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DIEFFENBACHIA SEGUINE INGESTION: IT MAY NOT BE AS BAD AS IT HAS BEEN LABELED IN RECENT TIMES

Moses W. Bariweni*[1], Raymond I. Ozolua[2], Oboma I. Yibala[3], Gideon O. Alade[4] and Imomoton J. Ogoina[1]

[1]Department of Pharmacology and Toxicology, Faculty of Pharmacy, Niger Delta University, Wilberforce Island, Bayelsa State, Nigeria.
[2]Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Benin, Benin City 300001, Nigeria.
[3]Department of Medical Laboratory Science, Faculty of Basic Medical Science, Niger Delta University, Wilberforce Island, Bayelsa State, Nigeria.
[4]Department of Pharmacognosy and Herbal Medicine, Faculty of Pharmacy, Niger Delta University, Wilberforce Island, Bayelsa State, Nigeria.

***Corresponding Author: Dr. Moses W. Bariweni**
Department of Pharmacology and Toxicology, Faculty of Pharmacy, Niger Delta University, Wilberforce Island, Bayelsa State, Nigeria.

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ABSTRACT
Exposure of humans and animals to certain plants has often resulted in cases of poisoning. The dumb cane plant, *Dieffenbachia seguine* (Jacq.) Schott (Araceae), is a popular indoor ornamental plant in Nigeria with trending fears regarding its very poisonous nature upon acute eating of the leaves. Fresh leaves of *D. seguine* were extracted in70% methanol and the dried extract (DSME) was employed in in the estimation of LD_{50} in rats. Subacute toxicity was evaluated at doses of 625, 1250 and 2500 mg/kg/day of the extract for 14 consecutive days. At the end of treatment, whole body and organ weights, hematological, biochemical and histological parameters were evaluated. The oral median lethal dose (LD_{50}) value was estimated to be 3808 mg/kg. Changes in whole body weights were not significantly different from control but the relative liver weight increased in the treated groups. There was a significant ($P<0.5$) reduction in white blood cell count, platelet count and the percentage of lymphocytes but the percentage of neutrophils increased significantly ($P<0.01$) in the treated groups. There was significant ($P<0.05$) reduction in urea, creatinine and bilirubin levels. The serum levels of alanine transaminase and aspartate transaminase increased significantly ($P<0.05$). Serum levels of total protein and albumin decreased significantly. Histology showed potentials for liver and kidney damage. The oral LD_{50} of DSME does not seem to agree with recent fears associated with oral ingestion of the leaves. Chronic ingestion of large quantities of the leaves may be toxic to the kidneys, liver, and the immune system.

KEYWORDS: Dieffenbachia, acute toxicity, sub-acute toxicity, liver damage, renal toxicity.

1. INTRODUCTION
Poisoning due to the consumption of plants by man and animals has been known since prehistoric times. The risk of poisoning becomes higher when the plant in question is used for ornamental purposes in homes. The dumb cane plant, *Dieffenbachia seguine* (Jacq.) Schott of the family Araceae is a popular indoor and outdoor perennial and easy-to-propagate ornamental plant. The leaves are simple with surfaces patterned by many irregular yellowish or cream-green splotches or white spots and flecks. It is hardly distinguishable from the other *Dieffenbachia* species. Aside from their ornamental use, *Dieffenbachia* species also possesses other uses as well. For example, the stem and root extracts are believed to possess narcotic, gastric and kidney irritant properties for which they were used as arrow poison and ordeal poison in the West Indies.[1] Other ethnomedicinal uses of the plant include treatment of gout, dropsy, and impotency.[2]

The poisonous nature of the plant has been a subject of several reports, in recent times, reports of severe destruction of respiratory structures including respiratory symptoms in an infant have been associated with the species.[3] It seems that all parts of the species are poisonous when ingested.[2] Their leaf juice causes keratoconjunctivitis, mastication of portions of leaf or stem has been reported to result in immediate and extreme irritation of the mouth, tongue, and lips followed by a painful burning sensation, intense salivation, edematous swelling, and temporary loss of speech for which the common name "Dumb cane" was given.[1] The oral effects have been associated with the presence of high amounts of calcium oxalate crystals which could pierce the oral mucosa but other reports indicate the presence of a proteinaceous agent. Other constituents include a proteolytic enzyme, a cyanogenic glycoside, a substance which causes contraction of smooth muscles[2],

asparagines and protoanemonin which causes swelling of the pharynx and larynx.[4]

Recently, reports of the poisonous nature of the leaves have trended in the media and have caused some worries with some persons discarding the plant from their homes. This indicated a need for the re-evaluation of the toxicological profile in spite of previous experimental studies. In this study, we used doses of its aqueous-methanol extract far in excess of what may be practicable in real life situations to evaluate its acute and sub-acute toxicological profile.

2. MATERIAL AND METHODS

2.1. Plant collection, authentication and preparation

Fresh leaves of *D. seguine* were collected from Yenagoa in Bayelsa State, Nigeria, in October, 2017. The plant was identified by Dr. A.T. Oladele of the Department of Forestry and Wildlfe Management, University of Port Harcourt, Port Harcourt, Nigeria and was authenticated at the Herbarium of the Department of Pharmacognosy and Herbal Medicine, Faculty of Pharmacy, Niger Delta University, Wilberforce Island, Nigeria by Prof. K.K. Ajibesin where a voucher specimen (NDUP 300) has been deposited. The fresh leaves were ground using a mortar and pestle to obtain a smooth paste. It was thereafter extracted cold in 70% methanol for 72 h. The extract was then concentrated to dryness *in vacuo* at 30^{o}C to give an extract (yield; 65.4% w/w), packed into an airtight amber-colored container, labelled DSME and kept in a refrigerator (2-8°C) until needed for experiments. Fresh stock solutions of DSME were prepared in distilled water each day for the experiments.

2.2. Animals

Adult Wistar rats of both sexes (160-200 g) bred in the animal house, Department of Pharmacologyand Toxicology, Faculty of Pharmacy, Niger Delta University, Wilberforce Island, Nigeria, were used for the study. The animals were housed (males separate from females) in standard plastic cages with 12 h light/dark cycle in a room with temperature of 26.0±2.0^{o}C. The animals were fed with standard rodent feed (Livestock Feeds Plc, Nigeria) and had free access to water. All animals were handled in accordance with European Union directive (2010/63/EU) for animals and ethical approval (NDU/PHARM/PCO/AEC/025) was obtained from the Animal Ethics Committee of Faculty of Pharmacy, Niger Delta University, Wilberforce Island, Bayelsa State, Nigeria.

2.3. Chemicals

Reagents and chemicals used for this study were of analytical grade and manufactured by Sigma-Aldrich (Germany), BDH (UK) and Randox Laboratories (UK). They were all freshly prepared before use.

2.4. Phytochemical screening

Phytochemical screening of DSME for the presence of classes of secondary plant metabolites such as alkaloids, tannins, saponins, anthraquinones and glycosides was done using standard methods.[5]

2.5. Acute toxicity testing

The Lorke (1983)[6] method for determining oral median lethal dose (LD_{50}) was modified for the acute toxicity testing of DSME. All animals were fasted overnight prior to the experiment but had free access to water until an hour before the experiment. Feeding resumed two hours after commencement of experiment. The test involved two phases. In the first phase, twelve rats were randomly allotted into four groups of three rats each and were given 0, 10, 100 and 1000 mg/kg of DSME orally. The rats were observed continuously for the first 4 h, and after 24 h for signs of toxicity and death. In the second phase of the study, the procedure was repeated using four rats randomly allotted into four groups of one rat each. Group one received 10 mL/kg of distilled water while the other groups received 1600, 2900 and 5000 mg/kg DSME respectively and observed as in phase 1. LD_{50} was calculated using the formula:

$$LD_{50} = \sqrt{(D_0 \times D_{100})}$$

Where, D_0= highest dose that gave no mortality; D_{100}= lowest dose that produced mortality

2.6. Fourteen-day oral toxicity evaluation

Twenty adult rats were randomly allotted into four groups (n=5). Animals in group A received distilled water 10 mL/kg and served as controls while those in groups B, C and D received 625, 1250 and 2500 mg/kg/day of DSME (being fraction of the minimum dose to establish safety) respectively for 14 days. The animals were monitored closely for symptoms of toxicity.

2.7 Hematological studies

On the 15th day blood samples were collected directly using a 5 mL syringe from abdominal aorta of the rats under diethyl ether anesthesia. About 0.5 mL of blood was immediately transferred into EDTA-laced sample bottles and properly mixed. Small sample volumes (13-18 µL) were withdrawn using an automated aspirator and fed into an automated hematology system (Sysmex Haematology Coagulation Systems®, Model KX-21N, Sysmex Incorporation, Kobe, Japan). The parameters measured include packed cell volume (PCV), white blood cell count (WBC), red blood cell count (RBC), platelet count (PLT), percentage neutrophil (NEU), and percentage lymphocyte (LYM).

2.8 Biochemical studies

The remaining volume from each sample of blood withdrawn from each aorta was transferred into a plain bottle (without anticoagulant), allowed to clot and then centrifuged at 5000 rpm to obtain serum for biochemical assays. Commercially available test kits (Randox Laboratories, UK) were used for these biochemical tests.

2.8.1 Urea determination

Ten micro liters of serum was mixed with 100 µL reagent 1 (EDTA, sodium nitroprusside and urease mixture) in a test tube and incubated for 10 min at 37°C. Equal volumes (2.5 mL) of reagent 2 (dilute phenol) and reagent 3 (dilute sodium hypochlorite) were added to the test tube, mixed and incubated immediately at 37°C for 15 min. The procedure was repeated with a blank and standard solution to obtain control values. The absorbance each of sample and standard was read against the blank at 546 nm in a 1 cm cuvette under controlled temperature (37°C). The serum urea concentration was calculated as shown below.[7]

$$\text{Urea concentration} = \frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times \text{Standard concentration}$$

2.8.2. Creatinine determination

A quantity of the serum (0.1 mL) was mixed with 1.0 mL of standard reagent (picric acid and sodium hydroxide solution). The absorbance was read after 30 s at 492 nm in a 1 cm light path at 37°C. The same procedure was repeated using blank and standard solutions. The concentration of creatinine was calculated using the same formula for estimating urea concentration.[8]

2.8.3. Assay of some serum enzymes

Serum alanine transaminase (ALT) and aspartate transaminase (AST) were measured using commercial kits (Randox Laboratories, UK) based on the method of Reitman and Frankel (1957).[9] For ALT measurement, 0.1 mL of serum was mixed with 0.5 mL of reagent 1 (phosphate buffer, L-alanine and α-oxoglutarate solution) and incubated for 30 min at 37°C. To this mixture 0.5 mL of reagent 2 (2,4-dinitrophenylhydrazone) was added, mixed and allowed to stand for 20 min at room temperature (25°C). This mixture was added to 5.0 mL of sodium and absorbance was read after 5 min at 546 nm in a 1 cm cuvette at 37°C against distilled water.

The procedure for serum ALT evaluation was used in determination of serum AST but reagent 1 contained L-aspartate instead of L-alanine. Serum AST activity was estimated from a standard reference table.

Commercial kits (Randox Laboratories, UK) were used for the assay of alkaline phosphatase (ALP) activity in which 0.05 mL of serum was mixed with 3.0 mL of reagent containing p-nitrophenylphosphate and diethanolamine buffer in a cuvette and the absorbance read at 405 nm maintained at 25°C. Other readings were taken at 1 min, 2 min and 3 min. The mean change in absorbance per minute was determined and this value was multiplied by 3300 to give the ALP activity.[10]

2.8.4. Albumin (ALB)

A quantity of serum (10 µL) was pipetted into a cuvette and mixed with 3000 µL of reagent (BCG concentrate, buffer and bromocresol green solution, Randox Laboratories, UK) and incubated at 37°C for 5 min. The absorbance was read at 578 nm in a 1 cm path length maintained at 25°C against the reagent blank. The procedure was repeated for blank and the standard and the absorbance calculated as shown above for urea.[11]

2.8.5. Total protein

Total protein (TP) was estimated using the Biuret method.[12] Serum (0.02 mL) was pipette into a cuvette and mixed with 1.0 mL of Biuret's reagent (sodium hydroxide, sodium-potassium tartrate, potassium iodide and copper sulfate solution), incubated for 30 min at 25°C and the absorbance measured at 546 nm in a 1 cm light path maintained at 25°C against distilled water. The process was repeated for the standard. The TP concentration was calculated with the same formula for urea.

2.8.6. Total bilirubin (TB) and conjugated bilirubin (CB)

The Jendrassik-Grof method was used to estimate TB and CB.[13] Briefly, 0.2 mL of serum was mixed in a cuvette with 0.2 mL reagent of 1 (sulfanilic acid and HCl solution), 0.05 mL of reagent 2 (sodium nitrite) and 1.0 mL of reagent 3 (caffeine). The mixture was allowed to stand for 10 min at 25°C. To this mixture was added 1.0 mL of reagent 4 (tartrate and sodium hydroxide solution) and allowed to stand for 30 min. The absorbance of the mixture was read at 578 nm in a 1 cm light path maintained at 25°C against the sample blank void of reagent 2. The absorbance multiplied by 10.8 gave the total bilirubin concentration (mg/dL).

2.8.7. Histological evaluation

Livers and kidneys obtained from sacrificed rats were rinsed in normal saline, blotted with Whatman's number 1 filter paper, observed for visible damage, weighed and then fixed in 10% formaldehyde-saline solution. Each organ was dehydrated in 70% ethanol and grossed with the aid of an automatic tissue processor (Leica TP 1020®). They were thereafter embedded in paraffin wax using an embedding panel (Leica EG 1160®), allowed to solidify and sectioned into 4 µm thickness with a microtome (Leica RM 2125®). The sections were floated out in warm water using a water bath (micro-Elisa®) and then stained with haematoxylin and eosin.[14] Photomicrographs of the stained tissue sections were produced using a digital microscope (Olympus®) at x 400 magnification.

2.8.8. Statistical analysis and data presentation

Data are presented as mean ± standard error of mean (SEM) and "n" represents the number of animals per experimental group. Inferential statistical analysis was done using one-way ANOVA followed by Dunnet's *post hoc* (GraphPad Prism 6 Software, San Diego, California, USA). Differences between compared data were considered significant at $P<0.05$.

3. RESULTS

3.1. Classes of secondary metabolites

Alkaloids, cardiac glycosides, flavonoids, reducing sugars and tannins were present while saponins and anthraquinones were absent (Table 1).

3.2. Oral acute toxicity of DSME

The extract did not produce any observable signs of toxicity at oral doses less than 1000 mg/kg except salivation. At 1600 mg/kg and above, writhing, restlessness and salivation occurred. Mortality occurred at 5000 mg/kg. The oral LD_{50} of DSME was estimated to be 3808 mg/kg.

3.3. Fourteen-day toxicity profile of oral DSME administration

3.3.1. Effects of DSME on weight indices

The weight indices of rats following 14 days of repeated oral administration are shown in Table 3. The changes in body weight were not statistically significant across the groups. However, there was significant increase in relative liver weight at 625 mg/kg/day ($P<0.05$), 1250 mg/kg/day ($P<0.01$) and 2500 mg/kg/day ($P<0.01$) compared to control. Relative weights of kidney and the tongue were not significantly different across the groups.

3.3.2. Effect on hematological parameters

There was a significant reduction in WBC, PLT and LYM while NEU significantly ($P<0.01$) increased in the treated groups. However, there was no significant difference in PCV and RBC in the treated groups when compared with control (Table 4).

3.3.3. Effect on biochemical parameters

Serum urea and creatinine levels reduced significantly ($P<0.01$) at 1250 and 2500 mg/kg/day respectively while total bilirubin concentrations reduced significantly ($P<0.01$) in all the treated groups (Table 5). The effects of DSME on serum ALP, ALT, AST, TP and ALB are shown in Figure 1. At 1250 and 2500 mg/kg/day, AST and ALT levels increased significantly ($P<0.01$) but the effect on ALP was comparable with control. Serum TP concentrations significantly reduced ($P<0.01$) at all doses of DSME while ALB concentration only reduced significantly ($P<0.05$) at 625 mg/kg/day (Figure 2).

3.3.4. Effect of kidney and liver histology

In the kidney, DSME caused proliferation of inflammatory cells at 1250 and 2500 mg/kg/day (Figure 3). The histology of the liver shows neutrophil infiltration occurring in all DSME-treated groups with further congestion of the central vein at 1250 mg/kg and portal triad at 2500 mg/kg (Figure 4).

Table 1: ***Identification of Classes of Compounds in DSME.***

Classes of Secondary Metabolites	Inference
Alkaloids	+
Anthracene Derivatives	-
Cardiac Glycosides	+
Flavonoids	+
Reducing Sugars	+
Saponins	-
Tannins	+

+ = present - = absent

Table 2: ***Oral acute toxicity profile of DSME.***

	Dose (mg/kg)	Writhing	Sedation	Diarrhea	Salivation	Death	Others
Phase I	0	0/3	0/3	0/3	0/3	0/3	0/3
	10	0/3	0/3	0/3	0/3	0/3	0/3
	100	0/3	0/3	0/3	0/3	0/3	0/3
	1000	0/3	0/3	0/3	2/3	0/3	0/3
Phase II	0	0/1	0/1	0/1	0/1	0/1	0/1
	1600	1/1	0/1	0/1	1/1	0/1	1/1
	2900	1/1	0/1	0/1	1/1	0/1	1/1
	5000	0/1	1/1	0/1	1/1	1/1	0/1

numerator = number of animals affected. denominator = number of animals in the group. LD50 = 3808 mg/kg.

Table 3: ***Weight Indices Following Daily Oral Administration of DSME for 14 days.***

	Weight Change (g)	Relative Liver Weight ($x10^{-3}$)	Relative Kidney Weight ($x10^{-3}$)	Relative Tongue Weight ($x10^{-3}$)
Control (DW)	33.1 ± 0.2	4.0 ± 0.5	0.6 ± 0.1	0.3 ± 0.1
DSME-625	33.5 ± 0.1	6.5 ± 0.4*	0.8 ± 0.1	0.4 ± 0.1
DSME-1250	33.3 ± 0.2	7.3 ± 0.9**	0.8 ± 0.1	0.3 ± 0.1
DSME-2500	32.9 ± 0.1	8.0 ± 0.1**	0.8 ± 0.1	0.4 ±0.1

doses of DSME are in mg/kg/day. The data represents mean ± SEM for the treatment groups, n=5. *P<0.5, **P<0.01when compared to control. DW: distilled water.

Table 4: Effect of daily (x14) oral Administration of DSME on Hematological Parameters.

	PCV (%)	RBC ($x10^6$/µl)	PLT ($x10^3$/µl)	WBC ($x10^6$/µl)	NEU (%)	LYM (%)
Control (DW)	43.6 ± 0.6	6.5 ± 0.2	734.0 ± 1.4	12.7 ± 0.5	19.5± 0.7	61.50 ± 0.3
DSME-625	44.4 ± 0.3	7.0 ± 0.2	656.0 ± 1.2**	12.2 ± 0.1	23.4 ± 1.0**	52.4 ± 0.3**
DSME-1250	44.7 ± 0.4	6.3 ± 0.1	647.0 ± 1.8**	11.3 ± 0.4*	22.4 ± 0.7*	55.4 ± 0.2**
DSME-2500	44.6 ± 0.3	6.9 ± 0.1	628.0 ± 1.6**	8.0 ± 0.2**	21.0 ± 0.7	57.6 ± 0.3**

doses of DSME are in mg/kg/day. The data represents mean ± SEM for the treatment groups, n=5. *P<0.05, **P<0.01 when compared with control a= less than control, b= higher than control. PCV: packed cell volume; WBC: white blood cell count; RBC: red blood cell count; PLT: platelet count; NEU: neutrophil; LYM: lymphocyte; DW: distilled water.

Table 5: Serum proteins and bilirubin levels of rats treated with oral doses of DSME for 14 consecutive days.

	Urea (g/dl)	Creatinine (g/dl)	TB (g/dl)	CB (g/dl)
Control (DW)	46.20 ± 0.92	0.76 ± 0.02	15.9 ± 0.4	3.9 ± 0.10
DSME-625	46.60 ± 0.54	0.72 ± 0.03	12.70 ± 0.20**	4.2 ± 0.20
DSME-1250	42.40 ± 0.67**	0.66 ± 0.02**	8.50 ± 0.50**	4.40 ± 0.40
DSME-2500	41.61 ± 0.31**	0.64 ± 0.02**	5.4 ± 0.4**	4.30 ± 0.10

Doses of DSME are in mg/kg/day. **P<0.01 compared with control. TB: total bilirubin; CB: conjugated bilirubin; DW: distilled water. n = 5 per group.

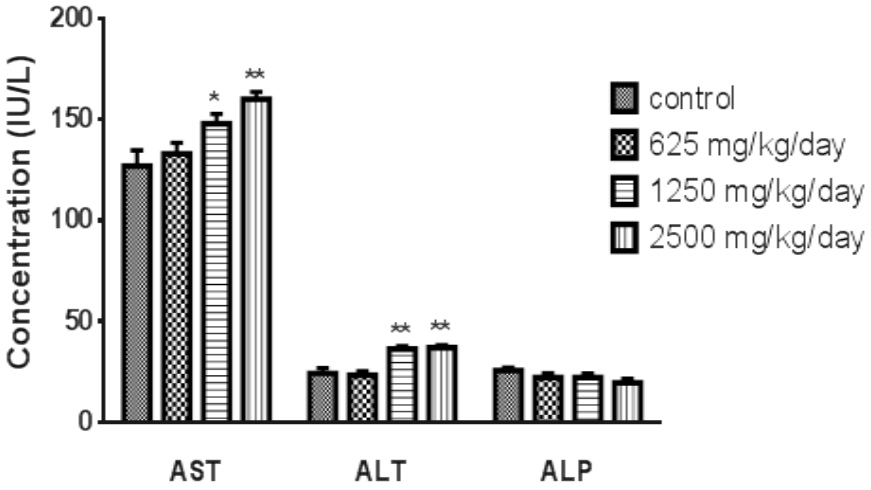

Figure 1: ***Effects of 14-day oral treatment of rats with methanol extract of D. seguine extract (DSME) on liver enzymes.*** *P<0.05, **P<0.01 versus control group. AST: aspartate transaminase; ALT: alanine transaminase; ALP: alkaline phosphatase. n = 5 per group.

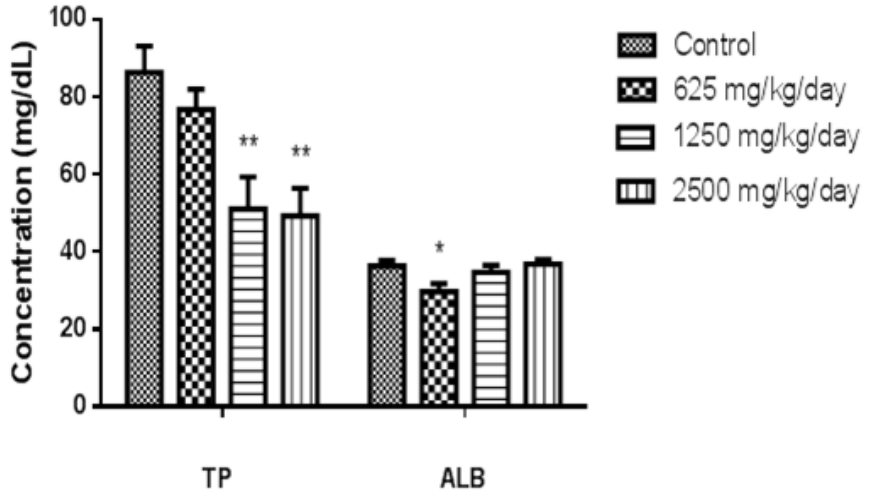

Figure 2: ***Effects of 14-day oral treatment of rats with methanol extract of D. seguine extract (DSME) serum proteins***. *P<0.05, **P<0.01 versus control group. TP: total protein; ALB: albumin. n = 5 per group.

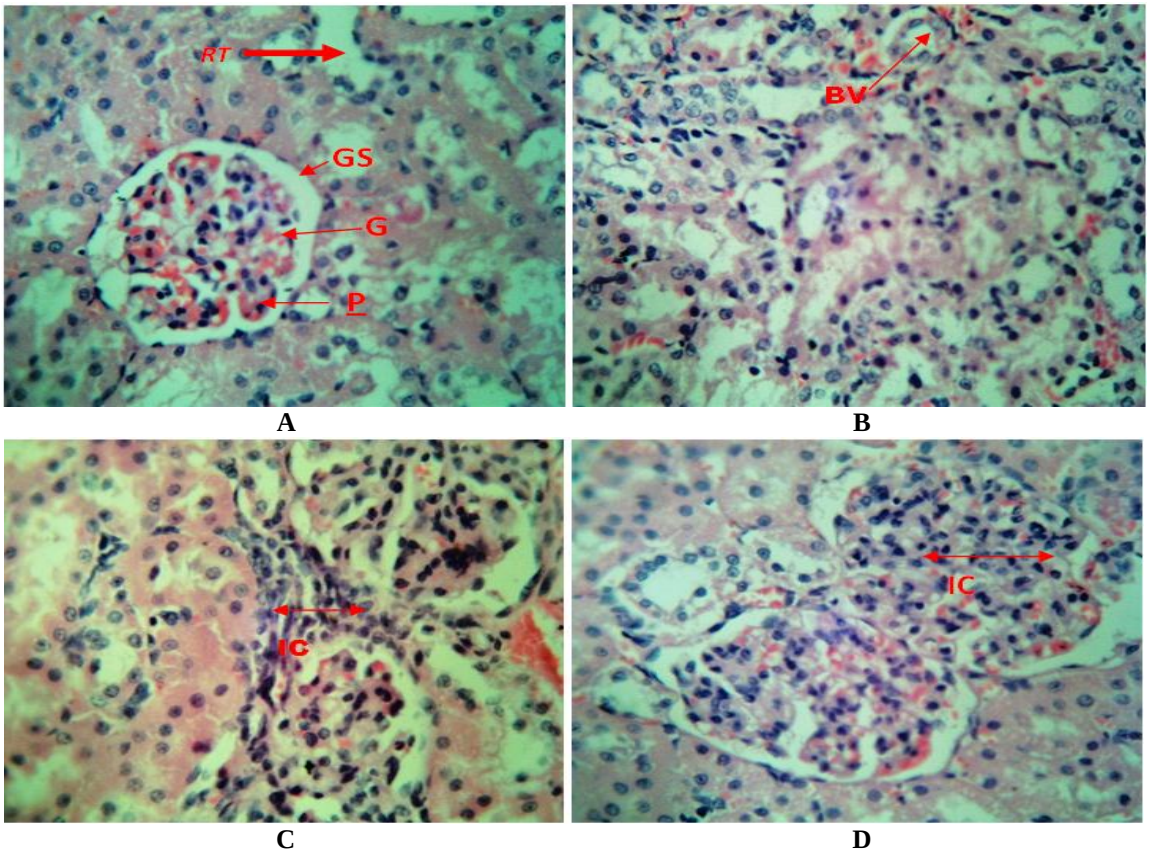

Figure 3: ***Effect of 14 days oral DSME on rat kidney histology***. **A:** Control, shows healthy kidney section with normal glomerulus (G), intact glomerular space (GS), podocytes (P) and renal tubules (RT). **B:** 625 mg/kg/day, shows normal kidney with blood vessels (BV). **C:** 1250 mg/kg/day and **D:** 2500 mg/kg/day respectively show inflammatory cell proliferation (IC). Magnification X400

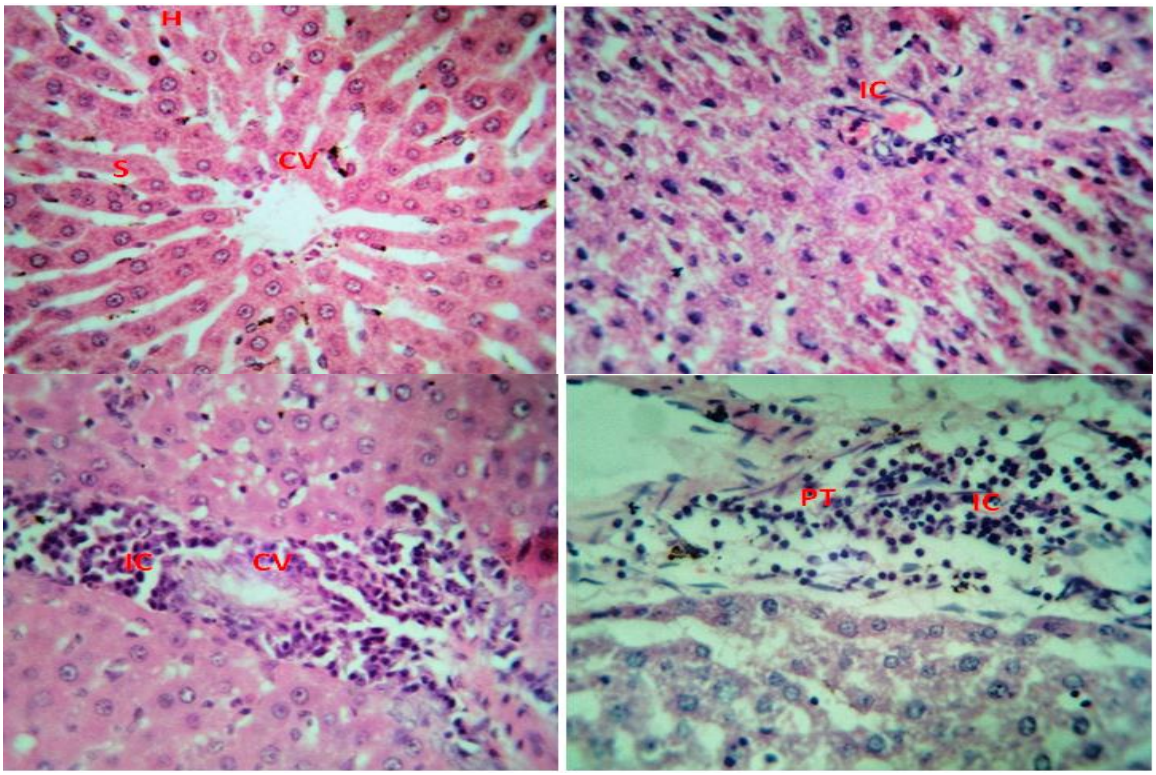

Figure 4: ***Photomicrographs of rat liver tissue (X 400 magnification) following 14 days of oral DSME administration***. **A:** control: shows a healthy liver section with normal liver histology displaying a central vein (CV)

radiating sinusoids (S) and cord of hepatocytes (H). **B:** 625 mg/kg shows mild infiltration of neutrophils within the central vein. **C** and **D** administered with 1250 mg/kg and 2500 mg/kg DSME respectively show moderate infiltration of neutrophils within the central vein in group C and within the portal triad in D.

4. DISCUSSION

The aqueous-methanol leaf extract of *D. seguine* (DSME) has shown acute and subacute toxicological profiles which nonetheless do not correlate with the level of panic that has been raised in recent times. The oral LD_{50} value of 3808 mg/kg places it in category of mildly toxic substances whereas it is feared that acute consumption of small quantities of the whole leaf can result in death. The Organization for Economic Cooperation and Development (OECD) under its Globally Harmonized Classification System (GHS) for chemical substances and mixtures categorizes substances with LD_{50} values between 2000 – 5000 mg/kg as unclassified or category 5.[15] Substances in category 5 have relatively low hazard due to acute exposure but may pose danger to vulnerable species. Ladeira *et al.* (1975)[16] had reported an intraperitoneal LD_{50} of 1000 mg/kg for *D. picta* in guinea pigs but oral doses did not cause any death. There have been several reports on the toxicity of *Dieffenbachia* species, such as the development of immediate burning and irritation of the oral mucosa in a child who chewed the leaves and airway symptoms following the ingestion of the plants but cases of death are rare and could occur if the symptoms are poorly managed.[3]

Repeated administration of substances in toxicity testing is useful in assessing target organ effects which are mostly not observable in acute toxicity testing. Statistically significant body weight changes are an indication of the general health status of experimental animals[17] but absence of such significant changes in body weight of the rats in this study suggest that although there were internal injuries, they had not started affecting the animal's nutritional state. Organ weight changes which may result from hypertrophy or necrosis are vital indicators of deleterious effects of test substances on enzymes, physiologic disturbances and target organ injury.[18] In this study, relative liver weights of treated rats increased significantly following 14-day administration indicating that DSME is hepatotoxic at doses greater than 625 mg/kg/day. Hematological indices are frontline parameters in toxicological studies because they often indicate the pathological status of the subjects. These indices can be altered by secondary plant metabolites contained in ingested plants.[18] White blood cells serve as the first line of defense against invasion by foreign substances, tissue injury and inflammation.[19] Platelets play an important role in blood hemostasis. An abnormally high platelet count may point to haemostatic disorders which may result in cardiovascular risks while a decrease may infer liver disease.[20] Lymphocytes are dynamic cells that mediate immune response to foreign substances. Lymphocytes also produce antibodies enabling the destruction of intracellular microbes and cancer cells. Neutrophils play a defensive role in microbial attacks and are also mobilized in acute inflammatory conditions.[21] In this study, the oral administration of DSME for 14 consecutive days did not adversely affect circulating red blood cell indices such as PCV, RBC and HBG. However, WBC, PLT and LYM reduced significantly although NEU increased significantly. This may suggest an immunosuppressive effect while the increase in NEU points to a pro-inflammatory effect of DSME which supports the inflammatory effect of *D. seguine* as reported by Barnes and Fox (1955).[1] Secondary metabolites such as tannins contained in plants are known to be responsible for activating and modulating the natural defense system in these plants, they are also known to be pro-inflammatory.[22] The presence of tannins in DSME may account for the increase in NEU.

The status of liver and kidneys can be assessed by serum biochemical assays. For the liver, estimation of the aminotransferases (ALT and AST) levels which describe the cellular integrity; alkaline phosphatase (ALP) levels which describe its link with the biliary tract; and protein levels which describe its functionality, are important.[23] In this study, AST and ALT levels increased significantly thereby indicating hepatotoxic effect of the extract. This may be due to structural or secretory alterations occurring in the liver.[23] Apart from liver enzymes, the status of the liver can also be assessed by the serum protein levels since they are synthesized and metabolized by the liver. A reduction in serum proteins levels is a sign of reduced synthetic function, which occurs in liver disease or damage but an increase occurs in cancerous conditions, or following high protein diet.[24] The significant reduction in serum protein levels as seen in this study corroborates the hepatotoxic effect of DSME. Tannins are substances found in most plant parts. Their polyphenolic nature confers on them the ability to form complexes with proteins which may result in allergic reactions and inflammatory responses. Tannins have been reported to cause damage to the liver of animals.[22] Tannins present in DSME may be responsible for the necrotic effect on the liver.

Kidney function is often assessed by the assay of urea, creatinine and bilirubin levels as these are indicators of glomerular filtration rate. Reduction in serum urea, creatinine and bilirubin levels indicates a protective effect on the kidneys, albeit also a sign of reduced glomerular function which depicts nephrotoxicity.[25] Lampe and Fagerstrom (1968)[26] had stated that *Dieffenbachia* species do not cause renal damage but in this study nephrotoxicity is one of the effects resulting from DSME administration. Proliferation of inflammatory cells occurs in benign conditions of the kidney which may lead to a reduction in glomerular filtration rates. An increase in inflammatory cells in most

cases result in increased urea and creatinine levels but the reverse is the case in liver failure[27] which is evidenced in this study and corroborated by alterations in the liver histology. Endogenously elevated bilirubin is known to offer protection against oxidative stress while low serum levels occur in accelerated progression of chronic kidney failure.[28] The low serum bilirubin levels in this study are an indicator of nephrotoxicty resulting from DSME administration. Flavonoids and alkaloids have been reported to possess liver and kidney protective properties by preventing lipid peroxidation.[29] The presence of flavonoids and alkaloids in DSME appear to offer some protection to the kidneys at 625 mg/kg but this effect is reversed at 1250 mg/kg and above. The nephrotoxic effect seen at higher doses may be attributed to the presence of tannins in the extract.

Histomorphological changes in target organs are first hand indicators of the toxicity of a chemical or biological substance in toxicity testing. In this study, doses of DSME resulted in proliferation of inflammatory cells in the kidney and also neutrophil infiltration and congestion seen in the liver histology. These histomorphological changes in the liver and kidney corroborate the toxic nature of DSME. Although tannins have been implicated in liver and kidney damage and mediation of inflammatory responses, other constituents of DSME such as saponins, oxalate crystals, and dumbain, a metalloproteinase which have been reported to cause proteolytic degeneration[30] may also play significant roles in the toxicity of the extract.

5. CONCLUSION

The aqueous-methanol leaf extract of *D. seguine* is considered slightly toxic as the oral LD_{50} in rats was estimated to be 3808 mg/kg. The extract is slightly toxic to the kidney and liver and may also possess immunosuppressant effects upon repeated administration to rats. Although the leaves of the plant are slightly toxic, this does not seem to correlate with the level of concern in recent times.

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