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Neuropharmacological evaluation of *Hibiscus rosa sinensis* roots in experimental animals

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Abstract

Objective: To investigate the effect of the methanolic extract of *Hibiscus rosa sinensis* roots (*H. sinensis*) on the central nervous system in mice and rats. **Materials and methods:** The methanolic extract of *H. sinensis* was studied on pentobarbital-induced sleeping time, elevated plus maze, hole board test, foot shock-induced aggression, haloperidol-induced catalepsy, lithium-induced head twitches, locomotor activity, acetic acid-induced writhing and hot plate analgesiometer at various doses (50 - 200 mg/kg), intraperitoneally for sedative, anxiolytic, antipsychotic, muscle relaxant and analgesic activities. **Results:** The extract prolonged pentobarbital-induced sleeping time indicating sedative effect. The extract increased the time spent in open arms and the number of head dips in elevated plus maze and hole board test respectively demonstrating anxiolytic activity. *H. sinensis* decreased number of fights in foot shock-induced aggression and potentiated haloperidol-induced catalepsy in dose dependent manner showing antipsychotic effect. The extract significantly inhibited head twitches induced by lithium suggesting its inhibitory effect on serotonergic system. The *H. sinensis* significantly decreased the locomotor activity in dose dependent manner. The extract showed potential analgesic activity in acetic acid-induced writhing and hot plate analgesiometer. The *H. sinensis* attenuated the behavior mediated by the dopaminergic and serotonergic system and potentiated the GABAergic actions. **Conclusion:** The results suggest that the methanolic extract of *Hibiscus rosa sinensis* has potential neuropharmacological activities.

Keywords: *Hibiscus rosa sinensis*, sedative, anxiolytic, antipsychotic, analgesic, catalepsy.

1. Introduction

Hibiscus rosa sinensis Linn (Malvaceae) is also known as shoe flower plant. The plant is evergreen woods, glabrous showy shrub, 5 - 8 Feet height. In traditional system of medicine *H. rosa sinensis* is used for the treatment of

cough, fever, dysentery, menorrhagia, pruritis, venereal diseases; as an abortifacient and is also applied topically to cancer swelling. Moreover, it is also known to have free radical scavenging effect, anti-pyretic, anti-inflammatory and anti-

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cancer activities [1, 2, 3, 4, 5]. The flowers and leaves of *H. rosa sinensis* found to exhibit significant hypoglycemic, lipid lowering activity and has significant protective effects in ischemic heart diseases [6, 7]. The plant has been reported to contain numerous compounds, including quercetin, carotene, niacin, riboflavin, malvalic acid, gentisic acid, margoric acid lauric acid [1, 4]. On the basis of traditional claims the plant has excellent medicinal properties and is used worldwide for the treatment of various diseases. In Indian system of medicine the plant has been mentioned to be brain tonic [1, 2]. However, there are very few investigative reports available pertaining to central nervous system, so it's our endeavour to evaluate the neuropharmacological profile of methanolic extract of *H. rosa sinensis*.

2. Materials and methods

2.1 Plant material

The roots were collected in the month of November from local area of Nashik (India) and authenticated by P. S. N. Rao (Director, Botanical survey of India, Pune). A voucher specimen of the plant has been deposited at Botanical survey of India, Pune (Voucher specimen no. NVHR3). The plant material was shade dried and coarsely powdered. The powdered plant material (1 kg) was defatted with petroleum ether (60-80°C) by Soxhlet extractor. The defatted marc was further extracted with methanol for 72 h. Extract was filtered and concentrated under reduced pressure. The yield of methanolic extract of *H. rosa sinensis* roots was found to be 6.2% w/w. The dried extract was suspended in 0.5% carboxy methyl cellulose in distilled water and administered intraperitoneally (i.p.).

2.2 Experimental animals

Swiss albino mice (18 - 25 g), Wistar rats (120 -180 g) of either sex were used for the study. Animals were housed in colony cages at ambient temperature of $25 \pm 2^\circ\text{C}$, 12 h light:

12 h dark cycle and $50 \pm 5\%$ relative humidity with free access to food and water *ad libitum*. Food but not water was deprived overnight and during the experiment. Each group consisted of 5 animals. All the experiments were carried out during the light period (08:00 - 16:00 h). The studies were carried out in accordance with the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi (India). The Institutional Animal Ethical Committee of N.D.M.V.P.S College of Pharmacy, Nashik approved the protocol of the study (CPN/IAEC/2007/02).

2.3 Phytochemical screening of the *H. rosa sinensis* roots

Phytochemical screening of the methanolic extract *H. rosa sinensis* roots for the presence of flavonoids, glycosides, saponins, tannins, alkaloids and triterpenes were carried out in accordance with procedures previously described [8, 9].

2.4 Drugs

Pentobarbital sodium (Siddharth Pharma, Mumbai, India), Diazepam injection I.P. (Ranbaxy Laboratory Ltd., Mumbai, India), Lithium sulphate (Glenmark Laboratories, India), Ondansetron (Alkem Mumbai, India), Haloperidol (RPG Life sciences Ltd., Ankeleshwar, India), Diclofenac (Novartis Mumbai, India), Glacial acetic acid (Ranbaxy Laboratories, Mumbai, India), Pentazocin (Ranbaxy Laboratory Ltd., Ahmedabad, India), were used in this study. All drugs were dissolved in distilled water and administered intraperitoneally (i.p.) unless stated otherwise. Distilled water was used as vehicle.

2.5 Acute toxicity test

The methanolic extract of *H. rosa sinensis* was administered orally and i.p. in doses of 100, 200, 400, 800, 2000 mg/ kg to groups of mice (n = 5) and percentage mortality was noted for 24 h upto the period of 7 days.

2.6 Neuropharmacological activities

2.6.1 Pentobarbital (PB)-induced sleeping in mice

The animals were divided into five groups consisting of five animals each. PB (50 mg/kg) was injected i.p. to mice pretreated with vehicle or *H. sinensis* (50, 100 and 200 mg/kg, i.p.) and diazepam (1 mg/kg, i.p.). The vehicle, *H. sinensis* and diazepam were administered 30 min prior to administration of PB. Immediately after PB administration, each animal was placed in an individual cage and observed. The latency to the loss of righting reflex (induction time in min) and the time required to recover righting reflex or awakening (sleeping time in min) were noted for each animal [10].

2.6.2 Anxiolytic activity in mice using elevated plus maze (EPM)

EPM is the simplest method to study anxiolytic effect of versatile types of anti-anxiety agents. The maze consisted of two open arms (35 x 5 cm) crossed with two closed arms (35 x 5 x 20 cm), which were connected together with a central square of (5 x 5 cm). The apparatus was elevated to the height of 25 cm in a dimly illuminated room. Mice (n = 5) were treated with *H. sinensis* (50, 100 and 200 mg/kg, i.p.) or diazepam (1 mg/kg, i.p.) or vehicle 30 min before placing individually in the centre of the EPM facing a closed arm and the time spent in both the open and closed arms was recorded for 5 min. The numbers of entries into open and closed arms were also counted during the test. An entry was defined as all four paws in the arm. Diazepam was used as a standard reference drug [11, 12].

2.6.3 Anxiolytic activity in mice using hole board apparatus

The apparatus consist of wooden box (40 x 40 x 25 cm) with 16 holes (diameter 3 cm) evenly distributed on the floor. The apparatus was

elevated to the height of 25 cm. The animals were divided into five groups (n = 5). The vehicle, *H. sinensis* in a dose of 50, 100 and 200 mg/kg, i.p. and diazepam (1 mg/kg, i.p.) were administered to mice 30 min before placing in the apparatus. The number and duration of head pokes during the 5 min period was recorded [13].

2.6.4 Foot shock - induced aggression in mice (FSIA)

FSIA behavior is induced in pair of mice by administering a train of impulses through an electronic stimulator to a grid floor for 3 min. The animals were divided into 5 groups of 10 mice (5 pairs of male mice) per group. The vehicle, haloperidol (1 mg/kg) as a standard and *H. sinensis* (50, 100 and 200 mg/kg) were administered i.p. 30 min before start of experiment. Aggressive behavior was noted in pair of mice by using two parameters viz. number of fights and latency to fight [14].

2.6.4 Haloperidol-induced catalepsy

Haloperidol (1 mg/kg) was injected intraperitoneally to mice pretreated with vehicle or *H. sinensis* (50, 100 and 200 mg/kg, i.p.). The vehicle or *H. sinensis* were administered 30 min prior to administration of haloperidol. The duration of catalepsy was measured at 0, 30, 60, 90, 120, 150 and 180 min using the Bar test. Both the forepaws of mouse were placed on a horizontal bar raised 3 cm from the table, and the time required to remove the forepaws from the bar was recorded as the duration of catalepsy and the cut-off time was 300 s. In all the experiments the scorer was blind to the treatment given to the mice. Between experiments, the animals were returned to their home cages [15].

2.6.5 Lithium-induced head twitches (serotonin mediated behavior)

Rats were divided into five groups and treated with lithium sulphate (200 mg/kg, i.p.). *H. sinensis* (50, 100 and 200 mg/kg, i.p.) or

Ondansetron (5 mg/kg, i.p.) or vehicle were administered 30 min before the test. The number of head twitches was counted for 60 min after lithium sulphate administration [16].

2.6.6 Locomotor activity in mice

The locomotor activity was measured using an actophotometer. The movement of the animal cuts off a beam of light falling on the photocell and the count is recorded digitally. Each mouse was placed individually in the actophotometer for 10 min and basal activity score was obtained. Subsequently, in five groups of animals, *H. sinensis* (50, 100 and 200 mg/kg), vehicle and diazepam (1mg/kg, i.p.) were administered. After 30 min the mice were placed again in the actophotometer for the recording of the locomotor activity [17].

2.6.7 Analgesic activity using hot plate analgesiometer

The mice were individually placed on a plate maintained at constant temperature ($56 \pm 1^\circ\text{C}$) and the time between placement and licking the paws or jumping was recorded as response latency. The animals were treated with graded doses of *H. sinensis* (50, 100 and 200 mg/kg, i.p.), pentazocin (10 mg/kg, s.c.) and vehicle before 30 min of test and the reaction time was noted at 0, 30, 60, and 90 min. The test was terminated at 15 s to prevent tissue damage and the percentage of inhibition was calculated [18].

2.6.8 Acetic acid-induced writhing in mice

Groups of mice were pretreated with *H. sinensis* (50, 100 and 200 mg/kg, i.p.) or diclofenac (30 mg/kg, i.p.) or vehicle 30 min before the injection of acetic acid (10 ml/kg of 0.6% solution, i.p.). The number of writhings was counted for 20 min after the injection of acetic acid. Abdominal writhing was considered as nociceptive behavior, and it was defined as an exaggerated extension of the abdomen combined with the outstretching of the hind limbs [19].

2.7 Statistical analysis

Results are expressed as mean \pm S.E.M., and the statistical analysis of data was done using one-way analysis of variance (ANOVA) followed by Dunnett's test. Probability level < 0.05 was considered statistically significant.

3. Results

3.1 Phytochemical analysis

The phytochemical screening of the *H. sinensis* has revealed the presence of flavonoids, glycosides, saponins and tannins.

3.2 Acute toxicity

The mice treated with oral and i.p. administration of *H. sinensis* up to 2 g/kg did not produce any toxic effects in mice. No mortality was observed and *H. sinensis* was found to be safe at given doses.

3.3 PB-induced sleeping in mice

Pretreatment with *H. sinensis* (100 and 200 mg/kg, i.p.) and diazepam (1 mg/kg, i.p.) produced significant ($p < 0.01$) prolongation of PB (50 mg/kg, i.p.) induced sleeping time as compared to vehicle treated group. Administration of the extract and diazepam also decreased the latency to sleep (Fig. 1).

3.4 Anxiolytic activity in mice using elevated plus maze (EPM)

The *H. sinensis* (200 mg/kg) and diazepam (1 mg/kg) showed significant ($p < 0.01$) increase in the occupancy in the open arm. The extract at 200 mg/kg and diazepam exhibited significant ($p < 0.01$) decrease in time spent in enclosed arm. The animals treated with diazepam and the extract (200 mg/kg) decreased preference to the closed arm entries and open arm entries were significantly increased ($p < 0.01$). The vehicle treated mice spent 34.0 ± 5.2 s in open arm and 253.4 ± 7.5 s in closed arm with 6.8 ± 1.1 entries in open arm and 15.8 ± 1.2 entries into enclosed arm (Table 1, 2).

Table 1. Effect of *H. sinensis* on time spend in open and enclosed arms of the elevated plus maze.

Treatment	Time spend in open arm (s)	Time spend in enclosed arm (s)	Ratio of time spend in open to enclosed arm
Vehicle	34.0 ± 5.2	253.4 ± 7.5	1 : 7.4
Diazepam (1 mg/kg, i.p.)	116.4 ± 9.6 **	178.8 ± 4.5**	1 : 1.5
<i>H.sinensis</i> (50 mg/kg, i.p.)	53.4 ± 9.4	242.6 ± 8.3	1 : 4.5
<i>H.sinensis</i> (100 mg/kg, i.p.)	74.0 ± 7.5	212.4 ± 12.6*	1 : 2.8
<i>H.sinensis</i> (200 mg/kg, i.p.)	119.4 ± 8.9**	187.8 ± 8.5**	1 : 1.5

n = 5. * p < 0.05, ** p < 0.01 vs. Vehicle (one-way ANOVA followed by Dunnett's test).

Table 2. Effect of *H. sinensis* on entries in open and enclosed arms of the elevated plus maze.

Treatment	Entries in open arm	Entries in enclosed arm	Ratio of entries in open to enclosed arm
Vehicle	6.8 ± 1.1	15.8 ± 1.2	1 : 2.3
Diazepam (1 mg/kg, i.p.)	21.6 ± 2.5**	11.8 ± 1.1	1 : 0.5
<i>H.sinensis</i> (50 mg/kg, i.p.)	8.4 ± 1.2	12.2 ± 0.8	1 : 1.4
<i>H.sinensis</i> (100 mg/kg, i.p.)	13.6 ± 1.5*	11.6 ± 1.5	1 : 0.8
<i>H.sinensis</i> (200 mg/kg, i.p.)	17.5 ± 1.1**	10.6 ± 0.9	1 : 0.6

n = 5. * p < 0.05, ** p < 0.01 vs. Vehicle (one-way ANOVA followed by Dunnett's test).

Table 3. Effect *H. sinensis* on foot shock-induced aggression.

Treatment	Latency to fight (s)	Number fights
Vehicle	16.4 ± 4.0	56.0 ± 4.3
Haloperidol (1 mg/kg, i.p.)	223.2 ± 9.8**	8.0 ± 1.9**
<i>H.sinensis</i> (50 mg/kg, i.p.)	31.8 ± 6.4*	22.0 ± 2.1**
<i>H.sinensis</i> (100 mg/kg, i.p.)	103.0 ± 13.5**	22.0 ± 3.2**
<i>H.sinensis</i> (200 mg/kg, i.p.)	116.6 ± 8.6**	17.0 ± 2.7**

n = 5. *p < 0.05, ** p < 0.01 vs. Vehicle (one-way ANOVA followed by Dunnett's test).

Table 4. Effect of *H. sinensis* on locomotor activity in mice using actophotometer.

Treatment	Locomotor activity (score) in 10 min		Reduction in activity (%)
	Before Treatment	After Treatment	
Vehicle	706.0 ± 40.3	635.8 ± 25.7	9.4 ± 2.5
Diazepam (1 mg/kg, i.p.)	696.4 ± 53.4	344.2 ± 17.35	49.6 ± 3.7**
<i>H.sinensis</i> (50 mg/kg, i.p.)	588.8 ± 41.5	475 ± 51.6	18.5 ± 7.6
<i>H.sinensis</i> (100 mg/kg, i.p.)	592.4 ± 39.6	346.2 ± 44.3	42.4 ± 3.7**
<i>H.sinensis</i> (200 mg/kg, i.p.)	610.4 ± 63.7	355.2 ± 69.6	42.9 ± 6.6**

n = 5. ** p < 0.01 vs. Vehicle (one-way ANOVA followed by Dunnett's test).

Table 6. Effect of *H. sinensis* on acetic acid-induced writhings.

Treatment	Number of writhings (per 30 min)	Inhibition (%)
Vehicle	63.4 ± 6.4	-
Diclofenac (30 mg/kg, i.p.)	12.0 ± 1.8**	81.0**
<i>H. sinensis</i> (50 mg/kg, i.p.)	21.6 ± 1.6**	65.9**
<i>H. sinensis</i> (100 mg/kg, i.p.)	18.8 ± 1.4**	70.3**
<i>H. sinensis</i> (200 mg/kg, i.p.)	13.6 ± 1.0**	78.5**

n = 5. **p < 0.01 vs. Vehicle (one-way ANOVA followed by Dunnett's test).

Table 5. Effect of *H. sinensis* on pain in hot plate analgesiometer.

Treatment	Response Latency (S)				Inhibition (%)		
	0	30	60	90	30	60	90
Vehicle	2.4 ± 0.5	3.8 ± 0.6	3.0 ± 0.8	4.2 ± 1.0	36.8	33.3	42.8
Pentazocine (10 mg/kg, s.c.)	1.8 ± 0.3	11.8 ± 1.4**	13.2 ± 0.9**	11.6 ± 0.6**	84.7	86.3	84.4
<i>H. sinensis</i> (50 mg/kg, i.p.)	2.4 ± 0.5	5.8 ± 0.5	6.4 ± 1.2	7.2 ± 0.9	58.6	62.5	66.6
<i>H. sinensis</i> (100 mg/kg, i.p.)	2.8 ± 0.3**	11.4 ± 0.5**	13.4 ± 0.8**	11.6 ± 1.0**	75.4	79.1	75.8
<i>H. sinensis</i> (200 mg/kg, i.p.)	2.4 ± 0.5	12.8 ± 0.8**	10.6 ± 0.97**	8.6 ± 0.9*	81.2	77.3	72.0

n = 5. **p < 0.01 vs. Vehicle (one-way ANOVA followed by Dunnett's test).

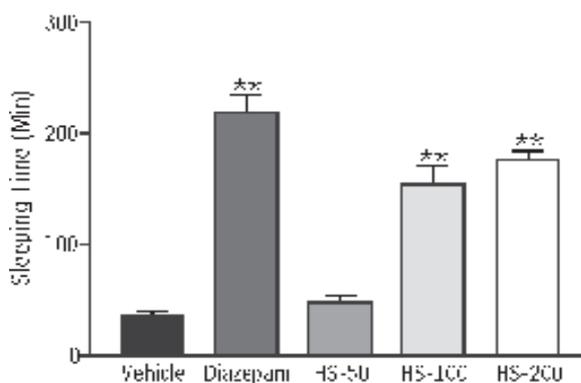


Fig 1. Effect of *H. sinensis* extract on pentobarbital-induced sleeping time. Each column represents mean ± S.E.M. (n = 5). ** p < 0.01, vs. Vehicle (one-way ANOVA followed by Dunnett's test).

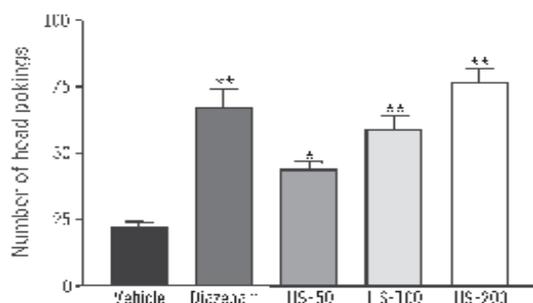


Fig 2a. Effect of *H. Sinensis* on number of head pokes. Each column represents mean ± S.E.M. (n = 5). *p < 0.05, **p < 0.01, vs. Vehicle (one-way ANOVA followed by Dunnett's test).

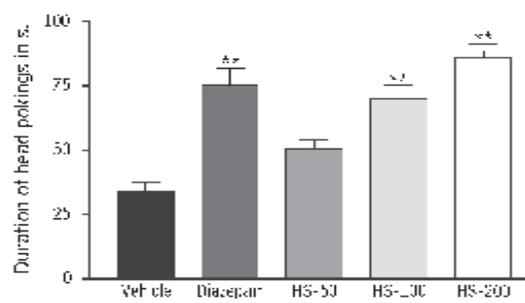


Fig 2b. Effect of *H. sinensis* on duration of head pokes. Each column represents mean ± S.E.M. (n = 5). **p < 0.01 vs. Vehicle (one-way ANOVA followed by Dunnett's test).

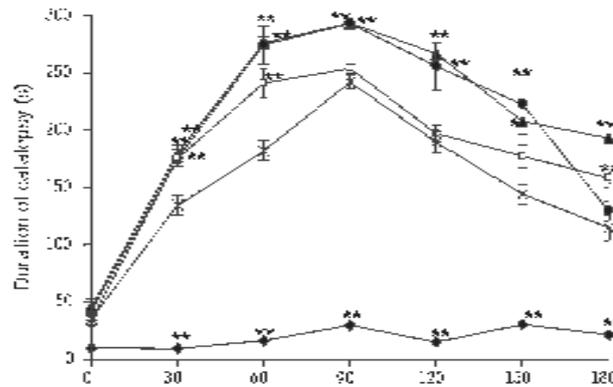


Fig 3. Effect of *H. sinensis* on haloperidol-induced catalepsy. Each point indicates mean \pm S.E.M. (n = 5). *p < 0.05, **p < 0.01, vs. Vehicle (one-way ANOVA followed by Dunnett's test).

- ✕ Vehicle + Haloperidol (1 mg/kg, i.p.)
 - *H. sinensis* (50 mg/kg, i.p.) + Haloperidol (1 mg/kg, i.p.)
 - *H. sinensis* (100 mg/kg, i.p.) + Haloperidol (1 mg/kg, i.p.)
 - ▲ *H. sinensis* (200 mg/kg, i.p.) + Haloperidol (1 mg/kg, i.p.)
 - ◆ *H. sinensis* (200 mg/kg, i.p.)
- *p < 0.05, **p < 0.01 vs Vehicle, (one-way ANOVA followed by Dunnett's test)

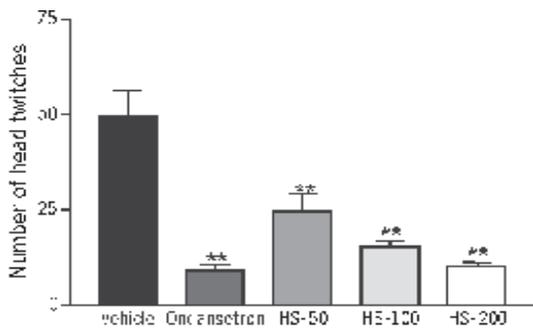


Fig 4a. Effect of *H. sinensis* on lithium-induced head twitches. Each column represents mean \pm S.E.M. (n = 5). **p < 0.01 vs. Vehicle (one-way ANOVA followed by Dunnett's test)

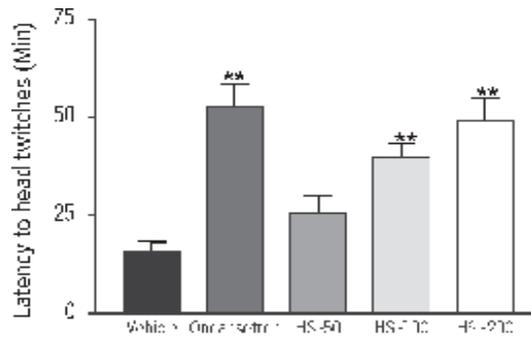


Fig 4b. Effect of *H. sinensis* on latency to lithium-induced head twitches. Each column represents mean \pm S.E.M. (n = 5). * p < 0.01 vs. vehicle (one-way ANOVA followed by Dunnett's test).

3.5 Anxiolytic activity in mice using hole board apparatus

The extract at all dose levels (100 and 200 mg/kg, i.p.) revealed statistically significant ($p < 0.05$, $p < 0.01$) increase in the number of head pokes. The duration of head poking was also increased as compared to control group. Diazepam (1 mg/kg, i.p.) showed significant ($p < 0.01$) increase in the exploratory activity (Fig. 2a, 2b).

3.6 Foot shock- induced aggression in mice

The administration of *H. sinensis* (50, 100 and 200 mg/kg, i.p.) showed significantly ($p < 0.01$) decrease in number of fights in foot shock-induced aggression compared with vehicle. Further the extract (100 and 200 mg/kg) significantly ($p < 0.01$) increased latency to fight. Haloperidol treated group showed statistically significant ($p < 0.01$) decrease in number of fights and increase latency to fight (Table 3).

3.7 Haloperidol-induced catalepsy

H. sinensis (50, 100, 200 mg/kg, i.p.) significantly potentiated haloperidol induced catalepsy at each time interval. The extract at dose 50, 100 and 200 mg/kg showed maximum cataleptic score of 253.8 ± 3.56 , 299.3 ± 0.896 and 293.4 ± 2.43 s. respectively at 90 min ($p < 0.01$) in haloperidol treated animals. In vehicle treated animals, haloperidol produced maximum catalepsy (236.2 ± 5.275 s) after 90 min (Fig.3).

3.8 Lithium-induced head twitches (serotonin mediated behavior)

H. sinensis at all dose levels (50, 100, 200 mg/kg) significantly ($p < 0.01$) decreased number of head twitches to 24.6 ± 4.6 , 15.2 ± 1.6 and 10.2 ± 1.06 respectively. In vehicle treated rats, Lithium sulphate produced 49.2 ± 7.2 head twitches whereas Ondansetron (a 5HT₃ antagonist) reduced the number of head twitches (9.0 ± 1.7) showing their effect on serotonergic system (Fig. 4a, 4b).

3.9 Locomotor activity in mice

H. sinensis (100, 200 mg/kg, i.p.) produced significant ($p < 0.01$) reduction in locomotor activity as compared to the control animals receiving only vehicle. The diazepam treated group revealed a statistically significant decrease in locomotor activity ($p < 0.01$) (Table 4).

3.10 Analgesic activity using hot plate analgesiometer

In hot plate analgesiometer, *H. sinensis* (100, 200 mg/kg, i.p.) significantly ($p < 0.01$) reduced the animal's sensitivity to pain by raising the threshold of reaction. Pentazocine showed significant ($p < 0.01$) increase in pain threshold indicating analgesic effect (Table 5).

3.11 Acetic acid induced writhing in mice

The administration of *H. sinensis* reduced the intensity of nociception, by decreasing the

writhing reaction induced by acetic acid. The extract at doses of 50, 100 and 200 mg/kg exhibited an inhibitory effect of 65.9, 70.3 and 78.5 % respectively. Diclofenac inhibited the pain sensation by 81.0 % (Table 6).

4. Discussion

The study was aimed to establish the potential neuropharmacological properties of *H. sinensis* based on its uses in traditional medicine, as reported earlier [1, 2].

The results of acute toxicity study indicated that *H. rosa sinensis* root extract did not produce any detectable toxicity either on oral or i.p. administration.

Pentobarbital-induced sleeping time is considered as a very sensitive way to assess agents for central nervous system depressant action [10]. The potentiation of pentobarbitone-induced sleeping time in mice suggests that the extract may have sedative effect. The compounds which inhibit hepatic metabolism of pentobarbitone may also prolong PB-induced sleeping time; the extract might be inhibiting hepatic metabolism of PB.

Several plants increase the exploration of the open arm in the EPM and are used to diminish anxiety in folk medicine [20]. In this study, the extract of *H. sinensis* increased the exploration and time spent into the open arms. Head dipping behavior in rodent has been recorded as a measure of anxiety. The anxiolytics increase the number and time spent in head-dips, while anxiogenic agents decrease head poking [21]. The extract significantly increased head-dipping behavior thus reinforcing the anxiolytic-like effect. In both of these tests diazepam is used as positive control as expected, it increased the exploration in the open arms of the EPM and hole board test, confirming its anxiolytic action.

The aggressive behavior in animals is regulated by central monoaminergic neurons. Central D₂ dopamine receptors are involved in the modulation of foot-shock aggression in mice and brain dopamine level has been reported to increase in foot shock-induced aggression [22]. *H. sinensis* reduced the number of fighting attacks and increased latency to fight, therefore indicating a possible antidopaminergic activity. The catalepsy is a phenomenon defined as the long-term maintenance of an animal in an abnormal posture [15]. Haloperidol induces catalepsy by blockade of D₂ receptor. Sanberg (1980) showed the cataleptic effect of the haloperidol [23]. The extract of *H. sinensis* potentiated haloperidol-induced catalepsy in a dose dependent manner indicating antidopaminergic activity. Drugs decreasing dopaminergic transmission are known to prolong barbiturate-induced sleep. Haloperidol, a typical antipsychotic is reported to potentiate barbiturate induced sleep [24]. In this study, pretreatment with *H. sinensis* was found to prolong pentobarbital-induced sleeping time in mice, therefore suggesting that *H. sinensis* may be involved in decreasing dopaminergic transmission. Thus, it may be suggestive that *H. sinensis* has antidopaminergic potential that needs further investigations in the treatment of psychosis.

The lithium-induced head twitches are due to increase in the release or formation of serotonin in the CNS, which stimulates serotonin receptors [16]. These head twitches are antagonized by drugs that blocks 5-HT receptors [22]. The result showed that *H. sinensis* significantly reduced lithium-induced head twitches, indicating an inhibitory effect on serotonergic system.

Locomotor activity is considered as an index of alertness and a decrease in it would indicate

CNS depressant activity [25]. The extract produced a reduction in motor activity showing a significant skeletal muscle relaxant and sedative effect.

The *H. sinensis* was found to have antinociceptive activity when assessed using the writhing and hot plate tests. The results obtained showed that the extract is effective in blocking the nociceptive effect induced by the chemical stimulus in the writhing test and by the thermal stimulus in hot plate test. It is well known that centrally acting drugs, for example morphine, have activity in both types of test while peripherally acting drugs, for example aspirin, have been reported to exhibit analgesic activity in the writhing test only [26]. The result clearly revealed that the extract of *H. sinensis* seems to possess both peripheral and central analgesic properties.

Phytochemical tests showed presence of flavonoids, glycosides, saponins, and tannins in methanolic extract of *H. rosa sinensis*. Thus presence of these chemical constituents may be responsible for diverse pharmacological effects of *H. rosa sinensis*.

Finally, we conclude that the *H. sinensis* produces a significant CNS depressant activity. The sedative, anxiolytic, antipsychotic and muscle relaxant activities of *H. sinensis* might be possibly mediated via facilitation of GABAergic transmission or inhibition of serotonergic as well as dopaminergic transmission. These results suggest that the roots of *H. rosa sinensis* have potential clinical application in the management of anxiety, psychosis, pain and muscle tension disorders.

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