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Original Article

Acute and chronic toxicity profiles of the methanol leaf extract of *Acacia ataxacantha* D.C (Leguminosae) in Wistar rats

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ABSTRACT

Acacia ataxacantha various parts have been reportedly used as herbal remedy for treatment of pains, microbial infections, ulcers, respiratory infection, mineral and vitamins supplements and dysentery. This study was conducted to ascertain the toxicity profile of methanol extract of *Acacia ataxacantha* in laboratory animals. The acute and chronic toxicity study was conducted according to the method of Lorke (1983) and OECD guidelines (2008) respectively. The oral lethal median dose (LD_{50}) of the extract was estimated to ≥5000 mg/kg. The extract significantly increases ($p \leq 0.05$) the liver (alanine transaminase, aspartate transaminase, alkaline phosphatase) and kidney (creatinine, urea and sodium ion) parameters at the dose of 400 mg/kg body weight. Histological examination revealed moderate glomerular necrosis and lymphocytes hyperplasia on the kidney (50, 200 and 400 mg/kg), the liver showed hepatocellular necrosis with kupffer cells hyperplasia, while mild mucosa necrosis was observed on stomach tissues. The extract is safe on acute administration, however prolong use may produce harmful effect on the liver, kidney and stomach.

1. Introduction

Medicinal plants have been used globally from ancient times as the major source of traditional medicine in the treatment of various diseases [1]. Report also shows that about 50% of orthodox drugs are derived from vegetable sources [2,3]. Presently, there is increasing popularity in the use of medicinal plant as they are believed to be natural, beneficial, safe, available, accessible and free from adverse effects [4,5]. These assumptions are based on long term use of the plants, with little or no scientific data to support information on the efficacy and safety profiles of these medicinal plants [1,6]. However, researches have proven that not all medicinal plants are safe, as they produce toxic effect on evaluation, which can result from inherent toxic effect of the active principle, overdosing, chronic use, interactions, allergies, contaminations [1,7,8]. The plant, *Acacia ataxacantha* is a shrub that is widespread in tropical Africa [9]. Traditionally, the plant was reported to be used in the management of painful inflammation of the respiratory tract, tooth, ulcers and skin sores [10]. The analgesic and anti-inflammatory activities of the plant had also been evaluated [11,12]. Hence, toxicity studies are necessary to assess the safety profile of the plant, as results obtained can be extrapolated to human on how to prevent or manage adverse effects relating to the plant use [13]. The present study was aimed at determining the chronic effect of extract in Wistar rats.

2. Materials and methods

2.1. Plant collection and identification

The fresh plant of *Acacia ataxacantha* D.C was collected from Bassawa area of Zaria, Kaduna State, Nigeria, in April 2015. It was identified and authenticated by Mallam Sanusi Namadi of the Department of Botany, Faculty of Sciences, Ahmadu Bello University-Zaria. Where herbarium number (Voucher Number 1924) was compared with existing specimen and deposited.

2.2. Experimental animals

Wistar rats (180–220 g) were obtained from Animal House, Department of Pharmacology and Therapeutics, Ahmadu Bello University, Zaria. The animals were maintained in a well-ventilated room under standard temperature and pressure, feeds and water administered *ad libitum*. Animals were handled in compliance with ARRIVE guidelines 2010 [14], while the experiments were conducted

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Table 1
Effect of extract on some renal biochemical parameters after 90 days of oral administration.

Biochemical Parameters	D/W 1 ml/kg	MEAA 50 mg/kg	MEAA 200 mg/kg	MEAA 400 mg/kg
Urea (mg/dL)	3.18 ± 0.45	5.53 ± 0.69*	5.80 ± 0.31*	5.67 ± 0.42*
Creatinine (mg/dL)	35.82 ± 0.66	47.00 ± 3.61*	45.50 ± 1.50*	49.67 ± 5.46*
Na^+ (mmol/L)	141.20 ± 2.71	148.67 ± 2.40	156.50 ± 7.50*	156.67 ± 0.88*
K^+ (mmol/L)	5.00 ± 0.07	5.50 ± 0.17	5.40 ± 0.50	5.40 ± 0.16
Cl^- (mmol/L)	101.92 ± 1.12	107.23 ± 2.92	101.25 ± 0.85	104.80 ± 2.77
HCO_3^- (mmol/L)	25.94 ± 0.91	25.40 ± 0.47	27.35 ± 0.95	25.83 ± 1.34

Data were presented as Mean ± SEM, n = 6, * = $P < 0.05$ level of significance
Statistical tool: ANOVA (one way analysis of variance) D/W = Distilled Water
MEAA = Methanol extract of *Acacia ataxacantha*.

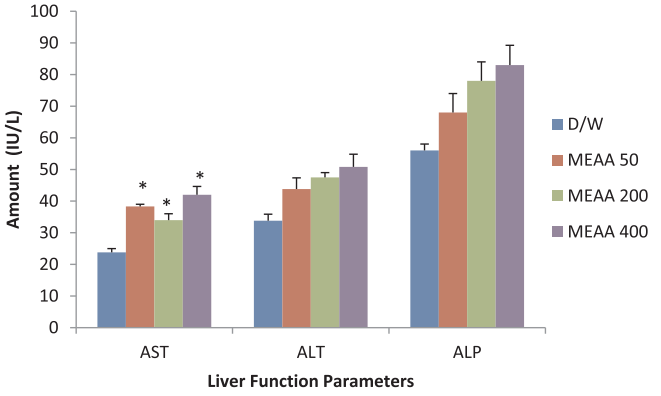

Fig. 1. Effect of extract on some liver function parameter in rats after 90 days of oral administration. Data were presented as Mean ± SEM, n = 6, * = P < 0.05 level of significance Statistical tool: ANOVA (one way analysis of variance), D/W = Distilled water MEAA = Methanol Extract of *Acacia ataxacantha.*

according to standard protocols of National Institute of Health (NIH, 2015) [15] guidelines for use and care of laboratory animals.

2.3. Equipment and instruments

Mortar and pestle, weighing balance "mg" (AE240 Switzerland), weighing balance "g" (Golden-Mettler USA), desiccator (Monax-Scotland), water bath, evaporating dish, plain bottles, EDTA bottles, capillary tubes, haematocrit centrifuge (Denley, BS400, UK), centrifuge (Techmel and Techmel, TT-645P, UK), haematology analyser (PIOWAY HY-3400, Japan).

Other materials include distilled water, methanol (BDH Chemical Ltd, Poole, England), chloroform (Sigma Chemicals Co. USA), 10% formalin.

2.4. Preparation of plant extract

The leaves of the plant were separated from the plant branches, cleaned, air-dried under the shade for fourteen days and crushed into coarse powder using pestle and mortar. Five hundred grams (500 g) of the coarse powder was cold macerated with 2.5 L of 70% $^{v}/_{v}$ methanol (in water) for 72 h. The resultant mixture was filtered using Whatman filter paper (No.1) and concentrated to dryness using evaporating dish over a water-bath, maintained at a temperature 40–50 °C to obtain a constant weight of the extract. The dried extract was packed in a clean air tight container and kept in a desiccator until used.

2.5. Acute toxicity study

The oral median lethal dose (LD_{50}) of the extract was determined in rats using the method described by Lorke 1983 [16]. Animals were fasted overnight and LD_{50} evaluation was carried out in two phases. In the first phase, nine rats were randomly placed into three groups of three mice each. Groups I, II and III were treated with the extract at doses of 10, 100 and 1000 mg/kg body weight orally respectively. The second phase design was determined by the result obtained from the first phase. In the second phase, three rats were put in three groups of one mouse each. Groups I, II and III were administered the extract at doses 1600, 2900 and 5000 mg/kg body weight respectively. In both phases, the rats were also observed for 24 h for signs of toxicity and mortality. The LD_{50} value was then calculated as the geometric mean of the highest non-lethal dose (with no death) and the lowest lethal dose (where death occurred).

2.6. Chronic toxicity study

According to OEDC 2008 [17], twenty four adult rats were randomly divided into four groups of six rats each. Group I (negative control) received distilled water while, groups II, III and IV received the extract at doses 50, 200 and 400 mg/kg body weight respectively. The treatment was done once daily (orally) for 90 days. All the animals were observed daily for signs of toxicity and mortality. The body weight of each rat was measured at 9 am once weekly using a sensitive balance before the commencement of dosing. At the end of the treatment period, the animals were euthanized in chloroform chamber. Vital organs like the liver, heart, kidney spleen and stomach were removed, weighed and preserved in 10% formalin until used for histological

Table 2
Effect of extract on haematological parameters after 90 days of oral administration.

Haematological indices	D/W 1 ml/kg	MEAA 50 mg/kg	MEAA 200 mg/kg	MEAA 400 mg/kg
WBC (10^9/L)	7.10 ± 1.05	8.63 ± 1.29	5.55 ± 1.25	5.07 ± 0.04
RBC (10^{12}/L)	7.58 ± 1.46	6.83 ± 0.67	7.70 ± 0.01	7.40 ± 1.73
HGB (g/dL)	13.86 ± 0.16	13.33 ± 0.22	14.50 ± 0.20	14.00 ± 0.12
PLT (10^9/L)	724.00 ± 2.90	699.00 ± 5.80	706.00 ± 9.00	773.00 ± 9.60
PCV (%)	50.80 ± 0.20	48.00 ± 1.70	50.50 ± 0.50	51.00 ± 0.60
MCV(fL)	65.90 ± 1.50	70.20 ± 3.60	65.60 ± 0.30	68.40 ± 2.00
MCH (Pg)	18.10 ± 0.30	19.50 ± 0.50	18.50 ± 0.10	18.80 ± 0.50
MCHC(g/dL)	27.20 ± 0.30	27.90 ± 0.70	28.20 ± 0.20	27.90 ± 0.40

Data were presented as Mean ± SEM, n = 6. Statistical tool ANOVA (followed by Dunnett post-hoc test) $p \leq 0.05$ level of significance. D/W = Distilled water. MEAA = Methanol Extract of *Acacia ataxacantha*.

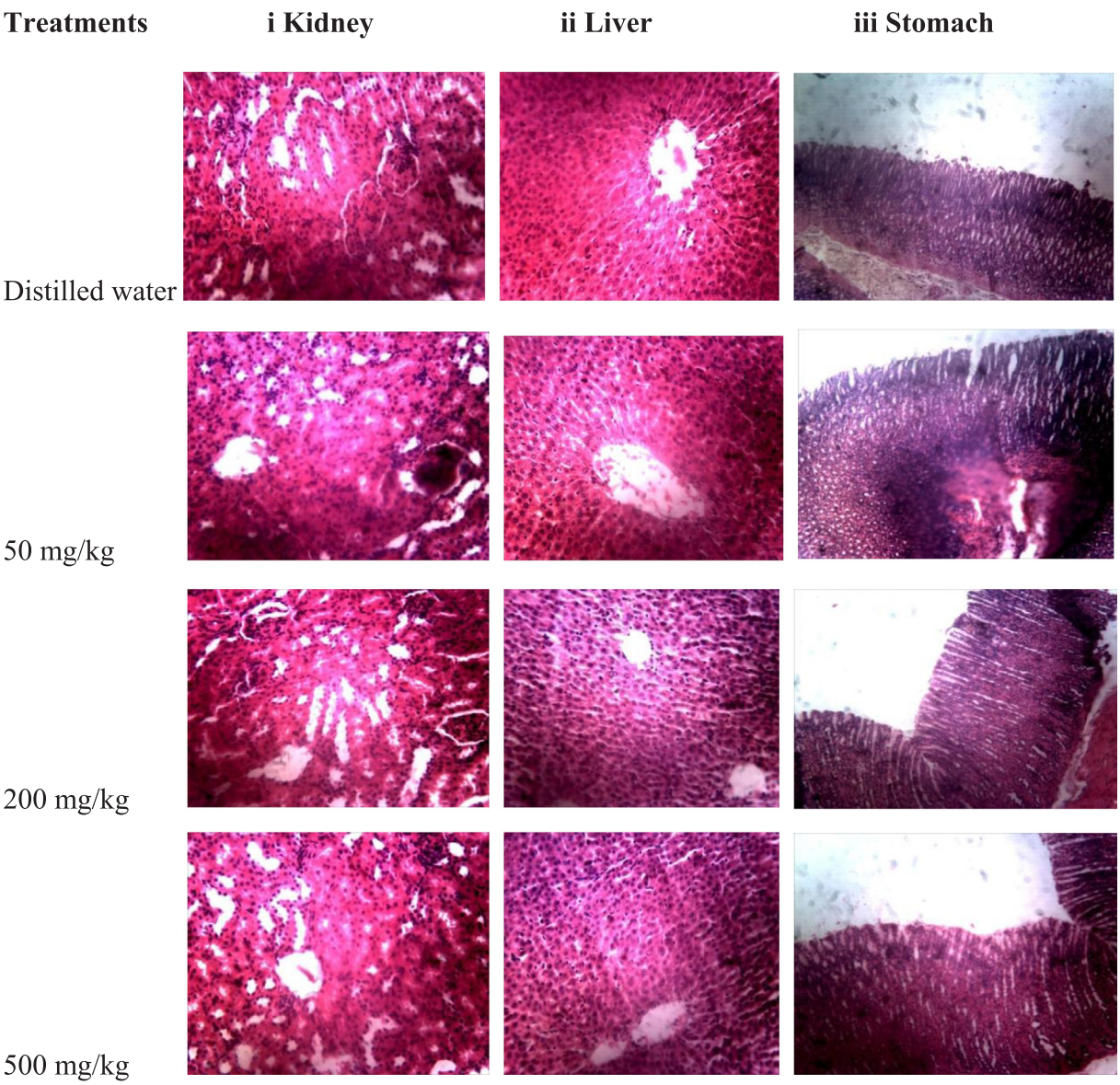

Plate 1. Photomicrographs of a section of tissues (H & E × 250) of Wistar rats administered with distilled water and methanol extract of *Acacia ataxacantha* for 90 days. (i) moderate glomeruli necrosis (50, 200 and 500 mg/kg) and lymphocyte hyperplasia (200 and 500 mg/kg) in the kidney. (ii) mild hepatocellular necrosis, accompanied with kupffer cell hyperplasia was observed at 500 mg/kg in the liver and (iii) stomach showed mild mucosa necrosis at 500 mg/kg.

examinations. Blood samples were collected for sera preparation by cardiac puncture. The sera preparations were used subsequently for biochemical (plain bottles) and haematological (heparinized bottles) studies.

2.7. Statistical analysis

The data were expressed as mean ± SEM and analyzed using one way analysis of variance (ANOVA) followed by Dunnett-t post-hoc test. Values of $p \leq 0.05$ were considered statistically significant

3. Results

The oral median lethal dose (LD_{50}) of the extract in rats was found to be greater than 5000 mg/kg body weight.

There was significant increase ($p \leq 0.05$) in body weight of the rats that received extract at doses of 200 and 400 mg/kg when compared with the negative control group. There was no significant difference in the relative organ weights of all the tested doses.

There was significant ($p \leq 0.05$) increase in the level of urea and creatinine, while the level of Na^+ ion significantly ($p \leq 0.05$) and dose dependently increase when compared with the negative control group in the liver function test (Table 1).

The level of aspartate transaminase increased significantly ($p \leq 0.05$) on evaluation of liver biochemical parameters, while there was no significant increase in the level of alanine transaminase and alkaline phosphatase (Fig. 1).

The extract on evaluation did not produce any significant difference in the level of haematological parameter (Table 2).

The photomicrograph revealed changes in kidney (moderate to severe glomeruli necrosis accompanied with lymphocytes hyperplasia) at doses tested. The liver architecture showed moderate hepatocellular necrosis with kupffer cell hyperplasia (400 mg/kg) on evaluation. Histopathology of the stomach showed mild necrosis of the mucosa lining (400 mg/kg). The heart and spleen did not show any cross changes in architecture on evaluation (Plate 1).

4. Discussion

Recent report showed that about 85% of people worldwide, particular in developing countries rely on traditional medicine and its practice for their primary health care need [18,19]. The oral median lethal dose (LD_{50}) of the extract in rats was found to be greater than 5000 mg/kg body weight. This shows that on acute administration (orally), the extract is practically non-toxic [16,20]. Reduction in body weight and relative organ weight is usually regarded as toxic effect of extract on animal, resulting to reduced food and water intake [21,22]. The extract caused an increase in body weight when compared with negative control group, hence showed relative safety of the extract on the rats.

The kidney is an important organ in the body that maintains homeostasis through its osmoregulatory function (regulation of electrolytes and blood pressure, maintenance of acid-base balance) [23]. Urea is a by-product of protein metabolism that is excreted solely in the kidney [24] while, creatinine is a by-product of muscle metabolism [25] which is also excreted exclusively by glomerular filtration. Hence, the levels of creatinine, urea, sodium, potassium or chloride, are parameters used as a measure of kidney function [26]. The significant increase in the serum level of urea and creatinine in the renal biochemical parameters tested is an indication of kidney dysfunction (may signify decrease in kidney function due to toxic effect of extract). Sodium is an extracellular fluid ion that is filtered and reabsorbed in the kidney [27]. The significant elevation in serum level of sodium ion in the serum electrolytes evaluated might be due to kidney dysfunction resulting from loss of excessive fluid (dehydration) and reduced water intake by the rats [28].

Liver is the major organ in the living system for metabolism of drugs and other xenobiotics. Alanine transaminase, aspartate transaminase and alkaline phosphatase are biomarkers used to evaluate liver function [29]. The significant increases in the level of AST might be an indication of liver damage, which is suggestive of hepatotoxic effect. Although, ALT and AST are largely used in the assessment of liver damage (as they cause hepatocytes inflammation, cellular leakage and damage to cell membrane) by drugs or any hepatotoxic substance [30,31], but elevated level of ALT is more specific for liver related injuries or diseases [32]. However, high level of AST is also indicative of liver damage, cardiac infarction and muscle injury [33]. ALT is present in the liver only in small quantities, the enzyme is secreted in the bile and substantial elevation of serum ALP is seen with mild intrahepatic or extra-hepatic biliary obstruction [24].

The analysis of haematological parameters is important in accessing toxic effect of substance because, it has a higher predictive value of toxicity in humans when tests involve rodents [34]. There was no significant difference in the haematological parameters when the extract treated groups were compared with the negative control group.

Histopathological evaluation of the 90 days oral administration of the extract in rats showed that the morphology of kidney, liver and stomach mucosa were adversely affected, suggesting that the extract could be toxic to the hepatocytes, kidney and stomach mucosa.

5. Conclusion

The methanol extract of *Acacia ataxacantha* is safe on acute administration, however prolong use may produce toxic effects on some organs (liver, kidney and stomach),

6. Conflict of interest

There are no literatures available on the toxicity profile of Acacia ataxacantha in Wistar rats.

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