Research Article

Toxicity Studies and Evaluation of *Phyllanthus amarus* and *Phyllanthus fraternus* Extracts on the Central Nervous System and Musculoskeletal Function

Atul R. Chopade* and FJ. Sayyad

Department of Pharmacology and Pharmacognosy, Government College of Pharmacy, Karad, Satara-415124, Maharshtra, India.

ABSTRACT

Ethnopharmacological relevance: A great number of preclinical and clinical studies have not only confirmed but have also extended the medicinal uses of species of the genus Phyllanthus mentioned in traditional medicine. **Aim of the study:** In the present study we have conducted the toxicity studies of the extracts of *Phyllanthus amarus* & *Phyllanthus fraternus* and also examined their effects on CNS and skeletal muscle function (motor coordination). **Materials and methods:** The toxicity studies of the extracts of *P. amarus* & *P. fraternus* was carried out by adopting the OECD Guideline 420 (Fixed Dose Procedure) as the first alternative to the conventional acute and chronic toxicity tests. The Locomotor activity was tested by using the digital actophotometer, while the rota-rod test was used to evaluate motor coordination. **Results:** The extracts of *P. amarus* & *P. fraternus* showed CNS depressant effect at high doses. The extracts of *P. amarus* & *P. fraternus* had no effect on the motor coordination. **Conclusions:** The current study suggests that the extracts of *P. amarus* & *P. fraternus* showed no significant signs of toxicity and have CNS depressive property without affecting the motor coordination.

INTRODUCTION

The plants belonging to the genus Phyllanthus (family- Euphorbiaceae) are widely distributed throughout the world.[1-3] These plants are used in folk medicine for treatment of several diseases, such as disturbances of kidney and bladder calculi, intestinal infections, diabetes and hepatitis B virus. A great number of preclinical and clinical studies have not only confirmed but have also extended the medicinal uses of species of the genus Phyllanthus mentioned in traditional medicine.[1-3]

However, until now there is no published data showing the effects of extracts of *P. amarus* & *P. fraternus* on CNS using digital actophometer and testing them side by side for their skeletal muscular performance.

In the present study we therefore attempt to examine the toxic potential along with their effects on the CNS and motor coordination in mice.

MATERIALS AND METHODS

Plant material

Phyllanthus amarus Schum and Thonn and *Phyllanthus fraternus* Webster family- Euphorbiaceae were obtained from different places in Karad western Maharashtra. The plant species were identified and authenticated by Botanical survey of India, Pune [Reference No:BSI/WC/Tech./2012/644].

Preparation of the *P. fraternus* extract

The dried leaves, stems and roots of *P. fraternus* was minced and extracted with 70% ethanol-water in the proportion of 70:30, being stirred and macerated at room temperature (22-28°C) for 15 days. The ethanol was evaporated and the extract (yield 5-7%) was concentrated to the desired level and stored in a refrigerator. The extract was dissolved in DMSO [dimethyl sulphoxide] to the desired concentration just before use.

Extracts of *Phyllanthus amarus*

The standardized extract of Phyllanthus amarus whole plant (water extract) Reference No: SR/KN/CL/1/2012-L12030241, was procured as a gift sample from Chemiloids Ltd., Vijaywada.

While the standardized methanolic extract of Phyllanthus amarus leaf (Methanol extract contains >2.5% of Phyllanthin and Hypophyllanthin) Report No: FP1112042-PA/11LOT05 and the standardized hydro methanolic extract of Phyllanthus amarus leaf (60% Methanol Hydroalcoholic extract contains >5% of Corilagen) - Report No: FP1102034 -PA/11LOT/02 were procured as a

gift sample from Natural Remedies Pvt. Ltd., Bangalore.

Experimental Animals

Swiss albino mice (20-30 g) were used for the study. The animals were maintained under standard environmental conditions and were fed with standard diet and water ad libitum. Food and water were freely available throughout the experiments.

A prior approval [Approval number- GCOPK/2011-12/ CPCSEA/616] was obtained from the Animal Ethics Committee of Govt. College of Pharmacy, Karad. (GCOPK) for the study [Protocol Reference No: CPCSEA-IAEC/2011-NOV/01 & CPCSEA-IAEC/2012-MAR/01]. GCOPK is registered under Committee for the Purpose of Control and Supervision of Experiment on Animals (CPCSEA), Govt. of India, CPCSEA Registration no- 209/GO/a/2000/ CPCSEA.

Preparation of standard Drug solutions and Phyllanthus infusions

The standard reference drug Diazepam (Campose) was obtained from the local market. The standardized aqueous extract of *P. amarus* whole plant (PAAE), standardized methanolic extract of *P. amarus* leaf (PAME), standardized hydro methanolic extract of *P. amarus* leaf (PAHME) the standardized hydro ethanolic extract *P. fraternus* (PFHEE) and the diazepam were dissolved in DMSO just before use.

Acute toxicity studie[4,5]

Fixed dose procedure (Guideline 420) of Organization for Economic Cooperation and Development (OECD) was carried out using male mice divided into five groups to identify any change in body weight; basic haematological and pathological parameters. To determine the acute toxicity of the extracts, the extracts of Phylanthus species were administered intraperitoneally (2.0 g/kg) (n = 4). The control group received vehicle DMSO. Mortality within 72 h was recorded for each group, and the animals then were observed for signs of toxicity. The animals were observed for 14 days for toxic symptoms such as body weight variation, consumption of food and water, behavioral changes, locomotion, convulsions and mortality.

Hematological Serum biochemical parameters

Collected blood was used for the estimation of hemoglobin (Hb) content; red blood cell count (RBC), white blood cell count (WBC) and glucose. Collected blood was also used for the estimation of serum biochemical parameters viz. serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT) and serum alkaline phosphatase (Span Diagnostics, Surat, India).

Measurement of motor performance using Rota rod apparatus[6,7]

In order to evaluate the possible non-specific effects of the extracts of *P. amarus* and *P. fraternus*, on the motor co-ordination, mice were tested on the rota-rod apparatus. The apparatus consists of a bar, subdivided into three compartments by disks (Rota Rod apparatus model- K19616-2 Inco, Ambala). The bar rotated at a constant speed of 22 rpm. The animals were selected 24 h before by eliminating those mice that did not remain on the bar for two consecutive periods of 50–60 s. After the selection, animals were treated with HE (200 and 400 mg/kg, intraperitoneally) or received the same volume of vehicle DMSO 30 min before the test. The standard drug Diazepam (1 mg/kg i.p.) was used for reference and was also administered 30 min before the test. The results were expressed as the time for which animals remained on the rota-rod. The cut-off time used was 60 seconds.

Locomotor activity using actophotometer[6,8]

The spontaneous locomotor activity of each mouse was recorded individually for 05 min using Digital Actophotometer [Dolphin Cat no. 1126]. Test group mice were pretreated 30 minutes before the experiment with Phyllanthus extracts (400 mg/kg intraperitoneally) dissolved in DMSO, the standard group mice were pretreated with Diazepam (1 mg/kg i.p.) and the control group mice were pretreated with vehicle (DMSO) and placed in the Digital Actophotometer one by one, which consist of a cage which is 30 cm long and 30 cm deep with wire mesh at the bottom. A continuous beam of light from about six lights was made to fall on corresponding photoelectric cell; the photoelectric cell got activated when an animal crossed the beam of light and thereby cut-off the rays of lights falling on it. These cutoffs were counted for a period of 05 minutes and the figure was taken as a measure of the locomotor activity of the animal.

RESULTS

Acute toxicity study

Different extracts like PAAE, PAME, PAHME and PFHEE were administered separately up to 2000 mg/kg body weight; none of the

extracts produced any toxic symptoms or mortality. Hence the phyllanthus extracts were considered safe for further pharmacological screening. So, according to the OECD-420 guidelines for acute oral toxicity, the LD50 dose of 2000 mg/kg and above is categorized as unclassified. There was no significant change in weight measured in the test groups of the phyllanthus extracts when compared with the control group. Table 1 shows the results of basic hematological parameters measured in mice treated with phyllanthus extracts. The results in table 2 show that there were no significant changes in the SGOT, SGPT and alkaline phosphatase levels when the values of the extracts were compared with the control.

Muscle co-ordination test

The extracts of P. amarus and P. fraternus (200 or 400 mg/kg), given before, did not significantly affect the motor response of the animals on the rota rod apparatus. The response presented by control and the phyllanthus extracts treated animals was almost similar. Results of motor coordination test revealed that the phyllanthus extracts did not exhibited marked reduction in motor coordination in mice. However, the diazepam treated group revealed a statistically significant decrease in motor coordination activity as compared with the control and phyllanthus extracts treated mice (Figure 1).

Test for locomotor activity

The phyllanthus extracts (400 mg/kg) showed a significant effect on the locomotor activity as determined by the actophotometer performance. But among them, PAME methanol extract showed significant effect when compared with the other extracts. All the phyllanthus extracts also showed the quick onset and longer duration of reduction of locomotor activity. However, the diazepam revealed a statistically significant decrease in motor coordination activity as compared with the control and phyllanthus extracts treated group (Figure 2).

DISCUSSION

Toxicity studies

The effects of the phyllanthus extracts were studied in the current study using some pharmacological and toxicity studies. The acute toxicity study was carried out as per OECD guidelines in mice by Pingale and Shewale.[9] The dose of 2, 4, 6 and 8gm/kg body weight plant material were administered orally in the form of aqueous slurry. Their results provide evidence that Phyllanthus amarus plant material was found to be nontoxic. No significant sign of toxicity was observed during the current study. Hence, a dose range of 200 to 400 mg/kg by intraperitoneal route was considered to be effective for the extracts. The hematological data showed no toxic implication. The values of RBC and WBC were quite similar to the control group. The observed effects of the extracts on different elements of the blood seem to suggest that the plant has no or very little haemopoietic functions. The insignificant effects of phyllanthus extracts on SGOT, SGPT, alkaline phosphatase and glucose levels after 14 days shows that the extracts are not toxic to major enzymes involved in basic metabolic activities in the tissues, liver and kidneys. In the acute toxicity studies, the hydro-ethanol extracts of phyllanthus species (2g/kg) did not produce any death in the animals. Sirajudeen et al determined the toxic side effects of aqueous extract of leaves of *P. amarus* (grown in Malaysia) following oral administration in rats.[10] Acute administration of *P. amarus* extract at a dose of 5 g/kg body weight did not produce any signs of toxicity or mortality. In the chronic study, no significant difference ($P > 0.05$) was observed between the control and *P. amarus* extract administered (male and female) rats (at the doses of 100, 400 and 800 mg/kg body weight for 6 weeks) in the total body weight gain as well as in the liver marker enzymes analyzed in serum. A single oral dose of the Phyllanthus amarus extracts at 5 g/kg b.w. did not produce mortality or any significant change in treated animals over a 14 day observation period. In the sub acute toxicity study, extracts were administrated (1 and 3 g/kg b.w.) daily by gavage to rats for 28 days. No significant differences were observed in body weight gain and blood glucose levels between controls and treated groups. Clinical biochemistry revealed no toxic effect. Neither gross abnormalities nor histopathological changes in liver, kidney and pancreas were observed. The study by Asare et al[11] was carried out to determine if the aqueous leaf extract of P. niruri administered to female Sprague-Dawley rats would illicit evidence of toxicity. From their study it can be concluded that the aqueous leaf extract of P. niruri has an LD50 greater than 5000 mg/kg b.w. with no adverse effect of this dose after a single administration.

CNS depressive potential of phyllanthus extacts

The results of the present study provided evidence that phyllanthus extracts reduced

locomotor activity confirming its CNS depressant activity. Locomotor activity is considered as an index of alertness and a reduction is indicative of sedative activity[12]. Gamma-aminobutyric acid (GABA) is the major inhibitory neurotransmitter in the central nervous system and different anxiolytic, muscle relaxant and sedative-hypnotic drugs exhibit their action via GABA. Therefore, it is possible that extracts of Tecoma stans flowers may act by potentiating GABAergic inhibition in the CNS via membrane hyperpolarization leading to a reduction in the firing rate of critical neurons in the brain or it may be due to direct activation of GABA receptors by the extracts[13]. Locomotor activity is considered as an increase in alertness and a decrease in locomotor activity is indicative of a sedative effect[14].

CONCLUSION

On the basis of results obtained from this investigation, we can conclude that the phyllanthus extracts have no or least toxicity and significant neuropharmacological activity as evident by significant reduction motor activity while no effect on muscle coordination. It is logical to suggest that it may be useful as CNS depressant agent in clinical conditions. Present work was a preliminary effort which will require further detailed investigation including characterization of active compounds and requires preformulation studies for development of a potential dosage form.

Table 1: Effects of phyllanthus extracts on basic haematological parameters

Groups	RBC (M/mm3)	TWBC (m/mm)	Hb (g/dl)	Glucose (mg/dl)
Control	6.145 ± 0.0403	7770.75 ± 256.81	11.75 ± 0.487	124.75 ± 2.016
PAAE	6.165 ± 0.105	8114.25 ± 67.169	11.25 ± 0.750	121.75 ± 2.869
PAME	6.130 ± 0.104	7965.25 ± 117.08	11.25 ± 0.629	153.75 ± 1.109
PAHME	6.120 ± 0.024	7962.25 ± 177.08	12.25 ± 0.629	147.75 ± 0.8539
PFHEE	6.217 ± 0.044	8353.0 ± 266.10	11.50 ± 0.645	145.75 ± 0.8539

Values are mean ± S.E.M of 4 mice. P<0.05 compared with the control (students t-test)

Table 2: Effects of phyllanthus extracts on SGOT, SGPT and alkaline phosphatase levels in mice

Groups	SGOT (U/L)	SGPT (U/L)	Alkaline phosphatase (U/L)
Control	129.25 ± 2.323	47.25 ± 1.109	48.75 ± 1.315
PAAE	134.75 ± 1.031	51.75 ± 0.853	52.75 ± 1.493
PAME	136.75 ± 2.689	44.75 ± 1.493	57.75 ± 1.031
PAHME	126.25 ± 1.377	40.75 ± 1.250	56.75 ± 0.750
PFHEE	136.0 ± 1.472	40.25 ± 1.843	61.75 ± 0.8539

Values are mean ± S.E.M of 4 mice. P<0.05 compared with the control (students t-test)

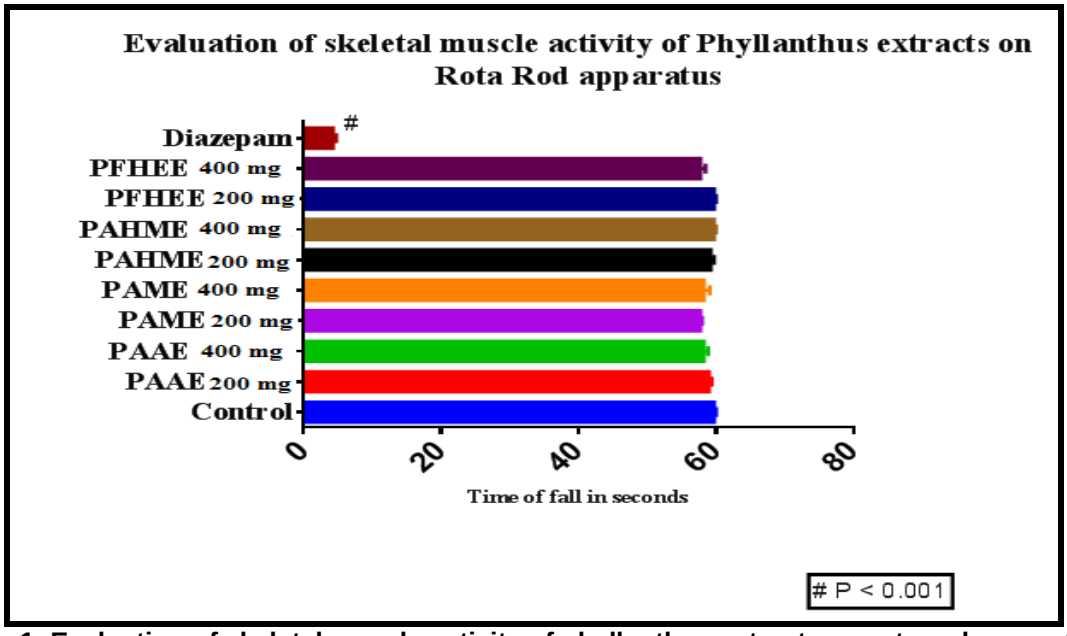

Fig. 1: Evaluation of skeletal muscle activity of phyllanthus extracts on rota rod apparatus

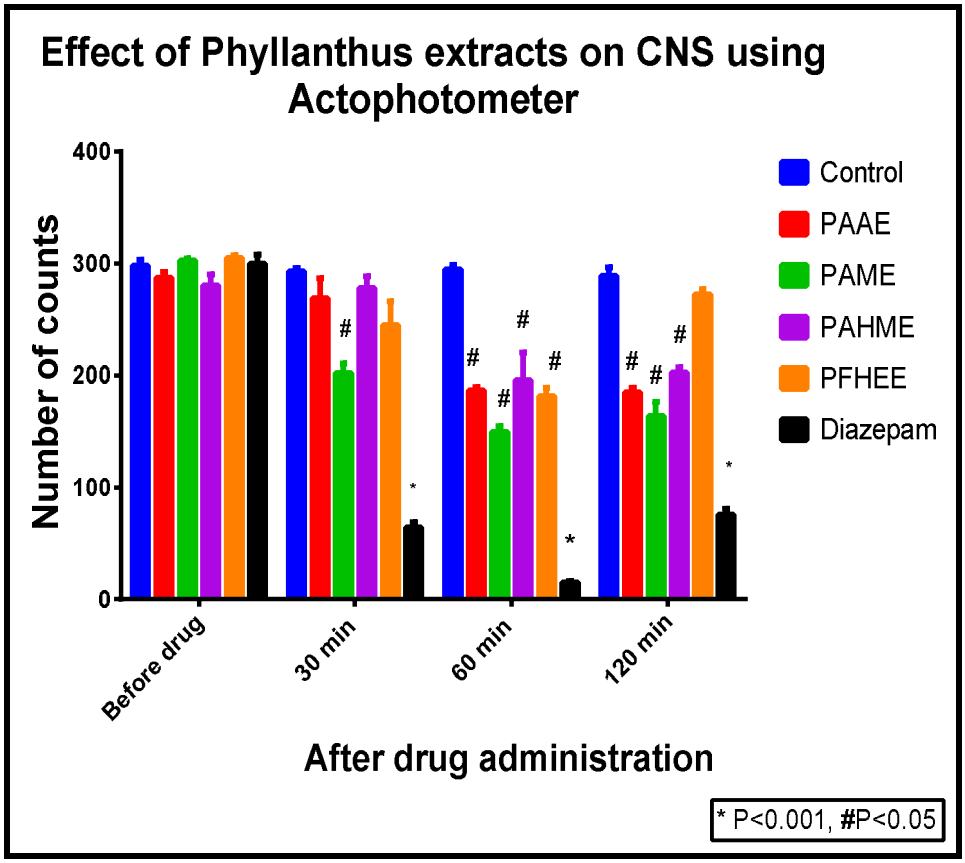

Fig. 2: Effect of Phyllanthus extracts on CNS using Actophotometer

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