

Hepato and nephroprotective effects of aqueous extract of *Citrullus lanatus* (Linn.) juice in gentamicin-overdosed commercial broiler chickens

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Abstract This study evaluated hepato- and nephroprotective potentials of *Citrullus lanatus* juice (CLJ) on gentamicin-overdosed broilers. Eighty broilers were used for this experiment; fifty broilers were used to confirm gentamicin toxic dose with liver function assay. While thirty broilers were reared from day old to thirty-five days, randomly distributed into six groups with five birds per group. Broilers in group A received 1.0mL/kg normal saline; group B received 45mg/kg gentamicin intramuscularly; group C received 200mg/kg Vitamin C orally, groups D, E, and F received 100, 200, and 400mg/kg of CLJ respectively orally for 14 days later received single dose 45mg/kg gentamicin intramuscularly. Statistical analysis was performed to assess significant differences among groups. Body weights were compared on days 35 and 49. Blood chemistry for liver and kidney functions were examined, liver and kidney pathologies were evaluated at day 49. There was significant increase in body weight of 400-treated birds unlike decreased body weight in GM-treated birds at day 49. There were significant decrease in Na⁺, Creatinine and Uric acids but increase in K⁺ in 400 mg/kg CLJ-treated birds compared with gentamicin-treated birds. There were liver and kidney pathological damages in gentamicin-treated birds. These impairments were resolved in 400 mg/kg CLJ-treated birds. The hepatoprotection and nephroprotection may be due to ascorbic acids and flavonoids present in CLJ. This may have provided antioxidant effects for restoration of liver and kidney pathological changes in 400 mg/kg CLJ-treated birds. This study shows that 400mg/kg CLJ may improve body weight and reversed gentamicin-overdose damages in liver and kidney of broilers.

Keywords: *Citrullus lanatus*, Gentamicin, Hepatoprotective, Nephroprotective, Broilers.

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1.Introduction

Gentamicin sulfate is an aminoglycoside antibiotic that is commonly used in poultry production. Gentamicin residue arising from overdose in poultry products is of great public health importance worldwide, which can lead to antibiotic resistance if it is not controlled. Gentamicin residue and or overdose can be associated with health risks in clinical cases such as hepatotoxic, nephrotoxic, and ototoxic effects [1]. The damages seen in kidney due to abuse of gentamicin in poultry industry in our community leading to nephrotoxicity requires great attention [2]. Gentamicin overdose can cause metabolic and nutritional abnormalities, hepatotoxicity, kidney failure as well as cancer leading to death.

Some studies have revealed that drug-induced toxicity had resulted in major causes of renal injury [3]. Gentamicin is normally used for treatment of different poultry diseases such as chronic respiratory disease, fowl cholera, salmonellosis to mention but a few [4,5] Gentamicin can be used to prevent early chick mortality due to bacterial infections by administration of gentamicin in day-old chicks. Although, in day old

chicks there are no observable effects of gentamicin at a dose of 10 mg/kg body weight, but, at higher dose different pathological changes such as enlarged and swollen kidneys, swollen liver, decreased serum total protein, albumin and higher concentrations of serum alanine aminotransferase (ALT), creatinine and urea were observed in dose dependent manners [6,7].

Serum electrolytes such as increased sodium, potassium and chloride are indication of nephrotoxicity which can be resolved with fruit juice [8,9]. Creatinine is produced in liver and it is breakdown to creatine phosphate in muscle. Creatinine is a good indicator of kidney damage [10]. Creatinine is removed mainly through the kidney, and the concentration in biological system is altered by various muscle toxicants or decreased muscular activity [11]. An increase in serum creatinine concentration is a biomarker of nephrotoxicity [12]. Uric acid is the final oxidative product of purine metabolism and is ready excreted by the kidney [13]. Thus, increased serum uric acid levels are seen in patients with reduced glomerular filtration rate (GFR) as well as in kidney failure [10].

Overdose of gentamicin causes nephrotoxicity arising from fat accumulation seen in renal proximal convoluted tubules could have led to loss of brush border integrity [14]. Although it is technically absurd to observe some farmers doing co-administration of gentamicin with killed vaccines for the treatment and prevention of bacterial infections. Major causes of kidney disease in birds are infectious nephritis, vitamins A and or C deficiency, heavy metal toxicity, and kidney tumor. This normally manifest with elevated blood uric acid in kidney disease when there is abnormality in the integrity of the urinary tract or blockade of urine flow [15]. Increased serum uric acid levels are a main risk factor for renal failure, which is the most nitrogenous metabolic product in birds [16, 17].

Measurement of blood creatinine concentration in mammals is used to evaluate the glomerular filtration rate [15]. Creatinine value is a vital test that can be used for examination of renal failure in birds [18]. This situation can arise due to increase in use of drugs, chemical toxins, and microbial agents, which can cause increase in serum biochemical indicators [15,19, 20].

Plants have been used for treatment and prevention of hepatotoxicity and nephrototoxicity in domestic animals [21]. The use of plants for treatment can serve as future approach to ethnopharmacology research and clinical sciences exploiting the knowledge of phytochemicals present in plants [22]. Watermelon also known as *Citrullus lanatus* fruit is a flowering plant belonging to the family, *Cucurbitaceae*. *Citrullus lanatus* seed has been shown to possess hepatoprotective effect in Wistar rats [23] and cardioprotective effects in rats [24]. *Citrullus lanatus* juice is rich in beta-carotene, vitamin C, and lycopene, which counteract free radicals effect in the body [25, 26]

Watermelon is a fruit with a juicy pulp that is red or pink with many seeds, the fruit is consumed worldwide due to the wellbeing benefits. Watermelon fruit contains water (91%) and sugar (6%), and is low in fat. Watermelon fruit has been identified as a good source of vitamin C [27]. The fresh watermelon juice contains 3.72 mg/100 g of Vitamin C in watermelon which varies with the difference in watermelon cultivars and environmental factors [28].

Vitamin C is found in Citrus fruit, vegetables and adrenal glands as hexuronic acid as a natural source. It is an essential nutrient that cannot be synthesized by the human body therefore, it has to be incorporated in the body through diet [28]. Vitamin C is a water-soluble essential nutrient that is frequently added to a variety of food products for nutrient enhancement and supplementation important for biosynthesis of collagen and certain hormones [29, 30]. Ascorbic acid is also an anticancer agent [31].

This study evaluated the hepatoprotective and nephroprotective potentials of *C. lanatus* juice in commercial broilers with gentamicin overdose.

2. Materials and Methods

2.1 Fruit collection and identification of *C. lanatus* fruit.

The *C. lanatus* fruit was collected from a fruit market in Alabata area very close to Federal University of Agriculture, Abeokuta. The *C. lanatus* fruit was deposited for authentication in the Herbarium unit of the Botany Department, University of Ibadan, Oyo State Nigeria and the voucher number UIH 22872 was issued.

2.2. Preparation of aqueous extract of *C. lanatus* Juice (CLJ).

The aqueous extract of CLJ was prepared from whole *C. lanatus* fruit while the rind and seeds were carefully removed. The CLJ was blended while water was removed using rotary evaporator until a

constant weight of aqueous extract of CLJ was obtained. This was done in the laboratory, Department of Veterinary Physiology and Biochemistry, Federal University of Agriculture, Abeokuta. The aqueous extract of CLJ was prepared according to the procedure previously described by Sofowora [32], it was stored in refrigerator at 4°C until it was used.

2.3 Ethical statement

This research was carried out with the approval of the ethical committee in the College of Veterinary Medicine, Federal University of Agriculture, Abeokuta, Nigeria with approval number FUNAAB / COLVET / CREC / 2021/09/01.

2.4. Experimental design: Birds Management

A total of Eighty (80) day-old commercial Arbor Acre broiler chicks were acquired from a reputable hatchery in Ibadan. The birds were kept in Poultry section of Teaching and Research Farm, College of Veterinary Medicine, Federal University of Agriculture Abeokuta, Ogun State, Nigeria, with longitude of 3°25'29"E, latitude of 7°13'27"N and an altitude of 76m above sea level. This is located in the tropical rainforest vegetation zone of South-Western Nigeria. The experimental site was a humid climate with mean annual rainfall of about 1037 mm, mean humidity and temperature of 83 % and 24.7 °C, respectively.

This experiment was divided into two studies, study one (1) was used to confirm the toxic dose of gentamicin with 50 pieces of day old broilers randomly distributed into 5 groups with 10 birds per group were observed for clinical signs, mortality twice daily for 28 days and evaluation of the liver function enzymes (Aspartate aminotransferase, AST; Alanine aminotransferase, ALT and Alkaline phosphatase, ALP). These groups were administered the following doses: 0, 40, 50, 60 and 80mg/kg body weight of gentamicin respectively according to the study of Javed [5] modified. The birds in study 1 were raised on deep litter management, fed with chick marsh and administered different doses of gentamicin at day old, the birds were observed for clinical signs, mortality and blood sample was collected on 28th day to evaluate liver function tests of the birds in different groups.

Birds in study 2, thirty (30) broilers were reared from day-old on deep litter management and later placed them in battery cage on day 30 for close monitoring. The study 2 was used to check protective effects of aqueous extract of CLJ on gentamicin-overdosed broilers. The birds in study 2 were initially given chick marsh from day old to 28th day and later fed with broiler finisher from 29th to 48th day, water and feed were provided *ad libitum* throughout the period of study. The birds in study 2, on day 33rd, were weighed and randomly distributed into six groups (A, B, C, D, E and F) with five birds per group in a battery cage system. The birds' weight was repeated on day 35th before extract administration at the onset of study 2. These thirty (30) pieces of broilers were observed and evaluated on day 49.

- Group A was given 1.0 mL/kg normal saline in pectoral muscles as control negative, NS.
- Group B was given 45 mg/kg gentamicin only, GM.
- Group C was given 200mg/kg of Vitamin C, VTM (Positive control).
- Group D was given 100 mg/kg of CLJ and 45 mg/kg gentamicin, GM.
- Group E was given 200 mg/kg of CLJ and 45 mg/kg gentamicin, GM.
- Group F was given 400 mg/kg of CLJ and 45 mg/kg gentamicin, GM.

On 35th day, birds in groups D, E and F were given different doses of aqueous extract of CLJ orally for fourteen (14) days, and later given 45mg/kg of Gentamicin (GM) intramuscularly on 42nd day. The birds in groups B were given 45mg/kg of Gentamicin intramuscularly while birds in group C were given 200mg/kg of Vitamin C (Ascorbic acid) on 42nd day, as described in section 2.5 below). This study was terminated on the forty-ninth (49th) day with samples collection. This experiment was designed with slight modification to the studies of [5, 6].

2.5. Drugs/ Vaccines administered

The birds were given antibiotics (ArserylR) for the first five days (days 1 to 5) to remove any known or unknown microbial infection, vaccinations were administered using Newcastle disease (Lasota strain) vaccine with Vaksimune NDR, 100 doses, batch number 201AL24801 was given on day 7. While Infectious bursal disease (Gumboro) vaccine B.P vet intermediate strain, 100 doses, batch number A19107 was administered twice on days 14 and 21 to the birds orally. The vaccinations were done to improve the immune status of the birds. GentanorR 100 (Gentamicin injectable containing Gentamicin 100mg/ml) (GM) was used to induce the broilers with mean body weight ranging from 1.38 to 1.50kg were given 0.62 to

0.68ml of GM intramuscularly that delivered 45mg/kg of GM in this study. While VcnorR Water Soluble Preparation (Vitamin C 100,000mg) in 100g per sachet, a multivitamin and supplement referred to as Vitamin (VTM) was given orally, broilers with mean body weight ranging from 1.38 to 1.5 kg were given 0.14 to 0.15ml, the stock solution was prepared by reconstituting 2,500mg/L of water that delivered 200mg/kg of Vitamin C to the broilers in this study. Arseryl, Gentamicin and Vcnor were manufactured and sold by Jubaili Animal Health, Nigeria.

2.6. Sample collection

At the end of the study, on 49th day all the birds were weighed, blood sample was collected from the jugular vein of each bird in plain sample bottle from each birds in all the groups. The blood samples, were held on ice until centrifugation at 5,000 revolutions at 0°C for 20 min. The serum biochemical evaluations (Total protein (TP), Albumin (Alb), Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), Alkaline phosphatase (ALP), and Creatinine activities were determined according to enzymatic procedures described in commercial test kits manufactured by Randox Laboratories, Crumlin, England. The serum uric acid was evaluated with PriestTM SYS kit manufactured by Robonik (India) PVT LTD. While the electrolyte analysis for Sodium, Potassium, Calcium and Chloride were determined using commercial colorimetric kits (Quimica ClinicaAplicada S A Amposta, Spain).

2.7. Histopathology of liver and Kidney

The birds were humanely sacrificed liver and kidney were carefully collected into 10% formalin solution for histopathological examination. The liver and kidney histopathology analysis were done in the laboratory of Department of Veterinary Pathology, Federal University of Agriculture, Abeokuta, Nigeria.

2.8. Statistical Analysis

The data obtained were statistically analyzed with one-way ANOVA in Graph pad InStat software version 7.0, California USA. The mean differences were considered statistically significant differences at $P < 0.05$ using the Tukey comparison postdoc test. The data generated were expressed as mean $\pm$ Standard Error of Mean and presented in tables and graphical forms.

3.0. Results

3.1. Mortality rate of commercial broilers treated with different doses of gentamicin, Study 1.

There was no mortality % in the broilers administered with 0 to 40 mg/kg body weight of gentamicin. There was single mortality on different days (9 and 18) in broiler given 50mg/kg body weight of gentamicin; two broilers died on day 26 this made 25% mortality in broilers given 50mg/kg body weight of gentamicin in study 1, (Table 1). Meanwhile broilers given 60mg/kg body weight of gentamicin showed 50% mortality with one mortality in day 4th, two died on day 9th, one died on day 17th and one died on day 22nd. Finally, broilers given 80mg/kg body weight of gentamicin showed 90% mortality rate with three mortalities on day 2, one on day 9, two on day 17 and three on day 24 in this group, (Table 1).

Table 1. Mortality rate of commercial broilers treated with different doses of gentamicin, Study 1.

Group	Day 0- 7	Day 8- 14	Day 15- 21	Day 22- 28	Nr. of total mortality	% of total mortality
A (0mg/kg)	0	0	0	0	0	0
B (40mg/kg)	0	0	0	0	0	0
C (50mg/kg)	0	1	1	2	4	25
D (60mg/kg)	1	2	1	1	5	50
E (80mg/kg)	3	1	2	3	9	90

3.2 Clinical Signs of commercial broilers treated with different doses of gentamicin, Study 1.

The broilers given 0 to 40mg/kg body weight gentamicin did not exhibit any clinical signs with no change in their behavior throughout the study period. Their interest in the feed and drinking water of these groups was normal. The broilers that received 50 to 60 mg/kg body weight of gentamicin showed depression with slight increased water intake. These signs were serious in broilers given 80 mg/kg body weight of GM with severe depression, sitting on hock joints shortly with 90% mortality after gentamicin

administration, (Table 1). Water intake was increased throughout the period of study and watery diarrhea was a regular feature. The birds progressively became emaciated and had prominent keel bone.

3.3. Result of serum liver function test of broilers treated with different doses of gentamicin, (Study 1).

The Aspartate aminotransferase, (AST) values of broilers treated with 50 and 60 mg/kg GM were significantly ($P<0.05$) high when compared with broilers given normal saline (NS) and 40mg/kg GM, but the Alanine aminotransferase, (ALT) values was significantly ($P<0.05$) high in broilers given 60mg/kg GM compared with broilers given normal saline (NS) and 40mg/kg GM. Considering Alkaline Phosphatase, ALP values the broilers given 60mg/kg GM were significantly ($P<0.05$) high than broilers given normal saline (NS), (Fig. 1).

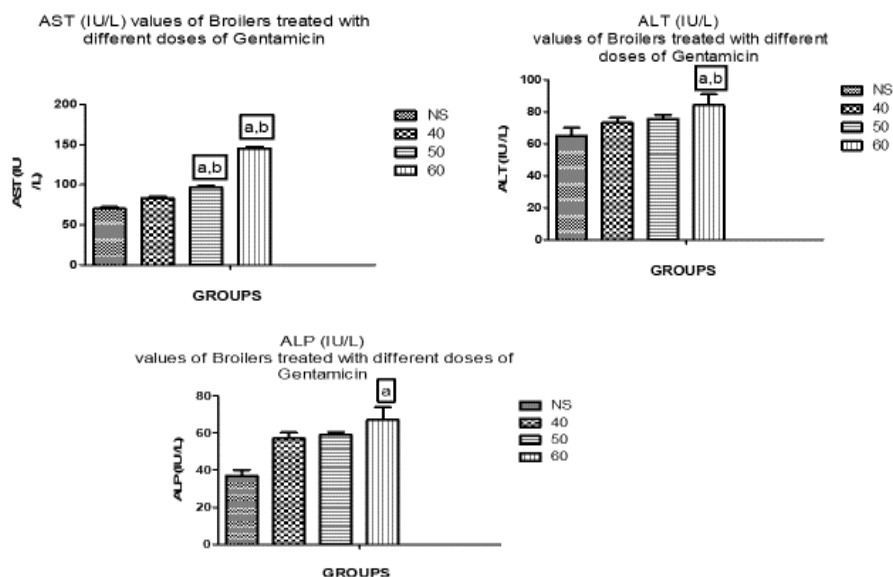


Figure 1. Serum liver test of broilers treated with different doses of gentamicin, (Study 1). NS= Normal saline; AST= Aspartate Aminotransferase; ALT= Alanine Aminotransferase; ALP= Alkaline Phosphatase. n= Number of Broilers used were 5 per group; a and b were significantly different ($P<0.05$) compared with NS and 40mg/kg groups respectively.

3.4 Result of body weight of gentamicin-overdosed Broilers within two week (35th and 49th day) treated with and without CLJ, (Study 2).

There was a significant ($P<0.05$) increase in body weight change in birds treated with 400 mg/kg/body weight of CLJ extract (14.67 %) on day 49 when compared with the body weight of birds on day 35. This similar trend was seen in VTM group, which showed a slight increase in body weight change (4.23%) when comparing body weight of these birds on days 35 and 49. While, there was a slight decrease in body weight change of birds treated with GM (4.17%) when compared birds on days 35 and 49. There was significant ($P<0.05$) increase in body weight of birds treated with 400 mg/kg/BW of CLJ extract (14.67%) when compared body weight of GM- treated birds (4.17%) at day 49. As well as significant increase in body weight change in 400 mg/kg/BW of CLJ extract (14.67%) compared with body weight change in normal saline-treated birds (5.63%), (Table 2).

Table 2. Result of mean weight of gentamicin- induced Broilers within 14 days (35th and 49th day) treated with and without CLJ, (Study 2).

Group/Weight (gm)	35 th day Treatment) kg	(Before 49 th day (After Treatment) kg	Differences in weight between 35 th and 49 th day (%)
NS	1.42± 0.05	1.50±0.00	5.63% (Increase)
GM	1.44± 0.02	1.38±0.07	4.17% (Decrease)

VTM	1.42± 0.02	1.48±0.04	4.23 % (Increase)
100 CLJ	1.38±0.05	1.38±0.04	No difference
200CLJ	1.40±0.06	1.44±0.02	2.86% (Increase)
400 CLJ	1.500± 0.00	1.72±0.00	14.67% ^{a, b} (Increase)

Mean± SEM; n= number of broilers per group is 5. The superscript a and b were significantly (P < 0.05) different when compared with NS and GM groups respectively. NS= Normal saline; GM= Gentamicin.

3.5. Result of Serum Liver Function Test values of GM-overdose Broiler treated with and without CLJ, Study 2.

The total protein concentration was significantly ($P<0.05$) increased in 200mg/kg of CLJ-treated broilers (5.60 ± 0.24 mg/dl) when compared with GM-treated broilers (4.6 ± 0.24 mg/dl), (Fig. 2). The albumin concentration was significantly ($P<0.05$) increased in broilers treated with 100, 200 and 400mg/kg of CLJ (3.10 ± 0.7 ; 3.00 ± 0.04 and 3.62 ± 0.09 mg/dl) respectively when compared with broilers treated with GM (2.6 ± 0.24 mg/dl) and severely significantly ($P<0.05$) decrease in albumin concentration when compared with broilers treated with normal saline (4.60 ± 0.24 mg/dl). The ALT concentration was significantly ($P<0.05$) low in broilers treated with 400mg/kg CLJ (66.60 ± 0.51 IU/L) when compared with GM-treated broilers (77.40 ± 0.93 IU/L); AST concentration were significantly ($P<0.05$) low in broilers treated with 400mg/kg CLJ (88.40 ± 0.51 IU/L) when compared with broilers treated with GM (112.4 ± 2.58 IU/L); While the ALP concentration was significantly ($P<0.05$) low in broilers treated with 400mg/kg CLJ (46.4 ± 0.24 IU/L) when compared with GM-treated broilers (57.60 ± 0.24 IU/L), (Fig. 2).

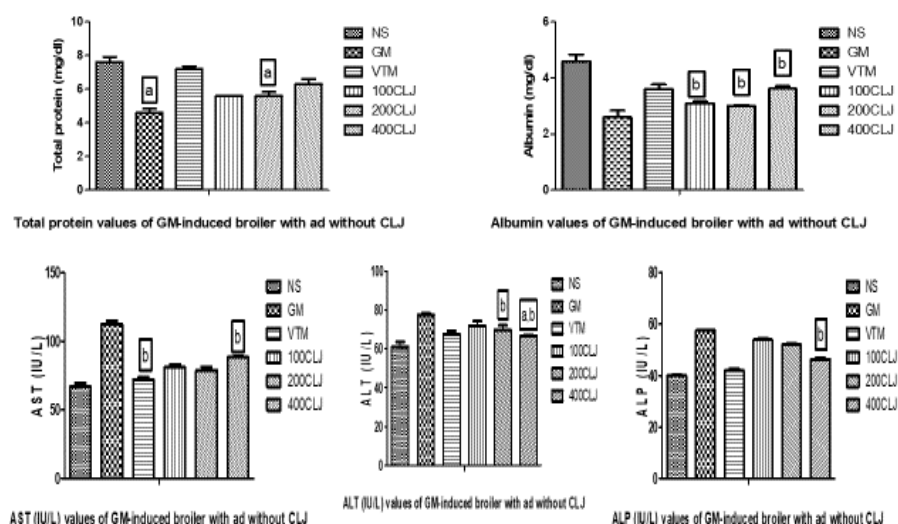


Figure 2. Serum Liver Function Test concentration of GM-overdosed Broiler treated with and without CLJ. NS= Normal saline; GM=Gentamicin; VTM=Vitamin; CLJ= *Citrullus lanatus* juice; 100CLJ= 100mg/kg of CLJ +GM; 200 CLJ = 200mg/kg of CLJ +GM; 400 CLJ = 400mg/kg of CLJ +GM; AST= Aspartate aminotransferase; ALT=Alanine aminotransferase; ALP= Alkaline phosphatase. a= significantly different compared to NS; b= significantly different compared to GM.

3.6. Result of Serum Kidney function Tests of gentamicin-overdosed broilers treated with and without CLJ.

There were significant ($P<0.05$) decrease in serum Na^+ concentration of broilers treated with 400mg/kg of CLJ (142.2 ± 0.51 mEq/L) when compared with serum Na^+ concentration of GM-treated broilers (158.0 ± 0.89 mEq/L). It was observed that the serum K^+ concentration of broilers treated with 200 and 400mg/kg of CLJ (3.8 ± 0.2 and 3.4 ± 0.24 mEq/L) respectively were significantly high when compared with serum K^+ concentration of gentamicin-treated broilers (2.5 ± 0.24 mEq/L). The serum Ca^{2+} concentration of broilers treated with 400mg/kg CLJ (7.20 ± 0.20 mEq/L) was significantly ($P<0.05$) higher than the serum Ca^{2+} concentration in normal saline-treated broilers (4.2 ± 0.2 mEq/L) but lower than the serum Ca^{2+} concentration of gentamicin-treated broilers (6.2 ± 0.37 mEq/L). Meanwhile the serum Cl^- concentration of broilers treated with 400mg/kg CLJ (63.00 ± 1.54 mEq/L) was significantly ($P<0.05$) higher than the serum Cl^- concentration of broilers treated with GM (55.83 ± 1.22 mEq/L), (Fig. 3). Considering creatinine, the serum

creatinine concentration of broilers treated with 400mg/kg CLJ ($1.12.00 \pm 0.04$ mg/dl) was significantly ($P < 0.05$) lower than the serum creatinine concentration of GM-treated broilers (1.86 ± 0.05 mg/dl). While the serum uric acid concentration of 200 and 400mg/kg treated broilers (5.74 ± 0.16 mg/dl and 5.78 ± 0.04 mg/dl) respectively were significantly ($P < 0.05$) lowered than the serum uric acid concentration of gentamicin-treated broilers (8.40 ± 0.12 mg/dl), (Fig. 4).

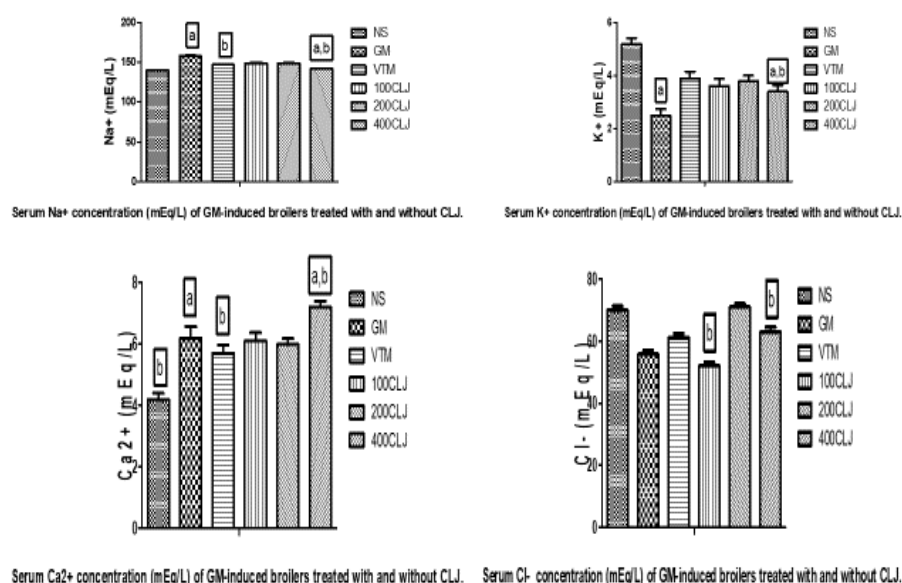


Figure 3. Serum electrolyte concentration of gentamicin-overdosed broilers treated with and without CLJ. NS=Normal saline; GM=Gentamicin only; VTM=Vitamin; CLJ= *Citrullus lanatus* juice; 100CLJ= 100mg/kg of CLJ+GM; 200CLJ = 200mg/kg of CLJ +GM; 400CLJ = 400mg/kg of CLJ +GM; Mean $\pm$ SEM with significant difference of $P > 0.05$ with a and b showed significant difference compared with NS and GM respectively.

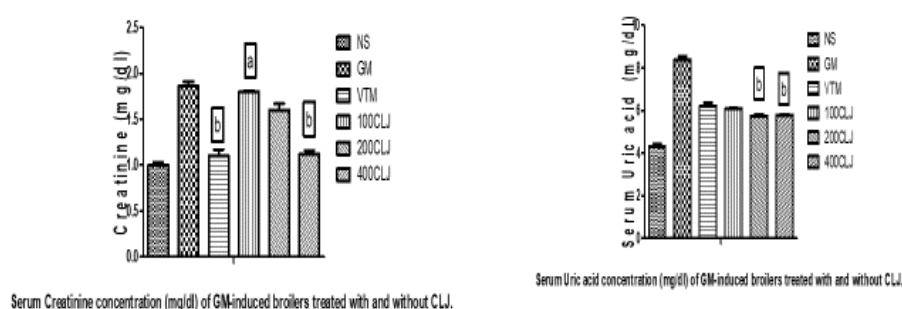


Figure 4. Serum Creatinine and uric acid concentration of gentamicin-overdosed nephrotoxic broilers treated with CLJ. NS= Normal saline; GM=Gentamicin; VTM=Vitamin; CLJ= *Citrullus lanatus* juice; 100CLJ= 100mg/kg of CLJ+GM; 200CLJ = 200mg/kg of CLJ+GM; 400CLJ = 400mg/kg of CLJ +GM; Mean $\pm$ SEM with significant difference of $P > 0.05$ with a and b showed significant difference compared with NS and GM respectively.

3.7. Histopathology of Liver in gentamicin-overdosed broilers treated with and without aqueous extract of CLJ.

There were no pathological lesions in the liver treated with normal saline and VTM. Liver treated with gentamicin showed moderate hyperplasia of the bile duct epithelial cells and moderate hepatic congestion. Meanwhile the liver treated with 100mg/kg of CLJ showed enlarged and congested central vein with mild mononuclear infiltration of the portal area. There were moderate congestion in liver of broilers treated with 200mg/kg CLJ with little or no pathological lesion in the liver of broilers treated with 400mg/kg CLJ, (Fig. 5).

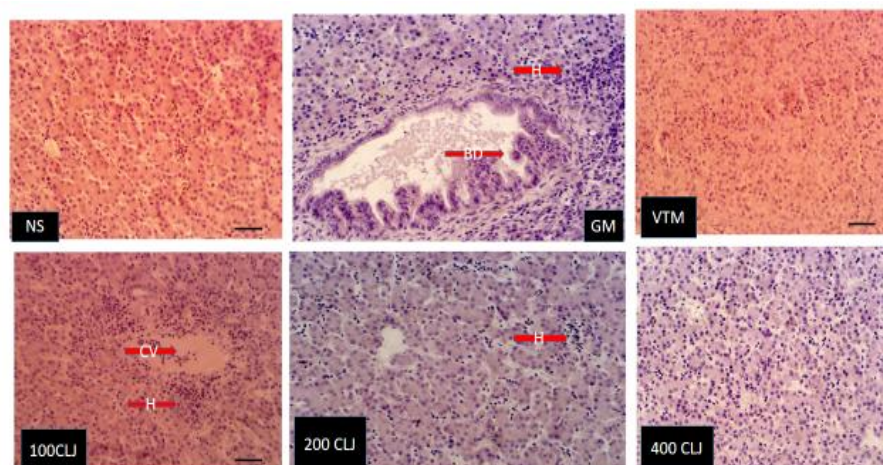


Figure 5. Histopathology of Liver in gentamicin-overdosed broilers treated with and without aqueous extract of CLJ. H&E Stain. Scale bar=50µm. NS=Control group; GM= Gentamicin-induced group; VTM= Vitamin C; 100CLJ= 100mg/kg of CLJ +GM; 200 CLJ = 200mg/kg of CLJ +GM; 400 CLJ = 400mg/kg of CLJ +GM; CV= Central vein; H=Hepatic; S= Sinusoid; Red Marked= Pathological CV, H and Bile duct, BD.

3.8. Histopathology of Kidney in gentamicin-overdosed broilers treated with and without aqueous extract of CLJ.

The kidney of broiler treated with GM showed presence of severe eosinophilic material on the intraluminal wall of the tubule indicated with thin red arrow. There were moderate eosinophilic materials around the tubular lumen and walls in kidney of broiler treated with 100mg/kg of CLJ. Meanwhile there were mild eosinophilic materials around the intraluminal wall of the kidney tubules in the broilers treated with 400mg/kg of CLJ. While there was no pathological lesion in the glomeruli and kidney tubules of broiler treated with normal saline and VTM, (Fig. 6).

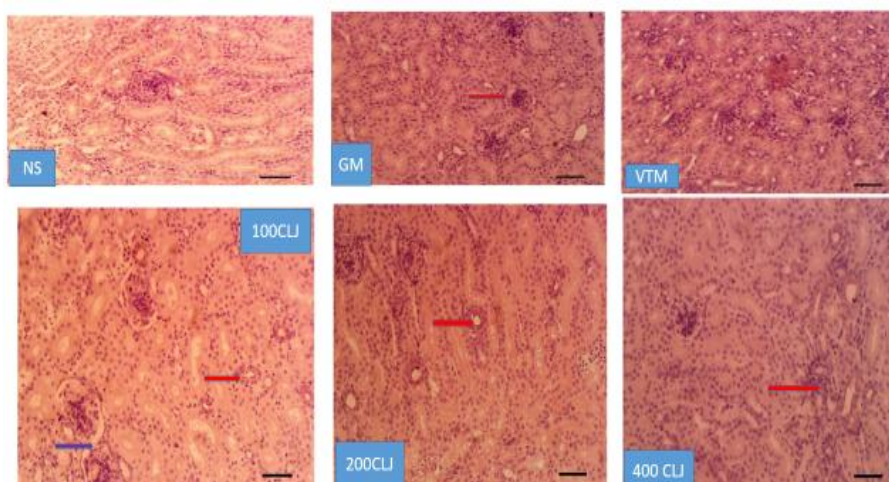


Figure 6. Histopathology of Kidney in gentamicin-overdosed broilers treated with and without aqueous extract of CLJ. H&E Stain. Scale bar=50µm. NS: Normal saline (NS) showing glomerulus and the renal tubules with no pathological lesion; GM: Gentamicin only (GM) showing eosinophilic material in the tubular lumen and haemorrhages in the interstitium, (red arrow); VTM: Vitamin C (VTM) showing the glomeruli and tubules with no pathological lesion. 100CLJ: 100mg/kg CLJ + GM showing mild presence of eosinophilic material in the tubular lumen and walls (red arrow); 200CLJ: 200mg/kg CLJ + GM showing mild presence of eosinophilic material on the intraluminal wall of the tubules (red arrow); 400CLJ: 400mg/kg CLJ + GM showing no pathological lesion.

4. Discussion

The clinical signs of low feed intake, emaciation, increased water intake, and watery diarrhea seen in this study 1 of broilers treated with 60mg/kg of gentamicin were similar to the observation seen by Javed

[5] when broilers were treated with 70 to 100mg/kg of gentamicin. The high mortality of broilers treated with 60 and 80mg/kg of gentamicin in study 1 may be due to hepatic and/or renal failure [18]. This confirmed the investigation and clinical signs seen during gentamicin toxicity in white leghorn birds by Islam [20] that reported increased mortality and toxicopathological alterations in the birds' kidneys and liver. The significant increase in body weight of birds treated with 400 mg/kg CLJ compared with the body weight of gentamicin-treated and normal saline-treated birds in this study. This may be attributed to the phytochemical compounds found in aqueous extract of CLJ such as ascorbic acids, flavonoids, phenolics, alkaloids, terpenes, triterpenoids, steroidal glycosides and vitamins [29, 30]. There could have been initiation of some antioxidant effects by the ascorbic acids found in CLJ that may have effected some improved biological activities in broilers treated with 400 mg/kg CLJ. This is similar to the previous observation by Rabiou [7] in rats treated with *C. lanatus* seed in lead-induced nephrotoxicity that showed increased body weight with some antioxidant effects on the rats.

Liver disease may occur due to hepatic membrane disruption that can lead to leakage of some enzymatic cytosolic contents which can invariably lead to increased serum liver function enzymes such as aspartate aminotransferase, alanine aminotransferase and alkaline phosphatase that are biomarkers of liver damage [7]. The increased in serum liver function enzymes values of gentamicin-treated broilers in study two of this experiment may be due to liver damage. This was invariably reduced in 400mg/kg CLJ-treated broilers which may be due to the ameliorative effects of some phytochemical substances like ascorbic acids, Citrulline, lycopene, alkaloids, glycosides, saponins, flavonoids, phenols, tannins and terpenes found in CLJ that may have caused some antioxidant activities in broilers administered with CLJ. This is similar to the assertions of Ebhohon [12] in investigation of hepato- and nephro-protective effects of methanol extract of CL rind in Wistar rats by relieving damages caused with motor engine oil in contaminated feed of Wistar rats with different doses of CL rinds.

The significant decrease in serum electrolytes of broilers treated with 400mg/kg of CLJ such as Na⁺ and Ca²⁺ concentration in study two of this experiment still suggests the ameliorative effects on the kidney of 400mg/kg CLJ-treated broilers. This is similar to the assertion of Onwuka [8] and Anacletus [9] that showed increased serum electrolytes in nephrotoxicity which were resolved with fruit juice. The serum concentration of creatinine of broilers treated with 400mg/kg CLJ was significantly lower than the serum concentration of creatinine of GM-treated broilers in this study. This may have been caused by antioxidant effects of CLJ that is rich in beta-carotene, vitamin C, and lycopene [25, 30]. This is confirming the assertion that creatinine is a good indicator of kidney damage leading to an increase in serum creatinine concentration as a biomarker of nephrotoxicity [12].

The reduction in serum creatinine in 400mg/kg CLJ-treated broilers may suggest that CLJ may be used to relieve nephrotoxicity in broilers. The serum uric acid values of 200 and 400mg/kg CLJ treated-broilers which were significantly lowered than serum uric acid values of gentamicin-treated broilers seen in this study. This may suggest that 200 and 400mg/kg CLJ-treated broilers may have reduced the serum uric acid levels in broilers by resolving the kidney damage since serum uric acids is known to be the major biomarker of most nitrogenous metabolic product in birds indicating kidney damage birds [16, 17]. The knowledge that optimal dosages and supplementation with Watermelon fruit as a good source of vitamin C, can be used in disease prevention [30]. This may be due to the phytochemistry nutraceutical importance of Ascorbic acids found in CLJ. This may be involved in liver drug and chemical metabolism that may relieve some hepatic damages in the body according to Takahashi [31] assertion that high-dose of intravenous vitamin C can improve quality of life in cancer patients. This was confirmed in this present study that the hepatic pathological lesions seen in gentamicin-treated broilers compared with expressions in liver of 200mg/kg CLJ-treated broilers totally disappeared in 400mg/kg CLJ-treated broilers. This is similar to the affirmation of Bazabang [23] that *Citrullus lanatus* had hepatoprotective effects in Wistar rats, while Akintunde [24] emphasized it is cardioprotective effects in rats.

Some of the major causes of kidney disease in birds are infectious nephritis, vitamins A and/or C deficiency, heavy metal toxicity, and kidney tumor. Also overdose of gentamicin can cause nephrotoxicity seen in this study that arose from fat accumulation in renal proximal convoluted tubules could have led to loss of brush border integrity as seen in previous study [14]. This present study revealed that kidney of broilers treated with gentamicin showed presence of severe eosinophilic material on the intraluminal wall of the tubules. The disappearance of these lesions seen in 400mg/kg CLJ treated-broilers and Vitamin C-treated broilers in this present study may be due to the presence of phytochemical substances like beta-

carotene, ascorbic acids and lycopene found in CLJ which may have played some antioxidant effects in the biological activities of the broilers kidney [31].

5. Conclusions

It may be concluded from this study that supplementation of aqueous extract of CLJ can cause increased body weight in commercial broilers. As well as involved in hepatoprotective and nephroprotective activities in gentamicin overdosed broilers. This is suggestive that aqueous extract of CLJ may be incorporated as a supplement in ameliorating gentamicin overdosed broilers.

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