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Control of Hepatic Coccidiosis in Rabbits Using *Calendula micrantha officinalis* and *Peganum harmala* Extracts

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CONTROL of hepatic coccidiosis in rabbits using two plant extracts (*Calendula micrantha officinalis* and *Peganum harmala)* was investigated.

The LD_{50} values of the two plant extracts against *Eimeria stiedae* oocyst viability *in vitro* were 31ppm for *C.m.officinalis* extract and 173ppm for *P.harmala* extract. The lethal effect of *C.m.officinalis* extract, which proved its potency in controlling *E.stiedae* oocysts as judged by LD_{50}, was probed by experimental infection of rabbits with *C.m.officinalis* treated oocyts. Occyst count and score lesions were considered as strong indications of plant extract efficacy in controlling hepatic coccidiosis. The curative effect of *C.m.officinalis* extract on hepatic coccidiosis was investigated by treating rabbits with a dose of 30ppm/ kg body weight.

The present study indicated that, this plant extract could be effective as a new biological control agent of hepatic coccidial oocysts.

Coccidiosis is recognized as the parasitic disease that has the greatest economic impact on poultry production. The world wide cost is estimated at about $ 800 million (Williams, 1998). Efforts to control coccidiosis have long been the objective of research.

Hepatic coccidiosis is caused by *Eimeria stiedae* (Lindemann, 1865). It causes a high losses in rabbits by mortality and affecting adversily in weight gain or feed conversion (Eckert *et al.*, 1995).

Anticoccidial drugs were used by many workers for controlling hepatic coccidiosis in rabbits. They reported that the failure of such program may be due to the presence of parasite resistance to the drug (Hassanian *et al.*, 1997). Also, infectious drug resistance possibility, constitute potential health hazard for human beings from using sulphonamides and other chemotherapy in controlling hepatic coccidiosis in rabbits (Kutkat *et al.*, 1998).

To avoid such hazards, alternative control including plant extracts was used. The botanical extracts were used, for a wide range, as insecticides (Thoresell *et al.,* 1970; Gayar and El Shazly, 1986; Mohamed *et al.,* 1996 and Mamdouh *et al.,* 1997), as antihelmintic (Hassanian *et al.,* 1991; Abdel-El-Rahman *et al.,* 1998 and Hassan, 2004) as well as molluscicides (Hostattmann and Marston, 1985; El-Emam *et al.,* 1986 and 1996; Motawe 1993; Shoeb *et al.,* 1994 and Abd El-Megeed, 1999).

The botanical extract were used for a limited extent in coccidiosis control. Artemisinin, isolated from *Artemisia annua,* has been found effective in reducing oocyst output from both *E.acervulina* and *E.tenella* infections when fed at levels of 8.5 and 17 ppm in starter diets (Allen *et al.,* 1997). Recently, extracts from 15 Asian herbs were tested for anticoccdial activity against *E.tenella.* Of the plant species tested, extract from *Sophora flavescens* was the most effective in reducing oocyst production (Youn and Noh, 2001; El Abasy *et al.,* 2003) suggested that sugar cane extract has immunostimulating and protective effects against *E.tenella* infection in chicken. Beta-glucan, extracted from Oat enhanced the resistance to *E.vermiformis* infection in mice (Yun *et al.,* 2003 ; Guo *et al.* 2005) investigated the effect of polysaccharide extract of two mush- rooms, *lentinus edodesand* and *Tremella fuciformis* and a herb, *Astragalus membranceus on Eimeria tenella* oocysts. Of the three extracts, both *lentinus edodesand* and *Astragalus membranceus* fed groups showed lower cecal oocysts output and the polysaccharide extracts may prove useful effect against avian coccidiosis particularly when they are used in conjunction with live oocysts.Artemisinin, isolated from *Artemisia annua,*has been found effective in reducing oocyst output from both *E.acervulina* and *E.tenella* infections when fed at levels of 8.5 and 17 ppm in starter diets (Allen *et al.,* 1997).

The present study aimed to investigate, for the first time, the effects of *Calendula micrantha officinalis* and *Peganum harmala* on *Eimeria stiedae in vitro* . Also, study coccidial properties of *C.m.officinalis* extract on the infectivity of *E.stiedae* oocysts in rabbits and evaluate the therapeutic effect of this plant species, which is commonly cultivated in Egypt (Tackholm, 1974) and used in medicine (Bailay, 1963).

This investigation considers as a trial for biological control of coccidiosis in a safe and non expensive manner. This trial can be help in development of control schemes for protozoa.

Materials and Methods

Preparation of E stiedae sporulated oocysts

The oocysts of *E.stiedae* were collected from gall bladders and necrotic hepatic lesions of naturally infected rabbits. The livers and gall bladders were removed, minced and digested in 0.25% trypsin in normal saline. The digested material were sieved, centrifuged at 2000 rpm for 10 minutes and washed several

times by saline solution. The oocysts were counted as the method described by (Ryley *et al.,* 1976). The oocyst were identified according to Levine (1985).The oocysts were incubated in 2.5% potassium dichromate dilution for 3 days at 28°C. The sporulated oocysts kept at 4°C until use.

Preparation of plant extracts

a- Preparation of C.m.officinalis extract

Leaves of *C.m. officinalis* were collected from campus yard at faculty of Agriculture, Cairo University. Leaves were ground by an electrical mixer. Then 100 gms leaves were added to one liter of ethanol. The mixture was left for about 48 hrs. at room temperature. Occasional shaking of mixture was carried out to get the maximum extraction. The extract was dried under reduced pressure using rotavapour till complete dryness.

b- Preparation of Peganum harmala extract

Peganum harmala seeds were crushed by an electrical mixer. Then 100 gms of crushed seeds were soaked in one litre of ethanol for about 48 hrs. at room temperature with occasional shaking. The extract was dried under reduced pressure using rotavapour till complete dryness.

In vitro exposure of E.stiedae oocysts to different concentrations of C.m. officinalis and P. harmala

E. stiedae oocysts were collected from gall bladders and necrotic hepatic lesions of infected rabbits. Four concentrations, 25, 50, 100and 200 ppm, of each plant extract *(C.m. officinalis and P. harmala)* were added to three replicates of oocyst group each, of one thousand oocysts. Oocysts were mixed and incubated at 28°C with plant extracts for 5 days and then examined under microscope to investigate development of oocyst sporulation.

Experimental infection of rabbits with C.m.officinalis treated oocysts

15 Newzealand white rabbits (5 weeks old) proved coccidia free, were used in this study. The animals were reared in metal wire floored cages. Faecal samples from all animals were examined daily for 2 successive weeks to confirm that animals were free from coccidial oocysts. The rabbits were divided into 3 groups, 5 rabbits in each. Each rabbit in the first group was experimentally infected with 50000 sporulated oocysts previously treated with 30 ppm *C.m.officinalis* , the third group was left as non infected control while each rabbit in the second group was infected with 50000 non treated sporulated oocysts as positive control group. Faecal samples were collected for determination the number of *E.stiedae* oocysts per gram by modified Mc Master technique for two weeks (Long *et al.,* 1976). At the end of observation period all rabbits were sacrificed for detecting the post mortem lesions.

Postmortem lesions

Focal lesions in livers were scored for severity from 0 to 4 for one replicate of 5 rabbits (Kutkat *et al.* 1998). It was recorded 0 (negative), 1 (1-2 focal lesions), 2 (3-5 focal lesions), 3 (6-8 focal lesions) and 4 (more than 8 focal lesions in liver).

The percentages of protection against lesions were calculated by using the formula described by Singh and Gill (1976). The percentages of protection were calculated by substracting average lesion score of the group from maximum expected score dividing the resultant figure by 4 and multiplying it by 100.

Treatment of hepatic coccidiosis infected rabbits with C.m. officinalis.

10 Newzealand white rabbits (4-5 week old) were infected with sporulated hepatic coccidial oocysts and divided into two groups Each rabbit in the first group was treated with 30 ppm/Kg body weight *C.m.officinalis* extract. While second group kept as control non-treated infected rabbits. Oocysts were collected from the feces of the two groups and counted by Mc master counting chamber (Long *et al.*, 1976).

Results

In vitro effect of *C.m.officinalis* and *P.harmala* on sporulation of *E.stiedae oocysts.*

The effect of ethanol plant extracts on the sporulation of *E stiedae* oocysts was probed by, *in vitro*, incubating oocysts with four different concentrations (25, 50, 100 and 200 ppm/1000 oocysts) of each of the plant extracts (*C.m officinalis* and *P.harmala*). The effect was examined under microscope by detecting and counting sporulated (Fig. 1) and degenerated oocysts (Fig. 2).

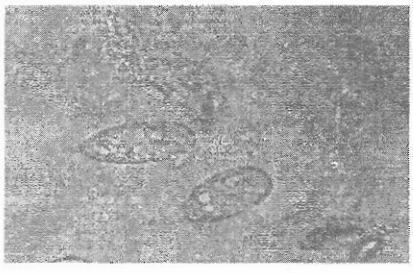

Fig. 1. Normal sporulated *E.stiedae* oocysts.

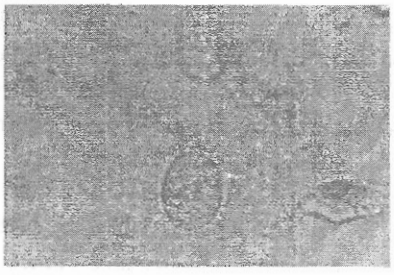

Fig. 2. Degenerated *E.stiedae* oocyst

As shown in Table (1) 200 ppm of *C.m.officinialis* extract exhibited a drastic effect on oocysts sporulation where only 9% of the total of 3000 oocysts were sporulated compared to nontreated control oocysts which exhibited 90% sporulation with LD_{50} 31ppm. This effect was also confirmed by the observed percentage of degenerated oocysts, at the same concentration, which reached 85% compared to only 1% of the control.

As depicted in Table 1 *P.harmala* extract showed lower effect in controlling sporulation than *C.m.officinalis* extract where the concentration of 200 ppm resulted in 50% sporulation with LD_{50} 173 ppm.

TABLE 1. Sporulation percentages of *Eimeria stiedae* oocysts treated with *Calendula micrantha officinalis* and *Peganum harmala* extracts.

Treatment / Oocysts	Control	*Calendula micrantha officinalis*					*Peganum harmala*				
		25	50	100	200	LD_{50}	25	50	100	200	LD_{50}
Sporulated oocysts	90%	57	35	20	9	31	81	73	57	50	173
Degenerated oocysts	1%	17	40	62	85		7	11	27	30	
Incomplete sporulated oocysts	9%	26	25	18	6		12	16	16	20	

Ability of C.m. officinalis treated oocysts in inducing coccidiosis in rabbits.

As shown in Table 2 oocyst count of rabbits infected with *C.m.officinalis* treated oocysts decreased from twenty day until twenty seven day post infection (22560 to be 3200) and then the count remained nearly constant up to 4 weeks post infection among the treated group.While, oocyst count of control rabbits infected with untreated oocysts reached to 107600 at twenty seven day. No oocyst were detected in non infected rabbits.

Lesion score.

Lesion score was used as a parameter of infections severity (Fig. 3) . It was found that number of focal lesions, in liver ranged between 0-2 in group 1 (rabbits infected with *C.m.officinalis* treated oocyst); 7-17 in infected non treated group (Table 3). It was clear that, rabbits infected with oocysts treated with *C.m.officinalis* extract showed high level of protection against lesion score compared to the protection percentage of rabbits infected with normal oocysts (Table 3). Hence, non infected rabbits exhibited no gross lesion.

TABLE 2. Mean oocysts count collected from rabbits experimentally infected with *C.m.officinalis* treated oocysts .

Groups / Day post infection	Mean oocysts count /gm		
	G_1 treated	G_2 control +ve	G_3 control –ve
14 th	-	-	-
15 th	-	-	-
16 th	3100	56130	-
17 th	4750	74050	-
18 th	4450	148160	-
19 th	19580	131720	-
20 th	22560	227130	-
21 th	21300	218360	-
22 th	15930	251750	-
23 th	12160	154260	-
24 th	8700	139260	-
25 th	8100	120500	-
26 th	3730	104070	-
27 th	3200	107600	-

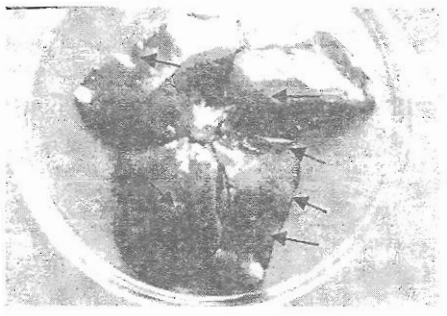

Fig. 3. Focal lesions in rabbit liver infected with E. stecdae oocysts.

TABLE 3. Lesion score of rabbits experimentally infected with *C.m.officinalis* treated oocysts.

Parameters / Groups	Mean lesion score	% protection
1st group infected with *C.m.officinalis* treated oocysts	1	75
2nd group control positive	3.6	10
3rd group control negative	0	100

Effect of C.m. officinalis extract on hepatic coccidiosis

Therapuetic effect of *C.m.officinalis* on hepatic coccidiosis was probed by treating infected rabbits with concentration of 30ppm/kg body weight. Oocyst count was used as a parameter for the healing effect.

Concentration of 30 ppm/kg rabbit body weight of *C.m.officinialis* extract produced mean oocyst count of 4660 in treating rabbits group compared to the mean oocyst count of control non treated rabbits 251750.

Discussion

Recently control of many infectious diseases threating poultry industry directed to use biological materials such as bacteria (Tawfik *et al.*, 1994 and Hassanain *et al.*, 1997), enzymes (Charles, 1993) and plant extract (Allen *et al.*, 1997, Youn and Noh 2001; El Abasy *et al.* 2003 and Yun *et al.*, 2003).

In our approach, the leathal effect of the botanical extracts on hepatic coccidiosis oocysts in rabbits, has been investigated *in vitro* and *in vivo* studies. Treatment of *E.stiedae* oocysts *in vitro* with different concentrations, 25, 50, 100 and 200 ppm, of both *C.m.officinalis* and *P.harmala* extracts revealed that damaging effect of *C.m.officinialis* extract was higher than *P.harmala* extract. This effect probably resemble that of *C.m. officinalis* against nematode worms *in vitro* (Hassanian *et al.*, 1991).Moreover treatment of *Moniezia expansa* worms *in vitro* using *P.harmala* and *Artemisia cina* revealed a successful results with *A. cina* rather than *P harmala* (Hassan, 2004). In this aspect, other plant extracts were used as antihelminthic *in vitro*, *Allium sativums* and *Nerium oleander* (Abd- El Rahman *et al.*,1998), *Flemingia vestita* (Tandon *et al.*, 1997).

C.m.officinalis has a marked effect on oocyst viability, it also affected the ability of oocyst to induce coccidiosis. *C.m.officinalis* extract which showed higher activity against sporulation process. It has been destroyed oocyst infectivity as judged by oocyst count and score lesion of infected rabbits. Oocyst count and score lesion recorded from rabbits experimently infected with *C.m.officinialis* treated oocysts were considered as a strong indication of the effectiveness of this plant extract. Both oocyst count and score lesion showed significant decreased level compared to control positive rabbits. Similar data was obtained by previous attempt of Youn and Noh (2001) when they studied the efficacy of extracts from 15 different herbs on chickens infected with *E.tenella* oocyst, they indicated that herb extract of *Sophora flavescens* was the most effective herb in decreasing level of score lesion and oocyst count. Also Giannenas *et al.*, (2003), declearted that Oregano essential oil exerted an anticoccidial effect against *E.tenella*. This effect was judjed by score lesions and oocysts excretion.

The important facet of the present study was to investigate *in vivo* the therapeutic effect of *C.m.officinalis* extract on rabbits hepatic coccidiosis. A dose of 30ppm/per kg body weight of *C.m.officinalis* was administered to infected rabbits. *C.m.officinalis* showed curative effect based on recovered oocyst count. Our results were coincided with those of Youn and Noh, (2001), who proved the therapeutic effects of the plant extract, *S.flavescens,* relied on the oocyst out put.

Our observation proved that *C.m.officinalis*, for the first time,may be used as a biological control agent against *E.stiedae* oocysts. Further studies are needed to isolate the different effective fractions of *C.m.officinalis* and evaluate its curative effect on other protozoal agents.

References

Abdel Megeed, Kadria N. (1999) Studies on the molluscicidal activity of *Calendula micrantha officinalis* (compositae) on fascioliasis transmitting snails. *J. Egypt. Soc. Parasitol.*, **29** (1), 183.

Abdel Rahman, Eman H., Kandil, Omnia M. and Abdel Megeed, Kadria N. (1998) Comparative studies of lethal effects of *Bacillus thuringiensis, Allium sativum* and *Nerium oleander* on trichostrongylidae parasites. *Egypt. J. Zool.*, **30**, 65.

Allen, P.C., Lydon, J. and Danforth. H.D. (1997) Effect of components of *Artemisia annua* on coccidia infections in chickens *Poult. Sci.*, **76**, 1156.

Bailey, I.H. (1963) Standard Gylopedia of Horticulture. The MacMillan Company, New York. Vol. I.

Charles, D.R. (1993) Air quality problems in poultry production: Ammonia, temperature and performance. Biotechnology: Recent advance in reducing environmental pollution while improving performance. 7th Annual European lecture, Toure., P. 10-20.

Eckert, J., Braun, R., Shirley, M.W. and Coudert, T. (1995) Biotechnology : Guidelines on techniques in coccidiosis research. Ecsc-Ec-EAEC, Brussels, Luxembourg.

El- Abasy, M., Moobu, M., Na, K.J. and Shimura, K. (2003) Protective effects of sugar cane extract (SCE) on *Eimeria tenella* infection in chickens. *J. Vet., Med. Sci.*, **65**. 865.

El-Emam, M.A., El-Amin, S.M., El-Sayed, M.M. and El-Nahas, H.A. (1996) Field trials to control the snail vectors of Schistosomiais and Fascioliasis by the plant, *Anagallis arvensis* (Primulaceae). *Egypt. J. Bilh.*, **18**, 89.

El-Emam, M.A., Shoeb, H.A., Ebid, F.A. and Refai, L.A. (1986) Snail control by *Calendula micrantha officinalis. J. Egypt. Soc. Parasitol.*, **16** (2), 563.

Gayar, F. and El-Shazly, A. (1985) Toxicity of certain plants to *Culex pipiens* larvae. *Bull. Soc. Entomol. Egypt.*, **52**, 467.

Giannenas, I., Florou Paneri, P., Papazahariadou, M., Christaki, E. (2003) Effect of dietary supplementation wit oregano essential oil on performance of broilers after experimental infection with Eimeria tenella. *Arch Tierernahr.*, **57** (2), 99.

Guo, F.C., Kwakkel, R.P., Williams,C.B., Suo, X., Li, W.K., Verstegen, M.W. (2005) Coccidiosis immunization : effect of mushroom and herb poysaccharides on immune response of chickens infected with *Eimeria tenella* . Avian Dis., **49** (1), 70 .

Hassan, E. Soad (2004) Ultrastructural and immunological studies of cestodes infecting sheep in Egypt. Ph.D. Thesis, Faculty of Science, Cairo University.

Hassanian, M.A., Abdel-Rahman, Eman, H, Abuel Ezz, Nadia, T. and Abdel-Aziz, M.M. (1997) Lethal effect of *Bacillus thuringiensis* preparation on cecal coccidiosis in ckickens. *J. Egypt. Ger. Soc. Zool.*, **24** (D), 85.

Hassanian, M.A., El Askalany, M.A. and Salwa, M. Rasahad. (1991) Anthelmintic activity of a wild Egyptian plant *Calendula micrantha officinalis. Vet. Md. Seni-suef*, (1), **65**, 71.

Hostettmann, K. and Marston, A. (1985) Review article No. 6. *Plant molluscicides Phytochemistry*, **24**, 39.

Kutkat, M.A., Zayed, A.A. and Nadia, M.T.N. Abouel Ezz (1998) A trial for immunization of rabbit against hepatic coccidiosis. *Zag. Vet. J.*, **26** (2), 70.

Levine, N.D. (1985) Veterinary protozoology. Iowa State University Press, Ames.

Lindemann, K. (1865) Weiteres uber gregainen. *Bull. Soc. Imp. Nat. Masceu*, **38**, 381

Long, P.L., Joyner, L.P., Millered, B.J. and Norton, C.C. (1976) A guide to laboratory techniques used in the study and diagnosis of avian coccidiosis. Folia Vet. Lat., **6**, 201.

Mamdouh, I., Nassar, O., Kandil, M. and Hassanian, M.A. (1997) The potentiality of *Sorghum bicolor* and *Nerium oleander* extracts in the control of the grey flesh fly *Parasarcophage argyrostroma. J. Egypt. Ger. Soc. Zool.*, **22** (E), 235.

Mohamed, M., El Shalzy, A., Mamdouh, I., Nassar, O. and El-Sherief, H.A. (1995) Toxic effect of ethanolic extract of *Nerium oleander* (Apocynaceae) leaves against different developmental stages of *Muscina stabulant* (Diptera-Muscidae) *J. Egypt. Soc. Parasitol.*, **26** (2), 461.

Motawe, H.M. (1993) Effect of some plant saponins on *Biomphalaria alexandrina* snails. *Egypt. J. Bilh.*, **15** (1-2): 189.

Ryley, J.F., Meade, R., Hazalhurst, J. and Robinson, T.E. (1976) Method in coccidiosis research: Separation of oocysts from feces. *Parasitol.*, **73**, 311.

Shoeb, H.A., Refahy, L.A., Saad, A.M. and Abd El-Motagaly, M.M. (1994) Screening of some dry powdered plants against *Biomphalaria alexandrina* snails. *Egypt. J. Bilh.*, **16** (1-2), 189.

Singh, G.J.P. and Gill, S.S. (1976) Activity of Amprolium against different of *Eimerta necatrix* infection and effect of medication on development of immunity. *Rev. Parasit.*, **37**, 63, 63.

Tackholm, V. (1974) Students, flora of Egypt. Cairo University Press.

Tandon, V., Pal P., Roy, B., Rao H.S.P. and Reddy K.S (1997) *In vitro* anthelmintic activity of root-tuber extract of *Flemingia vestita*, an indigenous plant in Shillong, *India. Parasitol Res.*, **38**, 492.

Tawfik, M. Adel, A., Nagwa, A. Shalaby and Hegazi, A.G. (1994) Biotechnical measures for controlling caecal coccidiosis in chickens. *J. of Egyp. Ger. Soc. of Zool.*, **14** (d),41.

Thoresell, W., Mikiver, A. and Malam, E. (1970) Plant extracts as protectants against disease-causing insects. *Entomol. Tidsker.*, **100**, 138.

Williams, R.B. (1998) Epidemiological aspects of the use of live anticoccidial vaccines for chickens. *Int. J. Parasitol.*, **28**, 1089.

Youn, H.J. and Noh, J.W. (2001) Screening of the anticoccidial effects of herb extracts against *Eimeria tenella. Vet. Parasitol*, **96**, 257.

Yun, C.H., Estrada, A., Van Kessel, A. and Park, B.C. (2003) Beta-glucan, extracted from oat, enhances disease resistance against bacterial and parasitic infection. FEMS *Immunol Med Microbiol.*, **35**, 67.

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مقاومة مرض الكوكسيديا الكبدية في الأرانب باستخدام مستخلص نبات الأقحوان ومستخلص نبات الحرمل

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تم في هذا البحث دراسة تأثير نوعين من المستخلصات النباتية { مستخلص نبات الكلندبولا ميكرنثا أوفيشينالس (الأقحوان) ومستخلص نبات بيجنم هرملا (الحرمل) } على حويصلات الايمريا استيدي وذلك بغرض مقاومة مرض الكوكسيديا الكبدية في الأرانب . وأثبتت الدراسة أن الجرعة المعملية التي تسبب ٥٠ % وفيات هي ٣١ مليجرام / لتر بالنسبة لمستخلص نبات الأقحوان و١٧٣ مليجرام / لتر بالنسبة لمستخلص نبات الحرمل .

وقد تم إجراء عدوى صناعية للأرانب باستخدام الحويصلات الناضجة للايمريا استيدي المعاملة بمستخلص نبات الأقحوان لدراسة التأثير المميت للمستخلص على عدد حويصلات الايمريا استيدي في براز الأرنب وكذلك على عدد الإصابات الضارة التي ظهرت في الكبد على شكل درنات (عقد) بيضاء اللون كمقياس لتقيم كفاءة هذا المستخلص للقضاء على مرض الكوكسيديا الكبدية في الأرانب .

كما تم دراسة التأثير العلاجي لمستخلص نبات الأقحوان على مرض الكوكسيديا الكبدية وذلك بمعالجة الأرانب المصابة بهذا المرض بجرعة ٣٠ مليجرام / كيلوجرام ولقد اتضح من هذه الدراسة أن مستخلص نبات الأقحوان يمكن أن يستخدم كعامل من عوامل المكافحة البيولوجية لمقاومة مرض الكوكسيديا الكبدية في الأرانب .