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Clitoria ternatea and the CNS

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Abstract

The present investigation was aimed at determining the spectrum of activity of the methanolic extract of *Clitoria ternatea* (CT) on the CNS. The CT was studied for its effect on cognitive behavior, anxiety, depression, stress and convulsions induced by pentylenetetrazol (PTZ) and maximum electroshock (MES). To explain these effects, the effect of CT was also studied on behavior mediated by dopamine (DA), noradrenaline, serotonin and acetylcholine. The extract decreased time required to occupy the central platform (transfer latency, TL) in the elevated plus maze (EPM) and increased discrimination index in the object recognition test, indicating nootropic activity. The extract was more active in the object recognition test than in the EPM. The extract increased occupancy in the open arm of EPM by 160% and in the lit box of the light/dark exploration test by 157%, indicating its anxiolytic activity. It decreased the duration of immobility in tail suspension test (suggesting its antidepressant activity), reduced stress-induced ulcers and reduced the convulsing action of PTZ and MES. The extract exhibited tendency to reduce the intensity of behavior mediated via serotonin and acetylcholine. The effect on DA- and noradrenaline-mediated behavior was not significant. In conclusion, the extract was found to possess nootropic, anxiolytic, antidepressant, anticonvulsant and antistress activity. Further studies are necessary to isolate the active principle responsible for the activities and to understand its mode of action.

Keywords: *Clitoria ternatea*; Cognitive behavior; Anxiety; Depression; Stress; Convulsions

1. Introduction

Clitoria ternatea Linn (Family Fabaceae) is commonly known as ''Butterfly pea.'' The plant is a twining evergreen herb, which will grow up to 3 m (9 ft) high, climbing over any available prop. The stems are pubescent and spindly. The compound leaves are made of three to nine oval or elliptical leaflets. The flowers are 2–4 cm long and in various shades of blue with a yellow throat or pure white with a big standard petal. The fruits are pods, resembling thin peas. Native to the island of Ternate in the Molluca archipelago, this species is now widely grown as ornamental, fodder or medicinal plant. The roots and seeds have powerful laxative effects, the flowers are used to make collyrium and the leaves are used in Madagascar to relieve joint pain. The plant may start flowering 4 months after sowing. Roots, seeds and leaves of *C. ternatea* are commonly used in the Ayurvedic system of medicine. The roots are bitter, refrigerant, laxative, intellect promoting, diuretic, anthelmintic and tonic and are useful in dementia, hemicrania, burning sensation, leprosy, inflammation, leucoderma, bronchitis, asthma, pulmonary tuberculosis, ascites and fever. The leaves are useful in otalgia and hepatopathy, whereas seeds are cathartic (Anonymous, 1995). *C. ternatea* contains antifungal proteins and has been shown to be homologous to plant defensins (Osborn et al., 1995). Rai et al. (2001), using passive avoidance test and spatial learning T-maze, have shown that the aqueous root extract of *C. ternatea* enhances memory in rats. Taranalli and Cheeramkuczhi (2000) reported that the alcoholic extracts of aerial and root parts of *C. ternatea* at 300 and 500 mg/kg po doses in rats attenuated electroshock-induced amnesia. The extract at 300 mg/kg dose produced significant memory retention, and the root parts were found to be more effective. The authors suggested that *C. ternatea* extract increased rat brain acetylcholine content and acetylcholinesterase activity in a similar fashion to the standard cerebroprotective drug pyritinol. Because the other activities of *C. ternatea* have not been studied, we investigated the nootropic, anxiolytic, antistress and anticonvulsant activities using conventional animal models and also the effects on behavior mediated via dopamine (DA) (haloperidol-induced catalepsy), noradrenaline (clonidine-induced hypothermia), serotonin (lithium-induced head twitches) and acetylcholine (sodium nitrite-induced respiratory arrest).

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2. Materials and methods

2.1. Extraction

The aerial parts of *C. ternatea* were collected from garden. Dr. D.R. Mahajan, a botanist at the KTHM College, Nashik, identified the plant material, and the specimen was deposited at the Botanical Survey of India, Pune (Voucher Specimen BSI 163826). The plant material was shade dried. One kilogram of the plant material was defatted with petroleum ether (60–80 °C) and then extracted with methanol. The methanolic extract of *C. ternatea* (CT, 6.36% w/w) was concentrated under reduced pressure. The dried extract was dissolved in distilled water and administered orally. The volume of injection in mouse was 0.1 ml and rat was 0.5 ml.

2.2. Animals

Male albino mice (Swiss, 22–25 g) and rats (Wistar, 125–150 g) were housed in groups of five under standard laboratory conditions of temperature, humidity and lighting. Animals had free access to food and water, except during experiment. They were deprived of food but not water 6 h before the drug administration. Each group consisted of five animals. All experiments were carried out during the light period (1100–1300 h). The studies were carried out in accordance with the guidelines given by the Indian Council for Medical Research and the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi (India) and the Institutional Animal Ethical Committee approved the study.

2.3. Drugs

Piracetam (Uni-UCB, India), clonidine (German Remedies, India), diazepam injections (Sigma, India), haloperidol (Searle, India), pentylenetetrazol (PTZ, Sigma, USA) and lithium sulfate (Glenmark Laboratories, India) were used in this study. Solvents used in this study were of analytical grade. All drugs were dissolved in distilled water.

2.4. Assessment of nootropic activity

The nootropic activity of CT was screened by using elevated plus maze (EPM) and object recognition test.

2.4.1. EPM test

The EPM consisting of two open arms (35 × 6 cm) and two enclosed arms (35 × 6 × 15 cm) was elevated to the height of 25 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 to either of the closed arms (transfer latency, TL) was noted (Itoh et al., 1990). On the first day, mice ($n = 5$) were allowed to explore the maze for 5 min after the measurement of TL. On the following day, mice received vehicle, piracetam (50 mg/kg ip) 30 min before or CT (100 mg/kg po) 60 min before the test, and the TL was noted for each animal. The TL was also measured on the ninth day. The TL was expressed as inflexion ratio (IR) using the formula described earlier by Jaiswal and Bhattacharya (1992):

$$\mathrm{IR} = (L_1 - L_0)/L_0$$

where L_0 = TL after 24 h or on the ninth day and L_1 = initial TL (s).

2.4.2. Object recognition test

The apparatus consisted of white colored plywood (70 × 60 × 30 cm) with a grid floor that could be easily cleaned with hydrogen peroxide after each trial. The apparatus was illuminated by a 60 W lamp suspended 50 cm above the box. The object to be discriminated was also made of plywood in two different shapes of 8 cm height and colored black.

On the day before test, mice ($n = 5$) were allowed to explore the box (without any object) for 2 min. On the day of test in the first trial (T_1), two identical objects were presented in two opposite corners of the box, and the amount of 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. During the second trial (T_2, 90 min after T_1), a new object replaced one of the objects presented in T_1, and mice were left individually in the box for 5 min. The time spent for exploring the familiar (F) and the new object (N) was recorded separately, and discrimination index (D) was calculated as $(N - F)/(N + F)$. Care was taken to avoid place preference and the influence of olfactory stimuli by randomly changing the role (familiar or new object) and the position of the two objects during T_2 and cleaning the apparatus with hydrogen peroxide (Bartolini et al., 1996). The animals received vehicle or CT (100 mg/kg po) 60 min prior to the first trial, whereas piracetam (50 mg/kg ip) was given 30 min before the first trial.

2.5. Assessment of anxiolytic activity

Anxiolytic activity was assessed by using EPM and the light/dark exploration test.

2.5.1. Elevated plus maze

The EPM as described earlier by Lister (1987) was used in this study. 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 on the open and closed arms was recorded. Mice ($n = 5$) were treated with vehicle, CT (30, 100, 200 and 400 mg/kg po) 60 min before and diazepam (1 mg/kg ip) 30 min before their placement on the maze.

2.5.2. Light/dark exploration test

The apparatus consisted of two boxes (25 × 25 × 25 cm) joined together. One box was made dark by covering its top

with plywood and a 10 W lamp illuminated the other box. The light source was placed 25 cm above the open box. The mice were placed individually in the center of the lit box and observed for the next 5 min for the time spent in lit and dark boxes (Crawley and Goodwin, 1981). Mice ($n=5$) were treated with vehicle, CT (30, 100, 200 and 400 mg/kg po) 60 min before and diazepam (1 mg/kg ip) 30 min before their placement in the lit box.

2.6. Assessment of anticonvulsant activity

The anticonvulsant activity was assessed using PTZ- and maximum electroshock (MES)-induced seizures in mice. Mice were treated with CT 100 mg/kg, the dose that exhibited significant anxiolytic activity in the EPM test and light/dark exploration test.

2.6.1. PTZ-induced convulsions

Male mice were divided in groups of five each. The animals were pretreated with vehicle, CT (100 mg/kg po) 60 min before or diazepam (1 mg/kg ip) 30 min before the ED_{95} dose of PTZ (80 mg/kg sc). The latency to seizures and the number of animals surviving after 24 h were noted for each animal (Swinyard and Woodhead, 1982).

2.6.2. MES-induced seizures

Mice were divided in groups of five each. They were treated as described for the PTZ-induced seizures and an electric shock (42 mA, 0.2 s) was applied through the corneal electrode. The duration of hindlimb extension was recorded for each animal (Swinyard and Woodhead, 1982).

2.7. Assessment of antidepressant activity

2.7.1. Tail suspension test

The method described by Steru et al. (1985) was used. Mice were divided in groups of five each. They were suspended by tying a thread to their tail from a height of 50 cm above the table top. Duration of immobility was recorded for 6 min (after discarding activity in the first 2 min because animals try to escape during this period). Mice were considered immobile only when they hung passively and remain motionless. Mice were treated with vehicle, CT (100 and 400 mg/kg po) 60 min before the test and fluoxetine (10 mg/kg ip) 30 min before the test.

2.8. Assessment of antistress activity

2.8.1. Cold restraint stress (CRS)-induced ulcers:

The rats were divided into groups of five each and fasted for 18 h. Rats were treated with vehicle, CT (100, 200 and 400 mg/kg po) 60 min before and diazepam (1 mg/kg ip) 30 min before the test. Immediately after vehicle or drug administration, each rat was subjected to CRS by strapping the rats on a wooden plank and keeping them for 2 h at 4–6 °C. The stomach of each animal was cut longitudinally along the grater curvature and the severity of gastric ulcers was assessed in terms of mean ulcer index as described earlier by Alphine and Word (1969).

2.9. Lithium-induced head twitches

The rats were divided into groups of five each. Piracetam (50 mg/kg ip) was administered 30 min before and CT (100 mg/kg po) was administered 60 min before the injection of lithium sulfate (3 mEq/kg ip). The number of head twitches was observed for 60 min after the administration of lithium sulfate as described earlier by Wielosz and Kleinrok (1979).

2.10. Clonidine-induced hypothermia

Clonidine (0.1 mg/kg ip) was administered 60 min after vehicle or CT (100 mg/kg po) and 30 min after piracetam (50 mg/kg ip) to groups of five mice and rectal temperature was recorded at 0, 30, 60, 90 and 120 min as described earlier by Drew et al. (1977).

2.11. Sodium nitrite-induced respiratory arrest

Sodium nitrite (250 mg/kg ip) was used to induce chemical hypoxia. Sodium nitrite reduces the oxygen-carrying capacity of blood by converting hemoglobin to methemoglobin. This dose produces death due to respiratory arrest in vehicle-treated mice (Hock, 1993). Drugs increasing cholinergic transmission delay or prevent the onset of respiratory arrest. CT (100 mg/kg po) was given 60 min

Table 1
Effect of piracetam and CT on TL and IR in EPM

Treatment dose (mg/kg)	TL (mean ± S.E.M.) (s)			IR	
	Day 1	Day 2	Day 9	Day 2	Day 9
Vehicle (5)	41.8 ± 1.98	20.16 ± 1.35	13.16 ± 1.16	1.07 ± 0.96	2.17 ± 0.70
Piracetam (50)	77.66 ± 2.38*	12.33 ± 1.78*	9.50 ± 1.23*	5.29 ± 0.33**	7.17 ± 0.93**
CT (100)	47.9 ± 2.02	14.25 ± 1.60*	7.33 ± 1.42*	2.36 ± 0.116	5.5 ± 0.44***

$n=5$ mice.

* $P<.05$, compared with the vehicle-treated group (one-way ANOVA followed by Dunnett's test).

** $P=.003$, compared with vehicle-treated group (Student's t test).

*** $P=.004$, compared with vehicle-treated group (Student's t test).

Table 2
Effect of piracetam and CT on T_1 and T_2 sessions and discrimination index in object recognition test

Treatment (mg/kg)	T_1 (mean ± S.E.M.)	T_2 (mean ± S.E.M.)		$D=(N-F)/(N+F)$
		N	*F*	
Vehicle (5)	24.5 ± 2.34	17.28 ± 1.79	10.88 ± 1.69	0.24 ± 0.03
Piracetam (50)	10.0 ± 3.5	15.25 ± 1.29	7.0 ± 1.20#	0.37 ± 0.03**
CT (100)	18.0 ± 2.98	13.25 ± 1.90	3.75 ± 1.80#	0.55 ± 0.03*

$n = 5$ mice.
* $P < .0001$.
** $P = .015$, compared with the vehicle-treated group (Student's t test).
# $P = .02$ compared with the new object (Student's t test).

before and piracetam (50 mg/kg ip) was administered 30 min before sodium nitrite. The time between injection of sodium nitrite and death was recorded. Each group consisted of five mice.

2.12. Haloperidol-induced catalepsy

Rats divided into groups of five each received vehicle or CT (100 mg/kg po) and haloperidol (1 mg/kg ip). Catalepsy was scored using "Bar test" at 0, 5, 15, 30, 45, 60, 90, 120 and 150 min after haloperidol as described by Ferre et al. (1990).

2.13. Statistical analysis

The observations are given as means ± S.E.M. The parametric data were assessed by Student's t test or one-way analysis of variance (ANOVA) followed by Dunnett's test. For nonparametric data, Kruskal–Wallis ANOVA was followed by Dunnett's test. $P < .05$ was considered significant.

3. Results

3.1. Assessment of nootropic activity

3.1.1. Elevated plus maze

On the first day, the mice entered the central platform (TL) 41.8 ± 1.98 to 77.66 ± 2.38 s after their placement in the EPM. On the second and ninth days, the TL was significantly reduced in all groups. The animals treated with piracetam (50 mg/kg) required least time on both second and ninth days ($F_{2,12} = 431.18$, $P < .0001$) to enter the central platform. The IR increased after piracetam on the second and ninth days significantly ($P = .003$), whereas CT increased the IR significantly on ninth day only ($P = .004$). The observations are given in Table 1.

Table 3
Effect of CT on time spent in open/enclosed arm in EPM

Treatment (mg/kg)	Time spent (mean ± S.E.M.) (s)	
	Open arm	Enclosed arm
Vehicle (5)	55.5 ± 3.37	235.50 ± 5.42
CT (30)	61.5 ± 2.62	230.33 ± 6.17
CT (100)	88.5 ± 4.87 *	204.00 ± 6.17 *
CT (200)	121.75 ± 6.18 *	168.00 ± 6.89 *
CT (400)	132.75 ± 5.92 *	165.50 ± 5.39 *
Diazepam (1)	157.50 ± 6.27 *	114.25 ± 6.78 *

$n = 5$ mice.
* $P < .0001$ (ANOVA followed by Dunnett's test).

Table 4
Effect of CT on time spent in lit/dark box in light/dark exploration test

Treatment (mg/kg)	Time spent (mean ± S.E.M.) (s)	
	Lit box	Dark box
Vehicle (5)	69.75 ± 9.43	229.50 ± 9.34
CT (30)	89.25 ± 18.32	207.50 ± 17.59
CT (100)	110.5 ± 6.00 *	189.00 ± 6.01 *
CT (200)	131.75 ± 10.00 *	166.00 ± 10.10 *
CT (400)	143.50 ± 10.10 *	154.75 ± 9.78 *
Diazepam (1)	176.00 ± 6.62 *	123.00 ± 6.64 *

$n = 5$ mice.
* $P < .0001$ (ANOVA followed by Dunnett's test).

3.1.2. Object recognition test

The mice in the first trial required 10 ± 3.5 to 24.5 ± 2.34 s to explore the objects. In the second trial, when a new object replaced one of the objects, CT- and piracetam-treated mice required significantly less time to explore the familiar object as compared with the new object. Both CT and piracetam significantly reduced the discrimination index. The observations are given in Table 2.

3.2. Assessment of anxiolytic activity

3.2.1. Elevated plus maze

Vehicle-treated mice spent 55.5 ± 3.37 s in the open arm. The oral administration of CT (100–400 mg/kg) dose-dependently increased the time spent in the open arm

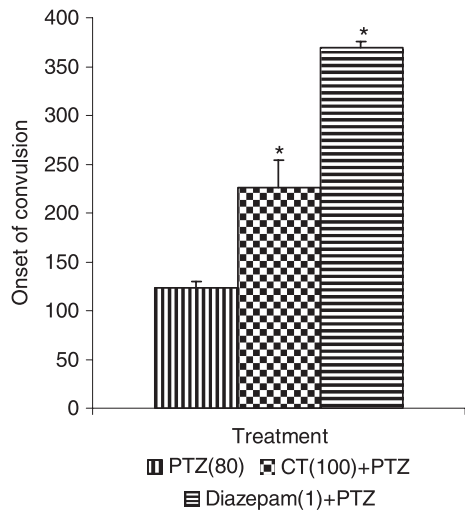

Fig. 1. Effect of CT on PTZ-induced convulsions in mice ($n = 5$). Values are means ± S.E.M. * $P < .05$, compared with vehicle-treated group (ANOVA followed by Dunnett's test).

Table 5
Effect of CT on time of immobility in tail suspension test

Treatment (mg/kg)	Time of immobility (mean ± S.E.M.) (s)
Vehicle	211.00 ± 8.35
CT (100)	165.60 ± 8.35 *
CT (400)	123.70 ± 8.80 *
Fluoxetine (10)	151.66 ± 8.90 *

$n = 5$ mice.
The duration of immobility was observed for 6 min after discarding activity during first 2 min.
* $P < .001$, compared with the vehicle-treated group (one-way ANOVA followed by Dunnett's test).

($F_{5,24} = 65.21$, $P < .0001$). A dose of 30 mg/kg was without any significant effect. The observations are given in Table 3.

3.2.2. Light/dark exploration test

The vehicle-treated mice spent 69.75 ± 9.43 s in the lit box and 229.5 ± 9.34 s in the dark box. CT (30 mg/kg) was without any significant effect on the time spent in lit or dark box, whereas the higher doses (100, 200 and 400 mg/kg) increased the time spent in the lit box ($F_{5,24} = 12.55$, $P < .0001$). The duration of time spent in the dark box decreased in a dose-dependent manner. The observations are given in Table 4.

3.3. Assessment of anticonvulsant activity

3.3.1. PTZ-induced seizures

The subcutaneous injection of PTZ produced convulsions after 123.0 ± 6.63 s and the CT and diazepam significantly delayed the onset of convulsions ($F_{2,12} = 47.48$, $P < .0001$). The animals that received vehicle died immediately after seizures. Three out of five animals died in the group that received CT, whereas only one animal died in diazepam-treated group (Fig. 1).

3.3.2. MES-induced seizures

In the vehicle-treated mice, the duration of tonic hindlimb extension was 34.7 ± 1.25 s. The animals treated with CT (100 mg/kg po) and diazepam (1 mg/kg ip) exhibited tonic hindlimb extension for 16.5 ± 2.62 and 9.28 ± 2.18 s ($P < .0001$), respectively.

Table 6
Effect of CT on CRS-induced ulcers in rats

Treatment (mg/kg)	Ulcer index
Vehicle	267.5 ± 6.29
CT (100)	207.5 ± 8.53 *
CT (200)	132.5 ± 10.30 *
CT (400)	27.5 ± 6.29 *
Diazepam (1)	20.0 ± 4.08 *

$n = 5$, $H = 17.65$, $P = .001$.
* $P < .05$, compared with vehicle-treated group (Kruskal–Wallis ANOVA followed by Dunnett's test).

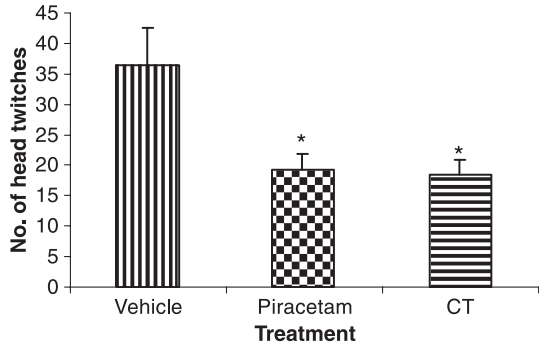

Fig. 2. Effect of CT on number of head twitches induced by lithium sulfate in rats ($n = 5$). * $P < .05$, compared with vehicle-treated group (one-way ANOVA followed by Dunnett's test). The values are means ± S.E.M. The doses of piracetam and CT were 50 and 100 mg/kg, respectively. The head twitches were counted for 60 min after lithium sulfate.

3.4. Assessment of antidepressant activity

3.4.1. Tail suspension test

The total duration of immobility in vehicle-treated mice was 211.0 ± 8.35 s. Oral administration of CT (100 and 400 mg/kg) significantly reduced the duration of immobility ($P < .001$). Fluoxetine (10 mg/kg ip) also reduced the duration of immobility. The observations are given in Table 5.

3.5. Assessment of antistress activity

After the CRS, the ulcer index in the vehicle-treated group was 267.5 ± 6.29. The treatment with CT (400 mg/kg) and diazepam significantly ($P < .05$) reduced the ulcer index. The effect produced by the lower doses of CT (100 and 200 mg/kg) was also significant. The observations are given in Table 6.

3.6. Lithium head twitches

Lithium induced 36.5 ± 6.0 head twitches in 1 h. Piracetam (50 mg/kg) and CT (100 mg/kg) reduced the number of

Table 7
Effect of CT on clonidine-induced hypothermia in mice

Time (min)	Change in temperature (°C)			
	Clonidine (0.1 mg/kg)	Piracetam (50 mg/kg) + clonidine (0.1 mg/kg)	CT (100 mg/kg)	CT (100 mg/kg) + clonidine (0.1 mg/kg)
0	37.63 ± 0.33	37.42 ± 0.18	37.45 ± 0.25	37.55 ± 0.26
30	35.19 ± 0.19	35.38 ± 0.35	36.50 ± 0.27	35.66 ± 0.21
60	34.56 ± 0.16	34.55 ± 0.35	36.88 ± 0.20	35.11 ± 0.30
90	35.72 ± 0.0.29	36.22 ± 0.35	36.44 ± 0.20	36.22 ± 0.21
120	36.22 ± 0.05	36.38 ± 0.23	36.22 ± 0.26	36.55 ± 0.21

$n = 5$, $H = 2.498$, $P = .648$. The differences were not significant (Kruskal–Wallis ANOVA).

Table 8
Effect of CT on duration of haloperidol-induced catalepsy in rats

Time	Duration of catalepsy (mean ln ± S.E.M.)	
	Treatment (mg/kg)	
	Vehicle	CT
0	1.38 ± 1.32	0.53 ± 0.0
15	2.15 ± 1.04	3.00 ± 0.71
30	3.03 ± 0.95	3.51 ± 0.09
45	3.19 ± 0.77	3.66 ± 1.13
60	3.6 ± 0.56	3.15 ± 0.92
90	3.2 ± 0.56	3.15 ± 0.92
120	3.06 ± 1.09	3.05 ± 0.67
150	2.97 ± 0.68	2.99 ± 0.05

$n = 5$. The differences were not statistically significant.

head twitches to 19.2 ± 2.7 and 18.5 ± 2.37, respectively. The differences were significant at P=.037 ($F_{2,12} = 4.4$) (Fig. 2).

3.7. Clonidine-induced hypothermia

Clonidine induced hypothermia in vehicle-treated mice. The peak effect was observed 60 min after clonidine. The CT per se was without any effect on the rectal temperature. Piracetam and CT did not significantly altered clonidine-induced hypothermia. The observations are given in Table 7.

3.8. Sodium nitrite-induced respiratory arrest

The mice treated with sodium nitrite died after 19.75 ± 0.85 min of its administration, whereas death occurred after 15.3 ± 0.41 and 16.71 ± 2.51 min in the CT- and piracetam-treated mice. The effects of CT and piracetam were not significant (P=.158).

3.9. Haloperidol-induced catalepsy

The haloperidol-induced catalepsy was measured until 150 min after haloperidol. The maximum catalepsy was noted 60 min after haloperidol. The intensity of catalepsy was more prominent in the group that received CT before haloperidol. In CT-treated group, maximum catalepsy was noted 45 min after haloperidol. The CT potentiated haloperidol-induced catalepsy only unto 45 min. However, after normalization of data (converting to ln) as suggested by Ferre et al. (1990), the difference between the effects produced by vehicle and CT were not significantly different. CT alone was without any cataleptic activity. The observations are given in Table 8.

4. Discussion

In the Ayurvedic system of medicine, the roots, seeds and leaves of *C. ternatea* have long been in clinical use. In many Ayurvedic formulations, the *C. ternatea* is used as a substitute for *Evolvulus alsinoids* (Anonymous, 1995). Recently, Taranalli and Cheeramkuczhi (2000) reported memory-enhancing activity of *C. ternatea*. The results of the present investigation suggest that *C. ternatea* possess a wide spectrum of CNS activity. The CT, though weak, exhibited nootropic, anxiolytic, antistress, antidepressant and anticonvulsant activities. The extract reduced lithium-induced head twitches, showed tendency (though statistically insignificant) to inhibit clonidine-induced hypothermia and reduce sodium nitrite-induced respiratory arrest. The effect on haloperidol-induced catalepsy was not significant.

The nootropic drugs facilitate intellectual performance, learning and memory (Giurgea, 1973). However, the neurological basis of such action is not known. Although involvement of cholinergic system is well established, the role of other neurotransmitters cannot be ignored (Hollander et al., 1986). We have reported decrease in brain contents of γ-aminobutyric acid (GABA) and DA after piracetam (Chintawar et al., 2002). It has been reported earlier that increase in the serotonergic transmission can interfere with learning acquisition and memory consolidation (Ogren, 1982). The role of DA is, however, controversial. Davis (1989) has shown that learning and memory can proceed normally despite depletion of brain DA. Piracetam, an established nootropic drug, is known to augment dopaminergic activity (Nyback et al., 1979). However, in another study, Bhattacharya et al. (1989) could not notice any significant effect of piracetam on DA levels. Brain DA level was found to decrease after administration of oil of *Celastrus paniculatus*, which possesses nootropic activity (Nalini et al., 1995). The increase in the IR and discrimination index by CT per se has proved that the plant possesses nootropic activity. The CT met a major criterion for nootropic activity, namely, improvement of memory in absence of cognitive deficit (Poschel, 1988). The decrease in TL by CT in the EPM is in accordance with the hypothesis of Itoh et al. (1990) that nootropic drugs decrease TL. The EPM test is useful in screening effect on long-term memory, whereas the object recognition test is useful to study the short-term memory. The improvement in IR by CT on the ninth day indicated its weak effect on long-term memory.

CT exhibited a weak anxiolytic activity in both animal models of anxiety, the EPM and light/dark exploration test. Increase in occupancy of the animals in the open arm or decrease in the time spent in the enclosed arm indicates the anxiolytic activity of drug (Pellow et al., 1985). Many researchers have shown an inverted U-shaped dose–response curve with anxiolytics (Weiss et al., 1981; Insel et al., 1984; Nutt and Glue, 1991), but this was not observed with CT. CT dose-dependently increased the time spent in the open arm. The light/dark exploration test measures natural aversion of mice and rats to brightly lit places. Several researchers have used this model for evaluation of anxiolytic agents (Imaizumi et al., 1996; Sanchez, 1995;

Bilkiei-Gorz et al., 1998). The observation that CT increased time spent in the lit box is in congruence with these studies.

An imbalance between excitatory and inhibitory neurotransmitters is responsible for seizures. Many drugs that increased the brain content of GABA have exhibited anticonvulsant activity against seizures induced by MES and PTZ. The MES is probably the best validated method for assessment of antiepileptic drugs in the generalized tonic-clonic seizures (Fisher, 1989; Loscher et al., 1991). The PTZ-induced seizures are similar to the symptoms observed in the absence seizures, and the drugs useful in the treatment of absence seizures suppress PTZ-induced seizures (McNamara, 1996). CT significantly delayed the onset of convulsions in PTZ-induced convulsions and also delayed the duration of tonic hindlimb extension in MES-induced convulsions. These observations suggest possibility of usefulness of CT in treatment of seizures.

Existence of cognitive problem in depressive illness is well known (Allain et al., 1990). Amitriptyline and imipramine have anticholinergic effects and this may attribute to the most adverse effects on memory. Because of increasing incidence of Alzheimer's disease and depression, there is a need for developing an antidepressant that could be useful in Alzheimer's disease and depression. A disturbed receptor balance could be contributing to the symptomatology of depression and cognitive impairment (Berendsen, 1995; Meneses and Hong, 1997). Fluoxetine, a selective serotonin reuptake inhibitor, reduces total duration of immobility. Both clinical and animal studies have strongly implicated a critical role of 5-HT_{1A}, 5-HT_{1B} and 5-HT_{2A} receptors in antidepressant response (Barnes and Sharp, 1999). Total duration of immobility was decreased by CT. In addition, CT did not produce sedation and behavioral toxicity but rather improved cognitive abilities.

Stress is known to alter the physiological homeostasis of the organism. Stress elicits various endocrinal and visceral changes including gastric mucosal integrity (Ander, 1984). Stress increases brain serotonin level (Bhattacharya and Bhattacharya, 1982) and leaves a lasting imprint on cognitive behavior (Jaiswal and Bhattacharya, 1993). Ray et al. (1991) have shown that peripheral and central injections of diazepam attenuate several stress responses like gastric ulcerogenesis. CT decreased ulcer index dose-dependently and showed antistress activity.

The increase in serotonergic transmission can interfere with learning acquisition and memory consolidation (Ichihara et al., 1993; Arnsten et al., 1997; Barnes et al., 1990). Lithium-induced head twitches are due to increased formation of serotonin in the CNS (Wielosz and Kleinrok, 1979). Both piracetam and CT reduced the head twitches significantly, and CT at the same dose exhibited increased IR in EPM, suggesting a link between cognitive improvement and decreased serotonergic transmission by CT.

It is well known that amphetamine, which markedly augments central noradrenergic activity, leads to mental confusion and retards memory consolidation. The amnesic effect of electroconvulsive shock, which is attenuated by piracetam, is known to produce marked increase in the turnover of rat brain NA (Bhattacharya et al., 1989). Clonidine, a presynaptic α-adrenoceptor agonist, induces hypothermia by reducing noradrenergic release (Drew et al., 1977). Piracetam and CT failed to reverse clonidine-induced hypothermia, indicating that noradrenergic mechanism was not involved in the central effects of CT.

Sodium nitrite is known to convert hemoglobin into methemoglobin, thereby reducing oxygen-carrying capacity and cholinergic transmission and ultimately leading to death (Hock, 1993). Piracetam and CT failed to decrease the effect of sodium nitrite. This indicated that CT did not increase the cholinergic transmission in the CNS. Anticholinergics are useful as initial drugs in Parkinson's disease, but they deteriorate cognitive behavior in Alzheimer's disease. This is the most important observation of the present study because the modern medicine does not have any drug that would be useful in treatment of Alzheimer's disease and Parkinson's disease simultaneously. This strongly suggests that there can be a category of drugs useful in Alzheimer's disease and Parkinson's disease. Thus, plants can provide a drug that would be useful in these diseases.

The striatum, the ventrorostral region and the nucleus accumbens septi have been implicated as the major brain structures involved in the antipsychotic-induced catalepsy (Duvoisin, 1967). Antipsychotic-induced catalepsy appears to be due to blockade of DA neurotransmission (Janssen, 1965; Carlesson, 1990; Seeman, 1980). In the present study, CT did not significantly increase haloperidol-induced catalepsy. Brain DA level was found to decrease after administration of oil of *C. paniculatus* (Nalini et al., 1995). This suggests that CT does not significantly alter DA levels in the substantia nigra. Thus, in conclusion, the extract was found to possess nootropic, anxiolytic, antistress and anticonvulsant activities. The present study is based on behavioral effects, and specific binding studies and measurement of neurotransmitter levels in freely moving animals are necessary to understand the mode of action and suitability of the extract for human use.

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