

# 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.

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

## 17 **MATERIALS AND METHODS**

### 18 **Plant materials**

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

### 25 **Extraction**

26 Fresh leaves of *S. officinarum* were washed, cut into smaller pieces and dried under shade for two  
27 weeks. The leaves were further pulverized to powder using electric grinder. The powdered leaf  
28 material (2 kg) was soaked in 50% ethanol (7.5 L) at room temperature ( $28 \pm 2$  °C) for 72 hours. It

1 was thereafter filtered and the liquid filtrate was concentrated and evaporated to dryness in *vacuo*  
2 40 °C using a rotary evaporator (BuchiLab Switzerland). The dry extract was stored in a refrigerator  
3 at -4 °C, until used for the proposed experiments.

#### 4 **Animals**

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

#### 9 **Sub acute toxicological study**

10 Adult wistar rats of both sexes were used in this study. They were weighed and randomly  
11 divided into four groups of 6 animals each and treated as follows; groups I, II, and III were  
12 administered 170, 340 and 510 mg/kg of the leaf extract respectively daily for 30 days. Group IV was  
13 administered with distilled water (10 mL/kg) for the same period of time. At the end of the treatment  
14 period, the animals were weighed again and sacrificed under light ethyl ether vapour.

15 The effect of the extract on some organs were studied. The organs, spleen, brain, ovary, testis, and  
16 heart of rats were surgically removed and fixed in 10% formalin. The organs were processed,  
17 sectioned and stained using hematoxylin and eosin (H&E) according to standard procedures.

#### 18 **Histopathological examination**

19 The organs of rats that were used in the study were surgically harvested and fixed in buffered  
20 formalin. They were then processed and stained with haematotoxylin and eosin (H&E) according to  
21 standard procedures at Department of Chemical Pathology, University of Uyo Teaching Hospital,  
22 Uyo. Morphological changes were observed and recorded in the excised organs of the sacrificed  
23 animals. Histologic pictures were taken as micrographs.

#### 24 **Statistical analysis**

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

## 5 RESULTS

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

7 The effect of leaf extract on body weight of rats treated with leaf extract of *S. officinarum* for  
8 30 days is shown in Table 1. There was considerably increase in body weights of rats treated the  
9 extract in all groups similar to that of the control group though non dose-dependently. The increase in  
10 body weight in the low dose (170 mg/kg) treatment group was significantly ( $p < 0.001$ ) lower than  
11 that of the control, while the weight increases of rats treated with higher doses (340 and 510 mg/kg)  
12 of the extract were higher than that of control but not statistically significant ( $p > 0.05$ ) (Table 1).  
13

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

15 Treatment of rats with the leaf extract (170-510 mg/kg) for 30 days did not cause any  
16 significant effect ( $p > 0.05$ ) on the weights of livers and kidneys of rats when compared to the control  
17 (Table 2).  
18

19  
20 Table 1: Effect of subacute administration of *S. officinarum* leaf extract on body weights of rats

Treatment	Dose	Initial body	Final body	Weight gain
R&G /Extract	(mg/kg)	weight (Kg)	weight (Kg)	(Kg)
Control	0.2mL	153.3 $\pm$ 9.40	207.6 $\pm$ 5.69	54.3 $\pm$ 2.81
<i>S. officinarum</i>	170	170.3 $\pm$ 6.88	201.0 $\pm$ 17.00	30.7 $\pm$ 3.86 <sup>a</sup>
	340	171.6 $\pm$ 1.66	232.0 $\pm$ 8.50	60.4 $\pm$ 2.74
	510	164.0 $\pm$ 9.53	209.6 $\pm$ 2.40	45.6 $\pm$ 3.41

21 Data are expressed as mean  $\pm$  SEM. Significant at <sup>a</sup> $p > 0.001$  when compared to control . n = 6.

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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 <sup>b</sup>
	510	0.71±0.02	1.73±0.01	0.65± 0.05	2.77± 0.18	0.04±0.01 <sup>b</sup>

Data is expressed as MEAN ± SEM, Significant at <sup>b</sup>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).

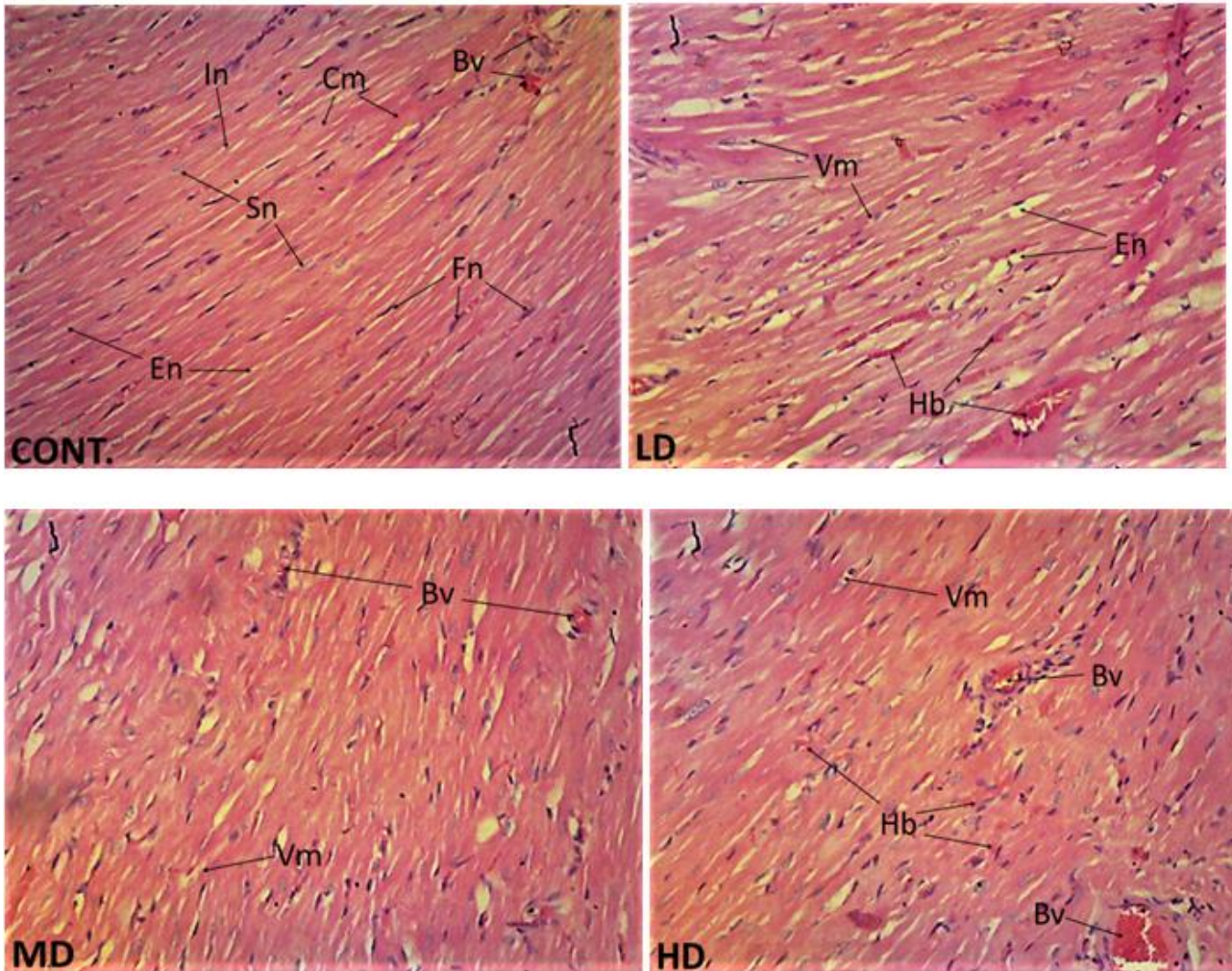


Figure 1: Photomicrograph of the transverse sections of hearts of rats treated with distilled water (**CONT**), leaf extract of *S. officinarum* at 170 mg/kg (**LD**), 340 mg/kg (**MD**) and 510 mg/kg (**HD**) heart tissue showing cardiac myocytes (Cm), intercalated disc (In), sarcoplasmic nuclei (Sn),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

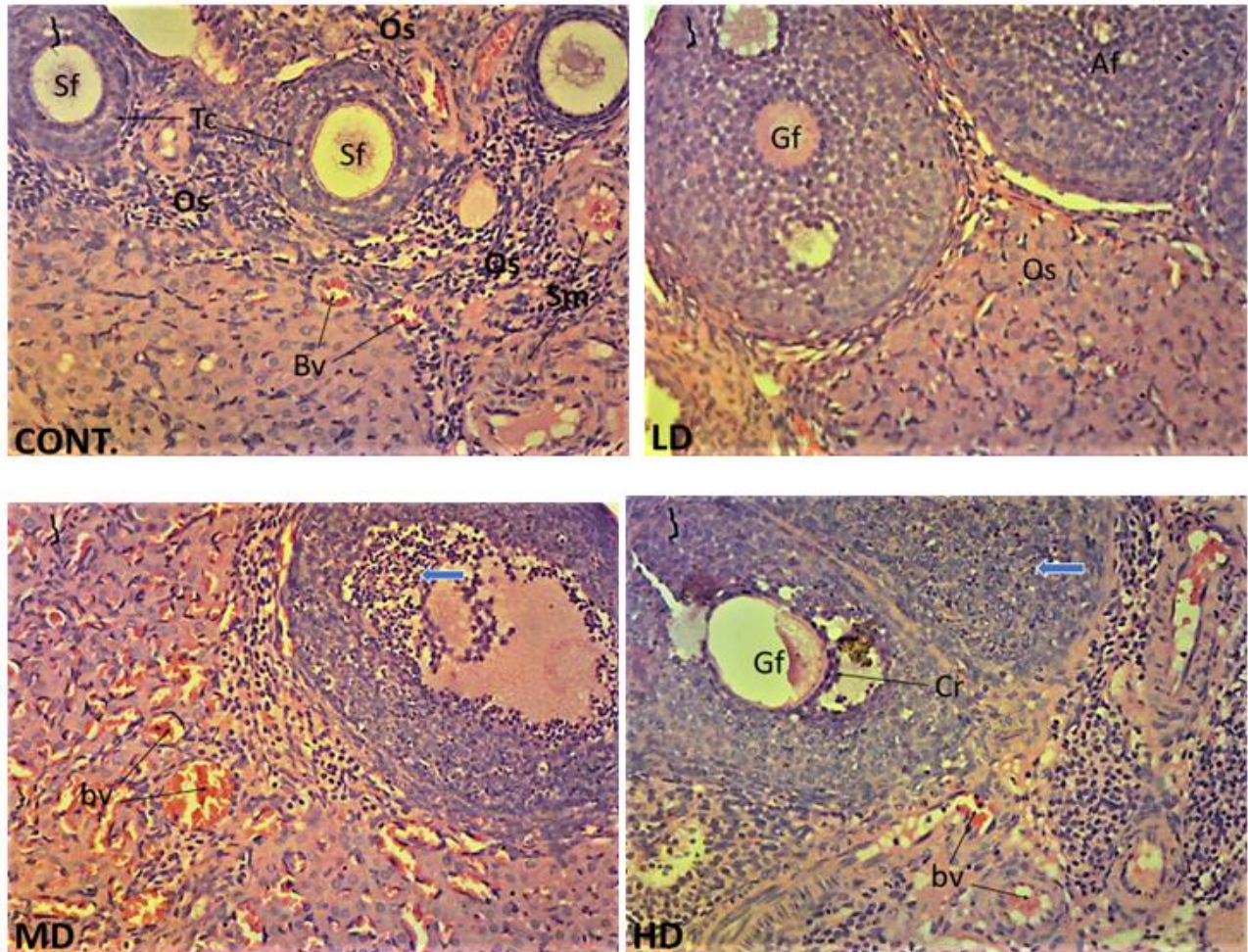


Figure 2: Photomicrograph of the transverse sections of ovaries of rats treated with distilled water (**CONT**), leaf extract of *S. officinarum* at 170 mg/kg (**LD**), 340 mg/kg (**MD**) and 510 mg/kg (**HD**) ovarin tissue showing secondary follicular cells (Sf) surrounded with Theca cells (Tc), blood 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).

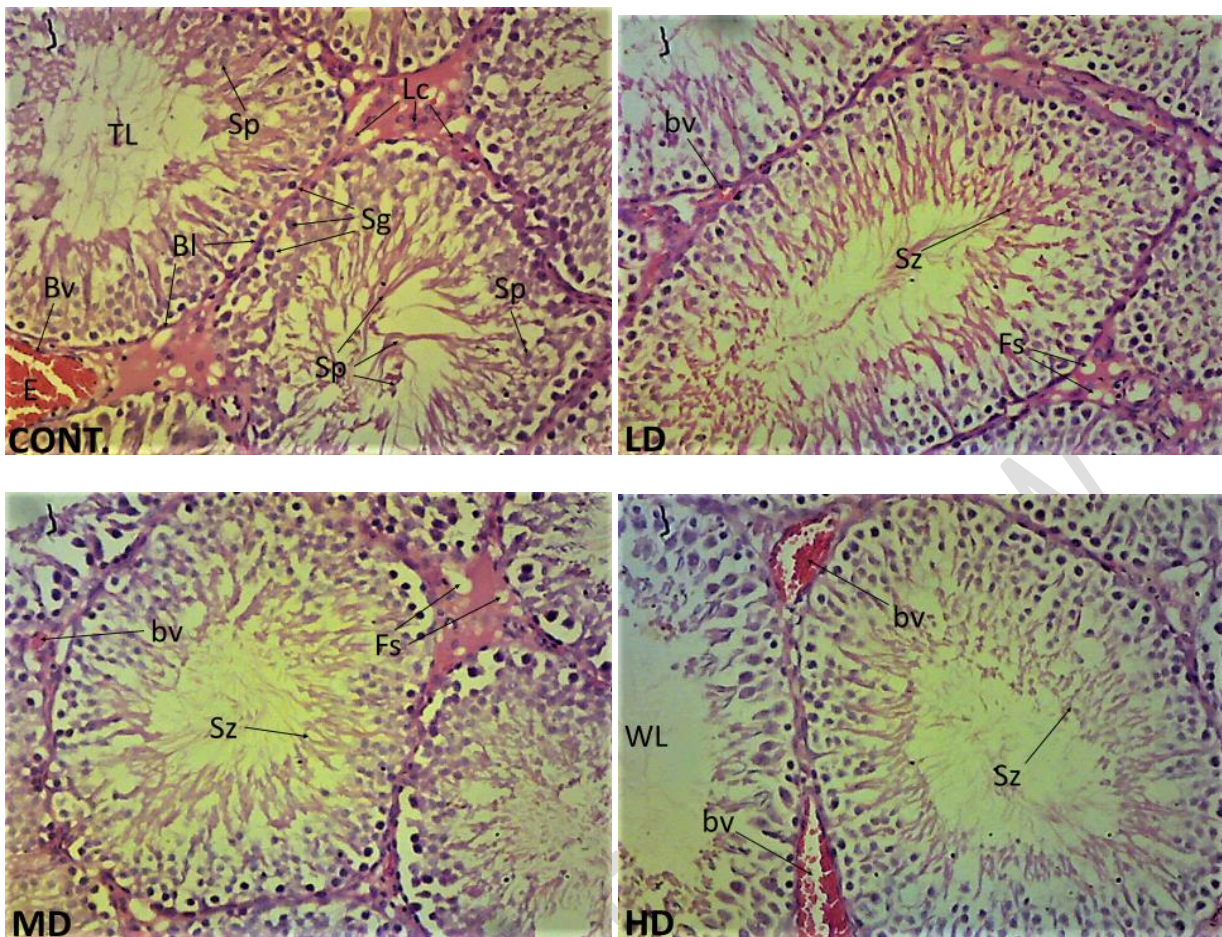


Figure 3: Photomicrograph of the transverse sections of testes of rats treated with distilled water (**CONT**), leaf extract of *S. officinarum* at 170 mg/kg (**LD**), 340 mg/kg (**MD**) and 510 mg/kg (**HD**) testicular tissues showing basement layers (B1), spermatogonia cells (Sg), radiating spermatozoa (Sz) within the tubular lumen (TL), spermatids (Sp), Leydig cells (Lc), blood vessels (Bv) with erythrocytes (E), spermatozoa (Sz), wider tubular lumen (WL). (x 100).

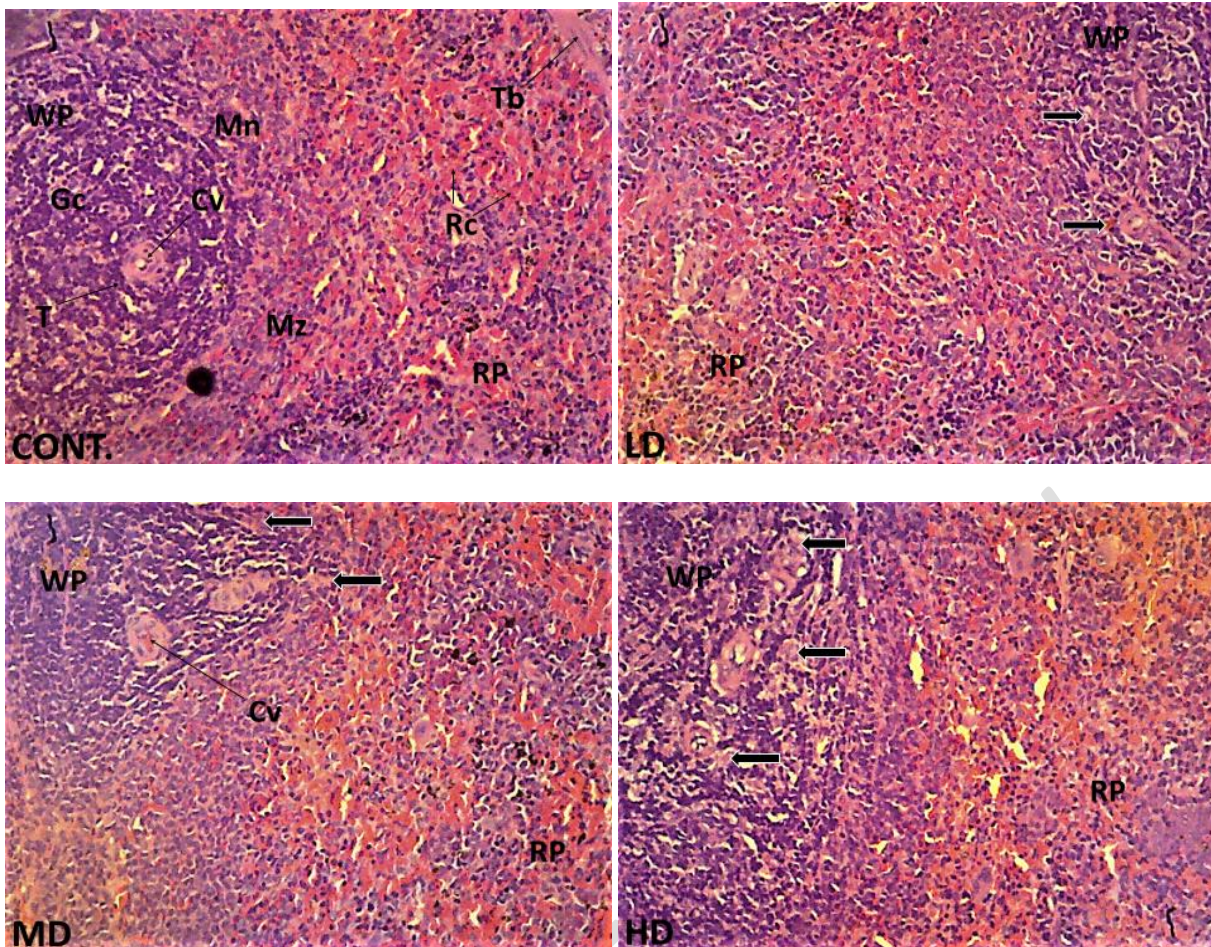


Figure 4: Photomicrograph of the transverse sections of spleens of rats treated with distilled water (**CONT**), leaf extract of *S. officinarum* at 170 mg/kg (**LD**), 340 mg/kg (**MD**) and 510 mg/kg (**HD**) spleen tissues showing white pulp (WP), germinal center (Gc), central vein (Cv), T-lymphocytes (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).

UNDER REVIEW

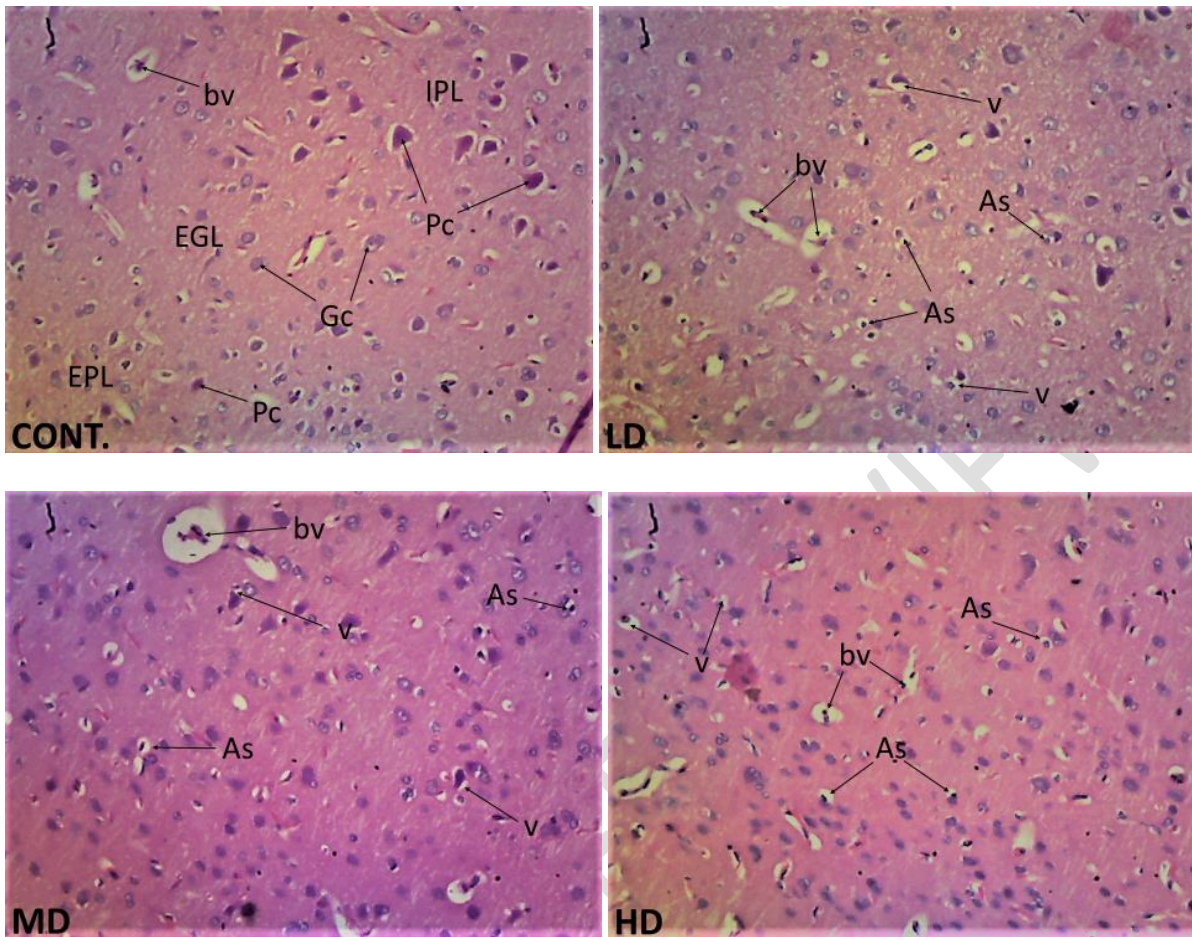


Figure 5: Photomicrograph of the transverse sections of brain tissues of rats treated with distilled water (**CONT**), leaf extract of *S. officinarum* at 170 mg/kg (**LD**), 340 mg/kg (**MD**) and 510 mg/kg (**HD**) brain tissues showing external pyramidal layer (EPL), external granular layer (EGL), internal pyramidal layer (IPL), blood vessels (bv), pyramidal cells (Pc) and granular cells (Gc), astrocytes (As).(x 100).

1 **DISCUSSION**

2 In this study, subacute administration of the leaf extract caused various degrees of  
3 weight gains in all the treatment groups which were insignificantly higher than control  
4 at higher doses (340 and 510 mg/kg) but significantly lower at the low dose (170  
5 mg/kg) when compared to control. Changes in body weights are markers of adverse  
6 effects of drugs and it is considered statistically significant if a body weight loss is  
7 more than 10% [16]. In this study, there were moderate increases in body weights of  
8 rats in all the extract treated groups but these increases were not significantly ( $p>0.05$ )  
9 different from that of the control group except at the low dose indicating that feeding  
10 habit of the rats was not adversely affected by the administration of the extract and  
11 there were no adverse effects of the extract on the body growth processes of rats.

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

22 On the histology, subacute administration of ethanol leaf extract of *S. officinarum* to  
23 rats for 30 days produced varying degrees of abnormalities ranging from mild to  
24 moderate defects on histologies of the heart, ovary, brain and spleen, with no  
25 deleterious effect on the male reproductive system, testis. The morphology of the  
26 testis was as normal as that of the control. Moderate effects were also observed on

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

## 18 **CONCLUSION**

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

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## 1 REFERENCES

- 2 1.Hartwell JL. Plants used against cancer. A survey. *Lloydia* 1967–1971; 30–34.
- 3
- 4 2. Palaksha MN, Ravishankar K, Girijasastry V. Phytochemical screening and  
5 evaluation of in-vitro antibacterial and anthelmintic activities of *Saccharum*  
6 *officinarum* leaf extracts. *World Journal of Pharmacy and Pharmaceutical Sci*,  
7 2013;2(6):5761-5768.
- 8 3. Ojewunmi O, Oshodi T, Ogundele O, Micah C and Adenekan S. Evaluation of the  
9 anti-diabetic and antioxidant activities of aqueous extracts of *Morinda lucida* and  
10 *Saccharum officinarum* leaves in alloxan-induced diabetic rats. *International*  
11 *Journal of Biochemistry Research and Review*, 2013; 3(3): 266-277.
- 12
- 13 4.Sun J, He X, Zhao M, Li L, Li C, Dong Y. Antioxidant and Nitrite-Scavenging  
14 Capacities of Phenolic Compounds from Sugarcane (*Saccharum officinarum* L.)  
15 *Tops Molecules* 2014;19: 13147-13160.
- 16
- 17 5.Palaksha MN, Ravishankar K, GirijaSastry V. Biological evaluation of in vivo  
18 diuretic, and antiurolithiatic activities of ethanolic leaf extract of *Saccharum*  
19 *officinarum*. *Indo American Journal of Pharmaceutical Research*. 2015; 5(06):  
20 2232-2238.
- 21
- 22 6.Okokon JE, Udoh AE, Nyong EE, Eno L, Udo NM. Psychopharmacological studies  
23 on leaf extract of *Saccharum officinarum*. *Tropical Journal of Natural Products*  
24 *Research*. 2019; 3(2):26-30.
- 25
- 26 7. Okokon JE, Davies K, Edem UA, Bassey AL, Udobang JA.(2021). Analgesic  
27 activity of ethanol leaf extract of *Saccharum officinarum*. *Tropical Journal of*  
28 *Natural Products Research*.. 2021; 5(6):1142-1145.
- 29
- 30 8. Okokon JE, Mobley R, Edem UA, Bassey AL, Fadayomi I, Horrocks P,  
31 Drijfhout F, Li WW. In vitro and in vivo antimalarial activities and chemical  
32 profiling of sugarcane leaves. *Science Reports* 2022;41598 Article No:14391 doi.  
33 org/ 10. 1038/ s41598 -022-14391-8.
- 34
- 35 9. Edem UA, Okokon JE, Bassey AL, Okokon PJ. Antioxidative stress and  
36 hepatoprotective activities of leaf extract and fractions of *Sacharum officinarum* in  
37 *Plasmodium berghei* infected mice. *Journal of Current Biomedical Research*.2022;  
38 2(4):317-337.
- 39
- 40 10. Edem UA, Udobang JA, Okokon JA.. Antiinflammatory and antipyretic activities  
41 of ethanol leaf extract of *Saccharum officinarum* in mice. *Journal of Medical and*  
42 *Pharmaceutical Sciences*.2023;10(8):29-36.
- 43
- 44 11. Edem UA, Okokon JE, Bassey AL, Iyanyi UL. Antiulcer activity of leaf extract of  
45 *Saccharum officinarum*. *Nigerian Journal of Pharmaceutical and Applied Sciences*  
46 *Research* 2023;12(2):22-27.

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12. Obidike IC, Amodu B, Emeje MO. Antimalarial properties of SAABMAL<sup>®</sup>: an ethnomedicinal polyherbal formulation for the treatment of uncomplicated malaria infection in the tropics. *Indian Journal of Medical Research*. 2015; 141(2): 221–227.

13. Akwetey GA, Achel DG. Ethnopharmacological use of herbal remedies for the treatment of malaria in the Dangme West District of Ghana. *Journal of Ethnopharmacology* 2010;129 (3): 367-376.

14. Singh A, Lal UR, Mukhtar HM, Singh PS, Shah G, Dhawan RK. Phytochemical profile of sugarcane and its potential health aspects. *Pharmacognosy Review*. 2015; 9(17): 45–54.

15. Coutinho ID, Baker JM, Ward JL, Beale MH, Creste S, Cavalheiro AJ. (2016). Metabolite profiling of sugarcane genotypes and identification of flavonoid glycosides and phenolic acids *Journal of Agriculture and Food Chemistry* 2016; 64,(21): 4198–4206.

16. Tepongning, R.N., Mbah, J.N., Avoulou, F.L., Jerme, M.M., Ndanga, E-K, K. and Fekam, F.B. . 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. Farah AO, Nooraain H, Noriham A, Azizah AH, Nurul HR. Acute and oral subacute toxicity study of ethanolic extract of *Cosmos caudatus* leaf in Sprague Dawley Rats. *International Journal of Biosciences, Biochemistry and Bioinformatics*. 2013; 3(4): 301-305.

18. Ping KY, Darah I, Chen Y, Sreeramanan S and Sasidharan S. Acute and subchronic toxicity study of *Euphorbia hirta* L. methanol extract in rats. *Biomedical Research International*. 2013;2: 1-14.