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Studies on Haematology, Serum Chemistry and Histology of Liver and Kidneys of Male Wistar Strain Albino Rats Fed Graded Levels of Raw Pigeon pea (*Cajanus cajan*) Seeds

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Received: April 10, 2017; **Published:** April 17, 2017

Abstract

The haematology, serum chemistry and histology of the liver and kidneys of male wistar strain albino rats fed with graded levels of raw pigeon pea (*Cajanus cajan*) seeds were investigated. Thirty male rats of average weights between 150g - 200g were assigned into six groups (A-F) of 5 rats each. The rats in Group A received 10% raw pigeon pea inclusion diets (rPPID), Group B (20% rPPID), Group C (30% rPPID), Group D (40% rPPID), Group E received 100% (raw Pigeon pea diets [rPPD]), while Group F (Control) received commercial rat feed. Haematology indices, serum chemistry and histology of the liver and kidneys were done according to established and standard protocols. Photomicrographs of the tissue lesions were taken using an olympus microscope fitted with an Amscope® camera unit. Data were analyzed using one-way analysis of variance (ANOVA). SPSS Version 15 for Windows (SPSS Inc, 2006) and Microsoft Excel Professional Plus (Microsoft Corporation, 2010) were also used. The PCV values for rats in group E (100% [rPP] diets) were significantly lower ($P < 0.05$) (35.0 ± 3.67%) than values for all the other groups A-D, including group F (Control) (43.8 ± 6.34%). White Blood Cells (WBC) counts differed significantly ($P < 0.05$) among treatment groups, with rats in group D (40% rPPID) recording the highest value (79.80 ± 5.38 × $10^9.l^{-1}$), followed by rats in group A (10% rPPID) (78.70 ± 7.46 ×$10^9.l^{-1}$). Results from this study showed that the rats in groups B, C and E showed some hepatic lesions like moderate lymphocytic portal inflammation (group B), coagulative necrosis of hepatocytes with a few hemosiderin-laden macrophages in the sinusoids (group B), moderate lymphocytic infiltration of the portal areas (group C) and marked lymphocytic infiltration of the portal areas (group E), No visible lesions were observed in the histology of kidneys of all the rats in groups A to F. It was concluded in this study that feeding raw pigeon pea seeds to rats is safe as it has no deleterious effect on the haemogram and serum chemistry, but it could interfere with the immuno-modulatory properties of the liver and this effect is dose/grade dependent.

Keywords: *Pigeon Pea; Haematology; Serum Chemistry; Histology; Liver; Kidney*

Introduction

The consumption of animal protein sources in developing countries is ever increasing [1], but the high cost of the conventional animal feed ingredients like fish meal, maize, groundnut cake and soyabean meal is a limiting factor, because of the pressing demands of humans and animals for these feed ingredients [1]. This has strongly compelled the need to explore alternative and cheaper and easily available sources of animal feed ingredients.

Legumes and cereal grains are the major protein and energy sources in non-ruminant diets [1]. Pigeon pea (*Cajuns cajan* L.), is a food legume crop, belonging to the Family *Phaseolea* [2]. In many developing countries of the semi-arid tropics and sub-tropics, it is used as a multipurpose legume crop [3], and plays an important role in the nutrition of humans and animals as a dietary protein source [4]. The

Cajanus cajan seeds has now been reported to be a non–conventional source of poultry feed [2]. Although pigeon pea is fairly well cultivated in Nigeria [5], it is an under-utilized and under-exploited legume in Nigeria [6]. The chemical composition of *C. cajan* plant seed has been reported by [7]. In spite of the fact that pigeon pea is a valuable crop for both human and animal nutrition, the seeds have received little attention in comparison to the leaves [8]. There is however the need to evaluate the effects of these alternative animal feed sources on the haematology, serum chemistry and histology of some vital organs like liver and kidneys of male Wistar strain albino rats.

Materials and Methods

Source of the Pigeon pea (*Cajanus cajan*)

The pigeon peas (*Cajanus cajan*) were bought from the popular Bodija market in Ibadan, Oyo State and identification was verified at the Department of Agronomy, University of Ibadan, Ibadan, Nigeria.

Feed Preparation

Pigeon pea (*C. cajan*) seeds (3kg) and the rat concentrate feed (3kg each) was ground into powdery form separately using an electric miller. The feed was then reconstituted into different percentage inclusions of raw pigeon pea seeds (10% rPPID, 20% rPPID, 30% rPPID 40% rPPID and 100% rPPD) and Control (Normal Concentrate Feed [NCF]), according to the method of [9,10]. Table 1 below shows the percentage inclusion of the raw pigeon pea (rPP) diets.

Groups	Normal feed(kg)	Pigeon pea inclusion (kg)	Total (kg)
A (10% rPPID)	2.7	0.3	3
B (20% rPPID)	2.4	0.6	3
C (30% rPPID)	2.1	0.9	3
D (40% rPPID)	1.8	1.2	3
E (100% rPPD)	_	3	3
F(Control) (NCF)	3	_	3

Table 1: *Graded levels of the Feed Inclusions.*

Experimental Animals

Thirty (30) Male Wistar strain of albino rats, divided into six groups (A-F), of five (5) rats each per group were used for the whole studies. The average weights of the rats were between 150 to 200g. The rats were housed individually in stainless-steel individual metabolic cages (Associated Crate Ltd), located in the animal house of the Department of Animal Science, University of Ibadan, Ibadan.

The rats were allowed to acclimatize with the environment and feeding of the rats was done for three weeks with each group receiving graded levels of pigeon pea meal, with group A receiving 10% rPPID, group B 20% rPPID, group C 30% rPPID, group D rPPID 40%, group E (100% rPPD and group F (control) ((NCF) and water was given *ad libidum*. The amount of feed taken by the individual rats was estimated daily by deducting the left-over feed from the 30g feed given daily.

Blood Collection and Preparation

At the end of the experiment, about 5 ml of blood was collected from the rats through the orbital veins. 2 ml of the blood from each rat were put into bottles containing sodium ethylene diamine tetraacetic acid (Na_2EDTA) and used to determine haematological parameters. Another 2ml of blood from each rat were collected into a sample bottle for serum biochemical analysis.

The blood for serum analysis was allowed to clot at room temperature in the clean sample bottles and then centrifuged for 10 minutes in a bench centrifuge at 2000 r.p.m. The clear serum was siphoned into clean sample bottles and stored immediately in the freezer until required for analysis. The sera separated from the clot by centrifugation were used to determine serum biochemical parameters.

2 ml blood for whole blood count (red blood cell count and white blood cell count and platelets) were collected into clean EDTA bottles, as anticoagulant to avoid clotting of the blood. Blood samples for serum chemistry was collected in clean non-heparinized sample bottles and were allowed to clot to obtain the serum. The serum separated from the clot and were decanted into clean bottles for serum chemistry analysis. The packed cell volume (PCV) and haemoglobin concentration were determined by the microhematocrit and cyan-methaemoglobin methods respectively, according to [11]. The erythrocyte count was determined by the haematocytometry method [11]. The complete white blood cell (WBC) counts were made in a haemocytometer using WBC diluting fluid and differential leucocytes counts were made by counting the different types of WBC from Giemsa stained slides viewed from each of the 30 fields on an oil immersion objective of a microscope [12]. The erythrocyte indices including; mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC), were determined from the values obtained from PCV values, red blood cells (RBC) count and haemoglobin concentration [13]. From the serum samples, the total protein was measured using biuret reaction, while albumin was measured by the colorimetric estimation with sigma diagnostic reagent (Sigma Diagnostic, UK.), containing bromocresol green (BCG). Globulin was obtained from the difference of total protein and albumin. Serum urea and creatinine levels were done using photoelectric colorimeter [12].

Aspartate amino transferase (AST) and alanine aminotransferase (ALT) were determined with the use of a photoelectric colorimeter [13], while the alkaline phosphatase (ALP) activity was determined according to [14]. Gamma glutamyl transferase (GGT) activity was measured using standard techniques of colorimetric analysis and spectrophotometric measurements using Sigma Aldrich Corporation (UK) kits.

After the three weeks feeding period, the liver and kidney tissues of the rats were harvested and the histological examination of the tissues was carried out by processing and staining of the tissues according to the method of [15]. Photomicrographs of the tissue lesions were taken using an olympus microscope fitted with an Amscope® camera unit.

Results

Table 2 below shows the haematology results, Table 3 reveals the mean values for the leukocyte changes, Table 4 shows the mean values for the serum proteins, Table 5 shows the mean values for the liver enzymes activities while Table 6 shows the mean values for Blood Urea Nitrogen (BUN) and Creatinine.

Parameters	Group A	Group B	Group C	Group D	Group E	Group F
PCV (%)	45.00 ± 2.40	46.40 ± 2.63	48.00 ± 2.40	49.40 ± 9.86	35.0 ± 3.67*	43.8 ± 6.34
HB (g/dl)	14.64 ± 0.95	15.22 ± 1.12	15.98 ± 0.84*	14.84 ± 6.4	15.3 ± 1.54	14.78 ± 4.23
RBC ($\times 10^{12}.l^{-1}$)	7.45 ± 0.06	7.57 ± 0.61	7.91 ± 1.05	8.00 ± 0.9547	7.53 ± 0.69	11.40 ± 7.57*
MCV (fl)	60.40 ± 2.46	61.29 ± 3.24	60.68 ± 3.45	61.75 ± 10.86	46.48 ± 4.36*	38.42 ± 13.91
MCH (pg)	19.59 ± 1.01	20.11 ± 1.73	20.20 ± 1.89	18.55 ± 7.35	20.32 ± 2.23	12.96 ± 11.80*
MCHC $g.dl^{-1}$	32.44 ± 3.35	32.80 ± 3.75	33.29 ± 3.24	30.04 ± 16.26	43.71 ± 5.11*	33.74 ± 10.57
Platelets ($x10^5$)	1.55 ± 1.17*	1.29 ± 6.08	1.05 ± 5.32	1.53 ± 5.00	1.06 ± 1.90	1.17 ± 6.05

Table 2: *Haematology results for the different groups fed graded levels of Pigeon pea diets.*

**refers to values with significant difference (P < 0.05)*

Mean Values for Packed Cell Volume (PCV), Haemoglobin (Hb), Red blood cell (RBC) count, Platelets.

GROUP A (10% rPPID), GROUP B (20% rPPID), GROUP C (30% rPPID), GROUP D (40% rPPID), GROUP E (100% rPPD), GROUP F (NCF).

Parameters	Group A	Group B	Group C	Group D	Group E	Group F
WBC ($\times 10^9.l^{-1}$)	78.70 ± 7.46*	54.66 ± 1.14	48.20 ± 2.99	79.80 ± 5.38*	45.30 ± 2.34	68.50 ± 1.90
Lymphocyte ($\times 10^9.l^{-1}$)	72 ± 4.38*	68 ± 10.32	67 ± 9.34	71 ± 9.70*	68 ± 9.1932	68.6 ± 4.92
Neutrophil ($\times 10^9.L^{-1}$)	24.8 ± 5.44*	27.2 ± 10.86	27.4 ± 8.83	25.2 ± 11.87	28.4 ± 8.94	27.4 ± 4.92
Monocyte ($\times 10^3.\mu l^{-1}$)	1.6 ± 1.07	1.6 ± 1.75	2.8 ± 0.88*	1.8 ± 0.88	2.0 ± 1.96	1.6 ± 1.07
Eosinophil ($\times 10^3.\mu l^{-1}$)	1.6 ± 2.23	2.4 ± 1.75*	2.0 ± 1.96	2.0 ± 1.96	1.6 ± 2.23	2.4 ± 1.75*

Table 3: *Mean values for leukocytes.*

**refers to values with significant difference (P < 0.05)*

White Blood Cell (WBC) count

Parameters	Group A	Group B	Group C	Group D	Group E	Group F
Total Protein (mg/dl)	8.26 ± 1.33	8.12 ± 0.91	8.12 ± 0.91	8.08 ± 0.51*	7.36 ± 0.74*	8.14 ± 0.80
Albumin (A) (mg/dl)	3.24 ± 1.09	3.14 ± 0.78	3.26 ± 0.45	3.42 ± 0.38	2.5 ± 0.95*	3.12 ± 0.51
Globulin (G) (mg/dl)	5.02 ± 0.51	4.98 ± 0.32	4.86 ± 0.42	4.66 ± 0.33*	4.86 ± 0.40	5.02 ± 0.40
A:G Ratio	0.6 ± 1.96	0.58 ± 0.16	0.64 ± 0.22	0.7 ± 0.22*	0.44 ± 0.22	0.58 ± 0.16

Table 4: *Mean values for the Serum proteins.*

**refers to values with significant difference (P < 0.05)*

Parameters	Group A	Group B	Group C	Group D	Group E	Group F
AST ($U.L^{-1}$)	39.8 ± 3.77	37.8 ± 2.91	37.4 ± 3.56	41.8 ± 4.02*	39.6 ± 2.23	42.0 ± 2.77*
ALT ($U.L^{-1}$)	28.8 ± 3.77	26.4 ± 4.29	27 ± 1.96	31.4 ± 2.23*	28.6 ± 4.51	30.8 ± 2.15*
ALP ($U.L^{-1}$)	110.2 ± 9.13	113.0 ± 19.45	113.2 ± 3.77	114.6±4.72*	108.4 ± 10.67	114.6 ± 20.54*
GGT ($U.mgprotein^{-1}$)	0.26 ± 0.11	0.42 ± 0.32	0.54 ± 0.55*	0.2±0.14	0.32 ± 0.09	1.32 ± 0.26*

Table 5: *Mean values for liver enzymes activities.*

**refers to values with significant difference (P < 0.05)*

Alanine-amino transferase (ALT), Aspartate-amino Transferase (AST), Alkaline Phosphatase (ALP) and Gamma-Glutamyl Transferase (GGT)

Parameters	Group A	Group B	Group C	Group D	Group E	Group F
BUN ($mg.dl^{-1}$)	15.58 ± 5.45	15.64 ± 5.41	15.56 ± 5.63	15.08 ± 5.30	10.66 ± 0.93*	15.90 ± 5.38
Creatinine ($mg.dl^{-1}$)	0.72 ± 0.16	0.66 ± 0.22	0.72 ± 0.16	0.72 ± 0.16	0.54 ± 0.11*	0.68 ± 0.09

Table 6: *Mean values for Blood Urea Nitrogen (BUN) and Creatinine.*

**refers to values with significant difference (P < 0.05)*

Kidney Histopathology

No visible lesions with deviation from the normal (control) were observed in the photomicrograph of all the kidney tissues in all the test groups.

Liver Histopathology

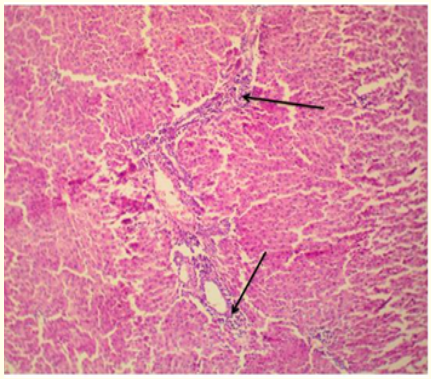

Figure 1: *Group B (20% rPPID) (Mag-x150). Moderate lymphocytic portal inflammation (black arrow) (H & E).*

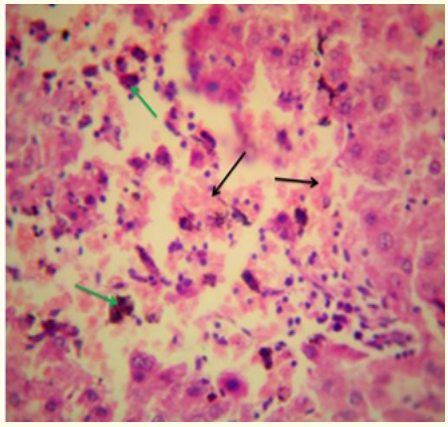

Figure 2: *Group B (20% rPPID) (x600). Coagluative necrosis of hepatocytes (black arrows) with a few hemosiderin-laden macrophages (green arrows) in the sinusoids (hemosiderosis) (H & E).*

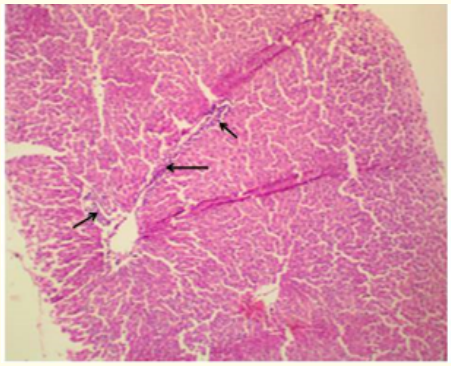

Figure 3: *Group C (30% rPPID) (x150). Moderate lymphocytic infiltration of the portal areas (arrows).*

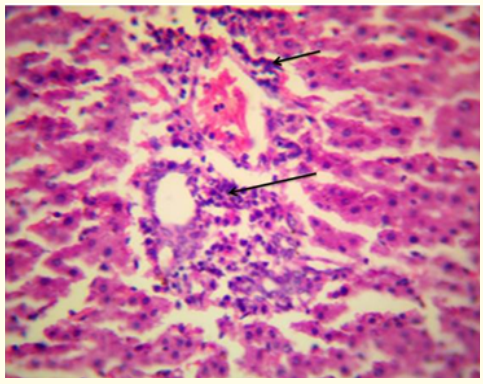

Figure 4: *Group E (100% rPPD) (x600). Marked lymphocytic infiltration of the portal areas (arrow).*

Discussion

The blood is a vital nutritional, physiological and pathological parameter in an organism [16]. Some of the blood parameters that are most usually affected by nutritional imbalancies and pathological conditions are PCV, HB, RBC, WBC, proteins and enzymes [17-19].

The hematological and biochemical values obtained for rats fed graded levels of pigeon pea based diets are presented in Tables 2,3,4,5,6. Hemoglobin (Hb) values (g/dL) did not vary significantly ($P > 0.05$) among the treatment groups, as compared with control rats except for rats in group C fed 30% (rPPID) (15.98 ± 0.84 g/dL). A low level Hb of the treatment diets would have implied poor dietary proteins [20], probably due to the presence of anti-nutritional factors (ANFs) in the raw pigeon pea (*Cajanus cajan*) diets. Hb concentration is highly sensitive to low protein intake and Hb values increases with increases in dietary protein intake, and nutrient imbalances and poor nutrient utilization could also lead to variations in Hb concentrations in animals [21-23].

Diets containing poor quality protein would usually affect transportation of oxygen from the respiratory organs to the peripheral tissues [24]. The PCV values for the rats in group E (100% rPPD) were significantly lower ($P < 0.05$) (35 ± 3.67%) than values for all the other groups A-D, including the control rats (NCF) (43.8 ± 6.34%). The PCV determination serves as a very accurate parameter to evaluate the level of RBC and to know the HB concentration [25,26] and the PCV is sensitive to the level of dietary protein [27]. PCV is also an index of toxicity and a reduction in the concentration of PCV in the blood usually would suggest presence of ANF like haemagglutinin, which has adverse effects on blood formation [28] and traces of haemagglutinin have been reported in pigeon pea [29,30].

In comparison with the rats in groups A to D, the rats in group E, fed (100% rPPD) recorded the significantly least ($p < 0.05$) MCV values (46.48 ± 4.36 fl), while rats in group F (control group [NCF]) recorded (38.42 ± 13.91 fl). For the MCH values, the group F (control group) recorded the significantly least ($p < 0.05$) value (12.96 ± 11.80 pg), and rats in group E fed (100% rPPD) recorded the significantly highest ($p < 0.05$) values for MCHC (43.71 ± 5.11 $g.dl^{-1}$). The Mean Corpuscular Haemoglobin Concentration (MCHC) values are highly sensitive to protein intake and their values increase with increase in the quality and concentration of dietary protein [31].

White Blood Cells (WBC) counts differed significantly ($P < 0.05$) among treatment groups, with rats in group D (40% rPPID) recording the highest value (79.80 ± 5.38 × $10^9.l^{-1}$), followed by rats in group A (10% rPPID) (78.70 ± 7.46 ×$10^9.l^{-1}$). High values of WBC suggests a good capacity of an animal to fight diseases [1] and a decrease in WBC count may be attributed partly to a reduction in protein intake [24], while nutritional stress has been reported to cause an increase in WBC count manifested as increased neutrophil count [32]. It has been reported that poor utilization of nutrients result in variation in WBC counts in animals [33]. Group A rats fed 10% rPPID) recorded the least value (24.8 ± 5.44 × $10^9.l^{-1}$) for neutrophil count and the value is significantly lower than all the other groups, including the control. Groups A and E rats fed (10% rPPID) and (100% rPPD) respectively, recorded the lowest values for eosinophils count, with both having the same value (1.6 + 2.23 × $10^9.l^{-1}$). Group A rats recorded the highest significant ($P < 0.05$) values for lymphocyte counts

(72 ± 4.38 ($\times 10^9.l^{-1}$), while monocyte counts did not show any significant difference among all the groups of rats, including the control group. Lymphocytes are linked to a variety of immunological function which includes immunoglobulin production and modulation of immune defense [34]. It was reported that an elevated lymphocyte infiltration is also a possible sequelae of chronic inflammation of the liver [35].

Rats in group E fed with (100% rPPD) recorded the signicantly ($P < 0.05$) lowest value for total serum proteins (7.36 ± 0.74 $mg.dl^{-1}$) and albumin (2.5 + 0.95 $mg.dl^{-1}$). Serum proteins are used as an indicator of the state of body cells, tissues and organs and also as indicator for the metabolism of ingested animal feeds [36, 37]. A high value of serum proteins indicate a better protein quality in the animal feeds, and the serum protein level reduces during severe malnutrition [32]. Serum albumin is a measure of the amount and type of protein in the blood, and it gives an index of the nutrition and health status [38] and is used as an indicator of protein status [39]. There exists a positive correlation between total serum proteins and dietary protein intake [37].

The serum activities of Alkaline transferase (ALT), Aspartate transferase (AST) and Alkaline phosphatase are usually used as indices in assessing the physiological status of the liver [36]. The level of these serum enzymes increases during liver damage and disease conditions. Among all the experimental diet groups, Group D rats fed (40% rPPID) recorded the significantly highest ($p < 0.05$) values for AST activities (41.8 ± 4.02 $U.L^{-1}$), ALT activities (31.4 ± 2.23 $U.L^{-1}$) and ALP activities (114.6 + 4.72 $U.L^{-1}$), while the group F rats (control group) also showed significant ($p < 0.05$) deviations as compared with the control groups. For GGT activities, group F rats (control group) showed a significantly ($p < 0.05$) increased values as compared to the experimental diet groups. The serum concentration of these enzymes are associated with the functions of the liver and they are used as index of feed quality [1]. It was however reported by [40] that liver enzymes have low diagnostic value for nutritional status because of their high variability in the blood and higher AST and ALT activities not beyond the threshold value could be an indicator for quality protein [31].

Group E rats fed with (100% rPPD) showed the significantly least ($P < 0.05$) values for BUN (10.66 ± 0.93 $mg.dl^{-1}$) and Creatinine (0.54 ± 0.11 $mg.dl^{-1}$). High blood urea levels are associated with poor protein quality [41] or excess tissue catabolism associated with protein deficiency. It was reported by [42,37] that there is an inverse relationship between total serum protein levels and serum urea concentration. A feedstuff with a good protein quality, upon ingestion will show a lower serum urea and creatinine values [43,44].

The concentration of serum urea may also increase despite low protein intake, in cases where dietary energy consumption is restricted, which is believed to indicate an increase in the breakdown of endogenous proteins for energy production [45,46].

Some alterations in the histology of the hepatic cells found in this study could also be associated with the raw form of the seed fed to the rats. Studies by [47] shows that feeding of the raw form of some legumes could interfere with some metabolic processes, like that of the liver, as they contain certain levels of anti-nutritional factors e.g saponin, trypsin inhibitors and haemagglutinin. Hepatic cells are critical targets in lesions caused by presence of excessive dietary components, because they are absorbed via the intestinal mucosa and they reach the liver through the portal vein [48]. There was no lesion in the renal tissues indicating a stable renal function.

Conclusion

Findings from this study show that pigeon pea (*Cajanus cajan*) seeds could be a good source of feed components for animals. However, feeding raw pigeon pea can induce some adverse effects on the hepatocytes of rats, hence necessary dietary precautions must be followed when using raw pigeon pea as a component of animal feeds and adequate processing must be done before feeding it to rats or other animals.

Further studies are needed towards investigating the effects of different processing methods of pigeon pea (*Cajanus cajan*) on the blood parameters and histology of vital organs of the rats. This will be of benefit in maximizing the potentials of pigeon pea as a source of animal feeds.

Conflict of Interest

There is no conflict of interest.

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