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Phytochemical screening and acute toxicity assessment of *Aloe vera* gel and extracts of turmeric, oregano, and lemon grass in broilers

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Abstract

This study aims to evaluate the high-dose effects of botanical extracts on blood profile, histopathologic appearance, and carcass quality in broiler chickens when given per orem. Fifty-two (52) broiler chickens were distributed in 6 treatment groups: T1 was given normal drinking water, T2 with commercial antibiotic growth promoter, T3 was treated with turmeric extract (10ml/head), T4 was given oregano extract (10 ml/head), T5 lemongrass extract (10ml/head), and T6 was given a 10 ml/head dose of *Aloe vera* gel. Experimental results showed that the high dose of herbal extracts did not have a direct effect on the liver and kidney and did not produce significant gross and bistopathologic changes. Results also suggest that the botanical extracts used in this study do not affect the hematological profiles (RBC and platelet) of broilers, as well as their serum biochemistry values. The results also showed the high dose of botanical extracts does not affect the relative internal organ weight and carcass quality of broiler chickens.

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Introduction

Today, there is a rising need to discover effective replacements for antibiotics to improve performance and maintain the health of livestock, especially poultry. Poultry production is considered at a high risk for the emergence of antibiotic resistance because poultry generally receives higher quantities of antibiotics than other livestock species and resistance is more likely to develop in conditions of overcrowding and poor sanitation (Rousham *et al.*, 2018). Ethnoveterinary medicine often offers cheaper alternatives than conventional Western drugs, and the products are readily available and more easily accessible (Zschocke *et al.*, 2000; Yineger *et al.*, 2007). These plants need to be studied for their effectiveness and toxicity. Those found acceptable with minimal harmfulness could be administered for use in veterinary practice (Gefu *et al.*, 2000). Research efforts are progressively directed toward using natural agents with comparable useful effects of growth promoters (Mehdi, 2018). Botanicals (herbs, spices, and essential oils) are pertained to as phytogenic feed additives containing many bioactive molecules with antioxidant and antimicrobial properties. Beneficial effects of phytogenic compounds have been observed on performance and antimicrobial activity in broiler chickens (Giannenas *et al.* 2005). Botanicals are also found to have stimulating effects on the digestive system (Windisch *et al.* 2008; Diaz-Sanchez *et al.,* 2015) and nutrient digestibility (Amad *et al.,* 2011; Mountzouris *et al.,* 2011). In contrast, other studies have described no effects or negative effects on performance, microbiota, and gut morphology (Hafeez *et al.,* 2015; Ahsan *et al.,* 2018). Certain botanicals are considered traditional medicinal plants due to their possible benefits due to the phenolic compounds present in them (Wasli *et al.,* 2018).

Oregano (*Origanum vulgare*) is an aromatic plant that has over 30 antioxidant components, most of which are phenolic and possess anti-microbial and anti-inflammatory characteristics (Alma *et al.*, 2003, Park *et al.*, 2015). Oregano can also enrich the antioxidant capacity of meat and meat produced from poultry (Forte *et al.*, 2018). Two phenolic monoterpene isomers extracted from thyme and oregano, namely thymol and carvacol, revealed antioxidant, antihypertensive, antimicrobial, immunomodulatory, and anticancer properties (Rathod *et al.*, 2021). In poultry, oregano supplementation has proven to increase production, lower mortality, regulate gastrointestinal microflora, suppress pathogens, and stimulate the immune system (Park *et al.*, 2015).

Curcuminoids are chemicals extracted from turmeric (*Curcuma longa*), a member of the *Zingiberaceae* family, and have been used in Ayurvedic medicine as a treatment for inflammatory diseases. The three chief components of curcuminoids discovered in turmeric are curcumin, dimethoxycurcumin, and bisdemethoxycurcumin. Curcumin (diferuloylmethane), is a powerful antioxidant, antibacterial, anti-inflammatory, anticarcinogenic, and has proapoptotic characteristics (Amalraj *et al.*, 2017). Turmeric contains curcuminoids and bioactive secondary metabolites that are essential for the health of poultry. Turmeric integration to feed also has major effects on blood biochemical markers, such as the activity of antioxidant and detoxifying enzymes. It also improves antibody titers after vaccination, neutralizes the adverse effects of aflatoxins in the diet, and decreases the number of potentially pathogenic microorganisms in hens' ileal contents (Guil-Gerrero *et al.*, 2017). However, turmeric can cause hepatic changes in chickens when fed excessively through the feeds, and these effects are not dependent on dose or time. Without obvious necrosis, the hepatic abnormalities in chicken seem to be predominantly confined to the bile ducts rather than the liver parenchyma (Al-Sultan and Gameel, 2004). Because of its wide margin of safety and pharmacological capabilities, (Khan *et al.*, 2012) determined that turmeric (Curcuma longa) can be utilized as a natural growth enhancer in poultry diet.

Lemon grass *(Cymbopogon citratus)* is a medicinal herb that is extensively used for its relaxing and sedative effects (Garcia *et al.*, 2017). Lemon grass has

flavonoids, phenolic compounds, terpenoids, and essential oils (such as citral, nerol, geraniol, citronellal, terpinolene, geranyl acetate, myrecene, and terpinolmethylheptenone) that have antibacterial, antifungal, antioxidant, and growth promoter properties (Sariözkan *et al.*, 2018). Studies had been conducted on the usage of lemongrass or its secondary metabolites for enhancement of performance in poultry, predominantly in broilers (Thayalini *et al.*, 2011; Sariözkan *et al.*, 2018).

Aloe vera (*Aloe barbadensis* Miller) is a familiar herb that has gotten a lot of attention from researchers. *Aloe vera* grows optimally in tropical and subtropical regions, and numerous countries have the right geographic environments for it. The leaf of *Aloe vera* is the most noteworthy portion of the plant, and it is divided into two components: latex and gel, which contains is 98.5% to 99.5% water (Fallah Husseini *et al.*, 2015). The remaining dry matter contains more than 75 biologically active chemicals (Boudreau and Beland, 2006) with medical benefits. *Aloe vera* contains anthraquinones, saccharides, vitamins, enzymes, and low-molecular-weight chemical. It also has anti-inflammatory, immunomodulatory, wound healing, anti-viral, anti-fungal, anti-tumor, anti-diabetic, and anti-oxidant properties (Christaki and Florou-Paneri, 2010). Darabighane *et al.* (2012) described that adding *Aloe vera* gel to broiler meals (at 1.5%, 2%, and 2.5%) increased antibody titer against Newcastle disease virus (NDV) on day 24 and 38. These findings agree with those of Paraso (2005), who stated that broilers given 2% *Aloe vera* gel in their drinking water had a considerable increase in antibody titer against NDV on days 37 and 52.

This study touches upon this issue and aims to evaluate the beneficial effects of specific plant extracts and their potential to stimulate signs of toxicity in broiler chickens when used as dietary supplements at a dose that could be considered high in their proportion with the other materials that comprise their feed. Four extracts were selected for their potential benefits to health, appetite, and digestion.

Materials and methods

Research design

This study employs a completely randomized design to determine the important variances of the experimental group (turmeric, oregano, lemon grass, and *Aloe vera*) and control groups. Fifty-four (54) one-day-old Cobb® broiler chicks were procured from a veritable local supplier. Female birds were used for this study due to their being generally more sensitive to toxic effects than their male counterparts. The chicks were randomly allocated among 6 treatment groups, with 3 replicates per treatment, blocked by pen into groups of 3 chicks. The birds were then randomly assigned to treatments containing high doses of aqueous medicinal herb extracts via drinking water on day 21 of rearing. Per orem (PO) administration was done to simulate the usual circumstance in which birds are exposed to toxic substances under field conditions (via ingestion). Feeds were withheld overnight and then, fed with the regular diet and administered a single oral dose of 10 ml per bird per medicinal herb in the drinking water. This high concentration was recommended by the Guideline OCSPP 850.2200 for substances that are expected to be of low toxicity. Distilled water was given to the control group orally. The treatment groups were as follows:

T1 – normal drinking water PO from days 0-35

T2 – 10 ml commercially av antibiotic growth promoter (tiamulin, doxycycline, retinol, and cyanocobalamine) PO at day 21

T3 – 10 ml turmeric extract per head (turmeric extract infused in drinking water) PO at day 21

T4 – 10 ml oregano extract per head (turmeric extract infused in drinking water) PO at day 21

T5 – 10 ml lemon grass extract per head (turmeric extract infused in drinking water) PO at day 21

T6 – 10 ml *Aloe vera* gel per head (turmeric extract infused in drinking water) PO at day 21

Preparation of plant extracts

The plant materials were then thoroughly rinsed with water to remove the calcium hypochlorite, cut into pieces approximately 2-3 cm in size, and rinsed once more with distilled water. The herbs were air-dried at

room temperature for 24 hours before being oven-dried at 50°C. The dried herbs were then ground into powder using mortar and pestles. To make the plant extract solution, 100g of dried plant material powder was dissolved in distilled water (1:10 w/v) in an Erlenmeyer flask. The powdered plant solution was then homogenized in a shaking water bath at 50°C for 48 hours before being filtered through cotton wool. The extracts were weighed and stored at -20°C until use. To prepare the *Aloe vera* gel, the researcher referred to a method by Ramachandra and Rao in 2008. Fresh *Aloe vera* leaves were washed and disinfected with calcium hypochlorite and then patted dry. The leaves were cut at about 0.5 inches from the base to drain out all the yellow sap material. Then the leaves were cut at about 1.5 inches from the apex. The spiny outer layer was stripped off and the upper epidermis of the leaves was also removed to expose the inner mucilaginous gel layer. The inner gel was scraped out, carefully avoiding any green leafy parts to get the gel chunks. The gel chunks were homogenized using an electric blender and the resulting crude *Aloe vera* gel was heated in a hot water bath at 70°C for 30 minutes to denature the enzymes that cause unwanted pigmentation of the solution. The aloe extract was flash cooled to 5°C within 10-15 sec to preserve the biological activity of the *Aloe vera* gel.

Phytochemical analysis of the botanicals used

The phytochemical profiling provided an empirical basis for the use of these plants since the biological or therapeutic activities of medicinal plants are closely related to their chemical compounds. The active compounds in plants vary widely depending on intrinsic factors, such as the plant part used, the harvest season, and the geographical origin, and extrinsic factors, such as the additive production technique (Gangguly, 2013). The crude aqueous extracts of turmeric rhizomes and leaves of *Aloe vera*, oregano, and lemon grass were tested for the presence of alkaloids, tannins, flavonoid saponins, and phenols utilizing standard methods of analysis. The intensity of the coloration determined the abundance of the compound present:

Alkaloid determination

5g of the sample was weighed into a 250ml beaker and 200ml of 20% acetic acid in ethanol was added and covered to stand for four hours. This was then filtered and the extract concentrated using a water bath to one-quarter of the original volume. Concentrated ammonium hydroxide was added dropwise to the extract until the preparation was complete. The whole solution was allowed to settle and the precipitate was collected by filtration and weighed.

Tannin determination

500 m of the sample was weighed into a 100 ml plastic bottle added with 50 ml of distilled water then shaken for one hour in a mechanical shaker. This was filtered into a 50 ml volumetric flask and made up to the 50 ml mark. Then 5 ml of the filtrate were pipetted out into a tube and mixed with 3 ml of 0.1M $FeCl_3$ in 0.1N HCl and 0.008M potassium ferrocyanide. The absorbance was measured in a spectrophotometer at 120nm wavelengths, within 10 minutes. A blank sample was prepared and the color also developed and read at the same wavelength. A standard was prepared using tannin acid to get 100 ppm and measured.

Flavonoid determination

100g of the plant sample was extracted repeatedly with 100 ml of 80% aqueous methanol at room temperature. The whole solution was filtered through Whatman filter paper No. 42 (125mm). The filtrate was later transferred into a crucible evaporated to dryness over a water bath and weighed.

Saponin determination

20g of each powdered plant samples were dispersed in 200 ml of 20% ethanol. The suspension was heated over a hot water bath at about 55°C for 4 hours with continuous stirring. The mixture was filtered and the residue was re-extracted with another 200 ml of 20% ethanol. The combined extract was reduced to 40ml over a water bath at about 90°C. The concentrate was transferred into a 250 ml separator funnel and 2ml diethyl ether was added and shaken

vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated. Then 60ml of n-butanol was added after which, the combined n-butanol extracts were washed twice with 10ml of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation, the samples were dried in the oven to a constant weight.

Phenol determination

For the extraction of the phenolic component, the fat-free sample was boiled with 50 ml of ether for 15 minutes; 5 ml of the extract was pipetted into a 50 ml flask, and then 10 ml of distilled water was added; 2 ml of ammonium hydroxide solution and 5 ml of the extract were pipetted into a 50 ml flask, and then 10 ml of distilled water were added, 2 ml of ammonium hydroxide solution and 5 ml of concentration amyl alcohol were also added. The samples were left to react for 30 minutes for color development.

Hematologic profile and serum biochemistry

Blood samples (5ml) were obtained from each bird for analysis on days 0 and 14 post-administration of oral treatments for erythrocytic and thrombocytic, profiling and biochemical analysis. The wing vein was punctured with sterile needles to collect 5 ml of blood from each bird, 0.5 ml of the collected blood was transferred into EDTA bottles, while the remaining 4.5 ml was allowed to settle to separate the serum from the blood. Measurements were made using a hematology analyzer which includes total counts (TC) of RBC and platelet. The coagulated blood was completely separated from the serum via centrifugation at 3000 rpm for 10 min and subjected to biochemical analysis. The following clinical biochemistry parameters were measured using a veterinary clinical chemistry analyzer: levels of total protein, albumin, uric acid, creatinine, and serum glutamic-oxaloacetic transaminase.

Macroscopic and microscopic morphology of the liver and kidney

On day 36, three birds were randomly selected and slaughtered by cervical dislocation. The liver and kidney were examined for anomalies such as changes in size or discoloration. Liver and kidney samples were also observed for cellular changes such as the size of the nucleus and the presence of inflammatory cells. Tissue fragments of the kidney and liver were fixed in 10% buffered formalin, dehydrated with successive concentrations of ethanol (70–100%), cleared in xylene, and embedded in paraffin. Slices of 5 µm were made with a microtome. Deparaffinization was performed with the following protocol: Xylol 4 min; 100% EtOH 2 min; 90% EtOH 2 min; 70% EtOH 2 min; 60%. The sections were stained with Mayer hematoxylin and eosin and mounted with mounting medium (DPX). Duplicate slides of each block were obtained. Sections were examined under LPO and HPO. Pathologic lesions associated with substance toxicity were assessed using criteria published by the Society of Toxicologic Pathology.

Determination of relative internal organ weight

Assessment of the internal weight of organs (gizzard, heart, liver, kidney, spleen, pancreas, and bursa of Fabricius) was done using a portable weighing scale.

Carcass quality evaluation

Broiler carcass evaluation was performed on day 35 on 18 birds randomly taken from each treatment. The birds were individually weighed and slaughtered. Eviscerated carcasses without the offal (feet and heads) were chilled in slush ice for 2 hours, allowed to drip for 2 minutes, and then weighed. Carcasses were cut into commercial parts: breasts, thighs, drumsticks, wings, and abdominal fat. All cuts have been individually weighed and examined for gross changes.

Ethics standards

An authorization from the university's Institutional Animal Care and Use Committee (IACUC) was acquired preceding experimentation. All animal trials in the study were done in compliance with IACUC recommendations. An animal research permit was requested from the Department of Agriculture, Regional Office 1 in La Union.

Statistical standards

To summarize the quantitative data obtained, the mean and standard deviation were computed. And to detect any significant differences in hematological profile, serum clinical chemistry values, and carcass quality between treatments. Analysis of Variance (ANOVA) or Kruskall-Wallis H test will be used. A list of differences was utilized for post hoc test analysis. All statements of significance were based on at least 95% confidence limits (i.e. $P<0.05$). The statistical test is conducted using SPSS version 27.

Results and discussion

Table 1 shows the results of the phytochemical analysis of the various botanicals used in this experiment. Note that turmeric contains an abundant number of flavonoids, some tannins, saponins, and phenols, but is lacking in alkaloids. Oregano contains a number of alkaloids, flavonoids, saponins, and phenols but contains no tannins. Lemon grass contains all the chemicals analyzed but its alkaloid component was not detected by Dragendorff's test, which may imply that the alkaloids are of trace amount that some phytochemical tests do not test positive for it. The same could be said with the *Aloe vera* leaf, but tannins also could not be detected in the sample. The implication of these results could be that the toxicity level of these materials is particularly low, so a high dose of double the recommended dietary amount may not show apparent pathogenic effects but may show changes in the cellular level. These results also suggest that the very low probability of toxicity from these botanical materials makes them viable as feed additives which may be used as substitutes for more expensive chemical components of the commercial growth promoters due to their biocompatibility and high margin of safety.

Table 1. Phytochemical analysis results for turmeric, oregano, lemon grass, and *Aloe vera* samples

Type of test	Component detected	Turmeric rhizome	Oregano leaves	Lemon grass leaf	*Aloe vera* leaf
Mayer's test	Alkaloids	-	+	+	+
Dragendorff's test	Alkaloids	-	+	-	-
Hager's test	Alkaloids	-	+	+	-
Wagner's test	Alkaloids	-	+	+	+
Gelatin test	Tannins	+	-	+	-
Alkaline reagent test	Flavonoids	++	+	+	+
Lead acetate test	Flavonoids	++	+	+	+
Froth test	Saponins	+	+	+	+
Ferric chloride test	Phenols	+	+	+	+

Table 2. Blood count showing RBC and PLT count of chickens given a high dose of varying plant extract per orem

Treatment	Complete blood count (CBC)	
	RBC (10^{12}/L)	PLT (10^{9}/L)
To (-)	2.50	12.33
To(+)	2.98	10.33
T1	2.58	14.67
T2	2.65	9.67
T3	2.75	9.67
T4	2.37	16.00

The effect of selected medicinal herb extracts on the blood values of broilers is summarized in Table 2. The mean RBC count (normal range = 2.5-3.9 × 10^{12}/L) is highest in T2 (commercial growth promoters with antibiotics) at 2.98 × 10^{12}/L, followed by T5 (lemon grass extract) at 2.75 × 10^{12}/L, and T3 (turmeric extract) (2.58 × 10^{12}/L). The mean platelet count (normal range = 3-33 × 10^{9}/L) is higher in T6 (*Aloe vera* gel) at 16.00 × 10^{9}/L and T3 at 14.67 × 10^{9}/L. in contrast, T4 (oregano extract) and T5 (lemon grass extract) have the same and lowest mean platelet count of 9.67 10^{9}/L. T2 (commercial growth promoters with antibiotics) having the highest mean RBC count supports the findings of Al-Saad *et al.* in 2014. T6 (*Aloe vera* gel) did not improve the RBC count and has the lowest mean. This finding agrees with those of Tariq *et al.* in 2014 but contradicts the findings of Arif *et al.* in 2022 that *Aloe vera* increases erythrocytes number in broiler chicken. Nonetheless, the results suggest that the RBC count of all treatments was within the normal range and did not cause any pathogenic change in cellular morphology or number.

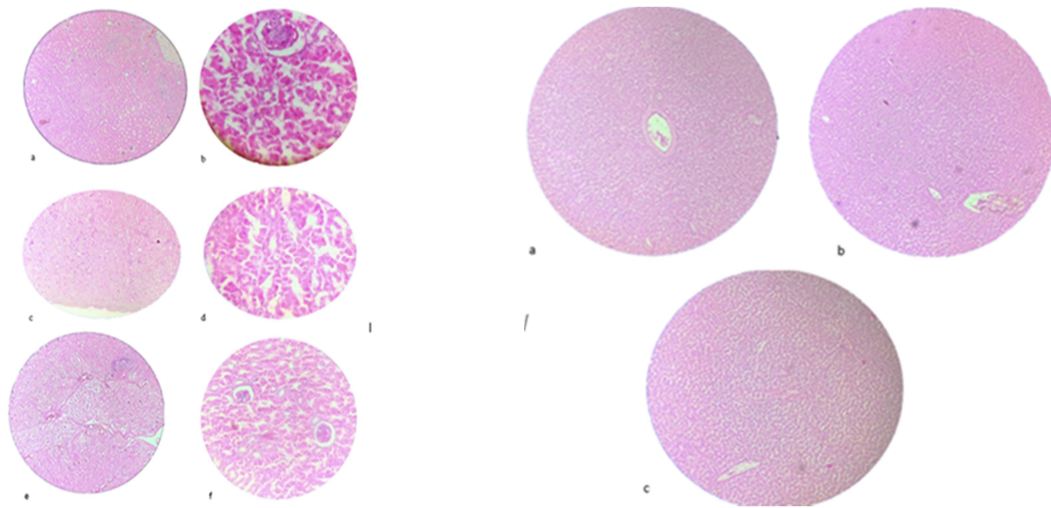
Table 3. Serum clinical chemistry of chickens given high dose of varying plant extract per orem

Tx	Serum biochemical values					
	Creatinine (umol/L)	AST(U/L)	Uric Acid (mg/dl)	Glucose (mg/dL)	Albumin (g/dL)	Total Protein(g/L)
To (-)	376.67	151.33	4.900	185.00	3.36	6.76
To(+)	330.00	147.33	.267	213.67	2.20	7.66
T1	470.00	81.93	.200	230.67	1.86	8.333
T2	193.33	134.30	.200	189.33	.66	10.53
T3	286.67	142.67	.167	160.00	.23	7.66
T4	326.67	171.00	.133	142.00	.53	5.36

Table 1 also shows that there is no comparable difference in the thrombocytic (platelet) count of the chickens across treatments. Despite variation in the data, there is no evidence to say that there is a statistical difference in the hematologic profiles across treatments at a 0.05 level of significance. T2 (commercial antibiotic growth promoter) shows the highest mean of creatinine (470.00 × umol/L), while T3 (turmeric extract) shows low mean of creatinine (193.33 × umol/L). T2 (commercial antibiotic growth promoter) has the lowest mean of AST (81.93 U/L; normal range = 70-316 U/L) and T1 (normal drinking water) has the highest AST (151.33 U/L), as well as the highest uric acid mean (normal range = 2.9-10.4 mg/dL) of (4.900 mg/dL). On the other hand, T2 (commercial antibiotic growth promoter) and T3 (turmeric extract) show the lowest value of mean uric acid (.200 mg/dL) among all the treatment groups. The mean of glucose (normal range = 136-464 mg/dL) is highest in T3 (turmeric extract) at 230.67 mg/dL. On contrary, the mean of T6 (*Aloe vera* gel) has the lowest mean at 142.00 mg/dL. The mean albumin (normal range = 2.52-2.81 g/dL) in T1 (normal drinking water) has the highest mean at 3.36 g/dL, and T5 (lemon grass extract) has the lowest (.23 g/dL). T4 (oregano extract) has the highest total protein value (normal range = 3.3- 4.9 g/dL) with 10.53 g/L and T6 (*Aloe vera* gel) has the lowest (5.36 g/L). The serum biochemical analysis of the chickens given various plant extracts per orem is summarized in Table 3.

The results suggest that the AST and glucose are at normal range across all the treatments. T1 (normal drinking water) has the normal uric acid level and there is a decrease in treatment groups with antibiotic growth promoters and extracts, according to (Harrison, 2022) the decrease of uric acid is caused by liver failure or starvation. The albumin of T1 (normal drinking water) is at normal range and there is a decrease in the remaining treatment groups. (Harrison, 2022) said that a decrease in albumin can caused by malnutrition, external blood loss, intestinal, malabsorption, etc. The results show that the increase in total protein is caused by hemolysis or dehydration. The results show varying effects upon the serum biochemistry of the sample animals which may give a good picture of the treatments' effects upon the physiology of the birds. These results, however, may be the culmination of varying external factors such as stress brought upon by crowding and ambient temperature, which broiler poultry are subjected to in conventional farms such as the setup used in this experiment, other factors such as human error and machine calibration, though kept to a minimum cannot be ruled out Instrument error such as old bulbs, slight variation in machine temperature, variation in reaction time or degraded reagents can cause decreased accuracy and precision. The changes in blood values due to the tropical environment are also a consideration. There are no statistical differences in the creatinine, mean AST, uric acid, and total protein across treatments at 0.05 level of significance. The glucose level of T3 (turmeric extract) is significantly different from T1 (normal drinking water), T5 (lemon Grass extract), and T6 (*Aloe vera* gel) at 0.05 level of significance. When it comes to albumin count, only T6 (*Aloe vera* gel) and T2 (commercial antibiotic growth promoter) had significant differences.

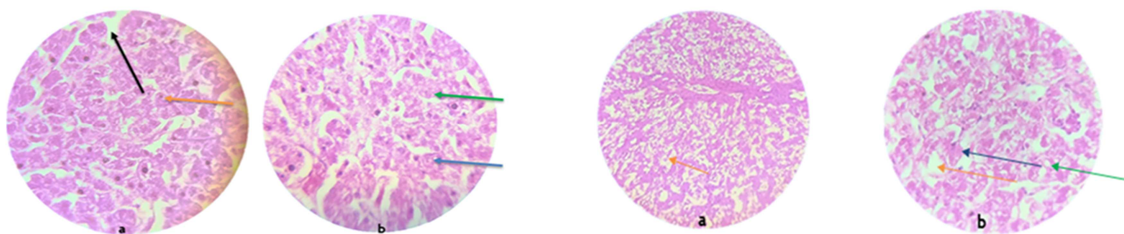
Histopathologic changes in the hepatic and nephrotic tissues were examined in all treatments (Fig. 1).



a. T3 (turmeric extract) under LPO (a) shows the renal cortex and some part of the renal medulla, T4 (oregano extract) under OIO (b) shows glomeruli, proximal and distal convoluted tubules, T5 (lemon grass extract) under LPO (c) showing the renal medulla and some part of the renal cortex and T6 (d) showing distal convoluted tubules and collecting duct under OIO. The renal cortex and medulla under LPO (e) and renal corpuscles with the convoluted tubules and collecting ducts under OIO (f) of T1 (normal drinking water).

b. Hepatic sections with mild, focal hyperemia, congestion, and edema. Low power view of the liver in a) T3 (turmeric extract) and b) T4 (oregano extract) shows the cross-section of the liver parenchyma with an eosinophilic appearance. T6 (*Aloe vera* gel; c) shows an increased amount of blood in capillaries.

Fig. 1a-b. Histopathologic changes in the hepatic and nephrotic tissues



a. OIO view of hepatic tissue from T5 (lemon grass extract, a) and T6 (*Aloe vera* gel), b) Sections showing pyknotic (orange); karyorrhectic (green); karyolitic (blue) cells.

b. HPO (a) and OIO (b) view of the liver parenchyma showing glycogen-like structures (pale pink stains inside the sinusoids – orange arrow) and sinusoidal dilation. glycogen-like structures (pale pink stains inside the sinusoids – orange arrow) and sinusoidal dilation cell swelling (blue arrow), sinusoidal dilation with amorphous material (orange arrow), and hepatocyte vacuolation (green arrow) were also observed.

Fig. 2a-b. Histopathologic changes of the liver samples from the 6 treatments observed under LPO show that all 3 samples from T3 (turmeric extract) and T4 (oregano extract) showed mild, focal hyperemia, congestion, and edema

Gross evaluation of each liver and kidney sample is not a sole tool to identify the uncharacteristic appearance of these organs, nor is it reliable in determining any presence of hepatomegaly or nephromegaly unless proven through histopathology. And discoloration in the liver and kidneys the naked

eye can only be considered pathologic when confirmed through further examination. All treatments presented normal nephrons with no evidence of hyperemia, congestion edema or any abnormal pigments observed on the entire high-power field (HPF) of the specimens (including the renal cortex and the renal medulla) Cellular swelling was also absent on all samples from the 6 treatments. Cellular swelling is a common observation in kidney cells, and this is the outcome of inflammation induced by the high-dose supplementation of toxic extracts. If the extent of cellular swelling is diffuse but the severity is mild, this occurrence is reversible. The cellular response to injurious stimuli depends on the type of injury, its duration, and its severity. For example, low doses of toxins or brief periods of ischemia may cause reversible injury, whereas larger toxin doses or more prolonged ischemia may result in irreversible injury and cell death. In the case of the study, birds were given high doses but the duration was brief since it was done on a single dose basis. The relative extent of the damage to the kidney if there is, was minimal. Additionally, the degenerative nuclear changes observed from all 6 treatments are a normal occurrence. Mild, focal pyknosis, karyorrhexis, and karyolysis are all present but not pathologic.

Histopathologic changes of the liver samples from the 6 treatments observed under LPO show that all 3 samples from T3 (turmeric extract) and T4 (oregano extract) showed mild, focal hyperemia, congestion, and edema (Fig. 2). Furthermore, all 3 samples from Treatment 6 (*Aloe vera* gel) showed mild, multifocal hyperemia, congestion, and edema. The 3 samples from T1 (normal drinking water) T2 (commercial antibiotic growth promoter), and T5 (lemon grass extract) did not show any relevant hyperemia, congestion, or edema.

Hyperemia is an active process that is part of acute inflammation, whereas congestion is the passive process resulting from decreased outflow of venous blood. Acute inflammation can be provoked by any noxious stimulus called an irritant, among which include chemical agents that are of endless variety such as toxins, acids, alkalies, and other caustic substances. It may be suggestive that high doses of compounds found in botanical extracts may indirectly contribute to such histopathologic changes. Congestion (passive) is a passive process resulting from decreased outflow of venous blood due to cardiac failure/cardiac defects or circulatory impairment of the liver. Edema on the other hand is a product of serous inflammation, may it be acute or chronic as mentioned earlier.

Histopathology examination showed normal size and number of Kupffer cells. However, several hepatocytes displayed degenerative nuclear changes such as varying nuclear sizes or anisokaryosis (Fig. 3). Diffuse nuclear shrinkage (pyknosis), nuclear fragmentation (karyorrhexis), and nuclear dissolution (karyolysis) were noted on samples from T5 (lemon grass extract). However, samples from T3 (turmeric extract), T4 (oregano extract), and T6 (*Aloe vera* gel) have only multifocal nuclear changes. These focal nuclear changes were seen on each sample from T1 (normal drinking water) and T2 (commercial antibiotic growth promoter) and may be considered as normal occurrences during cell apoptosis.

Based on these histopathology results, the cellular and tissue changes observed were diffuse but not severe. However, histology processing or preservation errors and limitations cannot be ruled out due to the degree of staining technique used as well as the available preservation materials. Most changes were mild, although their distribution is diffuse and some coagulative necrosis was observed, but these changes are reversible since there were no fibrosis and severe degenerative changes seen. Suffice it to say, the high dose of botanical used in this study did not have detrimental effects on the liver and did not produce significant pathological changes. Kidney histopathology showed no evidence of major degenerative changes. Apoptotic figures seen on the epithelia of the tubules, ducts, glomeruli, and other tubular organelles were mild and may be considered a normal part of the physiologic process. Necrosis or evidence of fibrosis was absent. Further, there were

no other significant histopathologic changes observed, therefore, the concentration of the botanical extracts that were used at a high dose, likewise, did not produce any significant effect on the kidneys.

Various internal organ weights were also measured across treatments. T3 (turmeric extract) has the highest mean relative liver weight at 62.80 g followed by T1 (normal drinking water) at 58.73 g, T2 (commercial antibiotic growth promoter) and T4 (oregano extract) has the same low mean relative liver weight of 52.26 g. T2 (commercial antibiotic growth promoter) has the lowest mean kidney weight at 14.80 g and T4 (oregano extract) (16.56) and T3 (turmeric extract) have the highest mean kidney weight at 16.56g and 16.23 g, respectively. For the mean spleen weight, T3 (turmeric extract) yielded the highest mean of 3.70 g. In the mean weight of the gizzard, T2 (commercial antibiotic growth promoter) and T5 (lemon grass extract) had the same mean of 29.73 g while T6 (*Aloe vera* gel) had the lowest of 25.50 g. The mean weight of the pancreas was highest in T3 (turmeric extract) with 6.26 g, T4 (oregano extract = 5.66 g), and T1 (normal drinking water = 5.50 g), while T2 (commercial antibiotic growth promoter) had the lowest mean weight of pancreas. T1 (normal drinking water) has the highest mean weight of lungs at 10.76 g, followed by T3 (turmeric extract) and T4 (oregano extract) with a similar mean of 10.73 g. T2 (commercial antibiotic growth promoter) has the lowest mean heart weight (9.43 g) and T5 (lemon grass extract) has the highest with 12.17 g. The bursa of Fabricius in T3 (turmeric extract) has the highest mean weight (3.87 g) while T2 (commercial antibiotic growth promoter) has the lowest (1.93 g). The effect of selected medicinal herb extract on relative internal organ weight is presented in Table 4.

Table 4. Internal organ weight of chickens given high dose of varying plant extract per orem

Tx	Relative internal organ weight							
	Liver	Kidney	Spleen	Gizzard	Pancreas	Lungs	Heart	BOF
To (-)	58.73	15.56	1.96	27.23	5.50	10.76	9.80	2.80
To(+)	55.26	14.80	1.70	29.73	4.63	9.20	9.43	1.93
T1	62.80	16.23	3.70	28.60	6.26	10.73	10.53	3.87
T2	52.26	16.56	1.53	26.83	5.66	10.73	10.93	3.80
T3	54.70	15.93	2.10	29.73	4.93	9.40	12.17	2.73
T4	55.46	15.20	1.63	25.50	4.80	9.86	10.70	2.87

Table 5. Weight of cut carcasses of chickens given high doses of varying plant extract per orem

Tx	Cut Carcasses						
	Live Weight (kg)	Carcass (kg)	Drumsticks (g)	Thighs (g)	Breast (g)	Wings (g)	Abdominal Fat(g)
To (-)	1.90	1.61	145.00	160.00	425.00	165.00	16.67
To(+)	1.78	1.58	160.00	195.00	381.67	208.33	15.00
T1	1.98	1.73	176.67	223.33	480.00	196.67	25.00
T2	1.76	1.55	166.67	190.00	406.67	175.00	14.33
T3	1.68	1.48	153.33	180.00	400.00	181.67	13.33
T4	1.61	1.36	145.00	183.33	348.33	150.00	8.33

This study showed that T3 (turmeric extract) has the highest mean weight in the liver in line with the study of (Mondal *et al.*, 2015) that the inclusion of turmeric increases the weight of the liver, gizzard, and heart. In contrast with the observation of Al-Sultan et al (2003) who reported that giving turmeric did not alter the weight of the liver, gizzard, and heart. Most of the mean of internal organs have a higher mean in T1 (turmeric extract) which is in line with the study of (Attia *et al.*, 2017). The results showed that turmeric improves the function of internal organs enhancing digestion, metabolic processes, and nutrient utilization for growth through stimulation of protein synthesis by the chicken enzymatic system. The effect of selected medicinal herb extract on the weight of cut carcasses of broiler is presented in Table 5.

The mean of T3 (turmeric extract; 1.98 kg), T1 (normal drinking water; 1.90 kg) has the highest mean live weight, and T6 (*Aloe vera* gel; 1.61kg) with the lowest. T3 (turmeric extract; 1.733 kg), and T1 (normal drinking water; 1.617 kg) has the highest carcass mean while T6 (*Aloe vera* gel) has the lowest mean of 1.367 kg. These results support the findings of Mondal (2015) who concluded that turmeric extract increased the carcass quality of broilers, but contradicts a study by Arif *et al.* in 2022 that says *Aloe vera* increases growth and improves weight gain in broilers. This may be affected partly by the appetites of the birds in a tropical setting, in that they tend to eat less in a high ambient temperature as an act of thermoregulation but leads to decreased weight gain. T3 (turmeric extract; 176.67 g), T4 (oregano extract; 166.67 g), T2 (commercial antibiotic growth promoter; 160.00 g) has the highest drumsticks mean weight, T1 (normal drinking water) and T6 (*Aloe vera* gel) has the same and lowest mean of 145.00 g. T3 (turmeric extract) has the highest mean weight of thighs (223.33 g), followed by the mean of T2 (commercial antibiotic growth promoter; 195.00 g) and T5 (lemon grass extract; 190.00 g), T1 (normal drinking water) has the lowest mean of 160.00 g. T3 (turmeric extract) has the highest mean weight of breast at 480 g, and T6 (*Aloe vera* gel) at 348.33 g has the lowest. T2 (commercial growth promoter with antibiotics; 208.33kg) has the highest mean weight of wings while T6 (*Aloe vera* gel; 150.00 kg) has the lowest. The highest mean of abdominal fat is in T3 (turmeric extract; 25.00 g) and T6 (*Aloe vera* gel; 8.33 g) the lowest.

Among all the treatments in the study, turmeric has the best results in terms of cut carcasses which agrees again with the study of Mondal in 2015 who concluded that turmeric improved the carcass yield of broiler chicken. These potential effects of turmeric could be attributed to its curcuminoids (3 to 5 %, as found in turmeric powder), bisdemethoxy curcumin, and demethoxy curcumin, the principal active compounds in turmeric that could improve carcass quality (Attia *et al.*, 2017).

There were no significant differences between the live weight and the cut carcass weights across treatments. The high dose of herbal extracts did not have adverse effects on the physiology and did not produce marked pathologic changes in the birds, as seen in their hematologic profile, serum biochemistry values, and the histopathology of their livers and kidneys. Treatment of the botanical extracts used also did not affect the production parameters, particularly the relative internal organ weight, and the carcass quality of the birds after slaughter.

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