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

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ABSTRACT

Objective: In the present study, three different extracts of Premna 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 is 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 (53.3%).

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, Pentylenetetrazol.

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, slow concomitant medicine 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 the literature survey, approximately 25,000 effective plant-based formulations used in folklore medicine and known to rural communities in India [11,12]. The roots of Premna 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 antihyperglycemic 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 were collected from Southern Ghats region and authenticated by Dr. K. Mathawa 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 comparisions 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 (LD50)

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, steriods, 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 PHP 200 mg/kg and 400 mg/kg were more significant (p<0.01) in this regard. Similar results were obtained for percentage mortality. PHP 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 steriods Tannins. Steroids, antraquione, 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

| Groups | Treatment | % Recovery |
|--------|-----------|------------|
| I      | Distilled water 10 ml/kg | 100 |
| II     | Phenyo in 25 mg/kg | 83.33 |
| III    | PHE 100 mg/kg | 83.33 |
| IV     | PHE 200 mg/kg | 79.92 |
| V      | PHE 400 mg/kg | 77.29 |
| VI     | PHP 100 mg/kg | 75.50 |
| VII    | PHP 200 mg/kg | 72.81 |
| VIII   | PHP 400 mg/kg | 68.22 |
| IX     | PHC 100 mg/kg | 65.64 |
| X      | PHC 200 mg/kg | 62.06 |
| XI     | PHC 400 mg/kg | 58.48 |

(*p<0.05, **p<0.01). SEM: Standard error of the mean. The reference standard phenytion 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              | ++  | -   | -   |
| Protein                    | +   | -   | -   |
| Amino acids                |     | -   | -   |
| Fats and oils              | -   | -   | -   |
| Steroids                   | +   | +   | +   |
| Volatile oils              | -   | -   | -   |
| Cardiac glycosides         | -   | -   | -   |
| Anthaquione glycosides     | -   | -   | -   |
| Cyangenic glycosides       | -   | -   | -   |
| Coumarin glycosides        | +   | -   | -   |
| Saponins                   | +   | -   | -   |
| Flavonoids                 | +   | -   | -   |
| Alkaloids                  | +   | -   | -   |
| Tannins and phenolic componds | + | + | + |

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

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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.

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