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EVALUATION OF ANTICONVULSANT ACTIVITY OF *PREMNA CORYMBOSA* IN EXPERIMENTAL MICE

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ABSTRACT

The drug is obtained from the dried whole plants of *Premna corymbosa* of family Verbenaceae. It is also called Munna, Arni, Agnimanthah etc. Literature survey revealed that the hydroalcoholic extract of roots of *Premna corymbosa* Rottl possess anti hyperglycemic, anti-inflammatory, antihyperlipidemic and antibacterial activity. The major chemical constituents obtained from it is flavanoids, alkaloids etc. The root is regarded as an alternative tonic and useful in the diseases of nervous system. To confirm the veracity of aforementioned claim, we have evaluated the anticonvulsant effect of the extract. In this investigation, the mice were pretreated with different doses of *Premna corymbosa* extract (200, 400mg/kg) for 10 days and then, they were subjected to Pentylenetetrazole (80 mg/kg) and maximal electroshock seizures (50mA, 0.2s) treatment. The methanolic extract of *Premna corymbosa* at the dose of 200 and 400 mg/kg significantly delayed the latency to the onset of first clonus of Pentylenetetrazole treated mice respectively. Whereas in case of maximal electroshock-seizures, the dose of 200 and 400 mg/kg significantly reduced the duration of hind limb extension and both the doses were statistically found to be equipotent. The reference standards, diazepam (2 mg/kg) and Phenytoin (25 mg/kg) provided complete protection. Thus, present study revealed anticonvulsant effect of *Premna corymbosa* against Pentylenetetrazole and maximal electroshock-induced convulsions in mice.

KEYWORDS: Anticonvulsant, *Premna corymbosa*, MES, PTZ.

INTRODUCTION

Herbal drugs constitute a major part in all traditional systems of medicine. Herbal medicine is triumph of all popular therapeutic diversity. Plants above all other agents have been used for medicine from time immemorial because they have fitted the immediate personal needs are easily accessible and inexpensive. For these and other reasons, the use of plants for medicines around the world still the preeminent synthetic chemist and that in plants particularly; there are almost infinite reserves of fascinating chemical constituents with actual and potential effects on human body. As such information accumulates; it becomes possible to better understand the traditional uses of plants. There are estimated to be at least, 250000 species of higher plants and around 20 million species in total; most of these have not been tested for biological activity [1].

In the traditional system of medicine, the whole plant of *Premna corymbosa* (Family: Verbenaceae.) have been in clinical use for centuries. Roots have antihyperglycemic [2] antihyperlipidemic [3], astringent, acrid, sweet, thermogenic, anodyne, bitter, pungent, laxative [4], stomachic, anaemia, inflammation, bronchitis, dyspepsia, piles, constipation and fever, cardiac disorder, cough, rheumatism, and tumor. Leaves have Stomachic,

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carminative, and galactogogue, dyspepsia, flatulence, rheumatalgia [5], neuralgia, hemorrhoids and tumors. In the traditional system of medicine, it is also used in nervous disorders *so Premna corymbosa is* recommended for the treatment of epilepsy [5].

Attempts to find out a common neurochemical basis for human or experimental epilepsy have been disappointing. Epilepsy is a fairly common neurologic disorder which is defined as a paroxysmal, self sustaining and self-limiting cerebral dysrrhythmia characterized by an abnormal and excessive EEG discharge & a loss of consciousness [6]. An imbalance between the excitatory and inhibitory neurotransmitters is responsible for seizures. At neuronal level, seizure activity often occurs when glutamatergic excitatory neurotransmitters overrides gamma-aminobutyric acid (GABA) mediated inhibition. Several animal models of convulsions have been developed to evaluate anti-seizure activity. Many drugs that increase the brain contents of GABA have exhibited anticonvulsant activity against seizures induced by maximum electroshock (MES), Pentylenetetrazole (PTZ). The MES is probably the best-validated method for assessment of anti-epileptic drugs in generalized tonic-clonic seizures. The PTZ induced seizures are similar to the symptoms observed in the absence seizures and drugs useful in treatment of absence seizures suppress PTZ induced seizures. The reproducibility of the responses to this treatment makes this a very useful model to investigate various facets of seizures [7].

MATERIALS AND METHOD

Preparation of the Extract

The dried roots of the plant were purchased and powdered mechanically to coarse powder. This powered drug was kept in air tight container until the time of use. About 1kg of the powered material was subjected to soxhlet extraction using 70% ethanol for 48hrs. The solvent was distilled off at low temperature under reduced pressure and evaporated at 40^{o} c. The yield of the product was approximately 18.25%w/w of PC. The methanolic extract of *Premna corymbosa* Rottl was stored in a refrigerator and reconstituted in water for injection just before use.

Phytochemical screening:

Qualitative tests for the presence of plant secondary metabolites such as flavonoids, carbohydrates, alkaloids, saponins and glycosides were carried out on the powdered drug using standard procedures [8].

Experimental animals

Male Swiss Albino mice (25-30g) were procured from Small Animal Breeding Station, College of Veterinary Science and Animal Husbandry, Mannuthi, Thrissur, Kerala and were used for the study. The animals were housed in polypropylene cages inside a well-ventilated room under standard conditions of light and dark cycle with free access of food and water. The Institutional Animal Ethical committee approved the protocol of the study.

Drugs

Phenytoin injection (Sun Pharmaceuticals PVT. LTD.), Pentylenetetrazole (PTZ) (Hi media Laboratories Pvt Ltd Mumbai.), Diazepam (Ranbaxy Pharma).

The drugs were used in this study was dissolved in water for injection and all the drugs were administrated intra peritoneally.

Acute Toxicity Studies

The acute toxicity studies were performed according to the OECD guidelines [14]. Male Swiss Albino mice (25-30g) maintained under standard laboratory conditions was used. A total of five animal were used which received a single oral dose (2000mg/kg body weight) methanolic extract of *Premna corymbosa* Rottl. Animals were kept overnight fasting prior to drug administration. After the administration of *Premna corymbosa* Rottl, food was with held for further 3-4 h. Animals were observed individually at least once during the first 30 minutes after dosing, periodically during the first 24h (with special attention during the first 4 h) and daily thereafter for a period of 14 days. Once daily cage side observations included changes in skin and fur, eyes and mucous membrane (nasal), and also respiratory rate, circulatory (heart rate and blood pressure), autonomic (salivation, lacrimation, perspiration, pilorection urinary incontinence, and defecation) and central nervous system (ptosis, drowsiness, gait, tremors and convulsion) changes.

ASSESSMENT OF ANTICONVULSANT ACTIVITY

1. Maximum electroshock induced seizures (MES)

The animals were divided into five groups of 6 mice each. Group I received saline,1ml/mice (p.o),group II received of Phenytoin 25mg/kg (i.p), group III & IV received 200mg/kg and 400mg/kg (p.o) of methanolic extract of *Premna corymbosa* respectively for 10 days. On tenth day, 60 minute after administration of last dose of *Premna corymbosa.* Maximum electroshock of 50mA current for 0.2 seconds was administered through ear electrodes to induce convulsions in the control and drug treated animals [9-11]. MES produce various phases of convulsions i.e., Flexion, Extension, Clonus & Stupor. The duration of tonic extension of hind limb was used as end point. i.e., prevention or decrease in the duration of hind limb extension was considered as protective action.

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2. Pentylenetetrazole (PTZ) induced seizures

The animals were divided into four groups of 6 mice each. Group I received Saline 1ml/mice (p.o.), Group II Diazepam 4mg/kg (i.p.) as reference standard, Groups III & IV received 200 mg/kg & 400 mg/kg (p.o) methanolic extracts of *Premna corymbosa* respectively. PTZ was administered (80mg/kg, s.c.) 45 minutes after administration of saline, standard drug & extract of *Premna corymbosa*. Animals were observed for 30 minutes after injection of PTZ. The anticonvulsant property of different doses of extract of *Premna corymbosa* in this model was assessed by its ability to delay the onset of myoclonic spasm and clonic convulsions. Protection against PTZ induced convulsions and percentage of mortality was measured [12,13].

Statistical Analysis

Statistical analysis of the results was carried out by one-way ANOVA followed by Dunnett's test. Results are expressed as mean ± SEM from six rats in each group. P values < 0.05 were considered significant.

RESULTS

Preliminary phytochemical studies

Preliminary phytochemical screening of the Methanolic extract shows the presence of alkaloids, flavonoids, saponins, tannins, amino acids, proteins and carbohydrates.

Acute toxicity studies

There is no mortality amongst the graded dose groups of mice up to a dose of 200 mg/kg. This finding probably suggests that the methanol extract is relatively safe or non- toxic in mice at the doses used for this study.

ASSESSMENT OF ANTICONVULSANT ACTIVITY

Effect on maximum electro shock induced seizures:

The result of anticonvulsant effect of *Premna corymbosa* against MES induced convulsions are shown in table 1.The methanolic extract of *Premna corymbosa* in doses of 200 and 400 mg/kg protect animals from seizures and duration of hind leg extension was reduced. The control treated mice showed extension and convulsions for 8.58±1.28 seconds where as mice treated with the methanolic extract (200 mg/kg-400mg/kg) exhibited hind leg extension for 2.83±1.16** & 2.5±0.54**Sec. respectively.

Effect on Pentylenetetrazole induced Seizures

The result of anticonvulsant effect of *Premna corymbosa* against PTZ induced convulsions are shown in table 2. In PTZ induced seizures, methanolic extract of *Premna corymbosa* of 200 & 400 mg/kg showed delayed onset of clonus (94.83±10.96 sec,97.83±7.19sec respectively) and extensor (366.2±115.50sec,394.83±11.47sec respectively) showed significant anticonvulsant activity as compared to control clonus 71.16±3.37sec and extensor 274.88±3.87sec. The methanolic extract protected all the animals in the group since there is no mortality was observed.

Table-1. Effect of methanolic extract of *Premna corymbosa* against MES induced seizures

DRUG	DOSE Mg/kg (B. Wt)	Time(sec) in various phases of convulsions				
		FLEXION	EXTENSION	CLONUS	STUPOR	RECOVERY
Group I control(saline 1ml/mice)	_	1.35 ± 0.3781	8.58 ± 1.28	11.75 ± 2.78	41.66 ± 6.32	118.66 ± 4.5
Group II standard drug (phenytoin)	25	0.1 + 0.00**	0.1 ± 0.126**	8.33 ± 1.66**	17.16 ± 6.32**	39.66 ± 7.39**
Group III methanolic extract of PC	200	1 ± 0.00**	2.83 + 1.16**	7.33 ± 1.169*	11.66 ± 2.06**	97.5 ± 11.27**
Group IV methanolic extract of PC	400	1.83 ± 0.25**	2.5 ± 0.54**	6.91 ± 3.51**	10.33 ± 1.03**	95.5 ± 3.27**

Values are expressed as mean ± SEM; One way ANOVA followed by Dunnett's test,Note n=6 in each group. *P <0.05, **P <0.01.

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Table-2. Effect of methanolic extract of *Premna corymbosa* against PTZ induced convulsions

DRUG	DOSE mg/kg (B. Wt)	Onset time in seconds			Recovery/Mortality
		Jerks	Clonus	Extensor	
Group I control(saline 1ml/mice	-	45.7±1.495	71.16±3.37	274.88±3.87	Mortality
Group II standard drug (phenytoin	4	0.00**	0.00**	0.00**	Recovery
Group III methanolic extract of PC	200	83±8.24**	94.83±10.96**	366.2±115.50**	Recovery
Group IV methanolic extract of PC	400	86.33±4.17**	97.83±7.19**	394.83±11.47**	Recovery

Values are expressed as mean ± SEM;One way ANOVA followed by Dunnett's test,Note n=6 in each group. *P <0.05, **P <0.01.

DISCUSSION

The present study revealed that *Premna corymbosa* extract possesses significant anticonvulsant activity against PTZ and MES induced convulsions. The effect was probably due to the flavonoids [15]. MES and PTZ models are of predictive relevance regarding the clinical spectrum of activity of experimental compounds. MES and PTZ tests are assumed to identify anticonvulsant drugs effective against generalized tonic-clonic partial seizures and generalized clonic seizures respectively. The effect of *Premna corymbosa* in these tests could therefore suggest anticonvulsant efficiency against the above mentioned type in man. It has often been stated that antiepileptic drugs that either presents or delays Pentylenetetrazole induced convulsions act by elevating the seizures threshold.

Since inhibition of the MES test predicts activity against generalized tonic-clonic and cortical focal seizures so activity against MES induced seizures suggests that the methanolic extracts of *Premna cormbosa* are useful in suppressing generalized tonic-clonic seizures by regulating GABA mediated synaptic inhibition through an action at distinct sites of this synopsis.PTZ test predicts activity against absence seizures. Since PTZ is a GABA- A receptor antagonist, the methanolic extract may be acting by increasing GABA concentration in brain [16].

In conclusion, the methanolic root extract of *Premna corymbosa* Rottl demonstrated possess anticonvulsant properties and less toxicity in experimental animals at the doses used. However, further studies still needed to be carried on exposure of the extract to human and its use in folk medicine for seizures control.

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