The therapeutic effect of *Nigella sativa L.* seeds oil on experimentally infected rabbits with hepatic coccidiosis

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Summary

The present study was carried out to investigate the therapeutic effect of *Nigella sativa* seeds oil emulsion as against *Eimeria stiedae* experimentally infected rabbits. After isolation of local *E. stiedae* strain from infected gallbladders, a total of 90 local rabbits (6-8 weeks age) and body weighing (750-1000 gm) were used. They were divided into 6 groups as follow: Group: 1 uninfected and untreated (control group), Group: 2 infected (untreated) with 10,000 sporulated oocysts of *E. stiedae*, Group: 3 uninfected and given 200mg/kg B.W. *N. sativa L*. oil emulsion, Group: 4 infected with 10,000 sporulated oocysts of *E. steidae*. and treated with *N. sativa L*. oil emulsion of 200 mg/kg B.W on day 16, post infection, Group: 5 uninfected and given 400 mg/kg B.W *N. sativa L*. oil emulsion, Group: 6 infected with 10,000 sporulated oocysts of *E. stiedae*. and treated with *N. sativa L*. oil emulsion of 400 mg/ kg B.W on day 16, post infection. Fecal sample were examined for oocyst count (16 days post infection) at each period of experiment (10, 20 and 30 days) 5 animals for each group were sacrificed, and specimens for liver, were excised for histopathological examination. The *E. stiedae* infected group showed deleterious pathological changes in infected livers. Both treated doses of *N. sativa* had significant anticoccidial activities as reflected by reduced fecal oocysts shedding and remarkable improvement of liver tissue histopathology. This improvement include restoration of normal hepatic architecture and increase of the binucleated hepatocytes, disappearance of hemorrhage between the hepatic lobules, formation of foreign body granulomas and reduction in the various stages of the parasites and oocysts in the bile ducts .The result showed that changes more rapid when animals were treated with 400 mg/ kg/ B.W of *N. sativa* seeds oil emulsion than when treated same emulsion at dose 200 mg/ k.g/ B.W without side effects. From the results obtained in the present study *N. sativa* seeds oil emulsion was safe without side effects and the dose 400 mg/kg/B.W was more effective against *E. stiedae* infection than dose 200 mg/kg/B.W which may be recommended for use as adjuvant therapy in clinical practices.

Keywords: *Nigella sativa L.*, Hepatic Coccidosis, Liver, Rabbits.

Introduction

Rabbit coccidiosis is a common and widespread protozoal infection in commercial operations and is responsible for major pathogenicity in definitive host (1). It is an important disease of rabbits cause economic loss, especially, in breeding and rearing establishments where sanitation measures are poor (2). *Eimeria stiedae* is one of the most pathogenic coccidian protozoans in domestic rabbits causing severe coccidiosis and increased mortality (3 and 4). It parasitizes the epithelial cells of the bile duct causing severe liver damage and great economic losses (5). The extensive use of chemical anti-coccidial drugs in controlling this disease has led to the development of drug-resistant parasites. Parasite resistance and the side effects of some of the anti-coccidial drugs will have serious consequences for future disease control (6). Therefore there is a pressing need to combat this disease to identifiy new effective drugs that are safe for the animals and the environment .Many studies have focused on the search for novel, safe and effective anti-coccidial drugs from natural medicinal plant or herbs. *Nigella sativa* (family: Ranunculaceae), also known as black cumin, is a herbaceous annual plant used in traditional medicine for thousands of years because of its and antioxidant effects and other various medicinal effects (7 and 8). Though 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 examined only the anti-schistosomal, trematodes, cestodes and nematodes activities of *N. sativa* (9-11**).** The aims of this study was to isolation and sporulation of *Eimeria stiedae* oocysts, evaluation the therapeutic effects of oil emulsion of *N.sativa L.* seeds on the pathological changes accompanied with the experimentally induced hepatic coccidiosis through fecal oocysts count and histopathological study.

Materials and Methods

Ninety male domestic local male rabbits (6-8 weeks) weighing between 750 - 1000 gm were used. During the experimental period, animals were kept in cages of experimental animal house of College of Veterinary Medicine - Baghdad University. Rabbits were individually housed in metal cages with grid on the bottom keeping rabbits from coming in contact with their feces. The rabbits were fed with commercial pellet food and water was supplied (12). The absence of *E. stiedae* and other coccidian oocysts prior to the experiments was confirmed by fecal and blood smear for parasites examination of rabbits.

Isolation and sporulation of *Eimeria stiedae* oocysts was done (13). Ten *Eimeria* –free rabbits of one month old were inoculated with 10000 freshly sporulated *E. stiedae* oocyst. Feces were examined daily until shedding of the first oocyst. Infected rabbits were then killed and oocyst were collected from their gallbladders. Oocyst were purified and allowed to sporulate in 2.5% potassium dichromate solution. Sporulated oocysts were used as a challenge material in the experiment.

Preparation of *N. sativa* Oil was done (14). Calculation of the dose of *N. sativa* oil was done (15). Ninety local breed rabbits were divided into 6 equal groups. Rabbits were administrated a single dose of 1×10^3 sporulated oocysts through a gastric tube for all experiment. On day 16 post-infection (Pi) rabbits were treated with *N. sativa* oil emulsions. One hour before feeding each rabbits was given a dose of 200mg or 400mg/ kg/ B.W oil emulsion of *Nigella sativa L.* seeds orally every morning after an overnight fast as follows, Group: 1 uninfected and untreated (control group). Group: 2 infected with 10,000 sporulated oocysts of *E. stiedae*. Group: 3 uninfected and given 200 mg/kg B.W *N. sativa L.* oil emulsion. Group: 4 infected with 10,000 sporulated oocysts of *E. stiedae*. and treated with *N. sativa L.* oil emulsion of 200 mg/ kg B.W on day 16.post inoculation. Group: 5 uninfected and given 400mg/kg B.W *N. sativa L.* oil emulsion. Group: 6 infected with 10,000 sporulated oocysts of *E. stiedae*. and treated with *N. sativa L.* oil emulsion of 400 mg/kg B.W on day 16 post inoculation. Counting of oocysts in feces was done (16).

At the end of each experimental period 10, 20 and 30 days, animals were sacrificed and specimens were taken from livers. The tissues have been kept in 10% formalin, processed and stained (17). PAS Stain was done (18). Statistical analysis of data was performed by using statistical package for social science (SPSS, 2008), Version 16. For the determination of the significant differences among means, one way analysis ANOVA was used. A p-value <0.05 was considered statistically significant.

Results and Discussion

The result of mean value of oocyst excretion in infected rabbits groups during the experiment was illustrated in (Table, 1).

Table, 1: Mean number of oocysts /g feces (mean ± SE) in infected and infected and treated groups

Groups / Periods	G2 Mean ± SE	G4 Mean ± SE	G4 %	G6 Mean ± SE	G6 %
10 days	86654.00 ± 9391.87 A	68547.20 ± 9280.09 B	20%89	62732.80 ± 8527.49 B	27,60%
20 days	47731.20 ± 6738.17 A	30611.70 ± 5758.25 B	35%86	20265.00 ± 4225.28 B	57,54%
30 days	10422.10 ± 2650.42	3542.00 ± 988.10	66,01%	809.70 ± 443.96	92,23%

Different capital denote significant (P<0.05) differences among groups.
Different small letters denote significant (P<0.05) within groups as pretreated period.
Reduction (%) = opg before treatment – opg after treatment × 100
Opg before treatment

The reduction of oocysts in the feces of both treated groups may due to the presence of powerful antioxidant in the seeds which are considered as potent immunity boosters (19). According to the above statement antioxidants of *N. sativa* seeds may limit the growth and development of the *Eimeria* within the body of the host leading finally to the reduction of the oocysts in the feces (20). The reduction percent was higher at the higher dose and that may be related to the presence of effective materials at higher concentration than the lower dose. Other researchers (15) concluded that the reduction or disappearance of fecal oocysts shedding is considered an indicator of recuperation from *E. stiedae* infection. These changes were reflected on histopathological pictures, which showed high reduction in the various stages of the parasite in the epithelial cells and immature oocysts in the bile ducts, resulting in reduced oocysts shedding and improved clinical symptoms, and that agreed with the present study.

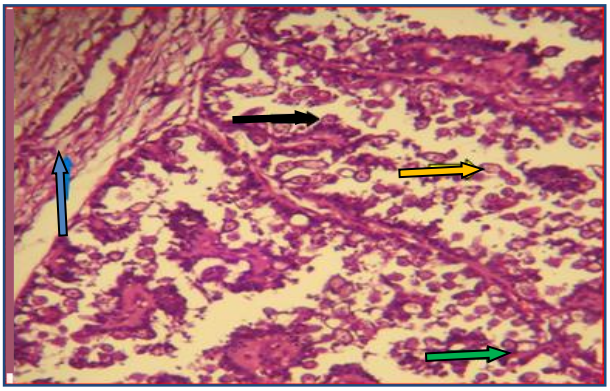
The livers of the control group showed normal tissue morphology, while in infected group: At 10 days of experiment, there was hyperplasia of epithelial lining of bile duct forming papillary projections with presence of sexual stages of development of the parasite (Fig. 1).The basement membrane thickened forming a loop-like structure **(**Fig. 2). The central veins showed severe dilation and congestion with infiltration of inflammatory cells in their lumina. Many of them undergo ruptured of their endothelial lining resulting in a wide areas of parenchymatous hemorrhages in 10 days post infection (Fig. 3). At 20 days period, there is severe dilation and telangiectasis of sinusoids contain RBCs and inflammatory cells with marked atrophy of hepatocytes (Fig. 4) accompanied by infiltration of inflammatory cells and large numbers of eosinophils (Fig. 5). At 30 days period, there is extensive periportal and interlobular fibrosis (Fig. 6). Other sections showed deposition of bile pigment within the hepatic parenchyma (Fig. 7).

Treated groups: At 10^{th} days period, the microscopic appearance was characterized by increase in the binucleated hepatocytes (Fig. 8). At 20 days period:-The tissue sections of both doses showed foreign body granulomas contain the destructed parasite in their centers surrounded by epithelioid cells and giant cells with formation of fibrous connective tissue capsule at the periphery (Fig. 9). Furthermore there is a decrease in the mononuclear cells within the portal areas especially at dose 400 mg/ kg/ B.W. of *N. sativa* oil emulsion (Fig. 10).

At 30 days period, the bile ducts in sections obtained from livers treated with 200 mg/kg/BW of *N. sativa* oil emulsion contained few oocysts (Fig. 11). At 400 mg/kg/BW of *N. sativa* oil emulsion the tissue sections showed decrease in sizes of hemorrhagic areas and restoration of hepatic tissue to nearly normal picture with hydropic degeneration of hepatocyes (Fig. 12). The main histopathological changes due to *E. stiedae* infection were the proliferation of the intrahepatic bile ducts as seen in tissue sections of infected animals and that may be attributed to the proliferation of *E. stiedae* in its predilection site within the epithelial cells lining these bile dusts. This result was in agreement with (21 - 24**).** The increase in the thickness of the basement membrane indicating its involvement in the pathogenesis of the papillary hyperplasia of the lining epithelium of the bile duct. It serves as a limiting substratum to support the overlying epithelium (25). The interlobular and peribiliary fibrosis was time and dose dependent. Recent studies have identified macrophages as critical regulators of fibrosis (26).

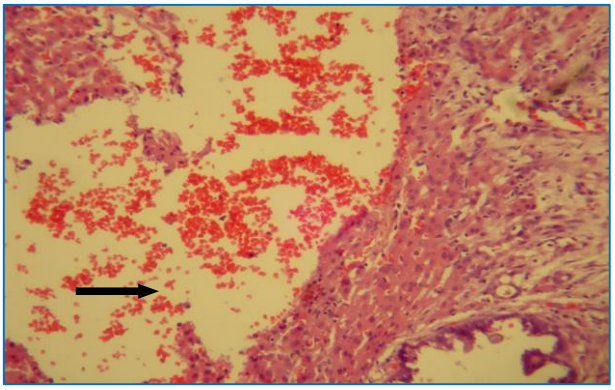
The present study suggested that *N. sativa* oil emulsion has a beneficial treatment effect against *E. stiedae* infection. (27) found that can be prevented liver fibrosis and cirrhosis, through immunomodulator and antioxidant activities and that explained the reduction in fibrous connective tissue in inflamed tissue. Tissue sections of treated groups showed destructive of the development stages of the parasite and that may be attributed to high concentration of alkaloid nigellicine in the oil emulsion of *Nigella sativa*, which has a deadly effect on parasites (11). The decrease in the intense of the inflammatory process in the treated groups may be due to both antioxidant and anti-eicosanoid effects of the oil, other than to the effect of thymoquinone which is active constituent (22). The restoration of

hepatocytes to the nearly normal structure was related to the *N. sativa* oil emulsion exceed the production of leukotrienes that cause damage to cells and tissue (28). In addition to the action of thymoquinone in preservation of the membrane integrity that disallowed the loss and leakage of enzymes from the cells (20 and 29). Moreover its ability to preserve the membrane integrity can by proven by the restoration of hepatic tissue (30).

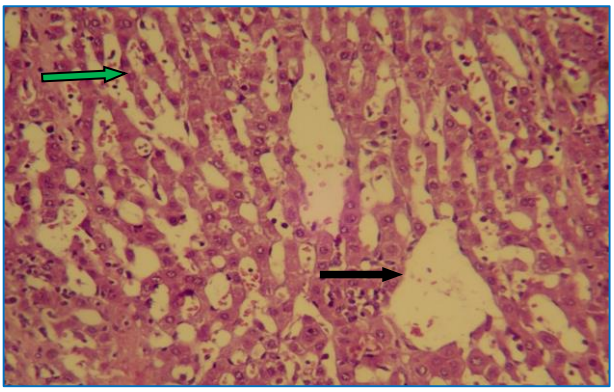


Figure, 1: G2: Liver of rabbit at 10 days post infection show hyperplasia of epithelial lining of bile duct forming arborizing papillary fronds ( ) with sexual develop-mental stages of the parasite ( ) and oocystes in the lumen ( ) with slight periportal fibrosis ( ) (H and E 40x) .

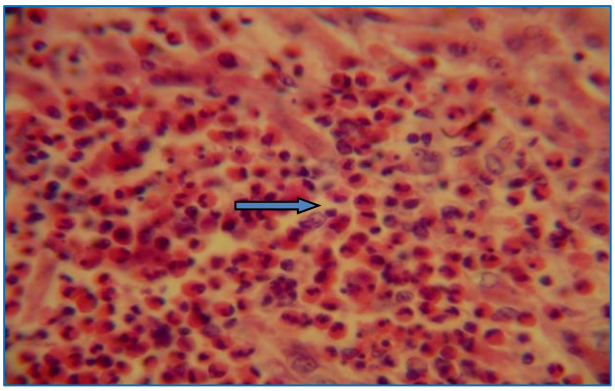
Figure, 2: G2: Liver of rabbit at 10 days post infection thickening of basement membrane ( ) over which reside epithelial cells with detachment of the looped basement membrane along with biliary epithelium containing macrogametocytes detachment of ( ) (PAS 40 x).



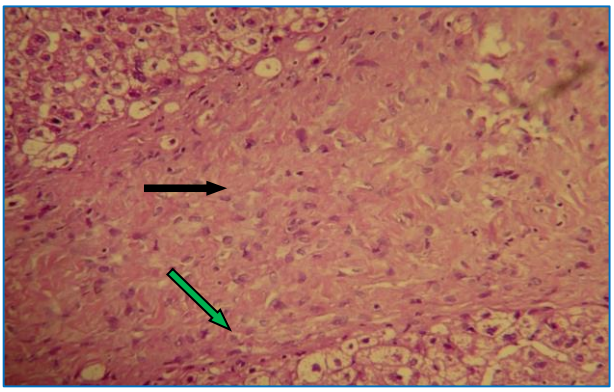
Figure, 3: G2: Liver of rabbit at 10 days postinfection show severe hemorrhage ( ) (H and E 40 x).



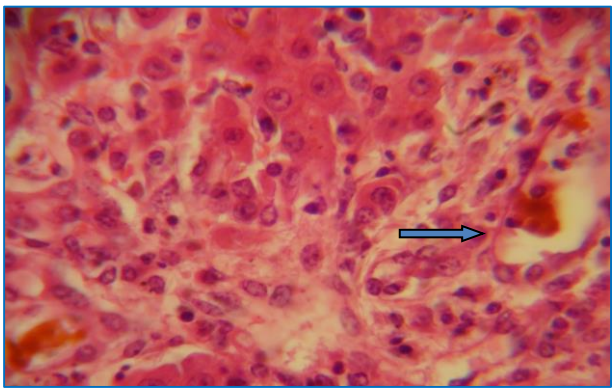
Figure, 4: G2: Liver of rabbit at 20 days post infection show telangiectasis ( ) with marked atrophy of hepatocytes ( ) (H and E 40 x).



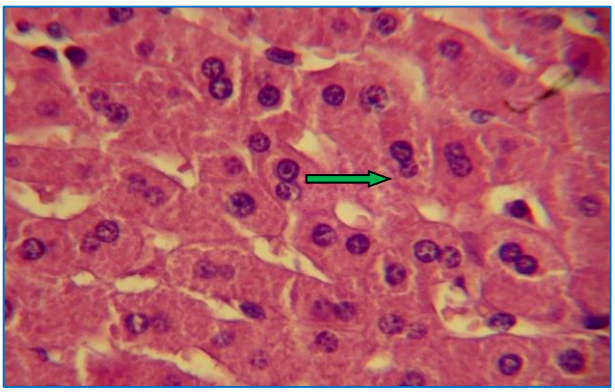
Figure, 5: G2: Liver of rabbit at 20 days post infection show infiltration of large numbers of eosinophils within the hepatic parenchyma ( ) (H and E 40x).



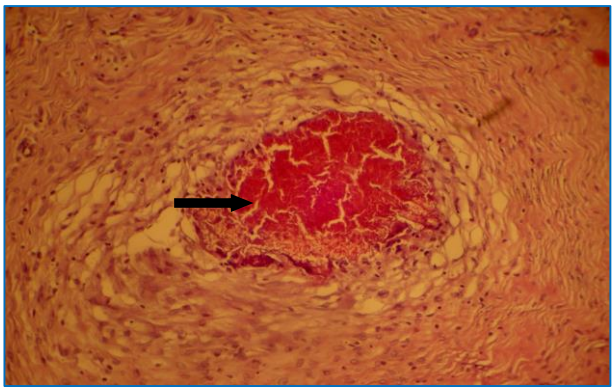
Figure, 6: G2: Liver of rabbit at 30 days post infection show marked interlobular fibrosis ( ) with vacuolar degeneration of hepatocytes ( ) (H and E 40x).



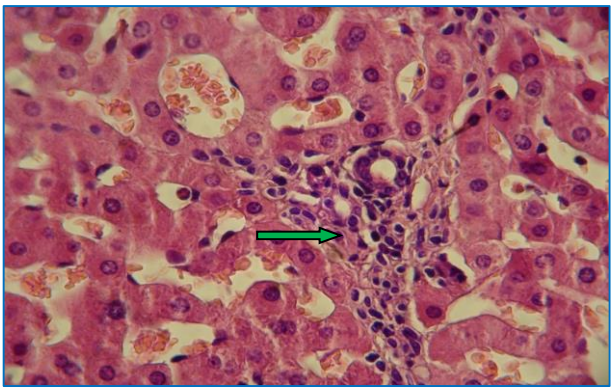
Figure, 7: G2: Liver of rabbit at 30 days post infection show deposition of bile pigment ( ) (H and E 40x).



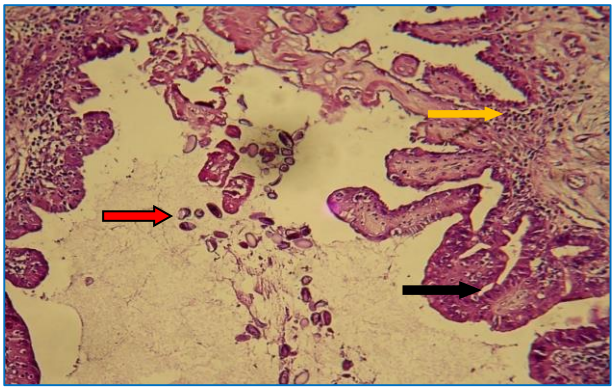
Figure, 8: G4:-Liver of rabbit at 10 days post infection show increase in the numbers of binucleated hepatocytes ( ) (H and E 40X).



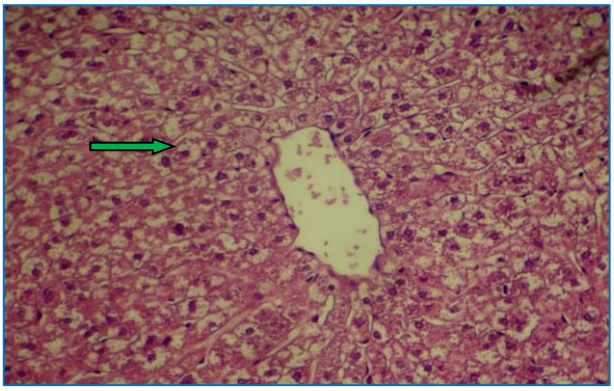
Figure, 9: G6: Liver of rabbit at 20 days post infection show foreign body granuloma contain the destructed parasite in the center ( ) (H and E 10x).



Figure, 10: G6: Liver of rabbits at 20 days post infection show slight infiltration of mononuclear cells in the portal areas ( ) (H and E 40x).



Figure, 11: G4: Liver of rabbit at 30 days post infection showing bile duct contain few oocysts in the lumen ( ) (H and E) with moderate hyperplasia ( ) and mild infiltration of mononuclear cells in the lamina properia of mucosa ( ) (H and E 10x).



Figure, 12: G6: Liver of rabbits at 30 days post infection showing restoration of hepatic tissue to nearly normal picture with hydropic degeneration of hepatocytes ( ) (H and E 40x).

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التأثير العلاجي للمستحلب الزيتي لبذور الحبة السوداء على الأرانب المصابة تجريبا بداء الاكريات الكبد

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الخلاصة

هدفت الدراسة الحالية إلى معرفة تأثير المستحلب الزيتي لبذور الحبة السوداء (*Nigella sativa*) كعلاج لاكريات الأرانب المتسبب عن طفيلي *Eimeria stiedae* بعد عزل العترة المحلية لطفيلي *E. stiedae* من *أكياس الصفراء المخمجة*تم استخدام 90 أرنب ذكر من سلالة محلية تتراوح أعمارها بين|(6-8 أسابيع وأوزانها بين (750 -1000 غم) حيث قسمت إلى 6 مجاميع متساوية من الحيوانات.المجموعة الأولى: غير مصابة وغير معالجة عدت مجموعة سيطرة.المجموعة الثانية: مصابة وغير معالجة حيث جرعت ب 10,000ألف كيس بيضه ناضج لطفيلي *E. stiedae*. المجموعة الثالثة: غير مصابة وجرعت ب 200 ملغم/كغم من وزن الجسم لمستحلب الزيت من بذور الحبة السوداء *N. sativa*. المجموعة الرابعة: مصابة ب 10,000 ألف كيس بيضه ناضج لطفيلي *E. stiedae* وجرعت بـ 200 مغلم/كغم من وزن الجسم بالمستحلب الزيتي لبذور الحبة السوداء *N. sativa*. بعد 16 يوم من الإصابة. المجموعة الخامسة: غير مصابة وجرعت ب 400 ملغم/كغم من وزن الجسم بالمستحلب الزيتي لبذور الحبة السوداء *N. sativa*. المجموعة السادسة: مصابة وجرعت ب 400 ملغم/كغم من وزن الجسم لمستحلب بذور الحبة السوداء *N. sativa*. تم فحص نماذج البراز لغرض عد أكياس البيض المطروحة في البراز بعد 16 يوم من الإصابة .ثم تم أجراء الصفة التشريحية ل 5 حيوانات من كل مجموعة وأخذت عينات أكبادها وأرسلت للفحص النسيجي عند كل فترة من فترات التجربة (10 و20 و30 يوم). اظهر الخمج التجريبي تغيرات مرضية ضارة في الأكباد المصابة. أظهرت النتائج أن كلا الجرعتين المعالجة ل *N. sativa* كان لها تأثيرات معتدة انعكست على قلة معدل طرح أكياس البيض، مع تحسن متميز في التشريح المرضي النسجي للأكباد، هذا التحسن شمل أعادة الهيكل الكبدي إلى الحالة الطبيعية وزيادة في إعداد الخلايا ذات النواتين مع اختفاء النزوفات بين الفصيصات الكبدية وقلة ملحوظة في تليف النسيج وأعداد الخلايا الالتهابية في متن الكبد وفي الباحات البابية مع تكوين أورام حبيبية ذات الجسم الغريب وقلة إعداد المراحل المختلفة من تطور الطفيلي وأكياس البيض في القنوات الصفراوية. أظهرت النتائج الحالية إن التغيرات المذكورة في أعلاه كانت أسرع في الحيوانات المعالجة بجرعة 400 ملغم/كغم من وزن الجسم بالمستحلب الزيتي لبذور الحبة السوداء.عن الحيوانات المعالجة بـ 200 ملغم/كغم من وزن الجسم بالمستحلب الزيتي لبذور الحبة السوداء.وبدون وجود أي تأثيرات جانبية على الحيوانات. لقد تبين من النتائج الحالية إن استعمال المستحلب الزيتي لبذور الحبة السوداء آمن وبدون ظهور تأثيرات جانبية وان جرعة المستحلب الزيتي 400 ملغم/كغم من وزن الجسم أكثر تأثيرا على الخمج ب *E. stiedae* من الجرعة 200 ملغم/كغم من وزن الجسم وبذالك يمكن استخدامها للأغراض السريرية كعلاج مساعد.

الكلمات المفتاحية: الحبة السوداء، داء الاكريات الكبدي، الكبد، الارانب.