Chenopodium ambrosioides oil extract as broad spectrum Parasiticides

Marwa M. Khalifa¹, Lilian, N. Mahrous² and Sabah H. El-Gayed ³
¹ Department of Parasitology, Faculty Veterinary Medicine, Cairo University, Giza, Egypt.
² Department of Parasitology, Faculty Veterinary Medicine, Beni-Suef University, Egypt.
³ Department of Pharmacognosy, Faculty of Pharmacy, Cairo University, Egypt.

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 relieving 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 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.

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 as an infectious protozoan disease caused by gut 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, and medication (Castañeda and González, 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 severes 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 a source of atmospheric pollutions as well as development of resistance in the treated hosts after several administration of chemical drugs 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 in Department 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 ºC), 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 1 per 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 by 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.

c. *Hymenolepis diminuata*: After 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 Petri-dishes 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 28ºC 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 oocysts under high magnification power. One hundred of C. parvum oocysts was counted using the Neubour haemocytometer slide according to Kuczynkaniel (1999) and percent of mortality was calculated (3 replicate). Testing the infectivity of these oocysts after this technique was done by inoculation of mice 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 10^6 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 2nd 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 m^2 = 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 15 dpi. The difference in EPG was 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

\[
\text{FECR} (%) = \frac{\text{Pre Treatment EPG} - \text{Post Treatment EPG}}{\text{Pre Treatment EPG}} \times 100.
\]

6- Reference chemicals:

Amprolium were used as reference material. Their concentration and exposure time was obtained according to the manufacturer 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 LD$_{50}$ versus *Eimeria* spp. in experimentally infected chicks, while its LD$_{100}$ 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 *H. diminuta* EPG 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 materials during all experiments. 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 which is mainly because of the 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 advances in the field 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 LD 100 was recorded at 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 LD_{100} versus Eimeria spp. in experimentally infected chicks, 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
To eradicate the worms present in the intestine of mice may be due to the nature of this plant described by (Batanouny, 1999). As C. ambrosioides oil extract (rich by Ascaridole) has sedative effect valuable in pain relieving 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 C. 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. parvum oocysts in vitro

<table>
<thead>
<tr>
<th>Tested conc.</th>
<th>Mean mortality in C. parvum oocysts after exposure to</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3h</td>
</tr>
<tr>
<td>100ppm</td>
<td>-</td>
</tr>
<tr>
<td>200ppm</td>
<td>30.66</td>
</tr>
<tr>
<td>300ppm</td>
<td>70.33</td>
</tr>
</tbody>
</table>

Concentration before these concentrations did not induce any mortalities.

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

<table>
<thead>
<tr>
<th>Tested conc.</th>
<th>Mean mortality in Eimeria spp. oocysts after exposed to</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3h</td>
</tr>
<tr>
<td>100ppm</td>
<td>-</td>
</tr>
<tr>
<td>200ppm</td>
<td>13.66</td>
</tr>
<tr>
<td>300ppm</td>
<td>70</td>
</tr>
<tr>
<td>400ppm</td>
<td>100</td>
</tr>
</tbody>
</table>

* Concentration before these concentrations did not induce any mortalities.

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

<table>
<thead>
<tr>
<th>Tested conc.</th>
<th>Mean mortality in H. diminuta eggs after exposure to</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3h</td>
</tr>
<tr>
<td>100ppm</td>
<td>-</td>
</tr>
<tr>
<td>200ppm</td>
<td>-</td>
</tr>
<tr>
<td>300ppm</td>
<td>9.66</td>
</tr>
<tr>
<td>400ppm</td>
<td>49</td>
</tr>
</tbody>
</table>
* Concentration before these concentrations did not induce any mortalities.

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

<table>
<thead>
<tr>
<th>Tested doses (mg/kg)</th>
<th>Mean number of oocysts shed during 3-5day PI</th>
<th>Efficacy %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Treated oil group</td>
<td>Control positive</td>
</tr>
<tr>
<td>100</td>
<td>5500</td>
<td>7000</td>
</tr>
<tr>
<td>150</td>
<td>4000</td>
<td>6500</td>
</tr>
<tr>
<td>200</td>
<td>2100</td>
<td>7200</td>
</tr>
<tr>
<td>250</td>
<td>0</td>
<td>6800</td>
</tr>
<tr>
<td>300</td>
<td>0</td>
<td>7000</td>
</tr>
<tr>
<td>350</td>
<td>0</td>
<td>5800</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Tested doses (mg/kg)</th>
<th>Mean number of oocysts shed during 3-5day PI</th>
<th>Efficacy %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Treated oil group</td>
<td>Control</td>
</tr>
<tr>
<td>80</td>
<td>13200</td>
<td>16000</td>
</tr>
<tr>
<td>100</td>
<td>8000</td>
<td>15000</td>
</tr>
<tr>
<td>120</td>
<td>7000</td>
<td>15500</td>
</tr>
<tr>
<td>140</td>
<td>2000</td>
<td>15000</td>
</tr>
<tr>
<td>160</td>
<td>0</td>
<td>15000</td>
</tr>
<tr>
<td>180</td>
<td>0</td>
<td>15200</td>
</tr>
</tbody>
</table>

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

![Effect of different oil concentration and exposure time on *C. parvum* oocysts in vitro](image1)

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

![Effect of different oil dose & exposure time on *Eimeria* spp oocysts](image2)
Plate (3): efficacy of Chenopodium oil versus *H. diminuta* eggs in vitro

![Graph showing the effect of different oil concentrations and exposure time on *H. diminuta* eggs in vitro.](image-url)
References


Salama, M. M., Taher, E. E. and El-Bahy, M. M., 2012: Molluscicidal and


الملخص

أصبح البحث عن بدائل غير كيميائية صديقة للبيئة لعلاج الطفيليات، من الاستراتيجيات الحديثة المتصلة لتجنب المشاكل الناتجة عن استخدام الأدوية الكيميائية التي تسببت في هذا المجال. يعتبر نبات الشينوبوديم المعروف باسم Zizyphus sp. من النباتات العشبية الغنية بمركبات الأسيتالد وهي تساهم مهددة ومسطحة للعالم علاوة على تأثيره المضاد للطفيليات وبعض الطفيليات.

تم في هذه الدراسة اختيار التأثير القاتل لمستخلص النبات على بعض الطفيليات داخل وخارج أجسام عوالاتها المعدية طبيعيا أو تجريبيا، وقد اتضح أن مستخلص نبات Zizyphus sp. قاتل على بعض الطفيليات تسبب طرديا مع زيادة التركيز المختبر وكذلك مدة التعرض.

اثبت التجربة أن جرعة 100 جزء في المليون قادرة على قتل جميع حيوانات الكريتوسپوديوم بارفم بعد تعريضها لمدة 12 ساعة، حيث فشلت هذه الحيوانات في إحداث العدوى في الفئران عن طريق الفم بعد تعريضها لهذه الجرعة في ظل أن كمية نبات Zizyphus sp. كانت نسبية لونية مع زيادة مدة التعرض حتى 48 ساعة، قادرية على قتل أجنة حيوانات الأسيتالد والتي تم تجميعها من الفئران بعد انتهاء فترة الخبر سمعها. وفي نفس الوقت أدت الجرعة 30 جزء في المليون لعدة 48 ساعة إلى حدوث التأثر لحركة أجنة بويضات اليمينييين ديمونتا عند ملاحظتها تحت المجهر الضوئي.

وباختيار التأثير العلاجي لهذا المركب داخل أجسام العوالات المصابة، أدت الجرعة 112 ملجم / كجم عن طريق الفم لمدة 3 أيام متتالية وكذلك الجرعة 160 ملجم / كجم نفس الفترة إلى أحدث انخفاض 50% عن طريق الفم. كذلك أدت الجرعة 180 ملجم / كجم لمدة 3 أيام في مياة الأسيتالد وجرعة 350 ملجم / كجم نفس الفترة إلى أحدث انخفاض في عدد حيوانات الأسيتالد الإمبريروسية الموجودة في الفئران. مزيدا من الخلايا نوعية بنسبة 50% و100% على التتابع. وفي المقابل لم تؤدي زيادة الجرعة حتى 400 ملجم / كجم لمدة 5 أيام متتالية عن طريق الفم إلى ريد إيحاء خاصيتين ديمونتا من أحياء الفقار الطبيعية، ولكن مانع لهذا الطيف.

ومع كل هذا المستوى من الجرعات و معدلات أعدادها، لم تظهر أي أعراض سمية على كائنات المتعالة كذلك لم يكن هناك تغير في عدد الحيوانات والبيوضات التي تم تشخيصها في المجموعات المضمنة الغير معالجة خلال نفس وقت التجربة. وفيما يخص الضوابط الإيجابية التي تم علاجها سبيلا لداء مريح، ادى استخدام أمبوليو مزيج 24/0% في الكتالك وتوزع على ملايين الفئران لمدة 3 أيام إلى الاختفاء التام للحيوانات ومعدلات 0.5 - 10 مل يوميا في الفئران. وجرعة 3 أيام إلى انخفاض أعداد الحيوانات ومع ذلك لا يوجد من علاج مع للأمبوليون المتضمنين لداء هذه العوالات.

وباختيار أن الجرعات السابقة لم تكشفها باستخدام المستخلص الخام لذات النبات، فإنه يمكن توقع نقص كبير في مستويات هذه الجرعات مستقبلا بعد استخلاص المادة الفعالة (أسيتالد) صناعيا من هذا النبات. *****