HISTOPATHOLOGICAL STUDY OF SUBACUTE ADMINISTRATION OF *Saccharum officinarum* LEAF EXTRACT ON SOME ORGANS OF RAT

ABSTRACT

Saccharum officinarum (Family-Poaceae) is used traditionally for the treatment of malaria and fever among others. Evaluation of subacute administration ethanol leaf extract of *Saccharum officinarum* for possible effect on organs of rats was carried out. The leaf extract (170, 340, 510 mg/kg body weight) was orally administered to male and female wistar rats daily for 30 days and the rats were sacrificed under light diethyl ether anesthesia at the completion of the administration. Subacute administration of *S. officinarum* leaf extract resulted in insignificant increase in body weights of rats without any significant ($p>0.05$) effect on the weights of the studied organs (brain, heart, spleen, testis and ovary) when compared to control. The leaf extract exerts mild to moderate effect on the histologies of brain, heart, spleen and ovary of rats, but had no effect on the testis. Chronic study is advocated to investigate the effect of prolonged administration of the extract organs and systems of rats.

Keywords: *Saccharum officinarum*, subacute, toxicity, organ weights

INTRODUCTION

The use of medicinal plants in the treatment and management of diseases is on increase all over the world. Herbal preparations are believed and claimed to be natural and safe. In spite of these assumptions, their usage are not without side effects and toxicities, which have been attributed to toxic potentials of the main constituents. Herbal preparations usage has been implicated variously in clinical cases of organs toxicities and dysfunctions. These have been attributed to paucity of scientific information on the toxic potentials of these herbal medicines. In spite of the assumed safety of African medicinal plants, studies have shown that many plants used as food or traditional medicines are also potentially toxic.

Saccharum officinarum (Family-Poaceae) also called sugarcane thrives throughout tropical and subtropical regions worldwide. In traditional medicine, it is used in the treatment of diarrhoea, dysentery, eyes, fever, arthritis, bedsores, boils, cancer, colds, cough, opacity, skin sores, sore throat, hiccups, inflammation, laryngitis, spleen, tumors, and wounds [1]. the leaf extract possesses some biological activities such as antibacterial and anthelmintic [2], anti-hyperglycaemic, anti-hyperlipidaemic [3], antioxidant [3,4], diuretic and antiurolithiatic [5], antidepressant and anticonvulsant [6], analgesic [7] and antimalarial [8], antioxidative stress and hepatoprotective [9], anti-inflammatory and antipyretic[10], antiulcer [11] activities. SAABMAL®: a polyherbal preparation containing *S. officinarum* is utilised as malarial remedy in Nigeria [12]. The leaves are employed in Ghana for the treatment of malaria locally [13]. Phytochemical screening of leaf extract of *Saccharum officinarum* revealed the presence of glycosides, phytosterols, saponins, tannins, flavonoids [5,14]. Some flavones and phenolics as well as their derivatives from the leaves of *S. officinarum* have been identified [8,15]. The medicinal potentials of the plant have been widely reported, but there is paucity of information on its toxicological potentials. In this study, subacute toxicity potential of the leaf extract of *S. officinarum* on histopathologies of some organs of rats is reported.

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 electric grinder. The powdered leaf material (2 kg) was soaked in 50% ethanol (7.5 L) at room temperature (28 ± 2 $^{\circ}$C) for 72 hours. It

was thereafter filtered and the liquid filtrate was 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 used for the proposed experiments.

Animals

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

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 of time. At the end of the treatment period, the animals were weighed again and sacrificed under light ethyl ether vapour.

The effect of the extract on some organs were studied. The organs;, 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 eaosin (H&E) according to standard procedures.

Histopathological examination

The organs of rats that were 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 collected were analyzed using one way analysis of variance (ANOVA) followed by Tukey's kramer multiple comparison post-test (Graph pad prism software Inc. La Jolla, CA, USA). Values were expressed as mean ± SEM and significance relative to control were 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 considerably increase in 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, while the weight increases of rats treated with higher doses (340 and 510 kg/kg) of the extract were higher than that of control but not statistically significant ($p>0.05$) (Table 1).

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

TREATMENT	DOSE(mg/kg)	Heart (mg)	Brain (mg)	Spleen (mg)	Testes (mg)	Ovary (mg)
Control	10 mg/ml	0.66± 0.07	1.65±0.06	0.62± 0.04	2.41±0.21	0.07±0.01
Crude extract	170	0.70±0.04	1.65±0.15	0.77± 0.11	3.07± 0.36	0.06±0.01
	340	0.66±0.02	1.71±0.06	0.74±0.02	3.31± 0.47	0.04±0.01[b]
	510	0.71±0.02	1.73±0.01	0.65± 0.05	2.77± 0.18	0.04±0.01[b]

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

Effect of subacute administration of leaf extract on histology of organs

Figures 1- 5 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 on the histology of the organs. Moderate effects were observed on the histo-structure of cardiac tissues of the treated rats at all the doses employed (170- 510 mg/kg) with pre-nuclear sarcoplasmic vacoulation (Vm), hemorrhagic blood vessels (Hb) and wide spaced endomycium (En) observed within the cardiac tissue (Figure 1). The low dose of the extract (170 mg/kg) did not affect the ovaries of the treated rats, while higher doses (340 and 510 mg/kg) caused moderate effects on the ovaries with sections showing abnormal cyto-structure with infiltrating neutrophils in developing secondary follicle (Sf) and infiltrating neutrophils in atrophying follicle (Figure 2). However, the leaf extract did not affect the testes of the treated rats adversely (Figure 3). Moderate effect of the leaf extract on the cyto-structure of the spleen were observed following subacute administration of the leaf extract (10-510 mg/kg) with treated rats' spleen tissues showing an abnormal splenic cyto-structure with areas of degenerative immunal cells within the white pulp, and numerous blood cells within the red pulp (Figure 4). The leaf extract administration affected the brain tissue of the treated rats moderately, with the lateral prefrontal cortex of the cerebral hemisphere having abnormal histo-structure with vacuolated neural cells, widespread activated and necrotic astrocytes within the cortical matrix. (Figure 5).

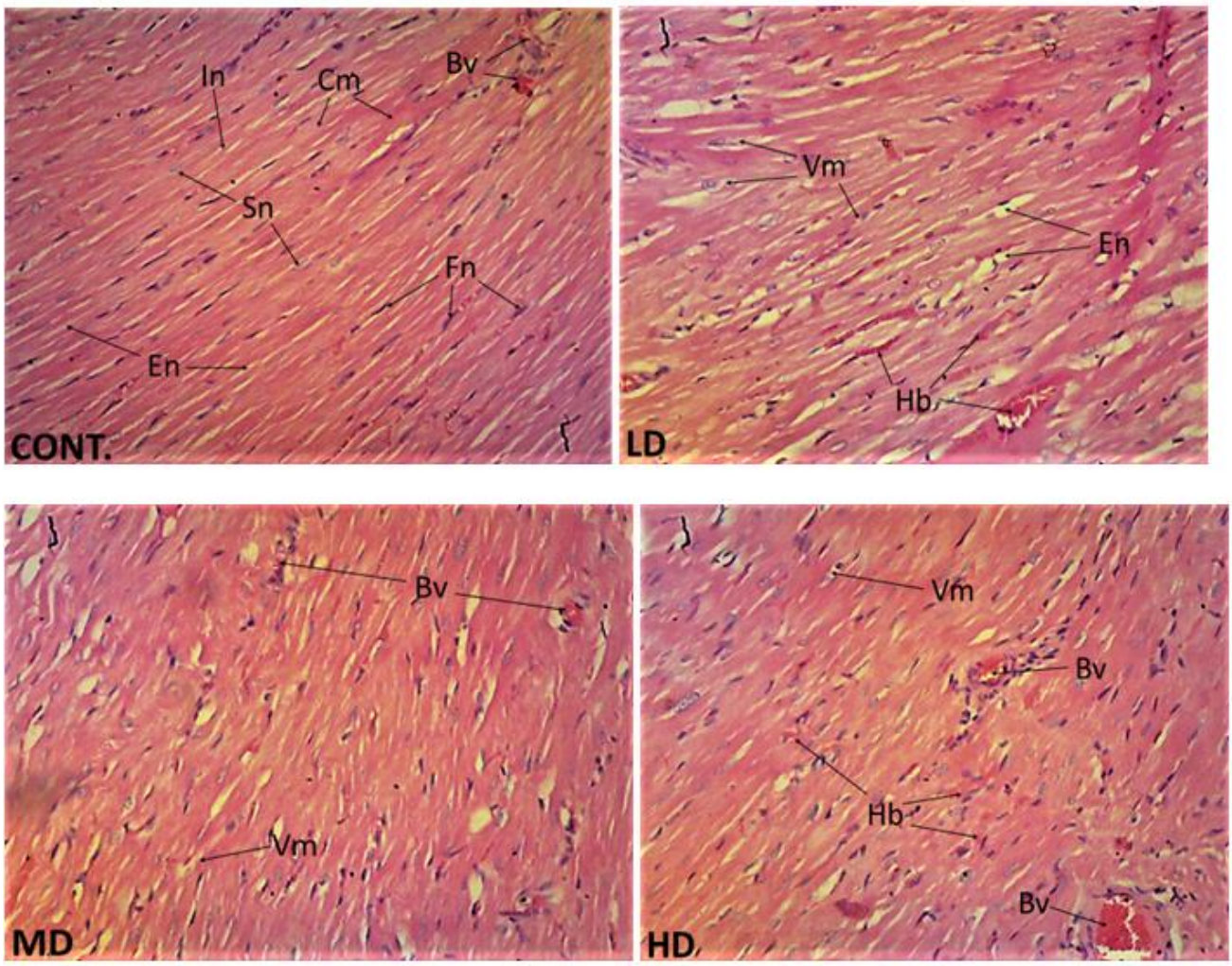

Figure 1:Photomicrograph of the transverse sections of hearts of rats treated with distilled water (**CONT**), leaf extract of *S. officinarum* at 170 mg/kg (**LD**), 340 mg/kg (**MD**) and 510 mg/kg (**HD**) heart tissue showing cardiac myocytes (Cm), intercalated disc (In), sarcoplasmic nuclei (Sn), fibrocyte nuclei (Fn) of fibrocytic cells within the endomycium (En), sarcoplasmic vacoulation (Vm), and hemorrhagic blood vessels (Hb

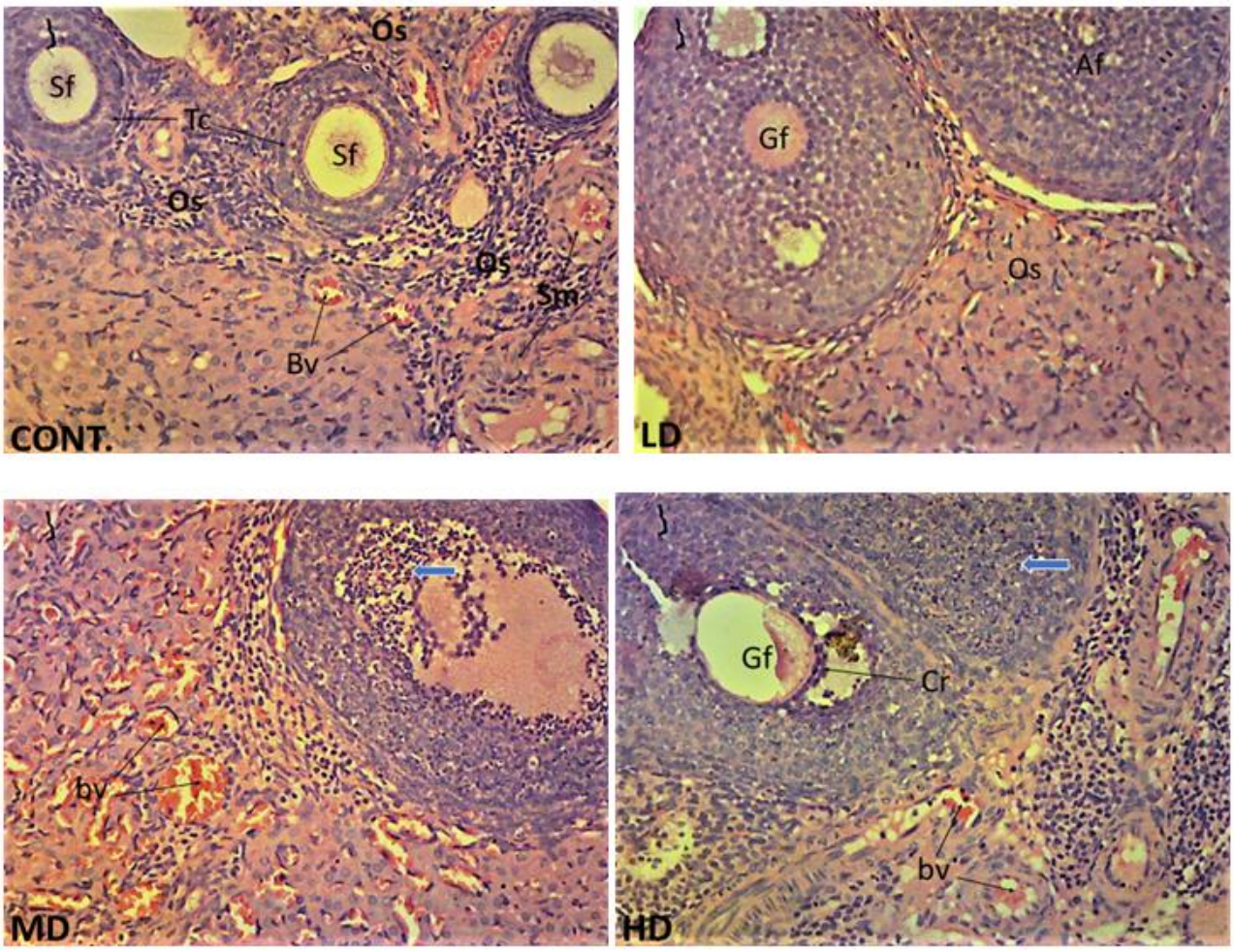

Figure 2: Photomicrograph of the transverse sections of ovaries of rats treated with distilled water (**CONT**),
leaf extract of *S. officinarum* at 170 mg/kg (**LD**), 340 mg/kg (**MD**) and 510 mg/kg (**HD**) ovarin tissue showing secondary follicular cells (Sf) surrounded with Thecca cells (Tc), blood vessels (Bv) with surrounding smooth muscles (Sm), developing graffian follicle (Gf) and an atrophying follicle (Af) within
the ovarian stoma (Os), infiltrating neutrophils (blue arrow) corona radiate (Cr) and blood vessels (bv).

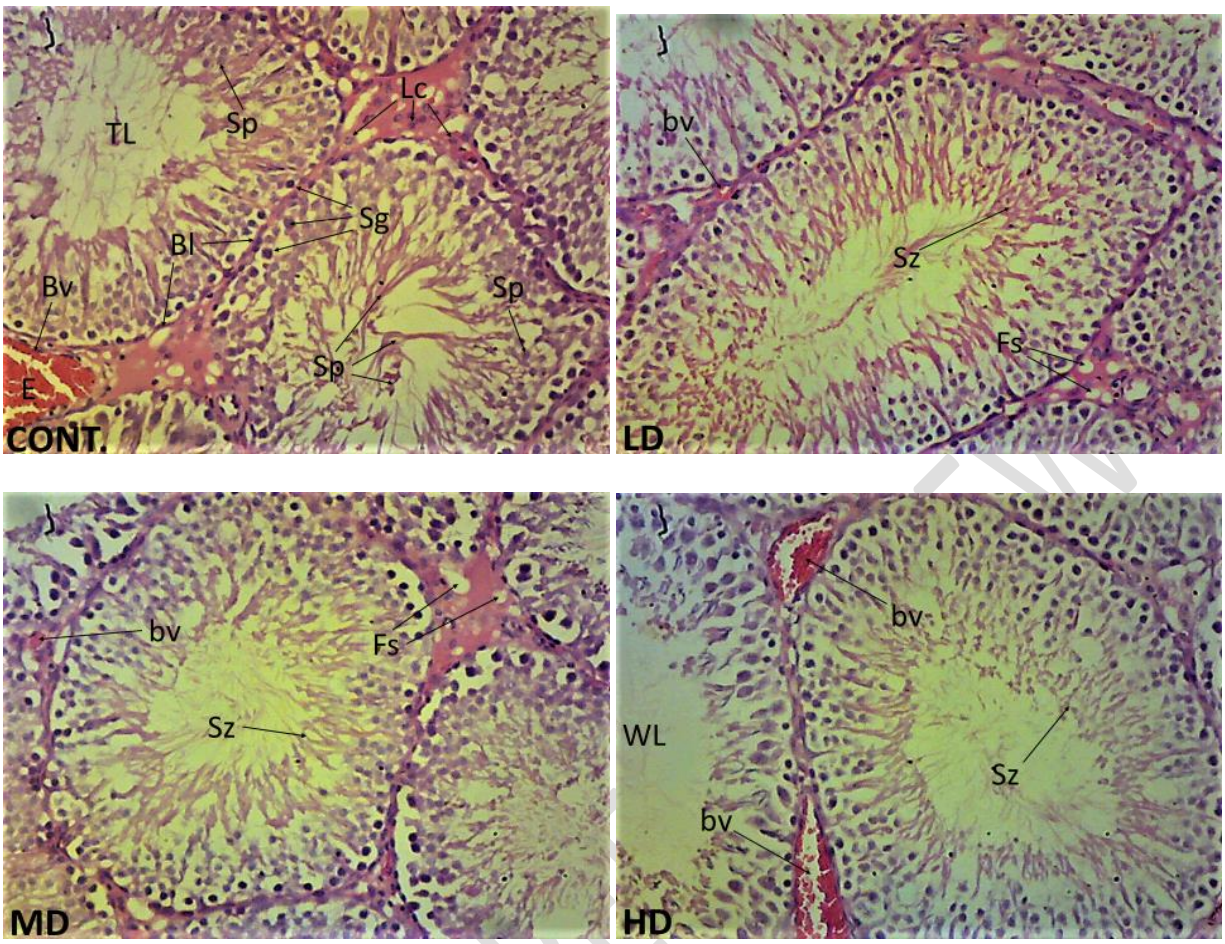

Figure 3: Photomicrograph of the transverse sections of testes of rats treated with distilled water (**CONT**), leaf extract of *S. officinarum* at 170 mg/kg (**LD**), 340 mg/kg (**MD**) and 510 mg/kg (**HD**) testicular tissues showing basement layers (Bl), spermatogonia cells (Sg), radiating spermatozoa (Sz) within the tubular lumen (TL), spermatids (Sp), Leydig cells (Lc), blood vessels (Bv) with erythrocytes (E), spermatozoa (Sz),widen tubular lumen (WL). (x 100).

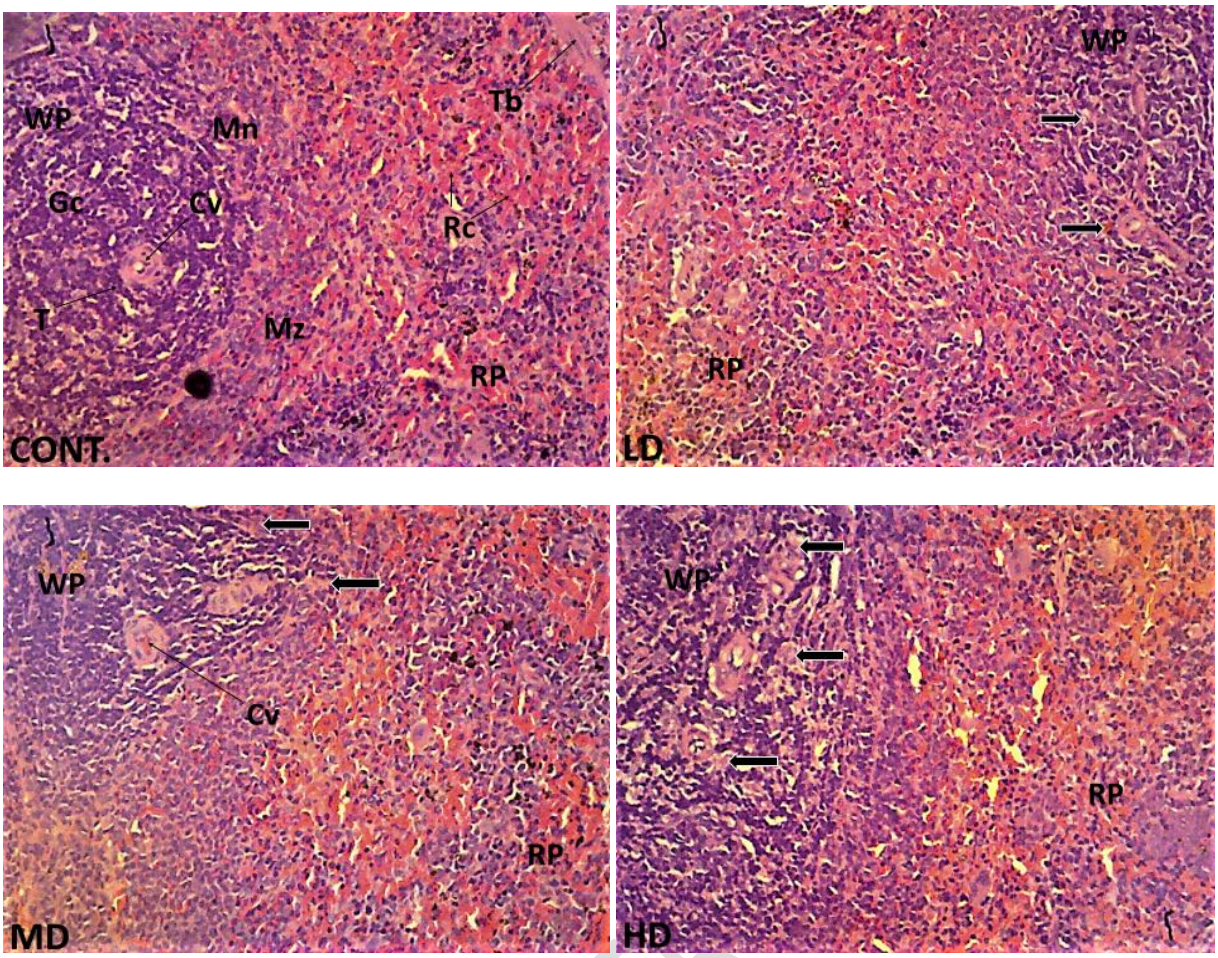

Figure 4: Photomicrograph of the transverse sections of spleens of rats treated with distilled water (**CONT**), leaf extract of *S. officinarum* at 170 mg/kg (**LD**), 340 mg/kg (**MD**) and 510 mg/kg (**HD**) spleen tissues showing white pulp (WP), germinal center (Gc), central vein (Cv), T-lymphocytes (T), mantle layer (Mn), marginal zone (Mz), the red pulp (RP), red blood cells (Rc) and tubercular tissue (Tb), degenerative immunal cells (black arrow) and the central vein (Cv).

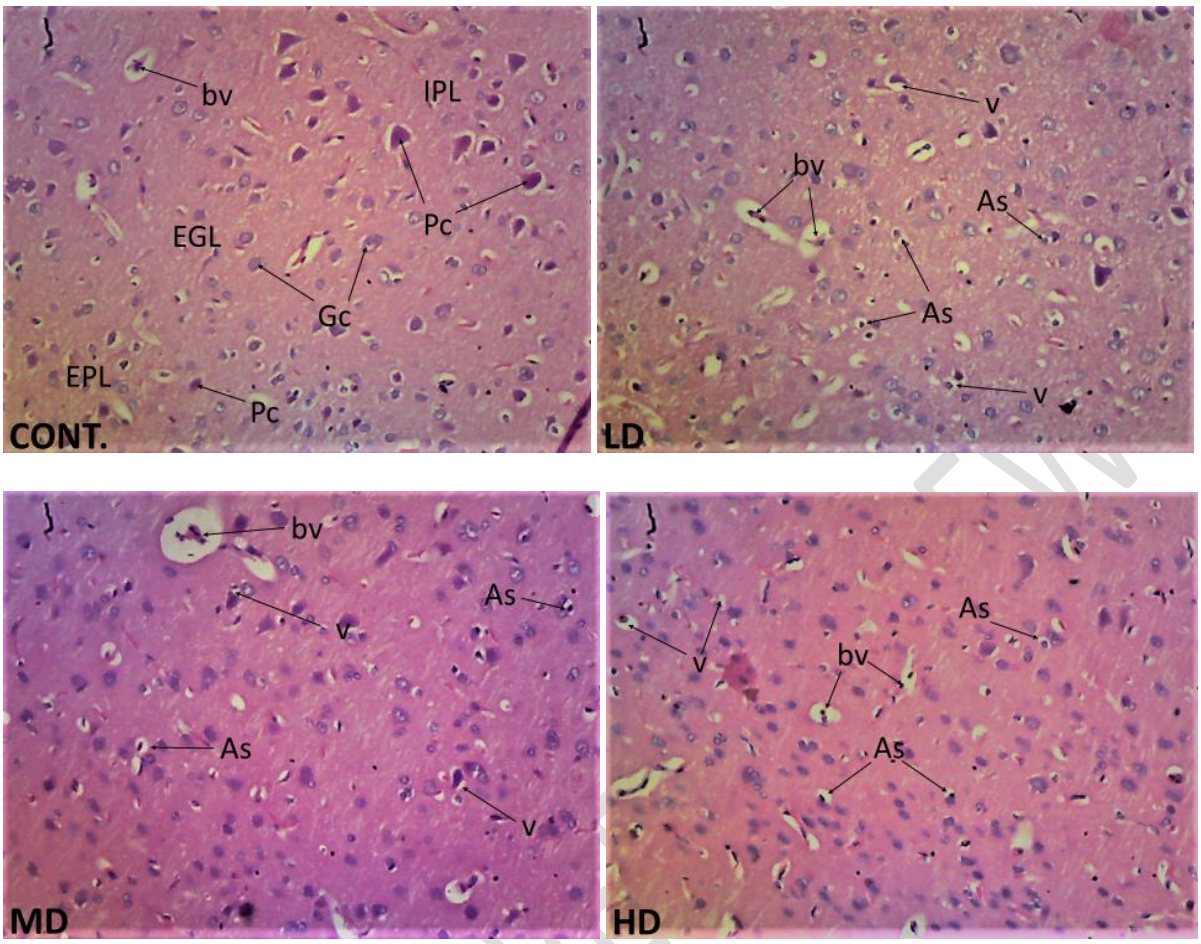

Figure 5: Photomicrograph of the transverse sections of brain tissues of rats treated with distilled water (**CONT**), leaf extract of *S. officinarum* at 170 mg/kg (**LD**), 340 mg/kg (**MD**) and 510 mg/kg (**HD**) brain tissues showing external pyramidal layer (EPL), external granular layer (EGL), internal pyramidal layer (IPL), blood vessels (bv), pyramidal cells (Pc) and granular cells (Gc), astrocytes (As).(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 weights are markers of adverse effects of drugs and it is considered statistically significant if a body weight loss is more than 10% [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 except at the low dose 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.

Treatment of rats with the leaf extract (170-510 mg/kg) for 30 days did not cause any effect on the weights of heart, brain, spleen, pancreas, testes and ovary. However, insignificant ($p>0.05$) increases in spleen weights of rats treated with the leaf extract were observed when compared to control. Generally, internal organs weights are considered as important indicator to injury and toxicities [17]. Hypertrophy of organs often indicates toxicity and damaged to organ [18]. This often results from oedema due to inflammation of the organs which will result in weight increases of the affected organs. The insignificant increases in spleen weight does not suggest a serious harmful effect and maybe a reflection of the moderate effect observed in the histopathology of this organ.

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 histologies of the heart, ovary, brain and spleen,with no deleterious effect on the male reproductive system, testis. The morphology of the testis was as normal as that of the control. Moderate effects were also observed on

the histo-structure of cardiac tissues of the treated rats with pre-nuclear sarcoplasmic vacoulation (Vm), hemorrhagic blood vessels (Hb) and wide spaced endomycium (En) within the cardiac tissue. This suggest a mild effect on the heart which might be reversed on withdrawal of the treatment. The ovaries were observed to be affected moderately by higher doses (340 and 510 mg/kg) of the extract causing abnormal cyto-structure with infiltrating neutrophils in developing secondary follicle and infiltrating neutrophils in atrophying follicle, depicting a mild effect, while the low dose had no effect on the ovaries. Moderate effect of the leaf extract on the cyto-structure of the spleen were also observed following subacute administration of the extract with treated rats' spleen tissues showing an abnormal splenic cyto-structure with areas of degenerative immunal cells within the white pulp (WP), and numerous blood cells within the red pulp. This suggest a mild toxic effect on the spleen. The leaf extract administration affected the brain tissue of the treated rats moderately, with the lateral prefrontal cortex of the cerebral hemisphere having abnormal histo-structure with vacuolated neural cells, widespread activated and necrotic astrocytes within the cortical matrix. This results indicate mild adverse effects on the brain cells which might also affect some functions of the CNS.

CONCLUSION

The results of this study show that subacute administration of leaf extract of *Saccharum officinarum* has no effect on the testis but can cause mild toxic effects to the heart, spleen, brain and ovary which are due to the activities of its phytochemical constituents.

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