

Ethanol Leaf Extract of *Synsepalum dulcificum* Schumach. & Thonn. (Sapotaceae) Induces Distortions in Renal, Hepatic and Reproductive Indices in Sprague-Dawley Rats

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ABSTRACT

Background and Purpose: *Synsepalum dulcificum* (Sapotaceae) is used in traditional medicine for treatment of diabetes, cough and cancer. This study was designed to investigate the *in vivo* toxicological endpoints of the ethanol leaf extract of *S. dulcificum* in male and female rats.

Methods: After estimating the LD₅₀ in mice, forty-eight rats were randomly assigned to four groups of twelve rats each in a sex ratio of 1:1 and were orally administered 10 mL/kg distilled water (control) and 118, 237, 475 mg/kg/day of the extract respectively for forty-five days. The animals were thereafter euthanized and blood samples collected for haematological, biochemical and antioxidant assays. Sperm parameters were evaluated and testis, liver, heart and ovary were harvested for histopathology. Constituents of the extract were determined by GC-MS.

Result: The LD₅₀ (oral) was estimated to be 4750 mg/kg in mice. There was a significant decrease in haemoglobin but lymphocytes and monocytes were significantly increased compared to control. Aspartate transaminase and alanine transaminase were significantly increased compared to control. Creatinine increased, high density lipoprotein (HDL) and albumin decreased significantly in treated rats. There was a significant increase in enzymatic and non-enzymatic antioxidants and a significant reduction in malondialdehyde (MDA). There were significant increases in reproductive markers of sperm count, motility and abnormality. Renovascular congestion and preserved cytoarchitecture in other organs were observed. Twenty-one compounds including n-hexadecanoic acid, oleic acid, 2,3-dihydro-3,5-dihydroxyl-6- methyl-4H-pyran-4-one (DDMP) and phytol were identified.

Conclusion: Prolonged use of the ethanol leaf extract of *S. dulcificum* could result in hepatic and renal dysfunctions and may increase the risk for male infertility and cardiovascular diseases.

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INTRODUCTION

Substances derived from herbs and herbal products constitute a large proportion of the conventional pharmacotherapeutic agents (Saad *et al.*, 2005). The increase in the use of herbal products especially in low- and middle-income economies is due to the perceived notion that these products are safe and effective (Awodele *et al.*, 2012). According to the World Health Organization (2005), herbal medicine can be referred to as herbal preparations and finished herbal products that contain whole plants, parts of plants or plant materials and/or their extracts which are utilized to confer therapeutic benefits in man and animals.

Synsepalum dulcificum Schumach & Thonn, Daniell (Sapotaceae) is commonly used locally for various disease conditions. It is also known as miracle berry and it is native to tropical West Africa. It grows up to 1.8 – 4.5 m in height and has a dense foliage. The leaves are 5 – 10 cm long and 2.0 – 3.7 cm wide (Inglett *et al.*, 1965; Akinmoladun *et al.*, 2020). *S. dulcificum* and all its component parts most especially the leaves, roots, barks, stems and fruits have been used in the management of a number of human diseases and infirmities (Akinmoladun *et al.*, 2020). In African traditional medicine, the leaves are used for the treatment of tuberculosis, malaria, asthma, diabetes and cancer (Oumorou *et al.*, 2010). It has also been reported that the fruit is used to sweeten sour foods and beverages while the bark is used for the treatment of erectile dysfunction (Fandohan *et al.*, 2017). Adaptation of *S. dulcificum* to various interventional roles in traditional medicine has resulted in many commercial pharmaceutical and food industry products (Akinmoladun, 2016). In spite of the many benefits of the plant, there is paucity of scientific information on its safety. Hence, this study was carried out to evaluate the toxicological indices following forty-five days administration of its ethanol leaf extract to Sprague-Dawley rats; and to identify the bioactive constituents of the extract.

MATERIALS AND METHODS

Collection and Identification of Plant Material

The leaf of *S. dulcificum* was collected in January 2019 from a forest in Ago-Iwoye, Ogun state, Nigeria by Mr. T.I. Adeleke of the Department of Pharmacognosy, Faculty of Pharmacy, University of Lagos and was duly authenticated by Dr. George Nodza at the Department of Botany, Faculty of Science, University of Lagos, Akoka, Nigeria where a voucher specimen (LUH 8417) was deposited.

Preparation of Extract

The leaves of *S. dulcificum* were air-dried to a constant weight and pulverised using a hammer mill and then weighed. A quantity (517 g) of the powder was macerated in 2.7 L of absolute ethanol for 72 h. It was then filtered using Whatman filter paper (125 mm pore size) and muslin cloth. The filtrate was dried at 40°C to a constant weight and was then concentrated using a rotary evaporator to yield a residue. The weight of the extract was measured and the percentage yield calculated using the formula:

$$\text{Percentage yield} = \frac{\text{weight of extract (g)}}{\text{weight of pulverized leaf sample (g)}} \times 100$$

Experimental Animals

Thirty Balb/c mice weighing 15 – 25 g and 48 male and female rats of ratio 1:1 weighing 150 – 200 g were procured from the Laboratory Animal Centre of the College of Medicine, University of Lagos, Lagos, Nigeria. The animals were kept under natural conditions of temperature and light and were fed with standard rodent chow (Livestock Feed Plc, Lagos, Nigeria) and clean water. They were acclimatized to the extant laboratory conditions for two weeks before the commencement of the study. Animal handling and procedures for experimentation in this study were in conformity with institutional ethical guidelines and the Guide for the Care and Use of Laboratory Animals published by the U.S. National Institute of Health (NIH Publication No. 85 – 23, revised 1996) for studies involving experimental animals.

Experimental Design

Acute Toxicity

The Miler and Tainter (1944) method as reported by Ikumawoyi *et al.* (2019) was adopted. Thirty Balb/c mice obtained for the study were randomly assigned to 6 groups of 5 mice each. They were fasted for 12 h and were administered doses of 500, 1000, 2000, 4000 and 5000 mg/kg *p.o.* They were first observed for 2 h for behavioural changes and mortality. Follow-up observations were made in 24 h and for a further 14 days for signs of delayed toxicity.

Sub-chronic Toxicity Study

Forty-eight rats were randomly allotted to 8 groups of 6 rats each. They were assigned as groups 1A, 1B, 2A, 2B, 3A, 3B and 4A, 4B. Animals in groups A were males while female animals were in groups B. Group 1 served as control and were administered 10 mL/kg distilled water while groups 2 – 4 were respectively administered 118, 237, and 475 mg/kg of ethanol leaf extract of *S. dulcificum* *p.o.* daily for 45 days. On termination of administration, animals were sacrificed by cervical dislocation. Blood

samples were obtained via the retro-orbital plexus of the eye into EDTA and lithium heparin bottles for biochemical and haematological assessments respectively. Blood samples were also collected into plain bottles for determination of oxidative stress indices. Sperm specimens were collected for reproductive viability assessment and vital organs were harvested for histopathology.

Biochemical Assessments

Liver and Renal Function Tests

The method reported by Awodele *et al.* (2014) was adopted. Blood samples collected into plain bottles were allowed to clot for 30 min, and serum was separated by centrifugation at 3000 rpm for 15 min. Serum samples were analyzed for aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), total bilirubin (T.BIL), total protein (T.PRO.), albumin (ALB), total cholesterol (T.CHOL), triglyceride (TG), creatinine (CREA) and urea using Roche Cobas commercial kits (Roche Diagnostics, Indianapolis, IN, USA) and a Roche-Hitachi 904 Chemistry Analyzer (MedTech Trade AG, Uster, Switzerland).

Oxidative Stress Indices

Reduced glutathione (GSH) was determined by the method of Sedlak and Lindsay (1968); glutathione peroxidase (GPx) was determined using the method of Hafemann *et al.* (1974); catalase (CAT) activity was assayed as described by Usuh *et al.* (2005); glutathione-S-transferase (GST) was determined according to the method of Habig *et al.* (1974); superoxide dismutase (SOD) activity was determined as described by Sun and Zigma (1978); while malondialdehyde (MDA) was determined using the method of Buege and Aust (1978).

Haematological Assessment

Red blood cell count, haemoglobin, haematocrit, platelet count, white blood cell count, percentage granulocyte, percentage lymphocyte, mean corpuscular haemoglobin concentration and percentage mean corpuscular haemoglobin concentration were determined using an automated haematology analyzer (Beckman Coulter, CA, USA).

Sperm Analysis

After sacrificing, male rats were strapped astride on their back on a dissecting board. A sterile surgical blade was used to make an incision on the right scrotum, and the right testis was removed with its ipsilateral epididymis into a beaker. The semen was expelled out of the epididymis into another beaker placed in a water bath at 36°C. Seminal fluid obtained from male animals across the different treatment groups were analyzed to determine

sperm motility, count and morphology using the methods of Cheesbrough (2000) and Ogli *et al.* (2009).

Sperm Motility

A drop (10 – 15 µL) of semen was placed on a slide such that the spermatozoa were evenly distributed and covered with a glass. Several fields of the specimen were properly examined using the ×40 objective of microscope. A total of 100 spermatozoa were counted and the number of motile cells was noted.

Sperm Count

A 1:20 dilution of semen was carried out with sodium bicarbonate-formalin (35% v/v formaldehyde) diluting fluid and thorough mixing was done. Using a Pasteur pipette, an improved Neubauer ruled chamber was filled with well-mixed diluted semen. After 5 min, the number of spermatozoa in an area of 2 mm² was counted using the ×10 objective of the microscope. The number of spermatozoa in 1 mL of fluid was calculated by multiplying the number counted by 100,000.

Sperm Morphology

A thin smear of the liquefied well-mixed semen was made on a slide. The smear was fixed with 95% (v/v) ethanol for 10 min while still wet and allowed to air-dry. The smear was washed with sodium bicarbonate-formalin (35% v/v formaldehyde) solution to remove possibly present mucus. The smear was then rinsed several times with water, covered with dilute (1:20) carbon fuchsin and allowed to stain for 3 min. The stain was then washed off with water. Counterstaining was done by covering the smear with dilute (1:20) Loeffler's methylene blue for 2 min. The stain was washed off with water, drained, and air-dried. The preparation was examined for normal and abnormal spermatozoa using the ×40 objective of the microscope. One hundred spermatozoa were counted, and the percentages showing normal and abnormal morphology were estimated.

Chromatographic Analysis

GC-MS analysis of the ethanol leaf extract of *S. dulcificum* was done using a Shimadzu GP-2010 gas chromatograph equipped with Rtx-5MS (30 m × 0.25 mm, 0.25 µm) column. Helium was used as the carrier gas at a flow rate of 1mL/min using an injection volume of 1.0 µL. The sample was injected in a split mode of 10:1. Mass spectral scan range was set at 35 – 550 (m/z). The injector temperature was kept at 250°C while the ion source temperature was at 200°C. The oven temperature was maintained at 40°C, and the interface temperature was at 250°C. Relative quantity of the identified compounds present in the extract was expressed as a percentage based on peak area produced in the chromatogram.

Statistical Analysis

Statistical analysis was done using One-Way Analysis of Variance (ANOVA) followed by Tukey's *post-hoc* using GraphPad Prism 6.0 (GraphPad Software, CA, USA). Results were considered significant at $P < 0.05$.

RESULTS**Oral Acute Toxicity in Mice**

There was no mortality observed at 500, 1000, 2000 and 4000 mg/kg *p.o.* However, at 5000 mg/kg, 60% mortality was observed. Behavioural signs of toxicity observed included piloerection, skeletal muscle relaxation, tachypnoea and sedation. The LD₅₀ was estimated to be 4750 mg/kg.

Effects of 45 Days Administration of Ethanol Leaf Extract of *S. dulcificum***Haematological Parameters**

Table 1 shows that in male rats, lymphocytes were significantly ($P < 0.05$) increased at 237 mg/kg/day and 475 mg/kg/day compared to control. Haemoglobin (HGB) was significantly ($P < 0.05$) reduced at 237 mg/kg/day compared to control. All other parameters were not significantly different from controls.

In female rats, monocytes were significantly ($P < 0.05$) increased at 118 mg/kg/day, 237 mg/kg/day and 475 mg/kg/day compared to control. Mean corpuscular haemoglobin concentration (MCHC) was significantly ($P < 0.05$) increased at 475 mg/kg/day compared to control (Table 2).

Hepatic and Renal Function

Table 3 shows aspartate transaminase (AST) was not significantly increased at 118 mg/kg/day and 237 mg/kg/day but significantly ($P < 0.05$) increased at 475 mg/kg/day compared to control. There was non-significant increase in alkaline phosphatase (ALP) at 118 mg/kg/day, 237 mg/kg/day and 475 mg/kg/day compared to control.

Total bilirubin was significantly ($P < 0.0001$) increased at 237 mg/kg/day and 475 mg/kg/day ($P < 0.01$) compared to control. Creatinine was not significantly increased at 118 mg/kg/day, 237 mg/kg/day and 475 mg/kg/day compared to control.

In female rats (Table 4), AST was not significantly increased at 118 mg/kg/day, 237 mg/kg/day but significantly ($P < 0.05$) increased at 475 mg/kg/day. Creatinine was non-significantly increased at 118 mg/kg/day, 237 mg/kg/day and 475 mg/kg/day compared to control.

Lipid Profile

In male rats, HDL was significantly ($P < 0.05$) reduced at 475 mg/kg/day compared to control. The values of T. CHOL, LDL and TG were not significantly different from controls (Table 5).

In female rats, T. CHOL, HDL, LDL and TG were non-significantly different from controls (Table 6).

Sperm Parameters

In Table 7, there was significant ($P < 0.001$) increase in sperm motility at 118 mg/kg/day, 237 mg/kg/day and 475 mg/kg/day compared to control. Sperm count was significantly ($P < 0.01$) increased at 118 mg/kg/day, 237 and 475 mg/kg/day compared to control. There was significant ($P < 0.05$) increase in percentage abnormality at 118 mg/kg/day and at 475 mg/kg/day compared to control.

Table 1: Effect of 45 days administration of ethanol leaf extract of *S. dulcificum* on haematological parameters in male rats

Dose	WBC ($\times 10^9$ /L)	RBC ($\times 10^{12}$ /L)	LYM (%)	MON (%)	GRAN (%)	HGB (g/dL)	HCT (%)	MCV (fL)	MCH (pg)	MCHC (%)	PLT ($10^5/\mu\text{L}$)
10 mL/kg DW	7.00 $\pm$ 2.60	7.93 $\pm$ 0.42	37.05 $\pm$ 0.55	20.50 $\pm$ 2.70	46.80 $\pm$ 3.80	14.80 $\pm$ 0.35	50.57 $\pm$ 2.28	63.57 $\pm$ 0.93	18.57 $\pm$ 0.64	28.80 $\pm$ 0.90	661.00 $\pm$ 10.00
118 mg/kg SD	14.60 $\pm$ 4.10	8.36 $\pm$ 0.31	42.30 $\pm$ 2.80	20.45 $\pm$ 1.55	43.10 $\pm$ 1.50	15.03 $\pm$ 0.32	55.20 $\pm$ 0.97	66.33 $\pm$ 2.97	17.93 $\pm$ 0.46	27.60 $\pm$ 0.70	785.51 $\pm$ 6.50
237 mg/kg SD	7.50 $\pm$ 1.30	7.39 $\pm$ 0.21	54.00 $\pm$ 1.50*	33.85 $\pm$ 0.85	33.85 $\pm$ 0.85	13.77 $\pm$ 0.68*	52.43 $\pm$ 1.41	71.07 $\pm$ 1.70	19.17 $\pm$ 0.22	27.70 $\pm$ 0.20	725.05 $\pm$ 6.00
475 mg/kg SD	7.85 $\pm$ 0.25	7.78 $\pm$ 0.11	52.25 $\pm$ 3.65*	14.85 $\pm$ 0.75	31.05 $\pm$ 0.25	14.43 $\pm$ 0.60	54.07 $\pm$ 2.04	69.47 $\pm$ 2.12	18.50 $\pm$ 0.51	27.95 $\pm$ 0.25	840.56 $\pm$ 7.50

* $P < 0.05$ versus 10 mL/kg DW (Control). Results are mean $\pm$ SEM, n=6, One-Way ANOVA followed by Tukey's *post hoc* test. DW; distilled water, SD; *S. dulcificum*, WBC; white blood cell, RBC; red blood cells, LYM; lymphocytes, MON; monocytes, GRAN granulocytes, HGB; haemoglobin, HCT; haematocrit, MCV; mean cell volume, MCH; mean corpuscular haemoglobin, MCHC; mean corpuscular haemoglobin concentration, PLT; platelet count.

Table 2: Effect of 45 days administration of ethanol leaf extract of *S. dulcificum* on haematological parameters in female rats

Dose	WBC ($\times 10^9$ /L)	RBC ($\times 10^{12}$ /L)	LYM (%)	MON (%)	GRAN (%)	HGB (g/dL)	HCT (%)	MCV (fL)	MCH (pg)	MCHC (%)	PLT ($10^5/\mu\text{L}$)
10 mL/kg DW	6.65 $\pm$ 1.65	8.63 $\pm$ 0.09	45.40 $\pm$ 4.60	10.90 $\pm$ 1.70	39.55 $\pm$ 7.05	14.97 $\pm$ 0.18	54.85 $\pm$ 1.75	65.10 $\pm$ 4.10	17.45 $\pm$ 0.65	26.40 $\pm$ 0.67	891.00 $\pm$ 41.00
118 mg/kg SD	9.45 $\pm$ 1.95	9.05 $\pm$ 0.23	35.90 $\pm$ 6.80	28.25 $\pm$ 0.55*	39.15 $\pm$ 3.95	15.57 $\pm$ 0.65	56.35 $\pm$ 3.25	63.55 $\pm$ 0.25	17.75 $\pm$ 0.35	28.07 $\pm$ 0.39	710.00 $\pm$ 127.00
237 mg/kg SD	9.55 $\pm$ 0.55	8.45 $\pm$ 0.04	39.75 $\pm$ 2.15	26.80 $\pm$ 3.20*	35.05 $\pm$ 0.55	13.93 $\pm$ 0.69	51.50 $\pm$ 2.70	64.25 $\pm$ 0.35	18.15 $\pm$ 0.15	28.50 $\pm$ 0.31	761.50 $\pm$ 43.50
475 mg/kg SD	12.45 $\pm$ 5.75	7.48 $\pm$ 0.32	41.25 $\pm$ 4.85	30.60 $\pm$ 3.40*	32.15 $\pm$ 5.45	11.93 $\pm$ 2.42	48.35 $\pm$ 1.05	64.75 $\pm$ 1.35	19.05 $\pm$ 0.35	29.27 $\pm$ 0.73*	591.50 $\pm$ 13.50

* $P < 0.05$ versus 10 mL/kg DW. Results are mean $\pm$ SEM, n=6, one-way ANOVA followed by Tukey's *post hoc* test. DW; distilled water, SD; *S. dulcificum*, WBC; white blood cell, RBC; red blood cell, LYM; Lymphocytes, MON; monocytes, GRAN; granulocytes, HGB; haemoglobin, HCT; haematocrit, MCV; mean cell volume; MCH; mean corpuscular haemoglobin, MCHC; mean corpuscular haemoglobin concentration, PLT; platelet count.

Table 3: Effect of 45 days administration of ethanol leaf extract of *S. dulcificum* on biomarkers of hepatic and renal functions in male rats

Dose	ALT (U/L)	AST (U/L)	ALP (U/L)	T.PRO (g/dL)	ALB (g/L)	T.BIL (mmol/L)	UREA (mg/dL)	CREA (mg/dL)
10 mL/kg DW	52.37±2.89	160.60±12.91	242.00±29.43	79.54±1.40	79.54±1.40	0.93±0.23	6.03±0.10	77.07±8.62
118 mg/kg SD	46.63±2.86	163.70±15.48	289.30±5.39	86.42±1.85	45.28±3.42	1.03±0.03	6.03±0.05	79.90±1.75
237 mg/kg SD	45.43±1.16	202.20±11.26	288.60±6.63	79.12±1.99	45.28±3.42	2.47±0.03****	5.13±0.47	83.30±2.44
475 mg/kg SD	47.13±10.67	222.60±2.07**	291.60±7.11	82.88±4.14	40.04±0.19	2.40±0.00**	7.07±0.99	90.17±2.95

** $P < 0.01$, **** $P < 0.0001$ versus 10 mL/kg DW. Results are mean $\pm$ SEM, n=6, One-Way ANOVA followed by Tukey's *post hoc* test. DW; distilled water, SD; *S. dulcificum*, ALT; alanine aminotransferase, AST; aspartate aminotransferase, ALP; alkaline phosphatase, T.PRO; total protein, ALB; albumin, T.BIL; total bilirubin, CREA; creatinine.

Table 4: Effect of 45 days administration of ethanol leaf extract of *S. dulcificum* on biomarkers of hepatic and renal function in female rats

Dose	ALT (U/L)	AST (U/L)	ALP (U/L)	T.PRO (g/dL)	ALB (g/L)	T.BIL (mmol/L)	UREA (mg/dL)	CREA (mg/dL)
10 mL/kg DW	63.93±1.94	185.40±4.14	280.80±11.49	77.00±7.90	47.60±3.27	1.13±0.27	7.03±0.78	74.90±5.70
118 mg/kg SD	69.40±10.58	214.60±16.51	298.00±13.90	78.80±4.62	36.90±1.91	1.07±0.18	5.67±0.52	82.90±0.10
237 mg/kg SD	60.80±5.00	264.40±20.13	267.90±16.97	81.50±4.07	43.07±3.17	1.60±0.24	6.13±0.03	85.20±0.10
475 mg/kg SD	75.53±6.34	328.40±48.45*	257.10±7.54	76.47±2.56	47.37±3.45	1.37±0.35	7.43±0.35	84.45±3.95

* $P < 0.05$ versus 10 mL/kg DW. Results are mean $\pm$ SEM, n=6, One-Way ANOVA followed by Tukey's *post hoc* test. DW; distilled water, SD; *S. dulcificum*, ALT; alanine aminotransferase, AST; aspartate aminotransferase, ALP; alkaline phosphatase, T.PRO; total protein, ALB; albumin, T.BIL; total bilirubin, CREA; creatinine.

Table 5: Effect of 45 days administration of ethanol leaf extract of *S. dulcificum* on lipid profile in male rats

Dose	T. CHOL (mg/dL)	HDL (mg/dL)	LDL (mg/dL)	TG (mg/dL)
10 mL/kg DW	2.39±0.09	1.44±0.21	0.66±0.34	0.79±0.02
118 mg/kg SD	2.45±0.10	1.26±0.06	0.93±0.03	0.65±0.23
237 mg/kg SD	2.43±0.17	0.69±0.11	0.53±0.09	0.77±0.07
475 mg/kg SD	2.17±0.14	0.50±0.29*	0.50±0.09	0.79±0.15

* $P<0.05$ versus 10 mL/kg DW. Results are mean $\pm$ SEM, $n=6$, One-Way ANOVA followed by Tukey's *post hoc* test. DW; distilled water, SD; *S. dulcificum*, T. CHOL; total cholesterol, HDL; high density lipoprotein, LDL; low density lipoprotein, TG; triglyceride.

Table 6: Effect of 45 days administration of ethanol leaf extract of *S. dulcificum* on lipid profile in female rats

Dose	T. CHOL (mg/dL)	HDL (mg/dL)	LDL (mg/dL)	TG (mg/dL)
10 mL/kg DW	2.39±0.09	1.44±0.21	0.66±0.34	0.79±0.02
118 mg/kg SD	2.45±0.10	1.26±0.06	0.93±0.03	0.65±0.23
237 mg/kg SD	2.43±0.17	0.69±0.11	0.53±0.09	0.77±0.07
475 mg/kg SD	2.17±0.14	0.50±0.29*	0.50±0.09	0.79±0.15

No significant difference versus 10 mL/kg DW. Results are mean $\pm$ SEM, $n=6$, One-Way ANOVA followed by Tukey's *post hoc* test. DW; distilled water, SD; *S. dulcificum*, T. CHOL; total cholesterol, HDL; high density lipoprotein, LDL; low density lipoprotein, TG; triglyceride.

Table 7. Effect of 45 days administration of ethanol stem bark extract of *S. dulcificum* on reproductive parameters in male rats

Dose	Motility (%)	Count ($\times 10^6$)	Morphology (% Abnormality)
10 mL/kg DW	66.50±7.79	51.35±2.25	11.00±0.00
118 mg/kg SD	100.00±0.00***	67.02±0.00**	17.00±2.00*
237 mg/kg SD	97.50±1.44***	70.75±1.35**	4.50±0.50*
475 mg/kg SD	95.00±2.89**	68.90±0.40**	19.50±0.50*

* $P<0.05$, ** $P<0.01$, *** $P<0.001$ versus 10 mL/kg DW. Results are mean $\pm$ SEM, $n=6$, One-Way ANOVA followed by Tukey's *post hoc* test. SD; *S. dulcificum*.

Antioxidant enzymes

Figures 1-6 show that in male rats, there was non-significant increase in GSH at 118 mg/kg/day compared to control. There was significant ($P<0.05$) increase in SOD at 118 mg/kg/day, 237 mg/kg/day and 475 mg/kg/day compared to control. CAT was significantly ($P<0.05$)

increased at 237 mg/kg/day and 475 mg/kg/day ($P<0.001$) compared to control. GPx was significantly ($P<0.01$) increased at 118 mg/kg/day and significantly ($P<0.05$) reduced at 237 mg/kg/day compared to control. MDA was not significantly different from control.

In female rats, GSH was non-significantly increased at 237 mg/kg/day and 475 mg/kg/day compared to control (Figures 7-12). SOD was not significantly increased at 118 mg/kg/day, 237 mg/kg/day and 475 mg/kg/day compared to control. CAT was significantly ($P<0.001$) increased at 118 mg/kg/day and 475 mg/kg/day compared to control. MDA was significantly ($P<0.05$) increased at 118 mg/kg/day and 237 mg/kg/day compared to control.

Histology

In the kidneys of male rats, there were normocellular glomerular tufts disposed on a background containing viable tubules in control animals while there were congested blood vessels in animals administered the extract at all doses (Figure 13a-d).

Figure 14a-d shows that in female rats, the kidney showed normocellular glomerular tufts disposed on a background containing renal tubules and no abnormalities in control and in animals administered 118 mg/kg/day of the extract but at 237 mg/kg/day and 475 mg/kg/day, congested blood vessels were observed. There were no abnormalities observed in the testis, liver, heart and ovary.

Gas chromatography-Mass Spectrometry Analysis

The GC-MS chromatogram of the ethanol leaf extract of *S. dulcificum* including the retention times and percentage peak areas of the compounds are shown in Table 8 and Figure 15.

Twenty-one compounds were identified in the extract. The most abundant constituents are N-hexadecanoic acid (25.84%), oleic acid (17.37%), 2,3-dihydro-3,5-dihydroxyl-6-methyl-4H-pyran-4-one (6.76%), phytol (6.25%) and hexadecanoic acid methyl ester (4.49%). Others include 9-octadecenoic acid (3.74%), myristoyl chloride (3.71%), 1-ethyl-2-pyrrolidinone (3.53%), pentadecanoic acid (3.51%), squalene (3.38%), methyl stearate (2.92%), 9,12-octadecandien-1-ol (2.81%), 2,3-dibenzofuran (1.99%), 1,3-dioxolane-2,4,5-trimethyl acetic acid (1.94%), neophytadiene (1.91%), octadecanoic acid (1.77%), ethyl-9,12,15-octadecatrienoate (1.60%), 9,12-octadecadienoic acid (1.44%), N-acetyl pyridine (1.38%), allantoinic acid (1.08%) and cis-13-octadecenoic acid (0.98%).

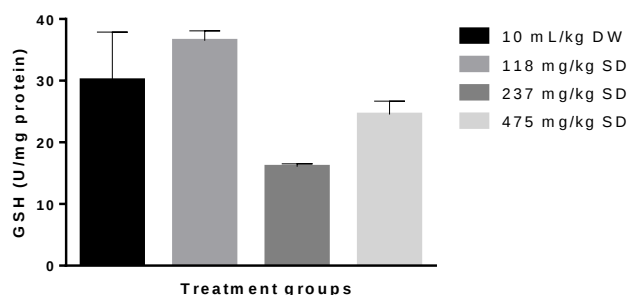


Figure 1: Effect of 45 days administration of ethanol leaf extract of *S. dulcificum* on GSH level in male rats. No significant difference versus 10 mL/kg DW. Results are mean $\pm$ SEM, (n=6), One-way ANOVA followed by Tukey's *post hoc* test. GSH; reduced glutathione, DW; distilled water, SD; *S. dulcificum*.

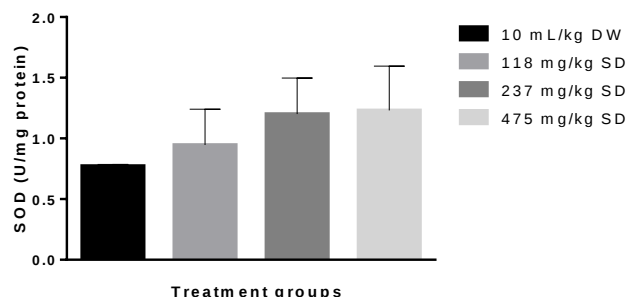


Figure 2: Effect of 45 days administration of ethanol leaf extract of *S. dulcificum* on SOD level in male rats. No significant difference versus 10 mL/kg DW. Results are mean $\pm$ SEM, (n=6), One-Way ANOVA followed by Tukey's *post hoc* test. SOD; superoxide dismutase, DW; distilled water, SD; *S. dulcificum*.

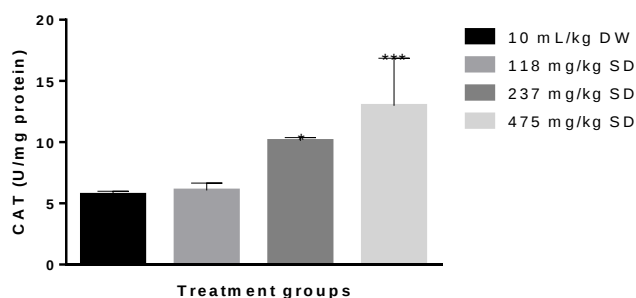


Figure 3: Effect of 45 days administration of ethanol leaf extract of *S. dulcificum* on CAT level in male rats. * $P<0.05$, *** $P<0.01$ versus 10 mL/kg DW. Results are mean $\pm$ SEM, (n=6), One Way ANOVA followed by Tukey's *post hoc* test. CAT; catalase, DW; distilled water, SD; *S. dulcificum*.

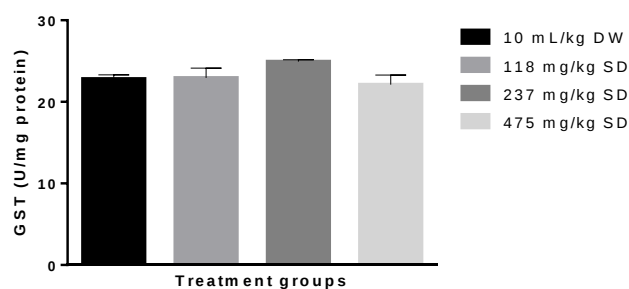


Figure 4: Effect of 45 days administration of ethanol leaf extract of *S. dulcificum* on GST level in male rats. Results are mean $\pm$ SEM. (n=6) No significant difference versus 10 mL/kg DW. One Way ANOVA followed by Tukey's *post hoc* test. GST; glutathione-S-transferase, DW; distilled water, SD; *S. dulcificum*.

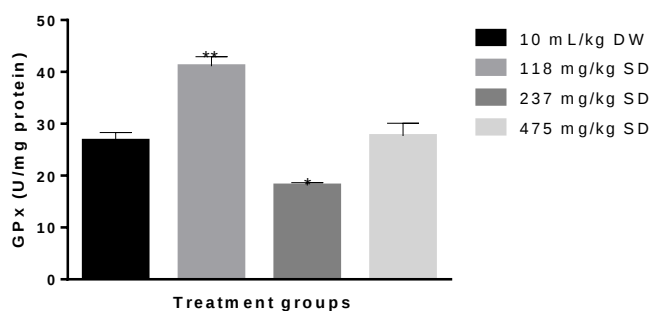


Figure 5: Effect of 45 days administration of ethanol leaf extract of *S. dulcificum* on glutathione peroxidase level in male rats. * $P<0.05$, ** $P<0.01$ versus 10 mL/kg DW. Results are mean $\pm$ SEM, (n=6), One-Way ANOVA followed by Tukey's *post hoc* test. GPx; glutathione peroxidase, DW; distilled water, SD; *S. dulcificum*.

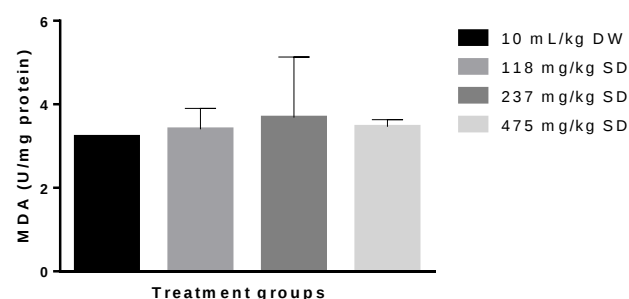


Figure 6: Effect of 45 days administration of ethanol leaf extract of *S. dulcificum* on MDA level in male rats. No significant difference versus 10 mL/kg DW. Results are mean $\pm$ SEM, (n=6), One-Way ANOVA followed by Tukey's *post hoc* test. MDA; malondialdehyde, DW; distilled water, SD; *S. dulcificum*.

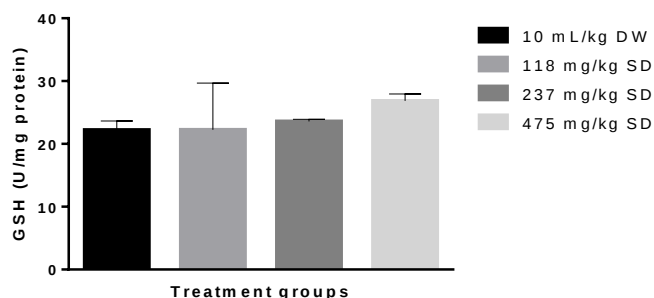


Figure 7: Effect of 45 days administration of ethanol leaf extract of *S. dulcificum* on GSH level in female rats. No significant difference versus 10 mL/kg DW. Results are mean $\pm$ SEM, (n=6), One-Way ANOVA followed by Tukey's *post hoc* test. GSH; reduced glutathione, DW; distilled water, SD; *S. dulcificum*.

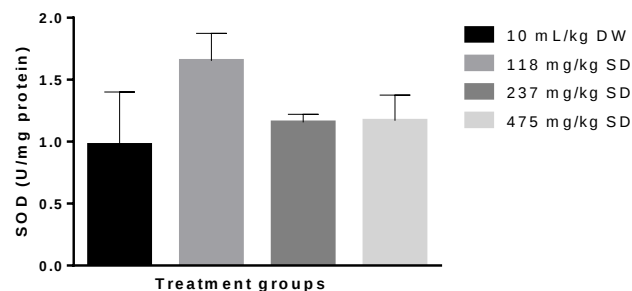


Figure 8: Effect of 45 days administration of ethanol leaf extract of *S. dulcificum* on SOD level in female rats. No significant difference versus 10 mL/kg DW. Results are mean $\pm$ SEM, (n=6), One-Way ANOVA followed by Tukey's *post hoc* test. SOD; superoxide dismutase, DW; distilled water, SD; *S. dulcificum*.

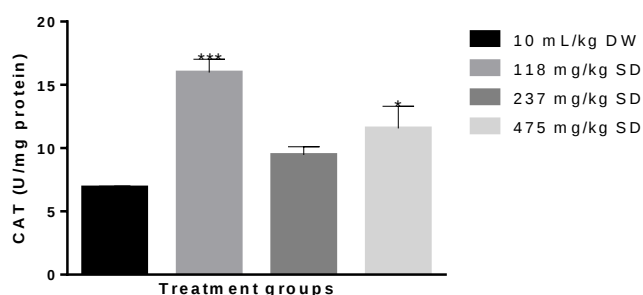


Figure 9: Effect of 45 days administration of ethanol leaf extract of *S. dulcificum* on CAT level in female rats. * $P<0.05$, *** $P<0.001$ versus 10 mL/kg DW. One-Way ANOVA followed by Tukey's *post hoc* test. CAT; catalase, DW; distilled water, SD; *S. dulcificum*.

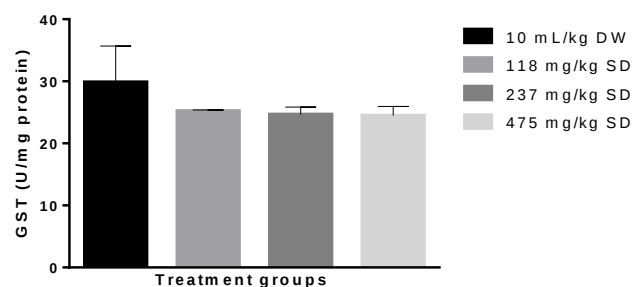


Figure 10: Effect of 45 days administration of ethanol leaf extract of *S. dulcificum* on GST level in female rats. No significant difference versus 10 mL/kg DW. Results are mean $\pm$ SEM, (n=6), One-Way ANOVA followed by Tukey's *post hoc* test. GST; glutathione-S-transferase, DW; distilled water, SD; *S. dulcificum*.

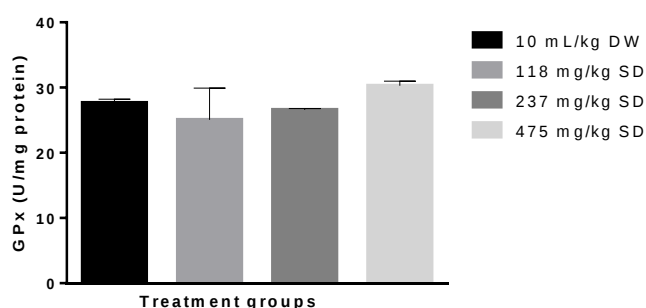


Figure 11: Effect of 45 days administration of ethanol leaf extract of *S. dulcificum* on GPx level in female rats. No significant difference versus 10 mL/kg DW. Results are mean $\pm$ SEM, (n=6), One-Way ANOVA followed by Tukey's *post hoc* test. GPx; glutathione peroxidase, DW; distilled water, SD; *S. dulcificum*.

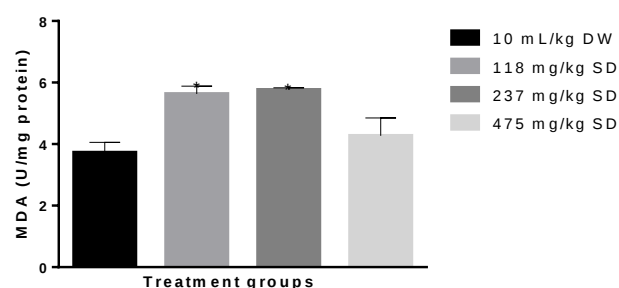


Figure 12: Effect of 45 days administration of ethanol leaf extract of *S. dulcificum* on MDA level in female rats. * $P<0.05$ versus 10 mL/kg DW. Results are mean $\pm$ SEM, (n=6), One-Way ANOVA followed by Tukey's *post hoc* test. MDA; malondialdehyde, DW; distilled water, SD; *S. dulcificum*.

DISCUSSION

This study was conducted to investigate the toxicological profile of the ethanol leaf extract of *S. dulcificum* following acute and 45 days repeated exposure in rodents. On acute administration to mice, behavioural signs of

toxicity elicited by the extract included piloerection, skeletal muscle relaxation, tachypnoea and sedation. The LD₅₀ oral was estimated to be 4786 mg/kg. This portends that the extract is slightly toxic on acute oral exposure in mice according to the Hodge and Sterner (2005) toxicity scale.

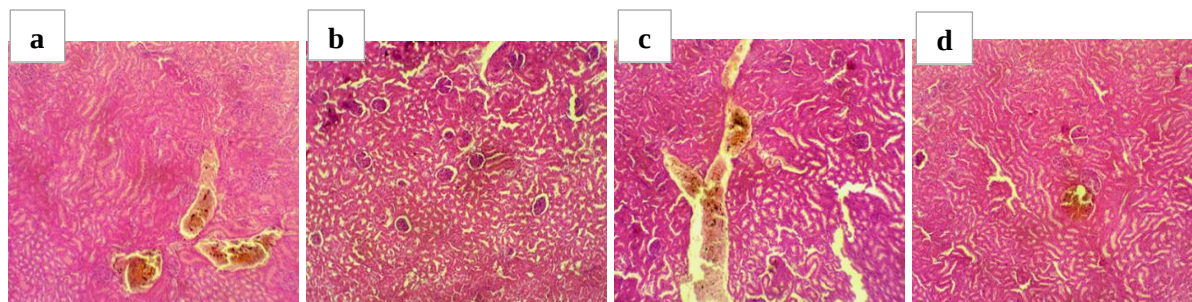


Figure 13a-d: Histological presentation of the kidney of control and *S. dulcificum* treated male rats. **a. 10 mL/kg DW** – showing normocellular glomerular tufts disposed on a background containing renal tubules and no abnormalities **b. 118 mg/kg SD** – showing normocellular glomerular tufts disposed on a background containing viable tubules and congested blood vessels. **c. 237 mg/kg SD** – showing normocellular glomerular tufts disposed on a background containing viable tubules and congested blood vessels. **d. 475 mg/kg** – showing normocellular glomerular tufts disposed on a background containing viable tubules and congested blood vessels.

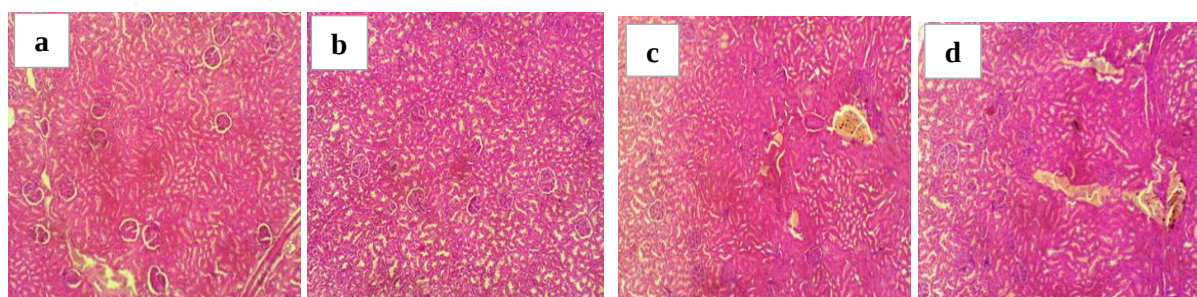


Figure 14a-d: Histological presentation of the kidney of control and *S. dulcificum* treated female rats **a. 10 mL/kg DW** – showing normocellular glomerular tufts disposed on a background containing renal tubules and no abnormalities **b. 118 mg/kg SD** – showing normocellular glomerular tufts disposed on a background containing viable tubules and no abnormalities. **c. 237 mg/kg SD** – showing normocellular glomerular tufts disposed on a background containing viable tubules and congested blood vessels. **d. 475 mg/kg** – showing normocellular glomerular tufts disposed on a background containing viable tubules and congested blood vessels.

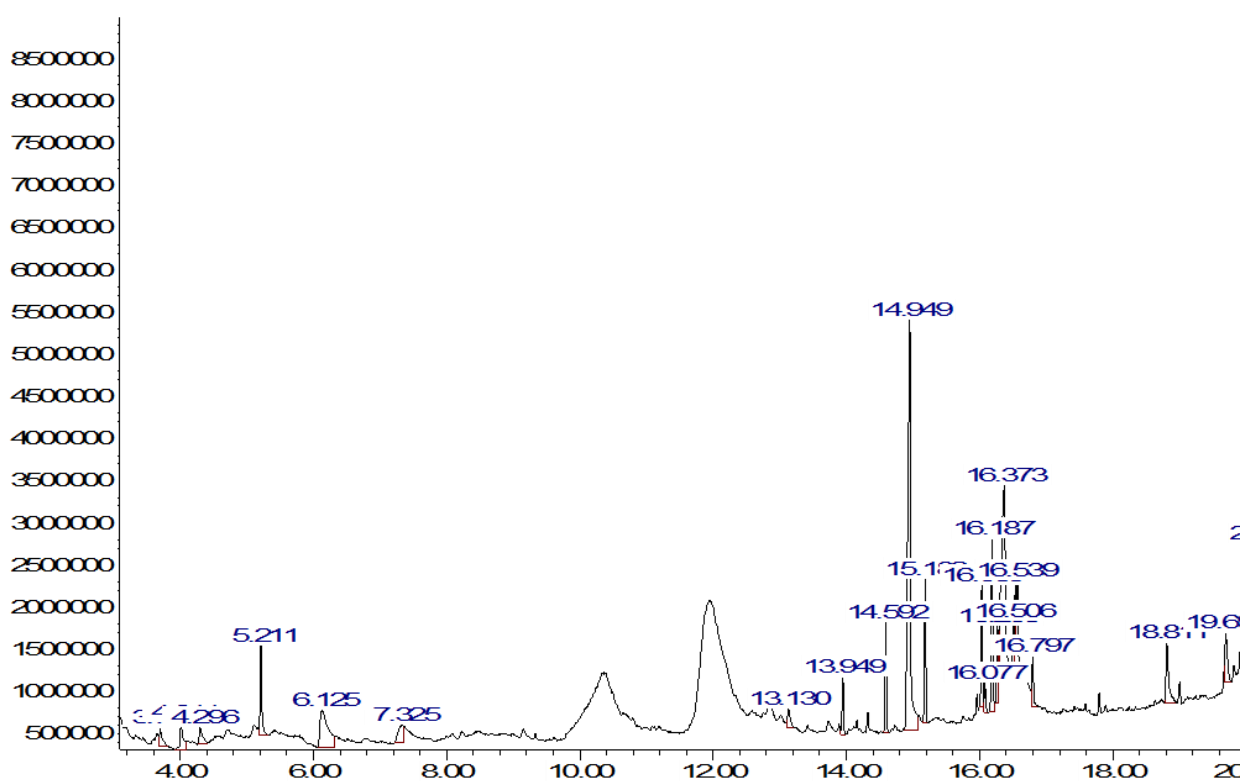
Findings from haematological assessment showed a significant increase in lymphocytes and reduction in haemoglobin compared to control. Monocytes and MCHC were significantly increased compared to control. The increase in monocytes portends a haematological insult which may be induced by extract administration while the observed increase in MCHC suggests that the extract could stimulate the production of RBCs but could negatively alter HGB production demonstrated by the observed reduction reported (Ashafa et al., 2011; Jorum et al., 2016).

Enzymes such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) are sensitive markers used to detect diseases of the liver and bone because they are cytoplasmic and are released into circulation after cellular damage. Increase in AST is secondary to hepatic insult which may be as a result of disruption to hepatocytes membrane architecture elicited by toxicants (Olson et al.,

2000). ALP is used as a biomarker for plasma membrane integrity of tissues (Khailova et al., 2020). Serum ALP is an osteogenic marker (Wu et al., 2017) and is elevated as a consequence of bone disease (Sarac and Saygili, 2014). In this study, AST and ALP were significantly increased compared to control. This suggests a perturbatory effect of the extract on hepatocytes cytoskeleton and predictive osteotoxic effect (Ahmad et al., 2002; Giannini et al., 2005; Davidson et al., 2019; Khailova et al., 2020). Bilirubin is the product of haemoglobin catabolism. Bilirubin levels increase when the excretory capacity of the liver has significantly diminished (Giannini et al., 2005). It was observed in this study that total bilirubin was significantly increased compared to control. This phenomenon correlates with the suggestive hepatotoxic effect of the extract. It has been reported that bilirubin level is elevated in hepatotoxicity because of inefficient bilirubin clearance (Amang et al., 2020).

Table 8: GC-MS-identified compounds in the ethanol leaf extract of *S. dulcificum*

S/N	Name of Compound	Retention Time	Peak Area (%)
1	Hexadecanoic acid methyl ester	15.182	4.49
2	Myristoyl chloride	18.811	3.71
3	9, 12-Octadecandien-1-ol	19.692	2.81
4	Allantoic acid	13.130	1.08
5	2,3-dihydrobenzofuran	7.325	1.99
6	Neophytadiene	13.949	1.91
7	Pentadecanoic acid	14.592	3.51
8	Methyl stearate	16.268	2.92
9	n-Hexadecanoic acid	14.949	25.84
10	Oleic acid	16.373	17.37
11	Phytol	16.187	6.25
12	Ethyl 9, 12, 15-Octadecatrienoate	16.539	1.60
13	9,12-Octadecadienoic acid	16.506	1.44
14	Octadecanoic acid	16.797	1.77
15	Squalene	20.344	3.38
16	Cis-13-Octadecenoic acid	16.077	0.98
17	9-Octadecenoic acid	16.035	3.74
18	2,3-dihydro-3,5-dihydroxyl 6-methyl-4H-pyran-4-one	6.125	6.76
19	1-ethyl-2-pyrrolidinone	5.211	3.53
20	N-acetyl pyridine	4.296	1.38
21	1,3-dioxolane,2,4,5-trimethyl acetic acid	4.011	1.94

Figure 15: GC-MS chromatogram of ethanol leaf extract of *S. dulcificum*

Creatinine, a waste product of muscle cell metabolism is excreted by the kidneys into the urine. Its concentration is usually measured to assess the functional status of the kidney, with elevations indicating kidney problems (Omage and Azeke, 2019). Impairment in the glomerular filtration rate results in increases in serum creatinine; an index of kidney dysfunction (Atangwo *et al.*, 2013; Mika and Guruvayoorappan, 2013; Ogbonna *et al.*, 2016; Ikumawoyi *et al.*, 2018)) characterized by damage to the nephrons (Gross *et al.*, 2005). In this study, the extract elicited an increase in creatinine level in both male and female rats compared to the control which indicates nephrotoxicity. Also, the urea level increased compared to the control. Serum urea tends to accumulate in renal dysfunction resulting in uraemia because the rate of its production exceeds the rate of clearance (Mayne, 1994; Adeneye *et al.*, 2008). The observed increase in urea is suggestive of kidney injury (Chinnappan *et al.*, 2019). These findings portend that the extract may be nephrotoxic on repeated exposures. Elevated concentrations of LDL-cholesterol and triglycerides (TG) are responsible for hypercholesterolemia and vascular dysfunction (Adaramoye *et al.*, 2008; Peng *et al.*, 2017; Farnier *et al.*, 2021). The effect of the extract on lipid profile showed a significant reduction in HDL and a non-significant change in CHOL, TG and LDL compared to control. Although LDL levels were not significantly altered, the reduction in HDL portends that the extract may have elevated the risk of derangement of lipid metabolism in treated animals hence, its interventional exposure may pose a risk for cardiovascular diseases (Umesh *et al.*, 2005; Selvan *et al.*, 2008).

Ighodaro and Akinloye (2018) have reported the ability of superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) to suppress or prevent the formation of free radicals or reactive species in cells. These enzymes are very fast in neutralizing molecules with the potential of developing into free radicals or any free radical with the ability to induce the production of other radicals. On assessment of antioxidant factors, there was a significant increase in the level of glutathione peroxidase at 118 mg/kg. In addition, an increase in GSH and significant increases in SOD and CAT were observed in treated animals compared to control. This suggests that the extract has the potential to induce antioxidant molecules and inhibit or reduce oxidative damage to biological macromolecules e.g., proteins, lipids and DNA probably mediated through its interplay in several pathways involved in cellular protection (Banerjee *et al.*, 2018). Malondialdehyde (MDA) is an index of cellular disruption which had been reported by Ayala *et al.* (2014) to be the most mutagenic product of lipid peroxidation. There were no significant changes in MDA levels at 475 mg/kg of the

extract in this study lending credence to the observed antioxidant effect.

On assessment of sperm parameters, there was significant increase in the number of abnormal sperm cells while sperm count and motility were significantly increased compared to control. Although the extract was able to positively affect sperm indices of count and motility, the reduction in normal sperm cells suggests that it may possess the ability to induce aberration in control mechanisms associated with testicular or pre and post testicular factors hence resulting in male infertility (Plachot *et al.*, 2002; Wamoto *et al.*, 2007; Sabra and Al-Habri, 2014).

Histopathological assessment of the kidney of treated rats showed congested blood vessels. This observation correlates with the results obtained as increased creatinine levels and supports the assertion of the risk for renal dysfunction posed by the extract.

Several compounds were identified when extract of *S. dulcificum* was subjected to analysis using gas chromatography and mass spectrometry. The most abundant constituents identified are n-hexadecanoic acid, oleic acid, phytol and DDMP. n-hexadecanoic acid has been reported to be anti-androgenic (Kumar *et al.*, 2010; Aparna *et al.*, 2012). The observed effect of the extract on sperm abnormality could be attributed to the anti-androgenic action of n-hexadecanoic acid because high levels of intra-testicular testosterone is required for spermatogenesis (Lombardo *et al.*, 2005). Antioxidant, cytotoxic and anti-inflammatory principles were also identified in the extract hence the antioxidant effect of the extract could be further explained to be a consequence of these compounds. Oleic acid (Parthipan *et al.*, 2015; Elagbar *et al.*, 2016), phytol (Lee *et al.*, 1999), squalene (Lou-Bonafonte *et al.*, 2018) and DDMP (Hwang *et al.*, 2013; Yu *et al.*, 2013) are reported to possess antioxidant and anti-inflammatory activities.

CONCLUSION

Findings from this study suggest that the ethanol leaf extract of *S. dulcificum* has potential to improve antioxidant indices. It is however, nephrotoxic, hepatotoxic and toxic to the testes in rats on long term administration. Hence, caution should be exercised in the duration of use to prevent significant organ dysfunction.

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CONFLICTS OF INTEREST

There is no conflict of interest with respect to the study.

FUNDING STATEMENT

None.

AUTHORS' CONTRIBUTIONS

All authors contributed to the development of the manuscript and approved the final draft. AAB, AAS and OA conceptualized and supervised the study. VOI designed, supervised and analyzed the data. OSY and ARA carried out the experiments.

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