ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES
Knowledge to Innovation

Vol 11, Issue 12, 2018

Online - 2455-3891
Print - 0974-2441

Research Article

EVALUATION OF ANTICONVULSANT ACTIVITY OF *PREMNA HERBACEA* (ROXB.) EXTRACTS IN PENTYLENETETRAZOL AND MAXIMAL ELECTROSHOCK-INDUCED CONVULSIONS IN MICE

ASHISH DATTATRAYA CHIMBALKAR[1]*, VYAWAHARE NS[2]

[1]Department of Pharmacy, Ponnaiyah Ramajayam Institute of Science and Technology University, Thanjavur, Tamil Nadu, India.
[2]Department of Pharmacy, Dr. DY Patil College of Pharmacy, Pune, Maharashtra, India. Email: ashishchimbalkar@gmail.com

Received: 20 June 2018, Revised and Accepted: 17 August 2018

ABSTRACT

Objective: In the present study, three different extracts of *Pemna herbacea* (Roxb.) were evaluated for its anticonvulsant activity against pentylenetetrazol (PTZ) and maximal electroshock (MES)-induced convulsions in mice.

Methods: The shed-dried powder of *P. herbacea* roots was passed through a sieve and subjected to extraction using Soxhlet apparatus with 70% ethanol, petroleum ether, and chloroform to get respective extracts named as ethanolic extract of *P. herbacea*, petroleum ether extract of *P. herbacea*, and chloroform extract of *P. herbacea* (PHC). Preliminary phytochemical analysis and acute oral toxicity study were done. Thereafter, the extracts were analyzed for PTZ- and MES-induced convulsions.

Results: The results revealed that PHC at the doses 200 and 400 mg/kg was effective against both, i.e., PTZ- and MES-induced convulsions. Overall PHC 400 mg/kg was most effective, as it significantly delayed onset of convulsions ($p<0.01$) and reduced % mortality (50%) in PTZ model, while in MES model, it showed the highest reduction in duration of hind limb extension ($p<0.01$) and percentage protection (33.33%).

Conclusion: The results reported anticonvulsant potential of PHC against both PTZ- and MES-induced convulsions suggesting mixed mechanism of action which may be attributed to different phytochemicals acting simultaneously.

Keywords: Anticonvulsant, *Premna herbacea* (Roxb.), Maximal electroshock, Pentylenetetrazole.

 DOI: http://dx.doi.org/10.22159/ajpcr.2018.v11i12.28006

INTRODUCTION

Convulsion is one of the most common and chronic neurological disorders in human beings with an incidence rate of approximately 1% of the total population [1,2]. It is characterized by recurrent and unpredictable interruptions of normal brain function that is epileptic seizures [3]. The current therapeutic treatment of epilepsy with modern anticonvulsant drugs is associated with variety of side effects, dose-dependent toxicity, especially, on chronic administration and teratogenic effects [4-6]. Moreover, approximately 30% of the patients exhibit reoccurrence of the symptoms which is the most important concern to address [7]. This increasing occurrence of epilepsy is attributed to an increase in stress, change in lifestyle, altered food habits, excessive alcohol consumption, and concomitant drug administration [8,9]. Suggesting need for the discovery of new drug. India has one of the richest medical plant traditions in the world, and the traditional Indian medicinal system has always exemplified the phenomena of symbiosis [10]. As per literature survey, approximately 25,000 effective plant-based formulations used in folk medicine and known to rural communities in India [11,12]. The roots of *Pemna herbacea* are traditionally being used to prevent and control convulsions but have not been well documented scientifically. On the other hand, the scientific validation for its other claims such as analgesic, anti-inflammatory, and antiulcer [13] suggests conducting preclinical evaluation for scientific validation of its anticonvulsant potential to give newer, safer, and effective anticonvulsant drug.

MATERIALS AND METHODS

Preparation of extracts

P. herbacea (Roxb.) plant material, i.e., roots was collected from Southern Ghats region and authenticated by Dr. K. Madhawa Chetty at Sri Venkateswara University, Tirupati. These roots were shade dried and powdered. The powder of *P. herbacea* roots was passed through a sieve (No. 40) and subjected to extraction using Soxhlet apparatus with 70% ethanol, petroleum ether, and chloroform at a temperature <20°C to get respective extracts named as ethanolic extract of *P. herbacea* (PHE) and petroleum ether extract of *P. herbacea* (PHP). After filtration, dark brown extracts were evaporated at 50°C [14].

Animal selection

Swiss albino mice (18–25g) of either sex were procured from M/s. National Toxicological Center, Pune. The mice were housed separately in the animal house and were fed on a standard pellet diet and provided water *ad libitum*. After approval of the Institutional Animal Ethics Committee from the National Toxicology Center, Pune, the studies were performed.

Drugs and chemicals

Pentylenetetrazol, Sigma, St. Louis, USA, diazepam Ranbaxy, India, and phenytoin sodium, M. J. Pharmaceuticals, Gujarat.

These drugs and chemical were purchased from local vendor.

Statistical analysis and calculations

Data were expressed as mean±standard error of the mean and statistically analyzed using one-way analysis of variance followed by Tukey–Kramer multiple comparisons test.

Methods

Preliminary phytochemical analysis

Preliminary phytochemical analysis was carried out using established methods to record the presence of phytochemicals [15].

Determination of acute toxicity study (LD_{50})

The acute toxicity of the extracts (PHE, PHP, and chloroform extract of *P. herbacea* [PHC]) was performed using albino mice as per the OECD guideline no 423 [16].

Antiepileptic activity of PHE, PHP, and PHC on pentylenetetrazol (PTZ)-induced convulsions

The 66 pre-selected mice of either sex (18–22 g) were divided into eleven groups each consisting six mice. All these mice were subjected to respective treatment as mentioned in Table 1, for the period of 7 days. On the 7th day 60 min after dosing, all mice were subjected to intraperitoneal injection of PTZ (80 mg/kg), and the onset of convulsions with percentage of mortality of mice were recorded and compared against vehicle-treated control mice. Diazepam (5 mg/kg) was used as a reference standard [17,18].

Antiepileptic activity of PHE, PHP, and PHC on maximal electroshock-induced convulsions

The 66 pre-selected mice of either sex (18–22 g) were divided into eleven groups each consisting six mice. All these mice were subjected to respective treatment as mentioned in Table 2, for the period of 7 days. On the 7th day 60 min after dosing, all mice were subjected to maximal electroshock treatment (40 mA for 0.2 s) and the reduction in duration of hind limb extension(the mice showing extension <10 s) was considered as protected as well as percent protection of mice was recorded and compared against vehicle-treated control mice. Diazepam (5 mg/kg) was used as a reference standard [19-21].

RESULTS

Preliminary phytochemical screening of the extracts showed the prominent presence of phenolic components, steroids, tannins, and glycosides (Table 3).

The acute oral toxicity study revealed that all the three extracts 100, 200, and 400 mg/kg were safe when administered until the dose of 2000 mg/kg. Based on pilot study and previously published papers, three doses, i.e., 100, 200, and 400 mg/kg were selected for anticonvulsant study [22].

In the PTZ model, the mice treated with PHE 400 mg/kg and PHP 400 mg/kg significantly delayed the onset of convulsions and were found to be equipotent ($p<0.05$), whereas PHC 200 mg/kg and 400 mg/kg were more significant ($p<0.01$) in this regard. Similar results were obtained for percentage mortality. PHC 400 mg/kg extract was the most effective test dose in this regard.

DISCUSSION

Medicinal plants are moving from fringe to mainstream use with a greater number of people seeking remedies and health approaches with lesser side effects caused by synthetic chemicals. Recently, considerable attention has been paid to utilize eco-friendly and bio-friendly plant-based products for the prevention and cure of different human diseases [23]. Considering the adverse effects of synthetic drugs, the world's population is looking for natural remedies which are comparatively safer and equally effective [24-26]. It is documented that about 80% of the world's population has faith in traditional medicine, particularly plant-based drugs for their primary health care [27].

Plants consist of numerous constituents called phytochemicals with diverse medicinal values. Moreover, the claims made in the traditional literature are vague [28]. Hence, scientific validation of these claims is vital to make therapy more effective and patient-friendly. The present study has been conducted to coordinate the traditional claim, experimental observations, and role of phytochemicals to establish its close relationship toward the actual therapeutic outcome [28-30]. In the present investigation, preliminary phytochemical analysis of PHE showed the presence of glycosides, flavonoids, tannins, and steroids, while petroleum ether extract showed the presence of steroids Tannins. Steroids, anthraquinone, and coumarin glycosides were found in chloroform extract. The earlier scientific studies have revealed that these phytochemicals are chiefly responsible for the pharmacological actions [29-31] and thereby suggested worth to explore the traditional claims [32]. Toxicity is one of the most important aspects of any medication to govern the extent of therapeutic utility. Since preliminary phytochemical results gave indication of further pharmacological

Table 1: Effects of PHE, PHP, and PHC on PTZ-induced convulsions

Groups	Treatment	Onset of convulsion (s)	% Mortality
I	Distilled water 10 ml/kg	247.06±1.15	100
II	Diazepam 5 mg/kg	485.86±2.66	0.00
III	PHE 100 mg/kg	245.18±1.22	83.33
IV	PHE 200 mg/kg	244.47±1.49	83.33
V	PHE 400 mg/kg	253.93±1.47*	66.66
VI	PHP 100 mg/kg	246.98±1.31	100
VII	PHP 200 mg/kg	247.41±0.87	83.33
VIII	PHP 400 mg/kg	246.51±2.01*	83.33
IX	PHC 100 mg/kg	248.96±1.12	100
X	PHC 200 mg/kg	257.34±0.86**	66.66
XI	PHC 400 mg/kg	261.87±1.34**	50.00

(*$p<0.05$, **$p<0.01$). The values are represented as mean±SEM (n=6). SEM: Standard error of the mean. In maximal electroshock MES-induced convulsions, PHE 400 mg/kg and PHP 400 mg/kg were found to be equally significant ($p<0.05$) to reduce the hind limb extension phase, whereas PHC 200 mg/kg and 400 mg/kg showed dose-dependent action ($p<0.05$ and $P<0.01$) in this regard. On the other hand, PHC 400 mg/kg dose only showed 33.33% protection, and rest all doses of extracts were ineffective. PHE: Ethanolic extract of *P. herbacea*, PHP: Petroleum ether extract of *P. herbacea,* PHC: Chloroform extract of *P. herbacea*

Table 2: Effect of PHE, PHP, and PHC in MES convulsion. The values are represented as mean±SEM (n=6)

Groups	Treatment	Reduction in hind limb extensor	% Recovery
I	Distilled water 10 ml/kg	26.18±1.26	0
II	Phenytoin 25 mg/kg	07.84±1.80	83.33
III	PHE 100 mg/kg	26.57±1.73	0
IV	PHE 200 mg/kg	25.99±1.47	0
V	PHE 400 mg/kg	22.85±1.46*	0
VI	PHP 100 mg/kg	27.30±1.48	0
VII	PHP 200 mg/kg	26.17±1.42	0
VIII	PHP 400 mg/kg	22.42±1.98**	0
IX	PHC 100 mg/kg	25.99±2.20	0
X	PHC 200 mg/kg	22.74±0.91*	0
XI	PHC 400 mg/kg	13.64±3.63**	33.33

(*$p<0.05$, **$p<0.01$). SEM: Standard error of the mean. The reference standard phonytoin was most effective in both these parameters. PHE: Ethanolic extract of *P. herbacea,* PHP: Petroleum ether extract of *P. herbacea*, PHC: Chloroform extract of *P. herbacea*

Table 3: Preliminary phytochemical analysis

Phytochemical	PHC	PHP	PHE
Carbohydrates Carbohydrates	++	-	-
Protein	+	-	-
Amino acids	-	-	-
Fats and oils	-	-	-
Steroids	+	+	+
Volatile oils	-	-	-
Cardiac glycosides	+	-	-
Anthraquinone glycosides	-	-	-
Cyanogenetic glycosides	-	-	-
Coumarin glycosides	+	-	-
Saponins	+	-	-
Flavonoids	-	-	-
Alkaloids	-	-	-
Tannins and phenolic compounds	+	+	+

PHE: Ethanolic extract of *P. herbacea*, PHP: Petroleum ether extract of *P. herbacea*, PHC: Chloroform extract of *P. herbacea*

screening, it becomes mandatory to evaluate these extracts for their toxicity profile and confirm its safety [33]. As per the principles of general pharmacology, drug shall not only be pharmacologically effective but also free of toxicity or else its undesirable effect shall be within acceptable range. The maintenance of desirable risk and benefit ratio is prerequisite to label any compound as a drug [34]. The acute oral toxicity studies of PHE, PHP, and PHC of *P. herbacea* revealed that all these extracts were found to be safe up to the dose of 2000 mg/kg. The results of acute oral toxicity study suggested that these extracts can be screened preclinically for validation of its claim in accordance with the methods mentioned in previously published reports. Further, the pilot study and dose range were found to be effective without any toxic outcome in earlier published reports, and three different doses, i.e., 100, 200, and 400 mg/kg for each extract were selected for anticonvulsant activity [35,36]. Though the PTZ model of all three dose have reported significant anticonvulsant activity, overall PHC was found to be more effective. The PTZ induces convulsions through inhibition of gamma-aminobutyric acid (GABA) transmission which resembles petit mal type of epilepsy in humans [37], and hence, this extract suggested GABA potentiating as a possible mechanism of action. For the petit mal type of epilepsy delayed onset, reduction in duration ultimately leading to prevent serious outcomes such as mortality is prime objective. The PHC at both the doses, i.e., 200 mg/kg and 400 mg/kg showed significant improvement in the onset of convulsions and mortality. The prevention of mortality is the most important outcome of this study as it provides chance to manage the recurrence in more better manner. In MES-induced convulsions, highest doses of all extract were effective, but the level of significance was more with PHC 400 mg/kg. In addition, PHC 200 mg/kg was also endorsing effective suggested it as a most potent extract.

The MES induces convulsions chiefly by the inhibition of Na^+ channels and represents human grand mal type epilepsy. The current results indicated activation of sodium channels by the extract. The PHC showed effectiveness in both the models which may be due to the presence of more than one active phytochemical acting simultaneously. The exact role of these phytochemicals will be revealed on further detailed investigation; however, it is very much clear that the extract has potential to act in a mixed type of epilepsy for which modern medicine has limitation.

CONCLUSION

The chloroform extract of the root of *P. herbacea* showed significant anticonvulsant activity against both PTZ- and MES-induced convulsions and thereby validated its traditional claim. The study also suggested a mixed mechanism of action which may be attributed to different phytochemicals acting simultaneously.

ACKNOWLEDGMENT

Authors are thankful to the National Toxicological Institute for the provision of facility to carry out experiments as well as PRIST University for their valuable support.

AUTHORS' CONTRIBUTIONS

Performed collection of sample, extraction, analysis, and statistical analysis of data and wrote the manuscript. The second author supervised the progress of work and helped in the evaluation of the manuscript and its language corrections.

CONFLICTS OF INTEREST

Authors declare no conflicts of interest.

REFERENCES

1. Delgado-Escueta AV, Treiman DM, Walsh GO. The treatable epilepsies. N Engl J Med 1983;308:1508-14.
2. Blume WT, Lüders HO, Mizrahi E, Tassinari C, van Emde Boas W, Engel J Jr, *et al.* Glossary of descriptive terminology for ictal semiology: Report of the ILAE task force on classification and terminology. Epilepsia 2001;42:1212-8.
3. Fisher RS, van Emde Boas W, Blume W, Elger C, Genton P, Lee P, *et al.* Epileptic seizures and epilepsy: Definitions proposed by the international league against epilepsy (ILAE) and the international bureau for epilepsy (IBE). Epilepsia 2005;46:470-2.
4. Yaro AH, Anuka JA,Salawu OA, Magaji MG. Anticonvulsant activities of methnol extract of *Chrysanthellum indicum* Linn, vatke in mice and chicks Nig. J Pharm Sci 2007;6:22-7.
5. Poole K, Moran N, Bell G, Solomon J, Kendall S, McCarthy M, *et al.* Patients' perspectives on services for epilepsy: A survey of patient satisfaction, preferences and information provision in 2394 people with epilepsy. Seizure 2000;9:551-8.
6. Samrén EB, van Duijn CM, Koch S, Hiilesmaa VK, Klepel H, Bardy AH, *et al.* Maternal use of antiepileptic drugs and the risk of major congenital malformations: A joint European prospective study of human teratogenesis associated with maternal epilepsy. Epilepsia 1997;38:981-90.
7. Sander JW. Some aspects of prognosis in the epilepsies: A review. Epilepsia 1993;34:1007-16.
8. World Health Organization (WHO). Neurological Disorders: Public Health Challenges. Geneva, Switzerland: WHO Library Cataloguing-in-Publication Data; 1993. p. 256-69
9. Holmes LB, Harvey EA, Coull BA, Huntington KB, Khoshbin S, Hayes AM, *et al.* The teratogenicity of anticonvulsant drugs. N Engl J Med 2001;344:1132-8.
10. Linnaeus C. Mantissa Plantarum. Stockholm: Laurentius Salvius; 1771. p. 587.
11. Harley RM, Atkins S, Budantsev PD. In: Kubitzki K, editor. The Families and Genera of Vascular Plants: Flowering Plant-Dicotyledons. Labiatae, Germany: Springer-Verlag; 2004. p. C267-8.
12. Fletcher HR. The Siamese Verbenaceae. Bulletin of Miscellaneous Information. Kew: Royal Botanic Gardens; 1938. P. 401-45.
13. Narayanan N, Thirugnanasambantham P, Viswanathan S, Kannappa Reddy M, Vijayasekaran V, Sukumar E, *et al.* Antipyretic, antinociceptive and anti-inflammatory activity of *Premna herbacea* roots. Fitoterapia 2000;71:147-53.
14. Dhamija I, Kumar N, Manjula SN, Parihar V, Setty MM, Pai KS, *et al.* Preliminary evaluation of *in vitro* cytotoxicity and *in vivo* antitumor activity of *Premna herbacea* roxb. In Ehrlich ascites carcinoma model and Dalton's lymphoma ascites model. Exp Toxicol Pathol 2013;65:235-42.
15. Khandelwal KR, Practical Pharmacognocy. Mumbai: Pragati Books Pvt. Ltd.; 2008.
16. OECD. Guideline on Acute Oral Toxicity, Environmental Health and Safety Monograph Series on Testing and Adjustment No 423; 2001.
17. Vyawahare NS, Bodhankar SL. Neuropahrmacological profile of piper betel leaves extract in mice. Pharmacologyonline 2007;2:146-62.
18. Dhingra D, Parle M, Kulkarni SK. Memory enhancing activity of *Glycyrrhiza glabra* in mice. J Ethnopharmacol 2004;91:361-5.
19. Kulkarni SK. Hand book of Experimental Pharmacology. 3rd ed. New Delhi: Vallabh Prakashan; 2005. p. 137-40.
20. Kulkarni SK, Verma A. Protective effect of – BR – 16A (Mentat), a herbal preparation on alcohol abstinence induced anxiety and convulsions. Ind J Expt. Boil, 1993; 31: 435-39.
21. Gupta YK, Malhotra J. Adenosinergic system as an endogenious anticonvulsant mechanism. J Phys Pharm 1997;41:329-43.
22. Viswanatha GL, Venkataranganna MV, Prasad NB, Ashok G. Evaluation of anti-epileptic activity of leaf extracts of *Punica granatum* on experimental models of epilepsy in mice. J Intercult Ethnopharmacol 2016;5:415-21.
23. Thai Plant Names Tem Smitinand. Bangkok: The Forest Herbarium. Royal Forest Department revised edition; 2001. p. 810.
24. Gijtenbeek JM, van den Bent MJ, Vecht CJ. Cyclosporine neurotoxicity: A review. J Neurol 1999;246:339-46.
25. Johnson WC, Williams OW. Warfarin toxicity. J Vascul Surg 2002;35:413-21.
26. Henry D, Sylvia A, Chidambaranathan S, Nirmala P. A prospective study of drug utilization pattern of anti-epileptic drugs and their adverse effects in a tertiary care hospital. Int J Curr Pharm Res 2017;9:42-5.
27. Pathare YS, Wagh VD. Herbal medicines and nutritional supplements used in the treatment of glaucoma: A review. Res J Pharm Biol Chem Sci 2012;3:331-9.
28. Joy PP, Thomas J, Mathew S, Skaria BP. In: Bose TK, Kabir J, Das P, Joy PP, editors. Medicinal Plants. Tropical Horticulture. Vol. 2. Calcutta: Naya Prokash; 2007.
29. Kokate CK. Practical Pharmacognosy. 4th ed. New Delhi: Vallabh

Prakashan; 1997.
30. Madhavi E, Gopala RK, Ruckmani A, Umar D. Evaluation of antiepileptic activity of ethanolic extract of *Azima tetracantha* root in mice. Int J Curr Pharm Res 2016;8:76-9.
31. Chaulya NC, Haldar PK, Mukherjee A, Khanra R, Chakraborty A. Anticonvulsnt acivity of methanolic extract of Rhizomes of *Cyperus tegetum* Roxb. J Pharm Sci Tec 2011;1:1-5.
32. Nimbal SK, Vekatrao N, Basaavraj P. Evaluation of anticonvulsant activity of alcoholic extract of *Beninkasa hispida* (Thunb) cogn. Fruit extracts. Int Res J Pharm 2011;2:166-68.
33. Atigari DV, Gundamaraju R. Evaluation of antiepileptic activity of methnolic extract of *Celastrus paniculatus*, Wild whole plant in rodents. Int J Pharm Phytopharm Res 2012;2:20-5
34. Gogte HN. Ayurvedic Pharmacology and Therapeutic Uses of Medicinal Plant, Drvyagunavidnyana: Chaukhamba Publication; 2009. p. 450-3.
35. Rang HP, Ritter RJ, Flower G. Pharmacology. 5th ed. London: Churchill livingstone; 2003.
36. Macnamara JO, editors. Goodman Gilman's the Pharmacological Basis of Therapeutics Pharmacotherapy of Epilepsy. 11th ed. New York: McGrewhill publiahers; 2001. p. 45-65.
37. Abubakar K, Ugwah-Oguejiofor CJ, Usman MN, Abubakar SB, Abdulkadir R. Evaluation of anticonvulsant effect of the methanol extract of *Evolvulus alsinoides* in mice, Sch. Acad J Pharm 2013;2:436-41.