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In vivo assessment of the toxic potential of *Dissotis rotundifolia* whole plant extract in Sprague–Dawley rats

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ABSTRACT

Objective: To assess the toxic potential of *Dissotis rotundifolia* (*D. rotundifolia*) whole plant extract in Sprague–Dawley rats within a 2-week period of administration.
Methods: Methanolic extract of *D. rotundifolia* was administered orally once daily at dose levels of 0, 100, 300 and 1 000 mg/kg body weight for 14 days. Toxicity was assessed using mortality, clinical signs, body and organ weights, hematological indices, serum chemistry parameters and histopathological analyses.
Results: There were no treatment-related mortalities or differences in clinical signs, hematology and serum biochemistry. This was confirmed by micrographs obtained from histopathological analysis.
Conclusions: The results obtained from the sub-acute toxicological assessment of *D. rotundifolia* extract suggest that the extract is non-toxic at doses up to 1 000 mg/kg/day administered for a period of 14 days.

1. Introduction

Medicinal plants have played a major role in the development of modern orthodox medicines and continue to be widely used in their original form [1]. Published data indicate that about 80% of the world's population depend on plant medicine for healthcare delivery and about 70%–95% of developing countries rely on plant medicine for primary healthcare [2]. Implicitly, the practice of herbal medicine is gradually becoming the mainstream throughout the world. This may be due in part to the recognition of the value of traditional medical systems and the identification of medicinal plants from indigenous pharmacopeias that have been shown to have significant pharmacotherapeutic effect, either in their natural state or as a source of new pharmaceuticals.

In the Ghanaian setting about 80% of the populace rely on plants for healthcare [2]. This is an indication that plant medicine continues to play a key role in maintaining healthcare. In Ghana, plants have been used as food and treatments for several ailments for decades with little or no scientific data on their safety. The practice has rested largely on clinical experience. Phytomedicines are widely patronized and perceived to be of low risk in comparison to synthetic drugs [3], because they are natural though they are not completely free of toxic or other adverse effects [4]. The popularity of plant medicines coupled to scarcity of evidence-based data on their safety has raised serious concerns regarding their therapeutic value. The evaluation of toxic effects of plant medicines in rodents and other

animals is indispensable to ensure safety. The increasing usage of plant preparations as cosmetics and medicine demands that they are assessed for their toxicity profile.

Dissotis rotundifolia (*D. rotundifolia*) is a member of the family Melastomataceae and geographically native to some parts of Africa including Western and Eastern Africa [5,6]. The plant is among the most widely used medicinal plants in tropical Africa, and has been reported to have various ethno-medicinal uses. Considering the phytoconstituents of the whole plant [7] and its reported pharmacological effects on worm infection, diarrhea, cough, dysentery, peptic ulcer and other diseases [8–13], proof of toxicological assessment of the whole plant extract is necessary in order to assure consumers of its safety. Thus, this study was designed to assess the potential toxicity of *D. rotundifolia* extract (DRE) in male and female Sprague–Dawley (SD) rats.

2. Materials and methods

2.1. Collection of plant material and preparation of extract

The *D. rotundifolia* whole plant was collected from the environment around the Kakum National Park, Cape Coast, Ghana in January 2012 and authenticated by the Curator at the Herbarium of the Department of Environmental Science, School of Biological Sciences, University of Cape Coast. A voucher specimen (No. 107346) was prepared and deposited at the herbarium. The whole plant was washed thoroughly, shade-dried for three weeks, oven-dried at 40 °C for 3 h then pulverized into powder.

The method of extraction described by Rath *et al.* [7] involving the use of dichloromethane and 70% methanol was employed in the preparation of the crude whole plant extract. One hundred and fifty gram of powder was transferred into a 2 L flask and 1.5 L of dichloromethane was added in a fume chamber. The flask was tightly corked with a cotton wool plug, which was covered with an aluminium foil and placed on an orbital shaker (IKA HS/ KS260 basic orbital shaker-Werke-GmbH & Co. KG Germany) at a speed of 200 r/min for 48 h. The resulting mixture was then filtered into a 2 L flat bottom flask using a Whatman No.1 filter paper. The residue was dried at 40 °C on a water bath and the filtrate was discarded appropriately. A volume of 1.5 L of 70% methanol was added to the dried residue corked with a cotton wool plug and placed on an orbital shaker at 200 r/min for 48 h. The resulting mixture was again filtered using a Whatman No.1 paper. The filtrate was subsequently concentrated using a rotary evaporator (BÜCHI rotavapor R-200, Germany) and later dried at 40 °C in an oven to obtain a brown coffee-colored extract and this was labeled as DRE. This extraction process was repeated on 2000 g of powdered plant sample. The crude DRE was stored at −20 °C until ready for use.

2.2. Experimental animals and maintenance

Male and female SD rats (200–270 g) were obtained from the Centre for Plant Medicine Research. The experimental protocol was approved by the Faculty of Pharmacy and Pharmaceutical Sciences Animal Experimentation Ethics Committee of the Kwame Nkrumah University of Science and Technology, Kumasi, Ghana and the experiments were conducted according to the guidelines on the use and care of experimental animals as provided by the Organization for Economic Cooperation and Development [14]. Animals were fed with commercial rat feed supplied by Ghana Agro Food Company Tema, Ghana and

water *ad libitum*. They were maintained at a 12 h light/dark cycle at (25 ± 2) °C. All animals were acclimatized for two weeks before start of any experiment.

2.3. Treatment and toxicity assessment

The median lethal dose (LD_{50}) values of the extracts were determined according to the method of Finney [15]. DRE at a maximum dose level of 5000 mg/kg (orally) was used for acute oral toxicity study. A total of 30 males and 30 females SD rats were selected and divided into 6 groups of 10 rats each (5 males and 5 females) per group. Group 1 served as control and received distilled water only. Groups 2–6 were treated with extracts at different doses (10, 100, 500, 2500 and 5000 mg/kg body weight). Each rat in a group received the same extract dose administered as a single oral dose. Mortality, if any, was determined within 24 h and observation continued for a 2-week period. In addition, observations including changes in colors of the skin, fur, eyes, salivation, lacrimation, perspiration, piloerection, urinary incontinence, defecation, drowsiness, tremors, and convulsions were made.

In the 14-day sub-acute oral toxicity assessment study, animals were divided into four groups of male rats and four groups of female rats. Each group comprised either 4 males or 4 females. Group 1 of each sex received 2 mL of distilled water and served as control. Groups 2, 3 and 4, received daily oral doses of 100, 300 and 1000 mg/kg, respectively at a volume of 2 mL. The extract was administered daily for a period of 14 days at the same time, 9:00 am and observed at least twice daily for morbidity and for physical signs of toxicity. Body and organ wet weights, biochemical and hematological indices were assessed in all animals at baseline and at the end of treatment. Animals were fasted overnight prior to necropsy and blood collection. Blood samples from each animal were collected into two tubes, one containing ethylenediamine-tetraacetate and the other gel separator. At termination, organs such as liver, kidney, spleen, and stomach were excised, weighed and used for histopathological studies.

2.3.1. Measurement of hematological indices

Blood samples were earlier collected into ethylenediamine-tetracetate-2K tubes and analyzed for red blood cell (RBC), hemoglobin (HGB), pack cell volume or hematocrit (HCT), mean corpuscular volume, mean corpuscular, mean corpuscular HGB concentration, red cell distribution width-standard deviation, platelets, mean platelet volume, platelet distribution width, white blood cell (WBC) counts, neutrophils, lymphocytes using a Sysmex-KX-2IN hematology auto-analyser.

2.3.2. Determination of clinical biochemistry indices

Blood samples collected earlier into gel separator tubes were processed for plasma by centrifuging for 5 min at 3000 r/min to ensure separation of plasma. Plasma samples were stored at −20 °C until ready for use. The plasma was analyzed for levels of total protein (TP), albumin (ALB), globulin (GLB), total bilirubin (TB), indirect bilirubin (IDB), direct bilirubin (DB), alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferases (AST), total cholesterol (TC), triglycerides (TG), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), very low-density lipoprotein cholesterol (VLDL-C), urea, creatinine (CRE) and lactate dehydrogenase (LDH) using an automated

chemistry analyser [Elan ATAC 8000 random access chemistry analyzer (WS-ATAC8000)]. The plasma was also analyzed for levels of K and Na^+ using Selectra Junior autoanalyzer.

2.3.3. Histopathology analysis

Immediately, after the collection of blood samples, animals were opened up and various body organs were harvested. These internal organs which included heart, liver, spleen, kidneys and stomach tissues were cleared of fat and connective tissues, blotted with clean wipes, examined macroscopically and individually weighed to obtain the organ wet weights. All tissues were preserved in a 10% neutral buffered formalin solution.

For histopathological examinations, excised internal organ tissues of rats were cleaned in physiological saline, fixed in Bouin's fluid, dehydrated in increasing concentrations of ethanol and embedded in paraffin wax. Thereafter, sections of tissues were cut at 5 µm with a rotary microtome and mounted on clean glass slides. Sections were stained with haematoxylin and eosin (H & E) as described by Bancroft and Gamble [16]. The stained tissues were observed through an Olympus microscope and photographed by charecouple device camera at total magnifications 100×.

2.4. Data analysis

Data collected were analyzed using GraphPad Prism, windows version 4.02 (GraphPad Software, San Diego, CA, USA). Descriptive and inferential statistics including means, *t*-test and ANOVA were employed in analysis of data except for histopathology results where micrographs were shown. A probability value of less than 5% was used to determine significant differences. Bonferroni *post-hoc* multiple test were performed where a significant difference was recorded for ANOVA.

3. Results

3.1. LD_{50} and acute toxicity studies

No mortality was recorded at all dose levels (10–5 000 mg/kg body weight) of DRE over the 24-h period. Furthermore, compared to the control group, the animals treated with all dose levels of DRE did not show signs of aggressiveness, vomiting, respiratory distress, salivation, sedation and diarrhoea. Daily observation over the 14 days post-treatment did not reveal evidence of latent DRE-related toxicities.

3.2. Effect of DRE on organ and body weight

The results presented in Table 1 showed that liver, heart, kidney, lungs, stomach and spleen from DRE-treated animals did not show significant changes ($P > 0.05$) compared to organs

Table 2

Body weights in rats orally treated with DRE during sub-acute studies.

Treatment	Body weight (g)		*P* value
	Initial	Final	
Control	238.12 ± 12.62	256.00 ± 13.70	0.000
100 mg/kg	252.12 ± 13.11	239.00 ± 8.93	0.267
300 mg/kg	237.50 ± 6.25	234.12 ± 8.27	0.664
1 000 mg/kg	235.00 ± 8.67	251.89 ± 15.37	0.088

Values are mean ± SEM, ($n = 8$); data analyzed using paired *t*-test.

from vehicle-treated animals. There were no changes in the body weight in all extract-treated groups with the exception of control group after 14 days of treatment (Table 2).

3.3. Effect of DRE on hematological parameters

The results indicated that most of the hematological parameters measured remained unchanged within the two-week experimental period when compared to control groups. The significant changes observed in WBC counts were not dose-related ($P > 0.05$) (Table 3).

3.4. Effect of DRE on serum biochemical parameters

The data for the biochemical parameters were presented in Table 4. The effect of the extract on serum biochemical parameters after a period of 14 days of treatment showed no significant differences in levels TP, ALB, GLB, TB, DB, ALP, ALT, AST, TC, LDL-C, HDL-C, urea, CRE, LDH and K (Table 4) ($P > 0.05$). The differences observed in levels of IDB, VLDL-C, and TAG though significant were not dose-dependent. Na^+ concentration was markedly lower in animals treated with DRE dose of 1 000 mg/kg ($P = 0.030$).

3.5. Photomicrograph of liver cells

Histopathological examination of the liver confirmed no cellular damage in all the treated groups, compared to control group. Intact liver tissue with plates of hepatocytes, sinusoids that confluence with the central vein was observed. No obvious proliferation of Kupffer cells to remove debris nor other forms of toxicity were observed (Figure 1A–D). Results shown are for males, similar results were obtained for females.

3.6. Photomicrograph of the kidney

Histopathological examinations of the kidneys showed no significant changes in the kidney in extract-treated groups

Table 1

Organ weights in rats orally treated with DRE during sub-acute studies (g).

Parameters	Liver	Kidney	Heart	Spleen	Lungs	Stomach
Control	1.620 ± 0.120	1.300 ± 0.070	0.790 ± 0.040	0.506 ± 0.040	1.230 ± 0.050	6.890 ± 0.610
100 mg/kg	1.510 ± 0.040	1.250 ± 0.080	0.780 ± 0.050	0.538 ± 0.030	1.210 ± 0.030	6.670 ± 0.430
300 mg/kg	1.470 ± 0.110	1.210 ± 0.040	0.750 ± 0.030	0.506 ± 0.040	1.250 ± 0.034	6.690 ± 0.260
1 000 mg/kg	1.450 ± 0.050	1.280 ± 0.050	0.730 ± 0.030	0.640 ± 0.120	1.240 ± 0.040	6.750 ± 0.330

Values are mean ± SEM, ($n = 8$) (14 days).

Table 3

Hematological indices in rats orally treated with DRE.

Parameters	Hematological values				*P* value
	Control	100 mg/kg	300 mg/kg	1 000 mg/kg	
RBC (M/μL)	7.86 ± 0.18	8.05 ± 0.18	7.54 ± 0.16	7.79 ± 0.16	0.235
HGB (g/dL)	14.88 ± 0.25	15.04 ± 0.30	14.49 ± 0.30	14.54 ± 0.19	0.389
HCT (%)	46.70 ± 0.83	47.80 ± 0.97	45.64 ± 1.10	45.43 ± 0.88	0.287
MCH (pg)	18.95 ± 0.23	18.66 ± 0.14	19.23 ± 0.13	17.83 ± 0.98	0.265
MCV (fL)	59.46 ± 0.62	59.41 ± 0.40	60.33 ± 0.30	59.31 ± 0.57	0.055
MCHC (g/dL)	31.86 ± 0.11	31.50 ± 0.13	31.76 ± 0.20	32.05 ± 0.24[c]	0.143
RDW-SD (fL)	29.16 ± 0.46	29.55 ± 0.25	30.16 ± 0.36	29.64 ± 0.50	0.379
Platelets × 10^2 (K/μL)	9.74 ± 75.95	9.98 ± 56.70	8.59 ± 108.58	9.02 ± 52.87	0.564
MPV (%)	6.99 ± 0.09	7.03 ± 0.09	7.05 ± 0.12	6.88 ± 0.11	0.650
PDW (fL)	8.29 ± 0.14	8.19 ± 0.11	8.38 ± 0.14	7.99 ± 0.16	0.243
WBC counts (K/μL)	11.93 ± 1.22	11.79 ± 0.99	9.35 ± 0.38	16.05 ± 1.12[abc]	0.001
Lymphocytes (%)	75.33 ± 3.03	74.55 ± 4.58	72.55 ± 3.01	68.44 ± 2.81	0.501
MID (%)	7.04 ± 0.64	6.41 ± 0.64	7.08 ± 0.33	8.34 ± 0.45	0.098
Neutrophils (%)	17.71 ± 2.45	14.14 ± 1.19	20.04 ± 2.68	23.23 ± 2.42	0.054

Values are mean ± SEM, (*n* = 8); [a]: Significant difference between 1 000 mg/kg body weight and control; [b]: Significant difference between 1 000 mg/kg body weight and 100 mg/kg body weight; [c]: Significant difference between 1 000 mg/kg body weight and 300 mg/kg body weight; PCV: Pack cell volume; MCV: Mean corpuscular volume; MCH: Mean corpuscular; MCHC: Mean corpuscular HGB concentration; RDW-SD: Red cell distribution width-standard deviation; MPV: Mean platelet volume; PDW: Platelet distribution width.

compared to control (Figure 2A–D). Intact glomerulus in Bowman's capsule with capsular space, proximal and distal convoluted tubules with simple cuboidal epithelium, blood vessel were seen. There were no obvious signs of damage to nephrons.

3.7. Photomicrograph of the spleen

Histopathological examinations of the spleen tissues showed no significant changes in spleen (Figure 3A–D). Normal splenic tissue with white pulp of lymphoid cells, surrounding an arteriole was observed. Surrounding the white pulp is the red pulp traversed by venous sinuses, which indicated the absence of no sign of damage or toxicity.

3.8. Photomicrograph of the stomach

There were no salient pathological changes in the stomach tissues of extract-treated animals as compared to controls (Figure 4A–D). Intact simple columnar epithelium and blood vessel in submucosa and smooth muscle were seen. Ulceration of the epithelia was not evident.

Table 4

Serum biochemistry parameters in rats orally treated with DRE.

Parameters	Biochemical values at indicated dosages				*P* value
	Control	100 mg/kg	300 mg/kg	1 000 mg/kg	
AST (IU/L)	166.48 ± 6.06	157.39 ± 4.17	159.92 ± 5.10	158.96 ± 7.78	0.716
ALT (IU/L)	49.00 ± 2.56	47.88 ± 3.30	53.50 ± 1.86	56.13 ± 3.07	0.142
ALP (IU/L)	513.88 ± 82.79	483.25 ± 71.07	521.75 ± 41.38	589.25 ± 36.59	0.663
TP (g/L)	133.12 ± 6.63	140.82 ± 7.98	136.15 ± 4.44	143.57 ± 9.95	0.764
ALB (g/L)	32.36 ± 0.60	31.71 ± 1.39	33.27 ± 1.75	31.22 ± 2.53	0.849
GLB (g/L)	100.76 ± 6.34	109.11 ± 8.10	102.88 ± 4.10	112.35 ± 10.02	0.672
A/G	0.33 ± 0.02	0.30 ± 0.03	0.33 ± 0.02	0.30 ± 0.04	0.786
DB (μmol/L)	2.60 ± 0.40	1.96 ± 0.33	2.13 ± 0.40	2.17 ± 0.28	0.631
IDB (μmol/L)	1.01 ± 0.23	2.07 ± 0.72	0.67 ± 0.22	2.86 ± 0.79	0.038
TB (μmol/L)	3.62 ± 0.44	4.04 ± 0.92	2.80 ± 0.33	5.02 ± 0.84	0.162
CRE (μmol/L)	21.70 ± 2.01	22.42 ± 1.48	25.92 ± 1.50	21.02 ± 1.54	0.166
UREA (mmol/L)	10.64 ± 0.36	10.91 ± 0.88	9.91 ± 0.38	10.85 ± 0.68	0.650
K (mEq/L)	5.84 ± 0.47	4.57 ± 0.34	5.43 ± 0.68	5.30 ± 0.74	0.488
Na$^+$ (mmol/L)	166.27 ± 16.55	114.17 ± 13.91	117.89 ± 19.47	101.38 ± 9.65[a]	0.030
LDH (IU/L)	1 313.1 ± 128.73	1 539.5 ± 186.77	1 832.1 ± 163.88	1 916.7 ± 160.53	0.050
TAG (mmol/L)	1.48 ± 0.07	1.12 ± 0.06	1.53 ± 0.13	1.81 ± 0.14[b]	0.045
TC (mmol/L)	4.07 ± 0.26	3.28 ± 0.39	3.31 ± 0.20	3.56 ± 0.32	0.247
LDL-C (mmol/L)	3.07 ± 0.27	2.28 ± 0.33	2.30 ± 0.16	2.40 ± 0.24	0.123
HDL-C (mmol/L)	0.32 ± 0.01	0.50 ± 0.16	0.31 ± 0.03	0.33 ± 0.05	0.428
VLDL-C (mmol/L)	0.29 ± 0.01	0.22 ± 0.01	0.30 ± 0.02	0.36 ± 0.02[b]	0.002

Values are mean ± SEM (*n* = 8); [a]: Significant difference between 1 000 mg/kg body weight and control; [b]: Significant difference between 1 000 mg/kg body weight and 100 mg/kg body weight.
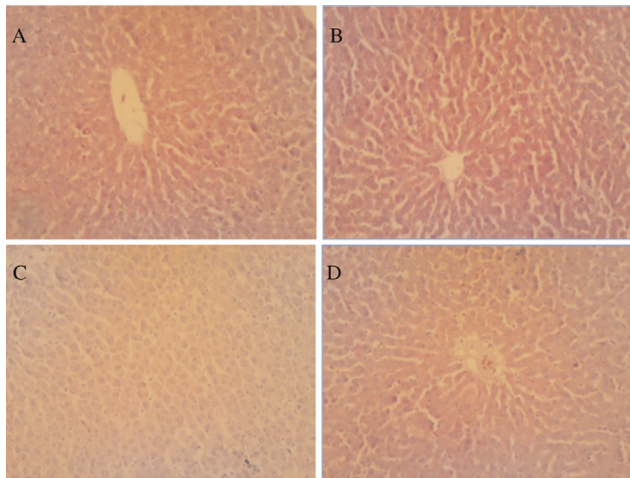
Figure 1. Micrographs of the liver tissues for control (A), 100 mg/kg (B), 300 mg/kg (C), 1 000 mg/kg (D) groups at the end of treatment period of 14 days with DRE.

Figure 2. Micrographs of the kidney tissues for control (A), 100 mg/kg (B), 300 mg/kg (C), 1 000 mg/kg (D) groups at the end of treatment period of 14 days with DRE.
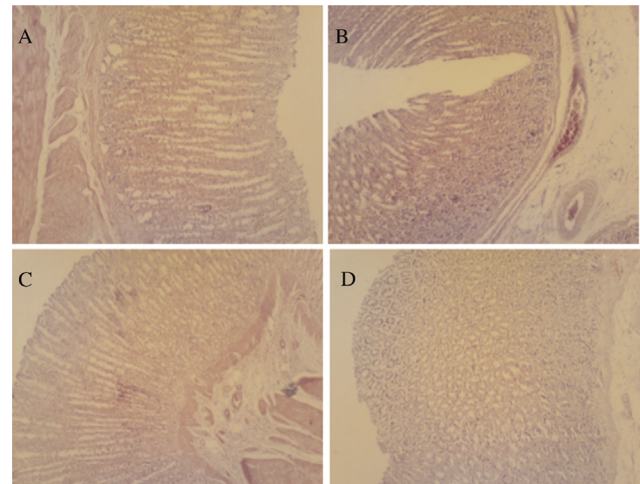

Figure 3. Micrographs of the splenic tissues for control (A), 100 mg/kg (B), 300 mg/kg (C), 1 000 mg/kg (D) groups at the end of treatment period of 14 days with DRE.

Figure 4. Micrographs of the stomach tissues for control (A), 100 mg/kg (B), 300 mg/kg (C), 1 000 mg/kg (D) groups at the end of treatment period of 14 days with DRE.
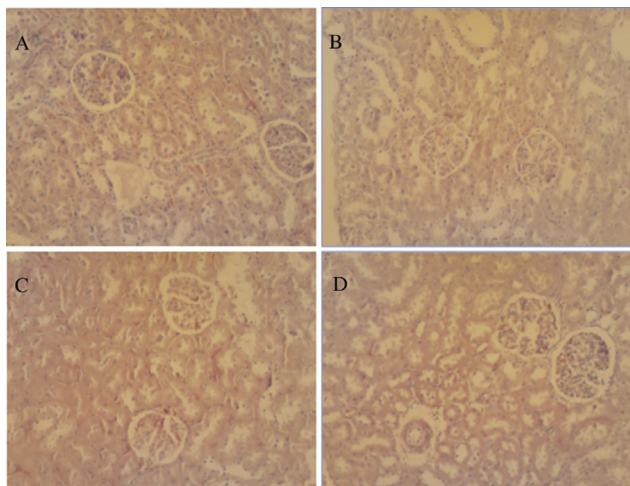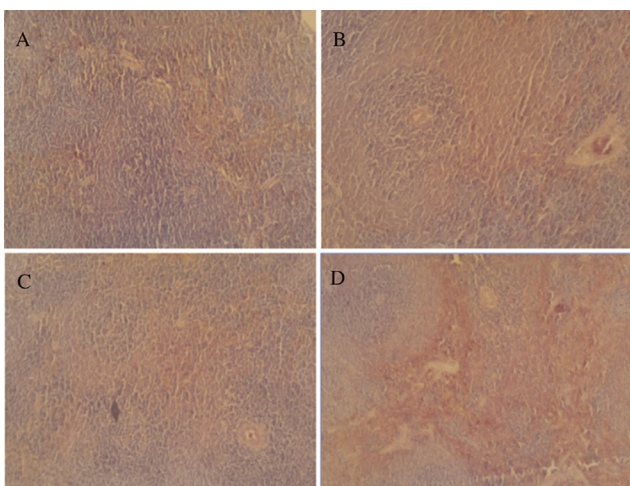
4. Discussion

In any pharmacological studies, proof of safety is necessary. No potential drug is used clinically without its toxicity and clinical trial studies data. Toxicological studies assist in deciding whether a new drug should be adopted for clinical use or not. Toxicity studies done in appropriate animal models are generally used to assess the potential health risk of drug to humans [17]. Such data provide information on health hazards likely to arise from a repeated exposure of the test substance to mammals, as is being used repeatedly for the management of several diseases. Depending on the duration of exposure of animals to drug agents, toxicological studies may be put into four categories; acute, sub-acute, sub-chronic and chronic studies.

In the present study, acute and sub-acute toxicity profile of the extract of the whole plant of *D. rotundifolia* was evaluated in both sexes of adult SD rats. Animals were subjected to administration of DRE orally. This mode of administration was considered the most suitable because it is the intended clinical route of DRE administration in humans and has been used in previous non-clinical studies.

The absence of deaths in the acute toxicity study, places the LD_{50} above 5 000 mg/kg. According to Oslon *et al.* [18], a substance that shows LD_{50} at 1 000 mg/kg body weight is considered safe or of low toxicity. Implicitly, DRE could be said to be of low toxicity. Organ and body weight are indices that are often used in toxicological evaluations may be altered in tumors, hyperplasias and other disease states although definite genotoxicity is mostly required to evaluate carcinogenic potentials of drug agents. The results in this study show that organ and body weight indices of liver, heart, kidney, spleen and stomach were not significantly altered by the 14-day extract treatment regimes. This may give an indication of the potential harmless effect of the extract to these internal organs.

This study also did not reveal any overt adverse effects on hematological parameters in the DRE-treated animals. Hematological changes such as anemia are often accompanied with bone marrow toxicity among other causes [19]. Hematological parameters are usually associated with health status and are of diagnostic importance in clinical assessment of the state of

health of animals and humans. Blood parameters are good indicators of physiological, pathological and nutritional status. Changes in these parameters have implications on therapeutic drug testing and toxicological factors in animals [19]. The hematological data shows that DRE has no significant adverse effect on blood. This may suggest that DRE neither altered the incorporation of hemoglobin into RBCs nor the morphology and osmotic fragility of RBCs. HCT is a measure of the volume of blood consisting of solid cells. The HCT which was unaffected by the extract in all the treatment groups indicates that the volume of RBC in the blood remained constant. Although WBC count was elevated at the highest DRE dose level (1 000 mg/kg body weight), the normal neutrophils would suggest that the extract may not have induced an inflammatory process since these cells are usually elevated in the course of inflammation [20].

A predominance of unconjugated bilirubin is suggestive of hemolytic jaundice, whereas a preponderance of conjugated bilirubin is generally associated with hepatic disease and extra-hepatic biliary obstruction [21]. The changes in IDB may be of no consequence as ALT, ALP and AST levels were not significantly affected. Elevated levels of AST and ALT are often diagnostic of underlying cellular injuries [22]. This is further supported by the normal levels of the bile pigments observed in this study suggesting lack of adverse DRE effects on the liver. There was also no change in the levels of urea in this study, suggesting that kidneys were not adversely affected. It is also interesting to note that the plant extract did not cause any impairment on the cardiovascular system as shown by the insignificant changes in the levels of electrolyte and lipid profile indices.

In order to confirm the absence of damage in the internal organs, histopathological assessment was done as shown in Figures 1–4. Histopathological evaluation showed no sign of tissue alteration or damage, thus the extract did not cause any toxic effect on the liver, kidney, stomach and spleen over the 14-day administration.

In conclusion, the study demonstrates that DRE is safe up to a dose of 1 000 mg/kg body weight within a 14-day period.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgments

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