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Alterations in Antioxidant Defense System in the Plasma of Female Khat Chewers of Thamar City, Yemen

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Abstract

Chewing Khat leaves (*Catha edulis*) is highly prevalent in Yemen and East African countries. Unfortunately, farmers use to apply pesticide for the better product of Khat. The present study has been designed to investigate the activity of plasma butyrylcholinesterase (BChE) and to assess the antioxidant defense system in the plasma of female Khat chewers in Thamar city, Yemen. Plasma of twenty female Khat chewers and twenty controls (non Khat chewers) were prepared and the activities of BChE and catalase (CAT) were estimated along with the measuring levels of reduced glutathione, total thiols and cholesterol. At biochemical level a significant decrease in the activities of BChE and CAT were observed in the plasma of female Khat chewers ($P < 0.05$) concomitant with reductions in the levels of reduced glutathione and total thiols ($P < 0.05$) as compared to non Khat chewers. This alterations on the antioxidants resulted in decrease of plasma cholesterol in Khat chewers group ($P < 0.05$). The present data show that the production of oxidants which are responsible for reduction in antioxidant defense system might be due to chewing Khat plant with more attention to the pesticide applied to the plant.

Keywords: Khat; organophosphates; butyrylcholinesterase; antioxidant; oxidative stress.

1. Introduction

Chewing Khat leaves (*Catha edulis*) is highly prevalent in Yemen and East African countries (Manghi *et al.*, 2009). The three main alkaloids present in Khat leaves are cathinone, cathine and norephedrine (Kalix, 1992). There are also small amounts of sterols and triterpenes, together with 5% protein and ascorbic acid. Khat leaves also contains tannin and minute amount of thiamin, niacin, riboflavin, iron and amino acids. Thus, only freshly picked leaves have the full efficacy (Lugman and Danowski, 1976).

Taken in excess, Khat causes extreme thirst, a sense of exhilaration, talkativeness, hyperactivity, wakefulness, and loss of appetite. It also can cause damage to the nervous, respiratory, circulatory, and digestive systems. Khat is reported to produce constipation and antispasmodic action (Makonnen, 2000). Chewing Khat has been linked with increased oxidative stress (Aleryani *et al.*, 2011). Oral administration of total aqueous Khat extract or of its alkaloid fraction exacerbated the oxidative stress in restrained rats due to the decreased activity of antioxidant enzymes, superoxide dismutase, catalase, glutathione-S-transferase (Kalix *et al.*, 1990). Similarly, Khat induced an increase in reactive oxygen species (ROS) and a depletion of intracellular glutathione in the cell cultures of human keratinocytes and fibroblasts, the reactions that could be opposed by addition of exogenous antioxidants (Lukandu *et al.*, 2008). But on the other hand, the flavonoid fraction of the Khat enhanced the activity of the antioxidant enzymes in rats and thus could provide a protection against the oxidative stress (Al-Qirim *et al.*, 2002). It was estimated by world health organization (WHO) that 30–50% of adult females consume Khat on a regular basis (WHO 2007). Unfortunately, farmers use to apply pesticide for the better product of Khat. The pesticides have two actions; it help the humans to remove harmful insect from the plants; on the other hand, it have negative effect for the human body especially for human's body enzymes . The fundamental toxicological activity of organophosphorus compounds (OPs) in human is due to the inhibition of esterases. Butyrylcholinesterases (BChEs) are enzymes belonging to a group of hydrolases classified by Aldridge (1953) as B-type esterases, they are inhibited by OPs and carbamate pesticides. As well as being present in the plasma, BChE is found in central nervous system, liver and other organs. As a result, along with acetylcholinesterase, BChE inhibition has been used as an indicator of exposure in biomonitoring programs of pesticide contamination. The exact physiological function of BChE is not yet clear, they appear to have a protective function by sequestering

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* Abbreviations used: BChE, Butyrylcholinesterase; GSH, Reduced glutathione; T-SH, Total thiols; OPs, Organophosphate compounds; CAT, Catalase

circulating OPs, thereby decreasing the toxic effect of these compounds on brain AChE (Russell and Overstreet, 1987). It has been reported that exposure to OPs induces oxidative stress by enhancing generation of ROS and/or by alterations in antioxidant defense system (Banerjee *et al.*, 2001). Oxidative stress occurs when the production of ROS overrides the antioxidant capability of the target cell (Klaunig *et al.*, 1998). ROS are potentially very damaging to cells, leading to oxidation of essential cellular constituents including proteins, lipids and DNA (Paradies *et al.*, 2002). The present study has been designed to investigate the pesticide effect of Khat via measuring plasma BChE and to assess the antioxidant defense system in the plasma of female Khat chewers in Thamar city, Yemen.

2. Materials and Methods

2.1. Chemicals

All chemicals used were of highest grade commercial products. Buryrylthiocholine iodide and DTNB (Ellman reagent) were from HiMedia, India. Kits of other tests were purchased from Spinreact, Spain.

2.2. Population and grouping

Thamar city female populations were divided into two groups each having n=20. Both group participants included in the present study fulfill the following criteria: healthy, non diabetic volunteers and aged between 20 and 35 years those excluded are suffering from hepatitis, carcinoma and diabetes:

1. Khat chewers group: local females with habit of chewing Khat.
2. Non-Khat chewers (control) group: local females never chew Khat.

Non-Khat chewers are those who never chew Khat, whereas, chewers are those who chew Khat daily. The study was performed in accordance with the Helsinki Declarations and was approved by Institution.

2.3. Sample collection

Blood samples of 40 individuals (20 each group) were collected between 8-10 A.M. 12 hours after end of Khat session, plasma of all samples were separated and were stored at -4 C° till the day of analysis.

2.4. Butyrylcholinesterase assay

Plasma BChE activity was determined colorimetrically by the method of Ellman *et al.* (1961). Briefly, plasma was pre-incubated with 10 mM of 5, 5-dithiobis-2-nitrobenzoic acid (DTNB or Ellman reagent) in 0.1 M phosphate buffer (pH 8.0) before the substrate butyrylthiocholine iodide was added (10 mM). Variations in optical density were recorded at 412 nm for 2 min at 25 C° using a spectrophotometer. Plasma BChE activity was expressed as nano-moles of substrate hydrolyzed per minute per mg protein using an extinction coefficient of DTNB (13,600 $cm^{-1}M^{-1}$).

2.5. Total thiols

Total thiol groups were quantified in the plasma according to the method of Ellman (1959) as modified by Sedlak and Lindsay (1968). Briefly, reaction mixture containing 0.2 M Tris-HCl and 0.02 M EDTA buffer (pH 8.2), plasma and 0.01 M DTNB (in methanol) was incubated for 15 minutes at room temperature then was centrifuged at 1,200 x g for 5 minutes. The supernatant was collected and the absorbance was read at 412 nm. Results were expressed as nmoles of T-SH/mg protein using molar extension coefficient of DTNB (13,600 $cm^{-1}M^{-1}$).

2.6. Low molecular weight thiols

Low molecular weight thiols, LMW-SH (primarily GSH) were measured in the plasma according to the method of Ellman (1959). Briefly, proteins in the plasma were precipitated by 4 % (w/v) sulphosalicylic acid followed by centrifugation at 1200 x g for 5 minutes. To the supernatant, 0.1 mM DTNB in 0.1 M phosphate buffer (pH 8.0) was added and the absorbance was read at 412 nm after 2 minutes. Results were expressed as nmoles of LMW-SH /mg protein using molar extension coefficient of DTNB (13,600 $cm^{-1}M^{-1}$).

2.7. Catalase activity

Catalase activity was assayed in the plasma following the method of Luck (1971). Appropriate amount of plasma was added to 12.5 mM H_2O_2 in 0.067M phosphate buffer (pH 7.0). The decrease in absorbance was followed at 240 nm for 3 minutes. Results were expressed as μmoles of H_2O_2 decomposed/min/mg protein using molar extinction coefficient of H_2O_2 (71 M-1 cm-1).

2.8. Cholesterol level

Cholesterol level was measured in the plasma according to the protocol provided by commercial kit, Spinreact, Spain. Results were expressed as mg/dl.

2.9. Protein content

Protein content was measured in the plasma according to the protocol provided by commercial kit, Spinreact, Spain. Results were expressed as g/dl.

2.10. Statistical analysis

Data were expressed as mean ± S.D. and were analyzed by student t-test. Differences between groups were considered significant when $P < 0.05$. All analyses were performed using the sigma-stat software (version 3.5).

3. Results

A decrease of 30.87% in the activity of BChE of Khat chewers group was observed as compared to the non Khat chewers group (Fig. 1), whereas, catalase activity was inhibited by 11.62% in the Khat chewers group (Fig.2). The thiol contents of Khat chewers group were also decreased as compared to non Khat chewers. It was observed that 14.42% of T-SH levels were less in Khat chewers concomitant with 49.12% decrease in the level of LMW-SH of Khat chewers group (Table 1). The increase in oxidative stress which is marked by the above results was affect the cholesterol level which was found to be reduced by 47.26% in Khat chewers group as compared to non Khat chewers group (Fig.3).

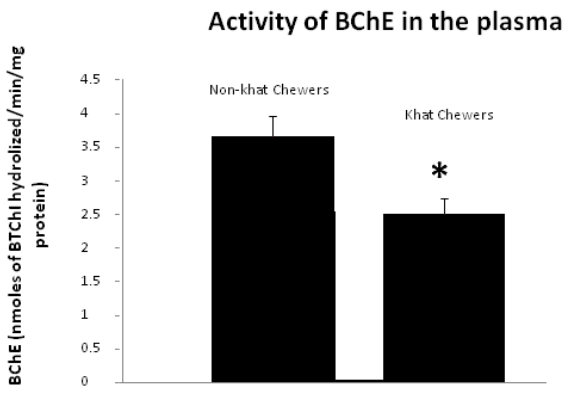

Figure 1. Activity of butyrylcholinesterase in the plasma of non-Khat chewers and Khat chewers. Results are expressed as mean ± S.D.; n= 20. Data were analyzed by student-t- test. *p<0.05 was considered significant from control group.

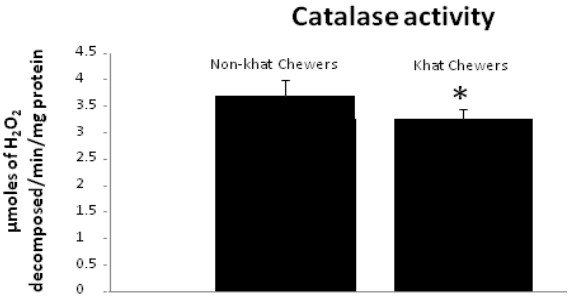

Figure 2. Activity of catalase in the plasma of non-Khat chewers and Khat chewers. Results are expressed as mean ± S.D.; n= 20. Data were analyzed by student-t- test. *p<0.05 was considered significant from control group.

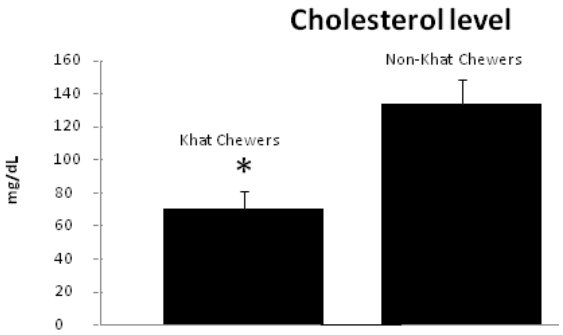

Figure 3. Levels of cholesterol in the plasma of non-Khat chewers and Khat chewers. Results are expressed as mean ± S.D.; n= 20. Data were analyzed by student-t- test. *p<0.05 was considered significant from control group.

Table 1. Effect of Khat chewing on thiol contents

	nmoles/mg protein	
	Non Khat Chewers	**Khat Chewers**
Total Thiols (T-SH)	256.73 ± 15.04	219.69±18.72*
LMW-SH (GSH)	122.23 ± 10.26	62.19±6.77*

Results are expressed as mean ± S.D; n= 20. Data were analyzed by Student-t- test.
*p<0.05 significantly different from control

4. Discussion

Khat is now widely chewed in Yemen and East African countries, however, only the immigrant communities from these countries are Khat chewers in Western countries (Manghi *et al.,* 2009). BChE and antioxidants CAT, GSH and TSH were decreased concomitant with decrease in cholesterol level in the present study in the plasma of female Khat chewers. BChE inhibition has been used as an indicator of exposure to pesticide. These findings are in agreements with those of Al-Akwa *et al.* (2009) who reported that chewing Khat increases the capacity of oxidant production and they also reported inhibition of AChE in the plasma of Khat chewers. ROS and the end products of LPO are believed to be largely responsible for the cytotoxic effects observed during oxidative stress (Cassarino and Bennett 1999). Moreover, cellular oxidative stress and cancer have been linked with pesticide exposure, particularly exposure to carbamate and OPs (Kassie *et al.,* 2001; Ranjbar *et al.,* 2002). The cell detoxifies free radicals via its antioxidant defense system, which includes non-enzymatic antioxidants like GSH and antioxidant enzymes; superoxide dismutase, catalase, glutathione peroxidase and glutathione reductase (Cassarino and Bennett, 1999). Thiols are organic sulfur derivatives characterized by the presence of sulfhydryl groups (-SH). Thiols are classified as large molecular weight (protein) thiols and low molecular weight thiols (GSH, cysteine and homocysteine). GSH is an important water soluble antioxidant that is central to cellular defense against oxidative stress and potentially toxic chemicals (Meister and Anderson 1983). It directly quenches reactive hydroxyl radicals and other oxygen-centered free radicals, and conjugates to the xenobiotics to water soluble products (Kidd, 1997). Low levels of GSH could be due to enhanced generation of ROS which are scavenged by GSH or decreased activity of GR enzyme, which converts oxidized glutathione (GSSG) to its reduced form. The relationship between the reduced and oxidized state of glutathione, the GSH/GSSG ratio or glutathione redox status, is considered as an index of the cellular redox status and a biomarker of oxidative damage, because glutathione maintains the thiol-disulphide status of proteins, acting as a redox buffer. Glutathione depletion was reported to induce apoptotic cell death which occurs through the upregulation of novel protein kinase C and activator protein-I (Domenicotti *et al.,* 2000). Therefore, disruption of the GSH redox status by OPs can alter transcriptional responses to induce programmed cell death. CAT is an antioxidant enzyme that appears to be less significant as it has relatively low affinity for H_2O_2 but is an important enzyme at higher H_2O_2 concentration (Chance *et al.,* 1979). The activity of CAT was decreased in the plasma of Khat chewers as compared to control group this will lead to accumulation of H_2O_2 that will contribute to high hydroxyl radical. Cholesterol is involved in the clustering of particular lipids within the membrane bilayer to form rafts, which in turn influences the distribution of proteins within the membrane. Cholesterol might exert its affect on membrane proteins viz. adenylate cyclase, GABA uptake transporters and the nicotinic acetylcholine receptor (Fong and McNamee, 1986; North and Fleischer, 1983). Alterations in cholesterol contents might alter the activity

of various membrane proteins. Inhibition of critical antioxidant enzymes and scavenger proteins may be the mechanism by which free radicals reduce antioxidant capacity (Grzelak *et al.*, 2000). It has been reported that Khat leaves reduced significantly the cholesterol levels in rabbit adrenal (Ahmed and el-Qirbi, 1993) and rabbits blood (Al-Habori and Al-Mamary, 2004). This decrease is attributed to the increase level of cAMP and increase adrenocorticotrophic hormone which is believed to be mediated by the activation of adenylyl cyclase, hence, increase cAMP level. The increase in cAMP concentration has an inhibitory effect on cholesterol synthesis (Mayes, 2000). However, the present finding are in contrast with those reported by Al-Zubairi *et al.* (2003) were the total cholesterol and LDL- cholesterol were non-significantly affected by Khat chewers as well as Khat chewers who also smoke. Our results are in agreement with the findings of some researchers, Al-Akwa *et al.* (2009) have reported an increase on the levels of free radicals of serum of male Khat chewers. Al-Qirim *et al.* (2002) have reported that Khat consumption increases circulating free radicals in rats. Lukandu *et al.* (2008) showed similar observations in keratinocytes and fibroblasts. Oral administration of Khat extract to rats induced lipid peroxidation and oxidative stress in hepatic and renal tissues as shown by significant increases in lipid peroxidation and significant decreases in levels of CAT and GSH (Al-Hashem *et al.*, 2011).

It is concluded that applying pesticides for better Khat production might be responsible for some of the changes in the antioxidant defense system which needs to be eliminated in future studies by using appropriate animal model and chemical-free Khat.

5. Conflict of Interest

No financial, personal, or other conflict of interest to be mentioned.

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