antioxidant activity. Analysis of serum liver enzymes, uric acid, albumin, total and prostatic ACP and testosterone revealed no changes in the treatment and control groups. These results suggest that the subchronic administration of catha edulis crude extract has no hepatotoxicity and prostate adverse effects in male rats, but may have antioxidant property due to its phenolic compounds.

Key words: Khat, *Catha edulis*, Lipid peroxidation, Lipid profiles, liver enzymes

INTRODUCTION

The habit of khat chewing represents a major socio-economic problem in the countries of Southern Arabia and the Horn of Africa. The leaves of *Catha edulis* (Celastraceae) (khat), a plant growing wild and cultivated in Eastern Africa and Southern Arabia are chewed for their stimulating and sympathomimetic effects (Alem *et al*., 1999). A large number of people chew khat leaves because of its pleasurable and stimulating effects. Although the use of khat has spread worldwide, it has until recently remained mostly confined to the regions where the plant is grown since only fresh leaves have the potency (of cathinone) to produce the desired effects. The fact that cathinone has a closer structural similarity with amphetamine and both share common pharmacodynamic features, led to the conclusion that cathinone is the most important active ingredient of khat which causes the major pharmacological effects (Hollister, 1995).

The chemical constituents of khat leaves include in addition to cathinone, norpseudoephedrine, merucathinone, ethereal oils, sterols, triterpenes, flavonoids, a significant amount of ascorbic acid and tannins (Kalix and Braenden, 1985). Many different compounds are found in khat including alkaloids, terpenoids, flavonoids, sterols, glycosides, tannins, amino acids, vitamins and minerals (Kalix and Braenden, 1985; Cox and Rampes, 2003). The phenylalkylamines and cathedulins are the major alkaloids present in khat leaves. The cathedulins are based on a poly-hydroxylated sesquiterpene skeleton and are basically polyesters of euonyminol and recently, 62 different cathedulins from fresh khat leaves were characterized (Kite *et al*., 2003).

The alkaloid fraction of khat is very efficiently extracted by chewing and the major compounds are absorbed in the oral cavity (Toennes *et al*., 2003). The detrimental effects of the active principle of khat on man and animals and the common adverse effects include insomnia, anorexia, irritability, hyperthermia, mydriasis

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and endocrinological disturbances have been described (Brenneisen *et al*., 1990; Nencini *et al*., 1993). Khat-induced analgesia has also been reported (Connor *et al*., 2000), although it is not known whether the mechanism is central or peripheral. Acute autonomic responses, such as elevated blood pressure and tachycardia, have been reported (Wilder *et al*., 1994). Clinical trials have shown khat to delay gastric emptying period after khat chewing (Heymann *et al*., 1995).

Administration of khat to rat reported to decrease the activities of free radical metabolizing enzyme systems and glucose (Al-Qirim *et al*., 2002) and ethanolic extract in rats revealed reduction in serum glucose and alkaline phosphatase and increase total bilirubin, acid phosphatase and lactate dehydrogenase levels (Tariq *et al*., 1983). Triglycerides and α-lipoproteins were significantly decreased in khat chewers as compared to their normal equivalents; however cholesterol and other lipids showed less marked changes (Ramadan *et al*., 1979; Al-Zubairi *et al*., 2003). Total serum protein levels reduced in high khat consumers whereas urea and creatinine greatly increased although this non-significant (Rmadan *et al*., 1979). Decreased blood ammonia and less change in uric acid were reported by Farag and Qirbi in khat fed rabbits. Recently in a long term study in animals, a decrease in plasma levels of cholesterol, triglycerides and glucose, in addition to increase in plasma level of HDL cholesterol and plasma uric acid were reported by Al-Habori and Al-Mamary (2004).

The widespread chewing of Khat in Yemen is a habit that has a deep-rooted sociocultural tradition in which consumers spend part of their time chewing khat. The present study was undertaken to investigate the biochemical changes associated with sub-chronic administration (90 days) of a crude extract of khat leaves in a manner and doses similar to human khat users. For the first time we estimated lipid peroxidation products (Malondialdehyde (MDA), measured as plasma TBARS) in rats, in addition we estimated lipid profiles, liver enzymes, total and prostatic Acid Phosphatase (ACP), uric acid, albumin and testosterone levels using male Sprague-Dawley rats as a model.

MATERIALS AND METHODS

Experimental animals: Thirty six randomly selected Sprague-Dawley male rats weighing 200-250 g and 5-7 weeks old were provided locally from the Animal House of Faculty of Veterinary Medicine and housed at the Animal House, Faculty of Medicine and Health Sciences, University Putra Malaysia. Rats were caged individually and were allowed free access of water and pelleted food *ad libitum*. They were acclimatized to laboratory conditions for 1 week prior to the experiment and were kept in polypropylene cages with wood shavings as bedding in 12 h light/dark cycle at 27°C. The experimental procedures were carried out in strict compliance with the Animal Ethics Committee's rules and regulation followed in this institute. Rats received either the khat extract treatments or distilled water (in the control group) daily for 13 consecutive weeks.

Study design: The animals were randomly allocated into 3 groups and exposed by forced feeding to 0, 1000 and 2000 mg kg^{-1} body weight/day crude *catha edulis* leaves extract for 13 consecutive weeks (90 days). Dose selection was based on the average daily consumption of khat leaves by the khat users in Yemen according to Nencini and Ahmed (1989) and Kalix (1990, 1994). Cage-side clinical observations were performed each morning on all animals and body weights were recorded at the study initiation and weekly. At the end of the study, food was withheld for 12 h to provide fasting blood samples. The rats were sacrificed under chloroform anesthesia and blood was collected by cardiac puncture procedure. Blood samples were collected into heparinized and plain tubes. Plain tubes were left at room temperature for clot to form and centrifuged for 5 min at 1500 rpm. Heparinized tubes were centrifuged immediately and plasma and serum were separated and stored at -80°C for later analysis. The procedures of this research were conducted in accordance with the OECD guidelines (Repeated dose 90-days oral toxicity study in rodents, guideline 408) and European Community guidelines (EEC Directive of 1986; 86/609/EEC) and approved by the Animal Care and Use Committee (ACUC), Faculty of Medical and Health Sciences, University Putra Malaysia.

The clinical chemistry parameter evaluated on separated plasma samples was MDA, measured as TBARS. The parameters evaluated on separated serum samples included total cholesterol, High Density Lipoprotein (HDL) cholesterol, triglycerides, albumin, uric acid, Alkaline Phosphatase (ALP), Aspatate Aminotransferase (AST), Alanine Aminotransferase (ALT), Lactate Dehydrogenase (LDH), Gamma Glutamyl Transferase (GGT), Total Acid Phosphatase (Total-ACP), prostatic ACP and testosterone levels.

Determination of MDA: Malondialdehyde (MDA) estimation was based on the reaction of MDA with two molecules of TBA to give a colored complex. MDA was determined in the form of TBARS and estimated according to the procedure of Yagi (1998) in which 0.1 mL of plasma sample was combined with 0.1 of 8.1% SDS

(Luka, BioChemika) followed by 2.5 mL TBA buffer (pH: 3.5) containing 1.06% TBA (Sigma), 20% acetic acid (Bendosen) and sodium hydroxide (Amresco). The mixture was then boiled at 95°C for 60 minutes followed by 10 min cooling on ice bath to room temperature. Samples were mixed and centrifuged at 3000 rpm for 15 min. A standard curve was prepared by diluting 1 mM tetraethoxypropane (TEP) (Sigma) in 0.1N HCl. Absorbance of the clear supernatant was measured at 532 nm in spectrophotometer (Pharmacia). Readings of MDA was extrapolated from the standard curve.

Clinical chemistry: Serum cholesterol, triglycerides, HDL-cholesterol, uric acid, albumin, liver enzymes, total and prostatic ACP levels were measured using the Chemistry Analyzer (Hitachi 902 Automatic Analyzer, Japan) while testosterone levels were estimated using electrochemiluminescence immunoassay kit from Roche, using ElecSys 2010 analyzer (Roche).

Statistical analysis: Statistical tests were performed using SPSS version 12. Results were expressed as mean±STD. To test for significant differences between groups, we used one-way Analysis of Variance (ANOVA) followed by Dunnett's test.

RESULTS

The effect of crude khat extract administration on body weight changes in rats is represented in Fig. 1. Treatment of rats for 13 weeks induced significant decrease in plasma MDA levels in the two treatment groups compared to the control group, while treatment of rats in the second group with 1000 mg kg^{-1} body weight induced significant increase in serum total cholesterol and HDL cholesterol. Control group which received distilled water plasma levels of MDA were found to be 2.26±0.73 μmol and serum value of total cholesterol and HDL cholesterol in rats were found to be 0.99±0.17 and 0.67±0.11 mmole, respectively (Table 1). Rats in the second group, which received 1000 mg kg^{-1} body weight had significantly decreased ($p<0.05$) MDA with plasma levels 1.62±0.48 μmol and serum total cholesterol and HDL cholesterol values 1.17±0.15 and 0.77±0.10 mmol, respectively. Rats in group 3 which received 2000 mg kg^{-1} body weight khat extract had significantly decreased ($p<0.05$) MDA plasma levels with values 1.57±0.34 μmole, while no effects on total cholesterol and HDL cholesterol ($p>0.05$).

Serum values of liver enzymes, triglycerides, LDL cholesterol, albumin, uric acid, total and prostatic acid phosphatase and testosterone from the two treatment groups were found to be similar to the control group ($p>0.05$).

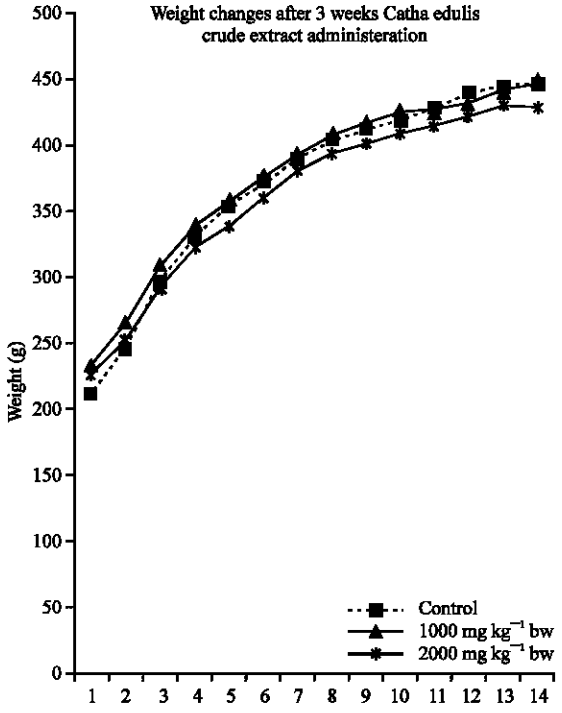

Fig. 1: Weekly weight gain changes in the group of rats fed *Catha edulis* crude extract administration for 13 weeks compared to the control group. Control group is represented by the solid square, 1000 mg kg^{-1} body weight treatment group represented by the triangle and 2000 mg kg^{-1} body weight shown by the asterisk. No significant differences were observed between the three groups

Table 1: Plasma levels of MDA, lipid profiles, uric acid, albumin, liver enzymes activities, total and prostatic ACP and Testosterone after feeding rats for 13 weeks

	Control group	1000 mg kg^{-1} bw	2000 mg kg^{-1} bw
MDA (μmol L^{-1})	2.26±0.73	1.62±0.48*	1.57±0.34*
Cholesterol (mmol L^{-1})	0.99±0.17	1.17±0.15*	1.13±0.15
Triglycerides (mmol L^{-1})	0.62±0.22	0.67±0.23	0.64±0.13
HDL-cholesterol (mmol L^{-1})	0.67±0.11	0.77±0.10*	0.75±0.11
LDL-chol (mmol L^{-1})	0.20±0.08	0.27±0.08	0.25±0.05
Uric acid (mmol L^{-1})	77.9±41.9	89.7±24.5	80.5±24.3
Albumin (mmol L^{-1})	36.8±3.46	37.1±3.21	36.5±2.36
AST (U L^{-1})	116.84±18.0	104.1±15.0	106.3±9.1
ALT (U L^{-1})	45.34±4.46	49.5±7.5	48.55±6.34
LDH (U L^{-1})	686±88.1	542.5±253.6	519.3±159
ALP (U L^{-1})	125±44.2	162.7±50.4	154.3±38.3
GGT (U L^{-1})	0.21±0.67	0.22±0.42	0.16±0.29
Total ACP (U L^{-1})	17.4±4.5	16.0±3.3	18.2±4.4
P-ACP (U L^{-1})	12.6±4.0	11.3±3.0	13.9±4.0
Testosterone (ng mL^{-1})	1.10±1.07	1.85±0.99	1.77±1.15

*$p<0.05$

DISCUSSION

In this study, plasma MDA, the most frequently used marker of lipid peroxidation (Janero, 1990) and a

biomarker of oxidative stress (Nielsen *et al*., 1997), have been used to investigate the effect of khat crude extract administration on plasma lipid peroxidation products in the form of TBARS. The reduced levels of plasma MDA in the treatment groups could be attributed to the long term effects of khat polyphenolic compounds. These results were found to be in the same line with our previous observation (Al-Zubairi *et al*., 2003) that khat chewing may not provoke lipid peroxidation and hence may have some antioxidative property. This would follow, since khat has been known to contain polyphenolic (proanthocynidines) constituents that have been reported to play a role as antioxidants (Koga *et al*., 1999). In addition the flavonoids content of khat leaves may afford some antioxidants activity, however the consumption of flavonoids was linked to protection against heart disease and cancers (Ross and Fuster, 1996) and it has been reported that they exert multiple biological effects, including anti-inflammatory, antiviral, anti-allergic, antioxidant and free radical-scavenging abilities (Sahu and Gray, 1996). Many studies carried out over the past few years have shown that polyphenolic fractions from plants inhibit oxidative stress (Sun *et al*., 2002). Flavonoids have also been suggested to act as antioxidant through free radical scavenging (Tirkey *et al*., 2005).

Uric acid is an important serum antioxidant, it represents a final product in the metabolism of the purines, acting as a potent free radical scavenger and inhibitor of lipid peroxidation (Halliwell and Gutteridge, 1985). The increase of serum uric acid is considered to be one of the body's natural responses to scavenge excessive free radicals produced, as uric acid is one of the quenchers of free radicals (Davies *et al*., 1986) while albumin plays a major role in quenching metal ions through their transport in plasma. Results of uric acid in this study were found to support the antioxidative effects of khat components, the major components, polyphenolic compounds. Our results were in controversy with results of Al-Qirim *et al*. (2002) who observed that intragastric administration of khat or its alkaloid significantly decreased the circulating activities of free radical metabolizing enzymes and glucose while increased the circulating level of uric acid. In the same study they observed khat consumption under stressful situation may enhance free radicals scavenging enzyme activities like glutathione-S-transferase and catalase if given alone, or were capable of preventing the stress induced decrease of enzyme levels.

Serum total cholesterol and HDL-cholesterol were increased after administration of khat extract at 1000 mg kg^{-1} body weight compared to the control group in contrast to the results of Ahmed and El-Qirbi (1993). While after 2000 mg kg^{-1} body weight khat extract administration there were no differences in total cholesterol or HDL cholesterol, however in rats plasma cholesterol is mostly carried by high density lipoproteins, while LDL plays a negligible role and carries more triglycerides than cholesterol (Nichols, 1967), this controversy with the results of Ahmed and El-Qirbi (1993), may be explained by the species differences.

The expression of toxicity of xenobiotics is usually determined biochemically by monitoring of some plasma enzymes and lipids. A rise in AST, ALT, ALP, TG and cholesterol are commonly measured as indices of the damage of the liver cells (Abdel-Baset *et al*., 1997). These enzymes are mainly localized in the cytoplasm and any damage in hepatic cells may result in alteration in the serum levels. Thus, the changes in activity and concentration of tumor marker enzymes like AST, ALT, ACP and ALP in tissue such as liver could reflect the state of hepatotoxicity (Vinitha *et al*., 1995). Our results failed to show effects of khat extract administration on liver enzymes in the treatment groups. This suggests that sub-chronic administration of *Catha edulis* crude extract has no hepatotoxic effects in rats. In the same line Mwenda *et al*. (2006) concluded that there were no observed histopathological changes in the testis, epididymis, liver, kidney and pituitary gland of the experimental animals fed with crude *Catha edulis* extract. In contrast to the results of Al-Habori *et al*. (2002) in which they reported that long term (6 months) feeding of khat leaves to New Zealand white rabbits increased liver enzymes levels and concluded that prolonged exposure to *Catha edulis* leaves may lead to toxic hepatocellular jaundice. This later observation by Al-Habori *et al*. (2002) depended on mixing an amount of khat up to 50% of the animal food, while human khat users are not chewing such high amount khat. However, the results of the present study seem to be more reasonable with the results observed by Mwenda *et al*. (2006) regarding the lack of hepatotoxic effects of khat extract administration.

The effect of khat on human reproduction remains an area of considerable interest. Khat administration for 13 weeks was observed to have no effects on rat total and prostatic ACP however khat chewers claim to have spermatorrhea on khat chewing. In this study we used air dried khat leaves in which the active component (cathinone) mostly undergone transformation into cathine which has a lesser potency compared to that of cathinone, which is the predominant stimulant in the fresh *Catha edulis* leaves. Controversy remains regarding the potential effects and mechanisms by which khat may affect reproductive functions. Limited studies that have been done provided conflicting results, with some

investigators suggesting that khat produce an increase in libido but also spermatorrhea and precocious ejaculation and others suggesting that it increases sexual desire and can be used as an aphrodisiac (Islam *et al*., 1990). In contrast, alternative findings suggest khat may have the opposite effect and may result in impairment of sexuality (WHO Advisory Group, 1980). Islam *et al.* (1990) reported loss of libido and decreased semen output in people who chew khat and Taha *et al.* (1995) reported no evidence that cathinone could be as an aphrodisiac. Khat has also been shown to decrease sperm volume, sperm count and fertilization of eggs in roosters (Hammouda, 1978) and increase in the frequency of abnormal sperm in mice (Qureshi *et al*., 1988).

A recent histopathological examination of sections of the male reproductive tract suggested that *Catha edulis* leaves had stimulated spermatogenesis and the cauda epididymides and Leydig cells were unaffected, when compared with equivalent sections from untreated rabbits (Al-Mamary *et al*., 2002; Al-Habori *et al*., 2002). The phenylpropanolamines cathine and norephedrine (the immediate metabolites of cathinone), at concentrations similar to those detected in blood samples of individuals who chewed khat leaves for 1 h (Toennes *et al*., 2003), were demonstrated *in vitro* to directly affect mammalian sperm function, accelerating capacitation and inhibiting spontaneous acrosome reactions (Adeoya and Fraser, 2005). More recently, in a study by Mwenda *et al.* (2006) who used the olive baboon, observed that khat administration causes a significant increase in the mean levels of testosterone while prolactin and cortisol levels were reduced. They concluded that khat may exert a transient effect on male fertility by interfering with the hormonal profile. In addition (Nyongesa *et al*., 2006) postulated that khat extract at high concentrations may cause reproductive function impairment in the user but at low concentrations it may enhance testosterone production with accompanying effects on reproductive functions in male mice.

Our results were found to be in agreement with that reported by a number of previous studies that *Catha edulis* can be used as an aphrodisiac. However we observed the serum values of testosterone to be increased non-significantly after feeding for 13 weeks while significantly increased when freeze dried khat leaves were administered for 6 weeks (unpublished manuscript). In the same line, results of Mwenda *et al.* (2006) reported that *Catha edulis* administration for 2 months in baboon monkey was associated with increase in plasma testosterone levels. In addition, these results also can be correlated with the results of Al-Mamary *et al.* (2002) who concluded that *Catha edulis* leaves administration had stimulated spermatogenesis. This is in contrast to some earlier observations in humans (Kalix and Braenden, 1985) and mice (Islam *et al*., 1990) in which khat consumption (in humans) or treatment with cathinone (in mice) resulted in a decrease in plasma testosterone levels. Cathinone, which is one of the active ingredients in khat, was reported to be responsible on the decrease in testosterone levels (Balint *et al*., 1991). The results of this sub-chronic study revealed that khat (*Catha edulis*) leaves oral administration to male rats significantly reduced plasma levels of lipid peroxidation product (MDA) with no hepatotoxicity or prostate adverse effects.

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