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Effect of Dietary Inclusion of *Garcinia kola*, *Gongronema latifolium* and *Vernonia amygdalina* on the Nutritional Quality of a Complementary Diet

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Abstract: The growth response and haematological profile of albino rats to complementary diets 1-3 containing *Garcinia cola*, *Gongronema latifolium* and *Vernonia amygdalina* respectively at 10% w/w level was investigated. Diet without plant materials served as control. The chemical composition of the plants and test diets were determined. Results show that the protein, fat, crude fibre and ash contents of the test diets on dry matter basis range between 15.44-18.30, 7.93-9.22, 7.65-9.55 and 8.99-10.80%, respectively. Potassium, magnesium, calcium and sodium were the major minerals while zinc, copper and phosphorus are the least in the diets. Phytic acid, oxalates and tannins are generally higher in the test diets. The feed consumption rates of the rats on the test diets were low and can be described using regression equations. Feeding time and quantity consumed are positively correlated in Diets 2 and 3 while Diet 1 and the control diet show little or no relationship. Rats fed on Diet 3 had the highest packed cell volume, red blood count, white blood count and haemoglobin contents. Diet 1 group had the highest mean corpuscular haemoglobin and mean corpuscular volume while Diets 2 and 3 had lower values. The control group had lower heart and kidney weight. The heart, liver and kidney colour of rats fed with the control diet were normal red. The heart colour was normal red while liver and kidney colour varies from very light brown to chocolate brown in rats fed the test diets.

Key words: Growth response, chemical composition, plant materials

INTRODUCTION

In many developing countries, weaning foods are commonly prepared from cereals, while commercial weaning foods are composed of cereal grains and soybean. The high incidence of protein energy malnutrition among weaning age children in this region may be due to

- Ffeeding solely on cereal-based diets.
- Tthe high cost, viscous character and overdilution of commercial weaning foods. There has been considerable interest on the use of legumes and oilseeds available locally like soybean, cowpea and groundnut in various food fortification and supplementation schemes[1,2]. The high utility of these legumes in various agro allied products and human consumption has continually led to increasing cost.

Reports by various authors have shown that some tropical plants like *Gongronema latifolium*, *Vernonia amygdalina*, *Chromolina odorantum*, *Ipomoea asterifolia*, *Emilia santifolia* and *Naulea latifolia* and *Garcinia cola* are widely cultivated and are rich in protein and minerals[3-6]. The successful utilization of these plant materials may represent a practical and sustainable way of addressing food security. However, before a definitive conclusion can be reached on the suitability of these plant materials in food systems, their biological and haematological properties need to be evaluated.

The objective of this study was to evaluate the food value and haematological properties of a complementary diet containing *G. latifolium, V. amygdalina* and *G. kola*.

MATERIALS AND METHODS

Materials: The major raw material used in this study were freshly harvested *G. latifolium*, *V. amygdalina* and *G. kola* obtained from a local farm in Akure, Ondo State, Nigeria. The samples were collected randomly (complete randomized design) and taken to the Department of Crop, Soil and Pest Management, Federal University of Technology, Akure, Nigeria for identification and packed in clean sterile sample bags.

Sample preparation: The leafy parts of the plants (*G. latifolium* and *V. amygdalina*) were cut, rinsed with distilled water, air-dried and oven dried at 50°C until the leaves were crispy. The dried leaves were milled, vacuum

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Table 1: Basal composition of experimental diets

	Diets			
Ingredients	1	2	3	Control
Maize	56	56	56	62
Groundnut cake	28	28	28	31
Oil	3	3	3	3
Bone meal	2	2	2	2
Vitamin mix*	1.8	0.8	1.8	1.8
NaCl	0.2	0.2	0.2	0.2
G. kola	10	0	0	0
G. latifolium	0	10	0	0
V. amygdalina	0	0	10	0
Total	100	100	100	100

*The vitamin mix contained Vit A, Vit D, Vit E, choline, P-amino benzoic acid, inositol, niacin, calcium panthothenate, riboflavin, Vit B_1, Vit B_6, folic acid, biotin and Vit B_{12} in proportions specified by AOAC[7].

packed in 5mm thick high density polythene sachet, labelled and stored in a cool, dry, dark place. *G. kola* seeds were first loaded in a conventional laboratory oven set at 50°C for 10-12 h, manually dehulled, dried (50°C for 24-36 h), pulverized, milled, vacuum packed in 5 mm thick high density polyethylene bag, labelled and stored in a refrigerator (3±1°C) until used in preparation of the experimental diets.

Preparation of experimental diets: Diets were formulated as shown in Table 1 and mixed homogenously with 10% (w/w) *G. kola* (Diet 1), *G. latifolium* (Diet 2) and *V. amygdalina* (Diet 3) respectively, sealed and stored in the refrigerator till they were needed for chemical analyses and feeding trials.

Proximate and mineral analysis: Dry milled plant materials and the diets were analysed for crude protein, fat, crude fibre and total ash as described by[7]. The nitrogen free extractives was obtained by difference. The gross energy values were estimated by multiplying the proportions of carbohydrate, protein and fat present in each diet by 4, 4 and 9 respectively. The moisture content was determined by drying the wet sample to a constant weight in an air circulating oven at 70-8°C. The mineral contents, namely: calcium (Ca), potassium (K), magnesium (Mg), manganese (Mn), copper (Cu), lead (Pb), sodium (Na), zinc (Zn) and iron (Fe) contents were determined as described by[8] using a Pye Unicam SP9 Atomic Absorption Spectrophotometer connected to an SP9 computer (Pye Unicam Ltd, York Street, Britain). Sulphur (S) content was determined turbidiometrically and absorbance was measured at 420 nm using spectrophotometer. Total phosphorus was determined by the spectrophotometric molybdovanadate method as described by AOAC[7]. The Burns[9] method was used to estimate tannins[10], method was used for the determination of phytic acids while oxalate content was obtained by the procedures of[11].

Feeding trials: Forty 28-day-old male (20) and female (20) albino rats (Winster strain) obtained from Department of Animal Science, University of Ibadan, Nigeria, were acclimated to diets and housing conditions for one week. The rats were housed singly in wire bottomed metabolic cages at ambient temperatures (22±1°C) under a light-dark cycle of 12 h light 12 h dark with facilities for separate collection of urine and faeces. On the first day of the second week, 16 of the rats were removed, divided into 4 treatment groups of 4 rats per group such that the mean group weights were identical and randomly assigned to each diet. Animals were maintained and utilized in accordance with standard guide for the care and use of laboratory animals. One group was fed on the control diet while the other three groups were fed diets 1 to 3 ad libitum for 21 days and had free access to water. During this period, the daily feed consumption and group weight changes were measured and changes in body weight over the period were calculated. Faeces and urine of the individual groups was collected in the last five days.

Growth performance and nutrient utilization of the rats were determined in terms of final mean weight (g), specific growth rate (SGR, $\%day^{-1}$), food intake, feed conversion ratio (FCR) and protein efficiency ratio (PER). These growth responses were calculated as follows: Weight gain (%) = 100(final body weight-initial body weight/initial body weight); SGR ($\%\ day^{-1}$) = 100 ($\log_e$) final body weight-$\log_e$ initial body weight/time (days); FCR = dry weight of feed fed (g)/weight gain (g); PER = weight gain (g)/protein fed (g).

Haematological analysis: At the end of the feeding trial, rats from each group were anaesthetised, sacrificed and blood samples collected into labelled sterile heparinized tubes containing a speck of EDTA. These samples were used determine the following set of hematological parameters: erythrocyte sedimentation rate (ESR), packed cell volume (PCV), red blood cells (RBC), white blood cells (WBC), hemoglobin (Hb) concentration, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) as described by[12]. The liver, heart and kidney were physically examined, weighed and expressed as g Kg^{-1} body weight.

RESULTS AND DISCUSSION

Chemical composition of plant materials: Table 2 shows the proximate composition and antinutritional factors of dry milled *G. latifolium*, *V. amygdalina* and *G. kola* on percent dry matter (DM) basis. The crude protein, lipid extract, ash, crude fibre and nitrogen free extractives of *G. latifolium*, *V. amygdalina* and *G. kola* are 27.20, 6.07,

Table 2: Proximate and antinutritional composition of *G. latifolium*, *V. amygdalina* and *G. kola*

Composition	*G. latifolium*	*V. amygdalina*	*G. kola*
Proximate (% DM)			
Crude protein	27.20 d±0.02	21.69 c±0.15	4.38 a±0.34
Crude Fat	6.07±0.03	5.66±0.05	4.79±0.05
Ash	11.62 c±0.29	12.48 c±0.31	1.26 a±0.10
Crude Fibre	10.81 ab±0.25	8.8 a±0.23	12.63 b±0.50
Nitrogen free extractives	44.30	51.37	76.95
Antinutritional			
Oxalates mg g	0.07±0.01	0.06±0.01	0.04±0.01
Phytates mg 100 g	263.25±18.81	169.23±8.24	714.54±18.83
Tannins mgTA g	1.30±0.10	5.68±0.10	4.30±0.13
Lead	ND[1]	ND[1]	ND[1]
Mercury	ND[1]	ND[1]	ND[1]
Oxalates mg g	0.07±0.01	0.06±0.01	0.04±0.01

Means followed by different lowercase letters are significantly different ($p<0.05$), Values are means of three replicate readings. ND: Not detectable

11.62, 10.81 and 44.30%; 21.69, 5.66, 12.48, 8.80 and 51.37%; 4.38, 4.80, 1.26, 12.63 and 76.94% DM respectively. The crude protein contents of *G. latifolium* 27.20% DM and *V. amygdalina* 21.69% DM are comparable with the percent DM values reported for commonly consumed vegetable protein sources, namely: melon 28.4%, peanut flour 24.3%, canola 25.0%, chickpea 24.0%, cowpea 24.7%, lentil 26.1% and greenpea (24.9%)[13-15]. The high crude protein composition of *G. latifolium* and *V. amygdalina* suggests that they may be able to contribute to the 23.6 g 100 g recommended for adults by the US National Research[16]. It also indicates their potential for use as cheap and readily available complementary protein sources. However, bioavailability of proteins of *G. kola* seed and hull has to be determined via *in vivo* studies before use in food/feed formulation. The crude fat content obtained for *G. latifolium, V. amygdalina* and *G. kola* are comparable with those reported for *Brachystegia eurycoma, Tamarindus indica* and *Mucuna flagellipes*, 5.87, 7.20 and 3.77% DM respectively[], higher than *A. triangularis* leaves, spinach and mustard, 0.1, 0.2 and 0.2% DM, respectively[17], *Gnetum africanum*, 2.4-9.6% DM[18] and most green vegetables, 0.1-0.7% [19]. The crude fibre and ash contents obtained are high when compared with that of commonly consumed leafy vegetables like lettuce 0.5 and 0.4% DM, spinach 0.6, 0.7% and chomte 2.8 and 1.7%[20]. They however compare favourably with values reported for fluted pumpkin leaves 12 weeks after planting, 14.20 and 12.4% DM[15].

The oxalate content of *G. latifolium*, *V. amygdalina* and *G. kola* are 0.07, 0.06 and 0.04 mg g, respectively. These values are lower than those reported by[21] for *X. sagittifolium* 254-381 mg 100 g *X. sagittifolium* 302-323 mg 100 g and *C. esculenta* 328-460 mg 100 g. The presence of high quantities of oxalates in foods has been associated with acridity and toxicity, especially when such commodities are consumed in high quantities[21], hence the presence and amount of oxalates present in a food material are important in the assessment of its nutritional status. However, the risk of calcium deficiency due to the consumption of oxalate-rich plants has been reported to be very minor because humans are able to efficiently use very low amounts of calcium in food[22,23]. The low levels of oxalates obtained in this study, coupled with the fact that many tropical African diets are commonly well-cooked before consumption suggests that it may not be a critical factor since oxalates are heat labile[23].

The total phytate contents of *G. latifolium*, *V. amygdalina* and *G. kola* are 263.25, 169.23 and 714.54 mg 100 mg, respectively. These values are lower than those reported for seed kernels of Gila beans (1370 mg 100 g) and other commonly consumed legumes like faba bean and soybean[24]. Tannin contents of *G. latifolium*, *V. amygdalina* and *G. kola* (1.30, 5.68 and 4.30 mgTA g, respectively) are low compared with values reported for sundried and oven dried cassava leaves, 47 and 59 mgTA g, respectively[25]. These values are however comparable with 2-4 mgTA g reported for *G. africanum*[18]. Phytates and tannins bind with proteins and minerals to form insoluble complexes, thereby reducing protein and mineral bioavailability[26]. Their control is therefore desirable. Since most phenolics and tannins are water-soluble, they can be significantly reduced by indigenous processing techniques such as soaking and/or cooking. Although phenolics and tannins are generally viewed as antinutritional factors due to their abilities to inhibit the activities of digestive enzymes, they are currently all considered (at low concentrations) to be health-promoting factors[24]. Phytate may also have possible health benefits in human nutrition, such as in the management of diabetes and obesity[24]. So its complete elimination during processing may actually be undesirable. The absence of toxic metals, namely: mercury and lead in the plant materials suggests that their consumption may not lead to mercury or lead poisoning.

Table 3 shows the proximate composition, energy content, minerals and antinutritional factors present in the experimental diets. The experimental diets had significantly higher protein, fat, ash and crude fibre contents: 15.44-18.30, 7.93-9.22, 7.65-9.55 and 8.99-10.80%, respectively when compared with the control. Protein is highest in Diet 2 (containing *G. latifolium*). This observation suggests that the plant materials used are capable of enhancing protein contents of conventional diets in view of the high crude protein contents of *G. latifolium* and *V. amygdalina* Table 2. Thus, it is expected that the experimental diets may be capable of meeting the protein need of consumers. However, before

Table 3: Proximate (% DM), energy (Kcal g), mineral (ppm) and antinutritional composition of experimental diets

Parameters	Diet 1	Diet 2	Diet 3	Control
Proximate composition				
Protein	15.44b	18.30c	18.06c	13.93a
Fat	9.22c	7.93a	8.67b	7.29a
Fibre	7.65b	9.55d	8.39c	6.81a
Ash	8.99ab	10.80c	9.65b	8.73a
Dry matter	88.91	87.97	88.17	88.10
Nitrogen free extractives	63.27a	60.52a	60.53a	67.62b
Energy (Kcal g)	379.57	358.28	371.15	374.28
Mineral composition (ppm)				
Na	557.06a	587.34c	577.12b	599.86c
K	711.53c	637.16b	625.20b	511.41a
Ca	584.23b	550.01a	535.98a	538.65a
Mg	637.35b	733.84c	701.16c	504.49a
P	4.46	5.84	6.48	ND[1]
Fe	44.66d	34.38c	24.77b	3.01a
Cu	3.04b	2.91b	6.95c	2.05a
Mn	44.60b	51.82c	39.72a	40.72ab
Zn	35.11a	65.24b	34.43a	43.21c
Pb	ND	ND	ND	ND
Hg	ND	ND	ND	ND
Antinutritional factors				
Phytic acid (mg 100 g)	3.37b	2.21a	5.17c	ND
Oxalate (g 100 g)	2.07b	1.89a	2.52c	ND
Tannin (mg 100 g)	3.64b	3.41b	2.06a	ND

Means within the same column followed by different lowercase letters are significantly different ($p<0.05$), Means are replicates of three readings; NFE, nitrogen free extract; MC moisture content; ND[1]: Not determined; ND: Not detected

a conclusion can be reached on its suitability or otherwise, its effect on biological systems has to be investigated.

Mineral analysis show the presence of high amounts of a wide range of beneficial elements, notably: potassium 511.41-711.53 ppm, magnesium 538.65-584.23 ppm, calcium 535.98-584.23 ppm and sodium 557.06-599.86 ppm. Zinc, copper and phosphorus are the lowest in the diets. The presence of these beneficial minerals in the experimental diets suggests that they may be capable of supporting healthy growth. Lead and mercury were not detected in all the samples.

Phytic acid and oxalates are significantly higher in Diet 3 5.17 mg 100g and 2.52 g 100 g, respectively while tannin is highest in Diet 1 3.64 g 100 g. Tannins, phytates and oxalates are generally regarded as antinutritional factors when present in foods. The antinutritional activity of tannins, oxalates and phytin lies in their ability to form complexes with metals (like Ca, Zn, Mg and Fe) and proteins thereby reducing protein and mineral bioavailability[26,27]. The beneficial effects associated with polyphenolic compounds suggest that their complete elimination may not be desirable[24].

Growth performance and feeding pattern: The observed feeding and growth pattern in rats fed the experimental diets are shown in Table 4. The feed consumption rates of the rats on the experimental diets were low compared to

Table 4: Growth performance and nutrient utilisation of rats fed on experimental diets

Parameters	Diet 1	Diet 2	Diet 3	Control
Initial weight (g)	50.03±0.02	50.95±0.03	52.09±0.03	53.04±0.03
Final weight (g)	57.09±0.03	44.91±0.03	48.75±0.05	57.99±0.01
Mean weight gain (g)	7.06±0.03	(6.04)	(3.34±0.04)	4.95±0.15
Mean weight gain (%)	14.12	(11.86)	(6.42)	9.34
Mean daily weight gain (mg)	340±0.11	(290±0.1)	(160±0.05)	240±0.1
Mean daily Feed Intake (g)	8.51±0.12	6.91±0.10	7.53±0.10	10.8±0.13
Protein efficiency ratio	0.25	(0.33)	(0.13)	0.25
Feed conversion ratio	0.04	(0.04)	(0.02)	0.02
Specific growth Rate (%day^{-1})	13.20	(12.61)	(6.23)	8.92

Values are expressed in mean±SEM of three test animals per group; Values in parenthesis are negative

the control diet. This may be due to variation in the taste of the test diet from that which the test animals are familiar with. This may, in turn, impact on the growth characteristics of the test animals. Only Diet 1 and the control diet gave positive weight 14.12 and 9.34%, respectively gain after three weeks of feeding. This observation may be due to either the low mean daily feed intake or the presence of antinutritional factors (especially phytic acid and tannin) or both. The astringent taste associated with the consumption of *V. amygdalina* affects its intake as food/feed[28]. However, boiling prior to use has been shown to decrease the content of secondary plant compounds, making it more palatable[28,29]. Similar report on the use of *V. amygdalina* at 30% (w/w) level in broiler feed did not affect their food intake, body weight gain and feed efficiency. These parameters decreased in the albino rats used in this study. Physiological differences in the test animals used in previous work[28], (broilers) may account for this observation. Diets 2 and 3 had the highest tannin and phytic acid contents respectively. Phytic acid and tannin binds with divalent mineral and proteins, thus reducing mineral and protein bioavailability[26,30]. This may, in turn, lead to low growth parameters, namely: mean weight gained, protein efficiency ratio, feed conversion ratio and the specific growth rate in the test animals.

The feeding pattern of the rats on the experimental diets can be described using regression equations shown in Table 5. These equations provide a means for assessing feeding pattern over the entire feeding period. The closer the coefficient of determination (R^2) is to 1, the stronger the proposed equation will be for describing the observed relationship[31]. The low R^2 values obtained for Diets 1 and the control (0.03 and 0.01, respectively) suggests that the equations obtained for Diet 1 and the control diet may not adequately describe observed feeding pattern on the diets. The R^2 values obtained for Diets 2 and 3 shows that the equation has 59.7 and 58.1%

Table 5: Regression equations showing feed consumption pattern and feeding period

Parameter	Regression equation	R^2	R
Diet 1	$Y = 0.0010x^2-0.0112x+8.473$	0.030	0.155
Diet 2	$Y = -0.0094x^2+0.3054x+5.035$	0.597	0.686
Diet 3	$Y = - 0.007x^2+0.2377x+6.02$	0.581	0.692
Control	$Y = 0.0014x^2-0.0217x+10.83$	0.007	0.062

Y= quantity of diet consumed; x = feeding period (days)

Table 6: Haematological profile, organ weight and colour of rats fed on experimental diets

Parameters	Diet 1	Diet 2	Diet 3	Control
Haematological profile				
ESR	1.29b±0.15	2.77c±0.15	1.80b±0.44	1.33ab±0.17
PCV	28.00ab±1.15	25.00a±0.58	33.00bc±2.89	29.67b±0.33
RBC	151.33b±0.88	121.67a±7.26	265.33b±24.61	283.33b±9.67
WBC	94.33c±1.20	47.00a±2.08	85.67bcd±18.48	107.67c±6.67
HB	11.05cd±0.08	8.51a±0.16	11.23d±1.02	10.57c±0.07
MCHC	39.43b±1.91	34.45a±0.49	34.28a±0.11	35.62a±0.35
MCH ($x10^{-3}$)	7.30c±0.08	7.52c±0.27	6.05bc±1.01	3.73ab±0.12
MCV ($x10^{-3}$)	1.85c±0.07	2.18c±0.10	1.77c±0.31	1.05ab±0.05
Relative organ weight (g Kg^{-1} body weight)				
Heart	0.63b±0.01	0.54b±0.01	0.50ab±0.01	0.40a±0.05
Liver	2.84a±0.01	2.77a±0.12	2.73a±0.04	2.97a±0.04
Kidney	0.55b±0.01	0.54b±0.01	0.49ab±0.02	0.41a±0.03
Organ colour				
Heart	Normal red	Normal red	Normal red	Normal red
Liver	Very light brown	Reddish brown	Chocolate brown	Normal red
Kidney	Light brown	Dark brown	Dark brown	Normal red

Values are expressed in mean±SEM of three test animals per group, Means within the row under each parameter followed by different letters are significantly different ($p<0.05$), ESR: erythrocyte sedimentation rate; PCV: packed cell volume; RBC: red blood count; WBC: white blood count; HB: haemoglobin; MCHC: Mean corpuscular haemoglobin count; MCH: Mean corpuscular haemoglobin; MCV: Mean corpuscular volume

chance, respectively of accurately describing observed feeding pattern. The Pearson's correlation coefficient (R) showed that feeding time and quantity consumed are positively correlated in Diets 2 and 3 while Diet 1 and the control diet show little or no relationship. This suggests that quantity of diet consumed and feeding time are mutually exclusive in rats fed on the control diet and Diet 1[31]. Thus, other factors other than the presence or absence of plant materials may have been responsible for the observed feeding pattern on Diet 1 and the control diet with time.

Haematological properties, organ weight and colour: Table 6 shows the haematological profile, weights and colour of some internal organs of rats fed on the experimental diets. Results show that rats fed on Diet 3 (containing *V. amygdalina*) had the highest packed cell volume (PCV), red blood count (RBC), white blood count (WBC) and haemoglobin (Hb) contents. The lowest value for these parameters was obtained for rats fed on Diet 1 (containing *G. kola*). However, they (Diet 1 group) had the highest mean corpuscular haemoglobin (MCH) and mean corpuscular volume (MCV) while Diets 2 and 3 had lower values. Rats fed the control diet had the lowest heart weight. Rats fed on Diets 2 and 3 had comparable heart weights, while those fed Diet 1 had the highest heart weight. The liver weight is comparable in all the treatment groups. Rats fed on Diets 1 to 3 had higher kidney weight than the control while those fed on Diets 3 had comparable values with the control group. The heart, liver and kidney colour of rats fed with the control diet were normal red. The heart colour was normal red in all the test groups. However, the liver had colour ranging from very light brown (Diet 1), reddish brown (Diets 2) to chocolate brown (Diet 3). Similar observations hold for the kidney colour where only rats fed the control diet had the normal red colour with others having colours ranging from light brown (rats fed Diet 1) to dark brown (rats fed Diets 2 and 3). Hence their blood may not be able to carry as much oxygen as it should. There is a 19.49 and 57.06% fall in the blood haemoglobin and red blood cell values respectively of rats fed on Diet 2. These observations suggests that the red blood cells are either too small or too few in number. As a result, the heart and lungs would be working harder than it normally should to make up for the lack of oxygen delivered to the tissues by the blood. Rats fed Diet 3 have comparable red blood cell values with the control. Rats fed on Diet 2 had the lowest packed cell volume, red blood count, white blood cells and haemoglobin contents. The only exception is the erythrocyte sedimentation rate where rats fed Diet 2 had the highest value. The red blood cells and white blood cells of rats fed Diets 1 to 3 were lower than that obtained for the control group. In terms of composition, Diet 1 had higher tannin while Diet 3 had higher phytic acid contents Table 3. Thus, the low haematological values obtained for rats fed Diet 2 ma not be directly attributed to the presence of antinutritional factors determined. Other bioactive components in *G. latifolium, V. amygdalina* and *G. kola* like sesquiterpene lactones and vernonisides[32] may have been responsible.

Rats fed Diets 1-3 had significantly higher mean corpuscular volumes (MCV) values compared with the control group. MCV is the average volume of the individual red blood cells. The high MCV values obtained for rats fed Diets 1-3 suggests that their blood cells are macrocytic while the control group is normocytic[12]. Macrocytic red blood cells are often associated with pernicious anaemias and folic acid deficiencies[33]. This deficiency may be induced by bioactive components of plants used in the formulation[34,35]. There is a marked variation in the mean corpuscular haemoglobin count (MCHC) obtained for the rats fed the test diets. MCHC is a measure of the amount of haemoglobin in an average cell. The MCHC value of those fed on Diets 2 and 3 are

comparable with the control group while rats fed Diet 1 had significantly higher values. Although rats fed on Diets 2 and 3 have comparable MCHC values, haemoglobin content is significantly lower in Diet 2 group and higher in Diet 3 group. This suggests that other factors, not immediately determinable, were responsible for the variation observed in the haemoglobin. The amount of haemoglobin determines how much oxygen the RBCs would be capable of carrying. The reddish brown and dark brown colours of the liver and kidney of rats fed Diets 2 and 3 suggests the absence of adequate amounts of oxygen. Although rats fed other diets had higher haemoglobin contents, their livers and kidneys were still brownish. Thus other factors, like the antioxidant properties of bioactive components present in the plants, aside from low haemoglobin content may have been responsible for the observations. Although all compounds present in the plants used in this work has not been fully determined chemically and structurally at this time, many report indicate the presence of saponins, sesquiterpene lactones, 3-methyl flavones, flavonoids and vernoniosides which have been shown to possess antioxidant properties in the plant materials used in this work [32,34-36]. These groups of compounds often lead to inferior haematological parameters and may account for the observed haematological profile. The brown colour observed for the liver and kidney suggests that the organs may have been competing with some oxygen scavenging bioactive components probably present in the plant materials. These observations presuppose the need for full chemical and structural elucidation of active principles present in the plant materials. This would aid a better understanding of biochemical processes taking place and their impact on overall health status.

These results suggest that internal organs, namely: liver and kidney are significantly affected by the ingestion of the plant materials over a long period. The pronounced effect of long term consumption on the kidney and liver may not be unconnected with their function as detoxifiers in biological systems.

CONCLUSION

This study has shown that some tropical underutilised plant materials have potentials for use in food fortification schemes. The limitations observed in the growth response of experimental animals to plant inclusion in diets can be addressed via further processing to reduce antinutritional factors. The isolation and characterisation of active principles in the plant materials would provide greater understanding of their role on in biological systems.

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