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Research Article

Investigation of Anticonvulsant Activity of *Phyllanthus urinaria L.* in Swiss Albino Rats

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ABSTRACT

Phyllanthus urinaria commonly known as *Bhumyamlaki* is an annual perennial plant indigenous to the Indian subcontinent and is used in traditional medicine as antidiabetic, antihypertensive, anti-inflammatory, antimalarial, and hepatoprotective. An ayurvedic text such as Bhavprakashnighantu describes *P. urinaria* is useful in reducing hyperactivity and beneficial in the treatment "*Pittajapasmara*" (type of epilepsy as described in ayurveda). The present study aimed to evaluate traditional claims in the folk medicine of bhumyamlaki (*P. urinaria*) which can be beneficial in the treatment of epilepsy using wistar albino rats. The anticonvulsant activity was carried out using the MES-induced seizure and PTZ-induced seizure model. In the MES-induced seizure model, animals were divided into a group of 5 (n=6). Group I was treated with saline, group II-IV was treated with ethanolic extract of *P. urinaria* (EPU) at the dose level of 100, 200 and 400 mg/kg. Standard drug phenytoin (25 mg/kg., *p.o*) was given to group V. Similar grouping was done in PTZ induced seizure model with deviation of the standard group which was treated with diazepam (4 mg/kg., *i.p*). Anticonvulsant activity was noted by accessing the parameters such as delay in the tonic hind limb extension, stupor, and decrease in mortality. It was observed that ethanolic extract of the whole plant of *P. urinaria* has significant ($p < 0.001$) anticonvulsant activity causing the delay in onset of tonic and clonic seizures and improved percentage protection against mortality. Further research is required to find out the exact mechanism of action.

Introduction

Epilepsy has become a major health problem worldwide, affecting a larger part of populations suffering from recurrent chronic seizures.[1] More than millions of new patients have been diagnosed with epilepsy every year.[2] Convulsions have become a serious neurological disorder worldwide, especially in socio-economically backward countries.[3] Social, neurobiological, and psychological consequences, conventional anti-epileptic drugs are associated with several untoward effects, and hence long-term treatment compliance is a major problem in the management of epilepsy. Herbal drugs have shown promising efficacy as potent anticonvulsants in the past few years. In light of this, the anticonvulsant effect of alcoholic extract of leaves of *Helianthus tuberosus* (AHT The morbidity rate of epilepsy is alarming, affecting more than 60 lakhs of the Indian population,[4]commonly called as Pigeon pea plant. The presence of phytoconstituents like flavonoids, the flavanone (substituted synthetic drugs available in the market cannot completely cure epilepsy and its efficacy rate is limited to 60–70%.[5] Epilepsy is commonly known as "Apasmara" in ancient ayurvedic text, signifying the occurrence of this disease for ages. Modern drugs which are used in the treatment of epileptic seizures work in the central nervous system by interfering with the generation and propagation of action potential and hence lead to potentially adverse effects.[6] In addition, these drugs have failed to prevent recurring seizures to provide a permanent cure for epilepsy. There is an immense need

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to explore new leads of herbal origin which can potentially prevent the occurrence and recurrence of seizures with no side effects.[7]

Phyllanthus urinaria is commonly known as *Bhumyamalaki* is an annual perennial plant indigenous to tropical Asia.[8] Whole plant as well as different parts of the plants have been traditionally used to treat different disease such as jaundice, hypertension and haemorrhoids.[9]

P. urinaria have been described in Ayurvedic text "*Bhavprakashnighantu"* under the chapter "*Guduchyadivarg*" as "*Pittashamak"* which can be useful in reducing the hyperactivity and may have potential effect in "*Pittajapasmara*" (type of epilepsy as described in Ayurveda).[10]

P. urinaria is widely and commonly available herb having well known and well documented phytochemical profile thus it will be economical to develop new antiepileptic formulation from the herbal origin. As plant is indigenous to the South Asia, it will be desirable and economical to develop herbal anticonvulsant medicine.

Lead from herbal origin possesses less adverse effects and yield desired result and gaining popularity.[11] The objective of the present study was to scientifically evaluate traditional claim of *P. urinaria* which was used as nervine tonic[12] and pave way for development of new drug lead in treatment of epilepsy.

Materials and Methods

Plant Material

Whole plants of *P. urinaria* were collected from Anmod region (GPS coordinates 15.438°N 74.308°E) in April 2017. The plant was authenticated by Dr. S. K. Das, Professor and Head, Gomantak Ayurvedic College and Hospital, Shiroda, Goa where voucher specimen has been kept with accession number GAM-32.

Preparation of Extract

The whole plant of *P. urinaria* was washed under the running tap water to remove mud and dust particles and was shade dried. Dried plants were coarsely powdered (1500 g), passed through the sieve and were defatted with petroleum ether to eliminate fatty substances. Defatted powder was further extracted with ethanol through soxhlet extraction. Solvent was recovered using rotary evaporator, and extract was vacuum dried and was kept in air tight container for further use.

Phytochemical Screening

Phytochemical screening was carried out to determine the presence of carbohydrate, flavonoid, tannins, alkaloids using standard methods.[13]

Chemicals

All the chemicals used in the present study were of analytical grade. Pentylenetetrazole was obtained from Sigma Aldrich, Phenytoin was obtained from Sigma laboratories. Chemicals were dissolved in saline prior to use.

Animals

Animals (Rats) were obtained from CPCSEA approved registered breeder A. Biosys Pvt. Ltd, Tumkur bearing registration number 1868/PO/Bt/16/CPCSEA, and were kept in animal house of PES RTB college of Pharmacy's animal house in polypropylene cages. Room temperature was maintained at about 24 ± 2°C having humidity of about 50–60% under standard 12 hours light/12 hours dark cycle. Animals had free access to pellet food and water *ad libitum.*

Approval of Animal Activity

Animal study for this protocol was approved by Institutional Animal Ethics Committee of RTBCOP under the guidelines of CPCSEA, with resolution number PESRTBCOP/ IAEC, Clear 2020-R-85.

Acute Oral Toxicity Studies

Acute oral toxicity studies were carried out as per OECD guidelines no 425. Ethanolic extract of *P. urinaria* (EPU) was administered to the mice in group (n=6) at dose level of 200, 400, 600, 800, 1600 and 2000 mg/kg to observe for any behavioural, autonomic, neurological changes every 30 minutes for next 3 hours and finally for mortality after 72 hours.[14]

Anticonvulsant Activity

MES Induced Seizure Model

Rats were divided into group of 5 having 6 animals each, group I was kept as control which received normal saline at dose level of 2 mL/100 g. Group II, III and IV served as test group and were administered EPU at dose level of 100, 200 and 400 mg/kg suspended in twin 80 v/v, respectively. Group V was kept as standard group which received standard drug phenytoin at dose level of 25 mg/kg. All the doses were given p.o 60 minutes prior to induction of electroshock. Convulsions were induced by giving electric shock of 150 mA for about 0.2 seconds through electrodes of electroconvulsiometer (Orchid Scientific). Electrodes were tied to pinna with the help of crocodile clips and cotton to prevent mechanical damage to skin. Parameters observed were onset and duration of tonic hind limb extension, onset of stupor and mortality and percentage protection from it.[15]

PTZ Induced Seizure Model

Animals were divided in to 5 groups as mentioned above; where in standard group was treated with diazepam (4 mg/kg, *i.p.*). Pentylene tetrazole (PTZ) was administered 30 minutes after respective group treatment at dose level of 80 mg/kg to induce seizure. Parameters observed were prolongation and duration of seizure latency, percentage protection from the seizure and mortality if any thereafter.

Table 1: Effect of EPU at different dose level on seizures induced by MES

Treatment	*Dose*	*Duration of Tonic hind limb extension(s)*	*Stupor (s)*	*Mortality*	*Protection against mortality*
Control (Group I)	1 mL/100g	11.55 ± 1.73	140.7 ± 8.37	5/6	16.66
EPU (Group II)	100 mg/kg	10.17 ± 1.37^{ns}	138.3 ± 14.6^{ns}	1/6	83.33
EPU (Group III)	200 mg/kg	3.84 ± 0.91***	119.1 ± 19.48*	0/6	66.66
EPU (Group IV)	400 mg/kg	2.30 ± 0.54***	70.61 ± 11.57***	0/6	100.00
Standard Drug (Phenytoin) (Group V)	25 mg/kg	No convulsions noted	53 ± 5.73***	0/6	100.00

All values are expressed as a mean ± SEM, n=6, *p<0.05, **p<0.01, ***p<0.001 as compared to control group (One Way Analysis of Variance (ANOVA) followed by multiple comparison Dunnet's test)

Results And Discussion

Phytochemical Parameters

Presence of phytoconstituents such as lignans, tannins, glycosides, flavonoids was found and reported earlier by the author.[8]

Acute Toxicity Study

The evaluation of toxicity studies neither revealed behavioural, autonomic or neurological changes nor any mortality. EPU was found to be safe even at the dose level of 2000 mg/kg. Dose of the extract was fixed up to 1/10th of highest dose that is at 200 mg/kg, study was evaluated at two more dose level i.e., lower of the therapeutic dose at 100 mg/kg and higher of the therapeutic dose that is at 400 mg/kg.

MES induced Seizure Model

It was observed that EPU significantly reduced the period of hind limb extension at increasing dose level as depicted in the Table 1. In control group tonic hind limb extension was exhibited for 11.55 ± 1.73 s, which was reduced to 2.30 ± 0.54 s in a group treated with EPU at 400 mg/kg as shown in Fig. 1. EPU at dose level of 400 mg/kg were significantly reduced onset of stupor 70.61 ± 11.57*** as compared to control group as shown in Fig. 2. There were no deaths observed in group III, group IV and group treated with standard, providing 100% protection against mortality.

PTZ Induced Seizure Model

In PTZ induced ulcer model it was observed that group treated with EPU at dose level of 400 mg/kg, onset of clonic seizure was delayed (84.42 ± 9.00***) as compared to control group (41.97 ± 4.43) as shown in Table 2. There was dose dependent delay in onset of clonic seizures in rats. Onset of tonic seizure was delayed in EPU at 400 mg/kg (152.1 ± 8.70***) as shown in Figs 3 and 4. Mortality rate was nil in standard diazepam treated group and group IV treated with EPU at 400 mg/kg.

CNS disorders such as convulsions hamper normal life of individual. Drugs from herbal origin are proved to be safe, potential, and are time tested for treatment of

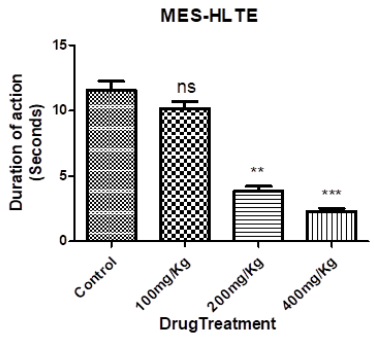

Fig. 1: Comparative effect of EPU on Hind limb tonic extension in Maximal electroshock induced seizure model at different dose level.

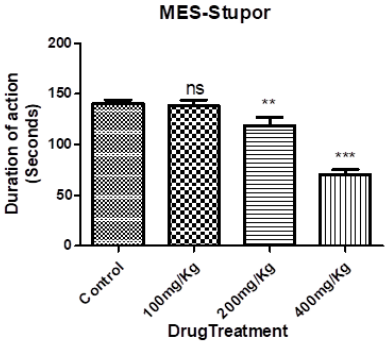

Fig. 2: Comparative effect of EPU on onset of stupor in Maximal electroshock induced seizure model at different dose level.

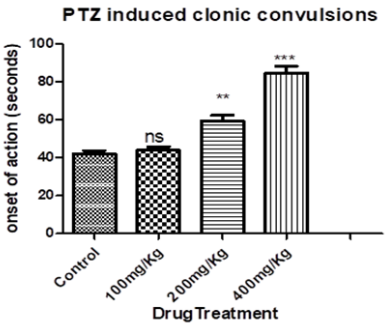

Fig. 3: Comparative effect of EPU on PTZ induced clonic convulsions at different dose level.

Table 2: Effect of EPU at different dose level on seizures induced by PTZ

Treatment	*Dose*	*Onset of clonic seizure(s)*	*Onset of tonic seizure(s)*	*Mortality*	*Protection against mortality*
Control (Group I)	1 mL/100g	41.97 ± 4.43	72.28 ± 5.86	4/6	33.33
EPU (Group II)	100 mg/kg	43.84 ± 4.84^{ns}	76.71 ± 8.74^{ns}	2/6	66.66
EPU (Group III)	200 mg/kg	59.11 ± 8.08**	98.66 ± 8.37**	1/6	83.33
EPU (Group IV)	400 mg/kg	84.42 ± 9.00***	152.1 ± 8.70***	0/6	100.00
Standard Drug (Diazepam) (Group V)	25 mg/kg	Absence of seizure	Absence of seizure	0/6	100.00

All values are expressed as a mean ± SEM, n=6, *p < 0.05, **p < 0.01, ***p < 0.001 as compared to control group (One Way Analysis of Variance (ANOVA) followed by multiple comparison Dunnet's test)

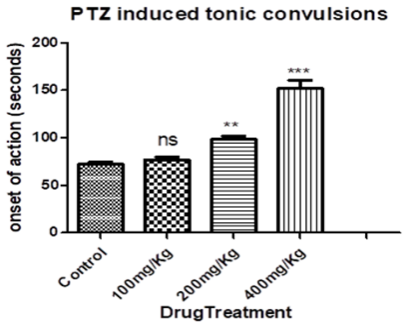

Fig. 4: Comparative effect of EPU on PTZ induced tonic convulsions at different dose level.

several disorder.[4] Commonly called as pigeon pea plant. The presence of phytoconstituents like flavonoids, the flavanone (substituted modern medicine exerts side effects as they interfere with the normal physiological mechanism of human body.[3] Social, neurobiological, and psychological consequences, conventional anti-epileptic drugs are associated with several untoward effects, and hence long-term treatment compliance is a major problem in the management of epilepsy. Herbal drugs have shown promising efficacy as potent anticonvulsants in the past few years. In light of this, the anticonvulsant effect of alcoholic extract of leaves of *Helianthus tuberosus* (AHT Thus there is a constant need to search potential drugs from herbal origin. In this purview this work had been taken to find out anticonvulsant effect of EPU against experimentally induced seizures in animals. Interpretation of the result indicates alcoholic extract of *P. urinaria* exhibits dose dependent inhibition of seizures which is remarkably seen through decrease in tonic hind limb extension, decrease in duration time of stupor in MES induced convulsion model. EPU at dose level of 400 mg/kg exhibited tonic hind limb extension for 2.30 ± 0.54***(S) as compared to control (11.55 ± 1.73) and stupor was restricted to 70.61 ± 11.57***(S) as compared to control (140.7 ± 8.37) with complete protection from mortality. Thus it was observed that there was decreased tonic hind limb extension, and decreased in mortality up to 100% in MES induced seizures in rats. EPU has also delayed the onset of tonic and clonic seizures as seen in PTZ induced seizures with significant increase in percentage protection against mortality. EPU at 400 mg/kg delayed the onset of clonic seizures (84.42 ± 9.00***) as compared to control (41.97 ± 4.43). Onset of tonic seizures was also delayed up to (152.1 ± 8.70***) as compared to control (72.28 ± 5.06) with complete protection from mortality. Our research work confirms traditional claim of the *Bhumyamlaki* (*P. urinaria*) in treatment of epilepsy.[10] Further research work is required to isolate the active constituents from the extract responsible for anticonvulsant activity and finding the mechanism thereof so as to pave way for development of antiepileptic herbal formulation.

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Abbreviations

EPU: Ethanolic extract of *Phyllanthus urinaria*
MES: Maximal electro shock
PTZ: Pentylenetetrazole
HLE: Hind limb tonic extension.
p.o.: Per oral
i.p: Intra peritoneal
CNS: Central nervous system

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