

# Sedative Potential of *Catha edulis* Forsk: Exploring In Vivo Model, Gene Expression, Molecular Docking, and GABAergic Pathway Involvement

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## Research Article

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# Abstract

## Background

Laboratory studies and traditional reports have linked *Catha edulis* (khat) to biopharmacological properties. This study aimed to assess the sedative effect of khat in mice and conduct computer-aided docking of GABA<sub>A</sub> receptors.

## Methods

Liquid chromatography-mass spectrometry (LC-MS) analysis confirmed the presence of cathinone and cathine, the active alkaloids, in the hydroalcoholic khat extract. An acute oral toxicity test in mice was used to determine the pharmacological dosage of the sedation models. In vivo sedative activity was evaluated using the open field test and rotarod test for motor coordination, along with the assessment of iatrogenic tolerance and the potential mechanism of action on the GABAergic system. Bicuculline and diazepam were used as the GABA<sub>A</sub> antagonists and agonists, respectively. Quantitative realtime PCR was used to quantify the expression of GABA<sub>A</sub> mRNA from mice brain. Molecular docking using AutoDockR Version 4.2 was performed on the GABA receptor (subunit A).

## Results

Khat extract significantly and dose-dependently reduced locomotor activity compared with the control group during the open field test. Administration of Khat extract at doses of 200 and 400 mg/kg, along with diazepam at 3 mg/kg, significantly decreased the latency time in the rotarod test. Pre-treatment with bicuculline (1 mg/kg) reversed the khat extract-induced decrease in locomotor activity (400 mg/kg) and modulate the level of GABA<sub>A</sub> receptor mRNA. No iatrogenic tolerance was observed within the ten-day period. The binding affinity of khat alkaloids to the GABA-A receptor was observed and compared to that of diazepam.

## Conclusion

These findings suggest that khat may have anxiolytic effects mediated through the GABAergic system. Future recommendations include further mechanistic studies, clinical trials, safety assessments, and standardization efforts. Overall, this study provides insight into the sedative properties of khat and its potential as an anxiolytic treatment option.

## Introduction

Khat (*Catha edulis* Forsk) is a psychoactive plant that grows in some areas of East Africa and the Arabian Peninsula. It is estimated that around 20 million people in countries such as Yemen, Somalia, Ethiopia, Saudi Arabia, Kenya, Tanzania, Eritrea, and Djibouti regularly chew Khat, which poses significant religious, political, social, economic, and health challenges (1, 2). Khat is typically consumed by gently chewing its leaves and buds to extract the juice, which is then swallowed with saliva. The remaining pulp is kept to the side of the mouth, and fresh leaves are constantly added. The residue is eventually spat out after the chewing session (3, 4).

For over a century, Khat has been used to combat fatigue, enhance productivity, promote alertness, and induce euphoria. It has also been valued for its medicinal and recreational purposes, often consumed during social gatherings known as "Khat sessions," where people engage in conversations and social interactions (5, 6).

Khat is commonly wrapped in banana leaves for commercial distribution to preserve its efficacy for a longer duration due to its short half-life. The central nervous system (CNS) effects of Khat typically last for about three hours in the human body (7). The euphoric effects of Khat are usually noticeable within an hour of consumption. The primary alkaloid in Khat, cathinone, reaches peak plasma levels 1.5–3.5 hours after chewing begins (8), with an average elimination half-life of approximately four and a half hours (9). Following chewing, the initial feelings of elation and alertness are often followed by increased anxiety, irritability, poor concentration, and insomnia (10). Khat has a low tolerance threshold, and its withdrawal symptoms may include nightmares, tremors, sadness, drowsiness, and hypotension. Chronic use of Khat has been associated with various health issues affecting the cardiovascular, digestive, genitourinary, endocrine, and oral systems (11).

Previous epidemiological studies have indicated a higher likelihood of psychiatric complications among Khat users (8). Additionally, some studies suggest that Khat may exacerbate or trigger primary psychotic disorders such as schizophrenia. Khat-induced psychosis is often characterized by short-lived symptoms of paranoia and mania (8, 12). However, the existing research is inconclusive and relies heavily on anecdotal evidence. Furthermore, many neurobehavioral studies on Khat have been conducted on individuals who have experienced trauma, making it challenging to determine whether the psychosis is caused by the trauma or the use of Khat. A better understanding of how Khat affects emotions is necessary to establish a solid evidence base for conducting experiments on this plant. Some studies have shown that Khat chewing has sedative effects among users (13), and it has been observed to decrease spontaneous motor activity in mice (14). Our previous study also demonstrated the antidepressant properties of Khat extract (15). Sedatives are typically used to reduce anxiety or induce sleep, and their mechanism of action often involves the GABAergic system [16]. Therefore, this study aims to explore the sedative activity of Khat extract in mice, investigate tolerance, and explore the potential mechanism of action on the GABAergic system (16). This study delves into the possible sedative activity of Khat extract in mice. We aim to objectively assess its effects on locomotor activity, balance, and coordination, providing concrete measures of its sedative-like properties, and determine whether these effects are mediated through GABA-A receptors, shedding light on its potential

mechanism of action. As the first investigation to specifically examine the sedative-like effects of Khat and its interaction with the GABAergic system, this research paves the way for exploring Khat's potential as a natural sedative compound. It offers valuable insights into its neurobehavioral effects.

## **Materials and Methods**

### **Drugs and Reagents**

Bicuculline, formic acid, methanol, acetic acid, and acetonitrile were purchased from Sigma-Aldrich, USA, while ethanol was obtained from Salehiya Medical, KSA, Jeddah. Diazepam was generously provided by the King Fahd Hospital Central Pharmacy, Ministry of Health, Jeddah, KSA. All other reagents were of analytical grade, unless otherwise specified. The Khat used in the study was collected from Faifa Mountains, KSA, and its botanical authentication was conducted at the Substance Abuse Research Center, Jazan University, KSA.

### **Extraction**

The extraction process was carried out at the King Fahd Medical Research Center, King Abdulaziz University, Jeddah, KSA. Three hundred grams of fresh Khat plant material, including leaves and stem tips, were cleaned and dried at room temperature in the shade for one night. The leaves were also freeze-dried over two nights. The dried leaves, weighing 88 grams, were then crushed using a pestle and mortar. The extraction procedures were based on a previously published method (2) with some modifications, where ethanol was used instead of methanol. The crushed leaves were placed in a flask, and ethanol (96%) was added to completely immerse the leaves. The mixture of Khat material and ethanol was gently stirred and left to stand overnight. Filtration was performed using a gauze roll to remove large particles, followed by filter paper to eliminate fine particles. The resulting filtrate was then evaporated using a rotary evaporator under vacuum conditions at 40°C to eliminate all traces of ethanol. The final ethanol-free extract weighed 12 grams, constituting approximately 4% of the original fresh material. The extract was stored in a refrigerator at -4°C.

### **Liquid Chromatography–Mass Spectrometry (LC-MS)**

The LC-MS system (Agilent Technology, Germany) consisted of an Agilent 1200 system, which included a solvent delivery module, a quaternary pump, an autosampler, and a column compartment. The column effluent was directed into an Agilent 6320 Ion Trap HPLC–ESI-MS. The column heater was set to 252°C. The system was controlled and the data were processed using ChemStation (B.01.03 SR2:204) and the 6300 Series Trap Control (version 6.2 Build No. 62.24) (Bruker Daltonik GmbH). Separation of the analytes was achieved using an Agilent Zorbax Extend-C18 column (80A, 150 mm length, 4.6 mm i.d., five m) and an Agilent-Zorbax Extend-C18 pre-column (Agilent Technologies, Palo Alto, CA, USA). The mobile phase consisted of 750 mL of 0.1% formic acid in water mixed with 250 mL of acetonitrile, pumped at a flow rate of 0.5 mL/min. The general MS adjustments included a capillary voltage of 3500 V, nebulizer pressure of 36 psi, drying gas flow rate of 12 L/min, desolvation temperature of 350°C, ion

charge control smart target of 150,000, and a maximum accumulation time of 150 ms. For sample preparation, 5 mL of methanol was used to extract 20 mg of powder, which was then filtered through a 0.45-micron nylon filter, dried with nitrogen gas, and reconstituted in 50 mL of methanol. For LC-MS analysis using the positive-Auto-MSN mode, a volume of 5  $\mu$ L was injected. The resulting spectra were then compared with the NIST2008 database.

## **Animal Models**

### **Experimental Animals**

A total of three hundred sixty-six albino healthy male mice weighing between 30–40 g were obtained from the Animal House of King Fahad Medical Research Center, King Abdulaziz University, Jeddah, KSA. The mice were housed in propylene cages under standard conditions with a 12/12 h light/dark cycle and provided ad libitum access to food and water. The experiments were conducted between 10.00 am and 4.00 pm, maintaining standard temperature, lighting, and noise conditions. Ethical approval was obtained from the Ethical Committee at the King Fahad Medical Research Center, King Abdulaziz University, Jeddah, KSA.

### **Administration of Drugs**

The drugs were administered to the mice via the intraperitoneal (i.p.) route. The injected volumes were adjusted to 0.2 mL. The Khat extract was dissolved in normal saline and stored in a refrigerator at -20°C prior to use. Bicuculline was dissolved in normal saline with a few drops of concentrated acetic acid (97%) and injected i.p. directly by adjusting the dose.

### **Acute oral toxicity test**

The acute oral toxicity test was conducted following the guidelines provided by the Organization for Economic Co-operation and Development (OECD) in guideline 425, using the Up and Down Procedure (17). Twenty-four mice were randomly divided into four groups, with six animals in each group. Different graded doses of the Khat extract (100, 200, and 400 mg/kg) were administered separately to the mice in each test group using an oral cannula. The control group received normal saline only. Prior to the experiment, all animals underwent a fasting period of 12 hours. Food was provided 3–4 hours after administering the test substance, and the animals were given free access to feed and water. The mice were periodically observed during the first 24 hours, with special attention given during the first four hours, and then daily for 14 days. Observations were made for any changes in the skin, fur, eyes, autonomic nervous systems, central nervous systems, somatomotor activity, and behavior patterns. Specific attention was given to the presence of tremors, convulsions, salivation, diarrhea, lethargy, sleep, and coma.

### **Open-field locomotor activity test**

The open-field test was conducted to assess behavioral responses, specifically locomotor activity (18). The locomotor activity of mice was recorded individually using an activity meter system (Optovarimex, Columbus, Ohio, USA) in an open field (44 x 44 cm Plexiglas cage) divided into 8 x 8 squares. The center-field occupancy, defined as the time spent in the area delineated by the 16 squares in the center of the open field, was measured in seconds per 25-minute period using the Auto-Track Multiple Zone Motion Monitor software. Forty-eight mice were divided into eight groups, with six mice in each group. Groups 1, 2, and 3 received different doses of the Khat extract (100, 200, and 400 mg/kg, i.p.), group 4 received diazepam (3 mg/kg, i.p.) as the positive control, and group 5 received the vehicle alone. All animals were administered the test substances or vehicles 30 minutes before being placed in the apparatus. Each mouse was placed individually in the activity cage, and their motor activity counts were automatically recorded for 25 minutes. To investigate the potential involvement of the GABAergic system in the sedative effect, mice were pre-treated with bicuculline (1 mg/kg, i.p.) 15 minutes before the administration of Khat extract at a dose of 400 mg/kg.

## **Rotarod Test**

The rotarod test was conducted based on the concept introduced by Dunham & Miya in 1957, with some modifications (19, 20). thirty mice were divided into five groups, with six mice in each group. Groups 1, 2, and 3 received different doses of the Khat extract (100, 200, and 400 mg/kg, i.p.), group 4 received diazepam (3 mg/kg, i.p.) as the positive control, and group 5 received the vehicle alone. Mice were trained on a fixed-speed rotarod (Economex; Columbus Instruments, Columbus, OH) until they could remain on the rotarod for 60 seconds. After the administration of the Khat extract, positive control, or vehicle, each mouse was placed on the rotarod, and the latency to fall was recorded.

## **Iatrogenic Tolerance Accompanies the Sedative Khat Extract**

To evaluate iatrogenic tolerance, an open-field test was conducted using the same procedures mentioned above. Mice were divided into two groups, with six mice in each group. One group received the vehicle, and the other group received the Khat extract at a dose of 200 mg/kg. The locomotor activity test was performed on the first, fifth, and tenth days of Khat extract administration.

## **RNA extraction and reverse transcriptase-polymerase chain reaction (RT-qPCR)**

In this study, we examined the expression of the GABA receptor gene using the RT-qPCR technique. Three groups of mice were used in this study. Group one received a dose of 400 mg/kg of Khat extract intraperitoneally (i.p.), while group two received a vehicle alone. The substances were administered 30 minutes before sacrificing and removing the mice's brains under liquid nitrogen. Each mouse was placed individually, and their motor activity counts were automatically recorded for 25 minutes. To investigate the potential involvement of the GABAergic system in the sedative effect of Khat extract, mice were pre-treated with bicuculline (1 mg/kg, i.p.) 15 minutes before receiving the Khat extract dose of 400 mg/kg

(Group three). To extract RNA from the cells of mice frozen brains, we utilized TRIzol reagent from Solarbio Life Science, China. The amount of extracted RNA was determined using a NanoDrop spectrophotometer. Subsequently, the first strand of cDNA was synthesized using the ABscript II cDNA First-Strand Synthesis Kit from AB clonal Technologies, China. We employed the 2X universal SYBR green fast qPCR mix from AB clonal Technologies, China, for the RT-qPCR analysis. The gene of interest was normalized using  $\beta$ -actin as the reference gene. The primer sequences for each gene were designed and obtained from Macrogen, Korea. The relative expression levels of these genes were calculated using the  $2^{-\Delta\Delta C_t}$  method (21). The primer sequences used for PCR amplification were as follows:

Murine  $\beta$ -Actin (22):

- Forward: AAGGCCAACCGTGAAAAGAT
- Reverse: GTGGTACGACCAGAGGCATAC

GABA<sub>A</sub>R (22):

- Forward: CCAAGTCTCCTTCTGGCTCAACA
- Reverse: GGGAGGGAATTTCTGGCACTGAT

## In Silico Molecular Docking

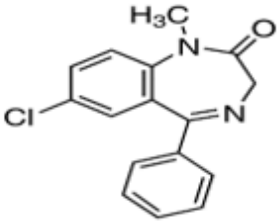
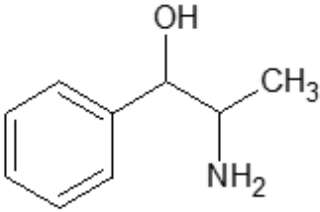
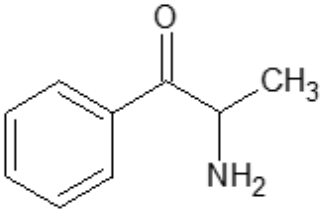
### Docking platforms

Molecular docking was performed using the AutoDockR Version 4.2 software. The AutoDock Tools (ADTR), a graphic user interface for AutoDockR 4.2, was used to prepare the protein and ligands for docking. The software was sourced from The Scripps Research Institutes in San Diego, CA, USA.

### Preparation of protein and ligand

The three-dimensional structure of the GABA-A receptor subunit A was obtained from the Protein Data Bank (PDB). The three-dimensional structure of the GABA-A receptor subunit A, along with its secondary structural details, was shown in Fig. 8. The structures of the ligands, namely diazepam (DIAZ), cathine (CATHI), and cathinone (CATHO), were obtained as SDF files from the PubChem database and converted to PDB formats using the Open Babel software. The chemical structures and systematic names of diazepam, CATHI, and CATHO were listed in Table 1. In the preparation of the protein and ligands for docking, nonpolar hydrogens were merged, and Gasteiger partial charges were assigned to all atoms. For the ligands, torsions were applied by rotating all rotatable bonds. The ligands were made flexible, while the protein was kept rigid. PDBQT files, which contain partial charges, torsion records, and atom coordinates, were generated for the protein and each ligand. These PDBQT files were used as input files for the docking experiments in the subsequent steps.

*Table 1. Chemical structures and IUPAC names of ligands, DIAZ, CATHI, and CATHO*

| Ligand    | Acronym | Structure                                                                         | Systematic Name                                       |
|-----------|---------|-----------------------------------------------------------------------------------|-------------------------------------------------------|
| Diazepam  | DIAZ    |  | 7-chloro-1-methyl-5-phenyl-3H-1,4-benzodiazepin-2-one |
| Cathine   | CATHI   |  | 2-amino-1-phenyl-1-propanol                           |
| Cathinone | CATHO   |  | 2-amino-1-phenyl-1-propanone                          |

## Docking

The docking procedure followed standard protocols for a rigid protein and a flexible ligand, as outlined in the AutoDockR 4.2 user guide. Firstly, a grid of 60x60x60 points with a spacing of 0.375 Å in the x, y, and z directions was constructed using AutoGrid, a component of the software. Electrostatic maps were calculated using a distance-dependent function of the dielectric constant, while all other parameters were set to default values as specified in the user guide. Docking simulations were carried out using the Lamarckian Genetic Algorithm (LGA), as described in the user guide. The process involved creating an initial population of 150 individuals, and random torsions were applied to each individual. In each docking run, a maximum of 2,500,000 energy evaluations were performed. At least 20 runs were performed for each of the three ligands. The best binding modes for each ligand obtained from the docking simulations were analyzed using LigPlot+, ADTR, and RasMolR programs..

## Statistical Analysis

The data are presented as mean  $\pm$  SEM (standard error of the mean). Multiple comparisons were conducted using one-way ANOVA, followed by the Tukey–Kramer post hoc test. A significance level of 0.05 was used as the criterion for determining statistical significance. All statistical analyses were performed using GraphPad InStat version 3.00, and the graphs were generated using GraphPad Prism version 4 (GraphPad Software, Inc., La Jolla, CA, USA).

## Results

### Acute oral toxicity study

In the acute toxicity tests, Khat extract was administered to the animals as single doses. Table 2 presents the data regarding body weight, general changes, and mortalities observed. It was found that all doses of Khat extract induced a change in sleep pattern within the first 24 hours. However, no significant variations were observed in the other findings. Notably, the results revealed that a dose of 400mg/kg of Khat extract caused death in 33% of the animals. Based on this information, the LD<sub>50</sub> (lethal dose for 50% of the animals) was determined to be greater than 400mg/kg.

## Liquid Chromatography–Mass Spectrometry

A representative mass spectral chromatogram, specifically an extracted ion chromatogram, demonstrated the presence of cathinone with a mass-to-charge ratio (m/z) of 134 at a retention time of 6.6 minutes. Similarly, cathine was detected with an m/z of 132 at a retention time of 7.5 minutes (Fig. 1). The average mass spectra were verified using the NIST2008 database, ensuring the accuracy of the identified compounds. In addition to the intact molecular ions, the mass spectral chromatogram also exhibited characteristic fragmentation patterns of cathinone (Fig. 1B) and cathine (Fig. 1C)..

Table 2  
Acute oral toxicity study

| Acute toxic sign | Control | Khat extract 100mg/kg          | Khat extract 200mg/kg          | Khat extract 400mg/kg          |
|------------------|---------|--------------------------------|--------------------------------|--------------------------------|
| Weight           | Normal  | Normal                         | Normal                         | Normal                         |
| Skin and fur     | Normal  | Normal                         | Normal                         | Normal                         |
| Eye              | Normal  | Normal                         | Normal                         | Normal                         |
| Tremors          | Absent  | Absent                         | Absent                         | Absent                         |
| Convulsion       | Absent  | Absent                         | Absent                         | Absent                         |
| Salivation       | Absent  | Absent                         | Absent                         | Absent                         |
| Diarrhea         | Absent  | Absent                         | Absent                         | Absent                         |
| Lethargy         | Absent  | Absent                         | Absent                         | Absent                         |
| Sleep            | Normal  | Increased during the first 24h | Increased during the first 24h | Increased during the first 24h |
| Coma             | Absent  | Absent                         | Absent                         | Absent                         |
| Mortality        | 0/6     | 0/6                            | 0/6                            | 2/6                            |

## Open-field locomotor activity test

As depicted in Fig. 2 the administration of Khat extract at doses of 100, 200, and 400 mg/kg, as well as diazepam at a dose of 3 mg/kg, resulted in a significant reduction in locomotor activity compared to the control group (p < 0.05) during a 25-minute period in the open field test. The data collected included

measurements of center-field occupancy and mean occupancy (in seconds) in each square, which were used to calculate total locomotor activity. Furthermore, when bicuculline (1 mg/kg) was administered prior to Khat extract (400 mg/kg), it reversed the decrease in locomotor activity observed in the test group (Fig. 3).

## Rotarod test

In the rotarod test, the administration of Khat extract at doses of 200 and 400 mg/kg, along with diazepam at a dose of 3 mg/kg, significantly reduced the latency time on the rotating rods ( $p < 0.05$ ). However, the 100 mg/kg dose of Khat extract did not exhibit a significant effect compared to the control group (Fig. 4). The values are presented as means  $\pm$  SEM of the latency time in seconds ( $n = 6$ ).

## Tolerance to sedative effect of Khat extract

The tolerance to the sedative effect of Khat extract was evaluated through daily administration of 200 mg/kg of Khat extract for ten days. The sedative activity was assessed on the 1st, 5th, and 10th days, and it revealed a consistent reduction in locomotor activity, as indicated by the licking time, on the first day (Fig. 5). The values are expressed as means  $\pm$  SEM ( $n = 6$ ). The asterisk (\*) signifies a significant difference compared to the control group at  $P < 0.05$ .

### Quantification of GABA<sub>A</sub> receptor gene

The study found that administering Khat extract at a dose of 400 mg/kg significantly reduced mRNA levels of GABAA. In the control group, the expression of GABAA mRNA was measured as  $1 \pm 0.01$ -fold of the control, indicating baseline levels. However, following treatment with Khat extract at 400 mg/kg, the fold control value increased to  $4.5 \pm 0.21$ , suggesting an upregulation of GABAA mRNA expression. Interestingly, when the mice were pre-treated with bicuculline (1 mg/kg) before receiving the Khat extract (400 mg/kg), the increase in GABAA mRNA expression observed in the test group was reversed. The fold control value in this group was measured as  $1.6 \pm 0.14$ , indicating a decrease in GABAA mRNA expression compared to the Khat extract-treated group. These findings suggest that the sedative effect of Khat extract may involve modulation of the GABAergic system, as indicated by the changes in GABAA receptor gene expression. The administration of Khat extract increased GABAA mRNA levels, which was reversed by pre-treatment with bicuculline.

## Docking

All binding parameters for DIAZ, CATHI, and CATHO, obtained after docking with the GABA-A receptor (subunit A), are listed in Table 3. The estimated total free energy of binding for the three ligands was  $-6.82$ ,  $-4.95$ , and  $-5.02$  kcal/mol, respectively. The estimated  $K_i$  values were 9.98, 234.47, and 209.86  $\mu\text{M}$ , respectively. DIAZ exhibited a lower  $K_i$  value, indicating a strong affinity, while the wider range of  $K_i$  values suggested a relatively weaker affinity of CATHI and CATHO towards GABA-A. The binding affinities of DIAZ, CATHI, and CATHO were nearly equal. LigPlot + software was used to analyze the

docked complexes of all inhibitors. Figures 7–10 display the LigPlots of the docked complexes of these three ligands with GABA-A. The LigPlot analysis revealed significant interactions between the ligands and the active site of GABA-A. The ligands were observed to bind in extended conformations within the active site and made extensive van der Waals contacts with the enzyme's active site residues (Figs. <link rid="fig7">8</link>–8). The benzene rings and side chains of these alkaloid ligands formed substantial hydrophobic contacts with active site residues, particularly Met-286, Met-261, Leu-285, Asn-265, and Phe-298. This similarity in interaction might contribute to the inhibitory effect of these ligands on the enzyme. Figures 8 and 9 depict different hydrogen bonding interactions between the active site residues and the two ligands. In Fig. 8, CATHI forms three hydrogen bonds with Asp-282, Asn-265, and Arg-269. Similarly, CATHO forms a hydrogen bond with Thr-262 (Fig. 8). DIAZ also forms hydrogen bonds, but the specific interactions are not mentioned in the provided text. hydrophobic contacts with active site residues, notably, Thr-262, Phe-289, Asn-265, and Met-286.

Table 3  
Interaction energies and inhibitor constants ( $K_i$ ) for the binding of daizepam, cathine, and cathinone with GABA-A receptor (subunit A)

| Parameters                                                   | DIAZ   | CATHI  | CATHO  |
|--------------------------------------------------------------|--------|--------|--------|
| (1) vdW + Hbond + desolvation energy (kcal/mol)              | -7.12  | -5.95  | -5.74  |
| (2) Electrostatic energy (kcal/mol)                          | + 0.00 | -0.20  | -0.17  |
| (3) Final intermolecular energy (kcal/mol) <sup>a</sup>      | -7.12  | -6.15  | -5.91  |
| (4) Final total internal energy (kcal/mol)                   | -0.43  | -1.13  | -1.02  |
| (5) Torsional free energy (kcal/mol)                         | + 0.30 | + 1.19 | + 0.89 |
| (6) Unbound system's energy (kcal/mol)                       | -0.43  | -1.13  | -1.02  |
| (7) Estimated free energy of binding (kcal/mol) <sup>b</sup> | -6.82  | -4.95  | -5.02  |
| (8) Estimated inhibition constant (298.15 K), $K_i$ (μM)     | 9.98   | 234.47 | 209.86 |
| (9) RMSD                                                     | 3.999  | 222.81 | 225.92 |

## Discussion

The objective of this study was to investigate the sedative effects of a hydroalcoholic extract obtained from fresh *Catha edulis* (Khat) using both mouse models and computer-aided models. Although the extract contains cathinone, previous studies have demonstrated its instability, as it undergoes hydrolysis to cathine during drying or extraction, rendering it physiologically inactive approximately 36 hours after harvest (23–25). Therefore, in this study, we specifically utilized fresh Khat for extraction purposes. By using fresh Khat, we aimed to ensure that we obtained the active compounds responsible for Khat's psychological effects before they degraded due to drying or prolonged exposure to ambient conditions.

Our previous research has demonstrated that the extract of Khat leaves possesses antidepressant properties (15) and may also exhibit sedative effects. In the present study, we observed that the administration of Khat extract at different doses significantly reduced locomotor activity compared to the control group. This was evident from the modulation of total locomotor activity in the open-field locomotor activity test, which is a widely accepted measure of central nervous system (CNS) excitability (26). This test is commonly used to assess anxiety-related behavior, as it measures the natural aversion of animals to open and brightly lit spaces. When animals are placed in this unfamiliar environment, they typically display signs of worry and fear, resulting in changes in various behavioral parameters. Anxiolytic treatments are known to alleviate such anxious behavior in animals tested in the open field (27, 28). Additionally, another study reported a decrease in locomotor activity of mice treated with Khat extract in a locomotor animal test (14).

The administration of Khat extract at doses of 200 and 400 mg/kg resulted in a significant reduction in the time that mice spent on the rotating rod, indicating its CNS-depressant effect. This effect was comparable to that of the reference drug diazepam (3 mg/kg) when compared to the control group. However, the administration of Khat extract at a dose of 100 mg/kg did not show a significant difference. The rotarod test is a well-known method for evaluating motor dysfunction induced by centrally acting drugs, allowing for the assessment of potential alterations in the animal's motor coordination ability, which can be caused by anxiolytic, sedative, and myorelaxant drugs (29). In this test, the difference in the time until the animal falls from the rotating rod between the vehicle-treated group and the treated group is used as an indicator of the sedative effect (30, 31).

The possible mechanism underlying the sedation effect of Khat was investigated in the open field test in the presence of bicuculline (a GABAA antagonist). Administration of bicuculline (1 mg/kg) prior to Khat extract reversed the decrease in locomotor activity observed in the test group, indicating a significant antagonistic property of bicuculline against the sedative effect of Khat. These findings suggest the involvement of the GABAergic system in the sedation produced by Khat extract. GABA antagonists, such as bicuculline, block the activity of GABA receptors and are known to have stimulant and convulsant effects, often used in the treatment of sedative drug overdoses (32). Bicuculline competitively inhibits the activation of GABAA receptors and interacts with the same binding sites as agonists (33).

The study revealed that administering khat extract at a dose of 400 mg/kg significantly reduced the mRNA levels of GABAA, indicating a sedative effect. Interestingly, the upregulation of GABAA mRNA expression observed in the khat extract-treated group was reversed by pretreatment with bicuculline, a GABA receptor antagonist. These findings suggest that the sedative effect of khat extract may involve modulation of the GABAergic system, as indicated by the changes in GABAA receptor gene expression. The upregulation of GABAA mRNA levels following khat extract administration implies an increase in GABAergic signaling, contributing to sedation. However, the reversal of this effect by bicuculline suggests that the sedative properties of khat extract may be mediated, at least partially, through the GABAergic system. Our previous research has suggested that khat exerts its effects through a

GABAergic mechanism (34). Nevertheless, this study constitutes a major exploration of the influence of khat on these receptors at a molecular level.

Furthermore, Khat extract has been shown to modulate GABA and dopamine transmissions, suggesting that it may utilize GABA and dopamine for its rewarding effects. Dopamine antagonists partially impede the enhancing properties of cathinone, a component of Khat extract. Moreover, administration of Khat extract in mice has been associated with locomotor symptoms resembling those seen in schizophrenia (35, 36). Additionally, glutamate may also play a role in the rewarding effects of Khat extract. Animals with activated glutamatergic cells in the ventral tegmental region have exhibited positive reinforcing behaviors (37). Notably, higher doses of Khat extract have been found to induce seizure-like signs in animal experiments, supporting this hypothesis (38). Glutamate initiates and spreads seizures, whereas GABA prevents their onset. These data collectively suggest that, in addition to dopamine, the effects of crude Khat extract on the rewarding response may be mediated through GABA and glutamate neurotransmission in the brain.

Benzodiazepines have been demonstrated to exhibit varying levels of tolerance in terms of their anticonvulsant, sedative, and skeletal muscle relaxant actions (39). In relation to tolerance, we assessed the impact of daily administration of Khat extract at a dose of 200mg/kg on open field tests. Interestingly, no changes in the sedative effects were observed on the 1st, 5th, and 10th days, suggesting that the development of tolerance to these effects did not occur.

This study is the first of its kind to compare the binding mode of CATHI and CATHO to GABA with that of DIAZ. Benzodiazepines exert their anxiolytic, anticonvulsant, muscle relaxant, and sedative-hypnotic effects by enhancing GABA's activity at GABAA receptors through their specific benzodiazepine-binding site. Interestingly, chronic habituation to Khat in mice resulted in decreased levels of GABA in the brain [8]. Table 3 provides the binding parameters obtained from docking DIAZ, CATHI, and CATHO with the GABA-A receptor (subunit A). The benzene rings and side chains of these alkaloid ligands form extensive hydrophobic contacts with key residues in the active site, particularly Met-286, Met-261, Leu-285, Asn-265, and Phe-298. Previous research has reported that Methionine 286 of the GABAA receptor controls the binding cavity for various anxiolytics and anesthetics (40).

## Conclusions

The study employed laboratory experiments using mice as the animal model. The hydroalcoholic extract of Khat was used for testing, and the presence of cathinone and cathine in the extract was confirmed through LC-MS analysis. Acute oral toxicity tests were conducted to determine the appropriate dosage for the sedation models. The sedative effects of Khat were evaluated using the open field test and the rotarod test. In the open field test, the locomotor activity of the mice was measured, and it was found that Khat extract significantly and dose-dependently decreased the locomotor activity compared to the control group. In the rotarod test, which evaluates motor coordination, administration of Khat extract at doses of 200 and 400 mg/kg, along with diazepam at a dose of 3 mg/kg, significantly decreased the

latency time. The study investigated the potential mechanism of action of Khat on the GABAergic system. Bicuculline, a GABA antagonist, was used to reverse the decrease in locomotor activity induced by Khat extract, indicating the involvement of GABA-A receptors. Molecular docking analysis using AutoDockR Version 4.2 was performed, which showed binding affinity between Khat alkaloids and the GABA-A receptor (subunit A). The study examined the development of iatrogenic tolerance, which refers to a reduced response to a drug with repeated exposure. It was observed that no iatrogenic tolerance was present within a ten-day period, suggesting that Khat may have a favorable safety profile in terms of sedation. Moreover, the study concluded that Khat exhibits sedative effects, potentially mediated by its interaction with GABA-A receptors. Future research recommendations include further mechanistic studies to understand the precise mechanism of action, clinical trials to evaluate the anxiolytic potential of Khat in humans, assessment of bioavailability and pharmacokinetics, exploration of combination therapy with other anxiolytic compounds, comprehensive safety assessments, and standardization and quality control measures. Overall, this study provides valuable insights into the sedative properties of Khat and highlights its potential as a natural compound for the treatment of anxiety-related conditions.

## **Declarations**

### **Acknowledgement**

Authors are grateful to staff in the Department of Pharmacology, Faculty of Medicine, King Abdulaziz University, Jeddah, Saudi Arabia.

### **Ethics approval and consent to participate**

Ethical approval was obtained from the Department of Pharmacology, Faculty of Medicine, King Abdulaziz University, Jeddah, Saudi Arabia.

### **Consent for publication**

Not applicable.

### **Availability of data and material**

The datasets used and/or analyzed during the current study available from the corresponding author on reasonable request.

### **Competing interests**

The authors declare that they have no competing interests.

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## Authors' contributions

HA, SIA, and MMET planned the investigation, evaluated the findings, wrote the article, and oversaw the administrative aspects. MMET, AB, HA, AHA, gathered the information and revised the text. Both authors have read and approved the manuscript.

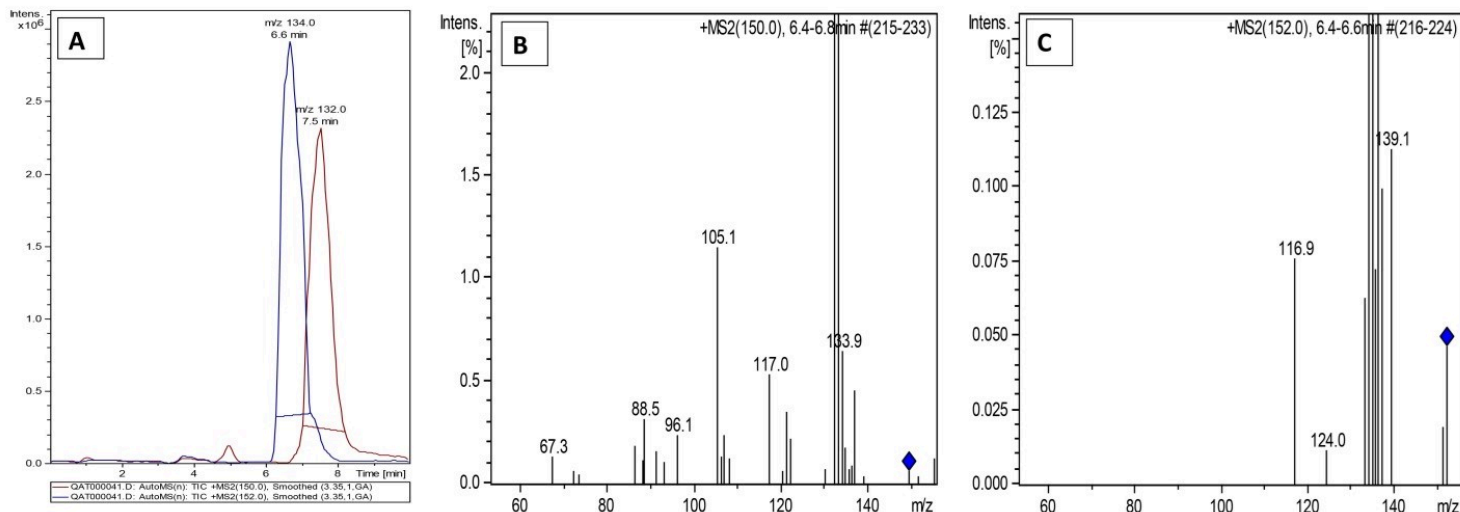
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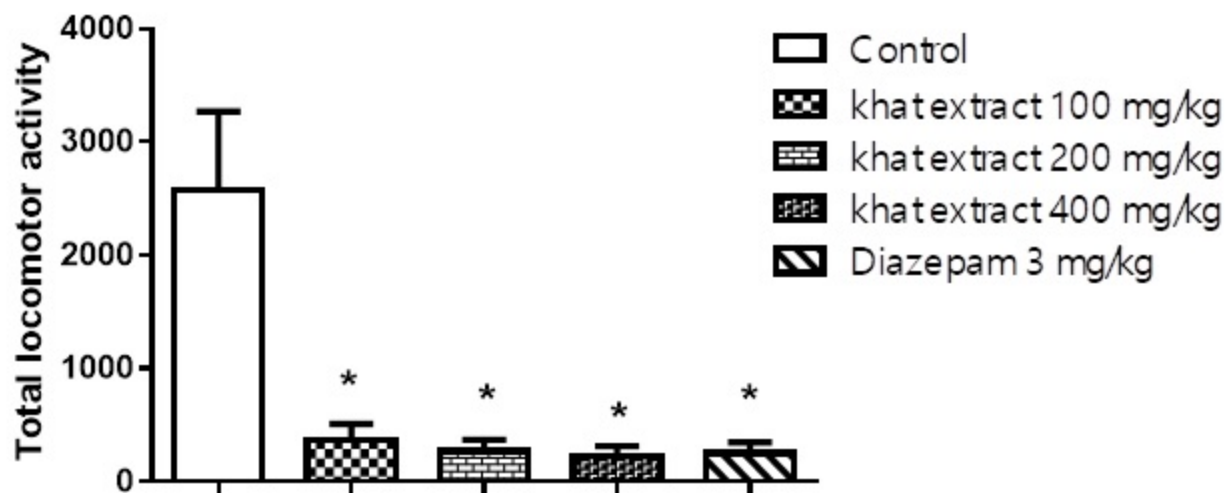
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## Figures



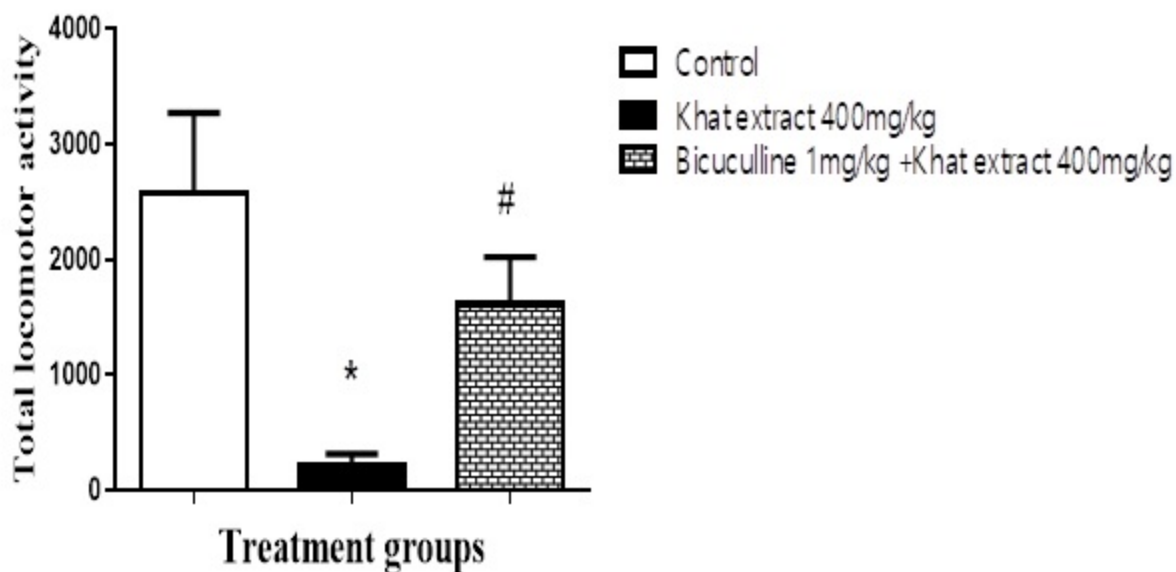
**Figure 1**

Mass spectral chromatogram. A: The presence of cathinone and cathine within the Khat extract. The MS/MS chromatogram (MS2) of cathinone is shown at a retention time of 7.5 minutes, with a precursor ion of  $m/z$  150 converting to a fragment ion of  $m/z$  132. Similarly, the MS/MS chromatogram of cathine is presented at a retention time of 6.6 minutes, with a precursor ion of  $m/z$  152 converting to a fragment ion of  $m/z$  134. B: The mass spectral fragments specific to cathinone, while Figure 1C showcases the mass spectral fragments associated with cathine.



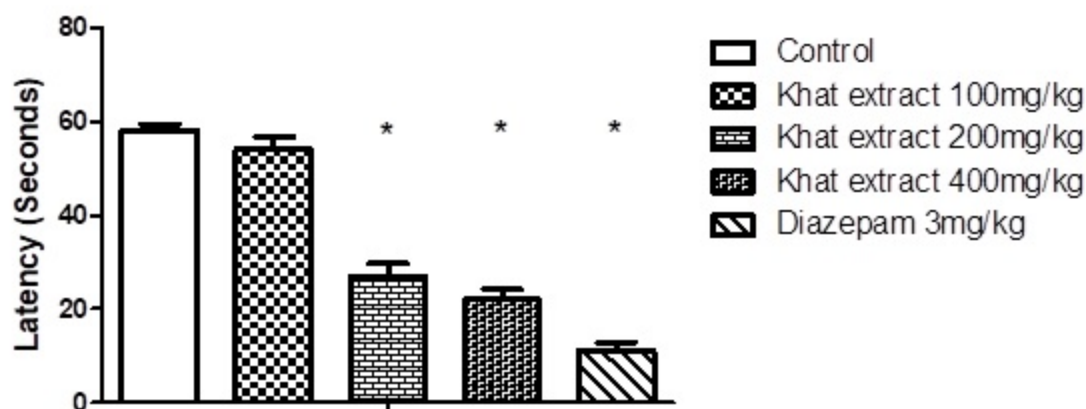
**Figure 2**

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**Figure 3**

The effect of the GABAA antagonist, bicuculline (administered intraperitoneally at a dose of 1 mg/kg), on the sedation induced by Khat extract (administered intraperitoneally at a dose of 400 mg/kg) in mice during the open field test. The drugs or saline (control) were administered 15 minutes prior to the administration of Khat extract. The values are expressed as means  $\pm$  SEM (n=6). The asterisk (\*) denotes a significant difference compared to the control group at  $P < 0.05$ , while the hashtag (#) denotes a significant difference compared to the Khat group at  $P < 0.05$ .



**Figure 4**

illustrates the impact of Khat extract and diazepam on the rotarod test in mice. It shows the effect of Khat extract (administered intraperitoneally at doses of 100, 200, and 400 mg/kg) and diazepam (administered intraperitoneally at a dose of 3 mg/kg) on the motor balance of the mice. The values

presented are the means  $\pm$  SEM of the latency time in seconds, and the sample size is  $n=6$ . The asterisk (\*) indicates a significant difference compared to the control group at  $P<0.05$ .

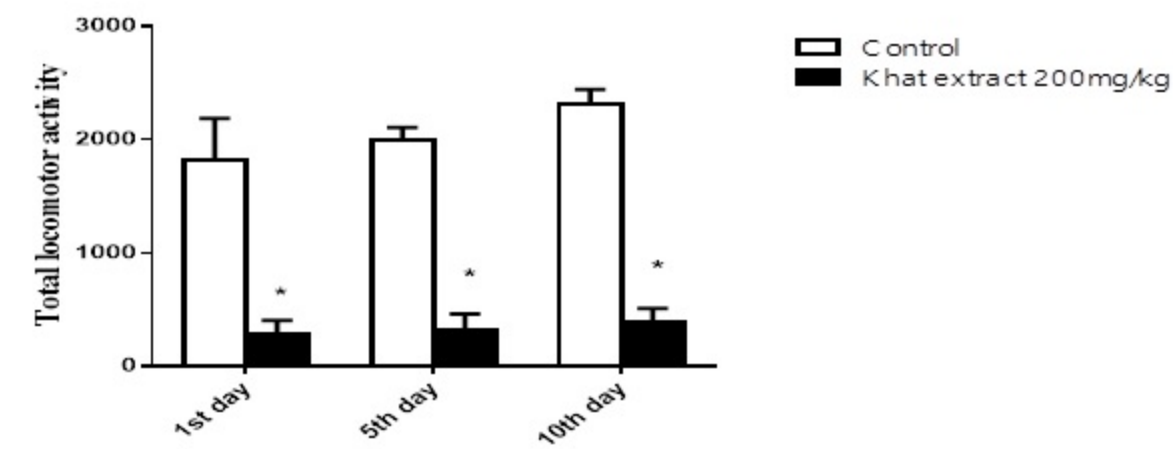


Figure 5

The tolerance to the sedative effect of Khat extract in mice. It demonstrates the effect of daily administration (over a period of 10 days) of Khat extract at a dose of 200 mg/kg (administered intraperitoneally) on the open field test in mice, specifically on the 1st, 5th, and 10th days. The values presented are the means  $\pm$  SEM ( $n=6$ ). The asterisk (\*) indicates a significant difference compared to the control group at  $P<0.05$ . B:

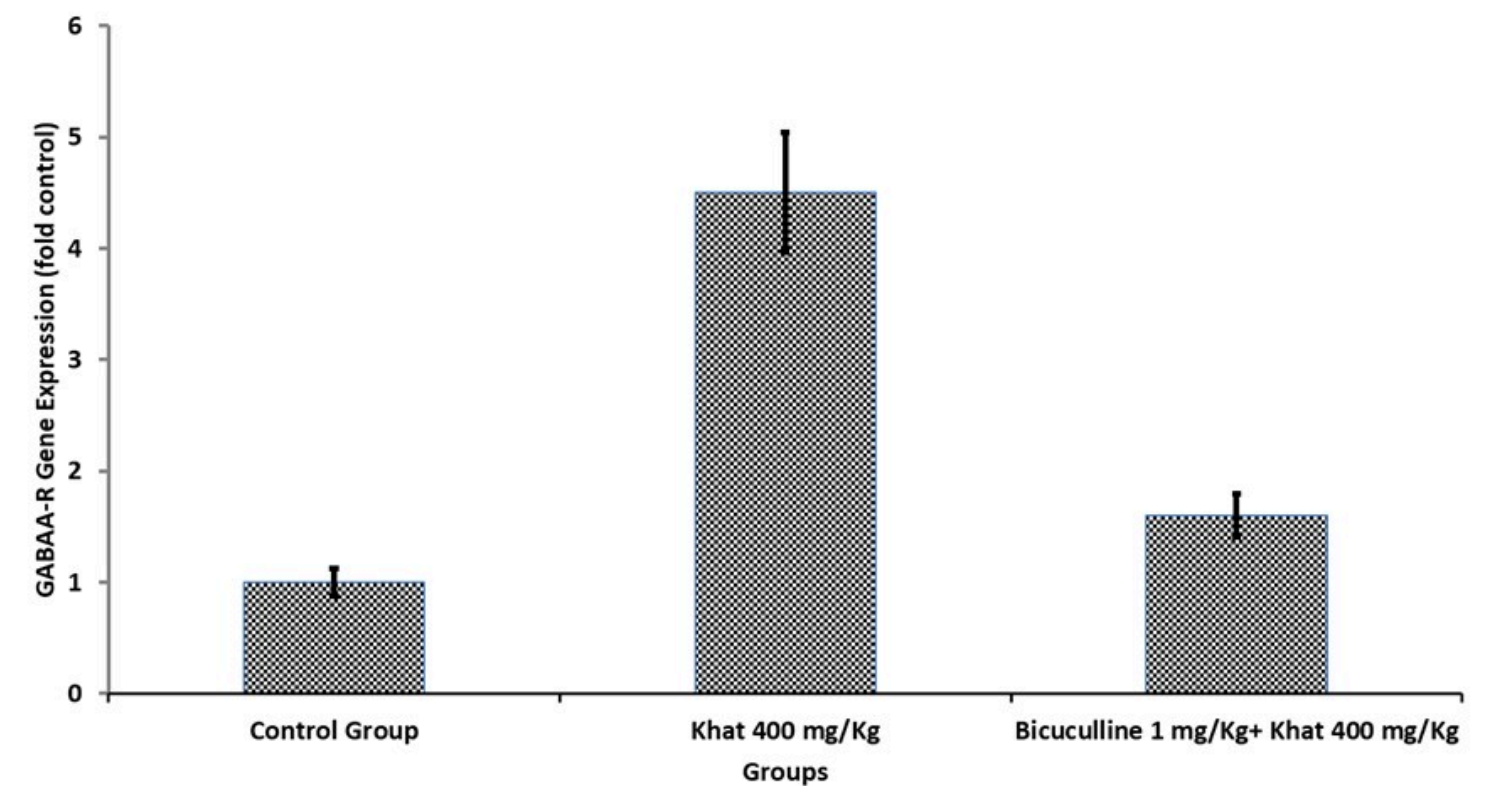
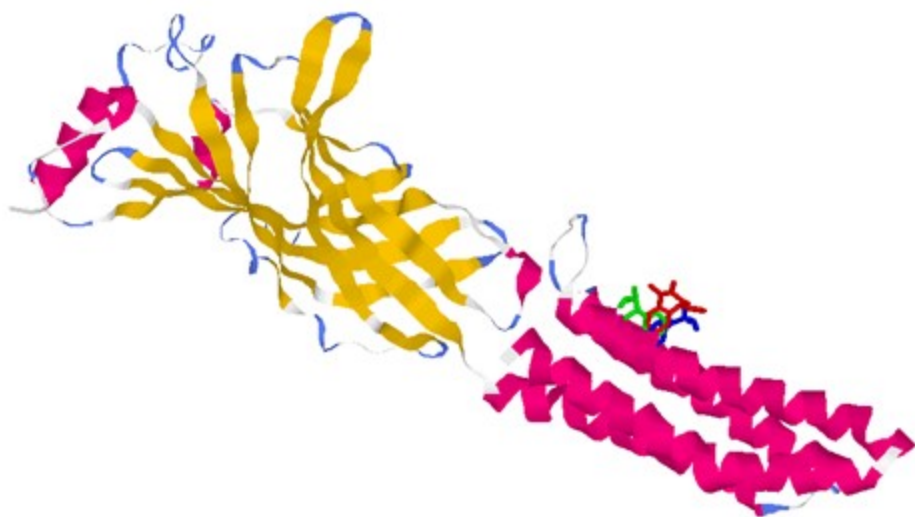


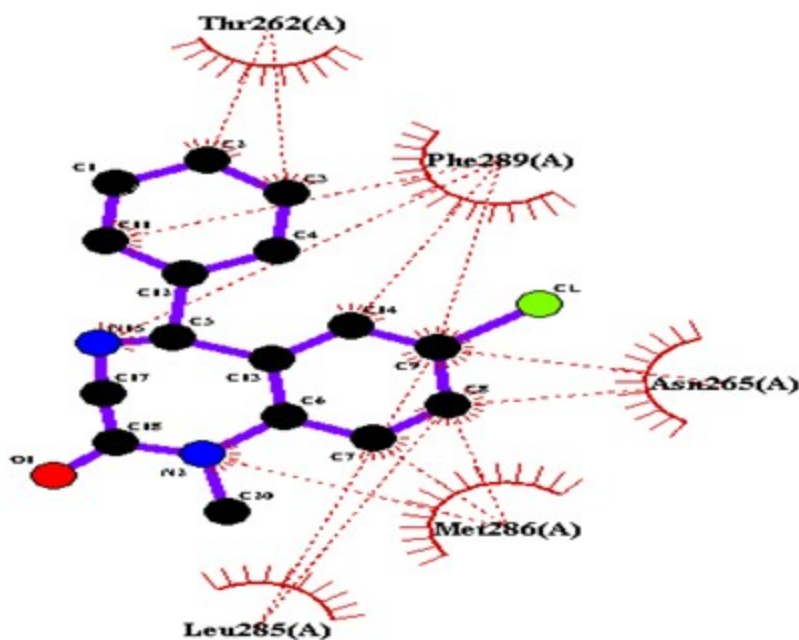
Figure 6

The effect of the GABA<sub>A</sub> antagonist, bicuculline (1 mg/kg), on the gene expression of GABA<sub>A</sub> receptor gene induced by Khat extract (400 mg/kg) in mice brain. The drugs or saline (control) were administered 15 minutes prior to the administration of Khat extract. The values are expressed as means  $\pm$  SEM (n=4).



**Figure 7**

All three ligands bound to the GABA-A receptor (subunit A). (DZP- red, CATHI-green, CATHO-blue).



**Figure 8**

LigPlot analysis of binding of diazepam to GABA-A receptor. Atoms are shown in CPK colors. Hydrophobic interactions are shown as red dotted lines and rays from residues towards ligand atoms.

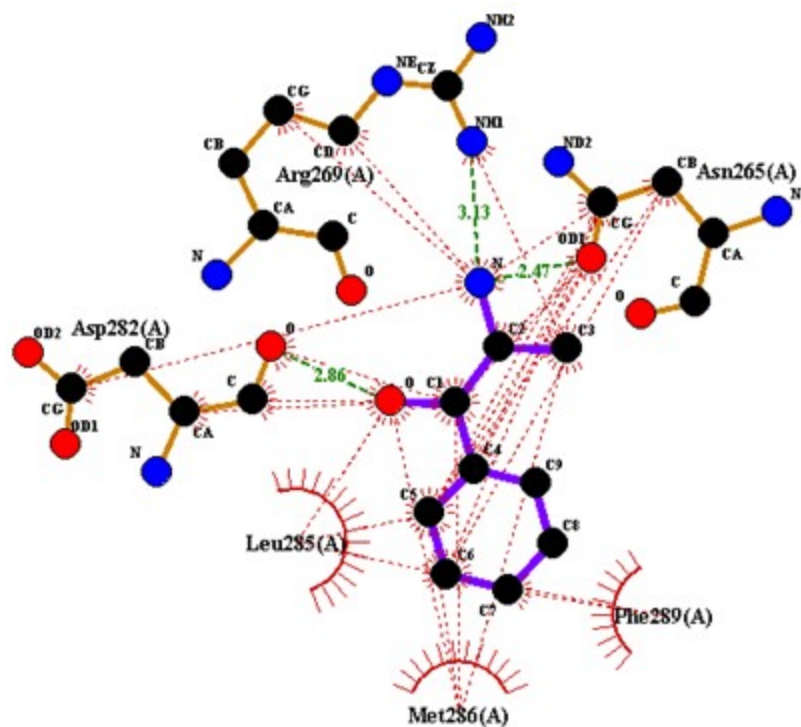


Figure 9

LigPlot analysis of binding of cathine to GABA-A receptor. Atoms are shown in CPK colors. H-bonds are shown as green dotted lines. Hydrophobic interactions are shown as red dotted lines and rays from residues towards ligand atoms.

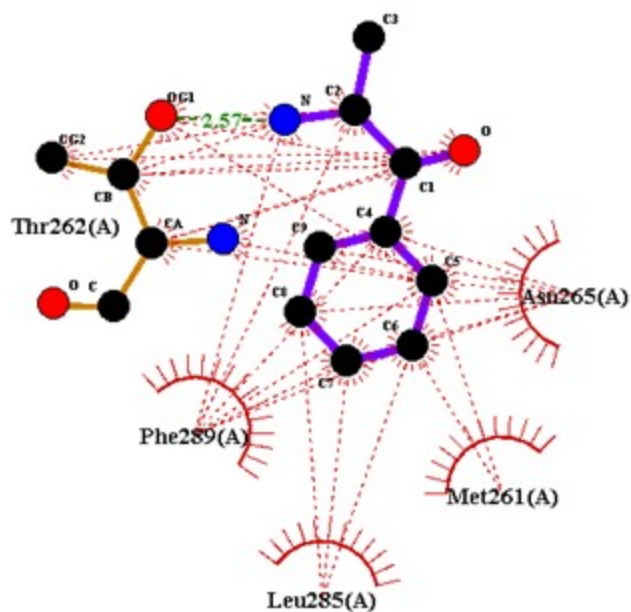


Figure 10

LigPlot analysis of binding of cathinone to GABA-A receptor. Atoms are shown in CPK colors. H-bonds are shown as green dotted lines. Hydrophobic interactions are shown as red dotted lines and rays from

residues towards ligand atoms.