



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

THESIS PRESENTED

By

Esraa Galal Mahmoud

(B.V. Sc., Fac. Vet. Med., South Valley University, Qena, 2006)

(M.V. Sc., Forensic Medicine & Toxicology, South Valley University, Qena, 2015)

For the degree of Ph.D.

(Forensic Medicine & Toxicology)

Under Supervision of

Prof. Dr. Abdel-Latif Shaker Seddek

Professor of Forensic Medicine & Toxicology
Department of Forensic Medicine & Toxicology
Faculty of Veterinary Medicine, Qena
South Valley University

Prof. Dr. Samy Abdel-Raouf Fahim

Professor of Pharmacology
Department of Pharmacology
Faculty of Veterinary Medicine, Qena
South Valley University

Dr. Dina Mohammed Waheed Shibat El-Hamd

Researcher of Poultry Diseases
Animal Health Research Institute, Qena
Animal Health Research Institute

To

Faculty of Veterinary Medicine

South Valley University

2022

CONTENTS

Item	Page
1- Introduction	1
2-The Aim of Work	3
3- Review of Literature	4
3.1 Etiological Agent of Avian Coccidiosis	4
3.2 Life Cycle of <i>Eimeria</i>	4
3.3 Economic Importance of Poultry Coccidiosis	5
3.4 Anticoccidial Drugs	5
3.5 Anticoccidial Drug Resistance	6
3.6 Plant Names	8
3.7 Plant Description	8
3.8 Plant Distribution	9
3.9 Phytochemistry	9
3.10 Economic Importance	12
3.11 Ethnopharmacology	13
3.12 Pharmacodynamic Studies	14
3.13 Safety And Toxicological Studies	21
4- Materials And Methods	27
4.1 Materials	27
4.2 Commercial Ration	27
4.3 Plant Material Collection	27
4.4 Experimental Animals	28

4.5 Chemicals Used for Detection and Counting of Oocysts	29
4.6 Materials For Hematological Analysis	29
4.7 Kits for Determination of Some Biochemical Parameters	30
4.8 Kits for Determination of Oxidative Stress Markers Tests	31
4.9 Chemicals Used for Histopathological Examinations	32
4.10 Equipments	32
4.11 Methods	33
4.12 Preparation of Aqueous Extract	33
4.13 Chemical Aanalysis of <i>Acacia nilotica</i>	33
4.14 Gas Chromatography – mass Spectrometry (GC-MS)	33
4.15 <i>In Vitro</i> Experiment	34
4.16 <i>In Vivo</i> Anticoccidial Study	35
4.17 Experimental Design	35
4.18 Collection of Sample	36
4.19 Performance Measurement	39
4.20 Hematological Analysis	39
4.21 Biochemical Analysis	42
4.22 Oxidative Stress Markers Analysis	45
4.23 Statistical Analysis	48
4.24 Histopathology	48
5- Results	49
5.1 GC-MS Analysis of Aqueous Seeds Extract of <i>Acacia Nilotica</i>	49

5.2 <i>In Vitro</i> Inhibitory Effect of Aqueous Seeds Extract of <i>Acacia Nilotica</i> on <i>Eimeria Tenella</i>	54
5.3 Inhibitory Effect of Different Concentrations of ANAE on Sporulation Rate	54
5.4 The Impact of ANAE on Sporulated Oocysts Count	56
5.5 Effect of Different Concentration of ANAE on Morphology and Size of <i>Eimeria Tenella</i> Oocyst	59
5.6 <i>In Vivo</i> Anticoccidial Efficacy of <i>Acacia Nilotica</i> Aqueous Seeds Extract In Broilers	60
5.7 Clinical Signs And PM	60
5.8 Growth Performance	64
5.9 Fecal Oocyst Count Results	66
5.10 Histopathology of Caecum	69
5.11 Hematological Results	77
5.12 Oxidative Stress Markers Results.	83
5.13 Histopathology of Liver	86
5.14 Biochemical Results	93
5.15 Determination of LD ₅₀ of Aqueous Seeds Extract of <i>Acacia Nilotica</i> In Male Albino Mice.	99
5.16 Acute Toxicity Study of Aqueous Seeds Extract of <i>Acacia Nilotica</i> In Broilers	100
5.17 Clinical Signs and PM	100
5.18 Growth Performance	103
5.19 Histopathology of Liver	105
5.20 Clinical Biochemistry of Liver	112
5.21 Diameter and Size of Hepatocyte Nucleus	114

5.22 Histopathology of Kidney	115
5.23 Clinical Biochemistry of Kidney	119
6- Discussion	122
7- Summary	154
8- conclusion	159
9- References	160
10- الملخص العربي	1

LIST OF TABLES

<u>Table no.:</u>	Page
Table 1: The bioactive compounds with their peak area, total area % and retention time (RT) present in aqueous extract of <i>Acacia nilotica</i>	50
Table 2: The bioactive compounds present in aqueous extract of <i>Acacia nilotica</i> with their action	53
Table 3: <i>In vitro</i> inhibitory effect of different doses of <i>Acacia nilotica</i> aqueous extract (6.25, 12.5, 25, 50, and 100 mg/ml) on oocyst sporulation for 5 days	55
Table 4: <i>In vitro</i> inhibitory effect of different doses of <i>Acacia nilotica</i> Aqueous extract (6.25, 12.5, 25, 50, and 100 mg/ml) on a number of sporulated oocysts (Means \pm SD) in control and challenged groups. **Highly significant (p < 0.01), *** Highly significant (p < 0.001), ****Highly significant (p < 0.0001)	57
Table 5: Clinical findings from 5th day post infection (DPI) in broilers infested with caecal coccidiosis	62
Table 6: Effect of different oral dose of <i>A. nilotica</i> aqueous extract (1.5, 3, and 6 g/litter water) for 8 days on Final body weight, body weight gain, feed intake and feed conversion ratio (Means \pm SE) in broilers of control and challenged groups. ****Highly significant (p < 0.0001)	65
Table 7: Fecal oocysts count (X1000 oocysts per gram) (Means \pm S.D.) in broilers of control and challenged groups. ****Highly significant (p < 0.0001)	67
Table 8: Histopathological findings of different experimental groups	69
Table 9: Effect of aqueous extract of <i>Acacia nilotica</i> (1.5, 3, and 6 g/litter water) for 8 days on erythrocytic count (Means \pm S.D.) in broilers of control and challenged groups. *Significant (p < 0.05) **Significant (p < 0.01) ****Significant (p < 0.0001)	78
Table 10: Effect of aqueous extract of <i>Acacia nilotica</i> (1.5, 3, and 6 g/litter water) for 8 days on Total WBCs count and differential leucocytes count (Means \pm SE) in broilers of control and challenged groups. *Significant (p < 0.05), ***Significant (p < 0.001), ****Significant (p < 0.0001)	81

Table(11: Effect of aqueous extract of <i>Acacia nilotica</i> (1.5, 3, and 6 g/litter water) for 8 days on oxidative stress markers (Means \pm SE) in broilers of control and challenged groups. *Significant (p < 0.05), **Significant (p < 0.01), ***Significant (p < 0.001), ****Significant (p < 0.0001)	84
Table 12: Effect of aqueous extract of <i>Acacia nilotica</i> (1.5, 3, and 6 g/litter water) for 8 days on liver function Tests (Means \pm SE) in broilers of control and challenged groups. *Significant (p < 0.05), **Significant (p < 0.01), ****Significant (p < 0.0001)	94
Table 13: Effect of aqueous extract of <i>Acacia nilotica</i> (1.5, 3, and 6 g/litter water) for 8 days on kidney function Tests (Means \pm S.D.) in broilers of control and challenged groups. *Significant (p < 0.05) **Significant (p < 0.01)	97
Table 14. Effect of different oral dose of <i>A. nilotica</i> aqueous extract (1, 3, 5, 7.5, 10, and 15 g/kg b.w.) on body weight, weight gain and feed intake (Means \pm S.D.) in broilers of control and challenged groups. ****Highly significant (p < 0.0001)	104
Table 15. Effect of different oral dose of <i>A. nilotica</i> aqueous extract (1, 3, 5, 7.5, 10, and 15 g/kg b.w.) on liver function (Means \pm S.D.) in broilers of control and challenged groups. *Significant (p < 0.05), ****Highly significant (p < 0.0001)	113
Table 16. Effect of different oral dose of <i>A. nilotica</i> aqueous extract (1, 3, 5, 7.5, 10, and 15 g/kg b.w.) on kidney function (Means \pm S.D.) in broilers of control and challenged groups. *Significant (p < 0.05), ***Highly significant (p < 0.001), ****Highly significant (p < 0.0001)	120

LIST OF FIGURES

<u>Figure no.:</u>	Page
Figure 1: GC-MS chromatogram for aqueous extract of <i>Acacia nilotica</i>	52
Figure 2: Sporulation index and IC50 of different doses of ANAE (6.25, 12.5, 25, 50, and 100 mg/ml) for 5 days. The values are presented as the mean \pm S.D. of 3 for each group. ***p < 0.001 compared with control group.	56
Figure 3: Sporulated oocysts count and Coccidial score of different doses of ANAE (6.25, 12.5, 25, 50, and 100 mg/ml) on the number of sporulated oocysts for 5 days. The values are presented as the mean \pm S.D. of 3 for each group. ***p < 0.001 compared with control group.	58
Figure 4: The Percentage Maximum Possible Effect (% MPE) was calculated as the percentage difference between the measured and baseline response and divided by the difference between the maximum response and the baseline response.	58
Figure 5: Morphological alterations of <i>E. tenella</i> oocysts after exposure to different ANAE concentrations (6.25, 12.5, 25, 50, and 100 mg/ml) at different time intervals. At varied doses of ANAE, <i>E. tenella</i> oocysts showed obvious morphological changes (alteration of oocyst, sporocyst, and sporozoite morphology) as well as aberrant sporulation.	59
Figure 6: Post mortem symptoms in control groups and different treated groups after 8 days post exposure to different doses of <i>Acacia nilotica</i> aqueous extract (1.5, 3, and 6 g/litter water).	63
Figure 7: The impact of different doses of <i>Acacia nilotica</i> aqueous extract (1.5, 3, and 6 g/litter water) for 8 days on body weight gain of broilers.	66
Figure 8: Oocyst count in control and different treated groups during 8 days post exposure to different doses of <i>Acacia nilotica</i> aqueous extract (1.5, 3, and 6 g/litter) water in broilers.	68
Figure 9: Microscopic examination of caecum from control negative group in therapeutic study.	71
Figure 10: Microscopic examination of caecum from control positive group in therapeutic study.	71
Figure 11: Microscopic examination of caecum from amprolium group in therapeutic study.	73

Figure 12: Microscopic examination of caecum from ANAE 1.5 g/L group in therapeutic study.	73
Figure 13: Microscopic examination of caecum from ANAE 3 g/L group in therapeutic study.	74
Figure 14: Microscopic examination of caecum from ANAE 6 g/L group in therapeutic study.	75
figure 15: Goblet cells stained with Alcian blue in non infected, infected, and all treated groups.	77
Figure 16: Erythrocytic count and hematological indices in control and different treated groups of broilers after 8 days post exposure to different doses of <i>Acacia nilotica</i> aqueous extract (1.5, 3, and 6 g/litter water) in broilers.	79
Figure 17: Total WBCs count and differential leucocytes count in control and different treated groups of broilers after 8 days post exposure to different doses of <i>Acacia nilotica</i> aqueous extract (1.5, 3, and 6 g/litter water).	82
Figure 18: Oxidative stress markers in control and different treated groups of broilers after 8 days post exposure to different doses of <i>Acacia nilotica</i> aqueous extract (1.5, 3, and 6 g/ litter water).	85
Figure 19: Microscopic examination of liver from control negative group in therapeutic study.	88
Figure 20: Microscopic examination of liver from control positive group in therapeutic study.	88
Figure 21: Microscopic examination of liver from amprolium group in therapeutic study.	90
Figure 22: Microscopic examination of liver from ANAE 1.5 g/L group in therapeutic study.	90
Figure 23: Microscopic examination of liver from ANAE 3 g/L group in therapeutic study.	91
Figure 24: Microscopic examination of liver from ANAE 6 g/L group in therapeutic study.	92
Figure 25: Liver function tests in control and different treated groups of broilers after 8 days post exposure to different doses of <i>Acacia nilotica</i> aqueous extract (1.5, 3, and 6 g/litter water).	95

Figure 26: kidney function Tests in control and different treated groups of broilers after 8 days post exposure to different doses of <i>Acacia nilotica</i> aqueous extract (1.5, 3, and 6 g/litter water).	98
Figure 27: Median survival of the oral mean lethal dose of aqueous extract of <i>Acacia nilotica</i> in male albino mice for 5 days with ascending concentrations from (25 mg/kg - 3000 mg/kg).	99
Figure 28: Oral mean lethal dose(LD₅₀) of aqueous extract of <i>Acacia nilotica</i> in male albino mice for 5 days was 800 mg/kg	100
Figure 29: Median survival of the oral mean lethal dose of aqueous extract of <i>Acacia nilotica</i> (1, 3, 5, 7.5, 10, and 15 g/ kg b.w.) in broilers for 10 days.	101
Figure 30: Gross pathological findings in broilers after 10 days post exposure to different doses of <i>Acacia nilotica</i> water extract (1, 3, 5, 7.5, 10, and 15 g/ kg b.w.) in acute toxicity study	102
Figure 31: The impact of different doses of <i>Acacia nilotica</i> aqueous extract (1, 3, 5, 7.5, 10, and 15 g/ kg b.w.)for five days on body weight gain and feed intake of broilers	105
Figure 32: Microscopic examination of liver from control negative group in acute toxicity study	107
Figure 33: Microscopic examination of liver from 7.5 g/kg group in acute toxicity study	107
Figure 34: Microscopic examination of liver from ANAE 10 g/kg group in acute toxicity study	108
Figure 35: Microscopic examination of liver from ANAE 15 g/kg group in acute toxicity study	109
Figure 36: Histological assessment of fibrosis in non infected and treated groups in acute toxicity study	111
Figure 37: Assessment of toxicological profile of <i>Acacia nilotica</i> on broilers (liver function) after five days of treatment with different doses of <i>Acacia nilotica</i> aqueous extract (1, 3, 5, 7.5, 10, and 15 g/ kg b.w.)	114
Figure 38: The effect of different doses of <i>Acacia nilotica</i> aqueous extract (7.5, 10, and 15 g/ kg b.w.) after 5 days post-exposure on diameter and size of hepatocyte nucleus in broilers.	115

Figure 39: Microscopic examination of kidney from control negative group in acute toxicity study	116
Figure 40: Microscopic examination of kidney from 7.5 g/kg group in acute toxicity study	116
Figure 41: Microscopic examination of kidney from 10 g/kg group in acute toxicity study	117
Figure 42: Microscopic examination of kidney from 15 g/kg group in acute toxicity study	118
Figure 43: Assessment of toxicological profile of <i>Acacia nilotica</i> on broilers (kidney function) after 5 days of treatment with different doses of <i>Acacia nilotica</i> aqueous extract (1, 3, 5, 7.5, 10, and 15 g/ kg b.w.).	121

LIST OF ABBREVIATIONS

ANAE	<i>Acacia Nilotica</i> 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 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 4×10^4 (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 subsidence 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 LD₅₀ 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 (LD₅₀) 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.