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ORIGINAL ARTICLE

Catha edulis Forsk. (Khat): Evaluation of its Antidepressant-like Activity

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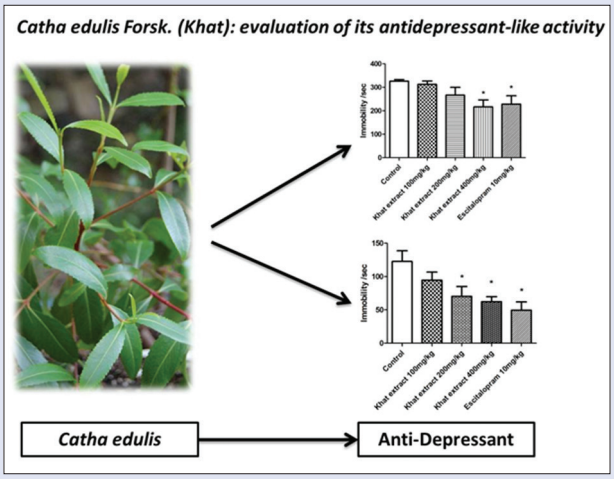
ABSTRACT

Background: *Catha edulis* Forsk. (Khat) is traditionally used for treating various disorders. Nevertheless there are no reports of any scientific assessment of its psychopharmacological properties. **Objective:** Therefore, the current study was designed to evaluate the antidepressant-like activity of Khat ethanolic extract using established animal models of depression and stress. **Materials and Methods:** Ninety healthy male albino mice were used in this study. Forced swim, tail suspension and head poking tests were utilized to evaluate the antidepressant-like activity of the ethanolic extract of Khat (100, 200 and 400 mg/kg, i.p.) and escitalopram (standard drug) which were administered 30 min prior to the tests. Phytochemical analysis of the standardized extract was conducted using liquid chromatography-mass spectroscopy (LC-MS). **Results:** A significant decrease in the head-dipping behavior was noticed after administration of 100, 200 and 400 mg/kg of Khat extract. Moreover, the extract significantly decreased the immobility time in tail suspension and forced swim tests. The presence of cathinone and cathine were detected in the extract using LC-MS. **Conclusion:** The current results suggest that the extract of Khat leaves has acute antidepressant properties and may have sedative effects.

Key words: Animal models, antidepressant-like activity, *Catha edulis*, depression

SUMMARY

- Antidepressant-like activity of Khat established *in vivo*
- The extract decreased the immobility time in tail suspension and forced swim tests
- Liquid chromatography-mass spectroscopy data revealed the presence of cathinone and cathine in Khat extract.



Abbreviations used: LC-MS: Liquid chromatography-mass spectroscopy; NIST: National institute of standard technology; SSRI: Serotonin reuptake inhibitors; FST: Forced swim test; TST: Tail suspension test.

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INTRODUCTION

Catha edulis Forsk. (Khat) is an evergreen dicotyledonous flowering shrub and psychostimulant plant that grows wildly and also commercially cultivated in Africa and Yemen, which belongs to the family *Celastraceae*.[1] Khat use has globally become a prominent and widespread habit.[2,3] Regular users of this plant get a sense of comfort, euphorbia, mental attentiveness, and pleasure.[4,5] Cathine, cathinone, and norephedrine are the major active constituents of Khat. These chemicals are structurally associated to amphetamine and noradrenaline. In terms of the distribution of Khat's phytochemical constituents, fresh plant is reported to contain an average of 36, 120, and 8 mg from cathinone, cathine and norephedrine, respectively, per 100 g of leaves. Cathinone has noticeably the strongest effects compared to cathine and norephedrine.[6,7]

Khat chewing is reported to ease social communications, develop individual performance, and augment work competence. Reported medicinal uses consist of curing asthma, lessening hunger and fatigue. Unfavorable health, environmental and socioeconomic outcomes have also been linked with regular Khat chewing.[8-10]

Khat chewing has been reported for the traditional self-medication of depression and the management of obesity.[11,12] Odenwald *et al*. reported the chewing and over-use of Khat is practiced by Somali immigrants in

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Norway, in reaction to experiences of depression, hopelessness, anger and frustration.[13] While the psychological influences of continuous Khat use have been the subject of much deliberation on its effect of social structure, there is now rising reason for fear over the healthiness effects on a broad range of peripheral organs. Psychosis and depression happen mainly among heavy chewers.[14] In a study with adult healthy Yemenis volunteers, functional mood disturbances were noticed during Khat chewing sessions (based on Hospital Depression and Anxiety Scale). The outcome on anxiety and depression was impermanent and had gone the subsequent day. Many Yemenite chewers, however, consider that Khat chewing enhances their sexual desire and pleasure.[10,15]

Obviously, in terms of pharmacological and toxicological impacts, several decades of concentrated clinical and experimental research on Khat have established a rich database. Although the scientific database on Khat is reasonably widespread and many studies have documented the impacts of Khat on mental and psychiatric health, few animal studies exist to substantiate those ethno-claims in animal models. Therefore, an immediate research effort is needed to investigate the role of Khat on animal models of stress and depression.

MATERIALS AND METHODS

Plant material and extraction

C. edulis (stem tips and leaves) was collected in April 2014 from Jazan, Saudi Arabia. The voucher specimen (CE-E-2010-32) was botanically identified and kept at Substance Abuse Research Centre, Jazan University. Three hundred gram of fresh plant were cleaned and dried at room temperature in shade for one night. The leaves also dried in freeze dryer over two nights. The dried leaves (weighed 88 g) were then crushed with pestle and mortar. Extraction procedures were conducted based on Alsalahi *et al.*, with some modifications where ethanol was utilized as extracting solvent.[16] The crushed leaves were placed into a flask and ethanol (96%) was added to dip the leaves entirely. The mixture of Khat material and ethanol were stirred smoothly and then left overnight. Filtration was performed, first by use of gauze roll to isolate the big particles followed by filter paper to eliminate the fine particles. The filtrate was then evaporated using rotary evaporator under a vacuum at 40°C to remove all traces of ethanol. The resulting ethanol-free extract (12 g) constituted about 4% of the original fresh material.

Liquid chromatography–mass spectrometry

The liquid chromatography-mass spectroscopy (LC-MS) system consisted of an Agilent 1200 system, a solvent delivery module, a quaternary pump, an autosampler, and a column compartment (Agilent Technology, Germany). The column effluent was connected to an Agilent 6320 Ion Trap high-performance liquid chromatography (HPLC)–electrospray ionization-MS. The column heater was set to 25 ± 2°C. The control of the HPLC system and data processing were performed using ChemStation (Rev. B.01.03 SR2 [204]) and 6300 Series Trap Control version 6.2 Build No. 62.24 (Bruker Daltonik GmbH, Bremen, Germany). The analytes were separated using an Agilent Zorbax Extend-C18 column (80A°, 150 mm length × 4.6 mm, i.d., 5 µm) an Agilent-Zorbax Extend-C18 precolumn (Agilent Technologies, Palo Alto, CA, USA). The mobile phase was prepared by mixing 750 mL of 0.1% formic acid in water with 250 mL acetonitrile and was pumped at a flow rate of 0.5 mL/min. General MS adjustments were set as follows: Capillary voltage, 3500 V; nebulizer, 36 psi; drying gas, 12 L/min; desolvation temperature, 350°C; ion charge control smart target, 150,000; and maximum accumulation time, 150 ms. To prepare sample a weight of 20 mg of powder was extracted by 5 ml of methanol, filtered through 0.45 micron Nylon filter, dried with nitrogen gas, and the residue was reconstituted in 50 µL methanol. A volume of 5 µL was injected for LC-MS analysis applying positive-Auto-MSn mode (library for matching). The average MS spectra were confirmed by NIST2008 database.

Animal models – Experimental animals

Ninety healthy male albino mice (30–40g) were procured from the Experimental Animal Centre of King Fahad Medical Research Center, King Abdulaziz University. Mice were maintained in polypropylene cages under a standard condition with 12/12 h light/dark cycle with free access to food and water *ad libitum*. Experiments were performed between 10.00 AM and 4.00 PM under standard conditions of temperature, lightening, and noise as was practicable. Escitalopram (Sigma-Aldrich, KSA) and Khat extract were administered by intraperitoneal (i.p.) route in mice. They were dissolved in normal saline and kept in refrigerator at −20°C prior to use and volumes injected were adjusted to 0.2 ml. The study was conducted after obtaining approval from Institutional Ethics Committee, King Abdulaziz University, Saudi Arabia.

Forced swimming test

Mice were randomly divided into five groups, each group consisting of six animals. The mice were treated with Khat extract (100, 200, and 400 mg/kg, i.p.), escitalopram (10 mg/kg, i.p.), or normal saline (i.p.) 30 min before the experiment. Animals were prepared to swim individually in a polypropylene vessel (30 cm × 15 cm × 30 cm) with a water level of 15 cm at 25 ± 2°C. The duration of immobility (time), distinguished by total cessation of swimming with the head just floating above water level was detected during the final 6 min period of test.[17] A reduction in the duration of immobility was an indicator of an antidepressant effect.

Tail suspension test

Tail suspension test (TST) was conducted using five groups of mice each consist of six animals. The mice were administered with Khat extract (100, 200, and 400 mg/kg, i.p.), escitalopram (10 mg/kg, i.p.), or normal saline (i.p.) 30 min before test. Mice both acoustically and visually isolated were suspended on the edge of a table 50 cm above the floor by the adhesive tape placed approximately one cm from the tip of the tail. Immobility time was recorded during a five min period.[18] The animal was considered to be immobile when it did not show any movement of the body and hung passively.

Head poking test

The hole-board model indicates that the head-dipping behavior is sensitive to the emotional state of animals and suggests that the expression of the anxiolytic state in animals may be reflected by an increase in head-dipping behavior. The hole board apparatus consisted of a wooden chamber (40 cm × 40 cm × 25 cm) with 16 holes (each of 3 cm diameter) evenly distributed on the floor. The apparatus was elevated to a height of 25 cm from the ground so that the mice could peep through the holes. The mice were treated with Khat extract (100, 200, and 400 mg/kg, i.p.), escitalopram (10 mg/kg, i.p.), or normal saline (i.p.) 30 min prior to test and kept in the apparatus. The numbers and the duration of head poking were recorded during the 5 min observation period.[19]

Statistical analysis

Data are presented as mean ± standard error mean multiple comparisons were performed using one-way ANOVA followed by Dunnett's test. $P < 0.05$ was considered significant. Statistical analyses were done using GraphPad Instat 3.0 (GraphPad Software, Inc., La Jolla, CA, USA).

RESULTS

Liquid chromatography–mass spectrometry

A representative MS chromatogram (extracted ion chromatogram) exhibited cathinone m/z 134 at 6.6 min and cathine m/z 132 at 7.5 min [Figure 1]. The average MS spectra were confirmed by NIST2008 database. MS chromatogram (data now shown) also exhibited fragments of cathinone and cathine.

Forced swimming test

As shown in Figure 2, significant ($P < 0.05$) reduction in the immobility time was observed with Khat extract (400 mg/kg) treatment as compared to control. Similarly, positive control escitalopram (10 mg/kg) also produced antidepressant action as indicated by a significant decrease in the immobility time. However, Khat extract (100, 200 mg/kg) was able to reduce immobility time but without significant difference ($P > 0.05$).

Tail suspension test

In the TST, there was significant ($P < 0.05$) reduction in the immobility time in the groups treated with Khat extract (200, 400 mg/kg) as compared to control [Figure 3]. Similarly, positive control escitalopram (10 mg/kg) also produced antidepressant action as indicated by a significant ($P < 0.05$) reduction in the immobility time. However, Khat extract (100 mg/kg) was able to reduce immobility time but without a significant difference ($P > 0.05$).

Head poking test

In head poking tests, there was significant decrease in the number of head poking after administration of Khat extract (100, 200, and 400 mg/kg) as compared to the control [Figure 4]. However, escitalopram (10 mg/kg) was capable to increase the number of head poking.

DISCUSSION

Although the scientific database on Khat is reasonably widespread and many studies have documented its potential impacts on mental and

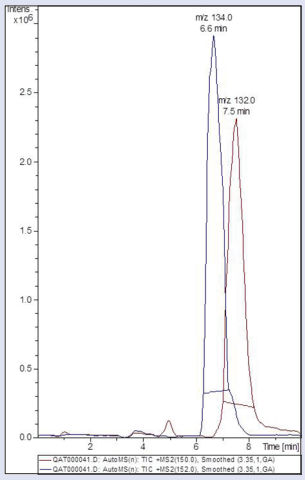

Figure 1: Mass spectroscopy chromatogram of cathinone and cathine from Khat extract. Mass spectroscopy/mass spectroscopy chromatogram of cathinone at 7.5 min m/z 150 (◊ 132) and cathine, at 6.6 min m/z 152 (◊ 134)

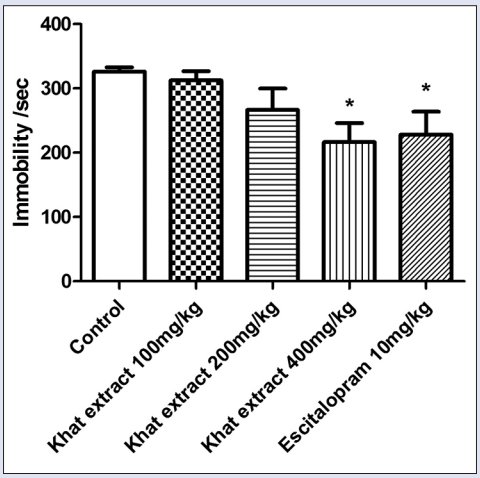

Figure 2: Effects of Khat extract and escitalopram on immobility time in forced swimming test. Each bar expressed as mean ± standard error mean ($n = 6$). One-way analysis of variance followed by Dunnett's test; *$P < 0.05$, as compared to control

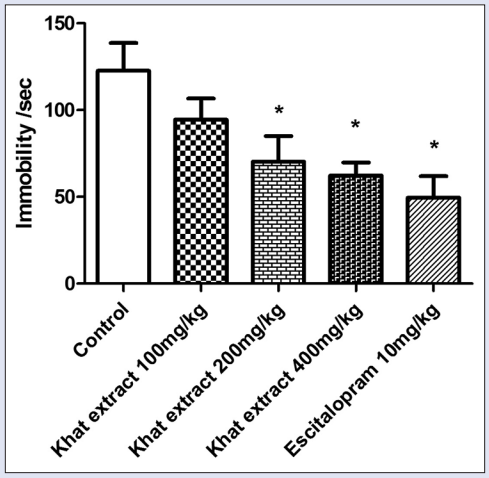

Figure 3: Effects of Khat extract and escitalopram on immobility time in tail suspension test. Each bar expressed as mean ± standard error mean ($n = 6$). One-way analysis of variance followed by Dunnett's test; *$P < 0.05$, as compared to control

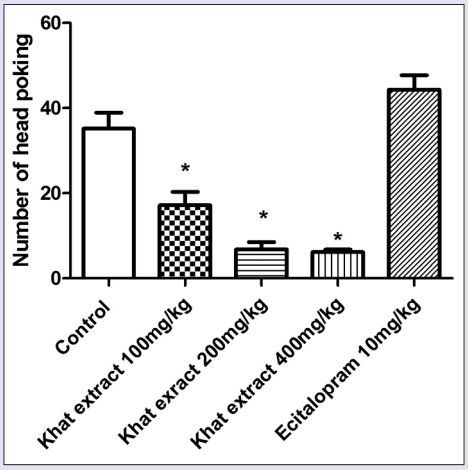

Figure 4: Effects of Khat extract and escitalopram on head pokes in head poking test. Each bar expressed as mean ± standard error mean ($n = 6$). One-way analysis of variance followed by Dunnett's test; *$P < 0.05$, as compared to control

psychiatric health, few experimental studies exist to substantiate those effects in animal models of stress and depression. The application of whole animal model is believed to be a quick technique for the recognition of neuropsychopharmacological effect of new phytomaterials.[20,21] Therefore, the current study was designed to investigate the role of Khat on animal models of stress and depression using forced swim test (FST), TST, and head poking tests. Although a lot of studies demonstrated that cathinone is unstable and undergoes degradation during drying or extraction and becomes physiologically inactive after about 36 h of harvesting,[8,22,23] the current investigation used standardized extraction method followed by spectroscopic confirmation. The phytochemical analysis (MS of Khat extract) indicated the presence of cathinone and cathine as shown in Figure 1. In this study, escitalopram was employed as a standard drug. The potency of escitalopram to inhibit serotonin reuptake (SSRI) and to induce antidepressant-like effects in applicable animal paradigms (FST; chronic mild stress; stress-induced ultrasonic vocalization) is noticeably augmented as compared with citalopram and other SSRI.[24]

The FST and TST models are currently the most extensively used tests of depression, and have been validated for use with both mice and rats. Both of these models follow different pathophysiological mechanisms.[25,26] Previous pharmacological studies indicates that dopamine functioning is a necessity for performance of mice in the FST, whereas both dopaminergic and serotonergic systems are involved in TST model. Therefore, we selected these tests to examine the antidepressant potential of Khat. Current results show a significant reduction in the immobility time in Khat treatment group (400 mg/kg) as compared to control animals in the FST. On the other hand, the current results also revealed that pretreatment of stressed animals with Khat (200 and 400 mg/kg) produces a considerable decrease in their mobility in TST. Therefore, our results on both models reinforce that Khat has antidepressant-like activity. Similarly, positive control escitalopram (10 mg/kg) also produced antidepressant action as indicated by significant reduction in the immobility time in both models (FST and TST).

The hole-board model indicates that the head-dipping behavior is responsive to the emotional status of animals and suggests that the expression of the anxiolytic state in animals may be reflected by amplification in head-dipping behavior.[27,28] A significant decrease in the exploratory head-dipping behavior (number of head poking and the time of head dipping) was observed after treatment with 100, 200, and 400 mg/kg of Khat extract, thus reinforcing the hypothesis that it has antidepressant-like activity. However, positive control escitalopram (10 mg/kg) was able to increase the number of head poking but without a significant difference as depicted in Figure 4. Earlier researches reported that escitalopram amplifies intra-synaptic level of the neurotransmitter serotonin by blocking the reuptake of the neurotransmitter into the presynaptic neuron.[29,30]

Previously published studies on the chemical compositions of herbs and their bioactivity recommend that plants containing saponins, flavonoids, and tannins own bioactivity against many central nervous system ailments.[31] Phytochemical data on Khat revealed the presence of many phytochemicals.[32] It is probable that the biomechanism of anxiolytic action of Khat could be due to the binding of cathine or cathinone to various receptors involved in the anxiolysis processes, which has to be explored further. The results found in this study propose that the extract of the leaves of Khat possesses anxiolytic and muscle relaxant activities. Further, detailed studies deserve to explore the pharmacological mechanism(s) of action of the plant extracts, as well as the active chemical(s) accountable for its biological actions by focusing the possibility of both serotonergic and dopaminergic systems in the observed results in this research. Authors are highly recommending the performance of more animal's models to further investigate the anxiolytic-like effect of Khat with analysis of cellular proteins involved.

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Conflicts of interest

There are no conflicts of interest.

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