Research Article

Anticonvulsant Effect of Ethanol Extract of *Glycerrhiza glabra* and Hydroalcoholic Extract of *Centella asitica* in Albino Rats by Pentylene Tetrazol (PTZ) induced convulsion and Pentylene Tetrazol (PTZ) induced kindled seizures

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ABSTRACT

Epilepsy is one of the most common neurological disorders affecting the people across all nationalities. Despite the optimal use of available antiepileptic drugs, still the goal of the treatment of epilepsy to completely control the seizures is not yet pursued. Even though, it is possible at the expense of significant toxic side effects. The present study was undertaken to investigate the effect of *Centella asiatica* (CAE) hydro alcoholic extract and *Glycerrhiza glabra* ethanolic extract (GGE) on convulsions in albino rats. In PTZ induced convulsions, the parameter monitored was onset of convulsions (as indicated by Jerks, Clonus and Extensor). These parameters were analyzed by using One-way analysis of variance (ANOVA), expressed as Mean ± SEM, followed by Dunnet's t test. P-values <0.01 were considered as significant. PTZ (70 mg/kg s.c.) was used for inducing convulsions in all three groups. In CAE +GGE groups, onset of time (seconds) to show convulsions such as Jerks and Clonus were 3.5±0.34 and 13.50 ± 3.4 as compared to control group 6.000±0.25 and 45.50 ± 8.40 respectively. The animal is CAE + GGE treated group showed a significant difference in delaying the onset of convulsions. Co-administration of *C. asiatica* and *G. glabra* extracts showed a significant protection against PTZ induced seizures in rats by reducing the severity of the convulsions when compared with control rats ($p<0.05$). Marked reduction in the mortality (33.33%) further evidenced the preventive effect of extracts.

Keywords: Anticonvulsant effect, *Centella asiatica, Glycerrhiza glabra,* Pentylene tetrazol induced convulsion, Pentylene tetrazol induced kindled seizures**,** PTZ.

INTRODUCTION

Epilepsy is most common neurological disorders affecting people across all nationalities.[1] The word epilepsy in derived from the Greek verb epilamvanein (to be seized", "to be taken hold off", or "to be attacked" indicating that the person having a seizure is 'possessed' or at least out of control.[2] Epilepsy includes a group of heterogeneous and diverse conditions. The terms epilepsy and seizure are not synonymous and the distinction must be made clear. 'A seizure is an abnormal behaviour (with symptoms or signs) resulting from abnormal discharges of cortical neurons and it is an observable phenomenon that is finite in time. Epilepsy refers to chronic conditions characterized by recurrent seizures.[3] Epilepsy is one of the most common neurological disorders characterized by sudden, transient alterations of brain function usually with motor, sensory autonomic or psychic symptoms often accompanied by loss of, or altered consciousness.[4] Coincidental pronounced alteration in the electro encephalogram (EEG) might be detected during these episodes. Epilepsy occurs due to abnormal activity of brain tissue. It may be Idiopathic (primary / genetic) or symptomatic (secondary / cryptogenic / reactive) epilepsy. The symptomatic or secondary epilepsy may be caused by several factors such as:

Birth trauma and head injury. Toxicities include drug intoxication, lead poisoning, and consumption of alcohol and psychotropic drugs. Degenerative cerebral diseases Metabolic disorders such as hypercalcaemia and hypoglycaemia.CNS infections. Cerebrovascular diseases (e.g. infarction). Systemic diseases such as Struge-Weber syndrome, tuberose sclerosis. Miscellaneous factors. In susceptible individuals, even physical stimuli such as sound, touch or stroboscopic light may precipitate seizures.[5]

In spite of intensive investigations, the path physiology is still poorly understood.[5] Several biochemical hypotheses suggest the involvement of decreased activity of inhibitory GABA ergic system or increased activity of excitatory amino-acids (glutamate and aspartate system) in epilepsy. And also there are various other factors which cause seizures, such as oxidative stress developed by the free radical generation. Epilepsy by itself means "idiopathic" in contrast to the commonly used but incorrect meaning of cause unknown. Epilepsy is treated mainly with drugs; though brain surgery may be used for severe cases. The antiepileptic drugs (AED's) like Valproate, Phenytoin and Carbamazepine are associated with osteoporosis and other disorders of bone and mineral metabolism including hypocalcaemia, serum concentrations of vitamin D metabolites hypophosphatemia, reduced and Secondary hyperparathyroidism. In addition, increased biochemical markers of bone formation and re sorption have been reported. These biochemical changes may place people treated with AED's at increased risk for low bone mineral density (BMD), osteoporosis, oesteomalacia, and

fractures. Only limited information is available regarding newer AED's such as lamotrigine.[6,7]

Keeping these complications in mind, various herbal medicines have been tried in the past for their potent anticonvulsant properties. We turn to ayurveda. Ayurveda is the knowledge of healthy living and not merely confined to the treatment of diseases or disorders. It is an ancient and holistic system of diagnosis and treatment involving nutrition, hygiene and rejuvenation originating in India more than 5000 years ago.[8]

Brahmi is a well known Ayurvedic medicine, consisting of the dried aerial parts, preferably leaves, of *Centella asiatica* Linn. (Apiaceae). It has traditionally been used for central nervous system (CNS) ailments including failing memory, insomnia, depression, stress and epilepsy.[9] Its clinical use in India is still as brain tonic and sedative. The marketed formulation which had *C. asiaitca* as one of its active ingredient showed a significant reduction in frequency of generalized tonic-clonic seizures, partial seizures and maximal electroshock (MES) induced convulsions, psychogenic attacks and alcoholic excess. The hydroalcoholic extracts of *C. asiaitca* showed significant protection against PTZ and maximal electroshock induced seizures. In our previous study, we have reported the protective action of *Centella asiatica* Linn. (Brahmi) against PTZ kindled seizure and increasing current electroshock tests (ICEST).[10,11]

Glycerrhiza glabra (Leguminosae) used in traditional system of medicine have been in clinical use for centuries. It possesses wide range of CNS activities such as antipyretic, anxiolytic and memory enhancing properties.[12] *G. glabra* is traditionally recommended for treatment of epilepsy. Along with its existed scientific report for its anticonvulsant profile against pentylenetetrazol seizure and lithium pilocarpine induced status epilepticus

With this background of information, the current study was designed to explore the combined effects of *Centella asiatica* and *Glycerrhiza glabra* extracts on secondarily generalized seizures and on seizure threshold current by ICEST.[13]

MATERIALS AND METHODS

Methods

The convulsive models such as PTZ and PTZ kindled seizures were used for the .Male wistar rats were used in pentylenetetrazol (PTZ) kindled seizures and female spergue dwelly rats with body weight 150-200 mg were used for PTZ Induced seizures. Dose of *Centella asiatica* 200 mg kg^{-1} b.w. and *Glycerrhiza glabra* 300mg kg^{-1} b.w. orally in combination was used in all the models.

Materials

Plant Materials

Leaves of *Centella asiatica* were collected from medicinal plant garden of K.L.E.S' College of Pharmacy Belgaum (India). *Glycerrhiza glabra* roots and rhyzomes were collected from Saswad and surrounding areas of Saswad, Pune district, Maharashtra (India) and both drugs were authenticated by Dr. Harsha Hegade, Regional Medical Research Center, ICMR, Belgaum.

Preparation of extracts

The collected drugs were shade dried and powdered. The powder of *Centella asiatica* Leaves was passed through sieve no 40 and extracted by percolation using 70% ethanol (100 gm in 500 ml) at room temperature for 24 h. After filtration, dark green coloured solution obtained from the *Centella asiatica* was evaporated at 50°C under reduced pressure, and then lyophilized (1mg of dry extract of *C.asiaitca* leaves is equivalent to 5.26 mg of dried leaves of *C. asiaitca*)

The roots and rhizomes of *Glycerrhiza glabra* were crushed to coarse powder and extracted with ethanol (70% v/v) using soxhlet extractor for 24 h. The extract was concentrated under reduced pressure and air dried. The semisolid mass obtained and stored in an air tight container in refrigerator for further use.[14]

Animal selection

Male albino Wistar rats (150-200g) and albino mice (18-25g) of either sex procured from M/s. Venkateshwara Enterprises, Bangalore (CPCSEA Reg. No. 276) were used with the approval of the Institute Animal Ethics committee. Animals were reared and maintained at the animal house of the institution and were on standard pellet diet and water *ad libitum*. They were initially acclimatized to the laboratory environment for one week prior to their use. Each group of animals was housed separately, with a distinct identity throughout the study.

Drugs used

Pentylene tetrazol , Sodium valproate (Sigma, St.Louis, USA), Diazepam (Ranbaxy)

Preparation of drugs and their administration

Centella asiatica extract (200mg/kg and 140mg/kg for rats and mice, respectively) was administered 2/4/7 hours before the respective convulsive stimuli. Diazepam (4mg/kg and 20mg/kg i.p.) and phenytoin sodium (20mg/kg., i.p.) was administered 45 min/60 min. before the respective convulsive stimuli either alone or in combination with other drugs. Sodium valproate (300mg/kg i.p.) was administered 15 min before the pentylenetetrazole (30mg/kg, i.p.) challenge.

Glycerrhiza glabra ethanol extract (GGE) (300mg/kg b.w. orally) was prepared freshly in the form of suspension using 0.5% W/V carboxyl methylcellulose. Diazepam (4mg kg^{-1} i.p.) and Phenytoin sodium (25mg kg^{-1} i.p.) were

administered 60 min and 30 min either alone or in combination with other drugs before the respective convulsive stimuli were given. Sodium valproate (300mg/kg i.p.) was administered 15 min before the pentylenetetrazole (30mg kg^{-1}, i.p.) challenge either alone or in combination with other drug. All the drugs were prepared in the form of solution using distilled water except GGE (Table 3).

Doses and calculations

Extract solution, to be administered, was prepared fresh by dissolving 200mg of the crude extract in distilled water to make 10ml of the solution. This represents 20mg/ml of the extract for 100mg/ml of the crude plant material.

Statistical analysis and calculations

All drug concentrations were represented as mg/ml. One way ANOVA, followed by Dunnet 't' test and Krushal Wallis H-test were performed for statistical analysis $P<0.05$ was considered statistically significant.

A. PTZ induced seizure

The best known convulsant is PTZ. In this kind of model PTZ is administered, intravenously or subcutaneously usually to rats or mice. Female Sprague dwelly rats with a body weight between 150 to 200 gm are used. Rats were divided into 3 groups of 8 rats each viz, control (1 ml per rat), diazepam (4mg kg^{-1}) treated, and extracts (CAE 200 mg kg^{-1} b.w. p.o. and GGE 300 mg kg^{-1} b.w. p.o.) treated. Seizure were induced by injecting pentylenetetrazol (70 mg $kg^{-1,}$ ip.) to the group of the rat which previously treated with vehicle (45 min), diazepam (30 min) and extracts (2h) for control diazepam and extracts treated groups respectively. Each animal is placed into an individual plastic cage for observation lasting 1 h. Seizures and tonic-clonic convulsions are recorded. Onset of seizures and recovery time of the animals is recorded (Table 1).

Table 1: Drugs and their concentrations given for the PTZ induced seizure

Drugs	Concentration	Dose in mg/kg body weight and route of administration	Time of administration prior to maximal electroshock
PTZ		70mg/kg, po	30 minutes
Diazepam	4mg/ml	4mg/kg, i.p.	30 minutes
CAE + GGE	40mg/ml, 60mg/kg	50mg/kg, po & 300mg/kg., po	2 hours

CAE – *Centella asiática*; GGE- *Glycerrhiza glabra*

The severity of convulsions was assessed by the duration of colonic flexion, tonic extensor, clonus, stupor and recovery phase for each animal. The duration of each phase for each animal (in second) was measured by using Stop watch.

The starting time for each phase was noted and then converted to duration of each phase by deducting starting time of one phase from the starting time of the previous phase

B. Pentylenetetrazole induced Kindling in Rats

The anti-convulsant activity in this model was assessed by its ability to protect against pentylenetetrazole induced kindling seizures. Male Wistar rats were first weighed and were selected for the experiment depending on the weight.

Establishment of PTZ Kindled Seizures in Rats

'Pentylenetetrazole' was dissolved in saline. Kindled seizures were induced by intraperitoneal injection of sub convulsant doses of PTZ i.e., 30 mg/kg, in rats, on alternate days, three times a week for nearly 10 weeks. The rats were observed for a period of 30 min. after subs convulsant PTZ manually and seizure activity scored using scoring system ranges from 0 to 5 (Table 2).

Animals showing five stage 5 seizures were considered to be kindled after which, the PTZ treatment was topped. To ascertain whether the increased sensitivity to PTZ is persistent, the rats were rechallenged with sub convulsant PTZ is persistent, the rats were rechallenged with sub convulsant PTZ (30mg kg^{-1}, ip), in 3rd and 10th day after PTZ treatment had ended. Only rats which had stage 5 seizures on both the days were used for experiments with different drugs. The selected rats were then divided into 5 groups of 8 rats each.

The rats selected from the animals showing all the 5 stage seizure, divided into 4 groups of 10 rats each. Group I received normal saline. Group II received GGE (300mg kg^{-1} b.w., p.o.,) and CAE (200 mg kg^{-1} p.o.). Group III received sodium valproate (300mg kg^{-1} b.w. ip.) and (4 mg kg^{-1} ip.) diazepam and group IV received GGE (300mg kg^{-1} b.w., p.o.,) + CAE (200mg kg^{-1} b.w.,p.o.,) sodium valproate (300mg kg^{-1} ip.)

Table 2: Scoring System for Pentylenetetrazole Kindled Seizures

State	Symptoms
0	No change
1	Hyper activity, restlessness, vibrissae twitching
2	Head nodding, head clonus, myoclonus jerks
3	Unilateral or bilateral limb clonus
4	Fore limb clonic seizures
5	Generalized clonic seizures, with loss of righting reflex.

Subconvulsant dose of PTZ (30mg kg^{-1} ,i.p) was administered to control and drug treatment animals. Subconvulsant does of PTZ (30mg kg^{-1}, i.p.) was

administered to control and drug treatment animals, which produce different stages of seizure (Table 3).

The drugs and chemicals were prepared fresh, the concentration, dose, route and the time of administration before PTZ challenge (Subconvulsant Dose, 30mg kg^{-1}., i.p) were as follows:

The effect of drugs on seizure assessed by the presence or absence of 5 stages of seizures in each rat that was confirmed by observing each rat for 30 minute after subconvulsant dose of PTZ (30mg kg^{-1}b.w., ip).

Table 3: Dose and concentrations use for the administration

Drugs	Concentration	Dose (mg/kg b.w.) and route of administration	Time of administration of drug prior to subconvulsant dose of PTZ challenge
Normal saline	-	1ml/rat, po	60 minutes
Diazepam+ sodium valproate	5mg/ml 00mg/ml	4mg/kg,ip 300mg/kg, i.p.	60 minutes 15 minutes
CAE+GGE + Sodium valproate	40mg/ml 6omg/ml 200mg/ml	200mg/kg and 300mg/kg, po, 300mg/kg, ip	45 minutes 15 minutes
CAE +GGE	40mg/ml & 60mg/ml, po,	200mg/kg and 300mg/kg, po,	45 minutes

Table 4: Effect of co-administration of *C.asiatica* and *G.glabra* extracts on pentylenetetrazol induced convulsions

Group	Treatment (mg/kg b.w.)	Mean score (mean ± SEM)		% Mortality
		Onset of jerks	Onset of clonus	
1	Control	6.000±0.25	45.50±8.40	100
2	Diazepam	2.5±0.500*	0.0±0.0*	0
3	CAE(200) + GGE(300)	3.5±0.34*	13.50±3.4*	33.33

CAE: *C. Asiatica extract*, GGE: *G. glabra* extract; *P< 0.05 when compared with control rats

Table 5: Effect of combination of *c. asiatica* and *g. glabra* on pentylenetetrazol kindled seizures

Seizure score	Control		CAE + GGE		Sodium valproate+Diazepm		Sodium Valproate + (CAE +GGE)	
	A	B	A	B	A	B	A	B
1	10	0	10	0	5	50	6	40
2	10	0	10	0	3	70	1	90
3	10	0	4	60	0	100	0	100
4	10	0	2	80	0	100	0	100
5	10	0	1	90	0	100	0	100

A: No. of animals showed seizure score; B: % reduction in seizure score; CAE: *C asiatica extract*, GGE: *G. glabra* extract

RESULTS AND DISCUSSION

The effect of *Glycerrhiza glabra* ethanol extract and *centella asiatica* hydroalcoholic extract on various animal models was observed by monitoring different parameters during the study.

A. Pentylene Tetrazol (PTZ) Induced Convulsion

In PTZ induced convulsions, the parameter monitored was onset of convulsions (as indicated by Jerks, Clonus and Extensor). These parameters were analyzed by using One-way analysis of variance (ANOVA), expressed as Mean ± SEM, followed by Dunnet's t test. P-values <0.01 were considered as significant. PTZ (70 mg/kg s.c.) was used for inducing convulsions in all three groups. In CAE +GGE groups, onset of time (seconds) to show convulsions such as Jerks and Clonus were 3.5±0.34 and 13.50±3.4 as compared to control group 6.000± 0.25 and 45.50±8.40 respectively. The animal is CAE + GGE treated group showed a significant difference in delaying the onset of convulsions. Co-administration of *C. asiatica* and *G. glabra* extracts showed a significant protection against PTZ induced seizures in rats by reducing the severity of the convulsions when compared with control rats ($p<0.05$). Marked reduction in the mortality (33.33%) further evidenced the preventive effect of extracts. In this model, the reduction in number of scores or abolition in scores is considered for the evaluation of anticonvulsant activity of drugs in all groups. (Table 4)

B. PTZ Induced Kindled Seizures in Rats

Establishment of PTZ kindled seizures in rats

Development of fully kindled, stage 5 seizure, i.e. generalized clonic seizures with loss of righting reflex in rats occurred after approximately 10 weeks of treatment

with subconvulsant doses of PTZ. To ascertain whether the increased sensitivity to PTZ is persistent, the rats were challenged with subconvulsant PTZ (30mg/kg), on 3rd and 10th day after PTZ treatment had ended. The progression of kindling from normal to stage 5 seizures, in rats during the course of kindling was desirable. There was no mortality during the induction of kindling. In this model, the reduction in number of scores or abolition in scores is considered for the evaluation of anticonvulsant activity of drugs in all groups (Table 5).

Control Group

All the animals in the control group showed all the stages of seizures from 0 to 5.

Sodium valproate + diazepam treated group

The animals which received sodium valproate (300mg/kg b.w. i.p.) and diazepam 40 mg/kg b.w. ip) i.e. 30 min before subconvulsant dose of PTZ (30mg/kg ip) showed marked reduction in the seizure score 3, 4, 5.where as 50% and 70% reduction in scores of 1 and 2 stage respectively. (Table 5)

CAE + GGE treated group

The animals which received GGE (300mg/kg, b.w. p.o.), 2 hrs before the subconvulsant dose of PTZ (30mg/kg, b.w., i.p.) showed the seizure scores. But no animals showed all the seizure scores from 0 to 5. Out of 8 animals only one animal showed stage no. 4 and 5. The drug showed significant reduction of scores in comparison with control groups, which is given as percentage as follows.

- 60% of animals showed state no. 3 (restlessness, vibrissae twitching and hyperactivity)
- 80% of animals showed stage no. 4 (head nodding, head clonus and myoclonic jerks).
- 90%of animals showed stage no. 5 (unilateral and bilateral limb clonus.)

Animals showed seizure scores within 15-20 min after subconvulsant dose of PTZ. Co-administration of *C. asiatica* and *G. glabra* extracts exhibited marked reduction in seizure scores 60%, 80% and 90% in stages 3, 4, 5 respectively. (Table-5)

Combination of sodium valproate and CAE + GGE

The synergistic effect of CAE [(200 mg/kg p.o.) (120 min pre-treatment of PTZ) + GGE [(300mg/kg p.o.) (120 min pre treatment of PTZ)] and sodium valproate [(300mg/kg., i.p.) (15 min pre-treatment)] Combination with sodium valproate, the extracts exhibited further reduction in seizure scores (40% and 90% in seizure score 1 and 2 and 100%in seizure score 3, 4 and 5). Thus co administration of the extracts with sodium valproate showed marked reduction in the seizure scores (Table 5).

CONCLUSION

The current experimental findings suggest that the co-administration of *C. asiaitca* and *G. glabra* extracts depicted the potential anticonvulsant property against PTZ-induced seizures, PTZ- kindled seizures and ICES test. The results suggest that the extracts may be useful for the treatment of various types of seizures, including petit mal, secondarily generalized and grand mal seizures. These findings are in agreement with earlier findings of our laboratory as well as other scientific reports. Apart from anticonvulsant profile the extracts also reported for the various CNS aliments.

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