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Original Article

Acute and subchronic toxicity evaluation of methanol stem-bark extract of *Ximenia americana* Linn (Olacaceae) in Wistar rats

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ABSTRACT

Ximenia americana is used in African ethno-medicine for spasmodic bowel diseases with stem bark particularly used for ulcers. This study evaluated the toxicity-profile of methanol stem-bark extract. Extract doses were selected from estimated oral median lethal dose (LD_{50}) of acute toxicity test. Ten male Wistar rats in 4-groups, weekly weighed and daily treated orally per body-weight for 28 days with normal-saline and extract-doses (250, 500, 1000 mg/kg) respectively were euthanized. Blood for biochemical and haematological analyses were collected into plain and anticoagulated (EDTA) sample-bottles respectively from each group. Vital-organs were isolated, weighed and fixed in buffered-formalin fixatives for histo-analyses. Mean ± standard-error of mean and statistical-significance at ($p \leq 0.05$) of obtained-data were evaluated. The extract at up to 5000 mg/kg caused no mortality or behavioural toxic-signs and thus, oral LD_{50} was estimated to be greater than 5000 mg/kg. No changes in organ-sizes, body-weights or anatomy of brain, heart, liver and stomach occurred, but at 1000 mg/kg, kidney showed vascular-congestion with polymorphonuclear cells, lungs had consolidated areas of polymorphs infiltration, while spleen had distorted germinal-centres. Liver enzymes and urea levels were not altered significantly, but a dose dependent significant increase in total-protein only at 1000 mg/kg; and significant reduction in albumin level at 500 and 1000 mg/kg were observed. The observed dose-dependent reduction in creatinine was not significant. Total-calcium and chloride ion concentrations increased significantly only at 250 mg/kg. In conclusion, acute oral administration of methanol stem-bark extract of *Ximenia americana* was relatively non-toxic in mice, but minimal anatomical changes in kidney, lungs and spleen occurred when used for few weeks in rats.

1. Introduction

Ximenia americana (Linn) of the family, Olacaceae and genus, *Ximenia* has common names as sour or monkey plum, sea lemon or false sandal wood and is known in the Northern part of Nigeria as 'Tsada', and in Eastern part (Igbo land) as 'Anya Nwona'; while the in Western part (Yoruba) it is called 'Igo' [1]. The various parts of the plant are used traditionally for several ailments, but the stem-bark is particularly used for stomach ulcers [2]. The practice of using medicinal plants to maintain and promote health, prevent and/or cure a wide variety of diseases is being carried out over ages [3]. Quite a number of medicinal plants used traditionally or locally had proven to be important sources of potential therapeutic agents. Plant or herbal preparations contain active chemical principles which in addition to being beneficial could also be toxic, but most often little or no attention is given in identifying the dose or duration related toxic effects of their use [4]. Toxicological evaluation correlates duration of exposures to the amounts of drugs used, and the effects on various organs and their physiologic processes as to provide information on the safety of use of drugs. Such toxicity findings are also as valuable for therapeutic dose selections and drug designs as the efficacy evaluations. In this study, the acute and subchronic toxicity properties of *Ximenia americana* were investigated with the aim to establish the acute and subchronic effect on body and organ weights, blood components and some biochemical parameters in rat following few days repeated intake of methanol stem bark extract of the plant.

2. Materials and methods

2.1. Collection and identification of plant material

Ximenia americana plant collected in June 2014 from Tashan-yari area of Makarfi in Kaduna State was identified and

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authenticated by a taxonomist, Mallam Sanusi of the Department of Biological Sciences, Ahmadu Bello University, Zaria by comparing with an existing voucher number 099.

2.2. Experimental animals

Adult male Swiss albino mice (18–29 g) and male Wistar rats (100–200 g) obtained from the Animal House Facility of Department of Pharmacology and Therapeutics, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria were used for the study. The animals were handled in compliances with the ARRIVE guidelines (2010) [5] and the experiments were conducted in accordance with the National Institute of Health guide for the care and use of laboratory animals [6].

2.3. Equipments and instruments

Weighing balance (Wet. Avery Ltd, Birmingham, England)
Heamatocrit centrifuge (Denley, BS400, UK)
Ion Selective Electrode-Electrolyte analyser (Pioway, XI-921D, Japan)
Haematology analyser (Pioway HY-3400, Japan)
Centrifuge (Techmel and Techmel, TT-645P, UK)
Other materials include mortar and pestle, dissecting kit, pasteur pipette, evacuated (EDTA) tubes and other sample bottles, capillary tubes; normal saline (0.9 g NaCl in 100 ml of Distilled Water), formaldehyde and chloroform (BDH Chemical Ltd., Poole England).

2.4. Preparation of plant extract

The stem bark of *Ximenia americana* were scraped off from the stems of the plant and dried under shade until a constant weight was obtained and then crushed into powder. Soxhlet extraction method was used whereby the obtained powdered material (759 g) was packed into a filter thimble underneath a conical flask containing the solvent (methanol) which is heated, to evaporate and condensed through the condenser into the thimble for 72 h extraction. The extract mixture was then filtered to obtain the filtrate which was concentrated to dryness on a rotary evaporator over water bath at 55 °C.

2.5. Acute toxicity study

Lorke's method (1983) [7] was adopted for the acute toxicity test in mice and rats. Thirteen (13) each of the animal species were used. Nine (9) of either of the species in 3 groups of 3 per group for three graded doses of 10, 100, 1000 mg/kg were treated orally per body weight and observed for 24 h for signs of changes in behavioural and/or death. In a second phase of the experiment, the remaining animals of each of the species in 4 groups of one animal per group were respectively given lower or higher doses of the extract depending on occurrence of death or no death in the first phase and observed again for 24 h. The oral median lethal doses were then calculated as the geometric mean of the highest non-lethal and the lowest lethal doses as: $LD50 = \sqrt{maximum\ nonlethal\ dose \times minimum\ lethal\ dose}$ for both animal species.

2.6. Subchronic toxicity study

Twenty-four (24) rats were divided into 4 groups of 6 rats each and treated daily per body weight with normal saline (1 ml/kg), extract doses of 250, 500, 1000 mg/kg respectively for 28 days. The animals were euthanized on the 29th day and vital organs including brain, liver, heart, kidneys, lungs, spleen and stomach were isolated, weighed and placed into fixative (formalin = 10% formal saline) for histological examinations. Blood samples were also collected via cardiac puncture into both EDTA tubes and non-heparinised tubes for haematological and biochemical tests respectively [8].

2.7. Statistical analyses

The obtained data were presented as tables and plates. The difference between means was analysed using one way analysis of variance (ANOVA) and Dunnett post hoc test was used to obtain statistical significant differences at $p \leq 0.05$.

3. Results

3.1. Estimation of the oral median lethal dose (LD_{50}) of the methanol stem bark extract of Ximenia americana

No death was recorded in the first-phase of the study for both animal species. In the second phase, doses of 1600, 2900 and 5000 mg/kg were used and no death was also recorded. The oral median lethal dose (LD_{50}) for the methanol stem bark extract of *Ximenia americana* was therefore estimated to be greater than 5000 mg/kg in both mice and rat and no signs of behavioural changes were also observed.

3.2. The subchronic (28 days) toxicity evaluation of Ximenia americana extract on body and organ weights, blood components and some biochemical parameters in rat

Slight variations of either an increase or decrease in the body and organs weights were observed, but none was significant ($p \leq 0.05$) except for the lungs at 250 mg/kg extract (Tables 1 and 2). There was no significant alteration in the concentrations of haematological indices (Table 3). The alteration in the concentration of the liver enzymes were not significant but the levels of total protein was increased in a dose dependent manner and was significant only at 1000 mg/kg with respect to the control group and no significant difference amongst the treatment groups. The albumin level was reduced in a dose dependent manner and significant ($p \leq 0.05$) for the two higher extract dose groups with respect to the control group. There changes in the urea level were not

Table 1
Weekly body weight changes of rats in 28 days daily administration with *Ximenia americana* methanol extract.

Treatments Per kg	Mean ± SEM of body weights in weekly intervals				
	DAYI	DAY8	DAY15	DAY22	DAY29
N/S (1 ml)	122.00 ± 8.00	145.00 ± 10.20	149.00 ± 10.84	160.00 ± 11.18	161.00 ± 9.51
EXT 250 mg	108.00 ± 2.50	122.00 ± 2.64	140.00 ± 8.94	148.00 ± 5.00	141.00 ± 1.68
EXT500 mg	116.00 ± 9.11	142.00 ± 10.26	144.00 ± 11.60	147.00 ± 13.40	158.00 ± 12.78
EXT1000 mg	120.00 ± 6.60	136.00 ± 7.25	134.00 ± 10.06	141.00 ± 10.06	144.00 ± 12.32

Data presented as mean ± SEM; Statistical tool: one way ANOVA and * = $p \leq 0.05$ (Dunnett post hoc test); Ext = Extract; N/S = normal saline; n = 5.

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