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ANTICOCCIDIAL ACTIVITY OF BLACK CUMIN *(NIGELLA SATIVA)* IN RABBITS

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ABSTRACT

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A total of 60 males New Zealand white rabbits (free from coccidian infection as determined by coccidian-free fecal samples over 7 consecutive days) were weighed and distributed into five equal groups of 12 rabbits each. First group (G1) was left as uninfected and untreated (negative control), second group (G2) was left as uninfected and treated with *N. sativa* seed oil. Rabbits of three other groups (G3, G4) and G5) were inoculated with $1x10^4$ sporulated oocysts of *Eimeria stiedae* per each rabbit through a gastric tube. The third group (G3) was left untreated and considered as positive control. At day 14 post inoculation (PI), the rabbits of fourth group (G4) were treated with *N. sativa* seed oil in a dose of 500 mg/kg BW for successive 14 days. Meanwhile, the rabbits of fifth group (G5) were given *N. sativa* seed oil for 7 days before infection and continued for 28 days PI with the same dose. Fecal oocysts were collected and counted using a modified McMaster technique on days 0, 7, 14, 21, 28 and 35 PI. At 28 day PI, blood samples were collected and the serum was prepared and kept in deep freezer at -20 °C for determination of serum AST, ALT, total cholesterol, creatinine, total protein and albumin. On the 28th day PI, bilary oocysts were counted; liver lesions were scored and the relative liver weights were reported in five rabbits from each group. As well as fresh liver tissue specimens of the sacrificed rabbits were fixed in 10% formalin. Sections 5-7 µm thickness were prepared and stained with hematoxylin and eosin (H&E) for the histopathological examination. Rabbits were weighed at the beginning of the experiment (0 day) and on days 7, 14, 21, 28 and 35 PI. The fecal and bilary oocysts output and liver lesion scoring in treated rabbits of G5 were decreased when compared with those of G4 on day 28 PI (14 days PI). Serum levels of liver enzymes (ALT and AST), total cholesterol and creatinine were decreased in G5 when compared with G4 and G2. However, the levels of total protein, albumin and globulin in G5 were increased and directed toward the normal level when compared with G4 and G2. By day 35 PI, the treated rabbits with the *N. sativa* seed oil for 28 days PI (G5) gained more weight and weight gain than those treated for 14 days PI (G4). Liver weights drastically decreased in the G5 animals as compared with G4 and positive control (G3) on day 28 PI. Number of developmental stages of *E. stiedae* was decreased in G5 when compared to G4 and G2. Mortality % of infected rabbits was 8.33 % in G5, 16.67% in G4, 41.67% in G3 however there were no mortalities in Groups 1 and 2. Following treatment with *N. sativa* seed oil, visible improvement was present in the liver structure of G5 on day 28 PI. It could be concluded that *N. sativa* seed oil is safe and gives a good effectiveness in control of *E. stiedae* infection in rabbits. Also it gives an improvement of growth performance, liver and kidney functions. Although presence of hyperplasia in the columnar epithelial cells of the bile ducts that may be recovered by time.

Key Words: *Nigella sativa, Rabbits, anticoccidial*

INTRODUCTION

Nigella sativa (*N. sativa*) (Family: *Ranunculaceae*), also known as black cumin, is a herbaceous annual plant used in traditional medicine for thousands of years because of its anticonvulsive (Rahman *et al.*, 2008), antibacterial (Ali *et al.*, 2012), antiinflammatory (Juhas *et al.*, 2008) and antioxidant (Tripathi *et al.*, 2012). It has beneficial effects in nervous system in rats (Hosseini *et al.*, 2012), hyperlipidemia (Sabzghabaee *et al.*, 2012), liver malignancy (Abdel-Hamid *et al.*, 2012) and renal injury after acetaminophen treatment (Ahmed and El-Mottaleb, 2012).

Although numerous studies have been conducted to determine the effects of *N. sativa* seeds on numerous diseases and microbes, only a few studies have examined their effect on parasitic infections. Most of these studies indicated that treatment with *N. sativa*

reduces the rate of parasitic egg lying and improves liver functions of infected animals with schistosomiasis (El-Shenawy *et al.*, 2008). Additional studies indicated that *N. sativa* is effective against trematodes, cestodes and nematodes (Abu-El-Ezz, 2005). *N. sativa* oil has anthelmintic effect in rats infected with *Trichinella spiralis* (Abu-El-Ezz, 2005) and *Aspiculuris tetraptera* and *Hymenolepis nana* (Ayaz *et al.*, 2007).

E. stiedae is one of the protozoal parasites with severe infectivity in rabbits and causes hepatic coccidiosis affects body weight and feeding rates that can result in death (Omata *et al.*, 2001). *N. sativa* seeds can be used in oil emulsions to safely and effectively treat *E. stiedae* infection in rabbits with no side effects (Baghdadi and Al-Mathal, 2011). El-Sayed *et al.* (2005) reported that seeds of *N. sativa* contain thymolhydroquinone and volatile oil in pharmacologically effective amounts. They found that feeding of chicks with seeds (till 10 %) yielded no differences of blood plasma chemistry which are indicative of liver and kidney functions, higher body weight and could not prevent signs of coccidiosis and shedding of oocysts of *E. tenella.*

Black cumin seeds up to 2 % improved performance and immunity of birds (Nasir, 2009) and in form of oil (0.5%), seeds (1%) or meal (10%) improved physiological performance, body reaction and biochemical parameters of chicks (Hermes *et al.*, 2011). Combination of *N. sativa* seeds oil and aqueous garlic extract had potent effect on AST, ALT, urea and creatinine in mice (El-Shenawy *et al.*, 2008). *N. sativa* improved activities of liver related enzymes resulted in better feed conversion rate (FCR) (Nasir, 2009) and reduced the adverse effects of benzo[a]pyrene-induced immunotoxicity in broilers (Latif *et al.*, 2011). Omega 3 and *N. sativa* seeds oil might prevent oxidative stress in rats (Attia *et al.*, 2011).

Black cumin (2 or 3%) decrease cholesterol in egg yolk (Aydin *et al.*, 2008), had a beneficial effect on growth performance in chicks (Durrani *et al.*, 2007) and improved broiler health if mixed with Artemisia leaves (Khalaji *et al.*, 2011). Also, it improved liver through disappearance of bleeding between hepatic lobules with inflammatory cells in portal area and reduction in various stages of parasites in bile ducts of rabbits (Baghdadi and Al-Mathal, 2011).

This study was undertaken to investigate, anticoccidial effect of *N. sativa* seeds oil by studying parasitological, biochemical, economical parameters and examining liver tissue structure of rabbits infected with *E. stiedae.*

MATERIALS and METHODS

Preparation of *E. stiedae* oocysts:
E. stiedae oocysts were collected from liver and gallbladder of infected rabbits and concentrated using flotation method (Heelan and Ingersoll, 2002), sporulated and stored in 2.5% pot. dichromate at 4°C until used.

***Nigella sativa* seed oil:**
Nigella sativa seed oil used in this study was produced by Pharco Pharmaceutical Company (Egypt, Alexandria) in the form of soft gelatinous capsules. Each capsule contains 450 mg of *N. sativa* seed oil. *N. sativa* seed oil was given to the rabbits by stomach tube after its extraction from the capsules.

Animals:
A total of 60 male New Zealand white rabbits, aged approximately 2 months and weighing of 954.92±3.79 gm (mean ± SE), were bred at Faculty of Agriculture, Moshtohor, Benha University. Rabbits were free from coccidian infection as determined by coccidian-free fecal samples over 7 consecutive days using the concentration flotation technique. The rabbits were weighed and distributed by ranking method (Gardiner and Wehr, 1950) into 5 equal groups of 12 rabbits each. Rabbits were separately housed in wire-floored batteries under sanitary conditions with controlled humidity, temperature, light periods (12-h light, 12-h dark cycles) and fed balanced diet *ad libitum.*

Experimental design:
First group (G1) was left as uninfected and untreated (negative control), second group (G2) was left as uninfected and treated with *N. sativa* seed oil. Rabbits of third (G3), fourth (G4) and fifth (G5) groups were inoculated with $1x10^4$ sporulated oocysts of *E. stiedae* per each through a gastric tube (Toulah, 2000). Third group (G3) was left untreated and considered as positive control. At day 14 post inoculation (PI), the rabbits of fourth group (G4) were treated with *N. sativa* seed oil in a dose of 500 mg/kg BW (Farah *et al.*, 2004) for successive 14 days (PI). Meanwhile, the rabbits of fifth group (G5) were given *N. sativa* seed oil for 7 days before infection and continued for 28 days PI with the same dose. Administration of *N. sativa* seed oil was applied orally every morning after an overnight fast and before feeding.

Fecal oocysts:
Fecal oocysts were collected, concentrated using the flotation technique and counted using a modified McMaster technique (Long and Rowell, 1975) on days 0, 7, 14, 21, 28 and 35 PI. The reduction % of oocysts was determined based on the number of oocysts per gram of feces in control and treated groups.

Bile oocysts:
Bile samples were then collected from the gall bladders of the sacrificed rabbits of all groups at 14 days post treatment (PT) and the oocysts of *E. stiedae* were counted per 1 ml bile (Peeters *et al.*, 1981).

Blood parameters:
Blood samples were collected from ear veins of rabbits at 28 day PI. The serum was prepared and kept in deep freezer at -20 °C until analysed. The biochemical parameters of the blood were determined in the serum by using commercial kits for total protein, albumin, cholesterol, AST and ALT. Serum globulin was calculated as difference between total protein and albumin.

Body weight:
Rabbits were weighed at the beginning of the experiment (0 day) and on days 7, 14, 21, 28 and 35 PI and their growth performances (body weights and body weight gains) were recorded (Davis *et al.*, 1986).

Pathological study:
Liver lesions were scored according to Peeters and Geeroms (1986) and the livers were weighed after they were removed from five slaughtered rabbits on day 28 PI. The relative liver weight for each rabbit was calculated as the percentage of the total body weight (Gomez-Bautista *et al.*, 1986). Fresh liver tissue specimens of the sacrificed rabbits on the 28th day PI were fixed in 10% formalin, dehyderated with ethanol, cleared and embedded in paraffin wax. Section of 5-7 µm thickness were prepared and stained with hematoxylin and eosin (H&E) for the histopathological examination (Erdogmus and Eroksuz, 2006).

Statistical analysis:
The differences among experimental treatments were tested at $P \leq 0.05$ by one-way ANOVA according to Duncan (1955) using the computer software program called.

RESULTS

Table 1: Mean number of oocysts/g feces (mean ± SE), overall mean and reduction percent in five groups of rabbits under experiment (n = 12).
Oocyst output (---x10^4 oocysts/gram feces)

Group / Days PI	G1	G2	G3	G4	G5	LSD at $P \leq 0.05$
0	$0.00^{a} \pm 0.00$	$0.00^{a} \pm 0.00$	$0.00^{a} \pm 0.00$	$0.00^{a} \pm 0.00$	$0.00^{a} \pm 0.00$	NS
7	$0.00^{a} \pm 0.00$	$0.00^{a} \pm 0.00$	$0.00^{a} \pm 0.00$	$0.00^{a} \pm 0.00$	$0.00^{a} \pm 0.00$	NS
14	$0.00^{d} \pm 0.00$	$0.00^{d} \pm 0.00$	$27.42^{a} \pm 3.55$	$13.58^{b} \pm 0.91$	$6.75^{c} \pm 0.64$	6.75*
21	$0.00^{d} \pm 0.00$	$0.00^{d} \pm 0.00$	$59.67^{a} \pm 3.46$	$25.92^{b} \pm 1.22$	$15.83^{c} \pm 1.24$	10.08*
28	$0.00^{c} \pm 0.00$	$0.00^{c} \pm 0.00$	$95.50^{a} \pm 2.72$	$14.67^{b} \pm 0.36$	$0.67^{c} \pm 0.19$	14.00*
35	$0.00^{c} \pm 0.00$	$0.00^{c} \pm 0.00$	$63.50^{a} \pm 3.49$	$7.25^{b} \pm 0.41$	$0.00^{c} \pm 0.00$	7.25*
Overall mean	$0.00^{c} \pm 0.00$	$0.00^{c} \pm 0.00$	$41.01^{a} \pm 4.26$	$10.24^{b} \pm 1.11$	$3.33^{c} \pm 0.73$	6.36*
Reduction %	100 %	100 %	0.00 %	75.03 %	91.88 %	---
Reduction (%) =	$\frac{\text{OPG of +Ve control group} - \text{OPG of treated group}}{\text{OPG of +Ve control group}}$					X 100

-G1=Not infected and not treated (-Ve control) -G2= Not infected and treated with *N. sativa* seed oil
-G3=Infected and not treated (+Ve control) -G4= Infected and treated with *N. sativa* seed oil for 14 days PI.
-G5= Given *N. sativa* seed oil for 7 days before infection and continued for 28 days PI
-Means with different alphabetical superscripts in the same row are significantly different (at $P \leq 0.05$) and vice versa. -
LSD = Least significance difference. - NS = Non significant. - (*) = Significance at $P \leq 0.05$. - 0 day = Day of infection.

Table 2: Mean number of oocysts/ml bile (mean ± SE) in five groups of rabbits under experiment (n = 12).

Group / Parameter	G1	G2	G3	G4	G5	LSD at P ≤ 0.05
Oocyst output per 1ml of bile ($x10^4$)	0.00^{d} ± 0.00	0.00^{d} ± 0.00	96.08^{a} ± 3.36	22.08^{b} ± 0.80	7.58^{c} ± 0.47	7.58*
Production	0.00 %	0.00 %	100.00 %	22.98 %	7.89 %	----
Reduction	0.00 %	0.00 %	0.00 %	77.02 %	92.11 %	----
Reduction (%) =	Oocyst per ml bile of +Ve control group – Oocyst per ml bile of treated group / Oocyst per ml bile of +Ve control group					X 100

-G1=Not infected and not treated (-Ve control) -G2= Not infected and treated with *N. sativa* seed oil
-G3=Infected and not treated (+Ve control) -G4= Infected and treated with *N. sativa* seed oil for 14 days PI.
-G5= Given *N. sativa* seed oil for 7 days before infection and continued for 28 days PI.
-Means with different alphabetical superscripts in the same row are significantly different (at P ≤ 0.05) and vice versa.
- LSD = Least significance difference. - (*) = Significance at P ≤ 0.05.

Table 3: Lesion scoring in five groups of rabbits under experiment (n=10). (Mean ±SE).

Group / Parameter	G1	G2	G3	G4	G5	LSD at P ≤ 0.05
Lesion scoring	0.00^{d} ± 0.00	0.00^{d} ± 0.00	3.80^{a} ± 0.13	2.30^{b} ± 0.15	1.80^{c} ± 0.20	0.50*

-G1=Not infected and not treated (-Ve control) -G2= Not infected and treated with *N. sativa* seed oil
-G3=Infected and not treated (+Ve control) -G4= Infected and treated with *N. sativa* seed oil for 14 days PI.
-G5= Given *N. sativa* seed oil for 7 days before infection and continued for 28 days PI.
-Means with different alphabetical superscripts in the same row are significantly different (at P ≤ 0.05) and vice versa.
- LSD = Least significance difference. - (*) = Significance at P ≤ 0.05.

Table 4: Some biochemical parameters in five groups of rabbits under experiment (n=10). (Mean ±SE).

Group / Parameter	G1	G2	G3	G4	G5	LSD at P ≤ 0.05
ALT (U/L)	12.49^{d} ± 0.05	12.26^{d} ± 0.05	20.16^{a} ± 0.26	16.59^{b} ± 0.21	13.94^{c} ± 0.12	1.50*
AST (U/L)	23.94^{d} ± 0.06	23.81^{d} ± 0.24	46.17^{a} ± 0.08	33.95^{b} ± 0.18	27.82^{c} ± 0.17	3.88*
Total Protein (gm/dl)	5.44^{a} ± 0.02	5.40^{a} ± 0.06	4.53^{d} ± 0.03	4.92^{c} ± 0.03	5.16^{b} ± 0.04	0.11*
Serum albumin (gm/dl)	2.79^{a} ± 0.01	2.76^{a} ± 0.03	2.39^{d} ± 0.01	2.61^{c} ± 0.02	2.70^{b} ± 0.01	0.06*
Serum globulin (gm/dl)	2.65^{a} ± 0.03	2.64^{a} ± 0.08	2.14^{d} ± 0.02	2.31^{c} ± 0.05	2.46^{b} ± 0.04	0.13*
A/G ratio	1.05	1.05	1.12	1.13	1.10	-----
Total cholesterol (gm/dl)	126.42^{d} ± 0.19	126.59^{d} ± 0.20	155.84^{a} ± 1.49	140.04^{b} ± 0.24	129.89^{c} ± 0.23	3.30*
Creatinine (mg/dl)	0.876^{c} ± 0.010	0.860^{c} ± 0.007	1.266^{a} ± 0.022	0.962^{b} ± 0.006	0.886^{c} ± 0.005	0.08*

-G1=Not infected and not treated (-Ve control) -G2= Not infected and treated with *N. sativa* seed oil
-G3=Infected and not treated (+Ve control) -G4= Infected and treated with *N. sativa* seed oil for 14 days PI.
-G5= Given *N. sativa* seed oil for 7 days before infection and continued for 28 days PI.
-Means with different alphabetical superscripts in the same row are significantly different (at P ≤ 0.05) and vice versa.
- LSD = Least significance difference. - (*) = Significance at P ≤ 0.05. - NS = Non significance at P ≤ 0.05.

Table 5: Growth performance parameters (gm) (body weight and body weight gain) in five groups of rabbits under experiment (n=20). (Mean ±SE).

Days PI	Group / Parameter	G1	G2	G3	G4	G5	LSD at P ≤ 0.05
0	Body weight (gm)	953.67 ^{a} ± 3.86	954.00 ^{a} ± 3.63	957.33 ^{a} ± 3.99	954.67 ^{a} ± 3.70	955.23 ^{a} ± 4.67	NS
7	Body weight (gm)	1151.65 ^{b} ± 2.83	1169.34 ^{a} ± 4.08	1017.01 ^{b} ± 8.49	1131.64 ^{c} ± 2.55	1134.00 ^{c} ± 3.59	17.67*
	Body gain (gm)	195.67 ^{a} ± 9.90	195.33 ^{a} ± 14.24	59.93 ^{b} ± 6.26	176.33 ^{a} ± 3.89	174.67 ^{a} ± 5.24	114.8*
14	Body weight (gm)	1392.00 ^{b} ± 6.78	1456.35 ^{a} ± 5.98	1097.50 ^{e} ± 6.13	1281.66 ^{d} ± 3.74	1326.33 ^{c} ± 4.04	44.67*
	Body gain (gm)	431.67 ^{b} ± 8.22	486.67 ^{a} ± 10.28	168.33 ^{e} ± 28.41	327.00 ^{d} ± 6.47	373.00 ^{c} ± 7.10	46.00*
21	Body weight (gm)	1643.67 ^{b} ± 4.74	1748.00 ^{a} ± 5.71	1211.33 ^{e} ± 5.49	1479.34 ^{d} ± 4.59	1547.00 ^{c} ± 5.09	67.66*
	Body gain (gm)	690.02 ^{b} ± 6.57	794.11 ^{a} ± 6.27	254.60 ^{e} ± 5.57	524.67 ^{d} ± 5.53	591.65 ^{c} ± 7.43	67.00*
28	Body weight (gm)	1949.33 ^{b} ± 7.59	2062.67 ^{a} ± 8.52	1318.32 ^{e} ± 5.89	1699.67 ^{d} ± 8.84	1752.66 ^{c} ± 6.58	53.00*
	Body gain (gm)	999.00 ^{b} ± 10.72	1108.67 ^{a} ± 11.53	361.67 ^{e} ± 7.41	734.33 ^{d} ± 6.32	814.67 ^{c} ± 12.42	80.33*
35	Final Body weight (gm)	2078.33 ^{b} ± 6.61	2196.67 ^{a} ± 6.45	1491.71 ^{e} ± 10.58	1809.32 ^{d} ± 9.88	1965.00 ^{c} ± 7.48	95.67*
	Cumulative Body gain (gm)	1124.33 ^{b} ± 8.13	1176.33 ^{a} ± 9.34	535.33 ^{e} ± 12.35	922.67 ^{d} ± 12.35	1004.33 ^{c} ± 8.08	58.00*

-G1=Not infected and not treated (-Ve control) -G2= Not infected and treated with *N. sativa* seed oil
-G3=Infected and not treated (+Ve control) -G4= Infected and treated with *N. sativa* seed oil for 14 days PI.
-G5= Given *N. sativa* seed oil for 7 days before infection and continued for 28 days PI.
-Means with different alphabetical superscripts in the same row are significantly different (at P ≤ 0.05) and vice versa.
- LSD = Least significance difference. - (*) = Significance at P ≤ 0.05. - 0 day = Day of infection.
- NS = Non significance at P ≤ 0.05. -Growth performance according to: Davis *et al* (1986)

Table 6: Relative liver weights in five groups of rabbits under experiment (n=10).

Parameter	Group	G1	G2	G3	G4	G5	LSD at P ≤ 0.05
Relative liver weight 28 days PI	L.W.	30.47 ^{d} ± 0.30	30.53 ^{d} ± 0.29	113.34 ^{a} ± 1.01	90.53 ^{b} ± 0.71	76.05 ^{c} ± 0.60	14.48*
	L.W./ B.W.	0.015	0.014	0.076	0.050	0.039	----

$$\text{Relative liver weight} = \frac{\text{Liver weigh}}{\text{Total body weight}} \times 100$$

-L.W. = Liver Weight -L.W. /B.W. = Liver weight / Body weight
-G1=Not infected and not treated (-Ve control) -G2= Not infected and treated with *N. sativa* seed oil
-G3=Infected and not treated (+Ve control -G4= Infected and treated with *N. sativa* seed oil for 14 days PI.
-G5= Given *N. sativa* seed oil for 7 days before infection and continued for 28 days PI.
-Means with different alphabetical superscripts in the same row are significantly different (at P ≤ 0.05) and vice versa.
- LSD = Least significance difference. - (*) = Significance at P ≤ 0.05.

Table 7: Mean number of developmental stages (per microscopic field) in lumen of bile duct in five groups of rabbits under experiment (n=10). (Mean ±SE).

Group / Parameter	G1	G2	G3	G4	G5	LSD at P ≤ 0.05
Developmental stages of *E. stiedae* / field	0.00 ^{c} ± 0.00	0.00 ^{c} ± 0.00	74.50 ^{a} ± 5.01	9.10 ^{b} ± 0.55	2.00 ^{c} ± 0.37	7.10*

-G1=Not infected and not treated (-Ve control) -G2= Not infected and treated with *N. sativa* seed oil
-G3=Infected and not treated (+Ve control) -G4= Infected and treated with *N. sativa* seed oil for 14 days PI.
-G5= Given *N. sativa* seed oil for 7 days before infection and continued for 28 days PI.
-Means with different alphabetical superscripts in the same row are significantly different (at P ≤ 0.05) and vice versa.
- LSD = Least signifieanee difference. - (*) = Significance at P ≤ 0.05.

Table 8: Mortality in five groups of rabbits under experiment.

Group / Mortality	G1	G2	G3	G4	G5
Rate	0/12	0/12	5/12	2/12	1/12
Percent	0.00 %	0.00 %	41.67 %	16.67 %	8.33 %

-G1=Not infected and not treated (-Ve control) -G2= Not infected and treated with *N. sativa* seed oil
-G3=Infected and not treated (+Ve control) -G4= Infected and treated with *N. sativa* seed oil for 14 days PI.
-G5= Given *N. sativa* seed oil for 7 days beforc infection and continued for 28 days PI.

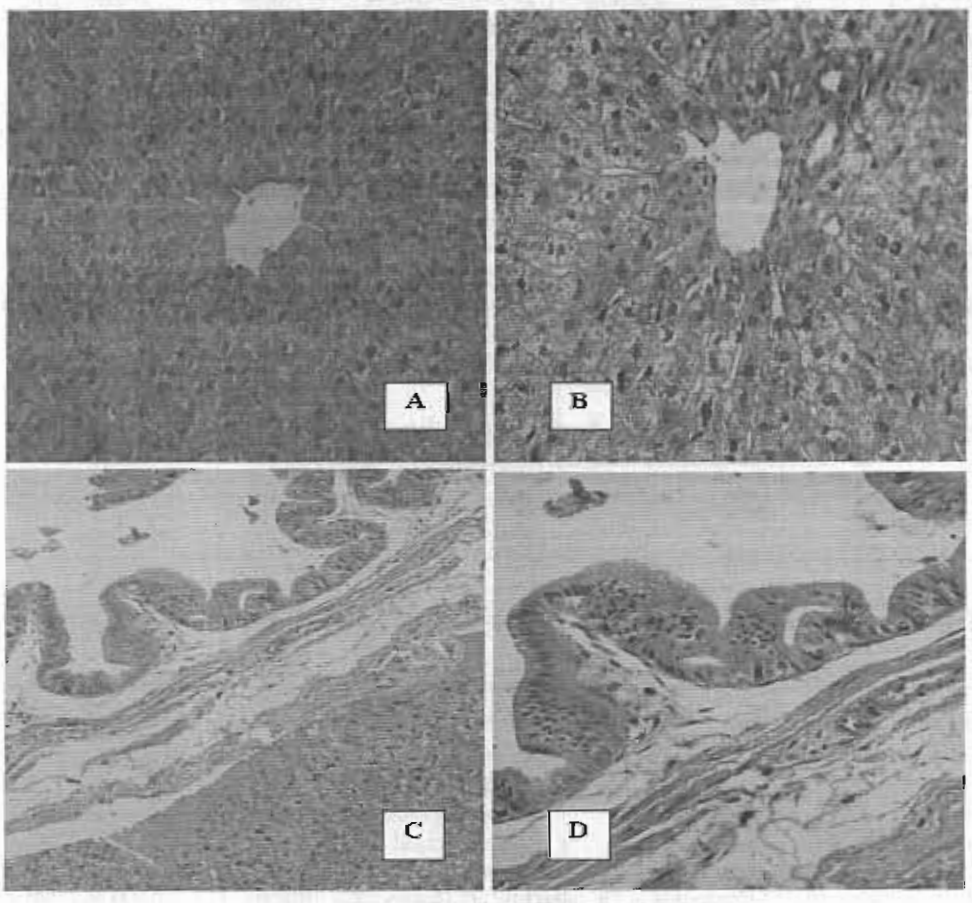

Figure 1: Photomicrograph of rabbit liver tissue of none infected (normal) and treated with *N. sativa* seed oil group (G2): showing a normal central vein (A) (H&E, 400 X), showing a normal portal area containing portal vein, hepatic artery and bile duct (B) (H&E, 400 X), showing a hyperplastic proliferation of the epithelial lining of bile duct (C) (H&E, 200 X) and (D) (H&E, 400 X).

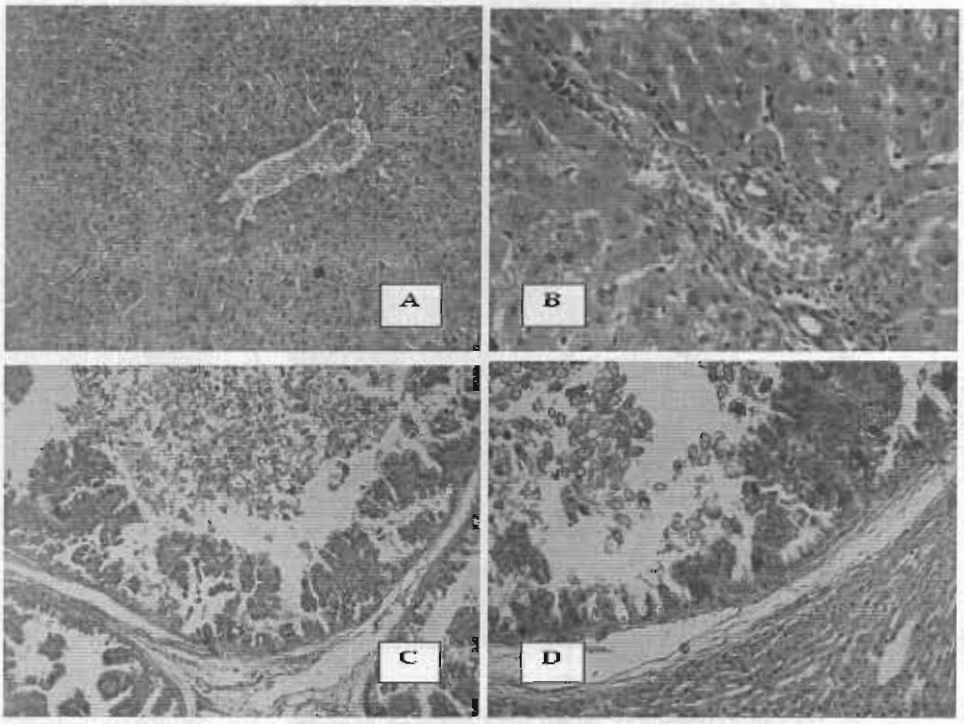

Figure 2: Photomicrograph of rabbit liver tissue of positive control group (infected and untreated) (G3) on day 28 PI: showing a dilated, congested central vein with necrosis of its endothelial lining (A) (H&E, 200 X), showing necrosis of hepatocytes and hemorrhages in portal area with inflammatory reaction (B) (H&E, 400 X), showing papilliform projections of epithelium into the lumen of bile duct containing very large number of developed oocysts (C) (H&E, 200 X), and showing different developmental stages of *E. stiedae* in the lumen of bile duct (D) (H&E, 400 X).

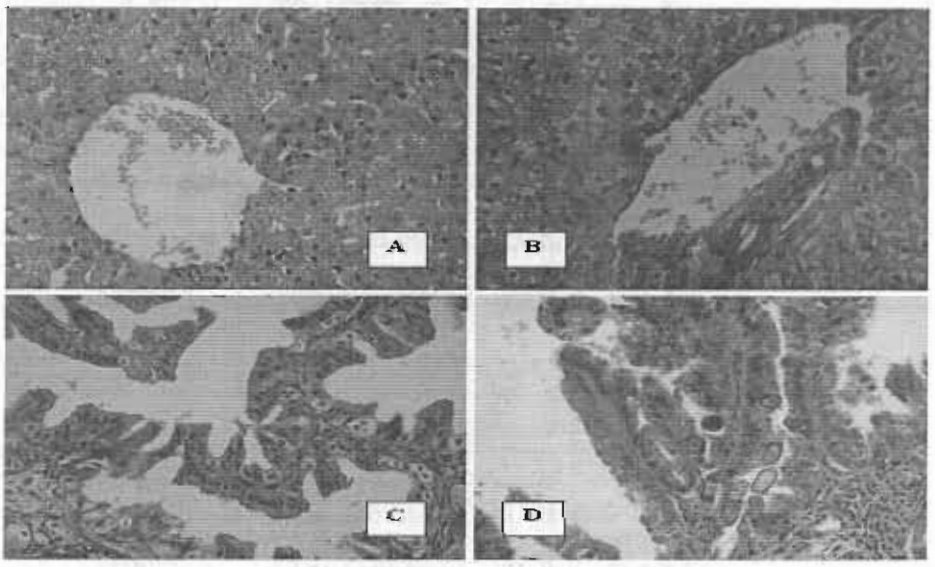

Figure 3: On day 28 PI, photomicrograph of rabbit liver tissue of infected group and treated with *N. sativa* seed oil for 14 days PI (G4): showing incomplete healing of its endothelial lining and healing of hepatic tissue (A) (H&E, 400 X), presence of little hemorrhages in the portal area with mild vacuolar degeneration of hepatocytes (B) (H&E, 400 X), multiple papillary fronds in the bile ducts with the presence of moderate number of the parasite stages from the lumen of bile duct (C) (H&E, 200 X), and (D) (H&E, 400 X).

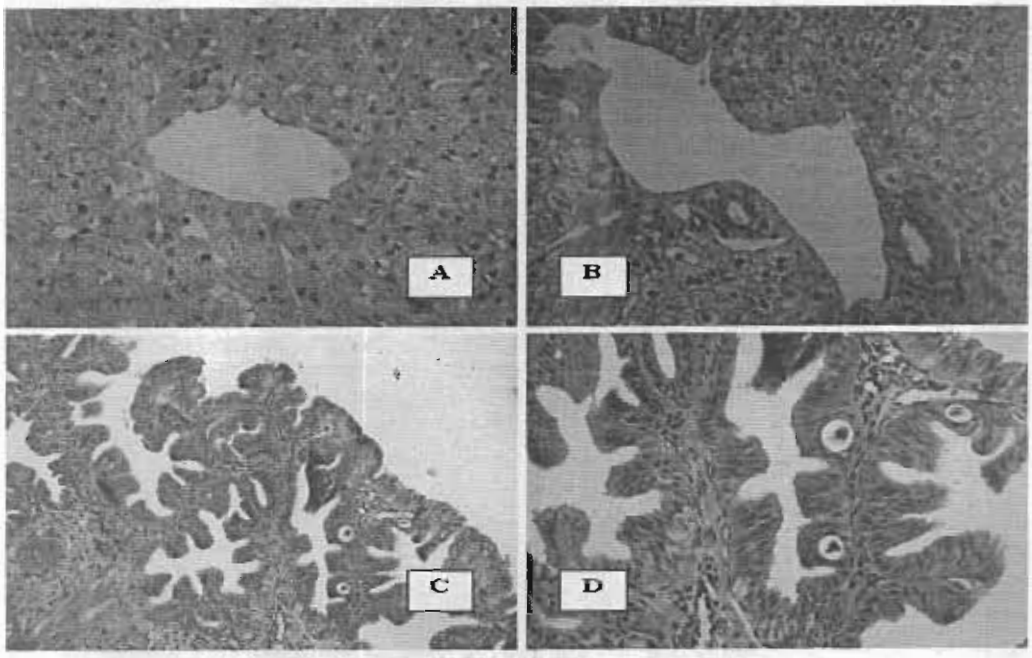

Figure 4: On day 28 PI, photomicrograph of rabbit liver tissue of infected group and given *N. sativa* seed oil for 7 days before infection and continued for 28 days PI (G5): showing healing of the central vein endothelium. The hepatocytes laminate regained their original radial shape (A) (H&E, 400 X), disappearance of hemorrhages from the portal area as well as disappearance of vacuolar degeneration of hepatocytes (B) (H&E, 400 X), showing almost disappearance of the parasite stages from the lumen of the bile duct (C) (H&E, 200 X), and (D) (H&E, 400 X).

DISCUSSION

This study indicates effectiveness of *N. sativa* for control of coccidiosis in rabbits by a dose of 500 mg/kg BW. At day 28 PI, infected rabbits treated with *N. sativa* seed oil recovered their appetite, started to gain weight, became active again and recovered from diarrhea with feces returning to its beady form. Similar results were reported by Al-Gamdi (2003) when used 250-500 mg/ kg BW; Baghdadi and Al-Mathal (2011) when used 400 mg/ kg BW in rabbits and El-Dakhakhny *et al.* (2000) when used 800 mg/kg BW orally for 4 weeks in albino rats. However, dose of 20, 40 and 100 mg/kg BW were effective in treating schistosomiasis through decreasing of number of shedding eggs and reducing of liver damage in mice (Tantawi and Mostafa, 2003).

At 28 day PI, Table 1 showed a marked decrease in fecal oocysts count in group G5 (0.67±0.19) when compared with those of G3 (95.50±2.72) and G4 (14.67±0.36). Meanwhile, at 35 day PI, there is disappearance of oocysts in the feces of group G5 (0.00±0.00) when compared with those of G3 (63.50±3.49) and G4 (7.25±0.41). Overall mean of oocysts output in G5 was 3.33±0.37 with reduction of 91.88% versus to 41.01±4.26 (reduction of 0 %) in G3 and 10.24±1.11 (reduction of 75.03%) in G4. This may be attributed to presence of powerful antioxidants and alkaloid nigellicine in *N. sativa* seed oil that could limit the growth and development of *E. stiedae* stages leading to reduction of oocysts formation and their appearance in the feces. The effective material in *N. sativa* seed oil in G5 may have been more concentrated than in G4, therefore having a more rapid and robust effect. In G5, the *E. stiedea* parasite exposed to the *N. sativa* seed oil for long time (28 successive days) versus short time (14 days) in G4. Similar results were reported by Abu-El-Ezz (2005) on *Trichinella spiralis* in rats, Ayaz *et al.* (2007) on *Aspiculuris tetraptera* and *Hymenolepis nana* in naturally infected mice, El-Shenawy *et al.* (2008) on *S. mansoni* in mice and Baghdadi and Al-Mathal (2011) on *E. stiedae* in rabbits.

At 28 day PI, Table 2 showed significant reduction of oocyst count in the bile of group G5 (7.58±0.47) with reduction of 92.11% when compared with those of G3 (96.08±3.36) (reduction of 0 %) and G4 (22.08±0.80) (reduction of 77.02 %). This may be due to the effect of thymoquinone substance present in the *N. sativa* seed oil that could inhibit the growth and development of *E. stiedae* invasive stages (sporozoaites and merozoites) resulted in reduction of oocysts formation in the bile. These results are supported by findings of Mahmoud *et al.* (2002), Rahman *et al.* (2008) and Baghdadi and Al-Mathal (2011) on *E. stiedae* in rabbits.

At 28 day PI, Table 3 showed significant decrease in the liver lesion scoring in G5 (1.80±0.20) when compared with that of G3 (3.80±0.13) and G4 (2.30±0.15). This indicates that the *N. sativa* seed oil was highly effective at reducing the liver lesions in G5 when compared with groups G3 & G4. Furthermore, rabbits infected with *E. stiedae* showed remarkable improvement in liver tissue histopathology on day 28 PI {in rabbits given *N. sativa* seed oil for 7 days before infection and continued for 28 days PI (G5)}. In addition, there was a huge reduction in various stages of parasite in liver tissue, resulting in reduced lesions in the liver (Table, 3); consequently the fecal and bilary oocyst output were markedly reduced. Similar results were reported by Baghdadi and Al-Mathal (2011) on *E. stiedae* in rabbits.

In this study, Table 4 showed that liver enzymes {ALT (13.94±0.12), AST (27.82±0.17)}, total cholesterol (129.89±0.23) and creatinine (0.886±0005) were significantly decreased in G5 when compared with G4 {ALT (16.59±0.21) and AST (33.95±0.13)}, total cholesterol (140.04±0.24), creatinine (0.962±0.006) and with G2 {ALT (12.26±0.05), AST (23.81±0.24)}, total cholesterol (126.59±0.20), creatinine (0.860±0.007). However, total protein (5.16±0.04), albumin (2.70±0.01) and globulin (2.46±0.04) in G5 were increased and directed toward normal level when compared with G4 (4.92±0.03, 2.61±0.02& 2.31±0.05, respectively) and G2 (5.40±0.06, 2.76±0.03 & 2.64±0.08, respectively). This may be attributed to presence of thymoquinone group that protected liver cells from damage consequently liver functions as AST and ALT were improved. Similar results were reported by Mahmoud *et al.* (2002) and El-Shenawy *et al.* (2008) in mice. However, *N. sativa* seeds (till 10 %) yielded no differences of blood plasma chemistry in chicks (El-Sayed *et al.*, 2005). Black cumin at level of 2 or 3% would decrease cholesterol in egg yolk (Aydin *et al.*, 2008). Addition of *N. sativa* in form of oil (0.5%), seeds (1%) or meal (10%) to broiler diets under stress increased total protein, albumin and globulin; reduced mortality, cholesterol, AST; and ALT and improved RBCs, WBCs and Hb (Hermes *et al.*, 2011), prevented oxidative stress and improved biochemical parameters (Attia *et al.*, 2011), decreased cholesterol (Zaoui *et al.*, 2002 and Isik *et al.*, 2011) and counterbalanced renal injury in rats (Ahmed and El-Mottaleb, 2012). Also daily administration of *N. sativa* seed oil (800 mg/kg orally for 4 weeks) improved AST, ALT and bilirubin but caused a significant decrease in cholesterol in albino rats (El-Dakhakhny *et al.*, 2000).

In Table 5, the uninfected rabbits that received *N. sativa* treatment for 35 days (G2) showed significant body weight and cumulative weight gains starting from day 14 till end of experiment (2196.67±6.45, and 1176.33±9.34, respectively) when compared with (G1) (2078.33±6.61 and 1124.33±8.13, respectively). This may be due to the *N. sativa* seeds contain appetizing materials that may increase food intake. Similar results were reported by El-Abhar *et al.* (2003). In groups (G5) and (G2), rabbits that received the *N. sativa* seed oil gained weight more rapidly. Specifically, rabbits in G5 (1965.00±7.48) increased in weight by 22.77 % as compared to G3 (1491.71±10.58), whereas G2 rabbits (2196.67±6.45) increased in weight by 33.91 % on day 35 PI. The difference in weight gain was very significant between the two treated groups (G5 and G4) (1004.33±8.08 versus 922.67±12.35, respectively). This may be attributed to the higher percentage of fatty materials in *N. sativa* seed oil. This agrees with that of El-Abhar *et al.* (2003) and Khalaji *et al.* (2011). Broilers receiving 40 g kg (-1) of black seed in feed had a significant effect on body weight gain, feed intake and FCR (Durrani *et al.*, 2007). *N. sativa* seeds (up to 2 %) improve performance, health and immunity of birds with less mortality (Nasir, 2009). In this study, disease symptoms were improved and body weights increased within 2 weeks of treatment, indicating recuperation from *E. stiedae* infection. Similar results were reported by Ghanem *et al.* (2008).

In Table 6, the mean liver weights and the relative liver weights of the infected and untreated rabbits (G3) (113.43±1.01 and 0.076, respectively) were significantly higher than the uninfected and untreated rabbits (G1) (30.47±0.430 and 0.015, respectively), and uninfected and treated rabbits (G2) (30.53±0.29 and 0.014, respectively) throughout the duration of the experiment. The liver weights were not significantly different between the infected groups (G1 and G2) (30.47±0.30 and 30.53±0.29, respectively) on day 28 PI. However, following *N. sativa* treatment, liver weights were drastically decreased in G5 (76.05±0.60) as compared with G4 (90.53±0.71) and G3 (113.34±1.01) on day 28 PI. Similar results were reported by Baghdadi and Al-Mathal (2011).

In Table 7, the mean number of developmental stages of *E. stiedae* per microscopic field was significantly decreased in G5 when compared to G4 and G3 (2.00±0.37 versus to 9.1±0.55 and 74.5±5.01, respectively). This attributed to the anticoccidial effect of *N. sativa* seed oil on *E. stiedae* parasite after use for long time (28 days PI). Similar results were mentioned by Baghdadi and Al-Mathal (2011).

In Table 8, the mortality of infected rabbits with *E. stiedae* was 8.33 % in G5 (1/12), 16.67% in G4 (2/12), 41.67% in G3 (5/12) PT. However, there were no mortalities in Groups 1 & 2. Similar results were reported by Hermes *et al.* (2011) in poultry and Baghdadi and Al-Mathal (2011) in rabbits.

Normal tissue structure of hepatocytes, portal area, and bile ducts was present in liver samples from uninfected and treated rabbits with *N. sativa* seed oil (G2), regardless of treatment, on day 28 PI (Fig. 1, A-D). This ensures that there may be no toxic effect of *N. sativa* seed oil on liver cells even for a long time treatment. Similar results were reported by Baghdadi and Al-Mathal (2011) in rabbits and Attia *et al.* (2011) in rats. Classical pathological changes of hepatic coccidiosis in liver of infected rabbits (G3) were present on day 28 PI (Fig. 2, from A to D). Rabbit liver of G3 showed a dilated congested central vein with severe necrosis of endothelial lining and haemorrhagic areas (Fig. 2, A), necrosis of hepatocytes and haemorhages in portal area with inflammatory reaction (Fig. 2, B), papilliform projections of epithelium into the lumen of bile duct containing very large number of developed oocysts (Fig. 2, C), and different developmental stages of *E. stiedae* in the lumen of bile duct (Fig. 2, D). Similar results were reported by Baghdadi and Al-Mathal (2011).

On day 28 PI, liver of treated rabbits with *N. sativa* seed oil for 14 successive days (G4) showed incomplete healing of congested central vein and endothelial lining encircling central vein (Fig. 3, A), presence of little hemorrhages from portal area with mild vacuolar degeneration of hepatocytes (Fig. 3, B), multiple papillary fronds in the bile ducts were recorded with the presence of moderate number of the parasite stages from bile duct lumen (Fig.3, C&D). Similar results were reported by Abdel-Hamid *et al.* (2012) in rabbits who reported that the methanolic extract of *N. sativa* has a chemopreventive effect against progression into liver malignancy. Meanwhile, the liver of the rabbits given *N. sativa* seed oil for 7 days before infection and continued for 28 days PI (G5) showed complete healing of the congested central vein (Fig.4, A), disappearance of hemorrhages from portal area (Fig.4, B), and almost disappearance of the parasite stages from the lumen of the bile duct although presence of hyperplasia in the columnar epithelial cells of bile ducts which may be recovered by time (Fig.4, C&D). Similar results were reported by Baghdadi and Al-Mathal (2011) on *E. stiedae* in rabbits, El Shenawy *et al.* (2008) on *S. mansoni* in mice.

The present results indicate that *N. sativa* seeds oil can be used to safely and effectively against *E. stiedae* in rabbits with no side effects although presence of hyperplasia in the columnar epithelial cells of the bile ducts that may be recovered by time. In addition to, improvement of growth performance as well as liver and kidney functions. Future studies would be focused on determining the active materials within *N. sativa* treatments, optimal doses and mode of action.

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تأثير الحبة السوداء المضاد للكوكسيديا فى الأرانب

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أجريت هذه الدراسة على ٦٠ أرنب (من ذكور الأرانب النيوزيلاندي الأبيض). تم فحص الأرانب يوميا ولمدة أسبوع للتأكد من خلوها من الإصابة بالكوكسيديا. وقسمت الأرانب إلى خمسة مجموعات متساوية بكل مجموعة ١٢ أرانب. المجموعة الأولي (G1) استخدمت كضابط سالب للتجربة (لم يتم عدواها ولم تعالج)، والمجموعة الثانية (G2) لم يتم عدواها بطفيل الأيميريا ستيدى وعولجت بزيت حبة البركة (٥٠٠ مجم / كجم وزن حى) لمدة ٢٨ يوم متتالية تجريعا بالفم. وباقي المجموعات (الثالثة، الرابعة والخامسة) تم عدواها معملياً بحويصلات الأيميريا ستيدى المتجرثمة بجرعة ١ x ١٠^٤ حويصلة لكل أرنب بإستخدام أنبوب اللى المعدى وذلك بعد أسبوع التأكد من خلوها من الكوكسيديا. المجموعة الثالثة (G3) لم تعالج (ضابطة موجبة). المجموعة الرابعة (G4) تم علاجها بعد أسبوعين من العدوى بزيت حبة البركة (٥٠٠ مجم / كجم وزن حى) لمدة ١٤ يوم متتالية تجريعا بالفم. المجموعة الخامسة (G5) تم تجريعها بالفم زيت حبة البركة (٥٠٠ مجم / كجم وزن حى) قبل العدوى بأسبوع وإستمر العلاج لمدة ٢٨ يوم متتالية من العدوى. تم فحص عينات البراز للأرانب لكل المجموعات لتحديد عدد حويصلات الأيميريا لكل جرام من البراز بعد ٠، ٧، ١٤، ٢١، ٢٨، ٣٥ يوم من العدوى. تم فحص السائل المرارى لتحديد عدد حويصلات الأيميريا لكل ١سم^٣ عند ٢٨ يوم من العدوى. تم قياسALT, AST، والبروتين الكلى، والألبيومين والجلوبيولين، والكوليسترول الكلى، والكرياتينين لعدد خمسة ارانب من كل مجموعة عند اليوم الرابع عشر من بداية العلاج. وتم ذبح خمسة أرانب من كل مجموعة فى اليوم الثامن والعشرين من العدوى لتحديد درجة الإصابة في الكبد، وتم وزن الكبد لكل أرنب منهم، كما تم إجراء الفحوص الهستوباثولوجية عليه. تم وزن الأرانب كل أسبوع وحساب الوزن المكتسب لكل أرنب. أظهرت نتائج الدراسة أن زيت حبة البركة بجرعة ٥٠٠ مجم / كجم وزن حى كأسلوب وقائى له كفاءة عالية وذلك من خلال تقليل عدد حويصلات الأيميريا فى كل من البراز والسائل المرارى بعد ٢٨ يوم من العدوى، كما قلل من درجة الإصابة فى نسيج الكبد. كما أنه قلل من نشاط إنزيمات الكبد (ALT, AST) ومستويات الكوليسترول، والكرياتينين بمصل الدم. كما وجد أن له تأثير يزيد من مستوى البروتين الكلي والألبيومين والجلوبيولين بمصل الدم، و يزيد من وزن الجسم ووزن الجسم المكتسب وذلك عند مقارنته الأسلوب العلاجى. بالفحص الهستوباثولوجي تلاحظ تلاشى حدوث ضرر على انسجة الكبد بعد إستخدام زيت حبة البركة بجرعة ٥٠٠ مجم / كجم وزن حى كأسلوب وقائى. وفى الوقت نفسه تلاحظ وجود ضرر على أنسجة الكبد بعد إستخدام زيت حبة البركة (٥٠٠ مجم / كجم وزن حى) كأسلوب علاجى. نستخلص من هذه الدراسة أن زيت حبة البركة بجرعة ٥٠٠ مجم / كجم وزن حى له كفاءة وقائية للحالات المصابة بطفيل الأيميريا ستيدى في الأرانب، وله تأثير إيجابى على أوزان الحيوانات وعلى وظائف الكبد ونسيجه ويعد بديلاً فعالاً وآمناً عن الأدوية الكيماوية ذات الآثار الجانبية وذلك لوقايتها وحمايتها من الإصابة بالكوكسيديا.