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Evaluation of Anti-Depressant Property of *Hibiscus rosasinensis* Plant Extracts

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Abstract

Aim: Flowers of Hibiscus rosa-sinensis Linn (Malvaceae) popularly known as "China-rose flowers" contain flavonoids. Flavonoids have been found to have antidepressant activity. The research work is concentrated about the extractions, phytochemical investigation and biological evolution of the antidepressant activity of Hibiscus Rosa sinensis in the experimental animal (mice) model i.e., forced swimming test tail suspension test and sleep induced test in the mice respectively. **Methods**: Anti-Depressant activity of ethyl acetate extract containing Hibiscus Rosa sinensis flower and leaf evaluated in(30 and 200mg/kg),tail suspension test (30 and 60mg/kg), forced swim test and (30 and 200mg/kg) mice as compared to vehicle control. **Results**: The tail suspension and forced swimming tests, sleep induced method three behavioral testes in rodent that predicted the clinical efficacy of many new types of antidepressant medication. *Hibiscus rosasinensis* extracts consists of this are the constituents are responsible for controlling the oxidation. The extract at oral doses 100 and 200 mg/kg for 14 days significantly P≤0.01 decreased the duration of immobility as dose-dependent manner in the forced swim test, sleep induced method and tail suspension test in the mice. **Conclusion** The finding of the present investigation suggests that antidepressant-like effect of *H. rosa-sinensis* is mediated through dopaminergic, adrenergic, and serotonergic mechanisms. In our further study, we will try to explore selective mechanism of action of *H. rosa-sinensis* flowers responsible for antidepressant-like activity

Keywords
Hibiscus Rosa sinensis, Tail Suspension, Forced swimming, Anti-depressant activity

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1. INTRODUCTION:

The character of depression is an emotional disorder, feelings of sadness, sleep disturbances, eating disorders, anxiety, and unexplained physical problems [1], and it is the second most common chronic condition in clinical practice [2] More than 300 million people of all ages have depression worldwide, which is the second leading cause of premature death or disability, according to the 2017 World Health Organization report [3, 4]

Depression of pathology is difficult to understand because various symptoms of depression cannot be explained under a single hypothesis [5] and this condition is now explained by reduced monoamine signaling and monoamine metabolite levels have been found in cerebrospinal fluid (CSF) of depressed individuals; likewise, neither serotonin, nor

epinephrine or dopamine depletion show pro-depressive effects. [6, 7] Generally, people are exposed to a different type of stresses throughout their lives. If the stress is continued to exist, which can badly affect the body's immune

system, cardiovascular system, central nervous system, and nerve transmission [8], because of monoamine oxidase (MAO) enzyme in brain is augmented, which in turn low levels of monoamines. [9, 10] Flowers of *H. rosa-sinensis* Linn (Malvaceae) (**Fig.1**) are reported to have cardio protective, hypertensive, antidiabetic, anticonvulsant, and antioxidant activity. [11-15]. These flowers are known to have flavonoids like anthocyanin and quercetin.[16] Flavonoids have been shown in antidepressant activity [17,18] and anxiolytic activity. [19,20] Pallavi et al., (2012). He was studied and developed on the anthocyanidins, flavonoids from Hibiscus rosa-sinensis flowers in tail suspension test and forced swim test to evaluate the antidepressant activity [21] Kim et al., (2018). He was suggested that the antidepressant-Like and Neuroprotective Effects of Ethanol Extract using the Root Bark of *Hibiscus syriacus* L, which is used to control neuronal cell damage and depressive disorder caused by chronic stress.[22]

Therefore, the current research was aimed to the research work is concentrated about the extractions, phytochemical analysis and biological evolution of the antidepressant activity of Hibiscus Rosa sinensis in the experimental using animal (mice) model i.e., forced swimming test tail suspension test and sleep induced test in the mice respectively.

Figre.1 Flowers of *H. rosa-sinensis* Linn (Malvaceae)

1. MATERIALS AND METHODS

1.1. Animals

Adult female albino mice (25g) and male Albino mice (25±2) were purchased from the laboratory animal center (Mahaveer, Hyderabad, CPCSEA Reg No: 146/99/CPCSEA) warehoused in a quiet room under a 12-h light and 12-h dark cycle at 25±2ºC for 5 days prior to experimentations. Ad libitum (AL) supply of standard chow is the feeding method to the animal, except during observation periods. The experimental procedure has been performed in the CPCSEA approved laboratory at the Mother Teresa pharmacy college, sankethika Nagar, Kothuru, Sathupallly, 507303, Dist. Khammam, Telangana. India (Regd.1769/PO/E/S/14/CPCSEA) with the approval no 01/2017 of the Institutional animal ethics committee.

2.1.1 Methods

The animals were selected into control group and experimental group, and they were divided into five groups. One group (I) of animals were administered at the concentration of 0.9% Tween 80. Second group animals were administered with Imipramine at the dose of 20 mg/kg, and third, fourth group of animals administered with the extract of hibiscus rosa sinensis at the doses of 30, 60 and 200 mg/kg. All were administered orally at 16:00/16:30 h for 14 days, except in case of groups 2, which was administered with Imipramine only at one day, seventh day and 14th days, respectively.

The behavioral tests were conducted at one hour after the last treatment, respectively. The prepared fine powders of flower and leaf were taken by the weight of 50g and 30g and which were extracted with methanol (70%) with help of soxhlation method. Where the total consumption of organic solvent, methanol to extraction of (flower and leaf) were required 230 mL and 180 mL in Soxhlet apparatus at the time of 18 h, to extract the active constituents from them. Final obtained extract was allowed to dry at room temperature, the dried product was stored 4°C.was shown in (**Fig.2**)

Figure. 2 Extraction of Hibiscus rosasinanisis

Figure 3. Forced swimming test

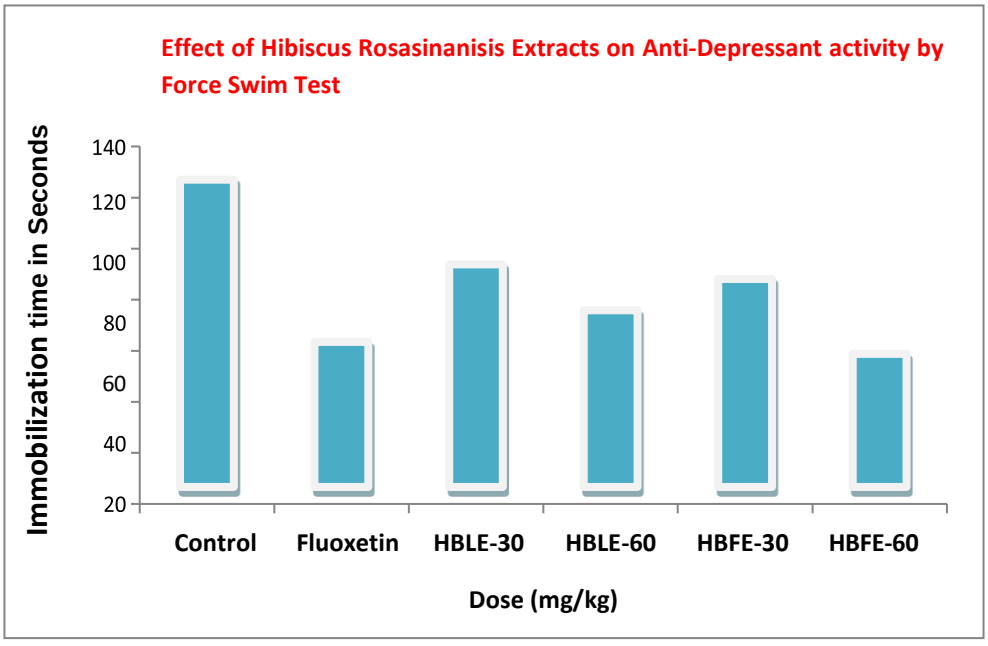

Figure.4 Effect of HRS extracts on antidepressant activity by forced swim test

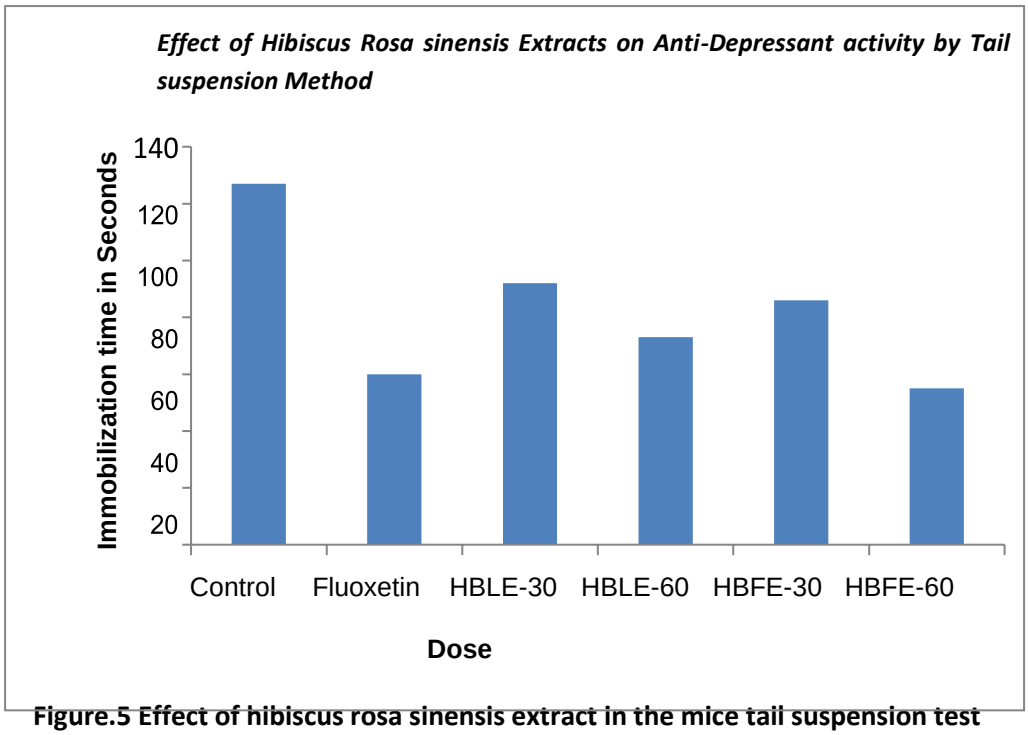

Figure.5 Effect of hibiscus rosa sinensis extract in the mice tail suspension test

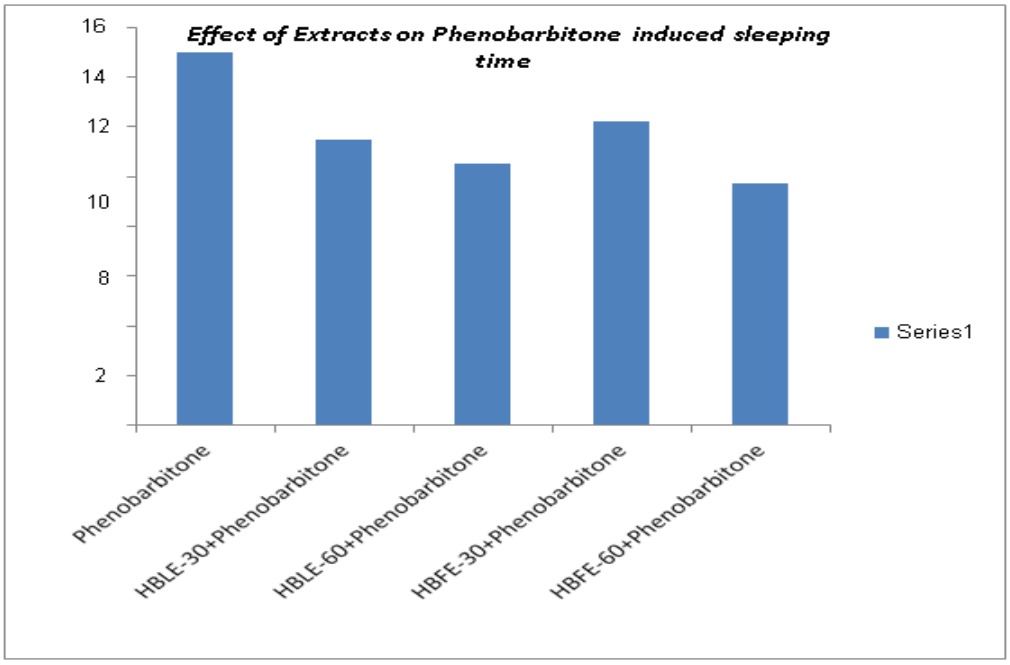

Figure .6 Effects of Extracts on Phenobarbitone induced sleeping time

2.1.2 Forced swimming test

The mouse was dropped into glass cylinder has (20 cm in height), 12 cm in diameter containing 8 cm deep water. The cylinder was maintained at temperature of 24-25°C and left it for 6 min. At the time of immobility for the final 4 min, interval of the swimming test was measured. At the control group was treated with tween 80 + 0.9% (w/v) saline solution. The other groups received an I.P. injection of extracts (30-200 mg/kg) in tween 80 plus 0.9% (w/v) saline solution and imipramine (20mg/kg), and one hour prior to experiment and the repeated doses for each individual groups contain animals and from group one to group four, in the experiments, respectively.

2.1.3 Sleep inducing method

The mice were affected with administration of ketamine to inducing sleeping time and it was measured. A group of animals received an injection of I.P of extract (30-200mg/kg), then after the 30 min and they were again injected with I.P. ketamine (20mg/kg), the interval between the administration of ketamine and the loss of the righting reflex was considered as the time to onset of sleep, while the time from the loss to regaining of the righting reflex was taken as the duration of sleep. All the individual groups of animals were in repeated doses during the experiments.

2.1.4 Tail suspension test

The mouses are allowed to house in cages which was made by plastic material for at least 10 days, before start the testing at a 12 h light cycle, with standard food and water were freely available for them by this experiment. The animal was transported from the housing room to the testing area by its own cages and had allowed them to new environment for 1 h prior to testing. The group of animals treated with the extract (30-200mg/kg) I.P injection at 30 min prior to testing. The test of the mice suspended on the edge of a shelf at 58cm above the top of the table by keeping the adhesive tape placed approximately 1cm long from the tip of the tail of mice. The duration of immobility recorded for a period of 5min. Mice were considered immobile when they hang passively and completely immobility for at least 1min. Imipramine (20mg/kg), which was used as control and then repeated doses to the individual groups possess animals in this experiment.

2.1.5 Statistical analysis

All data were expressed as Mean ± SEM. Statistical analysis was carried out by one-way ANOVA followed by Dunnett's test. The values were significant at $P < 0.05$ when compared with control group.

3. RESULT

The effect of oral administration of the H. rosa sinensis and the duration of immobility in the mice being under the forced swim test was showed in **Table.1**. The extract that showed in significantly (**P≤0.01**) on one day treatment, and the capable of the reducing the immobility time after the seven days. Then, after a 14-day treatment, the extract of the dose of at the 30 mg and 60 mg/kg significantly (**P≤0.01**), and the reduced the time of duration of immobility by the dose dependent manner as compared to that the vehicle control group, and which the resulting was decreased, respectively.

However, the reference standard of antidepressant, agents like, the fluoxetine at the dose of 20mg/kg, which was resulted in significant (P≤0.01) reduction as compared to that of vehicle control group. Effects of oral administration of the H. rosa sinensis and fluoxetine duration of immobility in the mice, tail suspension test depicted in **Table.2** The extract showed significantly (**P≤0.01**) on one day treatment, capable of the reducing immobility time after seven days.

After a 14 days treatment, the extract of dose 30 mg and 200 mg/kg significantly **(P≤0.01**) decreased the duration of immobility in a dose dependent manner as compared to vehicle control group, resulting was showed reduction, respectively. However, the reference antidepressant fluoxetine at the dose of 20 mg/kg, which showed resulted in significant (P≤0.01) as reduction as compared to vehicle control group.

3.1Effect of hibiscus rosa sinensis extract in the mice sleep inducing test

Effect of oral administration of the H. rosa sinensis and duration of immobility in the mice sleep inducing test showed below **Table.3**. The extract showed significantly (**P≤0.01**) on one day treatment, capable of the reducing immobility time after seven days. After 14 days the treatment with the extract of dose 30mg and 60mg/kg significantly (**P≤0.01**) decreased the duration of immobility in a dose dependent manner as compared to vehicle control group, which was resulting showed reduction, respectively. However, the reference antidepressant fluoxetine at the dose of 20mg/kg was showed resulted in significant (P≤0.01) reduction as compared to that of vehicle control group.

Table .1 Effect of hibiscus rosa sinensis extract in the mice forced swim test

S. No	Group	Dose	Length of Immobility			Length of Immobility			Length of Immobility Average SEM	%Change
1	Control		125	128	125	127	126.25	1.5	126±1.5	
2	Fluoxetine	30	60	61	62	60	60.75	0.957427	60.75±0.95	48.12
3	HBLE-30	30	90	92	93	92	91.75	1.258306	91.75±1.25	72.67
4	HBLE-60	60	72	70	75	73	72.5	2.081666	72.5±2.08	57.43
5	HBFE-30	30	85	86	88	86	86.25	1.258306	86.25±1.25	68.32
6	HBFE-60	60	58	57	56	55	56.5	1.290994	56.5±1.29	44.75

Table.2 Effect of HRS extracts on antidepressant activity by Tail suspension method

S. No	Group	Dose	Length of Immobility			Length of Immobility			% Change	
1	Control		140	145	146	132	140.75	6.39661	140.75±6.3	
2	Fluoxetine	30	65	63	65	64	64.25	0.95743	64.25±0.95	45.65
3	HBLE-30	30	88	87	85	86	86.5	1.29099	865±1.29	61.46
4	HBLE-60	60	65	68	67	65	66.25	1.5	66.25±1.5	47.07
5	HBFE-30	30	85	80	79	75	79.75	4.11299	79.75±4.11	56.66
6	HBFE-60	60	57	58	57	58	57.5	0.57735	57.5±0.57	40.85

Table .3 Effect of HRS extracts on Phenobarbitone induced sleeping time

S.No	Group	Dose	Sleeping time			Length ofSleeping Time Immobility			Average SEM
1	Normal Control		0	0	0	0	0	0	
2	Phenobarbitone	40	15	16	15	14	15	0.816497	15±0.00
3	HBLE-30+Phenobarbitone	30	12	11	12	11	11.5	0.57735	11.5±0.57
4	HBLE-60+Phenobarbitone	60	10	11	10	11	10.5	0.57735	10.5±0.57
5	HBFE-30+Phenobarbitone	30	13	12	12	12	12.25	0.5	12.25±0.5
6	HBFE-60+Phenobarbitone	60	10	9	10	10	9.75	0.5	97.5±0.5

3.1.2 DISCUSSION:

The tail suspension forced swimming tests and sleep induced method are three behavioral testes in rodent that predicted the clinical efficacy of many new types of antidepressant medication. *Hibiscus rosasinensis* extracts are consisting of the active constituents, which are responsible for the controlling the oxidation. The extract and at oral doses of 100 and 200 mg/kg for 14 days significantly **P≤0.01** decreased in the duration of immobility as dose-dependent manner in the forced swim test, sleep induced method and tail suspension test in the mice.

Mono amino oxidase (MAO) is an important enzyme in the metabolism of a wide range of monoamine neurotransmitters, including noradrenalin, dopamine and 5-hydroxytryptamine. Mono amino oxidase (MAO) exists in two forms, MAO -A and MAO- B. Mono amino oxidase (MAO) -A is more important than MAO B in the metabolism of the major neurotransmitter monoamine.

4. CONCLUSION

The finding of the present investigation suggests that antidepressant-like effect of *H. rosa-sinensis* is mediated through dopaminergic, adrenergic, and serotonergic mechanisms. In our further study, we will try to explore selective mechanism of action of *H. rosa-sinensis* flowers responsible for antidepressant-like activity.

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6. CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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