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Pharmacology of *Centella asiatica* Extract in Convulsions

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ABSTRACT

Centella asiatica has been useful in epilepsy, however its complete anticonvulsant action on various types of epilepsies is not well documented and its mechanism of action has not been clearly understood. The hydroalcoholic extract of *Centella asiatica* leaves, were screened for anticonvulsant activity, anti-oxidant property and its effect on diazepam withdrawal hyperactivity by using different experimental models. The final mechanism of action seems to be similar to that of benzodiazepines (GABA agonists). The effect of *Centella asiatica* extract on diazepam withdrawal hyperactivity gave an additional evidence to its mechanism of action. Also, it has a potent anti-oxidant property, which may make its use in epilepsy more beneficial. These findings explicitly suggest that the hydroalcoholic extract of *Centella asiatica* possesses a potential anticonvulsant activity as diazepam.

Keywords: Pentylenetetrazole, Kindled seizures, Status-epilepticus, hyperactivity, Locomotor activity, Increasing Current Electroshock (ICES) test, Lipid peroxidation, Anti-oxidant property.

INTRODUCTION

Ayurveda (Ayur, life; Veda, Knowledge) is the knowledge of healthy living but not merely confined to the treatment of diseases or disorders, it is an original holistic system of diagnosis and treatment involving nutrition, hygiene and rejuvenation, developed in India over 5000 years ago.

Brahmi is a well-known ayurvedic medicine. Brahmi consists of dried aerial parts, preferably leaves of *Centella asiatica* Linn, (*Apiaceae*). It has been traditionally used for a number of CNS ailments including failing memory, insanity, insomnia, depression, stress and epilepsy [1,2]. Its clinical use in India is still restrained to as a brain tonic and sedative [3]. The hydroalcoholic extract has been claimed not to have any protection against PTZ and MES induced convulsions [4]. However, other authors point to significant protection against the same, in acute studies [4].

The anticonvulsant mechanism of action has not been clearly understood and documented. Some authors say it may due to its anxiolytic or sedative effect [5], which appeared to be mainly through the cholinergic mechanism [5].

With this background information, the present study was undertaken to claim an effective anti-convulsant mechanism of action of a hydroalcoholic extract of *Centella asiatica* leaves in different experimental models.

Objectives

- To study Pentylenetetrazol – induced convulsions in rats
- To study Pentylenetetrazole induced kindled seizures in rats
- To Study Status epilepticus induced by Lithium – pilocarpine
- To study Diazepam withdrawal induced hyperactivity
- To study Increasing current electroshock (ICES) test in mice
- To study Lipid peroxidation estimation – Thiobarbituric acid reaction.

MATERIALS AND METHODS

Collection of plant material

The leaves of *Centella asiatica* used in this work were collected in November 2019 from the Botonical garden, ooty, India. The collected leaves were shade dried after authentication.

Preparation of extract

The extraction was carried out as specified [5]. Shade dried powdered leaves were comminuted until able to pass through sieve

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40 and extracted by percolation using 70% ethanol (100 gm in 500 ml) at room

temperature for 24 hr. After filtration, the dark green solution was evaporated at 50°C under reduced pressure, then lyophilized (1 mg of dry extract of *Centella asiatica* leaves is equivalent to 5.26 mg of the dried leaves of *Centella asiatica*). Phytochemical screening following standard procedure [6] showed the presence of flavanoid, saponins, tannins and essential oils.

Experimental animals

Albino mice (18-25 g) and rats (150-200 g) of Wistar strain, of either sex, obtained from Central Animal Facility, KSS college of Pharmacy Shikrapur, Pune. were used in the present study. Animals were housed in standard environmental conditions of light (12 h): dark (12 h) cycle. Rats and mice were given standard laboratory diet and water ad libitum.

Doses

Two percent solution of the extract was given orally to rats and mice at 2 and 4 hours before the experiment, in doses of 100 mg/kg and 140 mg/kg, respectively. Preliminary assay (data not shown) revealed that pharmacological response to 50 mg/kg was poor and that with 100 mg/kg was more significant in rats. Control groups were given 0.9% saline/distilled water in the same way.

Drug administration

Extract of *Centella asiatica* (100 mg/kg and 140 mg/kg for rats and mice, respectively) was administered 2/4/7 hours before the respective convulsive stimuli. Diazepam (4 mg/kg or 20 mg/kg, ip), was administered 45 min/60 min, before the respective convulsive stimuli either alone or in combination with other drugs. Sodium valproate (300 mg/kg, ip) was administered 15 min before pentylene tetrazol (30 mg/kg, ip) challenge. Lithium chloride (3 meq/kg, ip) was administered 21 hours before pilocarpine (30 mg/kg, sc) challenge.

Pentylenetetazol (PTZ) induced convulsions in rats

Pentylenetetrazol (80 mg/kg) intraperitoneally was used to induce convulsions in control and drug treated groups of 6 in each group. Rats were observed for the onset of forelimb tonic extension. Time required for the initiation of convulsions was noted and converted to a score as described [7].

Pentylenetetrazol induced kindled seizures in rats

Anti-convulsant activity in this model was examined by injecting the sub convulsant dose of pentylenetetrazol (30 mg/kg, ip) to the male wistar rats on alternative days, three times a week for nearly 10 weeks. The rats were observed for a period of 30 min after each sub convulsant dose of pentylenetetrazol manually and seizure activity scored according to scoring system ranges from 0 to 5 as described [7,8].

Animals showing five stage 5 seizures were considered to be kindled after which, the PTZ treatment was stopped. Ascertained the persistent increased sensitivity to PTZ by challenging the rats with sub convulsant PTZ (30 mg/kg), on 3rd and 10th day after PTZ treatment had ended. Only rats which had a stage 5 seizure on both the days were used for experiment.

Grouping of rats for the experiment

5 groups of rats, each consisting of 10 rats, were as follows:

Group 1 – received normal saline p.o.

Group 2 – received sodium valporate (300 mg/kg, i.p.)

Group 3 – received *Centella asiatica* extract (100 mg/kg, p.o.)

Group 4 – received Diazepam (4 mg/kg, i.p.) along with Sodium valporate (300 mg/kg, i.p.).

Group 5 – received *Centella asiatica* extract (100 mg/kg, p.o.) along with sodium valproate (300 mg/kg, i.p.).

The effect of drugs on seizures in the respective treated groups assessed by the presence or absence of seizure scores in each rat that was confirmed by observing each rat for 30 min after sub convulsant dose of PTZ (30 mg/kg, ip).

Status epilepticus induced by lithium-pilocarpine in rats

Grouping of rats for the experiment: 4 groups of rats, each consisting of 6 rats, were as follows:

Group 1 – received saline.

Group 2 – received diazepam (4 mg/kg, i.p.).

Group 3 – received CAE (100 mg/kg, p.o.),

Group 4 – received CAE (200 mg/kg, p.o.).

Status epilepticus was induced by method of Honchar et al. as [9], by intraperitoneal injection of 3 mEq/kg LiCl, followed 21 h later by 30 mg/kg pilocarpine. The animals were observed for a period of 90 minutes, for behavioural seizures. The latency in the onset of forelimb Colonus with rearing (FC+R) was observed. The extract was administered 2 h prior to pilocarpine challenge, while diazepam was administered 30 minutes prior to the same.

Effect of *Centella asiatica* on diazepam withdrawal induced hyperactivity

Diazepam withdrawal induced hyperactivity was studied as per the procedure [10]. To carry out the study the mice were divided into 4 groups, each consisting of 6 mice, where as follows:

Group 1 – received distilled water.

Group 2 – received Diazepam (20 mg/kg, i.p.).

Group 3 – received *Centella asiatica* extract (140 mg/kg, p.o.).

Group 4 – received Diazepam (20 mg/kg, i.p.) along with *Centella asiatica* extract (140 mg/kg, p.o.)

Hyperactivity induced by diazepam withdrawal done by abrupt cessation of chronic diazepam (20 mg/kg/day for 21 days)

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treated. The increase in activity was highest at 72 hr of the last dose of diazepam [10]. The effect of *Centella asiatica* extract on diazepam

withdrawal hyperactivity (locomotor activity) by concomitant administration along with diazepam using photoactometer [11].

Increasing Current Electroshock (ICES) test in mice

The anticonvulsant property of the drug (in different doses for different periods) in this model was assessed by its ability to increase in current (mA) required to induce the tonic hind limb extension (seizure threshold current) [12,13].

Grouping of mice for the experiment:

The mice were divided into 12 groups of 6 mice each. Among 12, only 9 groups received *Centella asiatica* extract at a dose of 70, 140 and 280 mg/kg, p.o. for a period of 7, 14 and 21 days.

Mice were challenged with ICES (2 mA/2 sec) 7 hours after the last dose of the extract starting with a current of 2 mA. Electroshock was delivered *via* ear electrodes, as a single train of pulses (0.2 sec duration) of linearly increasing current of intensity 2 mA/2 sec, until tonic hind limb extension (HLE) occurred or 30 mA current intensity was reached, depending upon which ever event occurred first. This current was recorded as the seizure threshold current (STC) for that animal.

Lipid peroxidation estimation – thiobarbituric acid reaction

Using the thiobarbituric acid reaction, the antioxidant property of the drug was assessed, where the ability to inhibit the formation of thiobarbituric acid reacting substance (TBARS) in brain homogenate, incubated in presence of different concentrations of the extract for 30 minutes [13-15]. The decrease in optical density, at 535 nm, was observed as a parameter of anti-lipid peroxidation effect.

Statistical analysis

Means, SEM, student t-test, ANOVA followed by Dunnet's test and Kruskal Wallis H test were used for analysis of results.

RESULTS

Pentylenetetrazol-induced convulsions in rats

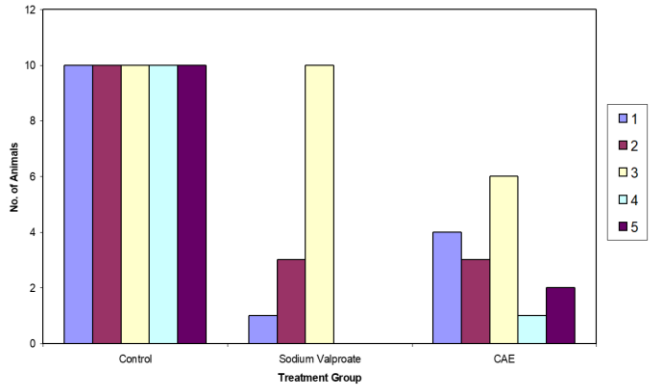
The hydroalcoholic extract of *Centella asiatica* showed significant anticonvulsant activity. *Centella asiatica* at 100 mg/kg (low dose), gave nearly a 50% protection with a mean score (S) of 0.49 0.23 and a % mortality of 50% (3/6) as compared to control. Combination of this dose with sub protective dose of diazepam protected the animals from tonic convulsions completely with 0% mortality. With a higher dose of CAE, 200 mg/kg (high dose), had aborted the onset of convulsions in 4 out of 6 animals and elicited a significant ($p<0.001$) mean score (S) of 0.92 0.05 with a 0% mortality as compared to control (Table 1).

Group	Treatment (mg/kg)	Mean Score (Mean ± SEM)	% Mortality
1	Control	0.0 ± 0.00	100%
2	Diazepam (4)	1 ± 0.00***	0%
3	Diazepam (0.5)	0.75 ± 0.03 ***	0%
4	CAE (100)	0.48 ± 0.22 *	50%
5	Diazepam (0.5) + CAE (100)	1.0 ± 0.00 ***	0%
6	CAE (200)	0.91 ± 0.05***	0%

Table 1: Pentylenetetrazol induced convulsions.

Pentylenetetrazole induced kindled seizures in rats

The *Centella asiatica* extract showed an excellent activity in this model. There was a marked reduction in the seizure scores by *Centella asiatica* extract (100 mg/kg, p.o.) alone and in combination with sodium valproate (300 mg/kg, i.p.) when compared to control group (Graphs 1 and 2).



Graph 1: Pentylenetetrazole induced kindled seizures.

Control: All animals showed all the seizure scores.

Sodium volproate: All animals showed seizure score 3, only 3 and 1 animal showed seizure score 2 and seizure score 1 respectively. But no animal showed seizure score 4 and 5.

CAE: 4 animals showed seizure score 1, 2 animals showed seizure score 2, 6 animals showed seizure score 3, 1 animal showed seizure score 4 and 2 animals showed seizure score 5.

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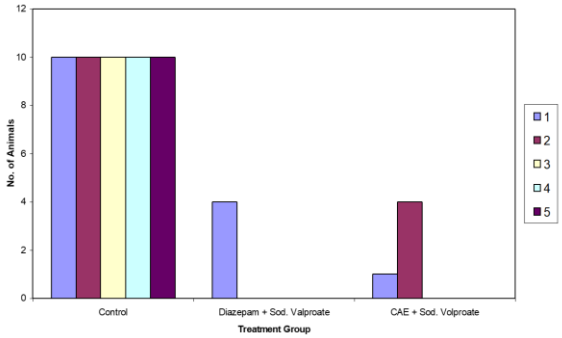


Graph 2: Pentylenetetrazole induced kindled seizures.

Control: All animal showed seizure scores 1 to 5.

Diazepam+Sod. valproate: only 4 animals showed seizure score 1 but no animal showed remaining seizure scores.

CAE+Sod. valproate: Only 1 animal showed seizure score 1 and 4 animals showed seizure score 2.

Status epilepticus induced by Lithium-pilocarpine

The extract at 100 mg/kg p.o. neither reduced the percentage mortality, nor the onset of fore limb clonus with rearing, however both these were delayed significantly at 200 mg/kg, p.o (Table 2).

Group	Treatment (mg/kg)	Number of Rats	Onset of Convulsions (Fc+R) (Min ± SEM)	% mortality
1	Control (Li+Pilo)	6	22.85 ± 0.84	100%
2	Diazepam (4)	6	90.00 ± 0.00***	0%
3	CAE (100)	6	27.33 ± 3.10	100%
4	CAE (200)	6	36.89 ± 3.48***	66.67%

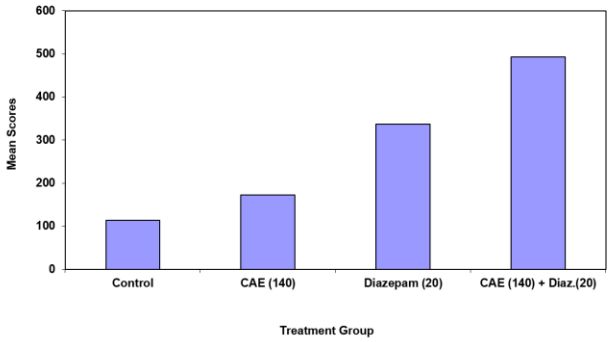
Table 2: Lithium – Pilocarpine induced status epilepticus.

Diazepam withdrawal induced hyperactivity

The *Centella asiatica* extract (100 mg/kg, p.o.) alone was not showed any significant difference in locomotor activity but showed a significant increase in locomotor activity along with diazepam i.e., at 72 hours after the abrupt cessation of the treatment compare to control group (p<0.01) (Table 3 and Graph 3).

Group	Treatment (mg/kg)	Locomotor Activity Mean ± SD	'F' value	P<	Dunnet's 't' value	P
1	Control	114.1 ± 28.55	6.498	0.01	0.617	>0.5
2	CAE (140)	172.6 ± 35.89			2.39	<0.05
3	Diazepam (20)	337 ± 115.38			3.98	<0.001
4	CAE (140) + Diazepam (20)	493.9 ± 304.55				

Table 3: Effect of CAE on Diazepam induced hyperactivity.



Graph 3: Effect of CAE on Diazepam induced hyperactivity.

Increasing current electroshock (ICES) test in mice

The significant increase in seizure threshold current (STC) was observed in all the groups of mice, treated by different doses (70, 140, 280 mg/kg, p.o.) of *Centella asiatica* extract on days 7 (p<0.01), 14 (p<0.002) and 21 (p<0.01) days (Table 4).

Group	Treatment (mg/kg b.w. & route of administration)	Seizure threshold current (mA) after CAE treatment (days)		
		7	14	21
1	Distilled water	158 ± 0.45 (6)	16.7 ± 2.98 (6)	18.8 ± 0.83 (6)
2	CAE (70mg/kg, po)	18.2 ± 0.70 (6)	20.1 ± 0.89 (6)	24.3 ± 0.51 (6)
3	CAE (140 mg/kg, po)	18.68 ± 0.66 (6)	21.35 ± 0.66 (6)	26.4 ± 0.42 (6)
4	CAE (280mg/kg, po)	20.5 ± 0.88 (6)	21.39 ± 0.66 (6)	26.9 ± 0.66 (6)
H value	-	11.85	10.12	17.32
P<	-	0.01	0.02	0.01

Table 4: Increasing current electroshock (ICES) test in mice.

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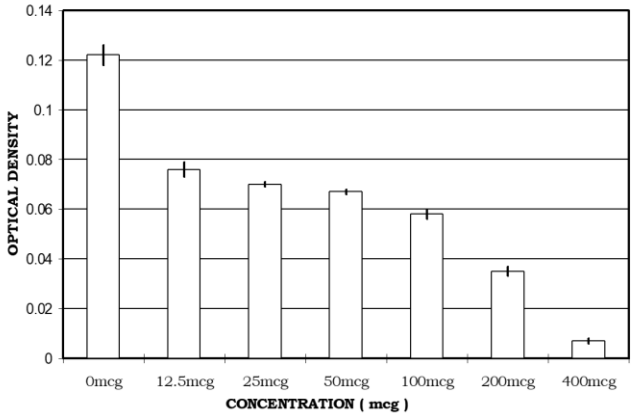
Lipid peroxidation estimation-Thiobarbituric acid reaction

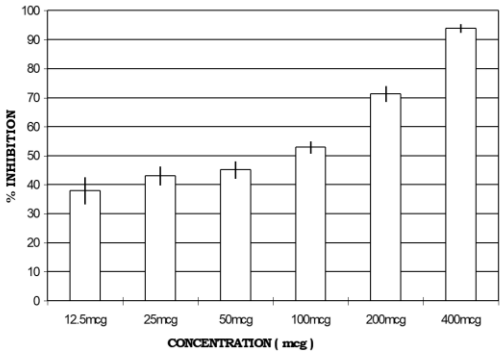
LPO production was inhibited in a concentration dependent manner (Figure 5 and Graphs 4 and 5). Almost complete inhibition was observed at 400 Hg, *viz.*, 0.007□0.001. The concentration giving 50% inhibition was at 94.48 Hg (Table 5).

Group	Concentration (mg/10ml)	Optical Density	% Inhibition
1	0	0.125 ± 0.004	-
2	12.5	0.078 ± 0.003	38.00 ± 4.38
3	25	0.078 ± 0.001	43.05 ± 2.95
4	50	0.065 ± 0.001	45.17 ± 2.78
5	100	0.055 ± 0.002	52.92 ± 1.8
6	200	0.030 ± 0.002	71.29 ± 2.52
7	400	0.007 ± 0.001	93.96 ± 1.26

Table 5: Lipid peroxidation inhibition.



Graph 4: Lipid peroxidation.



Graph 5: % inhibition of lipid peroxidation.

DISCUSSION AND CONCLUSION

The present experimental findings suggested that the hydroalcoholic extract of *Centella asiatica* showed its anti-convulsant effect in different experimental models.

Pentylenetetrazol induced convulsions: *Centella asiatica* extract at high dose aborted a fore limb extension in 4 out of 6 animals and significantly extended the time for the onset of convulsion.

Pentyletetrazol induced kindled rats: *Centella asiatica* at a dose 100 mg/kg, p.o. alone and in combination with sodium valproate (300 mg/kg, p.o.) showed a marked reduction in seizure scores.

Status epilepticus induced by Lithium – pilocarpine: *Centella asiatica* extract at high dose delayed the onset of convulsions in the model.

The findings in the above 3 models suggested that, the probable mechanism of action of *Centella asiatica* extract for its anti-convulsant activity is same as the mechanism of action of diazepam. It indicated that *Centella asiatica* explicit anticonvulsant action may be due to its interaction with GABA – coupled. Chloride ionophore complex (i.e., the mechanism seems to be related to the excitation of the inhibitory functions of the GABA neurotransmitter.

The combination of *Centella asiatica* extract along with diazepam resulted in significant increase in locomotor activity suggested that, the extract facilitates the action of diazepam. It is an additional evidence to say that the extract acts through similar mechanism as that of Benzodiazepines.

The extract showed a significant increase in seizure threshold current in KES test suggested that there may be prolongation of the Na^+ channel inactivation.

Cerebral tissue damage in brain ischemia is due to production of free radicals, which cause lipid-peroxidation in cellular membranes. The *in-vitro* analysis of this parameter by using brain homogenates at different concentrations of Centella extract have revealed its anti-oxidant property probably by capturing the free radicals, thus raising the importance of the use of the agent alone or in combination with other anti-epileptic drugs in cases of post traumatic epielkpsies, to prevent excessive cell necrosis and progression of the epileptogenic factor.

The extract at high dose delayed the onset of convulsions in the lithium – pilocarpine induced status epilepticus model, states its ability to antagonize the factors that influence the beginning and end point of a status – epilepticus which are unknown, but a role has been proposed is the glutamate mediated excitation and lack of GABA mediated inhibition. Diazepam and other diazepam like drugs abort these seizures. Hence, proving that *Centella asiatica* extract may act in the same mechanism as benzo diazepine.

The models, PTZ induced convulsions and PTZ induced kindled seizures revealed that the extract showed a protection against generalized clonic and tonic seizures. Activity of the extract in lithium-pilocarpine model revealed its ability to invalidate some aspects of human temporal lobe or psychomotor epilepsy.

It has been reported that diazepam reduces the turnover rate of straital dopamine, cerebellar and hypothalamic norepinephrine. The dopaminergic neurons in the par's compacta of the substantia nigra are reported to be monosynaptically inhibited by GABAergic pathways. Co-incidentally the hydroalcoholic extract of

CA has shown to cause an overall decrease in the turnover of Centella monoamines. Hence, strongly suggesting a mechanism similar to that of benzodiazepine.

Aminobutyric acid (GABA) is a major inhibitory neurotransmitter in the mammalion CNS (16). The further studies are required to correlate GABA levels in different regions of the brain after the treatment of *Centella asiatica*.

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