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The evaluation of the safety and toxicological characteristics of Acacia nilotica in broiler

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Abstract

In this study, we evaluated the clinical, biochemical, and pathological changes induced by oral administration of Acacia nilotica aqueous extract (ANAE) in broilers. A total of 71-day-old broilers were separated into one control group and six groups of 10 broiler chicks, which were subjected to a challenge. Various amounts of ANAE (1, 3, 5, 7.5, 10, and 15 g/kg b.wt) were given orally to broilers. Over a period of 10 days, indicators such as consumption of feed, alterations in body weight, and occurrences of poisoning or death were monitored. Blood samples were taken on day 5 after treatment for the purpose of evaluating biochemical parameters. Histopathological examination was performed on liver and kidney samples taken. The group that received ANAE at a dose of 15 g/kg showed decreased appetite, decreased locomotion, and increased hypersensitivity to touch, ultimately resulting in 100% mortality. The major lesions of histological liver tissue were cholestasis and adipose changes associated with significant increases in aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP); Indicating liver impairment. Renal tissues exhibit marked inflammation, accompanied by glomerular atrophy and changes in urea levels. Based on the above data, *Acacia nilotica* is toxic to broilers only at 15 g/kg, demonstrating the safety profile of ANAE in broilers.

Keywords: *Acacia nilotica*, Safety, Toxicological Profile, Biochemical, Histopathological, Broilers

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Competing interest: The authors have declared that no competing interest exists.

Introduction

Acacia is a medium aromatic, thorny, and nitrogen tree that grows in height to 15–18 m and has a 2–3 m diameter. With around 1350 species, Acacia is the second biggest genus in the Leguminosae family (Seigler and ecology, 2003). The species is native to Egypt and can be found in different tropical and subtropical regions globally, including Asia, Africa, America and Australia (Calder and research, 2002). Alkaloids, phenols, phenolic glycosides, terpenes, and volatile essential oils are among the complex phytoconstituents in Acacia nilotica. These phytoconstituents demonstrate high therapeutic potential and can be utilized to avoid, mitigate and treat various infectious diseases (Sadiq et al., 2015). In human health, Acacia nilotica is utilized for treating several health problems, including thoracic pains, bronchitis, colds, diarrhea, dysentery, fever, hemorrhages, tooth rage, and pneumonia (Chhabra and Uiso, 1991). Syphilis, oral candida, fungus skin infections, and malaria are also diseases managed by the plant (Kubmarawa et al., 2007, Kambizi and Afolayan, 2001). Other properties as antiviral (Subhan et al., 2018, Hussein et al., 2000), antibacterial (Srinivasan et al., 2001), and pesticides have been reported in the plant in recent studies (Sharma et al., 2014, Jigam et al., 2010). It is a safe medicinal herb that modifies numerous therapeutic effects without causing harm. Thus, medicinal plants influence the production and progress of modern research on the biological activities of substances. Therefore, the current investigation aimed to assess the toxicological profile of ANAE on broilers,

Materials and Methods

Plant Material Collection

Plants were collected from the state of Qena. Botanical permission was obtained from the Faculty of Veterinary Medicine, South Valley University, Qena, Egypt. Fresh samples were thoroughly washed and dried for 5–7 days in the shade at 32–35 °C and 50–60% Relative humidity. Dried samples were mechanically ground into a fine powder using a stainless steel industrial electric mixer.

Aqueous Extract Preparation

ANAE was prepared by boiling a 100 g powder (pod) in 1 L of distilled water for 15 min, filtering through a muslin cloth, and filtering paper. The extract was evaporated at 40°C. The dried extract (chocolate-colored crystals) was weighed, labeled, and stored in clean glass vials at 4°C until use

Experimental Animals

Seventy broiler chicks (one-day-old broiler chicks weighing 40-50 g) obtained from the University of South Valley Agriculture Department farm, were randomly selected for acute toxicity studies. Broiler chicks were housed in separate cages and cleaned daily to avoid reinfection and contamination. Water and food were available *ad libitum* at appropriate temperatures and a 12-hour light-dark cycle.

Experimental Design

A total of seventy broiler chicks were divided into seven groups consisting of ten chicks each. Broilers in Group 1, acting as the control, were administered oral distilled water. Concurrently, groups 2 through 7 were given ANAE in varying doses via oral administration for ten days.

The dosages given to each group were 1, 3, 5, 7.5, 10, and 15 g/kg, respectively. The toxicity and mortality indicators of the broiler chicks were attentively observed.

Growth Performance Parameters

Each group of animals was weighed independently. Body weights were measured from the outset to identify any alterations in their weight. The determination of feed intake (FI) and body weight gain (BWG) was assessed.

Biochemical Examinations

Blood samples were allowed to clot to obtain sera. Then they centrifuged at 3000 rpm for 15 minutes to get the supernatant. Then, the supernatant was transferred into sample bottles to estimate the activity of serum ALT, AST (Reitman and Frankel, 1957), and ALP (Belfield and Goldberg, 1971). Serum uric acid (Haisman and Muller, 1974), urea (Marsh et al., 1965, Young and Friedman, 2001) and creatinine (Young and Friedman, 2001).

Statistical Analysis

Data presented as mean and standard deviation. GraphPad Prism performed ANOVA and created graphs. Data are shown as mean ± SD.

Histopathology

Broilers belonging to both control and experimental groups were subjected to euthanasia. The liver and kidney specimens were collected and subsequently treated with a buffered formalin solution with a concentration of 10%. The tissues underwent three cycles of immersion in 70% ethanol, later underwent a graded ethanol series for dehydration, and were then impregnated with paraffin wax after 24 hours. The paraffin-embedded sections were sliced to a thickness of 5 microns and underwent staining with Hematoxylin and Eosin (H&E) as well as Picro Sirius Red stain to facilitate evaluation through the use of light microscopy. The sections were examined and photographed using a digital Japanese-origin microscope, specifically the Olympus BX50 model (Suvarna et al., 2018).

Results

Results of clinical and Post-mortem Examination

In the acute toxicity study, the clinical evaluation of broiler chickens that were orally administered varying dosages of ANAE (1, 3, 5, 7.5, and 10 g/kg) for a period of 10 days revealed no discernible changes in their physical characteristics or mortality rates. Following the administration of ANAE 15 g/kg for a period of 24 hours, evidence of toxicity manifested in various signs including convergence behavior, depression, diminished food intake, weight loss, emaciation, reduced sensitivity to tactile stimuli, and diminished locomotion, alongside a mortality rate of 100%. Early deaths occurred within 24 hours following administration, while late deaths were observed on day 5. These observations are depicted in Figure 1. Intrinsically, the majority of post-mortem lesions observed exhibited hepatic steatosis, accompanied by visibly congested renal structures (refer to Figure 2).

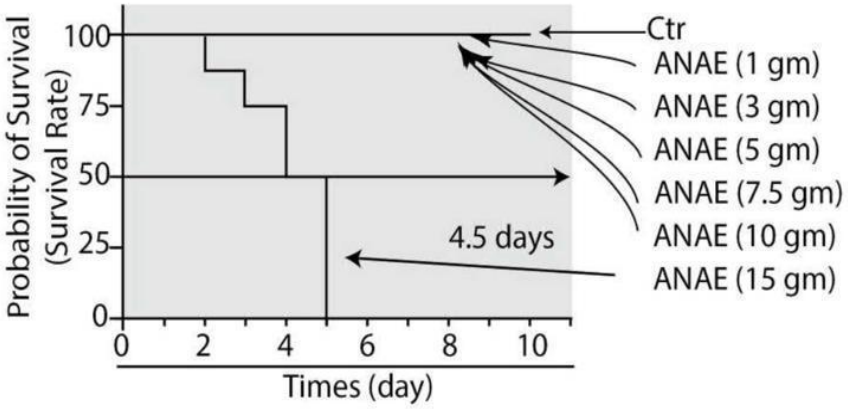

Figure 1: Median survival rate of aqueous extract of *Acacia nilotica* in broilers for 10 days. Broilers were treated with various doses; 1, 3, 5, 7.5, and 10 g/kg b.wt. survived to the last day of treatment (10 days). After 24 hrs of administration of 15 g/kg ANAE, signs of toxicity and mortality were observed in broilers (early deaths reported after 24 hrs and late deaths on day 5).

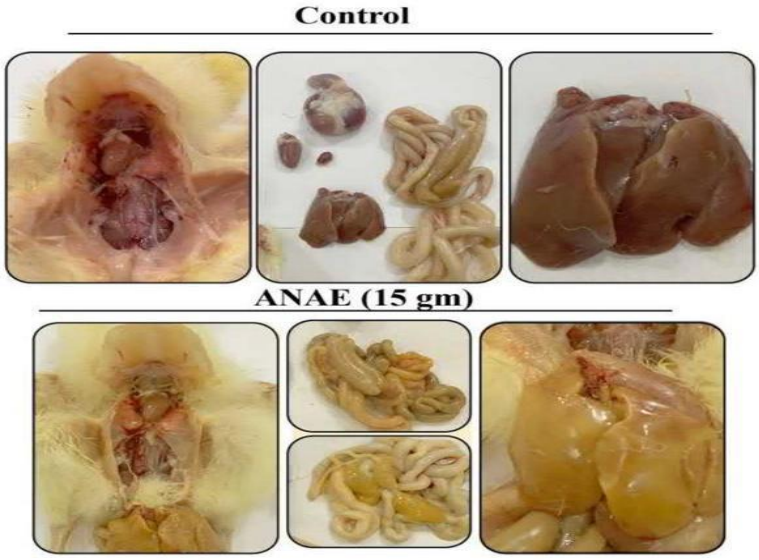

Figure 2: Gross pathological findings in broilers after 5 days post-exposure to ANAE 15 g/kg b.wt. In acute toxicity study. ANAE 15 g/kg treated group showed fatty liver with congested kidneys.

Growth Performance

The effect of ANAE administration on the mean body weight, weight gain, and FI of treated broilers at varying doses; 1, 3, 5, 7.5, 10, and 15 g/kg b.wt. is presented in (Figure 3). A daily oral dose of 1, 3, 5, and 7.5 g/kg of ANAE over 10 days did not demonstrate a significant difference in body weight, BWG, and FI. The challenged broilers with 15 g/kg for 5 days resulted in a substantial reduction ($P < 0.001$) in body weight and weight gain (39.20 ± 1.30 and 6 ± 4.24 g), respectively. The observed decreases were highly significant ($p < 0.001$) in 10 g/kg challenged broilers on day 10 following treatment (109.33 ± 7.87 and 65.0 ± 8.24 g), respectively, compared to the control. FI in treated groups with 10 and 15 g/kg was significantly ($P < 0.001$) decreased when compared to control group.

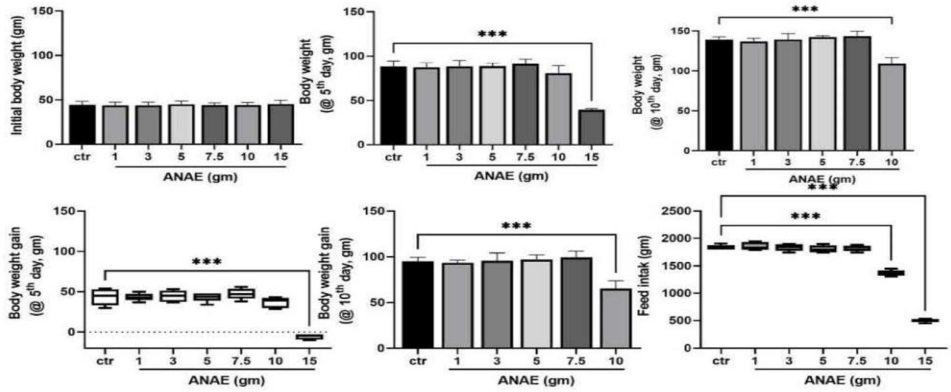

Figure 3: The impact of different doses of ANAE (1, 3, 5, 7.5, 10, and 15 g/kg b.wt.) on BWG and FI of broilers. The results are the mean ± SD of six broilers for each group. ***p < 0.001 compared to the control group.

Clinical Biochemistry

The impact of repeated ANAE oral dose on the biochemical parameters of the treated broilers is shown in Figures 4 and 5. Biochemical parameters due to oral administration of the aqueous extract at different doses of 1, 3, 5, 7.5 and 10 g/kg b.wt. for 5 days to broilers did not demonstrate statistically significant differences compared to control group. ANAE 15 g/kg challenged broiler chicken showed a highly significant increase in the level of AST ($P < 0.0001$) (Figure 4A), ALT (Figure 4B), and ALP (Figure 4C), with values of (267.2 ± 20.38 µ/l, 25.85 ± 3.38 µ/l, and 75.60 ± 6.66 µ/l) respectively, in comparison to the control group (194.2 ± 9.44 µ/l, 16.37 ± 1.35 µ/l, and 55.60 ± 6.84 µ/l). To assess the effect of ANAE on the kidney, the level of urea (Figure 5A), creatinine (Figure 5B), and uric acid were evaluated (Figure 5C). ANAE led to a significant elevation in creatinine level ($P < 0.05$) (0.54 ± 0.05 mg/dl) in the 15 g/kg treated group, as shown in (Figure 3). Urea values elevated significantly ($P < 0.001$) in the treated broilers with 15 g/kg (12.21 ± 2.62 mg/dl) than the regular control group (6.79 ± 0.47 mg/dl). Uric acid values were highly significant ($P < 0.001$) increased in the treated broilers with 15 g/kg (12.21 ± 2.62 mg/dl) than the regular control group (6.79 ± 0.47 mg/dl).

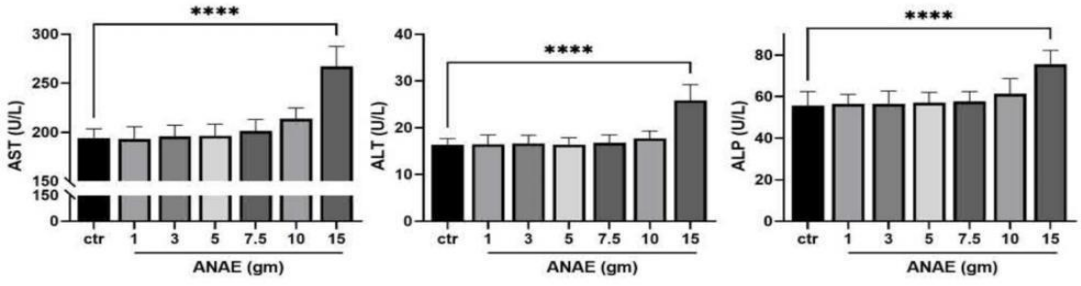

Figure 4: Assessment of toxicological profile of *Acacia nilotica* on broilers (liver function) after 5 days of treatment with different doses of ANAE (1, 3, 5, 7.5, 10 and 15 g/kg b.wt.). AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; ALP: Alkaline Phosphates. The findings are demonstrated as the mean ± SD of six broilers for each group. ****P < 0.0001 compared to the control group.

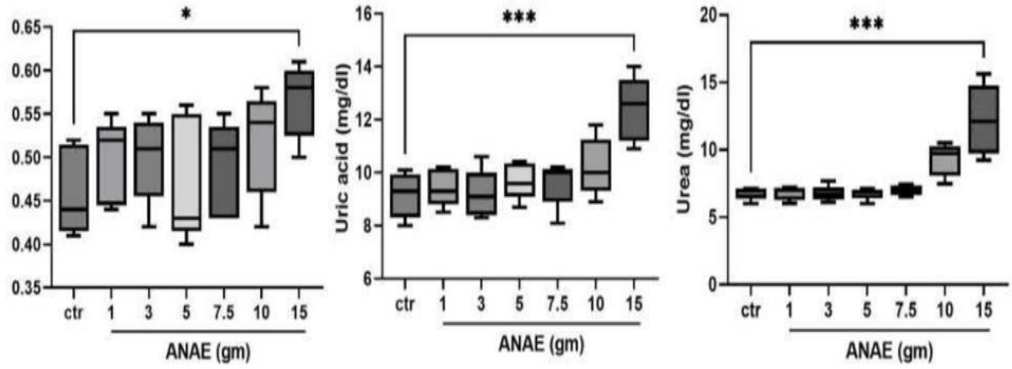

Figure 5: Assessment of toxicological profile of *Acacia nilotica* on broilers (kidney function) after 5 days of treatment with different doses of ANAE (1, 3, 5, 7.5, 10 and 15 g/ kg b.wt.). The results are the mean ± SD of six broilers for each group. *p < 0.05, ***p < 0.001 compared to the control.

Histopathology

Microscopically, H&E staining for the hepatic section is shown in Figure 6A. Microscopic examination of the liver from the control group showed normal hepatic parenchyma with large polyhedral and angular-shaped hepatocytes and a centrally located central vein without any inflammatory reaction around the portal area. ANAE 7.5 g/kg group showed mild infection in the liver with focal infiltration of mononuclear cells and inflammatory cell around the portal area. Liver from ANAE 10 g/kg group revealed dilation and congestion of the central vein, dilation and congestion of the portal area, and lymphocytic infiltration in the portal area. The liver from ANAE 15 g/kg treated group revealed severe congestion of the blood sinusoid and central vein, fibrosis in the portal area, lymphocytic infiltration with necrosis in cells, and fatty degeneration (steatohepatitis). The impact of a repeated oral dose of ANAE 7.5, 10, and 15 g/kg b.wt. on diameter (Figure 6B) and size of hepatocyte nucleus (Figure 6C) for 5 days in broilers is shown. ANAE 15 g/kg challenged broiler chickens showed a significant decrease in the hepatocyte nucleus's diameter and size ($P < 0.0001$). ANAE 10 g/kg induced a highly significant decrease in diameter ($P < 0.01$), with a significant decrease in the size of the hepatocyte nucleus ($P < 0.05$). The liver damage severity score of ANAE 10 and 15 g/kg recorded a highly significant ($P < 0.001$) increase compared to the control group, but the changes were found to be insignificant after treatment with ANAE 7.5 g/kg compared to the control (Figure 6D).

The results of Picro Sirius Red staining for the liver are depicted in Figure 7A. The histopathological evaluation of fibrosis was conducted following the administration of ANAE at a dosage of 15 g/kg. The analysis was carried out on day 5 post-treatment and revealed the presence of fibrosis in the portal triad, portal vein, and central vein. The findings of the present investigation indicate that ANAE dosages of 10 and 15 g/kg are associated with a marked reduction in Fibrosis Ishak Score, as evidenced by the results of statistical analysis which revealed a significant decrease in this metric when compared to the control group ($P<0.001$). This conclusion is supported by the data presented in Figure 7B. The administration of ANAE at a dosage of 10 g/kg resulted in

a statistically significant elevation in Alternatively, ANAE treatment at a dosage of 15 g/kg exhibited a markedly significant Relative Fibrosis (%) ($P < 0.05$). rise in Relative Fibrosis (%) ($P < 0.001$), as depicted in Figure 7c.

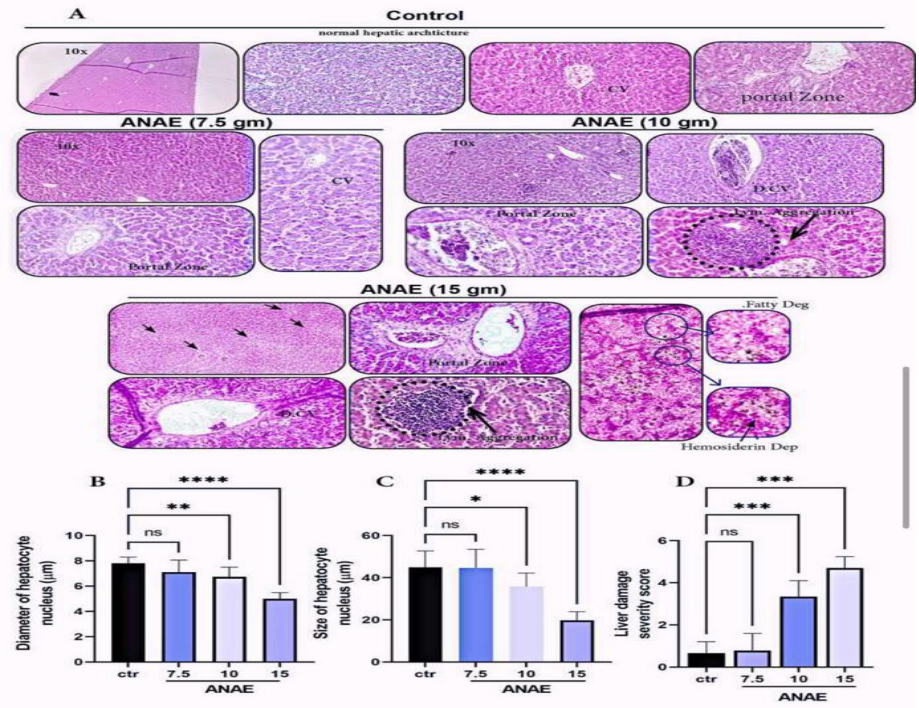

Figure 6: Histopathologically examined liver during treatment with ANAE; 7.5, 10, and 15 g/ kg b.wt.) after 5 days post-exposure, stained with H&E stain in control and treated groups. ANAE 7.5 g/kg group showed mild histological changes in the liver with focal infiltration of mononuclear cells and inflammatory cell around the portal area. Liver from ANAE 10 g/kg group revealed dilation and congestion of the central vein, dilation and congestion of the portal area, and lymphocytic infiltration in the portal area. The liver from ANAE 15 g/kg treated group revealed severe congestion of the blood sinusoid and central vein, fibrosis in the portal area, lymphocytic infiltration with necrosis in cells, and fatty degeneration (steatohepatitis). The effect ANAE on the diameter and size of hepatocyte nuclei in broilers are demonstrated as the mean ± SD of six broilers for each group. *p < 0.05, **p < 0.01, ***p < 0.001 and ****p < 0.0001 compared to the control.

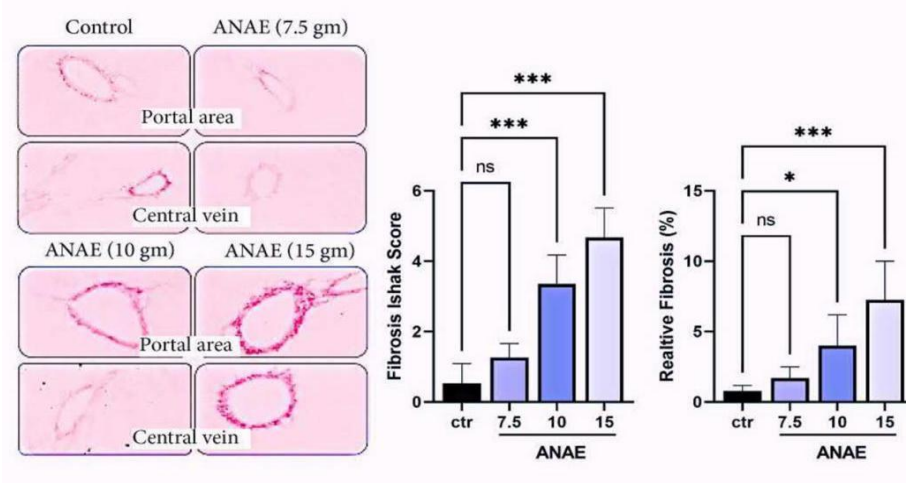

Figure 7: Histological assessment of fibrosis during treatment with aqueous extract of *Acacia nilotica* on day 5 post-treatment in the portal triad, portal vein, and central vein stained with Picro Sirius Red stain in control and treated groups. ANAE 15 g/kg treatment showed fibrosis in the portal triad, portal vein, and central vein. The effect ANAE on Fibrosis Ishak Score and Relative Fibrosis (%) in broilers are presented as the mean ± SD of six broilers for each group. *p < 0.05 and ***p < 0.001 compared to the control.

Figure 8 depicts the microscopic visualization of H&E staining for a renal section. The microscopic evaluation of the kidney specimens procured from the control group revealed a structurally intact renal architecture, devoid of any observable histological aberrations or abnormalities in the renal tubules and glomeruli. The group subjected to ANAE at the dose of 7.5 g/kg exhibited typical morphology of renal tubules and glomeruli. Renal specimens obtained from ANAE-treated subjects exhibited conspicuous inflammation characterized by lymphoid aggregation and congestion, along with notable dilation of Bowman's space and glomerular atrophy. The group treated with a dosage of 15 g/kg in ANAE demonstrated notable histological changes, including dilation of Bowman's space, glomerular atrophy, severe cellular infiltration, dilated tubules, vacuolar degeneration, and loss of tubular bush border.

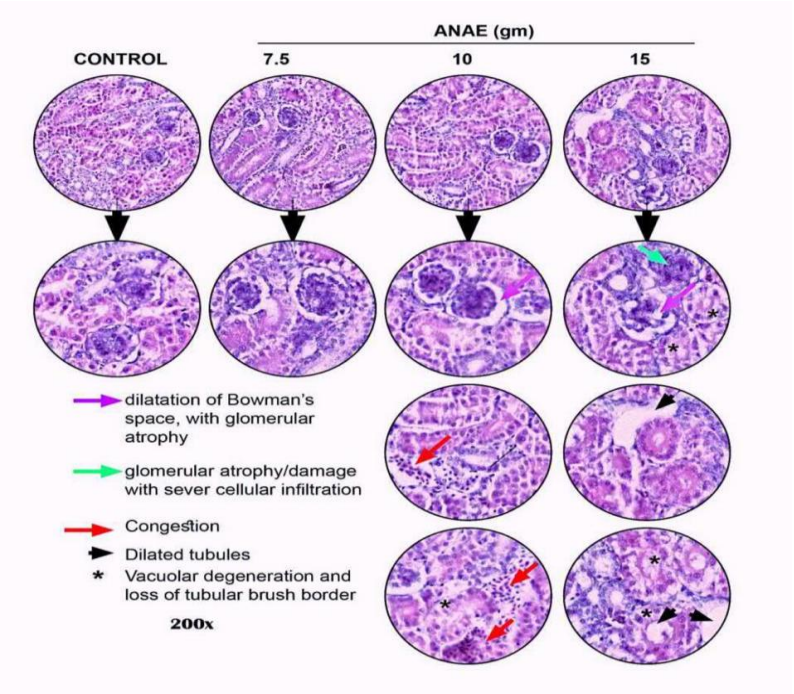

Figure 8: Histopathological examined kidney during treatment with ANAE 7.5, 10, and 15 g/kg b.wt.) after 5 days post-exposure stained with H&E stain in control and treated groups. ANAE 7.5 g/kg challenged group showed normal renal tubules and glomeruli. Kidney from ANAE 10 g/kg showed marked inflammation with lymphoid aggregation, congestion, dilation of Bowman's space, with glomerular atrophy. ANAE 15 g/kg treated group revealed histological alterations with dilation of Bowman's space, glomerular atrophy, severe cellular infiltration, dilated tubules, vacuolar degeneration and loss of tubular brush border.

Discussion

Medicinal plants are a precious reservoir of contemporary and traditional pharmaceuticals, and the world is progressively reverting to natural remedies. The potential adverse impacts of herbal medications are characterized by two predominant deleterious outcomes: hepatotoxicity and nephrotoxicity. (Colson and De Broe, 2005, Asif, 2012, Nwachukwu et al., 2009). Acacia nilotica represents a significant plant species, possessing therapeutic properties that can be employed for overall health maintenance. Numerous investigations have documented the pharmacological properties of the substance. Nonetheless, the plant exhibits potential hazards akin to

those of pharmaceutical drugs, rendering the inquiry into its toxicity profile paramount in the context of broiler farming. The viability of broilers, subjected to varying doses of 1, 3, 5, 7.5, and 10 grams per kilogram body weight, was evaluated. As of the tenth day, evidence suggests that the extract derived from Acacia nilotica is deemed safe. The acute toxicity of ANAE was demonstrated through administration at a dosage of 15 grams per kilogram of body weight. Over five days, observable alterations in behavior were evident, including but not limited to the onset of depression, weight loss, a reduced inclination to consume sustenance or fluids, diminished physical activity, and mortality. The result agreed with (Nesa et al., 2014), who reported locomotive ataxia, diarrhea and weight loss with methanol seeds extract of Acacia nilotica at doses of 50, 100, 200, 500, and 1000 mg/kg body weight in an acute toxicity study in mice. After 12-18 hours of treatment, a reduction in locomotion, sensitivity to touch, and prostration were considered as clinical symptoms of Acacia nilotica leaf extract toxicity in rats (Tanko et al., 2015a). Also, it was documented the typical clinical indications in rats treated for 14 days with n-Butanol and ethyl acetate extracts of Acacia nilotica leaves, with early deaths reported following 12 hours and late deaths reported 48 hours following fraction administration (Tanko et al., 2015b). Clinical toxicity markers were observed in Nubian goats given whole Acacia nilotica pods at doses of 5 g/kg/day for 35 days, including salivation, staggering gait, intermittent voice loss, reduced appetite, and death (between days 4 and 8) (Medani et al., 2016). Broiler chicks with ANAE 15 g/kg on day 5 and ANAE 10 g/kg on day 10 significantly reduced body weight and weight gain ($p < 0.001$). Many parts of Acacia nilotica have been mentioned to significantly influence the loss of appetite and, therefore, reduce BWG and weight loss. The toxicity of aqueous pod extract from Acacia nilotica was studied in rats who preserved 2% and 8% Acacia diet for 2 and 4 weeks (Al-Mustafa and Dafallah, 2000). The research demonstrated a substantial reduction in weight gain in treated animals, indicating the presence in Acacia pods of growth impairing substances, including tannin. Another study found a decreased body weight in rats fed 2%, and 8% leaves aqueous extract of Acacia nilotica in the diet for 2 and 4 weeks (Mohan et al., 2014). In contrast, the methanol root extract of Acacia nilotica has been shown to contribute significantly to a reduction in body weight in mice fed for 5 weeks (Jigam et al., 2010). ANAE 15 g/kg challenged group may have hepatotoxic and nephrotoxic effects, which were observed with a highly significant increase in AST, ALT, ALP, and urea ($p < 0.0001$). ALT, ALP levels, and AST in treated rats with aqueous extract of *Acacia nilotica* for 28 days were substantially higher than control levels at 500 mg/kg b.wt. (Alli et al., 2015). It was also reported a significant elevation in ALP, urea and creatinine concentrations ($P < 0.05, 0.01$) in goats dosed with the Acacia nilotica extract at 1g/kg/day (Medani et al., 2016). A study used aqueous stem bark extracts to test the toxicity of six plants, including *Acacia nilotica*, in mice given orally with 1000 mg/kg b.wt. for 28 days (MUKUNDI, 2015). There has been a significant increase in uric acid levels, a key indicator of kidney function. The toxicity of ethanol fruit extract of Acacia nilotica was investigated in rats given doses of 75, 100,

112.5, 125, 187.5, 250 and 500 mg/kg for 21 days (El-Hadiyah et al., 2011). Creatinine, ALT, elevated urea and AST in plasma were used to assess hepatotoxicity and nephrotoxicity. No behavioral alterations were observed on day 21. However, a three-week treatment caused a significant elevation in urea and ALT. The elevation in biomarker levels (ALT and urea) indicated that liver and kidney functions were impaired, respectively, especially at high doses. Histopathological examinations showed fatty degeneration, fibrosis and cholestasis in the liver with marked inflammation and destruction of glomerulus and tubules in the kidney. The result agreed with (Jigam et al., 2010), who revealed feathery hepatocyte degeneration and nephron damage. It was also indicated fatty vacillation of the hepatocytes with generalized necrosis and necrotic renal convoluted tubules in goats given 5 g/kg/day of Acacia nilotica extract (Medani et al., 2016).

Conclusions

Based on the results obtained in this study, it can be concluded that using Acacia nilotica extract (in aqueous form) can be deemed medically safe for broiler chickens at dosages below 15 grams per kilogram of body weight.

Conflict of interest

The authors have no conflicts of interest to declare. All co-authors have seen and agree with the manuscript's contents and there is no financial interest to report. We certify that the submission is original work and is not under review at any other publication.

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