International Journal of Agricultural Policy and Research Vol.5 (2), pp. 53-62, February 2017
Available online at https://www.journalissues.org/IJAPR/
https://doi.org/10.15739/IJAPR.17.006

ISSN 2350-1561

Original Research Article

Effect of *Mallus domestica* and *Moringa oleifera* on haematological and some biochemical parameter in female wistar rats fed cassava-based diets

Received 27 December, 2016 | Revised 6 February, 2017 | Accepted 13 February, 2017 | Published 23 February, 2017

***[1]Madukosiri CH,**
[2]Opara DC
and
[3]Amos Tau-tua BMW

[1]Department of Biochemistry, Faculty of Basic Medical Sciences, Niger Delta University, Wilberforce Island, PMB 071, Bayelsa State, Nigeria.
[2]Department of Community Medicine, Faculty of Clinical Sciences, College of Health Sciences, University of Uyo, Nigeria.
[3]Department of Chemistry, Faculty of Science, Niger Delta University, Wilberforce Island, PMB 071, Bayelsa State, Nigeria.

*Corresponding Author
E-mail: gginl@yahoo.com

Information on the toxicity of cassava-cyanide and of the side effects of treatment-diets on haematological parameter is scarce and had not been fully assessed. The indispensability of such data is obvious in regions where anaemia is rampant, particularly among the female population. The present study investigates haematological and biochemical profile of female Wistar rats fed *Manihot esculenta*-based diets and ameliorative effects of the treatment diets fortified with *Malus domestica* and *Moringa oleifera*. Twenty four (24) rats aged 9 to 12 weeks and weighing between 126.05±2.08 – 192.75±1.58g were randomised into 3 groups with 8 rats per group, and fed a*d libitum* with heat-treated cassava-cyanide diets for 28 day, after seven days acclimatization in control feed and climate condition. Full blood count was done using Automated Analyser; while the serum glucose, cholesterol, creatinine, and protein were measured spectrophotometrically. Results of haemoglobin (Hb) together with other haematological findings indicated the presence of microcytic hypochromic anaemia among the experimental rats. Higher values of cholesterol, glucose, and lower levels of serum protein were obtained from the test rats than those of the control and treatment animals and so support the toxicity effects of the cassava–cyanide. The comparatively higher Hb, serum protein and lower cholesterol concentration in the treatment group as against those of the test rats were positive indication of the ameliorative potential of the plant agents.

Key words: Cassava *Manihot esculenta*-based diet, haematological, amelioration, plant agents

INTRODUCTION

Cassava (*Manihot esculenta* Crantz) is the third-largest source of food carbohydrates in the tropics, after rice and maize; and a major staple food in the developing worlds, providing a basic diet for over half a billion people (FAO, 1995). Nigeria is the world's largest producer of cassava (FAO, 2013). Due to its characteristically rich energy source, year-round availability, tolerance to extreme stress conditions, and suitability to present farming and food systems in Africa, it is an important crop when discussing the issues of African Food Crisis Alleviation (Hahn and Keyser, 1985). Cassava has been considered to be the major human food crop with a high content of cyanogenic glycoside (Delange et al., 1982). Interestingly the health benefits of a diversified diet include reduction in adverse factors (such as hydrogen cyanide) which may exceed acceptable thresholds in a narrow diet; and provision of a wide spectrum of food components macronutrients, micronutrients and phytochemicals, which humans'

physiology requires (Whalquist, 2005). Malnutrition, with its two constituents of protein–energy malnutrition and micronutrient deficiencies, continues to be a major health burden in developing countries. A high prevalence of poor diet and infectious disease regularly unites into a vicious circle. Micronutrient deficiencies would best be addressed through food-based strategies such as dietary diversification (through home gardens and small livestock) (Muller and Krawinkel, 2005).

Previous studies had implicated hydrogen cyanide, in cassava diets, in the development of glandular and organ lesions such as thyroiditis, goiters, pancreatitis, renal and hepatic malfunctions in susceptible organisms (Madukosiri, 2013; Amar et al., 2006; Tulsawani et al., 2005). Toxicity syndromes includes Histotoxic ataxia ascribed to inactivation of the tissue enzyme - cytochrome oxidase by hydrogen cyanide which combines with the Fe^{3+} /Fe^{2+} component of the enzyme (Tulsawani et al, 2005). Others are respiratory problems resulting from inhibition of copper /zinc enzyme - carbonic anhydrase by the toxicant, hydrogen cyanide (E.P.A., 1990). Hydrocyanide or its detoxification product, thiocyanate, (SCN), can inhibit iodide oxidation, that is, the conversion of iodide, I^-, to iodine radical, I_2, by inactivating the enzyme, thyroid peroxidase (TPO) (Devlin, 2011). Reports with animal experiments revealed an increase in fasting blood sugar, change in myocardial morphology, elevation in blood aminotransferases and alkaline phosphatases and behavioral changes during sub-lethal dietary intake of cassava cyanide. These suggest an implication for dietary diversification with phytochemicals of plants in populations who are exposed to cassava toxicity (Jackson, 1998; Osuntokin, 1969).

A study on apple fortified diet concluded that the polyphenol rich feeds have the potential to positively influence the intestinal flora, blood parameters, and WBC mRNA gene expression pattern of immunological marker genes (Sehm et al., 2011). Other studies reported that apples potentially have a positive influence on blood lipid parameters and blood pressure in humans (Weichselbaum et al., 2010) and have an antigenotoxic potential, both for organically and conventionally grown apples (Briviba et al., 2007). Ethanolic extract of *M. oleifera*, has been shown to mitigate aluminium (A1C13) - induced anaemia in Albino rats (Osman et al., 2012). Moringa trees have been used to combat malnutrition, especially among infants and nursing mothers. However, further study is needed to investigate the effect of these plant agents on iron bioavailability and of anaemia in cassava consuming population (Leone et al., 2015; Kumar and Cooper, 2004). Information on the haematotoxicity of cassava-cyanide consumption and the effects of *Malus domestica* and *Moringa oleifera* on haematological parameters of cassava consumers is scarce and has not been fully determined. The present study was therefore designed to, evaluate the toxicity of cassava-cyanide consumption and the effects of fortifying the diet with the plant agents *Malus domestica* and *Moringa oleifera,* on some haematological and biochemical parameters in female wistar rats.

MATERIALS AND METHODS

Collection and Preparation of Cassava Feeds

Seven varieties of cassava traditionally known as Oguru, Yomugha, Janet, Accra, Agric sweet /bitter and Rowaina which were commonly grown and consumed in Bayelsa State, Nigeria were used for the preparation of rat feeds. The varieties were obtained from Okutukutu farms along Tombia-Yenagoa region of Bayelsa State; and no known agrochemical product was used in those farms. The taxonomist's identification had already been reported in an earlier study (Madukosiri and Amos Tau-tua; 2010). The tubers were washed with distilled water, outer brown peels manually removed and the pulp chopped into cube-sized pieces before subjection to heat treatment using dry air oven at 50°C (until total loss of moisture) and at 100°C for 30 minutes in order to mimic the usual effect of heat on toxicant level during processing. Each dry cassava sample was milled and stored in a desiccator until required for use. Equal weights of the varieties were pooled and used for feed formulation as explained in literatures (Akinowor et al., 2003; Madukosiri and Opara, 2016).

The feed was prepared using mineral, non-nutritive cellulose, vitamins and palm oil purchased from the local market in Tumbia, Yenagoa, Bayelsa. Each of the 20g of powdered *Moringa oleifera* seed and apple *Malus domestica* cultvars – Duchess, Gold delicious and Northwest Greening was introduced into the respective cassava treatment feeds. Formulated diet consisted of cassava flour, casein protein, sucrose, mineral, vitamins, non nutritive cellulose, palm oil, apple and moringa powder in proportions as given in Madukosiri and Opara (2016).

Apples, *Malus domestica* cultivars (of the family Rosaceae) - Duchess, Golden delicious, and North-west greening were purchased from Opolo market, Bayelsa State; while Moringa - *Moringa oleifera* Lam*,* also referred to as the "Miracle Tree," "Horseradish-tree," or "Ben oil tree", the best known and most widely distributed species of Moringaceae family, were purchased from Songhai-Amukpe, Sapele, Delta State. All plant agents were identified and authenticated by an expert in the Department of Crop Science, Faculty of Agriculture, Niger Delta University, Bayelsa. The apple samples (equal weight of cultivars) were similarly washed with distilled water, chopped into cube size and dried to constant weight in an oven (50°C). Exactly 100g (Wet Weight) each of the apple cultivars - Duchess, Golden delicious, and Northwest greening (in three replicate samples) were dried to constant weight of 77.6, 65.89, and 72.81 (g) respectively (Table 1). Also, the seeds of *M. oleifera* were cracked, and dried as above (Table 1). All samples were milled using Waring blender (Model No. BD0161DA-819FP, AKAI-

Table 1. Moisture Content of Plant Samples (%) (Mean ± SD)

Plant Samples	Moisture	Dry matter
Malus domestica		
Duchess	22.45 ± 0.12	77.55
Golden delicious	34.11 ± 0.73	65.89
Northwest greening	27.19 ± 0.22	72.81
Moringa oleifera	23.83 ± 0.42	76.17
Cassava varieties		
Accra	5.40 ± 2.12	94.06
Agric.	9.22 ± 0.99	90.78
Agric. Rowaina	6.73 ± 0.65	93.27
Janet	7.56 ±1.83	92.44
Oguru	8.55 ± 1.89	91.45
Rowaina	9.36 ± 3.12	90.64
Yomugha	5.80 ± 2.06	94.20

TOKIO, JAPAN). Formulated cassava diets consisted of cassava flour, casein protein, sucrose, minerals, vitamins, non-nutritive cellulose, palm oil, apple and moringa mills (g/ Kg, DW) in the respective portions for groups 2 and 3 feeds. The control diet was *Zea-maize*-based standard rat feed, purchased from Top Feeds, Eastern Premier Feed Mills Ltd, A subsidiary of Premier Feeds, Delta State.

Animals / Treatment

Experimental protocols observed were according to our Institutional Animal Ethics Committee guidelines as obtained from internationally recommended practices for use and care of laboratory animals (NIH, 1992).

Twenty-four (24) adult female albino rats of the wistar strain (*Rattusnorvegicus*) aged from nine to twelve weeks old and with individual weight from126.05 ± 2.08 – 192.75 ± 1.58g, were used for the present investigation. Rats were bred in the animal farm at Famgbe, Yenegoa region of Bayelsa State. Animals were acclimatized at room temperature and control diet for four weeks prior to experimental feeding. All animals (in the test and control groups) were fed *ad libitum* on diet and water. The clean metal cages housing the animals were placed in a well ventilated room and exposed to 12-hour day light and 12 hour-powered light at night with a relative humidity of 45-50%. Clean ceramic cups and plates were used; and as part of the measures or precautions taken to minimize contamination of feeds with urine and faeces, the cages were constructed to allow faecal pellets and urine to escape from the base or floor of cages, while the food and water compartments provided limited access so as to avoid spilling of food materials during feeding. In addition, food and water were replaced twice daily- morning and evening. Cages were cleaned weekly while daily chart of weight gain (or loss) versus feeds consumed (after correction for feeds wasted) were done using a weighing balance (Asearchtech Instrument, FA2104). Weekly mean (±SD) body weight was obtained and presented in Tables 3 – 6.

Animal Grouping

Animals were distributed randomly into three groups (of eight rats each) – 1, 2, and 3 for control, test and treatment diets respectively. At the end of 14 days feeding four rats were sacrificed from each of group 1 and 2 (representing sub-groups 1a and 2a); while the rest (1b and 2b) continued till the end of 28 days. Group 3 animals ate cassava test feed for the first 2 weeks, followed by the respective treatment diets - apple (for sub-group 3a) and moringa (for sub-group 3b) feed in the last 2 weeks before sacrifice.

Sacrificing the Animals

At the end of 14 days feeding periods, the 8 animals were anaesthetized under chloroform vapour and the blood immediately withdrawn via cardiac puncture (with sterile syringe) and placed into labelled sample tubes - lithium heparinised tube (for haematological parameters and plasma separation) and plain tube (and allowed to clot for serum collection).The rest 15 animals (excluding the one lost from test group 2 during the feeding period) were treated in the same manner after 28 days feeding.

Preparation of the Blood Samples

Blood specimen was placed in a plain sample container and allowed to clot at room temperature after which a gentle ringing was carried out to dislodge the clot from the tube. The tube was centrifuged for ten minutes at 628.88g at the end of which a clean dry Pasteur pipette was used to transfer the supernatant (serum) from the centrifuge tube into a clean dry labelled sample bottle. The sample bottle was properly closed and kept at -20°C till required for analysis. On the other hand the plasma was obtained by centrifugation to separate the cells from the heparinised blood sample.

Table 2.Composition of Formulated Cassava Diets (g/kg) Fed to the Various Rat Test Groups

Diet Components	Basal negative control diet (g/Kg) Group 1(a & b)	Test diet (g/Kg[1]) Group 2a	Test diet (g/Kg[1]) Group 2b	Test diet (g/Kg[2]) Group 3a	Test diet (g/Kg[3]) Group 3b
Standard Diet	1000	-	-	-	-
Sucrose	-	80	80	80	80
Vitamins	-	40	40	40	40
Mineral	-	20	20	20	20
Palm oil	-	40	40	40	40
Cellulose	-	20	20	-	-
Casein	-	40	40	40	40
Apple supplement	-	-	-	20	-
Moringa oleifera supplement	-	-	-	-	20
Heat –treated cassava flour	-	760	760	760	760
Total diet (kg)	1.0	1.0	1.0	1.0	1.0

Madukosiri and Opara(2016)

Table 3. Weekly Body Weight of Female Wistar Rats versus Standard Feeds Consumed (g,Mean±SD)

	Body weight	Standard feed consumed	Net weight gain/loss	Net weight gain/loss %
GROUP 1a				
1st week	163.72 ± 1,56	233.17 ± 2.30		
2nd week	180.36 ± 0.91	236.83 ± 1.37	16.64	10.16
3rd week	191.62 ± 1.48	238.21 ± 3.69	11.26	6.24
GROUP 1b				
3rd week	192.75 ± 1.56	238.21 ± 2.03		
4th week	226.69 ± 1.03	241.64 ± 3.75	33.94	17.61
5th week	281.92 ± 0.47	247.73 ± 2.38	55.23	24.36

Control groups 1a and 1b rats were fed standard feeds for 2 weeks and 4 weeks respectively (N=8).

Blood Analysis

Full blood count was performed by an Automated Analyzer (Model: Sysmex KX-21N). The blood was gently mixed and placed on a rack in the analyzer. Results were displayed on the computer for review. On the other hand the Automated Haematology Analyzer also measured the amount of haemoglobin in the blood. This was done by lysing the cells before transferring them into spectrophotometric measuring cuvettes.

Determination of Cholesterol, Glucose, Creatinine and Total Protein

Cholesterol was determined by the kit method of Randox according to the principle explained in Geetha (Geetha, 2011).To single reagent containing all enzymes (cholesterol ester hydrolase, cholesterol oxidase and proxidase) was added 3mL plasma and incubated under controlled condition(at 37°C) as specified by the manufacturers. The pink colour developed was read at 500nm in a UV/VIS spectrophotometer (Spectrum Lab, 752S). Cholesterol content was calculated as:

$$\frac{Absorbance\ of\ test}{Asorbance\ of\ standard} \times Concentration\ standard$$

Glucose determination was based according to glucose oxidase method (Geetha, 2011). In this method glucose is converted to glucoronic acid and hydrogen peroxide by the enzyme glucose oxidase. Hydrogen peroxide splits to form water and nascent oxygen – the later which combines with a chromogen (4-aminophenazone + phenol) to give a pink colour measured at 515nm in a spectrophotometer. To 0.8mL distilled water, 0.2mL plasma sample was added and mixed, out of which was withdrawn 0.2mL solution. Exactly 5mL of chromogen was added and the mixture incubated at 37°C for 15 minutes. Absorbance was measured against a reagent blank and plasma glucose (mg/dL) calculated as: absorbance of test / absorbance of standard x 100.

Spetrophotometric determination of creatinine (Geetha, 2011) was carried out using de-proteinated plasma sample.

Table 4. Weekly Body Weight of Female Wistar Rats versus Heat-treated Cassava Feeds Consumed (g, Mean ± SD)

	Body weight	HTCF consumed	Net weight gain/loss	% weight gain/loss
GROUP 2a				
1st week	146.50 ± 2.65	233.94 ± 0.67		
2nd week	125.75 ± 1.49	172.27 ± 1.30	-20.75	14.16
3rd week	131.07 ± 2.70	181.874.03 ±	5.32	10.53
GROUP 2b				
1st week	153.27 ± 0.39	224.98 ± 1.48		
2nd week	117.91 ± 2.14	163.76 ± 2.63	-35.36	23.07
3rd week	125.83 ± 3.48	181.00 ± 0.93	7.92	6.72
4th week	134.00 ± 0.73	196.29 ± 1.62	8.17	6.49
5th week	139.52 ± 2.15	217.04 ± 1.95	5.52	4.12

HTCF, heat-treated cassava feeds. 1st week was used for acclimatization with standard feed. Control group 1a and 1b were fed cassava feeds for 2 weeks and 4 weeks respectively (N=8).

Table 5. Weekly Body Weight of Female Wistar Rats versus Treatment Feeds Consumed (g, Mean ± SD)

	Body weight	TF consumed	Net weight gain/loss	% weight gain/loss
GROUP 3a				
1st week	126.05 ± 2.08	209,09 ± 2.01		
2nd week	102.75 ± 2.57	152.22 ± 1.81	-23.3	22.63
3rd week	111.42 ± 0.82	173.65 ± 1.43	8.7	8.44
4th week	108.23 ± 3.01	166.30 ± 2.47	-3.19	2.95
5th week	119.56± 3.38	184.73 ± 0.98	11.33	10.47
GROUP 3b				
1st week	138.25 ± 1.58	217.14 ± 3.01		
2nd week	115.98 ± 1.79	165.39 ± 2.85	-22.27	19.2
3rd week	129.50 ± 2.40	194.47 ± 2.47	13.52	11.66
4th week	122.00 ± 0.97	187.26 ± 0.92	-7.5	5.79
5th week	137.08 ± 1.39	213.57 ± 1.84	12.36	10.09

TF = Treatment feeds. Sub-group 3a and 3b were fed for 14 days wth cassava feeds, followed by apple and moringa diets (in that order) for the rest 14 days (N=8).

Into 4mL of acid tungstate solution was added 0.5mL of plasma and centrifuged at 905.58g for 10minutes to obtain a protein free supernatant. To 3mL supernatant was added 1.0mL each of picric acid and sodium hydroxide, shaken after each addition. The tubes were allowed to stand at room temperature for 10 minutes before absorbance reading at 520nm in a spectrophotometer. Concentration of creatinine (mg%) was calculation as, absorbance of test / absorbance of sample x concentration of standard (mg%) x100 / volume of sample.

Total protein level was determined using Biuret method (Geetha, 2011) based on the formation of purple coloured complex with cupric ions in alkaline solution. Into the sample tube were placed 100µL serum, 2.5mL sodium chloride (0.9g%), and 3mL biuret reagent. The test solution, blank and standard (containing 0.5mg/mL protein instead of serum) were incubated in a water bath (Thermo Electron Corporation, 2836) at 37^0C for 10 minutes and absorbance reading taken against a reagent blank at 555nm in a spectrophotometer. Protein in sample was calculated as absorbance of test / absorbance of standard x 100 / volume of serum.

Analysis of variance (ANOVA) among groups and Pearson's Product Moment correlation (PPMC) coefficient were done using the Statistical Package for Social Sciences (SPSS), version 17.0. The level of significance was

expressed at p = .05.

RESULTS

The weekly weight gain (%) determined was from 6.24 to 10.16 and 17.69 to 24.36 (g) for control groups 1a and 1b respectively (Table 3); while those of the test and treatment groups were from 4.12 to 10.53 and 8.44 to 10.47 respectively (Table 4 & 5). At the third week of feeding period, rats in the treatment sub-groups 3a and 3b lost 2.95 and 5.79 (%) of their weights respectively (Table 6).

The levels of haemoglobin (Hb) and red blood cell (RBC) determined from the treatment group were higher than those from the counterpart control and test groups but the differences were not significant (P= .05). On the other hand, the control values of the other haematological parameters – packed cell volume (PCV), platelets, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and mean corpuscular haemoglobin concentration (MCHC), were higher when compared to the levels obtained from the treatment and test groups (Table 7).

The result of correlation coefficient determined showed a negative but significant correlation between Hb and the immune component - neutrophils within group1b (P= .05). However, the negative correlation found between Hb versus other immune components such as white blood cells (WBC), lymphocytes, and Mon (monocytes), was not significant, (P= .05), (Table 8).

The biochemical parameters, cholesterol and glucose values, obtained from the test rats ranged between 155.2 - 172.0 and 74.0 to 80.5 (mg/dl) respectively, and were significantly higher than those of their corresponding controls and treatment groups, (P=.05), (Table 9). Values for total protein ranged from 0.29 to 0.86; 0.43 to 0.57; and 2.8 to 0.75 (mg/dl) for control, test and treatment groups in that order. The creatinine level determined from treatment group 3b (1.4±1.3) was significantly higher than its counterpart groups, (P= .05).

DISCUSSION

Although there was no significant difference determined between Hb values, the test group (which was administered the cassava-cyanide diet) had lower levels than those in the control and treatment groups. The level determined in the treatment group was an indication that the administration of the plant agents (particularly *M. oleifera*) could play an ameliorative role.

The PCV levels in the test and treatment groups were found below the normal range of 36 – 54(%) given in literature (Ita and Udofia, 2011), while the control values fell within the lower level reported in the cited literatures. The plant agents did not show evidence of amelioration of PCV component. This is in line with previous findings (Makanjuola et al., 2014). The decrease in MCV, MCH and MCHC values was suggestive of microcytic, hypochromic anaemia associated with iron deficiency. Hydrocyanide in cassava, when consumed can interact with haem iron (Guriprassanna et al., 2005) deactivating it. Although none of the MCH values determined from the rat groups was found below the lower limit, 27Pg, given in the literature (Burtis et al., 2012), the MCHC values determined in the test and treatment groups were below the lower limit of 30g/dl given in the cited reference. Those findings lent support to the presence of microcytic, hypochroic anaemia. The values of RBC determined were consistent with those of Hb results. The RBC values obtained for Moringa-treatment group was not statistically higher than those of the counterpart groups (P=.05). Cell deformity is one major parameter that determines RBC life span. Some toxicants could compromise the capacity of RBC to withstand internal stressors (Ojo et al., 2006). Toxicants could alter the membranes of erythrocytes as well as other cell types as a consequence of oxidative damage.

The significant difference determined between the values of WBC in the control versus test/ treatment groups support the presence of pathology following the consumption of cassava diets and to which the immune system responded by increasing its (WBC) production. Negative correlation was found between Hb and neutrophils (neu) / WBC / lymphocytes (lym) / Mon and support the relationship between anaemia and infection (Ita and Udofia, 2011). Particularly the increase in WBC, along with the decrease in the Hb concentration (as shown by the negative correlation determined between them), support the link between anaemia and susceptibility to pathology / infection. However all values fell within the range given in TRFC (2014).

The lack of significant difference between the other parameters; nuetrophils, and monocytes was taken as low level damage / inflammation resulting from acute rather than chronic toxicity. Nuetrophils are the first responders to inflammation and cell damage (Ear and McDonald, 2008). The levels of lymphocytes observed, particularly in groups 2b and 3a was suggestive of lymphocytes membranes oxidation as the rats were subjected to cassava–cyanide feeds. Low normal to low absolute lymphocyte concentration is associated with increased rates of infection after trauma (Abbas and Lichtman, 2003). The lack of significant difference between the lymphocyte values in apple / moringa-treatment and the control groups supported the ameliorative role of the former. The effectiveness of apples and moringa as potential disease-preventing agents against a range of degenerative diseases, inflammation, and immune system dysfunction corresponds to their content of powerful anti-oxidant phytochemicals such as phenolics, flavonoid, caratenoids and tannins (Reis et al, 2012). The eosinophils are primarily associated with parasitic infections and an increase in their number suggests such function (Albert, 2005). In the present case it was attributed to infection arising from depressed immunity.

Table 6. Net Weight Gain / Loss (%) Per Rat Group

Rat Groups	Mean Body Weight Gain (g) (%)		Body Weight Loss (g)	(%)	Net Weight Gain (g)	(%)
Group 2b	21.61	15.49	0	0	21.61	15.49
Group 3a	15.81	8.56	3.19	2.95	12.62	10.56
Group 3b	21.10	9.88	7.50	5.79	13.60	9.92

Net weight gain was calculated as the difference between mean body weight gain and body weight loss. (N=8).

Table 7. Haematological Parameters Determined from Female Wistar rats Fed with the Various*Diets.

	Parameters	Group 1a control	Group 1b Control	Group 2a Test	Group 2b Test	Group3a	Group3b
1	WBC	6.83 ± 0.73^{ab}	8.003 ± 1.36^{b}	8.23 ± 1.08	8.23 ±1.12^{cd}	9.48 ± 1.20^{d}	9.23 ± 1.17^{ac}
2	neutrophil	40.75 ± 12.34	31.00 ± 5.60	39.25 ± 7.76	31.00 ± 12.68	33.00 ± 4.08	38.25 ± 4.27
3	Lymphcyte	50 ±6.83 ^{a}	56.75 ± 11.32	52.75 ± 10.97	37.25 ± 13.52	36.25 ±3.20^{d}	42.75 ± 2.06
4	Monocyte	7.25 ± 1.71	7.75 ±0.96	7.75 ± 1.89	6.75 ± 1.71	6.00 ± 1.41	5.75 ± 1.26
5	Eusinophil	4.50 ± 1.29^{abcde}	4.50 ± 1.29	5.25 ± 2.50^{b}	3.50 ±1.73^{c}	3.25 ± 1.26^{d}	2.50 ± 0.58^{e}
6	RBC	6.85 ± 2.27	7.97 ± 2.7	8.887 ± 1.56	7.66 ± 0.94	7.15 ±1.56	8.2 ± 1.05
7	Haemoglobin	9.28 ± 0.97	8.68 ± 1.88	8.90 ± 0.92	7.79 ±2.04	10.78 ± 2.33	11.5 ± 2.65
8	PCV	36.75 ± 6.24	38.25 ± 2.22 ^{a}	27.00 ± 2.94^{a}	27.75 ± 3.86	24.5 ± 3.42	27.75 ± 2.22
9	MCV	60.38 ± 17.10	5 1.25 ± 10.72	60.68± 1 8.21 ^{a}	36.36± 15.91^{a}	31.81 ± 3.99	35.8 ± 8.39
10	MCH	41.3 ± 13.27	27.88 ± 3.28	33.93 ± 8.96	33.55 ± 9.09	28.65 ± 6.58	32.75 ± 5.19
11	MCHC	27.6 ± 6.29	30.1 ±4.15^{ab}	39.62 ± 6.72 ^{a}	28.42 ± 2.86^{b}	28.62 ± 8.25	25.15± 6.02
12	Platelet	33.0 ± 6.67^{a}	32.82 ±4.74	39.92 ± 4.39	31.07 ± 7.74	39.3 ± 4.68^{a}	30.27 ± 7.88

N=8 for each group,(N=4 for sub-group) ; *groups 1 & 2 ate the standard and cassava-cyanide diets respectively; sub-groups a & b ate the respective diets for 2 & 4 weeks, in that order. Group 3 ate cassava diet for two weeks followed by the treatment diets made with apples (a) & moringa (b) for the remaining 2 weeks. Figures with the same superscript in the horizontal axis are significantly different, (P= .05).

Table 8. Correlation Coefficient (r) determined between Haematological Parameter in Groups of Female Wistar Rats Fed Various Formulated Cassava Diets.

Parameters	Group1a	Group1b	Group2a	Group2b	Group3a	Group3b
Hb/PCV	-0.089	-0.381	0.098	0.844	-0.777	0.018
Hb/RBC	-0.666	0.538	-0.634	0.586	0.421	0.733
Hb/MCV	0.051	-0.459	-0.696	-0.913	-0.779	0.018
Hb/MCH	0.953*	0.396	0.778	-0.816	-0.165	0.449
Hb/MCHC	0.235	0.964*	0.526	0.824	0.859	-0.488
Hb/Platelet	0.166	-0.912	-0.655	0.460	0.668	-0.408
Hb/Neutrophil	0.277	-0.976*	0.098	0.779	0.091	-0.783
RBC/MCH	-0.362	-0.542	-0.979*	-0.895	-0.515	-0.089
RBC/PCV	0.996**	-0.951	0.385	0.077	-0.595	0.731
PCV/MCV	-0.996*	-0.074	-0.664	-0.6478	0.218	-.0.767
Monocyte/Eusinophil	0.987*	0.547	0.933	0.958*	0.937	0.688

Values with *, and ** were significant at p = .05 and p =.01 respectively

The biochemical parameters determined were consistent with our previous investigation as to the effects of cassava-cyanide consumption on thyroid status and organ enzymes of wistar rats (Madukosiri and Opara, 2016). For example, there was an increase in blood glucose and cholesterol levels and a decrease in protein value when cassava-cyanide was fed. The reversal of those effects when the plant agents were administered supported their ameliorative role. The above mentioned anti-oxidant phytochemicals are known to lower blood cholesterol and glucose levels, and decrease lipid peroxidation (Boyer and Liu, 2004). The level of creatinine determined in the moringa treatment group could be explained in terms of increased muscle breakdown and therefore support the weight reduction determined in that group. Our findings were in line with literature reports on the weight reductive role ascribed to those phytochemicals (Azzi, 2013a and b; Medjakovic, 2013).

The hydrogen cyanide content of the control (maize-based) diet had already been determined (Madukosiri and

Table 9. Biochemical Parameters Determined in Female Wister Rats Fed with the Various*Diets

Groups	Protein(mg/dL)	Creatinine(mg/dL)	Cholesterol(mg/dL)	Glucose(mg/dL)
1a	0.59±0.3	0.3±0.1[e]	150.0±3.4[b]	65.64±3.0
1b	0.66±0.2[ab]	0.6±0.3[d]	153.9±1.0	67.2±1.8[a]
2a	0.47±0.1	0.6±0.2[c]	167.4±12.2	74.9±0.90
2b	0.45±0.020[a]	0.7±0.3[b]	170.2±1.8[ab]	79.5±1.0[a]
3a	0.48±0.20[b]	0.6±0.3[a]	164.90±1.9	74.9±2.50
3b	0.55±0.2	1.4±1.3[abcde]	158.5±2.8[a]	78.6±1.30

N=8 for each group,(N=4 for sub-group) ; *groups 1 & 2 ate the standard and cassava-cyanide diets respectively; sub-groups a & b ate the respective diets for 2 & 4 weeks, in that order. Group 3 ate cassava diet for two weeks followed by the treatment diets made with apples (a) & moringa (b) for the remaining 2 weeks. Figures with the same superscript along the vertical axis are significantly different, (P=.05).

Opara, 2016) and was shown to be higher than their counterpart test / treatment feeds and therefore was expected to have given rise to more toxicity symptom but the results were contrary. According to the cited reference the hydrocyanide load of the cassava diet could not have represented the sole /major anti-thyroid agent in the feed – a report consistent with the observation of Amar et al., (2006) who demonstrated the role of thiocyanate (which is a metabolic product of HCN) in TPO inhibition and hypothyroidism. According to them, thiocyanate played a major role in cassava toxicity since its level of content and toxicity were unaffected by heat processing. It was possible that processing by heat might have reduced the HCN content but increased the thiocyanate level of our feeds. Thiocyanate, together with other related compounds are the hydrolytic product of HCN metabolism in plants and animal tissues (Schone et al., 2001). These and other factors such as the nature and the capacity of antioxidant factors (as already highlighted) and other components present in the body (or consumed along with the toxicant) determine the extent to which the consuming organism handles its HCN load (Madukosiri and Opara, 2016).

CONCLUSION AND RECOMMENDATION

Heat treated cassava-cyanide diet could be toxic to the consuming animals and precipitate anaemia. Decrease in levels of MCV, MCH and MCHC support the presence of microcytic, hypochromic anaemia resulting from iron deficiency.

Considering the fact that anaemia has remained a problem in the developing worlds, future toxicity studies should incorporate haematological investigations not only to assess the toxicity of test components but the side effects of treatment agents on haemopoietic factors. Also further studies should be designed to assess the effect of chronic toxicant ingestion which would involve a longer period of experimental feeding. A dose-dependent effect of the treatment agents should be included to enable the determination of the concentration of plant agents with maximum therapeutic and haematological action.

Conflict of interests

The authors declare that they have no conflicting interests

REFERENCES

Abbas AK, Lichtman AH (2003). Cellular and Molecular Immunology, 5th ed. Saunders, Philadelphia.

Akaninwor JO, Onyeike EN (2003). Compounding of Diets and Techniques of Animal Feeding Studies (eds) In: Onyeike EN, Osuji JO. Research Techniques in Biological and Chemical Sciences. Springfield Publishers Ltd Owerri (Nigeria). 3: 246-260.

Albert B (2005). Leucocytes function and percentage breakdown. Molecular Biology of the Cell, 4-7.

Amar KC, Dishari G, Sanjukte M, Smritiratan JC (2006). Effect of cassava (*Mannihot esculenta* Crantz) on thyroid status under Conditions of varying iodine intake in wistar rats. Afr. J. Traditional, Compl. Altern. med., 3(3): 87-99.

Azzi A (2013a). Molecular Aspects of the Metabolic Syndrome: Lipid metabolism, genes, and their regulation by natural compounds, A lecture presented at the Advanced School of International Union of Biochemistry and Molecular Biology (IUBMB), Mashhad University of Medical Sciences, Iran 11 – 15th March.

Azzi A (2013b). Molecular Aspects of the Metabolic Syndrome: Oxidative stress, oxidants and antioxidants and metabolic dysregulation, A lecture presented at the Advanced School of International Union of Biochemistry and Molecular Biology (IUBMB), Mashhad University of Medical Sciences, Iran 11 – 15th March.

Boyer J, Liu R (2004). Apple phytochemicals and their health benefits. Nutrition Journal, 3, 5.

Briviba K, Stracke BA, Rüfer CE, Watzl B, Weibel FP,Bub A (2007).Effect of Consumption of Organically and Conventionally Produced Apples on Antioxidant Activity and DNA Damage in Humans. J. Agric. Food Chem., 55 (19): 7716–7721.

Burtis CA, Ashwood ER, Bruns DE (2012). Tietz Fundamentals of Clinical Chemistry, Elsevier, India.

Delange F, Bourdoux P, Colinet E, Courtois P, Hermart P, Lagasse R, Mafuta M, Seghers P, Tilly C, Vanderpas J, Yunga Y, Ermans A (1982). Nutritional factors involved in the goitrogenic action of cassava. In: Delange F, Ahluwaiia R (eds) Cassava toxicity and thyroid research and public health issues. Otawa, Canada: International Development Research Centre (IDRC-207e), 17 – 26.

Devlin TM (2011). Textbook of Biochemistry with Clinical Correlations.7th ed.Danvers(MA): John Wiley, & Sons, Inc.

Ear T, McDonald PP (2008). Cytokine generation, promoter activation and oxidant-independent NF-kappaB activation in a transfectable human neutrophilic cellular model. BMC Immunol., 9:14-14.

Environmental Protection Agency (E.P.A) (1990). Summary review of health effects associated with hydrogen cyanide, Environ. Protection Research Agency North Carolina, USA, 309:175-198.

Food and Agricultural Organization (FAO) of the United Nations (1995). Dimension of Need: An atlas of food and agriculture, pp 23–27.Available from:<http://www.fao.org/docrep/t0207e/T0207E08.htm#Cassava%20toxicity>. [14 March 2005].

Food and Agricultural Organization (FAO) of the United Nations(2003). The state of food security in the word. Rome, Italy.

Geetha DK (2011ed). Practical Biochemistry. Jaypee Brothers Medical Publishers (P) Ltd, UK.

Gouriprasanna R, Sarma BK, Phadnis PP, Mugesh G (2005). Selenium-containing enzymes in mammals: Chemical perspective', J. Chem. Sci., 4:287–303.

Hahn SK, Keyser J (1985). Cassava: A basic food of Africa. Outlook on Agriculture 4**:** 95-100.

Hahn SK, Mahungu NM, Oto JA, Msabaha MA, Lutaladio NB, Dahniya MT (1987). Cassava and African food crisis. pp 17-23.

Ita SO, Udofia UA (2011). Comparative Study of some Haematological Parameters in Rats Following Ingestion of Crude oil (Nigerian Bonny Light) Petrol, Kerosine, and Diesel', Asian J. Biol. Sci., 4(6):498–505.

Jackson LC (1998). Behavioural effects of chronic sublethal dietary cyanide in an animal model: Implications for humans consuming Cassava. Hum. Biol., 60:597–614.

Kumar HD, Cooper EL, Yamaguchi Nobuo (2004), Management of Nutritional and Health Needs of Malnourished and Vegetarian People in India. Advances in Experimental Medicine and Biology. Springer US. pp. 311–321.

Leone A, Spada A, Battezzati A, Schiraldi A, Aristil J, Bertoli S (2015). "Cultivation, Genetic, Ethnopharmacology, Phytochemistry and Pharmacology of Moringa oleifera Leaves: An Overview". Int. J. Mol. Sci., 16(6):12791–835.

Madukosiri CH (2013). The effect of Heat-treated cassava diets and various levels of protein intake on some biochemical parameters in Wistar rats. Int. J. Bio, Pharm. Allied Sci., 2(4):815-826.

Madukosiri CH, Amos-Tuatua BM (2010). Mineral elements and toxicants in segments of Manihot esculenta grown in Bayelsa state. Act SATEC Life and Phy. Sci., 3(2): 115–118.

Madukosiri CH, Opara DC (2016). Effect of Malus domesticus and Moringa oleifera on Thyroid Status and some Enzyme activities in Female Wistar Rats Fed with Manihot esculenta-based Diets, Book of Abstracts, Annual General Meeting and Scientific Conference of Nutrition Society of Nigeria, Makurdi, pp 29 (abs).

Makanjuola BA, Obi OO, Olorungbohunmi TO, Morakinyo OA, Oladele-Bukola MO, Boladuro BA (2014). Effect of *Moringa oleifera* leaf meal as a substitute for antibiotics on the performance and blood parameters of broiler chickens. Livestock Research for Rural Development, 26(8):144. www.Irrd.org/Irrd16/8/maka26144.htm.

Medjakovic S. Jungbouer A (2013). Pomegranate: A Fruit that ameliorates Metabolic Symdrome: Food and Function, 4:19–39.

Muller O, Krawinkel M (2005). Malnutrition and health in developing countries CMAJ. 173(3):229.

National Institute of Health (NIH) (1992). Institutional animal care and use committee guidebook. NIH Publication, Washington D.C. USA. Government Printing Office, 92–3415.

Ojo OO, Kabutu FR, Bell M, Babayo U (2006). Inhibition of paracetamol-induced oxidative stress in rats by extracts of lemongrass (*Cymbropogon citrates*) and green tea (*Camellia sinensis*) in rats. Afri. J. Biotechnol., 5(12):1227-1232.

Osman HM, Shayoub Mohamed E, Babiker EM, Osman B, Elhassan AM (2012). Effect of Ethanolic Leaf Extract of Moringa oleifera on Aluminum-induced Anemia in White Albino Rats. Jordan J. Biol. Sci., 5(4):255.

Osuntokin BO (1969). Chronic cyanide intoxication and degenerative neuropathy in Nigeria. Ph.D. Thesis, Ibadan.

Reis SF, Rai DK, Abu-Ghannam N (2012). Water at room temperature as a solvent for the extraction of apple pomace phenolic compounds. Food Chemistry, 135:1991-1998.

Schone F, Leiterer M, Hartung H, Jareis G, Tischendorf F (2001). Rapseed glucosinolate and iodine in sows affect the milk iodine concentration and iodine status of piglets. Br. J. Nutr., 85(6):659-70.

Sehm J, Treutter D, Lindermayer H, Meyer H D, Pfaffl MW (2011). The Influence of Apple- or Red-Grape Pomace Enriched Piglet Diet on Blood Parameters, Bacterial Colonisation, and Marker Gene Expression in Piglet White Blood Cells. Food and Nutrition Sciences, 2(4):366-376.

The Rat Fan Club (TRFC) (2014). Normal Lab Values.17th October.

Tulsawani RKM, Debnath SC, Pant OM, Kumar AO, Prakash R, Viyayaraghavan R (2005). Effects of sub-acute oral cyanide administration in rats; Protective efficacy of alpha-ketoglutarate and sodium thiosulfate. Chemico-Biological Interaction, 1(12):156-160.

Weichselbaum E, Wyness L, Stanner S (2010). Apple polyphenols and cardiovascular disease – a review of the

evidence. 35(2): 92-101.
Whalquist M (2005). Diversification in Indigenoius and ethnic food culture. Diet Diversification and Health Promotion Forum Nutr. Basel, Karger, 57:52-61.