ISSN: 2231-6078
INTERNATIONAL JOURNAL OF DRUG DISCOVERY AND HERBAL RESEARCH (IJDDHR)
10(3): July.-Sept.: (2020), 778-785

Research Article

Pharmacological Screening of *Hibiscus Rosa* in rodent Models

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Abstract

To investigate the ant anxiety and anti depressant effects of hibiscus rosa in rodent model. The screening was performed by extraction of hibiscus rosa using continuous hot percolation "soxhalation". Obtained drug extract is used with standard drug (imipramine for anti depressant and diazepam for anti anxiety) in different doses comparative studies, the antidepressant effect of drug was investigated in the FST (forced swimming test), the animal were divided into four groups and each group has 6 animals. Group I was vehicle control received normal saline, group II received drug Impiramine (30 mg / kg), group III was treated with HRE extract 200 mg / kg body weight and group IV was treated with HRE extract 400 mg / kg body weight, the total duration of immobility was recorded, in further behavioral assay was performed in mice by using Elevated plus maze test, for this animals were divided into four groups and each group has 6 mice. Group I was vehicle control received normal saline. Group II received drug diazepam (1.5mg / kg), group III was treated with HRE extract 200 g / kg body weight and group IV was treated with HRE extract 400mg / kg body weight and mice behavior in elevated plus maze was recorded. The FST results showed that, compared with the control group, drug at a dose of 400 mg / kg significantly decreased the duration of immobility while animals administrated drug at doses of 200 mg / kg demonstrated no statistically significant increase in the duration of immobility. Furthermore, in Elevated plus maze, the animal treated orally with 200 mg / kg and 400 mg / kg of HRE extract showed changes indicating significant improvement in learning and memory, more time spent in open arm. The results obtained in this study suggest that the extract of leaves of Hibiscus rosa sinesis possess anti anxiety and anti depressant activity. Thus, HRE has potential clinical application in the management of anxiety and depression disorder. Further investigation of mechanism / mechanism of action of plant extract as well as the active substances responsible for its biological actions is necessary

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.

Pathophysiology 5-6

Pathophysiology of anxiety is associated with multiple regions of the brain such as (a) Amygdala, a temporal lobe structure which assesses the fearul stimuli and produces responses to fear. (B) Locus ceruleus a noradrenaline (NA) involving site located in the brain stem with widespread projections to areas responsible for producing fear responses (vagus, lateral and paraventricular hypothalamus). (c) Hippocampus region. (d) The hypothalamus is the principle area for generating neuroendocrine and autonomic responses to fear. The neurochemical theory of anxiety includes the abnormal functioning of several neurotransmitters such as NA, gamma aminobutyric acid (GABA) and serotonin (5-HT). The autonomic nervous system of anxious patient is hypersensitive and over reacts to various stimuli such as threat or fear, in such condition the locus ceruleus acts as an alarm center causing NA release and stimulate the sympathetic and parasympathetic nervous system producing anxiety symptoms. 5- HT is primarily an inhibitory neurotransmitter and the abnormalities in its function through release and uptake at the presynaptic autoreceptors (5-HT1A / 1D), the serotonin reuptake transporter site or effect of 5-HT at the postsyna plays a major role in the development of anxiety.

Stress is an important factor causing anxiety disorder. The stress related anxiety disorder is produced by persistent changes in the stress responsive CNS -CRF system.

other layer are consist of push layer (Fig.1). The drug layer mainly consists of drug along with two or more different agents. So this drug layer comprises of drug

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Cell bodies containing CRF are highly found in medial parvocellular region of hypothalamic para ventricular nucleus (PVN). The parvocellular region of hypothalamic PVN is considered as the neuroendocrine stress response system. The stress response in humans involves a series of hormonal events by the activation of hypothalamus pituitary adrenal axis which leads to the increased release of cortisol and adrenaline (stress hormones).

These stress hormones reacts with body and brain in various complicated mechanisms producing anxiety disorders (a maladaptive emotional state causing fear, anxiety and excessive stress characterized by physiological arousal, unpleasant tension and feeling of apprehension.

Causes of Anxiety Disorders :-

- Most research indicates that anxiety disorders are complex and arise from intricate neurochemical, neuroanatomic, neuroinflammatory, genetic, neuroendocrinologic and psychoimmunologic factors.
- Cognitive-behavioral theory
- Neurochemical theory
- Psychoimmunologic or immune-mediated theory.
- Environmental factors and stress theory.

Specific Anxiety Disorders :-

- Separation Anxiety Disorder
- Panic Disorder
- Agoraphobia
- Generalized Anxiety Disorder

Specific Phobias :-

- Social Anxiety Disorder
- Post traumatic Stress Disorder

Management of Anxiety :-

- People with anxiety disorders can benefit from a variety of treatments and services. Following an accurate diagnosis, possible treatments include psychological treatments and mediation.
- **Psychological treatments :**
- Behavioral therapies :
- Cognitive-behavioral therapy (CBT)
- Psychotherapy
- Psychodynamic therapy
- Family therapy and parent training
- **Drug Therapy :**
- Antianxiety drugs :

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. 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,

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.

Symptoms :

• 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 dissolution medium. The samples withdrawn were analyzed by UV spectrophotometer using multi component mode of analysis
• Complaints of or evidence of diminished ability to think/concentrate
• Recurrent thoughts of death, suicidal ideation, wish to be dead or attempted suicide.

- Depression can be care by optimize framewor:

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

Management of Depression:-

- People with depression disorders can benefit from a variety of treatments and services. Following an accurate diagnosis, possible treatments include psychological treatments and mediation.
- Psychological treatments:
- Behavioral therapies :
- Cognitive-behavioral therapy (CBT)
- Psychotherapy
- **Drug Therapy :**
- **Antidepressant drugs :**

Material and method:-

Plant profile:-

SCIENTIFIC CLASSIFICATION:

Kingdom:		Plantae
Clade	:	Magnoliopsida
Order	:	Malvales
Family	:	Malvaceae
Genus	:	Hibiscus
Species	:	Hibiscus rosa-sinensis

Extraction process:-

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:

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

Methodology

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.

Pharmacological Activity

Experimental Animals

Strain:Swiss mice

Sex :Either

Body weight :25 ± 5 gm

Housing condition :As per CPCSEA guidelines

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. 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

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

Forced swimming test (FST) 90

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 measure of 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.

Elevated plus-maze test 91

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.

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.

Requirements: Elevated plus maze

Grouping: Swiss albino mice weighing between 25-30

gms were randomly divided into 4 groups each containing 6 mice.

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 tothe 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.

RESULT:-

Plant Extraction

Formula: [weight of extract / weight of powdered drug] X 100

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

Solubility Determination

Table 3: Solubility determination of extract

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

Phytochemical Testing

Table 4: 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

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 5: Representative results of acute oral toxicity of *Hibiscus rosa sinensis*

S. No.	Groups	Observations/ Mortality
1.	5 mg/kg	0/3

	Bodyweight	
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 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 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.

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

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

Table 6 : 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 1h 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.

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	Extract 200mg/kg	3.5±0.5	4.16±0.37	115.8±7.2	110.8±7.94
4	Extract 400mg/kg	4.5 ±0.5	3.5 ±0.5	119.3 ±4.14	104.8 ±5.6

Table 7: Effect of administration of *Hibiscus rosa sinensis* on mice behaviour 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 e 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 are widely 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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