Research Article

Anticonvulsant action of aqueous extract of *Centella asiatica* and sodium valproate—A comparative study in pentylenetetrazole-induced seizures

Megaravalli R Manasa[1], Idoor D Sachin[2]

[1]Department of Pharmacology, Pushpagiri Institute of Medical Sciences and Research Center, Thiruvalla, Kerala, India.
[2]Department of General Surgery, Pushpagiri Institute of Medical Sciences and Research Center, Thiruvalla, Kerala, India.

Correspondence to: Megaravalli R. Manasa, E-mail: dr.manasamr@gmail.com

Received November 11, 2015. Accepted December 1, 2015

Abstract

Background: Antiepileptics available currently cause teratogenicity and chronic toxicity. Several plant extracts exhibit the potential to be developed into newer antiepileptics. **Aims and Objective:** To evaluate the anticonvulsant action of aqueous extract of *Centella asiatica* and compare it with sodium valproate in pentylenetetrazole (PTZ)-induced seizures in albino mice. **Materials and Methods:** Twenty-four male albino mice weighing 18–30 g were divided into four groups. Group I was administered distilled water, group II sodium valproate (300 mg/kg i.p.), and groups III and IV aqueous extract of *C. asiatica* (100 mg/kg and 300 mg/kg), respectively. Seizures were induced by giving PTZ (80 mg/kg s.c.) 1 h after administration of the respective treatments. Suppression of clonic seizure was considered as an indicator of anticonvulsant action of the compound. **Result:** The aqueous extract of *C. asiatica* at both doses (100 mg/kg and 300 mg/kg) suppressed the clonic seizures in mice, and this was statistically significant. The anticonvulsant action of the extract at a dose of 300 mg/kg was comparable to that of sodium valproate in this study. **Conclusion:** The aqueous extract of *C. asiatica* at a dose of 300 mg/kg has shown anticonvulsant action comparable to sodium valproate in PTZ-induced seizures.

KEY WORDS: *Centella asiatica*; Pentylenetetrazole; Sodium Valproate

Introduction

Epilepsy is characterized by a group of disorders with recurrent episodes of seizures owing to a chronic underlying process.[1,2,3] The lifetime risk of seizure is 5%, but highest risk is at extremes of age.[2] More than 20% of the patients exhibit uncontrolled seizures in spite of availability of a number of antiepileptics.[4,5] Current antiepileptics cause side effects such as teratogenicity, chronic toxicity, and adversely affect cognition and behavior.[6–8] Hence, there is a need for development of new antiepileptics.

Access this article online	
Website: http://www.njppp.com	Quick Response Code:
DOI: 10.5455/njppp.2016.6.0211201595	

In traditional systems of medicine, *Centella asiatica* (Sanskrit—Brahmi) has been used for various skin diseases, leprosy, and malaria.[9,10] It presents wound-healing and ulcer-healing properties, antinociceptive and anti-inflammatory properties, protective effect in psoriasis, cardioprotective property, immunomodulatory, cytotoxic, and antitumor properties, anxiolytic properties, and antioxidant and radioprotection properties.[11–23] It is extensively used for epilepsy in Ayurveda.[9,10] This study is done to evaluate the anticonvulsant action of *C. asiatica* and compare it with sodium valproate in mice.

Materials and Methods

Animals

Male albino mice weighing 18–30 g were obtained from Central animal house, KIMS, Hubli. They were housed in standard laboratory conditions and had free access to food and water ad libitum. Food and water were withdrawn just before

experimentation. They were divided into four groups, consisting of six animals each. The study protocol was approved by the institutional animal ethics committee.

Drugs and Chemicals

Sodium valproate (Sun Pharmaceuticals) and pentylenetetrazole (PTZ) (HiMedia Laboratories) were used in this study. All drug solutions were freshly prepared in distilled water at room temperature.

Plant Material

C. asiatica plants were obtained from Ayurvedic Mahavidyalaya, Hubli. The identification of the plant was done by the head of the department of Rasayanashastra, Ayurvedic Mahavidyalaya, Hubli.

Preparation of Aqueous Extract

The aqueous extract was prepared by cold maceration method. The plants were air dried and powdered. Thirty grams of the dry powder was soaked in 200 mL of cold water at room temperature. The extract was filtered, and the filtrate was dried at room temperature in a steady air current.[24]

The test solution of *C. asiatica* was prepared by dissolving 2 g of aqueous extract in 100 mL of distilled water at room temperature. It had a concentration of 20 mg/mL.

Preliminary Phytochemical Screening

A preliminary phytochemical screening of the extract revealed that the major components are the triterpenes—asiatic acid and madecassic acid, and their derived triterpene ester glycosides, asiaticoside, madecassoside, and centelloside. It contains other components including volatile oils, flavonoids, tannins, phytosterols, amino acids, and sugars.[10,25]

Assessment of Anticonvulsant Activity

PTZ-induced seizures. The animals were divided into four groups (n = 6). Group I received distilled water orally, and it served as the control. Group II received 300 mg/kg of sodium valproate intraperitoneally, and it was the standard group.[26] Groups III and IV were the test groups that received 100 mg/kg and 300 mg/kg of aqueous extract of *C. asiatica*, respectively. After 1 h, PTZ (80 mg/kg) was administered subcutaneously. The duration of various phases of ensuing convulsions were noted and subsequent mortality recorded. The suppression of clonic seizure was taken as an indicator of anticonvulsant action.[27]

Statistical Analysis

The results were expressed as mean ± standard error of mean (mean ± SE). Results were analyzed by one-way ANOVA, followed by Bonferroni test as post hoc test. A p value less than 0.05 was considered significant.

Result

All animals in the control group (group I) developed seizures. All animals in standard group (group II) were protected by sodium valproate and did not develop seizures. Group III animals, which received 100 mg/kg of aqueous extract of *C. asiatica*, exhibited significant delay in the onset of seizures ($p < 0.001$) and suppression of clonic seizure ($p < 0.01$) when compared with control, and it was statistically significant [Table 1]. The aqueous extract of *C. asiatica* at a dose of 300 mg/kg (group IV) has exhibited complete suppression of seizures, and its anticonvulsant activity is comparable to sodium valproate [Table 1].

Sodium valproate (group II) and aqueous extract of *C. asiatica* at a dose of 300 mg/kg (group IV) afford 100% protection from PTZ-induced seizures [Table 2]. Aqueous extract of *C. asiatica* at a dose 100 mg/kg (group III) has significantly increased the latency of seizure when compared with control group [Table 1].

Discussion

In this study, the anticonvulsant action of aqueous extract of *C. asiatica* was screened by PTZ method. The anticonvulsant action was compared with sodium valproate, which is considered as the standard drug for PTZ-induced seizures model. PTZ model is useful in screening of drugs effective in absence seizures.[27] In this study, the aqueous extract of *C. asiatica* at both doses (100 mg/kg and 300 mg/kg) has exhibited anticonvulsant action in the PTZ-induced seizure model. The anticonvulsant action of aqueous extract of *C. asiatica* at a dose of 300 mg/kg is comparable to

Table 1: Comparison of mean durations (in seconds) of different parameters in PTZ method

Parameters (duration in seconds)	Group I	Group II	Group III	Group IV
Latency of seizure	330.66 ± 10.8	0	419 ± 8.73***	0
Tonic hind limb flexion	1.5 ± 0.23	0	1.5 ± 0.23^{NS}	0
Tonic hind limb extension	10.66 ± 0.61	0	10.66 ± 0.43^{NS}	0
Clonic seizure	5.5 ± 0.44	0	3.5 ± 0.44**	0
Postictal depression	340.16 ± 3.78	0	189.16 ± 1.15***	0

Data expressed as mean ± SE.
n = 6, $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ (compared with control), NS, not significant.

Table 2: Percentage protection from clonic seizures in PTZ method

Group	% protection
Group I	0
Group II	100
Group III	0
Group IV	100

sodium valproate. Hence, the aqueous extract of *C. asiatica* may play a role in the treatment of absence seizures. Many studies have reported the anticonvulsant action of *C. asiatica* in other models of epilepsy. In a study by Gupta et al.,[28] the cognitive impairment and the oxidative stress induced by PTZ kindling was attenuated by *C. asiatica*. Katare and Ganachari[29] reported that *C. asiatica* has antilipidperoxidative and antiepileptic actions in the lithium–pilocarpine model of status epilepticus.

Visweswari et al.[30] found that one of the facets of anticonvulsant action of *C. asiatica* was by causing perceptible changes in the cholinergic system. In another study by Visweswari et al.,[31] there was a decrease in Na^+, K^+-ATPase, Mg^{2+}-ATPase, and Ca^{2+}-ATPase activities in brain during PTZ-induced epilepsy. The levels of these ATPases were increased in brain by pretreatment with *C. asiatica* extracts except with aqueous extract. PTZ induces seizures by antagonizing the inhibitory gamma-amino butyric acid (GABA)ergic neurotransmission.[32] Terpinoids, particularly triterpinoids and flavonoids, present in various plant extracts are reported to show anticonvulsant action in various epilepsy models such as PTZ model.[33] The phytochemical screening of aqueous extract of *C. asiatica* has revealed that it contains triterpines and flavonoids. Hence, the anticonvulsant action of aqueous extract of *C. asiatica* is probably owing to the triterpines and flavonoids present in it. The mechanism of anticonvulsant action may involve cholinergic system, the GABAergic neurotransmission, and by modulation of ATPases (Na^+- K^+, Mg^{2+} and Ca^{2+}) activities. However, there is a need for further studies to establish the exact mechanism of action.

Conclusion

The aqueous extract of *C. asiatica* exhibits anticonvulsant action comparable to sodium valproate in PTZ-induced seizures. However, further studies are needed to elucidate the exact mechanism of anticonvulsant action of this extract.

Acknowledgments

We thank Dr. Kamdod MA, Professor of Pharmacology, SDM Medical College, Dharwad, Karnataka, India, and staff of Department of Pharmacology, Karnataka Institute of Medical Sciences, Hubli, Karnatka, India, and Mrs. Nisha Kurian, Assistant Professor of Biostatistics, Pushpagiri Institute of Medical Sciences and Research Center, Thiruvalla, Kerala, India, for their help and support.

References

1. Lowenstein DH. Seizures and epilepsy. In: (Eds.). *Harrison's Principles of Internal Medicine*, vol 2, 19th edn. , New York: McGraw-Hill, 2015. pp. 2542–59.
2. Leach JP, Davenport RJ. Neurological disease. In: (Eds.). *Davidson's Principles and Practice of Medicine*. 22nd edn, New York: Churchill Livingstone Elsevier, 2014. pp. 1137–230.
3. Aminoff MJ, Kerchner GA. Nervous system disorders. In: (Eds.). *Current Medical Diagnosis and Treatment,* 54th edn, New York: McGraw-Hill, 2015:954–1026.
4. Liao WP, Chen L, Yi YH, Sun WW, Gao MM, Su T, et al. Study of antiepileptic effect of extracts from *Acorus tatarinowii* Schott. Epilepsia. 2005;46(Suppl 1):S21–4.
5. Sinoriya P, Irchhaiya R, Sharma B, Sahu G, Kumar S. Anticonvulsant and muscle relaxant activity of the ethanolic extract of stems of *Dendrophthoe falcata* (Linn. F.) in mice. Indian J Pharmacol. 2011;43(6):710–3.
6. Mathur S, Sen S, Ramesh L, Satish Kumar M. Utilization pattern of antiepileptic drugs and their adverse effects in a teaching hospital. Asian J Pharmaceut Clin Res. 2010;3(1):55–9.
7. Aldenkamp AP. Cognitive side effects of antiepileptic drugs. Neuropsychol Childhood Epilep. 2006;50:257–67.
8. Sutar RC, Kasture SB, Kalaichelvan VK. Evaluation of anticonvulsant activity of leaf extracts of *Holoptelea integrifolia* (Roxb.) planch in experimental animals. Int J Pharm Pharm Sci. 2014;6(4):308–11.
9. Nadkarni AK. *Hydrocotyla asiatica* Linn. In: *Dr. K. M. Nadkarni's Indian Materia Medica*, vol 1, 3rd edn. Bombay: Popular Book Depot, 1954:663–4.
10. World Health Organization. *WHO Monographs on Selected Medicinal Plants*, vol 1. Geneva: WHO, 1999. pp. 77–85.
11. Sunilkumar, Parameshwaraiah S, Shivakumar HG. Evaluation of topical formulations of aqueous extract of *Centella asiatica* on open wounds in rats. Indian J Exp Biol. 1998;36:569–72.
12. Chatterjee TK, Chakraborty A, Pathak M, Sengupta GC. Effects of plant extract *Centella asiatica* (Linn) on cold restraint stress ulcer in rats. Indian J Exp Biol. 1992;30(10):889–91.
13. Guo JS, Cheng CL, Koo MW. Inhibitory effects of *Centella asiatica* water extract and asiaticoside on inducible nitric oxide synthase during gastric ulcer healing in rats. Planta Med. 2004;70(12): 1150–4.
14. Cheng CL, Guo JS, Luk J, Koo MW. The healing effects of Centella extract and asiaticoside on acetic acid induced gastric ulcers in rats. Life Sci. 2004;74(18):2237–49.
15. Cheng CL, Koo MW. Effects of *Centella asiatica* on ethanol induced gastric mucosal lesions in rats. Life Sci. 2000;67(21):2647–53.
16. Somchit MN, Sulaiman MR, Zuraini A, Samsuddin L, Somchit N, Israf DA, et al. Antinociceptive and antiinflammatory effects of *Centella asiatica*. Indian J Pharmacol. 2004;36(6):377–80.
17. Natarajan S, Paily PP. Effect of topical *Hydrocotyle asiatica* in psoriasis. Indian J Dermatol. 1973;18(4):82–5.
18. Pragada RR, Veeravalli KK, Chowdary KP, Routhu KV. Cardioprotective activity of *Hydrocotyle asiatica* L. in ischemia-reperfusion induced myocardial infarction in rats. J Ethnopharmacol. 2004; 93:105–8.
19. Jayathirtha MG, Mishra SH. Preliminary immunomodulatory activities of methanol extracts of *Eclipta alba* and *Centella asiatica*. Phytomedicine. 2004;11(4):361–5.
20. Babu TD, Kuttan G, Padikkala J. Cytotoxic and anti-tumour properties of certain taxa of Umbelliferae with special reference to *Centella asiatica* (L) Urban. J Ethnopharmacol. 1995;48(1):53–7.

21. Wijeweera P, Arnason JT, Koszycki, Merali Z. Evaluation of anxiolytic properties of Gotukola—(*Centella asiatica*) extracts and asiaticoside in rat behavioral models. Phytomedicine. 2006;13(9–10):668–76.
22. Jayashree G, Muraleedhara GK, Sudarslal S, Jacob VB. Anti-oxidant activity of *Centella asiatica* on lymphoma-bearing mice. Fitoterapia. 2003;74(5):431–4.
23. Sharma J, Sharma R. Radioprotection of Swiss albino mouse by *Centella asiatica* extract. Phytother Res. 2002;16(8):785–6.
24. Okeke MI, Iroegbu CU, Eze EN, Okoli AS, Esimone CO. Evaluation of extracts of the roots of *Landolphia owerrience* for antibacterial activity. J Ethnopharmacol. 2001;78(2–3):119–27.
25. *Centella asiatica*—Monograph. Altern Med Rev. 2007;12(1):69–72.
26. Tandon VR, Gupta RK. An experimental evaluation of anticonvulsant activity of Vitex-negundo. Indian J Physiol Pharmacol. 2005;49(2):199–205.
27. Mittal R. Antiepileptics. In: (Ed.). *Drug Screening Methods*, 2nd edn. New Delhi: Jaypee Brothers Medical Publishers, 2009:400–22.
28. Gupta YK, Veerendra Kumar MH, Srivastava AK. Effect of *Centella asiatica* on pentylenetetrazole-induced kindling, cognition and oxidative stress in rats. Pharmacol Biochem Behav. 2003;74(3):579–85.
29. Katare SS, Ganachari MS. Effect of *Centella asiatica* on hypoxia induced convulsions and lithium pilocarpine induced status epilepticus and antilipid peroxidation activity. Ind J Pharmacol. 2001;33(2):128.
30. Visweswari G, Prasad KS, Chetan PS, Lokanatha V, Rajendra W. Evaluation of the anticonvulsant effect of *Centella asiatica* (gotu kola) in pentylenetetrazole-induced seizures with respect to cholinergic neurotransmission. Epilepsy Behav. 2010;17(3):332–5.
31. Visweswari G, Siva Prasad K, Lokanatha V, Rajendra W. The antiepileptic effect of *Centella asiatica* on the activities of Na^+/K^+, Mg^{2+} and Ca^{2+}-ATPases in rat brain during pentylenetetrazol-induced epilepsy. Indian J Pharmacol. 2010;42(2):82–6.
32. Olsen RW. The GABA postsynaptic membrane receptor-ionophore complex. Site of action of convulsant and anticonvulsant drugs. Mol Cell Biochem. 1981;39:261–79.
33. Sinoriya P, Irchhaiya R, Sharma B, Sahu G, Kumar S. Anticonvulsant and muscle relaxant activity of the ethanolic extract of stems of *Dendrophthoe falcata* (Linn. F.) in mice. Indian J Pharmacol. 2011; 43(6):710–3.

How to cite this article: Manasa MR, Sachin ID. Anticonvulsant action of aqueous extract of *Centella asiatica* and sodium valproate—A comparative study in pentylenetetrazole-induced seizures. Natl J Physiol Pharm Pharmacol 2016;6:128-131

Source of Support: Nil, **Conflict of Interest:** None declared.