



Research Article

Haematological and Histopathological parameters of rats fed with Aerial yam (*Dioscorea bulbifera*)

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The anti-nutrition contents and effects on haematological and histopathological profile of boiled and roasted preparations of *Dioscorea bulbifera* were evaluated to assess possible toxicity effects. The study was carried out in the Physiology Laboratory of Michael Okpara University of Agriculture, Umudike between July and December, 2014. *Dioscorea bulbifera* was obtained from National Root crop Research Institute, Umudike, and processed into boiled and roasted flour varieties. The anti-nutritional compositions of the two preparations were estimated following standard procedures. For the haematological and histopathological studies, 25 matured albino rats were divided into 5 groups of 5 rats. Group 1 animals were fed with normal feed and water and served as the control. Groups 2 and 3 were fed with 20% and 40% combinations of boiled variety with feed, while groups 4 and 5 were given 20% and 40% combinations of the roasted variety with feed respectively. At the end of 28 days all animals were sacrificed for blood and visceral organs collections used for haematological and histopathological studies. Results obtained revealed the presence of significant amounts of saponins, hydrocyanide, phenols and tannins in the boiled and roasted preparations of *Dioscorea bulbifera* when compared to the standard feed used. All animals fed with various dose concentrations of *Dioscorea bulbifera* showed no significant changes in their red blood cell (RBC), packed cell volume (PCV), Haemoglobin (Hb), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) with slight reduction in white blood cells (WBC) counts, suggesting no form of blood toxicity. The liver, kidney and lung histology also showed no necrosis and histopathological alterations and were not significantly different from those of the control rats. The consumption of *Dioscorea bulbifera* contain tolerable amounts of anti-nutrition factors and is safe for both human and animal consumption.

Keywords: Haematological, Histopathological, Rat, *Dioscorea bulbifera*, Anti-nutrients.

INTRODUCTION

The role of nutrition in the maintenance, development, reproduction and overall well-being of man and other animals cannot be over emphasized and has made the search for specific nutrient sources that will ultimately add more value to life a crucial task. Several animal experimental models have been employed to verify the claimed nutritional values of plants products before recommending the use of such food substances. The common use of small mammals such rats for such experimental purposes may be attributed to their body physiology and nutrient requirements which closely resembles that of humans. The state of health of humans including systemic toxicity is however usually based on developmental indicators which are most often restricted to the blood and a number of visceral organs including the liver, heart, kidneys, pancreas, gonads etc. Determination of haematological parameters may be means of clinically accessing the nutritional values and or toxicity of food substances. Hence with good nutrition, it is expected that the blood parameters will also be significantly improved.

Yam is the common name for some plant species in the genus *Dioscorea* (family) including *D. alata* (purple yam), *D. cavenensis* (yellow yam), *D. dumetorum* (biter yam), *D. rotundata* (white yam), *D. opposita* (Chinese yam), *D. trifida* (cush-cush-yam) and *Dioscorea bulbifera* (Aerial yam). These varying species all form edible tubers and over the years have been a rich source of carbohydrate and other nutrients to man. Many of the yam tubers are reported to be toxic and have to be detoxified by cooking before consumption in order to remove the toxic compound presents in them (Bhandar and Kawabata, 2005). Ezeocha and Ojmelukwe (2012) reported the presence of alkaloid (2.77%), flavanoid (1.38%), saponin (2.71%), tannin (0.21%) and phenol (1.91%) in *Dioscorea alata*. Anhwange et al., (2011) recorded 49.70 mg/100g and 79.40mg/100g of hydrogen cyanide in *Dioscorea bulbifera* and *Dioscorea dometorum* species respectively.

The aim of this work was to evaluate the haematological and histopathological effect of Albino rat feed with boiled and roasted *Dioscorea bulbifera*.

MATERIALS AND METHODS

Collection of *Dioscorea bulbifera* tubers and preparation of sample

Aerial yam (*Dioscorea bulbifera*) tubers were collected from Nsukka town in Nsukka Local Government Area of Enugu State, Nigeria. The tubers were sorted to remove bad ones and then divided into two parts. The first part weighing 300g was boiled in little amount of water which was thereafter drained off. The tubers were then peeled and sliced into fine pieces, dried in an oven and then milled into fine flour which was packaged in air tight container and stored at room temperature. The second part of the tubers also weighing 300g was roasted in charcoal fire, peeled and also milled into fine flour and properly stored until needed (Fig. 1)

Determination of anti-nutritional factors of samples

Tannin content was determined using the method of Pearson (1976), Saponin by the spectrophotometric method of Brunner (1984), Phenol content by Follin method described by Pearson (1976), while Hydrocyanide content was determined by the alkaloid pikrate colour meter method of Balagopalancy et al., 1998.

Animals and treatment

A total of 25 adult albino rats (*Rattus norvegicus*) were used for the work. The rats, obtained from the animal house of the College of Veterinary Medicine, University of Nigeria, Nsukka were housed in Aluminum cages and allowed to acclimatize for two weeks. They were fed at liberty with rat feed (vital feed finisher, Nigeria) and clean water. All animal experiments were conducted in compliance with NIH guidelines for care and use of laboratory animal (pub. No. 58-23, Revised 1985) as reported by Akah et al., 2009. The study was conducted in the Physiology Laboratory of the Department of Physiology and

Pharmacology, College of Veterinary Medicine, Michael Okpara University of Agriculture, Umudike, Nigeria.

The rats divided into 5 groups of 5 rats each and assigned daily treatments in the following order:

Group 1: 100% of normal feed and water and served as the control.

Group 2: 20% of boiled yam mixed with 80% feed.

Group 3: 40% of boiled yam mixed with 60% feed.

Group 4: 20% of roasted yam mixed 80% of feed.

Group 5: 40% of roasted yam mixed with 60% of feed.

The rats were kept in aluminum cages and allowed access to the feed and water while ensuring at the same time that the highest level of hygiene was maintained through-out the 28 days treatment period.

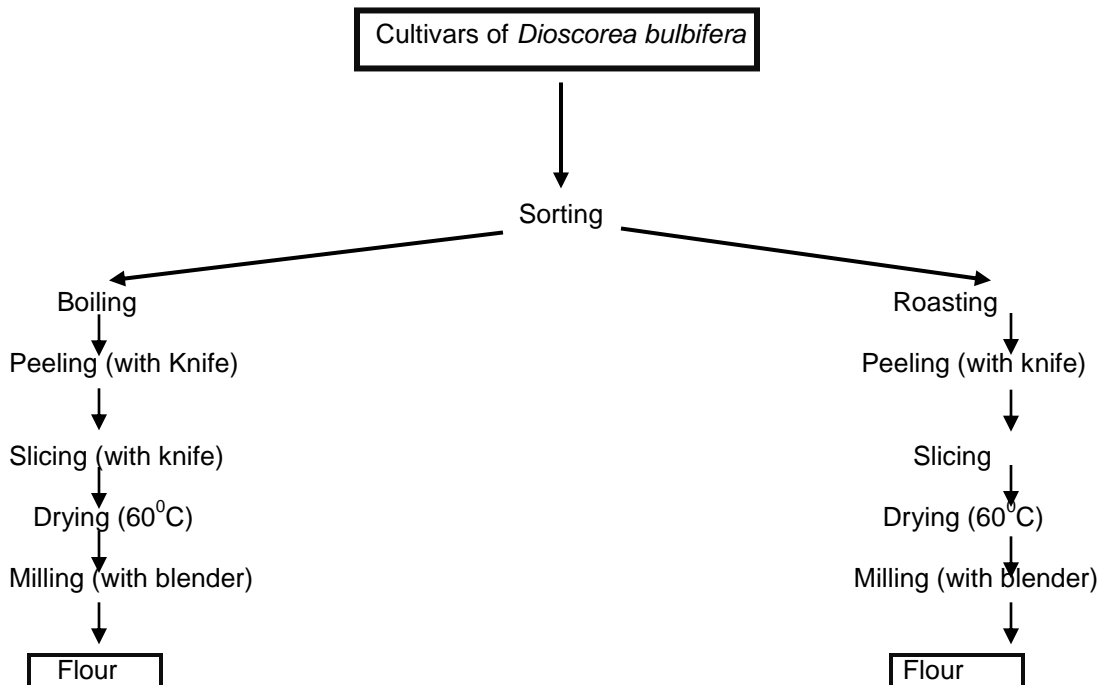


Fig. 1 Flow chart for the preparation of boiled and roasted *Dioscorea bulbifera* flour

Determination of blood parameters:

At the end of 28 days of treatment, the rats in all groups were sacrificed and blood samples collected by cardiac puncture into ethylene diamine tetracetic acid (EDTA) bottles for hematological assay. Red blood cell count (RBC), Packed cell volume (PCV), Hemoglobin concentration (HC), white blood cell count (WBC) and differential WBC counts were determined at once for each blood sample using an Automated Haematology Analyser, following standard procedures outlined by the producer, Mindray Company, China.

Liver, kidney and lung histology

Histological sections of the isolated liver, kidney and lung were prepared using the methods of John and Alan (1977) and Clayden, (1967). The tissues were dehydrated in graded levels of ethanol, cleared in xylene, and embedded in paraffin wax for sectioning. The 5µm thick sections were cut, mounted on glass slides and stained with hematoxylin and eosin for light microscopy. The photomicrographs of slides were

observed under the microscope with magnifications of x400 and x100. Selected images were captured using a moticam 2.0 digital camera attached to a computer.

Statistical Analysis

Results were expressed as means \pm standard error of mean (SEM). Student's t-test at 95% level of significance was used to establish statistical difference between control and treated groups. Computer software package SPSS version 17 was employed.

RESULTS

Anti-nutritional characteristics of *Dioscorea bulbifera*

The control feed, boiled and roasted Aerial yam tubers (*Dioscorea bulbifera*) were found to contain tannins, phenols, saponins, hydrocyanide in varying proportions. The boiled aerial yam contained more tannins phenols, saponins and hydrocyanide when compared to control feed while the roasted aerial yam had the same phenol content with the control but with higher tannin, saponin and hydrocyanide content. Thus, the boiled and roasted samples contained more anti nutritional factors than the control feed (Table 1).

Table 1. Anti-nutritional characteristics of boiled and roasted *Dioscorea bulbifera*

Parameters	Control Feed	Boiled	Roasted
Saponins	3.87 \pm 0.37*	4.14 \pm 0.70**	3.90 \pm 0.57*
Hydrocyanide (mg)	0.18 \pm 0.08*	0.38 \pm 0.02**	0.38 \pm 0.01**
Phenol%	0.89 \pm 0.02*	3.87 \pm 0.37**	0.89 \pm 0.03*
Tannin%	0.14 \pm 0.02**	0.17 \pm 0.06**	0.29 \pm 0.03**

Data are mean \pm S.D (n=5). Mean in the same column with difference superscript letters are significantly difference (p=0.05) for test compared to control.

**, * shows significant difference between the samples

Table 2: Effect of aerial yam (*Dioscorea bulbifera*) on the RBC and RBC Indices

Treatment	RBC x 10 ¹² /L	HC (g/dl)	PCV (%)	MCH (pg)	MCHC(g/L)	MCV (fl)
Control	5.96 \pm 0.68 ^a	11.08 \pm 0.61 ^{ab}	33.40 \pm 2.07 ^a	18.74 \pm 1.29 ^a	33.29 \pm 3.13 ^a	56.66 \pm 7.73 ^a
20% D.B+80% feed	5.44 \pm 0.50 ^a	10.72 \pm 0.80 ^{ab}	33.80 \pm 3.27 ^a	19.87 \pm 2.70 ^a	31.80 \pm 1.85 ^a	62.59 \pm 8.49 ^a
40% D.B+60% feed	5.72 \pm 1.05 ^a	9.84 \pm 0.47 ^b	30.80 \pm 3.11 ^a	17.55 \pm 2.80 ^a	32.15 \pm 2.85 ^a	57.76 \pm 6.97 ^a
20% D.B+80% feed	5.66 \pm 0.39 ^a	11.72 \pm 1.02 ^a	35.80 \pm 3.11 ^a	20.77 \pm 2.12 ^a	32.69 \pm 2.12 ^a	63.65 \pm 4.54 ^a
40% D.B+60% feed	5.30 \pm 0.86 ^a	10.52 \pm 1.86 ^{ab}	33.80 \pm 6.41 ^a	20.25 \pm 4.3 ^a	31.47 \pm 4.19 ^a	66.10 \pm 20.60 ^a

Data represent the mean \pm S.D for each group of rat (N=5) (number of animal per group). Mean in the same row with different superscript letters are significantly different (P<0.05) for test versus control.

Effect of *Dioscorea bulbifera* on RBC and RBC Parameter

Treatment with *Dioscorea bulbifera* at all dose levels and forms of preparation did not significantly affect the red blood cell counts and haemoglobin contents of the treated rats when compared to the control group. However 40% feed combination of boiled *Dioscorea bulbifera* slightly decreased PCV values while 20% feed combination of the roasted variety increased same. Treatment with boiled variety also did not significantly affect the MCH, MCHC and MCV values, while the roasted variety significantly ($P=0.05$) increased MCH and MCV values in all treated rats (Table 2).

Effect of Aerial yam (*Dioscorea bulbifera*) on the WBC and differential WBC counts

Treatment with both forms of *Dioscorea bulbifera* preparations significantly ($P=0.05$) lowered total WBC and increased neutrophils counts in all treatment groups when compared to the control. Lymphocytes and eosinophils values of the rats were also not significantly affected by treatment with the boiled variety of aerial yam but were slightly affected by the roasted variety. The number of monocytes was also significantly ($P=0.05$) increased in all treatment groups when compared to the control group (Table 3).

Table 3: Effect of Aerial yam (*Dioscorea bulbifera*) on the WBC and differential WBC

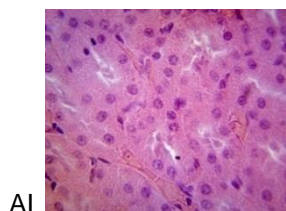
Group Treatment	BC X 10 ⁹ /L	Neutrophils %	Lymphocyte%	Eosinophils%	Monocytes %
Control feed	3.00±0.62 ^b	26.80±4.14 ^b	66.20±7.19 ^a	0.60±1.34 ^a	7.40±4.77 ^a
20% boiled D.B+80% feed	2.46±0.58 ^a	28.20±1.6 ^b	67.00±1.58 ^a	0.60±1.34 ^a	4.40±1.1 ^{ab}
40% boiled D.B+60% feed	2.48±0.13 ^a	34.00±7.9 ^{ab}	67.00±5.90 ^b	0.40±0.89 ^a	3.40±1.14 ^b
20% roasted D.B+80% feed	3.20±1.5 ^a	29.60±3.30 ^{ab}	68.00±1.58 ^a	0.20±0.44 ^a	3.60±1.14 ^b
40% roasted D.B+60% feed	1.92±0.42 ^a	36.20±6.61 ^a	60.00±3.55 ^a	1.00±0.72 ^a	4.80±0.83 ^{ab}

Data represent the mean± S.D for each group of n=5 (number of animals per group). Mean in the same row with different superscript letters are significantly different ($p<0.05$) for test versus control

Effects of *Dioscorea bulbifera* on Liver, Kidney and Lung histology in rats

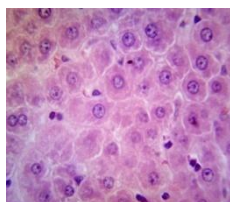
The histology of lungs showed no evidence of interstitial pneumonia and interlobular oedema. The hepatocytes in the liver showed no form of necrosis and no histopathological alterations and distortions, such as mid-cell oedema, degeneration of the hepatocytes, enlargement of the alveoli and alveoli hemorrhage where observe in the lungs. The kidney also did not show any sign of glomerular shrinking nor hemorrhage. Necrosis, glomerular cells shrinking and interstitial pneumonia.

Kidney



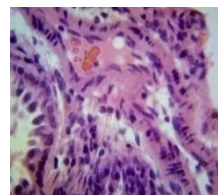
A1

Liver



B1

Lung



C1

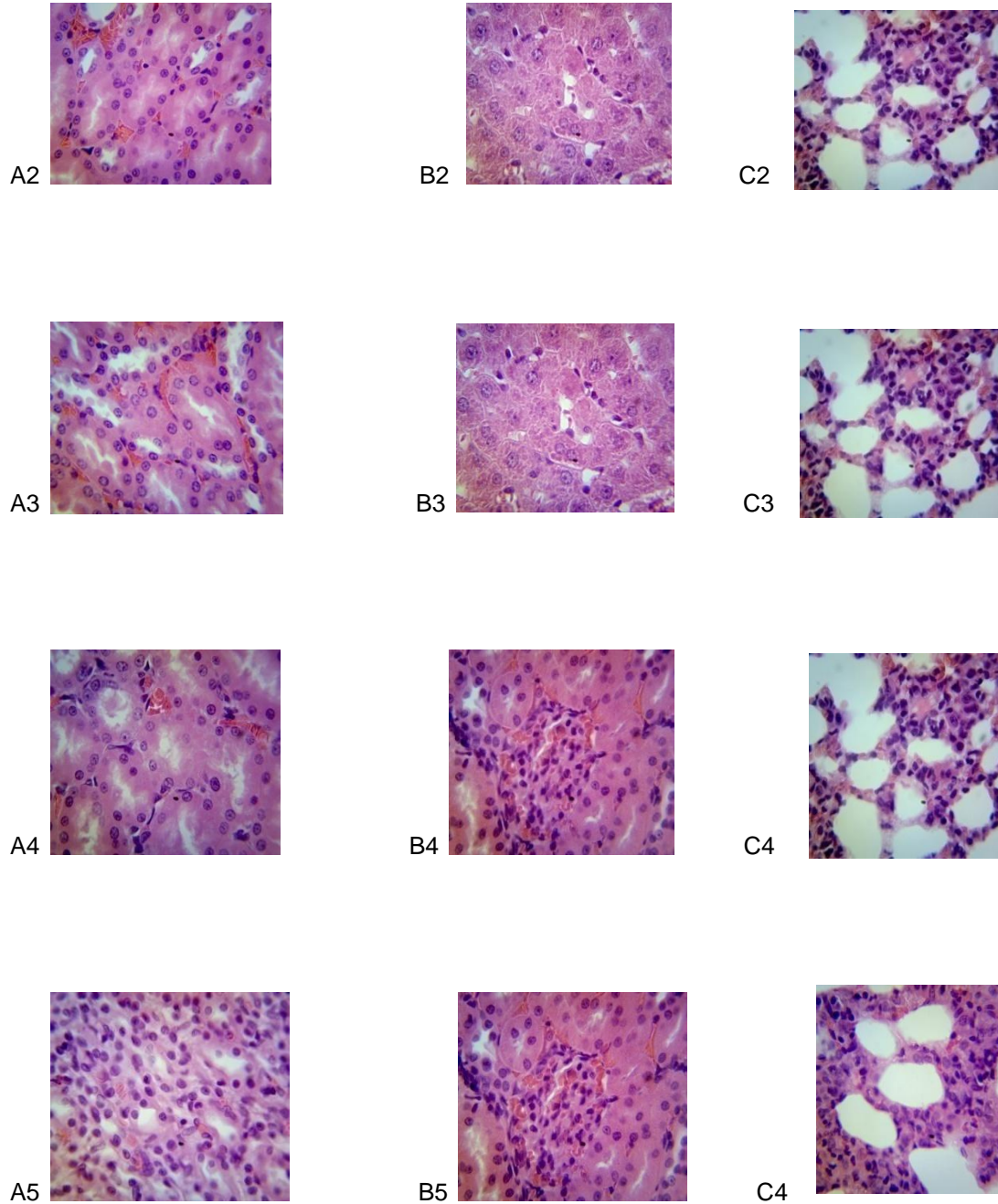


Plate A1: Photomicrograph of the kidney of normal rat with no form of necrosis and interlobular oedema.

Plate A2: Photomicrograph of the kidney of rats fed with 20% boiled *Dioscorea bulbifera* and 80% feed, showing no necrosis.

Plate A3: Photomicrograph of the kidney of rats fed with 40% boiled *Dioscorea bulbifera* and 60% feed also showing no necrosis

Plate A4: Photomicrograph of the kidney of rat fed with 20% roasted *Dioscorea bulbifera* and 80% feed with normal appearance

Plate A5: Photomicrograph of the kidney of rat fed with 40% roasted *Dioscorea bulbifera* and 60% feed showing normal appearance

Plate B1: Photomicrograph of the liver of normal rat with no form of necrosis.

Plate B2: Photomicrograph of the liver of rat fed with 20% boiled *Dioscorea bulbifera* and 80% feed showing no form of necrosis.

Plate B3: Photomicrograph of the liver of rat fed with 40% boiled *Dioscorea bulbifera* and 60% feed showing also no form of necrosis.

Plate B4: Photomicrograph of the liver of rat fed with 20% roasted *Dioscorea bulbifera* and 80% feed with normal profile

Plate B5: Photomicrograph of the liver of rat fed with 40% roasted *Dioscorea bulbifera* and 60% feed with normal presentation

Plate C1: Photomicrograph of the lungs of normal rats showing no evidence of interstitial pneumonia and interlobular oedema.

Plate C2: Photomicrograph of the lungs of rats fed with 20% boiled *Dioscorea bulbifera* and 80% feed with same presentation as in C1

Plate C3: Photomicrograph of the lungs of rat fed with 40% boiled *Dioscorea bulbifera* and 80% feed with same presentation as in C1

Plate C4: Photomicrograph of the lungs of rat fed with 20% roasted *Dioscorea bulbifera* and 80% feed with same presentation as in C1

Plate C5: Photomicrograph of the lungs of rat fed with 40% roasted *Dioscorea bulbifera* and 60% feed with same presentation as in C1

DISCUSSION

Results of this study have shown that all rats fed with both boiled and roasted varieties of *Dioscorea bulbifera* showed no sign of toxicity within the 28 days of treatment period. The rats instead had normal dispositions mechanically, physically, emotionally and appeared very health. These results suggest that the levels of the anti-nutritional factors in aerial yam can be well tolerated by the body after consumption and does not pose any threat of toxicity when consumed. The quantities of these anti-nutritional in *Dioscorea bulbifera* may instead be of health benefits to the consumer. Tannin is reported to be anti-carcinogenic, reducing mutagenic activity of a number of mutagens due to their anti-oxidative property which is important in protecting cellular oxidative damage including lipid peroxidation. Tannin also inhibits the generation of superoxide radicals and have shown evidence of possessing antimicrobial activities (Chung et al., 1998). The ability of tannins to react with protein to provide a typical tannin effect which is important for the treatment of inflammatory or ulcerated tissues has also been reported Parekh et al., (2005). Most plants that contain tannin as their main component have been found to be astringent in nature and are used for treating intestinal disorders including diarrhea and dysentery. Saponin is reportedly been used to alleviate cardiac problems associated with hypertension (Trease and Evan, 1989) and in the management of hypercholesterolemia in humans as it binds to cholesterol in the body to inhibit its reabsorption thereby facilitating its excretion from the body. These anti-nutritional factors may however become toxic to the system when intolerable quantities are ingested (Hallek et al., 2008).

Feeding with both boiled and roasted preparations of *Dioscorea bulbifera* did not significantly affect the RBC, HC, PCV, MCV, MCHC and MCH values of the experimental rats when compared to the control group (Table 2) and suggests that the consumption of aerial yam is safe and has no potential blood toxicity effect. Blood toxicity is usually accompanied with significant changes in the values of hematological parameters including falls in values of RBC, HC, PCV and other RBC indices due to the possible suppression of erythropoietic processes or haemolysis of the available RBC and resulting in liver necrosis and anemia with changes in blood biochemical parameters (Uhmacher et al., 2010). Since no significant decline in RBC values was observed following chronic consumption of aerial yam, the food substance can be adjudged to be safe for the feeding of man and other animals. The slight fall in WBC counts following long term consumption of *Dioscorea bulbifera* suggest possible immune suppression since the WBCs are known to be the key actors in immune responses as they form the first line of defense against invading microorganism and the lowering of WBC values usually indicates fall in immune strength (Sembulinggam, 2010). This observed physiological effect of *Dioscorea bulbifera* is however open for further evaluation, since the histopathological studies revealed no form of toxicity as the kidneys, livers and lungs showed no evidence of necrosis, histopathological alterations or distortions, suggesting a high margin of safety following chronic consumption of *Dioscorea bulbifera*.

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