PHYSICAL, CHEMICAL ANALYSES AND POSSIBLE EFFECT OF *ENTEROLOBIUM CYCLOCARPUM* SEED MEAL ON GROWTH, HAEMATOLOGICAL AND HISTOLOGICAL PROPERTIES OF WISTAR RAT

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Abstract
The physical, chemical and nutritional analysis of Enterolobium cyclocarpum seeds were investigated for nutritional purposes and the effect on the growth performance and blood parameters on wistar rat. The experiment involved 40 wistar rats divided into four groups of 10 rats each. The groups were labeled A (control), B, C and D (experimental groups). The rats were fed 8 weeks. The protein content in the prepared feed increased gradually within the groups due to the high value of crude protein in the Enterolobium cyclocarpum seeds flour. Ca and Mg decreased gradually within the experimental groups. The growth parameters of the rats decreased considerably in the four groups. Rats in group B had the highest weight gain. A gradual decrease in the weight of the rats was observed with C and D. A slight decrease was obtained in MCV, PVC and WBC while no significant difference was revealed in other haematological and biochemical parameters of the blood. Histological results obtained showed no observable lesion in the section of the heart while sections of the kidney and liver were significantly different in both 20 % and 30 % groups. The research revealed that Enterolobium cyclocarpum seed flour was not toxic at 10 % (w/w) and might be suitably used to fortify conventional seed flour less in protein for better application if properly processed at 10 % (w/w).

Keywords: *Enterolobium cyclocarpum*, compounded diets, haematology and nutritional evaluation

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INTRODUCTION

Food crops is globally known in nutrition as one of the important energy and proteins source for a large number of population in the world, especially in the developing countries where protein from animal and other sources is not sufficient to satisfy the protein intake of the growing populace (Ajayi et al., 2016). The high risk of cardio-vascular diseases and some types of cancer that emanated from the high intake of meat has necessitated contemporary research on the food properties and prospective application of protein sourced from less utilized and disregarded food crops (Enujugha et al., 2000). Forest tree species and many parts savanna have many applications in Africa such as food, wood, traditional and orthodox medicinal. Despite this usage, the lack of proper chemical knowledge and information on compositional constituents of these species limit their rational application in the sustainable progress (Cook et al., 2000).

E. cyclocarpum from *Leguminosae* family is known as parota in western Mexico. It is recognized in tropical America as one of the most significant leguminous species with possible high nutrient such as protein (Castro et al., 2006). *E. cyclocarpum* leave and pods was reported to provide high-quality forage for Cattle. The seed are locally utilized for human consumption (Castro et al., 2006). It is a very tall tree with up to 30 meters height and a trunk of 2 meters in diameter. Dark bright to void seeds with 10 -15 seeds in ear-shaped pods. The tree produces 86 kg of pods per tree. *E. cyclocarpum* seeds have been reported to have 15.6 % crude protein (Andrade et al., 2008) while 26.3 % of crude protein, 2.8 % of ether extracts and 63.1 % of nitrogen free extracts (Serratos et al., 2008). This plant with high nutritional value can now be found in other tropical regions in the world and has equally been subjected to quite number studies.

E. cyclocarpum, distributed all over the tropics grows approximately in large area of about 29 million hectares globally (FAO, 2010). *E. cyclocarpum* trees are found along the Gulf of Mexico coastal area and along streams and rivers in the Pacific coast (Pennington and Sarukhan, 2005). *E. cyclocarpum* seeds could be used for substituting important conventional forage. They were found to augment the ruminant food intake and reduced the feed stress of the dry season and thereby enhance their performance and productivity (Idowu et al., 2013). The leaf contains more saponins than other parts, leading to its low in vitro gas production parameters therefore suggesting its less acceptability by livestock. The high net gas production, metabolisable energy, organic matter digestibility and short chain fatty acids suggested the application and utilization of *E. cyclocarpum* seeds and the whole fruit as supplement (Babayemi. O. J. 2006). The aim of the present study therefore was to investigate the chemical composition and the effect of feeding graded levels of *E. cyclocarpum* seed flour on feed intake, weight, growth, haematological and histological properties of wistar rat with the view of incorporating it in human food chain.

MATERIALS AND METHODS

Physical characteristics of *E. cyclocarpum* seed

E. cyclocarpum seeds were examined for physical characteristics according to the method described by Fermia et al. (1995) and Ajayi et al. (2006). The number of seeds in 100g of seeds, the length (L), the width (W), thickness (T), loose and pack bulk density were measured to an accuracy of 0.01 mm. These data were then used to calculate the seed geometric diameter (Dg), arithmetic diameter (De), sphericity (Ø) using the following mathematical relationships as reported by Rodriguez-Miranda et al. (2010).

$De = (LWT)^{1/3}$; $Ø = (LWT)^{1/3} / L$; $Sa = \pi De^2$

where π is *3.14.*

Preparation of *E. cyclocarpum* seed flour

E. cyclocarpum seeds were obtained at the Department of Veterinary Medicine, University of Ibadan and were authenticated at the Herbarium Unit of Botany Department, University of Ibadan, Nigeria. *E. cyclocarpum* seeds were cleaned by removing extraneous particles such as stalks, broken seeds, immature seeds and other unwanted materials. They were air dried and pulverized to fine powder. The *E. cyclocarpum* seed flour obtained was stored in a properly labelled polythene bag as ECSF in readiness for subsequent experiments.

Feed formulation and preparation

Four different feeds labelled A, B, C and D according to the rats' groups were used in this experiment. These feeds were formulated based on earlier reported methodology described by Ajayi et al. (2013). The fundamental ingredients considered in compounding feed A which served as control feed were: maize (40 %), soybeans (18.20 %), bone (3.3 %), salt (0.80 %), groundnut cake (14.2 %), palm kernel cake (7.10 %), wheat (7.10 %), corn bran (7.10 %) and oyster shell (2.20 %). Feed A prepared was thoroughly mixed with different concentrations of ECSF to obtain three other experimental feeds labelled B, C and D. Feed B contained 90% of A and 10% of ECSF seed flour. Feed C contained 80% of A and 20 % of ECSF while feed D contained 70 % of A and 30 % of ECSF.

Experimental animals

Forty matured wistar rats (eight weeks old) with 60-80 g weight were used for the effect of *E. cyclocarpum* seed meal on the growth, haematological and histological properties of albino rat. The rats were purchased from Anatomy Department, University of Ibadan, Nigeria, divided into four groups (A, B, C and D) with 10 rats each and housed in the experimental animal house of the Anatomy Department, University of Ibadan. The animals were acclimatized for seven to get use of the environment before starting the experiment. They were fed with four categories of diets and tap water for eight weeks, maintained under normal room condition and 12 h light/dark cycle. The rats were sacrificed in accordance with standard laboratory animal care and ethical guideline of the University of Ibadan. Their weights were recorded at the starting point as zero (0) day and subsequent at a weekly interval. The weight of the feed consumed was also documented daily. Rats in groups B, C and D were fed with experimental feed (feed prepared with 10 %, 20 % and 30 %

of ECSF) while those in group A were fed with control feed (feed prepare without ECSF).

Nutritional assessment and Organs weight

The growth performance parameters of the experimental rats evaluated include Mean weight gain (MWG), Specific growth rate (SGR), Feed conversion ratio (FCR), feed intake (FI) and Average daily growth (ADG) (Ajayi et al., 2016). The liver, kidneys, lung and brain were quickly removed after the animals were sacrificed, cleaned with saline and weighed. The relative organ weight of each animal was then calculated relating the absolute organ weight and body weight of the animal on the day of sacrifice. Relative weight of organs = *(Weight of organ / Rat's body weight) X 100* (Khairy et al., 2014).

Weight gain:

$$(MWG) = Initial\ weight - Final\ weight$$

Average daily growth:

$$(ADG) = \frac{Initial\ weight - final\ weight}{experimental\ period\ in\ days}$$

Specific growth rate:

$$(SGR) = \frac{(ln\ W_2 - ln\ W_1)\ x\ 100}{experimental\ period}$$

Where: Ln = Natural log,
W_1 = *initial mean weight,*
W_2 = *final mean weight,*

Feed conversion ratio

$$(FCR) = \frac{total\ weight\ of\ the\ food\ given}{total\ weight\ gained\ by\ the\ rat}$$

Feed efficiency ratio

$$(FER) = \frac{Weight\ gain}{Total\ feed\ intake.}$$

Haematological examination

Blood (3 ml) were collected by cardiac puncture into EDTA bottles and stored at 10 º C. The packed cell volume (PCV), haemaglobin (Hb) concentration, red blood cell (RBC) and white blood cell (WBC) counts were determined using standard techniques as described by Dacie and Lewis, (2001). Other parameter such as MCV (mean corpuscular volume) and MCHC (mean corpuscular haemoglobine concentration) were equally determined (Dacie and Lewis, 2001).

Blood biochemical analyses

Blood sample collected in a test tube was remained at normal temperature for 30 min to clot. Theclotted blood sample was centrifuged at 3 000 r/min for 10 min with a table centrifuge to facilitate absolute partition of the serum from the clotted blood. A clear serum supernatant obtained was cautiously aspirated with syringe and needle and kept for the biochemical analysis. Blood biochemical parameters such as blood glucose level, total protein, albumin, AST (aspartate aminotransferase), ALP (serum alkaline phosphatase), ALT (alanine aminotransferase), creatinine and urea were determined (Raphael et al., 2014).

Histological studies

Little sections of the excised tissues were weighed and kept in 10 % formalin before being fixed and dehydrated in a succession of xylene concentrations. The thin sections were embedded in wax, sectioned at 5 µl, stained with haemotoxylin and eosin (H and E) dyes before been examined under a light microscope for histological alterations, Jain (1986).

Statistical analysis

Results obtained in triplicate were analyzed and expressed as mean values ± standard deviation. Statistical analysis of other documented data was carried out with SPSS software (version 2010). Significant differences within individual means were established with one way analysis of variance (ANOVA) and Duncan Multiple Range Test. Probability ($p \leq 0.05$) was considered significant.

RESULTS AND DISCUSSION

Physical characteristics

Table 1 represents the physical properties of *E. cyclocarpum* seed and seed flour. The average weight of hundred seeds was 95.02 ± 9.02 g while 105.05 ± 1.70 seeds were in 100 g. The average weight of each seed was determined to be 0.95 ± 0.17. The percentage of the seed kernel was found to be 64.20 ± 8.53 %. The length and width of a seed was measured to be 1.73 ± 0.25cm and 1.13 ± 0.02cm.

Table 1: Physical properties of *E. cyclocarpum* seed

Parameters	
Length of a seed (mm)	1.73±0.25
Breath of a seed (mm)	1.13±0.02
Thickness of a seed (mm)	0.41±0.01
Number of seeds in 100g	105.05±1.70
Weight of 100 seeds (g)	95.02±9.02
Weight of a seed (g)	0.95±0.17
Weight of the kernel from a seed (g)	0.61±0.09
Percentage of the kernel (%)	64.20±8.53
Loose bulk density of ECSF	0.46±0.04
Pack bulk density of ECSF	0.64±0.05
Arithmetic diameter (De) (mm)	1.130±0.02
Sphericity (Ø)	0.65±0.13
Surface area (Sa) mm^2	4.01±0.08

Values are expressed as mean ± SD

The loose bulk density and pact bulk density of the seed flour were determined to be 0.46 ± 0.04 and 0.64 ± 0.05. The bulk density of flour is a function of its mass and volume. It depends on the size of the particles and the flour's initial moisture level. The flour's high bulk density makes it more appropriate for packaging, shipping, and usage in recipes. Low bulk density, on the other hand, is thought to be advantageous in the formulation of supplementary foods (Akpata and Akubor, 1999). The values obtained in this study are lower than those reported for undecorticated groundnut seed by Mustapha et al. (2015) but it compared very well with the values obtained for some commonly used cereal and legume flours reported by Haq Nawaz et al. (2015). The values obtained suggest the suitability of the seed flour in food preparation and products. The arithmetic diameter of a seed is 0.65 ± 0.13 mm while the average surface area was 4.01 ± 0.08 mm^2.

The proximate and mineral compositions of the diets prepared with *E. cyclocarpum* seed flour at different concentrations

The proximate and mineral analyses of the feeds prepared were presented on table 2. All diets were made with the same ingredients with the exception of the inclusion of 0 %, 10 %, 20 % and 30 % of ECSF seed flour to diets A - D respectively. Moisture contents of all the diets were significantly different ($P < 0.05$). It ranged from 10.83 ± 0.02 % in the control group A and reduces gradually to 8.39 ± 0.01 % in the experimental group D (group with higher concentration of ECSF). The dry matter obtained increases also from 89.17 ± 0.02 % to 91.60 ± 0.15 respectively in the control and group D. The presence of moisture in seeds aids the microbial growth and control the shelf life of the seed. The higher the moisture content, the lower the shelf life and the higher the microbial growth. This result is comparable to 90.97 % obtained on G. senegalensis (Muhammad and Garba, 2008) and (90.20-90.67) earlier reported for toasted E. cyclocarpum fed to West African dwarf sheep, (Idowu et al., 2013). The protein content observed in the control diet is 22.67 ± 0.36 % while a high value of 24.57 ± 0.45 %, 25.56 ± 0.01 % and 25.76 ± 0.58 % were obtained in the experimental groups B, C and D. The crude fat value and crude fibre of 4.80 ± 0.01 % and 8.40 ± 0.03; 4.89 ± 0.01 and 6.40 ± 0.01 % were recorded in the control group A and experimental group B. These values later decreased gradually in the experimental groups (C and D) as the percentage inclusion of E. cyclocarpum seed in them increased. The crude fibre across the diets was low and this support the report of FAO (e.g. FAO, 1983) which stipulated that concentrates used as feeds with high density of nutrients have usually low crude fibre content and high digestible nutrients. The carbohydrate value was higher in the experimental diets than in the control. The metabolic energies calculated respectively in the groups were 1328.11 ± 0.69, 1374.40 ± 0.72, 1393.88 ± 0.68 and 1399.48 ± 6.44. The high energy values in the experimental diet are due to their high carbohydrate and protein content. This positive variation obtained in the proximate parameters in the experimental diet when compared to the control diet might be due to the presence of ECSF.

The concentration of calcium in the control diet was 887.50 ± 2.50 mg/kg which increased to 1183.66 ± 92.52 mg/kg in the diet compounded with 10 % of ECSF. This value was found to decrease gradually to 860.08 ± 13.87 mg/kg in 30 % of ECSF. Sodium concentration was found to be 366.33 ± 0.14 mg/kg in the diet prepared without the seed flour; it increased to 765.83 ± 18.55 mg/kg in the diet with 10 % of ECSF and later decreased gradually to 725.83 ± 25.04 in the diets with 30 % of ECSF. The concentrations of magnesium and zinc were also found to gradually increase as the level of ECSF increased. It can be therefore observed that the concentrations of calcium and sodium reduced while those of Magnesium and Zinc increased in diets prepared with 20 % and 30 % inclusion levels of ECSF when compared to that of 10 %.

Table 2: Result of proximate (%), mineral analyses (kg/L) and proportion of Energy (%) of feed prepared with graded level of *E. cyclocarpum* seed flour.

Parameters	**Group A**	**Group B**	**Group C**	**Group D**
Proximate	**Analysis**			
Dry matter	89.17±0.02^{a}	90.62±0.03^{b}	90.95±0.17^{c}	91.60±0.15^{d}
Moisture	10.83±0.02^{c}	9.37±0.03^{b}	9.05±0.07^{a}	8.39±0.01^{a}
Protein	22.67±0.36^{a}	24.57±0.45^{b}	25.56±0.01b^{c}	25.76±0.58^{c}
Fat	4.80±0.01^{a}	4.89±0.01^{b}	4.72±0.07^{d}	4.66±0.05^{c}
Crude fibre	8.40±0.03^{b}	6.40±0.01^{a}	5.33±0.04^{a}	5.26±0.03^{a}
Ash	8.30±0.02^{b}	9.13±0.00^{a}	9.17±0.02^{a}	9.15±0.04^{a}
Carbohydrate	44.82±0.14^{d}	45.62±0.45^{c}	46.15±0.15^{b}	46.42±0.31^{a}
Energy	1328.11±0.69^{c}	1374.40±0.72^{b}	1393.88±0.68^{a}	1399.48±6.44^{a}
Proportion of	**Energy**	**based on:**		
Protein	28.81±0.57^{b}	30.40±0.54^{a}	31.17±0.08^{a}	31.49±0.84^{a}
Fat	13.18±0.30^{a}	13.18±0.03^{a}	12.53±0.20^{b}	12.39±0.17^{b}
Carbohydrate	56.63±1.47^{a}	56.40±0.16^{a}	56.29±0.16^{a}	56.00±0.32^{a}
Mineral	**Analysis**			
Ca	887.50±2.50^{c}	1183.66±92.52^{a}	925.00±25.00^{b}	860.08±13.87^{c}
Mg	64.25±0.25^{d}	235.75±0.25^{a}	203.83±0.14^{a}	178.16±0.14^{c}
K	834.33±5.05^{a}	825.03±25.00^{a}	857.50±5.00^{a}	885.00±6.61^{a}
Na	366.33±0.14^{b}	765.83±18.55^{a}	752.53±24.5^{a}	725.83±25.04^{a}
Mn	3.65±0.10^{b}	3.85±0.05^{ab}	4.11±0.11^{a}	4.23±0.02^{a}
Fe	22.41±0.40^{b}	27.38±0.12^{a}	26.00±0.05^{a}	25.28±0.33^{a}
Cu	0.75±0.02^{b}	1.41±0.01^{a}	0.88±0.01^{b}	0.78±0.03^{b}
Zn	6.92±0.07^{c}	7.18±0.14^{a}	8.90±0.05^{ab}	9.06±0.16^{a}
Na/K	2.26	1.07	1.14	1.20

Values are expressed as mean ± SD.
Values on the same row having the similar letter as superscripts are not significantly different at ($P < 0.05$*).*
Group A: Standard feed; Group B: 10 % (w/v); Group C: 20 % (w/v); Group D: 30 % (w/v) of Enterolobium cyclocarpum seed flour

Nutritional assessment and Organs weight of rat fed with ECSF

Table 3 presents the effect of ECSF on the body weight gain, feed-intake, growth rate, feed utilization, survival rate and organ weight of rats fed with varying percentage of the seed flour. The rats were measured at the beginning of the experiment with the initial weight of 78.63 ± 6.81 g, 72.48 ± 1.88 g, 72.48 ± 1.88 g and 72.76 ± 1.66 g respectively for the control diet A and experimental diets (B, C, D). The final weights recorded in gram after the experimental period was respectively 128.26 ± 17.08 %, 114.93 ± 12.24 %, 118.36 ± 5.04 % and 109.95 ± 6.00 %. There was a significant increase in the weight of the rats in all the treatments within the experimental period as shown in figure 1. The percentage weight gains of 62.78 ± 12.10 %, 58.53 ± 16.09 %, 56.35 ± 12.49 %, and 51.11 ± 7.23 % were recorded. Rats fed with 10 % ECSF seed flour had the highest percentage weight gain followed with 20 % among the experimental diet and slightly lower than 62.78 ± 12.10 in the control. The same trend of result was obtained with the protein efficiency ratio, daily growth rate and feed intake. There was no significant difference at $P \leq 0.05$ in feed conversion rate and survival rate. No mortality was recorded in all the experimental groups. The gradual reduction in the weight gain (as shown in figure 2), in the protein efficiency ratio and the daily growth rate observed as the level of ECSF incorporated in the rats diet increased showed the slight negative effect of ECSF on the growth performance of the rats. Similar results were reported by Iyayi et al. (2006) where soybean meals were replaced by *E. cyclocarpum* and M. prurient seed flours to broilers and Idowu et al. (2013), were graded level of toasted ECSF were fed to West African dwarf sheep.

Table 3: Effect of E. cyclocarpum seed flour on the body weight, feed-intake Growth, feed utilization, % survival rate and rats' organ weights.

Parameter	Group A	Group B	Group C	Group D
Nutritional	**Properties**			
Initial Weight	78.63±6.81^{a}	72.48±1.88^{b}	76.07±3.50^{ab}	72.76±1.66^{b}
Final Weight	128.26±17.08^{a}	114.93±12.24^{ab}	118.36±5.04^{ab}	109.95±6.00^{b}
Weight gain	49.63±11.73^{a}	42.50±11.79^{a}	42.45±7.89^{a}	37.26±5.33^{b}
% Weight gain	62.78±12.10^{a}	58.53±16.09^{a}	56.35±12.49^{b}	51.11±7.23^{c}
Mean Weight gain	49.63±11.73^{a}	42.50±11.79^{a}	42.45±7.89^{a}	37.26±5.33^{b}
Feed Intake (FI)g	10.65±1.71^{a}	10.95±2.08^{a}	10.65±1.48^{a}	10.58±1.36^{a}
Feed conversion ratio (FCR)	9.45±2.29^{b}	11.65±3.63^{a}	11.60±1.74^{a}	11.93±1.74^{a}
Specific growth rate (SGR)	1.15±0.17^{a}	1.08±0.24^{a}	1.05±0.19^{a}	0.98±0.11^{a}
Daily growth rate	1.18±0.28^{a}	1.01±0.28^{b}	1.01±0.18^{b}	0.88±0.12^{c}
Protein efficiency ratio (PER)	0.46±0.10^{a}	0.37±0.10^{b}	0.36±0.60^{b}	0.32±0.40^{b}
Survival	100.00	100.00	100.00	100.00
Mortality	0/10	0/10	0/10	0/10
Tissue/organ weight				
Kidney	1.00±0.00^{a}	0.90±0.00^{b}	0.73±0.05^{c}	0.70±0.00^{c}
Brain	1.56±0.11^{a}	1.46±0.11^{b}	1.40±0.00^{ab}	1.33±0.05^{c}
Liver	4.26±0.05^{b}	4.73±0.11^{a}	4.76±0.11^{a}	4.66±0.11^{a}
Spleen	0.83±0.11^{a}	0.60±0.00^{b}	0.50±0.00^{b}	0.60±0.00^{b}
Lungs	1.33±0.15^{a}	1.00±0.00^{b}	1.26±0.05^{a}	1.10±0.00^{b}
Heart	0.43±0.05^{a}	0.43±0.05^{a}	0.40±0.00^{a}	0.40±0.00^{a}

Values are expressed as mean ± SD
Values on the same row having the similar letter as superscripts are not significantly different ($P < 0.05$*).*

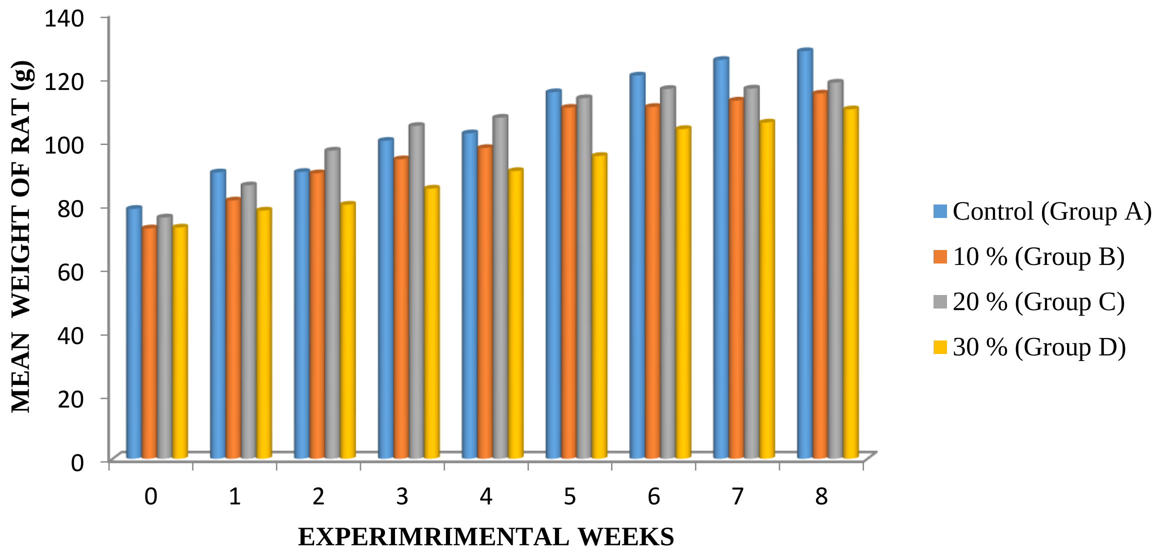


Figure 1: Mean weekly weight gain of rat fed on graded level of E. cyclocarpum seed flour

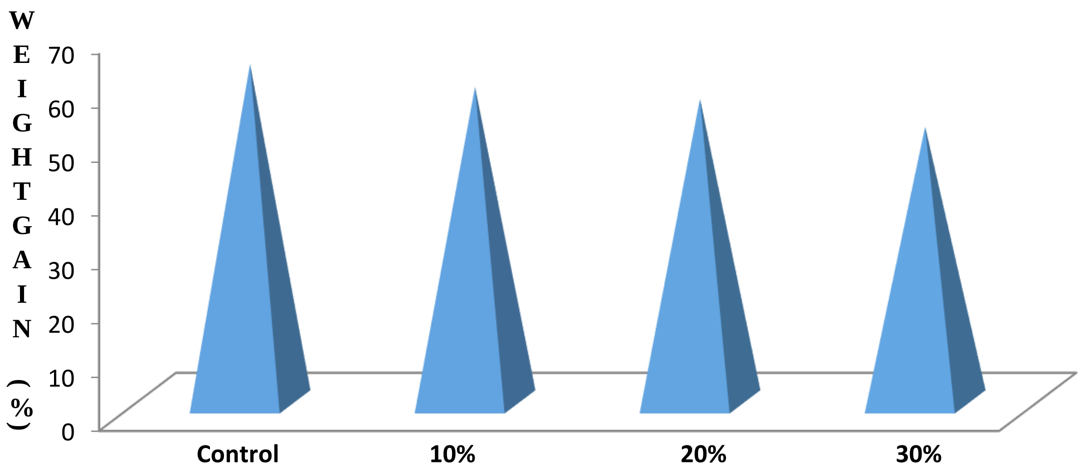


Figure 2: Percentage weight gain of rat fed on graded level of *E. cyclocarpum* seed flour

The organs weight as shown in Table 3 reveal a gradual reduction in the kidney weight across the groups with an increase in the liver weight with the highest in 10 % and 20 % experimental groups. The spleen weight was lowest in 20 % experimental group. The relative organ weight is fundamental in physiological and pathological analysis to establish the rate of exposure of the organ to the injury. The heart, liver, kidney, spleen, and lungs are the major organs affected by metabolic reaction caused by toxicant (Jothy et al., 2001). The liver, being a key organ in the metabolism and detoxification of xenobiotics, is vulnerable to damage induced by a huge variety of chemicals. Thus, the observed slightly increase in liver weight of the experimental groups to the control might be ascribed to high rate of metabolism of the liver due to the presence of some anti-functional nutrients in *E. cyclocarpum* seed.

Effect of *E. cyclocarpum* seed flour on the Haematological analyses of the rat blood

The results of the haematology and the blood biochemistry analyses were presented in table 4. Hematological parameters are important indices of the physiological and pathological status for both animals and humans (Adeneye et al., 2006).

Table 4: Result of haematological and blood biochemistry analyses of rats fed with varying percentage of E. cyclocarpum seed flour.

Parameters	Group A	Group B	Group C	Group D
Haematological	**Analysis**			
PCV (%)	45.00±1.00[a]	43.00±2.00[ab]	43.66±1.15[ab]	41.66±1.52[b]
Hb (g/dL)	14.90±0.43[a]	14.06±0.50[a]	14.46±0.28[a]	14.53±1.81[a]
RBC (10^6/µL)	7.33±0.11[a]	7.09±0.49[a]	7.39±0.12[a]	7.51±0.58[a]
WBC (10^3/µL)	6433.33±633.11[a]	6216.66±728.50[ab]	6016.66±104.08[ab]	5400.00±458.25[ab]
Platelet (x 10^4)	18.70.00±3.81[b]	15.53.33±7.08[b]	32.80±9.35[a]	14.40±3.64[b]
Lymphocyte (%)	70.66±2.51[a]	66.33±4.16[a]	61.00±1.73[b]	67.66±2.08[a]
Heterophyl (%)	23.00±3.60	30.66±2.51	34.33±1.15	28.00±2.64
Monocyte (%)	1.66±0.51[a]	1.66±0.50[a]	2.00±0.17[a]	1.33±0.49[a]
Eosinophyl (%)	3.00±0.85[a]	1.66±0.92[b]	2.66±0.66[a]	2.66±0.75[a]
MCV (fL)	61.37±1.53[a]	60.75±2.19[a]	59.08±1.49[a]	55.74±5.97[a]
MCHC (%)	33.10±0.41[b]	32.73±0.47[ab]	33.13±0.21[ab]	37.29±0.26[a]
MCH (pg)[a]	20.33±0.49[a]	19.85±0.48[a]	19.61±0.33[a]	20.71±0.60[a]
Basophils	3.00±0.85[a]	1.66±0.92[a]	2.66±0.66[a]	2.66±0.75[a]
Blood	**Biochemistry**	**Analysis**		
Total protein (g/dL)	7.10±0.17[a]	7.26±0.19[a]	6.50±0.10[b]	6.13±0.30[c]
Albumin (g/dL)	3.13±0.57[a]	3.36±0.11[a]	2.56±0.15[b]	2.33±0.20[b]
Globulin (g/dL)	3.96±0.11[a]	3.86±0.05[a]	3.83±0.25[a]	3.53±0.11[b]
Albumin/Globulin	0.78±0.00[a]	0.87±0.04[a]	0.85±0.08[a]	0.65±0.03[b]
AST (µL)	43.00±2.00[a]	40.00±2.00[a]	42.60±1.50[a]	41.33±0.59[a]
ALT (µL)	31.33±1.52[a]	27.66±2.51[b]	31.33±1.15[a]	29.66±1.52[ab]
ALP (µL)	111.00±7.93[a]	104.33±2.08[a]	106.06±5.50[a]	108.00±7.81[a]
Urea (mg/dL)	17.00±0.60[a]	16.86±1.15[a]	16.23±0.40[a]	16.30±0.52[a]
Creatinine (mg/dL)	0.63±0.05[a]	0.60±0.00[ab]	0.53±0.05[bc]	0.50±0.00[c]
Glucose Level	124.33±4.04[b]	128.33±0.57[a]	119.66±0.57[c]	114.66±0.57[d]

Values are expressed as mean ± SD
Values on the same row having similar letter as superscripts are not significantly different ($P < 0.05$*).*

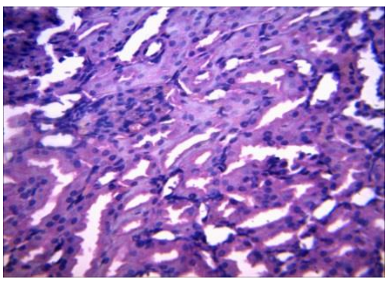
After the period of experiment, there was a slight reduction in the values of PVC, Hb and RBC among the experimental group when compared with the control group but not significant. PCV is involved in the transportation of absorbed nutrients. Hb and MCH are major indices for the diagnosis of anemia (Adeneye et al., 2006). WBC compared favourably within 10 % and 20 % groups to the control than the lowest value obtained in the 30 % group. Other haematology parameters such as MAV, MAH, MCHC, Lymphocye and Heterophyl compared favourably among the experimental groups and the control. Blood parameters analysis is relevant to risk evaluation and the changes in the haematological system that might be obtained in the analysis have a higher predictive value for human toxicity, when these data are translated from animal studies (Ibrahim et al., 2010). The deleterious effect of foreign compounds on the blood constituents of animals could be revealed by the assessment of haematological parameters (Odeyemi et al., 2009). They can also be used to look for changes in biomolecule levels, metabolic products, haematology, normal organ function, and organ histomorphology (Jothy et al., 2001). The lack of a significant change in the various haematological parameters investigated suggests that ECSF may not have a deleterious effect on the rat's blood in this investigation.

Histopathological analysis of organs

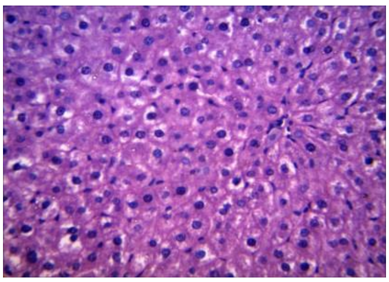
The results of the histopathological analysis of the kidney, heart and liver of rat fed with diet compounded with ECSF are shown in Table 5 while Figure 3 illustrated the photomicrograph of liver section for both the control and experimental groups. There was no abnormal activity (no visible lesion) seen around the heart of both the control and experimental groups. There was also no visible lesion seen in the kidney section with the control and group B, a very mild interstitial congestion in group C and severe congestion with a pink staining material in the collecting ducts and renal tubules were observed respectively in the kidney sections of the experimental group D. The kidney is crucial in the elimination of wastes from an animal's body, which could explain why severe interstitial congestion was observed at the maximum ECSF inclusion level. Rats with kidney abnormalities have also been documented (Ajayi et al., 2007). The histopathological section of the liver shows no visible lesion in the control. A mild to moderate diffuse vacuolar degeneration of the hepatocytes in group B, a moderate portal congestion and periportal cellular infiltration in group C and severe portal congestion with moderate periportal cellular infiltration in group D. The liver was so affected simply because it was an important organ involved in the metabolism, detoxification and excretion of chemicals in the body system (Pathan et al., 2010). This result reveals that E. cyclocarpum seed flour without any major lesion seen in both the control and group A might probably be safe at 10 % inclusion level.

Table 5: Results of histopathological studies of tissues of the rats fed with *E. cyclocarpum* seed flour

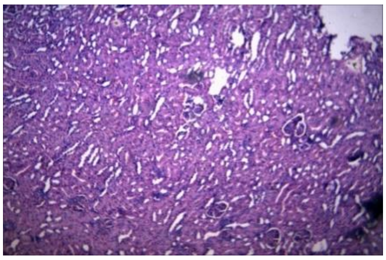
Organ	**Heart**	**Kidney**	**Liver**
Group A	No lesion seen	No lesion seen	Visible lesions not seen
Group B	No lesion seen	No lesion seen	There is a mild to modera diffuse vacuolar degeneration the hepatocytes
Group C	No lesion seen	There is a very mild interstitial congestion	There is a moderate portal congestion and periportal cellular infiltration
Group D	No lesion seen	There are pink staining material in the collecting ducts and renal tubules	There is a severe por congestion, moderate peripor cellular infiltration.



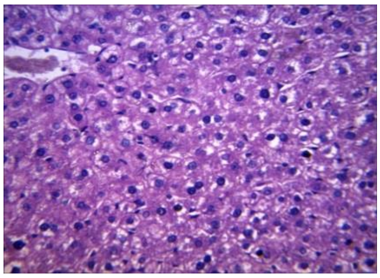
***Group A:** Kidney with no lesion seen*



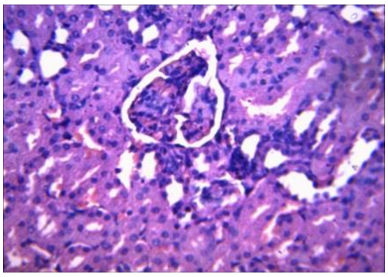
***Group A:** Liver with no visible lesion seen*



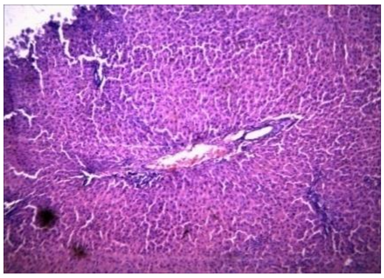
Group B: *Kidney with no lesion seen*



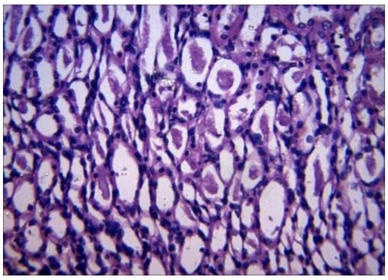
Group B: *Liver showing mild to moderate diffuse vacuolar degeneration of the hepatocytes*



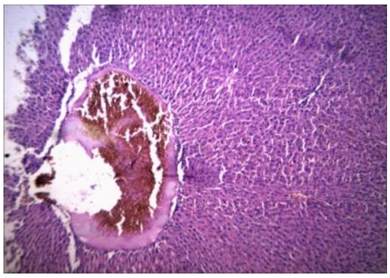
Group C: *Kidney showing a very mild interstitial congestion*



Group C: *Liver with moderate portal congestion and periportal cellular infiltration*



Group D: *Kidney showing pink staining material in the collection ducts and renal tubules*



Group D: *Liver showing a severe portal congestion, moderate periportal cellular infiltration*

Figure 3: Photomicrographs of the histological examination of the kidney and livers of the rat fed with graded level of *E. cyclocarpum* seed flour

CONCLUSION

The results obtained under this study revealed that *E. cyclocarpum* seed flour at 10 % level of incorporation into rat feeds showed good nutritional properties in term of crude protein, crude fibre, dry matter, weight gain percentage and feed intake when fed to rat.

CONFLICT OF INTEREST DECLARATION

The authors declare no conflict of interest in the cause of the research.

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