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Acute and Sub-Acute Toxic Effects of Aqueous Leaf Extracts of *Ximenia americana* (Linn.) and *Pappea capensis* (Eckl. and Zeyh.)

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ABSTRACT

Background: Although herbal extracts are often regarded as natural and without harmful effects, their application in the practice of modern medicine is usually viewed with skepticism because of many concerns, including toxicity. *Ximenia americana* and *Pappea capensis* are widely used in traditional treatment of various pathologies. However, the available data on their safety is still scanty. This study evaluated the acute and sub-acute toxicity effects of the two plants in mice. **Materials and Methods:** For acute toxicity studies, three groups were used. Group I mice were given normal saline; groups II and III mice were given a single dose of 2000 mg/Kg bw of *X. americana* and *P. capensis* extract, respectively. For sub-acute toxicity studies, seven groups of mice were used. Group I was given normal saline; groups II, III and IV were treated with *X. americana* extract, whereas groups V, VI and VII were treated with *P. capensis* extract. Extract doses of 250, 430 and 750 mg/Kg bw were used, respectively. Animals were treated daily for 28 days. **Results:** The extracts did not cause significant change in behavioral factors, body weights, red and white cell indices, and kidney functions in mice. However, at 2000 mg/Kg bw, *X. americana* extract caused a significant increase in relative pancreas weight. Additionally, at 750 mg/Kg bw, *X. americana* extract caused a significant increase in platelet distribution width, and total and indirect bilirubin. The extracts did not significantly alter kidneys functions. **Conclusions:** According to the current study findings, it was concluded that the extracts are devoid of neurotoxic, hematotoxic and nephrotoxic effects. However, *X. americana* extract possess mild hepatotoxic effects. It was also concluded that lethal dose of the extracts is greater than 2000 mg/Kg bw. Therefore, *X. americana* and *P. capensis* extracts are generally safe for use in traditional treatment of various diseases.

Keywords: Safety, Mice, Neurotoxic, Hematotoxic, Nephrotoxic.

INTRODUCTION

Although herbal extracts are often regarded as natural and without harmful effects, their application in modern medicine is usually viewed with a lot of skepticism. This is because of many concerns, including their safety and side effects [1]. Many studies have associated herbal preparation extracts with nephrotoxicity, hepatotoxicity, hematotoxicity and allergic reactions, particularly, when very high dosages have been administered [2]. The harmful effects of herbal preparations could be due to their constituents or contamination from the environment [3]. Therefore, scientific research on safety of the herbal extracts is strongly recommended so as to determine their therapeutic dose and possible toxicological effects.

Various previous studies have confirmed that *X. americana* and *P. capensis* extracts contain flavonoids, cardiac glycosides, tannins and hydrocyanic acid, which, when used at high dose levels, may cause toxicity [4;5]. A previous study by Maikai *et al* [6] associated acute toxicity observed in mice to tannins and anthraquinones. In contrast, Togbossi *et al* [7] indicated that *X. americana* hydroethanolic extract dose of 5000 and 1000 milligrams Kg^{-1} bw^{-1} showed no acute and sub-chronic toxicity, respectively, in rat models. Moreover, Muhammad *et al* [8], confirmed safety of methanolic extract of *X. americana* stem bark in rats.

Furthermore, Pendota *et al* [9], revealed that flavonoids have little to no cytotoxic effects on African green (vero) monkey kidney cells. Similarly, a previous study by Abdirahman *et al* [10], safety and hypoglycemic profiles of *Acacia nilotica* stem bark of aqueous extracts reported that tannins and flavonoids present in the extract could be associated with the slight toxicity.

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The available literature on the safety of *X. americana* and *P. capensis* still remains insufficient and contradicting. Moreover, there is no available data on their aqueous leaf extracts. It is against this background that the toxicity of their aqueous leaf extracts was determined in mice to assess their safety or otherwise, since the findings are important in validating their use by human beings.

MATERIALS AND METHODS

Collection of Plant Materials

Leaves of *X. americana* and *P. capensis* were collected from Siakago, Mbeere-North Sub-County, Embu County, Kenya, coordinates 0^0 33'17"N 37^0 36'18"E and 0^0 33'17"N 37^0 36'19"E for *X. americana* and *P. capensis,* respectively. The plant samples were collected during summer in the month of September. A certified taxonomist carried out botanical identification, and voucher specimens were deposited in the Herbarium of National Museums of Kenya in Nairobi for future reference. The specimens were assigned voucher numbers DMG001 and DMG002 for *X. americana* and *P. capensis,* respectively. The current study was conducted at the animal house in the department of biochemistry, microbiology, and biotechnology, Kenyatta University.

Plant Material Preparation

Away from direct sunlight, the leaves were thoroughly dried at room temperature and milled into a fine powder using an electric mill. The powder for each plant was stored separately in closed dry khaki bags at room temperature awaiting extraction.

Sample Extraction

Two hundred and fifty grams of ech powdered plant material was soaked in two liters of distilled water, placed in a water bath, and left to stand for two hours at 60 degrees Celsius. The resulting solution was decanted, and then filtered using filter papers (Whatmann No.1). The resulting filtrate was dried in a freeze drier [5]. The dry extracts were packaged separately and stored in airtight containers at -4°C [5].

Experimental Animals

This study employed female Swiss albino mice, weighing about 25±4 grams and 8–10 weeks old. For acute toxicity tests, fifteen nulliparous and non-pregnant female mice were employed, whereas for sub-acute toxicity thirty-five mice of either sex were used [11; 12]. The animals were randomly selected. They were hosted at Biochemistry, Microbiology and Biotechnology department breeding and experimentation facility at Kenyatta University. Standard propylene cages were used to house the animals and standard laboratory conditions, such as room temperature (25 ^{0}C) and twelve hours of light succeeded by twelve hours of dark cycles, and were maintained throughout the experiments. As recommended by Kirkham *et al* [13], mice were supplied with water *ad libitum* and fed on standard rodent pellets. With reference to Olfert *et al* [14], ethical consideration for the maintenance and treatment of research animals were observed during the study. Institutional Animal Ethics Committee, Kenyatta University as well as National Commission for Science, Technology and Innovation reviewed and approved the experimental protocols, and assigned approval numbers PKUA/006/006 and NACOSTI/P/21/8549, respectively.

Preparation of Experimental Doses

In line with Organization for Economic Development (OECD) guideline number 425, a single extract concentration of 2000 milligrams Kg^{-1} bw^{-1} was used for testing acute toxicity [15]. For determination of sub-acute toxicity, extract dose levels of 250, 430 and 750 mg Kg^{-1} bw^{-1} were used. Normal saline was used as the vehicle. Each mouse received 0.2 ml of the respective treatment as a single daily dose. All solutions were prepared daily.

Experimental Design

For acute toxicity testing, mice were randomized into two sets of five each. Group I (Control) mice were given the vehicle only (oral 0.9% w/v normal saline), whereas groups II and III (Experimental) mice were orally treated with *X. americana* and *P. capensis* extracts, respectively, at 2000 milligrams Kg^{-1} bw^{-1} (Table 1a). The mice were kept without food for about 3 to 4 hours before dosing but accessed water *ad libitum.* Following the treatment, the animals were closely monitored for toxic reactions within the first 6 hours followed by constant intervals, throughout the study duration of fourteen days. Food was provided after 1 to 2 hours of dosing [11].

Table 1a: Protocol for the Evaluation of Acute Toxic Effects of Aqueous Leaf Extracts of *X. americana* and *P. capensis*

Group	Treatment
I (Control)	Vehicle (0.9% w/v normal saline).
II (Experimental)	*X. americana* Extract (2000 milligrams kg^{-1} bw^{-1}).
III (Experimental)	*P. capensis* Extract (2000 milligrams kg^{-1} bw^{-1}).

In sub-acute toxicity analysis, animals were randomized into four groups of 5 each and treated daily for 28 days. In group I (Control), mice were orally given 0.9% w/v normal saline. Groups II, III and IV (Extract-treated A, B and C) consisted of rats treated with oral *X. americana* extract dose levels of 250, 430, 750 mg Kg^{-1} bw^{-1}, respectively, whereas groups V, VI and VII (Extract-treated A, B and C) comprised rats treated with oral *P. capensis* extract dose levels of 250, 430, 750 mg/Kg bw, respectively (Table 1b).

Table 1b: Protocol for the Evaluation of Sub-Acute Toxic Effects of Aqueous Leaf Extracts of *X. americana* and *P. capensis*

Group	Treatment
I (Control)	Vehicle (0.9% w/v normal saline).
II (Extract-treated group A)	*X. americana* Extracts (250mg/kg bw).
III (Extract-treated group B)	*X. americana* Extracts (430mg/kg bw).
IV (Extract-administered group C)	*X. americana* Extracts (750mg/kg bw).
V (Extract-treated group A)	*P. capensis* Extracts (250mg/kg bw).
VI (Extract-treated group B)	*P. capensis* Extracts (430mg/kg bw).
VII (Extract- administered group C)	*P. capensis* Extracts (750mg/kg bw).

Determination of Acute Oral Toxicity

Animals were closely observed throughout the study period for the changes in behavioral factors, including restlessness, confusion, withdrawal behavior, skin swellings, stool texture and color, teeth color, breathing, gait, fur condition and mortality. Their weights were noted at the beginning of the assay (day 1), and days 7 and 14. Total change in body weight (Δtotal) was day 14 body weight less day 1 body weight, and was expressed in grams.

At the final day of the test, animals were weighed and euthanized under isoflurane. Major organs including liver, pancreas, lungs, brain, kidney and heart were obtained, cleaned and weighed. The organ indices (relative organ to body weights) were derived following Lazic *et al* [16] formula;

$$\% \text{ Relative organ to body weight} = \frac{\text{Actual organ weight (grams)}}{\text{Individual rat body weight on sacrificng day (grams)}} \times 100$$

Determination of Sub-Acute Toxic Effects

The weights of animals were recorded on weekly interval across the test duration. At final day of the test, mice were euthanized with isoflurane, and vital organs harvested and weighed. The change in total body weight was given as animal body weight on the 28th day less body weight at the beginning of the study (day 1), whereas relative organ weights were derived as detailed in section 2.7. Blood for analysis of biochemical and hematological was obtained by cardiac puncture. Half portion of the blood was used for hematological analysis, whereas the other portion was centrifuged and serum collected for biochemical analysis.

Determination of Hematological Parameters

Chemistry analyzer (Mindray BS 120) was used to conduct a full haemogram. The hematological analysis was done according to the protocols described by Ayenew *et al* [17]. The analysis included red blood cell indices, white blood cell and platelet. Red cell distribution width (RDW), total hemoglobin (HB), mean corpuscular hemoglobin concentration (MCHC), hematocrit (HCT) and mean cell volume (MCV) include the analyzed red blood cell indices. Percentage of lymphocyte (LYM%), neutrophils (NE%), basophils (BA%), monocyte (MON%) and eosinophils (EO%) were analyzed for white blood cell parameter. Platelet distribution width (PDW), platelet count (PLT) and Mean platelet volume (MPV) were platelet variables tested.

Determination of Biochemical Parameters

Similarly, chemistry analyzer (Mindray BS 120) was used to carry out biochemical analysis, which included liver and kidney function tests, as outlined by Ayenew *et al* [17]. The analyzed biomarkers for liver function included total protein (T Prot), alanine aminotransferase (ALT), aspartate aminotransferase (AST), albumin (ALB), total bilirubin (BIL total), direct bilirubin (BIL direct), indirect bilirubin (BIL indirect) and globulin, whereas the evaluated biomarkers for kindey function included creatinine (CREAT), blood urea nitrogen (BUN), electrolytes including potassium, sodium, chloride and bicarbonate ions (K^+, Na^+, Cl^- and HCO_3^-).

Data Processing and Statistical Analysis

Data on biochemical and hematological indices were entered in the spread sheet (Microsoft® Excel) and then transferred to statistical software (Minitab, Version 19, NC, USA) for analysis. It was subjected to D'Agostino-Pearson test and conformed to assumptions of parametric data. Descriptive statistics were conducted and data presented as Mean ± Standard Error of Mean.

One-factor ANOVA was applied for inferential statistical analysis. Tukey's *post hoc* test was utilized for pairwise mean comparisons and separation. The statistical significance was considered at $p \leq 0.05$, whereas the results were organized in tables and visualized in graphs.

RESULTS

Effects of Aqueous Leaf Extracts of *X. americana* and *P. capensis* on Behavioral Factors in Mice

Generally, findings of the current study revealed that, at acute dose level of 2000 mg/Kg bw, *X. americana* and *P. capensis* leaf aqueous extracts did not cause any notable change on behaviour of mice. The normal behaviour was defined by the absence of restlessness, confusion, withdrawal behaviours, abnormal feeding, skin swellings, abnormal stool and teeth color, breathing difficulties, unusual gait and fur, and mortality (Table 2).

Table 2: Effects of acute dose of *X. americana* and *P. capensis* Extracts on Behavioral Factors in Mice

Factor	Outcome
Restlessness	Absent
Confusion	Absent
Withdrawal behaviors	None
Skin swellings	None
Stool texture and color Feeding behaviour	Normal Normal
Teeth colour	Normal
Breathing difficulties	Absent
Gait	Normal
Condition of the fur	Normal
Mortality	Nil

Effects of Aqueous Leaf Extracts of *X. americana* and *P. capensis* on Body weight in Mice

Overall, the current study findings showed that the acute oral dose level of 2000 mg Kg^{-1} bw^{-1} of *X. americana* and *P. capensis* extracts did not significantly change ($p > 0.05$) the body weight of extract-treated mice as compared with mice treated with the normal saline (Figure 1a).

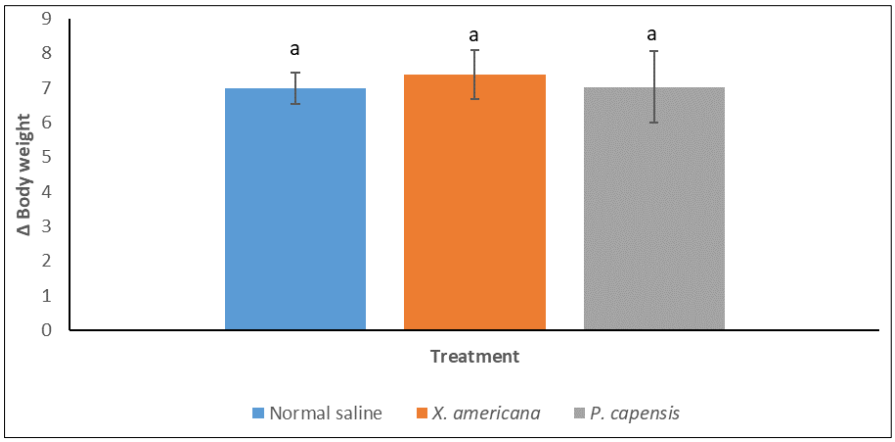

Figure 1a: Effects of Acute Oral Dose of Aqueous Leaf Extracts of *X. americana* and *P. capensis* on Body Weights of Mice. Bar graphs with the same alphabet are not significantly different ($p > 0.05$).

Similarly, the the sub-acute oral dose levels of 250, 430 and 750 mg Kg^{-1} bw^{-1} of *X. americana* and *P. capensis* extracts did not significantly alter ($p > 0.05$) the body weight of mice treated with the extracts as compared with mice treated with the normal saline (Figure 1b).

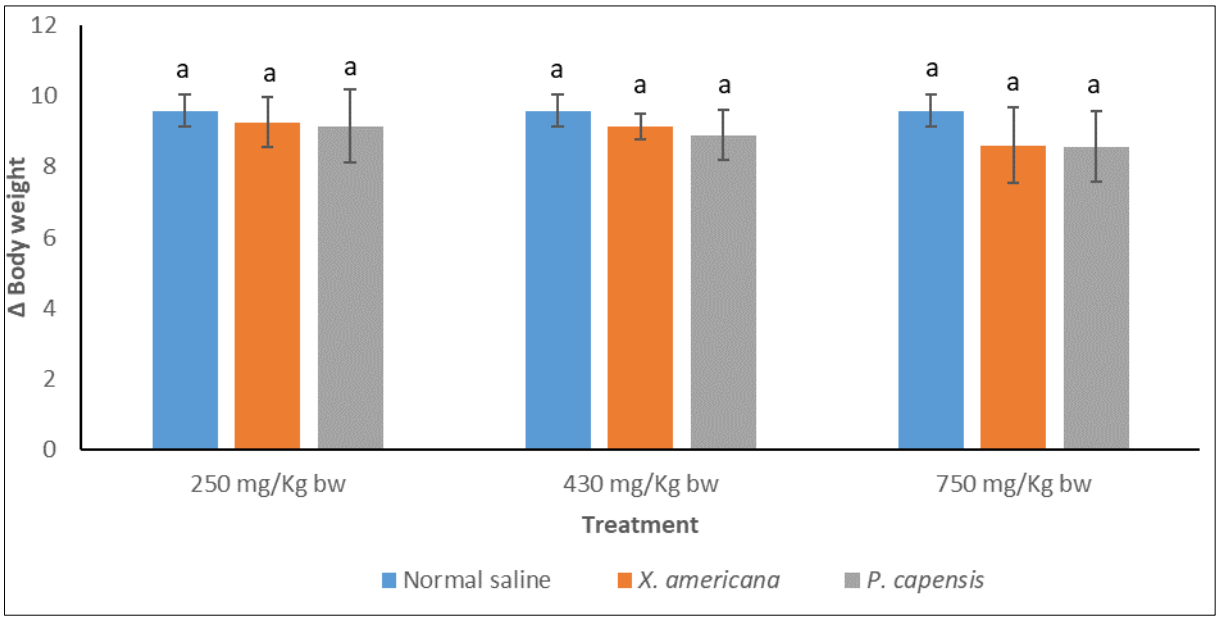

Figure 1b: Effects of Sub-Acute Oral Doses of Aqueous Leaf Extracts of *X. americana* and *P. capensis* Extracts on Body Weights of Mice. Bar graphs with the same alphabet are not significantly different ($p > 0.05$).

Effects of Aqueous Leaf Extracts of *X. americana* and *P. capensis* on Organ Indices in Mice

In general, this study revealed that the extracts did not induce significant changes ($p > 0.05$) in relative weights of kidneys, lungs, heart, brain and liver of extract-treated mice as compared with weights of control mice (Tables 3a and b). However, mice that received 2000 milligrams Kg^{-1} bw^{-1} dose level of *X. americana* extract showed significant increase ($p < 0.05$) in relative pancreas weight as compared with weight of control mice (Table 3a).

Table 3a: Effects of Acute Doses *X. americana* and *P. capensis* Extracts on Relative Organ Weights of Mice

Group	Relative Organ Weight					
	Heart	**Liver**	**Pancreas**	**Kidney**	**Lungs**	**Brain**
Control (0.9% NS)	0.53±0.05[a]	5.53±0.15[a]	0.97±0.10[b]	1.62±0.18[a]	0.86±0.07[a]	1.20±0.06[a]
XA 2000 mg/Kg bw	0.60±0.02[a]	5.74±0.09[a]	1.30±0.11[a]	1.60±0.14[a]	0.92±0.06[a]	1.29±0.13[a]
PC 2000 mg/Kg bw	0.58±0.03[a]	5.72±0.25[a]	1.07±0.08[b]	1.88±0.15[a]	0.67±0.04[a]	1.30±0.02[a]
Means with unique alphabet column-wise are significantly distinct ($p \leq 0.05$). **XA** = *Ximenia americana*; **PC** = *Pappea capensis*.						

Table 3b: Effects of Sub-Acute Doses of *X. americana* and *P. capensis* Extracts on Relative Organ Weights of Mice

Group	Relative Organ Weight					
	Heart	**Liver**	**Pancreas**	**Kidney**	**Lungs**	**Brain**
Control (0.9% NS)	0.63±0.10[a]	5.21±0.09[a]	0.98±0.09[a]	1.95±0.16[a]	0.81±0.07[a]	1.12±0.05[a]
XA Extract A	0.63±0.08[a]	5.56±0.13[a]	1.11±0.12[a]	1.94±0.15[a]	0.80±0.18[a]	1.17±0.13[a]
XA Extract B	0.77±0.12[a]	5.87±0.06[a]	1.15±0.19[a]	1.97±0.22[a]	0.90±0.04[a]	1.20±0.08[a]
XA Extract C	0.67±0.06[a]	5.26±0.20[a]	1.10±0.04[a]	1.84±0.09[a]	0.78±0.09[a]	1.11±0.15[a]
PD Extract A	0.74±0.09[a]	5.46±0.28[a]	1.02±0.06[a]	1.86±0.11[a]	0.89±0.15[a]	1.24±0.07[a]
PD Extract B	0.66±0.11[a]	5.50±0.29[a]	1.25±0.13[a]	1.96±0.10[a]	0.94±0.14[a]	1.27±0.12[a]
PD Extract C	0.69±0.05[a]	5.06±0.31[a]	1.14±0.07[a]	1.91±0.19[a]	0.83±0.06[a]	1.16±0.14[a]
Means with unique alphabet column-wise are significantly distinct ($p \leq 0.05$). **XA** = *Ximenia americana*; **PC** = *Pappea capensis*; **Extract A** = 250 mg Kg^{-1} bw^{-1}; **Extract B** = 430 mg Kg^{-1} bw^{-1}; **Extract C** = 750 mg Kg^{-1} bw^{-1}.						

Effects of Sub-Acute Doses of Aqueous Leaf Extracts of *X. americana* and *P. capensis* on Hematological parameters in Mice

Generally, findings of the current study revealed that *X. americana* and *P. capensis* extracts have no significant negative changes ($p >$

0.05) in the levels of red and white blood cell indices in extract-treated mice as compared with indices in the control mice (Table 4). Notably, the mice that received 750 milligrams Kg^{-1} bw^{-1} dose level of *X. americana* extract showed a significant increase ($p < 0.05$) in PDW levels as compared with the levels in control mice (Table 4).

Table 4: Effects of *X. americana* and *P. capensis* Extracts on Blood Cell Indices in Mice

Parameter	Control (0.9% NS)	XA Extract A	XA Extract B	XA Extract C	PC Extract A	PC Extract B	PC Extract C
RBC (10^6/uL)	7.97±0.16^b	9.37±0.14^a	10.03±0.12^a	9.97±0.59^a	8.16±0.50^{ab}	9.25±0.31^{ab}	9.96±0.28^a
HGB (g/dL)	11.90±0.93^b	14.29±0.98^{ab}	14.80±0.65^{ab}	15.74±1.05^a	12.74±1.09^b	13.66±0.7^{ab}	14.84±1.15^{ab}
HCT (%)	46.12±2.16^a	50.08±0.90^a	53.94±1.35^a	52.14±3.92^a	47.98±5.12^a	48.34±2.61^a	52.36±1.98^a
MCV(fL)	52.24±1.26^a	53.50±1.20^a	52.66±0.51^a	51.90±1.33^a	52.96±0.79^a	54.12±0.76^a	52.36±0.86^a
MCH (pg)	16.26±0.24^a	16.88±0.34^a	16.22±0.65^a	15.84±0.40^a	15.34±0.43^a	15.38±0.27^a	16.02±0.26^a
MCHC (g/dL)	30.14±1.05^a	30.41±1.06^a	31.88±1.03^a	32.04±1.34^a	29.04±1.05^a	28.88±0.39^a	30.28±0.84^a
RDW (%)	26.18±1.10^a	26.92±0.48^a	28.34±0.39^a	27.92±1.11^a	25.60±0.77^a	26.12±0.57^a	27.50±0.70^a
WBC (10^3uL)	12.41±1.00^a	11.03±0.92^a	10.75±0.67^a	10.16±1.19^a	11.77±1.05^a	10.35±1.29^a	11.09±0.58^a
NEU%	20.72±0.76^a	21.80±1.02^a	19.30±1.30^a	20.66±0.50^a	21.02±1.63^a	22.90±1.29^a	20.26±0.94^a
LYM%	66.36±2.37^a	67.14±3.51^a	66.04±0.84^a	63.38±2.21^a	65.36±0.70^a	64.78±3.93^a	66.08±2.40^a
MON%	15.52±0.70^a	14.52±1.42^a	15.10±0.76^a	17.22±1.47^a	15.88±1.14^a	16.98±0.92^a	15.64±1.61^a
EOS%	0.02±0.002^a	0.08±0.004^a	0.04±0.002^a	0.08±0.006^a	0.04±0.002^a	0.10±0.003^a	0.10±0.005^a
BAS%	0.48±0.07^a	0.34±0.07^a	0.28±0.04^a	0.26±0.05^a	0.40±0.010^a	0.42±0.058^a	0.34±0.060^a
PLT (10^3/uL)	674.2±23.0^a	672.80±44.4^a	690.0±56.5^a	681.8±32.9^a	660.8±90.5^a	683.8±43.1^a	674.2±23.0^a
MPV (fL)	8.52±0.81^a	7.78±0.32^a	8.38±0.41^a	8.24±0.33^a	8.00±0.71^a	7.66±0.3^a	8.04±0.83^a
PDW (fL)	12.94±1.08^b	15.04±0.77^{ab}	15.36±0.44^{ab}	16.28±0.59^a	13.12±1.51^b	14.3±1.57^{ab}	15.1±1.44^{ab}
PCT (%)	0.39±0.08^a	0.44±0.02^a	0.54±0.07^a	0.52±0.03^a	0.42±0.12^a	0.46±0.06^a	0.49±0.08^a

Means with unique alphabet row-wise are significantly distinct ($p \leq 0.05$). **XA** = *Ximenia americana*; **PC** = *Pappea capensis*; **Extract A** = 250 mg Kg^{-1} bw^{-1}; **Extract B** = 430 mg Kg^{-1} bw^{-1}; **Extract C** = 750 mg Kg^{-1} bw^{-1}.

Effects of Sub-Acute Oral Doses of Aqueous Leaf Extracts of *X. americana* and *P. capensis* on Organ Functions in Mice

Effects of Sub-Acute Oral Doses of X. americana and P. capensis Extracts on Liver Functions in Mice

In general, as table 5 shows, the three sub-acute doses of *X. americana* and *P. capensis* extracts did not significantly alter ($p > 0.05$) levels of most of the liver function biomarkers in extract-treated mice as compared with levels in control mice. Particularly, 750 mg/Kg bw dose level of *X. americana* extract induced a significant rise in the levels of BIL-total and BIL-indirect as compared ($p < 0.05$) with the levels in the control mice (Table 5). Moreover, the effects on BIL-indirect by 430 mg/Kg bw dose level of *X. americana* extract significantly differed ($p < 0.05$) when compared with control mice (Table 5).

Table 5: Effects of Sub-Acute Oral Doses of *X. americana* and *P. capensis* Extracts on Liver Functions in Mice

Parameter	Control (0.9% NS)	XA Extract A	XA Extract B	XA Extract C	PC Extract A	PC Extract B	PC Extract C
ALT (U/L)	55.78±6.79^a	53.9±5.02^a	51.3±5.51^a	54.88±3.11^a	54.84±3.13^a	57.92±6.0^a	55.22±2.43^a
AST (U/L)	204.3±13.7^a	208.5±11.7^a	204.8±9.67^a	215.8±22.6^a	201.1±21.1^a	217.7±23.9^a	225.9±13.1^a
BIL-total (µmol/L)	9.2±0.62^b	10.89±1.38^{ab}	12.61±0.33^{ab}	12.99±0.73^a	8.58±0.83^b	7.83±0.57^b	8.15±0.6^b
BIL-direct (µmol/L)	2.02±0.36^a	2.2±0.21^a	1.96±0.45^a	1.47±0.16^a	2.2±0.28^a	1.98±0.21^a	1.42±0.33^a
BIL-indirect (µmol/L)	8.82±0.48^b	9.72±0.95^{ab}	11.79±0.91^a	11.82±0.12^a	8.5±0.31^b	9.19±0.31^b	8.69±0.3^b
ALB (g/L)	25.1±1.49^a	26.34±0.94^a	23.76±1.93^a	25.7±2.45^a	27.22±2.42^a	25.86±2.43^a	24.63±1.41^a
T.prot (g/L)	57.8±1.56^a	58.34±1.24^a	55.0±2.94^a	58.88±2.18^a	63.96±7.0^a	61.19±5.44^a	59.61±4.1^a
Globulin (g/L)	31.06±1.35^a	30.19±1.26^a	31.07±1.64^a	31.53±2.94^a	32.72±0.77^a	33.24±3.23^a	34.16±3.47^a

Means with unique alphabet row-wise are significantly distinct ($p \leq 0.05$). **XA** = *Ximenia americana*; **PC** = *Pappea capensis*; **Extract A** = 250 mg Kg^{-1} bw^{-1}; **Extract B** = 430 mg Kg^{-1} bw^{-1}; **Extract C** = 750 mg Kg^{-1} bw^{-1}.

Effects of X. americana and P. capensis Extracts on Kidney Functions in Mice

Generally, findings of this study showed that, at 250, 430 and 750 milligrams Kg^{-1} bw^{-1} dose level, the *X. americana* and *P. capensis* extracts did not significantly alter ($p > 0.05$) functions of kidneys in extract-treated groups of mice as compared with the levels in control group of mice (Table 6).

Table 6: Effects of Sub-Acute Oral Doses of *X. americana* and *P. capensis* Extracts on Kidney Functions in Mice

Parameter	Control (0.9% NS)	XA Extract A	XA Extract B	XA Extract C	PC Extract A	PC Extract B	PC Extract C
CREAT (µmol/L)	26.18±1.94[a]	27.74±1.67[a]	29.38±1.81[a]	25.92±2.65[a]	23.54±2.09[a]	28.02±4.13[a]	26.98±4.30[a]
BUN (mmol/L)	6.78±0.42[a]	6.97±0.43[a]	6.73±0.72[a]	7.75±0.68[a]	7.62±0.37[a]	7.77±0.98[a]	7.42±0.94[a]
K^+ (mmol/L)	4.34±0.73[a]	4.36±0.74[a]	4.9±0.85[a]	4.64±0.79[a]	5.78±0.97[a]	6.34±0.57[a]	5.96±0.82[a]
Na^+ (mmol/L)	129.4±3.79[a]	132.6±4.07[a]	138.0±7.72[a]	138.4±8.29[a]	141.6±3.01[a]	143.4±3.67[a]	144.2±2.87[a]
Cl^- (mmol/L)	111.8±4.54[a]	112.6±8.96[a]	106.4±12.1[a]	110.0±8.77[a]	121.4±8.13[a]	125.4±3.74[a]	127.8±4.55[a]
HCO_3^- (mmol/L)	29.8±2.06[a]	31.4±1.47[a]	32.8±1.71[a]	33.2±1.98[a]	38.0±3.74[a]	40.2±1.43[a]	42.0±1.92[a]

Means with unique alphabet row-wise are significantly distinct ($p \leq 0.05$). **XA** = *Ximenia americana*; **PC** = *Pappea capensis*; **Extract A** = 250 mg Kg^{-1} bw^{-1}; **Extract B** = 430 mg Kg^{-1} bw^{-1}; **Extract C** = 750 mg Kg^{-1} bw^{-1}.

DISCUSSION

Globally, there is growing consumption of herbal plants as therapeutic agents or supplements in management of various human pathologies [18]. This is attributable to the belief that herbal drugs are safe and efficacious. Such herbal plants include *X. americana* and *P. capensis*, which are commonly utilized as remedies of various ailments [19]. However, the data about their safety is not sufficient. As a result, the current study, on acute and sub-acute toxicities of aqueous leaf extracts of *P. capensis* and *X. americana* in mice models, was carried out to provide data on their safety profiles.

Acute toxicity testing involves evaluation of general toxicological effects of a single dose of a substance over a short period of exposure. The lethal dose 50 (LD_{50}) and therapeutic index are derived from acute toxicological studies [6]. Following OECD guidelines 425, the current study employed a single oral extract dose of 2000 milligrams Kg^{-1} bw^{-1}. The present study evaluated behavioral parameters, body weights and relative organ weights as sensitive indices to indicate acute toxic effects of the extracts [20]. In the present study, extract-treated mice revealed no significant changes in their normal behavior. Abnormal behavior, including restlessness, confusion, withdrawal behavior, skin swellings, stool texture and color, teeth color, breathing, gait and fur condition were not noted in extract-treated mice. Moreover, no death was recorded. Therefore, it was suggested that the LD_{50} of *X. americana* and *P. capensis* extracts is higher than 2000 milligrams Kg^{-1} bw^{-1} in mouse models. As per the OECD criteria on Globally Harmonised Classification System for chemical mixtures and substances, substances that have an LD_{50} of between 2000 to 5000 mg kg^{-1} fall under category 5 [11]. Therefore, it was postulated that the two extracts are safe or are of low toxicity in mice models. These results concur with a study done by Bello *et al* [21], who reported acute and sub-acute toxicities of the *Alstonia scholaris* stem bark methanolic extract.

Moreover, the current study monitored body and relative organ weights as an aspect of determining the acute toxicity. Generally, the body weights and relative weights of liver, heart, lungs, kidney and brain of the extract-treated mice and the control rats were not significantly different. Therefore, it was postulated that the extracts may lack acute toxic effects in mice. The absence of toxicity in the studied extracts could be attributable to the poor absorption, in gastrointestinal tract, and rapid first-pass effects, in the liver, of the toxic phytocompounds in the extracts [21]. These findings concur with finding reported by Ugwah-Oguejiofor *et al* [15], who studied acute and toxicities of *Caralluma dalzielii* aerial section aqueous extract in rats and mice. However, the findings contradict those of reported by Maikai *et al* [6], who indicated that mice treated with 1600, 2900 and 5000 milligram Kg^{-1} bw^{-1} dose levels of *X. americana* extract significant reduced body weights as compared with the weights of control mice. These discrepancies could be attributable to the agro-ecology of the plant which influenced its phytochemical composition and proportion [22].

Notably, 2000 mg/Kg bw dose of *X. americana* extract significantly increased relative weight of pancreas in mice. This suggests that the extract may have caused acute pancreatitis. The phytochemical analysis revealed presence of tannins in *X. americana* extract, which is associated with activation of enzymes in the pancreas, such as trypsinogen, leading to inflammation [5; 23].

Usually, acute toxicity data is not sufficient for clinical application [24]. Thus, sub-acute toxicity test was also conducted in the present study. Most drugs applied to treat chronic illnesses are usually administered on repeated daily doses potentially leading to accumulation of remnants in the body with progressive toxic outcomes on body tissues and organs. Therefore, there is need for sub-acute toxicological evaluation [25]. Studies on sub-acute toxicity studies are useful in evaluating biochemical and hematological effects of the extracts, as it is not possible with acute toxicity studies [26]. Therefore, in the current study, the sub-acute toxicity profile of aqueous leaf extracts of *X. americana* and *P. capensis* was determined in mice using measurement of body weight, relative organ weight and haematological, as well as biochemical (kidney and liver function tests) parameters.

Modifications in animal body and relative organ weights are useful indicators of sub-acute toxicity of a substance [27]. In the current study, after sub-acute treatment, no significant differences were noted in body weight changes and relative organ weight changes among extract-treated and control mice. These results agree with a study done by Lulekal *et al* [28], who reported that essential oil of *Cymbopogon citratus* did not alter body and relative organ weights in mice and rabbits. However, the findings contradict those of a study done by Bello *et al* [21], who noted that rats administered with methanolic extract of *Alstonia scholaris* stem bark had a significant decrease in body and relative organ weights as compared to control rats. This discrepancy could be due to toxic secondary metabollites in *Alstonia scholaris*. The different composition of secondary metabolites in the plants vary due to varying environmental stress factors [22].

Moreover, the present study evaluated hematological parameters as an aspect of determining sub-acute toxicity of the studied extracts. Changes in hematological parameters are useful biomarkers of the extent of substance toxicity on hematopoietic system [29]. All blood cells are formed from the pluripotent stem cell present in the bone marrow. The stem cells have the potential to grow into RBCs, WBCs or platelets [15]. In the current study, *X. americana* and *P. capensis* leaf aqueous extracts did not significantly change hematological parameters except increased PDW caused by *X. americana* extract dose of 750 milligrams Kg^{-1} bw^{-1}. These findings support the safety of *X. americana* and *P. capensis* extracts, hence justifying that the extracts do not induce anaemia, immunological activity or thromboembolic disorders. These findings are similar to those of study done by Kifayatullah *et al* [30], who indicated that ethanolic extract of *Pericampylus glaucus* did not cause alterations of hematological parameters in mice. However, high PDW observed in mice that received *X. americana* extract dose of 750 mg Kg^{-1} bw^{-1} could be associated with conditions outside hematopoietic tissue [31].

Therefore, further studies are recommended to ascertain the effects of the extracts on platelet indices.

The liver and kidney are crucial organs by which substances are metabolized and excreted out of the body [32]. Therefore, evaluation of their functionality by measuring serum biochemical parameters is important in determining the toxicity of the extracts [25]. The present study determined the effects of *X. americana* and *P. capensis* extracts on the liver functions by measuring serum levels of ALT, AST, albumin, total, direct and indirect bilirubin, globulin and total protein. Principally, AST and ALT are found in the hepatocytes and any liver damage increases their serum levels, hence, they are useful markers for hepatocellular integrity [33]. The findings of the present study showed that AST and ALT levels in extract-administered mice did not significantly differ as compared with the levels in control mice. Therefore, it is suggested that the extracts do not cause injuries to the liver cells.

In addition, the functional integrity of the liver was assessed by measuring levels of albumin, total, direct and indirect bilirubin, globulin and total protein. Reduction in levels of albumin, total bilirubin, direct bilirubin, indirect bilirubin, total protein and globulin indicate decreased synthetic function of the liver, mainly due to liver injury or pathologies, whereas increase in their levels is commonly observed in malignant ailments, or after high protein intake [15]. In this study, total and indirect bilirubin were significantly elevated in mice administered with *X. americana* extract concentration of 750 mg Kg^{-1} bw^{-1}, as compared to the control mice. This suggests possible toxic effects of the extract on the hepatocytes hence interfering with the metabolism in mice. However, the levels of albumin, direct bilirubin, total protein and globulin were not significantly different in *X. americana* extract-administered mice as compared with the levels in the control animals, suggesting that the toxic effects of the extract were mild. For *P. capensis* extract-treated mice, no significant differences were observed in all the liver function biomarkers as compared to the control mice. This supports the belief on safety of the extract. These findings agree with those of a study done by Bariweni *et al* [25], who evaluated the toxicity of *Pavetta crassipes* leaf aqueous extract in rats.

Furthermore, the present study evaluated renal functions by measuring blood urea nitrogen, creatinine and selected electrolytes, such as potassium, sodium, chloride and bicarbonate ions. Abnormal levels of these biomarkers indicate a possible inability of the kidneys to filter blood. Abnormally elevated level of urea in serum suggests dysfunction of the kidneys, or dehydration, whereas abnormally reduced levels indicate acute renal failure or overhydration [21]. The studied extracts did not induce significant alterations on levels of the tested kidney function biomarkers as compared with the levels in the control group. This suggest that the plant extracts were non-toxic to kidneys of the mice. These findings support the argument that these extracts are safe. The findings concur with those reported by Agyigra *et al* [19], who evaluated acute and sub-chronic toxicity of *X. americana* stem bark methanol extract in rat models.

Therefore, according to the findings of this study, *X. americana* and *P. capensis* aqueous leaf extracts are generally safe for use in the traditional treatment of various pathologies in which the extracts have been evaluated. However, the said safe is only based on the evaluated acute and sub-acute oral extracts dose levels. Moreover, the current study could not particularly evaluate the cause of increased relative pancreas weight, PDW, and total and indirect bilirubin among the mice treated *X. americana* extracts, and therefore, further toxicological studies are recommended.

CONCLUSION

It was concluded that *X. americana* and *P. capensis* extracts are devoid of neurotoxic effects. In addition, it was suggested that the lethal dose of the extracts is greater than 2000 mg/Kg bw. Moreover, it was concluded that the extracts lacked hematotoxic effects. It was further concluded that, at 750 mg/Kg bw dose level, *X. americana* extract possess mild hepatotoxic effects through impairment of the liver's ability to metabolize some substances. Finally, it was concluded that the extracts are devoid of nephrotoxicity. Therefore, the findings of this study confirmed that *X. americana* and *P. capensis* extracts are generally safe for use in traditional treatment of various diseases.

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Conflict of interest

The authors declare that they have no conflict of interest.

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