Protective effect of Lyophilized Aqueous Extract of *Basella alba* Vegetable on Heamatological indices and selected Organs of Cyanide Induced Toxicity in Rats

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***Abstract: -* The protective effect of lyophilised aqueous extract of *Basella alba* (BA) vegetable on cyanide induced toxicity in rats was investigated. Twenty, 3weeks old male wistar strain albino rats were randomly distributed into one control and three treatment groups of five rats each; control group (no cyanide, no extract), group 2, treated with 3mg/kg body weight cyanide (CN only), group 3, treated with 3mg/kg body wt. cyanide and equivalent amount(3mg/kg body wt.) of extract (CN+BA) and group 4, treated with 3mg/kg body wt. ofextract only(BA only) were used for the investigation.Cyanide toxicity(when compared with the control) reduced food and water intake, while the food intake was improved in group 3 which was treated with equivalent concentration of cyanide and extract(CN+BA). Biochemical analysis of liver enzymes showed that cyanide (CN only) damaged the liver as there was significantly elevated activity of Aspartate aminotranferase (AST) and Alanine aminotransferase(ALT) which were considerably decreased by *Basella alba*. Heamatological analysis indicated a significant reduction in PCV, RBC and Hb of group treated with cyanide(CN only). The reduction effects were considerably cancelled by *basella alba* in the group treated with equivalent concentration of cyanide and extract(CN+BA). Lyophilized aqueous extracts of *Basella alba* showed good potentials as a safe antidote for cyanide toxicity when administered concominantly with sub-lethal dose of cyanide.**

***Keywords:* Lyophilized, *Basella alba*, Cyanide toxicity**

I. INTRODUCTION

Hydrogen cyanide (a colourless gas) and potassium cyanide (a solid) are common examples of cyanides. Cyanides enter air, water, and soil from natural processes (e.g. volcanoes, wildfire and microbiological activities) and industrial activities (e.g. electroplating, gold mining, production of textiles and plastics, etc.) (Johnny, 2015). It is a potential suicidal, homicidal and chemical warfare agent, and can be used for military or terrorism purposes. Occupational exposure, ingestion of cyanide-containing foods and combined inhalation of hydrogen cyanide (HCN) and carbon monoxide (CO) in fire smoke contribute to cyanide toxicity (Baskin, 2001).Cyanogenic glycosides are natural plant toxins that are present in several plants, most of which are consumed by humans. Cyanide is formed following the hydrolysis of cyanogenic glycosides that occur during crushing of the edible plant material either during consumption or during processing of the food crop (Bolarinwa *et al*., 2016). The production of cyanogenic glycosides in plant tissue is probably an important system of plant defense to minimize predation (Soto-blanco *et al*.,2002) and worldwide at least 2500 species of plants are known to be cyanogenic (Vetter, 2000). A common cyanogenic plant is Cassava(*Manihot spp*) which is a major dietary staple and contains a sufficient amount of cyanogenic glycosides which requires special processing to reduce the danger of toxicity.The cyanide level of cassava varies from about 75 to 350 ppm, but can be up to l000 ppm or more depending on the variety, plant age, soil condition, fertilizer application, weather, and other factors(Bolarinwa *et al*., 2016). Cyanide inhibits several cellular enzymes including cytochrome oxidase, which is a key enzyme in the cellular respiratorychain (Soto-blanco and Górniak, 2004; Leah *et al*., 2011). However, the general population may be exposed to cyanides by inhalation of contaminated air, ingestion of contaminated drinking water, and/or consumption of a variety of foods,though at a very low level (Soto-blanco *et al*., 2002)

There are, however, specific subgroups with higher potential for exposure. These subgroups include individuals involved in large-scale processing of cassava and those consuming significant quantities of improperly prepared foods containing cyanogenic glycosides, such as cassava, speciality foods such as apricot pits, bitter almonds and potatoes. The use of antidotes to reduce cyanide toxicity has long been realized (Bhattacharya, 2000). Such compounds which have been successfully used as antidote for cyanide poisoning are hydroxocobalamin,a natural form of vitamin B12 and sulphur containing dietary amino acids (cysteine and methionine) (Bolaji and Olabode, 2011).These are also part of the nutritional composition of some edible leafy vegetables available in the the tropics. Several vegetable species abound in sub-Saharan African countries especially Nigeria, where they are used as partly condiments or spices in human diets or as supplementary feeds to livestock such as rabbits, poultry and swine (Fasuyi and Alector, 2005 ;Bolaji and Olabode, 2011, Adeoluand Enesi, 2013).

Leafy vegetables are important items of diet in many Nigerian homes. These vegetables are harvested at all stages of growth and fed as processed, semi-processed or fresh to man (Bolaji and Olabode, 2011; Funda and Sevinc, 2018). *Basella alba* is one of the leafy vegetables, commonly known as Malabar spinach, vine spinach or ceylon spinach. It is branched, smooth, twining, herbaceous vine, reaching a length of several meters. The stems are green or purplish. The leaves are somewhat fleshy, ovate or heart-shaped, 5-12 centimeters (cm) in length, stalked, tapering to a pointed tip and heart shape at the base. (Sagar and Dattatraya, 2014). The spikes are axillary, solitary and 5-29 cm in length. The flowers are pink and about 4 millimeters (mm) long. The fruit is fleshy, stalkless, ovoid or nearly spherical, 5-6 mm in length and purple when mature (Akindahunsi and Salawu, 2005)

It is high in Vitamin A (400mg/100g), Vitamin C (102.0mg/100g), Cysteine (0.027mg/100g), Protein (1.8mg/100g),Calcium (109.0mg/100g), Magnesium (65.0mg/100g), Methionine (0.019mg/100g), Ash (1.4mg/100g) (National Institute of Nutrition, 2008) and several vital anti-oxidants required for cyanide detoxification.Recent studies shows that Basella alba exerts strong androgenic actions along with anti-ulcerative (Venkatalakshmi and Senthamaraiselvi, 2012), anti-bacterial, antioxidant, cytotoxic, anti-inflammatory (Krishna, 2012), nephron-protective, wound healing (Mohammed *et al.*, 2012) and anti-depressant effects. As per its known traditional use and values, it is known for its diuretic, demulcent and emollient property (Bolarinwa *et al*., 2016). The richness of the leaves in protein increases both packed cell volume and hemoglobin concentration in human and animal studies (Alada, 2000; Akindahunsi and Salawu, 2005). Inspite of the relative importance of this vegetable in the formation and composition of blood, there is little information encountered in the literature on the effect of this vegetable on cyanide toxicity when used as dietary supplement in animal model,hence this study investigates the toxicity of cyanide and whether co-administration of cyanide with extract of *Basella alba* leaves will reduce the extent of cyanide poisoning arising from oral cyanide ingestion.

II. MATERIALS AND METHODS

Animal model

Three-week old wistar strain albino rats were obtained at the animal breeding house located at Physiology Department of the University of Ibadan, Nigeria and transferred to the experimental laboratory of the Institute of Advanced Medical Research and Training of the College of Medicine, University of Ibadan. The rats were maintained on commercial rat chow and water *ad-libitum* until the weight range between 160g and 260g was attained. They were randomly distributed into one (1) control and three (3) experimental groups as follows: Group 1- no treatment (control); Group 2 was administered 3 mg/kg body weight of KCN; Group 3 was administered 3 mg/kg body weight each of both KCN and extract; Group 4 was administered 3mg/kg body weight of extract only. All treatments were maintained on commercial rat feed and water *ad-libitum* for the entire period of the experiment which was 30 days. Physical observations were made on the animals in the respective groups on day zero (when treatment commenced) and subsequently on daily basis. Before treatment was administered on each day, feed intake, water intake and body weight were measured.

Ethical Approval

The Animal Ethics Committee of the College of Medicine, University of Ibadan gave approval for the purchase of the rats with receipt number 158423 and housing of the rats in the animal house, Institute of Advanced Medical Research and Training (IAMRAT) . A veterinary doctor and a laboratory Scientist were also involved in the monitoring and analysis of the rats throughout the period of the experiment.

Preparation of the Basella alba

One kilogram fresh leaves of *Basella alba*(vine spinach) were purchased from Apeteagricultural farm in Ibadan, Oyo State and the vegetable was identified and authenticated by a Botanistat the Department of Botany,University of Ibadan, Nigeria.The vegetables was blended in warring blender and the paste was sieved through pre-washed white cloth in distilled water. The sieved liquid was then filtered through filter paper (Whatman no 1) to obtain a clear aqueous extract of the vegetable. The extract was lyophilized to obtain a dry powder. Protein concentration in the lyophilized extract of the vegetable was determined using bovine serum albumin (BSA) fraction V Sigma-Aldrichas standard protein.

Procedure for administration

The first group (NCNE) was administered with onlydistilled water.The second group (CN+DW) were fed with 3mg/kg body weight of cyanide salt solution with dose equivalent amount of distilled water.The third group (CN+BA) were administered with3mg/kg of both salts of cyanide and dose equivalent amount of*Basella alba* extract/body weight/day. The fourth group(NCN+BA) were fed with 3mg/kg of *Basella alba* extract/body weight/day with dose equivalent amount of distilled water for 30 days

Collection of blood samples and tissues

The rats were fasted for 24 hours before the blood samples were collected. Capillary tubes were used to collect blood samples from the rats while they were still alive using the occular puncture method.The blood samples were collected in lithium heparinized bottles and centrifuged at 1000rpm for ten (10) minutes using MSE table centrifuge at room temperature. Plasma was aspirated into universal bottles for liver function test analysis by assaying for a spartate aminotransferase (AST), alanine aminotransferase (ALT) and blood urea nitrogen (BUN) using automated analyzer (Rochecobas u 411 model) . The packed cell volume and other hematological parameters were also assayed using hematocrit analyzer (HemataStat 11).

Organ pathology

After the collection of blood samples, the rats were euthanized with chloroform. Confirmation of death was done through the cervical dislocation, dissected through the sternum. The testes, liver and brain tissues were harvested and placed inside labeled bottles containing 10% phosphate buffered formalin for 24 hrs for proper fixation. Testes were preserved in 10%Boiuns fluid. Thereafter, the organs were sectioned and each section was embedded in paraffin, prepared and stained with hematoxylin and eosin (H&E) for histopathological changes using light microscope (×100 magnification).

Statistical analysis

All data were presented in descriptive statistics. The one way ANOVA and post-hoc test (least significant difference) were used for comparison of the groups using statistical package for social science (SPSS version 20). The results were considered significant at p values of less than 0.05.

III. RESULTS

Table I: Effect of Cyanide and the lyophilized *Basella alba* leave extract on some morphological indices

Morphological indices	Control(NCNE)	CN+DW	CN+BA	BA+DW
Body weight (g)	159.89±22.08	148.57±19.6***a**	156.69±18.8**b**	151.83±18.1*****
Feed intake (g)	75.69±14.1	54.08±8.6*** a**	60.82±8.9*** b**	56.78±11.1*****
Water intake (ml)	216.20 ± 67.8	133.30 ± 36.8*****	143.40 ± 47.9*****	125.50 ± 53.6*****

***** ; Indicates significant difference at p< 0.05, (n = 5) from the control group

a,b; Indicates significantly different atP<0.05 along each row with alphabet(s) superscript.

NCNC; No Cyanide No Extract

CN+DW; Cyanide and Distill water

CN+BA; Cyanide and *Basella alba*

BA+DW; *Basella alba* and Distill water

Table II: Effect of Cyanide and Lyophilized *Basella alba* leave extract on some heamatological parameters

Heamatological indices	Control(NCNE)	CN+DW	CN+BA	BA+DW
PCV (g/dl)	50.67±2.5	44.67±2.5***a**	48.33±2.5	49.67±2.5**b**
RBC (g/dl)	8.59±0.2	7.38±0.3***a**	8.00±0.6	8.08±0.8**b**
Hb (g/dl)	16.53±1.1	14.50±1.1*****	15.66±1.2	16.16±1.2
Neutrophil (g/dl)	25.00±3.6	21.33±10.7**a**	29.69±4.1**b**	30.67±9.1**b**

***** ; Indicates significant difference at p< 0.05, (n = 5) from the control group

a,b ; Indicates significantly different at P<0.05 along each row with alphabet(s) superscript.

PCV=Packed Cell Volume;BRC=Red Blood Cell;Hb=Heamoglobin;WBC=White Blood Cell

Table III : Effect of cyanide and the lyophilized *Basella alba* leave extract on some biochemical indicators

Biochemical indicators	Control(NCNE)	CN+DW	CN+BA	BA+DW
AST (g/dl)	41.67±2.1	47.33±2.1*****	43.33±3.0	41.00±1.7
ALT (g/dl)	26.67±4.0	32.67±1.5***a**	29.00±3.6*****	24.00±2.6b
BUN (g/dl)	16.00±0.00	18.00±2.00*****	16.07±0.57	16.67±1.15

***** ; Indicates significant difference at p< 0.05, (n = 5) from the control group

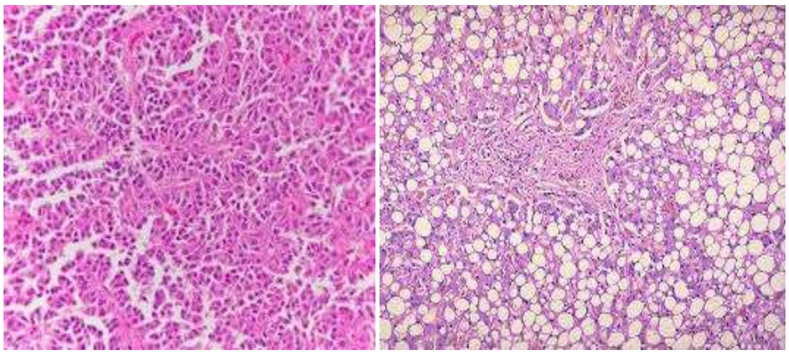
a,b ; Indicates significantly different at P<0.05 along each row with alphabet(s) superscript.

AST=Aspartate Aminotransferase Enzyme; ALT=Alanine Aminotransferase Enzyme; BUN=Blood Urea Nitrogen

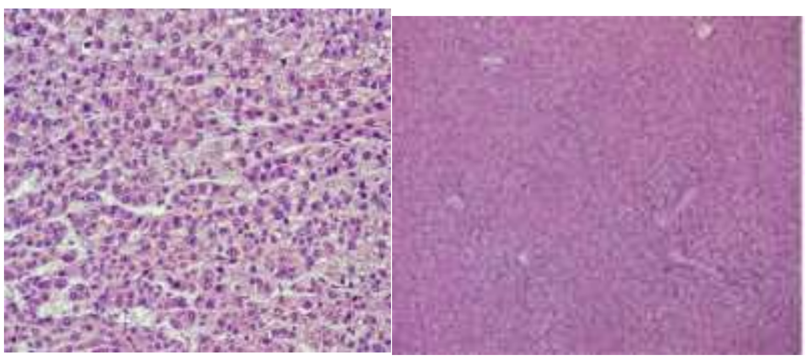
Table IV: Histopathology Analysis

Group	Control(NCNE)	CN+DW	CN+BA	BA+DW
Liver tissues and atrophy of hepatic cord of the liver	No visible lesion	Degeneration of fatty	No visible lesion	No visible lesion
Brain vacuolation and congestion of the brain	No visible lesion	Multifocal mal-lesion	No visible lesion	No visible lesion
Testes degeneration and severe testicular dissociation	No visible lesion	Multifocal testicular	No visible lesion	No visible lesion

FIGURES

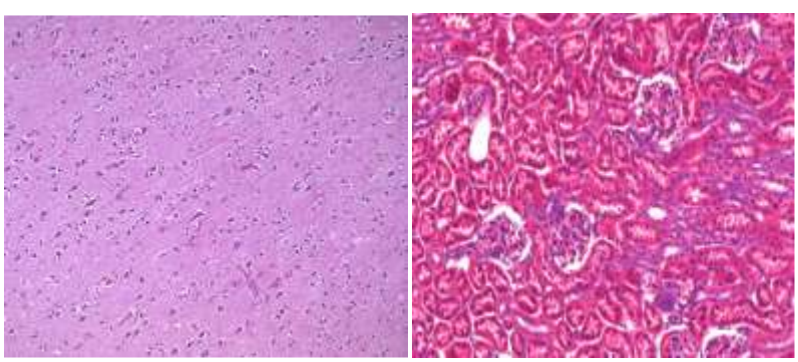


NCNE CN+DW

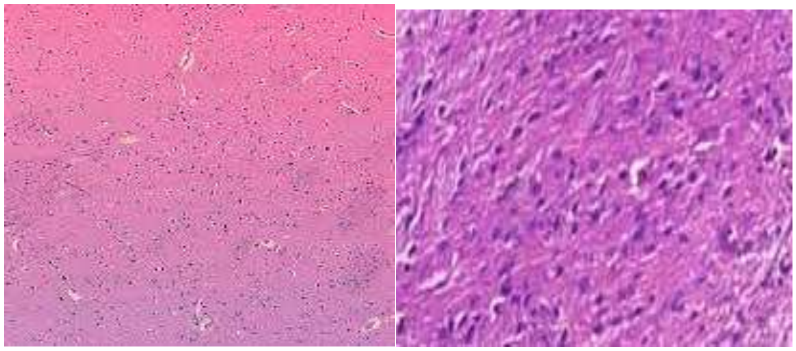


BA+DW CN+BA

FIG 1.Light microscopic micrographs of the rat liver tissue stained with hematoxylin and eosin(×100 magnification)

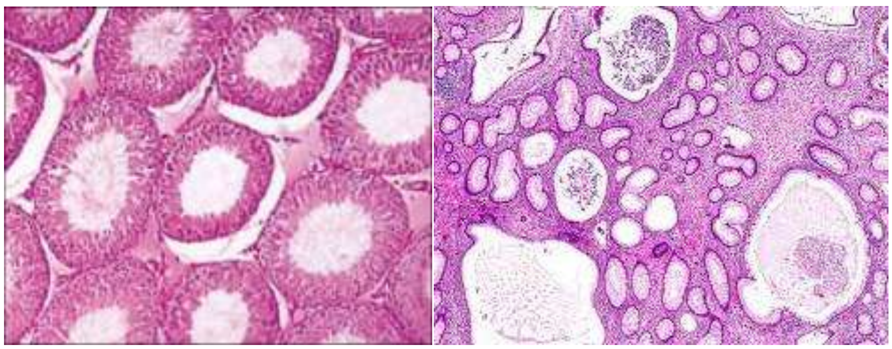


NCNE CN+DW

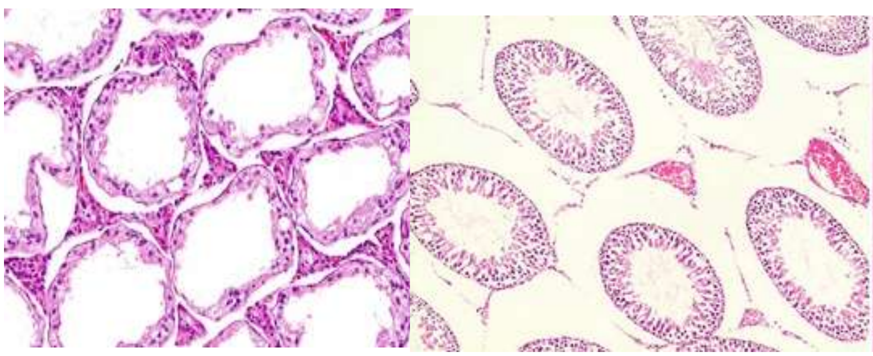


CN+BABA+DW

FIG 2.Light microscopic micrographs of the rat brain tissues stained with hematoxylin and eosin(×100 magnification)



NCNE CN+DW



CN+BA BA+DW

FIG 3. Light microscopic micrographs of the rat testicular tissues stained with hematoxylin and eosin(×100 magnification)

IV. DISCUSSION

The results showed that cyanide and leaf extracts of *B. alba* administered at the dosage used and for the period of the experiment have bothnegative and positive effects on the haemopoietic system and the findings of this study are similar with literatures on the potential health hazards inherent in the exposure to and ingestion of cyanide containing compound. However, it also showed the possible ameliorative effect of a plant used as vegetable in the communities in Nigeria where plants containing toxic amount of cyanogenic glycosides form the staple.

Oral administration of 3mg/kgbw KCN or more has been shown to result in decreased water and food consumption by rats and mice. This suggests poor palatability as initially confirmed by (Tulsawaniet al., 2005; Bolaji and Olabode, 2011). The data from table 1 indicated a significant reduction in the body weight and feed intake of group 2 compared with the control. This showed that cyanide reduced appetite,but this effect was corrected in group 3 with a significant increase in the mean values with respect to group 2.This indicated that *Basella alba*ameliorated the reduction effect of cyanide on body weight and food intake.This can be attributed to the detoxifying properties and the chemical composition of leaf extract which includes; proteins, fat, vitamin A, vitamin C, vitamin E, vitamin K, vitamin B9 (folic acid),B12, cysteine, niacin, thiamine and minerals such as calcium, magnesium and iron (Grubben and Denton, 2004;Bamideleet al., 2010; Sagar and Dattatraya, 2014).Most of these vitamins and minerals are well-known hematinic and are necessary for the detoxification of cyanide poison (Bolaji and Olabode, 2011). The vegetable alone did not have a significant effect on the body weight and food when compared with the control group.

In table 2, cyanide significantly reduced PCV,RBC and Hb which might suggest the occurence of anaemia.However, the elevated mean value observed in group 3 was due to the positive effect of the vegetable and the posibility of thevegetable's ability to increase heamopoiesis and reduces heamolysis in rats. The observed increases in the hemoglobin concentrations and packed cell volume in the rats treated with the aqueous *Basella alba* extractsis consistent with earlier reports that protein-rich diets increase both packed cell volume and hemoglobin concentrations in human and animal studies (Bamidele*et al*., 2010;Alada *et al*., 2004; Alada, 2000; Farhana *et al*., 2019).from the observed significant values of WBC, it is clear that an increase in the number of WBC is a normal reaction of rats to foreign substances, which alter their normal physiological processes. The leucocytosis observed in group 3indicated a stimulation of the immune system which protects the rats against infection that might have been caused by chemical and secondary infections. Leucocytosis, which may be directly proportional to the severity of the causative stress condition, may be attributed to an increase in leukocyte mobilization (Celik and Suzek, 2008;Adebayo *et al*,2010).

Biochemical indicators observed in this study (table 3) showed evidence of liver damage induced by treatment of rats with 3mgKCN/kg body weight (group 2). These indices of liver damage by cyanide were moderated by the treatment of the rats with aqueous extract of *Basella alba*. Liver enzymes(ALT and AST) are released into the blood whenever livercells are damaged

and enzyme activity in the plasma is increased (Aliyu*et al.*, 2006).The significant reduction in enzyme activities observed in group treated with aqueous extract only (group 4) suggests that *B. Alba*mightimproves hepatic functions.After protein metabolism, urea is the waste product releasedwhich is excreted with urine but the nitrogen contents of the urea are sometimes found the bloodstream as a result of kidney malfunctioning (Villalba *et al*., 2002). Cyanide significantly increased blood urea nitrogen which might suggest kidney damage, but this effect was considerably influenced by the aqueous extract of *Basella alba*.

Sub-acute exposure of male rats to 3mgKCN/kg body weight administered orally has been shown not only to produce significant change in parameters like organ body weight index (OBI), heamatology, level of urea, Aspatate Aminotransferase (AST) and alanine aminotransferase (ALT). It however produced various histological changes in the brain, heart and kidney (Tulsawani*et al*., 2005)

Histopathological analysis showed that the group treated with only 3mgKCN/kg body weight had degeneration of fatty tissues and atrophy of hepatic cord of the liver, Multifocal mal-lesion, vacuolation and congestion of the brain and also testicular degeneration and severe testicular dissociation (as shown in the figures below). However, no visible lesion was observed in the group treated with both the lyophilized vegetable extract and Potassium cyanide. This indicated the protective strength of the vegetable in neutralizing the toxic effect of cyanide poisoning as demonstrated by Nantia in 2017. In addition,the extract might have exhibited hepatoprotective activity due to its antioxidant properties which is attributable to flavonoids and carotenoids.

Basella alba is a rich source of sulphur containing amino acids and this may be partly responsible for the various ameliorative effects shown by this vegetable on cyanide toxicity (Manfo *et al*., 2014) It is also possible that such effects can be caused by any other molecule involving a different biochemical pathway from that of thiocyanate formation (Bromley *et al*, 2005). The major mechanism for removing cyanide from the body is through enzymatic conversion by the mitochondrial enzyme rhodanese (thiosulphate-cyanide sulphurtranferase EC2.8.1.1) to thiocyanate. The enzymatic conversion of cyanide to thiocyanate requires a source of sulphur, like polythionates, thiosulphates and persulphides. It is presumed that the sulphurtranferase binds first with the serum albumin to yield sulphanesulphur albumin complex which eventually react with the cyanide to form thiocyanates (Bolaji and Olabode, 2011; Leah *et al*., 2011).

V. CONCLUSIONS

The alterations in weight and certain heamatological, biochemical and histological parameters observed in the study point to selective toxicity of cyanide and the positive effect of the *Basella alba* extracts on the immune system of experimental animals.Thus, the results of this study suggests that *B.alba* leaves mighthave a promising role as a safe antidote for cyanide toxicity most especially if taken concomitantly with cyanide containing food(s) or shortly after ingestion of sub-lethal dose of cyanide and also in blood formation, hence the treatment and/or prevention of anaemia.

With the resurgence of interest on cyanide antidotes, a more effective prophylactic or therapeutic regimen can be anticipated in near future. Further research is recommended with the objectiveof ascertaining the efficacy and safety of the vegetable to a significant level which will be acceptable at global level. Finally,the availability of potential safe antidotes like *Basella alba* unveils the possibility of their value as first-line treatment, even in a complex clinical situation, where diagnosis is rapid and presumptive.

COMPETING INTERESTS

All Authors have declared that no competing interest exist.

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