Research Article

In vivo antianxiety and antidepressant activity of *Hibiscus sabdariffa* calyx extracts

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ABSTRACT

Objective: Different parts of *Hibiscus sabdariffa* have been used traditionally for treating several ailments including mental disorders. The present study was designed to evaluate the antianxiety and antidepressant potential of *H. sabdariffa* calyces using elevated plus-maze model and forced swim test respectively. **Material and Method:** Petroleum ether (60-80°C), chloroform, ethanol, and aqueous extracts were evaluated for antianxiety and antidepressant activity. **Results:** Among all the extracts, ethanol extract showed significant antianxiety and antidepressant activity at a dose of 400 mg/kg. Phytochemical screening of the bioactive ethanol extract demonstrated the presence of flavonoids, terpenoids, tannins, anthocyanins, and glycosides.

KEY WORDS: Calyces, Elevated plus maze, Flavonoids, Forced swim, *Hibiscus sabdariffa*

INTRODUCTION

Anxiety and depression are the most prevalent psychiatric disorders associated with diminished quality of life. According to the World Health report,[1] the number of people suffering from anxiety and/or depression has increased by 50% in the past two decades. Recent studies have shown that anxiety and depression may occur together; anxiety may predispose depression or vice versa.[2] Till date, benzodiazepines and selective serotonin reuptake inhibitors have been most widely prescribed medication for anxiety and depression, respectively. However, their adverse effects are a matter of concern. These considerations have lead researchers to investigate plants, in search of molecules which can combat anxiety and depression.

Hibiscus sabdariffa (Malvaceae) is popularly known as Gongura[3] or Roselle.[4] The plant is found in the tropical and subtropical regions of the world;[5] it is native to India, Malaysia,[6] and West Indies. In India, it is cultivated in Uttar Pradesh, Andhra Pradesh, West Bengal, Bihar, Punjab, Assam, and Tamil Nadu.[7] The plant is an annual, erect, bushy sub-shrub which grows up to 8 ft.[8,9] The leaves are 8-15 cm long, arranged alternately on the stems. The flowers are white to yellow with fleshy red calyx at the base.[10] *H. sabdariffa* has been used in folk medicine as a treatment for several ailments.[11] Almost all parts of the plant are used as remedies for various diseases,[12] but calyces are the most popular, both commercially and medicinally. These are used in folk medicine as sedative, diuretic, laxative, and tonic.[13] The plant is used in Ayurveda for treating hypertension, pyrexia, and liver dysfunction.[14] The major classes of phytoconstituents reported from *H. sabdariffa* include phenols/tannins, anthocyanins, flavonoids, phytosterols, saponins, and glycosides.[15,16] The plant is reported to be antimutagenic, anticonvulsant, antioxidant, anti-inflammatory, antimicrobial, laxative, chemopreventive, astringent, central nervous system (CNS) depressant, and sedative.[5,17-20] Despite widespread medicinal use of the plant, no significant reports pertaining to neuropharmacological properties are available. Therefore, the present study was planned to evaluate antianxiety and antidepressant activity of *H. sabdariffa* calyces.

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MATERIALS AND METHODS

Plant Material

Dried calyces of *H. sabdariffa* were purchased from Earth Expo Company, Gujarat, India. Morphologic and microscopic character of the procured sample

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of the calyces was found to match completely with those reported in the literature.[21] Voucher specimen (number 252) of the calyces has been deposited in herbarium-cum-museum of University Institute of Pharmaceutical Sciences, Panjab University, Chandigarh.

Chemicals and Reagents

Solvents used include petroleum ether 60-80°C (Merck India Ltd., Mumbai), chloroform (Thermo Fisher Scientific India Pvt., Ltd., Mumbai), ethanol (Panipat Sugar Mill, D-Unit, Panipat), and distilled water prepared in our laboratory.

Standard Drugs

Diazepam (Java Pharmaceuticals, Gurugram) and imipramine (Torrent Pharmaceuticals, Solan) were used, respectively, as standard antianxiety and antidepressant agents.

Preparation of Extracts

Coarsely powdered calyces of *H. sabdariffa* (1 kg) were soxhlet extracted successively with pet ether, chloroform, and ethanol. The marc was finally boiled with distilled water to prepare the water extract. Exhaustive extraction with each of the solvent was ensured. The extracts were dried using Eyela N 1100 rotary vacuum evaporator and were preserved in a vacuum desiccator containing anhydrous silica gel blue.

Experimental Animals

Laca mice (either sex), housed at the Central Animal House, Panjab University, were allowed standard pellet diet (Ashirwad, Chandigarh) and water *ad libitum*. Groups of 6 mice (20-30 g) were used in all sets of experiments. The animals were fasted for 18 h before use. Approval (PU/IAEC/S/16/112) from the Institutional Animal Ethical Committee of Panjab University, Chandigarh, was taken before carrying out biological studies.

Preparation of Doses

Tween 80 (5%) in aqueous carboxymethyl cellulose (CMC 0.5% w/w) was used as a vehicle for preparing the suspension of extracts/standard drugs. Doses of various test substances were prepared by suspending appropriate quantities in the vehicle so as to administer these to mice in volumes ranging between 0.20 and 0.30 mL per oral route.

Acute Toxicity Studies of the Extracts

Acute toxicity studies of pet ether, chloroform, ethanol, and water extracts of *H. sabdariffa* calyces were carried out on mice as per the OECD 423 guidelines.[22] After 12 h of fasting, different groups of mice were administered single oral dose (500, 1000, and 2000 mg/kg) of the four extracts. Immediately after dosing, animals were observed for signs of toxicity during the first 0.5, 1, 2, 4, 8, and 12 h and at every 24 h for 14 days. Behavioral parameters, tremors, lethargy, death, amount of water, and feed taken were observed.

Antianxiety Activity

Antianxiety activity was evaluated using elevated plus-maze (EPM) model.[23,24] The apparatus consisted of two open arms (16 cm × 5 cm) and two closed arms (16 cm × 5 cm × 12 cm) having an open roof. The apparatus was kept elevated (25 cm) from the floor for evaluating the anxiolytic behavior. Doses were administered orally using tuberculin syringe fitted with an oral cannula. The dose administration schedule was so adjusted that each mouse was having its turn on the EPM 60 min after the administration of the vehicle, diazepam (2 mg/kg), or the test extracts (100, 200, 400 mg/kg). Each mouse was placed at the center of EPM with its head facing toward the open arm. During 5 min duration of the experiment, behavior of the mouse was recorded as (a) the number of entries into the open arms and (b) mean time spent by the mouse in open arms.

Antidepressant Activity

Forced swimming test (FST) was used to evaluate antidepressant activity.[25,26] Mice were forced to swim in glass jar (25 cm × 12 cm × 25 cm) containing water to a height of 15 cm at room temperature (22°C ± 1°C). After an initial period of vigorous activity to escape, the animals assumed a typical immobile posture (ceased to struggle with minimal limb movements just sufficient to keep their head above the level of water). Mice were administered a single dose (100, 200, and 400 mg/kg) of the extract or the standard antidepressant imipramine (10 mg/kg, po) 60 min before the evaluation. Total immobility period during 6 min test session was noted.

Phytochemical Screening

The bioactive ethanol extract was screened for different classes of phytoconstituents using the standard procedures.[27]

Statistical Analysis

Results have been expressed as mean ± standard error mean. The significant difference among the groups was assessed by one-way analysis of variance (ANOVA) followed by Tukey's multiple range test. The results were considered statistically significant at $P < 0.001$. Statistical analysis was performed using the Graph Pad Prism 5.

RESULTS

Yield of Extracts

Yield of pet ether, chloroform, ethanol, and water extracts of *H. sabdariffa* calyces was observed to be 3.43, 6.72, 33.30, and 8.14% w/w, respectively.

Acute Toxicity Studies

No toxic effects were observed up to a dose of 2000 mg/kg for all the extracts of *H. sabdariffa* calyces.

Antianxiety Activity of Extracts

Administration of diazepam (2 mg/kg) significantly increased the number of entries and the time spent in the open arms compared to the control group. Among the four extracts of *H. sabdariffa* calyces, only the ethanol extract exhibited statistically significant ($P < 0.001$) antianxiety activity at a dose of 400 mg/kg (Figure 1).

Antidepressant Activity of Extracts

Among the four extracts, ethanol extract at a dose of 400 mg/kg demonstrated a statistically significant ($P < 0.001$) diminution of immobility time when the animals were subjected to FST (Figure 2). Results of imipramine (10 mg/kg) were similar to results of those observed with the ethanol extract.

Phytochemical Investigation

The bioactive ethanolic extract revealed the presence of flavonoids, terpenoids, tannins, anthocyanins, and glycosides.

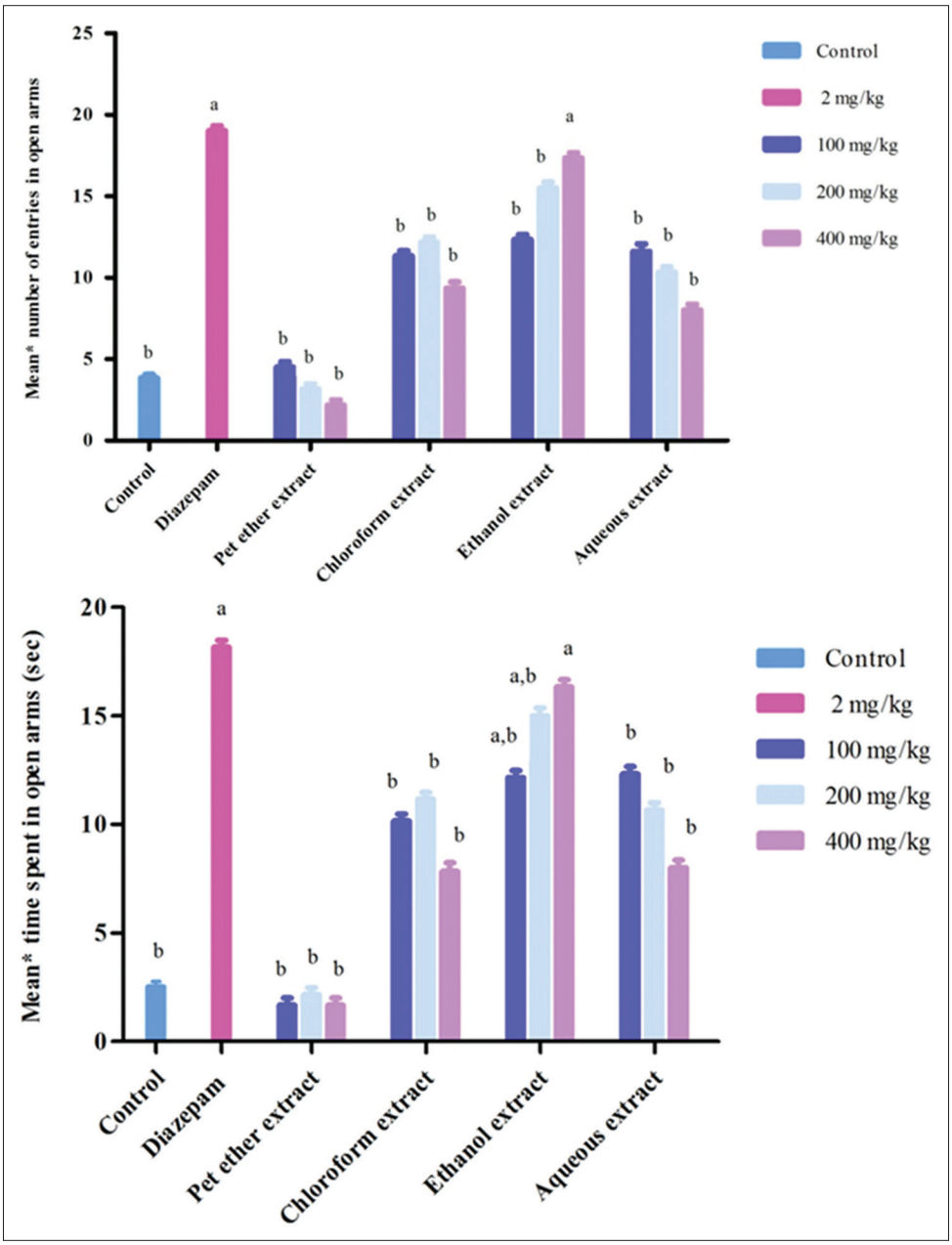

Figure 1: Antianxiey activity profile of different extracts of *Hibiscus sabdariffa* calyces using elevated plus maze. The data are expressed as mean ± standard error mean; **n* = 6; [a]*P* < 0.001 versus control; [b]*P* < 0.001 versus diazepam; one-way ANOVA followed by Tukey's multiple range test

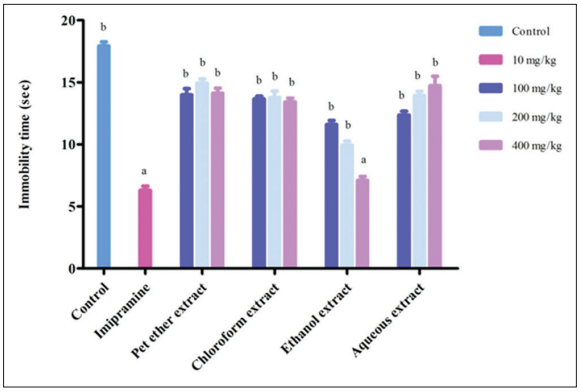

Figure 2: Antidepressant activity profile of different extracts of *Hibiscus sabdariffa* calyces using forced swim test. The data are expressed as mean ± standard error mean; **n* = 6; [a]*P* < 0.001 versus control; [b]*P* < 0.001 versus imipramine; one-way ANOVA followed by Tukey's multiple range test

DISCUSSION

Despite the traditional use of *H. sabdariffa* calyces for treating nervous disorders, there is an absence of scientific reports on the evaluation of its antianxiety and antidepressant effects. EPM model is considered to be etiologically similar to the anxiety observed clinically in human beings.[24] An anxiolytic agent increases both the frequency of entries and the time spent in open arms of the EPM and is thought to act through the γ-aminobutyric acid type A receptor complex, justifying the use of diazepam as a positive control in the study.[23] The FST has been used in preclinical tests to evaluate behavioral despair, i.e., measure of failure to escape from an aversive stimulus.[28]

The present study demonstrates that out of the four extracts, namely, petroleum ether, chloroform, ethanol, and water of *H. sabdariffa* calyces, the ethanol extract exhibited significant antianxiety and antidepressant activity, both at a dose of 400 mg/kg. Besides this, a dose-dependent decrease in both the activities was observed, which might be due to mild sedation at higher doses. Further, phytochemical investigation of the bioactive ethanol extract confirmed the presence of flavonoids, terpenoids, tannins, anthocyanins, and glycosides.

Previous biochemical and pharmacological reports have shown that flavonoids have significant effects on the CNS,[29] primarily due to their affinity for the central benzodiazepine receptors.[30] Thus, the antianxiety and antidepressant activity of the ethanol extract might be due to the flavonoids present in it.

CONCLUSION

Results of the present study indicate that the ethanol extract of the *H. sabdariffa* calyces has a significant antianxiety and antidepressant activity at a dose of 400 mg/kg. However, investigations are in progress to isolate the active constituent(s), elucidate their structure, and establish the mode of antianxiety and antidepressant activity.

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