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Role of *Catha Edulis* (Khat) in Free Radicals Formation *in Vivo* and *in Vitro* study

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I. ABSTRACT

The Khat, Catha edulis (Celastraceae) chewing resulted in many diseases and physiological alterations affecting man. This fact has been observed in several previous studies. The present work was evaluated for the first time to investigate the in vivo and in vitro mode of Khat action and how it causing the etiology of diseases. Thirty healthy male rabbits weighting 1600-1800gm. were divided into 3 groups with 10 animals in each, first group served as control animals; they received orally 10 ml. of normal saline daily period of 30 day, while animals in second and third groups received a single orally dose 5g/kg and 10g/kg respectively, of powdered khat leaves, dissolved in distilled water, period of 30 days. The level of lipid peroxidation (LPO) and Methylglyoxal (MG) in liver homogenate were measured, and the levels of hydroxyl and superoxide radicals' generation in the in vitro reactions with presence of khat were also measured. Results showed a dose dependent high significant (P<0.01) increase in the level of (LPO), (MG) in liver homogenate of animals treated with Khat as compared to control animals. Results of in vitro reactions showed increase in the superoxide and hydroxyl radicals' generation.

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II. INTRODUCTION

Khat is a common name given to an evergreen tree or large shrub with glabrous leaves. The first scientific notice of Khat was made by the Swedish botanist Peter Forsskal (1736-1763) who died in Arabia in July 1763; he described the plant and classified it as *Catha edulis*, family Celastraceae (Wood 1997).

Khat is widely cultivated in East Africa and Southern Arabia (Yemen); it grows wild at altitudes of 1500-2000 meters above sea level (Kalix 1996 and Wood 1997).

Khat chewing has been prevalent in all parts of Yemen, and has become a part of Yemeni traditions, as well as, one of the main reasons of serious socio-economic and health problems in Yemen (Abdo-Rabbo *et al.,* 2000).In recent years Khat has been spread in other areas of the world including Europe and USA (Dhaifalah and Santavy 2004).

Phytochemical and pharmacological studies have shown that khat contains two main active ingredients namely cathine and cathinone in addition to more than other 40 alkaloids, tannins, and terpenoids (Widler *et al.,* 1994 and kalix 1996). In addition to commonly damages observed in Khat chewers such as insomnia, anorexia, dyspepsia, constipation, weakness, and fatigue (Kalix and Braenden 1985), Khat chewing resulted in many diseases and physiological alterations, which were observed in several previous studies that carried out to evaluate the effects of Khat chewing.

The orally administration of Khat to the experimental rabbits in various doses resulted in:

a significant decrease in a level of hemoglobin (Al-Ahdal *et al.,* 1988), a significant decrease in serum triglycerides, total white blood cells count and high significant decrease with certain structural change in lymphocytes (Ahmed and El-Qirbi 1993), a decrease in serum total protein due to the damage of hepatocytes ability to protein synthesis (Al-Safadi and Al-Qirbi 1988), an increase in serum aminotransferases ALT and AST (Farag and Al-Qirbi 1991), a certain morphological change in epithelium of stomach, and pathological change in liver tissues (Al-Safadi and Al-Qirbi 1986a and 1986b).There have been controversial reports on the effect of Khat on the blood sugar; some found that Khat increased the fasting blood sugar (Luqman and Danowski 1976, and Mohyi and Fathi 1979), while others reported that khat has no any effect on blood sugar (Elmi 1983 and Al-Guneid 1984). Bastawi (1987) observed that Khat affects the carbohydrates metabolism, and leads to accumulation of toxic pyruvaldehyde (methylglyoxal) in mice liver.

Many controlled trials studies on Khat chewers showed that Khat chewing resulted in an increase of heart beat and blood pressure (Nencini *et al.,* 1984), dysfunction of bladder neck with decrease in maximum urine flow rate (Nasher *et al.*, 1995), a significant decrease in serum total protein and albumin as well as increase in a serum aminotransferase SGPT (Abdo-rabbo *et al.,* 2000).

Generally, the pathological effects of Khat chewing include CNS, cardiovascular system, blood, digestive system, lever, urinary system and metabolism.

In the last 10 years we have noted a decrease in the studies carrying out on Khat effects, while the average of Khat chewers are increasing specially among the youth of either sex in Yemen.

The present work was evaluated *in vivo* and *in vitro* for the first time to investigate the mode of Khat action, and the way, which Khat acting through, and causing the etiology of diseases and physiological alterations.

III. MATERIALS AND METHODS

Chemicals: Chemicals used in this experiment were obtained from Sigma, St.Louis, MO, USA. Kit of free radical's generation reactions was obtained from Ruschem, Russia.

Preparation of tested material (Khat): Under shade dried Khat leaves were powdered in electric mixer, the powder was dissolved in distilled water in ratio 1:2 (1gramm of powdered leaves: 2 ml.distilled water).

Animals: Thirty healthy male rabbits (1600-1800g) were divided into 2 treated groups and control, as follows:

- *Control group:* 10 animals treated with a single daily dose of 10ml.normal saline orally period of 30 days.
- *Second group:* 10 animals treated with a single daily dose of 5g/kg of Khat in 10ml. distilled water orally period of 30 days.
- *Third group:* 10 animals treated with a single daily dose of 10g/kg of Khat in 20 ml. distilled water orally period of 30 days.

All animals were maintained in standard environmental conditions and kept a standard commercial diet with water *ad libitum*.

All experiment was administrated in the Institute of Physiological Research- Azerbaijan Academy of Sciences.

After 30 days the animals were fasted over night for12hrs. Then they were sacrificed, the livers were removed immediately, perfused with normal saline containing heparin, homogenized with phosphate buffer saline (pH 7.2) using Ultra Turax homogenizer, centrifuged at 3000g for 30 min.The supernatant was removed and stored at -20°C.

Lipid peroxidation (LPO) Assay: The LPO level in liver homogenate was measured via the measuring of malondialdehyde (MDA) according to method described by Halliwell and Chirico (1993).

Methylglyoxal (MG) Assay: Methylglyoxal (also called pyruvaldehyde) formed as a side product of dihydroxyacetone phosphate in glycolysis. MG was determined in the liver homogenate according to the method described by Kaczmarski *et al,* (1999).

Hydroxyl radical assay: It was evaluated by the thiobarbituric acid reacting substance (TBARS) method described by Shylesh and Padikkala (1999). The efficacy of *C.edulis* to increase the TBARS formation generated by Fe^{3+}/ ascorbate/ H_2O_2 system degrading deoxyribose was assessed. Two concentrations of *C.edulis* 5µg/ml and 10µg/ ml were added separately into the reaction mixture containing deoxyribose (2.6mM), $FeCl_3$ (0.1mM), KH_2PO_4-KOH buffer (20mM, pH7.2), in a final volume of 1ml. The reaction mixture was incubated for 1h at 37°C. The formed TBARS were measured, and the level of hydroxyl generation was calculated in µg/ml. The reaction without addition *C.edulis* was used as control.

Superoxide radical assay: It was determined by the nitroblue tetrazolium (NBT) reduction method described by Shylesh and Padikkala (1999). Concentrations of C.*edulis* 5 and 10 µg/ml. were added separately into the reaction mixture containing 0.1mM EDTA (200 µl), 0.12mM riboflavin (50 µl) and 0.6 M phosphate buffer (pH7.4) in a final volume of 2ml. The optical density at 530 nm was measured, and the level of superoxide generation was determined in µg/ml. The reaction without addition *C.edulis* was used as control.

Statistical analysis: The statistical analysis was performed by SPSS software; continuous data are expressed as mean ± S.E. Data were compared using one – way ANOVA. P value <0.01 was considered to be statistically significant.

V. RESULTS

The obtained results (Table 1) showed a dose dependent high significant (P<0.01) increase in the level of MDA a product of lipid peroxidation LPO in the hepatocytes homogenate of the rabbits treated with 5g/kg and 10g/kg of Khat leaves as compared to control. Results also showed a dose dependent high significant increase in concentration of MG in the lever homogenate of rabbits treated with mentioned doses of Khat leaves.

Table 1: The level of MDA and MG after 30 days of orally administration of *C.edulis*

	Parameters	Treatments	
	Control	*C.edulis* 5g/kg	*C.edulis* 10g/kg
MDA (µM)	0.05±0.11	2.12±0.71*	3.83±0.55*
MG (µM)	1.01±0.34	18.51±1.12*	29.17±0.93*

Values are mean of 10 animals' ±S.E. * High significant at P<0.01 vs. Control.

Table 2: Effect of *C.edulis* leaves on *in vitro* free radicals generation

Free radicals	Control	*C.edulis* 5µg/ml	*C.edulis* 10µg/ml
Superoxide µg/ml	4.61	28.29	42.68
Hydroxyl µg/ml	6.29	62.86	94.11

Table 2 shows the results of superoxide and hydroxyl radicals' generation in the *in vitro* reactions. The separately addition of 5 and 10 µg/ml of *C.edulis* increased the superoxide radical generation in percentage 513% and 825% respectively as compared to control reaction (in absence of *c.edulis*). The hydroxyl radical generation increased in presence of *C.edulis* in the above mentioned concentration in percentage 899% and 1396% respectively as compared to control reaction.

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VI. DISCUSSION

Polyunsaturated fatty acids (PUFAs) are abundant in cellular membrane, and allow it fluidity. A free radicals attack organic molecules including PUFAs caused damage in cell function, initiating a free radicals attack on cell membrane known as lipid peroxidation (Halliwell and Chirico 1993).

Our results showed a significant increase in lipid peroxidation (measured as the amount of MDA), in the liver homogenate of the rabbits treated with Khat, clearly indicate to the excessive in free radicals formation resulted by Khat. Methylglyoxal (Pyruvaldehyde) formed as a side product of dihydroxyacetone phosphate in glycolysis (Chatterjea and Shinde 2005). In agreement with Bastawi (1987), who reported accumulation of MG in liver of Khat feed mice, our results showed a high significant increase in level of MG in the liver homogenate of the rabbits treated with Khat.The accumulation of MG in liver cells is due to the inhibition of glyoxalase I, and depletion of the antioxidant and antiglycation enzymatic defense system of the cell (Thornalley 2003).

The generation of superoxide and hydroxyl radicals in the specific *in vitro* reactions increased when Khat was added into it, this fact clearly indicates to the role of Khat in free radicals formation and ensured our *in vivo* results.

In order to that, liver playing a major in carbohydrates, proteins and lipids metabolism, and the damage of liver cells affected many functions within the body, we have *in vivo* tested the role of Khat in free radicals formation in the homogenate of liver, however, the destruction role of free radicals on the all cells is well known and deeply studied.

The free radicals have been implicated as playing a major role in the etiology of cardiovascular and blood diseases, tumors, Alzheimer, liver certain alterations, digestive system disorders, metabolism damages, and urinary system disorders (Droge 2002, Fong *et al,* 2002, Reiter *et al*, 2002, Scandalio 2002, and Crack and Taylor 2005).

Our *in vivo* and *in vitro* results investigated for the first time that, the free radicals formation, and the depletion of cells antioxidant enzymatic defense system, may the main mode of Khat action, and that could be the way which Khat acting through, and causes the etiology of diseases and alterations.

Further deep studies in this aspect are needed for more explanation the mode of Khat action, and it role in free radicals formation.

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