International Journal of Current Research and Academic Review

ISSN: 2347-3215 Volume 3 Number 7 (July-2015) pp. 23-30

www.ijcrar.com

Antiepileptic activity of ethanolic extract of *Biophytum sensitivum* (L.) DC. in Animal models

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KEYWORDS

Epilepsy, Anticonvulsant, Maximal electroshock seizures, Pentylenetetrazole

A B S T R A C T

Objective of the study is to study the anticonvulsant activity of ethanolic extract of *Biophytum sensitivum* L. in animal models. The anticonvulsant activity of ethanolic extract of leaves of *Biophytum sensitivum* L. (50, 100 and 200 mg/kg p.o) in rats was assessed using maximum electroshock seizure (MES) test and pentylenetetrazol (PTZ) using albino mice. Preliminary Phytochemical investigation of the ethanolic extract of *Biophytum sensitivum* (Linn.) leaves reveals the presence of flavonoids, saponins, tannins, terpenes, steroids, amino acids and polyphenolic compounds. The ethanolic extract of *B. sensitivum* was significantly and dose-dependently reduced the duration of tonic hind limb extension in both experimental models and also delayed the onset of tonic-clonic convulsions induced by pentylenetetrazol in mice. In this work the dose of 200 mg/kg afforded protection to all animals. The results obtained from this study indicate that the ethanolic leaf extract of *Biophytum sensitivum* L. may be beneficial in both tonic clonic and absence seizures.

Introduction

Epilepsy is one of the most common neurological disorders. Worldwide, the prevalence is estimated to be 0.5 – 1%, and there is a life time incidence of 1 – 3%. It has important medical, social and psychological consequences (White, 2003). Epilepsy is a heterogeneous symptom complex, a chronic disorder characterized by recurrent seizures. Seizures are finite episodes of brain dysfunction resulting from abnormal discharge of cerebral neurons (Katzung *et al.*, 2009). It is estimated that in India (with population more than 1 billion), there will be 6–10 million people with

epilepsy, accounting for nearly 1/5 of global burden (Devi et al.).

The current therapy of epilepsy with modern antiepileptic drugs is associated with side effects, dose-related and chronic toxicity, as well as teratogenic effects, and approximately 30% of the patients continue to have seizures with current antiepileptic drugs therapy (Smith and Bleck, 1991; Mattson, 1995; SamrJn *et al.,* 1997). Therefore, there is a great need for the development of cheap, effective and safe anticonvulsant agents from plants and other sources.

Traditional systems of medicine are popular in developing countries and up to 80% of the population relies on traditional medicines or folk remedies for their primary health care need (Akerele, 1988). Medicinal plants are believed to be an important source of new chemical substances with potential therapeutic effects (Farnsworth, 1989). Several plants used for the treatment of epilepsy in different systems of traditional medicine have shown activity when tested in modern bioassays for the detection of anticonvulsant activity (Raza *et al.,* 1999) and many such plants are yet to be scientifically investigated.

Biophytum sensitivum (L.) is an herbaceous plant belonging to Oxalidaceae family. This herb has a tropical distribution and is found in warmer parts of the world in, tropical Africa, Asia. In India is found in the wet lands of southern India. *B. sensitivum* has several medicinal properties like antiseptic properties, the plant parts are used in the treatment of asthma and phthisis (Pullaiah, 2002), inflammatory diseases, and diabetes (Puri *et al.,* 1997; Mitra and Ambasta, 1988; Kirtikar and Basu, 1984). The biological activity of the plant shows hypoglycemic (Puri and Baral, 1998), immunomodulatory (Guruvayoorappan and Kuttan, 2007), apoptotic effect (Guruvayoorappan and Kuttan, 2007), chemoprotective (Guruvayoorappan and Kuttan, 2007), cell-mediated immune response (Guruvayoorappan and Kuttan, 2007), hypocholesterolemic (Puri, 2003), antiinflammatory (Jachak *et al.,* 1999), antitumor (Bhaskar and Rajalakshmi, 2010), effects on prostaglandin biosynthesis (Bucar *et al.,* 1997; Bucar *et al.,* 1998), antibacterial activity (Natarajan *et al.,* 2010) and antioxidant activity (Guruvayoorappan *et al.,* 2006). But, its anticonvulsant activity is not yet validated scientifically as on date. Hence, the present study was carried out to evaluate the anticonvulsant activity of ethanolic extract of *B. sensitivum* (L.) in animal models.

Material and Methods

Plant materials and preparation of the extract

The leaves were collected from commercial source. The collected leaves were cleaned of extraneous matter shade dried and powdered until a constant weight was attained. The powder was macerated for 48 hr in ethyl alcohol; it was subjected to percolation by using methanol as a solvent. The menstrum collected and concentrated under reduced pressure. The dried extract was stored at 4°C until ready for use.

The ethanolic extract of *Biophytum sensitivum* (L.) leaves was subjected to the following investigations,

1. Preliminary phytochemical screening
2. Pharmacological activities

a). Determination of acute toxicity (LD_{50})
b). Anticonvulsant activity.

Experimental animals

Healthy adult albino rats of Wistar strain weighing 180–250gm and Swiss albino mice weighing 20–25gm were used for this study. The animals were housed in polypropylene cages, maintained under standard laboratory conditions (12 hr light: 12 hr dark cycle; 24±2°C; 30–70% humidity). They were fed with standard rat pellet diet (Hindustan Lever Ltd, Mumbai, India) and water ad libitum. The animals were acclimatized to laboratory conditions for 7 days. All the protocols and experiments were performed in accordance to guideline of CPCSEA. The experimental protocol was approved by the Institutional Animal Ethics Committee (Reg. No. GU/IAEC/2014/763) Geetanjali Medical College & Hospital, Udaipur.

Drugs and chemicals

Pentylenetetrazole (Himedia laboratories Pvt. Ltd. Mumbai), Phenytoin and diazepam (Sigma Chemical Co. Hyderabad) were used during the experimental protocol.

Preliminary phytochemical screening

The freshly prepared ethanolic extract of leaf was subjected to phytochemical screening tests for the detection of various constituents (Kokate, 1994).

Acute toxicity studies

Acute toxicity tests in albino rats have proven the LD_{50} of *Biophytum sensitivum* (L.) leaves extract to be more than 5g/kg. (Mascolo *et al.,* 1989) The stomach showed no histomorphological changes in any of the dose of extract studied. Based on the results obtained from this study, the dose of ethanolic extract of *Biophytum sensitivum* (L.) leave for anticonvulsant activity was fixed to be 50 mg/kg, 100mg/kg and 200mg/kg body weight.

Anticonvulsant activity

Maximal electroshock induced seizures

Seizures were induced in rats by delivering electroshock of 50mA; 50Hz for 0.2 seconds by means of an electro-convulsiometer through a pair of ear clip electrodes (Kumar, 2008). The test animals (n=6) received 50, 100 and 200 mg/kg of ethanolic extract orally as a suspension prepared in 2% Tween-80 solution; 1hr prior to induce the convulsion and standard group received phenytoin (25 mg/kg i.p.) (Manigauha *et al.,* 2009) and tested after 30 minutes for MES induced seizure response. Suppression of tonic hind limb extension was taken as a measure of efficacy and all the experimental groups were compared with the control (treated with vehicle) group (Mahendran *et al.,* 2011; Goyal *et al.,* 2009). All precautions were taken to minimize animal suffering and to reduce the number of animal used.

PTZ-induced seizures

PTZ at the dose of 80 mg/kg (minimal dose needed to induce convulsions) was injected i.p. to induce clonic convulsions in mice. Doses of 50, 100 and 200mg/kg of the extract were administered orally into test groups. Tween-80 solution and Diazepam (4 mg/kg) were administered orally into two groups of animals as control and positive control groups, respectively. PTZ was injected i.p. 60 min after the administration of drugs. Occurrence of HLTE and duration of seizures were noted. If no HLTE occurred during the time limit, the animals were considered protected. Percentage of inhibition of seizures relative to controls was calculated (Vogel and Vogel, 1997).

Statistical analysis

The results were expressed as Mean ± SEM. The data were analyzed using one-way analysis of variance (ANOVA) followed by Dunnett's test. P-values < 0.05 were considered as significant.

Results and Discussion

Phytochemical screening of EEBS

Phytochemical screening revealed that EEBS showed the presence of flavonoids, saponins, tannins, terpenes, steroids, amino acids and polyphenolic compounds.

MES-induced seizures

The percentage of reduction in the MES-induced seizures with Phenytoin (25 mg/kg, p.o) after 60 minutes was 96.14%, that is, there was a highly significant (P<0.000) decrease in seizures compared to the control, whereas, three different doses of EEBS (50, 100 and 200 mg/kg p.o) showed a dose-dependent decrease in the HLTE, that is, 38, 54.5 and 74.4% respectively, when compared to the control. Maximum anticonvulsant activity was observed with 200 mg/kg of *Biophytum sensitivum* (L.). The observations are shown in table 1.

PTZ-induced seizures

In this test, EEBS (50, 100 and 200 mg/kg p.o) showed alteration in the occurrence of HLTE and duration of seizures significantly as related to controls in the model of convulsion induced by Pentylenetetrazole. The standard drug (Diazepam) also showed a highly significant effect when compared to the control (P<0.000). Low dose of EEBS (50 mg/kg) showed a significant effect (P<0.05). Three different doses of EEBS (50, 100 and 200 mg/kg p.o) showed a dose-dependent percentage inhibition in PTZ-induced convulsions, that is, 17.31, 48.46 and 65% respectively, when compared to the control. Maximum anticonvulsant activity was observed with 200 mg/kg of *Biophytum sensitivum*. The values are depicted in table 2.

Epilepsy is a serious neurological disorder associated with recurrent episodes of seizures due to the abnormal episodic bursts of electrical activity in the brain. The currently available drugs used for treating epilepsy are associated with many adverse effects. Therefore, there is a great need for the development of cheap, effective and safe anticonvulsant agents from plants and other sources. Plants and their phytoconstituents have important role in the development of potent anticonvulsant agent.

Biophytum sensitivum DC (Oxalidaceae) is used as a traditional folk medicine in ailments such as inflammation, arthritis, wounds, tumors, burns, gonorrhea, stomach ache, asthma, cough, hypocholesterolemia, infectious diseases, degenerative joint disease, urinary calculi, diabetes, snake bite, amenorrhea and dysmenorrhea. The leaves of the plant have inhibition of prostaglandin synthesis and anti-oxidant activity; it may prevent generation of free radicals. Hence, it is considered a safe herbal medicine with only few and insignificant adverse effects.

MES-induced convulsion model is a widely used tool to screen drugs for generalized tonic-clonic seizures. MES causes several changes at the cellular level, disrupting the signal transduction in the neurons. MES causes cellular damage by facilitating the entry of Ca^{2+} into the cells in large amounts, prolonging the duration of convulsions (DeSarro *et al.,* 1999). Apart from Ca^{2+} ions, MES may also facilitate the entry of other positive ions like Na+, blockade of which, can prevent the MES-induced tonic extension (Gale, 1992). Currently available

anticonvulsant drugs like sodium valproate and phenytoin act by modulation of these ion channels (Rang *et al.,* 2003). On the other hand, drugs that antagonize NMDA receptors or potentiate opioids and GABA receptors are also reported to protect against MES-induced seizures (Inan and Buyukafsar, 2008).

Pentylenetetrazole (PTZ) exerting its convulsant effect by inhibiting the activity of gamma amino butyric acid (GABA) at GABA-A receptors (Bum *et al.,* 2010). Gamma amino butyric acid is the major inhibitory neurotransmitter which is implicated in epilepsy.

Table.1 Effect of ethanolic extract of leaves of *Biophytum sensitivum* (L.) on hind limb extension induced by MES in rats

Group No.	Group	Dose (mg/kg)	Hind limb extension (Sec)	Percentage inhibition of convulsions (%)
1	Control	–	18.69 ± 1.325	–
2	Phenytoin	25	0.72 ± 0.1045	96.14
3	EEBS	50	11.62 ± 1.128	37.82
4	EEBS	100	8.5 ± 0.7291*	54.52
5	EEBS	200	4.782 ± 0.4365*	74.41

Value are expressed as mean ± SEM (n = 6). EEBS – Ethanolic extract of *Biophytum sensitivum* (L.) leaves. * P < 0.001 when compare with control group

Table.2 Effect of ethanolic extract of leaves of *Biophytum sensitivum* (L.) on PTZ induced seizures in mice

Group No.	Group	Dose (mg/kg)	Onset time (Sec)	Duration of HLTE (Sec)	Percentage inhibition of convulsions
1	Control	–	51.4 ± 0.1702	35.47 ± 2.169	–
2	Diazepam	4	00 ± 00	00 ± 00	100
3	EEBS	50	53.5 ± 0.2362*	29.33 ± 1.067	17.31
4	EEBS	100	58.6 ± 0.164*	18.28 ± 1.188*	48.46
5	EEBS	200	62.33 ± 0.1932*	12.39 ± 1.281*	65.06

Value are expressed as mean ± SEM (n = 6). EEBS – Ethanolic extract of *Biophytum sensitivum* (L.) leaves. * P < 0.001 when compare with control group

The enhancement and inhibition of the neurotransmission of GABA will attenuate and enhance convulsion, respectively (Rang *et al.,* 2003). Diazepams a standard antiepileptic drug has been shown to exert their antiepileptic effects by enhancing GABA-mediated inhibition in the brain (Manocha *et al.,* 1997). This implies that *Biophytum sensitivum* (L.) may be effective as an anticonvulsant medicinal plant and its anticonvulsant effect by involve Gabergic inhibitory and glutaminergic excitatory mechanisms or inhibition of the voltage gated sodium channel (Löscher *et al.,* 1996).

Preliminary Phytochemical investigation of the ethanolic extract of *Biophytum sensitivum* (Linn.) leaves reveals the presence of flavonoids, saponins, tannins, terpenes, steroids, amino acids and polyphenolic compounds. The ethanolic extract of *B. sensitivum* was significantly

and dose-dependently reduced the duration of tonic hind limb extension in both experimental models and also delayed the onset of tonic-clonic convulsions induced by pentylenetetrazol in mice. In this work the dose of 200 mg/kg afforded protection to all animals. The anticonvulsant activity may be due to the presence of flavonoids and sterols in the extract.

Conclusion

The present study revealed that ethanolic extract of *Biophytum sensitivum* (L.) leaves possessed profound anticonvulsant activity in MES and PTZ-induced convulsive models. The present work did not include the identification of the active principal and its mechanism of action. Therefore, further research is required to elucidate specific mechanism(s) and active principles responsible for its anticonvulsant property.

Acknowledgment

The authors would like to thank the chairman and management of the Geetanjali Medical College and Hospital for providing laboratory facilities and supporting this research work.

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