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Research Article

HIBISCUS ROSA SINENSIS POSSIBLE PROTECTIVE AGAINST DEPRESSION IN MICE

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Abstract:

Aim of the present study was to evaluate the antidepressant activity of Hibiscus rosa sinensis in experimental models of depression using mice. To study the effect of Hibiscus rosa sinensis on behavior models of depression like forced swim test, tail suspension test. To study the effect of Hibiscus rosa sinensis on mechanism based models of depression like 5-HTP induced head twitches, clonidine induced aggression and L-Dopa induced hyperactivity and aggressive behavior. To study the effect of Hibiscus rosa sinensis on anti-oxidant levels of brain. The results from the present study confirm the antidepressant activity of hibiscus rosa sinensis, since it reduced the immobility in both FST and TST. In the present study, hibiscus rosa sinensis significantly increased the frequency of 5-HTP induced head twitches, Clonidine induced aggression and L-DOPA induced hyperactivity and aggressive behavior indicating its enhanced activity on serotonergic, noradrenergic and dopaminergic pathways respectively. Our results also confirm the involvement of serotonergic, noradrenergic and dopaminergic pathways in depression. Pretreatment with hibiscus rosa sinensis, also significantly increased the levels of SOD and Catalase with simultaneous decrease in LPO levels in mice brain, suggesting its strong antioxidant activity. Since oxidative stress is reported to play an important role in depression, the antioxidant activity of hibiscus rosa sinensis might be a part of the mechanism for its antidepressant activity. Results from behavioral experiments indicate that the antidepressant activity of hibiscus rosa sinensis, might be due to the facilitatory effect on serotonergic, noradrenergic and dopaminergic systems apart from the antioxidant activity.

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INTRODUCTION:

Depression is the most common of the affective disorders (disorders of mood rather than disturbances of thought or cognition); it may range from a very mild condition, bordering on normality, to severe (psychotic) depression accompanied by hallucinations and delusions. There are two types and they are Unipolar and Bipolar Unipolar depression is commonly (about 75% of cases) non-familial, clearly associated with stressful life-events and accompanied by symptoms of anxiety and agitation; this type is sometimes termed reactive depression. Other patients (about 25%, sometimes termed endogenous depression) show a familial pattern, unrelated to external stresses, and with a somewhat different symptomatology². This distinction is made clinically, but there is little evidence that antidepressant drugs show significant selectivity between these conditions.

The underlying pathophysiology of major depressive disorder has not been clearly defined. Clinical and preclinical trials suggest a disturbance in central nervous system (CNS) serotonin (5-HT) activity as an important factor. Other neurotransmitters implicated include norepinephrine (NE) and dopamine (DA) The role of CNS 5-HT activity in the pathophysiology of major depressive disorder is suggested by the efficacy of selective serotonin reuptake inhibitors (SSRIs) in the treatment of major depressive disorder [4].

Hibiscus is a genus of flowering plants in the mallow family, Malvaceae. The genus is quite large, comprising several hundred species that are native to warm temperate, subtropical and tropical regions throughout the world. Member species are renowned for their large, showy flowers and those species are commonly known simply as "hibiscus", or less widely known as rose mallow. Other names include hardy hibiscus, rose of sharon, and tropical hibiscus [7].

Each part of *H. rosa sinensis* contains a wide range of compounds. It was reported that phlobatannins, glycosides, saponins, flavonoids, terpenoids including other compounds such as thiamine, riboflavin and niacin are present in leaves, flowers, stem and roots. According to Patel and Adhav, whose study was conducted on four different morphotypes of *H. rosa sinensis*, glucosides, flavonoids, phytosterols, terpenoids, tannins, and phenolic compounds contributed to the pharmacological effects of the plant as they were present in all of them. This suggested that although the flower color differed, the phytochemical constitutions were very similar [8]. These findings also correlates with those

of another study carried out by thin layer chromatographic analysis.

The red hibiscus is the flower of the Hindu goddess Kali, and appears frequently in depictions of her in the art of Bengal, India, often with the goddess and the flower merging in form. The hibiscus is used as an offering to goddess Kali and Lord Ganesha in Hindu worship. In the Philippines, the gumamela (local name for hibiscus) is used by children as part of a bubble-making pastime. The flowers and leaves are crushed until the sticky juices come out. Hollow papaya stalks are then dipped into this and used as straws for blowing bubbles. Together with soap, hibiscus juices produce more bubbles. Also called "Tarukanga" in waray particularly in eastern samar province [10].

Drugs and Chemicals:

Thiobarbituric acid and DTNB reagent (Hi Media Laboratories Ltd., Mumbai), Trichloro acetic acid (Qualigens Fine Chemicals, Mumbai), Riboflavin (Astra IDL, Bangalore), Sodium dihydrogen phosphate and Disodium hydrogen phosphate (S.D. Fine Chemicals, Mumbai), Lorazepam (Ranbaxy, India), 1,1,3,3,-Tetraethoxy propane, O-Dianisidine, Imipramine hydrochloride, 5-Hydroxy Tryptophan (5-HTP), Clonidine and L-DOPA (Sigma, St. Louis, USA) were used in the study. The other chemicals and solvents used were of analytical grade and purchased from commercial suppliers. Imipramine (IMP), 5-HTP, clonidine, L-DOPA, Lorazepam was administered intraperitoneal by dissolving in normal saline.

METHODOLOGY:

Collection and Authentication of Plant Material:

The Aerial Parts (leaves and flowers) of *Hibiscus rosa sinensis* were collected and authenticated

Animal Ethics permission: the housing of the animals were carried out in the animal house of the Teja College of pharmacy-kodad, the treatment and sample collection, analysis of samples carried out in VYAS LABS, Medchal, Malkajgiri with approved CPCSEA registration number-2085/PO/RCBIBT/S/19/CPCSEA

Cold Extraction (Ethanol Extraction)[38]:

In this work the cold extraction process was done with the help of ethanol. About 45-60gms of powdered material was taken in a clean, flat bottomed glass container and soaked in 750 ml of ethanol. The container with its contents were sealed and kept for period of 7 days accompanied by continuous shaking with the shaker. The whole

mixture then went under a coarse filtration by a piece of a clean, white cotton wool.

Evaporation of Solvent:

The filtrates (ethanol extract) obtained were evaporated using Rotary evaporator in a porcelain dish. They rendered a gummy concentrate of greenish black. The extract was kept in vacuum desiccator for 7 days.

Preliminary Phytochemical Screening:

Preliminary phytochemical screening of the *Hibiscus rosa sinensis* extract was carried out for the analysis of Alkaloids, Carbohydrates, Tannins, Saponins, Steroids, Phenols, Flavonoids. As per the standard methods⁴⁰.

Animals:

Healthy Adult Male mice of 5 weeks old with Average weight in the range of 20-25gms were selected. Animals are housed 4 per cage in temperature controlled ($27^{\circ}\text{C} \pm 3^{\circ}\text{C}$) room with light/dark cycle in a ratio of 12:12 hrs is to be maintained. The Animals are allowed to acclimatize to the environment for seven days and are supplied with a standard diet and water *ad libitum*. The prior permission was sought from the Institutional Animal Ethics Committee (IAEC) for conducting the study.

Acute toxicity studies [3,12]:

Acute toxicity studies will be performed for ethanolic extract according to the acute toxic classic method as per OECD guidelines. Male mice were used for acute toxicity study. The animals were kept fasting for overnight providing only water, after which the extract will be administered orally at the dose of 300mg/kg and observed for 14 days. If mortality was observed in two animals out of three animals, then the dose administered was assigned as toxic dose. If the mortality was observed in one animal, then the same dose was repeated to confirm the toxic dose. If mortality was not observed, the procedure was repeated for further higher doses such 50,200 & 2000mg/kg body weight. The animals were observed for toxic symptoms for 72 h.

IN VIVO MODELS OF DEPRESSION EMPLOYED IN THE STUDY:

1. Forced swimming test (FST):

The procedure was described by Porsolt et al. (1978) was used. Swimming sessions were conducted by placing mice in individual glass cylinders (45 cm high×20 cm in diameter) containing ($25 \pm 2^{\circ}\text{C}$) water 38 cm deep, so mice could not support themselves by touching the bottom with their feet. Two swimming sessions were performed between 12:00 h and 19:00

h, an initial 15 min pretest followed 24 h later by a 6 min test.

Doses were given once daily for 7 days. On the 7th day mice were subjected to 15 min pretest. After 15 min, in the water the mice were removed and allowed to dry in a heated enclosure (32°C) before being returned to their home cages. They were again placed in the cylinder 24 h later and the total duration of immobility was measured during a 6 min test. Floating behavior during this 6 min period had been found to be reproducible in different groups of mice. An animal was judged to be immobile whenever it remains floating passively in the water in a slightly hunched but upright position, its nose just above the surface. The total immobility time for the period of 6 min was recorded with the help of stopwatch.

2. Tail suspension test (TST):

Doses are given once daily for 7 days. On the 7th day, 1hr after the administration of the test and standard drugs, mice were suspended on the edge of a table 50 cm above the floor by the adhesive tape placed approximately 1 cm from the tip of the tail. Immobility time was recorded during a 6 min period. [29]Animal was considered to be immobile when it did not show any movement of body and hanged passively.

3. 5-HTP induced head twitches in mice:

Doses were given once daily for 7 days. On the 7th day, 1hr after the administration of the test and standard drugs, mice were treated with 5-HTP (100 mg/kg i.p.) and the numbers of head twitches performed by each mice was counted by staggering method using three 2 min periods (19–21 min), (23–25 min), (27–29 min) after 5-HTP administration and number of head twitches were scored live by a blind observer.

4. Clonidine-induced aggression in mice:

Doses were given once daily for 7 days. On the 7th day, Clonidine was given 1 h after the administration of the test and standard drugs. The animals were then caged in bell shaped glass jar with a floor area of approximate 16 cm^2 . The biting/fighting episodes were recorded live by a blind observer over a period of 30 min, in each pair.

5. L-DOPA induced hyper activity and aggressive behavior in mice (LHA):

Mice were treated with L-DOPA (100 mg/kg i.p.) and the experiment was performed according to the method of. Mice were divided into 5 groups of 8 each ($n=8$), each group contain 4 pairs of mice, two pairs

from each sex (each pair contained same sex of mice).

Doses were given once daily for 7 days. On the 7th day, L-DOPA was given 1 h after the administration of the test and standard drugs. Stages of activity and aggressive behavior were recorded live every 10 min for 30 min after L-DOPA administration by the blind observer. The different parameters of observation were piloerection, salivation, increase in motor activity, irritability, reactivity, jumping squeaking, and aggressive fighting. The scores were graded in the following manner:

0—No effect; 1—Piloerection, slight salivation, slight increase in motor activity; 2—Piloerection, salivation, marked increase in motor activity and irritability; 3—Piloerection, profuse salivation, marked increase in motor activity, reactivity, jumping, squeaking and aggressive fighting.

Statistical analysis:

Results were expressed as mean \pm S.E.M. Statistical analysis was performed using one-way analysis of variance (ANOVA). If the overall *P*-value was found statistically significant (*P* < 0.05)

RESULTS AND DISCUSSION:

% Yield value of Ethanolic Extract from Aerial Parts of *Hibiscus rosa sinensis* was found to be **14.65%**

Preliminary Phytochemical Screening:

Investigation revealed the presence of steroid, Alkaloid, saponins, Tannins, phenols & Flavonoid in Ethanolic Extract of *Hibiscus rosa-sinensis*

Acute toxicity studies:

As per (OECD) draft guidelines 423 Female albino mice were administered *Hibiscus rosa-sinensis* and doses was be selected in the sequence (1.75- 5000) using the default dose progression factor, for the purpose of toxicity study. Animals are observed individually at least once during the first 30 minutes after dosing, periodically during the first 24 hours and daily thereafter, for a total of 14 days,. In all the cases, no death was observed within 14 days. Attention was also given to observation of tremors and convulsions, salivation, diarrhoea, lethargy, sleep and coma. Overall results suggested the LD₅₀ value as 2000 mg/kg. Hence therapeutic dose was calculated as 1/10th and 1/20th i.e. 100mg/kg and 200 mg/kg of the lethal dose for the purpose anti depressant investigations.

Forced Swim Test (FST):

The results (Table. 1) showed that both EEHRS (100, 200 and 400 mg/kg, p.o.) and imipramine (15 mg/kg, i.p.) significantly decreased the duration of immobility time in a dose dependent manner in FST model. Post-hoc analysis showed that the EEHRS (100, 200 and 400 mg/kg) and Imipramine (IMP) treated groups were significantly different (*p*<0.001) from the vehicle treated group (Fig. 1).

Table. 1. Effect of *Hibiscus rosa-sinensis* and imipramine (IMP) on forced swim test (FST) in mice.

Group no.	Treatment (dose in mg/kg)	Immobility period (sec) Mean \pm SEM
I	Control (0.3% CMC) + FST	136.3 \pm 7.3
II	EEHRS (100 mg/kg, p.o.) + FST	125.2 \pm 10.3
III	EEHRS (200 mg/kg, p.o.) + FST	98.5 \pm 8.6*
IV	EEHRS (400 mg/kg, p.o.) + FST	82.0 \pm 7.4*
V	Imipramine (15 mg/kg, i.p.) + FST	70.1 \pm 5.0*

Each column represents mean \pm S.E.M. of immobility period (sec), n = 6. * = *p*<0.001 compared to control

Tail Suspension Test (TST):

The results (Table. 2) showed that both EEHRS (100,200,400 mg/kg, p.o.) and imipramine (15 mg/kg, i.p.) significantly decreased the duration of immobility time in a dose dependent manner in TST model. Post-hoc analysis showed that the EEHRS (100, 200 and 400 mg/kg) and IMP treated groups were significantly different (*p*<0.001) from the vehicle treated group.

Table.2. Effect of *Hibiscus rosa-sinensis* and Imipramine (IMP) on tail suspension test (TST) in mice

Group no.	Treatment (dose in mg/kg)	Immobility period (sec)
I	Control (0.3% CMC) + TST	135.2±10.3
II	EEHRS (100 mg/kg, p.o.) + TST	110.6±10.2 ^a
III	EEHRS (200 mg/kg, p.o.) + TST	98.4±9.3 ^a
IV	EEHRS (400 mg/kg, p.o.) + TST	80.3±7.1 ^a
V	Imipramine (15 mg/kg, i.p.) + TST	63.4±5.5 ^a

Each column represents mean ± S.E.M. of immobility period (sec), n = 6. a = p<0.001 compared to control

5-HTP induced head twitches in mice:

Table.3. illustrates the effect of *Hibiscus rosa-sinensis* and IMP on 5-HTP-induced head twitches in mice. Post-hoc analysis revealed that three doses of *Hibiscus rosa-sinensis* (100, 200 and 400 mg/kg, p<0.01, p<0.001) significantly increased the 5-HTP-induced head twitches in comparison to control group. Further, the dose of 400 mg/kg was more effective than 100, 200 mg/kg. Similarly, IMP treated group showed significant increase (p<0.001) in the 5-HTP-induced head twitches compared to control. However, the effect of 400 mg/kg of *Hibiscus rosa-sinensis* was significantly higher than IMP (p<0.001) (Fig. 3).

Table. 3. Effect of *Hibiscus rosa-sinensis* on 5-HTP-induced head twitches in mice.

Group no.	Treatment (dose in mg/kg)	Head twitches Mean ± SEM
I	Control (0.3% CMC)	12.7±1.2
II	EEHRS (100 mg/kg, p.o.)	20.8±2.1 ^a
III	EEHRS (200 mg/kg, p.o.)	29.0±2.6 ^b
IV	EEHRS (400 mg/kg, p.o.)	37.8±3.5 ^b
V	Imipramine (15 mg/kg, i.p.)	24.0±2.2 ^b

Each column represents mean ± S.E.M. of number of head twitches, n = 6. a = p<0.01, b = p<0.001 compared to control

L-DOPA induced hyperactivity and aggressive behavior in mice:

The effect of *Hibiscus rosa-sinensis* and lorazepam on L-DOPA-induced hyperactivity and aggressive behavior is shown in Table 4. Post-hoc analysis revealed that three doses of *Hibiscus rosa-sinensis* (100,200 and 400 mg/kg, p<0.001) significantly increased the L-DOPA-induced hyperactivity and aggressive behavior (LHA) in comparison to control group.

Table.4. Effect of EEHRS and Lorazepam on L-DOPA-induced hyperactivity and aggressive behavior in mice.

Group o.	Treatment (dose in mg/kg)	Behavioral score
I	Control (0.3% CMC)	1
II	EEHRS (100 mg/kg, p.o.)	2.0 ± 0.2 ^a
III	EEHRS (200 mg/kg, p.o.)	2.0 ± 0.2 ^a
IV	EEHRS (400 mg/kg, p.o.)	2.6 ± 0.2 ^a
V	Lorazepam (2.5 mg/kg, i.p.)	2.1 ± 0.2 ^a

Each column represents mean ± S.E.M. of number of head twitches, n = 6. a = p<0.001, compared to control

Clonidine induced aggression in mice:

Table. 5. Indicates the effect of *Hibiscus rosa-sinensis* (100, 200 and 400 mg/kg, p.o.) and lorazepam (LA; 2.5 mg/kg) on the latency to first attack and the number of bouts in the clonidine induced aggressive behavior in mice. Post-hoc analysis showed that *Hibiscus rosa-sinensis* ($p < 0.001$) significantly increased the latency to first attack and decrease the no. of bouts compared to control.

Table 5: effect of *Hibiscus rosa-sinensis* on clonidine induced aggression in mice.

Group no.	Treatment (dose in mg/kg)	% Response (MEAN \pm SEM)	
		Latency to 1 st attack	Fighting response
I	Control (0.3% CMC)	102.1, \pm 10.1	100.6 \pm 8.3
II	EEHRS (100 mg/kg, p.o.)	122.6 \pm 12.1 ^a	89.5 \pm 8.6 ^a
III	EEHRS (200 mg/kg, p.o.)	130.5 \pm 12.5 ^b	67.9 \pm 6.6 ^b
IV	EEHRS (400 mg/kg, p.o.)	133.7 \pm 9.6 ^b	64.5 \pm 6.2 ^b
V	Lorazepam (2.5 mg/kg, i.p.)	140.0 \pm 8.0 ^b	42.6 \pm 4.8 ^b

Each column represents mean \pm S.E.M, n = 6. a = $p < 0.01$, b = $p < 0.001$ compared to control

CONCLUSION:

The results from the present study confirm the antidepressant activity of *hibiscus rosa sinensis*, since it reduced the immobility in both FST and TST. Results from behavioral experiments indicate that the antidepressant activity of *hibiscus rosa sinensis*, might be due to the facilitatory effect on serotonergic, noradrenergic and dopaminergic systems apart from the antioxidant activity.

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Conflicts of interest:

The authors express no conflicts of interest regarding the publication, all the authors worked and provided support equally and credited equally.

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