Acta Pharm. **68** (2018) 485–495 Original research paper
https://doi.org/10.2478/acph-2018-0038

Catha edulis active principle, cathinone, suppresses motor coordination, accelerates anxiety and alters the levels of dopamine and its metabolites in the limbic areas of male Swiss albino mice

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Accepted June 21, 2018
Published online September 24, 2018

Cathinone, the active principle of khat (*Catha edulis*), stimulates, excites and produces euphoric feelings in khat users. Locomotor and rearing activities, either individual or in groups, of male Swiss albino mice were decreased significantly compared to the control. Motor coordination tests (rotarod, rope climb and grip tests) have shown decreased motor performance in the mice treated with cathinone compared to the control. The elevated plus maze test has shown significant anxiety in the mice compared to the control. Contents of dopamine and its metabolite, homovanillic acid, were increased in the limbic areas compared to the control group. In contrast, contents of 3,4-dihydroxyphenyl acetic acid were depleted significantly and dose dependently compared to the control group in the limbic areas of mice. In conclusion, natural cathinone has depleted motor coordination, accelerated anxiety in mice and altered the contents of dopamine and its metabolites.

Keywords: cathinone, mice, behavioral activities, dopamine, 3,4-dihydroxyphenyl acetic acid, homovanillic acid, limbic area

Khat or qat (*Catha edulis,* Forsk) is an evergreen shrub belonging to the family *Celastraceae*. It is widely used as a stimulant in East Africa and the Arabian Peninsula. Its stimulating effect is due to the presence of its active constituents, cathinone and cathin (1). Chewing of khat is usually preferred in groups at social gatherings. During the chewing session, there is an atmosphere of euphoria, increased alertness, excitement and insomnia. About

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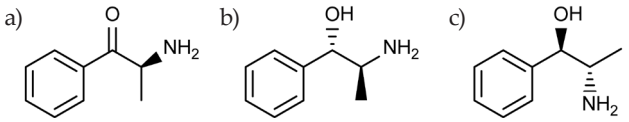

Fig. 1. Chemical structures of: a) cathinone, b) cathin and c) norephedrine.

2 hours after the end of the session, tension, emotional instability and irritability begin to appear, later leading to feelings of low mood and sluggishness (2). Chewers tend to leave the session feeling exhausted. Students chew khat in an attempt to improve mental performance before exams.

The active principle of khat, cathinone, is on the list of narcotics as a Schedule I drug and is found in fresh khat leaves. Cathinone decomposes within 48 h after leaf drying forming cathin and norephedrine, a Schedule IV drug (Fig. 1).

Various studies across the globe have reported that the chewing of khat is harmful to health and has impact on various body organs (cardiovascular, respiratory, endocrine and genitourinary systems) (2). Khat chewing is very popular among youths and its continuous use causes harmful side effects leading to a decrease of academic performance and increased risk of psychiatric disorders such as hopelessness and insomnia.

The various doses of cathinone and its routes of administration are reported. Qureshi *et al.* (3) have given 5, 20 and 40 mg kg^{-1} bm of cathinone orally to male albino mice for a period of 6 weeks. Safhi *et al.* (1, 4) have administered 0.125, 0.25 and 0.5 mg kg^{-1} bm of cathinone *i.p.* to Swiss albino mice for a period of 15 days. The latter dose has been reported to alter lipid peroxidation and glutathione levels and the activities of antioxidant enzymes. An altered level of brain lipids with 0.5 mg kg^{-1} of cathinone has also been reported but this concentration has not altered the content of total lipids (4).

Current research is an extension of our previous work (1, 4) where the effects of cathinone on the lipids (1) and oxidative stress (4) in the limbic areas of Swiss albino mice were investigated. Now, behaviors of Swiss albino mice and contents of dopamine and its metabolites are in the focus of our interest. This study is the first to address the effects of natural cathinone on motor coordination and anxiety in male Swiss albino mice.

EXPERIMENTAL

Chemicals

Potassium phosphate, methanol, sodium chloride, heptane sulfonic acid, dopamine [DA, 4-(2-aminoethyl)benzene-1,2-diol], 3,4-dihydroxy-phenyl acetic acid (DOPAC), homovanillic acid (HVA), perchloric acid and internal standard (IS) (3,4-dihydroxybenzylamine) were purchased from Sigma-Aldrich, Germany. Cathinone (reference standard ≥ 98 % purity, Sigma-Aldrich) was a generous gift from the Poison Control and Medical Forensic Chemistry Centre, Jazan, Kingdom of Saudi Arabia.

Experimental design

Animals (Swiss albino mice, 25–30 g) were obtained from the animal house of Jazan University, Jazan, KSA. Mice were handled every day for a week to acclimatize to the ex-

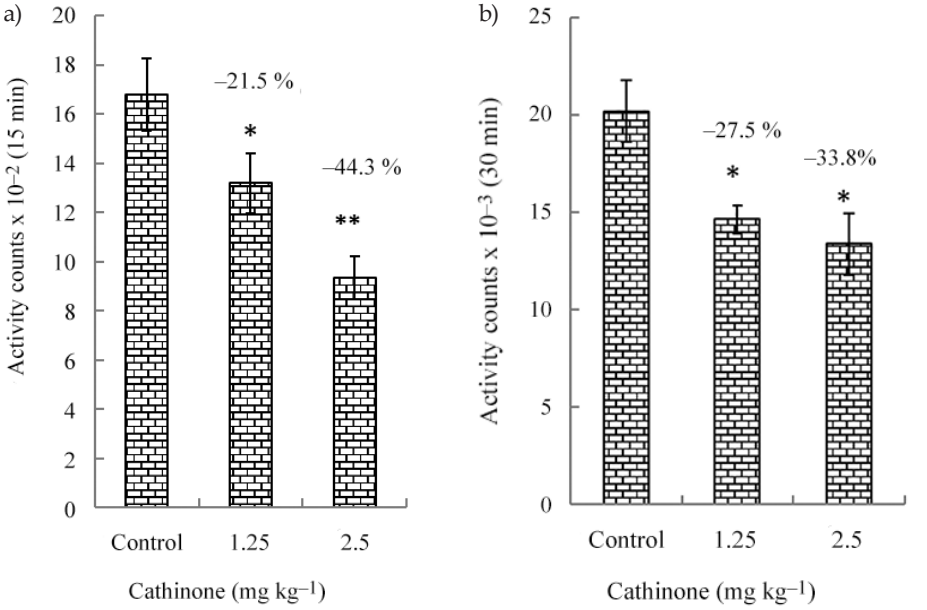

Fig. 2. The effect of cathinone (1.25 and 2.5 mg kg^{-1}) on day 13, one hour post dosing, on mean locomotor activity of: a) each animal for 15 min (n = 8) and b) the same animals in two subgroups (n = 4 × 2) for two alternate sessions of 30 min each. Data are expressed as mean ± SEM. Significantly different *vs*. controls: *p < 0.05, **p < 0.01.

perimental environment. The animals were divided randomly into three groups of eight animals each. Group 1 was the control group to which vehicle (normal saline) was given intraperitoneally (*i.p.*). Groups 2 and 3 were given cathinone 1.25 and 2.5 mg kg^{-1} *i.p.* once daily for a period of 15 days as reported (1, 4). On day 13, 1 h after cathinone dosing, the locomotor activity was observed for 30 min in each group using an actophotometer. Thereafter, 4 animals of each group were placed on a rotarod (with four compartments) for 300 s. After the rotarod, the locomotor activity of individual animals was measured in the actophotometer for 15 min. On day 14, the elevated plus maze, grip strength and hanging rope tests were performed. On day 15, after 1 h of cathinone administration, the animals were sacrificed and their brains were dissected out.

The study was performed according to international, national and institutional guidelines and was approved by the Institutional Ethics Committee of the College of Pharmacy, Jazan University (KSA).

Actophotometer for ambulatory and rearing activities

Spontaneous ambulatory and rearing activities were measured in a multiple activity cage (41 × 41 × 33 cm) made of clear Perspex (UGO, Italy) according to the method of Kulkarni (6). The activity chamber has horizontal (for ambulatory activity) and vertical (for rearing) sensors, which consist of two facing blocks containing an IR array of 16 emitters and respective receivers for the assessment of ambulatory and rearing activity. Height

of horizontal and vertical sensors can be adjusted according to the group/individual animals. The movement of animals interrupts one or more IR beam(s) inside the cage that are directly measured by the actophotometer, which displays the results on the screen. Interruptions are counted as the number of scores (1 interruption = 1 score) for ambulatory and rearing activities. The chamber was wiped with 70 % of alcohol after the trial of each group/individual animal. Ambulatory and rearing activities were measured on day 13, 1 h after cathinone administration (1.25 and 2.5 mg kg^{-1}), for each animal separately, for 15 min (n = 8) (Figs. 2a and 3a). The above activities were also counted for 30 min for the same animals divided into two subgroups for accurate counting, of 4 animals each, and activities were counted for two sessions (alternate) of each subgroup (Figs. 2b and 3b).

Rotarod test

Omni Rotor (Omnitech Electronics, Inc., USA) was used as described by Kelly *et al*. (7) to evaluate motor coordination on day 13. The animals were trained on the rotarod starting from 8 rpm for one day, then 10 rpm for 2 days and finally 15 rpm for 2 days before the start of the experiment. Each animal was given three, or more, trials a day until they were trained. The rotarod unit contained four compartments to allow four mice at a time. The time each mouse remained on the rotating bar was recorded for a maximum period of 300 s per trial. The apparatus automatically records the time when the mouse falls off the rotating shaft. The speed was set at 15 rpm. Data were expressed as mean time (s) on the rotating bar.

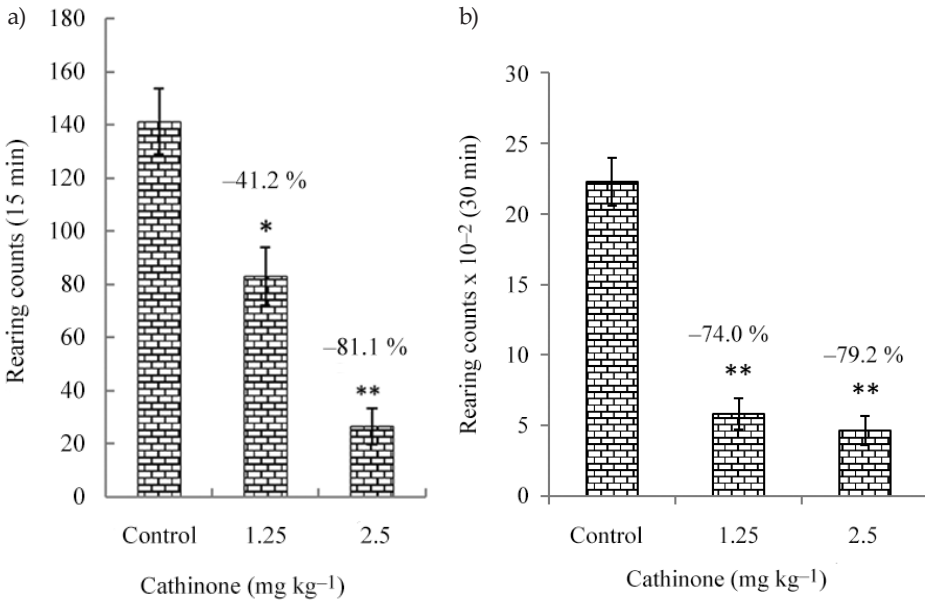

Fig. 3. The effect of cathinone (1.25 and 2.5 mg kg^{-1}) on rearing activity of: a) each animal put separately in actophotometer (n = 8) and b) the same animals in two subgroups (n = 4 × 2) for two alternate sessions of 30 min each. Data are expressed as mean ± SEM. Significantly different *vs.* controls: *p < 0.01, **p < 0.001.

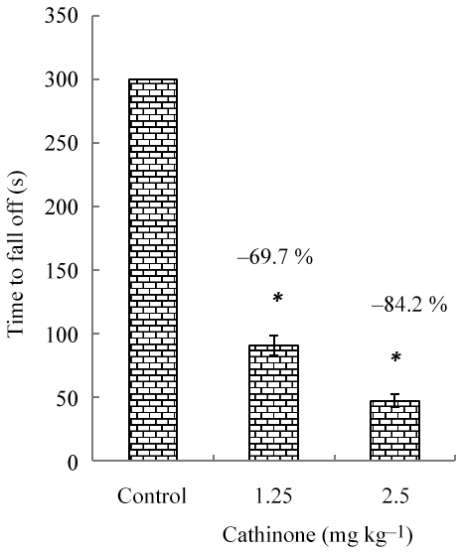

Fig. 4. The effect of cathinone (1.25 and 2.5 mg kg^{-1}) on motor coordination using rotarod. Data are expressed as mean ± SEM, $n = 8$. Significantly different *vs.* controls (none of control animals fell off the rotarod, SEM = 0): *$p < 0.001$.

Elevated plus maze

The elevated plus maze test was performed on day 14, 1 h after administration of natural cathinone. The method of Kulkarni (6) was used to measure the animal's anxiety in the elevated plus maze. The maze has two open arms (16 × 5 cm) and two closed arms (16 × 5 × 12 cm) with an open roof elevated 50 cm above the floor. An individual animal was placed in the center of the maze so that its head faced the open arm. The parameters were noted manually for 3 min. First, the mouse was placed on the open arm and then on the closed arm. The mice entered open and closed arms (arm entry defined as the entry of four paws into the arm); the average time (s) the animal spent in each arm (average time = total duration in the arm/number of entries) was recorded. Thereafter, natural cathinone (1.25 and 2.5 mg kg^{-1}) treated animals were placed one by one on open and closed arms and the entry in each arm was noted. The method of Garg *et al.* (8) was used to compare the number of entries and average time(s) spent in the open and closed arms of each group.

Hanging rope test

The animals were trained on a hanging rope for 2 days before the start of the experiment. The hanging rope test was performed on day 14. After completing the elevated plus maze, motor coordination of each mouse was assessed using the hanging rope test as described by Moran *et al.* (9). The apparatus had a rope of 50 cm length starting from the middle of the pole. The rope was fixed on a platform on the pole of 100 cm height. The rope test was assessed on the basis of time(s) taken to climb to the platform.

Grip strength test

The animals were trained for three days before starting the experiment. The grip strength test was performed on day 14. After completing the hanging rope test, motor coordination of each mouse was assessed using the grip strength test as described by Moran *et al.* (9). The apparatus uses a string of 50 cm length stretched between two vertical supports and is elevated. Grip strength was assessed on the basis of a six score system: 0: falls off; 1: hangs from the string with two forepaws; 2: as for 1 but attempts to climb onto the string; 3: hangs with two forepaws from the string plus one or both hind limbs; 4: hangs from the string with the tail, and 5: escapes.

Dissection of the limbic areas and isolation of neurotransmitters

On the last day of experiment (day 15), one hour after the dosing, the mice were sacrificed and brains were taken out quickly to dissect the limbic area for the estimation of dopamine and its metabolite. Brain portion between the posterior optic chiasma and anterior brain stem was used as limbic area as described earlier (1). The limbic area (20 %, *m*/*V*) was sonicated in perchloric acid (0.4 mol L^{-1}) with internal standard (3,4-dihydroxybenzylamine, 100 ng mL^{-1}) and centrifuged at 15,000x*g* for 10 min at 4 °C. The supernatant was filtered through a 0.22-μm membrane filter (Millipore, USA) and was used as the source for the assay of neurotransmitters.

Estimation of dopamine, DOPAC and HVA

Dopamine and its metabolites were estimated by the method of Zafar *et al.* (10) using Waters HPLC (Waters, USA) with an electrochemical detector. The filtrate was injected manually with a 20-μL loop into the ODS-C18 column. The potential of the electrochemical detector was set at 750 mV. The mobile phase consisted of 0.1 mol L^{-1} potassium phosphate (pH 4.0), 10 % methanol and 1.0 mmol L^{-1} heptane sulfonic acid. Samples were separated on the C18 column using a flow rate of 1.0 mL min^{-1}. Contents of dopamine, DOPAC and HVA were calculated using a standard curve generated by determining the ratio between analytes and internal standard peak areas for three known concentrations of dopamine, DOPAC and HVA and a constant concentration of IS. Millennium 32 software (Waters) was used and results are given as μg g^{-1} of tissue.

Statistical analysis

Results are represented as mean ± SEM. Data was analyzed by One-Way ANOVA followed by Tukey's Kramer test. The p-value < 0.05 was considered significant.

RESULTS AND DISCUSSION

Effects of cathinone

Animals treated with cathinone (1.25 and 2.5 mg kg^{-1}) were tested in comparison with the control group for behavioral parameters and contents of dopamine, HVA and DOPAC.

Locomotor and rearing activities. – Ambulatory activity decreased significantly ($p < 0.05$) and dose dependently after 1 h of dosing the cathinone compared to the control group (Fig.

2). One hour post cathinone dosing, the rearing activity also decreased significantly and dose dependently ($p < 0.01$) (Figs. 3a, b).

A range of behavioral responses such as locomotor activity, stereotyped movement, and loss of appetite has been reported with the administration of khat extract, cathinone or synthetic cathinone (bath salt) to rats and mice (11). There are contradictory reports on the behaviour of animals due to the treatment of *Catha edulis* and cathinone. Al-Mamary *et al*. (12) reported no change in the behaviour of animals after the treatment with *Catha edulis* whereas Marusich *et al*. (13) reported increased locomotor activity following the administration of synthetic cathinone (bath salts). In the present study, decreased locomotor and rearing activity was observed.

Motor coordination, rope climb and grip strength tests. – In rotarod, rope climb and grip tests, the animals treated with cathinone showed significantly decreased motor coordination ($p < 0.001$, $p < 0.01$, $p < 0.05$, resp.) in a dose dependent manner (Figs. 4, 5 and 6). In the rope climb test, the animals took significantly more time, dose dependently, to reach the platform and showed weaker motor coordination compared to the control. Similarly, Connor *et al*. (5) observed decreased spontaneous motor activity in mice after intragastric administration of khat extracts.

Elevated plus maze. – Cathinone-treated mice spent more time staying in the closed arms than open arms compared to the control group, significantly ($p < 0.05$) and dose dependently. We observed that each animal was moving in the closed arms but without crossing the line to enter the open arms, indicating anxiety. On the other hand, the time taken by cathinone-treated groups to stay in the open arms was significantly ($p < 0.001$) shorter than the time taken by the control group; a dose dependent effect was observed (Fig. 7). Thus, our observation has clearly shown that cathinone developed severe anxiety in mice.

During the khat chewing sessions, euphoria, increased alertness, hyperactivity, excitement and a general sense of well-being have been reported in humans (4). The chewing of khat has also caused depression (1). After 1–2 h, the euphoria, alertness, cheerfulness and excitement ends, later leading to feelings of low mood and sluggishness (4). In mice, the alertness, hyperactivity and excitement expired in 30 min. Thereafter, a phase of anxiety started, which caused fears in animals, due to which they were unable to enter into the open arms. The rotarod, grip strength and rope climb tests showed deterioration in motor performance of the mice. For this reason, mice were not capable of locomotion and rearing either in groups or individually.

Effects of cathinone on dopamine (DA) and its metabolites

Animals treated with cathinone for 15 days showed significant ($p < 0.05$) and dose dependent increase of dopamine. In contrast, the content of DOPAC decreased significantly and dose dependently ($p < 0.01$). On the other hand, the level of HVA increased significantly and dose dependently ($p < 0.05$) in the limbic areas of mice (Table I).

In the present study, the level of dopamine was increased with the administration of cathinone. This effect has already been reported (11). On the other hand, Wagner *at al*. (14) reported a significantly decreased level of DA with chronic administration of cathinone to rats. Dopamine is a primary neurotransmitter in the brain and plays an important role in

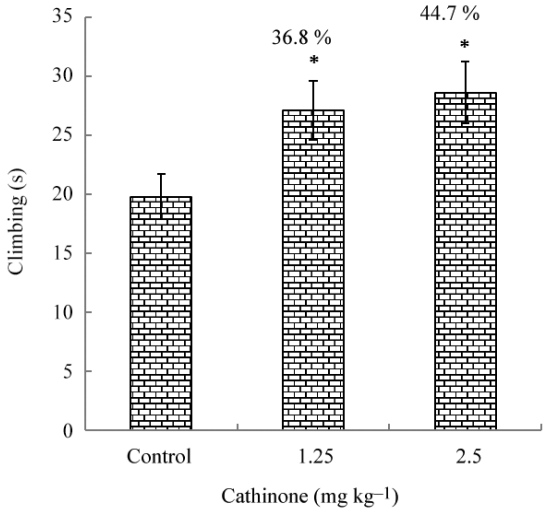

Fig. 5. The effect of cathinone (1.25 and 2.5 mg kg^{-1}) on motor coordination using the hanging rope climb test. Data are expressed as mean ± SEM, $n = 8$. Significantly different *vs.* controls: *$p < 0.001$.

regulating the movement, emotion, cognition and pleasure (15). Symptoms like euphoria, increased alertness, hyperactivity and excitement in khat chewers may be due to the increased DA content (16). Tyrosine hydroxylase (TH) is the rate limiting enzyme in the pathway of DA (17). Increased activity of TH may increase the content of DA, but no report of the effect of cathinone on TH is available in the literature.

We found that the content of DOPAC decreased significantly with cathinone treatment. No such previous report is available. Monoamine oxidase (MAO) acts on DA to metabolite it into DOPAC. Nielsen (16) has reported decreased activity of MAO with cathinone treatment. It could be inferred that, due to decreased MAO activity, the level of

Table I. Effect of different doses of cathinone (1.25 and 2.5 mg kg^{-1} bm) on the contents of dopamine, 3,4-dihydroxyphenyl acetic acid and homovanillic acid in limbic areas of male Swiss albino mice

Groups	Dopamine (μg g^{-1} tissue)	DOPAC (μg g^{-1} tissue)	HVA (μg g^{-1} tissue)
Control	14.26 ± 1.02	2.1 ± 0.16	1.95 ± 1.0
Cathinone (1.25 mg kg^{-1})	18.64 ± 1.48* (30.7 %)	1.26 ± 0.08** (-40.0 %)	2.56 ± 0.16* (31.3 %)
Cathinone (2.5 mg kg^{-1})	21.44 ± 1.95*** (50.4 %)	1.0 ± 06 *** (-52.4 %)	3.04 ± 0.24*** (55.9 %)

DA – dopamine, DOPAC – 3,4-dihydroxyphenyl acetic acid, HVA – homovanillic acid

Values are expressed as mean ± SEM ($n = 8$ mice/group). Significant difference *vs.* control group: *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. Values in parentheses are percentage changes compared to the control.

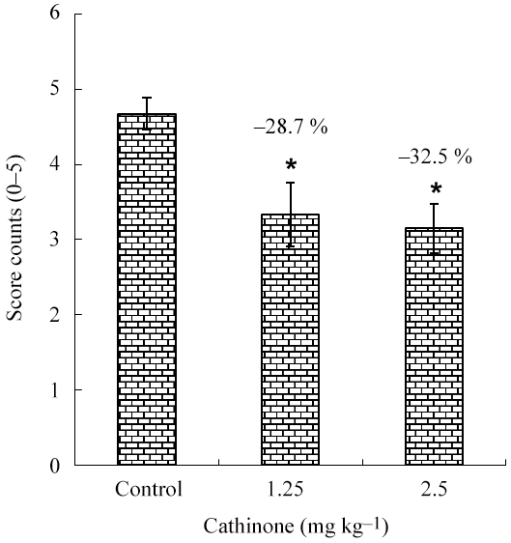

Fig. 6. The effect of cathinone (1.25 and 2.5 mg kg^{-1}) on motor coordination using the grip test. Data are expressed as mean ± SEM, $n = 8$. Significantly different *vs.* controls: *$p < 0.05$.

DOPAC decreased, which, in turn, may have caused the increased level of DA after cathinone administration. On the other hand, DOPAC acts as a substrate for the synthesis of HVA in the presence of catechol-*O*-methyltransferase (COMT). An increased level of HVA

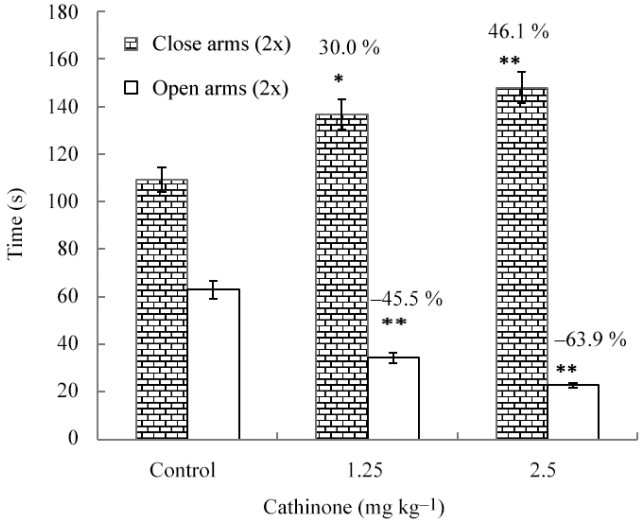

Fig. 7. The effect of cathinone (1.25 and 2.5 mg kg^{-1}) on elevated plus maze. Data are expressed as mean ± SEM, $n = 8$. Significantly different *vs.* controls: *$p < 0.05$, **$p < 0.001$.

may cause DOPAC depletion or, increased activity of COMT may break down DOPAC to HVA. This may lead to an increased content of HVA. Cathinone might have increased the activity of enzyme COMT, which breaks down DOPAC to HVA, but no literature reports on the effect of cathinone on the activity of COMT are available.

CONCLUSIONS

Natural cathinone depleted motor coordination and accelerated anxiety in Swiss albino mice and it also altered the contents of dopamine and its metabolites. To the best of our knowledge, the observed novelties in behavioral activities and dopamine metabolites, *i.e.*, DOPAC, are the first findings of the kind. A further study of cathinone and the activities of TH and COMT is required to understand the exact mechanism of DA metabolites changes.

Acknowledgements. – We are grateful to The Deanship of Scientific Research, Ministry of Education, Jazan University, Jazan, Saudi Arabia, for financial support.

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