NEUROPHARMACOLOGICAL PROFILE OF *TRICHOLEPIS GLABERRIMA* EXTRACT IN MICE

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Summary

The present investigation deals with the neuropharmacological investigations of different doses (100,300 mg/kg.) of methanol, chloroform, aqueous extract of aerial parts of *Tricholepis glaberrima.* The acute administration of extract reported increase in discrimination index in object recognition test, muscle relaxation, increase in reaction time in analgesic activity, potentiation of haloperidol induced catalepsy, The result points towards the potential activity of the *Tricholepis glaberrima extract* as a nootropic and increase in the dopaminergic transmission by the extracts.

Keywords: *Tricholepis glaberrima,* neuropharmacological study, analgesic activity,

Introduction

Neurological disorders constitute as much as 35% of the disease burden and over 1.5 billion people worldwide suffer from CNS diseases or disorders. Mood and anxiety disorders are the most common mental illnesses, each affecting up to 10% of the general population (1). The epilepsies are common and frequently devastating disorders, affecting approximately 2.5 million people in the United States alone (2). Many of the individuals with severe clinical depression display the suicidal behavior (3). The major disorders of mood include the syndromes of major depression and bipolar disorder. 3)These disorders commonly include distended autonomic functioning (e.g., altered rhythms of activity, sleep, and appetite) and behavior, as well as persistent abnormalities of mood. The psychoses are among the most severe psychiatric disorders in which there is a marked impairment of behavior, serious inability to think coherently and to comprehend reality. These common disorders typically include symptoms of false beliefs (delusions) and abnormal sensations (hallucinations) (4).

Tricholepis glaberrima DC (Asteraceae), commonly known as "Brahmadandi" is an important Medicinal plant used in our Traditional System of Medicine to treat various diseases. *Tricholepis glaberrima* is used in Ayurveda for nervine tonic, aphrodisiac, skin disease and in cough. It is used because of the broad area of biological activities like anti-inflammatory, urinary troubles; antiseptic activities (5), (6), (7).The plant is rich in many pharmaceutical active ingredients like flavonoids, triterpenoids, saponin glycosides and sterols (8), (9).

Materials and methods

Plant material

The plant *Tricholepis glaberrima* DC was collected from the Amravati region in month of Aug. 2008. Plant species identified and confirmed their authentification by Botanist Dr.Prabha Bhogaonkar, Vidarbh institute of science and humanities, (V.M.V), Amravati. A herbarium was prepared, authentified and deposited at Dept. of Botany, Vidarbh institute of science and humanities, (V.M.V), Amravati.

Preparation of extracts

The fresh aerial parts of *Tricholepis glaberrima* DC were dried under shade & powder in a mixture grinder. Powdered material first defatted by petroleum ether. Marc then extracted with chloroform and then by methanol for 6-8 hours with soxhlet extractor. Then each methanol and chloroform filtrate was concentrated separately on water bath to a thick paste & dried under vacuum. The aqueous extract was prepared by maceration process by treating 100g of fresh powder with 500ml of distilled water along with 10ml of chloroform as a preservative .The maceration process was carried for 7 days with occasional stirring. Both the extracts were filtered / condensed and evaporated to dryness under vacuum. This chloroform (CHE) methanoholic (MHE) and Aqueous (AQE) extract further use for study.

Chemicals and drugs

Haloperidols, sodium nitrate, were purchased from. Loba chemicals, Mumbai. Diazepam and pentazocin injection, piracetam suspension, Phenytoin tablets were purchased from the local market.

Preparation of drug solution

Accurately weighed quantity of powdered extract was dissolved in the distilled water to prepare the appropriate stock solution of the drug i.e. 100mg/ml and 300mg/ml respectively. The doses were administered orally by selecting the appropriate concentration of the stock solution. Phenytoin was suspended in 1% w/v acacia.

Animals

Swiss male albino mice (18-22g) were used. They were maintained at 25 + 2° C and relative humidity of 45 to 55% and under standard environmental conditions (12 h light 12 h dark cycle). The animals had free access to food (Chakan Oil Mills, Pune, India) and water ad libitum. Institutional Animal Ethical Committee approved the protocol. All experiments were carried out between 12:00- 16:00 h.

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 h for behavioral and autonomic profiles and for any sign of toxicity or mortality up to a period of seven days.(10)

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. 30 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 respective drug treatment. 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 (11), (12).

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 test drugs. 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 (13).

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 extract (100 and 300 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 (14).

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) were 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 PB (100, 200 and 400 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 (15), (16)

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 PB (100,200,400 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 (17).

Double unit Mirrored chamber

The mirrored chamber apparatus fabricated locally consisted of a mirrored cube (30× 30× 30cm) open on one side and placed in square box. The container box (40×40×30 cm) has 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 mirror chamber. The latency to enter the mirror chamber and time spent in mirror chamber during 5 min observation period was recorded 60 min after the drug administration. Diazepam (1mg/kg/i p) was used as a reference standard. These mice were not exposed to the apparatus before the test and evaluated only once to avoid habituation problem. The apparatus was washed after each evaluation to eliminate potential cues such excreta, urine left by the previous occupant (18), (19).

Haloperidol induced catalepsy

Mice were divided into four groups. The control group received vehicle (10 ml/kg p.o.) whereas the other group received PB (100 and 300 mg/kg) 60 min before haloperidol (1 mg/kg i.p). After the treatment, the forepaws of the mice were placed on rod of 0.9 cm diameter set at 2.5 cm from top. Duration for which the mice retains the forepaws on the elevated rod was noted down at 0, 15, 30, 60, 90 and120 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 (20), (21).

Sodium nitrite induced respiratory arrest

Mice were divided into four groups and were treated with vehicle (10 ml/kg) or CHE, MHE and AQE (100,300mg/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 (22).

Maximal electroshock induced seizures (MES).

Tonic clonic convulsions were induced 60 min after the respective drug treatment by giving maximal electroshock seizures (MES) (40mA for 0.2sec) using an electroconvulsiometer (INCO, Ambala, India) via crocodile ear clip 60 min after

administration of either vehicle (10 ml/kg), PB (100 and 300 mg/kg) or Phenytoin (20 mg/kg). 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 (23), (24).

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 1gm/kg. From this data, three different doses 100,300 mg/kg were selected for further study.

Effect of motor- co-ordination

Reduction in the mean change in fall off time was reported with CHE100 (113.09± 13.681**) and 300 (35.61± 1.742**) compared to that of vehicle treated mice (217.6± 12.258). The AQE dose (100 and 300 mg/kg) did not cause any significant change. Results are given in figure 1.

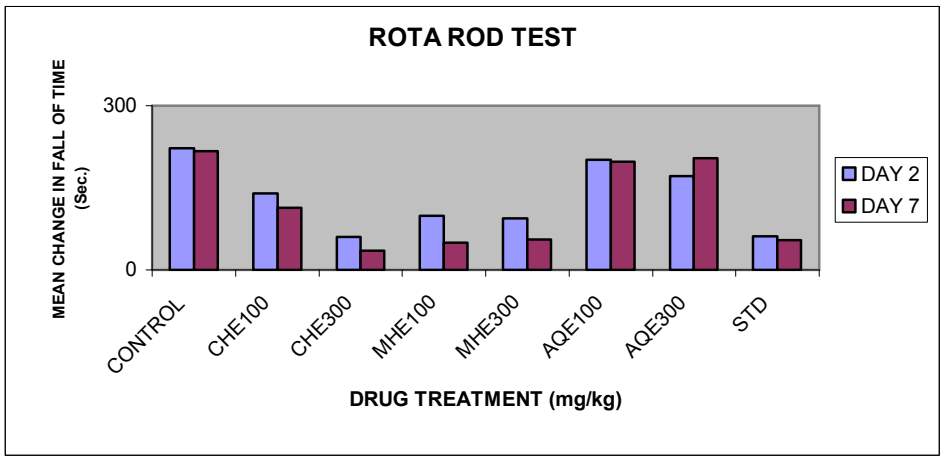

Figure 1: Effect of extract on the motor performance in mice.
n=6 Data was analyzed by one-way ANOVA followed by Dunnett's *P<0.05, **P<0.01, ***P< 0.001

Effect of Locomotor activity

CHE, MHE and AQE in a dose of 100 mg/kg and 300 mg/kg did not produce any significant change in locomotor activity as compared to control. Results are elaborated in Table 1.

Treatment(mg/kg)	**Basal Activity Score (Mean ± SEM)**
Control	386.2 ± 41.00
CHE100	292.2 ± 59.81
CHE 300	259.0 ± 11.74
MHE 100	265.0 ± 44.59
MHE 300	260.4 ± 17.90
AQE 100	298.4± 64.69
AQE 300	337.2± 42.27
Diazepam 2	60.8± 5.56**

Table 1: Effect of extract on mean change in locomotor activity in mice.
n=6 Data was analyzed by one-way ANOVA followed by Dunnett's *P<0.05, **P<0.01, ***P< 0.001

Analgesic activity

There was significant increase in reaction time in mice treated with different doses of CHE, MHE and AQE at 60 and 120 min time interval when compared against vehicle treated mice. Pentazocin was found to be effective in this regard. Effects of extract on mean change in reaction time in analgesic activity are shown in table 2.

Time	**Mean reaction time in second**							
	Control	**CHE 100**	**CHE 300**	**MHE 100**	**MHE 300**	**AQE 100**	**AQE 300**	**Pentazocin 30**
0 Min	7.54± 0.445	10.4± 0.4087	10.36± 2.140	7.68± 1.489	8.5± 1.722	9.42± 0.895	8.6± 0.988	9.48± 0.743
60 Min	7.65±0.3881	12.7± 0.517**	12.82± 0.468**	8.12± 0.697	8.14± 0.627	9.86± 0.537*	11.08± 0.527**	12.9± 0.545**
120 Min	6.82±0.193	9.5± 0.900**	10.36± 0.716**	7.1± 0.393	7.52± 0.351	10.38± 0.570**	10.6± 0.361**	12.34± 0.413**

Table 2: Effect of extract on mean change in reaction time in analgesic activity.
n=6 Data was analyzed by one-way ANOVA followed by Dunnett's *P<0.05, **P<0.01, ***P< 0.001

Elevated plus maze
The CHE, MHE and AQE treatment did not show any significant effect on the time spent in open or enclosed arm when placed on EPM. Diazepam significantly increased time spent in open arm and thereby showed anxiolytic action. (Data not shown).

Object recognition test
Increase in the discrimination index indicates nootropic activity. The mean discrimination index of the extract treated group of animals was found to be significantly greater than the vehicle treated group of animals which indicates significant nootropic activity. Both CHE (100 and 300 mg/kg) and piracetam significantly improved the discrimination index which shown in figure 2.

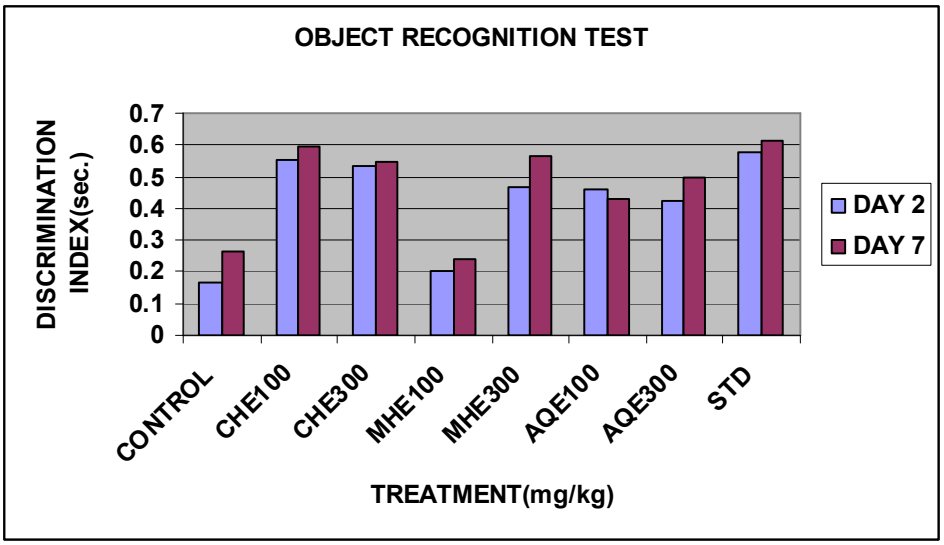

Figure 2: Effect of extract on the discrimination index in object recognition test mice. n=6 Data was analyzed by one-way ANOVA followed by Dunnett's *P<0.05, **P<0.01, ***P< 0.001

Double unit mirrored chamber
Pre treatment with different doses of CHE,MHE and AQE did not affect latency to first entry or time spent in mirror chamber when compared to vehicle treated mice; Diazepam, a reference standard showed significant anxiolytic effect. (Data not shown).

Haloperidol induced catalepsy
Maximum catalepsy was observed between 60 and 120 min (Table 3) and the extracts potentiated the catalepsy during 15 to 60 min. indicating increase in the dopaminergic transmission by the extracts.

Time	Mean duration of catalepsy						
	control	CHE 100	CHE 300	MHE 100	MHE 300	AQE 100	AQE 300
0 min	3.57±0.620	4.352± 0.816	3.73± 0.903	3.476± 1.074	3.812± 0.816	3.434± 0.826	3.478± 0.361
15min	23.8±2.129	24.49± 4.827	45.05± 6.32	24.49± 3.152	25.90± 4.293	25.90± 4.293	50.45± 7.815**
30min	57.29±4.903	79.12± 3.563*	99.76± 5.315**	43.49± 4.694	58.29± 6.366	95.45± 5.294**	78.87± 6.358*
60min	167.86±5.633	175.35± 6.965	173.12± 4.728	193.52± 7.461*	193.60± 5.396*	159.19± 7.471	165.15± 15.65
90min	211.73±10.016	195.1± 9.555	202.51± 16.157	210.18± 15.246	210.59± 14.341	213.81± 21.482	223.04± 23.593
120min	209.89±18.068	195.34± 30.346	203.98± 21.164	211.462± 14.830	199.61± 20.802	170.87± 17.358	222.57± 22.622

Table 3: Effect of extract on duration of haloperidol- induced catalepsy in mice.
n=6 Data was analyzed by one-way ANOVA followed by Dunnett's *P<0.05, **P<0.01, ***P< 0.001

Sodium nitrite induced respiratory arrest
Sodium nitrite is known to convert hemoglobin into methemoglobin, thereby reducing oxygen-carrying capacity and cholinergic transmission and ultimately leading to death. Extracts failed to decrease the effect of sodium nitrite. This indicated that extracts did not increase the cholinergic transmission in the CNS. (Data not shown).

Maximal electroshock induced seizures (MES).
Decrease in the duration of hind limb extension by the extract indicates anticonvulsant activity. The extracts did not significantly decrease the duration of hind limb extension in the extract treated animals when compared to that of the vehicle treated animals. This reveals that the extracts do not posses anticonvulsant activity. (Data not shown).

Discussion
Tricholepis glaberrima is claimed to be used as a nervine tonic, in Indian traditional system of medicine yet not documented scientifically in this regard. Despite extensive research, the neurological basis of learning and memory remains controversial. (25).

In the present investigation, CHE, MHE and AQE extract exhibited increase in discrimination index, muscle relaxation, increase in reaction time in analgesic activity and potentiation in haloperidol induced catalepsy. The effect on time spent in open arm and mirror chamber in elevated plus maze was not significant.

The improvement in discrimination index by the two doses (100 and 300 mg/kg) of extract proved major criteria of nootropic activity, improvement in memory in absence of cognitive deficit (26). Haloperidol induced catalepsy appears to be due to blockade of dopamine (DA) transmission(27),28),29).The depletion of brain DA content after

piracetam (30)) and oil of celastrus paniculatus administration,(31)which possess nootropic activity has also been documented. The extract in doses of 100 and 300 mg/kg has significantly potantiated haloperidol- induced catalepsy between time intervals of 15-60 min.

The extract did not produce any significant anxiolytic activity when tested on EPM and double unit mirror chamber too. Generally most of the anxiolytic agents have an adverse effect on memory as seen with the benzodiazepines, commonly used anxiolytic (32). Also the extracts significantly increase in reaction time in mice treated with different doses of CHE, MHE and AQE at 60 and 120 min time interval when compared against vehicle treated mice. CHE extract shows better analgesic activity than other extract.

Extract did not show anticonvulsant effect in MES model, also, the extracts failed to decrease the effect of sodium nitrite. This indicated that extracts did not increase the cholinergic transmission in the CNS.

Conclusion

It is thus apparent that different extract of *Tricholepis glaberrima* plant exhibited improvement in the discrimination index, potentiation of haloperidol induced catalepsy, and increase in reaction time in analgesic activity and muscle relaxant activity. These results suggest possible facilitation of dopaminergic transmission by the extracts which may be due to presence of phytoconstituents such as terpenoids and phenolic compounds (33).

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