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"PHYTOCHEMICAL SCREENING AND ANTI ANXIETY & ANTI DEPRESSANTS SCREENINGOF HIBISCUS ROSA FLOWERS EXTRACT"

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ABSTRACT

Hibiscus rosa sinensis Linn (Family: Malvaceae) contain flavonoids. Flavonoids have been found to have antidepressant activity. This research work is concentrated about the extractions, phytochemical investigation and biological evolution of the antidepressant activity of Flower extract of *Hibiscus rosa sinensis* in the Elevated plus-maze test and forced swimming test experimental animal model respectively. Anti-Depressant activity of Petroleum ether extract and methanolic extract containing *Hibiscus rosa sinensis* flower evaluated in plus-maze test and forced swimming test mice as compared to vehicle control. *Hibiscus rosa sinensis* extracts consists of this are the constituents are responsible for controlling the oxidation. The extract at oral doses 200 and 400 mg/kg for 14 days significantly $P \leq 0.01$ decreased the duration of immobility as dose-dependent manner in the flowers extract of *Hibiscus rosa sinensis* in the mice. In our study we explored selective mechanism of action of *Hibiscus rosa sinensis* flowers extract responsible for antidepressant activity

Keyword: *Hibiscus rosa sinensis,* Elevated plus-maze test, forced swimming test

1. INTRODUCTION

Anxiety disorders are present in up to 13.3% of individuals in the U.S. and constitute the most prevalent subgroup of mental disorders. The extent of their prevalence was first revealed in the Epidemiological Catchments Area study about 26 years ago. Despite their widespread prevalence, these disorders have not received the same recognition as other major syndromes such as mood and psychotic disorders; in addition, the primary care physician is usually the principal assessor and treatment provider. As a result of this management environment, anxiety disorders can be said to account for decreased productivity, increased morbidity and mortality rates, and the growth of alcohol and drug abuse in a large segment of the population.

Advances in anxiety research over the previous decade are likely to be reflected in modifications of diagnostic criteria in the upcoming DSM-5 planned for publication in May 2013. For instance, post-traumatic stress disorder (PTSD) and obsessive– compulsive disorder (OCD) have been reclassified in the separate domains of Trauma and Stressor Related Disorders and Obsessive–Compulsive and Related Disorders, respectively.

Depression

Depression is a disorder of major public health importance, in terms of its prevalence and the suffering, dysfunction, morbidity, and economic burden. Depression is more common in women than men. The report on Global Burden of Disease estimates the point prevalence of unipolar depressive episodes to be 1.9% for men and 3.2% for women, and the one-year prevalence has been estimated to be 5.8% for men and 9.5% for women. It is estimated that by the year 2020 if current trends for demographic and epidemiological transition continue, the burden of depression will increase to 5.7% of the total burden of disease and it would be the second leading cause of disability- adjusted life years (DALYs), second only to ischemic heart disease.

Worldwide, an estimated 4.4% of the population are living with depression . People with depression often do not get appropriate and timely care because health systems are not organised to deliver evidence-based treatments in an accessible format Collaborative care is a health service delivery framework developed to optimise depression care by using:

i) multidisciplinary approaches to working with input from two or more health care professionals, ii) structured evidenced-based case management, iii) proactive and scheduled patient follow-up, and iv) enhanced inter-professional communication systems.

Depression is a condition characterized by altered mood. An estimated 3-5% of the world's population experiences depression on any given date. There is a loss of interest in all usually pleasurable outlets such as food, sex, work, friends, hobbies or entertainment. Diagnostic criteria include presence of depressed mood nearly every day, markedly diminished interest/pleasure in most or all activities and three or more of the following:

- Poor appetite/significant weight loss/increased weight gain
- Insomnia/hypersomnia
- Psychosomatic agitation/retardation
- Feeling of hopelessness
- Loss of energy or fatigue
- Feelings of worthlessness, self-approach or excessive or inappropriate guilt
- Complaints of or evidence of diminished ability to think/concentrate
- Recurrent thoughts of death, suicidal ideation, wish to be dead or attempted suicide.

Community studies have shown that the prevalence rate of depression, defined by strict operational criteria, is approximately 6-8% for women and 3-5% for men. Depression is more common in lower social classes and among inner city dwellers. Women in their child bearing years are especially vulnerable. [30-31]

2. MATERIAL AND METHODS

2.1 Selection of plant and authentication

Fresh flowers of *Hibiscus rosa sinensis* was collected and authenticated bybotanist.

2.2 Extraction of Plant

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.. 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:

$$\% \text{ yield} = \frac{\text{Weight of extract}}{\text{Weight of plant material used}} \times 100$$

2.3 Phytochemical Screening

The flowers of *Hibiscus rosa sinensis* extract acquire was subjected to the precursory phytochemical analysis following standard methods by Khandelwal and Kokate. The extract was screened to identify the presence of various active principles of alkaloids, glycosides, phenols, flavonoids, Terpenoids, Saponins, Steroids.

2.4 Pharmacological Activity

Animals were selected at random from animal house of PBRI, Bhopal, India. Animals were further randomly divided into various treatment groups and kept in propylene 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. Fourranges of dose were used for toxicity studies, i.e 5mg/Kg, 50 mg/Kg, 300 mg/Kg, 2000 Mg / Kg. animals were observed individually for next 4 hours after dosing for thepresence of mortality during this period and 72 hours after sample administration.

2.4.1 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 Impiramine (30mg/kg), group III was treated with extract 200mg/kg bw and group IV was treated with extract 400 mg/kg bw.

2.4.2 Elevated plus-maze test

Elevated plus-maze is the most simple apparatus to study neuroprotective effects and anxiolytic 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.

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 1.5 mg/kg, i.p.)
Group III	Low dose of HRE (200 mg/kg, p.o)
Group IV	High dose of HRE (400 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).

3. Result

3.1 Plant Extraction

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

3.2 Result of 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

3.3 Result of 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
8.2	Libbermann-Burchard's test	Absent	Absent

3.4 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, 300 mg/Kg, 2000 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 rosa sinensis*

S. No.	Groups	Observations/ Mortality
1.	5 mg/kg Bodyweight	0/3
2.	50mg/kg Bodyweight	0/3
3.	300 mg/kg Bodyweight	0/3
4.	2000 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, 300, and 2000, 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 yellowingor 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 2000 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.

3.5 FST

The antidepressant effect of drug was investigated in the forced swimming test. Group I was vehicle control received normal saline, group II received Impiramine 30 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.

Table 5 :Effects of oral administration of *Hibiscus rosa sinensis* (200, 400 mg/kg) onthe duration of immobility in the forced swimming test in mice. The total duration ofimmobility was recorded 1h after the last administration.

S.no	Group	Mean±SD
1	Group - 1 (Vehicle) Control	221.6±13.42
2	Group - 2 (Impiramine -30mg/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

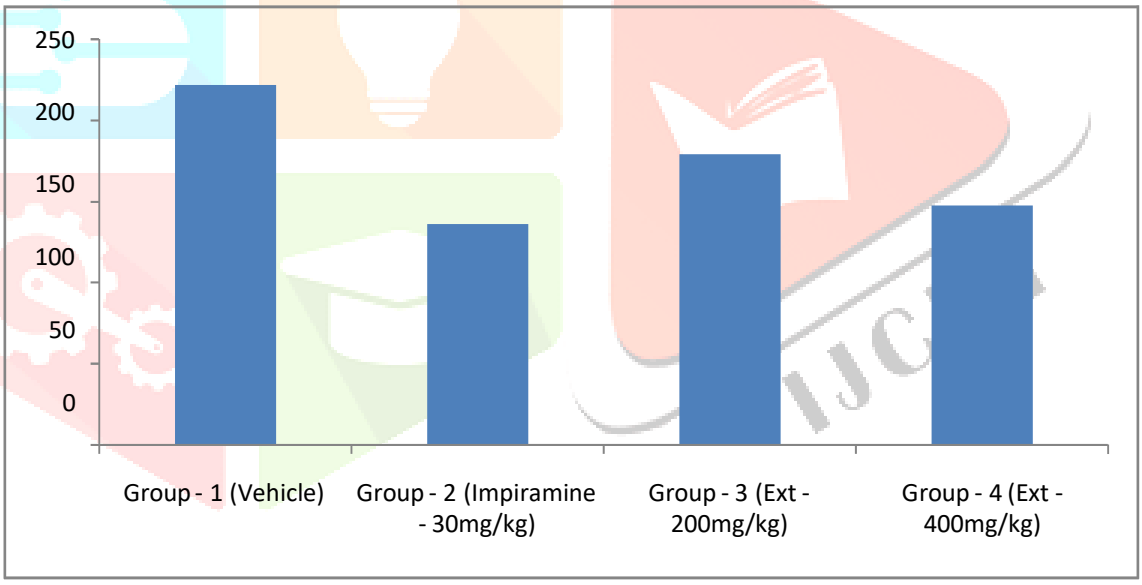

Figure 1: Effects of oral administration of *Hibiscus rosa sinensis* (200, 400, mg/kg) on the duration of immobility in the forced swimming test in mice. The total duration of immobility was recorded 1 h after the last administration.

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 behaviour 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	DZP	5.3±0.74	1.83±0.37	156±5.72	38.6±5.64
3	Extract200mg/kg	3.5±0.5	4.16±0.37	115.8±7.2	110.8±7.94
4	Extract400mg/kg	4.5±0.5	3.5±0.5	119.3±4.14	104.8±5.6

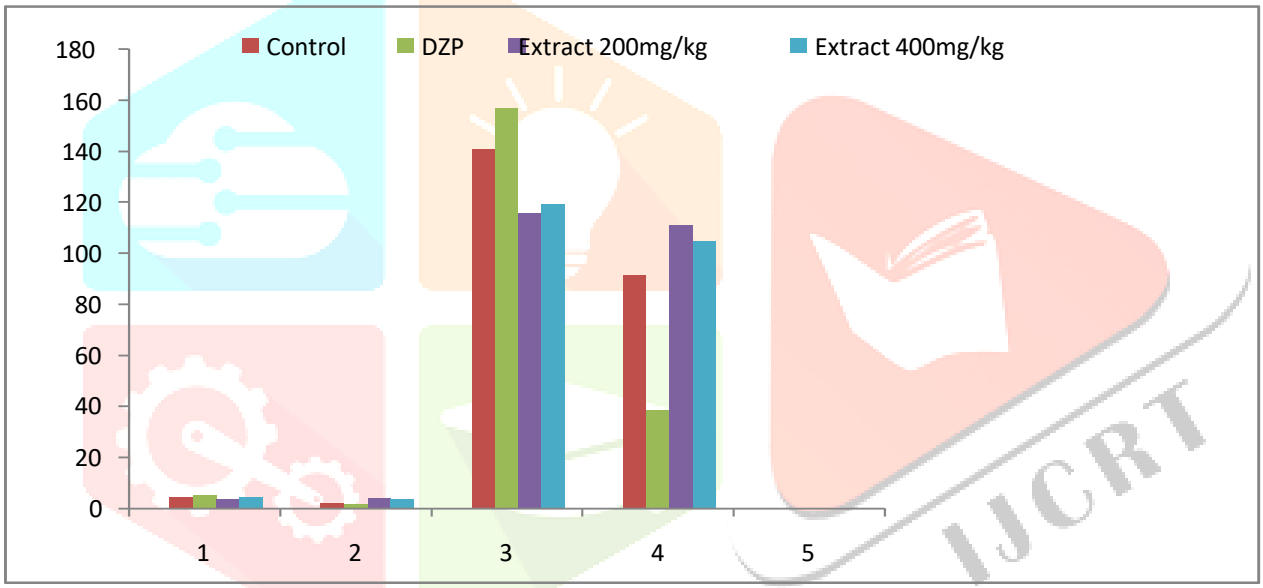

Figure 2: Effect of administration of *Hibiscus rosa sinensis* on micebehaviour in elevated plus maze.

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 test 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 or helplessness 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 ani 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.

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