

Blood profile as health indicator and histological changes in the liver and kidney of broiler chickens administered crude extract of *Lagenaria breviflora* fruit

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Abstract

Lagenaria breviflora Roberty is a potent plant that is commonly used as an alternative antibiotic by poultry farmers in Nigeria. There is information gap on the safe dose of its crude fruit extract in broiler chickens. Serum biochemical tests, liver and kidney histological studies were carried out to assess the health status of 120 broiler chickens administered crude extract of *Lagenaria breviflora* fruit (LBFE). The birds weighing 0.15 kg on the average were divided into 4 equal treatments (D₁–D₄) in 3 replicates of 10 birds each. D₁ (control) received 0.00 mL, D₂ received 2.50 mL, D₃ received 5.00 mL, while D₄ received 7.50 mL of LBFE/ 4litres of the drinking water respectively. The birds were offered broiler starter and finisher diets across the treatments. The experiment lasted for 42 d. The data collected were subjected to one - way ANOVA using SPSS. Total protein and albumin significantly increased with dose and were higher (4.55 g/dl) and (2.48 g/dl) in birds administered 7.50 mL of LBFE than the other treatments. Sodium and chloride ions were the only kidney function markers affected by the treatment. Sodium ion and chloride ion concentrations were significantly (p < 0.05) higher (130.60 mEq/L) and (82.03 mEq/L) in birds administered 7.50 mL of the LBFE respectively. Cholesterol was significantly lower (94.93 mg/dl) in birds on 5.00 and 7.50 mL of LBFE. SOD was higher (41.77 u/l) in birds on 5.00 and 7.50 mL of LBFE. Liver and kidney histology showed normal histologic architecture in all the treated groups, indicating absence of hepatic and kidney toxicity. Based on our result it was concluded that up to 7.50 mL of LBFE did not cause derangement in the liver and kidney function markers, lipid profile and antioxidant markers measured as health indicators. Our study has shown that crude extraction of *Lagenaria breviflora* whole fruit is a vital agricultural biotechnology that will offer a cheap, sustainable, eco-friendly and natural alternative that contains potent phytochemicals that could improve biological functions in broiler chickens.

Keywords: *Lagenaria breviflora* antioxidant, liver and kidney function

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Introduction

Herbs are natural sources of phytochemicals, which are used in parts or in whole in the treatment and management of diseases in farm animals (Ekunseitan et al., 2019). Research reports show that treatments of some illnesses are better with plant – based medicines, because modern pharmaceuticals either offer little alleviation or induce organ damage (Gajender et al., 2023). Osbourn and Lanzotti (2009) reported that coevolution has enhanced the preponderance of such secondary metabolites as alkaloids, saponins, and polyphenols in many plants. Incorporation of plants endowed with these potent secondary metabolites in the diet has been strongly recommended, because of their immense health promoting potentials (Polimati et al., 2022). For example, it was found that flavonoids, terpenoids and saponins have diverse biological actions such as cardioprotective, hepatoprotective, anti-inflammatory, antibacterial, antioxidant and anti-tumour (Huang et al., 2021; Perekh et al., 2024). *Lagenaria breviflora* Roberty also called 'Christmas melon' in English, 'Ahenyi,' 'Egusi ohia,' 'Ahu maa' (spiritual melon), and 'Ndise ekpo' in Nsukka (Enugu), Abia, Ebonyi and Akwa Ibom States of Nigeria respectively. The plant is cultivated in warm climates like Ghana and Kenya (Olakojo et al., 2024) but in Nigeria, it grows luxuriantly as weed and is majorly harnessed by the local people as medicine for humans and farm animals in many states. *Lagenaria breviflora* is one of the efficacious plants that is used in herbal medicine as an alternative antibiotic and anti-viral remedy in poultry disease management and control (Adeyemi et al., 2017; Olakojo et al., 2024). Ajani et al. (2015) evaluated the leaf of *Lagenaria breviflora* Roberty and reported that it contains flavonoids, tannins, phenolics, glycosides, saponins, alkaloids and terpenoids. Also, Adeyemi et al. (2017) reported the presence of oxalate, carotenoid, phytate, flavonoids, alkaloids, saponins, terpenoids, phenols and tannins in the fruit. Olakojo et al. (2024) evaluated the antibacterial activity of Christmas melon fruit extract on birds and reported that it significantly inhibited the growth of *Salmonella spp* colony in the gut and also reversed hepatic and renal hepatomegaly, hepatic parenchymal infiltration, hepatic necrosis, nephritis and tubular necrosis of the kidney, which accompany bacterial infections. Also, Ekunseitan et al. (2023) reported that *Lagenaria breviflora* whole fruit extract improved antioxidant capacity in meat broilers.

Although Olakojo et al. (2024) reported that methanolic extract of whole fruit of *Lagenaria breviflora* is a potential antibacterial nutraceutical, conflicting research reports on its protective effects on the liver and the kidney formed the basis of this research.

Balogun et al. (2014) reported severe damage to the liver ultrastructures in Wistar rats administered high dose of extract from sun – dried of *Lagenaria breviflora* fruit and ethanol extract of *Lagenaria breviflora* fruit. Also, Folorunsho et al. (2019) reported abnormal glomeruli with proliferated mesangial cell without capsular space, attenuated renal tubule and convoluted tubules with brush border in Wistar rats given high dose of methanol extract of whole *Lagenaria breviflora* fruit, indicating renal dysfunction. Inferences from earlier studies indicate that processing methods coupled with high dose and prolonged duration of administration could lead to organ damage due to loss of, or reduction in the concentration of some potent phytochemical components of the plant part(s).

For instance, Banjo et al. (2013) reported reduced concentrations of flavonoids, terpenoids, tannins, and absence of saponin and cynogeninoglucoside in extract of sun – dried *Lagenaria breviflora* fruit. In another study, Adepegba and Abu (2016) reported absence of flavonoids, phenols, tannins and anthraquinone in sun – dried, oven – dried, and hot water – soaked *Lagenaria breviflora* fruit.

Since research report on the crude extract of *Lagenaria breviflora* fruit, which appears to have the potential of retaining maximum concentrations of all the phytochemical constituents is rare, this research was designed to evaluate its safe dose margin in broiler chickens. Thus, this study seeks to assess the health status of broiler chickens administered crude extract of *Lagenaria breviflora* fruit. Some liver and kidney function markers as well as antioxidant markers, which are usually used as health indicators were evaluated in the experimental birds. The result of this study is intended to provide useful information to poultry farmers, who would want to use the crude fruit extract of *Lagenaria breviflora* fruit to raise broiler chickens.

Materials and methods

Ethical approval

Approval for this research was granted by the research and animal welfare committee of the College of Animal Science and Animal Production, Michael Okpara University of Agriculture, Umudike, reference number – 0016/ 003/002025/ APH. The animal welfare guidelines for handling experimental birds were strictly followed.

Location of the study

This experiment was conducted at the poultry facility of the College of Animal Science and Animal Production, Michael Okpara University of Agriculture, Umudike, Abia State.

Source and management of the birds

One hundred and twenty (120) broiler chickens raised from day-old were used for the study. The birds were sourced from a commercial farm in Nigeria and raised in deep litter under standard conditions. Broiler starter diet containing 23.11 crude protein and 2862.95 kcal/kg metabolizable energy, and a finisher diet containing 20.13 crude

protein and 2907.15 kcal/kg metabolizable energy (Table 1) were offered freely from 0-4 wk and 5-8 wk of age respectively. The birds were duly vaccinated as recommended by the National Veterinary Research Institute, Nigeria.

Table 1: Ingredients in the diet

Ingredients (%)	Starter	Finisher
Maize	48.00	55.50
Soybean meal	30.00	26.00
Wheat offal	3.00	3.00
Palm kernel cake (PKC)	10.20	10.20
Lysine	0.20	0.20
Methionine	0.10	0.10
Bone meal	5.00	2.00
Fish meal	3.00	3.00
Salt	0.25	0.25
Vitamin/mineral premix	0.25	0.25
Total	100.00	100.00
Calculated Composition		
Crude protein (CP) (%)	23.11	20.13
Metabolizable energy (ME) (Kcal/kg)	2862.95	2907.15
Crude fibre (%)	4.05	5.01
Ether (%)	4.17	5.45

*Sourcing/ identification of the *Lagenaria breviflora* fruit*

Fresh *Lagenaria breviflora* fruits were harvested at Michael Okpara University of Agriculture premises and identified by a botanist in the Department of Plant Science and Biotechnology, Michael Okpara University of Agriculture, Umudike.

*Preparation of the crude extract of *Lagenaria breviflora* fruit*

One and half kilograms (1.50 kg) of the fruits was weighed out with an electronic balance (XY2000 - 2C, PEC MEDICAL, China), washed with clean water and sliced into pieces with a sharp kitchen knife, and then blended with an electric blender to form the extract. The extract was purred into a clean 0.075 mm mesh sized sieve to remove chaffs. The sieved extract was transferred into a clean 1 litre plastic container and stored in a refrigerator at 4 °C. Fresh preparations were made after every 4 d till the end of the experiment.

Administration of LBFE

Administration of the fruit extract started at wk 3 of age. Daily doses of 0.00, 2.50, 5.00 and 7.50 mL of the *Lagenaria breviflora* fruit extract were drawn with a 10 mL syringe and added to 4 litres of the drinking water, and administered to D₁- D₄ respectively for 42 d.

Experimental design

The experiment was arranged in 4 equal treatments (D₁-D₄) in a completely randomized design (CRD). Each treatment had 30 birds in 3 replicates with 10 birds. The experimental model is as shown below:

$$Y_{ij} = \mu + D_i + e_{ij}$$

Where

Y_{ij} = Single observation of j^{th} observation on the i^{th} treatment.

μ = Overall mean

D_i = Effect of the i^{th} level of treatment

e_{ij} = Error

Data Collection

Blood sampling and analysis

At the end of the experimental period of 8 wk, 6 birds were randomly picked from each treatment, 2 birds per replicate for serum biochemistry. Blood collection was done by properly restraining the birds. The feathers around the neck region were parted

with the right thumb. The jugular vein was occluded at the base of the neck. With a 5 mL syringe, two milligrams of blood was drawn from the jugular vein of the bird and dropped in ethylenediaminetetracetic acid free sample bottles. Blood collection was done between 8.00 am and 10.00 am in the morning. The blood samples were kept for 30 min to clot and then centrifuged to harvest the serum. Randox kits (Randox Co. UK) were purchased and the procedures strictly followed during the laboratory analysis. Alanine aminotransferase, aspartate aminotransferase, and alkaline phosphatase levels and bilirubin level were assayed with Randox kit. Total protein (direct Biuret method) and albumin were assayed using Randox kit. Globulin was determined as described by (Colville, 2002). Urea, creatinine, Sodium ion, potassium ion, chloride ion and bicarbonate ion were assayed with specific Randox kit. Total cholesterol, triglyceride and high density lipoprotein cholesterol were assayed with a photospectrometer (Mindray, India) following Randox kit procedures. Low-density lipoprotein and very low-density lipoprotein cholesterol fractions were determined according to Friedwald's equation. The activities of superoxide dismutase (SOD), Catalase (CAT), Glutathione peroxidase (GPx), and Malondialdehyde (MDA) level were analysed with commercial Randox kits. Histological procedures for processing the liver and kidney sections were done as described by Ezike et al. (2023). The slides were

captured with a digital camera (Motic image camera Moti-Cam) connected to a laptop computer at x 200 magnification.

Data analysis

Data collected were analysed with one-way analysis of variance (ANOVA), using SPSS software package (Version 16.00). Significant means were separated using Duncan's Multiple Range Test (Duncan, 1955). Significantly different means were accepted at 5% confidence levels.

Results

Liver function markers of broiler chickens administered LBFE

Significant ($p < 0.05$) differences were observed only in total protein (TP), albumin (Ab) and globulin (Gb). TP and Ab were highest (4.55 g/dl) and (2.48 g/dl) in birds administered 7.50 mL of the LBFE compared to the other groups. Gb was significantly higher (2.07 g/dl) and statistically the same in bird on 5.00 and 7.50 mL of the fruit extract compared with the other treatments. ALT, AST, ALP activities and total bilirubin concentration did not differ significantly between the birds administered LBFE and the birds in the control group (Table 2).

Table 2: Liver function markers of broiler chickens administered LBFE

Parameters	D ₁	D ₂	D ₃	D ₄	SEM	p-value
Total protein (g/dl)	3.35 ^c	3.51 ^c	4.19 ^b	4.55 ^a	0.15	0.00
Albumin (g/dl)	1.77 ^c	1.85 ^c	2.15 ^b	2.48 ^a	0.88	0.00
Globulin	1.58 ^b	1.66 ^c	2.04 ^a	2.07 ^a	0.30	0.01
ALT (u/l)	29.33	28.00	29.00	26.67	0.46	0.16
AST (u/l)	41.33	40.67	41.00	41.33	0.60	0.98
ALP (u/l)	67.67	68.33	67.00	64.33	0.89	0.45
Bilirubin (mg/dl)	0.53	0.49	0.48	0.49	0.01	0.37

abc Means along the same row with different superscripts are significantly ($p < 0.05$) different. ALT= alanine aminotransferase, AST = aspartate aminotransferase, ALP = alkaline phosphatase. SEM= Standard Error of Mean.

Kidney function markers of broiler chickens administered LBFE

Significant ($p < 0.05$) differences were observed only in concentrations of sodium ion (Na^+) and chloride ion (Cl^-) in the treated groups. Na^+ concentration was significantly higher⁺ (129.27 mEq/L) and statistically the same in the treated birds

than the control birds (124.40 mEq/L). Cl^- was significantly higher (82.03 mEq/L) in birds administered 7.50 mL of the LBFE compared with the other treatments (77.27 mEq/L). Urea, creatinine, potassium and bicarbonate ions did not differ ($p > 0.05$) between the control birds and the birds administered LBFE as shown in Table 3:

Table 3: Kidney function markers of broiler chickens administered LBFE

Parameters	D ₁	D ₂	D ₃	D ₄	SEM	p-value
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Urea (mg/dl)	11.06	11.21	10.02	10.57	0.20	0.15
Creatinine (mg/dl)	1.07	1.01	0.98	1.02	0.02	0.22
Na ⁺ (mEq/L)	124.40 ^b	128.57 ^a	129.27 ^a	130.60 ^a	0.82	0.01
K ⁺ (mEq/L)	4.55	4.72	4.74	4.69	0.03	0.15
Cl ⁻ (mEq/L)	77.27 ^b	77.57 ^b	78.20 ^b	82.03 ^a	0.74	0.05
HCO ₃ ⁻ (mMol/L)	18.70	18.67	18.80	18.70	0.05	0.88

^{a-b} Means along the same row with different superscripts are significantly ($p < 0.05$) different. Sodium ion Na⁺ = Sodium ion, K⁺ = Potassium ion, Cl⁻ = Chloride, SEM= Standard Error of Mean.

Lipid profile of broiler chickens administered LBFE

Total cholesterol, triglyceride, and low-density lipoprotein - cholesterol, and very low - density lipoprotein cholesterol, were significantly affected ($p < 0.05$). Total cholesterol (98.37 mg/dl), triglyceride (72.20 mg/dl), low - density lipoprotein- cholesterol

(12.23 mg/dl) and very low - density lipoprotein cholesterol (14.31 mg/dl) were significantly lower in birds administered 5.00 and 7.50 mL of the LBFE than those in the other groups. High - density lipoprotein cholesterol concentration was the same between the birds in control and the birds administered LBFE (Table 4)

Table 4: Lipid profile of broiler chickens administered LBFE

Parameters	D ₁	D ₂	D ₃	D ₄	SEM	P-value
Cholesterol (mg/dl)	125.50 ^a	121.53 ^a	94.93 ^b	98.37 ^b	4.14	0.000
Triglycerides (mg/dl)	77.07 ^a	75.80 ^a	69.43 ^b	72.20 ^b	0.99	0.001
HDL-C (mg/dl)	66.80	67.67	71.07	71.83	1.00	0.203
LDL-C (mg/dl)	43.28 ^a	38.71 ^a	10.03 ^b	12.23 ^b	4.62	0.000
VLDL-C (mg/dl)	15.42 ^a	15.16 ^a	13.84 ^b	14.31 ^b	0.21	0.002

Means along the same row with different superscripts are significantly ($p < 0.05$) different. SEM= standard error of mean, HDL-C = high density lipoprotein cholesterol, LDL-C low-density lipoprotein cholesterol, VLDL-C, very low density lipoprotein cholesterol

Antioxidant parameters of broiler chickens administered LBFE

Gglutathione peroxidase (GPx) (41.30 μ /l), and superoxide dismutase (SOD) (31.13 μ /L) were significantly higher and statistically the same in birds administered LBFE than those in the control.

Catalase concentration was higher and statistically the same ((25.10 μ /l)) in birds on 5.00 and 7.50 mL LBFE than the birds in the other groups. The activity of malondialdehyde did not vary between the control birds and the treated birds as shown in Table 5 below:

Table 5: Antioxidant parameters of broiler chickens administered LBFE

Parameters	D ₁	D ₂	D ₃	D ₄	SEM	p-value
GPx (u/l)	34.47 ^b	37.90 ^{ab}	41.77 ^a	41.30 ^a	1.03	0.01
SOD (u/l)	25.77 ^b	29.20 ^{ab}	30.90 ^a	31.13 ^a	0.83	0.05
CAT (u/l)	17.67 ^c	22.50 ^b	25.13 ^a	25.10 ^a	0.96	0.00
MDA (mMol/L)	0.28	0.26	0.23	0.25	0.01	0.20

Means with different superscripts in a row are significantly different ($p < 0.05$). SEM= standard error of mean, GPx = glutathione peroxidase, SOD = superoxide dismutase, CAT= catalase, MDA= malondialdehyde.

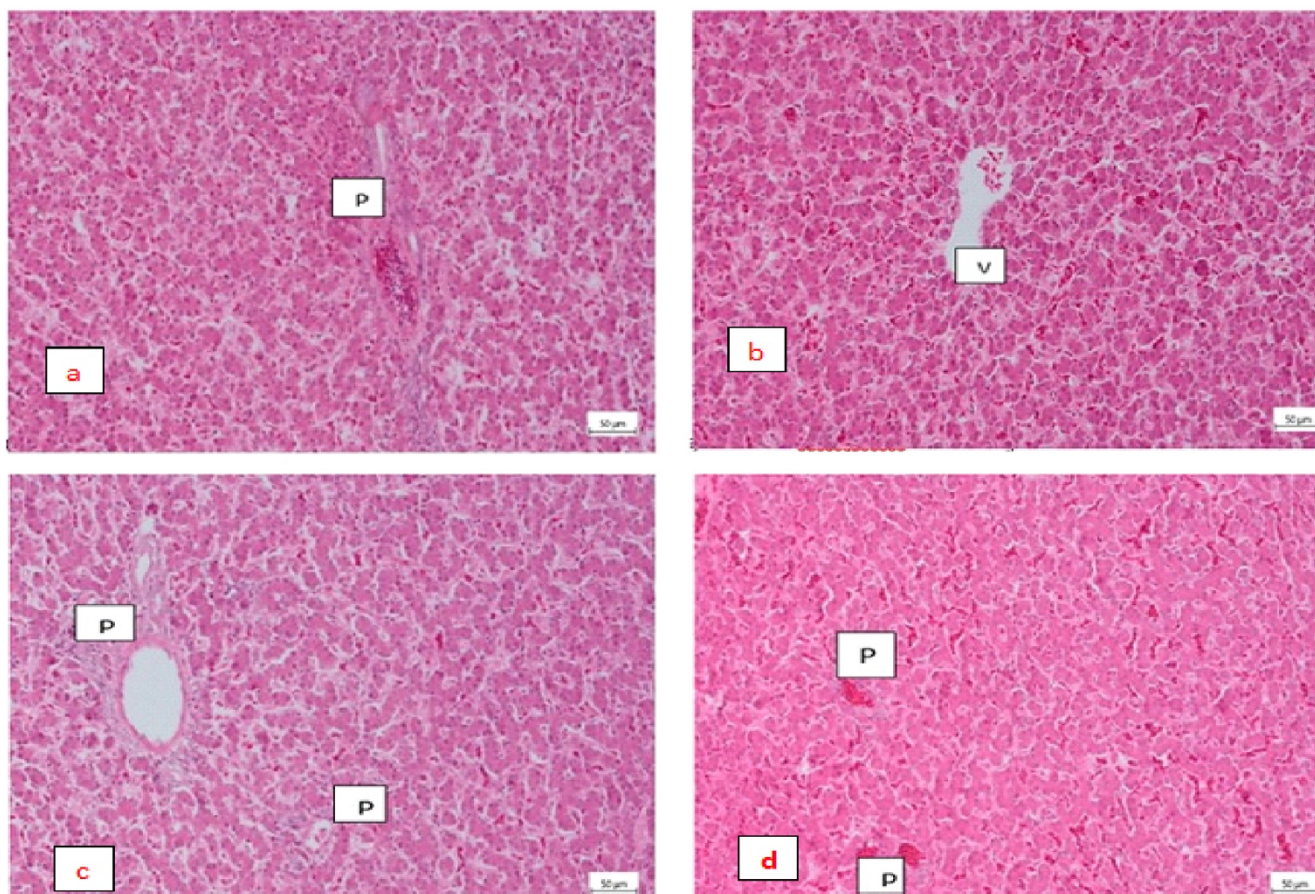


Figure 1: Photomicrographs (a-d) of the liver sections of broiler chickens administered LBFE **(a)** Liver section of broiler chickens administered 0.00 mL of crude *Lagenaria breviflora* fruit extract shows normal hepatic histomorphology. Hepatocytes arranged in two-cell thick interconnecting cords were observed. The normal structures of the portal triads were also observed. Portal area (P). HE x 200 **(b)** Liver section of broiler chickens administered 2.50 mL of crude extract of *Lagenaria breviflora* fruit shows normal hepatic histomorphology. Hepatocyte arranged in two-cell thick interconnecting cords were observed. The normal structures of the portal triads were also observed. Interconnecting cords were observed. Central vein (V), Portal area (P). HE x 200 **(c)** Liver section of broiler chickens administered 5.00 mL of crude extract of *Lagenaria breviflora* fruit shows normal hepatic histomorphology. Hepatocytes arranged in two-cell thick interconnecting cords were observed. The normal structures of the portal triads were also observed. Portal triad (P). HE x200 **(d)** Liver section of broiler chickens administered 7.50 mL of crude extract of *Lagenaria breviflora* fruit shows normal hepatic histomorphology. Hepatocytes arranged in two-cell thick interconnecting cords were observed. The normal structures of the portal triads were also observed. Portal triad (P). HE x 200.

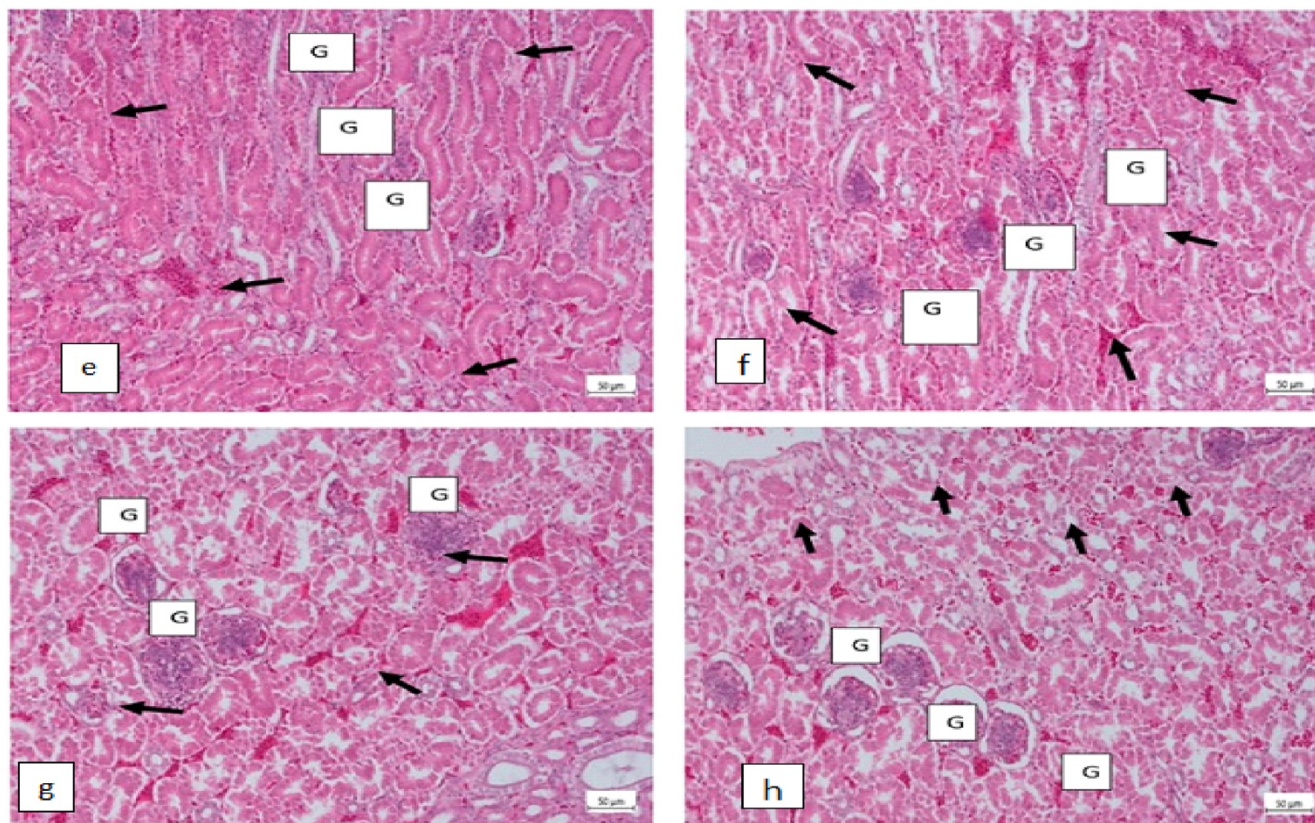


Figure 2: Photomicrographs (e-h) of the histological sections of the kidney of broiler chicken administered LBFE **(e)** Kidney section of broiler chickens administered 0.00 mL of crude extract of *Lagenaria breviflora* fruit shows the normal renal histomorphology. Glomeruli (G). Renal tubules (arrow). HE x200 **(f)** Kidney section of broiler chickens administered 2.50 mL of crude extract of *Lagenaria breviflora* fruit shows the normal renal histomorphology. Glomeruli (G). Renal tubules (arrow). HE x200. **(g)** Kidney section of broiler chickens administered 5.00 mL of crude extract of *Lagenaria breviflora* fruit shows the normal renal histomorphology. Glomeruli (G). Renal tubules (arrow). HE x 200 **(h)** Kidney section of broiler chickens administered 7.500 mL of crude extract of *Lagenaria breviflora* fruit shows the normal renal histomorphology. Glomeruli (G). Renal tubules (arrow). HE x 200.

Discussion

The ALT values recorded in this study across the treatments ranged from 26.67-29.33 IU/L, which is higher than the 20.67-44.33 and 18.00-21.98 IU/L reported by Kareem et al. (2024) and Ijioma et al. (2024) in Cobb 500 and ROS 308 finisher broiler chickens fed diets of different particle sizes supplemented with multienzyme and dietary West African black pepper respectively. AST values we recorded across the treatments were slightly higher than the 30.00-38.67 IU/L reported by Ijioma et al. (2024) but lower than the 119.67-162 IU/L in broiler chickens fed multienzyme and the ≤ 330 IU/L reference value reported for broiler chickens by Kareem et al. (2024). The ALP values recorded in our study were higher than the 20-50 IU/L reference range reported by Kareem et al. (2024). The reason for the differences between our results and of Ijioma et al. (2024) and Kareem et al. (2024) could be due to variations in season, breed, and nutrient composition of the diet (Nanbol et al., 2016). The non-significant differences in ALT, AST and ALP

levels observed in birds administered LBFE is in support of Ajani et al. (2014), Nworgu et al. (2018) and Iriboje and Olufayo (2023) on Wistar rats and broiler chickens respectively. This result suggests that the doses of LBFE used in this study did not disrupt the biological functions and regulatory roles of the liver. This agrees with Henry (2001) who reported that absence of any increases in ALT, AST and ALP activities above the normal range indicate absence of liver injury.

The observed increases in serum total protein, albumin and globulin in birds given (7.50 mL) of LBFE appear to be a direct positive effect of the active compounds in LBFE on hepatic function, especially on protein synthesis. The increase in globulin fraction in birds on the highest dose (7.50 mL) also indicates more immune stimulation. Similar observation was made on Wistar rats administered *Lagenaria breviflora* fruit extract (Saba et al., 2010).

The increase in total protein levels as dose increases across the treatments suggests that the LBFE did not

inhibit protein biosynthesis in the treated birds. Since it was postulated that increase in total protein augments the buffering capacity of the blood (Limbach et al., 2021), it implies that 7.50 mL of LBFE had more effect on the buffering capacity of the blood.

The non-significant increase in total serum bilirubin concentration recorded in birds administered LBFE in our study suggests intact functional integrity of the liver. This result is in agreement with Yang et al. (2021) who reported that increases in serum total bilirubin concentration indicates interference with the process of getting rid of ammonia from amino acid catabolism in the liver. This result shows that the potent compounds in the LBFE did not interfere with ammonia removal by the liver. The non-significant increases in urea, creatinine and potassium levels in birds administered LBFE indicate intact functional integrity of kidney. Our result is consistent with Sunmonu and Oloyede (2006) who reported that urea, potassium and creatinine levels in the serum are used to evaluate nephrotoxicity at glomerular and tubular levels. Sunmonu and Oloyede (2006) reported that increase in the levels of urea and creatinine above the normal range in the blood indicate renal dysfunction. Ighodaro and Omole (2010) reported that decrease in serum potassium ion concentration potentiates hypotonia and cardiac arrest.

Our result supports Ajani et al. (2015) who also found no nephrotoxicity in Wistar rats given high dose of ethanolic *Lagenaria breviflora* fruit extract. Our finding contrasts those of Balogun (2014) who reported liver and kidney damages in Wistar rats administered extract of sun-dried of *Lagenaria breviflora* fruit. The significant increase in Na^+ concentration in birds administered LBFE in our study appears to indicate a normal relative blood water to Na^+ ratio, which is an indication of normal function of the nephrons and enhanced membrane integrity of the kidney cells. This is so because Yang et al. (2021) reported that an increase in sodium (Na^+) ion concentration, which is accompanied by balanced water intake is a strong indicator of improved osmotic regulation. In our study, water was offered *ad libitum*, and the common salt content of the starter and finisher diets did not exceed the recommended 0.25% for broiler chicks and finisher broiler chickens. Since the birds were not dehydrated, the increase in sodium ion concentration in the treated birds could be attributed to a direct effect of the LBFE. This supports the report of Ajani et al. (2015) who found increased sodium concentration in Wistar rats administered high dose of ethanolic *Lagenaria breviflora* fruit extract. Since Browse (2005) also found a positive correlation between Na^+ reabsorption and Cl^- reabsorption, while Cl^- is associated with HCO_3^- excretion (Folorunsho et al., 2019). Glenn (2016) also reported that an increase in serum Chloride ion without a corresponding increase in bicarbonate ion

concentration could indicate that the renal tubules have the capacity to handle the excess Chloride ion load to prevent hyperchloremic metabolic acidosis, which occurs when bicarbonate fails to compensate. Thus, the significantly higher Cl^- concentration in birds on highest dose (7.50 mL) of LBFE without a corresponding, increase in HCO_3^- concentration could be an indication of normal tubular and glomerular functions. It has also been reported that decreases in Cl^- and HCO_3^- concentrations below the normal range suggest tubular and glomerular dysfunction (Chawla, 1999). In our study, Cl^- did not decrease with dose, while HCO_3^- did not differ between the control and the treated birds, which appears to indicate normal tubular and glomerular functions.

The significantly lower serum total cholesterol, triglyceride, low and very-low density lipoprotein cholesterol in birds administered 5.00 and 7.50 mL of the LBFE contrasts the report of Saba et al. (2010) on Wistar rats administered higher dose of the crude extract of *Lagenaria breviflora* fruit. Ekunseitan et al. (2019) reported that *Lagenaria breviflora* contains saponins, which have strong hypocholesterolemic and hypolipidaemic effects. The decreases in cholesterol, triglyceride, low density and very-low density lipoprotein cholesterol with increase in dose in our study could be attributed to higher concentration of saponins, alkaloids and flavonoids, which bind bile with cholesterol in the intestinal wall, causing a reduction in serum lipid levels and preventing its reabsorption into the blood stream (Adeyemi et al., 2017). The decrease in triglyceride concentration in our study could also be due to a reduction in lipoprotein lipase in the heart muscle, resulting in fewer uptake of triglyceride from the blood stream (Kareem et al. (2009). Higher reduction in lipid profile with increase in the quantity of flavonoids has also been recorded (Sharma et al., 2007).

The increases in SOD and CAT levels we recorded were also reported by Ajani et al. (2014) but with reduced MDA in Wistar rats. The absence of significant variation in MDA in birds administered LBFE compared to the control birds indicates that the LBFE did not induce lipid peroxidation in the treated birds. The significant increases in SOD and CAT corroborate earlier report of Ekunseitan et al. (2023) that *Lagenaria breviflora* is rich in antioxidants.

In this study, histological sections of the liver shows the normal hepatic histomorphology across the treatments. The hepatocytes are arranged in two-cell thick interconnecting cords with normal structures of the portal triads. This indicates absence of hepatic dysfunction, contrasting those of Balogun et al. (2014) who reported severe damage to the liver architecture in Wistar rats administered high dose of sun – dried, ethanolic extract of Christmas melon fruit. The disparity between our results and

those of Balogun et al. (2014) could be due to the processing method, which affected the concentrations of phytochemical constituents of *Lagenaria breviflora* fruit. Banjo et al. (2013) reported reduced concentrations of flavonoids, tannins and terpenoids and absence of saponin and cynogenenic glucoside in methanolic extract of sun-dried and pulverized *Lagenaria breviflora* fruit. Also, Adepegba and Abu (2016) reported absence of flavonoids, tannins, anthraquinone and phenols in extracts of sun-dried, oven-dried and hot water-soaked *Lagenaria breviflora* fruit. Thus, reduced concentration or complete elimination of these secondary metabolites, especially flavonoids terpenoids and saponins, which have anti-inflammatory, antioxidant, and hepatoprotective effects could have led to increased reactive oxygen species (ROS) production and the liver damage reported in those studies. In this study, crude *Lagenaria breviflora* fruit extract was used, which appears to suggest that the concentrations of flavonoids, terpenoids and other potent compounds that inhibit cellular path ways that induce inflammation and oxidative stress were high, resulting in the improved liver functional and its structural integrity we recorded in our study. Thus, the improved hepatic function recorded in this study across the treatments, which are evident in absence of increases in ALT, AST, ALP activities, bilirubin, and increases in total protein, albumin and globulin levels in the treated birds compared to the control birds could be due to the preponderance of flavonoids, terpenoids and other pro-liver health compound in the crude extract of *Lagenaria breviflora* fruit. Our result is consistent with Al-Garadi et al. (2022) who reported that lowered ALP, ALT, AST, bilirubin, and increase in total protein are indicators of minimal inflammation and hepatocyte damage. Our result suggests that the levels of the crude extract *Lagenaria breviflora* fruit we administered did not cause liver damage with respect to the liver health indicators we measured.

In this study, photomicrographs of the kidney sections show the normal renal histomorphology, with intact glomerular and renal tubules across the treatments, indicating absence of renal dysfunction across the treatments. In contrast, Folorunsho et al. (2019) observed abnormal glomeruli with proliferated messengial cells without capsular space, attenuated renal tubule and convoluted tubules with brush border in Wistar rats administered higher dose of whole fruit extract of *Lagenaria breviflora*, indicating renal dysfunction due to toxic substances. The difference between our result and those of Folorunsho et al. (2019) could be due to variations in dosage and processing methods used. Since no specific compound in *Lagenaria breviflora* fruit was implicated for the elevated kidney marker enzymes, altered electrolyte balance, and induced renal and tubular damage that were reported by Ajani et al. (2015) and Folorunsho et al. (2019), the kidney

damage they reported could be associated with losses of some phytochemicals in the cause of processing their *Lagenaria breviflora* fruit extract. Flavonoids, saponins and terpenoids, which are found in the leaf and fruit of *Lagenaria breviflora* (Ajani et al., 2015; Adeyemi et al., 2017) have been reported to improve kidney function by acting as antioxidant and anti-inflammatory agents, through inhibition of reactive oxygen species (ROS) production in tissue cells (Cao et al., 2022). On the other hand, Gajender, et al. (2023) reported that higher doses of flavonoids in excess of the body's requirements could also lead to organ damage. Thus, it appears that the doses used in this study were within tolerable limit that did not interfere with the physiological equilibrium of ROS. This result is in line with Sharifi-Rad et al. (2020) who reported that curcumin switched from being an antioxidant to a pro-oxidant at high dose, and modified an antioxidant enzyme (thioredoxin reductase), resulting in ROS production. This dose-dependent inter conversion from antioxidant to pro-oxidant in biological systems might not be limited to curcumin, but a general characteristic of most potent phytobiotics that contain antioxidant compounds. The result obtained in this study could be attributed to safe doses and a good processing method, which harnessed maximum phytochemical constituents in the *Lagenaria breviflora* fruit extract for the improved antioxidant capacity, liver and kidney function markers we recorded in the birds.

Conclusion/ Recommendation

Based on our result it was concluded that 2.50, 5.00 and 7.50 mL of LBFE did not cause derangement in the liver and kidney function markers, lipid profile and the antioxidant markers we measured as health indicators. Also, these doses did not alter the liver and kidney histologic architecture. Thus, up to 7.50 mL of LBFE appear to be safe and are recommended for use in broiler chickens. Our study has shown that crude extraction of *Lagenaria breviflora* whole fruit is a vital agricultural biotechnology that will offer a product that is cheap, sustainable, eco-friendly as well as a natural alternative that contains potent phytochemicals that could improve biological functions in broiler chickens.

Acknowledgement

We sincerely acknowledge the assistance of the Department of Animal Breeding and Physiology, Michael Okpara University of Agriculture, Umudike, for providing the facilities for this research.

Conflicts of Interest

There are no conflicts of interest.

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