EFFECT OF NINETY-DAY REPEATED ADIMINISTRATION OF LEAF EXTRACT OF *Solanum anomalum* ON RATS

Abstract

Solanum anomalum Thonn. ex Schumach, a plant whose fruits and leaves are used nutritionally is used traditionally for the treatment of malaria and fever among others. Evaluation of 90-day repeated administration of ethanol leaf extract of *S. anomalum* for possible effect on rats was carried out. The leaf extract (70, 140, 210 mg/kg body weight) was orally administered to male and female Wistar rats daily for 90 days and the rats were sacrificed under light diethyl ether anaesthesia at the completion of the administration. Subchronic administration of *S. anomalum* leaf extract did not affect the body weights of rats significantly ($p>0.05$) when compared to control. The leaf extract treatment did not affect the hemoglobin concentration, WBC, RBC and platelets counts significantly ($p>0.05$), percentages of PCV and eosinophils were also not affected by the treatment. However, percentages of neutrophils, monocytes and basophils were significantly ($p>0.05$-0.01) elevated at the highest dose (210 mg/kg), while lymphocytes percentage was reduced. The extract did not affect bleeding and clotting time significantly ($p>0.05$) when compared to control. The leaf extract non dose-dependently caused significant ($p<0.05$) decreases in ALT AST and ALP levels. However, total and direct bilirubin levels were significantly ($p<0.01$-0.001) decreased only at higher doses (140 and 210 mg/kg) of the extract. The leaf extract did not cause any significant ($p>0.05$) effect on uric acid, bicarbonate, chloride, potassium and sodium levels, while urea, creatinine, total cholesterol, triglyceride, HDL, VLDL and LDL levels of rats were significantly ($p<0.05$) reduced. The leaf extract did not exert any effect on the heart, testis, and spleen. Higher doses of the extract (140 and 210 mg/kg) produced some distortion in the histology of livers, kidneys, ovaries and brains of rats. High doses of the leaf extract should be avoided to prevent serious toxic effects.

Keywords: *Solanum anomalum*, subchronic, toxicity, organ weights

Introduction

Medicinal plants are use 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.(citation needed) 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 address to enhance proper use of these plants.

Solanum anomalum Thonn. ex Schumach, a plant whose fruits and leaves are used medicinally and nutritionally is commonly found growing in West and East Africa sub-regions. Its parts are utilised locally to treat diabetes, gastrointestinal disorders, infections, inflammation and pains [1]. Hypoglycemic and antidiabetic activities of the leaves have been reported[2]. Moreso, *in vivo* and *in vitro* antiplasmodial [3,4], anti-oedema [5], antioxidant and antiulcer [6], anticonvulsant and depressant [7], analgesic [8], antidiarrhoeal [9], hepatoprotective[10,11] and nephroprotective [12,13] properties of the leaf extract have also been reported. Phytochemical constituents such as alkaloids, flavonoids, saponins, tanins, diosgenin, a diosgenin glycoside (25(R)-diosgenin-3-O-α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranoside,

uracil, 5-methyluracil, 1-octacosanol, and octacosane have been reported on the leaves of the plant [2,3] .We report in this study the effect of chronic administration leaf extract of *Solanum anomalum* on rats.

MATERIALS AND METHODS

Plants collection

Fresh leaves of *Solanum anomalum* were collected in compounds in Uruan area, Akwa Ibom State, Nigeria in August, 2020. The plant was identified and authenticated by a taxonomist in the Department of Botany and Ecological Studies, University of Uyo, Uyo, Nigeria. Hebarium specimen was deposited at Department of Pharmacognosy and Natural Medicine Herbarium, University of Uyo (UUH.75a).

Extraction

Fresh leaves of *S. anomalum* were washed, cut into smaller pieces and dried under shade for two weeks. The leaves were further pulverized to powder using electric grinder. The powdered leaves material was (1.5 kg) was macerated in 50% ethanol (7.5 L) for 72 hours at room temperature (28 ±2 ˚C). This was thereafter filtered and the liquid filtrate was concentrated and evaporated to dryness in *vacuo* 40˚C using a rotary evaporator (BuchiLab, Switzerland). The extract was 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 administered 70, 140 and 210 mg/kg of the leaf extract respectively 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 and used immediately for haematological testing such as bleeding time, clotting time, full blood counts etc. Serum was separated from the remaining blood and stored at -20˚C until used for biochemical determinations such as liver function test, kidney function test, 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 surgically removed and fixed in 10% formalin. The organs were processed, sectioned and stained using hematoxylin and eosin (H&E) according to standard procedures.

Haematological Analysis

The following haematological parameters were determined; Haemoglobin level (Hb), Packed Cell Volume (PCV), Total and differential White blood Cell Count (WBC), Platelet Count. Full Blood Count. These parameters were determined at Haematology Department of University of Uyo Teaching Hospital using automated Haematology analyser.

Biochemical Analysis

Determination of the effect of the crude extract on the lipid profile (Serum TG, TC, HDL, LDL, VLDL levels) of the treated rats

Serum cholesterol, triglyceride and high density lipoprotein (HDL) levels of the diabetic rats were measured by enzymatic colorimetric methods using Randox diagnostic kits. The low and very low-density lipoprotein (LDL and VLDL) was estimated from the formula of Friedwald et al [14].

Liver Function Test

The following parameters were determined; Aspartate transaminase (AST), Alanine aminotransferase (ALT), Total Cholesterol, Alkaline phosphatase (ALP). Total plasma protein, Total and direct bilirubin. The determinations were done spectrophotometrically using Randox analytical kits according to standard procedures

of manufacturer's protocols [15] at the Chemical Pathology Department of University of Uyo Teaching Hospital.

Kidney Function Test

The following biochemical parameters were determined as markers of kidney function using diagnostic kits at the Chemical Pathology Department of University of Uyo Teaching Hospital; Levels of electrolytes (Na, K, Cl, and HCO_3), Creatinine and Blood urea

Histopathological Examination

The liver, kidney, spleen, brain, ovary, testis, and heart of each animal that was used in the study were surgically harvested and fixed in buffered formalin. They were then processed and stained with haematotoxylin and eosin (H&E) according to standard procedures at Department of Chemical Pathology, University of Uyo Teaching Hospital, Uyo.

Morphological changes were observed and recorded in the excised organs of the sacrificed animals. Histologic pictures were taken as micrographs.

Statistical analysis

Data obtained from this work were analysed statistically using students' T-test and ANOVA (one –way) followed by a post test (Tukey-Kramer multiple comparison test). Differences between means were considered significant at 5% level of significance ie $p \leq 0.05$.

RESULTS

Effect of leaf extract on Body weight

The effect of the extract on body weight of rats treated chronically with leaf extract of *S. anomalum* for 90 days is shown in Table 1. There was a no significant ($p>0.05$) effect on the body weight of the extract-treated groups when compared to that of control with the group administered with the middle dose of the extract (140 mg/kg) having the highest weight gain.

Effect on haematological parameters

The results of the effect of chronic administration of ethanol leaf extract of *S. anomalum* on haematological parameters rats is shown in Table 2. Chronic administration of leaf extract of *Solanum anomalum* to rats for 90 days did not affect the hemoglobin concentration, WBC, RBC and platelets counts significantly ($p>0.05$) when compared to control. Similarly, percentages of PCV and eosinophils were also

not affected by the treatment. However, percentages of neutrophils, monocytes and basophils were significantly ($p>0.05$-0.01) elevated at the highest dose (210 mg/kg) of the extract when compared to control (Table 2). The extract treatment further caused significant ($p>0.05$) reduction in lymphocytes percentage at 210 mg/kg dose of the extract (Table 2). Moreso, treatment of rats with leaf extract of *S. anomalum* for 90 days did not cause any significant ($p>0.05$) effect on the bleeding and clotting time of treated rats when compared to the control (Figures 1 and 2).

Effect of extract on liver function indices of rats

Administration of leaf extract of *S. anomalum* (70-210 mg/kg) to rats for 90 days caused a non dose-dependent and significant ($p<0.001$) reduction in ALT and AST levels of rats when compared to control (Table 3). The ALP levels were similarly reduced significantly ($p<0.001$) in groups treated with 70 and 140 mg/kg doses of the extract, while significantly ($p<0.001$) elevated ALP level was observed in the group treated with 140 mg/kg of the extract when compared to control (Table 3). Treatment with the extract further caused elevations in the levels of direct and total bilirubin. These elevations were only significant ($p<0.05$-0.001) at higher doses (140 and 210 mg/kg) of the extract 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, when compared to control (Table 3).

Effect on kidney function parameters of rats

Treatment of rats for 90 days with leaf extract of *S. anomalum* caused a non dose-dependent and significant ($p<0.01$-0.001) reduction in creatinine level of rats when compared to control (Table 4). Similarly, urea levels of the treated rats was only significantly ($p<0.05$) reduced at 70 and 210 mg/kg doses of the extract when compared to control (Table 4). However, the levels of uric acid and electrolytes (bicarbonate, sodium, potassium and chloride) were not affected by the extract treatment significantly ($p>0.05$) when compared to control (Table 4).

Effect of extract on lipid profile indices of rats

Treatment of rats with leaf extract of *Solanum anomalum* (70-210 mg/kg) caused dose-dependent but insignificant ($p>0.05$) decreases in the levels of total cholesterol, triglyceride, LDL and VLDL of rats treated for 90 days when compared to control. However, the HDL level was dose-dependently and significantly ($p<0.01$-0.001) reduced when compared to control (Table 5).

Effect on histology of organs

Figures 3 -11 show the effects of chronic administration of ethanol leaf extract of *S. anomalum* to rats 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 (Figures 4, 8 and 9). The morphologies of these organs were normal as that of the control. Higher doses of the leaf extract (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 (Figure 3),distortion of liver parenchyma, array of hepatocytes and multiple focal area with inflammatory infiltrates in the liver parenchyma depicting portal and lobular inflammation (Figure 5), atrophied glomeruli, congested blood vessels in the cortex and interlobular haemorrhage in the kidney (Figure 6), and vacuolation in the langerhans islet of the pancreas (Figure 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 (Figure 10). On the uterus, higher doses (140 - 210 mg/kg), caused mild diffused eosinophilic inflammatory infiltration and increase in myometrium layer (Figure 11).

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

Treatment R&G /Extract	Dose (mg/kg)	Initial body weight (Kg)	Final body weight (Kg)	Weight gain (Kg)
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 when compared to control $p>0.05$ .n = 6.

Table 2: Effect of chronic 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 extract	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
	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).

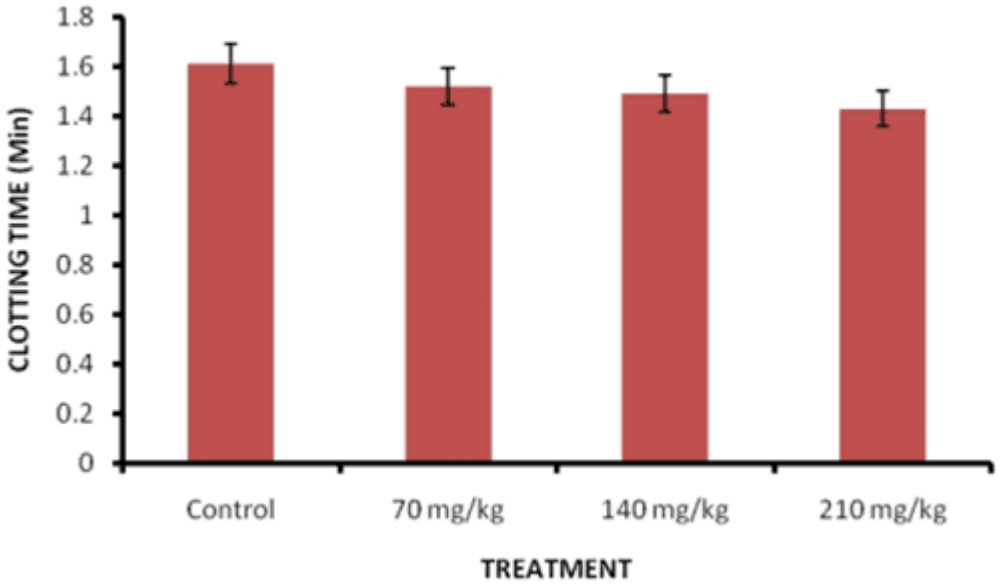


Figure 1: Effect of chronic administration of *Solanum anomalum* leaf extract on clotting time of rats

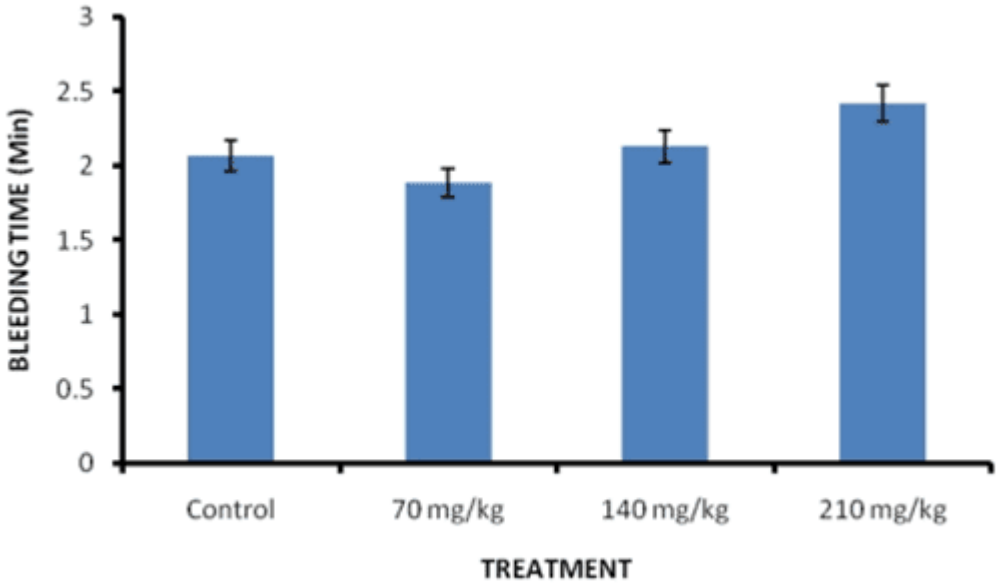


Figure 2: Effect of chronic administration of *Solanum anomalum* leaf extract on bleeding times of rats

Table 3: Effect of chronic 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 $^{a}p<0.05$, $^{b}p< 0.01$, $^{c}p< 0.001$, when compared to control. (n=6).

Table 4: Effect of chronic 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 chronic 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, when compared to control. (n=6).

DISCUSSION

In this study, chronic administration of the extract did not have any significant effect on the body weight of rats compared to untreated control, although there were insignificant weight gains with the different doses. Changes in body weights are markers of adverse effects of drugs or toxic agents and it is considered to be serious if a significant body weight loss [16]. In this study there were moderate increases in body weights of rats in all the extract treated groups but these increases were not significantly ($p>0.05$) different from that of the control group indicating that feeding habit of the rats was not adversely affected by the administration of the extract and there were no adverse effects of the extract on the body growth processes of rats.

Assessment of haematological parameters can be used to assess the degree of deleterious effect of foreign compounds including plant extract on the blood [17], as well as to explain hemopoitic effect of a chemical compound including those contained in plant extracts [18]. Chronic administration of leaf extract of *Solanum anomalum* to rats for 90 days did not affect RBC, PCV, hemoglobin concentration, WBC, eosinophils and platelets counts significantly ($p>0.05$) when compared to control. This might be an indication that there was no destruction of RBCs and/or inhibition of production of erythrocytes (erythropoiesis) [19] as well as leucocytosis.

This also indicates that the extract did not exert erythropoeitin potential, the humoral regulator of RBC production [20]. The lack of significant effect ($p > 0.05$) on the RBC and Hb also implies that the oxygen-carrying capacity of the blood was not negatively affected. However, percentages of neutrophils, monocytes and basophils were significantly elevated at the highest dose (210 mg/kg) of the extract when compared to control. The significant increase in the neutrophils, monocytes and basophils by the extract could possibly suggest stimulatory effect on the blood component to phagocytose, and therefore, an indication of inflammatory response perhaps in response to deleterious effect of the extract. The extract treatment further caused significant reduction in lymphocytes percentage at 210 mg/kg dose of the extract. Lymphocytes are the main effector cells of the immune system [21]. The reduction in the lymphocytes in this study may reflect an affect on the effector cells of the immune system. The non-definite effect of the extract on the platelets can be attributed to adjustment of the animals to the effect of the extract.

In our study, total protein and albumin decreased significantly in animals exposed to the extract for 90 days. The decreases observed in these serum proteins were indication of hepatic damage due to reduced synthetic potentials of the hepatocytes to synthesize enough serum proteins. Albumin is needed in the body to maintain many physiologic functions in the body such as fluid pressure in the arteries and

veins. Assay of serum albumin level is often used to test normal liver function as decreased hepatic synthesis of albumin is one of the features of end-stage liver disease or hepatic cirrhosis [22]. 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 is associated with hepatic damage [23]. In this study, significant decreases in serum total protein in rats that were administered with the extract were recorded. Decrease in serum total protein is known to be associated with defective capability of the hepatocytes to synthesize proteins [24,25] as was seen in this study. Assessment of bilirubin (total and conjugated) could be used to assess the excretory function of the liver [26]. Severe hemolysis causes the release of more bilirubin into the blood which manifests as elevated levels of direct and total bilirubin [27]. Bilirubin, is also one of the most commonly used liver function tests, a metabolic breakdown product of heme secreted into the bile, passed into the intestine and sometimes, reabsorbed from the intestine [28]. The reduction in the total and direct bilirubin as observed in this study with the leaf extract could be attributed to impairment in the secretory function of these proteins and an effect on the biliary system [29].

In this study, significantly reduced activities of AST, ALT and ALP were observed following chronic administration of the leaf extract of *S. anomalum*. Cellular leakage

of enzymes occurs often when the cell architecture and integrity is damage. Serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) are used as markers for acute and chronic hepatocellular damage [30]. Pyridoxal-5-phosphate (PLP) which is the active form of vitamin B6 is the coenzyme for both ALT and AST respectively [31]. Reduction in serum AST and ALT activities could result from metabolic, drug-induced, iatrogenic (unknown cause) as well as disease conditions leading to deficiency of vitamin B6 [32]. Decrease in the serum/plasma activities of aspartate aminotransferase has been shown to correlate with pyridoxal-5-phosphate deficiency [33], which is also prominent in epileptic patients on anticonvulsant drugs [34]. The extract may have affected the liver and caused pyridoxal-5-phosphate deficiency. Alkaline phosphatase (ALP) is a 'marker' enzyme for the plasma membrane and endoplasmic reticulum [35]. The significant reduction in alkaline phosphatase activities following chronic administration of leaves extract of *S. anomalum* can be attributed to either loss of membrane components (including ALP) into the extracellular fluid [36], inactivation of the enzyme molecule *in situ* [37], or inhibition of the enzyme activity at the cellular/molecular level. This can also be attributable to a reduction in concentration or total absence of specific phospholipids required by this membrane bound enzyme to work effectively [38].

Blood urea nitrogen (BUN) produced in the liver is derived from the diet or tissue sources and is excreted in the urine via the kidney. Serum urea accumulates in the blood in event of renal disease [39]. Serum creatinine is basically derived from endogenous sources by tissue creatinine breakdown [39]. Therefore, elevation of urea and creatinine levels in the serum is used as an index of nephrotoxicity [40]. 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 shows that the extract is toxic to the kidney as seen in the histology of the kidney especially at higher 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.

Alterations in the concentration of lipid profile indices such as cholesterol, high-density lipoprotein cholesterol, low density lipoprotein cholesterol and triglycerides serves as avenues to detect effect on the lipid metabolism as well as cardiac functions and diseases [41]. High blood cholesterol concentrations are an important risk factor for cardiovascular disease [42]. Therefore, the reduced levels of serum cholesterol by the extract though insignificant may be clinically beneficial to the animals as the extract is unlikely to be associated with cardiovascular risk at these doses. Similarly, the decreased levels of serum triacylglycerol by the extract may be

explained by a reduced lipolysis [41]. The reduction in the levels of VLDL, LDL and HDL in this study reveals a strong hypolipidemic activity of the leaf perhaps due to inhibitory activity on lipolysis which is due to the activities of its phytoconstituents and may be an indication that the extract may not predispose the animals to atherosclerosis and coronary heart diseases [43].

On the histology, chronic administration of ethanol leaf extract of *S. anomalum* to rats 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. Higher doses of the leaf extract (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 ALT, AST,ALT, total protein, albumin, total and direct bilirubin 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 highest dose (210 mg/kg) 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. Chronic administration of the leaf extract to rats was also found to caused vacuolation in the langerhans islet of the pancreas portraying an adverse effect on the pancreas. However, no mortality was recorded throughout the period of chronic study.

CONCLUSION

The results of this study show that 90 days administration of leaf extract of *Solanum anomalum* 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.

REFERENCES

1. Burkill HM. The useful plants of West Tropical Africa. 2nd Edition. Volume 5, Families S–Z, Addenda. Royal Botanic Gardens, Kew, United Kingdom. 2000,686 pp.

2.Okokon JE, Etuk IC, Thomas P, Drijfhout FP, Claridge TM, Li W-W. In vivo antihyperglycaemic and antihyperlipidemic activities and chemical constituents of leaf extract and fractions of *Solanum anomalum* in alloxan-induced diabetic rats. Biomedicine and Pharmacotherapy. doi.org/10.1016/j.biopha.2022.113153

3. Okokon JE, Opara KN, Azare B. Antimalarial activity of *Solanum anomalum* (Solanaceae). *Nigerian Journal of Pharmaceutical and Applied Sciences Research*. 2016; 5(1):1-6.

4. Okokon JE, Okokon PJ, Sahal D. In vitro antiplasmodial activity of some medicinal plants from Nigeria. *International Journal of Herbal Medicine*. 2017;5(5): 102 – 109.

5.Okokon JE, Davis K, Umoh EE, Amazu LU. Antiinflammatory activity of leaf extract of *Solanum anomalum*. Journal of Herbal Drugs. 2017; 7(4):243-249.

6. Okokon JE, Nyong EE, Thomas PS, Udoh AE. Antioxidant and antiulcer activities of ethanol leaf extract and fractions of *Solanum anomalum*. *Discovery Phytomedicine.* 2019;6(2):20-25.

7. Okokon JE, Nyong EE, Obot J, Davies K. Anticonvulsant and depressant activities of ethanol leaf extract of *Solanum anomalum. Nigerian Journal of Pharmaceutical and Applied Sciences Research*. 2019;8 (1): 73-79.

8. Okokon JE, Udoh AE, Nyong EE, Amazu LU. Analgesic activity ethanol leaf extract of *Solanum anomalum. African Journal of Pharmacology and Therapeutics.* 2020; 9(1):22-26.

9. Udobang J, Okokon JE, Ukpong BD, Akpan SJ. Analysis of ethanol extract of *Solanum anomalum* leaves for antidiarhoeal activity. *Journal of Current Biomedical Research*.2022; 2(2):145-157.

10. Etuk IC,Udobang JA, Ebong NO, Okokon JE. *Solanum anomalum* leaf extract and fractions attenuate oxidative stress and liver injuries in alloxan-induced diabetic rats. *Biology Medicine and Natural Product Chemistry*. 2023; 12(1):33-44.

11. Okokon JE, Whyks DW, Joseph SO. Hepatoprotective activity of ethanol leaf extract of *Solanum anomalum* against doxorubicin-induced liver toxicity. *Future Natural Products*. 2023; 9(2):57 - 62.

12.Etuk IC, Udobang JA, Daniel AO, Ekong O, Okokon JE,Oyepata SJ. Effect of leaf extract and fractions of *Solanum anomalum* on oxidative stress markers, kidney function indices and histology of alloxan-induced diabetic rats. *Journal of Current Biomedical Research.*2023; 3(1): 783-799.

13. Okokon JE, Onunkun JA, Anagboso MO, Udobang JA. Nephroprotective activity of ethanol leaf extract of *Solanum anomalum* against doxorubicin-induced kidney toxicity. *Asian Journal of Natural Products Biochemistry.* 2024; 22(2):59-66.

14. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clinical chemistry 1972; 18: 499-502

15. Tietz N, *Fundamentals of Clinical Chemistry*, Philadelphia, W.B. Saunders, 1976,pp.335-337.

16. Tepongning RN, Mbah JN, Avoulou FL, Jerme MM, Ndanga E-K, Fekam FB. Hydroethanolic extracts of *Erigeron floribundus* and *Azadirachta indica* reduced *Plasmodium berghei* parasitaemia in Balb/c Mice. *Evidence-based Complementary and Alternative Medicine*. 2018: 5156710. doi: 10.1155/2018/5156710.

17. Lawal IO, Grierson DO, Afolayan AI. Phytochemical and Antioxidant investigations of Ma clause a Aniston Hook; A south African Medicinal Plant. *African Journal of Traditional Complimentary Alternative Medicine*, 2015;12(1):28-37.

18.Bashir L, Shittu OK, Busari MB, Sani S, Aisha MI. Safety evaluation of giant African land snails (*Archachatina maginata*) haemolymph on hematological and biochemical parameters of albino rats. Journal of Advanced Medical and Pharmaceutical Science 2015; 3(3), 122-30.

19.Berinyuy EB, Lawal B, Olalekan AA, Olalekan IA, Yusuf AA, Sakpe S, Ossai PC. (2015). Hematological status and organs/body-weight parameters in Wister rats during chronic administration of *Cassia occidentalis*. *Int Blood Res Rev*, 2015; 4(3), 1-7.

20.Shittu OK, Lawal B, Alozieuwa BU, Haruna GM, Abubakar AN, Berinyuy EB. Alteration in biochemical indices following chronic administration of methanolic extract of Nigeria bee propolis in Wistar rats. *Asian Pacific Journal of Tropical Disease*, 2015; 5(8), 654-657.

21.McKnight DC, Mills RG, Bray JJ, Crag PA. Human Physiology, 4th ed. Churchill Livingstone, 1999,pp. 290- 294.

22.Sherwin JE, Sobenes JR. Liver Function. In: Kaplan LA, Pesce AJ, editors. Clinical Chemistry: Theory, Analysis And Correlation. 3rd Ed. Mosby; St. Louis, Mo: 1996; pp. 505–527.

23.Shin MO, Yoon S, Moon JO. The proanthocyanidins inhibit dimethylnitrosamine-induced liver damage in rats. *Arch Pharm Res*. 2010; 33:167-173.

24.Ahmed F, Urooj A. Hepatoprotective effects of *Ficus racemosa* stem bark against carbon tetrachloride-induced hepatic damage in albino rats. Pharmaceutical Biology 2010;48:210-216.

25. Najmi AK, Pillai KK, Pal SN, Akhtar M, Aqil M, Sharma M. Effect of l-ornithine l-aspartate against thioacetamide-induced hepatic damage in rats. Indian Journal of Pharmacology 2010; 42:384-387

26.Yakuba MT, Bilbis LS, Lawal M, Akanji MA. Evaluation of selected parameters of rat liver and kidney function following repeated administration of yohimbine. Biokemistri 2003; 15(2): 50-56.

27. Naganna B. Plasma proteins. In: Tawlar GP, Srivastava LM, Moudgil KD (Eds.).Textbook of Biochemistry and Human Biology. 2nd edn. India: Prentice-Hall of India Private Ltd. 1989. p. 172.

28. Yakubu MT, Akanji MA, Oladiji AT. Aphrodisiac potentials of the aqueous extract of *Fadogia agrestis* (Schweinf. Ex Hiern) stem in male albino rats. *Asian Journal of Andrology*,2005; 7(4), 399-404.

29.Ashafa AO, Yakubu MT, Grierson DS, Afolayan AJ. Effects of aqueous extract from the leaves of *Chrysocoma ciliata* L. on some biochemical parameters of Wistar rats. African Journal of Biotechnology 2009; 8 (8): 1425-1430.

30.Dufour DR, Lott JA, Nolte FS, Gretch DR, Koff RS, Seeff LB. Diagnosis and monitoring of hepatic injury: II. Recommendations for use of laboratory tests in screening, diagnosis and monitoring. Clinical Chemistry. 2000; 46:2050–2068.

31. Rej R. Review: the role of coenzymes in clinical enzymology. Annal of Clinical Laboratory Science 1977; 7: 455–468.

32. Lum G. Low activities of aspartate and alanine aminotransferases. Their significance in alcoholic liver disease. Lab Med. 1995; 26: 273–276.

33.Saori, N., Kimio, S., Makoto, M., Tadashi, H. and Yoshihide, O. (2003). Hepatic Alanine-glyoxylate Aminotransferase Activity and Oxalate Metabolism in Vitamin B6 Deficient Rats. The J Urology. 2003; 169:683-686.

34.Apeland T, Mansoor MA, Pentieva K, McNulty H, Strandjord RE. Fasting and post-methionine loading concentrations of homocysteine, vitamin B2 and vitamin B6 in patients on antiepileptic drugs. Clin Chem. 2003; 49: 1005–1058.

35. Sadiq FU, Abalaka ME, Babayi H. Sub-acute toxicity profile of methanolic leaf extract of *Securidaca longipedunculata* in Rats. *UMYU Journal of Microbiology Research* 2019; 4(1): 53 – 61.

36.Sadiq, F. U., Abalaka, M.E. and Babayi, H. Sub-acute toxicity profile of methanolic leaf extract of *Securidaca longipedunculata* in Rats. *UMYU Journal of Microbiology Research* 2019; 4(1): 53 – 61.

37.Akanji MA, Salau AK, Yakubu MT. Safety evaluation of aqueous extract of *Crateva adansonii* leaves on selected tissues of rats. Fountain Journal of Natural and Applied Sciences, 2013;2(1), 17-28.

38.Das N, Goshwami D, Hasan S, Raihan SZ. Evaluation of acute & subacute toxicity induced by methanol extract of *Terminalia citrine* leaves in Sprague Dawley rats. *Journal of Acute Diseases,* 2015; 4(4), 316-321

39. Mayne PD. The kidneys and renal calculi. In: Clinical chemistry in diagnosis and treatment. 6th ed. London:Edward Arnold Publications, 1994;pp.2-24.

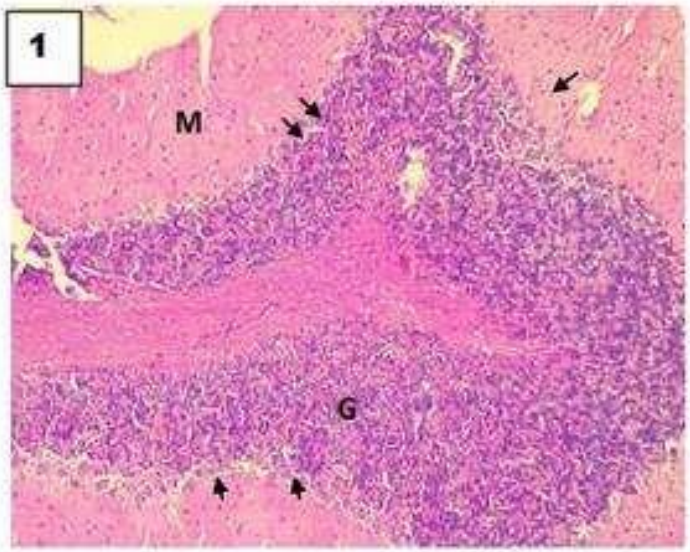
40. Flaoyen A, Hove K, Wilkins AL. Tolerance to nephrotoxic component of *Narthecium ossifragum* in sheep: The effects of repeated oral doses of plant extracts. Veterinary Research Communication. 2001; 25: 127-136.

41. Yakubu MT, Akanji MA, Oladiji AT. Alterations in serum lipid profile of male rats by oral administration of aqueous extract of *Fadogia argrestis* stem. Research Journal of Medicinal Plant. 2008; 2: 66-73.

42.Abolaji AO, Adebayo AH, Odesanmi OS. Effect of ethanolic extract of *Parinari polyandra* (Rosaceae) on serum lipid profile and some electrolytes in pregnant rabbits. Research Journal of Medicinal Plants 2007; 1: 121- 127.

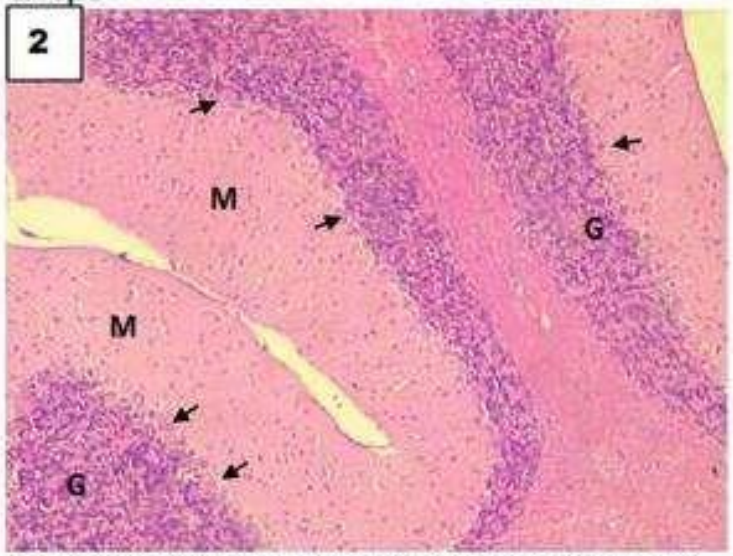
43. Panagiotakos B, Pitsavos C, Skoumas J, Chrysohoou C, Toutouza M, Stefanadis CI, Toutouzas PK. Importance of LDL/HDL ratio as a predicator for coronary heart disease events in patients with heterozygous familial hypercholesterolemia: A 15 year follow-up (1987-2002). Current Medical Research Opinion 2003;19: 89-94.

Group 1



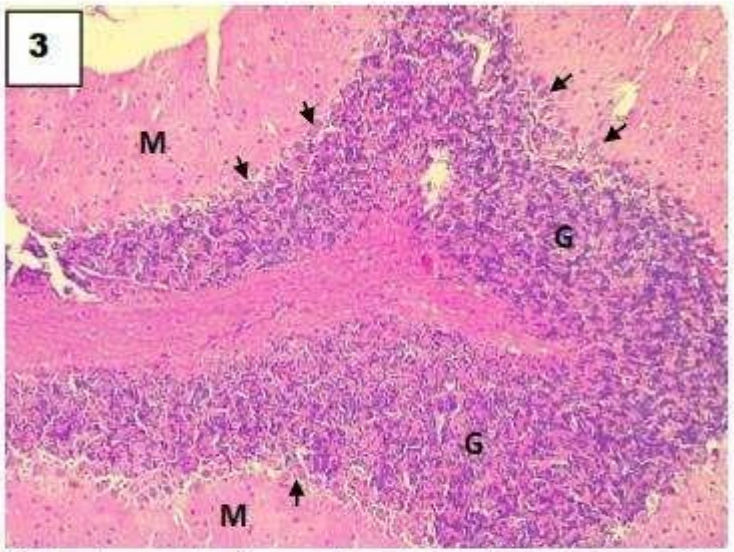
Photomicrograph of normal cerebella histology showed the densely granular cell layer (**G**), and molecular layer (**M**) consisting of stellate and basket cells. The granular cell layer border was lined by round to ovoid purkinje cells (arrows). No lesion seen. Haematoxylin and Eosin Stain, X100 magnification

Group 2



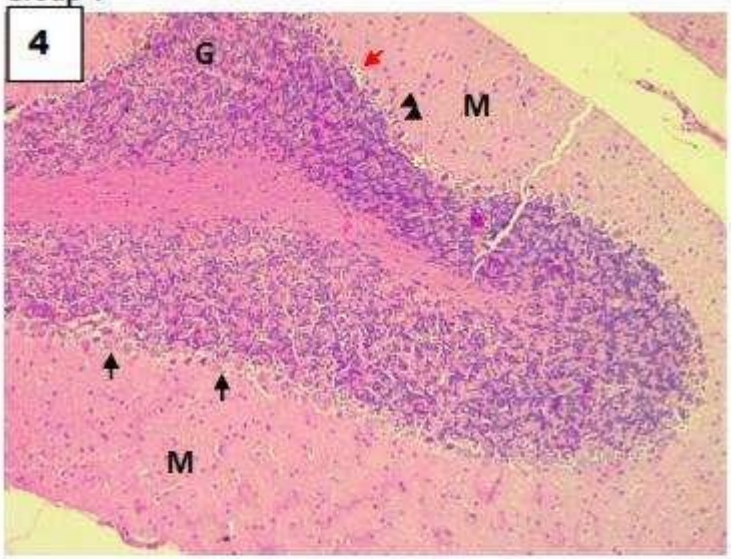
Photomicrograph of normal cerebella histology showed the densely granular cell layer (**G**), and molecular layer (**M**) consisting of stellate and basket cells. The granular cell layer border was lined by normal round to ovoid purkinje cells (arrows). No lesion seen. Haematoxylin and Eosin Stain, X100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

Group 3



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.

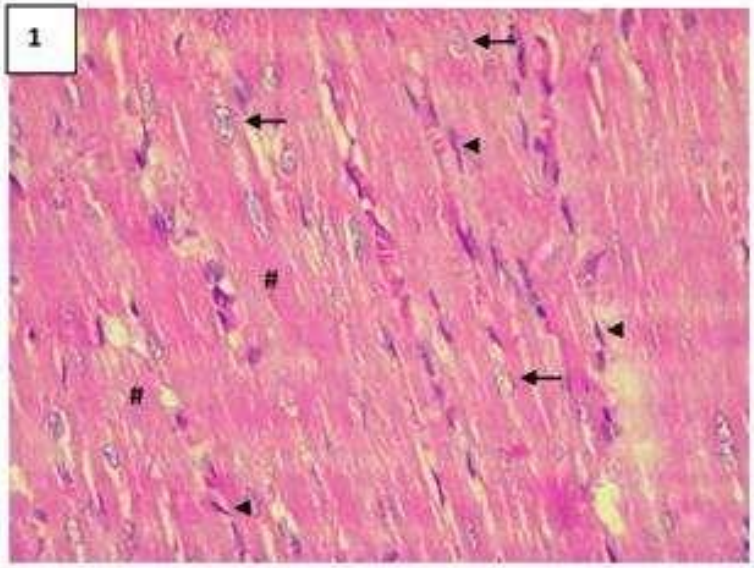
Group 4



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

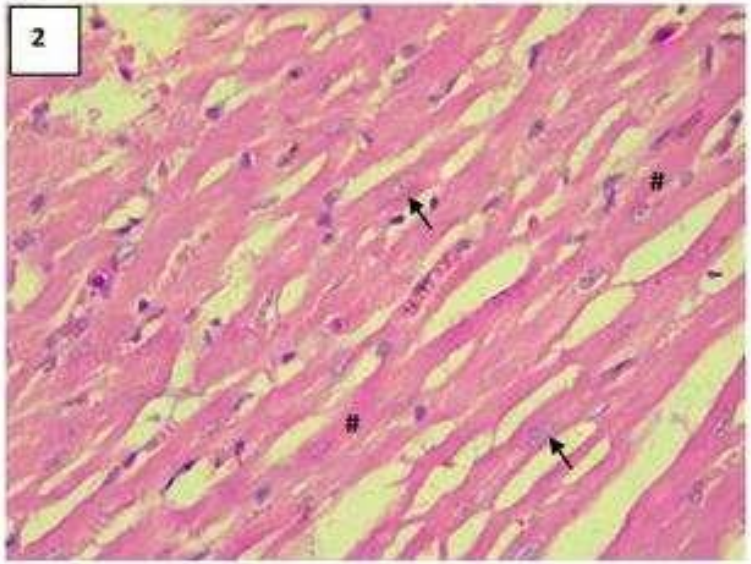
Figures 3: Histological sections of cerebella of rats treated with Normal saline 10 mL/kg(1), SA leaf extract 70 mg/kg bw (2), SA leaf extract 140 mg/kg bw (3), SA leaf extract 210 mg/kg bw(4) at Magnification (x100), stained with H&E Method.

Group 1



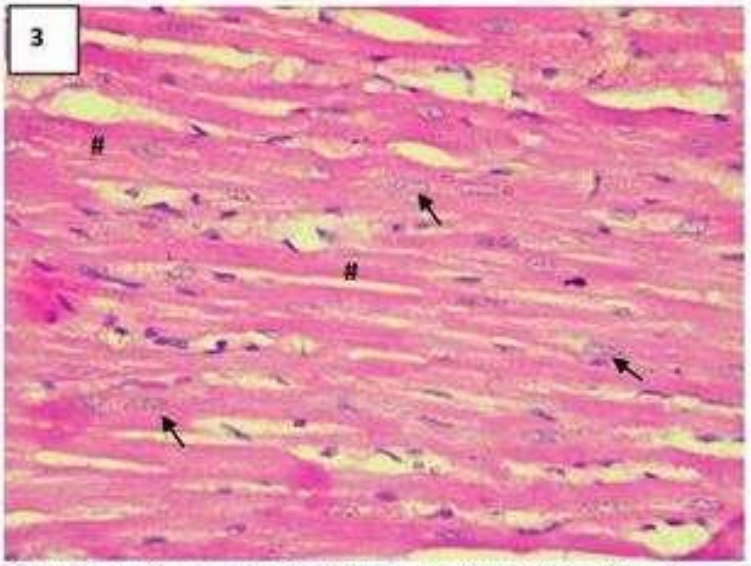
Normal heart histology architecture showing cardiac muscles evidence with spindle cardiac muscle cell nuclei (black arrows) and muscle fibre (#). No pathological changes seen

Group 2



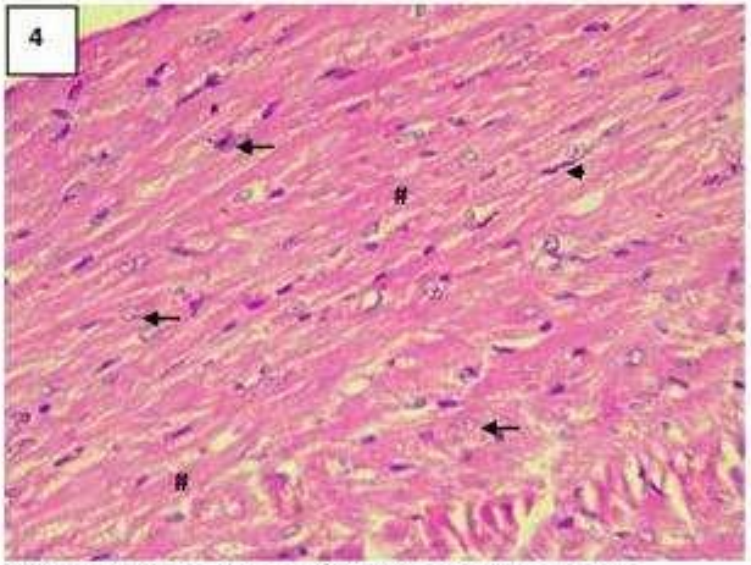
Normal heart histology architecture showing cardiac muscles evidence with spindle cardiac muscle cell nuclei (black arrows) and muscle fibre (#). No pathological changes seen

Group 3



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

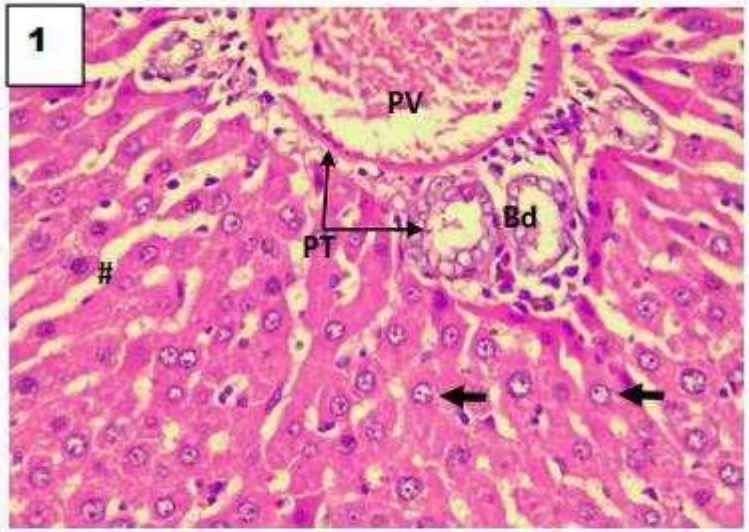
Group 4



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

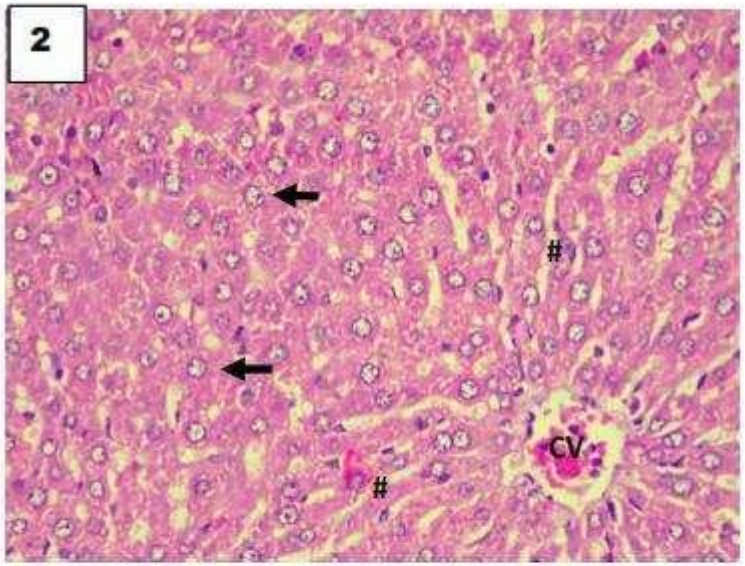
Figures 4: Histological sections of hearts of rats treated with Normal saline 10 mL/kg(1), SA leaf extract 70 mg/kg bw (2),SA leaf extract 140 mg/kg bw (3), SA leaf extract 210 mg/kg bw(4) at Magnification (x400), stained with H&E Method.

Group 1



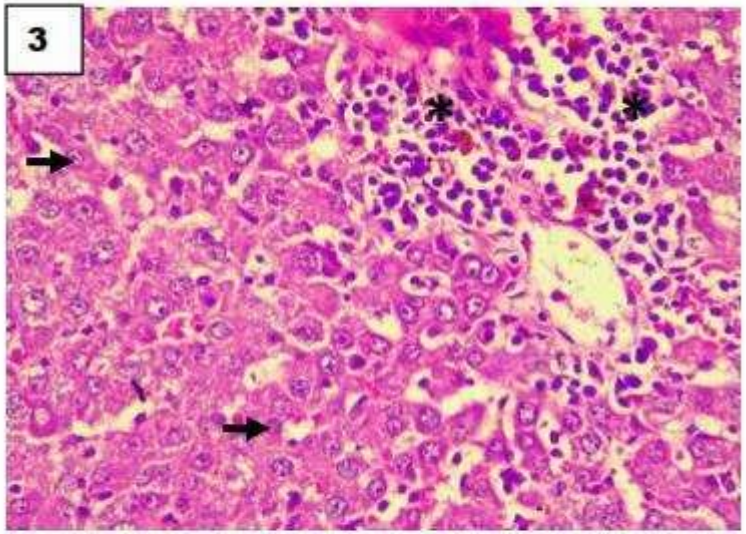
Liver section showing array of hepatocytes (thick black arrows) and some showed portal triad (PT) consisting of portal vein (PV)and bile duct (Bd), and the average size sinusoid (#). No pathological lesion seen.Haematoxylin and Eosin (H&E) stain. x400 magnification

Group 2



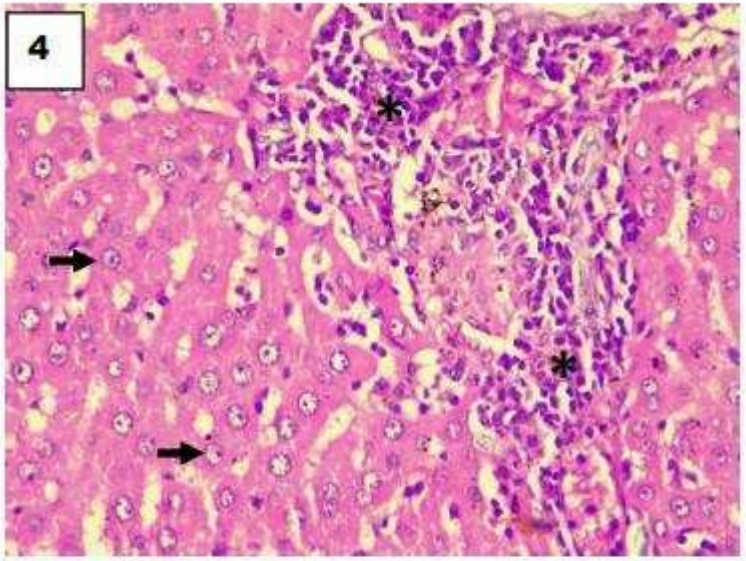
Section showed hepatocyte (Thick black arrows) with vacuolated nuclei, congested central vein (**CV**) and average sized sinusoidal spaces (#).No pathological lesion seen. H&E stain. x400 magnification

Group 3



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

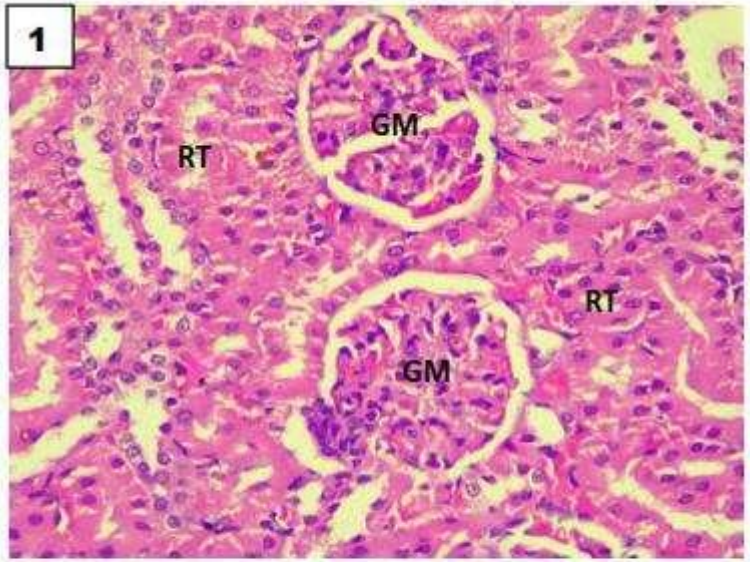
Group 4



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

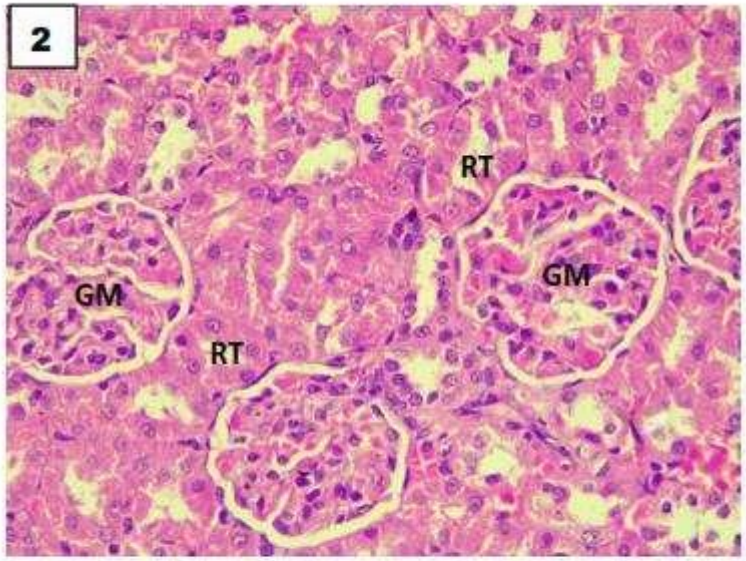
Figures 5: Histological sections of livers of rats treated with Normal saline 10 mL/kg(1),SA leaf extract 70 mg/kg bw (2), SA leaf extract 140 mg/kg bw (3),SA leaf extract 210 mg/kg bw(4) at Magnification (x400), stained with H&E Method.

Group 1



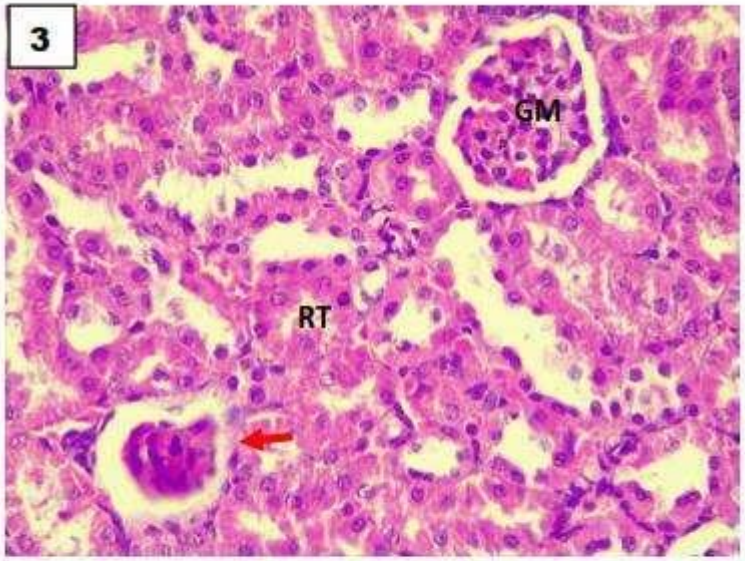
Kidney showed normal renal tissue histology architecture evidence with normal glomeruli (**GM**) and renal tubule (**RT**) . No pathological changes seen. Haematoxylin and Eosin (H&E) stain. X400 magnification

Group 2



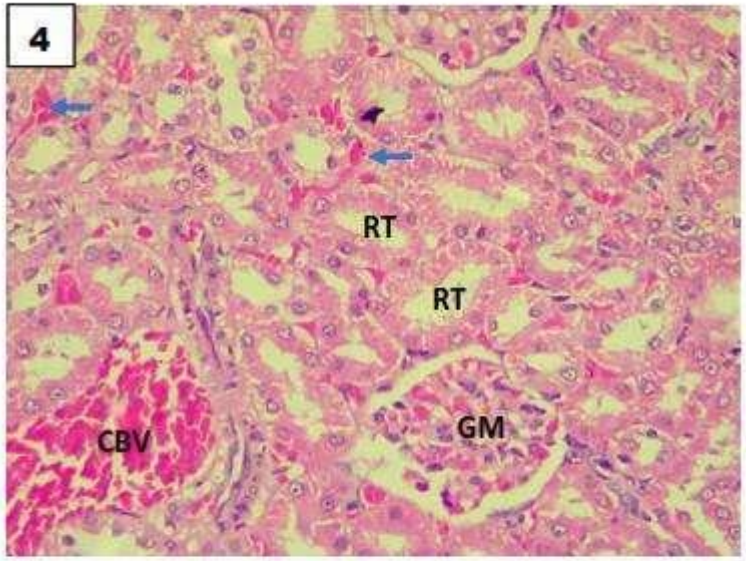
Kidney showed normal renal tissue histology architecture evidence with normal glomeruli (GM) and renal tubule (RT) . No pathological changes seen. Haematoxylin and Eosin (H&E) stain. x400 magnification

Group 3



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

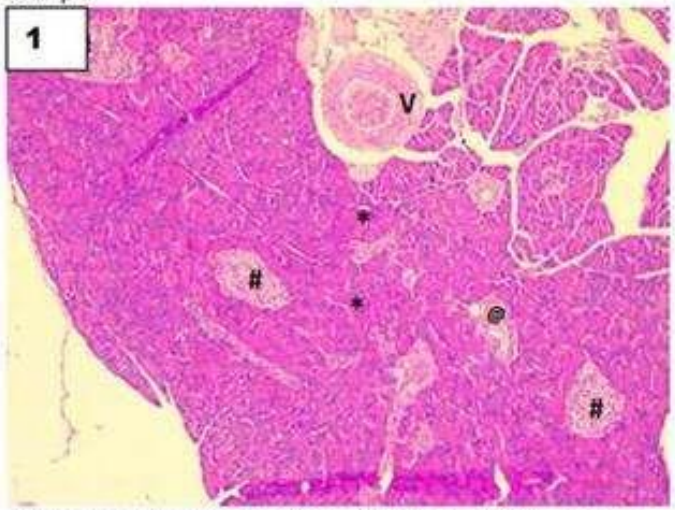
Group 4



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

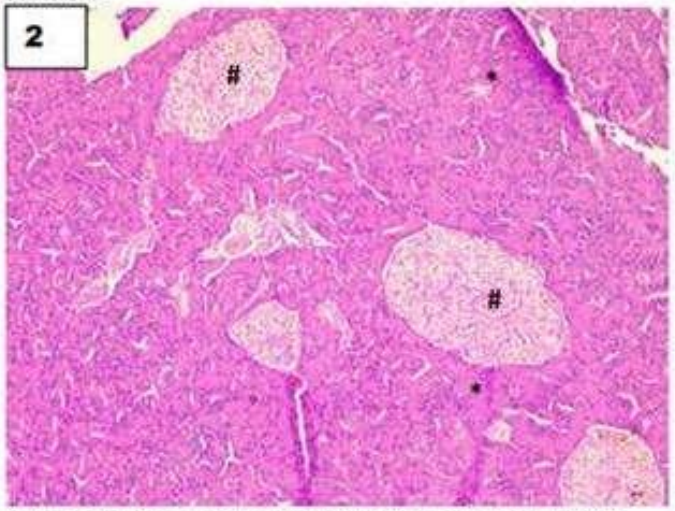
Figures 6: Histological sections of kidneys of rats treated with Normal saline 10 mL/kg(1), SA leaf extract 70 mg/kg bw (2),SA leaf extract 140 mg/kg bw (3), SA leaf extract 210 mg/kg bw(4) at Magnification (x400), stained with H&E Method.

Group 1



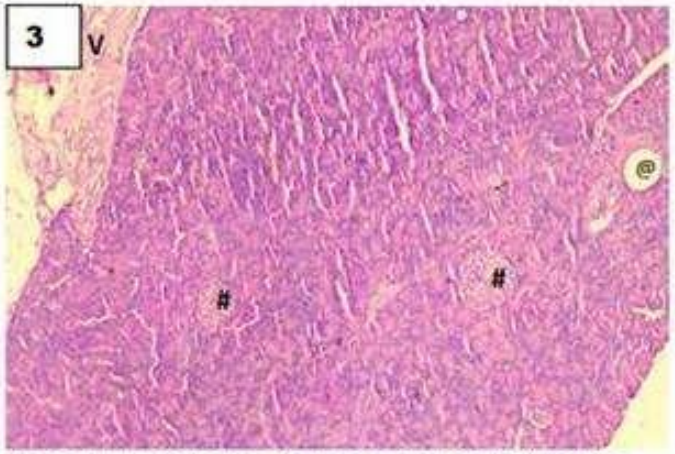
Pancreatic tissue section showed normal acini (*) showing acinar cells with basophilic cytoplasm. Several Langerhans islet (#) seen; the islet shows loosely packed pale-staining cells, also seen are interlobular duct (@) some with secretions. No lesion seen on the pancreas. H&E stain x100 magnification

GROUP 2



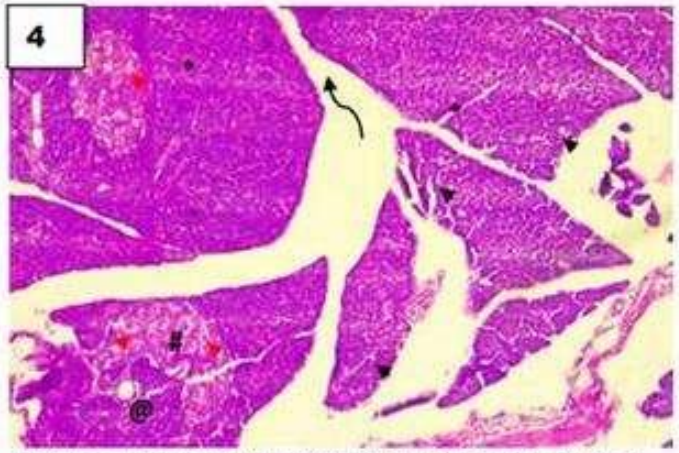
Pancreatic tissue section showed normal acini (*) showing acinar cells with basophilic cytoplasm. Several Langerhans islet (#) seen; the islet shows loosely packed pale-staining cells, also seen are interlobular duct (@) some with secretions. No lesion seen on the pancreas. H&E stain x100 magnification

Group 3



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

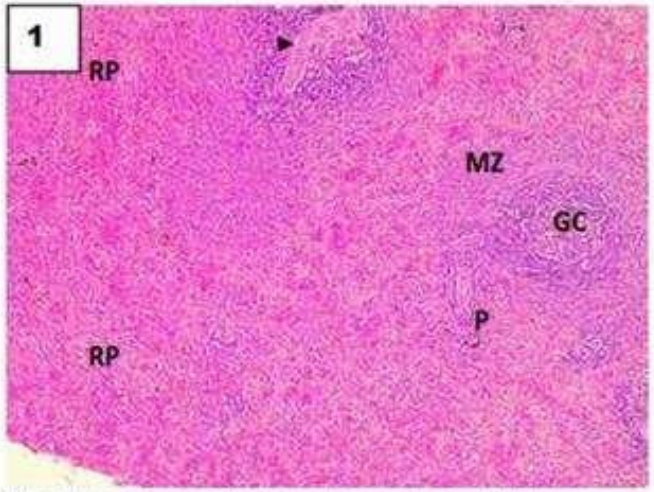
Group 4



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

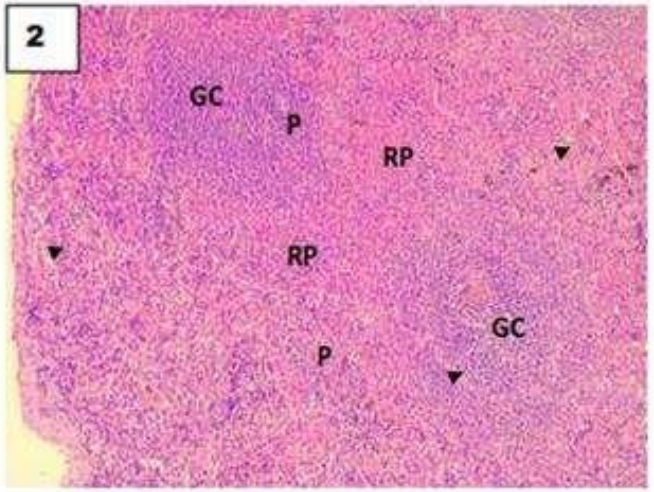
Figures 7: Histological sections of pancreas of rats treated with Normal saline 10 mL/kg(1), SA leaf extract 70 mg/kg bw (2), SA leaf extract 140 mg/kg bw (3), SA leaf extract 210 mg/kg bw(4) at Magnification (x400), stained with H&E Method.

Group 1



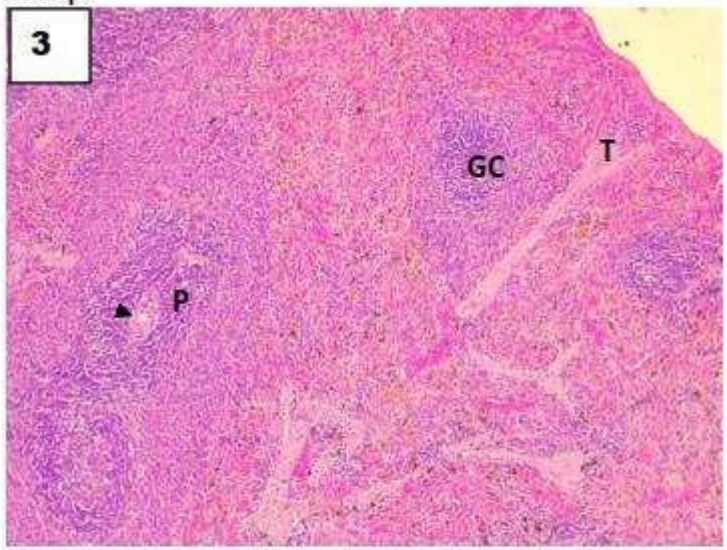
The photomicrograph showed normal splenic histology showing delineated white-pulp (WP), Central artery (arrowhead), and red –pulp (RP). The white pulp showed well organized marginal zone (MZ) and peri-arteriolar lymphocyte shealth (P) and geminal center (GC).

Group 2



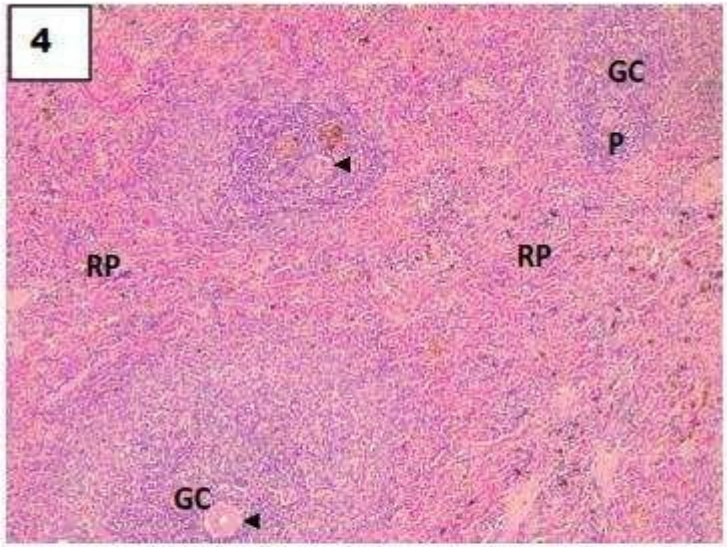
The photomicrograph showed normal splenic histology showing delineated white-pulp (WP) and red –pulp (RP). The white pulp showed well organized marginal zone (MZ) and peri-arteriolar lymphocyte shealth (P).

Group3



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

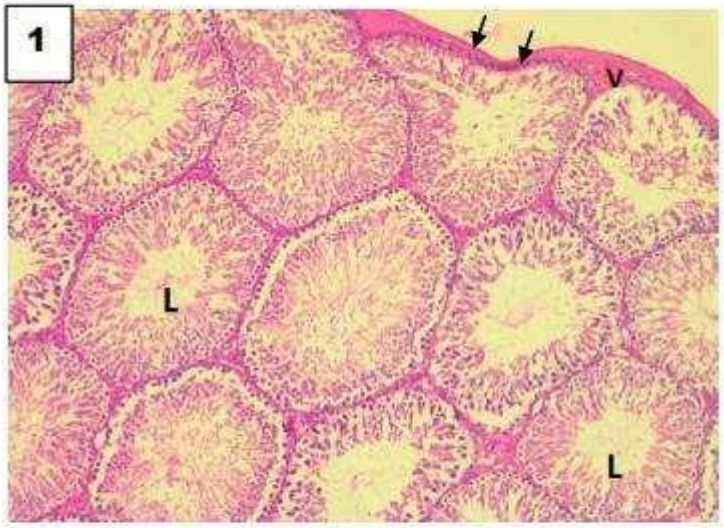
Group 4



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)

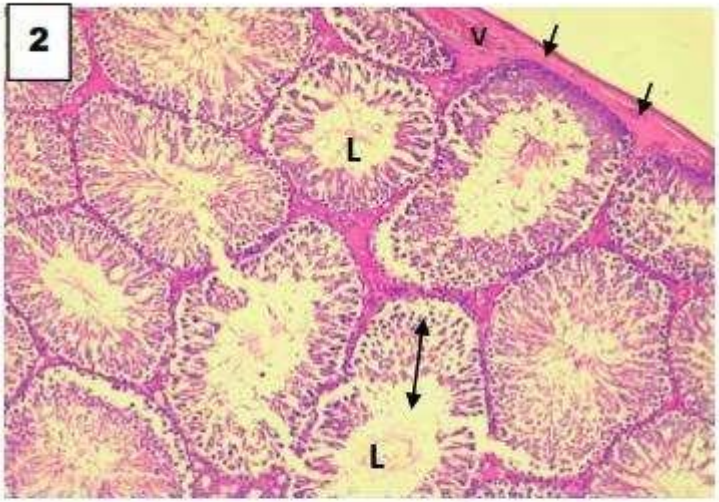
Figures 8: Histological sections of spleen of rats treated with Normal saline 10 mL/kg(1), SA leaf extract 70 mg/kg bw (2), SA leaf extract 140 mg/kg bw (3), SA leaf extract 210 mg/kg bw(4) at Magnification (x400), stained with H&E Method.

Group 1



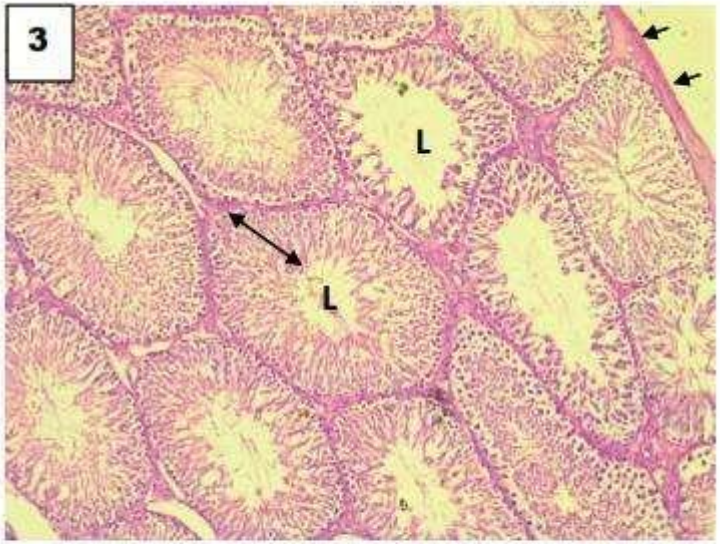
Testucular tissue showing normal seminiferous tubules, the sections showed different tubular stages and series of spermatogenic cells (double head arrow). The section showed normal tunical albuginea (short arrows). No pathologic changes seen on the cellular organization or the connective tissues. H&E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

Group 2



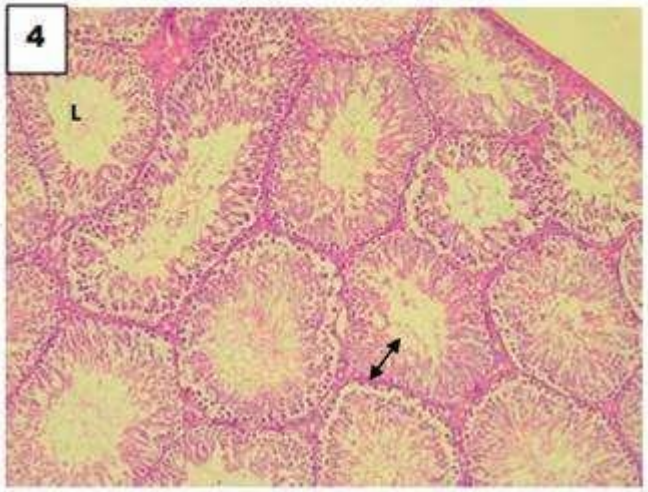
Testucular tissue showing normal seminiferous tubules, the sections showed different tubular stages and series of spermatogenic cells (double head arrow). The section showed normal tunical albuginea (short arrows). No pathologic changes seen on the cellular organization or the connective tissues. H&E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

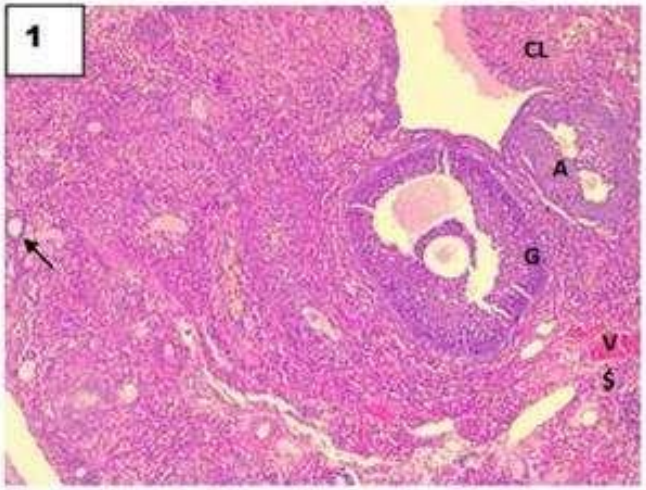
Group 4



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

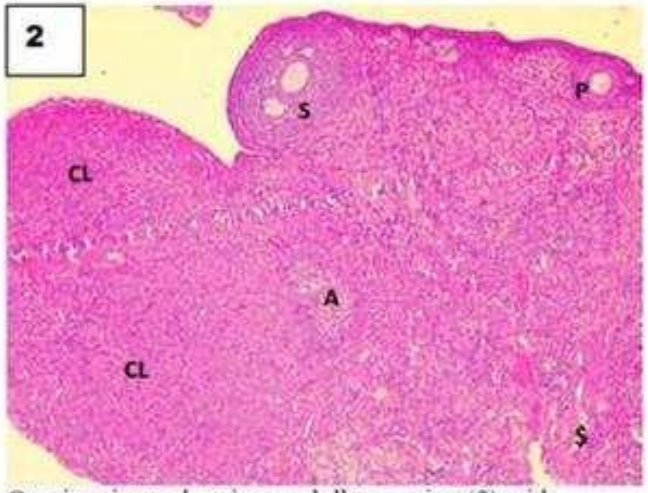
Figures 9: Histological sections of testes of rats treated with Normal saline 10 mL/kg(1), SA leaf extract 70 mg/kg bw (2), SA leaf extract 140 mg/kg bw (3), SA leaf extract 210 mg/kg bw(4) at Magnification (x400), stained with H&E Method.

Group 1

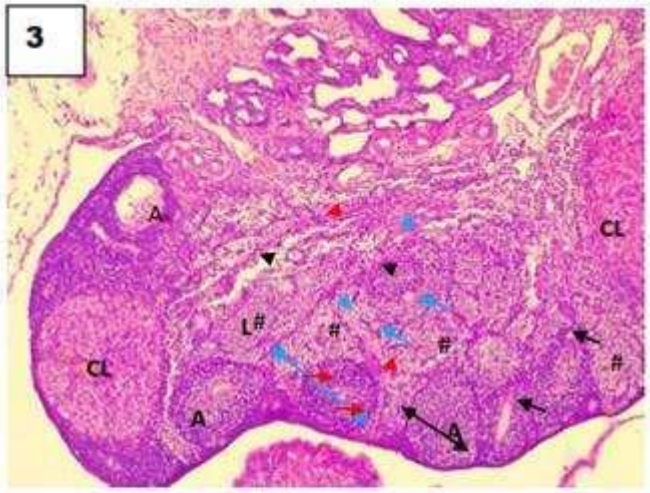


Ovarian tissue showing medullary region (M) with numerous blood vessel and the cortex with few developing follicles and a focal mature (grafian follicle) follicles and several corpus luteum (asterisks).

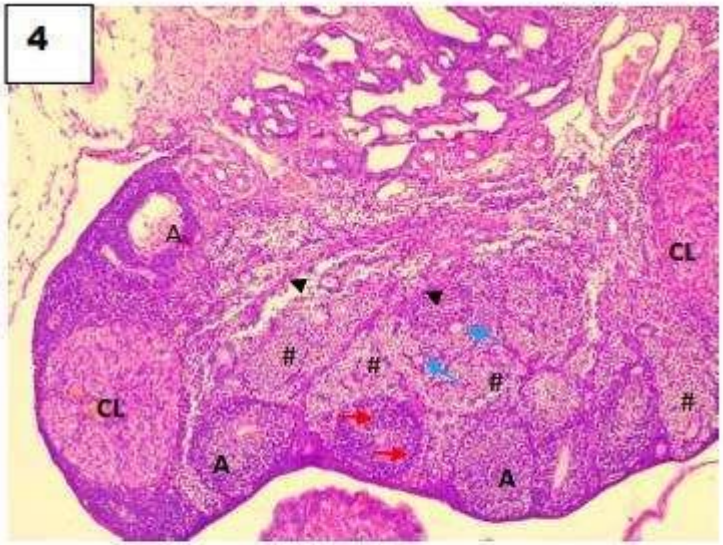
Group 2



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

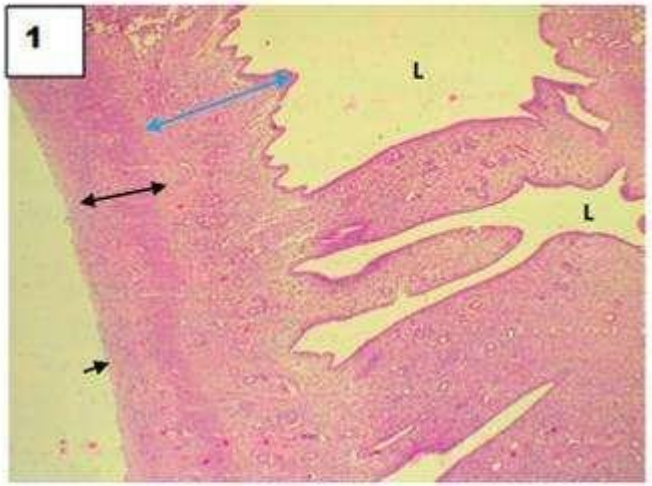


Section of ovarian tissue showed hyperplasia of stromal interstitial cells (#) associated with ovarian atrophy. It showed several atretic follicle (A) with a focal apoptotic cell (red arrow). Few developing follicle seen (blue arrow) and demarcating connective tissue septa (arrowhead)

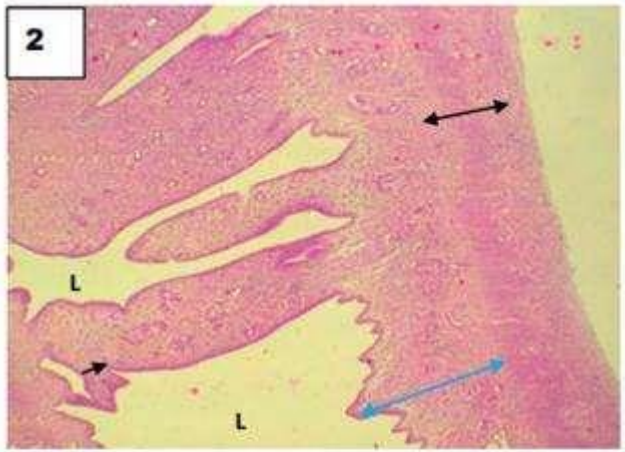


Section of ovarian tissue showed hyperplasia of stromal interstitial cells (#) associated with ovarian atrophy. It showed several atretic follicle (A) with a focal apoptotic cell (red arrow). Few developing follicle seen (blue arrow) and demarcating connective tissue septa (arrowhead)

Figures 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.

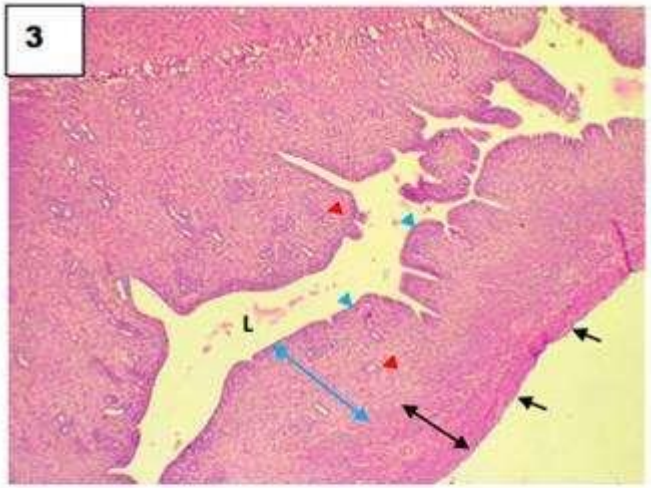


Section of uterine tissue showing normal architecture of Lumen (L), endometrium myometrium (blue two end arrow), myometrium (black two end arrow) and perimetrium (black arrowhead). Also seen average number of uterine glands in the endometrium. No lesion seen.

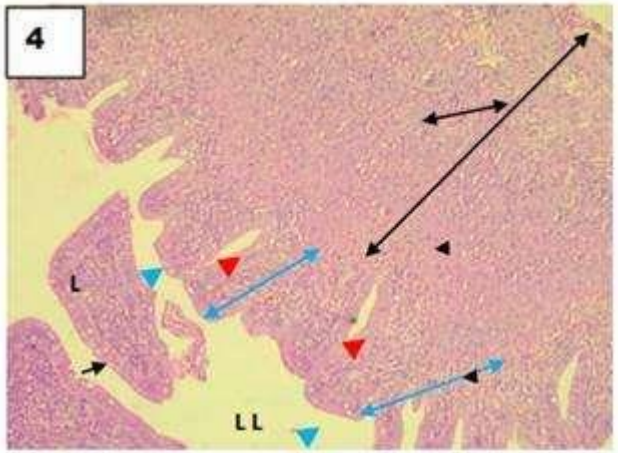


Section of uterine tissue showing normal architecture of Lumen (L), endometrium myometrium (blue two end arrow), myometrium (black two end arrow) and perimetrium (black arrowhead). Also seen average number of uterine glands in the endometrium. No lesion seen.

3



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 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.

Figures 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.