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ANTI ANXIETY AND ANTI DEPRESSANTS ACTIVITY OF HIBISCUS ROSA IN RATS

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ABSTRACT

Anxiety is a cardinal symptom of many psychiatric disorders and an almost inevitable component of many medical and surgical conditions. It is found that administration of 400 mg/kg *Hibiscus rosa sinensis* or 20 mg/kg DZP for 7 days significantly reduced the immobility time in FST. These results provide support for the potential anti anxiety and antidepressant activity of *Hibiscus rosa sinensis* and contribute towards validation of the traditional use of *Hibiscus rosa sinensis* in the treatment of emotional disorders. In this study, elevated plus maze test were used to evaluate the anti anxiety and anti depressant activity of extract *Hibiscus rosa sinensis* of in albino mice. The elevated plus maze is considered to be an etiologically valid animal model of anxiety. In the elevated plus maze, the open arms are more fear provoking than the closed arms. The reduction in entry and time spent in open arms are the indications of the high level of fear or anxiety. The results obtained in this study suggest that the extract of the leaves of *Hibiscus rosa sinensis* possesses anti anxiety and anti depressant activity. Thus, *Hibiscus rosa sinensis* has potential clinical application in the management of anxiety disorders.

KEYWORDS: *Hibiscus rosa sinensis, anti anxiety, Anti depressant,* Albino mice, Elevated plus maze.

INTRODUCTION

Anxiety is a normal emotional behavior, however, becomes pathological precipitating cardiovascular and psychiatric disorders when it is severe.[1] Many allopathic drugs are available to treat anxiety disorders, among which benzodiazepines are most commonly used which possess various systemic side effects.[2] Among the many mental illnesses and behavioral disorders, depression and anxiety are the two most prevalent psychiatric

disorders[3] Several classical anxiolytic and antidepressant drugs such as benzodiazepines, monoamine oxidase inhibitors, tricyclic antidepressants, selective serotonin reuptake inhibitors, serotonin-norepinephrine reuptake inhibitors, and noradrenergic and specific serotonergic antidepressants arewidely used in clinical practice to treat these disorders.[4,5]

Hibiscus Rosa sinensis has been used in Siddha medicine, a traditional Tamil system from South India, for many centuries.[6] Hibiscus extracts have been used for ages in Ayurveda to cure many ailments.[7] The plants have the natural health benefit that can be used to cure diseases naturally. They are used to cure ailments such as cough cold, hair loss and hair greying also.[8] The flowers and leaves of this plant play a major role in hair treatment.[9] These are ground into a fine paste with water and this is generally used as a shampoo plus conditioner.[10]

However, treatment by the mentioned drugs can also bring undesirable side effects including cardiovascular toxicity, sexual dysfunction, weight gain, and drug interactions. Therefore, there is an urgent need for the development of effective anxiolytic and antidepressant therapies without any or at least fewer adverse effects.

MATERIAL AND METHODS

Collection of Plant and Authentication: Fresh flowers of *Hibiscus rosa sinensis* was collected and authenticated bybotanist.

Extraction of plant: In the present study, extraction was performed using continuous hot percolation 'Soxhlation'. Dried pulverised flowers of *Hibiscus rosa sinensis* were placed in thimble of Soxhlet apparatus. Soxhlation was performed at 60°C using Petroleum ether (40 - 60ºC) as non polar solvent at first. Exhausted plant material (marc) was dried and then extracted with ethanol. For each solvent, soxhlation was continued till no colour was observed in siphon tube. For confirmation of exhausted plant marc (i.e. completion of extraction), colorless solvent was collected from siphon tube and evaporated for residue. Absence of residue confirmed the completion of extraction. Obtained extracts were evaporated using rotary vacuum evaporator (Bucchi type) at 40°C.Dried extract was weighed and percentage yield for each extract was determined using the following formula:

$$\% \text{ Yield } = \frac{\text{Weight of extract}}{\textit{Weight of plant Material}} X\ 100$$

Physical characteristics: Extract was investigated for its solubility in water, methanol, acetone, chloroform, ethylacetate, DMSO, petroleum ether.

Phytochemical investigation: Detailed phytochemical testing was performed to identify presence or absence of different phytoconstituents by using standard test methods.

Pharmacological activity experimental animals

Strain	:	Swiss mice
Sex	:	Either
Body weight	:	25 ± 5 gm
Housing condition	:	As per CPCSEAguidelines

Animals were selected at random from animal house of PBRI, Bhopal, India. Animals were further randomly divided into various treatment groups and kept inpropylene cage with sterile husk as bedding. Animals were housed in relative humidity of 30.7 % at 22 ± 20 C and 12:12 light and dark cycle. Animals were fed with standard pellets (Golden feeds, New Delhi, India) and water was available ad libitum (extra component was added in water as per protocol described below). All experimental animals were approved by Institutional Animals Ethics Committee (IAEC) of PBRI, Bhopal.

Acute oral toxicity: The acute oral toxicity study was carried out according to OECD 423 guidelines. Four ranges of dose were used for toxicity studies, i.e 5mg/Kg, 50 mg/Kg, 100 mg/Kg, 200mg/Kg. animals were observed individually for next 4 hours after dosing for the presence of mortality during this period and 72 hours after sample administration. No mortality was observed during acute toxicity experiment.

Forced swimming test (FST): The method was carried out on mice. Mice were placed in an open cylindrical container (diameter 10 cm, height 25 cm), containing 15 cm of water at 25 ± 1°C. The duration of observed immobility was recorded during the last 4 min of the 6-min testing period. Immobile time was defined as the absence of active/escape directed movements (mouse floating in the water without struggling) and was scored in a blind manner by an observer. Decrease in the duration of immobility during the FST was taken as a measureof antidepressant activity. The animals were divided in to four groups and each group has 6 animals. Group I was vehicle control received normal saline, group II received drug

Diazepam (1.50mg/kg), group III was treated with extract 100mg/kg bw and group IV was treated with extract 200 mg/kg bw.

Elevated plus-maze test: Elevated plus-maze is the most simple apparatus to study neuroprotective effects andanxiolytic responses produced by the test drugs. It is used to test almost all types of anxiolytic agents. Exposure of animals to novel maze alley evokes an approach- avoidance conflict which is stronger in open arm as compared to enclosed arm. Rodents (rats and mice) have an aversion for high and open space and prefer enclosed arm, therefore, spend a greater amount of time in enclosed arm. When animals enter open arm, they freeze, become immobile, defecate and show fear-like movements. The plasma cortisol level is also reported to be increased, as a true reflection of anxiety. Major advantages of this test procedure are: (a) It is simple, fast, and less time consuming, no prior training or noxious stimuli (sound or light) is required, and (c) it is predictable and reliable procedure for studying anxiety response as well as anxiolytic action drug.

The previous exposure of an animal to the elevated plane induced fear and to avoid the feeling of fear the animal occupies a safe position in the elevated plus maze. Latency to reach the central plateform of the elevated plus maze is indicative of the learning ability of an animal. The animal is said to have learnt if the latency to reach the central platform is reduced. The drug impairing memory, delays the entry of animal in the central platform.

Grouping: Swiss albino mice weighing between 25-30 gms were randomly divided into 4 groups each containing 6 mice.

Table 1: Grouping of HRE for Elevated plus maze.

Group I	Control (Vehicle treated group, p.o)
Group II	Standard (Diazepam 1.50 mg/kg, i.p.)
Group III	Low dose of HRE (100 mg/kg, p.o)
Group IV	High dose of HRE (200 mg/kg, p.o)

Procedure: The Elevated plus maze (EPM) test is suggested to be a simple method for the evaluation of learning and memory in mice by measuring transfer latency. EPM served as exteroceptive behavioral model in which stimulus exist outside the body. An elevated plus maze consisting of two open arms (16cm x 5 cm) and two enclosed arms (16cm x 5cm x12 cm) were connected to give the apparatus a plus sign appearance was used. The arms extended from central platform (5cm x 5cm) and maze was elevated to the height of 25 cm.

from the floor. On the first the day (7th day of drug treatment), each mouse was placed at the end of open arm, facing away from central platform. Transfer latency was taken as the time taken by the mouse to move into any one of the covered arms with all its four legs. TL was recorded on the first day for the each animal.The mouse was allowed to explore the maze for another 2 min and returned to its home cage. Retention of this learned task was examined 24 h after the first day trial (i.e. 8th day of drug treatment).

RESULTS AND DISCUSSION

The plant material was extracted by cold maceration and the percentage yield calculatedby the following formula was found to be 0.45 % (by petroleum ether) and 8.43 % (by ethanol).

Solubility determination

Table 2: Solubility determination of extract.

S. No.	Solvent	Solubility of Petroleum ether extract	Solubility of methanolic extract
1.	Water	Insoluble	Soluble
2.	Ethanol	Partial soluble	Soluble
3.	Petroleum ether	Soluble	Soluble
4.	DMSO	Soluble	Soluble

Phytochemical testing

Table 3: Phytochemical testing of extract.

S. No	Experiment	Presence or absence of phytochemical test	
		Pet. Ether extract	Methanolic extract
1.	**Alkaloids**		
1.1	Mayer's reagent test	Absent	Present
1.2	Wagner's reagent test	Absent	Present
1.3	Hager's reagent test	Absent	Present
2.	**Carbohydrates**		
2.1	Molish's test	Absent	Absent
2.2	Fehling's test	Absent	Absent
2.3	Benedict's test	Absent	Absent
2.4	Barfoed's test	Absent	Absent
3	**Proteins and Amino Acids**		
3.1	Biuret test	Absent	Present
4.	**Flavonoids**		
4.1	Alkaline reagent test	Absent	Present
4.2	Lead Acetate test	Absent	Present
5.	**Glycoside**		
5.1	Borntrager test	Absent	Present
5.2	Legal's test	Absent	Present
5.3	Killer-Killiani test	Absent	Present
6.	**Tannin and Phenolic Compounds**		

6.1	Ferric Chloride test	Absent	Present
6.2	Lead Acetate test	Present	Present
6.3	Gelatin test	Absent	Present
7.	**Saponin**		
7.1	Foam test	Absent	Present
8.	**Test for Triterpenoids and Steroids**		
8.1	Salkowski's test	Absent	Absent

Acute oral toxicity: The acute oral toxicity study was carried out according to OECD 423 guidelines. Four ranges of dose were used for toxicity studies, i.e 5mg/Kg, 50 mg/Kg, 100 mg/Kg, 200 mg/Kg. animals were observed individually for next 4 hours after dosing for the presence of mortality during this period and 72 hours after sample administration.

Table 4: Representative results of acute oral toxicity of *hibiscus rosasinensis.*

S. No.	Groups	Observations/ Mortality
1.	5 mg/kg Bodyweight	0/3
2.	50mg/kg Bodyweight	0/3
3.	100 mg/kg Bodyweight	0/3
4.	200 mg/kg Bodyweight	0/3

The AOT revealed that maximum toxic dose was above 5 g/kg in mice, which indicated that the plant extract was relatively safe. Administration of *Hibiscus rosa- sinensis* flower methanolic extract at doses of 5, 50, 100, and 200, mg/Kg in mice, did not produce any significant changes in behavior, skin effect, breathing, defecation, postural abnormalities, impairment in food intake and water consumption and yellowing or loss of hair. Dosing of animal's upto 500 mg/kg of all extracts caused no toxicity in rats. The oral acute and subacute toxicity of methanol leaf extract of *Hibiscus rosa- sinensis* were investigated in mice. In the acute treatment, a single oral dose of 200 mg/kg of extract gave to mice at 48 h intervals, did not reveal any signs of toxicity or mortality in any animal during the 14 days observation period.

Forced swimming test (FST): The antidepressant effect of drug was investigated in the forced swimming test. Group I was vehicle control received normal saline, group II received Diazepam 1.50 mg/kg, group III was extract HRE 200mg/kg treated group and group IV extract HRE 400 mg/kg treated group. The results showed that, compared with the control group, drug at a dose of 400mg/kg significantly decreased the duration of immobility while animals administrated with drug at doses of 200 mg/kg demonstrated no statistically significant increase in the duration of immobility. Effects of oral administration of *Hibiscus rosa sinensis* (200, 400 mg/kg) on the duration of immobility in the forced swimming test in

mice. The total duration ofimmobility was recorded 1h after the last administration.

Table 5: Effects of oral administration of *hibiscus rosa sinensis.*

S. No	Group	Mean ± SD
1	Group - 1 (Vehicle) Control	221.6±13.42
2	Group - 2 (Diazepam –1.50mg/kg)	135.83±8.23
3	Group - 3 (Ext HRE- 200mg/kg)	178.6±16.39
4	Group - 3 (Ext HRE- 400mg/kg)	147±7.21

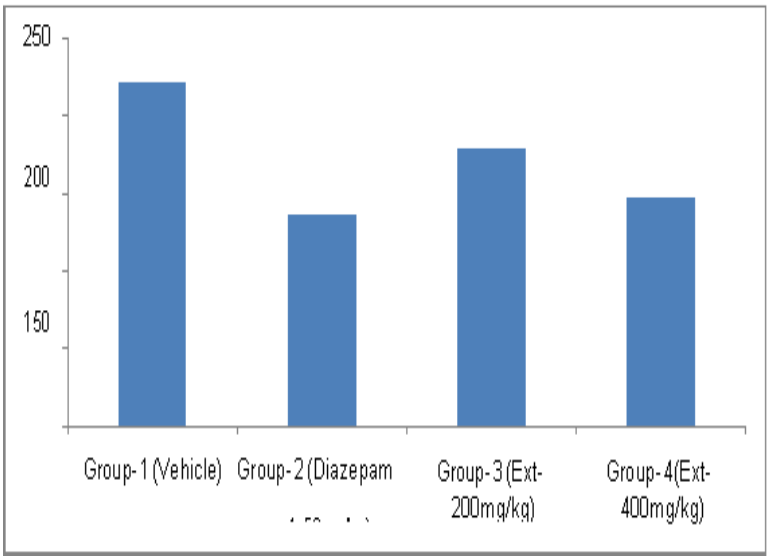

Figure 1: Effects of oral administration of *hibiscus rosa.*

Elevated plus-maze test: Dementia or cognitive problems are commonly seen in a large population. The factors such as emotions, stress and age are responsible for memory loss. Nootropics are the agents that improve memory or cognition. These are drugs, supplements, nutraceuticals, and functional foods that appeared to enhance mental functions such as cognition, memory, intelligence, motivation, attention, and concentration. EPM is a widely accepted model to study nootropic activity. In elevated plus maze, decrease in transfer latency time indicates the improvement of memory and vice versa. The Indian system of medicine focuses on utilization of herbs for controlling age-related neurodegenerative disorders. The animals treated orally with 200 mg/kg and 400 mg/kg of *Hibiscus rosa sinesis* extract showed changes indicating significant improvement in learning and memory.

Table 6: Effect of administration of *hibiscus rosa sinensis* on mice behavior in elevated plus maze.

S. No	Drug groups (n=6)	No. of open arm entries	No. of closed arm entries	Time spent in open arms	Time spent in closed arms
1	Control	4.5±0.763	2±0.81	140.8±4.77	91.5±4.4
2	Diazepam	5.3±0.74	1.83±0.37	156±5.72	38.6±5.64
3	Extract100mg/kg	3.5±0.5	4.16±0.37	115.8±7.2	110.8±7.94
4	Extract200mg/kg	4.5±0.5	3.5±0.5	119.3±4.14	104.8±5.6

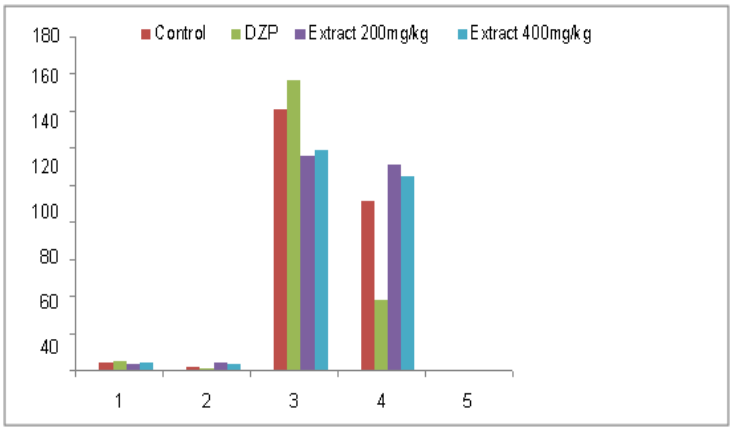

Figure 2: Effect of administration of *Hibiscus rosa sinensis* on micebehaviour in elevated plus maze.

DISCUSSION

Anxiety is a cardinal symptom of many psychiatric disorders and an almost inevitable component of many medical and surgical conditions. Indeed it is a universal human emotion, closely allied with appropriate fear presumably serving pyschobiologically adaptive purposes. Anxiety is a normal emotional behavior, however, becomes pathological precipitating cardiovascular and psychiatric disorders when it is severe. Although many drugs are available in allopathic medicine to treat anxiety disorders, they produce various systemic side effects or exhibit tolerance on chronic use.

Several classical anxiolytic and antidepressant drugs such as benzodiazepines, monoamine oxidase inhibitors, tricyclic antidepressants, selective serotonin reuptake inhibitors, serotonin-norepinephrine reuptake inhibitors, and noradrenergic and specific serotonergic antidepressants are widely used in clinical practice to treat these disorders. However, treatment by the above-mentioned drugs can also bring undesirable side effects including cardiovascular toxicity, sexual dysfunction, weight gain, and drug interactions. Therefore, there is an urgent need for the development of effective anxiolytic and antidepressant therapies without any or at least fewer adverse effects.

The animal models mentioned above are considered as the most widely validated tests for assaying anxiety and antidepressant substances such as benzodiazepines or amine uptake inhibitors. A natural conflict between the tendency to explore and the initial tendency to avoid an unknown risk occurs when a mouse is exposed to an unfamiliar environment. The exploratory activity reflects the combined effects of these tendencies in novel situations. In the elevated plus-maze model, based on the principle that is exposure to an elevated and open arm maze leads to a conflict, while the number of open arm entries and time spent in the open arm provide a measure of anxiety-induced inhibition of the normal exploratory activity. The forced swimming tests are behavioral despair models which give an indication of the clinical efficacy of various types of antidepressant drugs in rodents. These animal models are based on the despair orhelplessness behavior in response to some inescapable and confined space and are sensitive to various antidepressant drugs. The forced swimming state of immobility in animals are claimed to represent a condition similar to human depression and are amenable to be reversed by antidepressant drugs.

This study evaluated the anti anxiety and antidepressant activities of the in mice. We found that administration of 400□mg/kg *Hibiscus rosa sinensis* or 20□mg/kg DZP for 7 days significantly reduced the immobility time in FST. These results provide support for the potential anti anxiety and antidepressant activity of *Hibiscus rosa sinensis* and contribute towards validation of the traditional use of *Hibiscus rosa sinensis* in the treatment of emotional disorders.

In this study, elevated plus maze test were used to evaluate the anti anxiety and anti depressant activity of extract *Hibiscus rosa sinensis* of in albino mice. The elevated plus maze is considered to be an etiologically valid animal model of anxiety. In the elevated plus maze, the open arms are more fear provoking than the closed arms. The reduction in entry and time spent in open arms are the indications of the high level of fear or anxiety. The number of entries and time spent in the open arms have been found to be increased by anxiolytics and reduced by anxiogenic agents. A significant increase in the time spent in open arms was observed after treatment with all two doses of drug. A significant increase in both time spent in open arms and the entry into open arms is observed after treatment *Hibiscus rosa sinensis* extract.

CONCLUSION

Anxiety is a normal emotional behavior, however, becomes pathological precipitating

cardiovascular and psychiatric disorders when it is severe. Many allopathic drugs are available to treat anxiety disorders, among which benzodiazepines are most commonly used which possess various systemic side effects.

Among the many mental illnesses and behavioral disorders, depression and anxiety are the two most prevalent psychiatric disorders. Several classical anxiolytic and antidepressant drugs such as benzodiazepines, monoamine oxidase inhibitors, tricyclic antidepressants, selective serotonin reuptake inhibitors, serotonin-norepinephrine reuptake inhibitors, and noradrenergic and specific serotonergic antidepressants arewidely used in clinical practice to treat these disorders.

The results obtained in this study suggest that the extract of the leaves of *Hibiscus rosa sinensis* possesses anti anxiety and anti depressant activity. Thus, *Hibiscus rosa sinensis* has potential clinical application in the management of anxiety disorders. Further investigation of the mechanism/ mechanisms of action of the plant extract, as well as the active substance/substances responsible for its biological actions, is necessary.

Conflicts of interests

There are no conflicts of interests.

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