



Anti-coccidial Activity of *Moringa Oleifera* Plant

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Abstract: *Moringa Oleifera* leaves were screened for its toxicological effect and some pharmacological activities in broiler chicken. Toxicological effect was studied using upgrading oral doses of 75% ethanolic extract in different groups of mice with monitoring the toxicological symptoms and mortality rate. The anti-coccidial activity of *Moringa Oleifera* powdered leaves for prevention and its 75% ethanolic extract for treatment of coccidiosis were studied in broilers. The obtained result proved that there were no toxicological symptoms or mortalities obtained till oral administration of 6000 mg/kg b. wt. Inclusion of *Moringa oleifera* powder (0.5 and 1%) to broiler's feed were very efficacious and equal to diclazuril (1ppm) in preventing symptoms of coccidiosis associated with experimental infection with mixed *Eimeria* species. The anti-coccidial activity of *Moringa Oleifera* powdered leaves and its 75% ethanolic extract were studied on one-day old chicks. The experimental infected chicks with mixed *Eimeria* species induced severe coccidiosis in non medicated chicks and led to high mortality reached to (26.76%). Addition of 200 and 400ppm of *Moringa Oleifera* ethanolic extract induced marked curative effect of cocci diosis manifested by reduced mortality percent and improved PM lesion to be 13.3 and 10% respectively compared to 13.3% for group treated with the reference drug (25ppm) toltrazuril.

Keywords: *Moringa Oleifera*- prophylactic and treatment - Coccidiosis

1. Introduction

Poultry plays very important role for mankind through food supply, income and employment generation, providing raw materials to some industries. The direct or indirect contribution of poultry industry is very dangerous. Coccidiosis is ubiquitous where chickens are reared (traditional, industrial or organic/bio farms). It is recognized as the parasitic disease that has the greatest economic impact on poultry industries worldwide [1]. Coccidiosis in poultry is still considered as one of the main diseases affecting performance of poultry reared under intensive production systems. Coccidiosis results in intestinal lesions, impaired growth, poor feed utilization, poor flock uniformity and increased mortality. Because it damages the intestinal lining, coccidiosis predisposes birds to the development of necrotic enteritis, another intestinal disease caused by the bacterium *Clostridium perfringens*.

The intensive use of anticoccidial drugs has led to the development of resistance [2]. Increasing development of

drug-resistant coccidial species has stimulated searches for alternative control methods or new drugs. However, this has resulted in the increased cost of poultry products. Furthermore, drug or antibiotic residue in the poultry product is potentially offensive to the consumer. So the finding of natural replacer which improves the growth with prophylactic and curative effect against coccidiosis and without health hazard to human being is worthy.

The use of herbal remedies in the management of coccidiosis is not a new concept. For example, halofuginone, a quinazolinone alkaloid derived from *Dichroa febrifuga*, has been used as a coccidiostat, and the original extract from *D. febrifuga*, known as febrifugine, possesses antimalarial and anticoccidial activity [3]. The investigation of herbal materials as anticoccidial remedies therefore holds promise as an alternative in the control of coccidiosis. In addition to possibly lowering the cost of food production in different countries. *Moringa Oleifera* is one of the most

important medicinal plants and it is the most widely cultivated species of family Moringaceae. Every part of *Moringa* is said to have beneficial properties that can serve humanity [4]. In addition, various parts of *Moringa Oleifera* contain minerals and are a good source of protein, vitamins, β carotene, amino acids and various phenolics. The *Moringa* plant provides a rich and rare combination of zeatin, quercetin, β - sitosterol, caffeoylquinic acid and kaempferol. Various parts of this plant such as the leaves, roots, seed, bark, fruit, flowers and immature pods act as cardiac and circulatory stimulants, antioxidant, antibacterial, antimalarial and antifungal activities, and are being employed for the treatment of different ailments in the indigenous system of medicine, [5]. Furthermore, [6] reported the use of *Moringa Oleifera* acetone leaf extract on broiler chickens naturally infected with *Eimeria* species while [7], found that different parts of *Moringa Oleifera* were active against *Leishmania donovani* promastigotes and the flower extract of *M. oleifera* was found to be the most potent antileishmanial agent when compared to other parts of the plant like leaf, root, bark and stem. Little studies have examined the anticoccidial efficacy of the plant in livestock; thus, this study has provided evidence for the efficacy of *Moringa Oleifer* and its curative and prophylactic anticoccidial activity. In addition its effect on body performance was also investigated

2. Main Body

2.1. Plant Material

The fresh *Moringa Oleifera* (Moringaceae) were collected during November to December, 2012 from private farm in El Sadat city. The plant specimen was authenticated by the Department of Botany, Faculty of Science, and Cairo University, Egypt.

2.2. Plant Preparation

The plant leaves were washed with tap water dried at room temperature then chopped into small fragments and pulverized by using grinder. Cold extraction was done with 75% ethanol for 72 hour at room temperature with intermittent shaking [8]. The extracts were concentrated in a rotatory evaporator at 50°C and refrigerated until use. The plant was used in its powdered form to determine its effect as prophylactic anti-coccidial activity. The plant was used in its ethanolic extract form for determining its curative anti-coccidial activity.

2.3. Animals

Mice: Mature mice weighing (20-25 grams) of both sexes were used for the toxicological study.

Chicks: One day old unsexed chicks weighing (45-55grams) were used for studying the effect on anti-coccidial activity. The birds were kept under good hygienic measures. Chicks were fed on balanced commercial ration and water offered ad libitum. The chicks were floor reared in separate units along

the experimental periods.

Coccidia strains: Field strains of sporulated *Eimeria* oocysts were obtained from Department of Parasitology, Faculty of Veterinary Medicine, Cairo University. These strains include mixed *Eimeria* spp (*Eimeria acervulina* 19.5 x14.3 (4.5%), *Eimeria maxima* 29 x 23(29.5%), *Eimeria necatrix* 16.7 x14.2 (2.5%), *Eimeria tenella* 22.9 x 19.1(60%) and *Eimeria brunetti* 26.8 x 21.7(3.5%).

2.4. Drugs

Diclazuril obtained from Pharma-Swede Egypt as feed additive (0.2%) under trade name Diclomix®.

Toultrazuril obtained from Pharma Swede Egypt as oral solution (2.5%) under trade name Tolacox®.

3. Method

3.1. Toxicological Studies

The toxicological pattern of the ethanolic extract of *Moringa Oleifera* leaves was studied by determination of LD50 in mice.

3.2. Determination of LD50

LD50 of the studied extract was carried out in mice according to method described by [9]. For these purpose five groups of 5 mice in each, weighing 20-25 grams one of them served as control group. Other four groups were dosed orally the studied extract on ascending doses (30, 60, 90, and 120 mg/20g b.wt). Animals were put under observation for 24 hours for recording the toxic symptoms, mortality rate and post-mortem findings in each group. LD50 of the tested extract was calculated according to the following formula:

$$LD50 = "Dm -" \frac{\sum (zxd)}{n}$$

Where:

Dm = The largest dose which kill the animals.

z = Mean of dead animals between 2 successive groups.

d = The constant factor between 2 successive doses.

n = Number of animals in each group.

\sum = The sum of (a \times b).

3.3. Pharmacological Studies

3.3.1. Anticoccidial Effect

Propagation and isolation of oocytes of mixed *Eimeria* spp: Isolation and propagation of the coccidia was carried according to Long [10] and the experimental design were carried out according to table1.

Preparation of *Eimeria* oocytes: The methods for these procedures are best followed from the description by [11, 12, 13]. The calculated infective dose of culture (74000 oocytes/1ml) was given orally by insulin syringe which introduced intra-crop.

Table 1. Experimental design for the anticoccidial activity.

	Prophylactic capacity	Curative capacity
Groups	Standard (infected & treated with standard drug). • Control +ve (infected & non- treated). • Control -ve (non-infected & non-treated). Treated groups (<i>Moringa Oleifera</i> 0.5 & 1%).	• Standard (infected & treated with standard drug). • Control +ve (infected& non- treated). • Control -ve (non- infected & non-treated). • Treated groups(<i>Moringa Oleifera</i> 200 & 400 mg/liter)
Standard drug	Diclazuril 1ppm.	Toultrazuril 25ppm.
Onset & form of treatment	Onset: From 1 st day on feed Form: Powder.	Onset: After appearance of clinical signs on drinking water Form: Liquid.
Parameter	Clinical signs. Oocyst count. PM lesion. Mortality rate. Fecal score. Histopathological examination. Body weight &FCR.	• Clinical signs. • Oocyst count. • PM lesion. • Mortality rate. • Fecal score. • Histopathological examination.

3.3.2. Post Mortem Examination

It was carried out at 8th for both experiments. Lesion scores were determined according to [14] Lesion score was 0 when no evident lesions were detected while a score was 4 when the lesions were extremely sever.

3.3.3. Parasitological Examination (Oocyst Count)

Fecal samples were collected from freshly evacuated faeces on the litter which spread on the ground for each group from 5th to 11th dpi. The oocysts count was carried out according to the method described by [15] using the McMaster counting chamber.

3.3.4. Fecal Score

A fecal score (1 to 5) was recorded for each group of birds from 5th to 11th day dpi. a score of 1 indicate normal faeces and a score of 5 indicate the presence of severe diarrhea and /or a profuse amount of blood.

4. Result

4.1. Toxicological Studies

LD50: Oral administration of the 75% ethanolic extract on ascending manner 30, 60, 90, and 120 mg/20g body weight showed no toxicological symptoms, mortalities and

post-mortem changes. From this observation there is no LD50 recorded till oral administration of 6000 mg/kg body weight which corresponding to 15.5 g of air dried powdered leaves.

4.2. Anticoccidial Effect

4.2.1. Prophylactic Efficacy

Findings pertaining to survival percent, total oocyst count, lesion and fecal scores are presented in table (2). The experimental infected chicks with mixed *Eimeria* species induced severe coccidiosis in non-medicated chicks and led to high mortality reached to (26.76%). Addition of 0.5 and 1% of *Moringa Oleifera* powdered leaves reduced mortality to be 6.7 and 4.3% respectively. Inclusion of *Moringa Oleifera* powder (0.5 and 1%) to broiler's feed were very efficacious and equal to feed additive in preventing symptoms of coccidiosis associated with experimental infection with mixed *Eimeria* species and decreased significantly the total oocyst count and lesion score.

The effect of *Moringa Oleifera* as feed supplement on performance of control and coccidia infected chicken was presented in tables (3, 4&5). Use of *Moringa Oleifera* leaves powder in concentration of 0.5 and 1% induced an improvement in body weight with enhancement of the cumulative FCR in both control healthy and coccidia infected broilers.

Table 2. Anticoccidial prophylactic capacity of *Moringa Oleifera* in experimentally *Eimeria*-infected broiler chickens (n=15).

Group No.	Used concentration	Survival (%)	Total oocyst No(x 10 ³ /g)	Lesion score	Fecal score
Control groups	Control non- treated	73.33	629.75± 23.47 ^a	3.57 ±0.51 ^a	4.8± 0.42 ^a
	Control non-infected non-treated	100.0	0.0 ± 0.0 ^d	0.0± 0.0 ^d	1.0 ± 0.0 ^b
	Diclazuril 1 ppm	96.7	36.7± 5.62 ^c	0.37± 0.042 ^b	1.3 ±0.1
Treatment	<i>Moringa Oleifera</i> 0.5%	93.3	67.8 ±4.52 ^b	0.72 ±0.022 ^{bc}	1.5 ± 0.23 ^b
	<i>Oleifera</i> 1%	96.7	44.4 ± 3.38 ^c	0.40 ±0.11 ^{bc}	1.4 ± 0.21 ^b

Values with different letters (a, b, c, d) within the same column are significantly different at P value ≤ 0.05


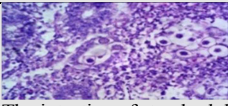

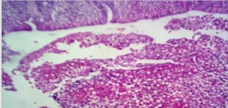

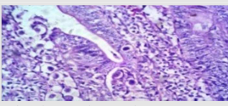

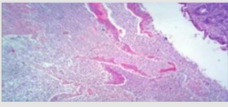

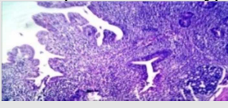
Table 3. The effect of *Moringa Oleifera* as feed additive on performance of control healthy broiler chicken (n=15).

Group	Final body weight g	Feed consumption g	FCR
Control non treated	1850	3348.5	1.81
Diclazuril 1ppm	1900	3002	1.58
<i>M. Oleifera</i> powder	0.5%	1950	1.63
	1%	2100	1.49

Table 4. The effect of *Moringa Oleifera* as feed additive on performance of coccidia infected chicken.

Group		Final body weight g	Feed consumption g	FCR
Infected non treated		1490	3085	2.1
Diclazuril	1ppm	1885	3092	1.64
M. <i>Oleifera</i> powder	0.5%	1818	2910	1.60
	1%	1850	2870	1.55

Table 5. Gross P. M and histopathological examination at 8th day of age of the anticoccidial prophylactic capacity.

Group	Gross P.M examination	Histopathological examination
Standard Diclazuril		 The intestine of standard diclazuril group showed low numbers of Eimeria in the epithelial lining of crypt and necrosis of the villar epithelium. Also Areas of 22haemorrhage were detected in the lamina propria and considerable numbers of inflammatory cells.
0.5%		 The intestine of 0.5% treated group showed large numbers of Eimeria was detected in lumen
1%		 The intestine of 1% treated group showed less pathological changes.
Control negative		 The intestine of control non infected non treated group revealed normal histological appearance of villus epithelium and crypts.
Infected non treated		 Large numbers of the Eimeria was detected in the lumen and the lamina propria was infiltrated with large numbers of inflammatory cells accompanied with areas of hemorrhages.

4.2.2. Curative Efficacy

Findings pertaining to mortality percent, total oocyst count, lesion and fecal scores, are recorded in table (6&7). The experimental infected chicks with mixed *Eimeria* species induced severe coccidiosis in non-medicated chicks and led to high mortality reached to (26.76%). Addition of 200 and


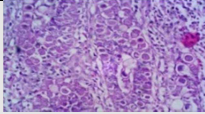

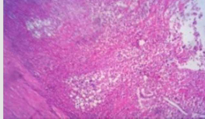

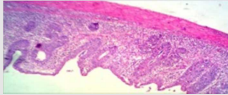
400ppm of *Moringa Oleifera* ethanolic extract reduced mortality to be 13.3 and 10% respectively compared to 13.3% for group treated with the reference drug (25 ppm toltrazuril) and decreased significantly the total oocyst count and lesion score.

Table 6. Anticoccidial curative capacity of *Moringa Oleifera* ethanolic extract in experimentally *Eimeria* infected-broiler chicken.

Used concentration	Survival (%)	Total oocyst No (x 10 ³ /g)		Lesion score	Fecal score
		During two days before treatment	During 5 days post treatment		
Control infected non-treated	73.33	7.5 ± 0.61 ^a	6.21.8 ± 17.38 ^a	3.57 ± 0.51 ^a	4.8 ± 0.42 ^a
Control non-infected non-treated	100.0	0.0 ± 0.0 ^b	0.0 ± 0.0 ^d	0.0 ± 0.0 ^d	1.0 ± 0.0 ^d
Toltrazuril	25 ppm 86.67	8.7 ± 1.27 ^a	256.75 ± 21.43 ^b	0.97 ± 0.067 ^c	1.4 ± 0.23
<i>Moringa Oleifera</i>	200 ppm 86.67	7.4 ± 0.59 ^a	285.2 ± 9.45 ^b	1.1 ± 0.12 ^b	1.9 ± 0.44 ^c
	400 ppm 90.0	7.9 ± 0.81 ^a	146.6 ± 8.57 ^c	0.81 ± 0.14 ^c	1.5 ± 0.31 ^b

Values with different letters (a, b, c, d) within the same column are significantly different at P value ≤ 0.05

Table 7. Gross P.M and histopathological examination at 8th day of age of the anticoccidial curative capacity.

Group	Gross P.M examination	Histopathological examination
Standard Toltrazuril		 The intestine of standard Toltrazuril treated group showed Eimeria in lamina propria of intestine accompanied with large numbers of inflammatory cells mainly macrophage and lymphocytes.
<i>Moringa Oleifera</i> 200 ppm		 The intestine of 200ppm treated group showed severe pathological changes in which large numbers of Eimeria in the epithelial lining of villi and crypt combined with large areas of hemorrhages.
<i>Moringa Oleifera</i> 400 ppm		 The intestine of 400ppm treated group showed Eimeria with low number and mainly found in the epithelial lining of crypt and areas of hemorrhage were detected in the lamina propria with considerable numbers of inflammatory cells.

5. Discussion

The result of acute oral toxicity (LD50) study of ethanolic leaf extract of *Moringa Oleifera* showed no mortality at the maximum dose of 6000 mg/kg/body weight. In an acute oral toxicity study by [16], *Moringa Oleifera* leaf extract was documented to be non-lethal in animals at 6400 mg/kg body weight. In addition, [17] found that *Moringa Oleifera* leaf extract was documented to be non-lethal in animals at 2000 mg/kg body weight. More so, the report of [18] revealed that the aqueous extract of *Moringa Oleifera* leaf is safe at dosage as high as 5000 mg/kg. These results may indicate safety of ethanolic or aqueous leaf extract of *Moringa Oleifera* when administered orally as the acute administration of 2 g/kg dose was reported to be the ceiling point for acute oral toxicity of medicinal plants [19].

Eimeria is an intracellular parasite, must adhere to and invade the intestinal epithelial cells in order to replicate. Gut-adapted microorganisms may compete for adhesion and invasion of cells. In the present study and after challenging the chicks with mixed Eimeria especially in the control non treated group, birds showed severe form of coccidiosis represented by a high mortality rate (26.7%), lesion score was 3.57 and fecal score was 4.8 (meaning severe watery diarrhea with obvious amount of voided blood in litter). Addition of 0.5 and 1% of *M. Oleifera* powdered leaves reduced mortality to be 6.7 and 3.3% respectively. In addition, Inclusion of *M. Oleifera* powder (0.5 and 1%) to broiler's feed were very efficacious and equal to diclazuril feed additive in preventing symptoms of coccidiosis associated with experimental infection with mixed Eimeria species. Birds supplemented with *M. Oleifera* (0.5 and 1%) and feed additive form of Diclazuril at a concentration of 1 ppm showed a significant reduction in the cumulative mean oocysts shed (67.8 ± 4.52 , and 44.4 ± 3.38 & $36.7 \pm 5.62 \times 10^3/g$, respectively) in

comparison with the control non-treated birds ($629.75 \pm 23.47 \times 10^3/g$). Oral supplementation of bird infected with mixed Eimeria species by powdered leaves of *M. Oleifera* (0.5 and 1%) showed significant decrease in lesion score (0.72 ± 0.022 and 0.40 ± 0.011 respectively). These findings were similar or better than those obtained in group treated with standard anticoccidial drug.

Our finding is matched with our histopathological study where the intestine of broilers supplemented with either 0.5 or 1% appeared devoid from Eimeria and the lamina propria was infiltrated with inflammatory cells mainly macrophages and lymphocytes

Birds infected with mixed Eimeria species and treated with ethanolic extract of *M. Oleifera* (200 and 400 mg/liter for 5 consecutive days) in drinking water showed a significant anticoccidial activity indicated by decreasing the total oocyst count (285.2 ± 9.45 and $146.6 \pm 8.57 \times 10^3/g$ respectively), decrease in lesion score (1.1 ± 0.12 and 0.81 ± 0.14 respectively) compared with infected non treated group (Total oocyst of 621.8 ± 17.38 and lesion score = 3.57 ± 0.51) and those treated with reference drug ((Total oocyst of 256.75 ± 21.43 and lesion score of 0.97 ± 0.067). Concerning the fecal score records, the results of *Moringa*. treated groups were 1.9 ± 0.44 and 1.5 ± 0.31 for 200 and 400 mg/liter compared to 4.8 ± 0.42 for the control non- treated group and 1.4 ± 0.23 for the reference group treated with 25 ppm toltrazuril for 2 consecutive days. The curative anticoccidial activity reported for *M. Oleifera* ethanolic extract in the present study is matched with our histopathological study where the intestine *Moringa* treated group showed presence of low number of Eimeria in the crypt and in the epithelial lining compared with the infected non-treated group and lamina propria was infiltrated by large numbers of inflammatory cells. Bloody colored diarrheal feces indicated by fecal score were seen in all groups following the onset of clinical signs in infected broiler chickens. The bloody diarrhea was milder in all the

treatment (toltrazuril and ethanolic extract of *M. Oleifera*) groups as the days progressed. This can be attributed to the reduction of oocyst shed in the feces of the broiler chickens as the day progressed. The reduction of oocyst shed in the feces will result in reduced damage caused on the intestinal lining and as a result the reduction in the level of hemorrhage. On the five day treatment of the present study, the feces of the birds treated with 200 or 400mg/kg body weight of *M. Oleifera* were devoid of diarrhea blood (fecal score, 1.9 ± 0.44 and 1.5 ± 0.31 respectively) compare to values of control non-treated group (4.8 ± 0.42).

The use of *M. Oleifera* is justified on the basis that it possesses anti-malaria properties [5] as well as anti-leishmanial activity [7] and *Eimeria* belongs to the phylum Apicomplexa as does the Plasmodium parasite. The result of the oocyst inhibition in the fecal matter shows that birds treated with powdered leaves of *M. Oleifera* at prophylactic study or ethanolic extracts at curative one were most promising with marked reduction of the total *Eimeria* oocyst shed in the feces. The anticoccidial activity of *M. Oleifera* either powdered leaves as prophylactic or ethanolic extract as treatment agents could be attributed to the antioxidant properties possessed by *M. Oleifera*. Antioxidant compounds are also well known to have a cellular protective action against oxidative stress and reduce the severity of *E. tenella* infections by altering the degree of intestinal lipid peroxidation [1]. The antioxidant properties of *M. Oleifera* have been reported previously by [20,21] which are ascorbic acid, flavonoids, phenolics and carotenoid. In different physio-pathological conditions, the systemic amounts of free radicals and reactive oxygen species are higher than normal [22]. Free radical oxidative species are known to be produced during the host's cellular immune response to invasion by *Eimeria* species [23], which play an important role in defending mechanism against parasitic infections. However, their high concentrations may become intolerable to cell causing tissue damage and cytotoxicity and partially contributing to the pathology of infection. In some parasitic infections, decrease in the content of antioxidants like Vitamine A, C and E occur in the host cell [24]. Another free radical oxidative species, nitric oxide enhance vasodilation and hemorrhages in coccidial infections and could be toxic to both parasite as well as to host cells harboring the coccidian parasite [25]. In addition, [26, 27] reported that *E. acervulina* oocysts motivate the lipid peroxidation, increase oxidative damage and imbalance in the antioxidant status in the infected birds by disturbing the oxidative balance. Therefore, to alleviate or reduce the oxidative stress by inclusion of antioxidants as feed supplements either natural as Vitamin E & Se or synthetic as butylated hydroxytoluene are commonly used in the poultry industry.

Inclusion of *M. Oleifera* either powder leaves (0.5 and 1%) to broiler's feed in prophylactic anticoccidial study or the ethanolic extract of *M. Oleifera* leaves (200 and 400mg/kg b.wt.) in curative study had a good dose related response to the improvement of body weight gain in broiler chickens infected

with mixed species of *Eimeria*. Our Findings showed that the infected non-treated group had 19.5% weight loss; this could be as a result of the damaging effect of the *Eimeria* species on the epithelial lining of the intestines which in turn impairs the absorption of nutrient and water from the intestine. The weight gain observed in the groups treated with *M. Oleifera* leaves could also be due to its nutritional value, having crude protein of 17.01%, carbohydrate (63.11%), crude fat (2.11%) and energy (1440.11 kcal/100 g) according to [28]. However, [29,30] reported that *M. Oleifera* leaf meal has 27.1 and 27.51% crude protein respectively. The improvement of the body weight could as well be attributed to the high levels of vitamins and amino acids in *M. Oleifera* leaves which improve recovery from the disease condition and increase feed intake and feed conversion rate.

6. Conclusion

The present study confirms that *M. Oleifera* possessed a marked anticoccidial activity and could be useful as alternative product for the control of avian coccidiosis in poultry production. It is possible of its use as prophylactic and curative agent for coccidia in addition to its growth promoting effect, safety as well as its antibacterial effect

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