

Assessment of the Sub-acute Toxicity Profile of *Vitex doniana* (Sweet) Leaf Fractions on Albino Rats.

ABSTRACT

Aim: To evaluate the sub-acute toxicity profile of fractions of *Vitex doniana* using adult albino rats.

Study design: An experimental study that lasted for 28 days.

Place and Duration of Study: Department of Medical Laboratory Sciences and Animal House Unit, College of Medicine, University of Nigeria Enugu Campus, between January 2019 and March 2019.

Methodology: Methanol, ethyl acetate and hexane fractions of *V. doniana* were orally administered to 6 groups of animals at doses of 150 mg/kg and 300 mg/kg body weight for each fraction, for 28 days. At the end of the administration, the animals were sacrificed. Body weight changes, Relative Organ Weights (ROW), haematological analysis, serum biochemical assay and histopathological changes of vital organs were used to evaluate the potential toxic effects of the fractions after treatments.

Results: The fractions had no adverse effects on the body weights, ROW and gross appearance of the tissues. There were no significant changes in the haematological and biochemical parameters of the treated animals compared to the controls. Histological assessment of the excised organs also showed no overt pathological changes apart from thickened alveolar septa in the lungs of the animals that received methanol and ethyl acetate fractions.

Conclusion: The polar fractions of *V. doniana* at the doses tested did not induce any toxic changes in the treated animals.

Keywords: *Vitex doniana*; toxicology; ethnopharmacology; medicinal plants

1. INTRODUCTION

Vitex doniana (Black Plum in English and *ucha kiri* in Igbo language) is a well-known plant that is widely distributed in South East Nigeria [1]. It has multiple uses as food (both leaves and fruits are edible), medicines and source of fuel. Various parts of the plants are used traditionally for the treatment of gastrointestinal disorders such as diarrhoea, haemorrhoids, constipation and dysentery [2].

Plants serve as major source of new drugs. In the process of drug discovery from plant sources, it is often necessary to determine the mechanisms of action of potential therapeutic compounds to aid proper pharmacological classification. Crude extracts of *Vitex doniana* have been shown to be safe at a dose of over 3000 mg/kg body weight and also to possess antiulcer activity in rodent model of ethanol and indomethacin induced gastric ulcers [3, 4].

Interest in cheaper, less toxic and readily available medicinal products from natural sources is on the rise. For this reason, a lot of attention has been shifted to research aimed at developing drugs from plant sources [5]. A key step in this area of research is the evaluation of the toxicity of these potential drug compounds. This study was thus designed to evaluate the toxicity profile of polar fractions of *V. doniana* using experimental animals. The aim of this study was therefore to assess the toxicity profile of the semi-purified fractions of *V. doniana*.

2. MATERIAL AND METHODS

2.1 Plant Materials, Extraction and Fractionation

Fresh leaves of *Vitex doniana* were collected in Enugu metropolis during the months of April and May 2018. They were sent to the Department of Plant Science and Biotechnology, University of Nigeria, Nsukka for authentication and confirmation of identity. The leaves were then air-dried under shade and grinded into fine powder using a gasoline powered mechanical grinder. The powder was weighed and stored as appropriate in sealed water and moisture proof bags.

Crude extraction was done using methanol as solvent with the aid of a Soxhlet apparatus. After complete evaporation, the extract obtained was completely dried in a rotary evaporator and stored in sterile universal bottles. A modification of the liquid-liquid solvent partitioning methods described by Aznir *et al.*, [6] and Brusotti *et al.*, [7] was employed. Briefly, the dried methanol extract was suspended in 500 ml methanol: water (9:1 solution) and partitioned with equal volume of n-hexane to extract the hexane fraction. The residue was again suspended in methanol: water (6:4 solution) and partitioned with 500 ml of ethyl acetate. The methanol: water suspended residue was then evaporated to dryness to serve as the methanol fraction. While the hexane and ethyl acetate partitions were also dried to obtain their respective fractions.

2.2 Acute toxicity

For the acute toxicity studies, a modification of the method by Dietrich Lorke [8] for the estimation of LD₅₀ was used with slight modifications. The experiment was carried out using mice. Three doses of the fractions were administered to three groups of three animals each (at doses of 10mg/kg, 100mg/kg and 1000mg/kg body weight respectively). The animals were observed for 24 hours for signs of acute toxicity (dullness, central nervous system depression, diarrhoea and death). This was followed by a repeat administration of the doses of 1500mg/kg, 2500mg/kg and 3500mg/kg body weight respectively and a further 24-hour observation for signs of toxicity.

2.2 Sub-acute toxicity

Seven groups (A – G) of five animals each weighing between 97g and 133g were used for this experiment. The treatment of the animals lasted for 28 days. The treated animals received either 150 mg/kg or 300 mg/kg body weight of the methanol fraction (Groups A and B); ethyl acetate fraction (Groups C and D); and n hexane fraction (Groups E and F) respectively. Group G served as the control and did not receive any treatment. At the end of the administration, the animals were weighed and blood samples were collected from each animal into an anticoagulated bottle (for haematological analysis – packed cell volume, total white cell count, platelet counts) and into a plain bottle (for biochemical analysis - estimation of serum levels of alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), urea and creatinine). The animals were then sacrificed under chloroform anaesthesia and the stomach, liver, kidney, lungs, spleen and heart were harvested and weighed to determine relative organ weights using the formula – weight of organ/weight of animal. The harvested organs were assessed for gross pathologies and subsequently processed into paraffin blocks for histological analysis [9].

2.2 Data analysis

All numerical data generated were subjected to preliminary exploratory statistics and where necessary have been expressed as mean ± standard error of mean. Statistical significance was determined by one-way analysis of variance (ANOVA) followed by Tukey post hoc test for multiple comparison. The significance level was set to $P < 0.05$. Results of the analysis are presented in tabular and graphic formats.

3. RESULTS

3.1 Oral acute toxicity study

Following the administration of up to 3500 mg/kg body weight (bw) of the fractions to the mice, no deaths or signs of distress were noticed within 24 hours of administration. Further observation for another 24 hours revealed no signs of distress or toxicity.

3.2 Gross appearance of harvested organs

The kidney, heart, liver, lung spleen and stomach of the animals from the various treatment groups after 28 days of oral administration of the various fractions did not reveal any overt pathologies with all the organs appearing normal.

3.3 Changes in body weight of animals and relative organ weights

Figure 1 is a bar chart showing the change in body weights of the animals in different fraction administration over a 28 day period of oral administration. All the animals in the treatment and control groups gained weight during the period of administration. The weight changes were converted to percentages and it was observed that all the treated groups, except group D, had higher percentage weight gains than the control group. Group A (150 mg/kg bw methanol fraction) had the highest percentage weight gain (78%) while Groups D (300 mg/kg bw ethyl acetate fraction) had the lowest percentage weight gain (64%).

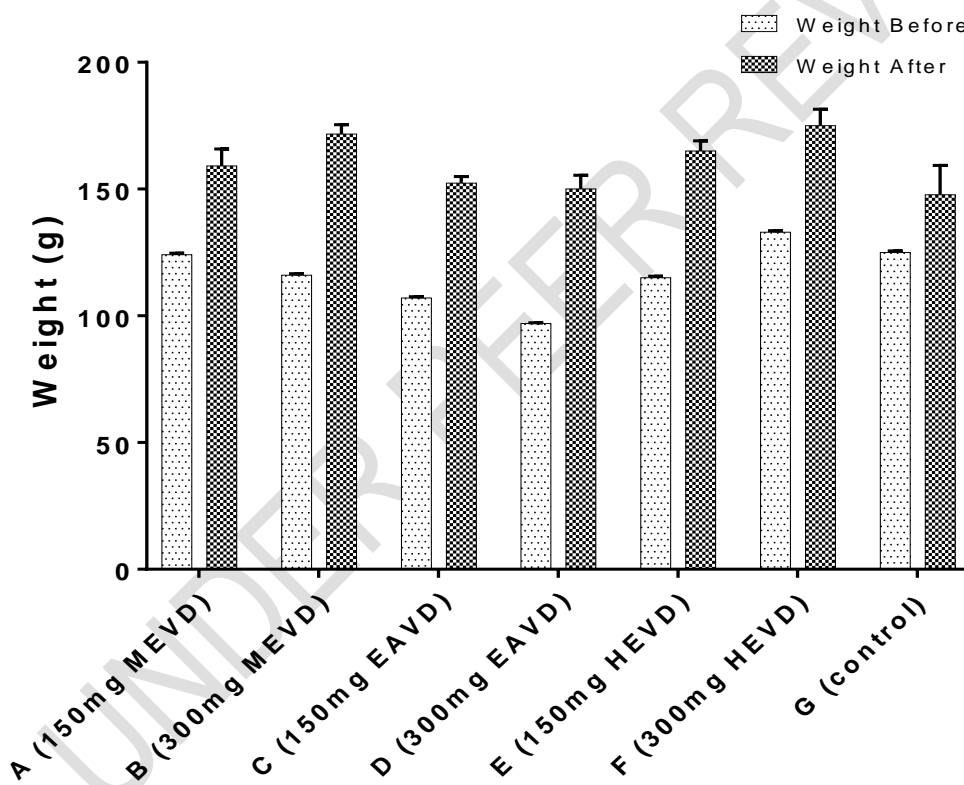


Figure 1: Bar chart showing the changes in body weight of animals before and after a 28 day oral administration of the various fractions. Each bars represent the mean for each group; the whiskers represent SEM.

The mean relative organ weights of the animals in the various treatment groups is presented in Table 1 below. After subjecting the weight data to analysis of variance and comparing the various groups, no statistically significant difference was observed between the various treatment groups.

Table 1: Relative organ weights of the animals in the various fraction treatment groups. Values presented are mean \pm SEM. F ratio and P values are from one-way analysis of variance with significance set at $P < 0.05$

Organs	Groups							ANOVA		
	A (150mg MEVD)	B (300mg MEVD)	C (150mg EAVD)	D (300mg EAVD)	E (150mg HEVD)	F(300mg HEVD)	G(control)	F ratio	P value	
Heart	0.004 0.000	\pm 0.004 0.000	\pm 0.004 0.000	\pm 0.004 0.000	\pm 0.004 0.000	\pm 0.003 0.000	\pm 0.003 \pm 0.000	0.004 \pm 0.000	1.474	0.267
Left lung	0.003 0.000	\pm 0.003 0.000	\pm 0.002 0.000	\pm 0.002 0.000	\pm 0.002 0.000	\pm 0.002 0.000	\pm 0.002 \pm 0.000	0.002 \pm 0.000	2.143	0.123
Right lung	0.004 0.000	\pm 0.004 0.000	\pm 0.004 0.000	\pm 0.004 0.000	\pm 0.004 0.000	\pm 0.004 0.000	\pm 0.003 \pm 0.000	0.005 \pm 0.000	1.153	0.391
Left kidney	0.004 0.000	\pm 0.004 0.000	\pm 0.003 0.000	\pm 0.003 0.000	\pm 0.003 0.000	\pm 0.003 0.000	\pm 0.003 \pm 0.000	0.003 \pm 0.000	2.084	0.132
Right kidney	0.004 0.000	\pm 0.003 0.000	\pm 0.004 0.000	\pm 0.003 0.000	\pm 0.004 0.000	\pm 0.003 0.000	\pm 0.003 \pm 0.000	0.004 \pm 0.000	1.699	0.204
Spleen	0.002 0.000	\pm 0.003 0.000	\pm 0.003 0.000	\pm 0.003 0.000	\pm 0.003 0.000	\pm 0.003 0.000	\pm 0.002 \pm 0.000	0.002 \pm 0.000	0.871	0.543
Liver	0.029 0.001	\pm 0.031 0.001	\pm 0.032 0.001	\pm 0.032 0.005	\pm 0.033 0.000	\pm 0.030 \pm 0.002	0.031 \pm 0.002	0.031 \pm 0.002	0.183	0.976
Stomach	0.014 0.002	\pm 0.028 0.003	\pm 0.014 0.001	\pm 0.02 \pm 0.004	0.027 0.002	\pm 0.024 \pm 0.005	0.013 \pm 0.003	4.579	0.012	

MEVD – methanol fraction of *V doniana*, EAVD – ethyl acetate fraction of *V doniana*, HEVD – n hexane fraction of *V doniana*.

3.4 Effect of administration of fractions on some haematological and biochemical parameters

The effect of oral administration of the various fractions on some haematological parameters is shown in Table 2. The mean total white cell count for animals in Group E that received 150mg/kg body weight (bw) of the hexane fraction was significantly lower than those of Group B (300mg/kg bw methanol fraction, $P = 0.023$) and Group C (150mg/kg bw ethyl acetate fraction, $P = 0.014$). All other parameters assessed did not significantly differ within the test groups and between test groups and control groups.

Table 2: Haematological parameters of test animals after 28 days of oral administration of the various fractions of *V. doniana*. Values are presented as mean \pm SEM. Level of statistical significance is set as $P < 0.05$

Groups	Haematological parameters		
	Packed cell volume	Total white cell count (mm^{-3})	Platelet count ($\times 10^9$)
A (150mg MEVD)	38.00 \pm 3.49	11.58 \pm 0.97	39.85 \pm 3.92
B (300mg MEVD)	47.75 \pm 5.65	14.98 \pm 1.77	24.03 \pm 2.99
C (150mg EAVD)	38.75 \pm 2.39	15.48 \pm 2.04	30.45 \pm 2.06
D (300mg EAVD)	37.25 \pm 1.03	10.35 \pm 1.30	37.55 \pm 5.25
E (150mg HEVD)	37.25 \pm 4.07	7.05 \pm 0.34 ^{*,b,c}	24.55 \pm 5.28
F(300mg HEVD)	38.00 \pm 3.39	12.83 \pm 1.62	25.90 \pm 4.85
G(control)	41.00 \pm 2.89	13.77 \pm 2.51	30.00 \pm 2.89
ANOVA			
F ratio	1.133	3.590	2.343
P value	0.379	0.014	0.071

MEVD – methanol fraction of *V doniana*, EAVD – ethyl acetate fraction of *V doniana*, HEVD – n hexane fraction of *V doniana*. * denotes statistically significant difference from groups corresponding to the superscript letter.

Table 3 shows the mean serum levels of some biochemical parameters assessed after 28 days of administering the fractions to test animals. There was no statistically significant difference in mean serum levels of urea, creatinine, AST, and ALT ($P > 0.05$). However, the animals in Group C (150mg/kg bw ethyl acetate fraction) had significantly lower serum ALP level when compared to Group F (300 mg/kg bw hexane fraction, $P = 0.015$) and Group A (150mg/kg bw methanol fraction, $P = 0.018$). Generally the fractions seem to cause an

increase in the serum levels of ALP of all treatment groups except Group C, when compared to the control group even though it was not statistically significant ($P > 0.05$).

Table 3: Biochemical parameters of test animals after 28 days of oral administration of the various fractions of *V. doniana*. Values are presented as mean \pm SEM. Level of statistical significance is set as $P < 0.05$

Biochemical parameters					
Groups	Urea (mmol/l)	Creatinine (umol/l)	Aspartate transaminase (iu/l)	Alanine transaminase (iu/l)	Alkaline phosphatase (iu/l)
A (150mg MEVD)	4.57 \pm 0.25	126.48 \pm 3.16	184.75 \pm 13.10	71.50 \pm 9.67	271.50 \pm 24.12 ^{*f}
B (300mg MEVD)	5.00 \pm 0.23	119.88 \pm 5.97	199.50 \pm 10.38	55.50 \pm 4.79	223.75 \pm 23.30
C (150mg EAVD)	4.80 \pm 0.12	117.65 \pm 5.96	194.75 \pm 25.44	57.50 \pm 5.48	187.25 \pm 10.69 ^{*a, f}
D (300mg EAVD)	5.00 \pm 0.09	105.03 \pm 3.00	218.25 \pm 36.44	52.75 \pm 2.87	211.25 \pm 13.49
E (150mg HEVD)	4.75 \pm 0.12	108.55 \pm 3.34	175.00 \pm 20.27	51.00 \pm 6.20	206.75 \pm 9.46
F(300mg HEVD)	4.60 \pm 0.26	115.53 \pm 5.15	188.75 \pm 22.21	51.00 \pm 6.20	211.25 \pm 13.49
G(control)	4.90 \pm 0.15	125.93 \pm 12.49	265.00 \pm 4.36	55.25 \pm 4.97	200.25 \pm 4.09
ANOVA					
F ratio	0.620	1.622	2.010	1.274	3.412
P value	0.712	0.190	0.110	0.311	0.016

MEVD – methanol fraction of *V doniana*, EAVD – ethyl acetate fraction of *V doniana*, HEVD – n hexane fraction of *V doniana*. * denotes statistically significant difference from groups corresponding to the superscript letter.

3.5 Effects of the administration of fractions on the histology of excised organs

Figures 2 (A – F) are micrographs of normal tissue sections of the kidney, heart, liver, lungs, spleen and stomach from the control group. Figure 3 (A – F) are representative tissue micrographs of animals in the various treatment groups showing observed pathological

changes. The kidney, heart and spleen sections of all treated animals appear normal. However, the liver sections of animals in Group B, C and D (that received 300 mg/kg body weight MEVD, 150 mg/kg body weight EAVD and 300 mg/kg body weight EAVD) showed congested central veins and mild periportal infiltration by inflammatory cells. Also, the lung sections of animals in group A appear to have mildly thickened alveolar septa while the animals in Groups E and F had moderately thickened alveolar septa.

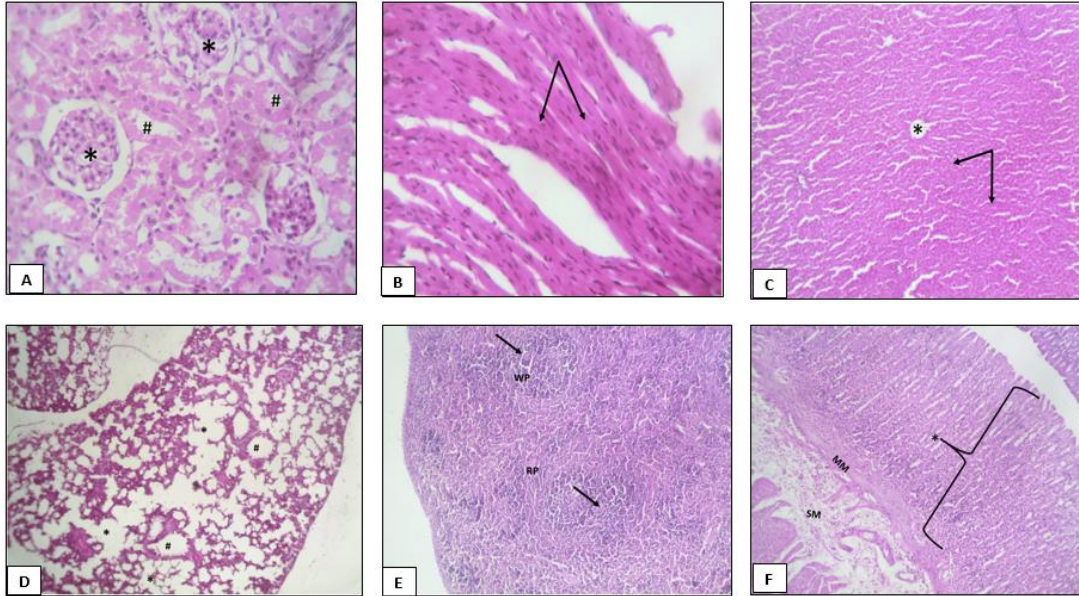


Figure 2: Micrographs of tissue sections from control animals showing normal histological features. A – Kidney section with normal glomeruli (*) and tubules (#); B – heart section with normal cardiac fibres (arrows); C – Liver section with normal hepatocytes (arrows) and central vein (*); D – lung section showing normal air sacs and alveolar septa (*) and terminal bronchioles (#); E – spleen section devoid of pathological features. RP- red pulp, WP – white pulp, arrows – splenic artery; F – Stomach section showing normal epithelia (*), muscularis (M) and submucosa (SM). Stain: H&E. Magnification: X100 – X400.

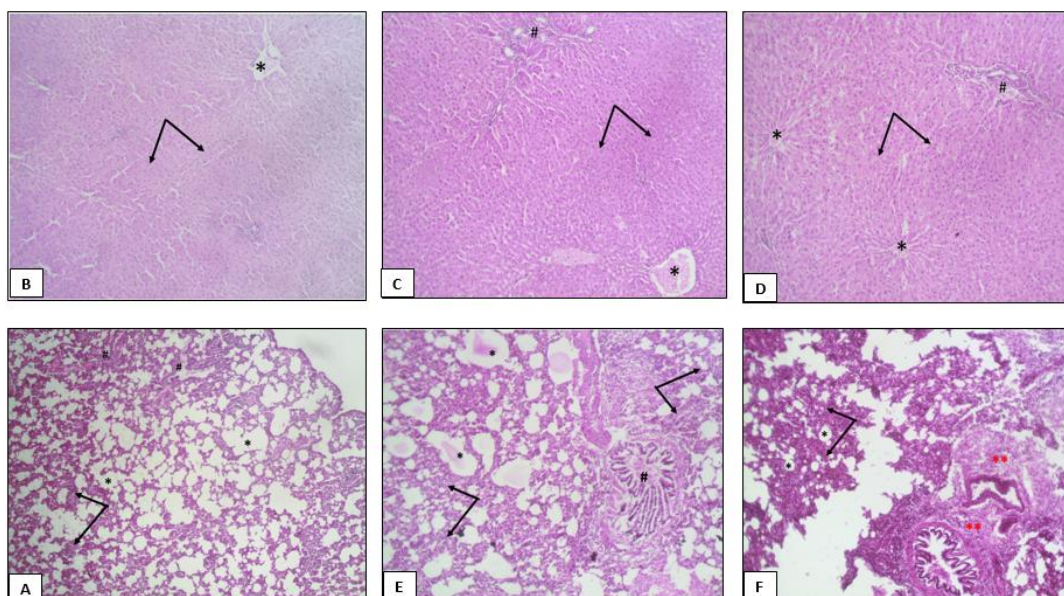


Figure 3: micrograph of selected liver and lung tissue sections from treated showing observed pathological changes. B – Liver section from an animal in Group B (300 mg/kg bw MEVD) the hepatocytes (arrows) are normal, central vein is mildly congested (*); C – Liver section from an animal in Group C (150 mg/kg bw EAVD) the hepatocytes (arrows) are normal, central vein is congested (*) and there is mild infiltration of the portal triad (#); D – liver section from an animal in Group D (300 mg/kg bw EAVD) the hepatocytes (arrows) are normal, central vein is congested (*) and there is moderate peri-portal infiltration (#); A – Lung section from an animal in Group A (150 mg/kg body weight of MEVD). The alveolar septa are slightly thickened (arrow) but the air sacs (*) and terminal bronchioles (#) are normal; E – Lung section from an animal in Group E (150 mg/kg b.w. of HEVD). The alveolar septa (arrows) appear thickened, both the terminal bronchiole (#) and air sacs (*) contains eosinophilic matter; F - Lung section from a representative animal in Group F (300 mg/kg b.w. of HEVD). The alveolar septa (arrows) appear thickened. There is evidence of peribronchial inflammation (**) with infiltration of inflammatory cells. The air sacs (*) appear smaller than normal. Stain: H&E. Magnification: X100 – X400

4. DISCUSSION

Establishing safety of potential drug compounds from plants is an important step in the process of drug discovery. In the acute toxicity study, the plant fractions did not cause any deaths or visible distress to the animals up to a dose of 3500 mg/kg suggesting that the plant leaf fractions are safe for potential therapeutic use [10, 11].

Blood is a tissue that is highly sensitive to toxic effects making it relevant to the evaluation of toxicity [12]. In this study, no major changes were observed in the haematological parameters assessed following administration of the fractions. However, animals that received the low dose of the hexane fraction were seen to have significantly lower total white cell count. Certain medicinal plants are known to cause adverse changes to haematological parameters [13, 14]. This decrease may thus be suggestive of a haematotoxic component within the hexane fraction. However, this observation is difficult to explain especially since the animals that received a higher dose of the fraction did not have decreased total white cell count.

In the serum biochemical analyses, no adverse changes were recorded in the serum levels of urea and creatinine in the treated animals. Both urea and creatinine are generally

accepted markers of renal integrity with overt changes in either of them being a strong indication of renal injury [15]. Serum levels of enzymes such as liver amino transaminases (AST and ALT) and other enzymes such as ALP are important for the assessment of hepatotoxicity of compounds. A rise in serum levels of activity of these enzymes is often indicative of damage to the liver [16].

In this study, the serum levels of the liver marker enzymes assessed were not adversely affected in the treated animals except for those that received the low dose of ethyl acetate fraction whose serum ALP levels were significantly lower. Alkaline phosphatase (ALP) is a group of isoenzymes found in many organs including the bones, liver, intestines, kidney and placenta [17, 18]. Given that the histological picture of the liver of animals in the group did not suggest any severe liver damage, it is possible that the reason for the decrease may be due other factors not covered in the present study. For instance, it is known that inflammatory conditions involving the intestinal region may give rise to changes in ALP levels especially since rats are known to have higher intestinal ALP levels than hepatic ALP levels [19].

At the end of the administration all animals were observed to have increased in weight. Changes in body weights can serve as useful indicators of toxicity [20]. However, the increased weights may have been as a result of normal physiological growth rather than a direct consequence of consuming the plant even though it is known that certain plant components induce weight changes in animals via various mechanisms [21,22]. In the same manner, there were no significant changes in the relative organ weights of the animals in the treatment groups compared to the control. Relative organ weight are also sensitive markers of toxicity of some substances [13]. The absence of adverse changes in organ weights by the fractions may be indicative of their safety and inability to cause abnormal growth in the organs of animals.

Both gross and histopathological assessment of the excised organs and tissue sections respectively did not reveal any overt signs of toxicity or pathology. Sections of the lungs from some of the animals showed varying degrees of thickening of the alveolar septa and inflammation. Such changes are more likely as a result of inhaled irritants or infections and not may not necessarily be as a result of fraction administration [23].

5. CONCLUSION

From these observations, it may be concluded that all three fractions of *V doniana* can be considered safe for consumption since it did not induce consistent, noticeable toxic changes to the animals tested. However the observed changes in the white cell count, ALP and liver and lung histology of some of the animals necessitates the need for cautious use of these plant leaves for medicinal purposes.

ETHICAL APPROVAL

Ethical approval for this study was obtained from the ethical committee Faculty of Veterinary Medicine, University of Nigeria Nsukka, Enugu State. Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee.

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