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Acute toxicity study and effect of prolonged administration (28 days) of crude ethanolic root extract of *Diospyros mespiliformis Hochst* (*Ebenaceae*) on clinical, haematological and biochemical parameters of albino rats

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ABSTRACT

Ethnopharmacological relevance: Different parts of the plant *Diospyros mespiliformis* have been used traditionally for the treatment of ailments in Nigeria particularly among the Kamwe people of Michika local government area of Adamawa State where the root has been used as an anti-malarial for ages. Most of the uses have been without any scientific evidence and toxicological assessment. The present study aimed to determine acute toxicity profile as well as the effect of prolonged administration of the extract on clinical, haematological and biochemical parameters of albino rats.

Materials and methods: Thirty and twenty-five Wistar rats of both sexes and of varying weights were, respectively, used for acute toxicity study and prolonged administration study of crude ethanolic root extract of *Diospyros mespiliformis*. The rats used for both studies were each administered graded concentrations of the extract (100, 200, 400, 800 and 1600 mg/kg) for acute toxicity testing and (50, 100, 200 and 400 mg/kg) for the study of the effect of prolonged administration. The rats used for acute toxicity study were observed for a period of 24 h for signs of toxicity and eventual death while parameters for prolonged study were recorded at weekly interval starting from day zero up to day 28 post administration.

Results: The extract produced an intraperitoneal LD_{50} of 570 mg/kg. Body weight changes were not statistically significant ($p > 0.05$) while haematological parameters (packed cell volume (PCV)), haemoglobin concentration (Hb), red blood cells (RBC), white blood cells (WBC) and differential leucocyte counts (DLC) were significantly modulated ($p > 0.05$) after administration. Haematological indices (mean corpuscular volume (MCV)), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentrations (MCHC) were similarly modulated significantly ($p > 0.05$).

Conclusions: The extract appeared to be moderately toxic while prolonged administration improved the blood parameters of rats, suggesting that the plant's extract at lower doses can be used for a prolonged period, without deleterious effect on the haematological profile and serum enzymes.

1. Introduction

Diospyros mespiliformis (*Hochst.ex A.Dc*) is a tall plant usually 15–50 m high with dense rounded and buttressed stem, belonging to the family Ebenaceae (Orwa et al., 2009). It occurs in woodlands, savannahs and along riverbanks preferring areas with permanent water that helps in natural regeneration. The mean annual temperature of 16–27 °C and annual rainfall of 500–1270 mm and an altitude of 350–1250 are ideal for the growth of the plant (Orwa et al., 2009).

In Nigeria, the plant is found in different parts of the country where it is called by different names such as Onye-Koji (Igbo), Kanya (Hausa) and Igidudu (Yoruba). The roots and the bark are used to stop purging, enhance fertility and against infections such as malaria, pneumonia, syphilis, leprosy, dermatomycoses and as an anthelmintic (Irvine, 1961; Gyang, 2001). Decoction of the root

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for the treatment of malaria is usually taken for one week or more by the locals of Tilly village of Michika local government of Adamawa State. Recently, the plant was also found to have potent trypanocidal activity against *Trypanasoma brucei-brucei* in rats (Mbaya et al., 2010).

Previous screening (Adeniyi et al., 1996; Shagal et al.,2012) of the leaves, roots and stem bark of this plant revealed the presence of several metabolites such as anthraquinones, tannins, triterpens, saponins, steroids and sugars, which have been reported to possess varied pharmacological activities. In view of the wide application of this plant in the folkloric medical practice of the people of Northern Nigeria in general and the Kamwe people in particular, this study evaluated the acute toxicity profile of the ethanolic root bark extract and the effect of prolonged administration of the extract on clinical, haematological and biochemical profiles of albino rats.

2. Materials and methods

2.1. Collection and preparation of experimental materials

2.1.1. Authentication and preparation of plant material

Leaves, fruits and root bark of *Diospyros mespiliformis* were obtained from traditional medicine market, opposite NEPA House, Maiduguri, Borno State, Nigeria. Professor S.S.Sanusi, a botanist at the Department of Biological Sciences, University of Maiduguri, carried out authentication of the plant. Voucher specimen (Vet 212A6) was deposited at the herbarium of the Department of Veterinary Physiology, Pharmacology and Biochemistry of the Faculty of Veterinary Medicine, University of Maiduguri.

2.1.2. Extraction of plant material

The dried root bark was pulverised into powder using pestle and mortar. One thousand (1000) grams was exhaustively extracted using soxhlet extractor and condenser using absolute (100%) ethanol as a solvent at the Department of Chemistry, University of Maiduguri, according to standard method (WHO, 1992).

2.1.3. Phytochemical screening

The extract was subjected to qualitative chemical screening for the identification of the various classes of the chemical constituents according to the method described by Sofowora (2006). Briefly, this is a qualitative screening based on reaction between metabolites within an extract, with some chemical compounds. The reaction is evidenced by color change and the degree of color change determines whether an extract contains low, moderate or high concentration of a specific metabolite. Color changes whether deep, moderate or faint correspond to high, moderate or low concentration of the metabolite within an extract, respectively.

2.1.4. Experimental animals

Wistar albino rats were obtained from the animal house of the Department of Biochemistry, College of Medical Sciences, University of Maiduguri, for the study. Fifty-five out-bred ten weeks old Wistar albino rats of both sexes and weighing 84 – 184 g were used for both acute and prolonged administration studies. The rats were housed in netted plastic cages under standard conditions (temperature 27 ± 2 °C, 70% relative humidity and 12 h daylight/night cycle). The experiment was conducted at the Parasitology laboratory of the Department of Veterinary Microbiology and Parasitology, University of Maiduguri. Pelleted poultry growers feed (Vital Feeds®, Jos Nigeria) and water were provided ad libitum. The experiment was carried out according to the care and use of experimental animals' protocol (Ochei and Kolhatkar, 2000) and was approved by the Faculty of Veterinary Medicine ethics and research committee.

2.2. Acute toxicity and safe dose determination

Thirty rats were used for this study. The rats were fasted overnight and randomly divided into six groups (A, B, C, D, E, and F) of five rats each. Groups A – E were, respectively, administered intraperitoneally (i.p), graded doses (100, 200, 400, 800 and 1600 mg/kg) of the crude ethanolic root extract of *Diospyros mespiliformis* resuspended in normal saline while group F (control) was administered normal saline equivalent to the highest volume of the extract given (Aliu and Nwude, 1982). Each group was housed in a separate netted plastic cage. The rats were observed for a period of 24 h for signs of toxicity (anorexia, depression, respiratory embarrassment, coma and death) and mortality. The lethal dose (LD_{50}) was calculated using the arithmetic method of Karber as modified by Aliu and Nwude (1982).

2.3. Determination of the effect of prolonged administration

Twenty-five Wistar albino rats were randomly divided into five groups (A, B, C, D and E) of five rats each, with each group assigned to a separate netted plastic cage. Rats in groups A–D were daily administered graded doses (50, 100, 200 and 400 mg/kg) of the ethanolic root extract of *Diospyros mespiliformis*, respectively, for 28 days by gavage using stomach tube, while those in group E were given normal saline by the same route for the same period of time. Body weight and haematological parameters were determined on the first day of administration of the extract (day 0) and thereafter weekly up to day 28 while serum enzymes were determined on day 29 of the experiment.

Body weight was measured using a sensitive measuring balance (Adam Equipment Company, United Kingdom). Blood for the determination of haematological parameters was obtained from tail vein while that for serum enzymes determination was obtained on day 29 of the experiment after anaesthetizing the rats using mild chloroform fumes and the blood collected through cardiac puncture. Haematological parameters (PCV, RBC, Hb, WBC, DLC) were determined using a standard criteria (Schalms et al., 1995) while serum enzymes (ALT, AST,) and (ALP) activities were determined using a commercial kit (Randox Laboratory Limited, Ardmore, UK) as described by Schmidt and Schimdt (1963) and Babson et al. (1966) respectively.

2.4. Statistical analysis

Data generated from the above were expressed as mean ± standard deviation (S.D.) using Analysis of Variance (ANOVA). Turkey–Kramer multiple comparison test was used to compare within and between groups and $p < 0.05$ was considered significant throughout the study (Graph pad Instant, 2009).

3. Results

The yield of the extract was 24.44% w/w, dark brown in color and soluble in water. Phytoconstituents found include resins, flavonoids, saponin glycosides, cardenolides, cardiac glycosides, anthraquinones, tannins, soluble starch and carbohydrates (Table 1).

3.1. Acute toxicity

Anorexia, depression, respiratory embarrassment, coma and eventual death were the signs of toxicity observed. All the rats treated with 400, 800 and 1600 mg/kg showed one form of toxicity sign or the other, while only one death was recorded in the group administered 200 mg/kg of the extract. No clinical sign or mortality was observed in group A (100 mg/kg). The clinical