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# Effect of Ninety-day Repeated Adiministration of Leaf Extract of *Solanum anomalum* on Rats

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***Authors' contributions***

*This work was carried out in collaboration among all authors. Authors JEO and ICE conceptualized and designed the research, and coordinated the animal studies. Authors CCO and JAU contributed to data analysis and interpretation. Authors UUF, JAU, and UPI were responsible for drafting the manuscript. Authors JEO, JAU, and UUF reviewed and approved the final version of the manuscript. All authors read and approved the final manuscript.*

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## ABSTRACT

The leaves and fruits of *Solanum anomalum* Thonn. ex Schumach are used locally for the treatment of pains, fever and malaria among others. Effect of 90-day repeated administration of *S. anomalum* leaf extract on rats was investigated. Oral administration of the leaf extract (70, 140 and 210 mg/kg) to rats (male and female) was carried out daily for 90 days and the rats were sacrificed after being anaesthesized with light diethyl ether at the completion of the administration. Oral treatment of rats subchronically with *S. anomalum* leaf extract had no significant ($p>0.05$) effect on rats' body weights, hemoglobin concentration, WBC, RBC, platelets counts, percentages of PCV and eosinophils relative to control. However, percentages of neutrophils, monocytes and basophils were elevated significantly ($p <0.05$-$0.01$) at the highest dose (210 mg/kg), while lymphocytes percentage was reduced. The leaf extract had no significant ($p>0.05$) effect on bleeding and clotting times relative to control. The leaf extract non dose-dependently caused significant ($p<0.05$) lowering of ALT, AST and ALP levels. However, total and direct bilirubin levels were elevated significantly ($p<0.01$-$0.001$) only at raised leaf extract' doses (140 and 210 mg/kg). The leaf extract exerted no significant ($p>0.05$) changes on uric acid, bicarbonate, chloride, potassium and sodium levels, but lowered urea, creatinine, total cholesterol, triglyceride, HDL, VLDL and LDL levels of rats significantly ($p<0.05$) relative to control. There was no observable distortion of heart, testis and spleen histologies. Distortion in the histology of livers, kidneys, ovaries and brains of rats were observed at raised extract doses (140 and 210 mg/kg). High doses of the leaf extract should be avoided to prevent serious toxic effects.

*Keywords: Solanum anomalum; subchronic; toxicity; organ weights.*

## 1. INTRODUCTION

Medicinal plants are used world over in the treatment and management of diseases. Inspite of claims that these plants are natural and safe, there are reports of associated toxic effects which sometimes are taken for granted but may result in serious consequences such as organ damages, which can be attributed to toxic potentials of the main constituents (Okokon et al., 2023). Information on the toxic potentials of some these medicinal plants are inadequate or does not exist at all. This paucity of information needs to be addressed to enhance proper use of these plants.

*Solanum anomalum* Thonn. ex Schumach, are found growing in West and East Africa sub-regions and its parts are employed nutritionally and medicinally for the treatment of diabetes, gastrointestinal disorders, infections, inflammation and pains (Okokon et al., 2023). Hypoglycemic and antihyperglyaemic activities of the leaves have been reported (Okokon et al., 2022). Also, *in vivo* and *in vitro* antimalarial (Okokon et al., 2016; Okokon et al., 2017), anti-oedema (Okokon et al., 2017), antioxidant and antiulcer (Okokon et al., 2019), antiepileptic and depressant (Okokon et al., 2019), antinociceptive (Okokon et al., 2020), antidiarrhoeal (Udobang et al., 2022), hepatoprotective (Etuk et al., 2023; Okokon et al., 2023), nephroprotective (Etuk et al., 2023; Okokon et al., 2024), genotoxic and cytotoxic (Okokon et al., 2023) potentials of the leaf extract are reported in literature. The leaves are rich in tannins, alkaloids, flavonoids, saponins, diosgenin and diosgenin glycosides (Okokon et al., 2022; Okokon et al., 2016). We report in this study the effect of subchronic administration of leaf extract of *Solanum anomalum* on rats.

## 2. MATERIALS AND METHODS

**Plants Collection:** *Solanum anomalum* leaves were collected fresh from bush areas around Uruan area, Akwa Ibom State, Nigeria in August, 2020. Identification and authentication of the plant was carried out by a taxonomist in the Department of Botany and Ecological Studies, University of Uyo, Uyo, Nigeria and hebarium specimen (UUH.75a) was deposited at Department of Pharmacognosy and Natural Medicine Herbarium, University of Uyo.

**Extraction:** The collected fresh leaves of *S. anomalum* were washed, chopped to smaller pieces and shade-dried for two weeks. The dried leaves were powdered using electric grinder. The powder (1.5 kg) was soaked in ethanol (50%) for three days at room temperature (28 ±2 $^{\circ}$C), and thereafter filtered. The liquid filtrate was concentrated to dryness in *vacuo* 40$^{\circ}$C using a rotary evaporator (BuchiLab, Switzerland) and

stored in a refrigerator at -4°C, until used for the proposed experiments.

**Animals:** Albino Wistar rats (138-150 g) of either sex were obtained from the University of Uyo animal house. They were maintained on standard animal pellets and water *ad libitum*. Permission and approval for animal studies were obtained from the College of Health Sciences Animal Ethics committee, University of Uyo.

**90-day toxicological study:** Adult wistar rats of both sexes were used in this study. They were weighed and randomly divided into four groups of 6 animals each and treated as follows; groups I, II, and III were respectively treated with *S. anomalum* leaf extract; 70, 140 and 210 mg/kg on alternate days for 90 days. Group IV was administered with distilled water (10 mL/kg) for the same period of time. At the end of the treatment period, the animals were weighed again and sacrificed under light ethyl ether vapour. Blood samples were collected by cardiac puncture into EDTA-bottles and used immediately for haematological testing such as bleeding time, clotting time, full blood counts etc. Sera samples were separated from the remaining blood samples and stored at -20°C until used for biochemical determinations such as assay of liver and kidney functions as well as lipid profile etc. The effect of the extract on some organs was studied. The organs; liver, kidney, spleen, brain, ovary, testis, and heart of rats were harvested and fixed in 10% formalin. The organs were processed, sectioned and stained using standard methods with hematoxylin and eosin (H&E).

**Haematological Analysis:** Haematological indices such as full blood count, total and differential White blood Cell Count (WBC), platelet count, haemoglobin concentration (Hb) and Packed Cell Volume (PCV) were estimated using automated Haematology analyser at Haematology Department of University of Uyo Teaching Hospital

## 2.1 Biochemical Determinations

**Determination of the effect of the crude extract on the lipid profile indices of the treated rats:** The various lipid profile indices such as total cholesterol, triglyceride and high density lipoprotein (HDL) levels of the treated rats were determined enzymatically in serum using Randox diagnostic kits by colorimetric methods. Friedwald et., (1972) formula was used to determine low and very low-density lipoprotein (LDL and VLDL).

**Effect of extract on Liver Function parameters:** Liver function parameters measured included liver enzymes (aspartate transaminase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP), total protein and albumin, total cholesterol, total and direct bilirubin. These were measured spectrophotometrically applying standard methods recommended by the manufacturer (Tietz, 1976) using Randox analytical kits at the Chemical Pathology Department of University of Uyo Teaching Hospital.

**Assay of Kidney function parameters:** The kidney function parameters determined were creatinine ,urea and electrolytes ( Na, K, Cl, and $HCO_3$ ) levels using diagnostic kits at the Chemical Pathology Department of University of Uyo Teaching Hospital;

**Histopathological Examination:** Buffered formalin was used to fix liver, kidney, spleen, brain, ovary, testis, and heart harvested from each rat that was used in this study. Standard methods of processing and staining with hematoxylin and eosin (H&E) were used at Department of Chemical Pathology, University of Uyo Teaching Hospital, Uyo to analyse the organs. Alterations in morphology were noted and recorded in each organ of sacrificed rat. Photomicrographs of the examined processed slides were taken.

**Statistical analysis:** Students' t-test and one –way analysis of variance followed by a post test (Tukey-Kramer multiple comparison test) were used to analyse data obtained from this study. Significant difference between means were considered at 5% ie $p \leq 0.05$.

## 3. RESULTS

**Effect of leaf extract on Body weight:** The effect of leaf extract on rats' body weights treated subchronically with *S. anomalum* leaf extract for 90 days is shown in Table 1. The extract did not produce any significant ($p>0.05$) effect on the body weight of the treated rats' groups relative to control although the group treated with the middle dose of the extract (140 mg/kg) had the highest body weight gain.

**Effect on haematological parameters:** Table 2 shows the effect of repeated administration of *S. anomalum* leaf extract on haematological indices of rats. Repeated treatment of rats with leaf extract of *Solanum anomalum* for 90 days did not affect the hemoglobin concentration, WBC, RBC and platelets counts prominently ($p>0.05$) relative to control. Similarly,PCV and eosinophils percentages were not affected by the treatment. However, percentages of neutrophils, monocytes and basophils were significantly ($p<0.05$-$0.01$) elevated in the rats' group treated with extract' highest dose (210 mg/kg) relative to control (Table 2). The extract treatment further caused significant ($p<0.05$) reduction in lymphocytes percentage in the group that received 210 mg/kg of the extract (Table 2). Moreso, treatment of rats for 90 days with *S. anomalum* leaf extract had no effect on the bleeding and clotting times of treated rats relative to control (Figs. 1 and 2).

**Effect of extract on liver function indices of rats:**There was a significant ($p<0.001$) non dose-dependent lowering of ALT and AST levels of rats following treatment with *S. anomalum* leaf extract (70-210 mg/kg) for 90 days relative to control (Table 3). Significant ($p<0.001$) lowering of ALP levels in groups treated with 70 and 210 mg/kg doses of the extract was also observed, while ALP level of rat group administered with the extract (140 mg/kg) was observed to be significantly ($p<0.001$) elevated relative to control (Table 3). Leaf extracts' treatment further elevated direct and total bilirubin levels. These high levels were only significant ($p<0.05$-$0.001$) at extract doses of 140 and 210 mg/kg in the case of total bilirubin and significant ($p<0.01$) in groups treated with 70 and 140 mg/kg of the extract in the case of direct bilirubin, relative to control (Table 3).

**Effect on kidney function parameters of rats:**Treatment of rats for 90 days with leaf extract of *S. anomalum* produced a significant ($p<0.01$-$0.001$) non dose-dependent lowering of rats' creatinine levels relative to control (Table 4). Similarly, treated rats' urea levels were only lowered significantly ($p<0.05$) at 70 and 210 mg/kg doses of the extract relative to control (Table 4). However, the levels of uric acid and electrolytes (bicarbonate, sodium, potassium and chloride) were unaffected by the extract treatment (Table 4).

**Effect of extract on lipid profile indices of rats:**Dose-dependent but insignificant ($p>0.05$) lowering of triglyceride, total cholesterol, LDL and VLDL levels were observed in rats following treatment with *Solanum anomalum* (70-210 mg/kg) leaf extract for 90 days relative to control. However, significant ($p<0.01$-$0.001$) dose-dependent lowering of HDL level was recorded following the same treatment relative to control (Table 5).

**Effect on histology of organs:** Figs. 3 -11 show the effects of repeated treatment of rats with *S. anomalum* leaf extract for 90 days on histology of some organs. The leaf extract (70-210 mg/kg) did not produce any defect on the histology of the heart, testis and spleen (Figs. 4, 8 and 9). The morphologies of these organs were normal as that of the control. Increased extract doses (140-210 mg/kg) were found to produce some defects such as mild defects observed as focal vacuolation in the purkinje layer and atrophied punkinje cells in the cerebella of the rats brains (Fig. 3),distortion of liver parenchyma, array of hepatocytes and multiple focal area with inflammatory infiltrates in the liver parenchyma depicting portal and lobular inflammation (Fig. 5), atrophied glomeruli, congested blood vessels in the cortex and interlobular haemorrhage in the kidney (Fig. 6), and vacuolation in the langerhans islet of the pancreas (Fig. 7), In the ovary, higher doses (140-210 mg/kg) caused hyperplasia of stromal interstitial cells which is associated with ovarian atrophy, atretic follicle with focal apoptotic cells. Few developing follicles were seen and demarcating connective tissue septa (Fig. 10). On the uterus, higher doses (140 - 210 mg/kg), caused mild diffused eosinophilic inflammatory infiltration and increase in myometrium layer (Fig. 11).

**Table 1. Effect of repeated administration of *S. anomalum* leaf extract on body weights of rats**

| Treatment R&G /Extract | Dose (mg/kg) | Initial body weight (g) | Final body weight (g) | Weight gain (g) |
|---|---|---|---|---|
| Control | 0.2ml | 148.2± 3.78 | 236.0 ±2.82 | 87.8±2.69 |
| *S. anomalum* | 70 | 151.0 ± 4.56 | 238.6± 5.82 | 87.6±2.18 |
| | 140 | 155.0 ± 2.87 | 249.0 ± 4.52 | 94.0±2.63 |
| | 210 | 156.5 ± 8.80 | 242.0 ± 9.19 | 85.5 ±3.38 |

*Data are expressed as mean ± SEM. Not significant relative to control p>0.05 .n = 6.*

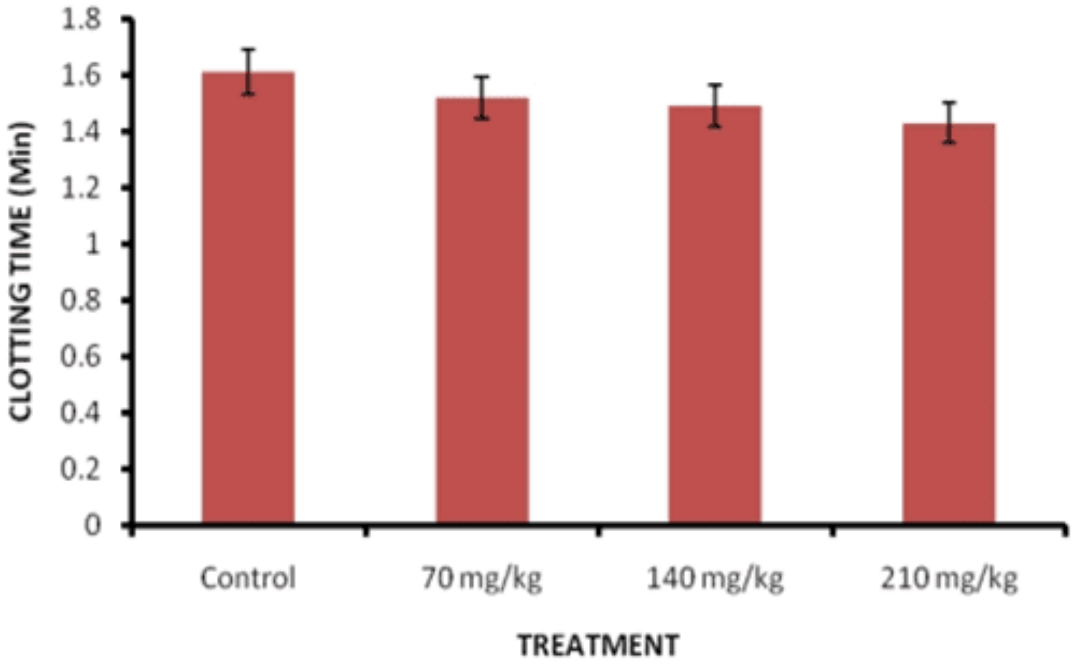



**Fig. 1. Effect of subchronic administration of *Solanum anomalum* leaf extract on clotting time of rats**

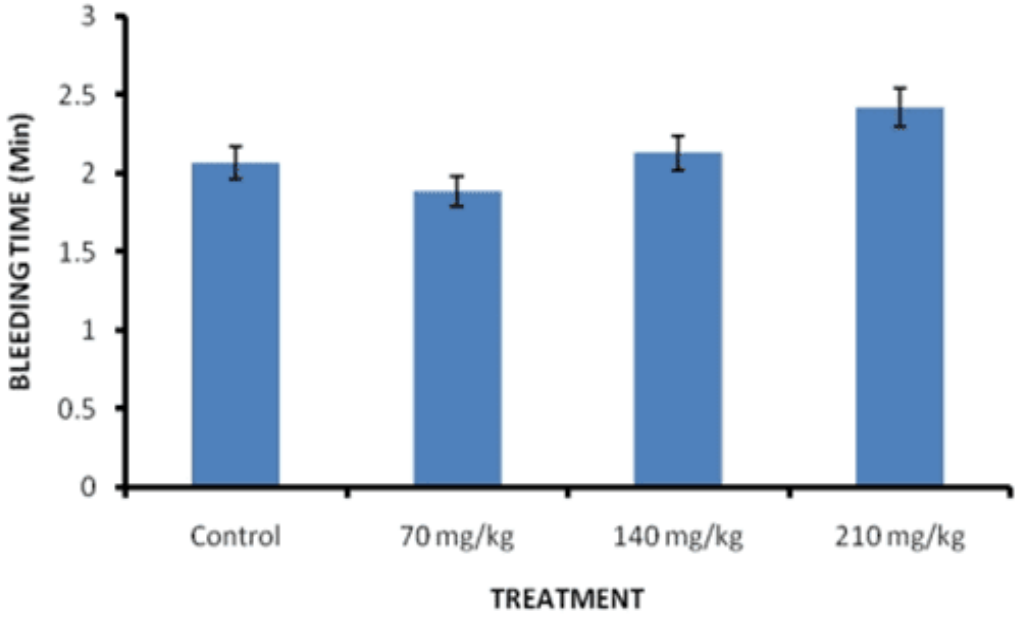
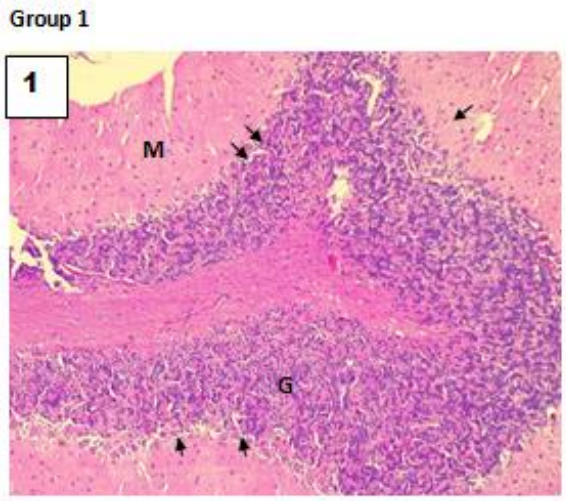
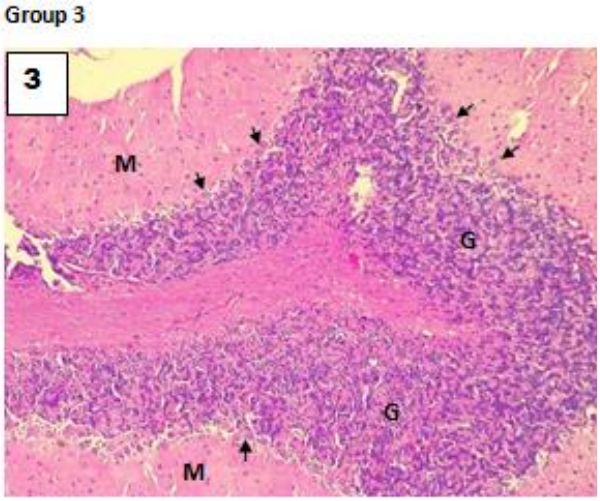


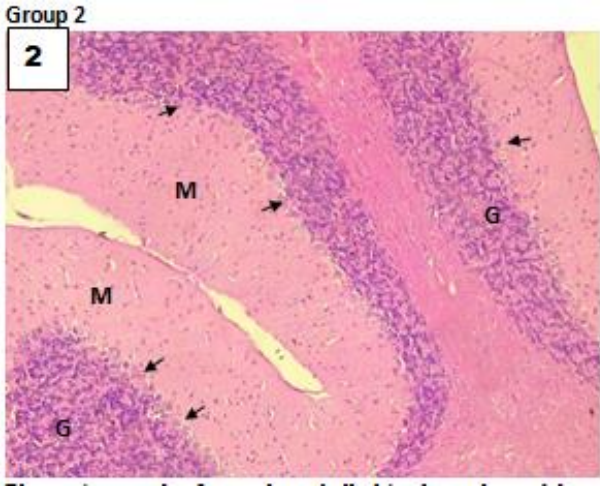

**Fig. 2. Effect of subchronic administration of *Solanum anomalum* leaf extract on bleeding times of rats**



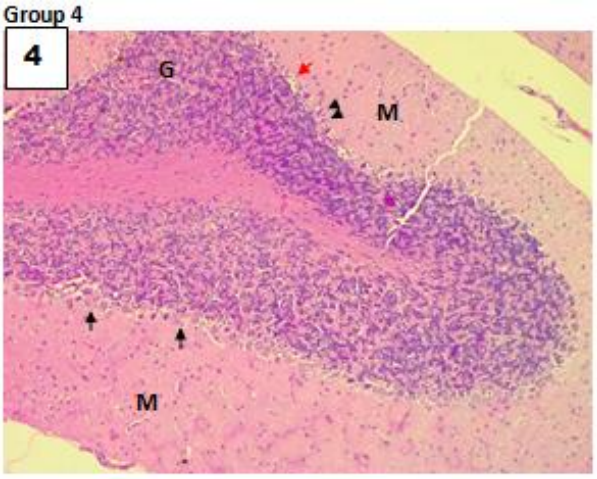

Photomicrograph of normal cerebella histology showed the densely granular cell layer (G), and molecular layer (M) consisting of stellate and basket cells. The granular cell layer border was lined by round to ovoid purkinje cells (arrows). No lesion seen. Haematoxylin and Eosin Stain, X100 magnification




Photomicrograph of normal cerebella histology showed the densely granular cell layer (G), and molecular layer (M) consisting of stellate and basket cells. The granular cell layer border was lined by normal round to ovoid purkinje cells (arrows). No lesion seen. Haematoxylin and Eosin Stain, X100 magnification.



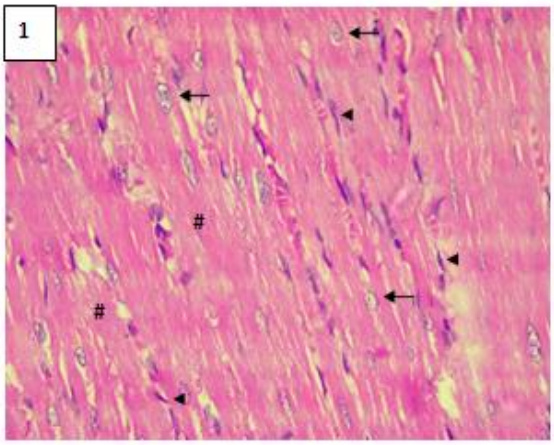

Photomicrograph of normal cerebella histology showed the densely granular cell layer (G), and molecular layer (M) consisting of stellate and basket cells. The granular cell layer border was lined by normal round to ovoid purkinje cells (arrows). No lesion seen. Haematoxylin and Eosin Stain, X100 magnification.




Section of normal cerebella histology showed the densely granular cell layer (G), and molecular layer (M) consisting of stellate and basket cells. The purkinje layer showed multiple focal vacuolation (red arrows) and atrophic punkinje cells (black arrowhead). Haematoxylin and Eosin Stain, X100 magnification

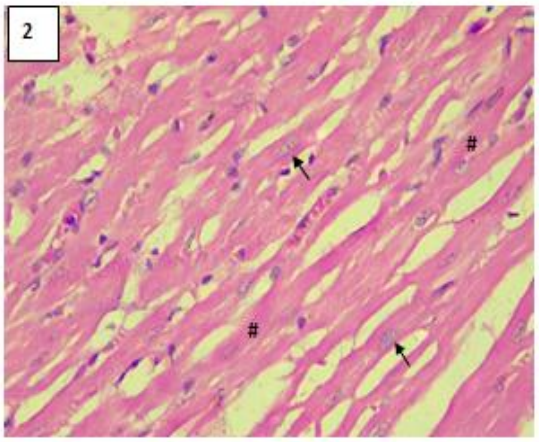
**Fig. 3. Histological sections of cerebella of rats treated with Normal saline 10 mL/kg(1), SA leaf extract 70 mg/kg bw (2), SA leaf extract 140 mg/kg bw (3), SA leaf extract 210 mg/kg bw(4) at Magnification (x100), stained with H&E Method**

Group 1



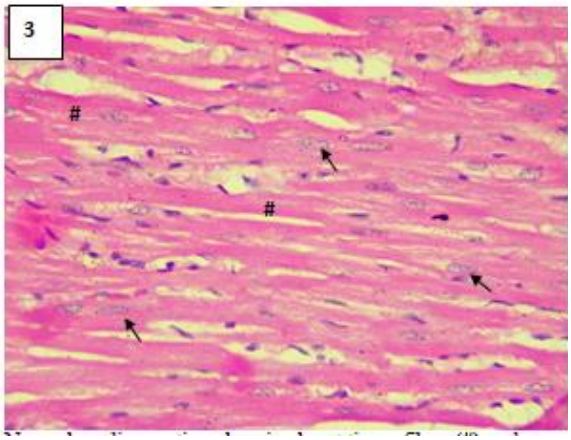

Normal heart histology architecture showing cardiac muscles evidence with spindle cardiac muscle cell nuclei (black arrows) and muscle fibre (#). No pathological changes seen

Group 2



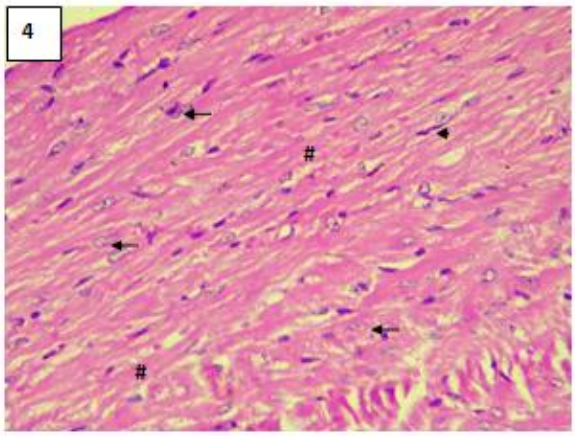

Normal heart histology architecture showing cardiac muscles evidence with spindle cardiac muscle cell nuclei (black arrows) and muscle fibre (#). No pathological changes seen

Group 3




Normal cardiac section showing heart tissue fibres(#) and nuclei (arrow). No pathologic changes seen.

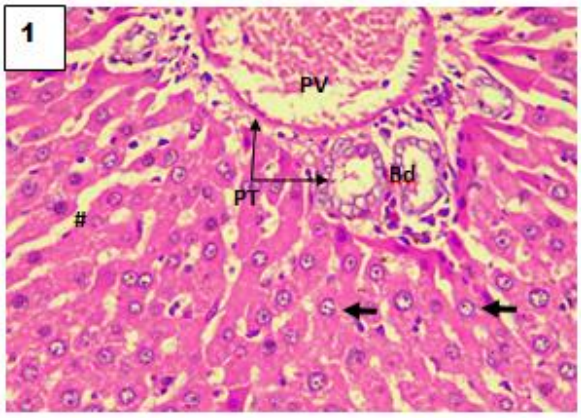
Group 4




Normal heart histology architecture showing cardiac muscles evidence with spindle cardiac muscle cell nuclei (black arrows) and muscle fibre (#). No pathological changes seen

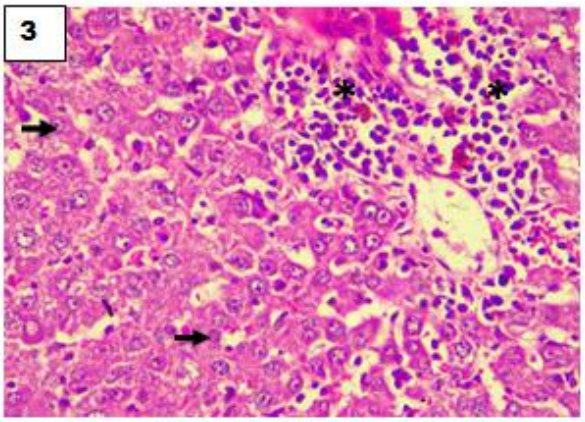
**Fig. 4. Histological sections of hearts of rats treated with Normal saline 10 mL/kg(1), SA leaf extract 70 mg/kg bw (2),SA leaf extract 140 mg/kg bw (3), SA leaf extract 210 mg/kg bw(4) at Magnification (x400), stained with H&E Method**

Group 1



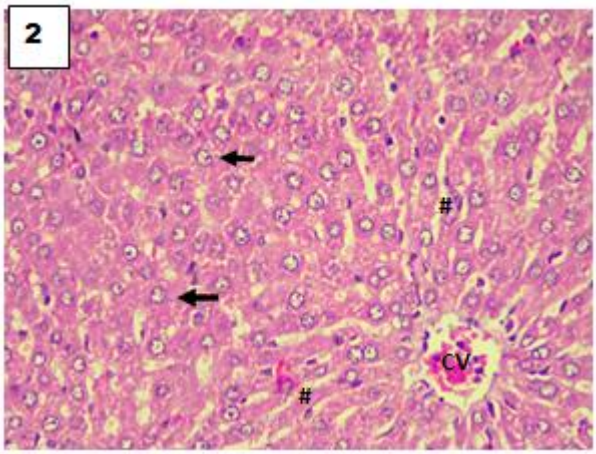

Liver section showing array of hepatocytes (thick black arrows) and some showed portal triad (PT) consisting of portal vein (PV)and bile duct (Bd), and the average size sinusoid (#). No pathological lesion seen. Haematoxylin and Eosin (H&E) stain. x400 magnification

Group 3



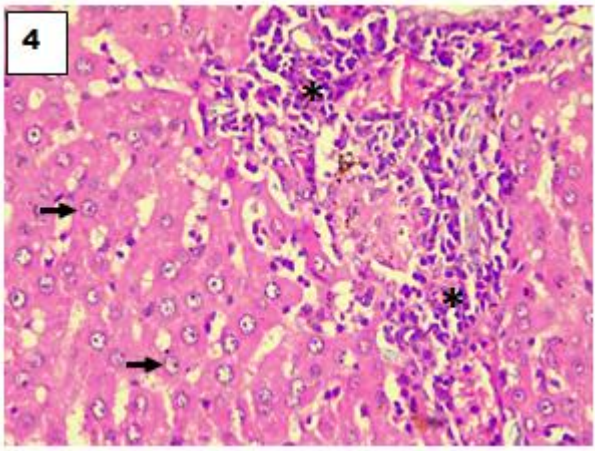

Section showed distorted liver parenchyma, array of hepatocytes (Thick black arrow), and multiple focal area with inflammatory infiltrate(astericks) in the liver parenchyma.

Group 2



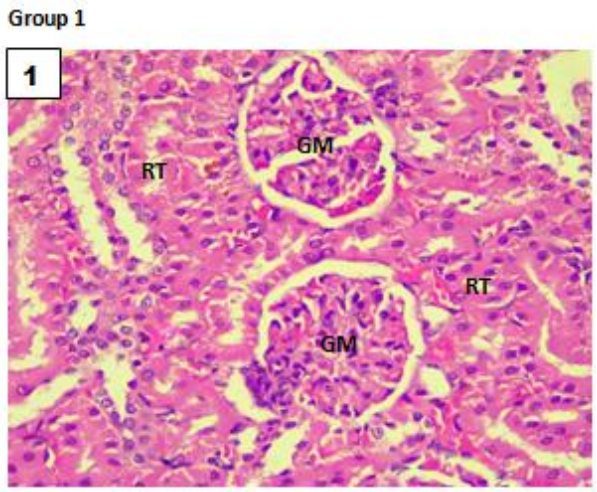

Section showed hepatocyte (Thick black arrows) with vacuolated nuclei, congested central vein (CV) and average sized sinusoidal spaces (#).No pathological lesion seen. H&E stain. x400 magnification

Group 4

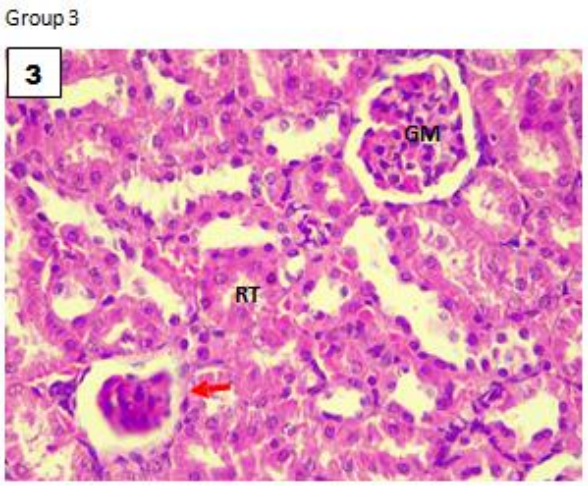



Section showing normal liver architecture, array of hepatocytes (black arrow), central vein (CV) and average sized sinusoid (read arrow). There was focal area of portal inflammatory infiltrate(asterisk) and in the liver parenchyma (portal and lobular inflammation). H&E stain. x400 magnification

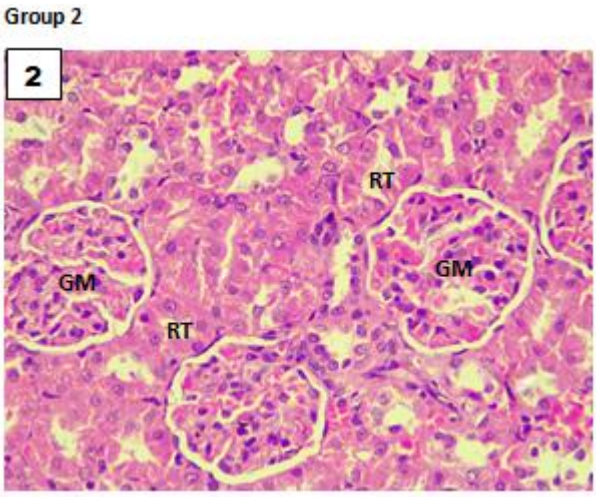
**Fig. 5. Histological sections of livers of rats treated with Normal saline 10 mL/kg(1),SA leaf extract 70 mg/kg bw (2), SA leaf extract 140 mg/kg bw (3),SA leaf extract 210 mg/kg bw(4) at Magnification (x400), stained with H&E Method**



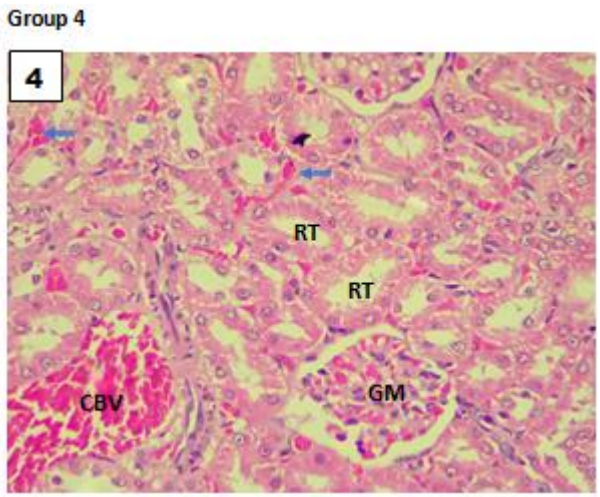

Kidney showed normal renal tissue histology architecture evidence with normal glomeruli (GM) and renal tubule (RT). No pathological changes seen. Haematoxylin and Eosin (H&E) stain. X400 magnification




Kidney showed normal renal tissue histology architecture evidence with normal glomeruli (GM) and renal tubule (RT). There are atrophic glomeruli (read arrow). Haematoxylin and Eosin (H&E) stain. X400 magnification

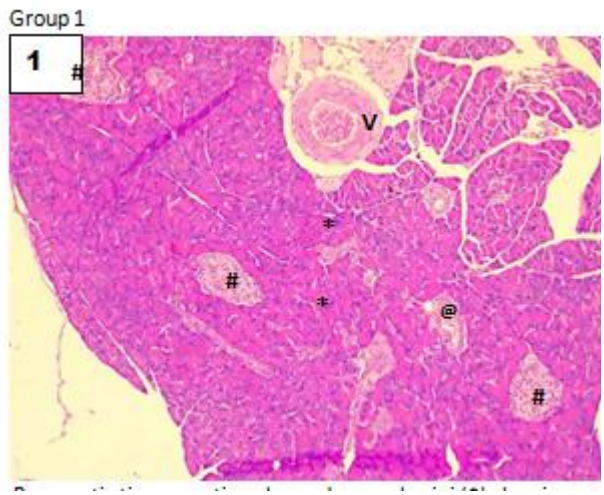



Kidney showed normal renal tissue histology architecture evidence with normal glomeruli (GM) and renal tubule (RT). No pathological changes seen. Haematoxylin and Eosin (H&E) stain. x400 magnification

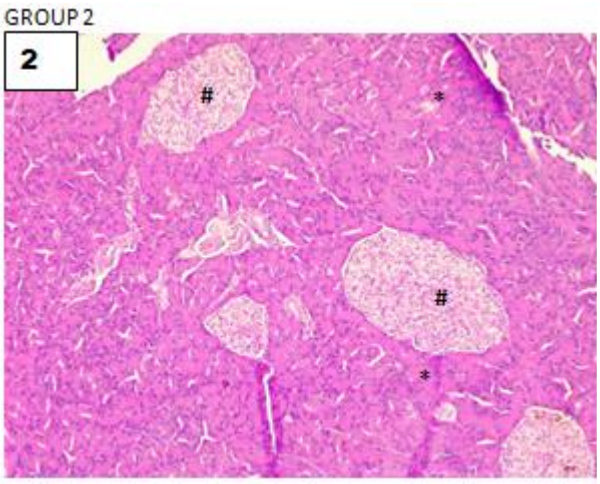



Kidney showed normal renal tissue histology architecture evidence with normal glomeruli (GM) and renal tubule (RT). There are congested blood vessel (CBV) in the cortex and intertubular haemorrhage(H). Haematoxylin and Eosin (H&E) stain. x400 magnification

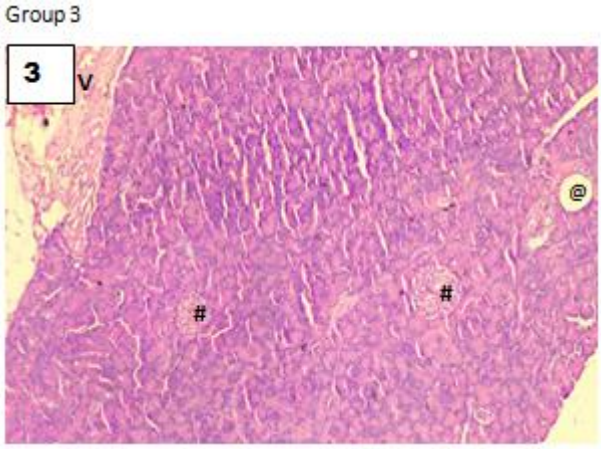
**Fig. 6. Histological sections of kidneys of rats treated with Normal saline 10 mL/kg(1), SA leaf extract 70 mg/kg bw (2),SA leaf extract 140 mg/kg bw (3), SA leaf extract 210 mg/kg bw(4) at Magnification (x400), stained with H&E Method**



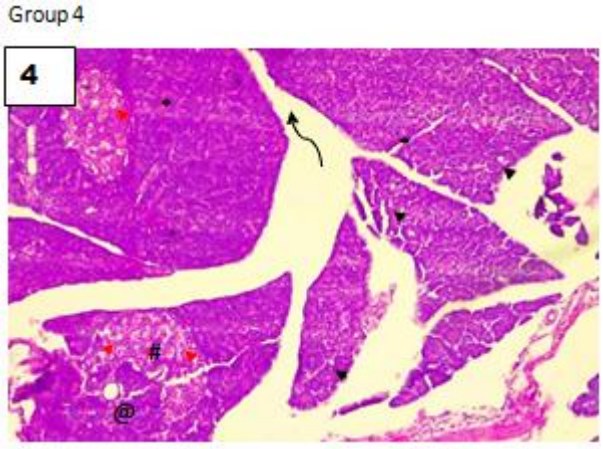

Pancreatic tissue section showed normal acini (*) showing acinar cells with basophilic cytoplasm. Several Langerhans islet (#) seen; the islet shows loosely packed pale-staining cells, also seen are interlobular duct (@) some with secretions. No lesion seen on the pancreas. H&E stain x100 magnification




Pancreatic tissue section showed normal acini (*) showing acinar cells with basophilic cytoplasm. Several Langerhans islet (#) seen; the islet shows loosely packed pale-staining cells, also seen are interlobular duct (@) some with secretions. No lesion seen on the pancreas. H&E stain x100 magnification

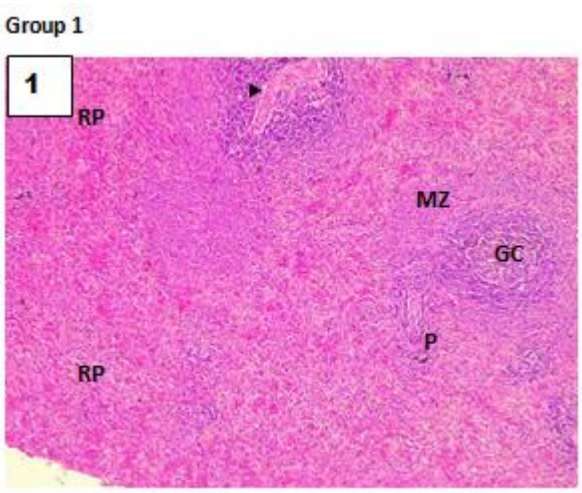



Pancreatic tissue section showed normal acini (*) showing acinar cells with basophilic cytoplasm. Very few and small Langerhans islet (#) seen: It shows loosely packed pale- staining cells, also seen are interlobular duct (@) some with secretions. H&E stain x100 magnification

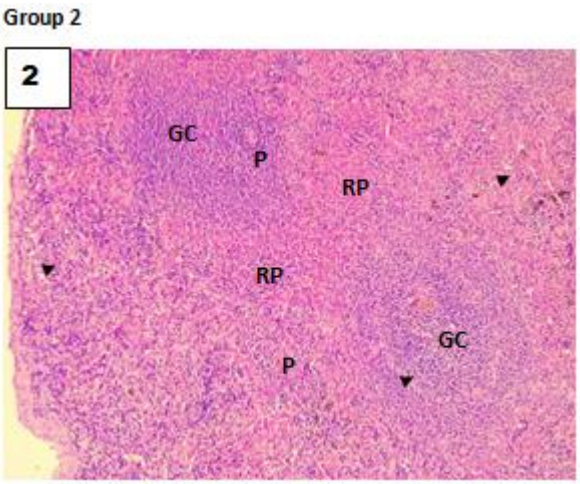



Pancreatic tissue section showed normal acini (*) showing acinar cells with basophilic cytoplasm. Langerhans islet with vacuolation (red arrowhead)was seen, there are also diffused fatty changes (black arrowhead). H&E stain x100 magnification

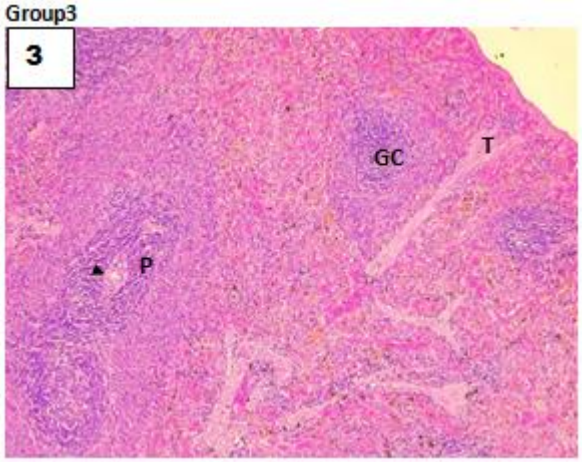
**Fig. 7. Histological sections of pancreas of rats treated with Normal saline 10 mL/kg(1), SA leaf extract 70 mg/kg bw (2), SA leaf extract 140 mg/kg bw (3), SA leaf extract 210 mg/kg bw(4) at Magnification (x400), stained with H&E Method.**



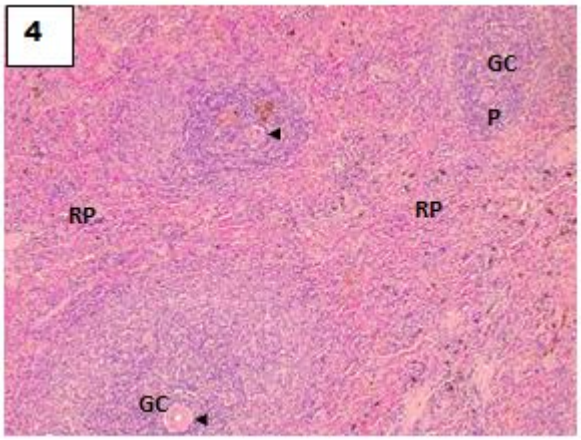

The photomicrograph showed normal splenic histology showing delineated white-pulp (WP), Central artery (arrowhead), and red –pulp (RP). The white pulp showed well organized marginal zone (MZ) and peri-arteriolar lymphocyte shealth (P) and geminal center (GC).




The photomicrograph showed normal splenic histology showing delineated white-pulp (WP) and red –pulp (RP). The white pulp showed well organized marginal zone (MZ) and peri-arteriolar lymphocyte shealth (P).



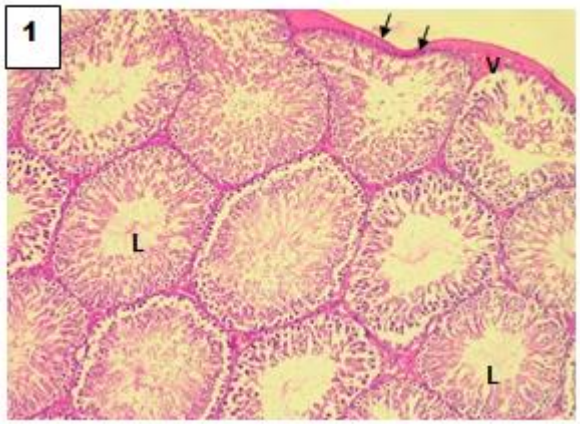

The photomicrograph showed normal splenic histology showing well delineated white-pulp (WP) and red –pulp (RP). The white pulp showed well organized marginal zone (MZ) and central artery(arrowhead) with peri-arteriolar lymphocyte shealth. The trabeculae in the parenchyma were distinctly seen




The photomicrograph showed normal splenic histology showing delineated white-pulp (WP) and red –pulp (RP). The white pulp showed well organized marginal zone (MZ) and peri-arteriolar lymphocyte shealth (P)

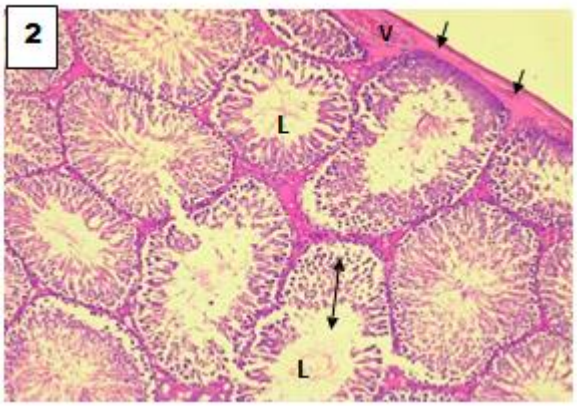
**Fig. 8. Histological sections of spleen of rats treated with Normal saline 10 mL/kg(1), SA leaf extract 70 mg/kg bw (2), SA leaf extract 140 mg/kg bw (3), SA leaf extract 210 mg/kg bw(4) at Magnification (x400), stained with H&E Method**

Group 1



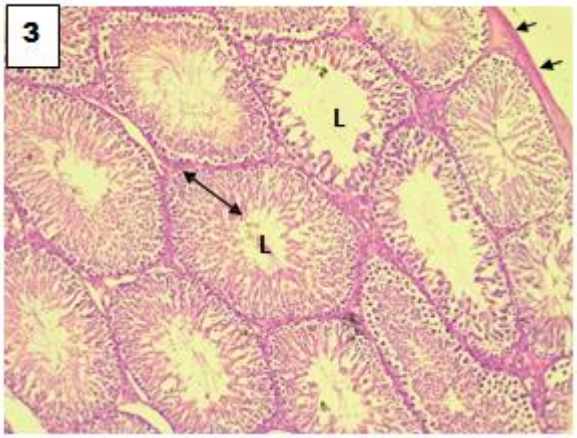

Testucular tissue showing normal seminiferous tubules, the sections showed different tubular stages and series of spermatogenic cells (double head arrow). The section showed normal tunical albuginea (short arrows). No pathologic changes seen on the cellular organization or the connective tissues. H&E stain X100 magnification

Group 2



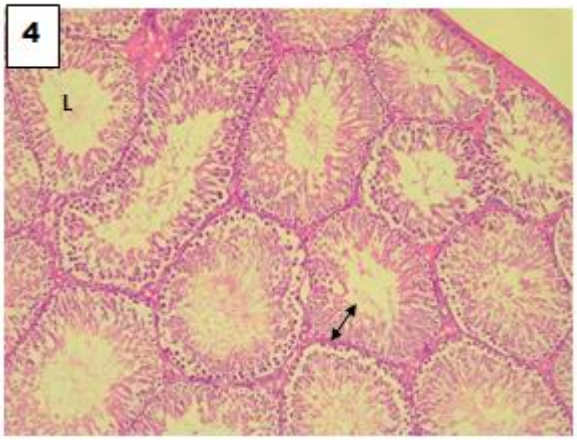

Testucular tissue showing normal seminiferous tubules, the sections showed different tubular stages and series of spermatogenic cells (double head arrow). The section showed normal tunical albuginea (short arrows). No pathologic changes seen on the cellular organization or the connective tissues. H&E stain X100 magnification

Group 3



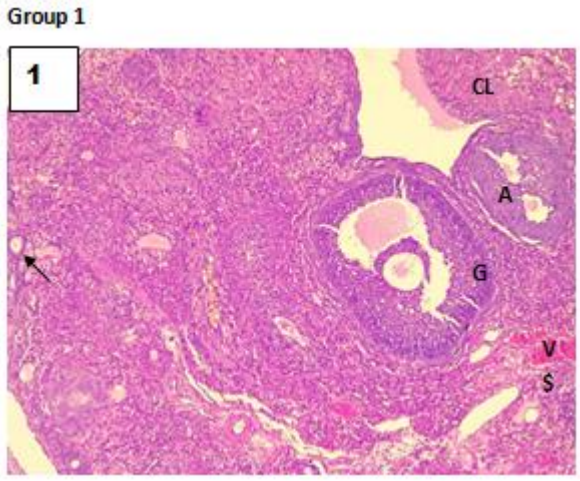

Testucular tissue showing normal seminiferous tubules, the sections showed different tubular stages and series of spermatogenic cells (double head arrow). The section showed normal tunical albuginea (short arrows). No pathologic changes seen on the cellular organization or the connective tissues. H&E stain X100 magnification

Group 4

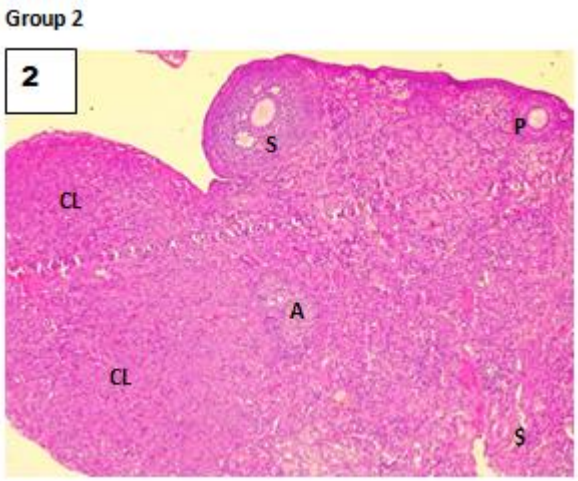



Section showed a well preserved cellular and connective tissue(CT) architecture. It showed seminiferous tubules with germinal cell layers (double heads arrows), some shows lumen (L). No evidence of pathologic changes. H&E x100 magnification

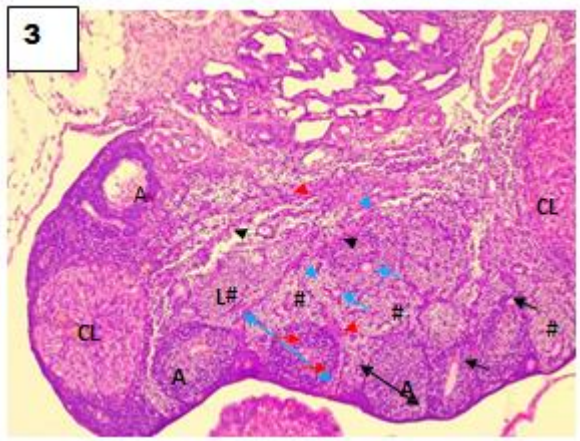
**Fig. 9. Histological sections of testes of rats treated with Normal saline 10 mL/kg(1), SA leaf extract 70 mg/kg bw (2), SA leaf extract 140 mg/kg bw (3), SA leaf extract 210 mg/kg bw(4) at Magnification (x400), stained with H&E Method.**



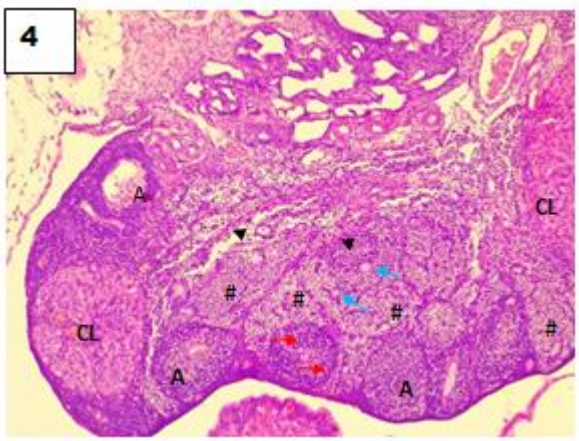

Ovarian tissue showing medullary region (M) with numerous blood vessel and the cortex with few developing follicles and a focal mature (grafian follicle) follicles and several corpus luteum (asterisks).




Ovarian tissue showing medullary region ($) with numerous blood vessel and the cortex with few developing follicles and a focal mature (grafian follicle) follicles and several corpus luteum (asterisks).

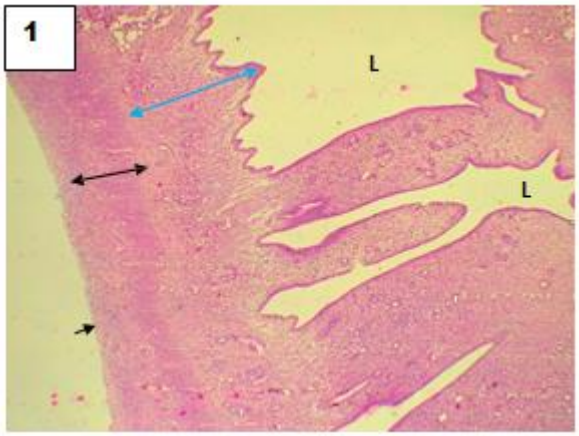



Section of ovarian tissue showed hyperplasia of stromal interstitial cells (#) associated with ovarian atrophy. It showed several atretic follicle (A) with a focal apoptotic cell (red arrow). Few developing follicle seen (blue arrow) and demarcating connective tissue septa (arrowhead)

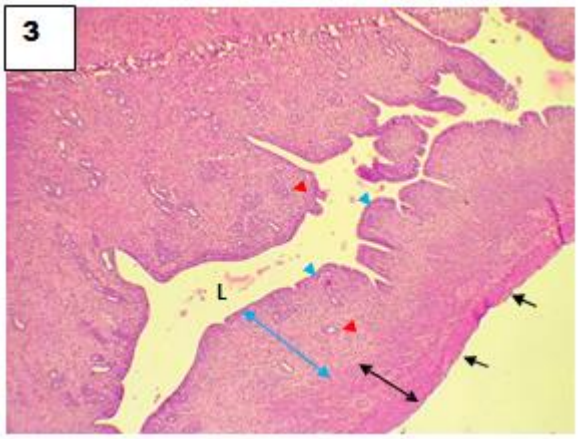



Section of ovarian tissue showed hyperplasia of stromal interstitial cells (#) associated with ovarian atrophy. It showed several atretic follicle (A) with a focal apoptotic cell (red arrow). Few developing follicle seen (blue arrow) and demarcating connective tissue septa (arrowhead)

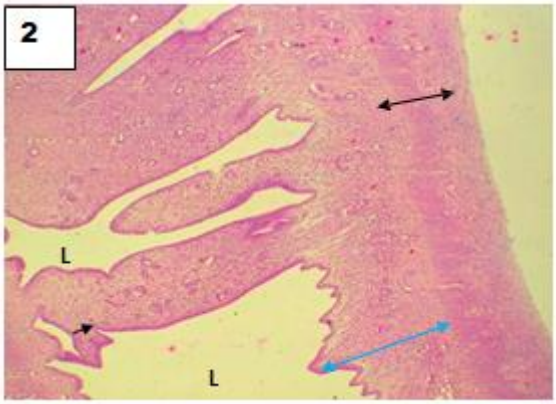
**Fig. 10. Histological sections of ovary of rats treated with Normal saline 10 mL/kg(1),SA leaf extract 70 mg/kg bw (2), SA leaf extract 140 mg/kg bw (3), SA leaf extract 210mg/kg bw(4) at Magnification (x400), stained with H&E Method**



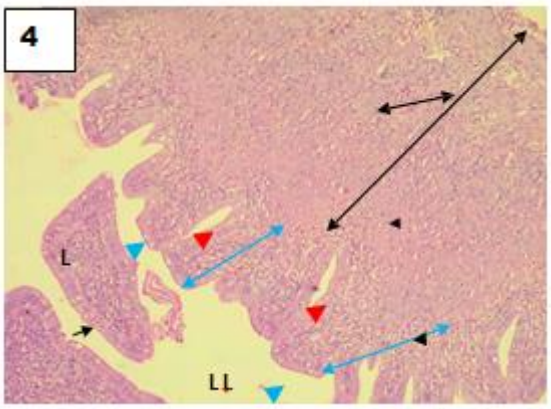

Section of uterine tissue showing normal architecture of Lumen (L), endometrium myometrium (blue two end arrow), myometrium (black two end arrow) and perimetrium (black arrowhead). Also seen average number of uterine glands in the endometrium. No lesion seen.




Section of uterine tissue showing normal architecture of Lumen (L), endometrium myometrium (blue two end arrow), myometrium (black two end arrow) and perimetrium (black arrowhead) with evidence of crypts (red arrowhead).




Section of uterine tissue showing normal architecture of Lumen (L), endometrium myometrium (blue two end arrow), myometrium (black two end arrow) and perimetrium (black arrowhead). Also seen average number of uterine glands in the endometrium. No lesion seen.




Section of Uterus showing showing lumen (L), endometrium (blue two end arrow), myometrium (black two end arrows). The endometrium has developed into several folds (blue arrowhead) with evidence of crypts (red arrowhead). There is also mild diffused eosinophilic inflammatory infiltration (black arrowhead) of the myometrium. There was increase in the myometrium layer.

**Fig. 11. Histological sections of uterus of rats treated with Normal saline 10 mL/kg(1), SA leaf extract 70 mg/kg bw (2), SA leaf extract 140 mg/kg bw (3), SA leaf extract 210mg/kg bw(4) at Magnification (x400), stained with H&E Method**

**Table 2. Effect of subchronic administration of *Solanum anomalum* leaf extract on heamatological parameters of rats**

| Treatment | Dose | WBC (L) | NEUT. (%) | LYM (%) | MONO (%) | ESINO (%) | BASO (%) | RBC (L) | HGB (g/dL) | PCV (%) | PLATELETS. (L) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Control | 10mg/ml | 12.61±0.50 | 21.40±1.30 | 75.76±1.86 | 1.83± 0.08 | 0.33± 0.05 | 0.35± 0.06 | 7.97± 0.30 | 13.70±0.46 | 44.08±1.20 | 847.5± 82.52 |
| Crude | 70 | 8.88±0.85 | 18.85± 1.79 | 76.71±2.61 | 2.76± 0.17 | 0.28 ±0.09 | 0.31± 0.07 | 8.13± 0.10 | 14.01±0.28 | 45.18±0.69 | 976.5± 68.85 |
| extract | 140 | 11.85±1.23 | 21.25±2.01 | 73.48±2.21 | 1.08± 0.09 | 0.35± 0.18 | 0.43± 0.05 | 8.27± 0.23 | 14.16±0.41 | 44.98±1.11 | 833.0± 47.49 |
| | 210 | 8.13±1.81 | 33.33 ± 2.60 [b] | 52.73±2.49[a] | 3.21±0.40[b] | 0.26± 0.11 | 0.78±0.09[b] | 7.41± 0.31 | 12.73±0.64 | 39.78±1.88 | 715.3± 85.68 |

*Data are expressed as MEAN ± SEM, Significant at [a]p<0.05, [b]p<0.01, when compared to control. (n=6).*

**Table 3. Effect of subchronic administration of *Solanum anomalum* leaf extract on liver function parameters of rats**

| TREATMENT | DOSE (mg/ kg) | ALT (IU/L) | ALP (IU/L) | AST (IU/L) | Total Bilirubin (µmol/l) | Direct Bilirubin (µmol/l) |
|---|---|---|---|---|---|---|
| Control | 10 mg/ml | 139.83± 3.31 | 416.66±87.15 | 144.83±14.60 | 1.65±0.11 | 0.83±0.06 |
| Crude extract | 70 | 67.88±11.51[c] | 277.83±28.70[c] | 85.00± 6.15[c] | 2.05± 0.15 | 1.46±0.12[b] |
| | 140 | 58.66±14.09[c] | 512.66±66.74[c] | 75.00±4.05[c] | 2.21± 0.13[a] | 1.45±0.12[b] |
| | 210 | 66.83±14.99[c] | 255.02±18.90[c] | 86.16± 5.96[c] | 2.63± 0.13[b] | 1.13±0.13 |

*Data are expressed as MEAN ± SEM, Significant at [a]p<0.05, [b]p< 0.01, [c]p< 0.001, relative to control. (n=6)*

**Table 4. Effect of subchronic administration of *Solanum anomalum* leaf extract on kidney function parameters of rats**

| TREATMENT | DOSE (mg/kg) | CREATININE (mg/kg) | UREA (mg/dl) | URIC ACID (mg/dl) | BICARBONATE (mMol/L) | SODIUM (mMol/L) | POTASSIUM (mMol/L) | CHLORIDE (mMol/L) |
|---|---|---|---|---|---|---|---|---|
| Control normal saline | 10 mg/ml | 81.66± 2.53 | 9.21± 1.41 | 0.19±0.03 | 12.00± 0.81 | 146.3±1.33 | 6.00± 0.20 | 103.0± 0.00 |
| Crude extract | 70 | 58.33± 5.45[c] | 4.70± 0.72[a] | 0.16± 0.03 | 14.50± 1.11 | 147.6±3.18 | 5.76± 0.08 | 102.0± 1.20 |
| | 140 | 58.88± 4.74[c] | 5.33± 1.07 | 0.22± 0.02 | 10.16± 1.19 | 146.0± 0.57 | 5.50± 0.17 | 101.0± 1.52 |
| | 210 | 61.66± 6.10[b] | 4.36± 0.69[a] | 0.28± 0.05 | 12.16±1.32 | 144.0±1.00 | 6.06± 0.40 | 99.0± 1.52 |

*Data are expressed as MEAN ± SEM, Significant at [a]p<0.05, [b]p< 0.01, [c]p< 0.001, when compared to control. (n=6)*

**Table 5. Effect of subchronic administration of *Solanum anomalum* leaf extract on lipid profile of rats**

| TREATMENT | DOSE mg/kg | TOTAL CHOLESTEROL (mMol/L) | TRIGLYCERIDE (mMol/L) | HDL-C (mMol/L) | LDL-C (mMol/L) | VLDL (mMol/L) |
|---|---|---|---|---|---|---|
| Control | 10 mL/kg | 1.70± 0.19 | 0.91± 0.17 | 0.63± 0.05 | 0.93± 0.11 | 0.35± 0.06 |
| Crude extract | 70 | 1.66± 0.10 | 0.78± 0.11 | 0.53± 0.02[b] | 0.75± 0.09 | 0.34± 0.05 |
| | 140 | 1.71± 0.10 | 0.73± 0.07 | 0.48± 0.04[c] | 0.77± 0.12 | 0.31± 0.03 |
| | 210 | 1.45± 0.16 | 0.63± 0.05 | 0.23± 0.04[c] | 0.81± 0.04 | 0.25± 0.02 |

*Data are expressed as MEAN ± SEM, Significant at [b]p< 0.01, [c]p< 0.001, relative to control. (n=6).*

## 4. DISCUSSION

In this study, subchronic administration of the extract did not exert any considerable effect on the body weights of rats compared to untreated control, although there were insignificant weight gains with the different doses. Alterations of body weights serve as indicator of toxic effects of drugs or toxic agents which is considered serious in cases of significant loss of body weight (Tepongning et al., 2018). In this study, moderately insignificant ($p>0.05$) improvement of rats body weights were observed in all the extract-treated groups relative to control group indicating that food intake and body growth processes of the rats were not affected negatively by the extract.

Determination of haematological indices is an important measure of intensity of toxic potentials of foreign compounds as well as plant extract on the blood (Lawal et al., 2015), as this gives information on hemopoitic effect of these foreign compounds (Bashir et al., 2015). Subchronic treatment of rats with *Solanum anomalum* leaf extract for 90 days did not affect RBC, PCV, hemoglobin concentration, WBC, eosinophils and platelets counts significantly ($p>0.05$) relative to control. This might be an indication that there was no destruction of RBCs and/or inhibition of production of erythrocytes (erythropoiesis) (Berinyuy et al., 2015) as well as leucocytosis. This also demonstrates that the extract has no erythropoeitin potential (Shittu et al., 2015). However, percentages of neutrophils, monocytes and basophils were significantly elevated at raised extract dose (210 mg/kg) relative to control. This suggests immunostimulatory effect of the extract to curb deleterious effect of the extract. The extract administration (at 210 mg/kg) further produced considerable lowering of lymphocytes percentage. Lymphocytes are important cells of the immune system (McKnight et al., 1999). The lowering of lymphocytes percentage observed in this study may suggest a depressive action on the immune system main cells. The inconsequential effect on the platelets suggest the adjustment of the animals to the extracts' effects.

In our study, exposure of rats to the leaf extract for 90 day lowered total protein and albumin levels significantly. The decreases observed in these serum proteins suggest liver damage resulting from compromised synthetic potentials of the hepatocytes. Albumin is needed in the body to maintain many physiologic functions in the body such as fluid pressure in the arteries and veins. Determination of serum albumin level gives information on the functionality of the liver as low level hepatic synthesis of albumin is indicative of end-stage liver disease or hepatic cirrhosis (Sherwin and Sobenes, 1996). In this study, the extract caused significant decrease in serum albumin level. This observation corroborates the histopathological findings and is in agreement with previous finding that reduced serum/plasma albumin level correlates with hepatic damage (Shin et al., 2010). In this study, significant lowering of serum total protein levels of rats that were treated with the leaf extract were recorded. Lowered serum total protein level reflects defective potentials of the hepatocytes to synthesize proteins (Ahmed and Urooj, 2010; Najmi et al., 2010) as was seen in this study. Evaluation of bilirubin (total and conjugated) level gives information on the excretory potentials of the liver (Yakuba et al., 2003). Raised levels of direct and total bilirubin results from severe hemolysis (Naganna, 1989). Bilirubin, is an important liver function index (Yakubu et al., 2005). The lowered total and direct bilirubin levels as observed in this study with the leaf extract suggest impairment of secretory function and an effect on the biliary system (Ashafa et al., 2009).

In this study, significantly reduced activities of AST, ALT and ALP were observed following subchronic treatment with *S. anomalum* leaf extract. Cellular enzymes often leak out of the cells when there is distortion of hepatocytes' architecture. Serum AST and ALT levels are used to determine acute and chronic hepatocellular damage (Dufour et al., 2000). Active form of vitamin B6, pyridoxal-5-phosphate (PLP), serves as a coenzyme for both ALT and AST (Rej, 1977). A number of factors such as metabolic, drugs and itrogenic activities as well as vitamin B6 deficiency could cause lowering of serum AST and ALT activities (Lum, 1995). Deficiency of pyridoxal-5-phosphate has been found to correlate with lowered AST level in plasma and serum (Saori et al., 2003), which is also prominent in epileptic patients on anticonvulsant drugs (Apeland et al., 2003). The extract may have affected the liver and caused pyridoxal-5-phosphate deficiency. Alkaline phosphatase (ALP) is an important enzyme and indicator for the plasma membrane and endoplasmic reticulum (Sadiq et al., 2019). The significant lowering of alkaline phosphatase activity after subchronic treatment of rats with leaves extract of *S. anomalum* can results from

either leakage of membrane components (including ALP) into the extracellular fluid (Sadiq et al., 2019), deanaturation of the enzyme molecule *in situ* (Akanji et al., 2013), or suspension of the enzyme activity at the cellular/molecular level. This can as well be due to gross lowering of concentration or complete absence of typical phospholipids needed for the proper functioning of the membrane bound enzyme (Das et al., 2015).

Blood urea nitrogen (BUN) is a product of metabolic activities in the liver and is removed from the body in the urine through the kidney. It is found in high amount in the serum when there is kidney injury (Mayne, 1994). Breakdown of tissue creatinine gives rise to serum creatinine (Mayne, 1994). Therefore, high levels of urea and creatinine in the serum is indicative of kidney injury (Flaoyen et al., 2001). In this study, there were significant lowering of both serum creatinine and urea levels in rats administered with the leaf extract for 90 days. This indicates that the extract can affect the kidney adversely as seen in the histology of the kidney particularly at raised doses (140 and 210 mg/kg). The electrolytes concentrations were not affected by the extract treatment suggesting that the glomerular filtration rate was not affected by the treatment.

Abnormal changes in the concentration of lipid profile indices such as cholesterol, HDL, LDL and triglycerides serves as avenues to diagnose effect on the lipid metabolism as well as cardiac functions and diseases (Yakubu et al., 2008). High blood cholesterol concentrations are an important risk factor for cardiovascular disease (Abolaji et al., 2007). Therefore, the serum cholesterol lowering effect of the extract though insignificant may be clinically significant as the extract is unlikely to caused cardiovascular problem at the doses used. The lowered serum triacylglycerol level observed in this study may be a result of reduced lipolysis (Yakubu et al., 2008). The lowering of VLDL, LDL and HDL levels in this study demonstrates a strong hypolipidemic potentials of the leaves perhaps due to inhibitory effect on lipolysis which is due to the activities of its phytoconstituents and may be an indication of the extracts' cardioprotective activity against lipid associated heart diseases (Panagiotakos et al., 2003).

On the histology, subchronic treatment of rats with *S. anomalum* leaf extract for 90 days did not produce any defect on the histology of the heart, testis and spleen, indicating that the extract has no deleterious effect on the heart, the male reproductive system and spleen. The morphologies of these organs were normal as that of the control. Raised extract doses (140-210 mg/kg) were found to produce some defects such as atrophied glomeruli, congested blood vessels in the cortex and interlobular haemorrhage in the kidney portraying a toxic effect on the kidney. These results are corroborated by the chemical pathology results which showed significant reductions in urea and creatinine levels. Furthermore, the leaf extract was found to cause distortion of liver parenchyma, array of hepatocytes and multiple focal area with inflammatory infiltrates in the liver parenchyma depicting portal and lobular inflammation, thus depicting hepatotoxic potential. These results corroborate the chemical pathology results which significant decreases in the levels of markers of liver functions were observed. In the ovary, higher doses (140-210 mg/kg) caused hyperplasia of stromal interstitial cells which is associated with ovarian atrophy, atretic follicle with focal apoptotic cells. Few developing follicles were seen and demarcating connective tissue septa. On the uterus, higher doses (140-210 mg/kg), caused mild diffused eosinophilic inflammatory infiltration and increase in myometrium layer. These findings depict adverse effect on the female reproductive system which also indicate contraceptive potentials. Also, the maximum dose (210 mg/kg) of the extract used in the study was found to produce mild defects observed as focal vacuolation in the purkinje layer and atrophied punkinje cells in the cerebella of the rats brains, indicating adverse effects on the brain cells. Subchronic administration of the leaf extract to rats was also found to cause vacuolation in the langerhans islet of the pancreas portraying an adverse effect on the pancreas. However, no mortality was recorded throughout the period of subchronic study.

## 5. CONCLUSION

The findings of this study show that 90 days oral treatment of rats with *Solanum anomalum* leaf extract can cause mild to moderate toxic effects to the liver, kidney, brain, pancreas, ovary and uterus but has no effect on the hematological parameters except differential WBC elevation, testis, heart, spleen and lipid profile.

## DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models

(ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

## CONSENT

It's not applicable.

## EHTICAL APPROVAL

Approval for the study was given by Faculty of Pharmacy Animal Ethics Committee, University of Uyo.

## ACKNOWLEDGEMENTS

The authors are grateful to Mr Nsikan Malachy of Pharmacology Department, University of Uyo for technical assistance.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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