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ANTICONVULSANT ACTIVITY OF *PHYLLANTHUS AMARUS* IN EXPERIMENTAL ANIMAL MODELS

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ABSTRACT

Objectives: The aim of this study was to investigate anticonvulsant effect of *Phyllanthus amarus* on maximal electroshock-induced seizures (MES) and pentylenetetrazole (PTZ) induced seizures.

Methods: The aqueous and ethanolic extracts of the leaves and stems of P. *amarus* (70 mg/kg, p.o) were studied for their anticonvulsant effect on MES and PTZ induced seizures in Swiss albino rats. The latency of tonic convulsions and the number of animals protected from tonic convulsions were noted.

Results: The aqueous and ethanolic extracts of the leaves and stems of P. *amarus* (70 mg/kg, p.o) significantly ($p<0.001$) abolished the hind limb extension induced by MES. The same dose also significantly ($p<0.001$) protected the animals from PTZ induced tonic convulsions.

Conclusions: The data suggests that the aqueous and ethanolic extracts of P. *amarus* may produce its anticonvulsant effects via non-specific mechanisms since it abolished the hind limb extension induced by MES as well as delayed the latency of seizures produced by PTZ.

Key Words: *Phyllanthus amarus*, aqueous extract, Ethanolic extract, Anticonvulsant, Rats

INTRODUCTION

Epilepsy is a major neurological disorder characterized by the occurrence of recurrent seizures (Hegde K, et al., 2009). All over the world a large population is affected by this progressive CNS disorder (Sankari M, et al., 2010). This chronic disorder involves alterations in the voltage-dependent ion channels, reduction in inhibitory, i.e. GABA-mediated drive or increase in excitatory, i.e. glutamate mediated inputs (Debnath S, et al., 2010). Even though there are many antiepileptic drugs in our drug armoury, still millions have uncontrolled epilepsy. More over the conventional drug therapy is associated with a lot of side effects, chronic toxic effects and teratogenicity (Yaro AH, et al., 2007). In developing countries, many people still depend on traditional medicines for various ailments including epilepsy (Nwabuisi C, 2002). Due to the clinical effectiveness, minimal side effect profile and relatively low costs, the herbal drugs are used for various applications in traditional medicine (Valiathan, 1998). In Indian traditional medicinal system several plants belonging to the family of Euphorbiaceae are used for the treatment of epilepsy (Porwal M and Sharma K, 2011).

Phyllanthus amarus (Syn.*Phyllanthus niruri*) is a common herbaceous plant of Euphorbiaceae family. This plant is widespread throughout the tropics and subtropics in sandy regions as a weed in cultivated and wastelands This herb which grows upto 10-60 cms tall, with terete stem and elliptic leaves (Bagchi GD, 1992) is used in India for curing a wide range of ailments like jaundice, urogenital problems, dysentery, dyspepsia, arthritis etc (Indian medicinal plants, 1995).

P. amarus possess hepatoprotective (Mary C and Parames CS, 2007), antioxidant (Bhattacharjee R, Parames CS,2006),antidiabetic activity (Halim BF and Ali AY,2002) and antiamnesic activity (Hanumanthachar J and Milind P,2007). It also has activity against hepatitis B virus (Thagarajan SP et al.,1988; Lee CD, et al., 1996) and hepatocellular carcinoma (Rajesh Kumar NV and Kuttan R 2000). The major ligans of this herb, namely, phyllanthin and hypophyllanthin, have anti-hepatotoxic against carbon tetrachloride- and galactosamine-induced hepatotoxicity in primary cultured rat hepatocytes (Syamsunder KV et al., 1995).

However there is no scientific data about the anticonvulsant activity of *P. amarus* till date. The aim of the present study was to evaluate the anticonvulsant property of aqueous and ethanolic extracts of *P. amarus* leaves in experimental animal models.

MATERIALS AND METHODS

Drugs and Chemicals

Phenytoin sodium (Abbott Group,Acme formulation Pvt Ltd,HimachalPradesh), Sodium valproate (SunPharma,Sikkim) was obtained from a pharmacy in Mangalore. Pentylene tetrazole(Sigma Aldrich,China) was obtained from Rajesh Chemicals,Mumbai

Instruments

Soxhlet apparatus was used to preparing the plant extracts.

Electro-convulsiometer for inducing convulsions.

Plant material

The young plants were cultivated during the month of June. The fresh leaves from the young *P. amarus* plants were collected in the month of September. They were authenticated by Dr.Noeline J.Pinto, Head of Botany department, St.Agnes College, Mangalore, Karnataka, India. They were shade dried, and then grinded into coarse powder.

Preparation of the extracts

P. *amarus* ethanolic extract (PAEE): A weighed quantity (500 g) of the coarse powder was taken and extracted with ethanol (90 %) in a Soxhlet apparatus. The extract was concentrated on a water bath at a temperature not exceeding 60ºc (yield 20 % w/w). The ethanol extract was dissolved in distilled water.

P. *amarus* aqueous extract (PAAE): A weighed quantity (500 g) of the coarse powder was taken and extracted with distilled water in a Soxhlet extractor. The extract was concentrated on a water bath at a temperature not exceeding 60ºc (yield 16 % w/w). The aqueous extract was dissolved in distilled water.

Animals

Adult Wistar albino rats of either sex weighing 150-200 g were used in this study after obtaining Institutional Animal Ethical Committee Clearance (IAEC), Yenepoya University . The rats were maintained under standard conditions in the Animal House (CPCSEA approved, Reg No: 347) under Dept of Pharmacology, Yenepoya University, Mangalore.The rats were kept in polypropylene cages (U.N.Shah manufacturers, Mumbai) under standard housing conditions and maintained on standard pellet diet (Amrut Lab Animal Feed, Pranav Agro Industries Ltd, Sangli, Maharashtra), and water *ad libitum*. The rats were maintained on a 12:12 hour light-dark cycle.

Acute toxicity study

Acute toxicity study was performed according to the OECD guidelines on Wistar Albino rats and the animals were kept overnight fasting providing water ad libitum, after which the extracts were, administered orally 2000 mg/kg and observed for the mortality.

Anticonvulsant Activity

a. Maximal electroshock (MES) seizure

Electrical stimulation was applied using ear electrodes. The electrodes were moistened with saline before application. All animals were stimulated with 150mA for 0.2 seconds, with constant voltage stimulators of 250 V (Vogel GH and Vogel WH 1997).

The animals were divided into four groups. Each group consisting of 6 males and 6 females (n=12).

Group I: **Normal Saline (**P.O for 10 days**) + MES on 10th day**

Group II: **PAEE** (70 mg/kg P.O for 10 days) **+ MES on 10th day**

Group III: **PAAE** (70 mg/kg P.O for 10 days) **+ MES on 10th day**

Group IV: **Phenytoin** (25mg/kg I.P for 10 days) **+ MES on 10th day**

On 10th day the test samples were given 1 hour prior to induction of convulsions. Suppression of tonic hind limb extension was taken as a measure of efficacy in this test.

b. Pentylene tetrazole (PTZ) induced convulsion

PTZ 70mg/kg I.P was administered to rats. The parameter noted was duration of convulsions (Vogel GH and Vogel WH 1997).

The animals were divided into four groups. Each group consisting of 6 males and 6 females (n=12).

Group I: **Normal Saline (**P.O for 10 days**) + PTZ on 10th day**

Group II: **PAEE** (70 mg/kg P.O for 10 days) + **PTZ on 10th day**

Group III: **PAAE** (70 mg/kg P.O for 10 days) **+ PTZ on 10th day**

Group IV: **Sodium valproate** (75mg/kg I.P for 10 days) + **PTZ on 10th day**

On 10th day the test samples were given 1 hour prior to induction of convulsions. Abolition of the convulsions was taken as a measure of efficacy in this test.

Statistical Significance

The results of the study is expressed as mean ± SEM, n = 12. One Way ANOVA was used to analyze and compare the data, followed by Tukey Krammer multiple comparison tests.

RESULTS

Acute toxicity study

There was no mortality among the graded dose groups of animals and they did not show any toxicity or behavioral changes at a dose level of 2000 mg/kg. This finding suggests that PAEE and PAAE were safe and non-toxic to rats up to 2000 mg/kg. Hence, a lower dose of 70mg/kg was selected for this study.

Antiepileptic activity: MES induced seizures

In the case of MES induced seizures there was no hind limb extension in PAAE, PAAE & Phenytoin groups on comparing with the control group($p<0.001$). There were 2 deaths in the control group. No mortality was observed in PAAE, PAAE & Phenytoin groups. (Table-1).

Table 1: Effect of PAEE & PAAE on MES seizures

Drugs	Dose (mg/kg)	Duration of Hind Limb Extension in seconds (mean±SD) n=12	Mortality
Normal Saline	-	14.441±0.643	2
PAEE	70	0±0.000***	0
PAAE	70	0±0.000***	0
Phenytoin	25	0±0.000***	0
Results are expressed as Mean ± SD, n=12. ***p<0.0001, Considered extremely significant on comparing with the normal group. One Way ANOVA followed by Tukey Krammer multiple comparison tests.			

Antiepileptic activity: PTZ induced seizures

In the case of PTZ induced seizures there was considerably significant decrease in the mean duration of convulsions in PAAE, PAAE & Sodium Valproate groups on comparing with the control group. There were 10 deaths in the control group. No mortality was observed in PAAE, PAAE & Sodium valproate groups. (Table 2)

Table 2: Effect of PAEE and PAAE on PTZ seizures

Drugs	Dose (mg/kg)	Duration of Convulsions in secs (mean±SD) n=12	Mortality
Normal Saline	-	12.161±0.55	10
PAEE	70	1.46±0.34***	0
PAAE	70	1.01±0.81***	0
Sodium Valproate	75	1±0.65***	0
Results are expressed as Mean ± SD, n=12. ***p<0.0001, Considered extremely significant on comparing with the normal group. One Way ANOVA followed by Tukey Krammer multiple comparison tests.			

DISCUSSION

The results demonstrated that the PAAE and PAAE have anticonvulsant activity in MES and PTZ induced seizure models. Table-1 & Figure-1 indicates that both the extracts of P.*amarus* leaves completely prevented the convulsions in MES models. Table-2 & Figure-2 indicates that both the extracts of P.*amarus* leaves have highly significant anticonvulsant activity in PTZ induced seizure models. The ethanolic and aqueous extracts of leaves of *P.amarus* increased the threshold of MES and PTZ induced convulsions in rats and offered protection against MES and PTZ induced seizures.

Among the tests used for evaluation of anticonvulsant activity, the MES and PTZ tests are the best validated method for assessment of antiepileptic drugs in human generalized tonic- clonic seizures and absence seizures respectively (Gopalakrishna HN et al., 2010). More over MES seizures (Rola et al., 2002) and PTZ induced seizure models (Rauca C et al., 1999) are associated with oxidative damage. MES induced seizures are abolished by the drugs that blocks voltage gated sodium channels like Phenytoin and Carbamazepine or by the drugs that block NMDA receptors like Felbamate. Whereas the drugs that block T-type Ca^{2+} current in thalamus like sodium Valproate or the drugs which possess $GABA_A$ agonistic like Diazepam prevents PTZ induced convulsions(Suresh Babu AR andKarki SS ,2011).

The complete abolition of MES induced seizures by the P.*amarus* extracts suggests an activity against generalized tonic clonic seizures. This may be due to voltage gated sodium channel blockade action or due to NMDA antagonistic property of the plant extracts. The activity against PTZ induced convulsions predicts a $GABA_A$ agonistic activity of P.*amarus* extracts. The activity against both MES and PTZ induced seizures can be due to the antioxidant property (Mary C and Parmes C, 2007) of P.amarus extracts.

In conclusion, results suggest that, the ethanolic and aqueous extract of leaves of P.*amarus* will be beneficial in the management of grandmal and petitmal epilepsies. Further studies are required to elucidate the exact mechanism by which this plant acts as an anticonvulsant agent.

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