



Original Research Article

Acute toxicity study and phytochemical screening of phyllanthus amarus ethanolic extract on wistar albino rats

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ABSTRACT

Objective: To analyse the acute toxicity study and phytochemical screening of phyllanthus amarus ethanolic extract on wistar albino rats.**Materials and Methods:** Acute toxicity study is done on the Wistar albino rats by dividing into 7 groups. Each group consisting of 2 animals [1male & 1 female]. Overnight fasted animals were kept in individual cages and were administered graded dose of Phyllanthus amarus ethanol extract. Preliminary phytochemical analysis of the ethanolic extract of the Phyllanthusamarus (PAEE) was done according to standard methods.**Results:** Evaluation Acute toxicity of Phyllanthusamarus in Wistar albino rats. Results- There was no signs of acute toxicity of any kind like stupor, convulsion, writhing or death after incremental dosing of Phyllanthusamarusethanolic extract (PAEE) to wistar albino rats during close monitoring for initial 2hrs and as well as at the end of 24hrs. Preliminary phytochemical analysis showed positive for Alkaloids, Phytosterols, Saponins, Carbohydrates, Flavanoids and negative for Triterpenoids, Lactones, Tannins, Proteins and Glycosides.**Conclusions:** Phyllanthus amarus doesn't seem to have acute toxicity in preliminary animal study.© 2020 Published by Innovative Publication. This is an open access article under the CC BY-NC license (<https://creativecommons.org/licenses/by-nc/4.0/>)

1. Introduction

The indigenous herb Phyllanthus amarus grows upto 10-60 cm tall is widely spread in our country. Phyllanthus amarus is a small weed, locally known as “Bhumyamalaki”. P. amarus is an important plant of Indian Ayurvedic system of medicine which is used in the problems of stomach, genitourinary system, liver, kidney and spleen.

1.1. Rationale of the study

Many indigenous medicinal plants are used for medicinal property and found to contain active constituents with protective properties against a variety of liver and kidney diseases. The phytochemicals like phenols, coumarins, lignans, essential oil, terpenes, carotenoids, glycosides, flavonoids, organic acids, lipids, alkaloids and xanthenes found in these herbs are known for hepatoprotective.

nephroprotective, antimicrobial, anti-inflammatory property.¹

This study is conducted to know safety profile and active metabolites of herb Phyllanthus amarus, a medicinal plant.

2. Materials and Methods

Drugs and chemicals

The chemicals used for biochemical analysis were of analytical grade and was procured from Himedia, India.

2.1. Instruments

Soxhelt apparatus

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Table 1: Drug, dose and number of animals are shown in²

| Drug | Dose (mg/kg bd.wt) oral | Number of animals | |
|--|-------------------------|-------------------|--------|
| | | Male | Female |
| Phyllanthus amarus (ethanolic leaves extract) | 100 | 1 | 1 |
| | 200 | 1 | 1 |
| | 400 | 1 | 1 |
| | 800 | 1 | 1 |
| | 1600 | 1 | 1 |
| | 3200 | 1 | 1 |

Table 2: Phytochemical analysis of Ethanolic extract of Phyllanthus amarus [EEPA]

| Test for | Test | End point |
|--------------------------------|--------------------------|--|
| Phytosterols | Salkowski reaction | Reddish brown colour |
| | Lieberman Burchardt test | Purple colour, which changes to green or blue colour |
| Triterpenoids | Tschugajeu test | Eosin red colour |
| | Brieskorn and Binar test | Red colour |
| Saponins | Foam test | Appearance of foam persisting for 10 minutes |
| Alkaloids | Dragendroff's test | Orange red precipitate |
| | Mayer's test | Creamy white precipitate |
| Carbohydrates | Fehling's test | Orange red precipitate |
| | Molisch's test | Reddish violet ring |
| Flavanoids | Ferric chloride test | Appearance of green colour |
| | Shinoda test | Appearance of magenta colour |
| Lactones | Baljets test | Appearance of yellow to orange colour |
| | Legal's test | Appearance of deep red colour |
| Phenolic compounds and tannins | Ferric chloride test | Appearance of bluish black precipitate |
| | | |
| Proteins | Biuret test | Appearance of red or violet colour |
| Glycosides | Baljets test | Appearance of yellow to orange colour |

Table 3: Phytochemical analysis result

| Phytochemical | Phyllanthus amarus |
|---------------|--------------------|
| Alkaloids | + |
| Phytosterols | + |
| Triterpinoids | - |
| Saponins | + |
| Carbohydrates | + |
| Flavanoids | + |
| Lactones | - |
| Tannins | - |
| Proteins | - |
| Glycosides | - |

3. Animals

Adult Wistar albino rats of either sex weighing 150-220 g were used in this study after obtaining Institutional Animal Ethical Committee Clearance (IAEC), Yenepoya University. The rats were kept under the normal standard conditions in the animal House (CPCSEA approved, Reg No: 347) under Department of Pharmacology, Yenepoya University, Mangalore. The rats were kept in cages (U.N.Shah manufacturers, Mumbai) in standard housing conditions and maintained on pellet diet (Amrut Lab Animal Feed, PranavAgro Industries Ltd, Sangli, Maharashtra), and

water ad libitum. The rats were maintained on 12:12 hour light-dark cycle.

3.1. Plant material and extract

The plants were grown during the month of June. The fresh leaves from Phyllanthus amarus were collected in the month of September. They were authenticated and approved 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.

3.2. Ethanolic extract of *Phyllanthus amarus* [EEPA]

A phyllanthus leaves were weighed quantity (500 g) of the coarse powder was taken and extracted with ethanol (90%) in a Soxhlet apparatus. The extract was concentrated in a water bath at a temperature not exceeding 60°C. The percentage yield of the extract was 10%. The ethanol extract was dissolved in distilled water.

3.3. Experimental protocol

3.4. Acute toxicity study

The Wistar albino rats were divided into 7 groups. Each group consisting of 2 animals [1 male & 1 female]. Overnight fasted animals were kept in individual cages and were administered graded dose of *Phyllanthus amarus* ethanol extract as shown in Table 1.²

The rats were observed for a period of 2 hours and there after at regular intervals for 24 hrs. for signs of acute toxicity of any kind like stupor, convulsion, writhing and death.

3.5. Phytochemical screening

Preliminary phytochemical analysis of the ethanolic extract of the *Phyllanthus amarus* (PAEE) was done according to standard methods safe.³ The tests are shown in Table 2.

4. Results

4.1. Acute toxicity study

There was no signs of acute toxicity of any kind like stupor, convulsion, writhing or death after incremental dosing of *Phyllanthus amarus* ethanolic extract (PAEE) to wistar albino rats during close monitoring for initial 2hrs and as well as at the end of 24hrs.

4.2. Phytochemical analysis of *Phyllanthus amarus*.

The phytochemical analysis of *Phyllanthus amarus* plant extract revealed the presence of alkaloids, phytosteroids, saponins, flavanoids which are suggested to have hepatoprotective function. (Table 3)

5. Discussion

The present animal study results reveals that medicinal herb *Phyllanthus amarus* does not produce any major acute toxicity. Our results are comparable with animal study done by Lawson Evi et al who conducted acute oral toxicity study of *Phyllanthus amarus* extract using the limit test procedure according to OECD Test Guidelines in mice which also

revealed there were no acute and subacute toxicity.⁴

The Phytochemical analysis of *Phyllanthus amarus* in our present study showed active metabolites like flavonoids, alkaloids and saponins. Various studies have similarly demonstrated these active metabolites like flavonoids, alkaloids, saponins and lignans which have antioxidant property and play a part in the hepatoprotective, nephroprotective, antimicrobial, anti-inflammatory, and anti-carcinogenic property.^{5,6}

6. Conclusion

Phyllanthus amarus doesn't seem to have acute toxicity in preliminary animal study. The active metabolites of *Phyllanthus amarus* plant extract which include alkaloids, phytosteroids, saponins, flavanoids are known to have medicinal value.

7. Source of Funding

None.

8. Conflict of Interest

None.

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