

Effect of Daily Chewing Soft Buds and Leaves of *Catha edulis* (Khat) on the Antioxidant Defense System and Oxidative Stress Markers in Blood

Khalid Mohammed Naji · Maher Ali Al-Maqtari ·
Adnan Ali Al-Asbahi · Qais Yusuf M. Abdullah ·
R. Nagesh Babu · Varadahally Rangaiah Devaraj

Received: 16 April 2014 / Accepted: 30 June 2014 / Published online: 16 November 2014
© King Fahd University of Petroleum and Minerals 2014

Abstract *Catha edulis* (Khat) is one of the major economic, social and health problems in Yemen. This paper aimed to study the effect of Khat on the oxidative status of Khat chewers by measuring the levels of enzymatic and non-enzymatic antioxidant as well as lipid peroxidation. The results exhibited significant reduction in erythrocytes superoxide dismutase (SOD, EC: 1.15.1.1), and catalase (CAT, EC: 1.11.1.6) in Khat chewers, in addition to elevation of serum glutathione-S-transferase (GST, EC: 2.5.1.18). Furthermore, non-enzymatic antioxidants glutathione (GSH) and vitamin C were significantly reduced ($p < 0.001$; $p < 0.015$), whereas malondialdehyde (MDA) was significantly elevated ($p < 0.001$). The depletion of GSH and vitamin C along with MDA elevation in Khat chewers compared with control reflects the obvious oxidative status, a result of enormous

reactive oxygen species (ROS) formation, leading to membrane damage. ROS possibly induced by active components of Khat or by pesticides added to the Khat tree. In addition, the reduction of SOD and CAT is indicative to cellular proteins damage which occurred by ROS. As well, the elevation of GST may due to a leakage of cellular GST to blood stream; this implies that GST active site was not affected. This study concludes that daily chewing Khat for long period certainly induce ROS production, leading to oxidative toxicity. Both enzymatic and non-enzymatic antioxidants are involved in the protection against this toxicity. People who habitually chew Khat for long term will be susceptible to the oxidative toxicity; therefore, they recommended giving up of Khat chewing.

Keywords Khat chewing · Antioxidant · Oxidative stress · *Catha edulis* · Antioxidant enzymes

K. M. Naji (✉) · M. A. Al-Maqtari
Department of Chemistry, Faculty of science,
Sana'a University, Sana'a, Yemen
e-mail: khalid.m.naji@gmail.com

M. A. Al-Maqtari
e-mail: Proteinase_613@hotmail.com

A. A. Al-Asbahi · Q. Y. M. Abdullah
Department of Biology, Faculty of Science,
Sana'a University, Sana'a, Yemen
e-mail: adnanalabahi@yahoo.com

Q. Y. M. Abdullah
e-mail: Qais.y.m.a@gmail.com

R. N. Babu
Department of Biochemistry, Mahrani Science College of Women,
Bangalore University, Bangalore, India
e-mail: nageshbabur@gmail.com

V. R. Devaraj
Department of Biochemistry, Central College Campus,
Bangalore University, Bangalore 560 001, India
e-mail: devaraj@bub.ernet.in

1 Introduction

The interruption of balance between oxidants and reductants within the body due to the excess production of peroxides and free radicals causes damage to cellular components and tissues leading to oxidative stress [1]. Oxidative stress occurs either due to the increasing level of the reactive oxygen species (ROS), or a decrease in the antioxidant defense. ROS molecules include hydrogen peroxide (H_2O_2), superoxide ($O_2^{\cdot-}$) and the hydroxyl radical ($\cdot OH$); they are harmful for all tissues [2]. The capacity of antioxidant defense systems to catch ROS is very crucial for protecting the tissues from oxidative damage. Cellular antioxidant defense systems including enzymatic [such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX), glutathione-S-transferase (GST) and glutathione reductase (GR)] and

non-enzymatic [such as tocopherols, carotenoids, flavonoids; ascorbate, urate, and glutathione (GSH)] act together to prevent disturbances in ROS homeostasis or to reduce the effect of oxidative stress in cells [3–5]. Pesticides also can induce oxidative stress, either by overproduction of free radicals or by alteration in antioxidant defense mechanisms, including detoxification and scavenging enzymes [6].

Catha edulis plant (Khat) is cultivated primarily in East Africa and southern west of Arabian Peninsula. Khat leaves and shoots of the shrub usually harvested and chewed to obtain a stimulant effect [7]. The active ingredients of *Catha edulis* are cathine (norpseudoephedrine) and cathinone (benzoylthamphetamine). These alkaloids are similar in structure and pharmacological activity to amphetamines [8]. More than 40 compounds have been identified in Khat extract [9]. The habit of Khat chewing has prevailed for centuries among populations including Yemen [10]. Khat consumption has increased during the last decades in Yemen, Eastern Africa, and has become a global phenomenon spreading to ethnic communities in the rest of the world, such as The Netherlands, UK, Canada and the USA [11].

Higher consumption of Khat in Yemen has led to an increasing demand, and this in turn needs an extensive use of pesticides to increase production; 95% of Khat farmers haphazard use hazardous and unofficially controlled pesticides for the Khat trees, risking the health of consumers [12]. The toxicity of various pesticides, including organochlorines and organophosphates (OPs), has been reported to induce the production of oxidative stress [13]. World Health Organization (WHO) was evaluated that 30–50% of adult females regularly consume Khat in Yemen [14]. Therefore, the relation between human health and Khat chewing habit extensively studied, but most of them have focused on the pharmacology of Khat and the Khat amphetamines, whereas relatively few reports have investigated potential cytotoxic effects and underlying mechanisms.

Oral administration of total aqueous Khat extract or of its alkaloid fraction exacerbates the oxidative stress in restrained rats due to the decreasing activity of antioxidant enzymes, SOD, CAT and GST [15]. On the other hand, the flavonoid fraction of the Khat enhances the activity of the antioxidant enzymes in rats and this could provide a protection against the oxidative stress [16]. Khat induces an increase in 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 [17]. Recently, it is reported that chewing Khat inhibits ROS scavenging enzymes in the human blood serum, resulting in significant elevations in free radical loads, which cause the increase in oxidative stress [18, 19].

In this study, we investigate the effect of chewing Khat in daily manner on the oxidative stress status by monitoring the activities of antioxidant enzymes (SOD, CAT and GST) and

measuring the levels of non-enzymatic antioxidants, (glutathione and vitamin C), as well as malondialdehyde (MDA) the final product of lipid peroxidation.

2 Materials and Methods

Fifty subjects were recruited after they have been written informed for this study, which principally performed in accordance with the Declaration of Helsinki. Twenty-five of them are Khat chewers of age range 24–40 years (mean 32.700 ± 5.079). They have been chewing a fresh Khat daily for at least 4 h for 3 years. The other 25 subjects used as a control are non-Khat-chewers of age range 22–45 years and mean age of 30.000 ± 4.691 . All subjects were not using any kinds of tobacco, healthy, did not suffered from any chronic disorder like hypertension, cardiac, renal or liver disease and did not have any history of health disorder.

2.1 Preparation of Plasma and Erythrocyte Lysate

Blood was collected with using EDTA anticoagulant and centrifuged at $700\text{--}1,000 \times g$ for 10 min at 4°C . The top yellow plasma layer was pipetted off without disturbing the white buffy layer. Then the plasma was stored on ice, and the white buffy layer (leukocytes) was removed and discarded. The erythrocyte portion (red blood cells) was lysed four times. The ice-cold double-distilled water was centrifuged at $10,000 \times g$ for 15 min at 4°C . The supernatant (erythrocyte lysate) was collected and stored on ice. This lysate was used for the determination of enzyme activities of SOD and CAT.

2.2 Enzyme Assay

2.2.1 Superoxide Dismutase (SOD)

The SOD activity was measured by a spectrophotometric method [20]. The hemolysate was mixed with ice-cold 0.05 M phosphate buffer containing 1 mM EDTA. The SOD was extracted from the supernatant by chloroform–ethanol and measured by a method, which uses the ability of the SOD enzyme to inhibit the reduction of nitro-blue tetrazolium (NBT) by superoxide radical that generated by the reaction of photoreduced riboflavin and oxygen. For each sample to be assayed, three tubes were set up containing 50, 100, 300 microliter of red blood cell extract plus 0.2 ml of EDTA/NaCN, 0.1 ml NBT, 0.05 ml riboflavin and potassium phosphate buffer to give a total volume of 3 ml. A tube containing no red blood cell extract was included in each run. After mixing, the tubes exposed to uniform illumination for 20 min. At 560 nm, optical densities were measured at room temperature. Results were given as units of SOD per gram of hemoglobin. One unit of SOD was defined as the amount

of enzyme causing 50 % of the maximum inhibition of NBT reduction.

2.2.2 Catalase (CAT)

Catalase activity was determined by the method of Aebi [21] by using a UV/visible spectrophotometer. Three milliliter reaction mixture volume contained 2.2 ml of 0.01 M pH 7.0 sodium phosphate buffers, 0.4 ml of 2 M H₂O₂ and 0.2 ml of erythrocyte lysate. The decomposition rate of substrate H₂O₂ was monitored at 240 nm. A molar absorptivity (ϵ) of 43.6 L mol⁻¹ cm⁻¹ was used to calculate the activity of CAT. One unit of CAT was defined as the amount of enzyme, which can decompose one μ mol of H₂O₂/min.

2.2.3 Glutathione-S-transferase (GST)

Activity of serum GST was assessed by the method of Habig [22], by measuring the conjugation of glutathione (GSH) and 1-chloro-2,4-dinitrobenzene (CDNB) as substrate. The reaction mixture contained 0.1 ml of serum sample, 0.1 ml of CDNB and 2.7 ml of phosphate buffer pH 6.25 in a total volume of 2.9 ml. The reaction was initiated by the addition of 0.1 ml of 29.93 mM GS. The absorbance of the mixture was read every 30 s at 340 nm against distilled water blank for 8 min by a spectrophotometer. The assay mixture without the extract served as the control to monitor non-specific binding of the substrates. The GST activity was calculated using the extinction coefficient of the product formed (9.6 mM⁻¹ cm⁻¹) and was expressed as nmoles of CDNB conjugated/minute.

Hemoglobin was determined by commercial kit according to the instructions of the manufacturer. Total protein was estimated according to Lowry [23] method by using bovine serum albumins as standard.

2.3 Determination of Glutathione and Vit. C

2.3.1 Glutathione (GSH)

Analysis of blood GSH concentration was performed with the method described by Ellman [24] and as modified by Ohkawa [25]. In brief, 0.2 ml of whole blood was added to 1.8 ml distilled water and was incubated for 10 min at 37 °C for complete hemolysis. After hemolysis, 3 ml of 4 % sulphosalicylic acid was added and tubes were centrifuged at 2,500 \times g for 15 min. Supernatant (0.2 ml) was mixed with 0.4 ml of 10 mM 5, 5'-dithiobis-(2-nitrobenzoic acid) (DTNB) and 1 ml phosphate buffer (0.1 M, pH 7.4). At the end of 5 min, absorbance of the yellow chromogen was measured at 412 nm and it was directly proportional to GSH concentration.

2.3.2 Vitamin C

Vitamin C (ascorbic acid) is a major soluble antioxidant in the body fluid and was estimated by the dinitrophenyl hydrazine (DNPH) method [26]. The serum was first deproteinized with 5 % TCA, incubated with the DTC reagent (0.1 M DNPH, 0.027 M of copper sulfate and 0.66 M of thiourea) for 60 min at 60 °C and then followed by the addition of 4.5 M sulfuric acid. The intensity of the formed color was read at 520 nm against a blank, which was similarly treated as the test sample except using distilled water instead of serum. Ascorbic acid concentration was expressed as mg/dl.

2.4 Determination of Malondialdehyde (MDA)

Serum MDA levels were estimated using thiobarbituric acid (TBA) method according to Beug [27]; 375 mg of TBA was dissolved in 2 ml of 0.25 N HCl, followed by 15 g of trichloroacetic acid (TCA) for a total volume of 100 ml. The solution was heated in a water bath at 58 °C to dissolve TBA properly. One milliliter of serum was added to 2 ml of TCA-TBA and mixed thoroughly. The solution was heated for 15 min in a boiling water bath. After cooling, the flocculent precipitate was removed by centrifugation. Sample absorbance was then determined at 535 nm against a blank that contained all reagents except the serum sample. Serum MDA concentration was calculated using extinction coefficient (ϵ) 1.56 \times 10⁵ M⁻¹ L cm⁻¹ for the malonaldehyde-TBA complex and expressed as nmol/ml.

2.5 Statistical Analysis

One-way analysis of variance (ANOVA) was used for statistical evaluation in all experiments by using GraphPad prism ver. 6. Data were shown as mean \pm SD. The value of $p < 0.05$ was considered statistically significant.

3 Results

Level of antioxidant enzymes activities exhibited various tendencies. Both erythrocyte SOD and CAT activities were significantly decreased in Khat chewers compared to the controls (i.e., non-Khat chewers) (Fig. 1), whereas serum GST activity of Khat chewers was significantly increased than that group of non-Khat chewers as a control (Fig. 2).

Non-enzymatic antioxidants and lipid peroxidation marker are illustrated in Table 1. Significant decrease in the serum glutathione (GSH) of the khat chewers compared to the non-Khat chewer's controls ($p < 0.001$) was observed. This reduction was also noticed in the level of serum vitamin C ($p < 0.015$). Both GSH and vitamin C reduced approximately by 32 % in the Khat chewer subjects comparing with

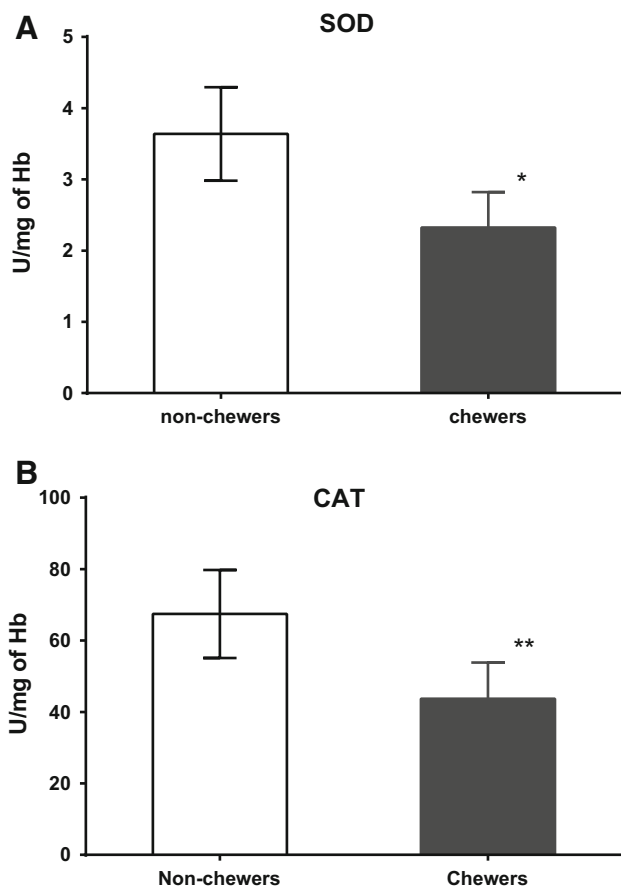


Fig. 1 a Activity of erythrocytes SOD and b CAT in blood of long-term daily Khat chewers and non-Khat chewers. Results expressed as mean \pm SD of triple replicate. (* $p < 0.05$; ** $p < 0.01$)

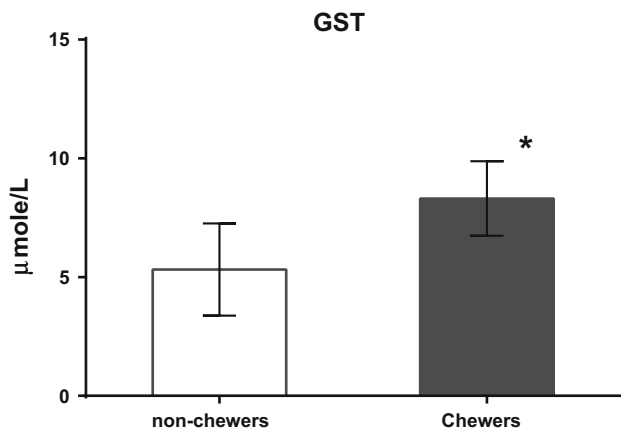


Fig. 2 Activity of serum GST in blood of long-term daily Khat chewers and non-Khat chewers. Results expressed as mean \pm SD of triple replicate. (* $p < 0.05$)

the non-Khat chewers. Malondialdehyde (MDA) is the final product of the lipid peroxidation, and it is considered as the intimate marker of the membrane leakage. The level of this marker was found to be significantly higher in the Khat chewers rather than non-Khat chewer controls ($p < 0.001$).

4 Discussions

Antioxidants are intimately involved in the prevention of cellular damage from that caused by unstable molecules known as reactive oxygen species (ROS), which can cause chain of reactions. Antioxidants terminate these chain reactions by removing free radical intermediates and inhibit other oxidation reactions by oxidizing themselves. Clinically, antioxidants were classified into two groups: preventive and chain-breaking antioxidants. SOD is one of the chain-breaking antioxidants, while CAT is the preventive antioxidant enzyme [28,29]. By comparing the activities of erythrocytes SOD and CAT in our results, we found that there was a significant decrement in the activities of erythrocyte SOD and CAT in daily Khat chewers relevant to the control (non-Khat chewers). The enzyme SOD breaks down the superoxide anion to hydrogen peroxide. This hydrogen peroxide converts by CAT, providing the first line of defense against reactive oxygen species toxicity.

This reduction of SOD and CAT was similar to the results presented in rat treated by Khat [16,30]. In contrast, the SOD and CAT activities increased in Chick embryos after being treated with Khat leaves that were treated by insecticides [31].

The decrease in SOD activity appeared to be associated with the decrease in the levels of SOD protein of the examined tissues [32]. In addition, this may be due to an increasing endogenous production of ROS as indicated by the increase in lipid hydroperoxides. In vitro studies indicate that all SOD enzymes are exquisitely susceptible to oxidative modification and inactivation through targeting of tyrosine [33] or histidine residues [34]. Depletion of CAT activity, observed in our study, might enhance the oxidative stress in patients by lowering the detoxification of H_2O_2 . Such conditions may accelerate the risk of cell components damage. It is well known that if the superoxide ions are not removed immediately, it may cause the inhibition of CAT activity [35]. Thus, the lower CAT activity we recorded might be a consequence of the decrease in SOD activity in hyperplastic and cancer patients.

Glutathione-S-transferase isozymes played a central role in human detoxification process. Human GSTs mainly consist of class Pi (GST π), Alpha (GST α), Mu (GST μ) and Theta (GST θ) enzymes, each subdivided into one or more isoenzymes. Both cytosolic and microsomal GSTs catalyze the conjugation of glutathione with wide variety of xenobiotics such as carcinogens, pharmacologically active agents, as well as reactive oxygen species (ROS). The conjugation may result in the formation of more water-soluble and less biologically toxic molecules that can be excreted easily [36].

Our results showed significant elevation in serum GST activity, reflecting the presence of high toxic compounds which may result from mixed pesticides commonly added

Table 1 Levels of antioxidants and lipid peroxidation marker of Khat chewers and non-chewers as a control

	Non-Khat chewer ^a (<i>n</i> = 25)	Khat chewers ^a (<i>n</i> = 25)	<i>p</i>
GSH (mg/100ml)	38.546 ± 1.544	26.354 ± 2.267	<i>p</i> < 0.001
Vit. C (μM/L)	42.284 ± 8.943	28.268 ± 13.234	<i>p</i> < 0.001
MDA (nM/ml)	2.8538 ± 0.4108	16.742 ± 1.4206	<i>p</i> < 0.015

^a All values are mean ± SD of three separated replicates

in large amount to khat trees by farmers or ROS produced. This elevation of GST in Khat chewer did not reported yet in human. However, the experimental animals were orally treated with Khat extract showed GST reduction [16]. Therefore, the elevation of serum GST activity suggests that cellular GST protein did not altered by ROS, which in turn leakage to bloodstream.

Glutathione (GSH) is an essential tripeptide found in mammalian cells. It maintains the intracellular thiol redox status and detoxifies exogenous and endogenous reactive molecules [3]. In addition to detoxification, GSH is important in storing and transporting of amino acids. The characteristic feature of the tripeptide GSH (γ -glutamylcysteinylglycine) is the presence of reactive sulphhydryl (–SH) group, which is oxidized to form GSSG, and then reduced to GSH by the NADPH-dependent glutathione reductase. In addition, glutathione peroxidase catalyzes the GSH-dependent reduction of H₂O₂ and other peroxides [37].

GSH in Khat chewer subjects is lesser than the control. The reduction of GSH in Khat chewers confirms the role of GSH in the detoxification of ROS, which produced as result of the Khat alkaloids continent effects or the effects of the pesticides added to Khat by farmers.

Our result confirms the previously reported studies, which exhibits the depletion of GSH in long-term Khat chewers or experimental animals treated with Khat extract [17, 30]. This enhancing the idea that GSH is strong cellular antioxidants, which effectively scavenges reactive oxygen species (e.g., hydroxyl radical, lipid peroxy radical and H₂O₂) and other free radicals directly and indirectly through enzymatic reactions [38]. Depletion of GSH can also activate apoptosis in the absence of such stimuli [3].

Level of serum ascorbate (vitamin C) was significantly decreased in Khat chewers against control (*p* < 0.001). Vitamin C has generalized antioxidant property for the more common ROS. Thus, the demand for vitamin C will be high unlike limited or specific antioxidants. This probably confirms the suggestion that vitamin C is the first line of defense in the antioxidant defense System [39].

The significantly reduced ascorbate level in Khat chewers compared to non-Khat chewers, most probably reflects the increasing demand for this antioxidant to counter the excessive free radical drain. ROS reversibly or irreversibly damage compounds of all macromolecules. When ROS are

not removed by natural scavengers, damage occurs through peroxidation of structurally important polyunsaturated fatty acids within membrane phospholipids. Lipid peroxidation has been used as an indirect marker for oxidant-induced cell injury [40].

Khat chewers showed higher level of malondialdehyde (MDA) than non-chewers; this significant elevation of MDA, the final products of lipid peroxidation, reflected the great status of oxidative stress (Table 1). This result confirms the similar elevation of MDA induced by Khat [16, 17, 30, 41]. Elevation level of MDA reflects the lipid peroxidation that occurred by radicals, from a polyunsaturated lipid abundant in the cell membranes, and this amplifies the damage of cell membrane.

5 Conclusion

It could be concluded from the present study that the significant decrease in both enzymatic and non-enzymatic antioxidants status in combination with increased presence of MDA in people daily chewing Khat for long period suggests that the reactive oxygen species(ROS) generates leading to damage of cellular proteins and peroxidation of cellular membrane lipids which causing damage of membrane permeability and leakage of cellular components to blood. These conditions increase the risk of oxidative toxicity. Antioxidants have protective effects against this toxicity. People who daily chew Khat for long term will be susceptible to the risk of oxidative stress; therefore, they are recommended to quit chewing Khat for better health benefits.

References

1. Chance, B.; Sies, H.; Boveris, A.: Hydroperoxide metabolism in mammalian organs. *Physiol. Rev.* **59**(3), 527–605 (1979)
2. Young, I.S.; Woodside, J.V.: Antioxidants in health and disease. *J. Clin. Pathol.* **54**, 176–186 (2001)
3. Valko, M.; Leibfritz, D.; Moncol, J.; Cronin, M.; Mazur, T.; Telser, M.: Free radicals and antioxidants in normal physiological functions and human disease. *Int. J. Biochem. Cell Biol.* **39**, 44–84 (2007)
4. Yu, B.P.: Cellular defenses from damage from reactive oxygen species. *Physiol. Rev.* **74**, 139–162 (1994)
5. Reiter, R.J.: Oxidative processes and antioxidative defense mechanism. *FASEB J.* **9**, 526–533 (1995)



6. Abdollahi, M.; Ranjbar, A.; Shadnia, S.; Nikfar, S.; Rezaie, A.: Pesticides and oxidative stress: a review. *Med. Sci. Monit.* **10**, RA141–RA147 (2004)
7. Dhaifalah, I.; Santavy, J.: Khat habit and health effect. A natural amphetamine. *Biomed. Pap. Med. Fac. Univ. Palacky Olomouc Czech Repub.* **148**, 11–15 (2004)
8. Wagner, G.C.; Preston, K.; Ricaurte, G.A.; Schuster, C.R.; Seiden, L.S.: Neurochemical similarities between d, l-cathinone and d-amphetamine. *Drug Alcohol Depend.* **9**, 279–284 (1982)
9. Halbach, H.: Medical aspects of the chewing of Khat leaves. *Bull. World Health Organ.* **47**, 21–29 (1972)
10. Kalix, P.: Review; the pharmacology of Khat. *Gen. Pharmacol.* **15**, 179–187 (1984)
11. Colzato, L.S.; Ruiz, M.; van den Wildenberg, W.; Hommel, B.: Khat use is associated with impaired working memory and cognitive flexibility. *PLoS One* (2011). doi:10.1371/journal.pone.0020602
12. El Hadrani, A.M.; Al Hoot, M.A.: An Association between Khat and Diabetes. *Egypt. J. Surg.* **19**(1), 16–19 (2000)
13. Ranjbar, A.; Pasalar, P.; Abdollahi, M.: Induction of oxidative stress and acetylcholinesterase inhibition in organophosphate pesticide manufacturing workers. *Hum. Exp. Toxicol.* **21**, 179–182 (2002)
14. WHO: Regional Office for the Eastern Mediterranean Country Cooperation. Country Cooperation Strategy for WHO and the Republic of Yemen (2007)
15. Kalix, P.; Geissshusler, S.; Brenneisen, R.; Koelbing, U.; Fisch, H.U.: Cathinone, a phenylpropylamine alkaloid from Khat leaves that has amphetamine effects in humans. *NIDA Res. Monogr.* **105**, 289–290 (1990)
16. Al-Qirim, T.M.; Shahwan, M.; Zaidi, K.R.; Uddin, Q.; Banu, N.: Effect of Khat, its constituents and restraint stress on free radical metabolism of rats. *J. Ethnopharmacol.* **83**(3), 245–250 (2002)
17. Lukandu, O.M.; Costea, D.E.; Neppelberg, E.; Johannessen, A.C.; Vintermyr, O.K.: Khat (*Catha edulis*) induces reactive oxygen species and apoptosis in normal human oral keratinocytes and fibroblasts. *Toxicol. Sci.* **103**(2), 311–324 (2008)
18. Al-Akwa, A.A.; Shaher, M.; Al-Akwa, S.; Aleryani, S.L.: Free radicals are present in human serum of *Catha edulis* Forsk (Khat) abusers. *J. Ethnopharmacol.* **125**(3), 471–473 (2009)
19. Aleryani, S.L.; Aleryani, R.A.; Al-Akwa, A.A.: Khat a drug of abuse: roles of free radicals and antioxidants. *Drug Test Anal.* **3**(9), 548–551 (2011)
20. Winterbourn, C.C.; Hawkins, R.E.; Brian, M.; Carrell, R.W.: The estimation of red cell superoxide dismutase activity. *J. Lab. Clin. Med.* **85**(2), 337–341 (1975)
21. Aebi, H.: Catalase in vitro. *Methods Enzymol.* **105**, 121–126 (1984)
22. Habig, W.H.; Pabst, M.J.; Jakoby, W.B.: Glutathione-S-transferases: the first enzymatic step in mercapturic acid formation. *J. Biol. Chem.* **249**, 7130–7139 (1974)
23. Lowry, O.H.; Rosenbrough, N.J.; Far, A.L.; Randel, R.J.: Protein measurement with Folin-phenol reagent. *J. Biol. Chem.* **193**, 265–275 (1951)
24. Ellman, G.L.: Tissue sulfhydryl groups. *Arch. Biochem. Biophys.* **82**, 70–77 (1959)
25. Ohkawa, H.; Ohishi, N.; Yagi, K.: Assay for lipid peroxides in animal tissues by TBA reaction. *Anal. Biochem.* **95**, 351–358 (1979)
26. Omaye, S.T.; Turnbull, J.D.; Sauberlich, H.E.: Selected methods for the determination of ascorbic acid in animal cells, tissues, and fluids. *Methods Enzymol.* **62**, 3–11 (1979)
27. Buege, J.A.; Aust, S.D.: The thiobarbituric acid assay. *Methods Enzymol.* **52**, 306–307 (1978)
28. Wayner, D.D.M.; Burton, G.W.; Ingold, K.U.; Barclay, L.R.C.; Locke, S.J.: The relative contributions of vitamin E, urate, ascorbate and proteins to the total peroxy radical-trapping antioxidant activity of human blood plasma. *Biochim. Biophys. Acta* **924**, 408–419 (1987)
29. Woodford, F.P.; Whitehead, T.P.: Is measuring serum antioxidant capacity clinically useful?. *Ann. Clin. Biochem.* **35**, 48–56 (1998)
30. Al-Hashem, F.H.; Bin-Jalial, I.; Dallak, M.A.; Nwoye, L.O.; Al-Khateeb, M.; Sakr, H.F.; Eid, R.A.; Al-Gelban, K.S.; Al-Amri, H.S.; Adly, M.A.: Khat (*Catha edulis*) extract increases oxidative stress parameters and impairs renal and hepatic functions in rats. *Bahrain Med. Bull.* **33**(1), 1–9 (2011)
31. Alhifi, M.: Oxidative stress in chick embryo brain, liver and heart treated with mixture of dimethoate and methidathion. *Sci. Technol. Vis.* **6**, 32 (2010)
32. Pejić, S.; Todorović, A.; Stojiljković, V.; Cvetković, D.; Lucić, N.; Radojčić, R.M.; Sačić, Z.S.; Pajović, S.B.: Superoxide dismutase and lipid hydroperoxides in blood and endometrial tissue of patients with benign, hyperplastic and malignant endometrium. *Anais da Academia Brasileira de Ciências* **80**, 515–522 (2008)
33. MacMillan-Crow, L.A.; Thompson, J.A.: Tyrosine modifications and inactivation of active site manganese superoxide dismutase mutant (Y34F) by peroxynitrite. *Arch. Biochem. Biophys.* **366**, 82–88 (1999)
34. Alvarez, B.; Demicheli, V.; Duran, R.; Trujillo, M.; Cervenansky, C.; Freeman, B.A.; Radi, R.: Inactivation of human Cu, Zn superoxide dismutase by peroxynitrite and formation of histidinyl radical. *Free Radic. Biol. Med.* **37**, 813–822 (2004)
35. Lardinois, O.M.: Reactions of bovine liver catalase with superoxide radicals and hydrogen peroxide. *Free Radic. Res.* **22**, 251–274 (1995)
36. Rajmakers, M.T.M.; Steegers, E.A.P.; Peters, W.H.M.: Glutathione-S-transferases and thiol concentrations in embryonic and early fetal tissues. *Hum. Reprod.* **16**, 2445–2450 (2001)
37. Lei, X.G.: In vivo antioxidant role of glutathione peroxidase: evidence from knockout mice. *Methods Enzymol.* **347**, 213–225 (2002)
38. Fang, Y.; Yang, S.; Wu, G.: Free radicals, antioxidants and nutrition. *Nutrition* **18**, 872–879 (2002)
39. Frei, B.; England, C.; Ames, B.N.: Ascorbate is an outstanding antioxidant in human blood plasma. *Proc. Natl. Acad. Sci. USA* **86**, 6377–6381 (1989)
40. Baynes, J.W.: Reactive oxygen in the aetiology and complications of diabetes. In: Ioannides, C., Flatt, P.R. (eds.) *Drug, Diet and Disease: Mechanistic Approach to Diabetes*, vol. 2, pp. 203–231. Ellis Horwood Limited, Hertfordshire (1995)
41. Al-Zubairi, A.; Al-Habori, M.; Al-Geiry, A.: Effect of *Catha edulis* (Khat) chewing on plasma lipid peroxidation. *J. Ethnopharmacol.* **87**(1), 3–9 (2003)