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Toxicological and Reversibility Assessment of *Dalbergia saxatilis* Root Extract on Body and Organ Weights, Hepatic Functions and Peroxidation in Rats

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Authors' contributions

This work was carried out in collaboration between all authors. Authors OKY and OOA designed the study. Author OKY performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors OKY and OOA managed the analyses of the study and the literature searches. Author MCI sectioned read, analysed and reported the micrographs of hepatic sections. All Authors read and approved the final manuscript.

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ABSTRACT

Aims: The aqueous root extract of *Dalbergia saxatilis* (DS) was investigated for its effects on the body and vital organs weights, liver toxicities and oxidative stress markers after 90 days treatment and 14 days reversibility in rats, with a view to ascertaining its safety claims.
Study Design: The study was 90 days subchronic toxicity test, followed by 14 days reversibility studies on Sprague-Dawley rats.
Place and Duration of Study: Toxicology Unit Laboratory, Department of Pharmacology, Therapeutics and Toxicology, College of Medicine, University of Lagos, Lagos, Nigeria, and

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Department of Pharmacology, Lagos State University College of Medicine, Ikeja, Lagos, Nigeria, between December 2007 and May 2012.

Methodology: Rats of both sexes were given daily doses of DS (40, 200 or 1000 mg/kg) for 90 days, during which the body weights were measured weekly. After 90 days, weights of vital organs- heart, kidneys, liver, lungs & spleen were measured; biochemical parameters- ALP, AST, ALT, bilirubin and uric acid were measured from blood, and oxidative stress markers- MDA, GSH, SOD & CAT were determined from liver and spleen tissues. Histopathological assessment of hepatocytes was also done. Additional 14 days reversibility tests were also carried out.

Results: There was significant (P = .05) body weight gain per week at 40 mg/kg & 200 mg/kg DS groups. Significant increases were also recorded in liver weight of female rats at 1 g/kg and spleen at 200 mg/kg for female & 1 g/kg for rats of both sexes. ALP was significantly elevated in female rats at 200 mg/kg, and in both sexes at 1/g/kg; direct bilirubin was elevated at 1 g/kg in both sexes; whereas LDH was elevated in female rats at 1 g/kg. Significant elevation of CAT, SOD & GSH, and significant lowering of MDA were recorded for female rats at 1 g/kg. Histopathology of the liver revealed lesions at DS dose of 1 g/kg.

Conclusion: The extract might cause weight changes, reversible splenotoxicity and irreversible hepatotoxicity in females, during prolonged oral use at 1 g/kg.

Keywords: Dalbergia saxatilis; subchronic toxicity; body weight; splenomegaly; hepatotoxicity; oxidative stress; reversibility test.

1. INTRODUCTION

Over the last two decades, the use of herbal remedies has expanded globally and traditional medicine has become very popular [1]. This assertion corroborates that of the World Health Organization, which states that about 80% of the world population, especially in developing countries, rely on plants for their health care [2]. Presently, it is common to notice that many people prefer to use medicinal plants rather than chemical drugs [3]; and this is especially true in regions of poverty, communal living and rich folkloric traditions, as commonly found in Africa, Asia, and some parts of Southern America, at least. Nevertheless, to benefit fully from the pharmacological uses of plants, the safety potentials vis-a-vis its toxicity profile on various body organs and systems need to be fully studied and reported as well.

The plant *Dalbergia saxatilis* (family: Leguminosae; sub family: Papilionaceae) is a woody shrub widely distributed in the forest and savannah regions. In West Africa, various parts of the plant are employed for medicinal uses [4]. This plant is fast gaining popularity due to its applicability by herbalists in some areas of Nigeria who employ the root decoction of the plant in the management of acute and chronic ailments including epilepsies, convulsions, tension and insomnia. The effectiveness of the aqueous extract of the plant has been confirmed in generalized seizures [5], anxiety and muscle relaxation [6] in our laboratory. Also, Uchendu et al. [7] reported that the plant has also been used to accelerate birth and to expel the placenta in human subjects, effects which they have corroborated through pharmacological investigations in animals. Furthermore, some of the other traditional uses of the plant include treatment of toothache, abdominal pain, suppression of cough, and in the treatment of yaws and small pox [8], which might warrant long-term usage, with possibility of self-administration. Despite its wide pharmacological usefulness, the traditional medical practitioners believe that the plant extract is beneficial, with little or no adverse health effects of concern to users [9], even when used chronically.

At present, there are no reported scientific studies on toxicity of the root extract of the plant, despite results from many studies that have disproved such claims for many medicinal plants, especially during or after prolonged use [10,11].

The data of the acute and subchronic toxicity studies on medicinal plants or preparations derived from them should be obtained in order to increase the confidence in their safety to humans, particularly for use in the development of pharmaceuticals [12]. Therefore, evaluating the toxicological effects of any medicinal plant extract intended to be used in animals or humans is a crucial part of its assessment for potential toxic effects [13]. Subchronic studies assess the undesirable effects of continuous or repeated

exposure of plant extracts or compounds over a portion of the average life span of experimental animals, such as rodents. Specifically, they provide information on target organ toxicity and are designed to identify no-observable adverse effect level [14].

The main objective of this research study was to subject the aqueous root extract of *Dalbergia saxatilis* to 90 days subchronic toxicological tests on body and vital organs weights, liver and peroxidation, with a view to assessing its safety potentials, and as such, provide information for likely users of the root decoction of the plant.

2. MATERIALS AND METHODS

2.1 Plant Materials

Fresh root parts of *Dalbergia saxatilis* were collected from secondary forests in Ikire, Osun State, Nigeria. It was authenticated by T.K. Odewo, who was then a senior superintendent of the Forestry Research Institute of Nigeria (FRIN) where a voucher specimen (FHI 106484) was deposited for reference; and confirmation was done by Professor J.D. Olowokudejo of the Botany and Microbiology Department, University of Lagos, Nigeria.

2.2 Extract Preparation

The root parts of the freshly harvested *D. saxatilis* were dried, ground into powdered form and boiled in distilled water (100 g/L) for 30 minutes. It was then left for 24 hours at room temperature for further extraction and filtered. The filtrate was evaporated to dryness in an oven set at 40℃, with extract yield: 12.6±1.3% (range: 11 - 14%) of weight of the dried root. The dried extract was stored in the refrigeration at 2℃ until ready for use. Administration was done by dissolving the extract in distilled water with a stock solution of 500 mg/ml concentration. Final administration given to each animal was calculated such that each animal was administered with extract solution of not more than 0.4 ml/100 g body weight.

Preliminary phytochemical screening revealed the presence of glycosides, saponins, tannins, oil and phenols [6].

2.3 Animals

The animals used for the experiments were young adult Sprague-Dawley rats of both sexes, 7-8 weeks (123 - 130 g), of which parents were mated and purpose-bred, and kept at the Laboratory Animal Centre of the College of Medicine of the University of Lagos, Idi-Araba, Lagos, Nigeria. The animals were maintained under standard environmental conditions, being fed with Pfizer-branded rodent feeds purchased from Livestock Feeds, Nigeria Ltd. and given water *ad libitum*. All animals were kept at room temperature in cross-ventilated rooms, without illumination at night to achieve 12h light/ 12h dark periods. The animals were acclimatized in the laboratory condition for 14 days prior to the experiment. The care and use of animals were conducted in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals [15]. Moreover, Ethical approval for animal use was obtained from the Experimental Ethics Committee on Animal Use of the College of Medicine of the University of Lagos, Idi-Araba, Lagos, Nigeria.

2.4 Subchronic Toxicity and Reversibility Test Schedule

Young adult rats were randomly allotted to four groups (10/sex/group), consisting of the control and three extract-treated groups, 40 mg/kg, 200 mg/kg and 1 g/kg (which represented one-fifth of the pharmacologically active dose, the pharmacologically active dose, and five times the pharmacologically active dose, respectively) in animals studies [16,17]. The doses were administered daily through drinking water throughout 90-day test and 14-day reversibility periods. After 90 days, a fraction of the rats (6/sex/group) were sacrificed and blood was collected retro-orbitally for measurement of biochemical as well as for oxidative stress parameters; vital organs- heart, kidneys, liver, lungs & spleen were also carefully excised for gross weight measurements; finally, sections of the hepatic cells were cut for histopathological assessment. Where significant changes were derived, the retained animals (4/sex/group) were subjected to re-measurement of the parameters for 14 days reversibility of toxic effects tests.

2.5 Body Weight Measurement

Eighty purpose-bred rats (40 males, 40 females, 7-8 weeks old, mean weight, 126 g approx.) were housed (10/sex/cage) in eight large cages (dimension, 60 x 75 cm). They were divided into four groups. Each group of rats consisted of 10 males & 10 females, which were housed separately to prevent mating. Groups I - IV rats

were given tap water only, 40 mg/kg, 200 mg/kg and 1 g/kg DS in tap water, respectively. Body weight of each member of the group was measured individually by aid of a sensitive digital balance on day 0, then once every 7th day till day 90. Mean weight ± SEM was recorded accordingly for male and female subgroups.

2.6 Vital Organs Measurement

At the end of the study, qualitative data on the weights of vital organs (heart, kidneys, liver, lungs and spleen) were assessed by carefully dissecting each organ from sacrificed animals into 10% formalin solution contained in a Petri dish. Isolated organs were dried with a blotting paper and weighed on a sensitive balance. Each weighed organ was then standardized for 100 g-body weight of each rat.

2.7 Biochemical Parameters Measurement

Whole blood of each rat was centrifuged at 2500 g for 20 min at 10℃ to separate the serum. Serum was mixed with reagents of Randox™ kits and read in a *Screenmaster* colorimeter set at 37℃. The activity of serum alkaline phosphatase (ALP) was determined at 405 nm using a standard method [18]; serum alanine amino transferase (ALT) and aspartate amino transferase (AST) were also determined at 340 nm [19]. Uric acid was measured using the urease cleavage Berthlot's reaction [20]. Lactate dehydrogenase (LDH) was measured by the method of Wroblewski and LaDue as indicated by Varley [21,22]. Sorbitol dehydrogenase (SDH) [23] and serum direct bilirubin [24] levels were also determined.

2.8 Measurement of *In vivo* Oxidative Stress and Peroxidation

From liver and spleen tissue samples of sacrificed rats, determination of catalase (CAT), superoxide dismutase (SOD), reduced glutathione (GSH), and malondialdehyde (MDA) was done according to the protocol of Sun and Zigma [25].

2.9 Histopathological Assessment of Cells

After the collection of blood, the liver was immediately excised, freed from adventitia, blotted, weighed and fixed in 10% formol saline for histological studies. Fixed sections were passed through xylene, alcohol and water to ensure that the tissue was totally free of wax and alcohol. Each section was then stained with haematoxylin and eosin for photomicroscopic assessment. To minimize bias, the pathologist was unaware of the doses and treatments given to the different groups of rats.

2.10 Statistical Analysis

Results are presented as mean ± S.E.M or percentages. Statistical significance between the groups was analysed by means of Student's *t*-test or analysis of variance (ANOVA) followed by Fisher's *post-hoc* PLSD multiple comparison tests. (P = .05) were considered significant.

3. RESULTS

3.1 Mean Body Weight of Rats

Steady increases in body weight were recorded throughout the study from day 1 – 90 in the control group. There were significant (P = .05, Student's *t*-test) increases in mean body weights on day 90 with 40 mg/kg extract and days 63 – 90 with 200 mg/kg extract group; there were however decreases in mean body weight on days 21, 28, 35 and 77 in the 1 g/kg groups, compared with the control. Cumulatively, there were significant (P = .05) weight gains, per week, in the 40 mg/kg and 200 mg/kg, extract groups, but not in the 1 g/kg group (Table 1).

3.2 Effect of Subchronic DS on Mean Weights of Vital Organs

There were no significant differences in the weights of the heart, lungs and kidneys at all doses tested. However, significant (P = .05) increases in the weight of liver in the female rats were recorded at 1 g/kg and spleen at 200 mg/kg for female and 1 g/kg for both sexes (Table 2).

3.3 Effect of Subchronic DS on Biochemical Parameters of Rats Treated with *D. saxatilis*

Significant (P = .05) changes were recorded in the levels of ALP at 200 mg/kg (F) and 1 g/kg (M, F), uric acid at 1 g/kg (M, F), direct bilirubin at 1 g/kg (M, F), and LDH (F) levels in the rat plasma at 1 g/kg of extract. However, no significant changes in the levels of AST, ALT and SDH were detected at all doses tested (Table 3a).

Table 1. Body weight of rats during 90 days of treatment with *D. saxatilis*

Treatment	Mean body weight (g)														
	Day 1	Day 7	Day 14	Day 21	Day 28	Day 35	Day 42	Day 49	Day 56	Day 63	Day 70	Day 77	Day 84	Day 90	Weight gain/ wk
Control	126.1±1.3	129.9±1.5	134.2±1.5	142.3±1.3	147.5±1.7	152.3±1.6	158.8±2.0	165.3±1.9	171.4±2.2	178.6±2.0	184.7±2.3	192.5±2.2	198.3±2.5	210.3±2.5	5 .88±1.88
DS 40 mg/kg	126.6±1.4	129.6±1.4	134.0±1.6	142.1±1.5	147.8±1.6	154.6±1.8	160.2±1.9	166.9±2.2	173.3±1.9	180.2±2.1	185.9±2.3	195.6±2.8	201.7±2.7	217.4±2.9[a]	6.37±2.10[a]
DS 200 mg/kg	125.9±1.5	128.4±1.4	133.8±1.7	141.9±1.6	149.2±1.7	156.6±1.8	162.4±2.0	170.6±1.8	175.8±2.4	186.2±1.8[a]	198.6±2.1[a]	206.0±2.5[a]	215.1±2.8[a]	230.1±2.8[a]	7.28±2.09[a]
DS 1 g/kg	126.5±1.3	128.3±1.5	132.2±1.8	137.1±1.6[b]	143.8±1.8[b]	153.5±2.0	160.8±1.9	167.4±1.8	172.4±1.9	178.1±2.3	182.5±2.0	189.9±2.4[b]	197.8±2.3	209.2±2.3	5.81±1.98

Table showing body weight changes in rats during the 90 days treatment with different doses of D. saxatilis or distilled water (control). a Significant increase; b Significant decrease in body weight (ANOVA, Fisher's post-hoc PLSD, F(0.05) 3, 1116 = 2.131) compared with the control.
DS = Aqueous root extract of Dalbergia saxatilis. Number of rat used = 20 (10/sex) per group

Table 2. Data on the organ weight per 100 g body weight of rats after 90-day subchronic treatment with *D. saxatilis*

Treatment		Mean organ weight per 100 g body weight ± S.E.M				
		Heart	Kidneys	Liver	Lungs	Spleen
Control	M	0.36±0.06	0.87±0.09	3.25±0.46	0.72±0.05	0.33±0.02
	F	0.35±0.06	0.84±0.10	3.31±0.33	0.71±0.04	0.32±0.03
DS	M	0.35±0.04	0.88±0.09	3.18±0.36	0.69±0.04	0.33±0.03
40 mg/kg	F	0.35±0.06	0.85±0.08	3.19±0.40	0.69±0.06	0.32±0.02
DS	M	0.37±0.07	0.87±0.11	3.22±0.39	0.72±0.05	0.36±0.03
200 mg/kg	F	0.37±0.05	0.85±0.09	3.36±0.36	0.70±0.04	0.48±0.04*
DS	M	0.36±0.05	0.89±0.09	3.31±0.43	0.71±0.06	0.46±0.03*
1g/kg	F	0.35±0.06	0.88±0.10	3.86±0.39*	0.71±0.05	0.53±0.03*

*Table showing weights of vital organs of rats after 90 days treatment with different doses of D. saxatilis or distilled water (control). * Significant (Two-way ANOVA, Fisher's post-hoc PLSD, F 7, 40 = 2.023) at P < 0.05 when compared with same sex control; M = male; F = female. Number of rats used = 12 (6 per sex) per group. DS = Aqueous root extract of Dalbergia saxatilis.*

Table 3a. Biochemical parameters of rats after 90-day subchronic treatment with *D. saxatilis*

Treatment		ALP (U/L)	AST (U/L)	Uric acid (mg/dL)	ALT (U/L)	Direct bilirubin (mg/L)	LDH (IU/L)	SDH (U/L)
Control	M	330.3±10.4	16.2±1.13	1.83±0.20	30.9±1.78	1.89±0.07	115.32±11.93	44.19±2.27
	F	333.2±13.0	16.2±1.39	1.71±0.31	31.6±1.23	1.78±0.06	116.23±10.39	43.16±1.78
D. saxatilis	M	331.1±12.9	15.3±0.86	1.78±0.32	31.3±1.23	1.97±0.11	114.94±11.24	44.56±2.65
40 mg/kg	F	333.0±13.8	15.7±0.50	1.69±0.33	31.3±1.24	1.85±0.08	115.43±13.26	44.03±1.97
D. saxatilis	M	361.3±12.8	16.2±0.88	1.83±0.40	32.3±1.33	2.06±0.12	115.75±12.68	43.88±1.94
200 mg/kg	F	447.9±14.5*	16.5±0.66	1.79±0.27	31.9±1.72	1.99±0.10	116.98±14.44	44.19±2.88
D. saxatilis	M	479.4±15.3*	17.6±0.85	1.89±0.34	32.2±1.89	3.31±0.15*	166.06±12.73	44.67±2.56
1 g/kg	F	482.8±14.5*	17.4±0.74	2.90±0.36*	32.6±1.56	3.42±1.78*	194.34±16.21*,†	44.96±2.11

Table showing values of blood biochemical parameters in rats after 90 days treatment with different doses of D. saxatilis or distilled water (control); n = 12 (6 per sex) per group. M = Male, F = Female.). Significant reduction compared to same sex control; † Significant (Two-way ANOVA, Fisher's post-hoc PLSD, F 7, 40 = 2.066) difference between opposite sex compared to controls. ALP- Alkaline phosphatase; AST- Aspartate aminotransferase; ALT- Alanine aminotransferase; LDH- Lactate dehydrogenase; SDH- Sorbitol dehydrogenase.*

3.3.1 Biochemical parameters after 14 days reversibility period

Reversibility tests did not show significant differences in the direct bilirubin and LDH levels compared with the control, but ALP and uric acid levels were significantly higher than that of control after the reversibility period (Table 3b).

3.4 Effect of Subchronic DS on Oxidative Stress and Peroxidation of Rat Liver

Significant (*P* = .05) decreases in CAT, SOD and GSH, as well as increase in MDA were recorded only in the female rats at 1 g/kg of extract compared with the control after 90 days (Table 4a).

3.4.1 Oxidative stress and peroxidation of rat liver after 14 days reversibility period

No changes in the significant (*P* = .05) decreases in CAT, SOD and GSH, as well as increase in MDA recorded in the female rats at 1 g/kg of extract compared with the control after the 14 days reversibility tests (Table 4b).

3.5 Effect of Subchronic DS on Oxidative Stress and Peroxidation of Rat Spleen

Significant (*P* = .05) decreases in CAT and SOD was recorded for female rats at 200 mg/kg, whereas significant decrease in CAT (M & F), SOD (M & F), GSH (M & F) and increase in MDA

(F) were recorded at 1 g/kg of extract compared with the control after 90 days (Table 5a).

3.5.1 Oxidative stress and peroxidation of rat spleen after 14 days reversibility period

There were no significant (P = .05) changes in oxidative stress markers between the treated and control after the 14 days reversibility tests (Table 5b).

3.6 Effect of DS on Histopathology of Liver

After 90 days of subchronic toxicity study, histopathological examinations of the control and 200 mg/kg extract-treated rats presented preserved lobular architecture, whereas at 1 g/kg, some forms of pathologic hepatocytes were recorded (Figs. 1-3).

4. DISCUSSION

The initial loss of appetite by the extract-treated rats could probably be due to the presence of saponins (due to bitterness), and tannins (due to astringent properties) of these phytochemicals, as previously reported [5]. The final body weight gain might be due to rebound increase in food consumption noticed from day 17 of the study. Generally, weight and body weight gain in rats often result from physiological variation such as food intake, and metabolism [26]. The significance of this is that changes in body weight

Table 3b. 14 days reversibility study on biochemical parameters of rats after treatment with *D. saxatilis*

Treatment		ALP (U/L)	AST (U/L)	Uric acid (mg/dL)	ALT (U/L)	Direct bilirubin (mg/L)	LDH (IU/L)	SDH (U/L)
Control	M	330.3±10.4	16.2±1.13	1.83±0.20	30.9±1.78	1.89±0.07	115.32±11.93	44.19±2.27
	F	333.2±13.0	16.2±1.39	1.71±0.31	31.6±1.23	1.78±0.06	116.23±10.39	43.16±1.78
D. saxatilis	M	NT	NT	NT	NT	NT	NT	NT
200 mg/kg	F	343.1±16.1*	NT	NT	NT	NT	NT	NT
D. saxatilis	M	347.4±13.6*	NT	NT	NT	1.93±0.20	NT	NT
1 g/kg	F	349.5±16.4*	NT	1.88±0.32*	NT	1.83±0.11	116.96±14.6	NT

*Table showing measured values of metabolic blood parameters in rats preserved for 14-day reversibility of effect test following 90 days treatment with different doses of Dalbergia saxatilis or distilled water (control). M = Male, F = Female. * Significant (Two-way ANOVA, Fisher's post-hoc PLSD, F 4, 15 = 2.730) reduction compared to same sex control; n = 8 (4 per sex) per group. NT = Not tested (because there was no initial significant difference compared with control in the 90 day test); ALP- Alkaline phosphatase; AST- Aspartate aminotransferase; ALT- Alanine aminotransferase; LDH- Lactate dehydrogenase; SDH- Sorbitol dehydrogenase*

Table 4a. Peroxidation (oxidative stress) of rat liver after 90-day subchronic treatment with *D. saxatilis*

Treatment		CAT (IU/mg protein)	SOD (IU/mg protein)	GSH (mg/g protein)	MDA (mmol/mg protein)
	M	53.83±4.17	2.82±0.21	4.57±0.20	0.79±0.02
Control	F	57.36±3.92	2.94±0.25	4.60±0.31	0.85±0.02
40 mg/kg	M	54.35±3.66	2.89±0.22	4.44±0.32	0.80±0.04
	F	58.05±4.01	2.89±0.22	4.57±0.33	0.82±0.03
200 mg/kg	M	55.12±3.64	2.91±0.23	4.63±0.40	0.84±0.03
	F	57.84±3.98	2.94±0.21	4.58±0.27	0.86±0.04
1 g/kg	M	58.20±4.03	2.89±0.24	4.63±0.34	0.85±0.03
	F	39.26±2.57*	2.03±0.18*	3.38±0.26*	1.87±0.04*

*Table showing measured values of liver cellular oxidative stress markers in rats after 90 days treatment with different doses of Dalbergia saxatilis or distilled water (control M = Male, F = Female); n = 12 (6 per sex) per group;). * Significant compared to same sex control; † Significant (Two-way ANOVA, Fisher's post-hoc PLSD, F 7, 40 = 2.066) difference between opposite sex compared to controls; CAT = Catalase, SOD = Superoxide dismutase, GSH = total glutathione, MDA = malondialdehyde*

can be used as the basis for the assessment of individual response to the effects of drugs and may indicate the side effects of a drug [27]. Significant changes in body and internal organ weights are considered sensitive indices of toxicity after exposure to toxic substance [28]. The recorded increases in weights of the liver and spleen could indicate hypertrophy. Hypertrophy of organs is first hand indication of toxicity of chemical or biological substance [13]. Liver and spleen are therefore susceptible organs of concern in this subchronic toxicity test.

Table 4b. 14 days reversibility study on peroxidation (oxidative stress) of rat liver after treatment with *D. saxatilis*

Treatment		CAT (IU/mg protein)	SOD (IU/mg protein)	GSH (mg/g protein)	MDA (mmol/mg protein)
Control	M	NT	NT	NT	NT
	F	56.55±3.73	2.96±0.22	4.37±0.36	0.86±0.03
1 g/kg	M	NT	NT	NT	NT
	F	44.26±2.57*	2.23±0.18*	3.48±0.26*	1.70±0.06*

*Table showing measured values of liver cellular oxidative stress markers in rats after 14 days reversibility test of effect following 90 days treatment with D. saxatilis or distilled water (control); n = 8 (4 per sex) per group. * Significant compared to same sex control; CAT = Catalase, SOD = Superoxide dismutase, GSH = total glutathione, MDA = malondialdehyde.; M = male, F = female; NT = Not Tested*

Table 5a. Peroxidation (oxidative stress) of rat spleen after 90-day subchronic treatment with *D. saxatilis*

Treatment		CAT (IU/mg protein)	SOD (IU/mg protein)	GSH (mg/g protein)	MDA (mmol/mg protein)
	M	54.83±4.17	2.92±0.21	4.67±0.20	0.89±0.02
Control	F	58.36±3.92	3.04±0.25	4.70±0.31	0.85±0.02
40 mg/kg	M	55.37±3.66	2.75±0.22	4.52±0.32	0.93±0.04
	F	59.04±4.01	2.97±0.22	4.66±0.33	0.90±0.03
200 mg/kg	M	54.13±3.64	2.93±0.23	4.45±0.40	0.96±0.03
	F	43.87±3.98*	2.05±0.21*	4.66±0.27	0.96±0.04
1 g/kg	M	40.22±4.03*	1.99±0.24*	4.73±0.34*	0.90±0.03
	F	35.24±3.57*,	1.95±0.22*	2.97±0.36*	1.95±0.04*

*Table showing measured values of liver cellular oxidative stress markers in rats after 90 days treatment with different doses of Dalbergia saxatilis or distilled water (control); * Significant compared to same sex control; † Significant (Two-way ANOVA, Fisher's post-hoc PLSD, F 7, 40 = 2.066) difference between opposite sex compared to controls; M = male, F = female; n=6 per group. CAT = Catalase, SOD = Superoxide dismutase, GSH = total glutathione, MDA = malondialdehyde*

Table 5b. 14 days reversibility of peroxidation oxidative stress of rat spleen after treatment with *D. saxatilis*

Treatment		CAT (IU/mg protein)	SOD (IU/mg protein)	GSH (mg/g protein)	MDA (mmol/mg protein)
Control	M	54.79±4.08	2.84±0.23	4.49±0.22	0.78±0.02
	F	56.48±3.88	2.96±0.23	4.64±0.20	0.86±0.03
200 mg/kg	M	NT	NT	NT	NT
	F	54.44±3.83	2.94±0.24	NT	NT
1 g/kg	M	52.20±4.03	2.79±0.22	4.33±0.35	NT
	F	53.36±3.36	2.73±0.20	4.47±0.33	0.81±0.03

Table showing measured values of liver cellular oxidative stress markers in rats after 14 days reversibility test of effect following 90 days treatment with D. saxatilis or distilled water (control). Significant (P < 0.05, Student's t-test) reduction compared to same sex control; M = male, F = female; n=6 per group. CAT = Catalase, SOD = Superoxide dismutase, GSH = total glutathione, MDA = malondialdehyde. NT = Not Tested*

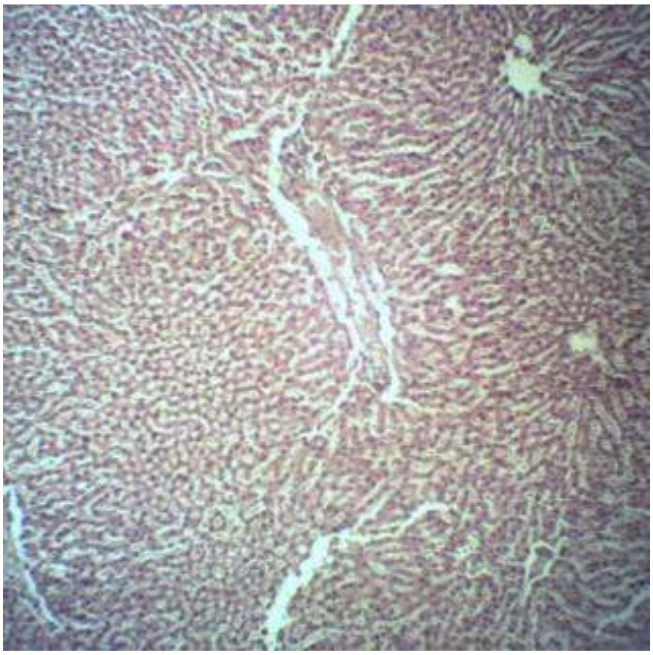

Fig. 1. The micrograph of hepatic section obtained from control rats given distilled water for 90 days. The hepatocytes are normal, sinusoids are dilated, the central veins and portal traits are free from infiltration by chronic inflammatory cells and congested vascular channels seen. Magnification: x 400

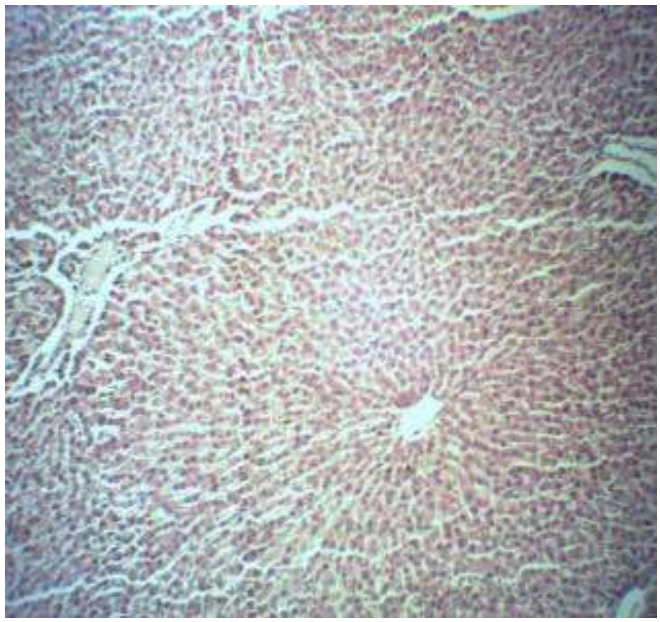

Fig. 2. The micrograph of hepatic section obtained from treated rats given *D. saxatilis* (200 mg/kg) extract for 90 days. The hepatocytes are normal, sinusoids are dilated, the portal traits show minimal infiltration by chronic inflammatory cells and the vascular channels are congested. Magnification: x 400

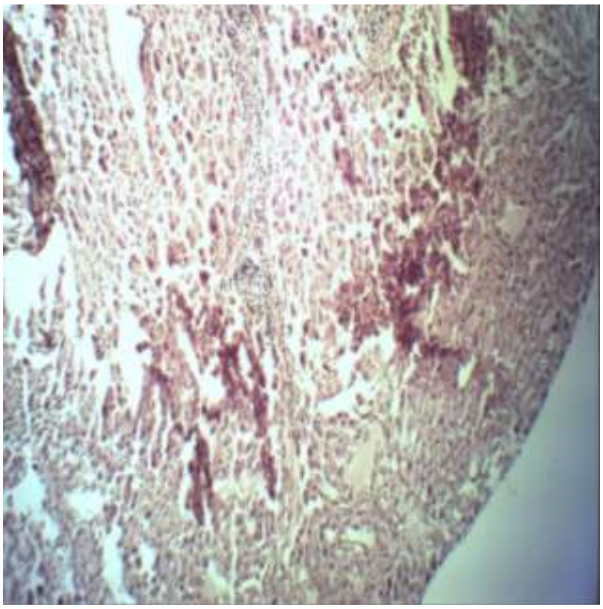

Fig. 3. The micrographs of hepatic sections obtained from treated rats given *D. saxatilis* (1 g/kg) extract for 90 days. The hepatocytes show hydropic degeneration, sinusoids are dilated, the portal traits show infiltration by chronic inflammatory cells and the vascular channels are being congested. Magnification: x 400

Increase in biochemical activities of AST and ALT, which are normal enzymes found in hepatocytes suggest leakage into the tissues, and as such, used as 'marker' to ascertain early toxic effects of administered foreign compounds causing injury to the liver and causing elevation of their activities in the bloodstream [29-33]. Furthermore, in this study, biomarkers such as ALP, uric acid, direct bilirubin and LDH which were significantly elevated at higher doses of extract suggest toxicity potential on the liver. The significant elevation of ALP level detected at 200 mg/kg and 1 g/kg of extract may be related to any of hepato-biliary cholestasis, bone toxicity, or anticonvulsants toxicities [34]. Although the extract of the plant has yet to be tested on all of these actions, its anticonvulsant actions have been reported [5]. This hypothesizes the possibility of the hepatotoxic effect of the aqueous root extract of the plant being linked to its anticonvulsant effect. ALP is a membrane bound enzyme while ALT and AST are cytosolic enzymes. These enzymes are highly concentrated in the liver and kidney and are only found in the serum in significant quantities when the cell membrane becomes leaky and even completely ruptured [35]. Since ALT and AST remained unchanged in this study, even at high doses of the extract, it therefore suggests that the toxic effect of the extract would have resulted from membrane leakage, while the cytosolic integrity of the liver is preserved, even at high dose of 1 g/kg.

Moreover, increased serum bilirubin activity caused by the extract is suggestive of cholestasis because it has been postulated that hyperbilirubinemia from a cholestatic process, results in elevated serum ALP activity [34], which is the observation in this study. In support of a possible hepatotoxicity, pathologic state of the liver was seen at 1 g/kg of extract, as hepatocytes showing continuing hydropic degeneration and infiltration by chronic inflammatory cells which was irreversible during additional 14 days of extract withdrawal. These effects were not seen in control and lower doses of extract.

Free radicals-induced lipid and protein peroxidation is believed to be one of the major causes of cell membrane damage leading to a number of pathological situations [36,37]. To combat these radicals, living organisms produce enzymes (e.g. catalase, superoxide dismutase, and peroxidase) and ultimately, reactive oxygen species (ROS) are formed in the human body in the cytosol, mitochondria, lysosomes, peroxisomes and plasma membranes under both physiological and pathological conditions [38].

Therefore, GSH, CAT SOD decreases indicate oxidative stress [39]. Furthermore, lipid peroxidation is one of the major outcomes of free radical-mediated injury that directly damages membranes and generates a number of secondary products including aldehydes, such as malondialdehyde (MDA) [40]. MDA is the most abundant individual aldehyde resulting from peroxidation; consequently, increased MDA could be used as important biomarkers of lipid and protein peroxidation [41,42] and as such, indicative of high oxidative stress [43,44]. The liver and spleen tissues showed levels of oxidative stress in this study. Despite showing oxidative stress, the spleen was able to regain its normal functions based on the reversal of the oxidative stress markers during the reversibility period. This phenomenal reversal within 14 days would require further investigation. Moreover, the antioxidant properties of phenols and tannins [45] which are confirmed phytochemicals [5,6] in the aqueous extract of *D. saxatilis* would be studied for this effect as well.

5. CONCLUSION

In conclusion, contrary to the belief that the aqueous root extract of *D. saxatilis* is devoid of toxicity, this subchronic study has revealed that body weight changes, liver and spleen, their functions, as well as hepatic histology are affected. But in as much as most of the markers of toxicity are reversible if extract is withdrawn, the liver architecture seems to be irreversibly damaged at high doses of the extract, and especially in the female. The higher susceptibility of female for selective toxicities compared to male animals in this study could be due to hormonal differences; but this would be studied more comprehensively, to ascertain this hypothesis.

CONSENT

It is not applicable.

ETHICAL APPROVAL

All authors hereby declare that “principles of laboratory animal care” (nih Publication no. 85-23, revised 1985) were followed, as well as specific National laws where applicable. All experiments have been examined and Approved by the research and ethics committee of the college of medicine of the University of Lagos, Lagos, Nigeria.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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