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Research Article
Acute and Sub-chronic Toxicity Evaluation of Crude Methanolic Leaves Extract of *Acacia nilotica* (Linn.)

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Abstract

Background and Objective: Although large amount of pharmacological studies has been conducted to ascertain the traditional uses of various medicinal plants, however, very few plants have been thoroughly evaluated for their toxic/side effects. The aim of this research was to evaluate the acute and sub-chronic oral toxicity of its methanolic leaves extract. **Materials and Methods:** The methanolic extract of *A. nilotica* leaves was administrated in a single dose of 3000 mg kg^{-1} via oral gavage and the animals were observed for any behavioral changes or mortality. In the sub-chronic toxicity study, the rats received control (10 mL kg^{-1} b.wt., distilled water) and 3 doses of the extract (150, 300 and 600 mg kg^{-1} b.wt.) for 28 days via oral gavage. Following the 28 day treatment, the rats were sacrificed for haematological, biochemical and histopathology studies. **Results:** In the acute toxicity study, *A. nilotica* was found to be non-toxic at a dose of 3000 mg kg^{-1} b.wt. In the sub-chronic toxicity study, there was significant ($p<0.05$) reduction in body and organ weights of the 3 treatment groups compared to control, starting from week 4. Haemoglobin level of 300 mg mL^{-1} b.wt., group was found to be significantly ($p<0.05$) lower than that of the control group. The levels of other parameters in the three treatment groups did not showed significant changes from that of the control. Histopathological studies revealed slight alterations in the liver and kidneys which was most expressed in the highest-dose group (600 mg kg^{-1}). **Conclusion:** These results demonstrate that, while a single dose and short term oral intake of *A. nilotica* leaves extract caused no toxicity, prolong intake/treatment was found to be associated with alterations in hepatic and renal tissues. Thus, prolonged use of high doses of *A. nilotica* orally should be discouraged and lower doses encouraged.

Key words: *Acacia nilotica*, toxicity, extract, methanolic, acute, liver

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Therapeutic evaluation of medicinal plants has recently witnessed a growing interest amongst researchers worldwide. Research on the therapeutic potential of plants has risen over the years, with volumes of scientifically documented information showing considerable potential for medicinal plants to be used in the treatment of several diseases[1]. However, while large amount of pharmacological studies have been conducted to ascertain the traditional uses of various medicinal plants, very few plants have been thoroughly evaluated for their toxic/side effects. Reports of efficacy are, by far, more numerous than those on toxicity[2,3]. There is, therefore, a need to further the investigation of herbal remedies and phytochemicals to incorporate the observations of short and long-term toxicity manifestations and to ensure effectual open communication of such findings.

Acacia nilotica (L.) also known as gum arabic tree, babul, Egyptian thorn or Prickly Acacia is multipurpose nitrogen fixing tree legume. It occurs from sea level to over 200 m and withstand extreme temperature (>50°C) and air dryness[4]. It is widely spread in subtropical and tropical Africa from Egypt to Mauritania southwards to South Africa and in Asia eastwards to Pakistan and India[5]. *Acacia nilotica* is a single stemmed plant, grows to 15-18 m in height and 2-3 m in diameter. *A. nilotica* is 1 of the most important tree and almost all its parts are used traditionally in medicine, including leaves, bark, root, flower, pods, gum, etc.[6]. The aim of this research was therefore to evaluate the acute and sub-chronic oral toxicity of crude methanolic leaves extract of *Acacia nilotica* in experimental rats.

MATERIALS AND METHODS

Study area: The research was carried out at Pharmacognosy and Pharmacology Laboratories of the Faculty of Pharmaceutical Sciences, Usmanu Danfodiyo University, Sokoto, Nigeria. The research was conducted in a 19 months period, from September, 2016 to April, 2018.

Plant collection and authentication: Fresh disease-free leaves of the plant used were separately collected from Bodinga, Sokoto State, Nigeria and was identified and authenticated by a Botanist at the Biological Sciences Department, Usmanu Danfodiyo University, Sokoto, Nigeria. The plant was identified as *Acacia nilotica* (Linn.) with voucher number UDUH/ANS/0247. The samples were shed-dried, ground and kept in air-tight containers till further use.

Preparation of plant extract: The methanolic crude extract was prepared by soaking a sample (100 g) of the powdered plant part material in 90% methanol (600 mL) for 72 h. The extracts were filtered using clean cloth and Whatman No. 1 filter paper. The filtrate was concentrated in vacuum at 30°C and stored in sterile sample containers at 4°C until further use.

Experimental animals: Female albino rats, weighing 160-200 g, were used in this toxicity study. They were obtained from the Animal Research Centre (ARC) of the Ahmadu Bello University (ABU), Zaria, Nigeria. The rats were kept in the Animal House of the Department of Pharmacology, Faculty of Pharmacy, Usmanu Danfodiyo University, Sokoto, Nigeria, where they were acclimatized to standard laboratory conditions for 7 days. They were housed in groups of 6 rats/group and maintained on 12 h light, dark cycle, with standard pellet diet and water *ad libitum.* An approval was obtained from the Animal Ethics Committee, Usmanu Danfodiyo University, Sokoto, Nigeria. The institutional animal ethical guidelines were strictly observed. The methods described by the Organization for Economic Co-operation and Development (OECD) for the acute and sub-chronic toxicity studies were used in the following toxicity studies of crude methanolic extract of *Acacia nilotica* on albino rats[7,8].

Oral acute toxicity study (limit test dose): The acute oral toxicity study was conducted in compliance with OECD[7] guideline 423, which stipulate the use of 5 animals. Five of the test animals were fasted overnight (~12 h) and weighed. Test doses of *Acacia nilotica* methanolic extract were calculated in relation to the body weight of every fasted animal, and administered via orally at 3000 mg kg^{-1}. The animals were regularly and individually observed for behavioural changes and general toxicity signs after dosing for the 1st 24 h, with special attention being given during the 1st 4 h and then for 12 h. Thereafter, observation was continued daily for a total of 14 days. Animals were also observed for the presence of toxic symptoms such as weakness, aggressiveness, food refusal, loss of weight, diarrhoea, noisy breathing, fluid discharge from eyes and ears, etc.

Sub-chronic toxicity studies: The rats were divided into 4 groups of 6 rats each for the sub-chronic toxicity study in which daily oral administration of different concentrations of *Acacia nilotica* methanolic extract was carried out for 28 days[7,8].

Group 1 : Received distilled water (10 mL kg^{-1} b.wt.) and would serve as the normal control

Group 2 : Received 150 mg kg^{-1} b.wt. of crude methanolic extract of *Acacia nilotica*

Group 3 : Received 300 mg kg^{-1} b.wt. of crude methanolic extract of *Acacia nilotica*

Group 4 : Received 600 mg kg^{-1} b.wt. of crude methanolic extract of *Acacia nilotica*

The treatment with the above doses was conducted for a period of 28 days. Clinical signs were observed at least twice a day during the 28 day treatment period. Body weights were measured once a week.

Collection of blood/serum samples and organs : At the end of the 28 day treatment period, the rats were fasted overnight, anaesthetized with chloroform and then decapitated. Paired blood samples were collected via cardiac puncture into EDTA and non-EDTA tubes. The EDTA blood was used for haematological evaluation, the non-EDTA blood used was allowed to coagulate, contents centrifuged and the serum separated was used for biochemical analysis[8].

Selected vital organs were dissected, cleansed of adhering tissues and rinsed in normal saline before their weights were measured. The kidneys and livers were immediately stored in 10% formalin for histopathological study[8].

Serum biochemical analyses: For biochemical analyses, diagnostic kits (Spectrum Diagnostics, Cairo, Egypt) were used to evaluate liver and renal parameters[9]. The parameters analyzed were: Aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), gamma glutamyl transferase (GGT), total and direct bilirubin, albumin (alb), total protein (tp), urea, uric acid, electrolytes and creatinine. The blood samples were centrifuged at 3000 rpm for 15 min and then used to analyse the following parameters:

Haematological analysis: Haematological analysis of the blood samples was conducted using an automatic haematology analyzer (XP-300, SYSMEX, UK). The parameters evaluated were: haematocrit (PCV), hemoglobin (Hb), red blood cell (RBC), mean cell volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), white blood cell (WBC), lymphocytes, granulocytes and platelets[8].

Histopathological study: Immediately after collection of blood samples, animals were opened up and some selected vital organs such as liver, lungs, heart, kidney and spleen were removed, weighed individually and fixed in 10% buffered formalin in labelled bottles. Tissues were processed routinely and embedded in paraffin wax. Sections of 4-5 µm thick were stained with haematoxylin-eosin dye for photomicroscopic examination. The microscopic features of the organs from the rats in treated group were compared with that of the control group[8].

Statistical analysis: The results were expressed as the Mean ± SD (n = 4). The results were statistically analyzed using version 20 of the IBM-SPSS statistical program (IBM Corp., Armonk, NY, USA). One-way ANOVA was used followed by Duncan's test for parametric multiple comparisons between the control and the treatment groups. Differences were considered significant at $p<0.05$.

RESULTS

Acute oral toxicity (LD_{50}) effects of *acacia nilotica* on female albino rats: There were no animal deaths in the first set of five female rats receiving 3000 mg kg^{-1} b.wt., of *Acacia nilotica* crude methanolic extract. No sign of toxicity was observed in the wellness parameters during the 14 day observation period. A similar observation was made in the second set of five female rats treated with 3000 mg kg^{-1} b.wt., of the extract. Therefore, the approximate acute lethal dose (LD_{50}) of *Acacia nilotica* extract in female rats was estimated to be higher than 3000 mg kg^{-1}.

Sub-chronic oral toxicity effects of *Acacia nilotica* extract on rats

Effect of oral administration of *Acacia nilotica* extract on general behavior: In the sub-chronic toxicity study, the female rats administered with the 150 mg kg^{-1} b.wt., of *A. nilotica* extract did not exhibit symptoms of toxicity. However, some signs of toxicity such as lethargy, self-isolation and weakness started manifesting at around days 20-22 and 25-26, respectively, in the groups treated with the 600 and 300 mg kg^{-1} b.wt., doses. Overall, mortality and changes in respiratory rhythm and fur patterns were not observed during the 28 day experimental period in the aforementioned groups.

Effect of oral administration of *Acacia nilotica* extract on body weights: The body weights and body weight gain of the rats treated with the extract doses (150, 300 and 600 mg kg^{-1}

Table 1: Effects of leaves extract of *Acacia nilotica* on body weights of experimental rats

		Acacia nilotica extract (mg kg^{-1} b.wt.)		
Body weight (g)	Control (10 mL kg^{-1} b.wt.)	150	300	600
Initial	186±20.4	190±24.2	183±21.6	191±20.2
Week 1	192±18.1	192±24.1	185±21.0	194±20.2
Week 2	194±17.6	195±23.5	186±18.3	190±19.3
Week 3	199±16.8	196±25.6	183±16.4	183±17.7
Week 4	219±38.2	195±24.4*	183±17.2*	177±15.9*

Data presented as Mean±SD (n = 6), *Significantly different from the control (p<0.05)

Table 2: Effects of leaves extract of *Acacia nilotica* on organ weights of experimental rats

		Acacia nilotica extract (mg kg^{-1} b.wt.)		
Body weight (g)	Control (10 mL kg^{-1} b.wt.)	150	300	600
Lungs	1.38±0.02	1.34±0.04*	1.36±0.02	1.36±0.03
Heart	0.56±0.03	0.54±0.04	0.55±0.02	0.56±0.03
Liver	5.53±0.24	5.58±0.18	5.59±0.13	5.56±0.04
Spleen	0.53±0.03	0.55±0.03	0.44±0.05*	0.40±0.06*
Kidneys	0.58±0.02	0.55±0.03*	0.55±0.02*	0.56±0.02*

Data presented as Mean±SD (n = 6), *Significantly different from the control (p<0.05)

Table 3: Effects of leaves extract of *Acacia nilotica* on haematological parameters in rats

		Acacia nilotica extract (mg kg^{-1} b.wt.)		
Parameters	Control (10 mL kg^{-1} b.wt.)	150	300	600
Hb (g dL^{-1})	15.2±2.36	15.3±1.97	12.3±1.88*	14.4±0.77
RBC (×10^9 L^{-1})	4.37±0.50	4.93±0.50	3.97±0.65	4.72±0.34
PCV (%)	41.0±5.10	41.2±5.95	40.0±1.90	43.2±1.94
MCV (fL)	89.4±5.03	88.9±2.81	87.5±0.78	90.6±1.67
MCH (pg)	33.2±0.98	33.5±1.38	32.6±1.41	33.6±0.48
MCHC (g dL^{-1})	32.9±0.57	33.4±0.62	32.5±1.88	34.4±1.42
WBC (×10^9 L^{-1})	13.0±3.15	12.7±1.29	12.8±2.79	13.2±3.31
LYM (%)	78.3±7.94	73.0±10.5	70.5±3.67	71.7±6.15
PLT (×10^9 L^{-1})	771±55.8	783±51.5	795±71.7	789±96.3
GRAN (%)	32.2±4.02	28.5±5.86	30.7±4.37	31.2±3.06

Data presented as Mean±SD (n = 6), *Significantly different from the control (p<0.05), Hb: Haemoglobin, RBC: Red blood cells, PCV: Packed cell volume, MCV: Mean corpuscular volume, MCH: Mean corpuscular hemoglobin, MCHC: Mean corpuscular hemoglobin concentration, WBC: White blood cell, LYM: Lymphocytes, PLT: Platelets, GRAN: Granulocytes

b.w.t.) are presented in Table 1. The rats showed slight statistically non-significant decrease in body weight compared with the control group from the beginning to the 3rd week of the experiment. However, at the 4th week of the experiment, the rats in the three treatment groups showed a significant (p<0.05) body weights reduction compared to control.

Effect of oral administration of *Acacia nilotica* extracts on organs weights: The rats' organ weights are shown in Table 2. The rats treated with the extract at the doses of 300 and 600 mg kg^{-1} b.wt., had spleen weights significantly (p<0.05) lower than those of the control. Furthermore, the mean organ weights of the lungs for 150 mg kg^{-1} group were significantly lower than the control group. There was also a significant (p<0.05) reduction in the weights of the kidneys of the 3 treated groups compared to control. No significant differences were observed in the weights of the other organs compared to their respective control measurements.

Effect of oral administration of *Acacia nilotica* extract on plasma haematological parameters: The haematological parameters of the control and treated groups were examined as shown in Table 3. Significant (p<0.05) reduction was observed in plasma haemoglobin levels in the rats treated with the 300 mg kg^{-1} b.w.t., of *A. nilotica* extract compared with the control. Other haematological parameters measured did not show statistically significant differences compared with the control groups.

Effect of oral administration of *A. nilotica* extract on serum electrolytes levels: The serum electrolytes levels of the control and treated groups are presented in Table 4.

Table 4: Effects of leaves extract of *Acacia nilotica* on electrolyte levels of experimental rats

		Acacia nilotica extract (mg kg^{-1} b.wt.)		
Body weight (g)	Control (10 mL kg^{-1} b.wt.)	150	300	600
Na^+ (mMol L^{-1})	138±1.60	136±1.97	133±2.37	135±1.94
K^+ (mMol L^{-1})	5.08±0.94	5.13±0.69	4.52±0.42	4.30±0.30
Cl^- (mMol L^{-1})	98.3±2.16	98.5±2.43	95.0±2.97	95.3±1.37
HCO^-_3 (mMol L^{-1})	21.8±1.47	23.8±1.72	23.2±1.94	24.5±1.87
Urea (mMol L^{-1})	6.27±0.60	5.57±0.48	5.73±0.44	6.20±0.88
Uric acid (mMol L^{-1})	1.30±0.13	1.35±1.10	1.42±0.15	1.57±0.20
Creatinine (mg dL^{-1})	0.50±0.10	0.57±0.10	0.65±0.10	0.67±0.10

Data presented as Mean±S.D (n = 6), *Significantly different from the control (p<0.05)

Table 5: Effects of leaves extract of *Acacia nilotica* on liver parameters of experimental animals

		Acacia nilotica extract (mg kg^{-1} b.wt.)		
Body weight (g)	Control (10 mL kg^{-1} b.wt.)	150	300	600
TP (g dL^{-1})	5.35±0.32	5.82±0.48	5.02±0.65	5.73±0.46
ALB (g dL^{-1})	3.28±0.50	3.38±0.18	2.97±0.51	2.95±0.41
AST (µ L^{-1})	157±10.9	158±19.2	162±22.6	164±7.33
ALT (µ L^{-1})	87.8±3.66	88.2±4.26	88.7±6.74	90.5±8.17
ALP (µ L^{-1})	380±14.00	384±19.5	387±6.74	391±7.71
GGT (µ L^{-1})	4.70±0.35	4.57±0.52	4.97±0.20	4.95±0.31
TB (mg dL^{-1})	0.61±0.05	0.61±0.06	0.67±0.13	0.65±0.07
DB (mg dL^{-1})	0.16±0.02	0.17±0.04	0.16±0.03	0.17±0.02

Data presented as Mean±SD (n = 6), *Significantly different from the control (p<0.05), TP: Total protein, ALB: Albumin, AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, ALP: Alkaline phosphatase, GGT: Gama glutamyl transferase, TB: Total bilirubin, DB: Direct bilirubin

Examination of serum electrolytes did not show much variation between the treated groups and their respective control groups, sodium, potassium, chloride, bicarbonate, urea, uric acid and creatinine concentrations in the treated groups were not significantly different from those in the control group.

Effect of oral administration of *A. nilotica* extracts on serum liver parameters: The serum liver parameters of the control and treated groups are presented in Table 5. The acute oral treatment of rats with the crude methanolic extract of *A. nilotica*, in general, did not induce significant modifications of the liver parameters when compared to control group. However, AST, ALT and ALP levels were mildly but dose-dependently increased in the rats treated with *A. nilotica* extract compare with the irrespective control groups.

Histopathological examination

Histopathological examination of liver tissue: Histopathological examinations were performed on the liver to assess whether or not liver tissues had been damaged. The photomicrographs of the experimental rats' liver treated with control and treated groups are presented in Fig. 1a-d. The liver of the control group shows normally distributed hepatocytes with normal orientation of sinusoid (green arrow) with normal clear central vein. While the 150 mg kg^{-1} treated group shows normally distributed hepatocytes with normal orientation of sinusoid (yellow arrow) with slightly congested central vein (black arrow) and normal clear central vein (CV). The 300 mg kg^{-1} treated group shows normally distributed hepatocyte with normal orientation of sinusoid (green arrow) with moderately congested central vein (CV). Lastly, the liver of 600 mg kg^{-1} (Group 4) treated group shows normally distributed hepatocyte with normal orientation of sinusoid (green arrow) with highly congested central vein (CV).

Histopathological examination of kidney tissue: Histopathological examinations were performed on the kidney to assess whether or not kidney tissues had been damaged. The photomicrographs of experimental of rats' kidney treated with control and treated groups are presented in Fig. 2a-d. The kidney of the control (Group 1) and 150 mg kg^{-1} (Group 2) treated group show normal glomerulus (black arrow) with normal collecting ducts. While the kidney of 300 mg kg^{-1} (Group 3) treated group shows normal glomerulus (black arrow) and moderately degenerated glomerulus with moderate cellular infiltrated collecting ducts. Lastly, the kidney of 600 mg kg^{-1} (Group 4) treated group shows moderately (black arrow) and highly (yellow arrow) degenerated glomerulus with moderate cellular infiltrated collecting ducts.

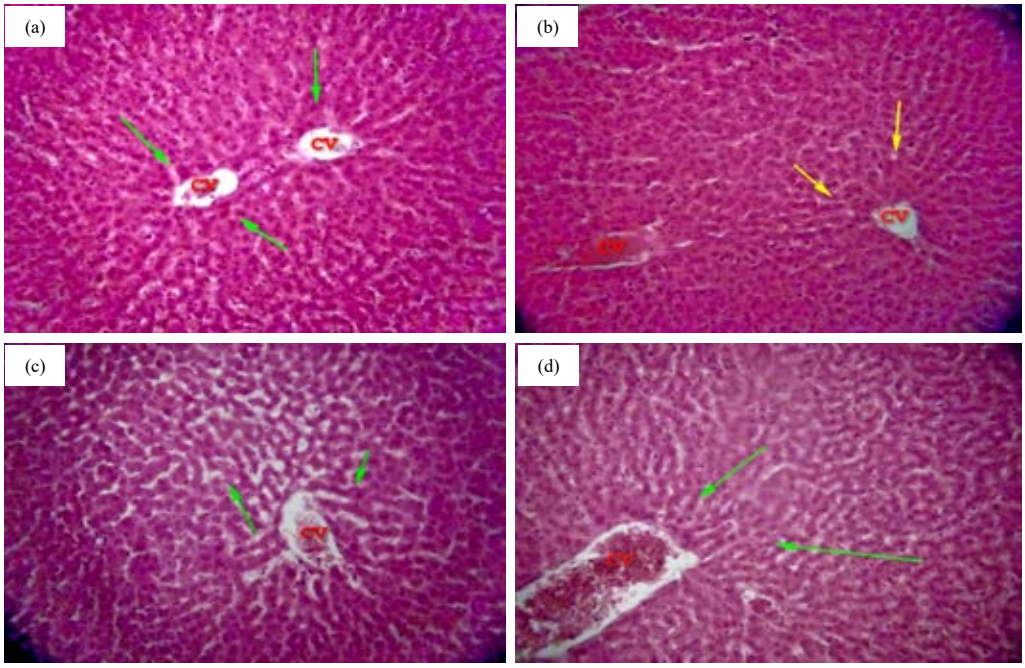

Fig. 1(a-d): Liver sections stained with hematoxylin and eosin (H and E-stained under 150× magnification power) showing the effect of *A. nilotica* methanolic extract in a 28 day sub-chronic toxicity study in female rats, (a) Control group, (b) 150 mg kg^{-1}, (c) 300 mg kg^{-1} and (d) 600 mg kg^{-1}
CV: Central vein

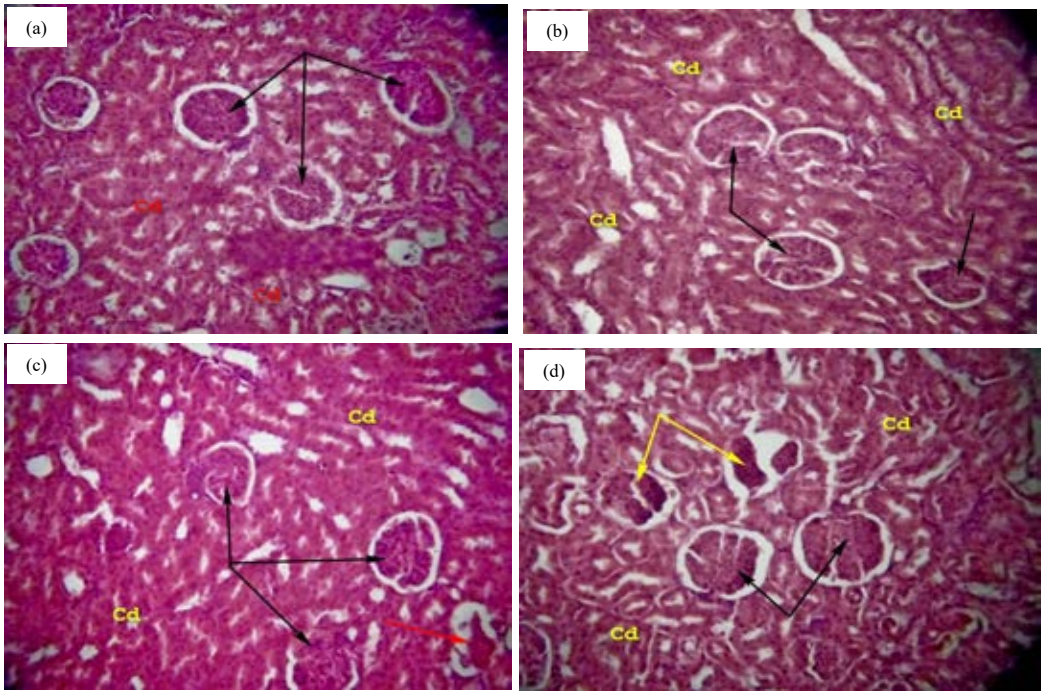

Fig. 2(a-d): Kidney sections stained with hematoxylin and eosin (H and E-stained under 150× magnification power) showing the effect of *A. nilotica* methanolic extract in a 28 day sub-chronic toxicity study in female rats, (a) Control group, (b) 150 mg kg−1, (c) 300 mg kg^{-1} and (d) 600 mg kg^{-1}
Cd: Collecting duct

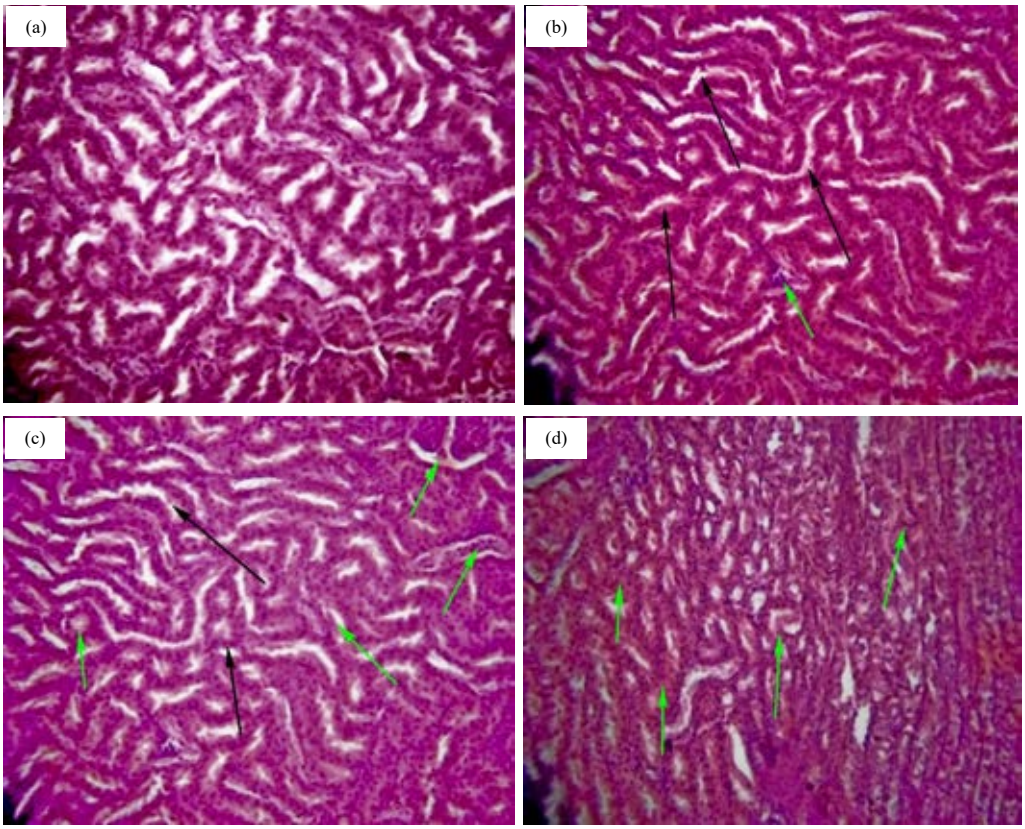

Fig. 3(a-d): Kidney medulla sections stained with hematoxylin and eosin (H and E-stained under 150×magnification power) showing the effect of *A. nilotica* methanolic extract in a 28 day sub-chronic toxicity study in female rats, (a) Control group, (b) 150 mg kg^{-1}, (c) 300 mg kg^{-1} and (d) 600 mg kg^{-1}

Histopathological examination of kidney medulla: Histopathological examinations were performed on the kidney medulla to assess whether or not collecting ducts had been damaged. The photomicrographs of experimental rats' medulla treated with control and treated groups are presented in Fig. 3a-d. The rat kidney medulla of the control (Group 1) shows normal collecting duct (black arrow) with normal cellular orientation, the rat kidney medulla of the 150 mg kg^{-1} (Group 2) shows normal collecting duct (black arrow) with slight cellular infiltrated collecting duct (green arrow), the rat kidney medulla of the 300 mg kg^{-1} (Group 3) shows normal collecting duct (black arrow) with moderate cellular infiltrated collecting duct (green arrow) and the rat kidney medulla of 600 mg kg^{-1} (Group 4) shows severe cellular infiltrated collecting duct (green arrow).

DISCUSSION

Considering the numerous therapeutic potentials of *Acacia nilotica* as an alternative medicine effective for a wide range of diseases and infections[10,11], it is very essential that a safety profile of the plant be established as a guide for the management of its applications and usage in herbal preparations. This should serve to prevent exposing human subjects to potential toxicity-related health risks while using *A. nilotica*. Toxicity studies in appropriate animal models are commonly used to assess potential health risks in humans. Such toxicity studies assess the hazard and determine the risk level by addressing the probability of exposure to that particular hazard at certain doses or concentrations[12].

In the present study, single-dose oral administration of crude methanolic leaves extract of *Acacia nilotica* in female rats at 3000 mg kg^{-1} b.w.t., had no effects on mortality, examined clinical signs, body weight or overall observation. Therefore, no acute toxicity was found in rats treated with the extract and the approximate lethal dose was determined to be higher than 3000 mg kg^{-1}. Yet, the lack of toxicity-indicative manifestations upon acute oral administration of the extract can be attributed to sub-sufficient absorption of the extract in the gastrointestinal tract, or a high first-pass metabolism rate in the liver, by which toxic components would have been converted to their harmless derivatives[12]. Nonetheless, the

knowledge gained from our acute toxicity study may serve for choosing more appropriately the test doses of *A. nilotica* extracts for later sub-chronic toxicity studies to report results of greater clinical relevance-as was the case in the present investigation.

The sub-chronic toxicity study, which involved rats given the extract orally at doses of 150, 300 and 600 mg kg^{-1} b.wt., has not demonstrated any significant changes in animal behaviour. There was no significant reduction in body weight in female rats at the three doses. But at the beginning of week 4 there was significant ($p<0.05$) reduction in body weight at doses of 300 and 600 mg kg^{-1} b.wt., when compared with the control. Similarly, it was also observed that, there was significant ($p<0.05$) reduction in the weight of kidneys of the three dose groups compared to control group. There was also significant ($p<0.05$) reduction in the weight of spleen of 300 and 600 mg kg^{-1} b.w.t., dose groups compared to control group. There was also a significant (p 0.05) reduction in lungs weight of 150 mg kg^{-1} b.wt., dose group compared to control. With the exception of these, no significant differences were found in the organ weights of the treated rats in comparison with the control groups.

It goes without saying that a decrease in body weight may be an indicator of adverse effects[13]. The liver and the kidneys are target organs for toxic chemicals due to their essential functions in bodily detoxification and excretion processes. Thus, they are considered highly useful in toxicity studies because of their sensitivity to harmful compounds and their potential to predict toxicity. Toxicity-related changes in the weights of these vital organs are often accompanied by corresponding histopathological findings. Changes in the weight of the lungs have less toxicity implications due to the lungs' limited role in the removal of harmful substances from the body[14,15]. Therefore, it could safely be claimed that the liver and the kidneys could serve as the primary target organs in investigations related to the sub-acute oral toxicity of a herbal extract.

Analysis of blood parameters in animal toxicity studies is important to report alterations in those parameters and evaluate the relative risk to the hematopoietic system when extrapolating those findings to humans[16,17]. Determining certain blood biochemical parameters and investigating major toxic effects on specific tissues, specifically the kidneys and the liver may provide useful information regarding the mechanisms of toxicity of an otherwise safe and therapeutic agent[18].

In regards to the observed haematological values, most of the values shown in the treated groups were normal in comparison with the control group. Nonetheless, the levels of Hb was significantly ($p<0.05$) different from control. Yet, some values were different from those of the control group, in non-significant manner such as those pertaining to RBC, LYM, PLT and GRAN. Reductions in these indices indicated that the extract interfered with the normal production of Hb and its concentrations within RBCs[19].

There were significant ($p<0.05$) changes in the levels of sodium, potassium and creatinine in comparison to control. Urea, uric acid and creatinine are biomarkers of possible malfunction of the kidneys[20]. In this study, the levels of most of the parameters tested were normal in comparison to their respective control. Nevertheless, the values were within the normal ranges of these parameters, which ruled out the possibility of precipitated abnormalities. Thus, these findings suggest that the extract does not affect the normal kidney function.

There was no significant change in any of the biochemical parameters tested as compared to its control levels. However, there were mild non-significant changes in the levels of some of the parameters in comparison with their respective controls. Due to its distinctive abundance in the cytoplasm of liver cells, ALT has been commonly used as a marker to quantify suspected liver cell damage[21,22]. AST is more ubiquitous in nature. Besides making up 80 and 20% of the total intracellular enzymes in hepatic mitochondria and cytoplasm, respectively, it is found in the heart, skeletal muscle, kidneys, brain, pancreas and blood cells[23,24].

To state another observation, mild and statistically-insignificant increases in some of the serum biochemical parameters were observed in animals of the highest-dose group. These findings could signal mild degeneration and the presence of lesions, which was confirmed by histopathological examination of the livers of the animals in the highest-dose group. These results suggest that the extract may have altered few hepatic functions and indicate that the rats' livers in the highest-dose group may have been injured upon sub-chronic administration.

The histological features of the liver were displayed in Fig. 1. The morphology of the hepatic cells in the control groups was normal. However, in the extract-treated group, morphological alterations in the structure were observed, which was most expressed in the highest-dose group. Furthermore, histopathological examinations revealed normally distributed hepatocytes with normal sinusoid orientation but with gradual congestion of the central vein (CV) especially in the 2 high doses (300 mg kg^{-1} and 600 mg kg^{-1}) when compared to control liver. Congestion of hepatic central vein (CV) is an effect which may lead to congestive hepatopathy[25]. The histological features of the kidney were displayed in Fig. 2. The morphology of the renal cells in control and 150 mg kg^{-1} treated groups showed

normal glomerulus with normal collecting ducts. While the kidneys of 300 mg kg^{-1} and 600 mg kg^{-1} treated groups showed moderately degenerated glomerulus and cellular infiltrated collecting ducts.

CONCLUSION

The results of the LD_{50}, acute and sub-chronic studies revealed that the leaves extract of *A. nilotica* is relatively safe. However, the histopathological evaluation showed some levels of alterations in liver and renal tissues of rats in the high dose groups. Therefore, caution and safety measures should be taken before oral ingestion of *Acacia nilotica* for therapeutic purposes or for other uses, since the integral safety does not seem to exist for this extract. Lower doses should be encouraged. Additional studies will be necessary to verify if the observed alterations are reversible and tissues recovery possible.

SIGNIFICANCE STATEMENT

This research discovered that although *Acacia nilotica* is safely used traditionally for various therapeutic purposes, however, prolong intake/treatment is associated with alterations in hepatic and renal tissues. Thus, prolonged use of high doses of *A. nilotica* orally should be discouraged and lower doses encouraged. The study will help the researchers to uncover the critical areas of natural product research, especially in deciding effective, therapeutic and safe dosage and treatment duration of this phyto-medicine. Thus, a new theory on the search for effective therapeutic agents, with less toxicity, may be arrived at.

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