Journal of Complementary and Alternative Medical Research

Volume 26, Issue 1, Page 12-20, 2025; Article no.JOCAMR.128776
ISSN: 2456-6276

Histopathological Study of Subacute Administration of *Saccharum officinarum* Leaf Extract on Some Organs of Rat

Jude E. Okokon [a*], John A. Udobang [b], Chinyelu C. Osigwe [c], Uwaeme Ugonma Florence [c], Uduak P. Ise [d] and Utibe A. Edem [a]

[a] Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Uyo, Uyo, Nigeria.
[b] Department of Clinical Pharmacology and Therapeutics, Faculty of Basic Clinical Sciences, University of Uyo, Uyo, Nigeria.
[c] Department of Pharmacology and Toxicology, Faculty of Pharmacy, Madonna University, Elele, Rivers State, Nigeria.
[d] Department of Pharmacology and Toxicology, Faculty of Pharmacy, Bingham University, Karu, Nigeria.

Authors' contributions

This work was carried out in collaboration among all authors. Authors JEO and UAE did the research concept and design. Authors JEO and UAE did the animal studies. Authors did the JEO and JAU did the data analysis and interpretation. Authors JEO, UUF, CCO and JAU did the writing the article. Authors JEO, JAU, UUF, CCO and UAE read and approved the final manuscript.

Article Information

DOI: https://doi.org/10.9734/jocamr/2025/v26i1611

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/128776

Original Research Article

Received: 27/10/2024
Accepted: 29/12/2024
Published: 03/01/2025

**Corresponding author: E-mail: judeefiom@yahoo.com;*

Cite as: *Okokon, Jude E., John A. Udobang, Chinyelu C. Osigwe, Uwaeme Ugonma Florence, Uduak P. Ise, and Utibe A. Edem. 2025. "Histopathological Study of Subacute Administration of Saccharum Officinarum Leaf Extract on Some Organs of Rat". Journal of Complementary and Alternative Medical Research 26 (1):12-20. https://doi.org/10.9734/jocamr/2025/v26i1611.*

ABSTRACT

The leaves of *Saccharum officinarum* (Family-Poaceae) is employed traditionally for ethnotherapy of malaria and fever among others. Evaluation of subacute administration of *Saccharum officinarum* leaf extract for possible effect on organs of rats was carried out. The leaf extract (170, 340, 510 mg/kg body weight) was administered orally to Wistar rats of both sexes daily for 30 days and the rats were sacrificed after being subjected to light diethyl ether anesthesia at the completion of the administration. Subacute administration of *S. officinarum* leaf extract resulted in insignificant improvement in the treated rats body weights, but the weights of brain, heart, spleen, testis and ovary were not affected significantly ($p>0.05$) when compared to control. The leaf extract exerted toxic effects which were mild at low dose (170 mg/kg) and moderate at higher doses (340 and 510 mg/kg) on the histologies of brain, heart, spleen and ovary of rats, but no pathological sign was seen on the testis. The effects were found to increase with increasing dose. These could have resulted from the selective toxicity potential of the phytochemical constituents of the leaf extract resulting in localised toxicity of organs. Chronic study is advocated to investigate the consequences of prolonged treatment of rats with the leaf extract on organs and systems of rats.

Keywords: Saccharum officinarum; subacute; toxicity; organ weights.

1. INTRODUCTION

The ethnomedicinal use of plants in the therapy 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. Many plants employed as food or medicine demonstrate toxic potentials despite the claimed safety of their usage.

Saccharum officinarum (Family-Poaceae) also called sugarcane thrives in most countries of the world. Ethnomedically, the leaves of the plant are employed in the therapy of various diseases and ailment (Hartwell, 1967–1971). The leaf extract has been reported to possess some biological activities such as antimicrobial and effect on worms (Palaksha et al., 2013), lipid and blood glucose lowering activities (Ojewunmi et al., 2013), free radicals scavenging activity (Ojewunmi et al., 2013; Sun et al., 2014), effect on the kidney (Palaksha et al., 2015), depression and convulsion prevention (Okokon et al., 2019), antinociceptive (Okokon et al., 2021), antiplasmodial (Okokon et al., 2022), antioxidative stress and liver protective (Edem et al., 2022), anti-oedema and antipyretic (Edem et al., 2023), antiulcer (Edem et al., 2023) activities. SAABMAL®: a polyherbal preparation containing *S. officinarum* is utilised as malarial remedy in Nigeria (Obidike et al., 2015). The leaves are employed in Ghana for the treatment of malaria locally (Akwetey & Achel, 2010). some secondary metabolites suh as glycosides, saponins, tannins, flavonoids (Palaksha et al., 2015; Singh et al., 2015), as well as some flavones and phenolics and their derivatives have been detected and identified from the leaves of *S. officinarum* (Okokon et al., 2022; Coutinho et al., 2016). 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 *S. officinarum* leaf extract on histopathologies of some organs of rats is reported.

2. MATERIALS AND METHODS

2.1 Plant Materials

Saccharum officinarum fresh leaves were gotten from a compound in Uyo village, Uyo LGA, Akwa Ibom State, Nigeria, in the month of June, 2024. Identification and authentication of the leaves were carried out by a taxonomist in the Department of Botany and Ecological studies, University of Uyo, Uyo, Nigeria and a voucher number (UUPH 215b) was assigned and specimen deposited at Department of Pharmacognosy and Natural Medicine hebarium, Faculty of Pharmacy, University of Uyo.

2.2 Extraction

S. officinarum fresh leaves were processed for extraction by being washed, chopped to smaller pieces and shade-dried for 14 days. The

chopped dry leaves were further powdered using electric grinder. The leaf powder (2 kg) which was soaked for 72 hours in ethanol (50%) at room temperature was filtered, the liquid filtrate concentrated to dryness in *vacuo* 40 ˚C using a rotary evaporator (BuchiLab Switzerland). This was stored in a refrigerator at -4 ˚C, for the proposed experiments.

2.3 Animals

The Wistar rats, both male and female, gotten from University of Uyo Animal house and sheltered in plastic cages, were used in this study. They were well fed on Guinea Feed pellets and given unrestrained access to drinking water. Approval for the study was given by Faculty of Pharmacy Animal Ethics Committee, University of Uyo.

2.4 Sub Acute Toxicological Study

Male and female adult Wistar rats used in this experiment were weighed and randomly shared into four groups (n=6); groups I, II, III and IV. Based on previously established median lethal dose (LD_{50}) by Okokon et al. (2019), the leaf extract, 170, 340 and 510 mg/kg, was respectively administered orally to the rats in groups I, II, and III, daily for 30 days. Distilled water (10 mg/kg) was administered orally to rats in group IV for the same number of days. Twenty-four hours after the last administration, the animals were weighed again and subjected to light ethyl ether vapour before being sacrificed.

The effect of the extract on some organs were studied. The organs; spleen, brain, ovary, testis, and heart of rats were surgically isolated and placed in 10% formalin. The organs were passed through standard processes, sectioned and stained with hematoxylin and eosin (H&E) stain according to standard procedures at Department of Chemical Pathology, University of Uyo Teaching Hospital, Uyo. The slides were examined, read and interpreted by an histopathologist in the above department. Changes in the morphology were observed and recorded in the harvested organs of the sacrificed rats. Histological micrographs were also taken.

2.5 Data Analysis

One way analysis of variance (ANOVA) and Tukey's kramer multiple comparison post-test (Graph pad prism software Inc. La Jolla, CA, USA), were used to analyse data from this study. Significant values (mean ± SEM) relative to control were considered at $p<0.05$.

3. RESULTS

3.1 Effect of Subacute Treatment with Leaf Extract on Rats' Body Weights

The body weight changes resulting from *S. officinarum* leaf extract treatment of rats for 30 days is shown in Table 1. There was considerable increase in body weights of the *S. officinarum* extract-treated rats in all experimental 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 though insignificant ($p>0.05$) statistically (Table 1).

3.2 Effect of Subacute Administration of Rats with Leaf Extract on Organs' Weights

Oral administration of *S. officinarum* leaf extract (170-510 mg/kg) to rats for 30 days did not affect liver and kidney weights of rats significantly ($p>0.05$) relative to the control (Table 2).

Table 1. Effect of subacute treatment of rats with *S. officinarum* leaf extract on body weights

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

3.3 Effect of Subacute Administration of Leaf Extract on Histology of Organs

Figs. 1- 5 show the effects of subacute treatment of rats for 30 days with *S. officinarum* leaf extract 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 (Fig. 1). Treatment of rats with the extract at the dose of 170 mg/kg, did not affect the ovaries of treated rats, while higher doses (340 and 510 mg/kg) showed signs of moderate effects on the ovaries such as abnormal cyto-structure with infiltrating neutrophils in developing secondary follicle (Sf) and infiltrating neutrophils in atrophying follicle (Fig. 2). However, the leaf extract did not affect the testes of the treated rats adversely (Fig. 3). The leaf extract demonstrated moderate toxic effect on the cyto-structure of the spleen following subacute treatment with the leaf extract (170-510 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 (Fig. 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. (Fig. 5).

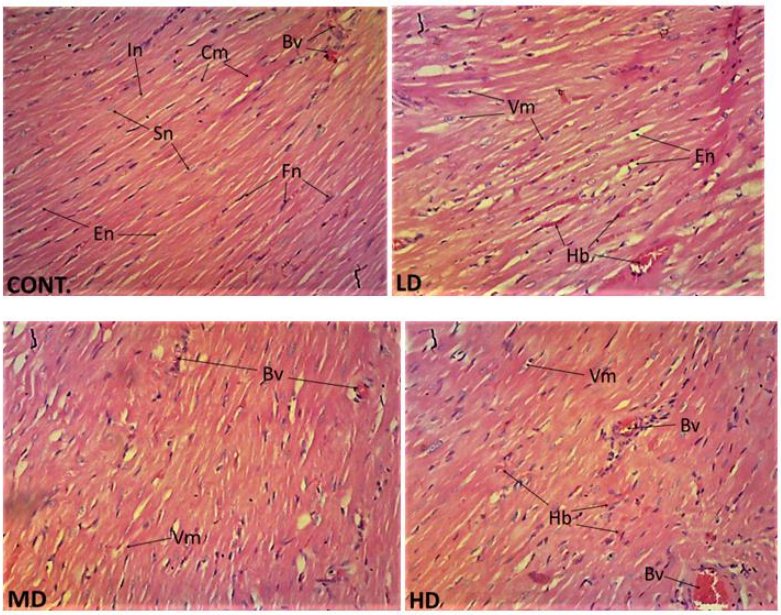

Fig. 1. Photomicrograph of the transverse sections of hearts of rats treated with distilled water (CONT), *S. officinarum* leaf extract at 170 mg/kg (LD), 340 mg/kg (MD) and 510 mg/kg (HD) heart tissue showing cardiac myocytes (Cm), intercalated disc (In), sarcoplasmic nuclei (Sn), fibrocyte nuclei (Fn)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

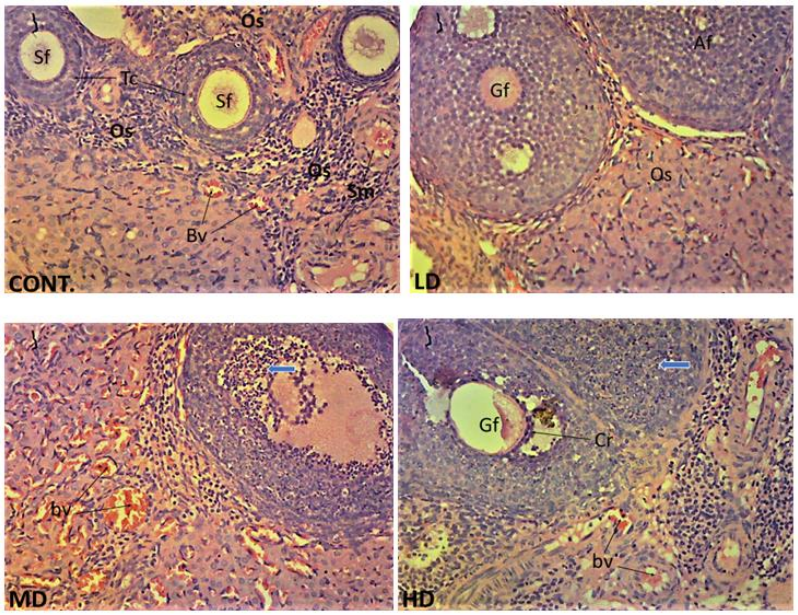

Fig. 2. Photomicrograph of the transverse sections of ovaries of rats treated with distilled water (CONT), *S. officinarum* leaf extract at 170 mg/kg (LD), 340 mg/kg (MD) and 510 mg/kg (HD) ovarin tissue showing secondary follicular cells (Sf) surrounded with Thecca cells (Tc), blood vessels (Bv) with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

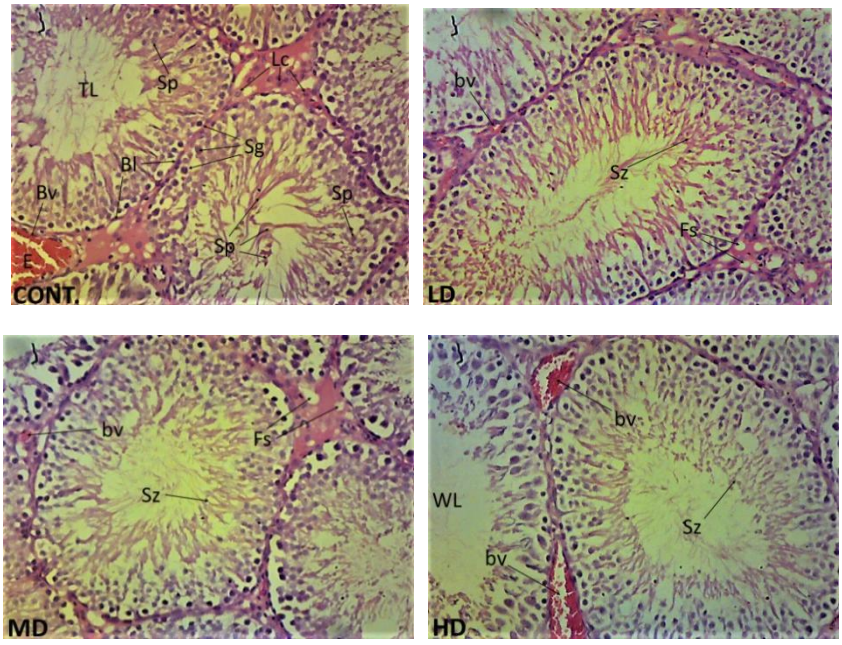

Fig. 3. Photomicrograph of the transverse sections of testes of rats treated with distilled water (CONT), *S. officinarum* leaf extract at 170 mg/kg (LD), 340 mg/kg (MD) and 510 mg/kg (HD) testicular tissues showing basement layers (Bl), spermatogonia cells (Sg), radiating spermatozoa (Sz) within the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

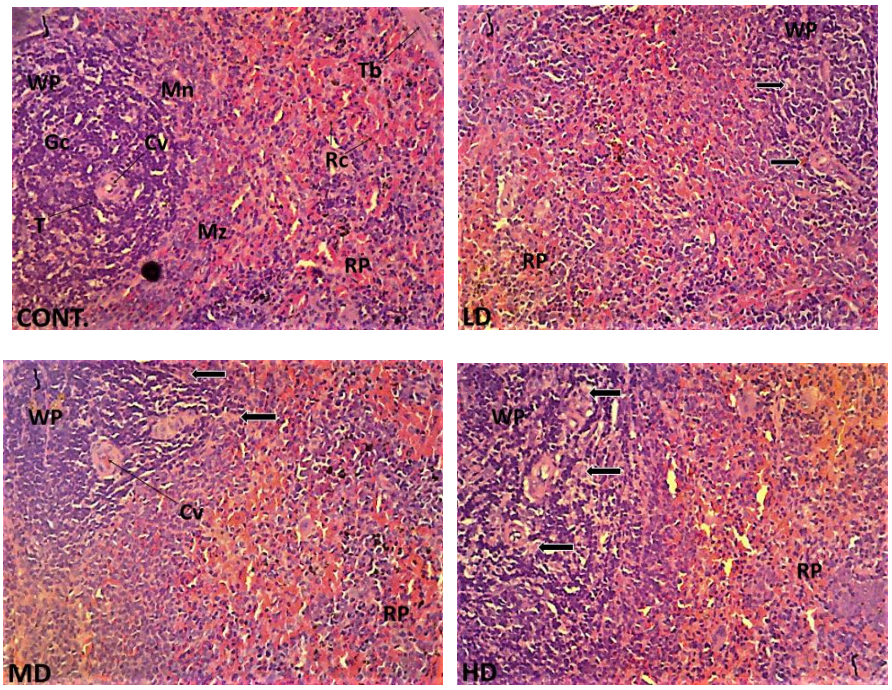

Fig. 4. Photomicrograph of the transverse sections of spleens of rats treated with distilled water (CONT), *S. officinarum* leaf extract at 170 mg/kg (LD), 340 mg/kg (MD) and 510 mg/kg (HD) spleen tissues showing white pulp (WP), germinal center (Gc), central vein (Cv), T-lymphocytes (T), mantle layer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

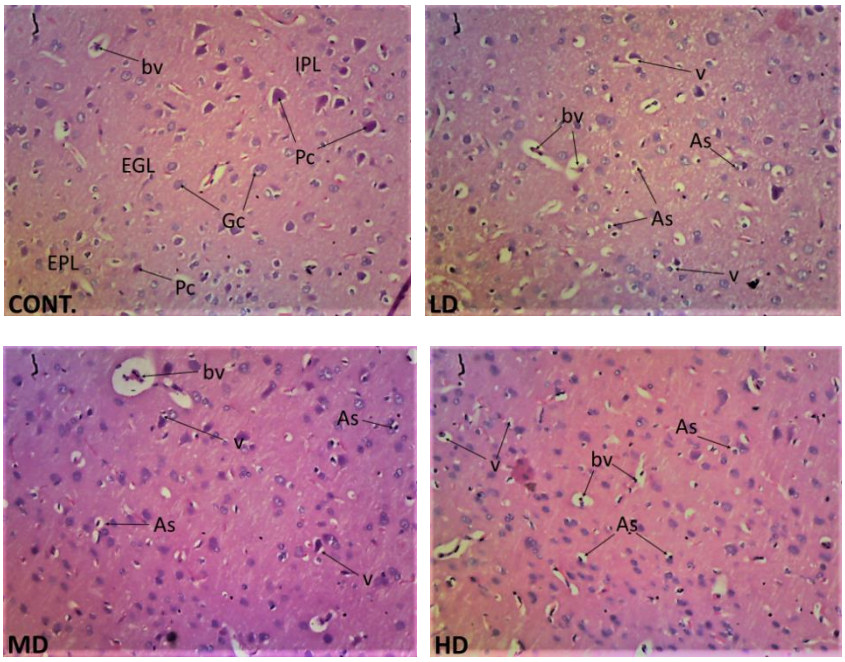

Fig. 5. Photomicrograph of the transverse sections of brain tissues of rats treated with distilled water (CONT), *S. officinarum* leaf extract at 170 mg/kg (LD), 340 mg/kg (MD) and 510 mg/kg (HD) brain tissues showing external pyramidal layer (EPL), external granular layer (EGL), internal pyramidal layer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

4. DISCUSSION

The findings of this study demonstrated that subacute administration of the leaf extract caused various degrees of weight gains in all the treatment groups which were insignificantly higher than that of control at higher doses (340 and 510 mg/kg) but significantly lower at the extract' dose of 170 mg/kg relative to control. Body weight changes are often used as a measure of adverse effects of drugs and other agents (Tepongning et al., 2018). The results of this study demonstrated that the leaf extract caused moderate improvement of body weights of rats in all treated groups though not different significantly ($p>0.05$) relative to that of the control group except at the low dose indicating that feeding habit of the rats did not change negatively as a result of the treatment with the extract, and hence body growth processes of rats were not adversely affected.

Repeated administration of leaf extract (170-510 mg/kg) to rats for 30 days did not cause any effect on the weights of heart, brain, spleen, pancreas, testes and ovary. However, statistically insignificant ($p>0.05$) elevation of spleen weights following treatment of rats with the leaf extract were observed relative to control. Generally, weights of internal organs are regarded as significant indicator of injury and toxicities (Farah et al., 2013). Abnormal enlargement of organs often reflects toxicity and damage to organ (Ping et al., 2013). This sometimes emanates from oedema resulting from inflammation of the organs with consequent 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 oral treatment of rats with *S. officinarum* leaf extract 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 extract doses (340 and 510 mg/kg), 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. The leaf extract following subacute administration, demonstrated moderate toxic effect on the cyto-structure of the spleen such as 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.

5. CONCLUSION

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

CONSENT

It is not applicable.

EHTICAL APPROVAL

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

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

ACKNOWLEDGEMENTS

The authors are grateful to University of Uyo management for providing enabling environment and Mr Nsikan Malachy of Pharmacology Department, University of Uyo for technical assistance.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

Akwetey, G. A., & Achel, D. G. (2010). Ethnopharmacological use of herbal remedies for the treatment of malaria in the Dangme West District of Ghana. *Journal of Ethnopharmacology*, 129(3), 367–376.

Coutinho, I. D., Baker, J. M., Ward, J. L., Beale, M. H., Creste, S., & Cavalheiro, A. J. (2016). Metabolite profiling of sugarcane genotypes and identification of flavonoid glycosides and phenolic acids. *Journal of Agricultural and Food Chemistry*, 64(21), 4198–4206.

Edem, U. A., Okokon, J. E., Bassey, A. L., & Iyanyi, U. L. (2023). Antiulcer activity of leaf extract of *Saccharum officinarum*. *Nigerian Journal of Pharmaceutical and Applied Sciences Research*, 12(2), 22–27.

Edem, U. A., Okokon, J. E., Bassey, A. L., & Okokon, P. J. (2022). Antioxidative stress and hepatoprotective activities of leaf extract and fractions of *Saccharum officinarum* in *Plasmodium berghei*-infected mice. *Journal of Current Biomedical Research*, 2(4), 317–337.

Edem, U. A., Udobang, J. A., & Okokon, J. A. (2023). Antiinflammatory and antipyretic activities of ethanol leaf extract of *Saccharum officinarum* in mice. *Journal of Medical and Pharmaceutical Sciences*, 10(8), 29–36.

Farah, A. O., Nooraain, H., Noriham, A., Azizah, A. H., & Nurul, H. R. (2013). Acute and oral subacute toxicity study of ethanolic extract of *Cosmos caudatus* leaf in Sprague Dawley rats. *International Journal of Biosciences, Biochemistry and Bioinformatics*, 3(4), 301–305.

Hartwell, J. L. (1967–1971). Plants used against cancer: A survey. *Lloydia*, 30–34.

Obidike, I. C., Amodu, B., & Emeje, M. O. (2015). Antimalarial properties of SAABMAL®: An ethnomedicinal polyherbal formulation for the treatment of uncomplicated malaria infection in the tropics. *Indian Journal of Medical Research*, 141(2), 221–227.

Ojewunmi, O., Oshodi, T., Ogundele, O., Micah, C., & Adenekan, S. (2013). Evaluation of the anti-diabetic and antioxidant activities of aqueous extracts of *Morinda lucida* and *Saccharum officinarum* leaves in alloxan-induced diabetic rats. *International Journal of Biochemistry Research and Review*, 3(3), 266–277.

Okokon, J. E., Davies, K., Edem, U. A., Bassey, A. L., & Udobang, J. A. (2021). Analgesic activity of ethanol leaf extract of *Saccharum officinarum*. *Tropical Journal of Natural Products Research*, 5(6), 1142–1145.

Okokon, J. E., Mobley, R., Edem, U. A., Bassey, A. L., Fadayomi, I., Horrocks, P., Drijfhout, F., & Li, W. W. (2022). In vitro and in vivo antimalarial activities and chemical profiling of sugarcane leaves. *Scientific Reports*, 41598, Article 14391. https://doi.org/10.1038/s41598-022-14391-8

Okokon, J. E., Udoh, A. E., Nyong, E. E., Eno, L., & Udo, N. M. (2019). Psychopharmacological studies on leaf extract of *Saccharum officinarum*. *Tropical Journal of Natural Products Research*, 3(2), 26–30.

Palaksha, M. N., Ravishankar, K., & Girijasastry, V. (2013). Phytochemical screening and evaluation of in-vitro antibacterial and anthelmintic activities of *Saccharum officinarum* leaf extracts. *World Journal of Pharmacy and Pharmaceutical Sciences*, 2(6), 5761–5768.

Palaksha, M. N., Ravishankar, K., & GirijaSastry, V. (2015). Biological evaluation of in vivo diuretic, and antiurolithiatic activities of ethanolic leaf extract of *Saccharum officinarum*. *Indo American Journal of Pharmaceutical Research*, 5(06), 2232–2238.

Ping, K. Y., Darah, I., Chen, Y., Sreeramanan, S., & Sasidharan, S. (2013). Acute and subchronic toxicity study of *Euphorbia hirta* L. methanol extract in rats. *Biomedical Research International*, 2, 1–14.

Singh, A., Lal, U. R., Mukhtar, H. M., Singh, P. S., Shah, G., & Dhawan, R. K. (2015). Phytochemical profile of sugarcane and its potential health aspects. *Pharmacognosy Review*, 9(17), 45–54.

Sun, J., He, X., Zhao, M., Li, L., Li, C., & Dong, Y. (2014). Antioxidant and nitrite-scavenging capacities of phenolic compounds from sugarcane (*Saccharum officinarum* L.) tops. *Molecules*, 19, 13147–13160.

Tepongning, R. N., Mbah, J. N., Avoulou, F. L., Jerme, M. M., Ndanga, E. K., & Fekam, F. B. (2018). Hydroethanolic extracts of *Erigeron floribundus* and *Azadirachta indica* reduced *Plasmodium berghei* parasitaemia in Balb/c mice. *Evidence-Based Complementary and Alternative Medicine*, 5156710. https://doi.org/10.1155/2018/5156710

Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle5.com/review-history/128776