"Toxicological studies and therapeutic aspects of *Acacia nilotica* seeds in broilers"

THESIS PRESENTED

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LIST OF ABBREVIATIONS

ANAE	*Acacia Nilotica* Aqueous Extract
L	Liter
KG	Kilo Gram
B.W.	Body Weight
EDTA	Ethylene Diamine Tetra Acetic Acid
GC-MS	Gas Chromatography-Mass Spectrometry
RPM	Round Per Minute
BWG	Body Weight Gain
FCR	Feed Conversion Ratio
RBCs	Red Blood Cell
WBCs	White Blood Cell
Hb.	Hemoglobin
PCV	Packed Cell Volume
MCV	Mean Corpuscular Volume
MCH	Mean Corpuscular Hemoglobin
MCHC	Mean Corpuscular Hemoglobin Concentration

AST	Aspartate Aminotransferase
ALT	Alanine Aminotransferase
ALP	Alkaline Phosphatase
NO	Nitric Oxide
MDA	Malondialdehyde
MPO	Myeloperoxidase
CAT	Catalase
GSH	Glutathione
SOD	Superoxide Dismutase
TAC	Total Antioxidant Capacity
ANOVA	One-way Analysis of Variance
SPSS	Statistical Package for Social Sciences
H&E	Hematoxylin and Eosin Stain

SUMMARY

Acacia nilotica which belongs to the family of Leguminosae, is a medium sized tree distributed in Egypt and used in folk medicine. Several authors reported that *Acacia nilotica* has various pharmacological activities. Therefore, the present study aimed to evaluate its anticoccidial activity and determine the safety and toxicological profile of *Acacia nilotica* aqueous seeds extract in broilers.

Extraction is the first step before component analysis to isolate bioactive phytochemicals from plant materials. GC-MS spectra of extract reported the presence of various constituents like fatty acids, esters, alkaloids, phenols, carbohydrates, and terpenes. The bioactive compounds exhibited anti-inflammatory, anti-oxidant, and anticoccidial activities. Due to the presence of the compounds mentioned above in the aqueous seeds extract of *Acacia nilotica*, it could be used to treat coccidiosis.

Our research assessed the in vitro inhibitory effect of escalated concentrations (6.25, 12.5, 25, 50, and 100 mg/ml) of *Acacia nilotica* aqueous seeds extract (ANAE) on *Eimeria tenella* sporulation. Statistical analysis revealed that ANAE decreased the percentage of oocyst sporulation in a dose-dependent manner. Furthermore, ANAE showed abnormal sporulation and morphological deterioration of *E. tenella* oocytes. Area Under the Curve (AUC) calculation was used to determine the efficacy of ANAE and revealed that ANAE concentrations significantly reduced the coccidial score index in ANAE 25, 50, and 100 100 mg/ml. At 100 mg/ml, ANAE completely suppressed the sporulation of *E. tenella* oocysts, with obvious changes to their morphology and size.

A total number of sixty broiler chicks at the age of fourteen were used for therapeutic investigation. Fifty experimental broiler chicks were

orally administered with sporulated oocysts (0.5 mL of suspension containing 4 x 10^4 (40000 oocysts). Three doses of aqueous extract of *Acacia nilotica* seeds 1.5, 3, and 6 g/L, respectively, and one dose of amprolium hydrochloride 20% at 1.5 g/L were used for 8 days three times daily. Clinical signs were recorded all over the experimental period. Fecal samples were collected every day for oocyst count.

Blood samples were collected for estimation of blood picture, and biochemical parameters. Samples from the liver and cecum were collected for histopathological examination and oxidative stress markers analysis at the end of the experiment. Broilers treated with amprolium 1.5 g/L and ANAE 1.5, 3, and 6 g/L appeared normal without any clinical signs or mortalities. All treated groups showed a highly significant decrease in the number of oocysts in feces ($P < 0.0001$). Hematological parameters of broilers with ANAE 1.5 g/L showed a non significant decrease in RBCs, Hb, and PCV with a highly significant increase of WBCs, monocytes and lymphocytes ($P < 0.0001$). Broiler chicks treated with amprolium 1.5 g/L, ANAE 3 and 6 g/L, did not show statistically significant differences compared to non-infected control and these values remained within normal reference ranges.

For biochemical parameters of broiler chicken treated with ANAE 1.5 g/L, they showed a highly significant increase in AST ($P < 0.0001$), with a significant decrease in serum albumin ($P < 0.05$), but these parameters have no statistical significance in treated broilers with amprolium 1.5 g/L, ANAE 3 and 6 g/L. There is no significance in serum urea, creatinine and uric acid for renal parameters at doses of aqueous extract of *Acacia nilotica* and amprolium group.

For oxidative stress markers, broiler chicks treated with amprolium and ANAE 1.5 g/L showed highly significant increases in NO, MDA, and MPO ($P < 0.0001$), and highly significant decreases in

CAT, GSH, SOD, and TAC ($P < 0.0001$). Broiler chicks treated with aqueous extract of *Acacia nilotica* at the dose of 3 and 6 g/L demonstrated reduced oxidative stress with increased anti-oxidant enzymes.

According to histopathological examination, the cecum and livers of ANAE 1.5 g/L challenged broilers showed less similar changes than those infected with coccidiosis. These changes in the cecum were expressed by intact mucosal epithelium, a slight reduction of the coccidial stages infiltrating the lamina propria and the glandular epithelium, as well as moderate inflammatory reaction with mononuclear cells infiltrations. Livers showed a moderate inflammatory reaction with mononuclear cells infiltrations in the portal area.

Amprolium-treated groups showed more moderate histological alterations and improvement than the positive control group in the cecum and liver. Cecum examination revealed intact mucosal epithelium, moderate reduction of the coccidial stages infiltrating the lamina propria and the glandular epithelium, as well as moderate inflammatory reaction with mononuclear cells infiltrations. The liver showed marked bile duct hyperplasia with the luminal projection of the proliferated epithelial lining in the portal area, and there were no signs of inflammatory reaction.

Cecum and livers of ANAE 3 g/L challenged broilers showed more liver and cecum histology improvement than amprolium treated groups. Cecum showed intact mucosal and cecal glands epithelium with moderate reduction of the coccidial stages, as well as hyperplasia of lymphoid tissue as a sign of increased immune response. Livers showed nearly the same normal hepatic parenchyma as the negative control group.

The histopathological examination of the cecum and livers of ANAE 6 g/L challenged broilers showed a complete subside in any

inflammatory reactions and the best immune response. The cecum showed intact mucosal and cecal glands epithelium with complete reduction of the coccidial stages, absence of inflammatory reaction, as well as goblet cells hyperplasia and hyperplasia of the lymphoid tissue as a good immune response.

Fifty-five male albino mice were used to determine LD_{50} by administering ascending concentrations of aqueous extract of *Acacia nilotica* from 25 mg/kg b.w to 3000 mg/kg b.w. for 5 days. The mortalities (response) in each group were recorded. The oral mean lethal dose (LD_{50}) of aqueous extract of *Acacia nilotica* seeds in male albino mice was 800 mg/kg b.w.

Seventy-one-day-old broiler chicks were used for the acute toxicity study. Different doses of aqueous extract of *A. nilotica* seeds (1, 3, 5, 7.5, 10, and 15 g/kg b.w) for 10 days were used. Clinical signs and postmortem lesions were recorded all over the experimental period. Blood samples were collected for estimation of the biochemical parameter. Samples from the liver and kidney were collected for histopathological examination.

Broiler chickens did not demonstrate death or change in physical appearance and morphological characteristics throughout the observation period (10 days) after oral administration of ANAE (1, 3, 5, 7.5, and 10 g/kg). ANAE 15 g/kg challenged broilers showed behavioral changes such as depression**,** weight loss, off food and water, decrease in locomotion, decrease in sensitivity to touch, and 100% mortality occurred. Various doses; 1, 3, 5, and 7.5 g/kg of aqueous extract of *A. nilotica* did not reveal a significant change in body weight and weight gain compared to control. Broiler chicks with ANAE 15 g/kg on day 5 and ANAE 10 g/kg on day 10 exhibited a highly significant decrease in body weight and weight gain ($P < 0.001$).

Oral doses of *A. nilotica* (1, 3, 5, and 7.5 g/kg) did not show statistically significant differences in biochemical parameters compared to control. Treatment with ANAE 10 g/kg induced a significant increase in ALP, urea, creatinine, and uric acid ($P < 0.05$), while AST and ALT non significantly increase. ANAE 15 g/kg challenged broilers induced highly significant increases in all biochemical parameters ($P < 0.0001$, $P < 0.001$).

Microscopic examination of diameter and size of hepatocyte nucleus in ANAE 15 g/kg challenged broilers, showed a highly significant increase ($P < 0.0001$). ANAE 10 g/kg induced a highly significant increase in diameter ($P < 0.01$), with a significant increase in the size of the hepatocyte nucleus ($P < 0.05$), but the change was not significant in ANAE 7.5 g/kg treatment when compared to the control.

Histopathological examination of the liver from ANAE 7.5 g/kg showed mild infection in the liver with focal infiltration of mononuclear cells and dilation of blood sinusoid. ANAE 7.5 g/kg challenged group showed normal kidneys with normal glomeruli. Liver from ANAE 10 g/kg revealed dilation and congestion of central vein, dilation and congestion of portal area and lymphocytic infiltration in the portal area. Microscopic examination of the kidney from ANAE 10 g/kg showed slight histological alterations with lymphoid aggregation and a mild effect on glomerulus and inflammatory cells in the kidney. Liver from ANAE 15 g/kg revealed severe congestion of blood sinusoid and central vein, fibrosis in portal area, lymphocytic infiltration with necrosis in the cell, fatty degeneration (steatohepatitis), intrahepatic and extrahepatic cholestasis. The kidney from ANAE 15 g/kg revealed marked inflammation with destruction of renal tubules and glomeruli.