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ANTICONVULSANT EFFECT OF ETHANOL EXTRACT OF *GLYCERRHIZA GLABRA* AND HYDROALCOHOLIC EXTRACT OF *CENTELLA ASIATICA IN ALBINO RAT AND MICE*

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ABSTRACT

The ethanolic extract of *Glycerrhiza glabra* and hydroalcoholic extract of *Centella asiatica* showed protective effect against convulsive models. In MES test, significant reduction was found in all phases by treatment of the combination. Increase seizure threshold current was observed by *Glycerrhiza glabra* and *Centella asiatica* treatment in ICEST. These findings suggest that co administration of *Glycerrhiza glabra* & *Centella asiatica* extract possess marked anticonvulsant effect against various in vivo experimental models suggesting that, the synergistic anti convulsant activity.

KEY WORDS

Epilepsy; Glycerrhiza glabra; Centella asiatica; Increasing current electroshock seizure test; Maximal electro Shock test.

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 behavior (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[1]. 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. Several biochemical hypotheses suggest the involvement of decreased activity of inhibitory GABA agric system or increased activity of excitatory amino-acids (glutamate and aspartate system) in epilepsy [2]. And also there are various other factors which cause seizures, such as oxidative stress developed by the free radical generation[5]. 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[9] including hypocalcaemia, serum concentrations of vitamin –D metabolites[12,11] hypophosphatemia,[13,14] reduced and Secondary hyperparathyroidism [12, 15, 16]. In addition AED's Use of antiepileptic drugs during pregnancy increases the risk for specific congenital malformations such as neural tube defects, cleft lip and palate and cardiovascular

malformations[17]. Even the current antiepileptic drugs such as Oxcarbazepine, gabapentin, tiagabine, topiramate, levotiracetam, lamotrigin, felbamate, and fosphenytoin have the drawbacks like limited spectrum or drug interactions with oral contraceptives. Three drugs of these gabapentine, lamotrigin and topiramate are approved for use in adults with partial seizure or without generalization. It is felbamate and lamotrigine have potential of significant side effects. fosphenytion and lamotrigine is parent pro-drug of phenytion that is more tolerable than parenteral phenytoin[18] . Therefore not surprising that the currently used antiepileptic drugs fail to provide satisfactory seizure control and toxicities associated with these drugs can further compromise quality of life while drug-drug interactions may complicate clinical management.

Keeping these complications in mind, various herbal medicines have been tried in the past for their potent anticonvulsant properties. 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.[25]

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 [19, 20, 21]. Its clinical use in India is still as brain tonic and sedative. [22] 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 [23]. The hydroalcoholic extracts of *C. asiaitca* showed significant protection against maximal electroshock induced seizures [24]. 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) [25.]

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 [6] and memory enhancing properties. [7, 26] G. *glabra* is traditionally recommended for treatment of epilepsy. [9] along with its existed scientific report for its anticonvulsant profile against pentylenetetrazol seizure and lithium pilocarpine induced status epilepticus [10].

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.

MATERIALS AND METHOD

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 colored solution obtained from the *Centella asiatica* was evaporated at 50^0 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*) [51, 65] 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.

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 and Chemicals

Pentylene tetrazole (Sigma, St.Louis, USA)Sodium valproate (Sigma, St.Louis, USA)Diazepam(Ranbaxy) Phenytoin sodium (M.J. Pharmaceuticals, Gujrat)

Preparation of Dose 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 admistered 45 min/60 min. before the respective convulsive stimuli either alone or in combination with other drugs.

Glycerrhiza glabra ethanol extract (GGE) (300mg/kg b.w. orally) was prepared freshly in the form of suspension using 0.5% W/V carboxy methycellulose. In all the models except ICEST model GGE was administrated 2hrs before the respective convulsive stimuli were given. In case of ICEST model GGE was administered 7 hrs before the shock treatment. 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. All the drugs were prepared in the form of solution using distilled water except GGE.

Doses and Calculations:

The dose of the Glycyrrhiza glabra extract was fixed as 300mg/kg. b.w. orally.[27] In ICEST model in addition to this dose, 500mg kg^{-1}. b.w. orally was also used

Extract 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.

METHODS USED

A. Maximal Electroshock (MES) – Induced Convulsions in Rats:

The anticonvulsant property of the drug in this model was assessed by its ability to protect against MES induced convulsions. The method used was described by Dandiya & Sakina, 1999.The animals were first weighed and were selected for the experiment depending on the weight. Rats of either sex were used. The rats were then divided into four groups of six rats each. Group 1 received saline; group 2 received 20mg/kg b.w. of phenytoin sodium; group 3 received CAE 200 mg/kg bw. And GGE 300 mg/kg b.w. of Maximal electroshock (Inco Electroconvulsiometer model # 100-3) of 150 mA

current for 0.2 Sec was administered through ear electrodes to induce convulsions in the control and drug treated animals. The drugs and chemicals were prepared fresh; the concentration, dose and the duration before induction of convulsion were as follows:

Table 1 Dose and concentrations use for the administration.

Drugs	Concentration	Dose in mg/kg body weight and route of administration	Time of administration prior to maximal electroshock
Saline	--	1ml/rat, po	30 minutes
Phenytion	25mg/ml	25mg/kg, i.p.	30 minutes
CAE + GGE	40mg/ml 60mg/kg	50mg/kg, po & 300mg/kg., po	2 hours

The severity of convulsions was assessed by the duration of clonic 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. **Increasing current electroshock seizure test in mice (ICEST):**

The anticonvulsant property of the drug (in different doses followed both acute and chronic administrations) in this model was assessed by its ability to increase in current required to induce the tonic hind limb extension (seizure threshold current).The animals were first weighed and were selected for the experiment depending on the weight. Mice of either sex were used. Albino mice were distributed in to 4 groups viz control (vehicle), CAE (200 mg kg^{-1} p.o.) treated, GGE (300mg kg^{-1} b.w., p.o.,) treated,CAE (200 mg kg^{-1} p.o.)+ GGE (300 mg kg^{-1} p.o.) treated and for a period of 7, 14, 21 days. One group each received distilled water for respective drug treated groups (Refer **Table 2**)

Table 2 Dose and concentrations use for the administration.

Group	Drug and its concentration	Dose in mg/kg b.w. and route of administration		
		7 days	14 days	21 day
1	Distilled water	0.5ml/mouse,po	0.5ml/mouse,po	0.5ml/mouse,po
2	CAE (8mg/ml)	200mg/kg,po	200mg/kg,po	200mg/kg,po
3	LE (12mg/ml)	300mg/kg,po	300mg/kg,po	300mg/kg,po
4	CAE(8mg/kg) +GGE (12mg/ml)	200mg/kgand 300 mg/kg ,po	200mg/kgand 300 mg/kg ,po	200mg/kgand 300 mg/kg ,po

Mice were challenged with ICEST (2mA/2sec) 7 hours after last dose of CAE i.e., starting with a current of 2mA, electroshock was delivered, via ear electrodes, as a single train of pulses (0.2 sec duration) of linearly increasing current intensity of 2mA/2sec., until tonic hind limb extension (HLE) occurred or 30mA current intensity was reached,

Depending upon whichever event occurred first. This was recorded as the seizure threshold current (STC) for that animal.

RESULT AND DISSCUSSION

Table 3: EFFECT OF CO-ADMINISTRATION OF *C. ASIATICA* AND *G. GLABRA* EXTRACTS ON MAXIMAL ELECTROSHOCK INDUCED CONVULSIONS IN RAT

Group	Treatment (mg/kg b.w.)	Time (Sec.) in various phases of convulsions			
		Flexion	Extension	Clonus	Stupor/Recovery
1	Control	2.210±0.2964	8.930± 1.263	8.10±0.7520	115.2±12.39
2	Phenytoin	4.294±0.4896	0.0± 0.0	7.100±0.7667	6.526±0.9409
3	CAE + GGE(200mg/kg and 300 mg/kg)	3.610±0.4004	6.970±0.8025	7.900±0.5044	10.49±1.427

CAE: *C. Asiatica extract*, GGE: *G. glabra* extract.

Table 4: EFFECT OF COMBINATION OF *C.ASIATICA* AND *G.GLABRA* ON INCREASING CURRENT ELECTROSHOCK SEIZURE (ICES) TESTS IN MICE

Groups	Treatment and route of administration (mg kg^{-1} bw)	Seizure threshold current (mA) after treatments (days)		
		7	14	21
1	Control	10.13±0.97	11.25±0.49	11.25±0.49
2	CAE	17.13±2.154	16.88±1.59	19.50±0.56
3	GGE	13.13±1.69	20.63±1.99	23.25±1.677
4	GGE+CAE	20.38±2.18	24.38±1.19	26.25±0.49
H value**H value obtained from Kruskal Wallis H-test		**13.98**	**19.12**	**25.27**
P<		**0.01**	**0.001**	**0.001**

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. Effect on Maximal Electroshock (MES) Induced Convulsions in Rats:

Centella asiatica extract at 200 mg/kg b.w. p.o. produced a more significant ($p<0.001$) effect in the phase of stupor, 10.49±1.427 minutes and recovery, minutes as compared to control, viz.,115.2±12.39 and It also didn't caused a decrease in the phase of flexion, which was just statistically significant ($p<0.01$) in comparison to control, minutes The MES model has served to identify antiepileptic drugs that are functionally similar to phenytoin and most of these compounds display, in common, the ability to inactivate voltage dependent Na+ channels in a dose dependent fashion, such compounds suppress sustained repetitive firing in cultured neurons. Hence, CAE +GGE may be expected to have a similar type of mechanism (Ramans and Jong, 1999).CAE + GGE at the above mentioned dose, administered acutely, might be effective against partial and secondary generalized seizure, as depicted by the protection by CAE in this model (Roman S. and Jong M.R., 1999)

B. Increasing current electroshock seizure test in mice (ICEST):

Albino mice were distributed in to 4 groups viz control (vehicle), CAE (200 mg kg^{-1} bw, po) treated, GGE (300mg kg^{-1} bw, po) treated, CAE (200 mg kg^{-1} bw, po) + GGE (300 mg kg^{-1} bw, po) treated. Anticonvulsant activity was assessed by subjecting the mice to increasing current electroshock seizure test (ICEST) and by measuring the increase in the current required to induced tonic hind limb extensor (seizure threshold current) for each mouse, 7h after the last dose. Mice of all groups individually challenged

with ICEST (2mA/2sec) 7 hours after last dose of drug i.e., starting with a current of 2mA, electroshock was delivered, via ear electrodes, as a single train of pulses (0.2 sec duration) of linear increase in current intensity of 2mA/2sec., until tonic hind limb extension (HLE) occurred or 30mA current intensity was reached, depending upon whichever event occurred first. This was recorded as the seizure threshold current (STC) for that animal. Combination of *C. asiatica* and *G. glabra* extracts exhibited their anticonvulsant potential by raising the seizure threshold current (20.38 ± 2.8, 24.38 ± 1.19 and 26.25 ± 0.49 for 7, 14 and 21 days drug treatment respectively) required to induce tonic hind limb extensor against electroshock.

Combination of CAE and GGE exhibited considerable anticonvulsant effect against MES induced convulsions by reducing the duration of flexion and clonus and doesn't show any change in the extensor phase. The current experimental findings suggest that the co-administration of *C. asiaitca* and *G. glabra* extracts depicted the potential anticonvulsant property against MES 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.

On the basis of various CNS activities and present data, exhibited by these extracts (*C. asiatica* and *G. glabra*) in experimental animals, it can be speculated that, the extracts containing chemical component(s) affect CNS and induce anticonvulsant properties. Thus, in conclusion, the combination *C. asiatica* and *G. glabra* possess potent anticonvulsant property against petit mal, secondarily generalized and grand mal seizures. Thus we conclude that the combination of these extracts is more beneficial than their individual effects in protecting the animals from various seizures. The combination found to be synergistic. But, further battery of test viz, *in vitro* tests and clinical studies are required to be carried out to confirm its anticonvulsant potential.

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