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Haemato-biochemical indices and gross pathology of broiler chickens orally administered *Phyllantus niruri* extract

Bamidele Innocent Osho[1]*, Imoleayo Sarah Olateju[1], Grace Tamunobaraboya Osho[1] and Abraham O. Fatoki [1]

*corresponding author: ibosho@futa.edu.ng, isoladeji@futa.edu.ng

Department of Animal Production and Health, School of Agriculture and Agricultural Technology, Federal University of Technology, Akure, P.M.B. 704, Nigeria

ABSTRACT

One-hundred-and-twenty-day-old mixed-sex marshall breed of broiler chicks was randomly assigned to four (4) treatment groups (30 birds/experimental oral dosage levels). The treatment groups comprised T1 (control), T2, T3, and T4 containing 5ml, 10ml, and 20ml of *Phyllanthus nuriri* extract (PNE) stock solution per litre of water which was provided to the birds as drinking water regularly for the six weeks of the experiment. The haematological, serum metabolites, serum proteins, and gross pathology of some internal organs of the birds were examined. No significant effect (P>0.05) was observed in the packed cell volume, red blood cells, haemoglobin concentration, mean cell haemoglobin concentration, lymphocyte, heterophil, monocyte, basophil, and eosinophil, except for mean cell volume and mean cell haemoglobin. Likewise, the serum proteins were not influenced (P>0.05) by PNE but alanine aminotransferase and aspartate aminotransferase were significantly reduced when compared to the control group. No significant differences (P>0.05) were recorded on the gross pathology of the liver, heart, spleen, caecum, crop, proventriculus, and kidney of the broiler chickens provided with the different dosage levels of PNE. Conclusively, PNE up to 20 mL support normal haemopoietic activity and confers hepato-protective effects on broiler chickens.

Keywords: medicinal plant, haematology, serum, organs, broiler.

INTRODUCTION

Herbs have been a good source of medicine and food for man, livestock, and birds since the beginning of creation. Medicinal plants are rich in phytochemicals which play a crucial role against chronic, metabolic, genetic dysfunctional disease, and infectious diseases (Ugboko et al., 2020). As a result, the use of plants in ethnomedicine have been encouraged and documented based on their defensive ability against multiple disease-causing microorganisms (Ogbole et al., 2018; Chikezie et al., 2015). In animal production, there has been an increased research interest in the use of medicinal plants as a potential substitute for antibiotics (Lillehoj et al., 2018). Due to the high cost of antibiotics and the residual effects they have on the end users of animal products (man), the application of plants and their extract due to their therapeutic properties in the poultry industry has gradually gained acceptance in most part of the world (Oladeji et al., 2019).

Phyllanthus niruri L. is a valuable herb found in South East Asia, which has been used for centuries in the treatment of pathological conditions such as bronchitis, dysentery, tumours, diabetes (Bagalkotkar et al. 2006), gallstones, and kidney stones (Freitas et al. 2002). It is rich in flavonoids and phenolic compounds (Shajib et al. 2015) and possess hepatoprotective activity (Pramyothin et al. 2007; Manjrekar et al., 2008). It also possesses antimicrobial property due to the presence of some bioactive compounds in it (Ates and Erdogrul, 2003) and its extract also serve as immunomodulators that improve immune system components and disrupted immune system function (Eze, 2014*). P. niruri L* extract orally administered to broiler chickens at 65% dosage has been reported to decrease the total number of leukocytes in broiler chickens infected with *M. gallisepticum* bacteria (Hidana et al. 2018). Since the efficacy of herbal extract on the health status of animal can be determined through the blood profile and internal organ examination, this study investigated the blood and serum profile of broiler chickens as well as internal organs condition of broiler chickens orally administered different dosage levels of ethanolic extract of *Phyllantus niruri.*

MATERIALS AND METHODS

Collection and processing of plant extract

Phyllanthus niruri was harvested within the Southwestern region of Nigeria, air dried at room temperature to a constant weight for two weeks and thereafter, pulverized using an electric blender. The powder was weighed and soaked at 400g per 1200 ml of 80% ethanol for 72 hours after which it was filtered using Buchner flask and funnel with Whattman filter paper. The filtrate from the blended *Phyllanthus niruri extract* was sieved with folded

muslin cloth. The extract recovered after squeezing through a muslin cloth was kept in a freezer at -10^{o}C until use. One percent concentration was reconstituted to the extract to make *P. niruri* stock solutions. Thus, the extract was reconstituted into 50, 100 and 200 mg/kg doses.

Animal management and experimental design

One-hundred-and-twenty-day-old mixed sex marshall breed of broiler chicks were used for the experiment. The chicks were allowed to acclimatize for 2 weeks after which they were randomly assigned to four (4) treatment groups (30 birds/experimental oral dosage levels; 10 birds/replicate) using completely randomized design. The treatment groups were T1 (control), T2, T3, and T4 containing 5ml, 10ml, and 20ml of *Phyllanthus nuriri* extract (PNE) stock solution per liter of water respectively was provided to the birds in the treatment group as drinking water regularly for a period of six weeks. Experimental broiler starter and finisher's diet formulated according to NRC (1994) requirement as shown in Table 1.0 were provided *ad-libitum* and the experiment lasted for 6 weeks.

Table 1. Ingredient composition of experimental broiler starter and finisher diet

Ingredients	Starter	Finisher
Maize	50.40	55.00
Wheat offal	3.00	5.00
GNC	16.00	8.00
Soyabean meal	17.00	21.25
Fish meal	5.00	3.00
Vegetable oil	5.00	4.00
Di-calcium phosphate	1.00	1.50
Limestone	1.50	1.50
Premix	0.30	0.30
DL-methionine	0.20	0.10
L-Lysine	0.10	0.10
Salt	0.30	0.30
Calculated analysis		
Crude protein (%)	22.43	19.57
Metabolizable energy (Kcal/kg)	3194.00	3137.00
Crude fibre (%)	3.20	3.34
Calcium (%)	1.12	1.10
Available Phosphorus (%)	0.46	0.49
Methionine (%)	0.57	0.43
Lysine (%)	1.21	1.14

A total of 12 birds were randomly selected from each treatment groups (4 birds/replicate) at the end of the experiment. They were sacrificed and blood samples were collected into ethylenediamine tetraacetic (EDTA) and plain bottles for haematological and serum biochemical assays respectively. The blood in the plain bottles was spined in a centrifuge and its serum was separated into another set of plain well-labelled bottles and frozen at − 20 °C prior to analysis. The haematological indices were determined within 120 min of post-collection (Shastry 1983). The serum proteins and enzymes (Aspartate aminotransferase (AST), and alanine aminotransferase (ALT) was analyzed based on the standard methods provided by the commercial kits utilized. The slaughtered birds were eviscerated and internal organs such as the liver, kidney, spleen, heart, caecum and crop were examined for pathological conditions. The internal organs were graded based on their physical appearances: normal (1), streaked (2), fatty infiltration (3), highly congested (4), and haemorrhage/inflamed (5). All data were subjected to a one-way analysis of variance using SPSS version 20 and the differences among the experimental treatment means were determined ($P < 0.05$) by the Duncan multiple range test of the same statistical package.

RESULTS AND DISCUSSION

Haematological indices of broiler chickens orally administered different dosage levels of Phyllantus niruri extract

The haematological indices of the broiler chickens orally administered different dosage levels of ethanolic extract of *Phyllantus niruri* are presented in Table 2.

Table 2. Heamatological indices of broiler chickens fed different dosage levels of *Phyllantus niruri* extract

Parameters	T1 (Control)	T2 (5ml PNE)	T3 (10ml PNE)	T4 (20ml PNE)
PCV (%)	26.83±0.31	27.83±0.87	26.83±2.37	30±0.45
RBC ($x10^{12}$/l)	3.77±0.07	4.07±0.2	3.58±0.72	4.59±0.15
HB (g/dl)	8.95±0.09	9.28±0.29	8.93±0.79	10.02±0.15
MCV (fl)	95.61±1.24^{b}	98.73±1.31^{b}	106.45±12.28^{a}	91.17±0.69^{b}
MCH (pg)	33.91±0.42^{b}	34.92±0.43^{b}	38.79±4.11^{a}	33.74±0.25^{b}
MCHC (g/dl)	33.36±0.04	33.35±0.04	33.29±0.05	33.39±0.02
LYM	63.5±0.22	63.33±0.33	64.17±0.31	64±0.37
HET	20.83±1.01	22.67±0.49	20.83±1.14	21±1.1
MONO	12.33±0.99	10.67±0.56	11.5±1.12	12±1.1
BAS	2.33±0.21	2.33±0.21	2.5±0.22	2.17±0.17
EOS	1.00±0.00	1.00±0.00	1.00±0.00	0.83±0.17

$^{a, b}$ = Means on the same row but with different superscripts are statistically ($P<0.05$) different; PCV- Packed cell volume, RBC- Red blood cell, HB- Haemoglobin, MCHC: Mean corpuscular

haemoglobin concentration, MCV: mean corpuscular volume, MCH: mean corpuscular haemoglobin, Lymph-lymphocytes, Mono-monocytes, HET = Heterophil; MON = Monocyte; BAS = Basophil; EOS = Eosinophil.

The packed cell volume (PCV), red blood cells (RBC), haemoglobin concentration (Hb), mean cell haemoglobin concentration (MCHC), lymphocyte (LYM), heterophil (HET), monocyte (MONO), basophil (BASO), and eosinophil (EOS) were not affected (P>0.05) by the oral administration of ethanolic *Phyllantus niruri extract* at different graded levels compared to the control. The non-significant heamolytic changes observed in the PCV, RBC, Hb, MCHC, LYM, HET, MONO, BASO, and EOS are indicative that PNE did not tend to cause anaemic conditions and other erythrocyte cell-related disorders such as polycythemia, in the broiler chickens. A similar result was reported by Omokore and Alagbe (2019) and Jimoh et al. (2020) that *Phyllanthus amarus* does not influence the haematology of rabbits nor the general performance and health status of animals. Likewise, the non-significant differences observed in the white blood cell indices (lymphocyte, heterophil, monocyte, basophil, and eosinophil) is in concordance with the result of Unigwe et al. (2020) which suggested that PNE had non-toxic effect thereby validating its anti-microbial property. However, the mean cell volume (MCV) and mean cell haemoglobin (MCH) were significantly increased (P<0.05) with the different dosage levels of the ethanol extract of *Phyllantus niruri* added to the drinking water of broiler birds, with broiler chickens orally administered 10ml of ethanolic *Phyllantus niruri extract* having the highest mean cell volume (106.45fl) and mean cell haemoglobin (38.79 pg), while birds in the control group had the lowest mean cell volume (95.61fl) and mean cell haemoglobin (33.91pg). All the mean values of MCV and MCH obtained fell in the normal haematological value range for chicken (Wikihow, 2013) and birds orally administered the three different dosage levels of PNE had higher MCV and MCH values compared to the control. This revealed that PNE contains bioactive compounds which enhance the erythropoietic process of broiler chickens

Effect of Phyllantus niruri extracts on the serum protein and metabolites of broiler chickens

Table 3 revealed the serum proteins and metabolites of broiler chickens orally administered with varied dosage levels of PNE. The result of serum protein revealed that albumin, globulin, and total protein of the broiler chickens were not significantly (P>0.05) influenced by the different PNE dosage levels compared to the control which contradicted the results of Unigwe et al. (2020). However, there was a gradual reduction in the mean values of the alanine aminotransferase (ALT) and aspartate aminotransferase

(AST) of broiler chickens orally provided the different doses of PNE. The broiler chickens placed on 10ml of PNE had the lowest mean value of 80.53 IU/L and 149.92 IU/L compared to the control which had the highest mean value of 102.1 IU/L and 195.92 IU/L for ALT and AST respectively. The decrease in ALT and AST levels in the birds orally provided with PNE compared to their control is evidence of the hepato-protective properties of Phyllantus niruri. (Manjrekar et al., 2008)

Table 3. Effect of different dosages of *Phyllantus niruri* extract on serum enzymes and proteins

Parameters	T1 (Control)	T2 (5ml PNE)	T3 (10ml PNE)	T4 (20ml PNE)
ALB (g/dl)	8.69±2.99[a]	12.02±2.79[a]	14.05±2.37	14.8±3.14[a]
TP (g/dl)	40.34±12.1[ab]	59.59±12.59[ab]	75.6±5.48	71.09±6.65[ab]
GLO (g/dl)	31.65±9.23[b]	47.57±10.27[ab]	61.55±4.91	56.29±7.27[ab]
ALT (U/L)	102.1±4.53[a]	80.53±16.48[a]	99.47±3.77	96.44±3.56[a]
AST (U/L)	149.92±24.43[a]	156.75±32.22[a]	195.92±10.82	187.9±21.61[a]

[a, b, ab,] = Means on the same row but with different superscripts are significant at P <0.05; ALB-albumin, TP- total protein, GLO-globulin, ALT-alanine transaminase, AST-aspartate transaminase.

Internal organs of the broiler chicken administered ethanolic extract of Phyllanthus niruri

Table 4 showed that no significant differences (P>0.05) were recorded on the gross pathology of the liver, heart, spleen, caecum, crop, proventriculus, and kidney of the broiler chickens provided with 5ml, 10ml, and 20ml of ethanolic extract of *Phyllanthus niruri* when compared with the control.

Table 4. Examination of the internal organs of the broiler chicken orally administered ethanolic extract of *Phyllanthus niruri*

Organs	T1 (Control)	T2(5ml PNE)	T3 (10ml PNE)	T4 (20ml PNE)
Liver	1.57±0.35	1.67±0.88	1.53±1.33	1.67±0.78
Heart	1.00±0.01	1.00±0.00	1.00±0.00	1.00±0.01
Spleen	1.00±0.02	1.00±0.00	1.00±0.00	1.00±0.00
Caecum	2.33±1.33	2.30±1.33	2.28±1.53	2.30±1.33
Crop	1.00±0.01	0.99±0.05	1.05±0.04	1.00±0.01
Proventriculus	1.01±0.00	1.00±0.05	0.92±0.01	0.99±0.01
Kidney	0.97±0.05	0.92±0.03	1.00±0.02	1.01±0.05

Means on the same row without superscripts are not significant at P >0.05.

The results of this study agreed with the findings of Unigwe et al. (2020) who reported that pathological lesions on similar internal organs examined were due to *Salmonella enteritidis* inoculated into the broiler chickens rather than *P. amarus* extract orally administered to the birds. Hence, the results of this study confirm the antibacterial activity of the bioactive compounds present in *P. niruri* as reported by Hidanah et al. (2018) and Hidanah et al. (2022).

CONCLUSION

It can be concluded that oral administration of ethanolic extract of *Phyllantrus niruru* up to 20ml does not alter erythropoiesis in broiler chickens. Rather, it confers hepato-protective and antibacterial effects on the broiler chickens due to the presence of bioactive compounds such as alkaloids, flavonoids, saponins, and tannins.

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