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Acute Toxicity Study of Ethanol Extract of *Phyllanthus Amarus* Leaves in Wistar Albino Rats

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ABSTRACT

Background and objective: The safety of plant-derived bioactive compounds has become a global concern. The present study investigated the acute toxicity of ethanol extract of Phyllanthus amarus (EEPA) leaves in Wistar rats.

Methods: Adult male Wistar rats (n = 13) weighing 150 – 180 g (mean weight = 165 ± 15 g) were used for this study. The ethanol extract of the plant leaves was obtained using cold maceration method. The method described by Lorke was used to determine oral LD_{50} of the extract. Signs of toxicity and possible death of rats were monitored for twenty-four (24) h.

Results: The major sign of toxicity observed within 24 h was general body weakness. No deaths were recorded in both phases and all the animals survived. The oral LD_{50} of EEPA leaves was greater than 5000 mg/kg body weight (bwt).

Conclusion: The results of this study indicate that ethanol extract of P. amarus leaves is not toxic at doses not exceeding 5000 mg/kg bwt.

Keywords: Acute Toxicity; Bioactive Compounds; Extract; Lethal Dose; Medicinal Plant

Introduction

Plant-derived substances have gained huge attention in recent times due to their many applications [1,2]. Medicinal plants are important sources of therapeutically active compounds [3]. These plant components exist as complex mixtures of many phytochemicals (alkaloids, glycosides, terpenoids, phenols, flavonoids and lignans) [4-6]. Phytomedicinal studies support the identification and characterization of bioactive compounds from natural sources [7,8]. Furthermore, the safety of plant-derived bioactive compounds remains a global concern [9-14]. *Phyllanthus amarus* is a herbaceous plant native to tropical regions of the world. It grows to a height of 30 - 60 cm with elliptic leaves. The plant is used to treat conditions such as jaundice, urogenital problems, diarrhoea, dyspepsia, arthritis, ulcers, genitourinary tract infections, hemorrhoids, gonorrhea, neurological debility, epilepsy, dropsy, hepatic and urolitic diseases, as well as Hepatitis B and C viruses [15]. *Phyllanthus amarus* has been demonstrated to possess a wide range of therapeutic and pharmacological properties. Its antiviral, antitumor, anti-inflammatory, hepatoprotective, diuretic and antioxidant effects have been established [16]. The present study investigated the acute toxicity of EEPA leaves in Wistar rats.

Materials and Methods

Collection of Plant Material

Fresh leaves of *Phyllanthus amarus* were collected from a forest area at Isiohor, Benin City, and identified at the Department of Plant Biology and Biotechnology, University of Benin, Benin City, Nigeria.

Plant Preparation and Extraction

The leaves were washed, and air-dried at room temperature for one month at the Department of Biochemistry, Faculty of Life Scienc-

es, University of Benin, Benin City, Nigeria. They were subsequently ground into powder using a mechanical blender. Exactly 500 g of the pulverized sample was cold macerated in absolute ethanol (5 L) for 72 h in a bell jar and filtered using a muslin cloth. The resultant ethanol extract was thereafter concentrated using rotary evaporator and freeze-dried with a lyophilizer [17-21].

Experimental Rats

Adult male Wistar rats (n = 13) weighing 150 – 180 g (mean weight = 165 ± 15 g) were obtained from the Department of Anatomy, University of Benin, Benin City, Nigeria. The rats were housed in metal cages under standard laboratory conditions: temperature of 25 oC; 55 – 65 % humidity and 12-h light/12-h dark cycle. They were allowed free access to rat feed (pelletized growers mash) and clean drinking water. The rats were acclimatized to the laboratory environment for one week prior to commencement of the study.

Acute Toxicity Test

Acute toxicity test was carried out on the extract using Lorke's method [22]. In the first phase, nine (9) rats were divided into three groups of three (3) rats each. Each group of rats was administered EEPA leaves at doses of 10, 100 and 1000 mg/kg bwt. The rats were placed under observation for 24 h to monitor their behaviour and mortality. In the second phase, 4 rats were randomly assigned to four groups of 1 rat each. The rats were administered higher doses of the extract (1600, 2900 and 5000 mg/kg bwt) orally, and then, observed for 24 h for signs of behaviour and mortality.

The lethal dose (LD_{50}) of EEPA leaves was calculated thus:

$$LD_{50} = \frac{\sqrt{D0 + D100}}{2}$$

where D_0 = Highest dose that gave no mortality; D_{100} = Lowest dose that produced mortality.

Statistical Analysis

Data are expressed as mean ± SEM, and statistical analysis was performed using GraphPad Prism Demo (6.07).

Results

Acute Toxicity of EEPA Leaves in Wistar Rats

No mortality was recorded even at 5000 mg/kg bwt of the extract (Table 1).

Table 1: Outcome of Acute Toxicity Study.

Dose (mg/kg bwt)	No. of rats	No. of deaths	Survival	Mortality ratio
Phase 1				
10	3	0	3	0/3
100	3	0	3	0/3
1000	3	0	3	0/3
Phase 2				
1600	1	0	1	0/1
2900	1	0	1	0/1
5000	1	0	1	0/1

Note: Data are number of death and survival of rats.

No. of deaths recorded = Nil

No. of rats that survived = All

Mortality ratio = no. of death/no. of survival

Oral LD50 > 5000 mg/kg bwt.

Discussion and Conclusion

There have been growing interests in the toxicity of substances isolated from plants. This study investigated the acute toxicity of EEPA leaves in Wistar rats. The results showed that no death was recorded in both phases after 24 h and all the rats in each group survived. Thus, the median lethal dose (LD_{50} oral) of EEPA leaves was greater than 5000 mg/kg bwt. These results suggest that the ethanol extract of *Phyllanthus amarus* leaves may be relatively safe [23]. One major and overriding criterion in the selection of herbal preparations for use in medical practice is safety. Phytochemicals present in plant extracts should not only be clinically effective, but safe for consumption. Therefore, screening of bioactive components present in plant extracts to identify their toxic potential is necessary for selection of plants for drug formulations [25-52].

Significant Statement

The results obtained in this study show that ethanol extract of *Phyllanthus amarus* leaves is not toxic at doses not exceeding 5000 mg/kg bwt.

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