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Research Article

EVALUATION OF NEUROPHARMACOLOGICAL ACTIVITY OF HYDROALCOHOLIC EXTRACT OF FRUITS OF TRAPA BISPINOSA IN LABORATORY ANIMALS

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ABSTRACT

The present investigation deals with evaluation of neuropharmacological activity of different doses (100, 250, 500mg/kg, p.o.) of hydroalcoholic extract of Trapa bispinosa (TB) in laboratory animals. The effects of extract on various parameters like motor coordination, spontaneous locomotor activity, object recognition, transfer latency, anxiolytic activity, analgesic activity, sodium nitrite induced respiratory arrest and hypoxic stress etc was studied. The TB (250 & 500mg/kg) found to decrease time required to occupy the central platform (transfer latency) in the elevated plus maze and to increase discrimination index in the object recognition test, indicating nootropic activity. TB (250 and 500mg/kg) showed significant increase in reaction time in hot plate analgesic activity. Moreover it also showed significant reduction in spontaneous locomotor activity and latency to death in sodium nitrite induced respiratory arrest. To conclude hydroalcoholic extract of TB possesses significant facilitation of learning and memory which may be due to enhanced cholinergic function. TB also showed significant analgesic activity.

Key words: Trapa bispinosa, Nootropic, Analgesic.

INTRODUCTION

Medicinal herbs constitute the cornerstone of traditional medicinal practice worldwide. These herbs are relatively cheap and available. These medicinal plants represent a great deal of untapped reservoir of drugs and the structural diversity of their component molecule makes a valuable source of novel lead compounds[1]

Trapa bispinosa (TB) is a floating aquatic herb. The acrid juice is used for diarrhea and dysentery. The fruits are used as intestinal astringent, aphrodisiac, anti-inflammatory, and in leprosy, urinary discharges, fractures, sore throat, bronchitis, and anemia. TB fruit is claimed nervine tonic and useful in nervous debility[2].

There has been no previous study on the Neuropharmacological effects of TB and this is the first study to examine the effects of TB experimentally for central nervous system. In the present study the effects of hydroalcoholic extract of TB on various neurobehavioral parameters like motor coordination, spontaneous locomotor activity, object recognition, transfer latency, time spent in open and closed arms and reaction time etc was studied.

MATERIALS AND METHODS

Plant material

The plant material collected from 'Maihar' region of Madhya Pradesh, India. Plant was authenticated by Botanical survey of India (Voucher specimen number-BSI/WC/Tech/2008-976). The hydroalcoholic extract of dried fruit was prepared by Green Chem. Herbals, Bangalore, India using following procedure

Preparation of extract

Fruits were extracted with 50% aqueous alcohol and concentrated. The concentrated mass was washed with petroleum ether several times to remove the resinous matter. Then the mass was diluted with 25% aqueous alcohol, filtered and concentrated, dried to get the powdered form of the extract.

Animals

Swiss male albino mice (18-22g) and male wistar rat (180-225g) were used. They were maintained at 25 + 2° C and relative humidity of 45 to 55% and under standard environmental conditions (12 hours light 12 hours dark cycle). The animals had free access to food and water ad libitum. Institutional Animal Ethical Committee approved the protocol (Approval number- CPCSEA/IAEC/PC-04/09-2K7). All experiments were carried out between 12:00- 16:00 hours.

Chemicals and drugs

Sodium nitrate was purchased from Loba chemicals, Mumbai. Diazepam injection, pentazocin injection, piracetam syrup, was purchased from the local market.

Acute toxicity test

Healthy adult male albino mice (18- 22g) were subjected to acute toxicity studies as per guidelines (AOT 425) suggested by the organization for economic co-operation and development (OECD-2001). The mice were observed continuously for 2 hours for behavioral and autonomic profiles and for any sign of toxicity or mortality up to a period of seven days[3].

Effect on motor coordination

The motor coordination was assessed 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. Thirty min interval was kept between two trails. Only those mice which have demonstrated their ability to remain on the rotating rod for at least 2 min were selected. These 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 of time followed by TB 100, 200 and 500 mg/kg, p.o. One hour following the administration of vehicle or drug, mice were 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 i.p.) was used as a reference standard[4-5].

Locomotor Activity

The locomotor activity (horizontal activity) was measured 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 and treated with TB 100, 200 and 500 mg/kg,p.o. 60 min after dosing; the mice were placed again in the actophotometer for recording the activity score as described earlier. The results were reported as mean change in the locomotor activity. Diazepam (2 mg/ kg/i.p.) preparation was used as reference standard[6].

Object recognition test

The apparatus fabricated locally consisted of white colored plywood (70 × 60 ×30 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 colored black.

One day before the test, mice were allowed to explore the box without any object for 02 min. On the day of test, in the first trial (T1) conducted 60 min after administration of vehicle (10 ml/kg) or TB (100, 250 and 500 mg/kg) or piracetam (150 mg/kg) two identical objects were presented in opposite corners 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 first (T1) and a new object replaced one of the objects presented in T1 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 discrimination index (D) was calculated as (N-F)/ (N+F). The object was changed randomly and apparatus was cleaned with hydrogen peroxide after each trial to avoid place preference and the influence of olfactory stimuli respectively[5].

Transfer Latency using Elevated plus maze test

Locally fabricated elevated plus maze consisting of two open arms (35 × 6 cm) and two enclosed arms (35 × 6 × 15 cm) was used. The maze was elevated to the height of 40 cm. Mice were placed individually at the end of an open arm facing away from the central platform and the time it took to move from the end of open arm to either of the closed arms (transfer latency, TL) was recorded. On the 1st day, mice were allowed to explore the plus maze after the measurement of TL. On the 2nd and 9th day, mice were placed again on the elevated plus maze as before and TL was noted again[5].

Analgesic activity

The analgesic effect was studied using digital hot plate (Columbus-USA) method wherein the reaction time (paw licking, jumping or any other sign of discomfort) was recorded at 0, 60, and 120 min after administration of vehicle (10 ml/ kg) or TB extract (100, 250 and 500 mg/kg). The temperature of the plate was maintained at 55°C ± 01° C. A cut off reaction time of 30 s was chosen in order to avoid injury. Pentazocin (30 mg/kg) was used as a reference standard[4].

Anxiolytic activity using elevated plus maze (EPM)

Locally fabricated elevated plus maze consisting of two open arms (35 × 6 cm) and two enclosed arms (35 × 6 × 15 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 05 min on the open and enclosed arm was recorded. The animals received vehicle (10 ml/ kg) or TB (100, 250 and 500 mg/kg) 60 min before and diazepam (1 mg /kg i.p.) 30 min before their placement on the maze. Increased exploratory activity in the open arm was taken as an indication of anxiolytic activity[7-8].

Sodium nitrite induced respiratory arrest

Mice were divided into four groups and were treated with vehicle (10 ml/kg) or TB (100, 250, 500mg/kg). Sixty min later, all mice were subjected to sodium nitrite treatment (250 mg/kg,i.p). The time between injection of sodium nitrite and death was recorded[9].

Hypoxic stress induced neurotoxicity in mice

Mice were subjected to hypoxia by putting them individually in a tightly closed 300ml glass container which was placed in aquarium of 25^{0}C temperature. The animals had convulsion and died from hypoxia. The latency for death was recorded. Mice received single dose of TB 100,250 and 500 mg/kg.

STATISTICAL ANALYSIS

The results are expressed as mean ± SEM. Comparison between the groups was made by one way analysis of variance (ANOVA) followed by Dunnett's test.

RESULTS

Acute oral toxicity test

All mice were free of any toxicity up to the dose of 2 gm/kg however sedation was noted above the dose of 1 gm/kg. From this data, three different doses 100, 250 and 500 mg/kg were selected for further study.

Effect on motor coordination:

All doses of TB (100, 250 and 500 mg/kg) were found to be statistically insignificant in reducing the fall off time. Diazepam (2 mg/kg) showed highest reduction in fall off time in both the experiments (P<0.01).

Locomotor Activity

TB in a dose of 100 mg/kg did not produce any significant effect in mean change in locomotor activity (9.6+1.23) as compared to control (10.32 + 1.05). However next two doses i.e. (250 and 500 mg/kg) produced significant (P<0.05) reduction (16.84+1.58; 17.67+1.52) respectively in locomotor activity.

Object recognition test

TB in a dose of 100 mg/kg did not produce any significant change in discrimination index. TB (250, 500mg/kg) treated mice showed significant increase in discrimination index (P<0.01) when compared against vehicle treated mice. TB 100 mg/kg was less significant in this regard. Piracetam (150 mg/kg) was also significant (P<0.01) in both the tests.

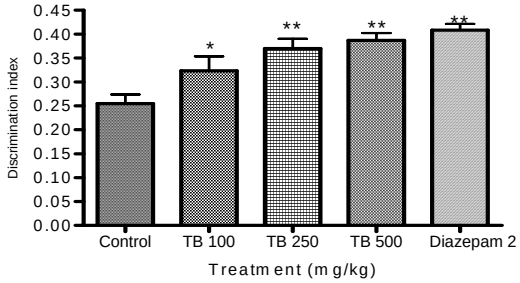

Fig. 1: Effect of TB extract and piracetam on discrimination index in object recognition test in mice.

Results are expressed as mean ± SEM. (n = 6). Data was analysed by one way analysis of variance (ANOVA) followed by Dunnetts test. *P<0.05, **P<0.01.

Analgesic activity

TB (100mg/kg) did not show significant change in analgesic activity. TB (250, 500 mg/kg) significantly (P<0.05) delayed the reaction time and thereby showed analgesic activity. Pentazocin significantly (P< 0.01) delayed the reaction time (Table 1).

Table 1: Effect of TB extract and pentazocin on analgesia induced by hot plate in mice

Treatment (mg/kg)	Mean reaction time (second)		
	0 minute	60 minute	120 minute
Control	7.28 ± 0.24	7.34 ± 0.29	6.65 ± 0.22
TB 100 mg/kg	7.62 ± 0.19	7.9 ± 0.17	7.61 ± 0.16
TB 250 mg/kg	7.42 ± 0.31	8.42 ± 0.33*	7.82 ± 0.19
TB 500 mg/kg	7.28 ± 0.20	8.49 ± 0.34*	8.43 ± 0.14*
Pentazocin 30	7.33 ± 0.21	12.31 ± 0.24**	12.00±0.27**

Results are expressed as mean ± SEM. (n = 6). Data was analysed by one way analysis of variance (ANOVA) followed by Dunnetts test. *P<0.05, **P<0.01

Elevated plus maze (EPM)

None of the dose of TB (100,250,500 mg/kg) showed significant change in either the time spent in open arm or enclosed arm when placed on EPM. Diazepam (1 mg/kg) significantly (P<0.01) increased time spent in open arm thereby showed and anxiolytic action.

Sodium nitrite induced respiratory arrest

TB (250,500 mg/kg) significantly (P<0.01) delayed the onset of respiratory arrest due to sodium nitrite compared to the control mice. The TB 100 mg/kg was also significant (P<0.05) in this regard (Figure 2).

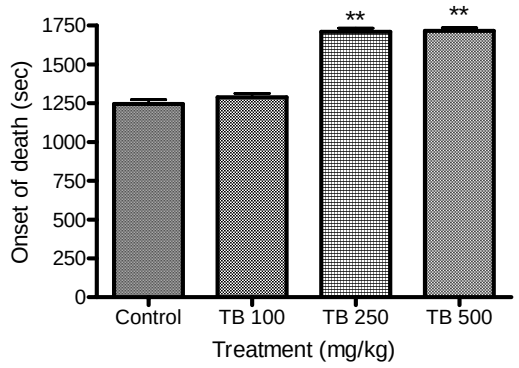

Fig. 2: Effect of TB on the onset of respiratory arrest induced by sodium nitrite in mice.

Results are expressed as mean ± SEM. (n = 6). Data was analysed by one way analysis of variance (ANOVA) followed by Dunnetts test. *P<0.05, **P<0.01.

Transfer latency using EPM

On 2nd day the TL was significantly (P<0.01) reduced in mice pretreated with TB 500 mg/kg and Piracetam 150 mg/kg when compared to those of control mice. The TL in mice pretreated with TB 100 and 250 mg/kg was insignificant. On the 9th day, in mice pretreated with TB 250, 500 mg/kg and Piracetam 150 mg/kg TL was significantly (P<0.01) reduced , While the TL in mice pretreated with TB 100 mg/kg was insignificant (Figure 3).

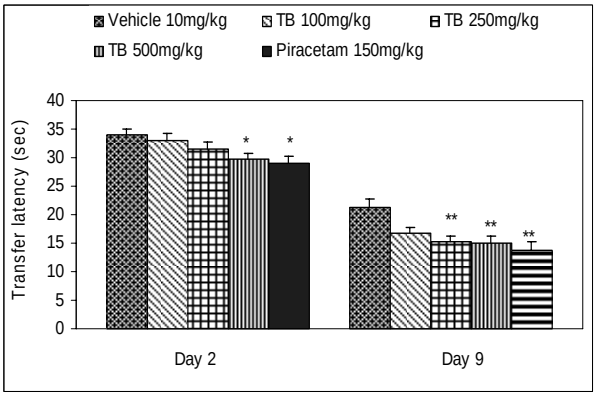

Fig. 3: Effect of TB and piracetam on TL (second) on 2nd and 9th day in mice using EPM.

Results are expressed as mean ± SEM. (n = 6). Data was analysed by one way analysis of variance (ANOVA) followed by Dunnetts test. *P<0.05, **P<0.01.

Hypoxic stress induced neurotoxicity in mice

TB 250 and 500 mg/kg significantly (P<0.05, P<0.01) prolonged the latencies for death following hypoxic stress. The TB 250 mg/kg was found to be less effective (P<0.05) than TB 500 mg/kg (P<0.01). TB 100 mg/kg was found to be insignificant to prolonged latencies for death.

DISCUSSION

It has been well reported that most of the drugs acting on CNS may lead to neurotoxicity especially when administered for prolong period and neuroprotective drugs usually recommended for chronic use. The neurotoxicity of any compound is easily reflected as muscular incordination and hence rota rod test is usually recommended[11]. In this study all doses of TB, (100,250,500 mg/kg) did not reduced the fall off time in rota rod model and thereby ruled out possible neurotoxicity.

TB 250 and 500 mg/kg possess significant (P<0.05) analgesic activity in hot plate analgesia. However, the mechanism of this action has not been investigated here. It is not known whether this action is opioid-like in nature or involves acetylcholine or other agents[12].

In our investigation, the extracts did not produce any significant change in the exploratory activity of the mice in the EPM model. Anxiolytic compounds, by decreasing anxiety, increase the open arm exploration time as well as the number of entries into the open arm. The extracts of the plant failed to demonstrate any such effect in the rats and hence we can conclude TB does not possess anxiolytic activity[13].

An important point to be noted is that recently the plus maze model is also being used to study learning and memory processes in rodents. With respect to our findings, in contrast to that of diazepam, the extracts did not cause an increase in the number of entries into the open arm. It could thus also be inferred that the rats retain the memory of the aversive quality of the open arm and this could probably be considered a significant finding with respect to the plant extracts[14].

In EPM TB 500 mg/kg on 2nd day was significantly (P<0.01) reduced the TL. On the 9th day mice pretreated with TB 250, 500 mg/kg and was significantly (P<0.01) reduced the TL. The significant (P<0.01) improvement in discrimination index by the two doses (250 and 500 mg/kg) of extract proved that TB met major criteria for nootropic activity, improvement of memory in absence of cognitive deficit[15]. This observation has been strengthened by the finding that TB has shortened the transfer latency in the elevated plus maze model indicating improvement in memory, which is in accordance with the hypothesis of Itoh *et al*[16]. TB (250 and 500 mg/kg) delayed the death due to sodium nitrite induced respiratory arrest. In this case, respiratory arrest is caused due to chemical hypoxia and thereby reduces oxygen carrying capacity. The drug that delay or abolish this arrest probably act by improved cholinergic transmission[9]. The neurological basis of learning and memory established the role of cholinergic system[17-18].

Hypoxia induced neurodegeneration is one of the prime pathological states in clinical practice. In modern life incidence of hypoxic stress are increasing day by day. These factors leave lasting imprints on cognitive behavior via induction of convulsion. In rare case it may result in death too. The major mechanism postulated for the hypoxic stress is increased levels of serotonin level. The drug that increases adenosine level in brain has shown to prolong aforementioned convulsion[10]. Antihypoxic effect of TB makes it a suitable candidate for treatment of stroke.

CONCLUSION

The results suggest that the hydroalcoholic extract of *Trapa bispinosa* possesses significant facilitation of learning and memory, antihypoxic and analgesic activity without affecting motor coordination. There by validating its claim as a nervine tonic in the Indian system of medicine.

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