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Evaluation of the Anti-Coccidial Effect of Cashew Oil and/or Toltrazuril in Chickens Experimentally Infected with *Eimeria tenella*

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Key words
Broilers, Coccidiosis, *E. tenella*, Experimental infection, Toltrazuril, Cashew oil

ABSTRACT

This research defined assessed the therapeutic effect of cashew oil, toltrazuril, and their combination to broiler chickens experimentally induced cecal coccidiosis. For this study, divided randomly one hundred broiler chicks at 15th-day old into equal five groups (20 each). kept G1 as control negative; G2, non-treated infected; G3, infected treated with cashew oil (1.5ml/liter drinking water); G4, infected treated with toltrazuril (2.5ml/liter drinking water); G5, infected treated with a mixture of cashew oil at a dose of 1ml and toltrazuril 1.25ml/liter drinking water. On the 15th the oocysts of the *Eimeria tenella* parasite were implanted orally into all but the negative control groups. According to the current findings, G2 demonstrated a significant increase in feed conversion rate, oocysts count, lesion score, mortality rate, count total leucocytic, ALP, ALT, AST, total bilirubin, creatinine, urea, uric acid, and MDA level, the body weight significally reduction, weight gain in the body, feed consumption, hematological parameters, lymphocytes%, cholesterol level, and Catalase enzyme activity. These results followed the histopathological findings. Treatment with the tested drugs improved all the tested parameters. Still, the control of coccidiosis caused by Eimeria tenell is highly effective by combination of cashew oil and toltrazuril.

INTRODUCTION

The administration of anti-coccidial drugs is a highly effective method of controlling coccidiosis (Soutter *et al*., 2020). The anti-coccidial drugs control coccidiosis by inhibiting *Eimeria* sp.'s proliferation (Craig *et al*., 2020). Toltrazuril is a highly effective anti-coccidial drug. It is effective against all *Eimeria* infecting chicken and has activity against all of the *Eimeria* sp.'s developing intracellular phases (Mathis *et al*., 2003).

The search for products derived from natural sources and plants has increased in the last years to produce drug-free birds. These natural origin compounds related to their ability to improve immune response, body weight gain, and destroy *Eimeria* oocysts. Also, the development of new drugs to avoid the main problem of drug resistance to control avian coccidiosis and make poultry valid for human consumption (Quiroz-Castañeda, 2018). Among the natural products is the cashew nut shell oil *(Anacardium occidentale* L.), which comprises anacardic acid, cardanol, cardol, and 2-methylcardol acids (Trevisan *et al*., 2006). These phenolic oil components biological activities have

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attracted considerable attention due to their activity as molluscicidal (Kubo *et al*., 1986), activity as anti-tumor (Itokawa *et al*., 1989), activity as antimicrobial (Kubo *et al*., 2003), and properties as antioxidant (Abreu *et al*., 2017). The synergistic antimicrobial property to cashew nut shell oil with other antibiotics is fascinating (Muroi and Kubo, 1996). The commercial mixture of castor oil and cashew nut liquid reduced the excretion of oocysts and increased the heterophile/lymphocyte ratio (Collares *et al*., 2018). The study of Toyomizu *et al*. (2003) during experimental coccidial infection provides the first evidence of the reducing effects to the severity of cecal lesions in chickens by cashew nut shell oil.

The aim of this study was estimation the effects of cashew oil compared to toltrazuril, administered alone or in combination at their recommended oral doses against cecal coccidiosis in broiler chickens. Moreover, the growth performance, hematological, biochemical, histopathological, and antioxidant effects in chicken were also examined.

MATERIALS AND METHODS

Chemicals

The diagnostic kits were obtained from Bio-Diagnostic Co., Giza, Egypt to assaying kidney and liver function tests, the lipid peroxidation levels, and antioxidants. Toltrazuril (Toltrasol 2.5 % oral solution) ® was obtained from ARABCOMED, Egypt. Cashew oil (Cardox 200) ® was obtained from Souter Trade Company for importing and trading veterinary pharmaceuticals, Egypt.

Collection and preparation of Eimeria tenella *oocysts*

The isolated oocysts from the ceci of chickens naturally infected. They were separated using sieving and sedimentation techniques (Soulsby, 1968). The sporulated *Eimeria* oocysts were kept in 2.5% potassium dichromate solution at 4°C until their use for experimental infection.

Experimental design and grouping

Using one hundred chicks broiler chicks of the Cobb strain, one-day old. The program of vaccination was conducted using the Hitchner BI strain (eye drops on the 7th day of age). At the 13th day of age, the Gambero vaccine and ND clone 30 were administered in drinking water. The birds fed on anti-coccidial drug free and balanced ration. Five groups of birds were formed. Five groups of birds were formed (20 chicks each). G1 (control negative), G2 (control positive), G3, infected + cashew oil at a 1.5ml/liter drinking water (according to Souter Trade company), G4, infected + toltrazuril at 2.5ml/liter drinking water (according to ARABCOMED company, Egypt) and G5: Infected + a mixture of cashew oil at a dose of 1ml and toltrazuril at 1.25ml/liter of drinking water. All except one of the groups (G1) were exposed to oocysts *E. tenella*. Infection was performed orally on the 15th day of age by a single dose of 5×104 sporulated oocysts. Administered the drugsfrom the 5th day post-infection for three successive days in the drinking water. All groups were kept under observation until the end experiment (36th day of age).

Criteria of the efficacy of the tested drugs

Growth performance parameters

Chickens of each group were individually marked and weighed before feeding at the beginning of the experiment, on 15th day of age the day of infection, and then weekly till the end of the investigation. The body weight (BW), body weight gains (BWG), feed intake (FI) and feed conversion ratio (FCR) were observed according to Davis *et al*. (1986) and Seddiek *et al*. (2008).

Oocysts reducing percentage

Daily collected three fresh fecal samples spread on the ground from each group from different parts of the litter for oocysts count at day 5th until day 20th post-infection. Stored the samples in 2.5 % potassium dichromate until counting by McMaster technique (Levine, 1988).

Hematological and biochemical analysis

Blood samples were collected on the 9th and 23rd days post-infection from vein of the wing randomly selected five birds from each group. The hematological analysis following Schalm *et al*. (1975). The serum enzymatic activities of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were assessed following Reitman and Frankel (1957), serum ALP activity according to Belfield and Goldberg (1971), serum cholesterol level according to Richmond (1973), serum bilirubin level according to Walter and Gerade (1970), serum uric acid level following Barham and Trinder (1972), serum creatinine level following Larsen (1972) and serum urea level following Fawcett and Soctt (1960). the colorimetrical estimations of hepatic malondialdehyde (MDA), the primary end of lipid peroxidation product, according to the protocol of Ohkawa *et al.* (1979) and catalase activity following Aebi (1984).

Histopathological examination

The kidney, cecum, and part of the liver for histopathological examination were put in 10% neutral buffered formalin and examined according to Lillie (1954).

Statistical analysis

Using graph pad prism software, one-way ANOVA

and Tukey multiple comparisons were used to evaluate the data. For this study, $P < 0.05$ considered an acceptable threshold of statistical significance.

RESULTS

Growth performance

In the infected non-treated group (G2), body weight, weight gain, and feed consumption decreased while feed conversion ratio increased (G1). G3, G4, and G5 showed significant growth improvement over G2, with G5 showing the best outcomes (Tables I, II).

Table I. The effect of cashew oil, toltrazuril, and their combination on the body weight in broiler chickens experimentally infected with *Eimeria tenella*.

Group	Day (age)			
	15th	22nd	29th	36th
G1	260.1± 7.71[a]	588.25± 11.20[a]	1097.66± 20.37[a]	1604.00± 19.20[a]
G2	267.30± 7.22[a]	430.71± 11.23[d]	800.00± 10.22[d]	1220.88± 22.23[d]
G3	253.00± 9.14[a]	500.00± 15.38 [bc]	920.66± 17.41 [bc]	1362.27± 25.19 [c]
G4	253.00± 6.75[a]	547.61± 13.34[b]	1004.00± 23.42[b]	1450.82± 22.91[b]
G5	265.00± 5.23[a]	589.12± 10.26[a]	1080.00± 18.34[a]	1580.00± 30.51[a]

Means within rows with different letters differ at $P \leq 0.05$. n = 20.
G1, negative control; G2, positive control; G3, infected + cashew oil at 1.5ml/l; G4, infected + toltrazuril at 2.5ml/l; G5, Infected + mixture of cashew oil at 1ml/l and toltrazuril at 1.25ml/l.

Oocysts count

The discharge oocyst in faeces developed on day 7, peaked on day 9, and dropped till day 19 post-infection. G2 produced more oocysts than the other groups. G5 had the largest reduction in faecal oocyst count, as seen in Table III.

Lesion score and mortality %

Table IV shows that G3, G4, and G5 reduced lesion score significantly compared to G2. At 9th and 23rd dpi, G5 was much lower than G2, G3, and G4. Mortality in G2 decreased 30% and in G3, G4, and G5 it decreased 15%.

Hematological parameters

G2, G3, G4, and G5 had lower RBCs, Hb, and PCV than G1. G3, G4, and G5 had significantly higher parameters than G2, with G5 showing the most improvement (Tables V, VI). There is significant improvement in the DLC in G3, G4, and G5 compared to G2.

Table II. The effect of cashew oil, toltrazuril, and their combination on the body weight gain (in g), feed consumption (in g) and feed conversion ratio (mean ± S.E.) in broiler chickens experimentally infected with *Eimeria tenella* n = 20.

Group	Day (age)		
	15-22	22-29	29-36
Body weight gain			
G1	328.15± 2.20[a]	509.41± 4.27[a]	506.34± 3.22[a]
G2	163.41± 2.50[d]	369.29± 4.14[d]	420.88± 1.21[c]
G3	247.00± 3.11[bc]	420.66± 1.28[bc]	441.61± 2.29[b]
G4	294.61± 1.12[b]	456.39± 2.17[b]	446.82± 4.12[b]
G5	324.12± 2.20[a]	490.88± 5.53[a]	500.00± 3.21[a]
Feed consumption (g)			
G1	687.50± 11.23[a]	840.61± 10.88[a]	1120.00± 12.58[a]
G2	555.35± 10.74[d]	760.25± 6.22[cd]	1090.00± 8.92[c]
G3	583.00± 9.23[c]	780.37± 11.44[c]	1100.00± 9.58[b]
G4	620.50± 5.45[b]	822.00± 12.25[ab]	1100.00± 13.34[b]
G5	660.00± 11.57[a]	845.50± 9.67[a]	1150.00± 16.55[a]
Feed conversation ratio			
G1	2.09± 0.02 [e]	1.65± 0.01 [d]	2.21± 0.01 [d]
G2	3.39± 0.03 [a]	2.06± 0.02 [a]	2.59± 0.08 [a]
G3	2.36± 0.03 [b]	1.86± 0.01 [b]	2.49± 0.03 [b]
G4	2.11± 0.01 [c]	1.80± 0.06 [b]	2.46± 0.05 [b]
G5	2.04± 0.05 [de]	1.72± 0.08 [cd]	2.30± 0.08 [cd]

Means within rows with different letters differ at $P \leq 0.05$. n = 20

Table III. The oocysts count (x 10^3/gm feces) (mean ± S.E.) from 7th to 19th day post-infection in broiler chickens experimentally infected with *Eimeria tenella* and treated with the tested anticoccidial drugs.

Day	Group			
	G2	G3	G4	G5
7th	24.6 ± 2.42 [a]	12.6 ± 2.58 [bc]	13.8 ± 1.66 [b]	11.5 ±1.22 [d]
8th	34.8 ± 3.16 [a]	10.6 ± 1.11 [bc]	12.2 ± 1.76 [b]	9.0 ± 1.66 d
9th	97.0 ± 3.58 [a]	9.6 ± 1.22 [bc]	10.4 ± 1.23 [b]	5.6 ± 0.45 [d]
10th	65.0 ± 2.68 [a]	9.0 ± 1.02 [bc]	9.8 ± 1.11 [b]	4.9 ± 1.18 [d]
11th	17.6 ± 1.12 [a]	8.4 ± 1.56 [bc]	9.0 ± 1.22 [b]	4.4 ± 1.23 [d]
12th	12.8 ± 1.76 [a]	7.5 ± 1.23 [bc]	8.1 ±1.22 [b]	4.0 ± 1.13 [d]
13th	6.8 ± 1.55 [a]	4.3 ± 1.02 [bc]	5.3 ± 1.10 [b]	2.9 ± 0.02 [d]
14th	6.1 ± 1.13 [a]	3.8 ± 0.88 [bc]	4.4 ± 1.01 [b]	2.6 ± 0.54 [d]
15th	5.8 ± 1. 08 [a]	2.2 ± 0.05 [b]	2.5 ± 0.44 [b]	2.0 ± 0.07 [c]
16th	2.8 ± 0.03 [a]	1.3 ± 0.03 [b]	1.5 ± 0.08 [b]	1.2 ± 0.06 [c]
17th	2.0 ± 0.02 [a]	0.0 ± 0.00 [b]	0.0 ± 0.00 [b]	0.0 ± 0.00 [b]
18th	1.8 ± 0.01[a]	0.0 ± 0.00 [b]	0.0 ± 0.00 [b]	0.0 ± 0.00 [b]
19th	1.5 ± 0.01 [a]			

Means within rows with different letters differ at $P \leq 0.05$. N= 3

Table IV. The effect of cashew oil, toltrazuril, and their combination on lesion score (mean ± SE) and mortality % in broiler chickens experimentally infected with *Eimeria tenella*.

Group	Lesion score		no. of deaths	Mortality %
	At 9th dpi	At 23rd dpi		
G1	00 ± 00	00 ± 00	0	00.00%
G2	3.60±0.11 [a]	2.60±0.09 [a]	6	30.00%
G3	2.60 ±0.03 [b]	2.20 ± 0.01 [b]	4	20.00%
G4	2.40 ± 0.03 [b]	1.80 ± 0.05 [bc]	4	20.00%
G5	1.60 ± 0.04 [c]	1.20 ± 0.05 [d]	3	15.00%

Means within rows with different letters differ at $P \leq 0.05$. n= 5

Biochemical parameters

Tables III and IV show a considerable increase in blood ALT, AST, ALP activity, total bilirubin, urea, creatinine, and uric acid levels in G2, G3, G4 and G5 compared to G1. G3, G4, and G5 showed considerable improvements over G2. G2, G3, G4, and G5 have lower Catalase enzyme activity and higher MDA levels than G1. G3, G4, and G5 have higher catalase activity and lower MDA levels than G2.

Histopathological results

At 1st sacrifice (9th dpi)

Histopathologically, the intestines (Ceci) Covering epithelium and intestinal crypts were normal in G1 control birds. In G2, the intestinal crypts were heavily infected with coccidial stages. Cashew oil-treated chickens decreased significantly (G3). Live coccidial schizont stages decreasedin toltrazuril-treated chicken (G4). In cashew oil + toltrazuril-treated hens (G5), the mucosa was normal with dead parasite stages (Fig. 1).Normal hepatocytes are radially organised in the portal area of uninfected, untreated hens (G1). Infected, untreated hens (G2) had necrotic foci and mononuclear cell infiltration. Cashew oil (G3)-treated chickens demonstrated periportal eosinophil infiltration. Toltrazuril (G4)-treated liver demonstrated periportal eosinophilic cell infiltration. Finally cashew oil + toltrazuril hens (G5) revealed mononuclear and eosinophilic cell infiltration (Fig. 2). Non-infected, untreated chickens (G1) had normal glomeruli and tubules. Infected, untreated chickens (G2) have interstitial nephritis. Cashew oil-treated hens (G3) had interstitial nephritis. In toltrazuril-treated chickens (G4), renal tubules revealed moderate degeneration. Cashew oil + toltrazuril hens (G5) showed minor renal tubule cloudiness (Fig. 3).

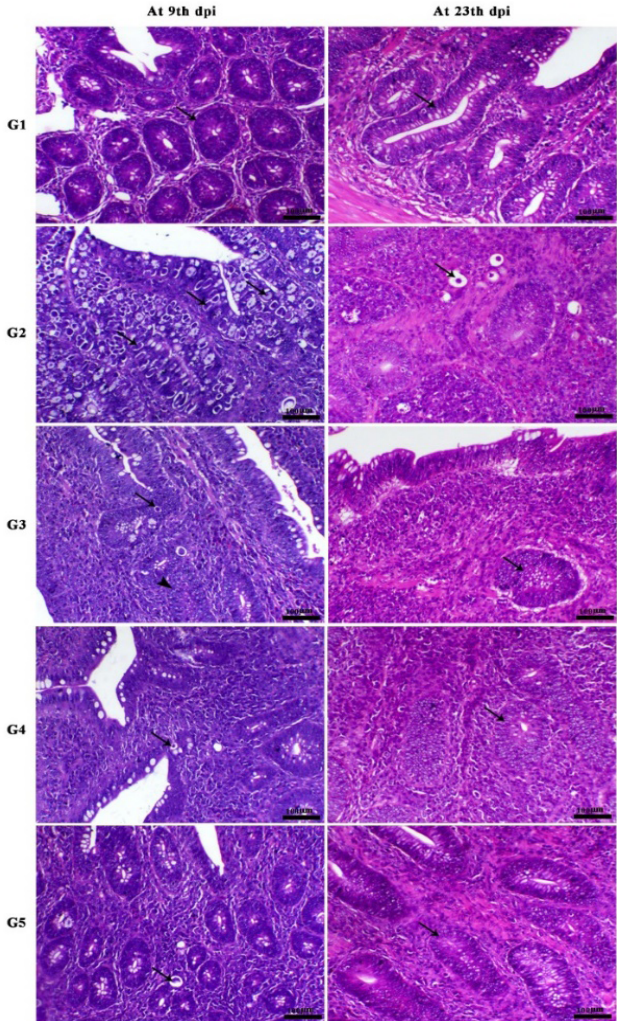

Fig. 1. Histological structure of intestine (cecum) showing normal both covering epithelium and intestinal crypts (arrow) at 9th and 23th dpi., in non-infected non-treated chicken (G1), heavily infestation of the intestinal crypts with different coccidial stages (arrow) at 9th dpi. and dead coccidial stages (arrow) at 23th dpi., in infected non treated chicken (G2), marked decrease of affected crypts which contained the schizont of the parasite (arrow) and associated with intestinal hyperplasia (arrowhead) at 9th dpi. and hyperplasia of the crypts lining epithelium (arrow) with mild interstitial mononuclear cells infiltration (arrow) at 23th dpi., in infected chicken treated with cashew oil (G3), marked decrease of the number of live coccidial schizont stages (arrow) at 9th dpi. and hyperplasia of the crypts lining epithelium (arrow) with slight interstitial mononuclear cells infiltration at 23th dpi., in infected chicken treated with toltrazuril (G4), normal mucosa with apparent dead parasitic stages within the intestinal epithelial cells (arrow) at 9th dpi. and slight hyperplasia of the crypts lining epithelium (arrow) at 23th dpi., in infected chicken treated with cashew oil + toltrazuril (G5), H & E, X200.

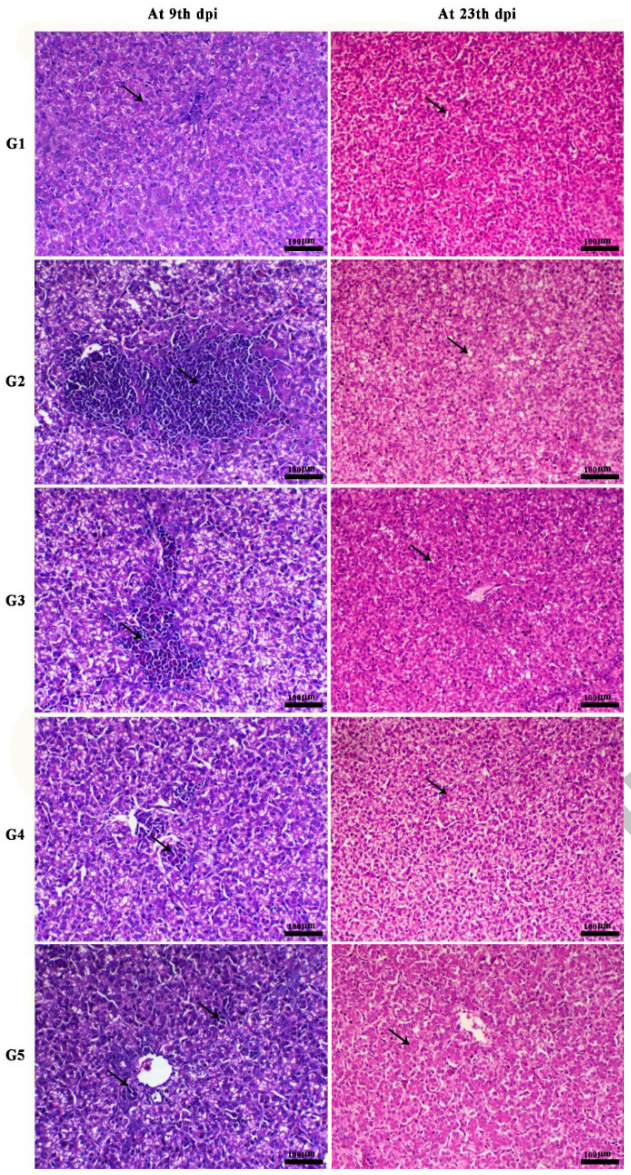

Fig. 2. Histological structure of liver showing normal hepatocytes (arrow) at 9th and 23rd dpi., in non-infected non treated chicken (G1), necrotic foci associated with mononuclear cells infiltration (arrow) 9th dpi. and a moderate degree of fat hepatic vacuolation (arrow) at 23rd dpi., in infected non treated chicken (G2), periportal eosinophilic cells infiltration (arrow) at 9th dpi. and a mild degree of hepatic vacuolation (arrow) at 23rd dpi., in infected chicken treated with cashew oil (G3), small area of periportal eosinophilic cells infiltration (arrow) at 9th dpi. and normal hepatocytes (arrow) at 23rd dpi., in infected chicken treated with toltrazuril (G4), a mild degree of mononuclear and eosinophilic cells infiltration (arrows) at 9th dpi. and normal hepatocytes (arrow) at 23rd dpi., in infected chicken treated with cashew oil + toltrazuril (G5), H & E, X200.

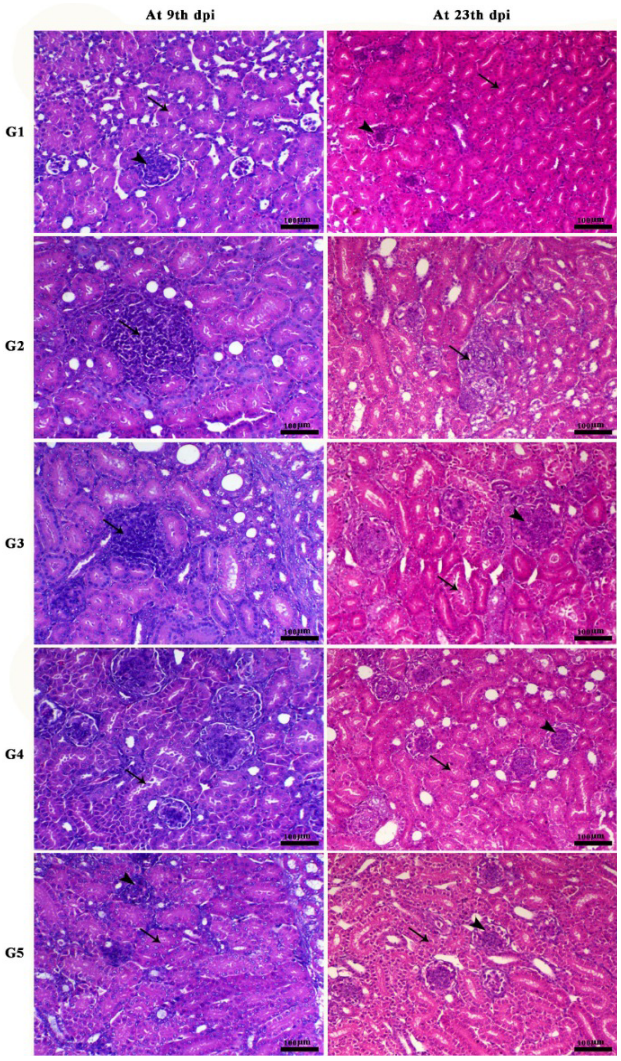

Fig. 3. Histological structure of kidney showing normal renal glomeruli and tubules (arrowhead and arrow, respectively) at 9th and 23rd dpi., in non-infected non treated chicken (G1), features of interstitial nephritis (arrow indicates mononuclear infiltration) at 9th dpi. and features of regenerative tubular basophilia (arrow) at 23rd dpi., in infected non treated chicken (G2), foci of interstitial nephritis (arrow indicates mononuclear infiltration) at 9th dpi. and mild degenerative changes within the renal tubular epithelium (arrow) (arrowhead indicates normal glomerulus) at 23rd dpi., in infected chicken treated with cashew oil (G3), mild degenerative and desquamative changes within the renal tubules (arrow) at 9th dpi. and normal renal glomeruli (arrowhead) and tubules (arrow) at 23rd dpi., in infected chicken treated with toltrazuril (G4), mild cloudy swelling of the renal tubules (arrow) (arrowhead indicates normal glomerulus) at 9th dpi. and normal renal glomeruli (arrowhead) and tubules (arrow) at 23rd dpi., in infected chicken treated with cashew oil+toltrazuril (G5). H & E, X200.

At the 2nd sacrifice (23th dpi)

Histopathologically, non-infected, non-treated hens had normal intestines (Ceci) (G1). Untreated chickens (G2) had dead coccidial stages. In cashew oil (G3) and toltrazuril (G4)-treated hens, epithelial crypts hyperplasia and interstitial mononuclear cell infiltration occurred. In cashew oil + toltrazuril-treated chickens (G5), crypt hyperplasia was seen (Fig. 1). Hepatocytes were normal in non-infected, non-treated control chickens (G1). Non-treated infected hens (G2) had moderate hepatic vacuolation. Hepatic vacuolation was modest in cashew-oil-treated chickens (G3). Hepatocytes were normal in toltrazuril-treated (G4) and cashew oil-treated (G5) chicken livers (Fig. 2). Uninfected, untreated hens (G1) had normal renal glomeruli and tubules. *E. tenella*-infected untreated hens (G2) had regenerated tubular basophilia. Cashew oil-treated kidney (G3) revealed modest degenerative tubular epithelial alterations. Kidneys treated with toltrazuril (G4) and cashew oil + toltrazuril (G5) had normal glomeruli and tubules (Fig. 3).

DISCUSSION

Anticoccidials and good management are essential for controlling and preventing coccidiosis in domestic chickens (Abdisa *et al*., 2019). Anti-coccidial medicines have been the subject of contemporary study. As a result, new medications must be developed to limit the spread of coccidiosis in animals that are consumed by humans (Quiroz-Castañeda, 2018).

Infection with *E. tenella* is characterized by rapid replication of the parasite in a host cell with extensive damage to intestinal mucosa within 4-7 days (Olanrewaju and Agbor, 2014). The feed efficiency and body weight gain in birds treated with toltrazuril better than infected non-treated birds. Toltrazuril act against all intracellular schizonts give increased weight gains by reducing in excretion oocyst and lesion scoring (Elkhouly *et al*., 2016). The cashew oil has the same effect of toltrazuril improved body weight gain and decreased the lesion score (Ferket *et al*., 2020) and oocysts count (Quiroz-Castañeda, 2018). Also, have antibacterial activity, preventing secondary bacterial infection after infection by coccidiosis (El-Sawah *et al*., 2020) and improved the immune parameters (Moraes *et al*., 2019; Yusuf and Aliyu-Paiko, 2020). It can reduce the intestinal microbial load, resulting the toxins associated with adverse morphology changes in intestine (Xu *et al*., 2003). The cashew oil-treated group showed a reduction in lesion score, fecal oocysts count, and mortality % similar to the toltrazuril-treated group, which coordinated with Collares *et al*. (2018). They concluded that mixture of cashew nut liquid and castor oil reduced the excretion of oocysts at 7and 14 days post-challenge. Cashew act as a natural anti-coccidial drug contains alkyl phenolic oil and anacardic acid, which showed in previous studies an improvement in energy utilization, livability, and reduced the severity of the lesions in infected chickens (Farias *et al*., 2017). Therefore, their potential protonophore/ionophore properties, anacardic acids have the potential to be used as anti-coccidial and/or anti-inflammatory drugs (Toyomizu *et al*., 2003). The oil's antioxidant effect on the host cell (Murakami *et al*., 2014). The *E. tenella* reduced RBCs count, Hb concentration, and PCV %; this may be due to cecal hemorrhages, bloody diarrhea, and loss of appetite, which leads to macrocytic hypochromic anemia (Hana *et al*., 2011). The reduction in Hb concentration may be attributed to hemorrhages in the caeca followed by the development of caecal lesions (Patra *et al*., 2010). According to Kavitha *et al*. (2018), on the other hand, the significant increase in total leucocytic count differential leucocytic count (monocytosis and eosinophilia) with a significant decrease in lymphocytes % confirmed by Bremner *et al*. (2021). The hematological parameters were markedly improved in the toltrazuril treated group (Harfoush *et al*., 2010; Youssef *et al*., 2015). Also, the hematological parameters in the cashew oil-treated group may be attributed to decreased cecal lesions and hemorrhage (Murakami *et al*., 2014; Sanches *et al*., 2019). Our study revealed a significant increase in serum creatinine, urea, uric acid, total bilirubin, ALT, AST and ALP in the infected non-treated group due to the harmful effect on liver function, liver injury and kidney parenchyma (Adamu *et al*., 2013). These findings are similar to those obtained by Harfoush *et al*. (2010), Mondal *et al*. (2011) and Patra *et al*. (2010). Moreover, Dovhiy *et al*. (2020) concluded that infestation with *Eimeria* showed a feasible increase in the total bilirubin content, urea, and AlAT enzyme activity. The current study revealed a significant decrease in serum cholesterol levels. The declined triglyceride levels may due to anorexia (Allen and McMurtry, 1984). There were no abnormalities in enzymes AST and ALT in the liver functions. These findings imply that cashews at the amounts tested were not harmful to laying hens (Braz *et al*., 2018). Flavonoids in cashew nut shell liquid can foster several mechanisms of action on lipid metabolism (Deszcz *et al*., 2000). Multiple pathological disorders are exacerbated by oxidative stress. Antioxidant defence mechanisms are reduced or absent in the presence of elevated levels of reactive oxygen species (ROS) (Heyman *et al*., 2011). Oxidative stress is a key player in a wide range of diseases. An increase in ROS or a decline in antioxidant defence mechanisms are two ways to describe it (Ighodaro and Akinloye, 2018). Various pathological disorders are exacerbated or even caused

by oxidative stress. An increase in ROS or a decline in antioxidant defence mechanisms are two ways to define this phenomenon (Koinarski *et al*., 2005). Oxidative stress is a key player in a wide range of disease states. It is characterised by a rise in ROS and/or a decrease in antioxidant defence systems (Yusuf and Aliyu-Paiko, 2020), cashew nut shell liquid increased broiler chicks' antioxidant enzyme activity. Cashew oil contains anacardic acid, which acts as coccistacis by blocking prooxidative enzymes involved in ROS generation (Green *et al*., 2007; Ha and Kubo, 2005; Sun *et al*., 2006), acting directly on the chelation of metallic acids but do not quench reactive oxygen species (Kubo *et al*., 2006; Tsujimoto *et al*., 2007), and preventing the generation of superoxide anion (Kubo *et al*., 2006). In the current study, contrast histopathological lesions revealed a heavy infestation of the intestinal crypts with different coccidial stages at 9th dpi and dead coccidial stages at 23rd dpi (Olabode *et al*., 2020). The trial boosted the cecal mucosa's ability to heal. This may be due to the chronic stage, when the host can regulate its defensive mechanisms by boosting local mucosa immune responses (Khalafalla, 2009). The parasite's life cycle in an infected host is self-limiting due to immunity from sickness and/or repeated infection. The groups treated with cashew oil or toltrazuril or cashew oil combined with toltrazuril showed a marked reduction of crypts affected which contained the parasite schizont and intestinal hyperplasia at 9th dpi, and slight hyperplasia of the lining crypts epithelium with mild interstitial mononuclear cells infiltration at 23rd dpi (Ashraf, 2011). Histopathological analysis of liver and kidney samples from the infected non-treated group exhibited necrotic foci associated with mononuclear cells infiltration 9th dpi, moderate hepatic fat vacuolation, and regenerating tubular basophilia 23th dpi., These findings match (Saber, 1995). Compared to the infected non-treated group, cashew oil, toltrazuril, and cashew oil combination improved livers and kidneys. Cashew oil and toltrazuril was best. At 9 dpi, cashew oil-treated mice had periportal eosinophilic cells and interstitial nephritis (mononuclear infiltration). 23 dpi, there was modest hepatic vacuolation and renal tubular epithelial degeneration. The toltrazuril-treated group had periportal esinophilic cell infiltration and significant degenerative and desquamative renal tubule alterations at 9 dpi, but normal hepatocytes, glomeruli, and tubules at 23 dpi. Cashew oil mixed with toltrazuril treated group showed slight mononuclear and esinophilic cells infiltration and mild hazy renal tubule enlargement with normal glomerulus at 9th dpi, and normal hepatocytes, renal glomeruli, and tubules at 23rd dpi.

CONCLUSION

Controlling avian coccidiosis requires collaborative efforts that will create new research avenues. Based on this study's results, toltrazuril (control medicine) and cashew oil had the same anti-coccidial action. Toltrazuril and cashew oil worked best.

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IRB approval

The experimental methods, procedure were approved by the Faculty of Veterinary Medicine, Kafrelsheikh University, Egypt.

Ethical statement

The rules for the care and use of the animals used in the experiments were authorized by the Research Ethical Committee of the Faculty of Veterinary Medicine at the University of Kafr El-Sheikh, Egypt.

Statement of conflict of interest

The authors have declared no conflict of interest.

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