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Full length research paper

Potential health effects of daily khat leaves chewing: Study on the biochemical blood constituents changes among adults in Sana'a city, Yemen

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The khat plant (Catha edulis) leaves is grown and consumed daily in Yemen as a natural stimulant by chewing the young buds and tender leaves that contain the stimulant "Cathinone" for the mild stimulant effect. Cathinone is believed to be the main active ingredient in fresh khat leaves. In Yemen this habit has a deep-rooted socio-cultural tradition in which consumers spend part of their time chewing khat (ranging between 6-8 hours per day). The effect of this habit on blood constituent has not been adequately studied in human. There is an extensive literature on khat which estimated the effectiveness and specificity for these substances on kidney and liver function test in animals than humans. The present study was undertaken to investigate the biochemical changes associated with chewing Khat.

Key words: Biochemical assay, Catha edulis, khat, oxidation, per-oxidation, Yemen.

INTRODUCTION

The khat plant (Catha edulis Forsk) is a tree of the family Celastraceae, frequently cultivated in certain areas of East Africa and the Arabian Peninsula. Worldwide about ten million People chew khat daily. Several authors have argued that regular consumption of khat seriously affects the social and economic life of the chewing khat (Penning et al., 2008). Usually, a person consumes 100–200 g of the leaves per day; young leaves are preferred because these have the highest stimulant activity. The leaves of the khat plant contain alkaloids structurally related to amphetamine. They are chewed daily by a high proportion of the adult population in Yemen for the pleasant mild stimulant effect. Khat has been used first as a drink prepared from dry leaves, but its effect is weak compared with coffee (El-Mahi, 1962). It was found later that drying the leaves results in loss of some active constituents (Revir, 1983), therefore the habit of chewing the green leaves was adopted. For many hundreds of years the custom of chewing khat leaves has been practised for the resulting central stimulant effects (Luqman, and Danowski, 1976). In Yemen, the habit is widespread with a deep-rooted socio-cultural tradition, forty four different types of Khat in different regions, each has different cathinon constituent (Halbach, 1972). The pleasurable central stimulant properties of khat are commonly believed to improve work capacity, and are used on journeys and by students preparing for examinations to counteract fatigue. In recent years, because of improved air transport, the consumption of fresh khat leaves has expanded considerably, even to communities in Europe.

Early clinical observation suggested that khat had amphetamine-like properties(Szendrei, 1980). Like coffee, tea, Khat has a stimulating dependence affect and no withdrawal symptoms. The active ingredient is alkaloid (cathinone)-pharmacological effect is increase energy, euphoria, concentration motivation and decreased appetite, increases metabolism decreases appetite CNS stimulant acts on adrenergic receptors. It may cause elevation of arterial blood pressure and pulse rate with

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subsequent increased cardiovascular risk, particularly in hypertensive patients (Al-Motarreb,et.,al 2010).
It seems to be a common cause of stomatitis and other problems in the mouth as well as gastro-oesophageal reflux. It may be associated with increased risk of carcinoma of the mouth and oesophagitis, may interfere with absorption of some orally administered antibiotics (Valko, et.,al 2007). It may have a toxic effect on the liver, possibly as a result of pesticides used in khat cultivation.(Anwar et al., 2012). It is associated with an increased risk of low birth weight infants in khat-chewing pregnant women. Subsequent chemical analysis confirmed that the fresh leaves contain a number of compounds, including phenylalkylamine compounds (alkaloids) such as nor pseudoephedrine(cathine) and alpha aminopropiophenone (cathinone), the later being structurally related and pharmacologically similar to amphetamine (Al-Motarreb et.,a l 2010 , Valko, et.,al 2007) .
Khat leaves also contain considerable amounts of tannins (7%–14% in dried material), vitamins, minerals and flavonoids (Halbach, 1972, Valko, et.,al 2007). As a plant containing amphetamine-like substances, the main effects of Khat are on the cardiovascular system, Gastrointestinal System, and nervous system, much of the concern raised about the harmful effects of khat are related to excessive use.
Recently (Penning et al., 2008) came to a similar conclusion: state that is khat dependence is low to mild, craving and tolerance to khat effects exists but there is no definite withdrawal syndrome. There is no strong, and even contradictory, evidence for a causal relation between khat use and psychiatric morbidity". As oral administration of khat in rats was associated with decreased serum free radicals metabolizing enzymes such as superoxide dismutase (SOD) and catalase (CAT) (Valko, et.,al 2007). The widespread chewing of khat in Yemen is a habit in which consumers spend part of their time chewing khat. There is an extensive literature on khat. Only a few studies, however, have investigated the effect of khat chewing on blood constituents. The present study was undertaken to investigate the biochemical changes associated with chewing and study the associated health factors.

MATERIALS AND METHODS

Study subject

This study involved random selection of 50 individual's educated male daily chewing khat since age of 15 years consuming at least 200 gram of fresh leaves. The age of the subject between 20-40 years old and 20 individuals never chew khat as a control from the community of the Sana'a University. All of individual's were adult male healthy and do not suffer from any hereditary diseases or chronic symptoms. According to their ages they divided into two groups. Group A representing those aged between 20-30 and B those aged between 30-40 years.

Sample preparation

Blood Sample of 2 ml has been collected from each subject after overnight fasting in heparinised tube, tested within 30 minutes using the facilities in biochemistry and the medical lab in military hospital Sana'a Yemen.

Biochemical Assays

Cholesterol, glucose, albumin, uric acid, Urea, Creatinine, and Total protein in the serum were estimated using chemistry analyzer (Automatic Analyzer, Japan). Estimation of Lipid Peroxidation and Oxidative Stress Parameters in serum collected was conducted by measuring the concentration of TBARS using the method described by (Wasowicz et al., 1993). The GSH content of serum collected was measured at 412 nm using commercially available kits (Randox Laboratories, UK). CAT activity in serum collected was assayed using commercially available catalase activity assay kits (Biovision, K 773-100).

Assay of CAT activity

Catalase activity of samples (chewing and non-chewing kath) was assayed by the method of (Aebi et al.,1987). The samples were mixed in 50 mM phosphate buffer (pH: 7.0), Decomposition of H_2O_2 was determined by the decrease in absorbance at 240 nm. One unit of this activity was defined as the amount of enzyme decomposing 1Qmol/ H_2O_2 / per min (U/mg of protein).

Assay of GSH-Px activity

Subjects (chewing and non-chewing kath) GPX activity was measured using a modification of the method of (Paglia and Valentina,1967). Samples were mixed in the phosphate buffer (50mM, pH: 7.2). A sample was added to the reaction mixture (0.3 M EDTA, 0.1 mM NADPH, 0.5 U glutathione reductase, 0.5 mM NaN_3), t-butyl hidroperoxide (t-BOOH) and reducted glutathione (GSH). The absorbance at 340 nm was recorded for 3 min. Reduced glutathione is oxidized by the GPx. Glutathione reductase is reduced to oxidize glutathione using to NADPH. The amount of protein was determined by the method of (Lowry et al., 1951).

Statistical analysis

Statistical tests results were expressed as means ± STD

Table 1. Levels of blood biochemical assay on the khat chewers and no-chewers.

Parameters	Control	Group A	Group B
Glucose mg/dl	95± 1.56	65.95±1.21*	64.87±1.43*
Total cholesterol mg/dl	185±0.22	187±0.15*	189±0.41*
Albumin (g/dl)	3. 63±0.219	3.11±0.11*	2.99±0.11*
Uric acid mg/dl	6.76± 0.23	9.54±0.41*	9.43±0.52*
Creatinine mg/dl	o.83±0.32	2.5±0.32*	2.1±0.32*
Urea (mg/dl)	32.3 ±1.92	44.64±0.43*	41.23±0.43*
Total protein (g/dl)	6.05± 0.206	4.86±0.19*	5.01±0.17*

Table 2. Clinical parameters of khat chewing and khat no chewing sides (The values are means ± SD).

Parameters	Control	Group A	Group B
(GSH)(mg/100µl of serum)	38.5± 1.54	23.3± 1.44*	24.1± 1.64*
TBARS (mM/100 µlof serum)	0.90 ± 0.004	1.87±0.005*	1.97±0.004*
(CAT) (U/mg of protein)	67.45±2.32	43.21±2.13*	44.2± 2.14*

Values are statistically significant at *$P<0.05$.

which performed using analysis of variance (ANOVA) using Minitab 2000 Version and data is reported as mean (n = 3 x 1) ± standard deviation (n = 3 x 1).

RESULTS

As shown in table 1 and 2; Khat chewing caused significant increases in the levels of serum, urea, bilirubin and creatinine, in khat chewer group more than the control, accompanied by significant increase in group A than group B, Significant decrease in the glucose ,albumin and protein level . Furthermore significant increase in lipid peroxidation biomarkers thiobarbituric acid reactive substances (TBARS) was indicated in table 2. Decrease in Levels of Catalase (CAT) and Glutathione (GSH).

DISCUSSION

In this study, there was a significant decrease in serum total protein and albumin of khat chewing in both groups as compared to control khat non-chewing, which may indicates decrease in liver function, decreased protein synthesis, either primary as in liver cell damage or secondary to diminished protein intake and reduced absorption of amino acids(Chawla, 1999). Some constituents of the *Catha edulis* might be been converted to pro-oxidant metabolites or the extract during chewing might have induced decreased synthesis or activity of the antioxidant system in khat chewing groups suggesting that the extract generated free radicals or directly inhibited synthesis of antioxidant enzymes. Oxidative stress is often defined as an imbalance of pro-oxidants and antioxidants, which can be quantified in humans as the redox state of plasma GSH/GSSG. The increase in serum urea, creatinine, and uric acid has been linked to kidney disease in much research (Chawla., 1999).

Both groups of Khat chewing had significantly increased serum creatinine, impaired renal function due to a reduced ability to excrete these products could originate from changes in the threshold of tubular re-absorption, renal blood flow and glomerular filtration rate (Chawla., 1999, Carvalho., 2003). These results are in broad agreement with previous studies that reported the khat induced cytotoxicity in livers and kidneys after oral administration of khat to animals (Al-Motarrebet al., 2002, Dimba et al., 2003, and .Gunaid et al., 1997).

The subject's age significantly affected the results and

played a role in the level of toxicity among younger's age in group A more than group B. The activities of GSH and CAT were decreased among khat chewers in group A more than group B. The fresh extract generated by chewing fresh leaves may release free radicals or directly inhibited synthesis of antioxidant enzymes.

The role of glutathione as a protective agent against oxidative organ damage has been the subject of extensive studies (Nasher et al., 1995, Baynes, 1995). The decrease in the control group renal activity was significant which might suggest the decrease in the renal activity could be accompanied with many cofounder factors. From the results presented in this paper in the biochemical constituent in most of the khat chewers specifically the age between 20-30 more than 30-40 years old. The decrease in glucose and lipid per oxidation could be related to oxidative stress in hepatic and renal tissues which indicated by the significant increase in lipid per-oxidation biomarkers (TBARS) and a significant decrease in levels of the antioxidant components.

CONCLUSION

A harmful activity of chewing khat on health has been reported in several studies. Studying the effect of daily dose intake of this plant in Yemen one of the most the people chewing khat in continuous manner. The study reveal that khat (Catha edulis) leaves have a toxic effects on the blood constituent, alterations in biomarkers of oxidative stress and biochemical could be an indicator for liver and the kidney toxicity . Further studies on the effect of khat each organ function and studying the co-founder that might affect the toxicity of the plant is recommended.

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