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Subacute Toxicity Study of Leaf Extract of *Saccharum officinarum*

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Abstract

Saccharum officinarum (Family-Poaceae) is used traditionally to treat malaria and fever, among others. Evaluation of subacute administration ethanol leaf extract of *Saccharum officinarum* for possible effect on hematological indices, liver and kidney functions, and lipid profile of rats was carried out. The leaf extract (170, 340, 510 mg/kg body weight) was orally administered to male Wistar rats daily for 30 days, and the rats were sacrificed under light diethyl ether anesthesia after the administration. Subacute administration of *S. officinarum* leaf extract resulted in an insignificant increase in the body weights of rats without any significant ($p>0.05$) effect on the weights of liver and kidney when compared to control. The leaf extract treatment did not affect WBC, lymphocytes, monocytes, eosinophil, and basophil percentages. However, it caused significant ($p<0.05$) decreases in RBC and platelet counts, hemoglobin concentration, and PCV percentage, especially at the middle dose (374 mg/kg), and also prolonged bleeding and clotting time significantly ($p<0.05$) when compared to control. The leaf extracts non-dose-dependently caused insignificant ($p>0.05$) decreases in total protein, albumin, and ALT levels. ALP was significantly ($p<0.05$) decreased at the highest dose (510 mg/kg). However, AST, total and conjugated bilirubin levels were significantly ($p<0.01$-0.001) decreased only at higher doses (340 and 510 mg/kg) of the extract. The leaf extract did not cause any significant ($p>0.05$) effect on urea, creatinine, potassium, and sodium as well as total cholesterol, triglyceride, VLDL, and LDL levels of rats, but the highest dose (510 mg/kg) significantly ($p<0.05$) increased Cl level and reduced HDL level of rats when compared to control. The leaf extract exerts mild to moderate effects on the histology of the livers and kidneys of rats. Chronic study is advocated to investigate the effect of prolonged administration of rats' extract organs and systems.

Keywords: *Saccharum officinarum*; haematological parameters; kidney function; liver function.

Abbreviations: ALT: Alanine aminotransferase, ALP: Alkaline phosphatase, AST: Aspartate aminotransferase, RBC: Red blood cell, WBC: White blood cell, PCV: Packed cell volume, Hb: Hemoglobin concentration, LDL: Low density lipoprotein, VLDL: Very Low Density Lipoprotein, HDL: High density lipoprotein, WHO: World Health Organisation, H & E: Heamatoxylin and eosin.

INTRODUCTION

Saccharum officinarum (Family-Poaceae), also called sugarcane, thrives throughout tropical and subtropical regions. In traditional medicine, it is used in the treatment of diarrhea, dysentery, eyes, fever, arthritis, bedsores, boils, cancer, colds, cough, opacity, skin sores, sore throat, hiccups, inflammation, laryngitis, spleen, tumors, and wounds (Hartwell, 1967-1971). the leaf extract possesses some biological activities such as antibacterial and anthelmintic (Palaksha *et al.*, 2013), anti-hyperglycaemic, anti-hyperlipidaemic (Ojewunmi *et al.*, 2013), antioxidant (Ojewunmi *et al.*, 2013; Sun *et al.*, 2014), diuretic and antiurolithiatic (Palaksha *et al.*, 2015), antidepressant and anticonvulsant (Okokon *et al.*, 2019), analgesic (Okokon *et al.*, 2021) and antimalarial (Okokon *et al.*, 2022), antioxidative stress and hepatoprotective (Edem *et al.*, 2022), anti-inflammatory and antipyretic (Edem *et al.*, 2023a), antiulcer (Edem *et al.*, 2023b) activities. SAABMAL®: Nigeria's polyherbal preparation containing *S. officinarum* is utilized as a malarial remedy (Obidike *et al.*, 2015). The leaves are employed in Ghana to treat malaria locally (Akwetey & Achel, 2010). Phytochemical screening of the leaf extract of *Saccharum officinarum* revealed the presence of glycosides, phytosterols, saponins, tannins, and flavonoids (Palaksha *et al.*, 2013; Singh *et al*., 2015). Some flavones and phenolics, as well as their derivatives from the leaves of *S. officinarum*, have been identified (Coutinho *et al*., 2016; Okokon *et al.*, 2022). The medicinal potentials of the plant have been widely reported, but there is a paucity of information on its toxicological potentials. This study reported subacute toxicity potential of the leaf extract of *S. officinarum* on haematological parameters, liver and kidney functions and lipid profile.

MATERIALS AND METHODS

Plant materials

Fresh leaves of *Saccharum officinarum* were collected in June 2020 from residential quarters in Uyo village in Uyo LGA, Akwa Ibom State, Nigeria. The leaves were identified and authenticated as *Saccharum officinarum* by a taxonomist in the Department of Botany and Ecological Studies, University of Uyo, Uyo, Nigeria, and a voucher specimen (UUPH 215b) was prepared and deposited at the herbarium of the Department of Pharmacognosy and Natural Medicine, Faculty of Pharmacy, University of Uyo.

Extraction

Fresh leaves of *S. officinarum* were washed, cut into smaller pieces and dried under shade for two weeks. The leaves were further pulverized to powder using an electric grinder. The powdered leaf material (2 kg) was soaked in 50% ethanol (7.5 L) at room temperature (28 ± 2 ˚C) for 72 hours. After that the liquid filtrate was filtered, concentrated, and evaporated to dryness in *vacuo* 40 ˚C using a rotary evaporator (BuchiLab Switzerland). The dry extract was stored in a refrigerator at -4 ˚C until it was used for the proposed experiments.

Animals

In this study, male albino Wistar rats were used. The animals were sourced from the University of Uyo Animal House and sheltered in plastic cages. They were fed with pelleted standard Feed (Guinea feed) and given unlimited access to water. The Faculty of Pharmacy Animal Ethics Committee, University of Uyo, approved the study.

Sub-acute 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 170, 340, and 510 mg/kg of the leaf extract, respectively, daily for 30 days. Group IV was administered with distilled water (10 mL/kg) for the same period. At the end of the treatment period, the animals were weighed again and sacrificed under light ethyl ether vapor. Blood samples were collected by cardiac puncture and used immediately for hematological testing, such as bleeding time, clotting time, complete 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, and lipid profile

The effect of the extract on some organs such as the liver and kidney, was studied. The organs, livers, and kidneys of rats were surgically removed, weighed, 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, and Full Blood Count. These parameters were determined at Haematology Department of the University of Uyo Teaching Hospital using an automated Haematology analyser.

Biochemical analysis

Liver function test

The following parameters were determined: Aspartate transaminase (AST), Alanine aminotransferase (ALT), Total Cholesterol, and 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 (Tietz, 1976) at the Chemical Pathology Department of the 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 and kidneys of rats used in the study were surgically harvested and fixed in buffered formalin. They were then processed and stained with hematotoxylin and eosin (H&E) according to standard procedures at the Department of Chemical Pathology, University of Uyo Teaching Hospital. Morphological changes were observed and recorded in the excised organs of the sacrificed animals. Histologic pictures were taken as micrographs.

Statistical analysis

The Data collected were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison post-test (Graph pad prism software Inc. La Jolla, CA, USA). Values were expressed as mean ± SEM, and significance relative to the control was considered at $p<0.001$ and $p<0.05$.

RESULTS

Effect of subacute administration of leaf extract on body weight of rats

The effect of leaf extract on body weight of rats treated with leaf extract of *S. officinarum* for 30 days is shown in Table 1. There was considerable increase in the body weights of rats treated the extract in all groups, similar to that of the control group, though non-dose-dependently.

The increase in body weight in the low dose (170 mg/kg) treatment group was significantly ($p<0.001$) lower than that of the control. In contrast, the weight increases of rats treated with higher doses (340 and 510 kg/kg) of the extract were higher than that of the control but not statistically significant ($p>0.05$) (Table 1).

Effect of subacute administration of leaf extract on organ weights of rats

Treatment of rats with the leaf extract (170-510 mg/kg) for 30 days did not cause any significant effect ($p>0.05$) on the weights of livers and kidneys of rats when compared to the control (Table 2).

Table 1. Effect of subacute administration of *S. officinarum* 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	153.3 ± 9.40	207.6 ± 5.69	54.3 ± 2.81
S. officinarum	170	170.3 ± 6.88	201.0± 17.00	30.7 ± 3.86[a]
	340	171.6 ± 1.66	232.0 ± 8.50	60.4 ± 2.74
	510	164.0 ± 9.53	209.6 ± 2.40	45.6 ± 3.41

Data are expressed as mean ± SEM. Significant at [a]$p>0.001$ when compared to control. n = 6

Table 2. Effect of subacute administration of *Sacharum officinarum* leaf extract on organ weights of rats.

Treatment	DOSE (mg/ kg)	Liver (mg)	Kidney(mg)
Control	10 mg/mL	6.00± 0.35	1.19±0.03
Crude extract	170	6.11±0.45	1.31±0.12
	340	6.29±0.25	1.14±0.03
	510	6.10±0.38	1.21±0.06

Data is expressed as MEAN ± SEM, Significant at [b]$p< 0.01$, compared to control. (n=6).

Effect of subacute administration of leaf extract on hematological parameters of rats

The effect of subacute administration of ethanol leaf extract of *S. officinarum* (170-510 mg/kg) on the hematological parameters of rats is shown in Table 3. Subacute administration of leaf extract of *S. officinarum* to rats for 30 days did not significantly affect the WBC counts, lymphocytes, monocytes, eosinophil, and basophil percentages ($p>0.05$) compared to control. However, the neutrophils percentage of the group treated with 170 mg/kg of the leaf extract was significantly ($p<0.05$) increased and significant ($p<0.05$-0.01) decreases in RBC and platelets counts, hemoglobin concentration and PCV percentage were observed in the group treated with the middle dose (340 mg/kg) of the extract when compared to control (Table 3). Moreover, treatment of rats with leaf extract of *S. officinarum* (170 - 520 mg/kg) for 30 days caused a significant ($p>0.05$-0.001) increase in the bleeding time of rats in all doses used when compared to control though non-dose-dependently, while significant ($p<0.001$) decreases in the clotting time of rats treated with doses of the extract (170 and 340 mg/kg) was observed when compared to the control (Figures 1 and 2).

Effect of subacute administration of leaf extract on liver function indices of rats

Administration of leaf extract of *S. officinarum* (170-510 mg/kg) to rats for 30 days caused a non-dose-dependent and insignificant ($p>0.05$) decrease in total protein, albumin, and ALT levels of rats when compared to control (Table 4). The ALP levels were similarly reduced, but this was only significant ($p<0.01$) in the group treated with the highest dose (510 mg/kg) dose of the extract. Similarly, AST, total and conjugated bilirubin levels of treated rats were decreased, but the reductions were significant ($p<0.01$-0.001) only at the higher doses (340 and 540 mg/kg) of the extract when compared to control (Table 4).

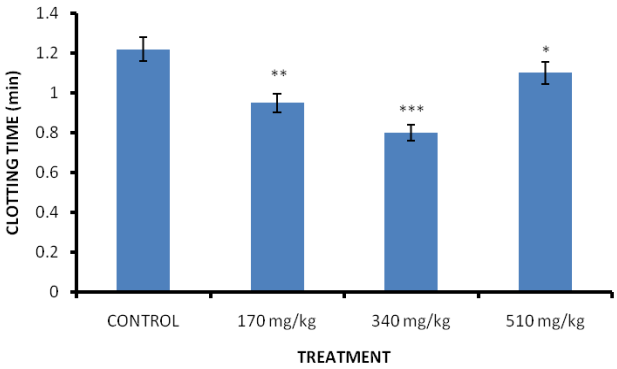

Figure 1. Effect of subacute administration of *Saccharum officinarum* leaf extract on clotting time of rats.

Data is expressed as MEAN ± SEM, Significant at *$p<0.05$, **$p<0.01$, ***$p<0.001$, when compared to control. (n=6).

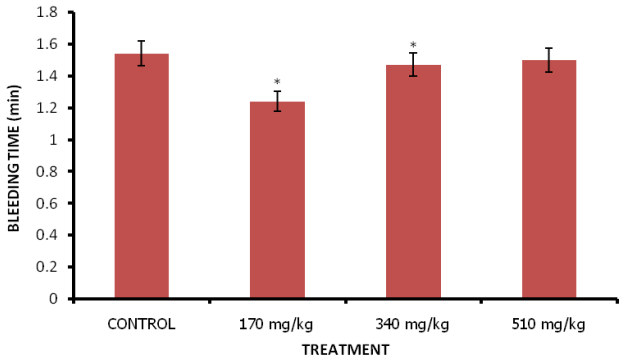

Figure 2. Effect of subacute administration of *Saccharum officinarum* leaf extract on bleeding time of rats.

Data is expressed as MEAN ± SEM, Significant at *p<0.01 when compared to control. (n=6).

Effect of subacute administration of leaf extract on kidney function parameters of rats

Treatment of rats for 30 days with leaf extract of *S. officinarum* (170-510 mg/kg) did not cause any significant effect (p>0.05) on the levels of urea, creatinine, potassium, and sodium of the treated rats when compared to control. However, the level of chloride was significantly (p<0.05) increased at the higher dose of the extract (510 mg/kg) compared to control (Table 5).

Effect of subacute administration of leaf extract on lipid profile indices of rats

Treatment of rats with leaf extract of *S. officinarum* (170-510 mg/kg) caused non-dose-dependent but insignificant (p>0.05) decreases in the levels of total cholesterol, triglyceride, LDL, and VLDL of rats treated for 30 days when compared to control. However, the HDL level was significantly (p<0.05) reduced at the highest dose (510 mg/kg) of the extract when compared to control (Table 6).

Effect of subacute administration of leaf extract on histology of organs

Figures 3 and 4 show the effects of subacute administration of ethanol leaf extract of *S. officinarum* to rats for 30 days on histology of some organs. The leaf extract (170-510 mg/kg) caused varying defects in the histology of the organs. Mild effects were observed on the hepato-architecture with micro and macro-vesicular steatosis areas, vacuolated hepatocytes, hemorrhagic blood deposits (H), and arrays of sinusoidal spaces within the hepatic lobule in all treatment groups. The history-architechture of renal tissues of treated rats was moderately affected with glomeruli (G) having widened bowman's space (Wb), vacuolated ductal cells, and hemorrhagic blood vessels (H) within the cortical matrix.

Table 3. Effect of subacute administration of *Saccharum officinarum* leaf extract on hematological parameters of rats.

Treatment	Dose	WBC (L)	NEUT. (%)	LYM (%)	MONO (%)	ESINO (%)	BASO (%)	RBC (L)	HGB (g/dL)	PCV (%)	PLATELETS. (L)
Control	10 mg/ml	6.27±0.05	17.26±1.46	77.96±2.43	2.93±0.52	0.33± 0.03	0.50± 0.05	6.12± 0.46	13..53±0.14	43.76±2.27	754.0± 35.90
Crude extract	170	7.81±0.98	23.63±1.46	73.73±1.93	1.43±0.68	0.66±0.17	0.66± 0.17	8.40 ± 0.15	14.63± 0.36	46.96±1.17	775.0± 82.21
	340	4.48±0.85	13.65±1.72	82.26±1.74	2.60±1.42	0.46± 0.32	0.46± 0.03	4.85± 1.63	9.13± 2.73	28.23±1.34	542.3± 30.52
	510	6.99±0.99	21.33±1.74	75.56±3.01	3.68±0.69	0.30± 0.17	0.36± 0.23	7.77± 0.27	12.44± 2.06	34.93±1.96	769.0± 95.04

Data is expressed as MEAN ± SEM, Significant at $^{a}p<0.05$, $^{b}p<0.01$, when compared to control. (n=6).

Table 4. Effect of subacute administration of *Sacharum officinarum* leaf extract on liver function parameters of rats.

TREATMENT	DOSE (mg/ kg)	Total Protein (mg/dL)	Albumin (mg/dL)	ALT (IU/L)	ALP (IU/L)	AST (IU/L)	Total Bilirubin (µmol/l)	Combined Bilirubin (µmol/l)
Control	10 mg/ml	67.33± 4.70	41.66±3.52	6.20± 3.31	52.38±3.28	21.0± 2.51	4.36±0.43	2.50±0.26
Crude extract	170	67.0±4.35	42.33±2.60	7.00±2.19	46.66±2.02	22.0± 2.05	4.43± 0.48	2.63±0.29
	340	61.0±4.04	36.33±2.40	5.83±1.20	50.33±5.60	14.0 ± 3.51^{c}	3.33 ± 0.58^{b}	1.73 ± 0.14^{a}
	510	65.66±2.72	42.33±1.45	5.46±0.08	39.0 ± 1.15^{b}	17.0 ± 1.52^{a}	3.90± 0.50	2.06±0.27

Data is 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 subacute administration of *Sacharum officinarum* leaf extract on kidney function parameters of rats.

TREATMENT	DOSE (mg/kg)	CREATININE (mg/kg)	UREA (mg/dl)	BICARBONATE (mMol/L)	SODIUM (mMol/L)	POTASSIUM (mMol/L)	CHLORIDE (mMol/L)
Control normal saline	10 mg/ml	4.56± 0.43	93.33± 8.41	28.33± 1.20	146.6±7.96	4.76± 0.29	76.0± 0.57
Crude extract	70	5.53± 0.98	107.6± 15.23	24.33± 1.76	153.3±4.37	4.93± 0.18	74.0± 2.08
	140	4.93± 0.71	100.0± 14.01	26.00± 1.15	121.3± 8.09	3.80± 0.32	68.0± 5.13
	210	3.43± 0.12	72.33 ± 1.45^{a}	26.0±2.64	134.3±8.37	4.26± 0.31	94.0 ± 5.50^{a}

Data is expressed as MEAN ± SEM, Significant at $^{a}p<0.05$, when compared to control. (n=6).

Table 6. Effect of subacute administration of *Sacharum officinarum* leaf extract on the 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	2.10± 0.05	0.84± 0.05	1.46± 0.03	1.02± 0.03	0.38± 0.02
Crude extract	70	1.90± 0.05	0.88± 0.08	1.44± 0.01	0.85± 0.07	0.40± 0.03
	140	2.16± 0.08	0.70± 0.06	1.52± 0.02	0.96± 0.08	0.31± 0.02
	210	1.96± 0.08	0.66± 0.01	0.96 ± 0.08^{a}	0.88± 0.02	0.30± 0.01

Data is expressed as MEAN ± SEM, Significant at $^{a}p<0.05$, when compared to control. (n=6).

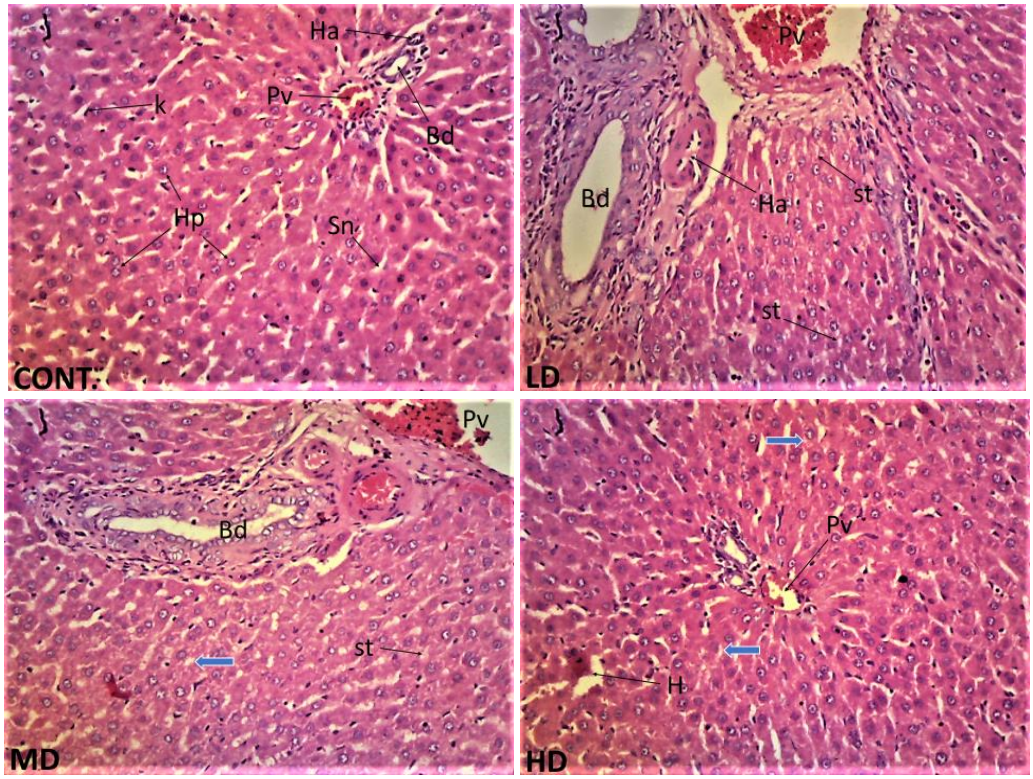

Figure 3. Photomicrograph of the transverse sections of livers of rats treated with distilled water (**CONT**), the leaf extract of *S. officinarum* at 170 mg/kg (**LD**), 340 mg/kg (**MD**), and 510 mg/kg (**HD**) liver tissue showing portal vein (Pv), bile duct (Bd) and hepatic artery (Ha) within the connective tissue (Ct) of the portal area, well-populated hepatocytes (Hp), kupffer cells (Kc) and arrays of sinusoidal spaces (Sn) within the hepatic lobule vacuolated hepatocytes (blue arrow), micro and macro-vesicular steatosis (st). (x 100).

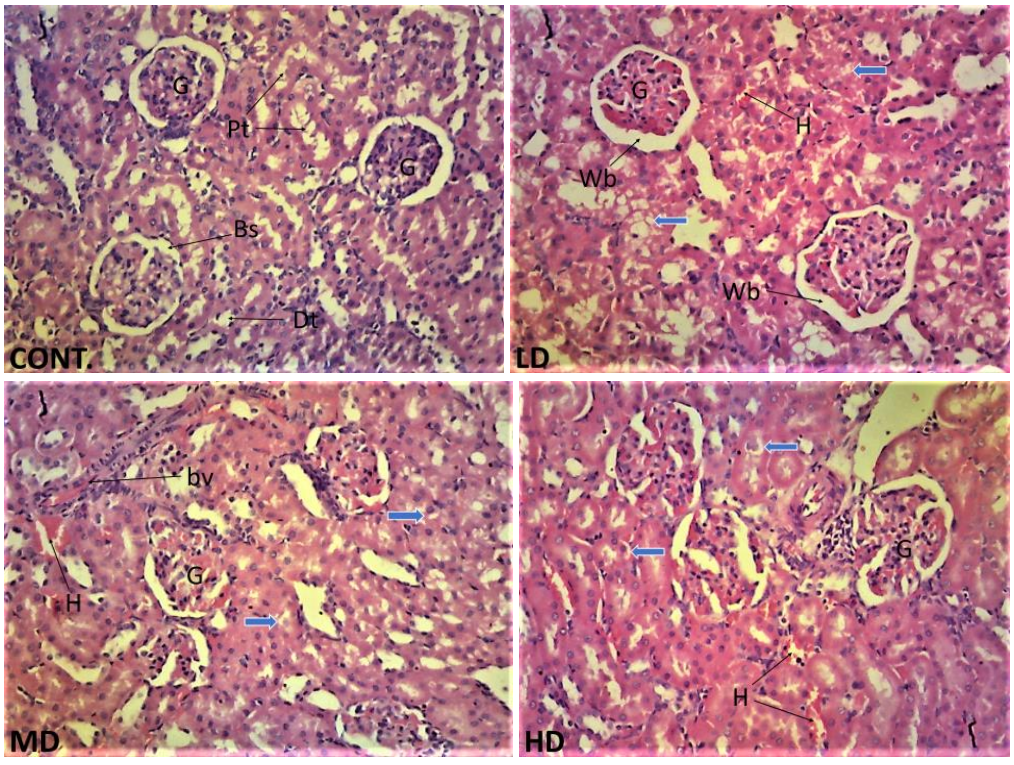

Figure 4. Photomicrograph of the transverse sections of kidneys of rats treated with distilled water (**CONT**), leaf extract of *S. officinarum* at 170 mg/kg (**LD**), 340 mg/kg (**MD**), and 510 mg/kg (**HD**) liver tissue showing glomeruli (G), Bowman's space (Bs), proximal convolated tubules (Pt), distal convolated tubules (Dt) and infiltrating interstitial connective tissues (Ic), widened Bowman's space (Wb), vacoulated ductal cells (blue arrow), and hemorrhagic blood vessels (H) (x 100).

DISCUSSION

In this study, subacute administration of the leaf extract caused various degrees of weight gains in all the treatment groups, which were insignificantly higher than control at higher doses (340 and 510 mg/kg) but significantly lower at the low dose (170 mg/kg) when compared to control. Changes in body weight are markers of adverse effects of drugs, and it is considered statistically significant if a body weight loss is more than 10% (Tepongning *et al*., 2018). In this study, there were moderate increases in the 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 except at the low dose, indicating that the feeding habit of the rats was not adversely affected by the administration of the extract. There were no adverse effects of the extract on the body growth processes of rats.

The Treatment of rats with leaf extract (170-510 mg/kg) for 30 days did not affect on the weights of the liver and kidneys. However, slight decreases in the liver weights of treated rats were observed but these were not significant when compared statistically to the control group. Generally, internal organ weights are considered an essential indicator of injury and toxicities (Farah *et al.,* 2013). Hypertrophy of organs often indicates toxicity and damage to organs (Ping *et al.,* 2013). This often results from edema due to inflammation of the organs, which will increase the weight of the affected organs. The decrease in the weights of the liver in the low-dose group does not suggest a severe harmful effect and may*be a reflection of the moderate effect observed in the histopathology of these organs.

Plant extracts and other chemical compounds are evaluated for possible effects on hematological parameters to assess their toxic potential in blood (Bashir *et al*., 2015). Subacute administration of leaf extract of *S. officinarum* to rats for 30 days did not significantly affect the WBC counts, lymphocytes, monocytes, eosinophil, and basophil percentages ($p>0.05$) compared to control. However, the neutrophils percentage of the group treated with 170 mg/kg of the leaf extract was significantly ($p<0.05$) increased and significant ($p<0.05$-0.01) decreases in RBC and platelets counts, hemoglobin concentration and PCV percentage were observed in the group treated with the middle dose (340 mg/kg) of the extract when compared to control. These suggest the hemolytic effect of the extract and/or suppression of erythropoiesis (the rate of production of erythrocytes) (Berinyuy *et al*., 2015), as well as thrombopoiesis. These imply that the oxygen-carrying capacity of the blood can be affected and indicative of the extract's anemia and thrombocytopenia-inducing potentials. The reduced platelet counts observed explain the significant increases in the bleeding and clotting times of the treated rats when compared to the control, indicating an effect in the blood clotting mechanism. However, the percentage of neutrophils, was significantly elevated by the extract when compared to the control. The significant increase in the neutrophils by the extract could suggest an enhancement in the ability of the blood component to phagocytose, and indicative of an inflammatory response perhaps in response to the deleterious effect of the extract, a demonstration of the immunogenic potential of the extract.

In this study, administration of leaf extract of *S. officinarum* (170-510 mg/kg) to rats for 30 days exerted insignificant ($p>0.05$) effects on total protein, albumin, and ALT levels of rats when compared to control. The ALP levels were similarly reduced, but this was only significant ($p<0.01$) in the group treated with the highest dose (510 mg/kg) dose of the extract. Similarly, AST, total and conjugated bilirubin levels of treated rats were decreased, but the reductions were significant ($p<0.01$-0.001) only at the higher doses (340 and 540 mg/kg) of the extract when compared to control. The results suggest that the extract does not affect the synthetic functions of the liver. Decreases in serum proteins indicate hepatic damage due to the reduced capability of the hepatocytes to synthesize enough serum proteins. Reduced serum/plasma albumin is associated with hepatic damage (Shin *et al*., 2010; Yousef *et al*., 2010). Assessment of albumin and protein in the liver could be used as an important indicator of the synthetic function of the organs, whereas bilirubin (total and conjugated) could be used to assess the excretory function of the liver (Kaplan *et al*., 1979; Yakuba *et al*., 2003). Severe hemolysis causes the release of more bilirubin into the blood, which manifests as elevated levels of direct and total bilirubin (Ngana,1989). Bilirubin is a metabolic breakdown product of heme derived from senescent red blood cells and is commonly used in liver function tests. It is removed from the blood by the liver, chemically modified by a process called conjugation (formation of bilirubin), secreted into the bile, passed into the intestine, and to some extent, reabsorbed from the intestine (Yakubu *et al*., 2005). The reduction in the total and direct bilirubin, as observed in this study with the leaf extract, could be adduced to impairment in the secretory function of these proteins and may also adversely affect the functional activity of the liver, especially the biliary system (Ashafa *et al*., 2009).

In this study, significantly reduced activities of AST and ALP were observed following subacute administration of the leaf extract of *S. officinarum*, especially at higher doses of the extract. Cellular leakage of enzymes occurs often when the cell architecture and integrity are damaged. Similarly, the presence of enzymes in the serum above their average level is a pointer to clinical diagnosis of various pathological conditions. Serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) have been reported by numerous authors as markers for acute and chronic hepatocellular damage (Dufour *et al.,* 2000). Pyridoxal-5-phosphate (PLP), the active form of vitamin B6, is the coenzyme for both ALT and AST, respectively (Rej,

1977). A Significant decrease in serum AST might be due to metabolic, drug-induced or iatrogenic (unknown cause). Pathophysiological conditions associated with a deficiency of vitamin B6 might lead to a decrease in serum AST and ALT activities (Lum, 1995). Decrease in the serum/plasma activities of aspartate aminotransferase has been shown to correlate with pyridoxal-5-phosphate deficiency (Waner & Nyska, 1991; Evans & Whitehorn, 1995; Hall, 2001; Saori *et al.,* 2003). 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; it is therefore an ectoenzyme of the plasma membrane and it is often used to assess the integrity of the plasma membrane (Shittu *et al.,* 2015). However, it was not affected by the extract treatment. The results further suggest mild toxic potential of the extract at higher doses.

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 serum in renal disease when the production rate exceeds that of excretion (Mayne, 1994). Serum creatinine is derived from endogenous sources by tissue creatinine breakdown (Mayne, 1994). Therefore, elevation of urea and creatinine levels in the serum had been taken as the index of nephrotoxicity (Ali *et al.,* 2001; Flaoyen *et al*., 2001). In this study, treatment of rats for 30 days with leaf extract of *S. officinarum* (170-510 mg/kg) did not cause any significant effect ($p>0.05$) on the levels of urea, creatinine, potassium, and sodium of the treated rats when compared to control. However, the level of chloride was significantly ($p<0.05$) increased at the higher dose of the extract (510 mg/kg) when compared to control. This shows that the extract is not nephrotoxic at the doses studied. The electrolyte concentrations were not affected by the extract treatment except chloride level at the highest dose, suggesting that the glomerular filtration rate was not affected by the extract treatment mostly at lower doses.

Alterations in the concentration of significant lipids like cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol and triglycerides can give helpful information on lipid metabolism as well as the predisposition of the heart to atherosclerosis and its associated coronary heart diseases (Yakubu *et al.,* 2008). High blood cholesterol concentrations are an essential risk factor for cardiovascular disease (Abolaji *et al.,* 2007). Therefore, the slightly reduced levels of serum total cholesterol, triglyceride, LDL, and VLDL especially at higher doses 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 reduced lipolysis may explain the decreased levels of serum triacylglycerol by the extract (Yakubu *et al.,* 2008). The reduction in the levels of VLDL, LDL, and HDL in this study reveals a robust 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 (Philip, 1995; Jackson, 1996; Mayes, 1996; Panagiotakos *et al.,* 2003).

On the histology, subacute administration of ethanol leaf extract of *S. officinarum* to rats for 30 days produced varying degrees of abnormalities ranging from mild to moderate defects on histology of the liver and kidney of rats. Higher doses of the leaf extract (340-510 mg/kg) were found to produce some defects such as areas of micro and macro-vesicular steatosis, vacuolated hepatocytes, hemorrhagic blood deposits, and arrays of sinusoidal spaces within the hepatic lobule in all treatment groups portraying a mild to moderate effect on the liver. This is corroborated by the reduced levels of AST and ALP which have no effect on ALT. The history-architechture of renal tissues of treated rats was moderately affected, with glomeruli having widened bowman's space, vacuolated ductal cells, and hemorrhagic blood vessels within the cortical matrix, portraying a mild toxic effect on the kidney. The mild effect of the extract on the kidney is further supported by the chemical pathology results in which the lack of any significant effect of the extract on levels of urea, creatinine, potassium, and sodium of the treated rats was observed when compared to the control.

CONCLUSION

The results of this study show that subacute administration of leaf extract of *Saccharum officinarum* can cause anaemia, thrombocytopenia, hypolipidemia, increased clotting and bleeding time and a mild toxic effect on the liver and kidneys, which are due to the activities of its phytochemical constituents.

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