

Insights into the Anorexic Potential of Khat: An Integrated in vivo, ex vivo, and in silico Investigation

Ahmed Ali Jerah

Jazan University

Manal Mohamed Elhassan Taha

mtaha@jazanu.edu.sa

Jazan University

Abdullah Farasani

Jazan University

Sivakumar Sivagurunathan Moni

Jazan University

Emad Sayed Shaheen

Jazan University

Andleeb Khan

Jazan University

Ibrahim A Khardali

General Directorate of Health Affairs

Magbool E Oraiby

General Directorate of Health Affairs

Heyam Sidahmed

Al-Neelain University

Siddig ABDELWAHAB

Jazan University

Research Article

Keywords: Catha edulis, Appetite, Leptin, Ghrelin, 5-Hydroxytryptamine, Molecular Docking

Posted Date: February 26th, 2024

DOI: <https://doi.org/10.21203/rs.3.rs-3978503/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License. [Read Full License](#)

Additional Declarations: No competing interests reported.

Abstract

Chewing khat (*Catha edulis*) releases cathine and cathinone, which may reduce appetite through an unknown mechanism. This study investigated the peripheral and central effects of fresh leaves and buds of *C. edulis* water extract (CEWE) on appetite and body weight, using *in vivo*, *ex vivo*, and *in silico* models. Rats of both sexes were used for three different experiments. Serotonin receptors were studied using isolated rat fundus samples and molecular docking. Significant differences in body weight were observed in the CEWE-treated groups compared with the controls over eight weeks. However, plasma leptin and ghrelin levels did not change significantly. The expression of the ghrelin and leptin genes was also unaffected, but the expression of the 5-HT gene decreased with CEWE treatment. CEWE antagonizes 5-HT receptors in isolated rat fundus samples. *In silico* docking indicated that the khat ligands inhibited 5-HT_{2C} receptors. Cathine and cathinone levels in rat plasma were measured. These findings suggest that the appetite-suppressing role of khat can be attributed to its antagonistic effect on 5-HT receptors. Further research is needed to fully understand the mechanism of action of the khat extract. These findings provide a basis for future studies to validate and expand on these discoveries.

1. Introduction

Khat (*Catha edulis*) has been widely chewed by the people of East Africa and the Arabian Peninsula for eight centuries [1]. This plant is native to the Hararghe Zone of Ethiopia [2]. The cultivation, marketing, and chewing of khat are legal in some countries such as Uganda, Ethiopia, Kenya, Yemen, and Djibouti. While Khat is considered a narcotic in Saudi Arabia, its circulation is prohibited in some Western countries [3]. Men and women chew fresh leaves and buds for various purposes. The effects of khat consumption include increased energy consumption, alertness, and euphoria. Users often experience a sense of well-being, heightened sociability, and desire to engage in conversations. Some individuals also report improved concentration and focus, making it popular among students and workers seeking enhanced productivity [4, 5]. Khat has been incorporated into traditional medicinal practices for various reasons. It has been used to treat conditions, such as depression, headaches, and toothaches [6–8].

In addition to cathinone and cathine, khat also contains other psychoactive components, particularly norephedrine, which has similar effects, although to a milder degree [9, 10]. These naturally occurring psychoactive substances are reported to cause excitement, euphoria, increased alertness, and augmented restiveness [7, 8, 11]. According to prevalent traditional beliefs, Khat was reported to reduce appetite, obesity, and body weight [7, 12, 13]. It is well known that amphetamine has appetite-suppressing properties, which may partially explain the low body mass index and malnutrition associated with amphetamine addiction. [14], little is known about their natural equivalents, such as cathine and cathinone. In addition, the action of amphetamine in reducing appetite is well known; it acts through a central mechanism in the *arcuate nucleus* of the hypothalamus [15], unlike Khat, which requires further investigation. Dexfenfluramine, structurally similar to amphetamine, is a serotonergic anorectic drug that decreases appetite by increasing extracellular brain serotonin levels [16, 17].

Leptin and ghrelin are two crucial hormones that are involved in the complex regulation of appetite. Leptin, primarily produced by the adipose tissue, acts as a satiety signal, relaying information about the body's energy stores to the brain. High leptin levels indicate sufficient energy reserves, leading to appetite suppression and increased energy expenditure. Leptin acts on receptors in the hypothalamus and other brain regions involved in appetite control, influencing neuronal activity and promoting feelings of fullness [18, 19]. Ghrelin, predominantly secreted by the stomach, plays a pivotal role in stimulating appetite and promoting food intake. Ghrelin levels increase before meals and decrease after eating. It acts on receptors in the hypothalamus, triggering hunger sensations and stimulating food-seeking behaviors. Ghrelin also influences reward pathways in the brain, potentially contributing to the pleasurable aspects of eating [19, 20]. Understanding the interplay between khat consumption and leptin-ghrelin interactions can contribute to a better understanding of the overall impact of khat consumption on energy balance and weight management. This may also have implications for potential therapeutic applications or interventions targeting appetite control and weight loss [5, 21, 22].

Previous studies have documented the anorexic effects of khat; however, the underlying mechanism(s) have not yet been explored [12, 23, 24]. An *in vivo* study using pure cathinone revealed that acute oral dosing caused a noteworthy reduction in food consumption and body weight in rats [5]. One study on appetite-regulating hormones reported that chewing khat reduced subjective feelings of hunger and increased feelings of fullness, although there was no change in PYY or ghrelin release [24]. Despite confirmation of khat's anorectic action through various experimental and clinical studies [7, 24, 25], the underlying mechanism remains unclear. Therefore, this study aimed to address the existing knowledge gap regarding the underlying mechanism of khat's anorectic effects. This study investigated the impact of acute and chronic oral administration of khat leaves on appetite-controlling hormones (ghrelin and leptin), and examined the effect of khat on serotonin receptors in the gastrointestinal tract using an *ex vivo* isolate of rat tissue. Additionally, molecular docking of cathine and cathinone on 5-HT_{2C} serotonin receptor subtypes was performed to provide further insights.

2. Materials and Methods

2.1 Materials and reagents

All solvents, reagents, and chemicals used in this study were analytical grade. Amphetamine, cathine, and cathinone reference standards were obtained from Lipomed (Al-Ashban Trading, Kingdom of KSA). Ethanol, chloroform, sodium chloride, potassium chloride, magnesium sulfate, calcium chloride, glucose, ammonium formate buffer, formic acid, and acetonitrile were purchased from Sigma-Aldrich and Thermo Fisher (St. Louis, MO, USA). Kits for ghrelin (Product Code: MBS2602044) and leptin (Product Code: MBS012834) were purchased from My BioSource (USA).

2.2 Plant material and extraction

The khat plant was obtained from the Substance Abuse and Toxicology Research Center (SARC), Jazan University, with all the required official approvals. Taxonomic identification was confirmed at the Medical Research Center of the Jazan University. A voucher specimen (MRC/CE/1442-1) was deposited in the botanical facilities at the Medical Research Center, Jazan University. In our study, CEWE (*Cathe edulis* water extract) was obtained using a specific extraction method [11, 26, 27]. Fresh leaves and young shoots of khat were collected and immediately frozen at -80°C . The frozen samples were minced using a mortar and pestle to achieve a uniform particle size. The minced plant material was subjected to extraction using distilled water to ensure efficient extraction of the active compounds. The resulting mixture was filtered to remove the solid particles, and the filtrate was concentrated through evaporation or freeze-drying. Extracted CEWE was promptly administered to the animals, ensuring that they received the extract immediately after extraction. This approach aims to maintain the bioactivity and integrity of the active compounds present in the extract, allowing for an accurate assessment of their effects on animals. The dose in all studies was calculated based on the fresh plant weight.

2.3 Animal study

2.3.1 Animals, husbandry, ethical approval, and sample collections

The rats utilized were Sprague-Dawley rats of both sexes, weighing 150–200 g ($n = 70$). All animals in this study were obtained from the Medical Research Center at Jazan University's Animal House, where permission for research ethics was obtained (approval number: MRC/1443/4/1). All techniques used in this study adhered to the guidelines for animal research as defined by the Institutional Ethics Board Guidelines of Jazan University on Animal Care and Use in Research. The rats were allowed to acclimatize to a specific temperature ($24 \pm 2^{\circ}\text{C}$) and humidity (50–60%) and were allowed free access to food and water. Rats were housed in polypropylene cages with a 12-hour light/dark cycle. Khat was orally administered to rats using a sterile syringe and curved stainless steel feeding needles. Blood samples were collected through heart acupuncture or cervical dislocation of living animals after anesthesia with diethyl ether. Serum and plasma samples were collected by centrifugation and stored at 4°C until further analysis. The weights of the animals were measured using a digitally sensitive balance.

2.3.2 Grouping and dosing of experimental animals

A preliminary experiment was conducted to determine the appropriate CEWE dose. Experimental doses ranging from 100 to 2000 mg/kg CEWE were administered to six groups of rats [28]. No signs of physical or behavioral toxicity were observed in these rats. Therefore, doses of 100, 300, and 500 mg/kg were chosen. Three experiments were conducted with different durations and doses to study the acute and chronic effects of CEWE (Fig. 1). The first study used both sexes of rats for long-term anorexic effects (8 weeks). Forty rats of both sexes were randomly divided into eight groups of five rats each. The normal control group consisted of two groups of male and female rats. Each group of male and female subjects received oral doses of CEWE at 100, 300, and 500 mg/kg/day for eight weeks, resulting in six groups. After 56 days, serum and plasma samples were collected to measure leptin and ghrelin levels. In the second study, which examined the acute effects of khat, 20 male rats were randomly divided into four equal groups. At the same time, the first group received only distilled water at a dose of 5 ml/kg of body weight. The other three groups were administered oral doses of 100, 300, and 500 mg/kg CEWE. In the third study, five male rats were orally administered (12.4 g). The dose of khat was calculated based on the average daily human intake of khat. Two hours later, plasma was collected for leptin and ghrelin measurements in studies two and three.

2.3.3 Body weight

In the first study (Fig. 1), which focused on the long-term effect of CEWE on the weight of rats, live weight was recorded on the first day and the 56th day. The final weights of the animals were determined using a sensitive digital balance. Changes in body weight were assessed using a previously reported method [29]. The initial body weights of all animals were measured before they were orally administered CEWE orally for eight weeks. Weight increase (as a percentage) was computed after the eighth week as follows:

Weight gain %	=	$\frac{\text{Final weight (gm)} - \text{Initial weight(gm)}}{\text{Final weight (gm)}} \times 100$
---------------	---	--

2.3.4 Determination of ghrelin

Ghrelin concentration in plasma was evaluated using a competitive inhibition enzyme immune assay [30]. All reagents and solutions were prepared according to the manufacturer's protocol (BioSource, USA). Standards and samples (100 µL) were placed on a microplate in their respective places. Then, 50 µL of reagent A was added to all wells. A plate sealer was used to cover the microplate. The microplate was then incubated for one hour at 37°C. After incubation, the plate was cleaned three times with wash buffer using a Biotek washer (Model 50TS). A 100 µL working solution of detection (Reagent B) was pipetted, and the microplate was covered with a sealer. After 30 min of incubation at 37°C, the microplate was washed five times with washing buffer. Then, the substrate (90 µL) was added to each well of the washed microplate, and which was sealed and incubated for 20 min at 37°C. The reaction was terminated by adding 50 µL of the stopping reagent. The color change was immediately detected at 450 nm using an ELISA microplate reader (Biotek 800TS) equipped with Gen 5 software.

2.3.5 Determination of leptin

Plasma leptin concentration was evaluated using a competitive inhibition enzyme immune assay [19] procured from MyBioSource, USA. All the reagents and solutions were prepared according to the manufacturer's instructions. Standards and samples (100 µL) were placed on microplates at their respective positions. The microplate was wrapped with an adhesive plastic film and kept in an oven at 37°C for 120 min. After incubation, 100 µL of the detection solution (Reagent A) was added to all wells, covered with an adhesive plastic film, and kept in an oven at 37°C for 60 min. After incubation, the plate was washed thrice with wash buffer using a Biotek washer (Model 50TS). Reagent B (100 µL of reagent B was pipetted into each well and incubated at 37°C for 60 min. The microplate was then rinsed five times. The substrate was incubated at 37°C for 20 min and then sealed with an adhesive plastic film. Then, a stopping solution was added to block the reaction. An ELISA microplate reader (Biotek 800TS) with Gen 5 software was used to determine the change in color at 450 nm.

2.4 Serotonin receptors study

2.4.1 Alkaloids rich extraction

In this experiment, a previously reported method [6] was used to extract khat, because the goal was to obtain an effective alkaloid concentration, specifically for cathine and cathinone. Briefly, 50 g of green material was macerated into small pieces in a plant mill. After adding 200 ml of methanol, the mixture was sonicated for 15 min. To distinguish the liquid from the solid plant material, the resulting mixture was filtered through a Buchner funnel using a Whatman filter paper. The alcohol solution was evaporated to dryness under steam. The small volume was dissolved in 0.02 N sulfuric acid before extraction and separation in chloroform. Saturated sodium bicarbonate was used to make the aqueous acidic layer basic and dichloromethane was used to remove it.

2.4.2 Rat fundus strip

The experiment was based on a previously reported method [31]. Five rats were used and the experiments were conducted on five different days. The animal was slaughtered by neck dislocation and left for exsanguination; therefore, no anesthetic drugs were used to affect the results. The gastric fundus was removed and placed in fresh Krebs Ringer solution. Two incisions were made on either side of the dome-shaped preparation in order to open the fundal end lengthwise. A strip was created from the tissue by alternating the transverse incisions on the opposing sides of the muscle. Reads were knotted around each end of the strip. One end was linked to a tissue holder and moved to an organ bath; the other was attached to the transducer; a 1-gram stretching weight was placed on the tissue, and it was allowed 45 min to equilibrate. Khat extract and 5-hydroxytryptamine (5-HT) were then added to assess their agonist and antagonist effects on the 5-HT receptors. The results are expressed as a percentage of inhibition.

2.4.3 RNA extraction and reverse transcriptase-polymerase chain reaction (RT-PCR)

In this study, we examined the expression of leptin, ghrelin, and 5HT markers using the RT-qPCR technique. To extract RNA from the cells, 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. For the RT-qPCR analysis, we employed the 2X universal SYBR green fast qPCR mix, also from AB clonal technologies, China. The genes of interest, including leptin, ghrelin, and 5HT, were normalized using β-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-ΔΔCt method [32]. The primer sequences used for PCR amplification were as follows:

Murine β -Actin:

- Forward: AAGGCCAACCGTGAAAAGAT
- Reverse: GTGGTACGACCAGAGGCATAC

Ghrelin:

- Forward: AAGTAACCACTGACAGGACTGGT
- Reverse: CAGGAGAGTGCTGGGAGTTGCA

Leptin:

- Forward: TGCTGCAGATAGCCAATGAC
- Reverse: GAGTAGAGTGAGGCTTCCAGGA

5-HT:

- Forward: CTCCTCCCGATCTCTTTGAAATCGC
- Reverse: TGTTGAGCTTTGCCAGTTTCG

2.5 Liquid chromatography–mass spectrometry

2.5.1 Sample preparation

The calibration and control samples were prepared by dissolving one mg/mL of real standards of cathinone, cathine, and amphetamine in a negative plasma sample to eliminate matrix effects. Reference samples of d,l-cathinone HCl (1 mg free base/1 mL acetonitrile/H₂O: 1/1) and d-cathine HCl (1 mg free base/1 mL methanol) were prepared in clean glass vials. The concentrations used to establish the calibration curve were 20, 50, 100, 200, 400, 800, and 1000 ng/ml. Using the solid-phase extraction technique, one mL of blood, calibrators, and control samples were extracted after treatment with amphetamine-D5 as an internal standard. The extracts were dried under a nitrogen stream before being reconstituted in a 150-mL mobile phase [33].

2.5.2 Instrumental analysis (LC-MS-MS)

Cathine, cathinone, and amphetamine-D5 were detected using an LCQ Fleet Ion Trap Mass Spectrometer (Thermo Fisher Scientific, Inc.) attached to an autosampler [34]. Thermo X-calibur® software was used to connect and control all instruments. At ambient temperature, liquid chromatography analysis of the control and test compounds was performed on a C18 column (Hypersil Gold; 150×3 mm I.D.; particle size, 5 μ m, Thermo Scientific). The mobile phase (20:80 v/v) consisted of ammonium formate buffer (10 M) with 0.1% formic acid. The mobile phase was run at a flow rate of 300 mL/min by using an isocratic technique. The detector settings were improved by directly injecting chemicals into a mass-selective detector. Autotuning of amphetamine was performed using a tune file stored for use in the acquisition technique. All substances were positively charged with $[M + H]^+$ at the LC-MS interface (ESI) using electrospray ionization. Compounds were identified using LCMS-MS in the full scan mode for m/z ranges of 85–200. Cathine, cathinone, and the internal standard amphetamine-D5 were identified and quantified using collision-induced dissociation of precursor ions at m/z 152, 150, and 141 to create fragment ions at m/z 134, 132, and 119, respectively.

2.6 Docking

2.6.1 Ligands' identification and preparation

The Khat-associated ligands cathine and cathinone and available drugs for weight control, obtusifolioside, and cassiaside B2 (Fig. 2), were obtained from PubChem (National Center for Biotechnology Information, National Library of Medicine, USA), an online database [35]. hemSketch was used to create the chemical structures of the ligands [36]. Open Babel software was used to produce three-dimensional structures of the ligands [37], which were then saved as SDF files for further preparation and molecular docking studies.

2.6.2 Identification and preparation of protein targets

The protein structures of serotonin receptors (5-HT_{2C}) are shown in Fig. 3. Their structures were downloaded from the Research Collaboratory for Structural Bioinformatics Protein Data Bank (PDB) [38]. Two 5-HT_{2C} serotonin subtype receptors were chosen as target proteins for the docking investigations, as shown in Table 1. These were later referred to as "G" (5-HT_{2C} in an agonist-bound active

conformation; chain-A of PDB ID: 6BQG) and "H" (5-HT2C in an antagonist-bound inactive conformation; chain A of PDB ID: 6BQH), respectively. MOE and PyMol software were used to visualize and analyze molecular structures in an interactive manner so that protein-ligand interactions, geometry optimization, and the pre-docking technique could be better understood. Hydrogens and Gasteiger charges were added to the protein structure, and all heteroatoms and water molecules were removed. MMFF94's force field parameters were used to optimize protonated proteins to eliminate unfavorable steric conflicts [39, 40]. The protonated and optimized structures were preserved for further preparation and analysis. Molecular Operating Environment (MOE) software was used to execute all minimizations (grade < 0.001).

Table 1
Selected 5-HT2C serotonin receptor

Protein target	Description
G	5-HT2C in an agonist-bound active conformation; chain A of PDBID: 6BQG
H	5-HT2C in an antagonist-bound inactive conformation; chain A of PDBID: 6BQH

2.6.3 Molecular docking and setting

MOE was used to conduct docking tests with ten poses for each ligand (the other parameters were kept at default). Superfluous chains were removed. Different types of ligand interactions with the X-ray co-crystallized protein were determined, so that the next docking technique could be used. Docking was accomplished using MOE's default DOCK settings, which were as follows: the scoring function was London dG., and the ten ligand conformers with the highest and best scores were preserved by default. The scoring configuration of the ligand-target complexes was determined based on energetic grounds (MM/GBVI). For each compound, the optimal positions with the lowest binding energies were chosen. Docking scores, binding energies, and chemical structures of the selected ligands are shown.

2.6.4 Toxicity and drug likeness prediction

A web server called ProTox was used to predict the acute oral toxicity of cathine and cathinone based on their chemical similarities with known toxic properties and presence of toxic fragments [41]. Toxicity classifications were defined using the admetSAR program, in line with the worldwide standardized system of chemical categorization and labeling (GHS) [35]. Classes I and II are fatal, class III is toxic, class IV is harmful, class V may be harmful, and class VI is non-toxic in nature. Toxic doses were specified as LD50 values in mg/kg body weight, where LD50 is an approach that yields an in silico drug compound for particular proteins. The pharmacokinetics and drug-likeness of the ligands were evaluated using SwissADME [42]. SwissADME is a web-based tool for this purpose.

2.7 Statistical analysis

The data were analyzed using SPSS version 21.00. The data are presented as mean $\pm$ S.E.M. One-way ANOVA and Student's t-test were used to compare the mean differences. A full factorial analysis of variance was used to analyze the body weight based on sex and group. The L.S.D. test was used for post-hoc analysis. Alpha was set at 0.05.

3. Results

3.1 Effects of CEWE on weight gain

Table 2 lists the final weights and weight gain percentages. A full factorial ANOVA revealed that sex and dose had significant effects ($P < 0.05$). Female rats (240.6 ± 16.1 g) were significantly ($P < 0.05$) different from male rats (295.2 ± 38.4 g). There was a significant change ($P < 0.05$) in the weight gain percentage of the CEWE-administered group (100 mg/kg) compared to that of the control group. No interaction ($P > 0.05$) was observed between sex and group in the model.

Table 2

The effects of khat extract administration on male and female rats' body weight. The duration of the experiment was 8 weeks (First study)

Dose (mg/kg body weight/day)	Sex of the rats		Total for both sexes			
	Female		Male			
	Final weights (gm)	Weight gain (%)	Final weights (gm)	Weight gain (%)	Final weights (gm)	Weight gain (%)
Control Groups	246.4 ± 12.1 ^a	18.26 ± 1.9 ^a	299.0 ± 42.4 ^a	50.60 ± 7.5 ^a	269.77 ± 8.95 ^a	32.63 ± 1.2 ^a
100 mg/Kg	233.8 ± 10.4 ^a	14.79 ± 2.6 ^a	270.6 ± 9.9 ^a	19.23 ± 2.5 ^b	252.20 ± 11.63 ^a	17.01 ± 0.9 ^b
300 mg/Kg	232.6 ± 17.1 ^a	14.14 ± 1.5 ^a	287.4 ± 18.4 ^a	24.55 ± 3.6 ^b	260.00 ± 3.38 ^b	19.34 ± 0.8 ^c
500 mg/Kg	251.8 ± 20.3 ^a	18.86 ± 0.9 ^a	324.4 ± 14.2 ^a	38.67 ± 5.8 ^c	292.11 ± 5.58 ^c	29.87 ± 2.5 ^a
Total	240.6 ± 16.1 ^a	16.39 ± 0.8 ^a	295.2 ± 38.4 ^a	32.35 ± 1.5 ^c	267.86 ± 10.10 ^d	24.37 ± 1.8 ^a

Groups with different superscript letters are significantly different ($P < 0.05$). SPSS software was used to analyze the data. ANOVA was used to test the differences between the groups' means, followed by *post hoc* analyses.

3.2 Levels of leptin and ghrelin

The effects of eight weeks of oral CEWE dosing on plasma levels of leptin and ghrelin in female and male rats were measured using ELISA (Fig. 4). The mean leptin levels in male rats are 0.58 ± 0.18 , 0.30 ± 0.15 , 0.76 ± 0.30 , and 0.76 ± 0.33 ng/mL for normal control groups at 100 mg/kg, 300 mg/kg, and 500 mg/kg, respectively, with no significant differences ($P > 0.05$). At the end of the study, CEWE did not cause any significant changes in plasma ghrelin levels in the different experimental groups (Fig. 4). As shown in Figs. 4A and 4B, oral administration of CEWE (100, 300, and 500 mg/kg) for two hours did not lead to any variation in the plasma levels of leptin and ghrelin. In addition, a high dose of CEWE (12.4 g/kg), comparable to the daily human dose, did not affect the levels of these two hormones (Figs. 4C and 4D). Figure 4E and 4F show the findings of the eight weeks experiment.

3.3 Antagonistic effect of Khat extract on 5-HT receptors

Figure 5 shows the concentration-response curve of the rat fundus strips ($n = 5$) in the presence of different concentrations of 5-HT. The highest contraction of gastric tissue was recorded at four ng/mL, and the response was concentration dependent ($P < 0.05$). Pre-administration of Khat extract (50 µg/mL) led to the complete antagonism of 5-HT (1 ng/mL). It can be concluded that khat inhibits the contractile effect of 5-HT on rat fundus tissue in a dose-dependent manner, as shown in Fig. 5. Nitrendipine was used as the control to inhibit the contractile effects of 5-HT.

3.4 Gene expression

Effects of two hours' oral administration of CEWE (100, 300, and 500 mg/kg body weight) on plasma levels of ghrelin, leptin, 5-HT are shown in Fig. 6. The gene expression levels of leptin, ghrelin, and 5-HT were analyzed in different treatment groups (Fig. 6). In the control group, the expression of leptin was measured as 1 ± 0.01 -fold of the control. Following treatment with CEWE at doses of 100 mg/Kg, 300 mg/Kg, and 500 mg/Kg, the fold control values for leptin expression were 1.1 ± 0.01 , 1.12 ± 0.009 , and 1.1 ± 0.008 , respectively. These results indicate that there were no significant changes observed in the expression of leptin in response to CEWE treatment at any of the tested doses. No significant differences were observed in the ghrelin mRNA levels between the groups (Fig. 6). The gene expression levels of 5-HT were analyzed in different treatment groups. Treatment with CEWE at doses of 100 mg/Kg, 300 mg/Kg, and 500 mg/Kg resulted in a significant decrease in 5-HT expression compared to the control group ($p < 0.05$). The decrease in 5-HT expression was dose-dependent, with the highest dose of 500 mg/Kg showing the most pronounced reduction.

3.5 LC-MS-MS quantification of Khat components in plasma

Cathine and cathinone were retained on the column with good peak shapes at retention times of 6.02 and 6.09 minutes, respectively, as shown in Fig. 7. These khat alkaloids were detected in the full scan mode with high sensitivity using LC-MS-MS. No significant endogenous peaks interfering with the cathine or cathinone peaks were identified in the chromatograms of blank plasma samples. The calibration curve between 20 and 1000 ng/mL exhibited excellent linearity with a Pearson's correlation coefficient of 0.9998. These compounds were identified in the plasma of rats treated with 300 mg/kg khat extract. Cathine and cathinone concentrations were 28.72 and 91.13 ng/mL, respectively.

3.6 Molecular docking and *in silico* prediction of toxicity and pharmacokinetics

3.6.1 Khat compound interactions with 5-HT_{2C}: Putative agonists and antagonists

Docking of the two khat ligands was performed on G (5-HT_{2C} in an agonist-bound active conformation) and H (5-HT_{2C} in an antagonist-bound inactive conformation) proteins to determine which ligands are most likely to act as agonists or antagonists. Figures 8 and 9 show how well cathine and cathinone docked to the G and H 5-HT_{2C} proteins compared to how well the reference drugs bound to those proteins. Both cathine and cathinone bound to G, but at different sites for cassiaside B2 and obtusifoliol, away from the hydrophobic pocket that resembles the agonist-binding site (Figs. 8A and 8B). However, cathine and cathinone had relatively higher binding affinities than that of obtusifoliol (Figs. 8C and 8D). Figures 8C and 8D show that cathine and cathinone bound to H at the same binding sites as those of the reference compounds (Figs. 9A and 9B). As a result, we examined the relative differences in the binding affinities of G and H to classify the khat ligands as acceptable agonists and antagonists. Inspection of the contacts between cathine and G protein indicated two residues with a combination of polar, non-polar, and acidic residues (Figs. 9A and 9B). The two reference compounds exhibited positive $\Delta\Delta G$ values greater than +1 kcal/mol (cassiaside B2) with $\Delta\Delta G = +1$ kcal/mol (obtusifoliol) and $\Delta\Delta G = +0.6$ kcal/mol. These results confirmed the agonistic activity of the reference compounds. However, cathine and cathinone exhibited negative $\Delta\Delta G$; cathine, with $\Delta\Delta G = -1.6$, and cathine and cathinone, with $\Delta\Delta G = -0.3$ (Table 4). These results indicated that the khat ligands had antagonistic activity against 5-HT_{2C}.

Table 4
Binding affinity and difference in $\Delta\Delta G$ (free energy) values between the predicted binding affinity of each Khat ligands to G and H

Ligand name	Formula	MW/ g/mol	Binding affinity (kcal/mol)		$\Delta\Delta G$ (kcal/mol) (H - G)
			G	H	
Cassiaside B2	C ₃₉ H ₅₂ O ₂₅	920.82	-10.2	-9.2	1
Obtusifoliol	C ₃₀ H ₅₀ O	426.72	-6.5	-5.9	0.6
Cathine	C ₉ H ₁₃ NO	151.21	-6.8	-8.4	-1.6
Cathinone	C ₉ H ₁₁ NO	149.19	-7.5	-7.8	-0.3

3.6.2 Proposed agonist and antagonist ligand protein interactions

In addition to the free binding energy, the molecular interaction between the ligands and the binding site of the two 5-HT_{2C} serotonin receptors is essential for determining whether the ligands can be recognized as agonists or antagonists. First, molecular interactions were observed between cassiaside B2 and obtusifoliol, the standard ligand, and two 5-HT_{2C} serotonin receptors. As shown in Fig. 10A, cassiaside B2 is shown to interact with the amino acids of the G protein as follows: Asp 1002, Arg 243, Asn 1006, and Ala 1001 via hydrogen bonds. Moreover, cassiaside B2 exhibits nonpolar interactions with Asp 1005, Asp 1039, Glu 1008, and Lys 1042, in addition to the polar side-chain acceptor Gln 244. In contrast, the molecular interaction of obtusifoliol with the G protein, as presented in Fig. 10B, displayed interactions with six amino acids via hydrogen bonds with the polar side chain acceptor Asn 158. In addition, there is a nonpolar acidic side-chain donor with Asp 1002 and a backbone of Arg 157, Arg 243, Lys 1042, and Ile 160. An inspection of the contacts for cathine with G protein, as shown in Fig. 11A, indicates that four residues interact via hydrogen bonding with a non-polar acidic side chain donor with Asp 134. In addition, cathine interacted with the backbone of Val 135 and phenylalanine residues, including Phe 327 and Phe 328. Similarly, inspection of cathinone contacts with the G protein, as shown in Fig. 11B, revealed seven combinations of non-polar and polar residues. Interestingly, cathinone displays π - π ring stacking interactions with Phe 327, polar side chains with Tyr 358, and non-polar interactions with Asp 134 and the backbone of Val 135, Val 354, Ile 131, and Trp 324.

Similarly, the molecular interactions between the ligands and binding site of the H protein were examined to determine whether cathine and cathinone could be recognized as antagonists. First, the molecular interaction between cassiaside B2 and obtusifoliol, the standard ligand, and H protein was observed. As shown in Fig. 12A, cassiaside B2 interacts with 14 amino acids of the H protein, forming hydrogen bonds with the polar side chain acceptor Asn 203 and backbone donor Leu 350. There are also contacts with non-polar interactions between the acidic side chain donors Glu 198 and Glu 347, backbone donors Leu 209, Val 354, Val 202, and Val 208, and two residues of phenylalanine, including Phe 201 and Phe 327. Moreover, cassiaside B2 was in contact with the polar side-chain acceptors Asn 210, Asn 351, Gln 343, and Tyr 118. In contrast, the molecular interaction of obtusifoliol with the H protein, as presented in Fig. 12B, displayed interactions with 12 amino acids and the polar side chain acceptors Asn 331 and Asn 210. In addition, there is a non-polar acidic side-chain donor with Asp 134 and Glu

347. In addition, there were interactions with the backbone donors Ile 131, Leu 209, Leu 350, Trp 130, Val 202, Val 208, Val 354, and Phe 327. An inspection of the contacts for cathine with the H protein, as shown in Fig. 13A, indicates that the two residues interact via hydrogen bonding with a non-polar acidic side chain donor with Asp 134 and the polar side chain acceptor with Tyr 358. In addition, cathine interacted with the backbone donors Trp 130, Val 208, and Val 354, and a phenylalanine residue, Phe 327. Similarly, inspection of cathinone contacts with the H protein, as shown in Fig. 13B, revealed four combinations of non-polar and polar residues and high ligand exposure to the aromatic and methyl groups. Interestingly, cathinone displays a hydrogen bond with the acidic side-chain donor Asp 117. In addition, there were contacts with the polar side-chain acceptors Asn 54, Gln 53, and Tyr 118.

3.6.3 Common residues interacting with khat compounds

Figure 14 and 15 show the residues that formed the most common contacts with the cathine and cathinone, respectively. As depicted in Table 5, a "1" marks the presence of a connection between the residue (rows) and the ligand (columns) if any atom of the residue or ligand lies within four angstroms of each other. The right-most column totals all these interactions for each residue and provides the total number of interacting molecules that come into contact with each residue.

Table 5

Common ligand-binding residues of selected ligands. 1: the presence of a contact between the residues (rows) and the ligand (columns) if any atom of the residue or ligand lies within 4 Å. Gray-colored rows indicate residues of remarkable importance.

Residues	Cathine - G	Cathinone - G	cassiaside B2 - H	obtusifoliol - H	Cathine - H	Cathinone - H	Sum
Asp 134	1	1		1	1		4
Asp 117						1	1
Asn 54						1	1
Asn 203			1				1
Asn 210			1				1
Asn 331				1			1
Asn 351			1				1
Gln 53						1	1
Gln 343			1				1
Glu 198			1				1
Glu 347			1	1			2
Ile 131		1		1			2
Leu 209			1	1			2
Leu 250			1				1
Leu 350				1			1
Phe 201			1				1
Phe 327		1	1	1			3
Phe 328	1						1
Trp 130				1	1		2
Trp 324		1					1
Tyr 358					1		1
Tyr 118			1			1	2
Val 135	1	1					2
Val 208			1	1	1		3
Val 202			1	1			
Val 354			1	1	1		3

3.6.4 Toxicity analysis and drug likeness

The Protox-II webserver was used to verify the toxicity of the compounds and determine their carcinogenic, immunogenic, hepatotoxic, and cytotoxic properties. Table 6 displays the results of the LD₅₀ toxicity class, indicating that cathine was more toxic than cathinone, in which they were in classes 3 and 4, respectively; their GI absorption was low. However, cathinone but not cathine showed probable carcinogenicity. In contrast, the reference substances, cassiaside B2 and obtusifoliol, showed carcinogenicity and immunotoxicity, respectively (Fig. 16).

Table 6
ADMET prediction

Compound	Lipinski	GI absorption	BBB permanent	LD ₅₀ Class	Predicted LD ₅₀ mg/kg	Carcinogenicity ~ Probability	Immunotoxicity	nrf2/ARE	HSE
Cassiaside B2	No; 3 violations: MW > 500, N or O > 10, NH or OH > 5	LOW	NO	5	5000	Active	Active	Inactive	Inactive
Obtusifolioside	Yes; 1 violation MLOGP > 4.15	LOW	NO	4	2000	Inactive	Active	Active	Active
Cathine	Yes; 0 violation	High	Yes	3	150	Inactive	Inactive	Inactive	Inactive
Cathinone	Yes; 0 violation	High	Yes	4	400	Active ~ 0.64	Inactive	Inactive	Inactive
nrf2/ARE: Nuclear factor (erythroid- derived 2)-like 2/antioxidant responsive element, HSE: Heat shock factor response element BBB: Blood Brain Barrier, LD ₅₀ classes: Class I: fatal if swallowed (LD ₅₀ ≤ 5 mg/kg), Class II: fatal if swallowed (5 < LD ₅₀ ≤ 50 mg/kg) Class III: toxic if swallowed (50 < LD ₅₀ ≤ 300 mg/kg), Class IV: harmful if swallowed (300 < LD ₅₀ ≤ 2000 mg/kg), Class V: may be harmful if swallowed (2000 < LD ₅₀ ≤ 5000 mg/kg), Class VI: nontoxic (LD ₅₀ > 5000 mg/kg),									

4. Discussion

This study describes the role of khat in appetite and body weight by using *in vivo*, *ex vivo*, and *in silico* models. This is the first study to include male and female rats fed varying durations and doses of fresh khat extract. We also focused on the effect of khat on 5-HT receptors, which was explained using the rodent fundus and bioinformatics tools. Fresh khat was used to link this research to the practical side because drying reduces the amount of active compounds [25]. It was also found that, in addition to other limitations, the available studies on the effect of khat on appetite are flawed owing to the use of dried khat [5, 7]. To further validate the results of this study, the plasma of CEWE-treated rats was examined using LC-MS/MS to quantify the levels of cathine and cathinone. LC-MS is currently regarded as the best method for the detection of amphetamine-like compounds in research and forensic laboratories [43].

The present study aimed to investigate the potential anti-obesity effects of CEWE in rats over an eight-week period. Treatment with CEWE at doses of 100, 300, and 500 mg/kg resulted in significant reductions in body weight gain compared with the control group. These findings align with those of previous studies that have explored the effects of khat consumption on weight loss, including earlier research [4, 7, 8, 25]. Studies have shown that khat decreases body weight in rat models. Previous research. These studies consistently demonstrated that khat administration leads to decreased body weight in rat models. Notably, sustained weight loss has been observed in trials utilizing higher doses or longer treatment durations, whereas lower doses have shown short-term weight loss, followed by the development of tolerance to this effect [44]. Moreover, a cross-sectional study conducted among healthy individuals in Ethiopia revealed that khat users exhibited a lower mean body weight than khat non-users [45]. These collective findings support the potential of CEWE as an agent for weight management, and highlight the need for further exploration of its mechanisms and long-term effects. In our study, we ensured the uniformity of key factors by selecting Sprague-Dawley rats of the same age, housing them in standardized cages, and maintaining consistent light, humidity, and temperature conditions. These measures were taken to minimize variations in growth, development, physical activity, and environmental

factors. By controlling for these confounding factors, we aimed to enhance the internal validity of our findings and provide a more reliable experimental experience.

Leptin and ghrelin are hormones that regulate appetite. The activation of leptin or ghrelin receptors in the hypothalamus triggers a series of signaling cascades that result in variations in food intake [18, 22]. Further studies in rodents and humans are needed to determine how khat affects leptin levels. There have been only two studies in rats and humans, but they did not provide sufficient evidence to support the leptin-lowering effect of khat. This may be due to the differences in the extraction method, treatment time, or dose. Both studies attempted to explain how gut hormones and stomach emptying influence changes in leptin secretion [21, 24]. In this study, CEWE did not result in significant changes in plasma leptin levels among rats in the different experimental groups. However, we observed a difference in hormone levels between the male and female rats. Furthermore, leptin levels in the blood follow a diurnal pattern and are regulated by sex, age, activity, and glucose intake [46]. The current study revealed no change in leptin levels after testing Khat for two hours. We noted that khat had no short-term effects on appetite through these two hormonal pathways. Similar findings have been reported previously [24]. In contrast, we found that khat did not affect ghrelin levels, regardless of the dose or time. It was found that there was a previous report in which Murray and his co-researchers administered fresh khat to six habitual users for three hours and found no changes in the level of ghrelin in the blood [24].

Serotonin (5-HT) is known to play a role in appetite regulation. Serotonin has been shown to influence appetite reduction in the hypothalamus by increasing the levels of two anorexigenic peptides [47]. In rats, 5-HT medications reduce food intake in a manner consistent with an increase in fullness. They have been proven to reduce calorie intake in humans, and are linked to decreased appetite and increased satiety. 5-HT_{2C} receptor subtype also plays a role in these effects. 5-HT-acting medications, such as fenfluramine, d-fenfluramine, and sibutramine, are effective anti-obesity therapies. Khat also possesses serotonin-related properties [48, 49]. Cathinone, for example, has been shown to increase serotonin release from the striatum *in vitro*, bind to serotonin receptors, and inhibit serotonin absorption after a single or repeated dose [50]. In this study, khat extract antagonized 5-HT in the rat fundus strips. The serotonin-antagonizing properties of khat may explain its anorexic properties of this psychoactive plant. Our results support the proposed mechanism of the appetite-suppressive action of khat, which alters dopamine and serotonin physiology. Fenfluramine is a weight loss medication that suppresses hunger by releasing serotonin and dopamine into the striatum [51].

The current study was designed to determine the ability of cathine and cathinone to act as agonists or antagonists of two 5-HT_{2C} receptors, G and H, subtypes of serotonin receptors that play an essential role in reducing appetite. Furthermore, cassiaside B2 and obtusifolioside were selected as the standard ligands for the 5-HT_{2C} receptor. This simulation shows that cathine and cathinone act as antagonists against 5-HT_{2C} receptors G and H with lower free binding energies than obtusifolioside but higher free binding energies than cassiaside B2. First, the highly conserved residue Asp 134's molecular contact with molecules is well defined as being essential for agonist activity [52]. Cathine and cathinone interacted with the G protein via Asp 134 and numerous polar and nonpolar hydrogen bonds with residues 3 and 6, respectively. However, none of these matched with the contacts of the reference compounds. Interestingly, these residues are well established as essential for the ligand-binding activity of small molecules [53]. These contacts suggest the probable agonistic activities of cathine and cathinone. Similarly, ligands contact the H protein through polar and nonpolar hydrogen bonds with six and four residues, respectively. Some of these, including Asp 134, Trp 130, Val 208, Val 354, Tyr 118, and Phe 327, are essential for the antagonistic activity of standard ligands against the H protein. Predicted and antagonistic interactions typically involve a combination of ring-stacking with aromatic residues supported by clusters of other direct hydrogen bonds and smaller non-polar, polar, and acidic residues. The current study acknowledged the number of chief residues involved in the appetite inhibition effect of cathine and cathinone, including Asp 134, Phe 327, Val 202, and Val 354. Together, the findings of *ex vivo* and *in silico* studies support the role of khat components as 5-HT antagonists.

SwissADME was used to study the pharmacokinetic properties of cathine and cathinone. This free website studies drug-likeness parameters based on Lipinski's rule of five, which helps define the level of absorption or permeability of lipid bilayers present in the human body, validating the oral bioavailability of a molecule [42]. Lipinski's rule is a promising bioavailable compound, where the maximum molecular weight is 500 Da, hydrogen bond acceptors are less than 10, hydrogen bond donors are less than five, and log P values are less than five [54]. Cathine and cathinone obey Lipinski's rules. Interestingly, the gastrointestinal absorption of the ligands is high, which means that cathine and cathinone more effectively bind to G and H, thereby affecting human appetite. Compounds with higher absorption rates are more effective [55]. In addition to gastrointestinal absorption, the ligands were examined for blood-brain barrier (BBB) penetration, which is explained as the concentration ratio of steady-state radiolabeled molecules in the brain (C_{brain}) to that in peripheral blood (C_{blood}) [56]. The prediction of BBB penetration for ligands is highly important because 5-HT_{2C} receptors are widely distributed in human brain tissue and are expressed in dopaminergic cell body regions of the substantia nigra and ventral tegmental area, as well as in terminal projection areas of the nucleus accumbens, striatum, and prefrontal cortex [57]. According to SwissADME, cathine and cathinone exhibit high BBB penetration, suggesting that they easily and effectively interact with their targets G and H. This *in silico* study also predicted the toxicity levels of cathine and cathinone using the Protox website, which revealed low LD₅₀ values. This result aligns with the World Health Organization (WHO)

classification of khat as a class C drug [58]. Long-lasting khat consumption leads to cancer in the human body through cellular oxidative stress, which is ascribed to the ability of khat leaves to undergo naturalization in the gut to produce nitrosamines [59].

Conclusions

In this study, we effectively measured the active components of khat in rat plasma using liquid chromatography-mass spectrometry. The study found that The water extract of khat caused a decrease in rat weight without affecting the levels of leptin and ghrelin, which are hormones related to appetite regulation. Both ex vivo and in silico experiments have revealed that khat has antagonistic effects on 5-HT receptors. The appetite-suppressing properties of khat can be attributed to its central serotonergic activity. This study presents the initial findings of a computational assessment of the pharmacokinetics and toxicity of cathine and cathinone. Further research is recommended to validate the appetite-suppressing effects of khat, explore its therapeutic applications, conduct toxicity evaluations, investigate the underlying mechanisms, and raise public health awareness. These include additional empirical studies, clinical trials, computational assessments, mechanistic studies, comprehensive toxicity evaluations, collaborative research, and public education. We recommend combining western blotting and immunofluorescence techniques in future studies to further validate and strengthen our findings.

Abbreviations

Å: Angstrom

$\Delta\Delta G$: Free Energy

µl: Microliter

5-HT: 5-hydroxytryptamine

ACN: Acetonitrile

ADMET: Absorption, Distribution, Metabolism, Excretion, Toxicity

Ala: Alanine

ANOVA: Analysis Of Variance

Arg: Arginine

Asn: Asparagine

BBB: Blood-Brain Barrier

CEWE: *Catha Edulis* Water Extract

ELISA: Enzyme-Linked Immunosorbent Assay

ESI: Electro-Spray Ionization

GHS: The Globally Harmonized System of Classification Of Labeling Of Chemicals

GI: Gastrointestinal Tract

Gln: Glutamine

HCL: Hydrochloric Acid

Kcal/Mol: Kilocalorie per Mole

KSA: Kingdom Of Saudi Arabia

LC-MS: Liquid Chromatography – Mass Spectroscopy

LD50: Lethal Dose 50%

LSD: Least Squared Differences

Lys: Lysine

M/Z: Mass-To-Charge Ratio

Mg/Kg: Milligram per Kilogram

MMFF94s: Merck Molecular Force Field Variant 94

MOE: Molecular Operating Environment Software

MRC: Medical Research Centre

MS: Mass Spectrometer

MSD: Mass Spectrometer Detector

ng/mL: Nanogram per Milliliter

RCSB-PDB: Structural Bioinformatics Protein Data Bank

RT-PCR: Real-Time Polymerase Chain Reaction

S.E.M.: Standard Error of the Mean

SDF: Single-User Geodatabase File Format

SWGDRUG: Scientific Working Group for the Analysis of Seized Drugs

Trp: Tryptophan

Val: Valine

Declarations

Acknowledgment

The authors extend their appreciation to the Deputyship for Research & Innovation, Ministry of Education in Saudi Arabia, for funding this research work through project number ISP23-82.

Disclosure

The authors report no conflicts of interest in this work.

Authors' contribution

MMET: Investigation, Roles, Writing – original draft, data curation. SIA: Data curation, Investigation, Supervision, Roles, Writing – original draft. HAY, AF, and AAJ: Formal analysis, investigation. SSM: Formal analysis, investigation. ESS: Investigation. BO, MO and IK: Investigation, Writing – review, and editing. HMAS: Computational work.

References

1. Kassim, S. The impact of protective psychosocial factors on khat chewing among male medical and dental future health-care providers in Yemen. *Journal of Dental Sciences* **2020**, *15*, 451-456, doi:10.1016/j.jds.2019.08.009.
2. Woldemariam, G.W.; Iguala, A.D.; Tekalign, S.; Reddy, R.U. Spatial Modeling of Soil Erosion Risk and Its Implication for Conservation Planning: the Case of the Gobeles Watershed, East Hararghe Zone, Ethiopia. *Land* **2018**, *7*, 25.

3. Adane, T.; Worku, W.; Azanaw, J.; Yohannes, L. Khat Chewing Practice and Associated Factors among Medical Students in Gondar Town, Ethiopia, 2019. *Substance Abuse* **2021**, *15*, 1178221821999079, doi:10.1177/1178221821999079.
4. Cox, G.; Rampes, H. Adverse effects of khat: a review. *Advances in psychiatric treatment* **2003**, *9*, 456-463.
5. Alshagga, M.A.; Alshawsh, M.A.; Seyedan, A.; Alsalahi, A.; Pan, Y.; Mohankumar, S.K.; Alkebsi, A.; Kassim, S.; Mohamed, Z. Khat (*Catha edulis*) and obesity: A scoping review of animal and human studies. *Annals of Nutrition and Metabolism* **2016**, *69*, 200-211.
6. Sabir, N.O.; Hamad, A.E.H.; Abdelwahab, S.I.; Taha, M.M.E.; Ahmed, B.M. Pharmacological studies of catha edulis forsk. on isolated animals tissues. *World Journal of Pharmaceutical Research* **2017**, *7*, 108.
7. Alshagga, M.A.; Mohamed, Z.; Seyedan, A.; Ebling, F.J.; Alshawsh, M.A. Khat (*Catha edulis*) upregulates lipolytic genes in white adipose tissue of male obese mice (C57BL/6J). *Journal of ethnopharmacology* **2020**, *262*, 113187.
8. Bereda, G. Catha Edulis Forsk and Its Adverse Effects On Health: Current and Ongoing Factuality. *Ann Clin Med Case Rep* **2021**, *7*, 1-10.
9. Abebe, W. Khat and synthetic cathinones: emerging drugs of abuse with dental implications. *Oral surgery, oral medicine, oral pathology and oral radiology* **2018**, *125*, 140-146.
10. Sporkert, F.; Pragst, F.; Bachus, R.; Masuhr, F.; Harms, L. Determination of cathinone, cathine and norephedrine in hair of Yemenite khat chewers. *Forensic science international* **2003**, *133*, 39-46.
11. Pendl, E.; Pauritsch, U.; Kollroser, M.; Schmid, M.G. Determination of cathinone and cathine in Khat plant material by LC-MS/MS: Fresh vs. dried leaves. *Forensic science international* **2021**, *319*, 110658.
12. Kalyanasundar, B.; Perez, C.I.; Arroyo, B.; Moreno, M.G.; Gutierrez, R. The Appetite Suppressant D-norpseudoephedrine (Cathine) Acts via D1/D2-Like Dopamine Receptors in the Nucleus Accumbens Shell. *Frontiers in neuroscience* **2020**, *14*.
13. Zelger, J.L.; Carlini, E. Anorexigenic effects of two amines obtained from *Catha edulis* Forsk.(Khat) in rats. *Pharmacology Biochemistry and Behavior* **1980**, *12*, 701-705.
14. Collins, J. Physical performance and muscle mass in the assessment of sarcopenia and its association with receipt and completion of planned treatment in non-small cell lung cancer. Cardiff University, 2017.
15. Baig, A.M. DARK side of amphetamine and analogues: pharmacology, syndromic manifestation, and management of amphetamine addiction. **2018**.
16. Thorat, V.M.; Khanwelkar, C.C.; Matule, S.M.; Salve, P.S.; Surle-Patil, S.A.; Sadanandan, S. Effect of Duloxetine Pretreatment on 5-Htp and Dexfenfluramine Induced Behaviours in Albino Rats. *Biomedical and Pharmacology Journal* **2019**, *12*, 1339-1343.
17. Foltin, R.W.; Evans, S.M. Sex differences in the anorexigenic effects of dexfenfluramine and amphetamine in baboons. *Experimental and clinical psychopharmacology* **2018**, *26*, 335.
18. Püschel, J.; Miehle, K.; Müller, K.; Villringer, A.; Stumvoll, M.; Fasshauer, M.; Schlögl, H. Beneficial effects of leptin substitution on impaired eating behavior in lipodystrophy are sustained beyond 150 weeks of treatment. *Cytokine* **2019**, *113*, 400-404.
19. Kroemer, G.; Zitvogel, L. Ghrelin and leptin regulating wound healing. *Trends in Immunology* **2022**.
20. Müller, T.D.; Nogueiras, R.; Andermann, M.L.; Andrews, Z.B.; Anker, S.D.; Argente, J.; Batterham, R.L.; Benoit, S.; Bowers, C.Y.; Broglio, F. Ghrelin. *Molecular metabolism* **2015**, *4*, 437-460.
21. Mahmood, S.A.; Lindequist, U. A pilot study on the effect of *Catha edulis* Frosk., (Celastraceae) on metabolic syndrome in WOKW rats. *Afr J Tradit Complement Altern Med* **2008**, *5*, 271-277, doi:10.4314/ajtcam.v5i3.31283.
22. Poher, A.-L.; Tschöp, M.H.; Müller, T.D. Ghrelin regulation of glucose metabolism. *Peptides* **2018**, *100*, 236-242.
23. Lemieux, A.M.; Li, B.; al'Absi, M. Khat use and appetite: an overview and comparison of amphetamine, khat and cathinone. *Journal of ethnopharmacology* **2015**, *160*, 78-85.
24. Murray, C.D.; Le Roux, C.W.; Emmanuel, A.V.; Halket, J.M.; Przyborowska, A.M.; Kamm, M.A.; Murray-Lyon, I.M. The effect of khat (*Catha edulis*) as an appetite suppressant is independent of ghrelin and PYY secretion. *Appetite* **2008**, *51*, 747-750.
25. Al-Motarreb, A.; Al-Habori, M.; Broadley, K.J. Khat chewing, cardiovascular diseases and other internal medical problems: the current situation and directions for future research. *Journal of ethnopharmacology* **2010**, *132*, 540-548.
26. Dhabbah, A.M. Determination of chiral cathinone in fresh samples of *Catha edulis*. *Forensic science international* **2020**, *307*, 110105.
27. Alfaifi, H.; Abdelwahab, S.I.; Mohan, S.; Elhassan Taha, M.M.; Syame, S.M.; Shaala, L.A.; Alsanosy, R. Catha edulis Forsk. (Khat): Evaluation of its Antidepressant-like Activity. *Pharmacogn Mag* **2017**, *13*, S354-S358, doi:10.4103/pm.pm_442_16.
28. Abdelwahab, S.I.; Mohan, S.; Abdulla, M.A.; Sukari, M.A.; Abdul, A.B.; Taha, M.M.E.; Syam, S.; Ahmad, S.; Lee, K.-H. The methanolic extract of *Boesenbergia rotunda* (L.) Mansf. and its major compound pinostrobin induces anti-ulcerogenic property in vivo: Possible involvement of indirect antioxidant action. *Journal of Ethnopharmacology* **2011**, *137*, 963-970, doi:https://doi.org/10.1016/j.jep.2011.07.010.

29. Akase, T.; Shimada, T.; Terabayashi, S.; Ikeya, Y.; Sanada, H.; Aburada, M. Antiobesity effects of *Kaempferia parviflora* in spontaneously obese type II diabetic mice. *Journal of Natural Medicines* **2011**, *65*, 73-80.
30. Yurtcu, N.; Caliskan, C.S.; Guvey, H.; Celik, S.; Hatirnaz, S.; Tinelli, A. Predictive and Diagnostic Value of Serum Adipokines in Pregnant Women with Intrahepatic Cholestasis. *International Journal of Environmental Research and Public Health* **2022**, *19*, 2254.
31. Kamar, S.S.; Latif, N.S.A.; Elrefai, M.F.M.; Amin, S.N. Gastroprotective effects of nebivolol and simvastatin against cold restraint stress-induced gastric ulcer in rats. *ACB* **2020**, *53*, 301-312, doi:10.5115/acb.20.055.
32. Arocho, A.; Chen, B.; Ladanyi, M.; Pan, Q. Validation of the 2- $\Delta\Delta C_t$ calculation as an alternate method of data analysis for quantitative PCR of BCR-ABL P210 transcripts. *Diagnostic Molecular Pathology* **2006**, *15*, 56-61.
33. Bouzas, N.F.; Dresen, S.; Munz, B.; Weinmann, W. Determination of basic drugs of abuse in human serum by online extraction and LC-MS/MS. *Analytical and bioanalytical chemistry* **2009**, *395*, 2499-2507, doi:10.1007/s00216-009-3036-x.
34. Mohan, S.; Abdelwahab, S.; Hobani, Y.; Syam, S.; Al-Zubairi, A.; Al-sanousi, R.; Oraiby, M. *Catha edulis* extract induces H9c2 cell apoptosis by increasing reactive oxygen species generation and activation of mitochondrial proteins. *Pharmacognosy Magazine* **2016**, *12*, 321-326, doi:10.4103/0973-1296.185732.
35. Cheng, F.; Li, W.; Zhou, Y.; Shen, J.; Wu, Z.; Liu, G.; Lee, P.W.; Tang, Y. admetSAR: a comprehensive source and free tool for assessment of chemical ADMET properties. **2012**.
36. Hunter, A.D. ACD/ChemSketch 1.0 (freeware); ACD/ChemSketch 2.0 and its tautomers, dictionary, and 3D plug-ins; ACD/HNMR 2.0; ACD/CNMR 2.0. **1997**.
37. O'Boyle, N.M.; Banck, M.; James, C.A.; Morley, C.; Vandermeersch, T.; Hutchison, G.R. Open Babel: An open chemical toolbox. *Journal of cheminformatics* **2011**, *3*, 1-14.
38. Rose, P.W.; Prlić, A.; Altunkaya, A.; Bi, C.; Bradley, A.R.; Christie, C.H.; Costanzo, L.D.; Duarte, J.M.; Dutta, S.; Feng, Z.; et al. The RCSB protein data bank: integrative view of protein, gene and 3D structural information. *Nucleic Acids Research* **2016**, *45*, D271-D281, doi:10.1093/nar/gkw1000.
39. Halgren, T.A. Merck molecular force field. I. Basis, form, scope, parameterization, and performance of MMFF94. *Journal of computational chemistry* **1996**, *17*, 490-519.
40. Halgren, T.A. MMFF VI. MMFF94s option for energy minimization studies. *Journal of Computational Chemistry* **1999**, *20*, 720-729.
41. Drwal, M.N.; Banerjee, P.; Dunkel, M.; Wettig, M.R.; Preissner, R. ProTox: a web server for the in silico prediction of rodent oral toxicity. *Nucleic acids research* **2014**, *42*, W53-W58.
42. Daina, A.; Michielin, O.; Zoete, V. SwissADME: a free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. *Scientific reports* **2017**, *7*, 1-13.
43. Attafi, I.M.; Albeishy, M.Y.; Oraiby, M.E.; Khardali, I.A.; Shaikhain, G.A.; Fageeh, M.M. Postmortem distribution of cathinone and cathine in human biological specimens in a case of death associated with khat chewing. *Arab Journal of Forensic Sciences & Forensic Medicine* **2018**, *1*, 922-930.
44. Aziz, H.A.; Peh, K.K.; Tan, Y.T.F. Herbal delivery system for treatment of obesity administration of encapsulated khat-extracts on body weight of rats. *Obesity Research & Clinical Practice* **2011**, *5*, e305-e312, doi:https://doi.org/10.1016/j.orcp.2011.03.008.
45. Girma, T.; Mossie, A.; Getu, Y. Association between body composition and khat chewing in Ethiopian adults. *BMC Research Notes* **2015**, *8*, 1-5.
46. Knutson, K.L. Impact of sleep and sleep loss on glucose homeostasis and appetite regulation. *Sleep medicine clinics* **2007**, *2*, 187-197.
47. Müller, T.D.; Blüher, M.; Tschöp, M.H.; DiMarchi, R.D. Anti-obesity drug discovery: advances and challenges. *Nature Reviews Drug Discovery* **2021**, 1-23.
48. Georgescu, T.; Lyons, D.; Heisler, L.K. Role of serotonin in body weight, insulin secretion and glycaemic control. *Journal of Neuroendocrinology* **2021**, *33*, e12960.
49. Kotańska, M.; Lustyk, K.; Bucki, A.; Marcinkowska, M.; Śniecikowska, J.; Kołaczowski, M. Idalopirdine, a selective 5-HT₆ receptor antagonist, reduces food intake and body weight in a model of excessive eating. *Metabolic brain disease* **2018**, *33*, 733-740.
50. Connor, J.D.; Rostom, A.; Makonnen, E. Comparison of effects of khat extract and amphetamine on motor behaviors in mice. *Journal of ethnopharmacology* **2002**, *81*, 65-71.
51. Schoonjans, A.-S.; Lagae, L.; Ceulemans, B. Low-dose fenfluramine in the treatment of neurologic disorders: experience in Dravet syndrome. *Ther Adv Neurol Disord* **2015**, *8*, 328-338, doi:10.1177/1756285615607726.
52. Carpenter, J.; Wang, Y.; Wu, G.; Feng, J.; Ye, X.-Y.; Morales, C.L.; Broekema, M.; Rossi, K.A.; Miller, K.J.; Murphy, B.J. Utilization of an active site mutant receptor for the identification of potent and selective atypical 5-HT_{2C} receptor agonists. *Journal of medicinal chemistry* **2017**, *60*, 6166-6190.

53. Yuen, H.; Hung, A.; Yang, A.W.H.; Lenon, G.B. Mechanisms of action of cassiae semen for weight management: A computational molecular docking study of serotonin receptor 5-HT2C. *International journal of molecular sciences* **2020**, *21*, 1326.

54. Mishra, S.; Dahima, R. In vitro ADME studies of TUG-891, a GPR-120 inhibitor using SWISS ADME predictor. *Journal of Drug Delivery and Therapeutics* **2019**, *9*, 366-369.

55. Hamza, M.; Mahmood, A.; Khan, S.; Rizwan, M.; Munir, A. Design and synthesis of novel inhibitor against Ser121 and Val122 mutations in P53 Cancer gene. *Advances in Pharmacology and Pharmacy* **2019**, *7*, 63-70.

56. Kovačević, S.Z.; Jevrić, L.R.; Kuzmanović, S.O.P.; Lončar, E.S. Prediction of in-silico ADME properties of 1, 2-O-isopropylidene aldohexose derivatives. *Iranian journal of pharmaceutical research: IJPR* **2014**, *13*, 899.

57. Higgins, G.A.; Fletcher, P.J. Serotonin and drug reward: focus on 5-HT2C receptors. *European journal of pharmacology* **2003**, *480*, 151-162.

58. Omare, M.O. Contemporary trends in the use of Khat for Recreational purposes and Its possible Health implications. *Open Access Library Journal* **2020**, *7*, 1.

59. Nigussie, T.; Gobena, T.; Mossie, A. Association between khat chewing and gastrointestinal disorders: a cross sectional study. *Ethiopian journal of health sciences* **2013**, *23*, 123-130.

Figures

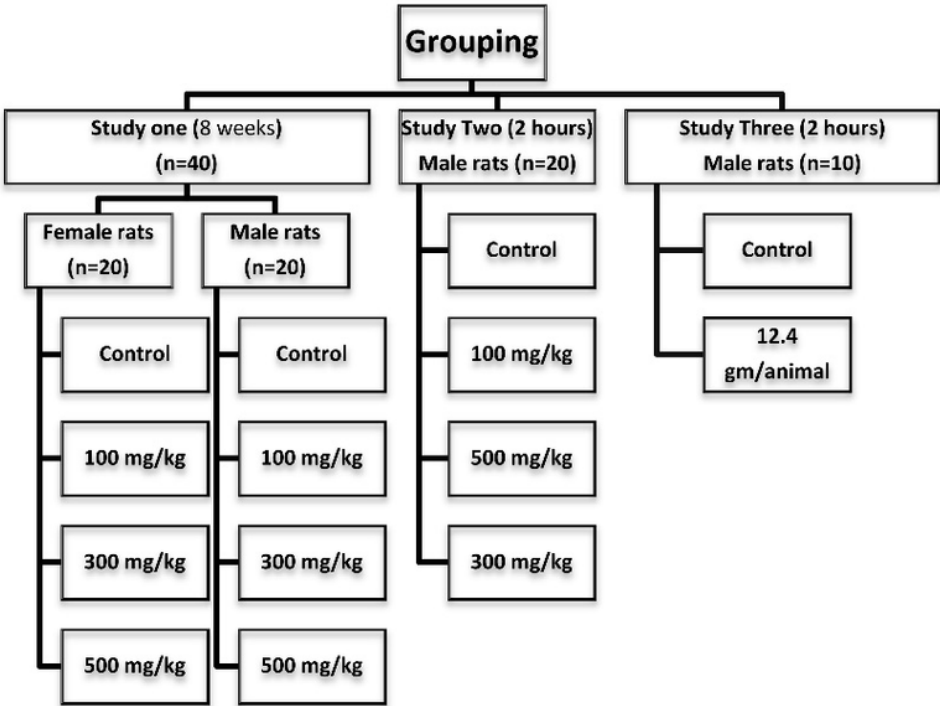


Figure 1

Experimental design of the *in vivo* study. The animals were given fresh khat water extract (CEWE) orally. Three experiments with different durations and doses were conducted to study the acute and chronic effects of CEWE. The first one used both sexes of rats (n = 40) for long-term anorexic effects (8 weeks).

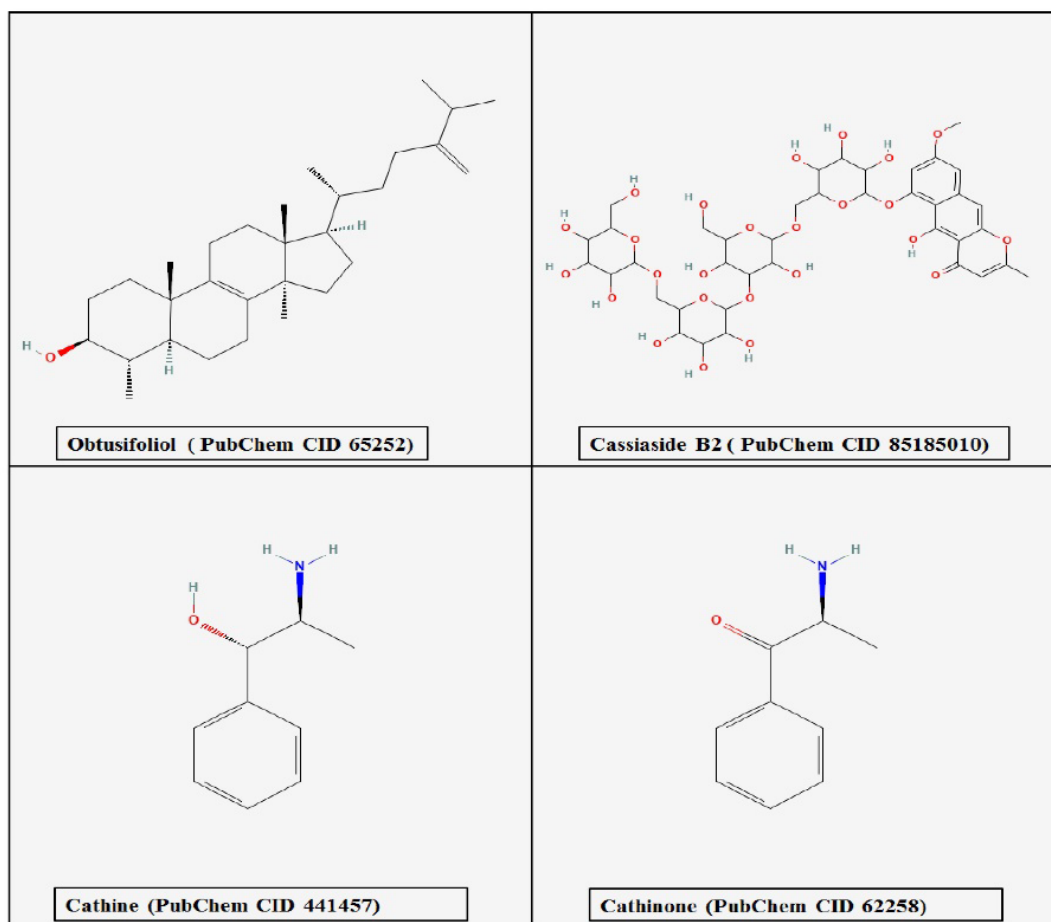


Figure 2

Chemical structures of study ligands.

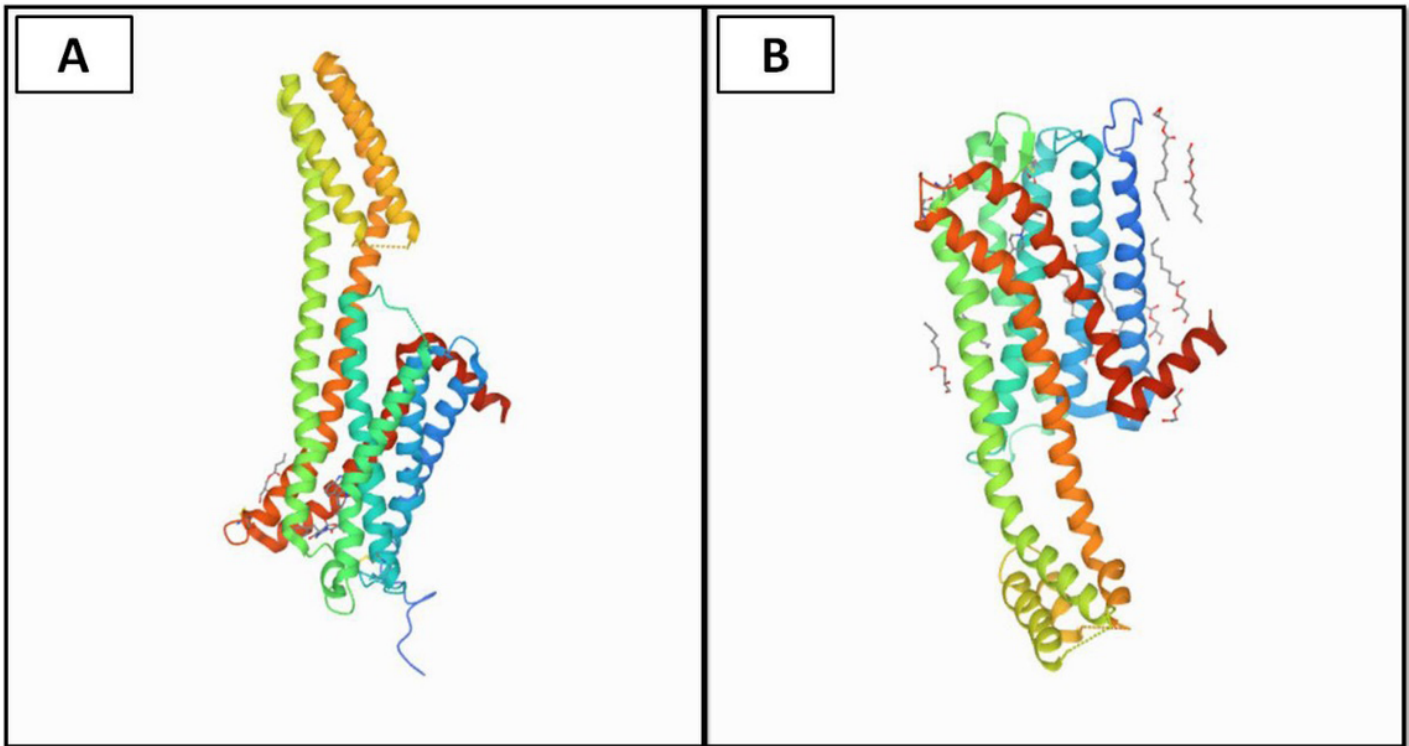


Figure 3

3D structures of the selected serotonin receptors (5-HT_{2C}) A: The 3D structure of "G" (5-HT_{2C} in an agonist-bound active conformation; chain A of PDB ID: 6BQG. B: The 3D structure of "H" (5-HT_{2C}) in an antagonist-bound inactive conformation; chain A of PDB ID: 6BQH.

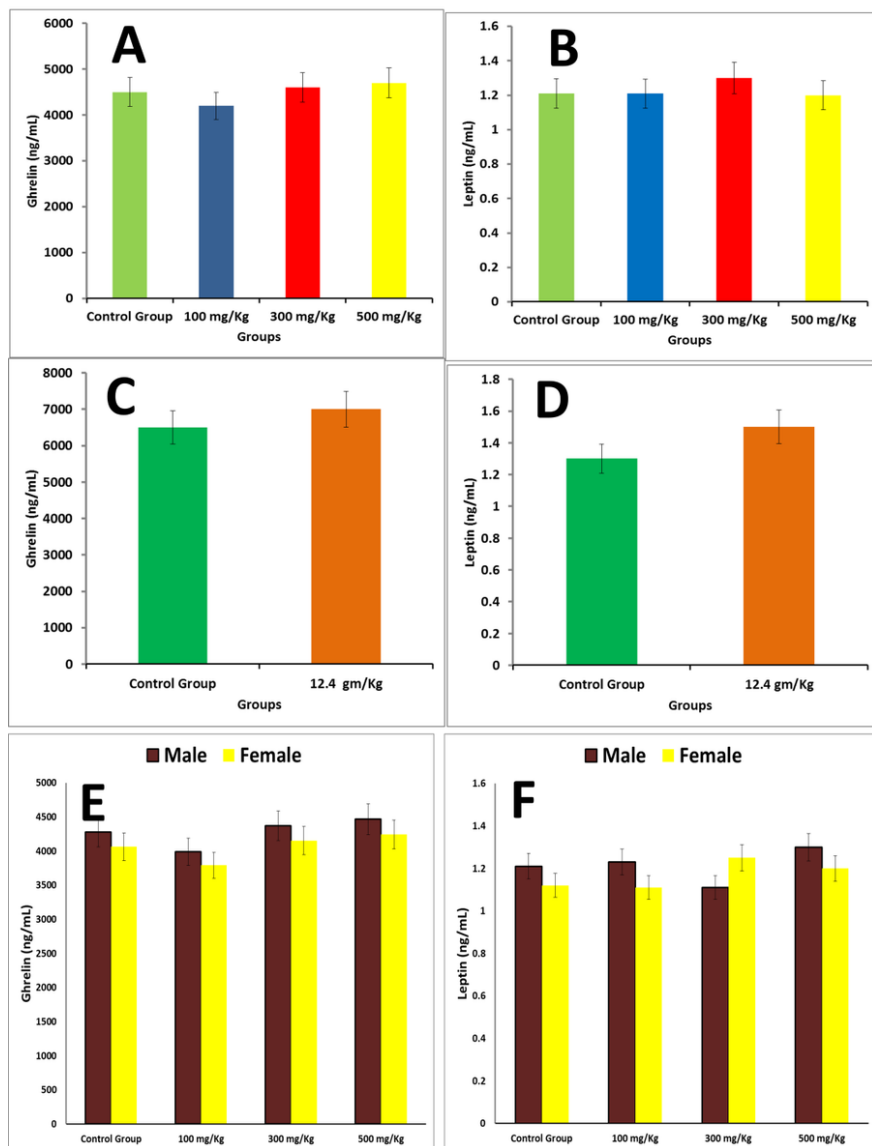


Figure 4

Effects of two hours' oral administration of CEWE (100, 300, and 500 mg/kg body weight) on plasma levels of ghrelin (A) and leptin (B). Figures 2D and 2C show the effects of a two-hour oral dose of CEWE (12.4 g/kg) on the plasma levels of ghrelin and leptin, respectively. Hormone levels in male rats were measured using enzyme-linked immunosorbent assay kits. The values are expressed as mean $\pm$ standard deviation ($n = 5$). ANOVA and SPSS were used to examine the differences ($P < 0.05$) between the different groups. Leptin and ghrelin levels were measured in ng/ml. Figures E and F show the findings for eight weeks.

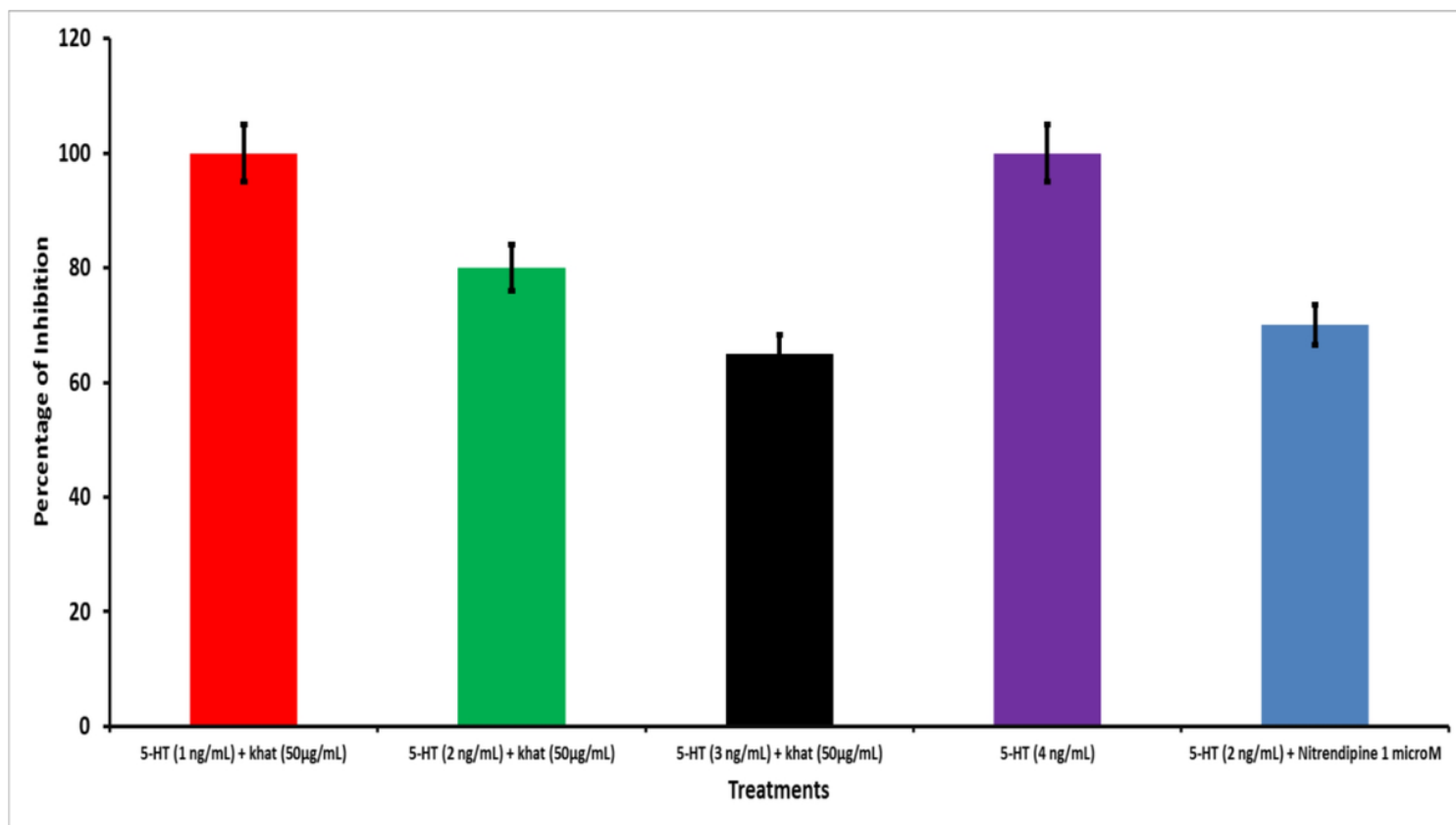


Figure 5

Rat fundus strips were prepared in the presence of various concentrations of 5-HT and khat. Five rats were used in the experiments, which were conducted on five different days. The gastric fundus was removed and placed in fresh Kreb's ringer solution. Khat extract was added to assess its agonist and antagonist effects on the 5HT receptors. Preadministration of khat extract (50 µg/mL) resulted in complete antagonism of 5HT (2 ng/mL). Khat inhibited the enzyme activity by 51% at 50 µg/mL.

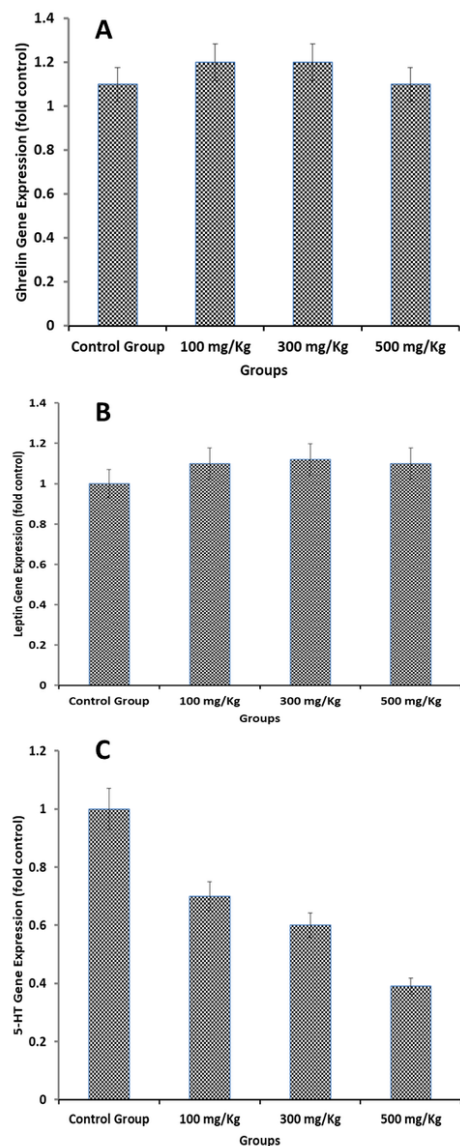


Figure 6

The relative expression levels of ghrelin (A), leptin (B), and 5-HT (C) were analyzed using RT-qPCR. No significant effect was observed in the expression of both ghrelin and leptin genes. However, treatment with CEWE resulted in a decrease in the expression of 5-HT gene. The data represents the mean $\pm$ standard deviation (N = 5). Statistical significance was determined using a one-way ANOVA test, with * indicating $p < 0.05$ compared to the control group.

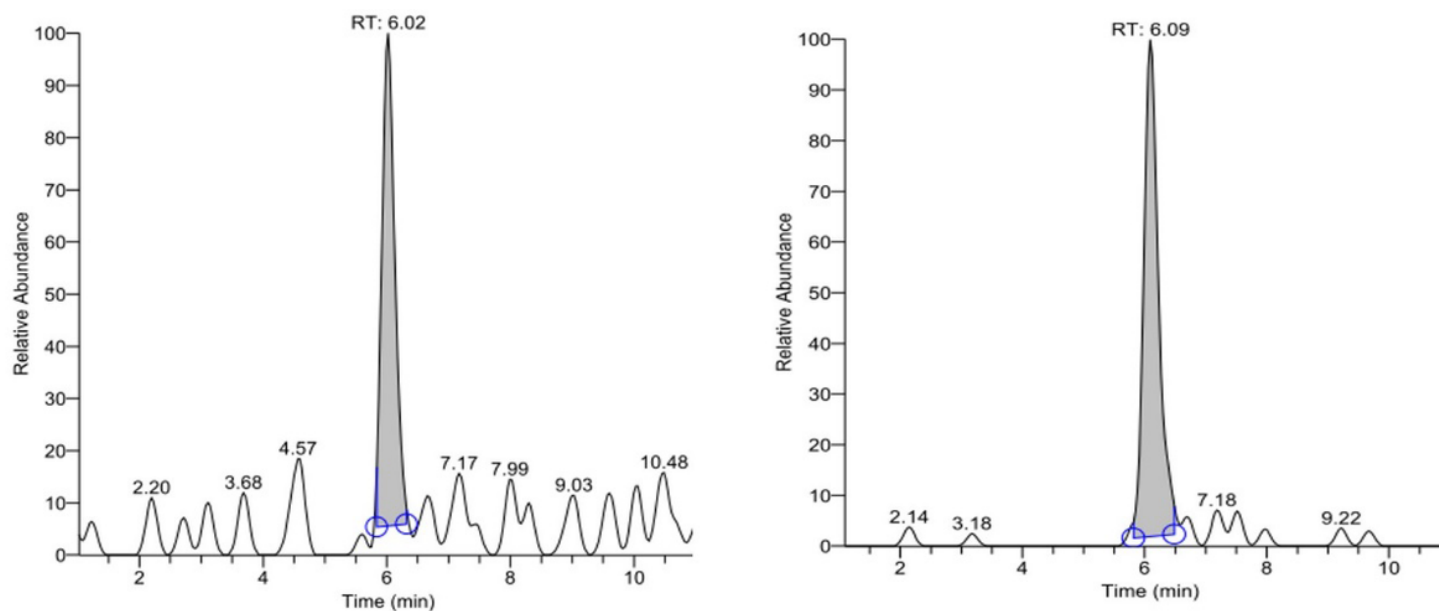


Figure 7

Liquid chromatography-mass spectrometry-mass spectrometry of cathine (left image) and cathinone (right image) alkaloids in rat plasma after administration of 300 mg/kg of khat extract. Mass spectrometry analysis of diluted (1:1000) STD cathine (a: Ion m/z 152) and STD cathinone (b: Ion m/z 150) shows specific ion scan spectra.

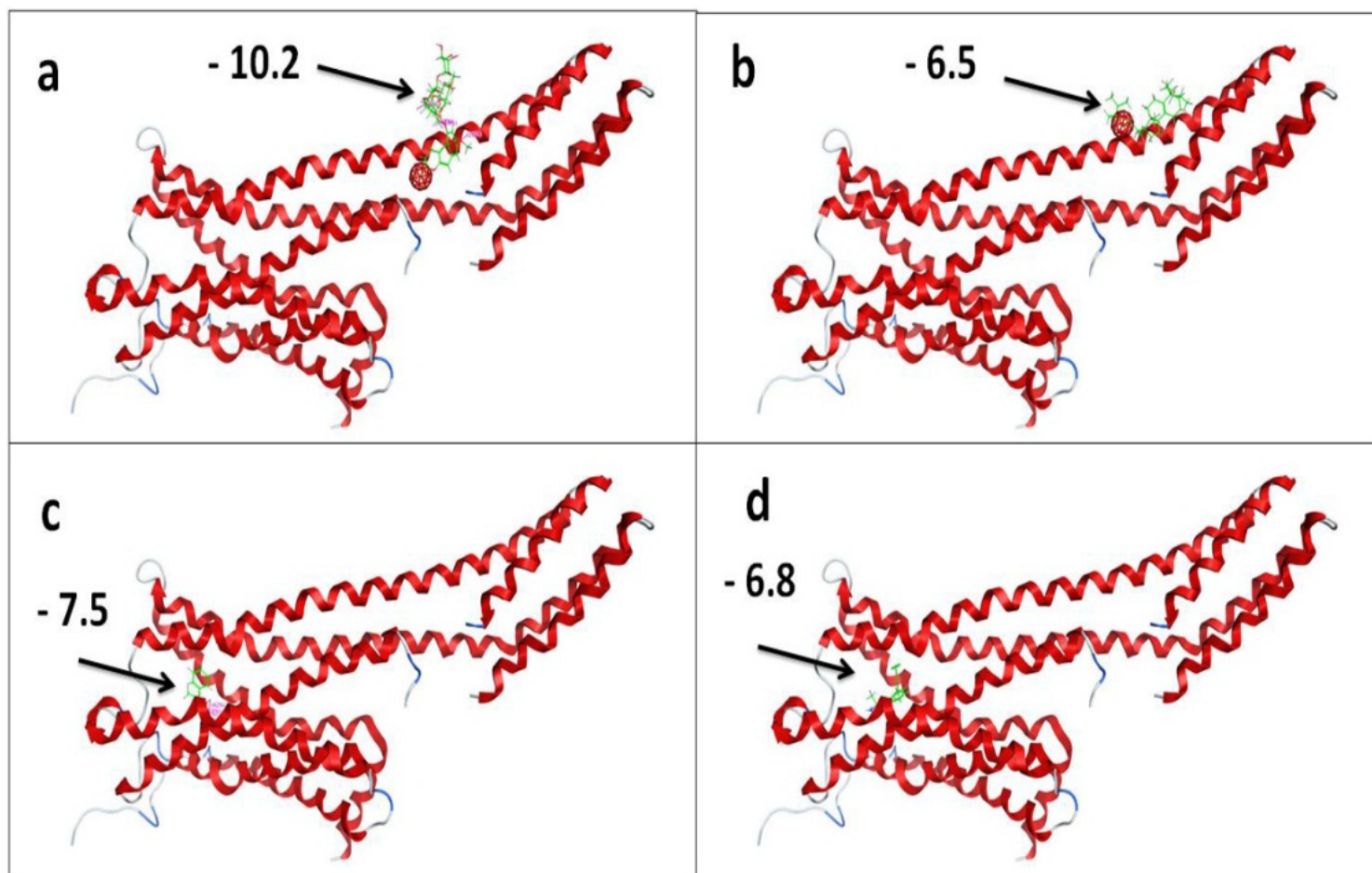


Figure 8

Binding of cassiaside B2 and obtusifoliol, cathine, and cathinone (a, b, c, and d, respectively) to structure G (5-HT_{2C} in an agonist-bound active conformation; chain A of PDBID: 6BQG).

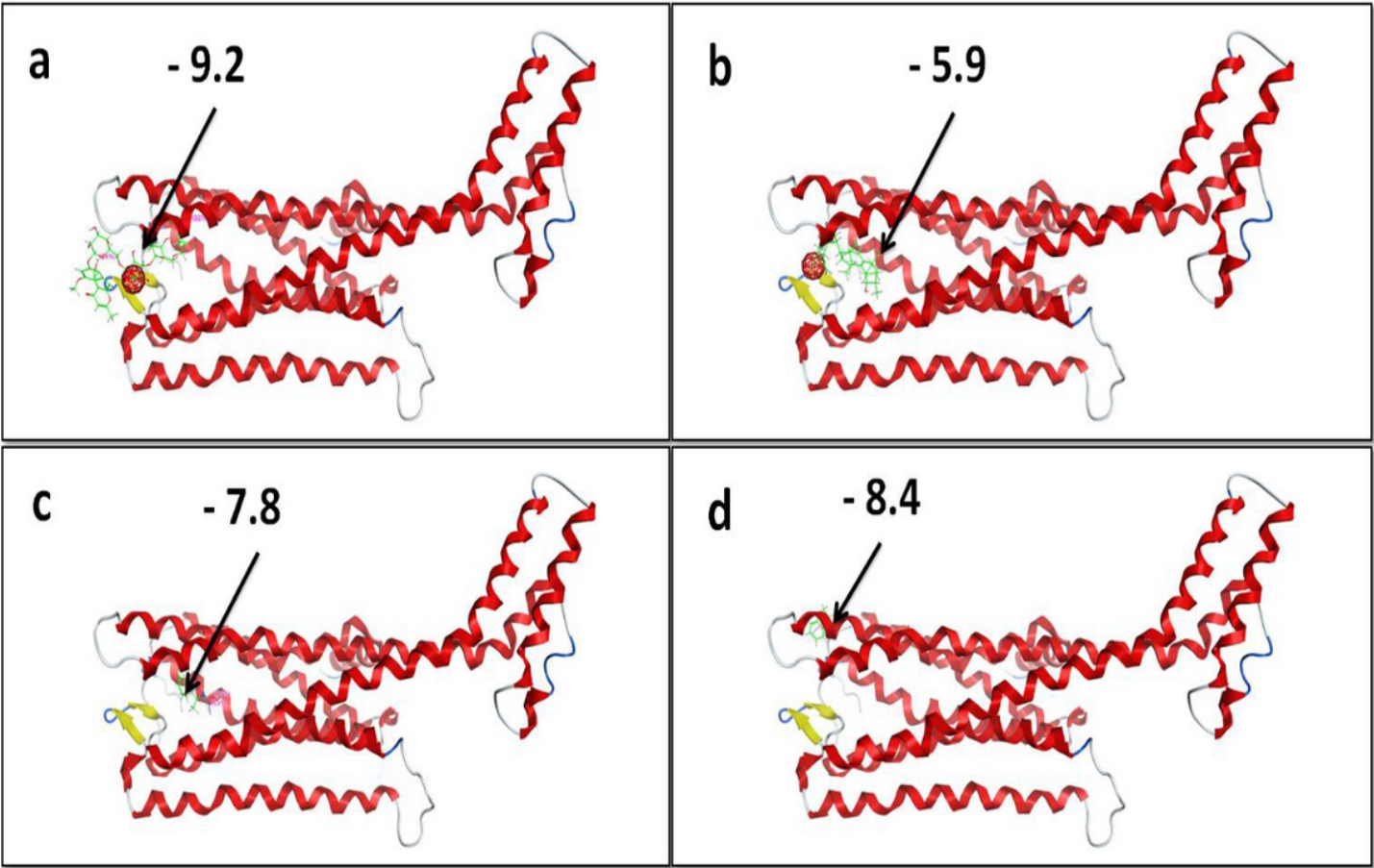


Figure 9

Binding of cassiaside B2 and obtusifoliol, cathine, and cathinone (a, b, c, and d, respectively) to structure H (5-HT_{2C} in an antagonist-bound inactive conformation; chain A of PDBID: 6BQH).

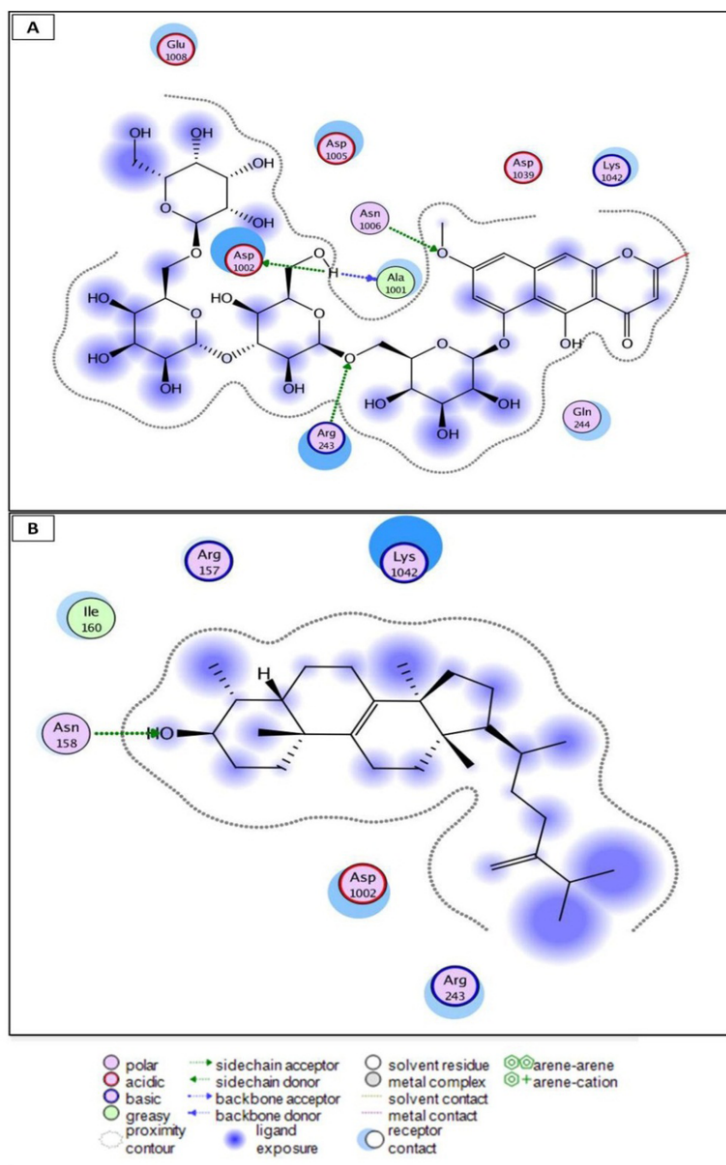


Figure 10

Cassiaside B2 and obtusifoliol interactions at the G binding site The 2D ligand-protein interactions of cassiaside B2 and obtusifoliol in the binding site of G (A and B) are indicated by green dashed arrows, indicating hydrogen bonds. Blue halos indicate solvent-exposed atoms.

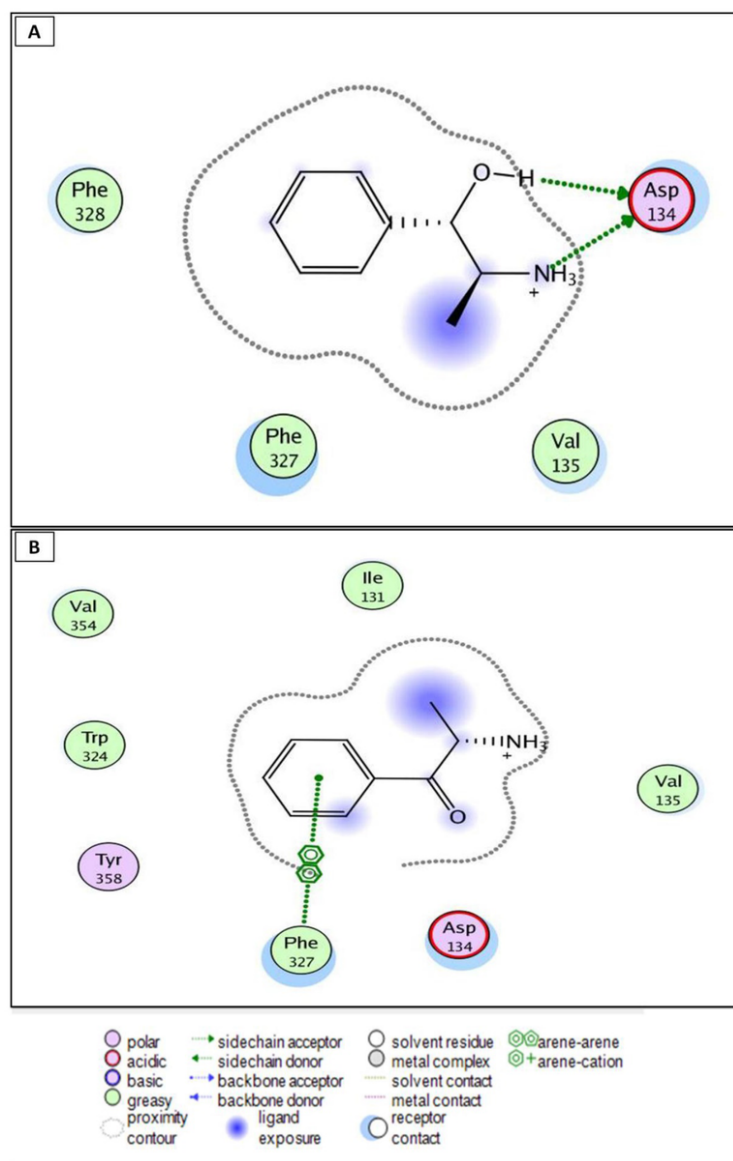


Figure 11

Cathine and cathinone interactions at the G-binding site. The 2D ligand-protein interactions of cathine and cathinone in the binding site of G (A and B) are indicated by green dashed arrows, indicating hydrogen bonds. The blue haloes show solvent-exposed atoms.

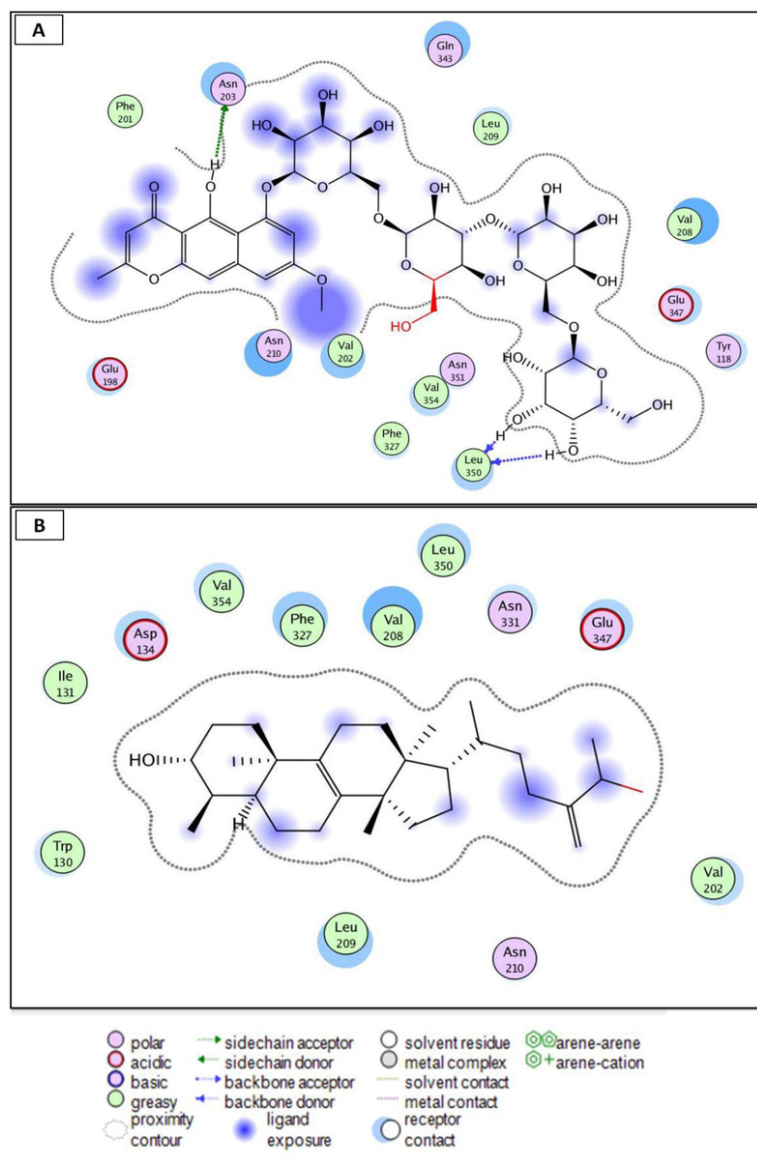


Figure 12

Cassiaside B2 and obtusifoliol interactions at the H-binding site. The 2D ligand-protein interactions of cassiaside B2 and obtusifoliol in the binding site of H (A and B) are indicated by green dashed arrows, which indicate hydrogen bonds. Blue haloes indicate solvent-exposed atoms.

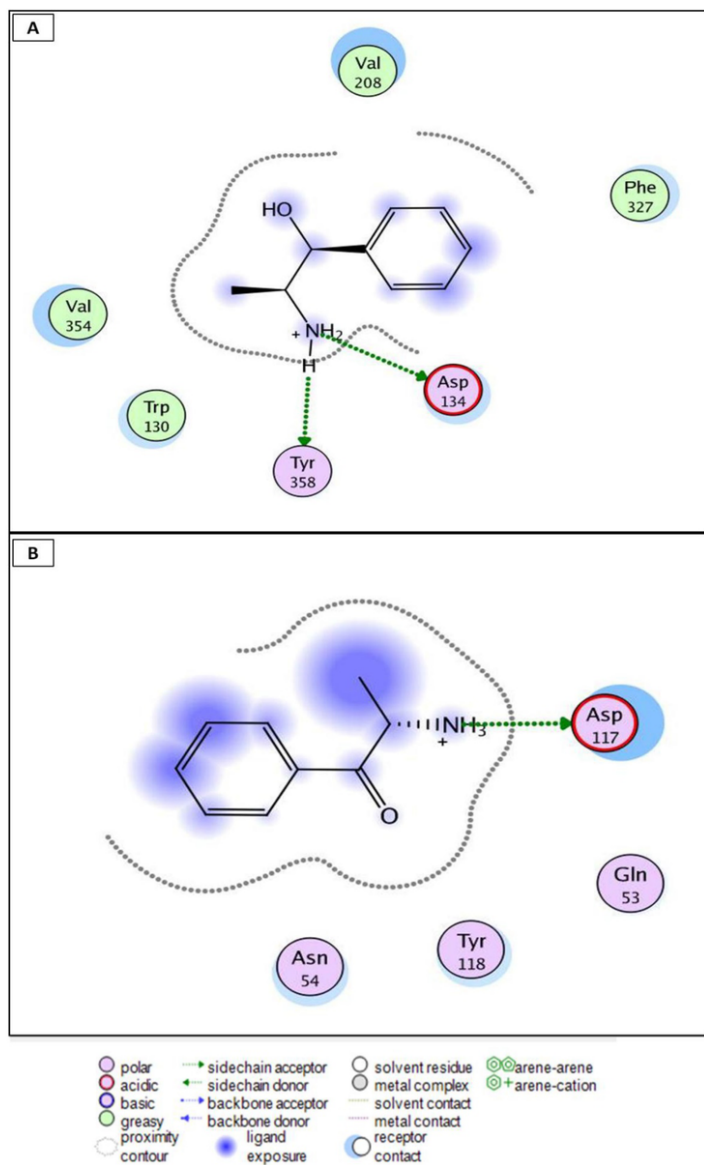


Figure 13

Cathine and cathinone interaction in the binding site of H. The 2D ligand-protein interactions of cathine and cathinone in the binding site of H (A and B) are indicated by green dashed arrows, which indicate hydrogen bonds. Blue haloes indicate solvent-exposed atoms.

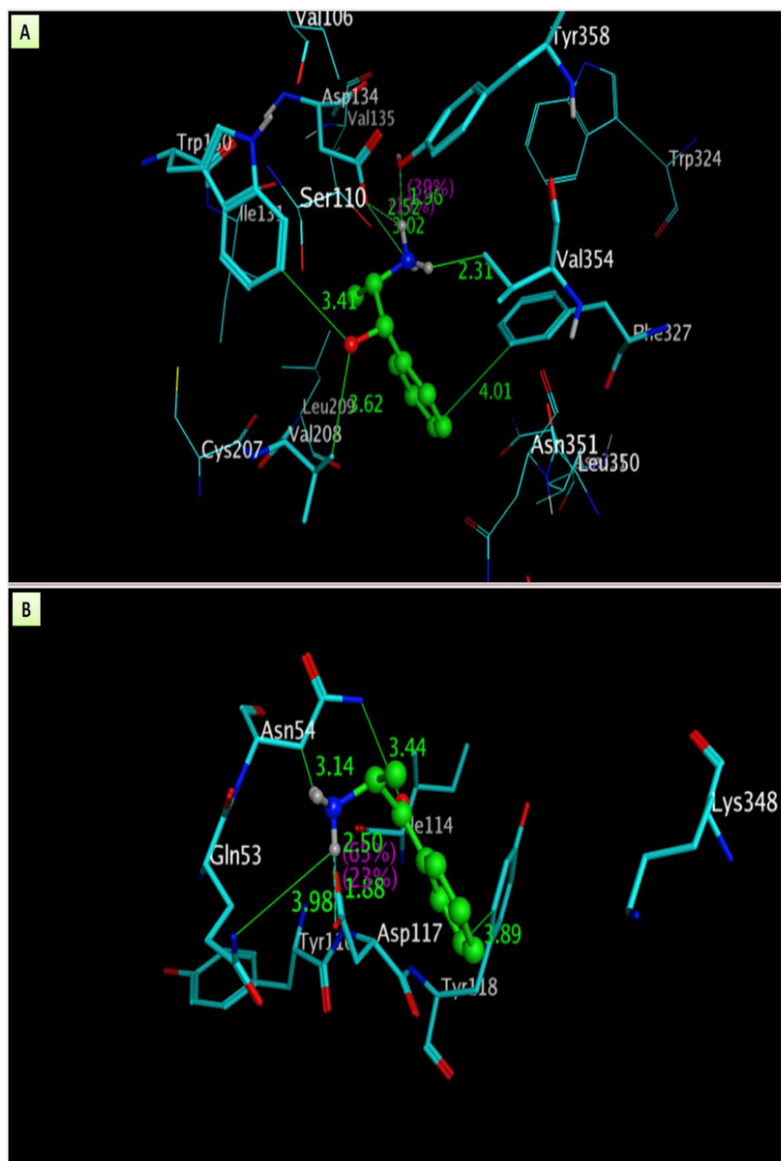


Figure 15

3D view of the interaction between cathine and cathinone in the binding site of H (A and B). The compounds are shown as green carbon balls and sticks, and the residues are shown as aqua sticks. The purple dashed lines indicate the hydrogen bonds, which are labeled with their strengths and lengths.

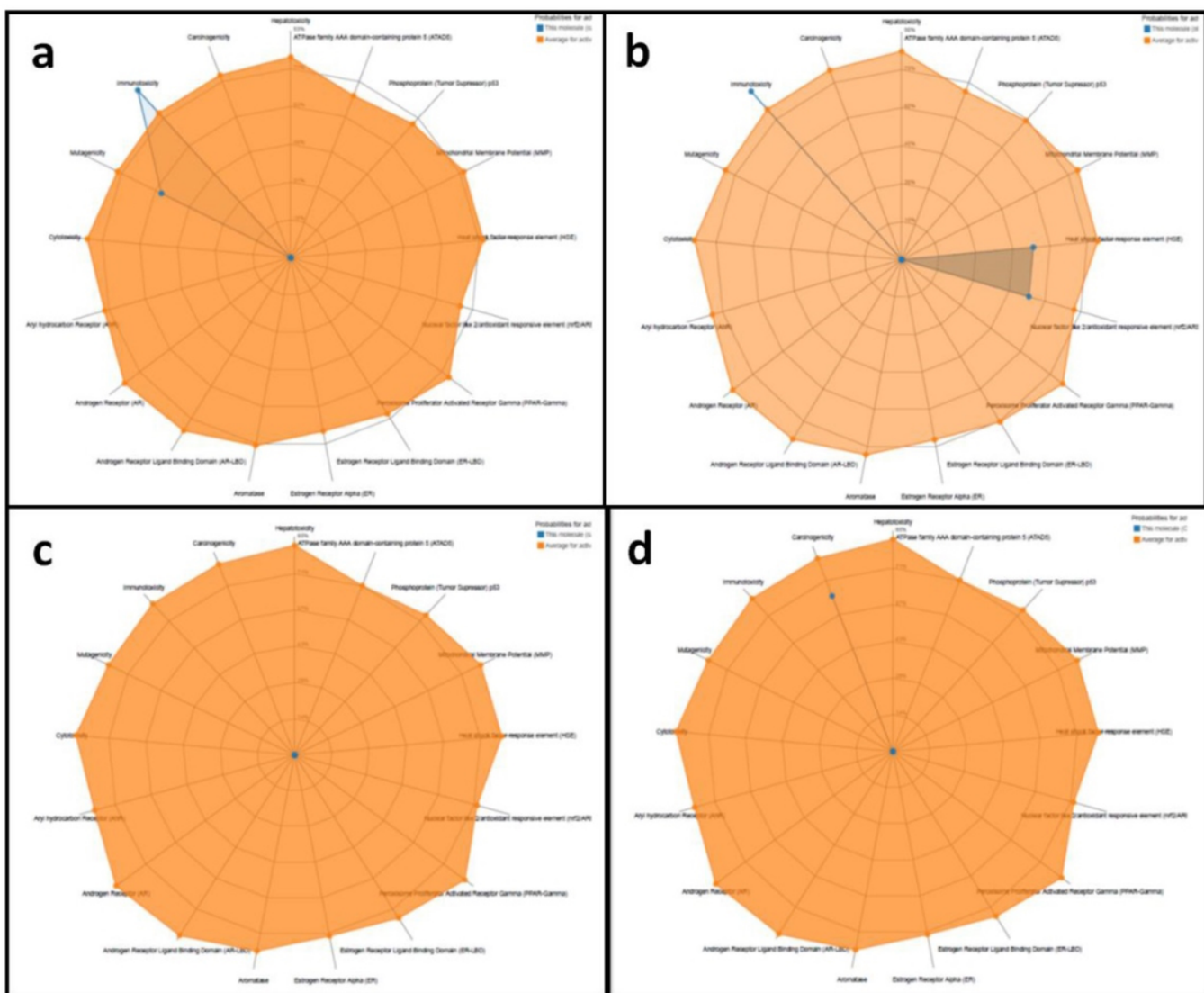


Figure 16

Acute oral toxicity prediction using Protox. The results indicate that the carcinogenicity of cassiaside B2, obtusifoliol, cathine, and cathinone indicates a moderately safe oral intake.