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Sub-acute Toxicity Profile of Aqueous Seed Extracts of *Dacryodes edulis*

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ABSTRACT

This study assessed the subacute toxicity effects of the aqueous seed extracts of *Dacryodes edulis* in albino rats. Twenty animals divided into four groups (A – D) of five animals each. Groups A - C were given daily oral doses of 800, 400 and 200 mg/kg bodyweight respectively for 28 days; group D served as the control. Changes in body weight, relative organ weight (ROW), haematological and biochemical assays and organ histology were used to assess toxicity of the extract at the end of administration. It was observed that the treated animals had increased ROW of their stomachs. The haematological parameters and biochemical parameters measured were normal except serum aspartate transaminase and urea which were significantly increased when compared to the control group. The extracts had no toxic effect on all assessed organs of animals that received 400 and 200 mg/kg body weight; however at a dose of 800 mg/kg bodyweight histopathological changes were observed in the kidney sections of the animals in that group. These results suggests that aqueous extracts of *D. edulis* may be largely safe for oral consumption however it should be administered with caution because of the few toxic effects observed.

Keywords: Medicinal plants, Toxicity studies, *Dacryodes edulis*, Ethnopharmacology, Animal studies.

INTRODUCTION

Dacryodes edulis is a plant found in the tropics belonging to the family of Burseraceae. It is commonly called African pear or Bush butter tree, Bush fruit tree in English, Safou (French), Atanga (Gabon), Ube (Ibo), Elemi (Yoruba) and Orumu (Benin) [1]. In Nigeria, particularly in the South east, the fruit of *Dacryodes edulis* can be roasted, eaten raw or boiled in salt water. The roasted or boiled fruit can also be consumed with roasted or boiled corn. Different parts of *Decryodes edulis* have several beneficial purposes including uses medicinal and cosmetic purposes [2].

The extract from the bark of this plant is used to cicatrize wound [3] and also used for the treatment of leprosy, dysentery, anaemia, spitting blood, debility, stiffness, tonsillitis and skin diseases. Skin diseases such as scabies, ringworm, rash and wound are treated with the juice made from the crushed leaves. The use of the stem or stem twigs as chewing stick helps to maintain a good oral hygiene. Also the leaves serve as an antiemetic. Infections of the ear, fever, headache, malaria and cephalgy are treated with the leaf sap [4]. Studies of the phytochemical investigations of the fruit, seed and leave has shown to contain alkaloids, tannins, Saponins, glycosides and flavonoids [5-9]. However, despite the numerous medical and pharmacological evidence on *Dacryodes edulis*, there is no detailed report on its subacute toxicity profile of the crude aqueous extracts of *D. edulis* in experimental animals. Therefore this study was designed to assess the subacute toxicity profile of *D. edulis*.

MATERIALS AND METHODS

Plant materials and crude aqueous extraction

Seeds of *D. edulis* were obtained by peeling off the flesh of mature ripe fruits of the pears. They were then washed and dried under shade and ground to fine powder. About 100 g of the powder were macerated in 400 ml of distilled water for 6 hours to extract. The mixture was intermittently shaken during this period to improve extraction. After 4 hours, the mixture was sieved using a muslin cloth and filtered thrice using a Whatman filter paper. The filtrate obtained was refrigerated till needed for administration.

Experimental animals

Twenty apparently healthy, disease free adult albino rats were used for the study. The animals were housed in clean metal cages and left for a period of two weeks to acclimatise to the laboratory ambient conditions (28 – 30°C temperature, 12 hour light-dark cycle). The animals were weighed and grouped into four groups (A – D) of five animals each according to their weights. Throughout the duration of the experiment, they were fed on standard commercially available rat feed (Guinea Feed®, Enugu, Nigeria) and unrestricted access to clean water. Animal handling and experimentation were supervised by the animal care staff of the College of Medicine, University of Nigeria to ensure conformity to the guidelines for the use of animals in research [10].

Experimental design

The animals in groups A to C served as the treatment group and received a daily single dose of the extract for 28 days while group D served as the control and received distilled water only. For the treatment groups, animals in groups A, B and C received 800 mg/kg, 400 mg/kg and 200 mg/kg bodyweight of the aqueous extract of *D. edulis* (DEAE) respectively. The animals were

monitored daily for visible signs of toxicity. At the end of administration (day 28), blood samples were collected via retro-orbital puncture into anticoagulated and plain bottles to be used for haematological and biochemical assays respectively. The animals were weighed again before being sacrificed using chloroform as anaesthesia. Visceral organs of interest – kidneys, stomach, intestines, liver, spleen, testes and heart– were harvested for further examination.

Gross examination of organs and relative organ weight measurements

The harvested organs were carefully examined for gross tissue changes. Each of the harvested organs were then gently blotted on a filter piece of filter paper and weighed. The percentage ratio of organ weight to the animal's total body weight (relative organ weights) was calculated.

Tissue histopathology

The excised organs were fixed in 10% formal saline for 24 hours and then processed using standard tissue processing protocol for producing formalin fixed paraffin embedded tissue blocks. The blocks were sectioned to obtain 5 micron thick tissue sections which were stained using the haematoxylin and eosin procedure. The tissue sections were then examined under an Olympus™ light microscope. Images of areas of interest in the sections were captured using a camera attached to the eyepiece of the microscope.

Haematological and biochemical assays

The complete blood count was performed on the anticoagulated blood samples of each animal using an automated haematology analyser (Mindray BC-5300, China). The sera from the blood samples collected in plain bottles were separated and used for the estimation of serum levels of alkaline phosphatase, alanine transaminase, urea, sodium, potassium, chloride and total bilirubin using an automated clinical chemistry analyser (Mindray BS – 230, China)

Statistical analysis

Numerical data generated in the study were analysed using a software (SPSS version 20). Results of the analysis were expressed as mean \pm standard error of mean (SEM). One way analysis of variance was used to measure variations in parameters according to treatment doses while Tukey's post hoc test was used for multiple comparisons between the groups. Statistical significance was set at probability level of $p < 0.05$.

RESULTS AND DISCUSSION

Throughout the 28 day period of the administration, no visible signs of distress were observed in the animals at all doses administered. All the animals survived the experiment. The effects of the DEAE on weights of the animals are presented in Table 1. Animals in groups A, C and D lost weight while those in B gained weight. However there was no significant difference in body weight change between the treatment groups and the control group ($p=0.530$). The observed weight loss in animals in groups A and C is not dose dependent and may be as a result of differences in adaption of the animals to their new environment

and not necessarily as a result of any toxic component in the extract [11]. This seems a more plausible explanation because animals in the control group also lost some weight.

Table 1: Effects of DEAE on the body weights of the animals in the treatment groups compared with the control group. Values are presented as mean \pm standard error of mean (SEM).

Groups	CEAE Treatment (Mg/Kg B.Wt)	Initial Body Weight (G)	Final Body Weight (G)	Body Weight Change (G)
A	800	168.00 \pm 10.21*	152.16 \pm 13.80*	-15.84 \pm 22.35
B	400	157.00 \pm 5.57*	164.46 \pm 8.02*	7.46 \pm 5.59
C	200	161.00 \pm 7.57*	142.40 \pm 11.68*	-18.60 \pm 13.41
D [Control]	--	239.20 \pm 9.66	231.28 \pm 9.44	-7.94 \pm 2.07
F-ratio	--	21.117	13.425	0.765
Sig.	--	0.000	0.000	0.530

Note: *- p<0.05 when compared with control (Group D)

On gross examination of the excised tissues, there were not changes suggestive of organ toxicity. All the organs appeared normal. Table 2 shows the relative organ weights of the animals in the various treatment groups compared to the control group. Animals treated with the different doses of DEAE were observed to have significantly higher relative stomach weights when compared to the control group. Measurement of relative organ weight provides useful information on substance toxicity on such organs [12, 13]. The observed increase in relative stomach and lung weights of some of the treatment animals may have been as a result of toxicity of DEAE to these organs [14]. However, it is also possible that the increased relative organ weights may be as a result of the decreased body weights of the animals especially those in groups A and C which received 800 mg/kg and 200 mg/kg body weights respectively [13].

Table 2: Relative organ weights of animals in DEAE treatment groups and control group Values are presented as mean \pm SEM

Groups	Relative Left Kidney Weight (G)	Relative Right Kidney Weight (G)	Relative Stomach Weight (G)	Relative Liver Weight (G)	Relative Spleen Weight (G)	Relative Left Testis Weight (G)	Relative Right Testis Weight (G)	Relative Right Epididymis Weight (G)	Relative Left Epididymis Weight (G)	Relative Heart Weight (G)
A	0.36 \pm 0.02	0.40 \pm 0.04	0.84 \pm 0.05*	4.4 \pm 0.38	0.29 \pm 0.05	0.60 \pm 0.86	0.67 \pm 0.10	0.21 \pm 0.01	0.22 \pm 0.03	0.38 \pm 0.04
B	0.41 \pm 0.01	0.39 \pm 0.03	0.88 \pm 0.01*	3.6 \pm 0.18	0.25 \pm 0.06	0.62 \pm 0.07	0.63 \pm 0.10	0.20 \pm 0.02	0.28 \pm 0.02	0.37 \pm 0.02
C	0.43 \pm 0.03	0.41 \pm 0.01	1.0 \pm 0.09*	4.7 \pm 0.59	0.30 \pm 0.02	0.62 \pm 0.04	0.62 \pm 0.04	0.26 \pm 0.04	0.32 \pm 0.03	0.36 \pm 0.00
D control	0.32 \pm 0.00	0.32 \pm 0.00	0.38 \pm 0.01	3.8 \pm 0.14	0.22 \pm 0.00	0.55 \pm 0.01	0.52 \pm 0.01	0.24 \pm 0.01	0.32 \pm 0.01	0.30 \pm 0.00

F-ratio	3.207	1.612	18.294	1.190	0.994	0.298	0.638	2.355	0.953	4.742
Sig.	0.059	0.235	0.000	0.352	0.426	0.826	0.604	0.119	0.444	0.019
Note: *- p<0.05 when compared with control (Group D)										

The result of the haematological parameters of animals in the treated and control groups is shown in Table 3. No significant difference was observed in any of the measured haematological parameter or index of the treated versus the control group. Blood is one of the tissue systems most affected by toxic compounds since it is responsible for the transport of drugs and their metabolites [15]. There were no significant changes in the haematological profile of the treated animals when compared to the control. Hence, DEAE extracts may not be toxic to the blood system.

Table 3: Effects of DEAE on the haematological parameters of the animals in the treatment groups compared with the control group. Values are presented as mean \pm SEM.

Group	Packed Cell Volume	Total White Blood Cell Count	Red Blood Cell Count	Haemoglobin Concentration	Mean Cell Volume	Mean Corpuscular Haemoglobin	Mean Corpuscular Haemoglobin Concentration	Platelet Count	Neutrophil Count	Lymphocyte Count	Monoocyte Count	Eosinophil Count	Basophil Count
A	43.4 \pm 1.8	9.7 \pm 2.3	7.7 \pm 0.11	14.3 \pm 0.64	55.8 \pm 1.7	18.3 \pm 0.59	32.8 \pm 0.12	1009.2 \pm 129.9	36.8 \pm 14.2	40.2 \pm 8.3	22.2 \pm 6.1	0.80 \pm 0.20	0.00 \pm 0.00
B	43.3 \pm 0.88	8.8 \pm .14	7.7 \pm 0.19	14.2 \pm 0.23	55.5 \pm 0.98	18.2 \pm 0.26	32.7 \pm 0.17	870 \pm 145.7	10.0 \pm 0.57	64.3 \pm 4.2	25.3 \pm 4.3	0.33 \pm .33	0.00 \pm 0.00
C	45.2 \pm 0.20	9.6 \pm 1.3	8.3 \pm 0.23	14.8 \pm 0.08	54.4 \pm 1.5	17.9 \pm 0.48	32.9 \pm 0.38	702 \pm 70.2	19.2 \pm 2.5	53.0 \pm 4.2	29.0 \pm 4.7	0.60 \pm .24	0.20 \pm 0.20
D	46.5 \pm 0.86	11.8 \pm 0.68	8.4 \pm 0.24	15.2 \pm 0.32	54.9 \pm 1.0	18.1 \pm 0.13	32.8 \pm 0.31	820.5 \pm 63.9	13.0 \pm 2.3	51.2 \pm 4.7	34.5 \pm 2.9	0.75 \pm .25	0.25 \pm 0.25
F-ratio	1.427	0.547	2.61	1.138	0.193	0.175	0.08	1.702	1.906	2.322	1.098	0.591	0.6
Sig.	0.28	0.659	0.096	0.37	0.9	0.912	0.97	0.216	0.179	0.123	0.385	0.632	0.626

Table 4 presents the result of the biochemical parameters measured. Animals treated with 800 mg/kg body weight of DEAE (Group A) had significantly higher mean serum aspartate transaminase when compared to the control group. Also, animals that received 800 mg/kg and 400 mg/kg body weight of DEAE had significantly higher mean serum urea levels compared to the control while Group C treated with 200 mg/kg body weight of DEAE was observed to have significantly higher serum sodium concentration compared to controls. Increased serum levels of aspartate transaminase (AST) in the animals that received the highest dose of DEAE suggest that the extract may have hepatotoxic components. The enzyme AST is found in the cytosol of hepatocytes hence an increased AST levels in the serum is indicative of damage to these hepatocytes [16,17]. Increased serum

urea levels observed in animals treated with 400 mg/kg and 800 mg/kg body weight of DEAE also suggests that the extracts may adversely affect kidney function. Serum urea is generally accepted as a good predictor of renal function. The serum levels of urea is usually found to increase with kidney injury because the impaired nephrons become unable to adequately clear urea produced in the body [18,19]. The elevated serum sodium level observed in animals treated with 200 mg/kg bodyweight of DEAE is difficult to explain because the rats that received higher doses were not affected. However it is possible that the increase is as a result dehydration [20]

Table 4: Effects of DEAE on the haematological parameters of the animals in the treatment groups and the control group Values are presented as mean \pm SEM

Group	Alkaline Phosphatase	Alanine Transaminase	Aspartate Transaminase	Serum Urea Levels	Sodium	Potassium	Chloride	Total Bilirubin
A	286.8 \pm 33.4	41.3 \pm 3.4	168.9 \pm 10.4*	25.3 \pm 3.4*	138.5 \pm 3.7	5.3 \pm 0.09	98.5 \pm 3.9	0.44 \pm 0.34
B	235.3 \pm 64.8	35.3 \pm 9.1	142.3 \pm 19.1	23.5 \pm 3.8*	135.2 \pm 7.6	5.5 \pm 0.29	101.8 \pm 3.4	0.55 \pm 0.32
C	239.8 \pm 22.2	45.0 \pm 6.2	120.6 \pm 8.4*	21.2 \pm .98	143.2 \pm 3.0*	5.3 \pm 0.18	101.1 \pm 1.0	0.62 \pm 0.33
D Control	142.0 \pm 24.8.	37.3 \pm 5.7	120.6 \pm 11.3	10.5 \pm 2.7	116.3 \pm 9.8	4.6 \pm 0.56	91.2 \pm 6.9	0.77 \pm 0.45
F-ratio		0.491	3.679	4.771	3.543	1.476	1.279	0.142
Sig.		0.694	0.038	0.017	0.043	0.264	0.32	0.933
Note: *- p<0.05 when compared with control (Group D)								

Figures 1 and 2 are representative micrographs of the histopathological examination of tissue sections from the excised organs. Examination revealed that the liver, heart, stomach, intestines and testes of the DEAE treated groups were normal with no pathological changes. However, histopathological changes were observed in the kidneys of the animals treated with 800 mg/kg and 400 mg/kg bodyweight of DEAE with sections showing eosinophilic tubular casts and mild glomerular constriction respectively. Eosinophilic intratubular casts as seen in some of the treated animals are characteristic of progressive nephropathy associated with increased glomerular permeability [21,22]. These histological changes correlate with the elevated serum urea already discussed. Several medicinal plants products have been reported to be toxic to the kidneys therefore it is possible that DEAE has renotoxic components [23].

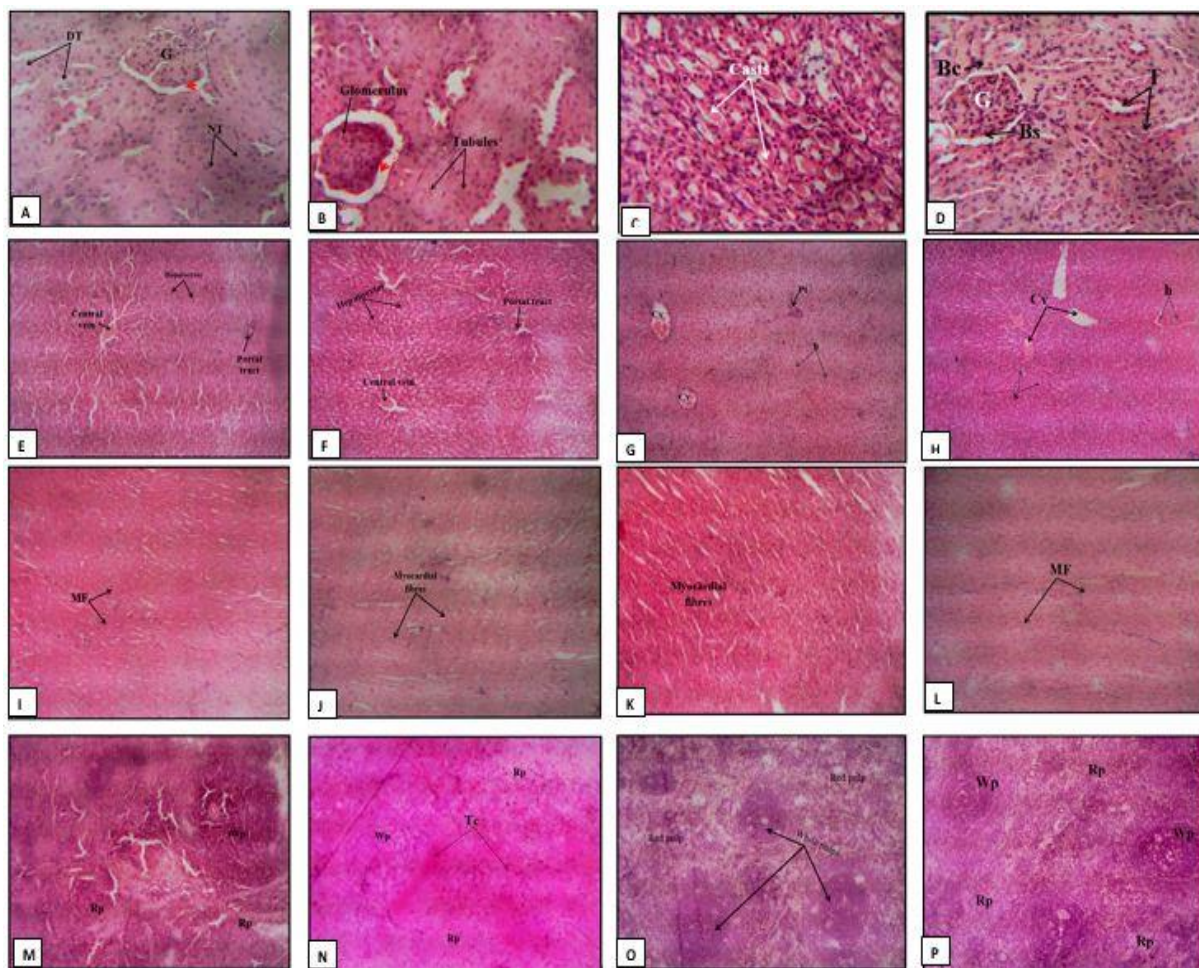


Figure 1: (A – P) Annotated photomicrographs of kidney (A – D), liver (E – H), heart (I – L) and spleen (M – P) of animals in Group A (A, E, I, M), Group B (B, F, J, N), Group C (C, G, K, O) and Control group (D, H, L, P). Key: NT – normal tubules, DT – distal tubules, G – glomerulus, BC – Bowman's capsule, BS – Bowman's space; CV – central vein, H – hepatocytes; MF – myocardial fibres; RP – red pulp, WP – white pulp. Stain: H & E.

Lastly, the spleen section of animals that received 200 mg/kg body weight of DEAE had slightly increased white pulp while those of the other treatment groups were normal. The observed increased cellularity of the splenic white pulp of animals given 200 mg/kg body weight of the extract may have been as result of several factors. Extra-medullary hemopoiesis is one of the major causes of such increases. This occurs normally in young rodents and to a lesser extent in adult rodent especially in response to toxic challenges to the haemopoetic system [24]. Also, certain therapeutic compounds like citral and elmiron are known to cause an increase in the cellularity of the splenic white pulp via unknown mechanism [25]. It is possible that DEAE causes spontaneous increase in white pulp cellularity.

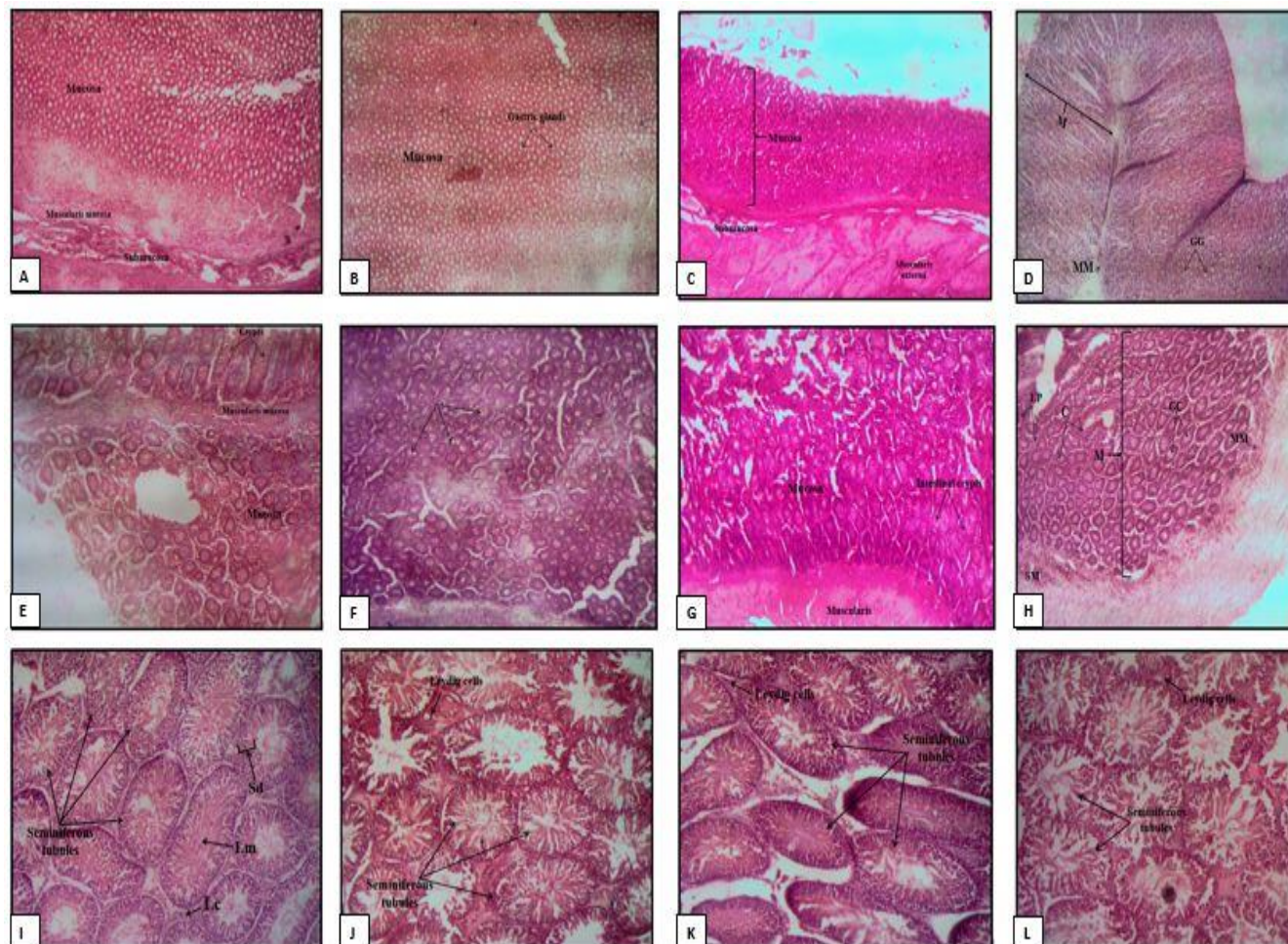


Figure 2: (A – L): Annotated photomicrographs of stomach (A - D), intestine (E - H) and testes (I - L) of animals in the treatment Group A (A, E, I), B (B, F, J), C (C, G, K) and control group D (D, H, L). M – mucosa, GG – gastric gland, MM – muscularis mucosa, SM – submucosa; Sd – spermatogenic unit, Lm – lumen, Lc – Leydig cells. Stain: H & E.

CONCLUSION

From the results of this toxicity study, DEAE did not induce toxic changes to majority of the parameters measured and organs assessed. However, the extracts may not be totally safe for consumption because of the observed adverse effects noticed in the kidney at higher doses. Traditional use for medicinal purposes should be cautious. It is thus important that further studies be carried out to determine possible toxic metabolites present in *D. edulis* seeds.

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