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Toxicological Assessment of Aqueous and Methanol Leaves Extracts of *Scoparia dulcis* Linn (Plantaginaceae) in Wistar Rats

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ABSTRACT

The leaf and whole part of *Scoparia dulcis* L. have been used in the management of different disorders in Nigeria and other parts of the world without documented scientific evidences of its safety to man. Hence, the current study was performed to assess the acute and sub-chronic toxicity of the aqueous and methanol leaves extracts of *Scoparia dulcis* in Wistar rats. The acute toxicity was conducted in two phases. In the first phase, doses of 10, 100 and 1000 mg/kg body weight of the extracts were administered orally. In the second phase, 1600, 2900 and 5000 mg/kg body weight of the extracts were administered, signs and symptoms of toxicity were monitored for 24 hours. Sub-chronic toxicity studies were carried out on Wistar rats for both extracts; haematological, biochemical and histopathological analyses after 28 days oral administration of the extracts at 500, 1000 and 1500 mg/kg body weight were conducted. The acute toxicity of all extracts was found to be greater than 5000 mg/kg which is practically non-toxic according to standard scale of toxicity. In sub-chronic test, there was significant difference (p<0.05) in the Aspartate Aminotransferase (AST) at 500 and 1000 mg/kg of aqueous extract and blood urea at 1000 mg/kg of methanol extract when compared to the control. The kidney and Liver of aqueous extract showed better physiological features when compared to that of the methanol extract. It could be concluded that prolonged oral administration of *Scoparia dulcis* leaf may be associated with increased risk of toxicity.

Keywords: Acute toxicity, Safety profile, *Scoparia dulcis*, Sub-chronic toxicity.

Introduction

Toxicity studies are conducted to provide greater understanding of the potential intrinsic hazard of the test item and to estimate safety margins. These safety margins are used to determine an initial safe starting dose for clinical trials, a safe dose for continued use in humans through longer clinical trials, and ultimately to achieve successful review of registration dossiers to support marketing approval and use of new medicines within the wider population.[1,2] The use of herbs as medicine has played an important role in nearly every culture on earth.[3] About 80% of the populations in Africa and Asia still rely on herbal medicine due to their lower costs, availability, fewer adverse effects and perceived effectiveness.[4,5] Despite the wide popularity of herbal medicines, there are still concerns associated with not only their use, but their safety. Less than ten percent of herbal products in the world market are truly standardized to known active components and strict quality control measures are not always diligently adhered to.[6,7] Therefore, there is need for the establishment of toxicological profiles of these herbal medicines.

Scoparia dulcis Linn (Plantaginaceae), commonly known as sweet broom weed is a perennial herb widely distributed in tropical and subtropical regions.[8] It is also known as rumafada in Hausa, Mesenmesengogoro in Yoruba and aiya in Ibo indigenous languages of Nigeria.[9] In these regions, fresh or dried *S. dulcis* plants have been traditionally used as remedies for stomach troubles, hypertension,[10] diabetes, bronchitis,[11,12] as analgesic and antipyretic [13]andantisicklingagents.[14,15]A number of different principles such as scoparic acid A, scoparic acid B, scopadulcic acid A and B, scopadulciol, and scopadulin have been shown to contribute to the observed medicinal effect of the plant.[16] Despite the widespread uses of *S. dulcis* in ethno-medicine, the safety profile of the leaves has not been established scientifically. Therefore, this study was undertaken to establish the toxicological profile of the aqueous and methanol leaves extracts of *S. dulcis* in Wistar rats.

Materials and Methods

Experimental animals

Forty-seven (47) Wistar rats of either sexes weighing 150 - 200 g were obtained from the Animal House, Department of Pharmacology and Therapeutics, Ahmadu Bello University, Zaria, Nigeria. The animals were housed in well ventilated cages under standard environmental conditions (22 ± 2°C, 12:12 h dark/light cycle, frequent air change), fed with normal feed and allowed free access to water ad libitum. Ethical approval for the use of laboratory animals was obtained from Animal Rights Ethical Committee with the approval number ABUCAUC/2018/002.

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Collection, Identification and Preparation of Scoparia dulcis
Scopariadulcis leaves were collected in the field around Yankarfe village, Sabongari Local Government Area, Kaduna State, Nigeria, in the month of August 2016. The plant was identified and authenticated by Mallam Namadi Sanusi, in the Herbarium Unit of the Department of Botany, ABU, Zaria, Nigeria and voucher specimen (no: 32034) was deposited. The leaves were air dried under shade, pulverized and stored in an air tight container for further use.

Extract preparation
Extraction of the plant material was done using the method described by Kokate.[17] Briefly, five hundred grams (500 g) of the pulverized plant sample was extracted with methanol in a soxhlet apparatus at 50ºC and the filtrate was further concentrated via rotary evaporator to recover solvent and final evaporation to dryness of the extract was done via the water bath after which it was stored in a desiccator for subsequent use. The aqueous extract of the sample was obtained by macerating 500 g of the plant material in distilled water with occasional shaking. The filtrate was freeze-dried and stored 4°C.

Acute toxicity studies
The acute toxicity study was carried out using rats of either sexes according to [18] as described below:
In the first phase, nine rats were divided into three groups of three rats each. The groups received 10, 100 and 1000 mg/kg of the methanol extract respectively. The toxicity study was carried out orally and observed for signs and symptoms such as loss in weight, behavioural changes and general distress of the animals for a period of 24 hours. In the second phase, three groups of one rat each were treated. The doses to be administered in the second phase are dependent on the outcome of the first phase; the groups received 1600, 2900 and 5000 mg/kg doses of methanol extract respectively. Signs and symptoms of toxicity were observed for 24 hours. The Acute toxicity was calculated as the geometric mean of the lowest lethal dose that caused death and the highest non-lethal dose that did not cause death. The procedure above was repeated for the aqueous extract.

Sub-chronic toxicity studies
The study was carried out with accordance to [19] and Organisation for Economic Cooperation and Development (OECD 407) guidelines.[20]
Thirty-five rats of both sexes were divided into seven groups, each group comprised of five Wistar rats, all groups were treated with the extracts (methanol and aqueous) orally daily for 28 days with adequate feed and water intake. Groups 1 - 3 was administered with methanol leaves extract of *S. dulcis* at doses of 500, 1000, and 1500 mg/kg while groups 4 - 6 were treated with the aqueous extract (500, 1000, and 1500 mg/kg) and the seventh group was taken as the control and treated with distilled water (1 mg/kg). The animals were observed daily for any sign of morbidity and mortality, their weights werealso taken once a week to determine any change in weight.[21] At the end of the treatment period of 28 days, the animals were sacrificed by adhering to the guidelines of animal rights and ethics, blood samples were collected from each group for heamatological, biochemical and histopathological analyses.

Haematological studies
Blood samples were collected into ethylene diamine tetra acetic acid (EDTA) bottles for estimation of packed cell volume (PCV), heamaglobin concentration (Hb), red blood cell (RBC), mean corpuscular concentration (MCHC), mean cell heamaglobin (MCH), mean cell volume (MCV), white blood cell (WBC) and differentials – neutrophils, lymphocytes, monocytes, basophils, eosinophils, erythrocytes sedimentation rate (ESR), using an automated haematological machine (Cell-DynTM Abbott, USA).[22]

Biochemical studies
The blood samples for biochemical analyses were collected into plain universal bottles, allowed to clot and centrifuged at 3500 rpm for 10 minutes from the three different extracts. The sera were separated and stored at -4°C and the biochemical parameters evaluated included alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), total protein, albumin, creatinine and urea as liver and kidney function tests by colorimetric method using Randox assay kits and automated Biochemistry analyser.[23,24]

Histopathology
The kidney and liver of the sacrificed rats were harvested and sliced into 3 to 4 cm thick and fixed in 10% formalin solution for sectioning. The fixed specimens were sliced, processed, and embedded into paraffin blocks. The blocks were cut into 5 µm thick, paraffin sections by rotary microtome. The sections were stained with haematoxylin and eosin H and E for histological observations. The method of [25] was used during the histopathology investigation for possible tissue lesions. Photomicrographs of the tissues were taken at the magnification of ×400.

Statistical analysis
Data were expressed as mean ± standard error of mean (S.E.M). Significant differences between means of different groups were determined using one way analysis of variance (ANOVA) followed by Dunnett t-test. Differences were considered significant at $p< 0.05$. All Statistical analyses were performed using SPSS (version 20) for Windows (SPSS Inc., Chicago, IL, USA).

Results and Discussion

Acute Toxicity Profile of the Aqueous and Methanol Leaves Extracts of Scoparia dulcis
The oral median lethal dose (LD_{50}) values of the aqueous and methanol leaves extracts of *S. dulcis* were estimated to be greater than 5000 mg/kg in rats. Administration of both extracts of *S. dulcis* orally in rats did not produce any behavioural sign of toxicity, there wasno death recorded at all the doses tested up to 5000 mg /kg in rats. This is in agreement with the earlier studies reported by Abdulsalaam and co-worker.[12] This suggests that the extract is practically non-toxic.[18] The median lethal dose (LD_{50}) value is used for assessing the safety margin of substances and it often gives an idea of the toxic level of a chemical substance or compound. It provides the prospect for discovering and maximizing the clinical benefits of test compounds.[26]

Sub-chronic toxicity studies
Effect of 28 days Oral Administration of Aqueous and Methanol Leaves Extracts of Scoparia dulcis on Haematological parameters in Rats.
The haematopoietic system is one of the sensitive targets for toxic compounds and an important index of physiological and pathological status in man and animals. Analysis of blood parameters is relevant to risk evaluation as the changes in haematological parameters have a high predictive value for human toxicity, when the data is translated from animal studies.[27] Sub-chronic oral administration of the extracts showed no significant ($p>0.05$) difference in the red blood cells, haemoglobin, HCT, MCV, MCH, MCHC and PLT in all the treatment groups in both extracts when compared with the control group (Table 1).However, There was significant increase ($p<0.05$) in the Lymph at 1000 and 1500 mg/kg, Minimum inhibitory dilution (MID) at 500, 1000 and 1500 mg/kg of MLESD and Lymph in 500 and 1000 mg/kg of ALESD when compared with control group (Table 2). Lymphocytes are one of the subtypes of white blood cell in a vertebrate's immune system, they are of fundamental importance in the immune system because lymphocytes are the cells that determine the specificity of the immune response to infectious microorganisms and other foreign substances, increase in lymphocytes may be an indication of an acute infection.[28] In human adults lymphocytes differential make up roughly 20 to 40 percent of the total number of white blood cells. The entire system of white blood cell focuses on host defense, the lymphocytes particularly are essential to immune defense system as their primary function is to respond to antigens by initiating the immune response.[22] The increases observed in lymphocytes for both extracts could also be attributed to the immune stimulatory activity of *Scoparia dulcis* as earlier reported.[12]

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Table 1: Effect of aqueous and methanol leaves extract of *Scoparia dulcis* on heamatological parameters in Wistar rats after oral administration for 28 days.

Treatment	RBC ($x10^{12}$/L)	HGB (g/dL)	HCT (%)	MCV (fL)	MCH (pg)	MCHC (g/dL)	PLT ($x10^9$/L)
D/Water (1mL/kg)	4.72 ± 0.26	12.62 ± 0.59	41.12 ± 1.29	83.60 ± 2.01	29.06 ± 3.11	30.70 ± 1.16	228.0 ± 25.04
ALESD 500 (mg/kg)	4.38 ± 0.14	10.64 ± 0.28	36.60 ± 0.27	84.78 ± 1.63	27.24 ± 1.48	31.22 ± 1.45	293.6 ± 42.92
ALESD 1000 (mg/kg)	5.38 ± 0.26	12.06 ± 1.14	40.78 ± 3.81	76.22 ± 6.91	22.48 ± 2.12	29.52 ± 0.24	315.8 ± 19.37
ALESD 1500 (mg/kg)	5.20 ± 0.40	14.14 ± 1.06	43.54 ± 4.46	90.14 ± 0.48	28.74 ± 1.06	33.10 ± 1.14	277.6 ± 19.37
MLESD 500 (mg/kg)	4.64 ± 0.18	11.62 ± 0.38	38.98 ± 1.60	87.18 ± 1.17	27.48 ± 1.43	30.98 ± 1.09	186.92 ± 18.53
MLESD 1000 (mg/kg)	4.33 ± 0.28	11.42 ± 0.70	36.94 ± 1.83	88.06 ± 1.42	28.10 ± 1.17	31.76 ± 0.93	318.40 ± 14.78
MLESD 1500 (mg/kg)	4.43 ± 0.11	11.22 ± 0.54	36.20 ± 1.68	84.96 ± 2.57	23.90 ± 0.36	30.30 ± 0.38	246.00 ± 10.55

Data are presented as Mean ± SEM (n=5); *p<0.05 compared with distilled water. One way ANOVA Dunnett's post hoc test. D/water: Distilled water. ALESD: Aqueous Leaves Extract of *Scoparia dulcis*; MLESD: Methanol Leaves Extract of *Scoparia dulcis*. RBC: Red Blood Cells, HGB: Haemoglobin, MCV: Mean Corpuscular Volume, mean cell heamaglobin (MCH), MCHC: Mean Corpuscular Haemoglobin Concentration mean corpuscular concentration (MCHC), PLT: Platelets.

Table 2: Effect of aqueous and methanol leaves extract of *Scoparia dulcis* on white blood cells and differential parameters in Wistar rats after oral administration for 28 days.

Treatment	WBC ($x10^9$/L)	LYMPH ($x10^9$/L)	MID ($x10^9$/L)	GRAN ($x10^9$/L)	LYMPH (%)	MID (%)	GRAN (%)
D/Water (1mL/kg)	5.9 ± 0.39	2.1 ± 0.22	0.40 ± 0.05	3.98 ± 0.06	39.4 ± 5.32	6.32 ± 1.34	54.38 ± 5.80
ALESD 500 (mg/kg)	6.52 ± 0.46	3.34 ± 0.26*	0.42 ± 0.06	3.48 ± 0.39	48.28 ± 1.99	6.94 ± 0.72	44.76 ± 2.54
ALESD 1000 (mg/kg)	6.92 ± 1.89	2.2 ± 0.34*	0.5 ± 0.07	4.22 ± 0.99	33.98 ± 6.36	8.00 ± 1.21	58.02 ± 7.27
ALESD 1500 (mg/kg)	4.84 ± 0.65	2.52 ± 0.39	0.42 ± 0.05	2.00 ± 0.16	51.38 ± 1.33	8.74 ± 0.56	39.68 ± 1.05
MLESD 500 (mg/kg)	6.52 ± 0.74	3.16 ± 0.69	0.48 ± 0.07*	2.62 ± 0.64	49.26 ± 10.09	7.78 ± 0.49	43 ± 10.09
MLESD 1000 (mg/kg)	9.15 ± 1.85	4.33 ± 0.47*	0.95 ± 0.23*	3.88 ± 1.34	51.45 ± 6.22	8.33 ± 0.94	40.23 ± 5.52
MLESD 1500 (mg/kg)	6.22 ± 1.18	2.36 ± 0.15*	0.44 ± 0.10*	3.42 ± 1.07	44.42 ± 5.00	6.08 ± 0.45	49.64 ± 5.27

Data are presented as Mean± SEM (n = 5); *p<0.05 compared with distilled water. One way ANOVA Dunnett's post hoc test. D/water: Distilled water. MLESD: Methanol Leaves Extract of *Scoparia dulcis*. WBC: White Blood Cells.

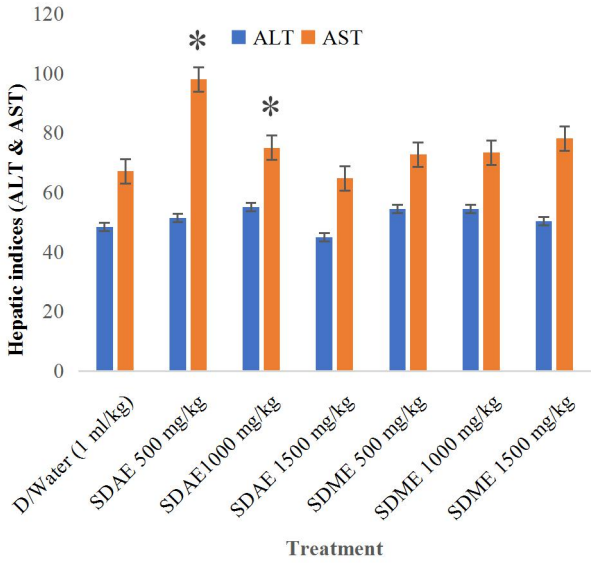

Figure 1: Effect of aqueous and methanol leaves extract of *Scoparia dulcis* on hepatic indices (ALT & AST) in Wistar rats after oral administration for 28 days.*p<0.05 compared with distilled water.

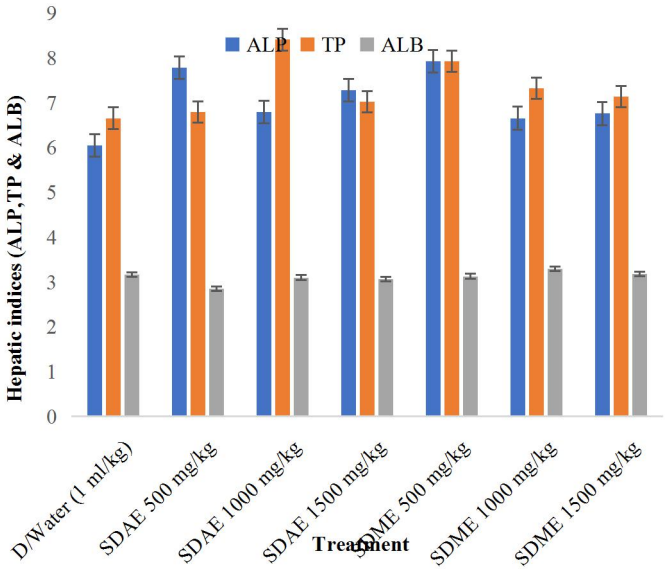

Figure 2: Effect of aqueous and methanol leaves extract of *Scoparia dulcis* on hepatic indices (ALP, TP & ALB) in Wistar rats after oral administration for 28 days.

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Effect of aqueous and methanol leaves extract of Scoparia dulcis on Biochemical parameters

Liver function tests involve evaluating serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), bilirubin and albumin levels. The most commonly used indicators of liver damage are the alanine aminotransferase and aspartate aminotransferase.[29] In this study, there were no significant difference ($p>0.05$) in the biochemical parameters test for ALESD, when compared to control in total protein, liver transaminase and phosphatase (ALP, ALT), albumin, urea and creatinine at all doses tested but a slight significant increase ($p<0.05$) was observed in AST at 500 and 1000 mg/kg (figure 1 and 2; Table 3). Generally, necrositic injuries of the hepatocytes from toxic agents primarily cause an elevation of enzymes found within the hepatocytes such as the amino transferases.[30] ALT and ALP are very specific for hepatic tissue and a sensitive indicator to hepatic damage unlike AST which is not specific for hepatic tissue and subsequently a not so reliable indicator of hepatocellular damage.[22]However, such injuries to the liver was noted in the histopathological studies for both extracts and may be responsible for the slight increase observed in the AST of the ALESD. Furthermore, For MLESD, there were no significant differences ($p>0.05$) compared to control in the liver transaminases and phosphatase (ALT, ALP, AST), albumin and creatinine in all the doses tested but a significant difference was observed in urea at 1000 mg/kg doses (Table 3). Usually, kidney damage is indicated by the level of excreted substances such as creatinine, urea, albumin etc. consistent elevation of blood urea level occurs only when the renal function, specifically the glomerular filtration rate (GFR) is reduced by 40 to 60 percent which is indicative of renal impairment.[31] This suggest a likelihood of reduction of glomerular filtration rate from administration of S. dulcis MLESD after an extended period of time.

Investigation for the toxic potential of a chemical is incomplete without gross and histopathological evaluation.[32]Thehistopathological finding of kidney of the ALESD in general showed slight tubular adhesion and necrosis with slight to moderate lymphocyte hyperplasia. While that of the MLESD in general showed slight glomerular necrosis, slight tubular necrosis and lymphocyte hyperplasia; which was not dose dependent because more damage was seen in the lowest dose (500 mg/kg) of the extracts administered. The kidney of ALESD showed better physiological features when compared to that of the MLESD, this corroborates the result obtained for the biochemical assessment. This change seen in the kidney for the MLESD could explain the increase in the urea levels previously noted in the biochemical analysis. However, low urea concentration could be an indication of low protein diets or kidney malfunction. The kidney comprises of the glomerulus and the tubules and it functions mostly in excretion of waste products. The urea is the totality of the protein metabolism waste product of the kidney in proportion to the concentrating power of the kidney.[33] The histopathology of liver of the ALESD showed slight cytoplasmic vacoulation, hepatocellular necrosis and kupfer cell hyperplasia while that of MLESD showed slight hepatocellular necrosis, kupfer cells and lymphocyte hyperplasia; the above changes were not dose dependent and this result is in agreement with the earlier studies reported by Abere and co-worker.[15] The liver's increased vulnerability to toxic attack is mostly due to its involvement in various important functions including direct involvement in the metabolism of toxic substances.[34]

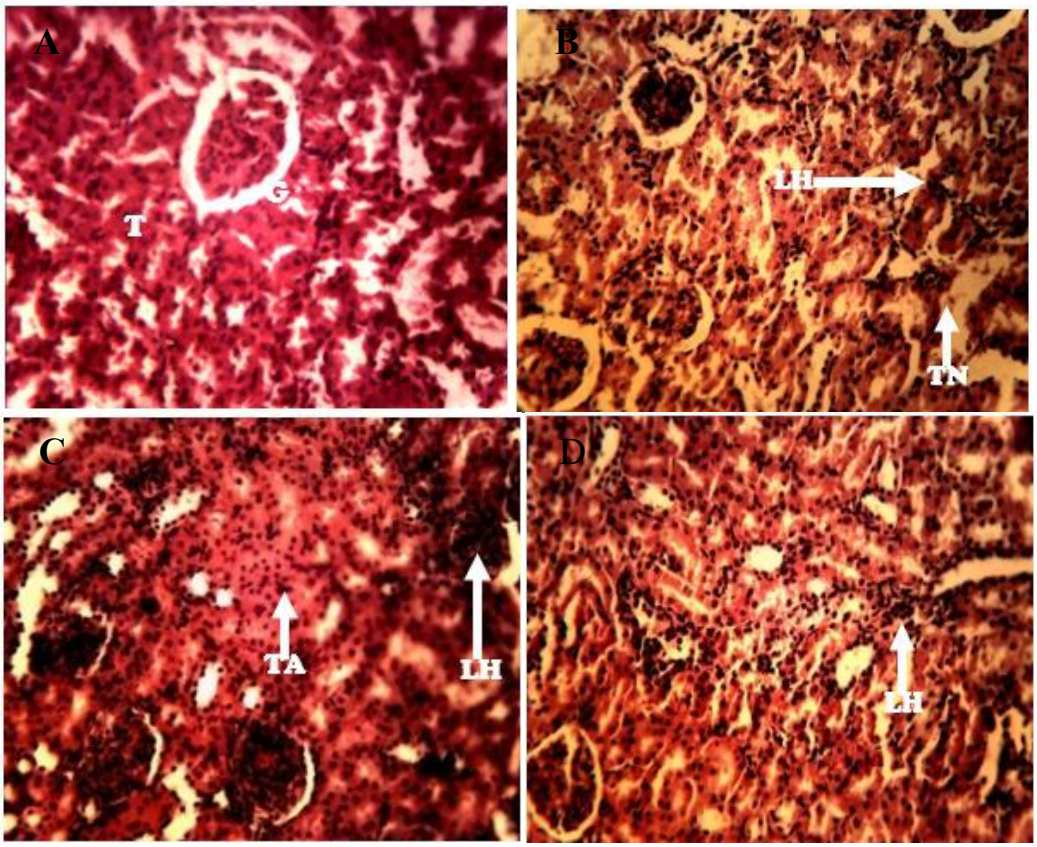

Figure 3: Photomicrograph from the kidney section of **(A)** control group (1 mL/kg distilled water) show normal features; glomerulus and tubules; **(B)** treated with 500 mg/kg of aqueous leaves extract of *S. dulcis* show slight tubular necrosis (TN) and slight lymphocyte hyperplasia (LH); **(C)** treated with 1000 mg/kg of aqueous leaves extract of *S. dulcis* show slight tubular adhesion (TA) and moderate lymphocyte hyperplasia (LH); **(D)** treated with 1500 mg/kg of aqueous leaves extract of *S. dulcis* showing slight lymphocyte hyperplasia(LH). (Hematoxylin and eosin stain; original magnification X400).

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Table 3: Effect of aqueous and methanol leaves extract of *Scoparia dulcis* on renal indices in Wistar rats after oral administration for 28 days.

Treatment	Urea (µmol/L)	Creatinine (µmol/L)
D/Water (1 mL/kg)	53.45 ± 13.93	0.84 ± 0.07
ALESD 500 mg/kg	60.47 ± 10.40	0.94 ± 0.7
ALESD 1000 mg/kg	65.36 ± 11.53	0.94 ± 0.12
ALESD 1500 mg/kg	49.25 ± 10.43	1.08 ± 0.10
MLESD 500 mg/kg	93.03 ± 17.13	1.00 ± 0.08
MLESD 1000 mg/kg	118.98 ± 8.20*	0.96 ± 0.12
MLESD 1500 mg/kg	103.23 ± 12.60	1.02 ± 0.10

Data are presented as Mean ± SEM (n = 5); *P<0.05 compared with distilled water. One way ANOVA Dunnett's post hoc test. D/water: Distilled water; ALESD: Methanol Leaves Extract of *Scoparia dulcis*; MLESD: Methanol Leaves Extract of *Scoparia dulcis*

Conclusion

The leaves of *Scoparia dulcis* is a good candidate for more biological studies, this is attributed to the low toxic effect seen on the organs tested; the aqueous extract of the plant was observed to have a lower toxic effect when compared to the methanol extract of the plant.

Conflict of interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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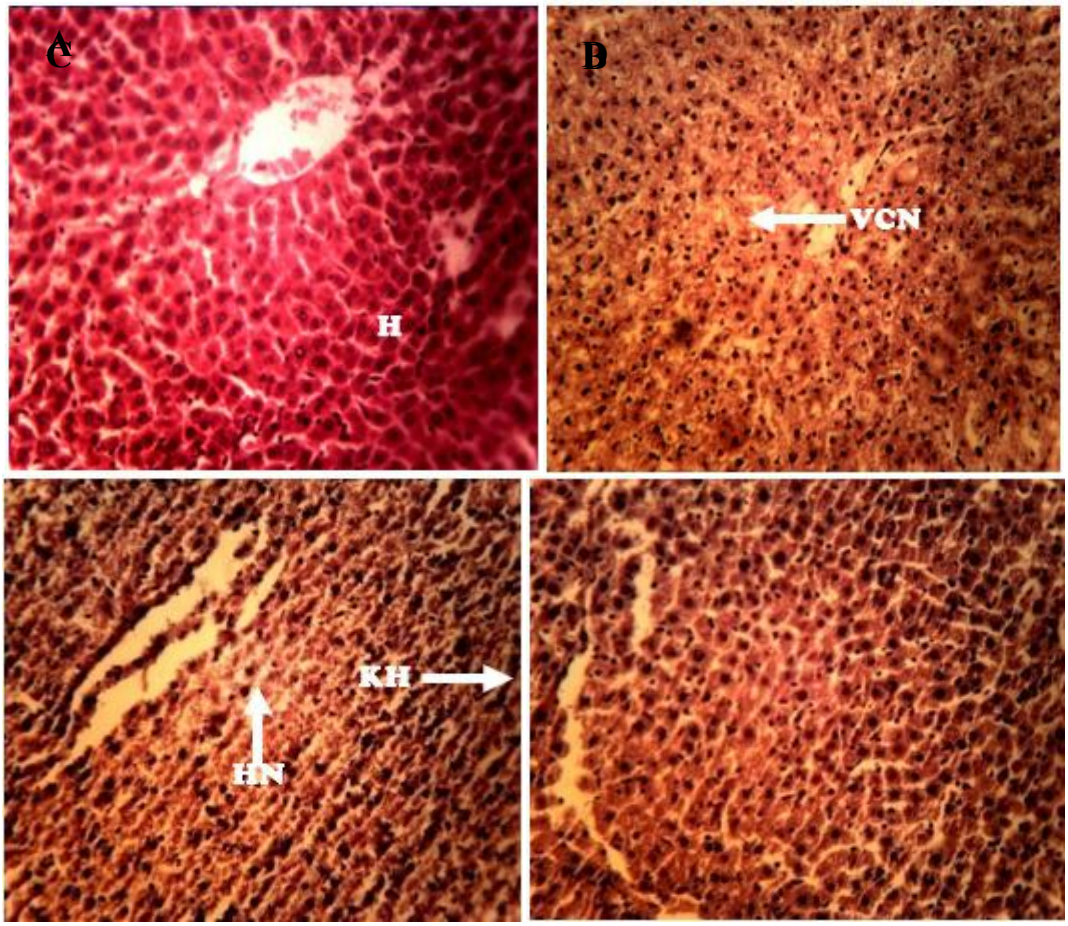

Figure 4: Photomicrograph from the liver section of **(A)** control group (1 mL/kg distilled water) show normal features; hepatocytes (H); **(B)** treated with 500 mg/kg of aqueous leaves extract of *S. dulcis* show slight cytoplasmic vacoulation and necrosis (VCN); **(C)** treated with 1000 mg/kg of aqueous leaves extract of *S. dulcis* show moderate hepatocellular necrosis (HN) and Kupfer cell hyperplasia (KH); **(D)** treated with 1500 mg/kg of aqueous leaves extract of *S. dulcis* show normal features. (Hematoxylin and eosin stain; original magnification X400).

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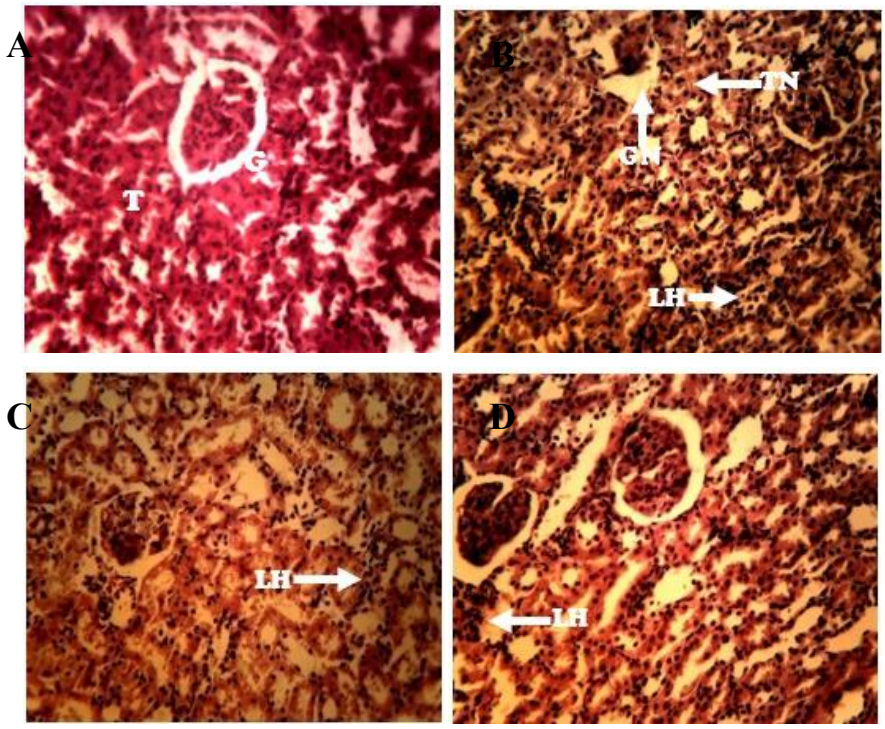

Figure 5: Photomicrograph from the kidney section of **(A)** control group (1 mL/kg distilled water) show normal features; glomerulus (G) and tubules (T); **(B)** treated with 500 mg/kg of methanol leaves extract of *S. dulcis* show slight glomerular (GN) and tubular necrosis (TN), lymphocyte hyperplasia(LH); **(C)** treated with 1000 mg/kg of methanol leaves extract of *S. dulcis* show slight lymphocyte hyperplasia (LH).; **(D)** treated with 1500 mg/kg of methanol leaves extract of *S. dulcis* showing slight lymphocyte hyperplasia (LH). (Hematoxylin and eosin stain; original magnification X400).

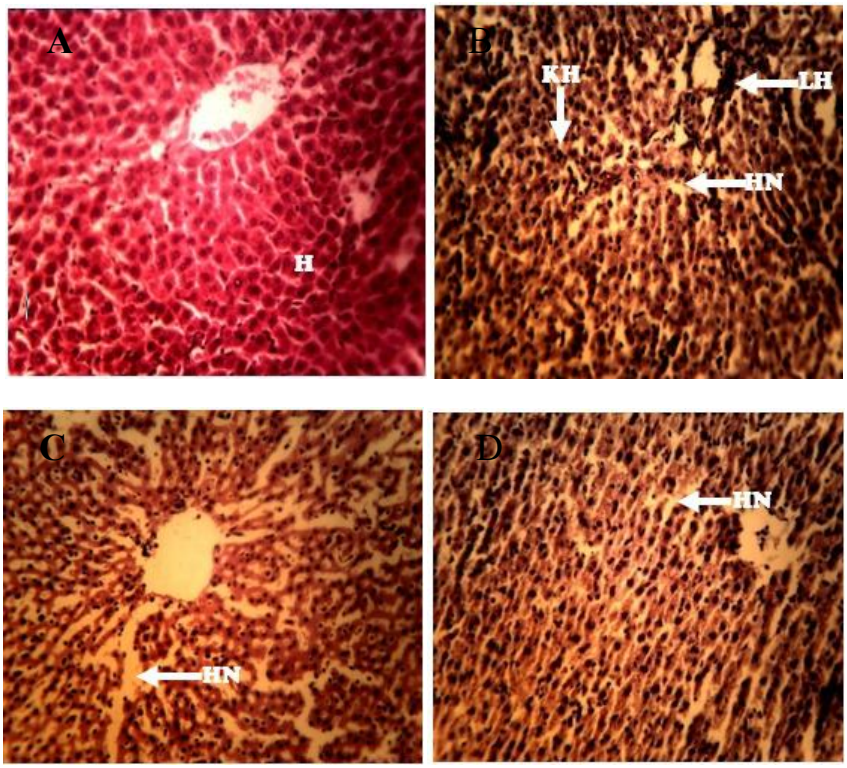

Figure 6: Photomicrograph from the liver section of **(A)** control group (1 ml/kg distilled water) show normal features; hepatocytes (H); **(B)** treated with 500 mg/kg of methanol leaves extract of *S. dulcis* show slight hepatocellular necrosis (HN), kupfer cell (KH) and lymphocyte hyperplasia (LH); **(C)** treated with 1000 mg/kg of methanol leaves extract of *S. dulcis* show slight hepatocellular necrosis (HN); **(D)** treated with 1500 mg/kg of methanol leaves extract of *S. dulcis* showing slight hepatocellular necrosis (HN). (Hematoxylin and eosin stain; original magnification X400).

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