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BEHAVIOURAL AND NEUROPHARMACOLOGICAL EFFECTS OF HEDYCHIUM SPICATUM IN EXPERIMENTAL ANIMAL

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ABSTRACT
Hedychium spicatu. (Family Zingiberaceae) is a rhizomatous herb, used in medicines, food, cosmetics and perfumery industries. Traditionally, it is widely used in treating inflammation, pain, asthma, foul breath, vomiting, diarrhoea, bronchitis, hiccough and blood diseases. This study systematically reviewed traditional and folk uses, pharmacological properties, bioactive compounds and market potential of H. spicatum. Research gaps and potential of future research have also been discussed here we studied neuropharmacological and Behavioral changes by this plant The acute oral toxicity study conducted as per the OECD guideline did not show any toxicity as per its acceptable range for both extracts of HS (EHS and PHS). The initial behavioral observations did not revealed any gross change in the animal behavior treated with the extracts of the plants. However i reduction in alertness, reaction to touch stimuli and righting reflex was reported evident in EHS. ethanolic extract of HS exhibited significant reduction in basal activity count as well as the fall off time. Ethanolic extract of both the plants did not show any significant change in latency period in hot plate method. Although the significant increase in the reaction time with ethanolic extract of HS was observed but absence of straub tail phenomena ruled out the narcotic analgesic action. The increased reaction time may be due to the sedative effect. ethanolic extract of HS showed significant dose dependent (dome shape curve) activity. The results of anticonvulsant activity evaluated against MES induced convulsions both acute as well as chronic studies revealed ineffectiveness of bothe the extracts towards prevention of convulsions. The antianxiety action of the rhizomes of HS may due to presence of saponin. The results further suggested the possible use of these plants in various related bahavioral and neurological complaints.

1 MATERIALS

1.1) Plant material and preparation of extracts

1.2 H. spicatum
The rhizomes of H. spicatum were purchased from local markets of amravati and, with voucher specimen no. AHMA R-079. The rhizomes were dried under shade, powdered and passed through 40 mesh sieve. The powdered material was extracted with petroleum ether and ethanol using soxhlet apparatus. The petroleum ether extract (PHS) obtained was dried in rotary vacuum evaporator at 40V, yielding a viscous mass (1.36% w/w). The ethanolic extract (EHS) obtained was dried in rotary vacuum evaporator at 40°C, yielding a dark brown coloured viscous mass (5.30 % w/w).

1.3) Chemicals and drugs
Haloperidol (Rajesh chemicals, Mumbai); sodium nitrate (Loba chemicals, Mumbai) were purchased from respective vendors. Diazepam injection (Calmpose®), pentazocin injection (Fortwin®), piracetam suspension (Nootropil®), and phenytoin tablets (Eptoin®) were purchased from the local market.

1.4) Preparation of drug solution
Accurately weighed quantity of extract was suspended in 1% acacia. The doses were administered orally by selecting the appropriate concentration of the stock solution. Diazepam injection, pentazocin injection and piracetam suspension were diluted with the distilled water and phenytoin was suspended in 1% w/v acacia. The doses were administered by selecting the appropriate concentration (10 ml/kg) of the stock solution.

1,5) Storage conditions
The dried extracts were preserved in the desicator and used for preparation of various doses. All the dosage of the extracts and drug solutions were prepared once a week as required and stored in airtight amber colored vials in the refrigerator.

1.6) Route of administration
All the extracts and piracetam were administered orally; diazepam, haloperidol, phenytoin and sodium nitrite were administered intraperitoneally and pentazocin was administered subcutaneously.

1.7) Volume of administration
The volume of administration was calculated based upon the body weight of animals and was kept constant with respect to their body weights (10 ml/kg).

1.8) Animals
Male Swiss albino mice (18-22 g) were used. They were maintained at 25 ± 2° C and relative humidity of 45 to 55% and under standard environmental conditions (12 hrs light 12 hrs dark cycle). The animals had free access to food (Amrut feed, Chakan oil mills, Pune, India) and water ad libitum. All experiments were carried out between 12:00-16:00 hrs. The experimental protocols were approved by the Institutional Animal Ethical Committee (lAEC) of Bharati Vidyapeeth University, Poona College of Pharmacy, Pune- 411038.

1.9) Daily acclimatization of animals
The animals were shifted from animal house to the laboratory one hour prior to the start of the experiment. The respective apparatus were cleaned with either hydrogen peroxide or damp cloth wherever necessary to avoid possible bias due to odor left by the previous animal.

2 METHODS

2.1) Phytochemical Analysis of Extracts
The standard procedures were adopted for testing the presence of various chemical constituents in the extracts

2.2 Acute toxicity study
Healthy adult male albino mice (18- 22 g) were subjected to acute toxicity studies as per guidelines (AOT 425) suggested by the Organization for Economic Cooperation and Development (OECD-2001). The mice were administered the different doses of extracts of HI or HS. The dose progression or reduction was carried out as suggested by the AOT-425 guidelines. The mice were observed continuously for 2 hrs for behavioral and autonomic profiles and for any sign of toxicity or mortality up to a period of 7 days.[98]

2.3 Treatment schedule for pharmacological experiments
Animals were divided into five groups; each consisting of six animals for each experiment. Control group I received vehicle (10 ml/kg), Group II, 111, IV received 30, 100 and 300 mg/kg of either HI or HS extracts, respectively. Group V received respective reference standard drug.[99]

2.4 Behavioral effects
Behavioral effects of HI and HS (30, 100 and 300 mg/kg) were assessed 60 min after the administration of vehicle, HI or HS for next 2 hour (Taber et al., 1957). The observations were recorded by an independent trained observer who was unaware of the treatment given to avoid subjective bias. The observation parameters consisted of body position, alertness, reactivity to touch stimuli, righting reflex and lacrimation.[100]

2.5 Motor coordination
The motor coordination was assessed on 2"^ and 7* day of treatment using digital rota rod (Inco - Ambala, India). Mice were trained by placing them on a rotating rod (20 rev/ min), twice daily for three consecutive days before the experiment. 30 min interval was kept between two trials. Only those mice which had demonstrated their ability to remain on the rotating rod for at least 120 seconds were selected. The selected mice were divided into five groups with 6 animals in each group. The mice were then tested for motor coordination to record basal fall off time per 300 sec after the respective drug treatment. One hour following the administration of vehicle or drugs {HI, HS or Standard), mice placed again on the rotating rod and the fall off time per 300 sec was recorded. The difference between mean fall of time before and after drug treatment was considered for evaluation. Diazepam (2 mg/ kg. ip) was used as a reference standard.[101]

2.6 Locomotor activity
The locomotor activity (horizontal activity) was measured on 2" and 7 day of treatment using a digital actophotometer (Space-lab, India). Each mouse was placed individually in the actophotometer for 05 min and basal activity score was obtained. Subsequently animals were divided into five groups separately for HI and HS groups. Sixty min after dosing, the mice were placed again in the actophotometer for recording the activity score. The observations were recorded as mean change in the locomotor activity. Diazepam (2 mg/kg, ip) was used as reference standard.[102]

2.7 Analgesic activity
The analgesic effect was evaluated on 2""^ and 7* day of treatment using digital hot plate (Columbus - USA) instrument wherein the reaction time (paw licking, jumping or any other sign of discomfort) was recorded at 0, 60, and 120 min after the administration of vehicle (10 ml/ kg), HI or HS. The temperature of the plate was maintained at 55°C ±1° C. A cut off reaction time of 15 second was chosen in order to avoid injury. Pentazocin (30 mg/kg, s.c.) was used as a reference standard.[103]

2.8 Elevated plus maze (EPM)
Locally fabricated elevated plus maze consisting of two open arms (35x6 cm) and two enclosed arms (35x6x15 cm) was used. The maze was elevated to the height of 40 cm. Mice were placed individually in the center of the EPM facing an enclosed arm. The time spent by the mouse during the next 5 min in the open and enclosed arm was recorded on 2""^ and 7* day of dosing schedule. The animals received vehicle, different doses of HI or HS 60 min before and diazepam (1 mg/kg, ip) 30 min before their placement on the maze. Increased exploratory activity in the open arm was considered as an indication of anxiolytic activity.[104]

2.9 Object recognition test
The apparatus, fabricated locally, consisted of white

colored plywood (70 x 60 x30 cm) with a grid floor. It was illuminated by a 40 W lamp suspended 50 cm above the apparatus. The object to be discriminated was also made of plywood in two different shapes of 10 cm height and coloured black. One day before the test, mice were allowed to explore the box without any object for 02 min. On the day of test, the first trial (Ti) was conducted 60 min after the administration of vehicle, HI, HS or piracetam (150 mg/kg). Two identical objects were presented in the opposite comers of the box and the time taken by each mouse to complete 20 s of object exploration was recorded (Exploration was considered as directing the nose at a distance less than 2 cm to the object and/or touching with nose). Second trial (T2) was performed 90 min after the first trial (Ti) and a new object replaced one of the objects presented in Ti and mice were left in the box for next 05 min. The time spent for exploring the familiar (F) and the new object (N) was recorded separately and the discrimination index (D) was calculated as (N-F)/ (N+F) on the second and seventh day of drug administration. The object was changed randomly and the apparatus was cleaned with hydrogen peroxide or damp cloth after each trial to avoid the place preference and the influence of olfactory stimuli respectively.[105]

2.10 Double unit mirrored chamber test

The mirrored chamber apparatus fabricated locally consisted of a mirrored cube (30x 30x 30cm), open on one side and placed in square box. The container box (40x40x30 cm) had a white floor and black wall making 5cm corridor, completely surrounding the mirrored chamber. A sixth mirror was placed on the wall of the box, positioned to face the open side of the mirrored chamber. The latency to enter the mirrored chamber and time spent in mirrored chamber during 5 min observation period was recorded on the 2"" and 7* day, 60 min after the respective drug administration. Diazepam (1 mg/kg, ip) was used as a reference standard. The mice were not exposed to the apparatus before the test and evaluated only once to avoid the habituation. The apparatus was washed with hydrogen peroxide after each evaluation to eliminate the potential cues such excreta, urine left by the previous occupant.[106]

2.11 Haloperidol induced catalepsy

Mice were divided into four groups. The control group received vehicle (10 ml/kg, po) whereas the other group received either HI or HS (30,100 and 300 mg/kg, po) 60 min before the haloperidol (1 mg/kg, ip). After the treatment, the forepaws of the mice were placed on a rod of 0.9 cm diameter set at 2.5 cm from top. Duration for which the mice retained the forepaws on the elevated rod was noted down at 0, 15, 30, 60, 90 and 120 min. the cut off time was 300 sec. The animals were tested twice at each time interval and only the greater duration of time was recorded. Between measurements, the mice were returned to their home cages. An animal was considered to be cataleptic if it remained on the bar for 60 seconds.[107]

2.12 Maximal electroshock induced seizures (MES)

Tonic clonic convulsions were induced by giving maximal electroshock seizures (MES) (40mA for 0.2sec) using an electroconvulsiometer (INCO, Ambala, India) via crocodile ear clip, 60 minutes after administration of either vehicle (10 ml/kg, po), HI or HS (30, 100 and 300 mg/kg, p.o.) and 90 minutes after phenytoin (25 mg/kg, ip). The number of animals protected from tonic hind limb extension seizure (abolition of tonic hind limb extension within 10 sec after delivery of the electroshock was considered as protected mice) and duration of tonic hind limb extension seizure was determined in each dose group.[108]

2.13 Sodium nitrite induced respiratory arrest

Mice were divided into four groups and were treated with the vehicle (10 ml/kg) or HI or HS (30, 100, 300 mg/kg, p.o). Sixty min later, all the mice were subjected to sodium nitrite treatment (250 mg/kg, ip). The time between injection of sodium nitrite and death was recorded.[109]

Results Phytochemical and neuropharmacological evaluation of ethanolic and petroleum-ether extracts of of Hedichium spicatum

1.1 Phytochemical analysis

Table 11.1: Phytochemical evaluation of ethanolic and petroleum-ether extracts of HS.

no.	Phytochemical test	Test	Ethanolic extract	Pet. ether extract
1	Test for Steroids	Salkowaski test	-	-
		Liebermann-Burchard reaction	-	-
2	Test for Triterpenoids	Salkowaski test	-	+
		Liebermann-Burchard test	-	+
3	Test for Glycosides	Balget's test	-	-
		Keller-Killiani test	-	-
		Legals test	-	+
		Bomtrager'stest	-	-
4	Tests for Saponin	Foam Test	+	-
5	Tests for Carbohydrate	Molisch's test	+	-
		Barfoed's test	+	-
		Fehling's test	-	-
		Benedict's test	-	-
6	Tests for Alkaloids	Mayer's test	+	+
		Hager's test	+	+
		Dragendorff s tes	+	-
7	Tests for Flavonoids	Ferric-chloride test	+	-
		Shinoda test	+	-
8	Tests for Tannins	Ferric-chloride test	-	-
		Gelatin test	-	-
9	Test for Proteins	Millon's test	-	-
		Xanthoproteic test	-	-
		Biuret test	-	-
		Ninhydrin tes	-	-

1.2 Acute oral toxicity test

All animals treated with ethanolic and petroleum ether extracts of roots of//. spicatum (EHS and PHS) were free of any toxicity as per acceptable range given by the OECD guidelines and no mortality was observed up to 2000 mg/kg. Hence three different doses 30, 100, 300 mg/kg were selected for further study.

1.3 Behavioral effects

Mice treated with the EHS (30, 100 and 300 mg/kg) or PHS (30, 100 and 300 mg/kg) were observed for 2 hours for behavioral signs.

EHS (30 - 300 mg/kg) decreased the alertness and reactivity to touch stimuli, whereas EHS 100 - 300 mg/kg decreased the righting reflex. EHS (30 - 300 mg/kg) did not show any effect on lacrimation at all doses tested. (Table 5.2)

Treatment with PHS (30 - 300 mg/kg) did not produced any difference in their behavior and general body posture during the observation period. The animals were alert, with normal grooming, touch response and pain response. Limb tone and grip strength were normal and the animals did not show staggering gait or contractions. (Table 5.2)

Table 11.2: Behavioral assessment of ethanolic and petroleum ether extract of spicatum in mice.

Behavioral signs	Control (10 ml/kg)	EHS 30	EHS 100	EHS 300	PHS 30	PHS 100	PHS 300
Alertness	-	↓	↓	↓	-	-	-
Body position	-	-	-	-	-	-	-
Reactivity to touch stimuli	-	↓	↓	↓	-	-	-
Lacrimation	-	-	↓	↓	-	-	-
Righting reflex	-	-	-	-	-	-	-

- : Normal, ↑: Increased, ↓: Decrease

1.4 Motor coordination

The mean fall off time of vehicle treated control group was 222.47±21.06 seconds and 217.60±12.26 seconds on 2"** and 7* day of the treatment respectively. This fall off time was significantly reduced after treatment with EHS (100 - 300 mg/kg) on both the experimental days as

compared to the control group. On 2"" day of the treatment EHS 300 mg/kg was more potent ($p<0.01$) than EHS 100 mg/kg ($p<0.05$), while both these doses were statistically equipotent ($p<0.01$) on 7* day of the treatment.

None of the doses of PHI (30 - 300 mg/kg) significantly changed the fall off time on both the experimental days when compared to the control group. Diazepam 2 mg/kg was significantly reduced the fall off time when compared with the control group on both the experimental days. (Table 5.3)

Table 1.3: Effect of ethanolic and petroleum ether extract ofH. spicatum on fall off time by rota rod test.

Fall Treatments (mg/kg)	2"" Day	7* Day
Control (10 ml/kg)	222.47±21.06	217.60112.26
EHS 30	195.43112.00	201.75114.08
EHS 100	158.98+18.60*	131.74+5.26**
EHS 300	136.83112.51**	127.8614.71**
PHS 30	191.65+0.12	203.8719.74
PHS 100	211.98119.90	184.4515.26
PHS 300	191.47114.24	127.86122.59
Diazepam 2	61.0219.66**	54.7816.99**

[Mean ± SEM)
Fall off time(Seconds)

1.5 The locomotor activity

(Horizontal activity) was measured on the 2" and 7 day of treatment. The mean basal activity score of vehicle treated control group was 284.5±26.39 and 326.2±21.62 on 2"" and 7* day of the treatment respectively. Pretreatment with EHS (30 - 300 mg/kg) significantly decreased the basal activity score on 7* day of treatment, but the results of 2"" day were insignificant in this regard as compared to the control group. There was no significant difference between the basal activity score of the vehicle treated control group and PHS (30 -300 mg/kg) treated animals on both the experimental days. Diazepam 2 mg/kg significantly decreased the locomotor activity on both the experimental days as compared to the control animals. Hence the results indicated significant decrease in the locomotor activity with EHS (30 - 300 mg/kg) on 7"' day as compared with control animals. Whereas EHS (30 - 300 mg/kg) on 2""* day and PHS (30 - 300 mg/kg) on both the experimental days did not significantly altered the locomotor activity at all doses tested as compared with control animals. (Table 5.4).

Table 1.4: Effect of ethanolic and petroleum ether extract of H. spicatum on basal activity score (locomotor activity) by digital actophotomet.

Basal activity score (Mean ± SEM)		
Fall Treatments (mg/kg)	2"" Day	7* Day
Control (10 ml/kg)	284.5±26.39	326.2±21.62
EHS 30	266.0±7.64	260.0±7.05*
EHS 100	205.6±28.02	185.8±23.32**
EHS 300	183.0±16.31	148.4±13.33**
PHS 30	285.2±16.50	295.41±1.33
PHS 100	269.2±16.70	280.4±34.16
PHS 300	256.8±23.10	297.6±25.62
Diazepam 2	56.2±6.21	60.8±5.56

1.6 Analgesic activity

The mean reaction time of vehicle treated control group was 7.12+ 0.52, 7.20± 0.44 and 6.96+ 0.43 seconds on 2"'^ and 7.54+ 0.45, 7.66+ 0.39 and 6.82± 0.19 seconds on 7* day of the treatment at 0, 60 and 120 minutes of observation period respectively.

The analgesic effect was evaluated on 2"^* and 7* day of treatment. On both the experimental days significant increase in reaction time was observed at 60 min after the treatment with EHS 300 mg/kg, when compared with the control group, while other doses were insignificant in this regards. On the other hand, none of the doses of PHS showed significant change in the reaction time on both the experimental days. Pentazocin 30 mg/kg showed significant increase in reaction time on both the experimental days. Results also showed that EHS 300 mg/kg statistically equipotent to that of pentazocin 30 mg/kg on 7^ day of the treatment. (Table 5.5 & 5.6)

Table 1.5: Effect of ethanolic extract of H. spicatum on reaction time for analgesic activity by digital hot plate.

Treatments (mg/k)	Reaction time (Sec (Mean ± SEM)		
	0 minute	60 minute	120 minut
Control (10 ml/kg)	7.12±0.52	7.66±0.39	6.96±0.43
EHS 30	6.48±0.31	6.34±0.46	7.18±0.24
EHS 100	6.28±0. 66	7.44±0.65	6.98±0.65
EHS 300	6.98±0. 83	9.60±0.99	8.42±0.39
Pentazocin 30	7.10±0. 36	11.86±0.50	11.86±0.54
7*" Day			
Control (10 ml/kg)	7.54±0.45	7.66±0.39	6.82±0.19
EHS 30	7.44±0.67	1 6.34±0.49	4.20±1.46
EHS 100	7.04±0.75	6.50±0.79	6.84±0.54
EHS 300	8.48±0.50	12.90±0.60	12.34±0.41
Pentazocin 30	8.48±0.50	12.90±0.60	12.34±0.41

Table 5.6: Effect of petroleum ether extract of H. spicatum on reaction time for analgesic activity by digital hot plate.

Treatments (mg/k	Reaction time (Sec (Mean ± SEM		
	0 minute	60 minute	120 minut
Control (10 ml/kg)	7.12±0.83	7.20±0.44	6.96±0.43
EHS 30	6.50±0.54	7.42±0.46	8.46±0.6
EHS 100	7.04±0.66	7.10±0.56	7.26±0.57
EHS 300	7.26±0.63	8.08±0.69	8.42±0.39
Pentazocin 30	7.10±0.3	11.86±0.50	11.86±0.54
7*" Day			
Control (10 ml/kg)	7.54±0.45	7.66±0.39	6.82±019
EHS 30	6.28±0.67	8.82±0.79	6.50±0.63
EHS 100	7.18±0.75	7.00±0.58	7.06±0.41
EHS 300	7.78±0.70	7.20±1.01	8.72±0.50
Pentazocin 30	8.48±0.50	12.90±0.55	12..34±0.41

1.7 Elevated plus mazes (EPM)

On 2"" and 7* day of the treatment the vehicle treated control group showed mean time spent in the closed arm as 215.87+15.24 seconds and 244.48±13.09 seconds and time spent in open arm as 36.88±9.47 seconds and 26.22±2.87 seconds respectively. Decrease in the time spent in the closed arm and increase in time spent in the open arm was significant after the treatment with EHS (30 & 100 mg/kg) in which the dose 30 mg/kg was found to be more significant than the dose 100 mg/kg as compared to the control animals on both the experimental days. None of the doses of PHS (30 - 300 mg/kg) significantly affected the time spent in closed and open arm on both the experimental days. On the other hand diazepam 1 mg/kg significantly decreased the time spent in the closed arm and increased the time spent in the open arm on both the experimental days. Hence EHS (30 & 100 mg/kg), but not PHS (30 -300 mg/kg), was significantly produced anxiolytic effect. (Table 5.23 & 5.24)

Table 1.7: Effect of ethanolic extract of H. spicatum on exploratory activities in EPM.

Time spent in closed arm (Seconds) (Mean ± SEM)		
Treatments(mg/kg)	**2"" Day**	**7* Day**
Control(10 ml/kg)	215.87±15.24	244.48±13.09
EHS 30	131.38 ±7.58	122.34±9.61
EHS 100	189.58±12.18	201.58±9.07
EHS 300	220.91±9.92	231.69±4.24
Diazepam	105.52±6.37	110.88±5.68

Time spent in open arm (Seconds) (Mean ± SEM)

Control (10 ml/kg)	36.88±9.47	26.22±2.87
EHS 30	70.26±5.08	83.2±4.96
EHS 100	51.02±7.71	54.06±7.13
EHS 300	39.53±4.71	46.07± 6.78
Diazepam 1	78.17±4.66	84.38±6.57

Table 1.8: Effect of petroleum ether extract of//, spicatum on exploratory activities in EPM.

Time spent in closed arm (Seconds) (Mean ± SEM).

Treatments(mg/kg)	2"" Day	7* Day
Control(10 ml/kg)	215.87±15.24	244.48±13.09
EHS 30	232.09±13.88	202.76±17.82
EHS 100	222.70±12.58	214.14±12.30
EHS 300	211.37±21.86	212.94±9.44
Diazepam	110.88±5.68	105.52±6.37

Time spent in open arm (Seconds) (Mean ± SEM).

Control (10 ml/kg)	36.8819.47	26.2212.87
EHS 30	43.8715.25	44.8116.02
EHS 100	43.9613.73	44.9015.90
EHS 300	48.4815.51	46.0716.78
Diazepam 1	78.1714.66	32.5414.21**

1.8 Object recognition test

Increase in the discrimination index indicates nootropic activity. The mean discrimination index of vehicle treated control group was 0.17+0.02 and 0.27+0.02 on 2"^ and 7^ day of the treatment respectively.

Pretreatment with EHS 300 mg/kg showed significant increase in the discrimination index on both the experimental days. Whereas, other doses of EHS and all doses of PHS (30 - 300 mg/kg) were showed insignificant effects on the discrimination index on both the experimental days. Piracetam 150 mg/kg was significantly increased the discrimination index on both the experimental days (Table 5.25). Hence EHS 300mg/kg showed significant nootropic activity**.**

Table 1.9: Effect of ethanolic and petroleum ether extract of//, spicatum on discrimination index in object recognition test.

Treatments (mg/kg)	Discrimination index (Mean ± SEM)	
	2"" Day	7*" Day
Control (10 ml/lig)	0.17±0.02	0.27±0.02
EHI 30	0.21±0.03	0.26±0.04
EHI 100	0.21±0.04	0.42±0.03
Em 30	0.34±0.05	0.46±0.07
PHI30	0.28±0.05	0.25±0.03
PHI 100	0.27±0.04	0.25±0.02
PHI300	0.21 ±0.04	0.31±0.03
Diazepam	0.58±0.04	0.62±0.03

1.9 Double unit mirrored chamber test

The mean discrimination index of vehicle treated control group was 104.37±11.35 seconds and 138.80l33.51 seconds on 2"** and 7* day of the treatment respectively.

EHS 30 mg/kg on 2"" day and EHS 30 & 100 mg/kg on ?"' day after the treatmenwere significantly decreased the latency to enter the mirrored chamber and increased the time spent in mirrored chamber as compared to the control animals. On the 7* day the dose of 30 mg/kg was more significant than the dose of 100 mg/kg. Whereas PHS (30 - 300 mg/kg) insignificantly affected the latency to enter the mirrored chamber and time spent in mirrored chamber at all doses tested on both the experimental days. Diazepam 1 mg/kg significantly decreased the latency to enter the mirrored chamber and increased the time spent in mirrored chamber on both the experimental days. (Table 5.26 & 5.27) Hence only EHS 30 & 100 mg/kg, but not EHS 300 and PHS (30 - 300 mg/kg), significantly produced anxiolytic effect**.**

Table 1.10: Effect of ethanolic of H. spicatum in double unit mirrored chamber test for anxiolytic activity.

Latency to enter mirrored chamber (Seconds) (Mean ± SEM		
Treatments (mg/kg)	**2"" Day**	**7* Day**
Control (10 ml/kg)	104.37±11.35	138.80±33.51
EHS 30	34.51±9.56**	25.38 ±6.98**
EHS 100	54.78±16.75	47.39±6.70**
EHS 300	74.38±20.93	79.67±16.14
Diazepam	21.71±4.05**	22.14±3.68**

Time spent in mirrored chamber (Seconds) (Mean ± SEM)

Control (10 ml/kg)	40.03±4.55	26.22±2.87
EHS 30	202.91±36.91	44.8±16.02
EHS 100	114.56±9.44*	44.90±5.90
EHS 300	51.27±10.01	46.07±6.78
Diazepam 1	223.33±13.06	32.54±4.21

Table 1.11: Effect of petroleum ether extract of//, spicatum in double unit mirrored chamber test for anxiolytic activity.

Latency to enter mirrored chamber (Seconds) (Mean ± SEM		
Treatments (mg/kg)	2"" Day	7* Day
Control (10 ml/kg)	104.37±11.35	138.80±33.51
EHS 30	90.30±21.38	81.80±22.71
EHS 100	80.50±26.11	77.61±13.86
EHS 300	69.72±13.82	79.67± 13.68
Diazepam	21.71±4.05**	22.14±3.68**

Time spent in mirrored chamber (Seconds) (Mean ± SEM)

Control (10 ml/kg)	40.03±4.55	35.12±4.35
EHS 30	45.77±2.99	47.09±4.57
EHS 100	36.73±9.44	43.97±4.42
EHS 300	50.70±10.31	48.86±4.96
Diazepam 1	33±13.06	256.61±6.98

1.10 Haloperidol induced catalepsy

On both the experimental days haloperidol 1 mg/kg significantly induced cataleptic behavior in control group between 60 - (30 - 300 mg/kg) PHS (30 - 300 mg/kg) was insignificantly affected the haloperidol induced catalepsy at aldoses tested on both the experimental days. Hence the results were indicated the ineffectiveness of EHS and PHS on dopaminergic transmission. (Table 5.28 & 5.29).

Table 1.12: Effect of ethanolic extract ofH. spicatum on haloperidol induced catalepsy.

Duration of catalepsy (Second) (Mean ± SEM)				
2"" Day				
Time	**Control(10ml/kg)**	**EHS 30**	**EHS 100**	**EHS 300**
0 min	3.58±0.45	3.28±0.56	3.45±0.35	3.92±0.346
15 min	25.63±2.94	21.06±2.84	22.46±1.16	17.29±2.36
30 min	52.29±5.42	51.98±3.59	53.97±4.14	49.41±4.50
60 min	167.87±5.66	167.90±7.77	169.07±6.69	159.09±5.80
90 min	210.13±4.14	207.85±6.62	232.03±15.71	249.31±16.57
120 min	184.44± 11.28	172.33±14.05	188.16±18.37	174.03±11.54
Day 7				
0 min	3.57±0.62	5.23±0.68	5.31±0.54	3.88±0.73
15 min	23.80±2.14	23.73±2.35	22.06±1.22	22.41±2.64
30 min	57.29±5.42	50.64±3.59	52.52±4.14	58.54±4.50
60 min	149.65±5.25	169.41±5.22	157.30±6.66	168.48±6.65
90 min	211.74±10.02	211.40±9.81	213.29±20.51	225.36±14.41
120 min	209.90±18.07	171.93±26.71	186.36±12.67	181.59±17.7

Table 1.13: Effect of petroleum ether extract ofH. spicatum on haloperidol induced Catalepsy.

Duration of catalepsy (Second) (Mean ± SEM)				
2"" Day				
Time	**Control(10ml/kg)**	**PHS30**	**PHS 100**	**PHS 300**
0 min	(10 ml/kg)	3.79±0.56	4.77±0.35	3.92±0.35
15 min	3.58±0.45	21.85±3.04	28.72±2.31	39.35±7.44
30 min	25.63±2.94	58.77±6.72	59.65±11.06	51.60±9.98
60 min	52.29±5.42	166.50±5.65	166.47±6.75	166.47±5.49
90 min	167.87±5.66	183.37±21.05	214.18±11.28	249.31±17.31
120 min	210.13±4.14	167.11±9.55	181.00±6.6	172.41±12

Day 7				
0 min	3.57±0.62	4.11± 0.89	4.51±0.86	4.29±0.74
15 min	23.80±2.14	26.38±2.35	31.43±5.92	42.31±6.16
30 min	57.29±5.42	54.40±5.42	44.13±4.81	60.83±6.05
60 min	149.65±5.25	167.99±5.22	162.78±5.46	166.85±8.48
90 min	211.74±10.02	222.53±13.04	230.30±9.92	238.91±6.82
120 min	209.90±18.0	169.55±9.9	198.36±9.89	189.81±14.22

1.11 Maximal electroshock induced seizures (MES)

The mean duration of hind limb extension in vehicle treated control group was 17.85±0.85 and 17.98±0.54 seconds on 2"" and 7* day of the treatment, respectively. On both the experimental days the duration of hind limb extension produced by maximum electroshock was significantly reduced after treatment with EHS (30 - 300 mg/kg), however it could not abolish the hind limb extension on both theexperimental days. PHS (30 - 300 mg/kg) showed insignificant effects in this regard. Phenytoin 2mg/kg significantly showed disappearance of the hind leg extensor tonic convulsion produced by maximum electroshock on both the experimental days. (Table 5.30 & 5.31)

Table 5.14: Effect of ethanolic and petroleum ether extract ofH. indicus on maximal electroshock induced seizures.

Group	Mice convulsed/Mice used
Control	6/6
EHS 30	6/6
EHS 100	6/6
EHS 300	6/6
PHS 30	6/6
PHS 100	6/6
PHS 300	6/6

Table 5.15: Effect of ethanolic and petroleum ether extract of H. spicatum duration of hind limb extension in MES test.

Treatments (mg/kg	2"" Day	7*" Day
Control (10 ml/lig)	17.85±0.85	17.98±0.54
EHI 30	15.54±1.19*	12.55±0.91**
EHI 100	13.89±0.94**	10.80±0.51**
Em 30	12.39±1.00**	10.21±0.51**
PHI30	16.85±1.39	18.48±0.69
PHI 100	17.89±1.18	17.13±0.51
PHI300	17.37±1.33	18.96±0. 1.24
Diazepam	0	Q

1.12 Sodium nitrite induced respiratory arrest

The mean onset of death in vehicle treated control group was 17.35±0.40 minutes and 16.39±0.72 minutes on 2"" and 7* day of the treatment respectively. On both the experimental days both EHI (30 - 300 mg/kg) and PHI (30 - 300 mg/kg) was insignificantly affected the time of onset of death due to sodium nitrite induced respiratory arrest at all the doses tested. (Table 5.32)

Table 1/16: Effect of ethanolic and petroleum ether extract of H spicatum on sodium nitrite induced respiratory arrest.

Treatments (mg/kg)	Onset of death (minute(Mean ± SEM)	
	2"" Day	7*" Day
Control (10 ml/lig)	17.35±0.40	16.3910.72
EHI 30	16.08± 0.39	16.891 0.71
EHI 100	18.06± 0.49	17.521 0.65
Em 30	19.51± 0.65	19.7410.35
PHI30	17.4710.69	17.2511.14
PHI 100	19.31± 1.21	18.931 0.93
PHI300	17.86+0.	18.1511.1
Diazepam	17.35±0.40	16.3910.72