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Original Article Chenopodium ambrosioides oil extract as broad spectrum Parasiticides

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Abstract:

Development a novel non-chemical approach that decrease the need for drug treatment, considered to be good realistic strategy to avoid the problem of drug resistance specially with using of biodegradable eco-friendly plant extracts.

Chenopodium ambrosioides (C. ambrosioides) is a weedy herb of the genus Chenopodium commonly known as Chenopodium oil. Its oil extract (rich by Ascaridole) has sedative, antifungal and pain reliving properties with documented anti-parasitic effects. In the present study the efficacy of different concentrations of chenopodium oil extract was evaluated as parasiticides versus Cryptosporidium parvum (C.parvum), Eimeria spp. oocysts and Hymenolepis diminuta (H.diminuta) in vitro and in vivo after induction of experimental infection in chicks and mice. The LD100 of the diluted extract was 200 ppm after 12 hours(h) versus C.parvum oocysts as the exposed stages failed to infect the mice after oral inoculation. The zygotes of Eimeria spp. oocysts were failed to sporulate after exposure to 200 ppm after 24h. The dose of 300 ppm succeeded to stop movement of *H.diminuta* onchospheres in the exposed eggs when examined under the microscope after 48h exposure time.

Per mouth administration of upgrade dose level of *C. ambrosioides* oil extract for 3-5 successive days induced marked reduction in the mean number of *C.parvum* and *Eimeria* spp. oocysts in experimental infected mice and two week old chicks. The reduction in diagnostic stages was increased with increasing the dose and replications. The oral dose of 112 mg/kg for 3 successive days was inducing 50% reduction (LD50) in the previously counted *C.parvum* oocysts infected mice while complete disappearance of the oocysts (LD100) was recorded after administration of 160 mg/kg for the same period. Higher dose as 180 mg/kg for 3 successive days were calculated as LD50 versus *Eimeria* spp. in experimentally infected chicks, while its LD100 was 250 mg/kg for 3 successive days also.

Complete eradication of the target parasites was recorded in control groups treated by amprolium 0.024% in drinking water for 3 days for chicks and after ingestion to 0.5 ml-1ml daily for 3 successive days in mice. Increasing the dose up to 400mg/kg for 5 successive days failed in expulsion or decreasing of the number of *H.diminuta* worms or eggs. No changes in the number of shed eggs or oocysts or in its vitality in control non exposed stages during all experiments.

In all cases no toxicity symptom was observed on the treated chicks and mice using the previous doses replications. Extraction of the active materials from this plant (Ascaridole) will be sharply reducing the previously recorded lethal doses versus these parasites in the future.

Key words: Chenopodium ambrosioides, Cryptosporidium, Eimeria, Hymenolepis in vitro - in vivo.

INTRODUCTION

Development a novel nonchemical approach that decrease the need for drug treatment, considered to be good realistic strategy to avoid the problem of drug resistance specially with using of biodegradable eco-friendly plant extracts.

Cryptosporidium is low host specific parasite, causes diarrhea in infected livestock and in human specially those of young age. The infective oocysts may be transmitted directly by the fecal-oral route, through contaminated water and raw food. The oocysts are commonly found on dairy farms and may be transmitted to humans through contaminated raw milk and dairy products (Minarovičová, et al., 2007).

Avian coccidiosis is characterized an infectious as protozoan disease caused bv aut parasites of the genus Eimeria. This disease annually causes a global loss of over 2.4 billion US dollars in the poultry industry, including poor growth performance, replacement of chicks, medication (Casta neda and and Gonz'alez, 2015). Year after year, new chemicals were characterized as the most specific anticoccidial drugs but after this it must change to overcome the resistant that develop against them.

Hymenolepis is a cestode parasites widely distributed among poultry and rodents. The worm cause severs losses as it interferes with the growth rate of the infected hosts. Among their several species all of them transmitted by arthropods intermediate hosts which facilitate their distribution to new individuals **(Solusby, 1986).**

In the absence of specific drug for treatment of cestode parasites in general as well as with the already knowledge about role of chemical preparations as а source of atmospheric pollutions as well as development of resistance the in treated hosts after several administration chemical druas of specially versus coccidian parasites, direction toward using of eco-friendly plant extract considered to be the approach prefers several advantages if applied for treatment of parasites. Chenopodium ambrosioides (*C*. ambrosioides) is a weedy herb of the genus Chenopodium commonly known as Chenopodium oil. Its oil extract (rich by Ascaridole) has sedative, antifungal and pain reliving properties with documented anti-parasitic effects (Patel, 2017). This plant was distributed in the Nile region including the Delta, Valley and Fayuom, the oases of the Western Desert and the entire Sinai Peninsula (Batanouny, 1999). From the previous facts, the present study aimed to evaluate the efficacy of C. ambrosioides oil extract as broad spectrum parasiticides in vitro and vivo on three selected parasites.

MATERIAL AND METHODS

1- Ethical approval:

All study steps and procedures were approved by the Institutional Animal Care and Use ethical Committee (IACUC) of Faculty of Veterinary Medicine-Cairo University.

2- Tested plant extract:

C. ambrosioides, oil extract was prepared Department in of Pharmacognosy, Faculty of Pharmacy, Cairo University, Egypt. Efficacy of the tested oil was evaluated using series of upgraded concentrations (100, 200, 300 and 400 part per million (ppm) each for (3 to 48) hours exposure time according to the nature of experiment. Each experimental concentration was set in triplicate. All experiments were conducted simultaneously at room temperature (26+2 °C), Control group in drug solvent was run with each experiment. According to Salama et al. (2012) 100 mg of the oil was dissolved in 1ml Tween 80. An amount of 99ml of distilled water was added. The solution contains 1per 1000 concentrate. This stock solution was used to prepare the required concentrations of the experiments by add of distilled water.

3-Tested Parasite:

a. *Eimeria spp.* oocysts were extracted from the ceci of natural infected freshly died chicken according to Amer et al, **2010**. The oocysts were cleaned by several washing and precipitation, then kept in 2.5% potassium-dichromate solution in refrigerator till use.

b. *Cryptosporidium* oocysts were collected from diahrric feces of naturally infected calves after examination by Modified Ziehl-Neelsen stain technique (Henriksen and Pohlenz 1981). The oocysts were harvested using concentration floatation technique. Oocysts in the surface layer were collected and cleaned bv several washing and sedimented by centrifugation at 3000 rpm for 5 minutes. *Cryptosporidium species* oocysts were measured 4.9 x 4.6 µm and identified as *C. parvum* according to **Khalil, 1993**.

Hymenolepis diminuata: After С. identification of natural infection by H. diminuta in mice in private farm in aborawash Giza. The required naturally infected mice were purchased for the experiments. Three mice were necropised to collect adult worms from its small intestine. Eggs obtained from its gravid segment and cleaned by sieving and kept for exposure according to Makki et al., 2011.

4- In vitro exposure:

Three 6 cm diameter Petridishes contained one layer of fresh cleaned C. *parvum* oocysts, *Eimeria* spp. unsporulated oocysts *and H*. *diminuta* eggs were used for each tested concentration and exposure time. At the end of the exposure time, tested solution was removed and the stages were cleaned by washed and sedimented several times

Determination of the oil efficacy post exposure:

The exposed *Eimeria* spp. were incubated at 28°C for 5-7days. Numbers of sporulated oocysts were counted in 100 oocysts in each time and percent of mortality was calculated (3 replicate).

For the exposed *C. parvum* oocysts, wet smears stained with carbol fuchsin were done to detect brilliance appearance of oocysts wall and

retractile residual body within the magnification oocysts under high power. One hundred of C. parvum oocysts was counted using the slide Neubour haemocytometer according to Kuczynkaniel (1999) and percent of mortality was calculated (3 replicate). Testing the infectivity of these oocysts after this technique was inoculation of mice done by in suspected cases

H. diminuta onchospheres movement of the exposed and control eggs were evaluated using sudden exposure to light using high power magnification. The number of dead eggs were counted in 100 inspected eggs and percent of mortality was calculated (3 replicate) in exposed and control dishes and the efficacy of the oil was calculated.

5- In vivo study:

a. efficacy of the oil versus *Eimeria* species

Used birds: chicks of 14 day age were obtained from a commercial hatchery. The chicks were weighed, divided into groups (3 chicks each) and housed on wire cages. All groups of birds were fed with standard ration and water adlibitum, they allocated into the following groups:

G (1): Control non-infected and non treated group (3 chicks).

- **G** (2): Control Infected with *Eimeria spp.* (5.000 sporulated oocyst/ bird) orally at 14th day of age and still without treatment (3 chicks).
- **G (3):** Infected as before then treated with chenopodium oil by day 5 pi and continued for 3 successive

days. (6 subgroups each of 3 chicks).

G (4): Infected as before and treated with Amprolium drinking water by 5 day post infection and continued for 3 successive days (3 chicks).

Feces of all birds were collected from second day post infection and continued for 7 successive days to count Oocysts per gram (OPG) using McMaster's technique according to **solusby, 1986.**

b. efficacy of the oil versus *C. parvum* oocysts

Used mice: mice with approximate body weight of 20-22 g albino-swiss mice were maintained under hygienic measures in the laboratory supported with dry food and water adlibitum. They allocated into the following groups:

G (1): Control non infected non treated group (2 mice).

G (2): Infected with 1 x 10 ⁶ oocysts /mouse (positive control) (2 mice).

G (3): infected with 1 x 10⁶ oocysts / mouse then treated by dose of amprolium for 3 successive days.

G (4): Infected with oocysts as mentioned before and treated with amprolium in drinking water by 2 dpi infection and continued for 3 successive days (2 mice).

Fecal samples of all mice were examined from the time of the 2^{nd} day to 7 day post inoculation. The diagnosed oocysts were counted as before. The pipette used for counting the white cells was filled with PBS as diluent and calculation was as follow: Number of oocysts / m m² = N x 10000. (N = number of oocysts in 16 small squares).

c. Testing the oil versus *H. diminuta*:

A number of 15 H. diminuta naturally infected mice were allocated in 5 groups, each group (3) four groups used for treatment and one left as control. Mice were inoculated orally by the following drug dose separately 100mg/kg, 200mg/kg, 300mg/kg and 400mg/kg. Feces of each mouse were examined and the present eggs were counted using McMaster's technique before treatment with oil then daily for The difference in EPG was 15 dpi. estimated at the day 15 then mice were scarified and the present worms were counted in both treated and non treated mice.

Efficacy of chenopodium oil in vivo was evaluated by Fecal Egg Count Reduction Test (FECRT) via count of eggs per gram (EPG) in feces according to **Kakar et al., 2013** using the following equation

FECR (%) = Pre Treatment EPG - Post Treatment EPG / Pre Treatment EPG x 100.

6- Reference chemicals:

Amprolium were used as reference material. Their concentration and exposure time was obtained according manufacturer to the guidelines. The drug was applied as 0.024% in drinking water for 3 days for chicks and after ingestion to 0.5 ml-1.0 ml daily for 3 successive days in mice.

RESULTS

Parasiticidal effect of C. ambrosioides oil extracts at different concentrations were evaluated versus C.parvum, *Eimeria* spp. oocysts and *H.diminuta* in vitro and in vivo after induction of experimental infection in chicks and mice. Concerning the in vitro effect of the extracts versus all stages of the exposed parasites in vitro, the data demonstrated that the Parasiticidal effect of the tested extracts was increased with increasing the concentration and the exposure time. The concentration of 100 ppm revealed gradual increasing in the % of mortality in the exposed C.parvum oocysts, reached to its maximum as 59.33% after 84 h exposure time. In the same time 100% mortalities, was recorded after exposure to 200ppm and 300 ppm for 12 and 6 h respectively. Oocysts exposed to these concentrations failed to infect the mice after oral inoculation (Table1, plate 1).

As described in table, plate (2), 50% mortalities in the exposed *Eimeria* spp oocysts was recorded after exposure to 100 ppm, and 200ppm after 24h and 6 h. respectively, while 100% mortalities was recorded in oocysts exposed to 200 ppm, 300 ppm and 400 ppm after 24h, 6h and 3 h respectively, as the zygots of these oocysts failed to sporulate after exposure to these concentrations.

Exposing of *H.diminuta* eggs in vitro revealed no movement for the exposed eggs onchospheres (100 % mortality) after exposure to dose of 300 ppm for 48h and also after exposure to 400 ppm for 18h (Table 3, plate 3). In the same time no mortalities were recorded in control non exposed stages during the same previous exposure time.

Per mouth administration of upgrade dose level of *C. ambrosioides* oil extract for 3-5 successive days induced marked reduction in the mean number of *C.parvum* and *Eimeria* spp. oocysts in experimental infected mice and two week old chicks.

The reduction in diagnostic stages was increased with increasing the dose and replications. The oral dose of 112 mg/kg for 3 successive days was inducing 50% reduction (LD_{50}) in the previously counted C.parvum oocysts infected mice while complete disappearance of the oocysts (LD_{100}) was recorded after administration of 160 mg/kg for the same period. Higher dose as 180 mg/kg for 3 successive days were calculated as LD₅₀ versus *Eimeria* spp. in experimentally infected chicks, while its LD₁₀₀ was 250 mg/kg for 3 successive days also. (Tables 4 & 5)

Complete eradication of the target parasites was recorded in control groups treated by amprolium 0.024% in drinking water for 3 days for chicks and after ingestion to 0.5 ml-1ml daily for 3 successive days in mice.

Concerning treatment of natural H.diminuta infection in mice. administration of oral doses from 100 to 400 mg/kg and following up the reduction in EPG in feces during 5-15day post infection revealed no marked reduction in number of

EPG H.diminuta that previously recorded from the same mice before treatment. Moreover, 400mg/kg for 5 successive days failed in expulsion or decreasing of the number of H.diminuta worms or eggs as three to five intact worms were extracted from the intestine of these mice at scarification of these mice at the end of the experiment (15d PI).

No changes in the number of shed eggs or oocysts or in its vitality in control non exposed stages as well as that exposed to the plant extract solvent during all experiments. materials Moreover no toxicity symptom was observed on the treated chicks and mice using the previous doses replications.

Discussion

Oil of C. ambrosioides has been used for many years in different parts of the world to treat parasite infections in animals and humans (Eguale and Giday 2009). Chenopodium oil is known for its anthelmintic property is mainly because of the which presence of ascaridole. It was one of the main antihelmintics used for the treatment of ascarids and hookworms in humans, dogs, pigs, horses and cats in the early 1900's. Ascaridole have sedative, pain-relieving properties and antifungal activity because of the presence of various types of monoterpenes (Patel, 2017).

Coccidiosis causes serious problems, such as malabsorption, dysentery and affected performance in broilers. Applying coccidiostat drugs and attenuated vaccines are the common approaches to prevent and control coccidiosis. The frequent use of anticoccidial medications leading to drug-resistant Eimeria strains, and the vaccines are expensive to produce (Abbas et al., 2011). Therefore, discovering new molecules as well as novel uses of the already available compounds especially those of known safety and without any side effects, considered to be the most promising the field advances in of drug development.

Parasiticidal efficacy of upgrade concentration of C. ambrosioides oil extracts were evaluated versus three selected parasitic stages include C.parvum, Eimeria spp. oocysts and H.diminuta in vitro and in vivo after induction of experimental infection in chicks and mice. The results show direct relation between the effective oil concentration and the thickness of the wall of the exposed stage. Thin wall stages such as that of C.parvum oocysts was broken at 200 ppm after 12 h. The same level of mortality was recorded versus Eimeria spp oocysts at 200 ppm but after 24h also due to special structure of *H.diminuta* eggs the recorded at LD 100 was high concentration (300 ppm) and after more prolonged exposure time as 48h. These results showing the effect of this oil as contact poisoning as it has special ability to kill these parasites, but this effect was related at the level of the present study to the structure of the exposed stages in vitro. On the contrary, Etewa and Abaza (2011) mentioned that Eimeria stiedae induced marked hepatic histopathological alterations in rabbits that were not treated with garlic, versus those treated with garlic.

Per mouth administration of upgrade dose level of *C. ambrosioides* oil extract for 3-5 successive days induced marked reduction in the mean number of *C.parvum* and *Eimeria* spp. oocysts in experimental infected mice and chicks.

The reduction in diagnostic stages was increased with increasing the dose and replications complete disappearance of the C.parvum oocysts (LD_{100}) was recorded after administration of 160 mg/kg for 3 successive days. Higher dose as 250 mg/kg for 3 successive days were calculated as LD₁₀₀ versus *Eimeria* spp. experimentally infected chicks. in Moreover, a the oral doses from 100 to 400 mg/kg and following up the reduction in H.diminuta EPG in feces during 5-15day post infection revealed no marked reduction in mean EPG that previously recorded from the same mice before treatment. There is no effect on the intact worms which present in the intestine of the treated mice at scarification. Moreover no toxicity symptom was observed on the treated chicks and mice using the previous doses replications. In the authors opinion successful treatment of these parasites in vivo with no detours effect on the general health condition of the infected chicken and mice may be considered as advantage for this plant extract and this may be related to that mentioned by Singh et al., 2011.

As a result of presence of materials such as carvacrol (isomer of thymol) this has antioxidant activities. Moreover in ability of high dose of the extract

(400mg/kg for 5 successive days) to eradicate the worms present in the intestine of mice may be due to the nature of this plant described by 1999). (Batanouny, As С. ambrosioides oil extract (rich by Ascaridole) has sedative effect valuable in pain reliving pain, so that appear as un-able to expel the tape worms that usually need contraction effects than relaxation

For conclusion. comparing the required dose used from this plant extract for eradication of these parasites with that of commercial drugs such as amprolium, the wide difference between them considered to be an interested matter. For this reason the authors advise separation of the effective molecules from С. ambrosioides crud extract and using them in purified form. This will prefer more reduction in the recorded doses. Moreover the authors and in agreement with Ghada et al.,2014 can accept her idea that suggest mixing of part from this plant extract with different percentage of some commercial drugs. By this way the reduction in the level of the calculated plant dose as well as production of new formulation from the commercial drugs were become available, the matter which reduce development of drug resistance versus the commercial drugs.

Table (1): Mean efficacy of Chenopodium oil versus C. parvun	oocysts in vitro
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Tested conc.	Mean mortality in <i>C. parvum</i> oocysts after exposure to						
Testeu conc.	3h	6h	9h	12h	18h	24h	48h
100ppm	-	9.33	9	30	44.33	50	59.33
200ppm	30.66	49	81.33	100	100	100	100
300ppm	70.33	100	100	100	100	100	100

Concentration before these concentrations did not induce any mortalities.

Tested conc.		Mean mortality in <i>Eimeria spp.</i> oocysts after exposed to						
rested conc.	3h	6h	9h	12h	18h	24h	48h	
100ppm	-	9.66	15	29	44.33	50	59.66	
200ppm	13.66	31	50	71.33	83.66	100	100	
300ppm	70	100	100	100	100	100	100	
400ppm	100	100	100	100	100	100	100	

* Concentration before these concentrations did not induce any mortalities.

Table (3): Mean efficacy of Chenopodium oil versus *H. diminuta eggs* in vitro

Tested conc.		Mean m	ortality in <i>I</i>	H. diminu	uta eggs a	after exposure to)
rested conc.	3h	6h	9h	12h	18h	24h	48h
100ppm	-	-	-	10	14.66	20	24.33
200ppm	-	9	19	24	29	44	71
300ppm	9.66	29.66	40.33	60	74.66	90	100
400ppm	49	69.66	79	89	100	100	100

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* Concentration before these concentrations did not induce any mortalities.

Tested doses (Mean number of oocysts shed during 3-5day PI						
mg/kg)	Treated oil group	Control positive	Efficacy %				
100	5500	7000	21.42 ±2.3				
150	4000	6500	38.46±3.6				
200	2100	7200	70.83±4.1				
250	0	6800	100				
300	0	7000	100				
350	0	5800	100				

Table (4): Efficacy of Chenopodium oil on Eimeria spp. infected chicks

Table (5): Efficacy of Chenopodium oil on C. parvum infected mice

Tested doses (Mean number of oocysts shed during 3-5day PI						
mg/kg)	Treated oil group	Control	Efficacy %				
80	13200	16000	17.5±1.3				
100	8000	15000	46.66±14.1				
120	7000	15500	54.83±16.55				
140	2000	15000	86.66±11.8				
160	0	15000	100				
180	0	15200	100				

Plate (1): efficacy of Chenopodium oil versus C. parvum oocysts in vitro

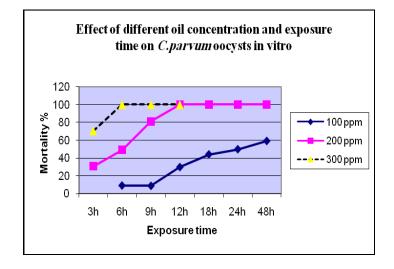


Plate (2): efficacy of Chenopodium oil versus Eimeria spp. oocysts in vitro

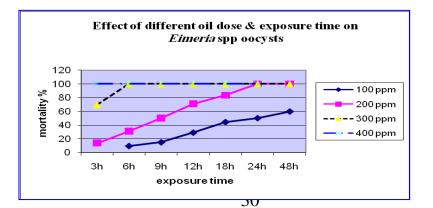
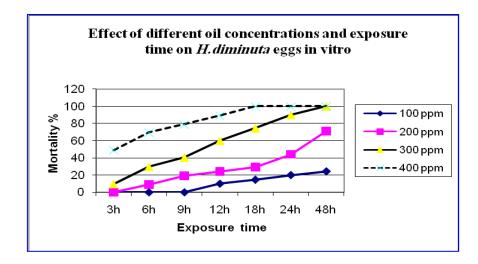


Plate (3): efficacy of Chenopodium oil versus H. diminuta eggs in vitro



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Soulsby, E. J. L. (1986): Helminthes, Arthropods and Protozoa of Domesticated Animals (7thedn) Bailliere Tindall London. مستخرج نبات الشينوبوديم الزيتى كمضاد طفيليات واسع الطيف مروة محمد عبد خليفة وليليان ناجى محروس وصباح حسن الجايد ليقسم الطفيليات - كلية الطب البيطرى- جامعة القاهرة قسم الطفيليات - كلية الطب البيطرى- جامعة بني سويف قسم العقاقير كلية الصيدلة جامعة القاهرة

الملخص

اصبح البحث عن بدائل غير كيميائية صديقة للبيئة لعلاج الطفيليات، من الاستر اتيجيات الحديثة المتبعة لتجنب المشاكل الناتجة عن استخدام الادوية الكيميائية في هذا المجال، يعتبر نبات الشينوبوديم المعروف باسم زيت الشينوبوديم، من النباتات العشبية الغنية بمركب الأسكاريدول الذي له خصائص مهدئة ومسكنة للالم علاوة على تأثيره المضاد للفطريات وبعض الطفيليات.

تم فى هذه الدراسة اختبار التأثير القاتل للمستخلص الزيتى لهذا النبات على بعض الطفيليات داخل وخارج اجسام عوائلها المعدية طبيعيا او تجريبيا، وقد اتضح ان لهذا المستخلص تأثير قاتل على هذه الطفيليات تناسب طرديا مع ازدياد التركيز المختبر وكذلك مدة التعريض.

اثبتت التجارب ان جرعة ٢٠٠ جزء في المليون قادرة على قتل جميع حويصلات الكريبتوسبوريديوم بارفم بعد تعريضها لمدة ١٢ساعة، حيث فشلت هذه الحويصلات في احداث العدوى في الفئر ان عن طريق الفم بعد تعريضها لهذه الجرعة. كذلك كانت نفس الجرعة ولكن مع زيادة مدة التعريض حتى ٢٤ ساعة، قادرة على قتل أجنة حويصلات الايميريا والتي لم تتمكن من النمو إلى ابواغ عند تتميتها بعد انتهاء فترة التعريض معمليا. وفي نفس الوقت أدت الجرعة حرعة المجرعة و المليون لمدة ٨٤ ساعة إلى حدوث انعدام لحركة أجنة بويضات الهيمنوليبيس ديمينوتا عند ملاحظتها تحت المجهر الصوئي.

وباختبار التأثير العلاجى لهذا المركب داخل أجسام العوائل المصابة، أدت الجرعة ١١٢ ملجم / كجم عن طريق الفم لمدة ٣ أيام متتالية وكذلك الجرعة ١٦٠ ملجم / كجم لنفس الفترة إلى أحداث انخفاض ٥٠% و ١٠٠% فى عدد حويصلات الكريبتوسبوريديوم الموجوده فى براز الفئران التى تم عدوتها تجريبيا على التتابع. كذلك ادت الجرعة ١٨٠ ملجم / كجم لمدة ٣ أيام فى مياه الشرب و الجرعة ٢٥٠ ملجم / كجم لنفس الفترة إلى أحداث فتص فى عدد حويصلات انواع الايميريا الموجودة فى براز الكتاكيت المعدية معمليا بنسبة ٥٠% و ١٠٠% على التتابع. فذلك ادت الجرعة ١٨٠ ملجم / كجم الايميريا الموجودة فى براز الكتاكيت المعدية معمليا بنسبة ٥٠% و ١٠٠% على التتابع. وفى المقابل لم نؤدى زيادة الجرعة حتى ٤٠٠ ملجم / كجم لمدة ٥ أيام متتالية عن طريق الفم، إلى طرد ديدان الهيمنوليبيس ديمينوتا من المعاء الفئران المصابة طبيعيا بهذا الطفيل.

ومع كل هذا المستوى من الجرعات ومعدلات اعطائها، لم تظهر اى اعراض سمية على العوائل المعالجة كذلك لم يكن هناك تغير فى أعداد الحويصلات والبويضات التى تم تشخيصها فى المجموعات الضوابط الغير معالجة خلال نفس وقت التجربة. وفيما يخص الضوابط الايجابية التى تم علاجها بدواء مرجعى، ادى استخدام أمبروليوم ٢٤. ٧٠ فى الكتاكيت وجرعة ٥. • مل ١- مل يوميا فى الفئران لمدة ٣ أيام الى الاختفاء التام للحويصلات من البراز هذه العوائل.

وباعتبار ان الجرعات السابقة تم تسجيلها باستخدام المستخلص الخام لهذا النبات، فانة يمكن توقع نقص كبير في مستويات هذه الجرعات مستقبلا بعد استخلاص المادة الفعالة (اسكاريدول) صناعيا من هذا النبات. *****