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DISCOVERY

Effect of aqueous *Piliostigma thonningii* leaf extracts on the hematological and serum biochemical indices of broiler chicken

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ABSTRACT

Three hundred one-day-old broiler chicks (Ross 308) were used to evaluate the effect of aqueous *Piliostigma thonningii* leaf extracts (PTE) on the some hematological and serum biochemical parameters of broiler chicken. The birds were randomly assigned to five treatments of four replicates consisting of 15 birds each in a completely randomized design. Birds in treatment 1 (T1) were given PTE at 0 % while T2, T3, T4 and T5 were fed PTE at 20ml, 40ml, 60ml and 80ml per liter of water. Feed and water were offered *ad libitum* throughout the experiment which lasted for 4 weeks. The hematological parameters examined are: pack cell volume (PCV),

hemoglobin (Hb), red blood cells (RBC), erythrocyte sedimentation rate (ESR), white blood cells (WBC) and its differentials while those of serum biochemical parameters are: albumin, globulin, total protein, cholesterol, creatinine, urea, serum glutamic oxaloacetate (SGOT) and serum glutamic phospho-transaminase (SGPT). Result obtained showed that all the hematological parameters were significantly ($P<0.05$) different among the treatments. Albumin, globulin, total protein, cholesterol, urea, SGPT and SGOT values were significantly influenced ($P<0.05$) by the inclusion of PTE in the water of birds. Creatinine level were not significantly ($p>0.05$) different among the treatments. It could be concluded that PTE at levels up to 80 ml have no deleterious effect on the blood profile of birds.

Keywords: *Piliostigma thonningii* leaf extract, hematology, broiler chicks

1. INTRODUCTION

The use of plants in traditional medicine (herbs) has been in existence for thousands of years because plants have proven to be a natural renewable resources with valuable bioactive compounds (Lina, 2017; Cherkupally *et al*., 2017) and also provided a clue on the discovery of new products of medicinal value for drug development (Senthilkumar *et al*., 2018). According to Rates (2001) out of the about 250,000– 500,000 plant species estimated by the WHO (1992), only a small percentage has been investigated phytochemically and even a smaller percentage has been properly studied in terms of their pharmacological properties. Among the underexplored leguminous plant is *Piliostigma thonningii* which is found to be loaded in several secondary metabolites such as flavonoids, phenol, tannins and alkaloids (Ighodaro *et al*., 2012; Akindahunsi and Salawu, 2005), minerals and vitamins (Jimoh and Oladiji, 2005). The plant belongs to the family caesalpiniacea and found in abundant in most part of the world including Africa and Asia. The plant leaves have been reported to contain 10.09 % protein, 2.81 % fat, 6.10 % ash, 5.23 % crude fibre, 72.17 % carbohydrates (Ighodaro *et al*., 2012) and also have several biological effects, exhibiting antibacterial (Akinpelu *et al*., 2000), anti-inflammatory (Togola *et al*., 2005), anthelmintic (Fokae *et al*., 2000), hypocholesterolemic (Igoli *et al*., 2005), immunomodulatory (Fakae *et al*., 2000), hypoglycemic (Dasofunjo *et al*., 2012) and hematopoietic properties (Dasofunjo *et al*., 2013).

Several studies have been carried out on different plant extracts on animal's performance. According to Bestami *et al*. (2009), clove extract at 400 ppm caused a significant ($P<0.05$) increase in final weight of broilers. *Piliostigma thonningii* leaf extract at 0.4g/kg of body weight has also been reported to reduce blood cholesterol level in Wistar rats. According to Alagbe (2019), *Parkia biglobosa, Delonix regia* leaf extract and garlic/ lemon grass extract have helped to improve weight gain, feed conversion ratio, blood profile and reduce mortality in birds. However, limited information is available on the effect of PTE on the blood profile of broilers. A timely evaluation of PTE will give a clue on the immune system of the animal and provides its safe recommended level in broiler chicks.

2. MATERIALS AND METHODS

Site of the experiment

The experiment was carried out at Division of Animal Nutrition, Sumitra Research Farm, Gujarat, India during the month of August to October, 2019.

Collection and processing of test material

Fresh and mature leaves (*Piliostigma thonningii*) were obtained from the premises of Sumitra research farm in India and was authenticated by a taxonomist Dr. Sharma Padash and grinded into fine particles using a laboratory blender. The extract (PTE) was prepared by putting 150 grams of the powdered sample in 1000 ml of distilled water for 2 days after which the extract was filtered using Whatmann filter paper No. 1and kept in refrigerator at 4ºC for further analysis. The powdered sample was also subjected to proximate analysis.

Experimental animals and management

Three hundred one-day old Ross 308 broiler chicks were used for the experiment. Prior to the commencement of the study, the pens were properly disinfected with Morigad at 10 ml to 50 liters of water, feeders and drinkers were thoroughly washed and all other electrical fittings were fixed. The birds were weighed on arrival to the farm and thereafter weekly and distributed randomly into five (5) treatments of 300 chicks of four replicates each consisting of 15 birds and electric brooders were used as source of heat. Light was also provided approximately 24 hours in a form of natural light during the day and artificial light during the night, ten bulbs (100 watt) was used for this purpose. The initial brooding temperature was 34ºC in the first week of age which was gradually

reduced by 2°C per week to 22°C. Birds were kept under similar conditions of management throughout the experimental period. Vaccination was done according to the prevailing disease condition in the environment. Water soluble multi-vitamins (Biovite super® at 1ml to 5 litres of water) was given to the chicks before 3 days of vaccination and 3 days after vaccinations in order to guard stress. Fresh, clean and cool drinking water was provided to the experimental bird's *ad-libitum*.

Feed formulation and experimental design

Birds were fed basal diet formulated according to NRC (1994). Starter diet was given between (0-4 weeks) containing a crude protein of 23.40 % and metabolizable energy of 2950.6 kcal/ kg. Treatment 1 (Control) was given 0 % PTE, treatment 2, 3, 4 and 5 were given PTE at levels 20 ml, 40ml, 60ml and 80 ml/ liter respectively. The experimental design that was used is a completely randomized design (CRD).

Blood analysis

At 4th weeks, twelve birds were randomly from each treatment for haematological and serum biochemical analysis. Selected animals were kept in a stress free environment to prevent oxygenated blood becoming deoxygenated during blood collection. The sampled birds were bled from punctured wing vein to aspire 5mls of blood from each birds out of which 2mls was collected into bijou bottle treated with Ethylene Diamine Tetra Acetate (EDTA) for haematological assay. Complete blood analysis was performed within three (3) hours of collection using a commercial diagnostic kits (Nosrac diagnostic analyzer, China). Pack cell volume was estimated by micro haematocrit method (Jain, 1986). Red blood cell, haemoglobin, haemoglobin, white blood cell and its absolute counts were determined by using Neubauer's chamber. Values of MCV, MCH and MCHC were calculated using:

MCV (fl) = PCV/RBC × 100
MCH (pg) = Hb/RBC × 10
MCHC (%) = Hb (100mg blood)/PCV × 100

Sera were stored at -20°C until it was used for biochemical analysis. Commercial diagnostic kits (Nosrac diagnostic analyzer, China) were used for determination of total protein (TP 32B-660), albumin (TP 34V-901), globulin (TP 54H-908), calcium (TP 01A-101), sodium (TP 12-01R-21), bicarbonate (TP 09-08A-44), phosphorus (TP 12-09RT), total cholesterol (TP-140-09T), low density lipoprotein (TP-093-YT), high density lipoprotein (TP-093-W2), glucose (TP-120P-01), triglycerides (TP-061-0R), urea (TP-671-R03), creatinine (TP-06R-10), total bilirubin (TP-097-LR5) along with the activity of the following enzymes: alanine transaminase (TP-091-OP5), aspartate transaminase (TP-067-HT), serum glutamic oxaloacetate transaminase (TP-11-OPL) and serum glutamic pyruvic transaminase (TP-056-EO).

Laboratory analysis

Proximate analyses of feed (crude protein, crude fiber, ether extracts and ash) were determined in accordance with the Official Methods of the Association of Official Analytical Chemists (AOAC, 2000). Phytochemical analysis of saponins, flavonoids, phenolics, alkaloids, steroids and glycosides were using standard methods described by Harbone (1973); Odebiyi and Sofowora (1978). Tannins (Van-Burden and Robinson, 1973) and flavonoids (Boham and Kocipai-Abyazan, 1974) contents were also determined in the extracts.

Statistical analysis

All data collected will be subjected to one-way analysis of variance (ANOVA) using SPSS (25.0) and significant means will be separated using Duncan multiple range tests (Duncan, 1955) significant will be declared if $P \leq 0.05$.

3. RESULTS

The ingredients composition of experimental diet is presented in Table 1. The proximate components of the diets revealed the presence of crude protein (23.40 %), crude fibre (4.02 %), ether extract (5.04 %) and metabolizable energy (2950.6 kcal/kg).

Table 1 Ingredient composition of the experimental diets

Ingredients	Starter (0-4 weeks)
Maize	54.00
Wheat offal	5.00
Soya meal	30.00

Groundnut cake	8.00
Oyster shell	2.00
Bone meal	3.00
Lysine	0.20
Methionine	0.20
*Premix	0.25
Salt	0.30
Toxin binder	0.10
Total	
Determined analysis (% DM)	
Crude protein	23.40
Crude fibre	4.02
Ether extract	5.04
Energy (Kcal/kg)	2950.6

* Premix supplied per kg diet: - Vit A, 13,000 I.U; Vit E, 5mg; Vit D3, 3000I.U, Vit K, 3mg; Vit B2, 5.5mg; Niacin, 25mg; Vit B12, 16mg; Choline chloride, 120mg; Mn, 5.2mg; Zn, 25mg; Cu, 2.6g; Folic acid, 2mg; Fe, 5g ; Pantothenic acid, 10mg ; Biotin, 30.5g ; Antioxidant, 56mg

Proximate components of *Piliostigma thonningi* leaves shows that it contained moisture, crude protein, crude fibre, ether extract and ash at 8.79 %, 11.21 %, 14.22 %, 0.31 and 7.22 % respectively as presented in Table 2.

Table 2 Proximate composition of *Piliostigma thonningi* leaves

Parameters	% Composition
Moisture	8.79
Crude protein	11.21
Crude fibre	14.22
Ether extract	0.31
Ash	7.22

Table 3 shows the phytochemical constituents of *Piliostigma thonningi* leaf extract. The extract revealed the presence of bioactive chemicals like alkaloids, saponins, flavonoids, tannins and steroids. The values obtained are 0.40, 4.38, 9.77, 1.67 and 0.02 (mg/100g) for alkaloids, saponins, tannins, flavonoids and steroids respectively.

Table 3 Phytochemical composition of *Piliostigma thonningi* leaf extract

Phytochemicals	Composition (mg/100g)	Permissible range
Alkaloids	0.40	3.50
Saponins	4.38	7.02
Tannins	9.77	31.50
Flavonoids	1.67	6.11
Steroids	0.02	1.30

Hematological parameters of broiler chicks give different levels of PTE are presented in Table 4. The parameters determined were pack cell volume (PCV), hemoglobin (Hb), Erythrocyte sedimentation rate (ESR), red blood cell (RBC), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) and white blood cell (WBC) with its differentials.

Table 4 Hematological parameters of broiler chicks fed different levels of PTE

Parameters	T1	T2	T3	T4	T5	SEM
PCV (%)	27.10^{b}	28.57^{b}	32.04^{a}	33.32^{a}	34.80^{a}	1.20
Hb(g/dl)	9.12^{c}	10.29^{b}	11.40^{b}	13.21^{a}	13.60^{a}	0.33
ESR (mm/hr)	1.97^{c}	2.14^{b}	2.87^{b}	3.47^{a}	3.79^{a}	1.44

RBC ($\times 10^6$/µl)	1.93[b]	2.03[a]	2.51[a]	2.88[a]	2.92[a]	1.01
MCV (fl)	140.4[a]	140.7[a]	127.6[b]	115.7[c]	119.2[c]	1.65
MCH (pg)	47.25	50.69	45.42	45.87	46.58	2.85
MCHC (%)	33.65	36.02	35.58	39.65	39.08	1.10
WBC ($\times 10^6$/µl)	18.73[c]	21.04[b]	23.56[a]	24.70[a]	25.32[a]	1.05
Lymphocytes (%)	5.02[c]	9.13[b]	9.68[b]	10.21[a]	10.45[a]	0.25
Monocytes (%)	0.60	0.71	0.78	0.71	0.73	0.01
Heterophils (%)	4.50[c]	5.11[c]	6.05[b]	7.18[a]	7.80[a]	0.03
Basophils (%)	1.27	1.99	2.07	2.10	2.20	0.02
Eosinophils (%)	0.58	0.66	0.64	0.67	0.61	0.04

[a,b,c] means with same superscript are significantly different (P<0.05)

RBC: red blood cell, WBC: white blood cell, MCV: mean corpuscular volume, MCHC: mean corpuscular hemoglobin concentration, MCH: mean corpuscular hemoglobin, SEM: Standard error of mean

PCV values obtained are 27.10 %, 28.57 %, 32.04 %, 33.32 % and 34.80 % for T1, T2, T3, T4 and T5 respectively while Hb values are 9.12, 10.29, 11.40, 13.21 and 13.60 (g/dl) for T1, T2, T3, T4 and T5. ESR values are 1.97, 2.14, 2.87, 3.47 and 3.79 (mm/hr) for T1, T2, T3, T4 and T5 respectively. PCV, Hb, ESR, RBC, MCV and WBC values were significantly ($p<0.05$) different among the treatments. Values for MCH are 47.25, 50.69, 45.42, 45.87 and 46.58 (pg) for T1, T2, T3, T4 and T5 respectively while those of MCHC are 33.65, 36.02, 35.58, 39.65 and 39.08 (%) for T1, T2, T3, T4 and T5. MCH, MCHC, monocytes, basophils and eosinophils were not significantly ($p>0.05$) influenced by the oral inclusion of PTE.

Table 5 shows the serum biochemical indices of broiler chicks given different levels of PTE. Albumin values obtained are 1.55, 1.71, 1.82, 1.76 and 1.83 for T1, T2, T3, T4 and T5 respectively while those of globulin are 1.00, 1.91, 1.98, 2.00 and 2.03 for T1, T2, T3, T4 and T5 respectively. Values obtained for creatinine 0.63, 0.60, 0.61, 0.66 and 0.64 (mg/dl) for T1, T2, T3, T4 and T5 respectively. Total protein, cholesterol, urea, SGPT and SGOT values were significantly ($p<0.05$) different among the treatments. Urea values obtained are 1.88, 1.71, 1.45, 1.20 and 1.10 (mg/dl) for T1, T2, T3, T4 and T5 while those of cholesterol are 95.18, 67.08, 66.10, 45.78 and 40.90 (mg/dl) for T1, T2, T3, T4 and T5 respectively. No significant difference ($p>0.05$) was observed in the creatinine values among the treatments.

Table 5 Serum biochemical indices of broiler chicks fed different levels of PTE

Parameters	T1	T2	T3	T4	T5	SEM
Albumin (g/dl)	1.55	1.71	1.82	1.76	1.83	0.04
Globulin (g/dl)	1.00[c]	1.91[b]	1.98[b]	2.00[a]	2.03[a]	1.01
Total protein (g/dl)	2.55[b]	3.63[a]	3.70[a]	3.76[a]	3.86[a]	0.09
Creatinine (mg/dl)	0.63	0.60	0.61	0.66	0.64	0.02
Cholesterol (mg/dl)	95.18[a]	67.08[b]	66.10[b]	45.78[c]	40.90[c]	3.07
Urea (mg/dl)	1.88[a]	1.71[a]	1.45[b]	1.20[c]	1.10[c]	0.04
SGPT (iu/l)	123.9[a]	120.1[a]	100.7[b]	98.03[c]	80.65[c]	1.35
SGOT (iu/l)	75.12[a]	74.30[a]	59.81[b]	50.44[b]	45.56[c]	1.50

[a,b,c] means with same superscript are significantly different ($p<0.05$)

Serum glutamic oxaloacetate (SGOT), Serum glutamic phospho-transaminase (SGPT), SEM: Standard error of mean

4. DISCUSSION

The crude protein (11.21 %), crude fibre (14.22 %), ash (7.22 %) obtained for *Piliostigma thonningi* leaves in this experiment were higher than those reported by (Ighodaro *et al.*, 2012; Ayoola *et al.*, 2016). These differences in the proximate components could be attributed to differences in soil type, age, species, season and location (Norton, 1994). Phytochemical constituents of *Piliostigma thonningi* leaf extract also reveals that tannin has the highest concentration, followed by saponin, flavonoids, alkaloids and steroids respectively. This composition agrees with the findings of Bello *et al.* (2006) but contrary with reports of Ighodaro *et al.* (2012) who reported a concentration of saponin is highest in ethanolic extraction of *Piliostigma thonningi* leaves. According to Alagbe (2019), extraction method and age of plants are some cardinals that could influence the phytochemical composition of plants. However, all the values reported in this experiment are below the lethal dose reported by Alagbe (2019); Kumar and amit (2010). According to Ojewuyi *et al.* (2014) each medicinal plant species has its own nutrient composition besides having pharmacologically important

phytochemicals. Phytochemicals are also bioactive chemicals or secondary metabolites which performs multiple biological activities such as antioxidant, anti-microbial effect, modulation of detoxification enzymes, stimulation of the immune system (Jothy *et al*., 2013; Chang *et al*., 2006; Saleem *et al*., 2006). Tannins have been reported to perform antibacterial and antiviral activities (Adisa *et al*., 2010).

According to Olorode and Longe (2000) blood are significant tools of accessing the clinical and nutritional health status of animals. Hemato-biochemical analysis has been found useful for disease prognosis and feed stress monitoring (Etim *et al*., 2013) and their variations in animals have been attributed to age and sex (Azeez *et al*., 2009), breed (Elagib and Ahmed, 2011), feed replacement (Oloyede *et al*., 2010) and environment (Azeez *et al*., 2009). RBC, Hb, PCV, ESR, WBC and MCHC values significantly ($P<0.05$) increased from treatment 1 to 5. Though the hemoglobin range (9.12 – 13.60 g/dl) reported in this experiment which is slightly higher than 8.00 – 9.60 g/dl recorded by Obikaonu *et al*. (2011), however, all values were within the reference range for broilers (Talebi *et al*., 2005); Abdi-Hachesoo *et al*. (2011). According to Etim *et al*. (2013) hematological values cannot be absolutely constant due to variations in age, breed, strain and sampling techniques.An increase in the RBC level is an indication of increased oxygen in the animal tissue (Isaac *et al*., 2013; Ugwuene, 2011). Isaac *et al*. (2013) also opined that a significant increase in PCV reveals a better transport of oxygen and absorbed nutrients, thus result in an increased primary and secondary polycythemia. PCV and MCH are also significant indices in the diagnosis of anemia and also serve as useful parameters of the bone marrow capacity to produce red blood cells in animals (Chineke *et al*., 2006).

White blood cells play a vital role in the prevention of disease or infection, thus animals with low WBC level stand a risk of disease infections. Animals in T_4 and $T_5$with high WBC are capable of generating antibodies and have a high degree of resistance to diseases (Isaac *et al*., 2013; Soetan *et al*., 2013). Leucocyte count and its differentials have also been reported to increase during stress and unfavorable conditions (Gotoh *et al*., 2001). Butterworth (1999) described basophils and eosinophils as important effector cells in allergy and host defense responses particularly against parasitic infections. Total protein, cholesterol, urea, SGPT and SGOT were significantly ($p<0.05$) different among the treatment, the total protein level increased significantly from treatment 1 to 5. This is a clear indication that the protein reserve in the diet is enough to maintain and support the growth of the animal. According to Vivian *et al*. (2015) fluctuation in serum albumin could be attributed to the quality of diet as well as the presence of diseases. Similarly, globulins play a significant role in fighting infections, hormone carrier as well as blood clotting process because of the presence of antibodies and enzymes in them (Vivian *et al*., 2015). Cholesterol and urea level decreased as the level of PTE increased. The trend in the cholesterol level is a sign that the meat is safe for consumption and reduces the risk of cardiovascular disease; it also shows that PTE contains hypolipidemic substances. This result agrees with the findings of Akintomide *et al*. (2018); Dey *et al*. (2011); Vivian *et al*. (2015) when banana leaf was used as a phyto additive in the diets of broiler chicks.

Urea is a useful indicator of kidney failure in birds, creatinine level was not significantly ($p>0.05$) influenced by PTE, this shows that the integrity of the animal's kidney is maintained. According to Borges *et al*. (2005), creatinine builds up in the blood can occur once the kidney is not filtering the blood effectively. However, all the values of the serum biochemical constituents studied fall within the ranges for broilers reported by Ibrahim (2012) on the serum biochemical values of indigenous chickens in Al-Ahsa, Saudi Arabia. SGPT and SGOT level decrease as the level of PTE increases in the treatment. According to Iyayi (1994); Alagbe (2017), SGPT and SGOT respond the presence of toxic substance in the blood. PTE has revealed to be non-toxic to the animal; similar observation was made by Alagbe (2019) when *Parkia biglobosa* leaf extract was feed to broiler chickens at different levels.

5. CONCLUSION

Secondary plant metabolites or bioactive chemicals gives plants the strength to perform multiple biological activities such as antimicrobial, anti-inflammatory, antiviral, antioxidant and antifungal properties. The feeding of PTE to broiler chicks up to 80 ml per liter water has no detrimental effect on the blood profile of birds.

Conflicts of Interest

None

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