Ethanolic Crude Extract of Roots of *Hibiscus rosa sinensis* Ameliorates Depression like Behaviour in Animal Models

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**Abstract:** The aim of the present study was to evaluate the antidepressant activity of the ethanolic extract of roots of *Hibiscus rosa sinensis* (HRS). The extract was tested on rats and mice at doses level of 250 and 500 mg/kg, Fluoxetine was used as a standard drug at dose of 15 mg/kg, by using forced swimming test, tail suspension test and open field test. HRS extract at dose level of 500 mg/kg caused high significant reduction in all activities when compared with the control group, HRS extract (250 and 500 mg/kg) respectively produced significant ($P<0.05$) effects at all the treatment doses. The results of the present study showed that the roots of China rose have significant antidepressant activity.

**Key words:** *Hibiscus rosa sinensis* • Animals • Forced Swimming Test • Tail Suspension Test • Open Field Test

**INTRODUCTION**

Medicinal plants are rich sources of therapeutic agents for curing of diseases. Several medicine systems in the world are present like Chinese medicine, Tibetan medicine and Hindu medicine. Unani is the science of life and comprehensive medical system [1]. In traditional medicine system, Unani is mostly used in Pakistan and other Asian countries, in which uses natural resources and herbs in the formulations [2]. In modern medicine systems, various people still rely on the medicinal plants for healing of diseases because of fewer side effects, low resistance and low cost [3].

*Hibiscus rosa sinensis* belongs to the family Malvaceae and commonly known as China rose [4]. It is a medicinal plant all parts are used in the traditional system from ancient time. It is used as antiviral, aphrodisiac, antipyretic, antispasmodic, antifungal, antiestrogegenic, antidiabetic, analgesic, spasmylotic, anti-inflammatory, antidepressant, antibacterial, anti-implantation, laxative, hypotensive, antiovulatory, juvenoid, antitumor and antifertility activity [5-7].

The world health organization has reported that almost 450 million persons have behavioral disorders or mental problems and in which small numbers of persons received basic treatment instead of all suffered patients. This amount is 12.3% of the universe burden level of diseases and in the year of 2020 will rise up to 15% [8, 9].

Depression disorder is a common disorder related with increase rates of physical impairment, recurrence, suicide rate, chronicity, psychosocial impairment and relapse [10].

Many effective antidepressant medicines such as selective nor-adrenaline reuptake inhibitors (SNRIs), selective serotonin reuptake inhibitors (SSRIs), monoamine oxidase (MAO) inhibitors and tricyclic antidepressants [11] are present that enhanced the level of neurotransmitters and decreased the level of depression but disadvantage of this medicine has more side effects. Due to this, alternative therapy of depression treatment is still needed because it is effective only in limited portion of the population [12, 13].

**Aims of the Study:** The aim of the present study is to evaluate the pharmacological activity of *Hibiscus rosa sinensis* which can be very helpful in diseases managements.

The ethanolic extract from *Hibiscus rosa sinensis* which have medicinal uses and evaluate the antidepressant activity.
MATERIALS AND METHODS

Plant Material: Roots of *Hibiscus rosa sinensis* were collected from Karachi and identified by Madam Afsheen Ather in Herbarium of the Department of Botany, Karachi University, Karachi, Pakistan, allotted the voucher specimen G.H. No: 92098 extraction of hibiscus rosa sinensis (roots)

The root parts of plant were taken and properly washed with distilled water, dried under shade and then grinded to collect coarse powder. Almost 850g of coarse powdered was subjected to the Soxhlet apparatus (Model: HMFT) for extraction, using ethanol as a solvent. The extract was concentrated in a rotary evaporator (Buchi, Switzerland) at 30–40°C to obtain semi-solid material [14]. Its percentage was calculated 3.29% (28g). The extract was stored at 4°C in a properly labeled airtight container. A fresh dilution of dried extract in 2% Tween 80 was prepared for the experiments and administered by oral route at two different doses of 250 and 500 mg/kg.

Study Animals: For the assessment of antidepressant effects (Of what??) both genders of SD rats and mice were used. Standard cages were used to keep the experimental animals [15] at Dr. HMI (Institute of Pharmacology) Hamdard University, Karachi, Pakistan to maintain the new environment of the animals under conditions of standard lighting (12 h of darkness and light) and temperature (27±2°C), for at least one week before the starting of the assessment. Animals were fed with standard diet and water. The departmental animal ethical committee approved standard procedures for the animals handling and uses of animals in antidepressant activities.

Chemicals: Absolute Ethanol (Merck, Germany) and Fluoxetine (Hilton, Pharmaceutical, Pakistan).

Evaluation of Antidepressant Activity

Forced Induced Swimming in Rats (FST): The rats were divided into four groups (I, II, III and IV) of six animals in each group (n=6), weighing between 150–250 g. Control group (Group I) orally received normal saline and at the same time group II rats were administered orally 14 days with standard drug (Flouxetine). Group III and IV were orally treated 14 days with extract of HRS at a dose level of 250 and 500 mg/kg. After 14 days of drug administration rats were forced to swim in a glass tank (Height 45cm, width 12cm) in which water level should not increase from 15cm for the duration of 06 minutes. Rats were recorded as immobile when floating motionless or making only those movements necessary to keep the head above water. A decrease in the duration of immobility during the forced swimming test was taken as a measure of antidepressant activity [16, 17].

Tail Suspension Test in Mice (TST): The mice were divided into four groups (I, II, III and IV) of six animals in each group (n=6), weighing between 20 – 30 g. Group I (Control group) orally received normal saline and at the same time group II were administered orally 14 days with standard drug (Flouxetine). Group III and IV were orally treated 14 days with extract of HRS at a dose level of 250 and 500 mg/kg. After 14 days of drug administration mice were suspended with their tails to the edge of the table, 35cm away from the bottom for 05 minutes interval. Animals were considered immobile when they don’t show any movement of body and remain hanging passively [18].

Open Field Test in Mice (OFT): Open field equipment was made of plywood that is white in colour and measured 72 by 72 and wall is 36cm long. In this test, mice were divided into four groups (I, II, III and IV) of six animals in each group (n=6), weighing between 20 - 30g. Control group (Group I) orally received normal saline and at the same time group II were administered orally 14 days with standard drug (Flouxetine). Group III and IV were orally treated 14 days with extract of HRS at a dose level of 250 and 500 mg/kg. After 14 days of drug administration mice were placed independently in the middle of the open field for 05 minutes to count Total Locomotion (TL) i.e. the total number of square crossed both outer and inner ones, Peripheral Locomotion (PL) and Central Locomotion (CL) respectively. The other factors, which were evaluated are rearing, grooming and defecation [19, 20].

Statistical Analysis: The results were represented as mean ± standard error mean (S.E.M). The calculations of the experimental collected data were done by using ANOVA followed LSD post hoc test when compare to control group. All the data were analyzed with SPSS software version no. 20 and the results were significant with \( p<0.05 \).

RESULTS

Forced Swimming Test in Rats: Two different doses of *Hibiscus rosa sinensis* (HRS) extract (250 and 500 mg/kg) were used to investigate the anti-depressant effect. The administration of Flouxetine (15 mg/kg) as positive control significantly \( (p<0.001) \) decreased immobility time
Fig. 1: Comparative graphical representation of antidepressant effect of *Hibiscus rosa sinensis* roots extract by forced swimming test response.

Table 1: Effects of *Hibiscus rosa sinensis* on duration of immobility time in tail suspension test (TST)

<table>
<thead>
<tr>
<th>Treatment groups</th>
<th>Dose (mg/kg)</th>
<th>Duration of immobility (sec)</th>
<th>% Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>-</td>
<td>180.33 ± 46.50</td>
<td>0.00</td>
</tr>
<tr>
<td>Flouxetine</td>
<td>15</td>
<td>101.66 ± 20.21</td>
<td>43.62</td>
</tr>
<tr>
<td>Extract HRS 250</td>
<td>250</td>
<td>115.33 ± 16.68</td>
<td>36.04</td>
</tr>
<tr>
<td>Extract HRS 500</td>
<td>500</td>
<td>95.50* ± 17.00</td>
<td>47.04</td>
</tr>
</tbody>
</table>

Values are expressed as Mean ± S.E.M (Standard error mean) (n=6). The statistical analysis was performed by one-way ANOVA followed by post hoc test. The asterisks (*) denote the significance levels (* P<0.05) compared with control groups.

(107.50 ± 9.49) respectively compared to the control group. While HRS extract (250 and 500 mg/kg) significantly (*p<0.025, *p<0.001) decreased immobility time (111.16 ± 6.52 and 93.33 ± 9.66 second) respectively compared with control group. Comparative graphical representation of antidepressant effect of *Hibiscus rosa* extract is presented in figure 1. When results were compared with standard drug Flouxetine, it was found that, extract dose 500 mg/kg had same pattern of activity as standard drug, because the result was found comparable to the result of Flouxetine. Whereas 250 mg/kg extract dose difference with Flouxetine was significant (*p<0.025*)

Effects of HRS on immobility time (Seconds) are represented on y-axis and treatment groups are represented on x-axis by using forced swimming test. Each point represents the mean ± S.E.M. (Standard error mean) for 06 animals. The asterisks (*) denote the significance levels (**p<0.025, ***p<0.001) of all test groups when compared with control group.

Tail Suspension Test in Mice: Two different doses of *Hibiscus rosa* extract (250 and 500 mg/kg) were used to investigate the antidepressant effect. The administration of Flouxetine (15 mg/kg) as positive control decreased immobility time (101.66 ± 20.21second) respectively compared to the control group. *Hibiscus rosa* extract (250 and 500 mg/kg) decreased immobility time (115.33 ± 16.68 and 95.50 ± 17.00second) respectively when compared with control group. The results of tail suspension test are mentioned in table 1. Extract dose of 500 mg/kg has significant results when compared with
control group \((p<0.05)\). When results were compared with standard drug Flouxetine, it was found that, extract doses results difference with Flouxetine were non-significant.

**Open Field Test in Mice:** Results of open field test were significant compared with control group. In control group, 13.00±1.88 number of central square crossed and in standard group (Flouxetine) was crossed 11.16±1.85, it was not significant but results of extract doses (250 and 500 mg/kg) were significant when compared with control group. Comparative graphical representations of *Hibiscus rosa* extract and Flouxetine on the duration of immobility are shown in figure 2. Significantly, decreased grooming of mice with standard group and treated groups as compared with control group and the increased number of rearing in test groups were not significant.

Effects of HRS on immobility time (Seconds) are represented on y-axis and treatment groups are represented on x-axis by using open field test. Each point represents the mean for 06 animals. Treated groups have significant different results when compared with control group.

**DISCUSSION**

Depression is a most common life-threatening problem with a high incidence of anxiety. Causes of lots of morbidity are related with depression. Many antidepressant medicines are available that have different types of mechanism of actions likes dopaminergic, noradrenergic and serotonergic systems [21].

Many herbs have been reported for their antidepressant effects like Hypericum perforatum, Curcuma longa, Centella asiatica, Crocus sativus, Clitoria ternatea, Ginkgo biloba, Mimosa pudica, Withania somnifera and Rhazya stricta decne [22].

The present study provided behavioral evidence for the antidepressant activities of *Hibiscus rosa* roots. In present study used animal models, forced swimming test (FST) in rats developed by Porsolt and Selvi [16, 17] and Tail suspension test (TST) in mice developed by Steru [18] are specific for all major classes of antidepressants and evaluation of antidepressant agents. Walsh and Syed developed open field test (OFT) in mice, it is a confirmation test for antidepressant activities of FST and TST [19, 20].

In present study, extract of HRS roots (250 and 500 mg/kg) and Flouxetine Hcl (Standard drug) orally administered for 14 successful days to mice and rats, produced significant \((p<0.05)\) antidepressant effects in forced swimming test, tail suspension test and open field test as compared with control group.

In FST, the rats are placed in an inescapable cylinder of water and note the observation when starts the escape oriented movements and become develop an immobile posture. Despair behavior gives up the hope to animal for escape. The passive behavior are developed its means the rodents loss the ability to cope with stressful stimuli [23, 24]. Present study results has shown diminution of immobility time or significant \((P<0.025, p<0.001)\) reduction of the number of head dipping, it is the sign of exploratory behavior [25].

TST is included in the despair model behavior. The immobility shown by mice when subjected to an unavoidable and inescapable stress has hypothesized to reflect behavioral despair. Antidepressants medications decrease the immobility when mice display after active and unsuccessful attempts to escape when suspended by tail. Decreased immobility time in FST and TST is due to increased level of catecholamine, neurotransmission and central 5-HT [26].

In TST, the decrease immobility time directly showed the antidepressant effect of HRS extract. HRS roots extract results compared with the control group showed significant \((P<0.05)\) differences and decrease of the time spent immobile by mice.

The open field test (OFT) is designed to examine responses of mice to a new and unfamiliar environment (Novel environment) and it is used to confirm the observed antidepressant effects is not due to activation of general motor activity. In OFT measure the locomotor activities such as numbers of line cross, frequency of grooming, rearing and defecation but are also measures of anxiety and exploration. These behaviors have increased level of frequency that indicates increased locomotion and exploration behaviors and decreased level of anxiety. Measure the exploratory behavior and anxiety when mice number of central square crosses and the duration of time spent in the central square [19].

It was observed that HRS can produce antidepressant activity at different doses level 250 and 500 mg/kg in a dose dependently manner. The findings from the present investigation indicated that HRS root have significant antidepressant activity as shown by its effects on different experimentally induced depress models in rats and mice.

Several different plants have antidepressant effect that are previously reported, glycosides and quercetin likes rutin, hyperoside and isoquercitrin have significant
results in forced swimming activity [27-29]. Previous study showed that the phytochemical analysis of HRS revealed presence of flavonoids, quercetin, glycosides and cyanidin [25], which may be responsible for its antidepressant activity.

It was observed that HRS can produce antidepressant activity at different doses level 250 and 500 mg/kg in a dose dependently manner. The findings from the present investigation indicated that *Hibiscus rosa sinensis* roots have significant anti-depressant activity as shown by its effects on different experimentally induced depressed models in rats and mice.

**CONCLUSIONS**

The results of present study showed that the ethanolic extract of HRS roots (250 and 500 mg/kg) have significant antidepressant activity. Studies of HRS in details are required to isolate constituents with their mechanistic action.

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**Conflict of Interest:** The all authors declare that there are no conflicts of interest in this study.

**REFERENCES**


