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Research Paper

Cathinone, an active principle of *Catha edulis*, accelerates oxidative stress in the limbic area of swiss albino mice

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ABSTRACT

Ethnopharmacological relevance: Cathinone hydrochloride is an active principle of the khat plant (*Catha edulis*) that produces pleasurable and stimulating effects in khat chewers. To the best of our knowledge no data of cathinone on oxidative stress in limbic areas of mice is available. This is the first study of cathinone on oxidative stress in limbic areas of the brain in Swiss albino male mice.

Materials and methods: The animals were divided into four groups. Group-I was the control group and received vehicle, while groups-II to IV received (−)-cathinone hydrochloride (0.125, 0.25 and 0.5 mg/kg body wt., i.p.) once daily for 15 days.

Results: The level of lipid peroxidation (LPO) was elevated dose-dependently and was significant ($p<0.05$, $p<0.01$) with doses of 0.25 and 0.5 mg/kg body wt. of cathinone as compared to control group. In contrast, the content of reduced glutathione (GSH) was decreased significantly ($p<0.01$, $p<0.001$) with doses of 0.25 and 0.5 mg/kg body wt. of cathinone as compared to control group. The activity of antioxidant enzymes (GPx, GR, GST, CAT, and SOD) was also decreased dose-dependently: the decreased activity of GPx, GR, catalase and SOD was significant with doses of 0.25 and 0.5 mg of cathinone as compared to control group, while the activity of GST was decreased dose-dependently and was significant with 0.5 mg of cathinone as compared to control group.

Conclusions: The results indicate that the cathinone generated oxidative stress hampered antioxidant enzymes, glutathione and lipid peroxidation.

1. Introduction

Khat (*Catha edulis*) is an evergreen shrub that grows at high altitudes in East Africa and the Arabian Peninsula. People chew fresh young khat leaves for its stimulant and pleasurable effects, which are attributed mainly to cathinone (Al-Motarreb et al., 2002; Belewe et al., 2000). Khat use has increased steadily over the last 50 years and has become a significant problem with both social and medical amifications. Its use as a stimulant is gradually spreading from source countries to other parts of the world, especially within immigrant communities (Al-Motarreb et al., 2002). Because of its general social acceptability and euphoric effects among khat usage, khat chewing often plays a dominant role in celebrations, meetings, marriages, and other gatherings. It has remained most deeply rooted in source countries because only fresh leaves have the potency to produce the desired effects. According to one survey, there are 5–10 million regular khat users worldwide (Kalix, 1984). While it can be abused, khat has regional medicinal uses for the treatment of depression, fatigue, hunger, obesity, and gastric ulcers.

In Saudi Arabia, the cultivation and consumption of khat is forbidden and the ban is strictly enforced. However, it is nonetheless by residents in the districts nearest to Yemen.

The active stimulant ingredients of fresh khat leaves are cathinone and cathin (Fig. 1). When khat leaves dry, cathinone, a more potent schedule I drug, converts to cathin, the less potent schedule IV substance. Cathinone is supposed to be a natural amphetamine and releases serotonin in the central nervous system, where it exerts its effects via two main neurochemical pathways: dopamine and noradrenalin. It has also been postulated that, like amphetamine, cathinone causes the release of serotonin in the central nervous system. Both cathinone and amphetamine induce the release of dopamine from the central nervous system's dopamine terminals; thus increasing the activity of the dopaminergic

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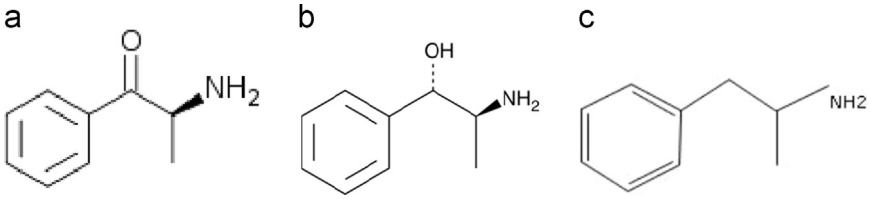

Fig. 1. Structure of cathinone (a), cathine (b) and amphetamine (c).

pathways (Kalix and Braenden, 1985). Cathinone also has a releasing effect on noradrenalin storage sites, which facilitates the transmission of noradrenalin.

Khat use has been reported to affect the cardiovascular, digestive, respiratory, endocrine, hepato-biliary and genito-urinary systems (Kalix, 1984). In regular khat chewers, it damages the kidneys by increasing urea and creatinine level (Kalix and Khan, 1984) and has reduced total serum protein levels. Recent studies have also demonstrated the toxic effects of short and long-term use of khat leaves on the liver function of rabbits.

Oxidative stress plays an important role in damaging the neurons in the brain. Indirect evidence via monitoring biomarkers such as reactive oxygen species, and reactive nitrogen species production, as well as antioxidant defenses indicates that oxidative damage may be involved in the pathogenesis of the brain disease, viz. Parkinson's disease, Alzheimer's disease, Huntington's disease, stroke, etc. (Patel and Chu, 2011). These diseases are also related to the cumulative oxidative stress that disrupts mitochondrial respiration and causes mitochondrial damage.

The limbic system is a complex set of brain structures that lie on both sides of thalamus under the cerebrum, consisting of the hypothalamus, thalamus, amygdala, hippocampus, fornix, column of fornix, mamillary body, septum pellucidum, habenular commissure, cingulate gyrus, parahippocampal gyrus, uncus, limbic cortex and limbic midbrain areas.

To the best of our knowledge, no scientific data is available on the effects of cathinone on oxidative stress in the limbic areas of mice, or on the effects of the lowest doses of cathinone. This study is the first to address low-doses effects of cathinone, as well as the first to report on oxidative stress in limbic areas of male Swiss albino mice.

2. Materials and methods

2.1. Chemicals and reagents

Glutathione reduced (GSH), glutathione oxidized (GSSG), glutathione reductase (GR), nicotinamide adenine dinucleotide phosphate reduced form (NADPH), 5,5′-dithiobis-2-nitrobenzoic acid (DTNB), 1-chloro-2,4-dinitrobenzene (CDNB), thiobarbituric acid (TBA), trichloroacetic acid (TCA), ethylene diamine tetraacetic acid (EDTA), (−)-epinephrine, sulfosalisylic acid, sodium azide and hydrogen peroxide used in this experiment were purchased from Sigma-Aldrich, Co., USA. Cathinone hydrochloride was a generous gift from the Poison Control & Medical Forensic Chemistry Center, Ministry of Health, Jazan, Kingdom of Saudi Arabia.

2.2. Experimental animals and design

Male Swiss albino mice (25–35 g) obtained from the Animal House of the College of Pharmacy were used in this study. The mice were divided into four groups of six animals per group. Group 1 was the control group, and vehicle was given via intraperitoneal (i.p.). Groups II–IV were the experimental groups, and cathinone hydrochloride (0.125, 0.25 and 0.5 mg/kg body wt., i.p., in normal saline) was given once daily for a period of 15 days. We declare that the present study was performed according to international, national and institutional guidelines governing animal experiments studies and biodiversity rights.

2.3. Dissection of the limbic areas

The brain portion between the posterior optic chiasma and anterior brain stem was used as a limbic area. Coronal sections of the brain were cut from the posterior of the optic chiasma, where the fornix starts, to the anterior of the brain stem, where the hippocampus ends.

2.4. Extraction of brain tissue

On the 15th day after the cathinone doses, the animals were sacrificed by decapitation and their brains were taken out quickly to dissect the limbic area. Each limbic area was weighed and homogenized at 4 °C in 10 mM Tris–HCl (pH 7.4) with 10 μl/ml protease inhibitor to get 5% (w/v) homogenate. The homogenate was centrifuged at 800*g* for 5 min at 4 °C to separate the nuclear debris. The supernatant-1 (S-1) was used for estimation of lipid peroxidation and the remaining S-1 was further centrifuged at 10,500*g* for 30 min at 4 °C to obtain post-mitochondrial supernatant (PMS), which was used for the estimation of reduced glutathione and antioxidant enzymes.

2.5. Estimation of protein

Protein was estimated by the method of Lowry et al. (1951) using bovine serum albumin (BSA) as standard.

2.6. Estimation of lipid peroxidation (LPO)

Lipid peroxidation was estimated according to the procedure of Utley et al. (1967) as modified by Islam et al. (2002). In brief, 0.25 ml of S-1 was pipetted in $13 \times 100\ mm^2$ test tubes and incubated at 37 ± 1 °C in a metabolic shaker (120 cycles/min) for 60 min. Another 0.25 ml of the same S-1was pipetted in a centrifuge tube and placed at 0 °C. After 1 h of incubation, 0.25 ml of 5% chilled TCA followed by 0.5 ml of 0.67% TBA was added to each test tube and centrifuge tube and mixed after each addition. The aliquot from each test tube was transferred to a centrifuge tube and centrifuged at 3000*g* for 15 min. Thereafter, supernatants were transferred to other test tubes and placed in a boiling water bath for 10 min. Thereafter, the test tubes were cooled and the absorbance of the color was read at 535 nm. The TBARS content was calculated by using molar extinction coefficient of $1.56 \times 10^5\ M^{-1}\ cm^{-1}$ and expressed as nmoles TBARS formed/h/mg protein.

2.7. Estimation of reduced glutathione (GSH)

GSH content was measured by the method of Jollow et al. (1974) with slight modification. PMS was mixed with 4.0% sulfosalisylic acid (w/v) in 1:1 ratio (v/v). The samples were incubated

at 4 °C for 1 h, then centrifuged at 4000g for 10 min at 4 °C. The assay mixture contained 0.1 ml of supernatant, 1.0 mM DTNB and 0.1 M phosphate buffer pH 7.4 in a total volume of 2.0 ml. The yellow color developed was read immediately at 412 nm (Shimadzu-1601, Japan). The GSH content was calculated as nmoles GSH mg^{-1} protein using a molar extinction coefficient of $13.6 \times 10^3\ M^{-1}\ cm^{-1}$.

2.8. Estimation of glutathione peroxidase (GPx)

GPx activity was determined by the method of Mohandas et al. (1984). The reaction assay consisted of phosphate buffer (0.05 M, pH 7.0), EDTA (1 mM), sodium azide (1 mM), glutathione reductase (1 EU/ml), glutathione (1 mM), NADPH (0.2 mM), hydrogen peroxide (0.25 mM) and 0.1 ml of PMS in the final volume of 2 ml. The disappearance of NADPH at 340 nm was recorded at room temperature. The enzyme activity was calculated as nmol NADPH oxidized/min/mg/protein using a molar extinction coefficient of $6.22 \times 10^3\ M^{-1}\ cm^{-1}$.

2.9. Estimation of glutathione reductase (GR)

Glutathione reductase activity was measured by the method of Carlberg and Mannerviek (1975) as modified by Mohandas et al. (1984). The reaction mixture consisted of phosphate buffer (0.1 M, pH 7.6), NADPH (0.1 mM), EDTA (0.5 mM), oxidized glutathione (1 mM) and 0.1 ml of PMS in total volume of 2 ml. The enzyme activity was quantified at room temperature by measuring the disappearance of NADPH at 340 nm and calculated as nmol NADPH oxidized/min/mg protein using a molar extinction coefficient of $6.22 \times 10^3\ M^{-1}\ cm^{-1}$.

2.10. Estimation of glutathione-S-transferase (GST)

Glutathione-S-transferase (GST) activity was measured by the method of Habig et al. (1974). The reaction mixture consisted of phosphate buffer (0.1 M, pH 6.5), reduced glutathione (1 mM), 1-chloro-2,4-dinitrobenzene (CDNB, 1.0 mM) and 0.1 ml of PMS in a total volume of 2.0 ml. The change in absorbance was recorded at 340 nm and enzyme activity was calculated as nmoles CDNB conjugate formed/min/mg protein using a molar extinction coefficient of $9.6 \times 10^3\ M^{-1}\ cm^{-1}$.

2.11. Estimation of catalase (CAT)

Catalase activity was assayed by the method of Claiborne (1985). Briefly, the assay mixture consisted of phosphate buffer (0.05 M, pH 7.0), hydrogen peroxide (0.019 M) and 0.1 ml PMS in a total volume of 2.0 ml. The changes in absorbance were recorded at 240 nm. Catalase activity was calculated in terms of nmol of H_2O_2 consumed/min/mg/protein using a molar extinction coefficient of $43.6 \times 10^3\ M^{-1}\ cm^{-1}$.

2.12. Estimation of superoxide dismutase (SOD)

Superoxide dismutase activity was measured as described by Stevens et al. (2000) by monitoring the auto-oxidation of (−)-epinephrine at pH 10.4 for 3 min at 480 nm. The reaction mixture contained glycine buffer (50 mM, pH, 10.4) and 0.2 ml of PMS. The reaction was initiated by the addition of (−)-epinephrine. The enzyme activity was calculated in terms of nmol (−)-epinephrine protected from oxidation/min/mg protein using a molar extinction coefficient of $4.02 \times 10^3\ M^{-1}\ cm^{-1}$.

2.13. Statistical analysis

Results are expressed as the mean ± SEM of six animals. Differences between the means of experimental and control groups were analyzed statistically using one-way analysis of variance (ANOVA), followed by Turkey's test for all parameters. A p value < 0.05 was considered statistically significant.

3. Results

3.1. Effect of cathinone on lipid peroxidation (LPO) and GSH

The content of TBARS increased dose-dependently and was significant ($p < 0.05$, $p < 0.01$) with doses of 0.25 and 0.5 mg/kg of cathinone as compared to control group (Fig. 2). GSH plays a crucial role in the antioxidant system. Conversely, the content of GSH decreased dose-dependently and its depletion was significant ($p < 0.01$, $p < 0.001$) with doses of 0.25 and 0.5 mg/kg of cathinone as compared to control group (Fig. 3).

3.2. Effect of cathinone on antioxidant enzymes

The activity of antioxidant enzymes (GPx, GR, SOD, CAT) decreased dose-dependently (Table 1) and the depletion was significant with doses of 0.25 and 0.5 mg of cathinone as compared to control group. The activity of GST also decreased dose-dependently and its depletion was significant ($p < 0.01$) with the

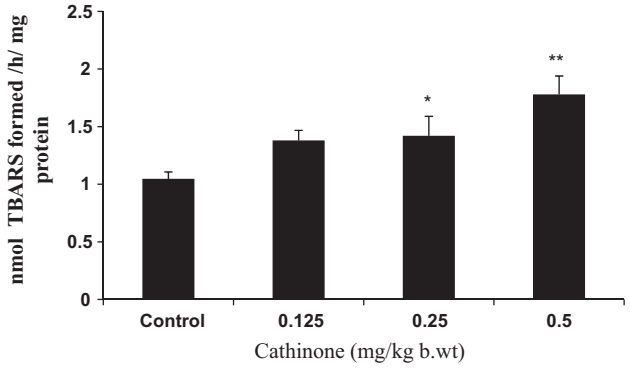

Fig. 2. Effect of cathinone on the content of LPO in limbic areas of mice. The level of TBARS increases dose-dependently as compared to control, and the increment is significant with 0.25 and 0.5 mg/kg doses of cathinone. The data are expressed as mean ± SEM of 6 animals. $^*p < 0.05$, $^{**}p < 0.01$ cathinone groups (0.25 and 0.5 mg/kg) vs. control group.

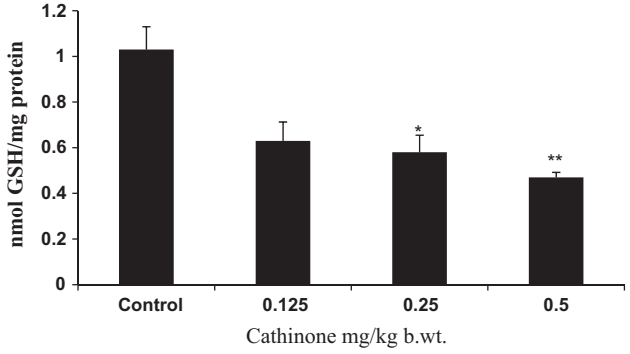

Fig. 3. Effect of cathinone on the content of GSH in limbic areas of mice. The level of GSH decreases dose dependently as compared to control, and the depletion is significant with 0.25 and 0.5 mg/kg doses of cathinone. The data are expressed as mean ± SEM of 6 animals. $^*p < 0.01$, $^{**}p < 0.001$ cathinone groups (0.25 and 0.5 mg/kg) vs. control group.

Table 1
Effects of cathinone on the activity of antioxidant enzymes in limbic areas of Swiss albino mice.

Enzymes	Control	0.125 mg/kg b.wt. of cathinone	0.25 mg/kg b.wt. of cathinone	0.50 mg/kg b.wt. of cathinone
GPx (nmol NADPH oxidized/min/mg/protein)	231.95 ± 14.34	214.75 ± 11.69 (−7.41%)	169.68 ± 10.01** (−26.84%)	138.19 ± 12.16*** (−40.42%)
GR (nmol NADPH oxidized/min/mg protein)	52.77 ± 5.08	51.56 ± 5.29 (−2.29%)	37.36 ± 1.80* (−21.43%)	35.88 ± 1.27* (−29.35%)
GST (nmol CDNB conjugate formed/min/mg protein)	429.25 ± 17.06	402.68 ± 21.84 (−6.18%)	372.71 ± 23.24 (−13.17%)	317.85 ± 15.21** (−25.95%)
Catalase (nmol H_2O_2 consumed/min/mg protein)	11.09 ± 0.97	9.81 ± 1.68 (−11.48%)	5.80 ± 0.67* (−47.40%)	5.15 ± 0.42** (−53.56%)
SOD (nmol epinephrine protected from oxidation/min/mg protein)	446.18 ± 30.5	362.18 ± 27.59 (−18.82%)	313.71 ± 7.86** (−29.69%)	296.46 ± 8.95*** (−50.50%)

Values are expressed as Mean ± S.E.M. of 6 animals. Values in parentheses show the percentage decrease with respect to the control.

* $p < 0.05$ cathinone groups vs. control group.
** $p < 0.01$ cathinone groups vs. control group.
*** $p < 0.001$ cathinone groups vs. control group.

dose of 0.5 mg/kg of cathinone in limbic areas as compared to the control group.

4. Discussion

The brain is very susceptible to damage caused by oxidative stress, due to its rapid oxidative metabolic activity, high poly-unsaturated fatty acids, relatively low antioxidant capacity, and inadequate neuronal cell repair activity (Halliwell, 2001; Cassarino and Bennett, 1999; Ishrat et al., 2009). Oxidative stress reflects an imbalance between the systemic manifestation of reactive oxygen species and the biological system's ability to readily detoxify the reactive intermediates or to repair the resulting damage. Lipid peroxidation has been used as an indirect marker of oxidant-induced cell injury (Al-Zubairi et al., 2003). The effect of cathinone on lipid peroxidation measured as TBARS causes demonstrated oxidative damage to membranes in the brain tissue of Swiss albino mice. A dose-dependent increment on the level of TBARS was observed in the limbic areas of mice when treated with cathinone and was significant with doses of 0.25 and 0.5 mg/kg body wt. (Fig. 2). There is no report on the effects of cathinone on brain lipid peroxidation, although an indirect study has shown significant changes in the levels of lipid peroxidation in hepatic and renal tissues of rats treated orally with khat extract (100 mg/rat) for 15 weeks (Al-Hashem et al., 2011). In contrast, Al-Zubairi et al. (2003) have reported insignificantly elevated levels of lipid peroxidation in the fasting plasma of groups of Yemeni males aged 20–45 years chewing 4% and 9.2% of khat for more than 2 years.

Glutathione (GSH) is an essential tripeptide found in mammalian cells, where it maintains the intracellular thiol redox status. It is a primary source of indigenous antioxidants that attenuates oxidative damage directly by scavenging reactive oxygen species (ROS) and indirectly through GPx and GST, which utilize GSH as substrate (Dringen et al., 2000; Adibhatla et al., 2003; Ahmad et al., 2012). It directly quenches reactive hydroxyl radicals and other oxygen-centered free radicals, and conjugates xenobiotics to water soluble products (Kidd, 1997). It also plays a central role in coordinating the body's antioxidant defense processes: the exposed sulfhydryl groups in glutathione bind to a variety of electrophilic radicals and metabolites that may cause cell damage (Chawla, 1999). Low levels of GSH can be due to enhanced generation of ROS, that are scavenged by GSH, or decreased activity of GR, which converts oxidized glutathione (GSSG) to its reduced form, which has been observed in this study. The relationship between the reduced and oxidized states of glutathione, the GSH/GSSG ratio or glutathione redox status, is considered an index of cellular redox status and a biomarker of oxidative damage, because glutathione maintains the thiol–disulphide status of proteins by acting as a redox buffer. Both, GSH and GSSG occur simultaneously in the cells. Protein glutathionylation maintains GSH inside the cells, while GSH oxidized to GSSG is rapidly exported (Ghezzi et al., 2005). GSSG can only be derived from GSH, while GSH can be biosynthesized (Go and Jones, 2013). Thus, a depleted concentration of GSH can alter the ratio of GSH:GSSG. Further, the main cause of GSH loss during oxidative stress in the brain is the formation of protein-glutathione mixed disulfides (Pr-SSG) and the loss of thiol proteins (Ravindranath and Reed, 1990). The decreased concentration of GSH also causes cell damage, which has been observed with the treatment of the various doses of cathinone in the limbic areas of mice (Fig. 3). Al-Meshal et al. (1991) have also reported a decreased content of GSH by *Catha edulis* extract leading to increased production of ROS and induction of oxidative stress in mice. A decreased content of GSH in hepatic and renal tissues in rats treated orally with khat extract (100 mg/rat) for 15 weeks has also been reported (Al-Hashem et al., 2011). Recently Masoud et al. (2012) have reported a decreased level of GSH in the plasma of female adult humans 12 h after taking khat. *In vitro* study of primary normal human oral keratinocytes and fibroblasts has shown an increased ROS in cytosol and depleted GSH within 1 h of khat exposure (Lukandu et al., 2008).

Oxidative stress is defined as a cytological consequence caused by imbalance between the production of free radicals and the ability to scavenge them. Oxidative stress also exhaust the various antioxidants enzymes such as GPx GR and CAT (Tabassum et al., 2013; Vaibhav et al., 2013; Ashafaq et al., 2012; Raza et al., 2011). GPx contributes a vital role in removing the free radicals and hydro peroxides. GPx mediates the breakdown of hydro peroxides by consuming reduced glutathione as substrate and as a result of this, reduced glutathione is converted into oxidized glutathione (Imam and Ali, 2000). Regeneration of reduced glutathione is also attributed to GR (Tabassum et al., 2013). The activity of these enzymes depleted dose dependently and was significant with the dose of 0.5 mg/kg. The study indicates that the chewing of the khat having such low quantity of cathinone may generate less free radicals than higher doses.

The SOD is another important enzyme in the brain which provides first line of defense. SOD with the help of GPx and catalase acts as a free radical scavenger which prevents tissue damage due to peroxide formation. Catalase is found in the brain at low level but crucially mediates the detoxification of hydrogen peroxide to water and oxygen, similar to glutathione peroxidase (Khan et al., 2009). In this study, the activities of SOD and catalase decreased dose dependently as their depletion was significant with the doses of 0.25 and 0.5 mg/kg body wt., respectively, in mice treated with cathinone, suggesting that the cathinone generated free radicals or directly inhibited the synthesis of antioxidant enzymes. This finding is similar to a recent study in which administration of *Catha edulis* extract or its alkaloid fraction was shown to have altered the activities of the free-radical

metabolizing/scavenging enzyme system. A decreased activity of superoxide dismutase (SOD) and catalase (CAT) was also reported in the liver and kidneys of rats treated orally with khat extract (100 mg/rat) for 15 weeks (Al-Hashem et al., 2011). A decreased activity of catalase was also observed in the plasma of female adult 12 h after taking khat (Masoud et al., 2012).

5. Conclusion

It is evident that very low doses of cathinone (less than 10 times the human dose in terms of per kg) accelerate oxidative stress in the limbic areas of Swiss albino mice.

Acknowledgments

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