NEW TRENDS IN TREATMENT OF TOXOCARA CANIS AFFECTING UNUSUAL SITES

By
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ABSTRACT
The efficacy of Zingiber officinale extract (ZOE) against toxocariasis was investigated in experimental mice and results were compared to those obtained using albendazole and combination of albendazole and Zingiber officinale extract (Albz). Sixty male albino mice, 10 weeks old, were classified into 6 experimental groups of 10 mice each:

Group I: None infected, non-treated (normal control) and received 0.1 ml of sterile distilled water.
Group II: Infected mice with T. canis eggs (infected control).
Group III: Infected mice by treated eggs with ZOE (T. canis eggs were incubated 24h with 500 mg/ml of ZOE before mice inoculation).
Group IV: Infected mice by T. canis eggs and treated by albendazole (drug control).
Group V: Infected mice by T. canis eggs and treated by ZOE.
Group VI: Infected mice by T. canis eggs and treated by combination of albendazole and ZOE. The present study showed that, Zingiber officinale extract (ZOE) had a significant inhibitory effect on the larval recovery rates in the brain and eye compared to the infected and drug controls. Combination of albendazole and Zingiber officinale extract (Albz) reduced the larval counts by higher percentages attaining the best results. The most obvious reduction in larval counts was observed in GVI to be 94.50% and 96.34% respectively. It was observed that the larvae in all treated groups showed sluggish movement Zingiber officinale improved the induced pathological changes by Toxocara in the studied organs that regressed to near normal picture after its combination with Albz. And Zingiber officinale seemed to be effective as albendazole against T. canis infection. However, a greater effect was obtained by their combination.
INTRODUCTION

Human toxocariasis is an accidental parasitic disease due to infection by larval stages of *Toxocara canis* and *T. cati*, the common roundworms of dogs and cats, respectively (Despommier, 2003). It results in at least three syndromes in humans: visceral larva migrans syndrome (VLM), ocular larva migrans syndrome (OLM), and covert toxocariasis (Magnaval et al., 2001). The treatment of VLM and OLM has been investigated in well-controlled studies (Abdelmeguid., 2010). The drugs available for their treatment are albendazole, thiabendazole, tinidazole and diethylcarbamazine (Musa et al., 2011). Several plant extracts were used as antihelminthic effect against *Toxocara canis* including *Nigella sativa* (Musa et al., 2011), and *C. ambrosioides* by reducing the inflammatory reaction produced by the infection of *T. canis* larvae in vivo (Reis et al., 2010).

*Zingiber officinale* (Ginger) belonging to the family Zingiberaceae is a perennial herb. It is widely distributed in tropical Asia. It is one of the most common spices, which is in use since centuries for its versatile medicinal actions (Imtiyaz et al., 2013). Numerous experimental and clinical trials have proven ginger for its range of therapeutic activities such as antibacterial, antidiabetic, antiemetic, hypolipidaemic, hepatoprotective properties (Imtiyaz et al., 2013).

Several investigations were done upon the antihelminthic activity of ginger and its constituents. Iqbal et al. (2006) showed that both crude powder and aqueous extract of dried ginger showed antihelminthic activity in sheep. Lin et al. (2010) suggested that the 6- gingerol, 10-shogaol, 10-gingerol, 6-shogaol and hexahydrocurcumin, a constituent isolated from the ginger might be used as larvicidal agents (Lin et al., 2010).

The aim of this work was to focus on traditional medicine *Zingiber officinale* to evaluate the histopathological changes in *T. canis*-infected mice after treatment with *Zingiber officinale*, albendazole and a combination of both.

MATERIALS AND METHODS

Ethics statement

The animal experiment was carried out according to the internationally valid guidelines and the research protocol was approved by Research Ethics Committee, Faculty of Medicine, Benha University, Egypt.

Adult *T. canis* were collected from the intestines of stray puppies from Cairo and Giza Provinces. Eggs were obtained from the uteri of adult female worms and purified by straining through a sieve with 0.5 mm pores, washed with saline several times, and then kept in 0.5% formal saline solution (99.5 ml physiological saline and 0.5 ml formaldehyde 40%) for 4–8 weeks in petri-dishes at 28-30°C to induce embryonation. Maturation of the eggs was checked every day after aeration and shaking of eggs to enhance maturation and to prevent sticking. Then, mature embryonated eggs were kept at 4°C until used.

A total of 60 laboratory-bred male Swiss albino mice, 10 weeks-old were classified into 6 experimental groups of 10 mice each:

**Group I:** None infected, non-treated (normal control) and received 0.1 ml of sterile distilled water

**Group II:** Infected mice with *T. canis* eggs (infected control)

**Group III:** Infected mice by treated eggs with ZOE (*T. canis* eggs were incubated 24h with 500 mg/ml of ZOE before mice inoculation)

**Group IV:** Infected mice by *T. canis* eggs and treated by albendazole (drug control)

**Group V:** Infected mice by *T. canis* eggs and treated by ZOE.

**Group VI:** Infected mice by *T. canis* eggs and treated by combination of albendazole and ZOE. Each mouse was inoculated with about 500 embryonated viable *T. canis* eggs counted by hemocytometer through the oral route (Fan et al. 2003a). This in-
effective dose was selected according to Al-Saeed and Mahmood, (2011) who found that the higher doses of T. canis eggs were between (125 - 500 eggs) showed lesions in liver, lungs, eyes and muscles of sacrificed mice after 4 weeks of infection. The efficiency of the experimental T. canis infection in mice was confirmed by the enzyme-linked immunosorbent assay (ELISA) for detection of T. canis IgG antibodies on day 14 PI. ELISA was carried as described by De Savigny (1975) with some modification.

**ELISA test for determination of IgG antibodies in the mice serum**

(Linbro, Flow Laboratories, Connecticut, USA).

Blood was collected from mice by tail puncture at 14th day PI before the administration of the treatment. T. canis excretion–secretion (TES) antigen (kindly provided) was diluted in 0.1 M sodium carbonate buffer (pH 9.6) and the concentration was adjusted to be 20 μg/ml. Procedures followed the manufacture’s procedure.

At the end of the experiment, blood was collected from all animals under ether anaesthesia. The blood was collected from the mouse’s orbital venous plexus (Hoff and Rlagnet 2000). The sera were separated by centrifugation at 3000g for 10-15 minutes and stored at -20°C until the determination of biochemical parameters. Eye, liver, lung and brain from each sacrificed mice were collected for detection of parasitological, histopathological studies.

**Albendazole in the form of Alzentral** (Epico Pharm Co., 10th Ramadan city, Egypt) as a white suspension of 100 mg/5 ml was given orally at a dose of 100 mg/kg once daily for 5 consecutive days, diluted in 0.1 ml distilled water on the 20th day of infection for 5 days. This dose was selected based on previous studies (Yarsan et al. 2003).

**Fresh rhizome of Zingiber officinal** was purchased from a local market and was identified in the Department of Medicinal and aromatic plants; Horticulture Research Institute, Giza, Egypt and the specimens were recorded for future reference.

The rhizome was dried and ground into fine powder using an electrical blender. Fine powder (100g) was homogenized in ethanol (95%; 500 mL) and left in a conical flask at room temperature for 3 days.

Then, the mixture was filtered through a fine muslin cloth and a filter paper (Whatman No. 1). Using rotary evaporator (Sigma-Aldrich, USA) the extract became concentrated.

The extract was then lyophilized and yielded ZOE.

Tween-20 (10%) was used to dissolve the extract in the concentration of 100mg/mL.

In this study, dose of 500mg/kg were selected for the oral administration of ZOE according to Abdulaziz Bardi et al., (2013).

**Parasitological study for T. canis larval recovery**

- Samples from the lung and liver tissues (0.5 g each) were sliced and digested in 50 ml pepsin-HCl solution (2.5 g pepsin, 3.5 ml HCl, and 500 ml water) and incubated at 37°C for 24 h (Horiuchi et al. 2005).
- After incubation, the digests were filtered through a sieve. The sedimental liquids then were centrifuged for 2 min at 1,500 rpm.
- The sediments were then collected, and examined for the presence of T. canis larvae and then the larvae were counted using a light microscope at × 10 magnification
- Larvae in the brain were counted directly, after squashing small amounts of fresh unstained brain tissue between two slides to calculate parasite load in 1 gm (Chung et al. 2004).
- The percentage of efficacy of the treatments was calculated from the count reduction of recovered larvae,
using the arithmetic mean of larvae count with the following formula:

\[
\text{Efficacy (\%)} = 100 \times \frac{\text{mean larvae recovered in controls} - \text{mean larvae recovered in treated mice}}{\text{mean larvae recovered in controls}}.
\]

**Histopathological study**

- The eye, lung, liver and brain tissues of each mouse were fixed separately in 10% buffered formalin, dehydrated in different concentrations of alcohol, cleared with xylol, and embedded in paraffin blocks.
- Tissue sections of 5 um thickness were stained with haematoxylin and eosin (H & E).
- The slides were observed under the light microscope at x10, x40, x100 magnifications for the presence of any pathological changes resulting from migration of *T. canis* larvae as destruction and necrosis of the cells, haemorrhage, cellular infiltration and granulomas.

**Biochemical Analysis:**

Hepatoprotective effect of ZOE was evaluated by liver function biochemical parameters. Serum levels of the liver enzymes, aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) were assessed in the sera of all groups by immunoassays. They were determined by commercial kits (spectrum, Germany) (Reitman and Frankel, 1957; Teitz and Shuey, 1986) according to the manufacturer's instructions.

**Statistical analysis**

The statistical analysis was conducted using STATA version 11 (STATA corporation, College Station, Texas). The corresponding P-values were obtained. A P-value < 0.05 was considered statistically significant (S), a P-value < 0.001 was considered statistically highly significant (HS), while a P-value > 0.05 was considered statistically non-significant.

**RESULTS**

In the present work, No *T. canis* larvae were detected in the brain examined tissues of the uninfected control mice (GI), (table 1). At the same time, there was more reduction in means of larval counts in both GIV and GV (treated with albendazole and ZOE) to be 87.56% and 85.34% respectively. The most obvious reduction in larval counts was observed in GVI (treated with a combination of albendazole and ZOE) to be 94.50%. It was observed that the larvae in all treated groups showed sluggish movement. The mean number of larvae in the brains was significantly decreased in GIII that was infected by *T. canis* eggs incubated with 1 ml of ZOE for 24 hours before animal inoculation (32.2±9.93) with a percentage of reduction of 57.83%. Table (2) shows that, No *T. canis* larvae were detected in the eye tissues of the uninfected control mice (GI), there was more reduction in means of larval counts in both GIV and GV (treated with albendazole and ZOE respectively) to be 83.22% and 70.51% respectively. The most obvious reduction in larval counts was observed in GVI (treated with a combination of albendazole and ZOE) to be 96.34%. It was observed that the larvae in all treated groups showed sluggish movement. The mean number of larvae in the eye was significantly decreased in GVI that was infected by *T. canis* eggs incubated with 1 ml of ZOE for 24 hours before animal inoculation (0.2±0.77) with a percentage of reduction of 96.34%.

The histological sections of brains form mice infected with 500 egg/ml after 4 weeks of infection showed pathological changes such as cellular infiltrate mainly of lymphocytes either diffuse or in aggregates, focal cerebral haemorrhage, haemorrhage in the meninges, numerous cross sections of *Toxocara* larvae deposited with the cerebral parenchyma, focal proliferation of glia cells, numerous perivascular cuffing of the blood vessel with glia cells and necrosis of neurons (Fig. 1).
In the present study, we notice that the eye is the least organ to be affected by *T. canis* infection. The uninfected control mice (GI) in which no abnormality was observed in the histopathologic sections of the retina. The histological examination of eyes for mice (GII) infected with 500 egg/ml of *T. canis* after 4 weeks showed retinal necrosis associated with inflammatory infiltrate. In GIII (infected mice by treated eggs with ZOE), there were moderate retinal necrosis. On giving albendazole (GIV), there were no histopathological changes. GV (infected and treated with ZOE) showed slight retinal necrosis. GVI (infected and treated with a combination of ZOE and ABZ) revealed no histopathological changes. (Fig.2).

Table (1): The means of larval counts in brains of treated and control groups of animals.

<table>
<thead>
<tr>
<th>Experimental Groups (No.=10)</th>
<th>Mean number of larvae± SD</th>
<th>% of Reduction</th>
<th>t</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>GI</td>
<td>0±0</td>
<td>-</td>
<td></td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>GII</td>
<td>76.4±17.0</td>
<td>57.83%</td>
<td>7.1</td>
<td>&lt;0.001**</td>
</tr>
<tr>
<td>G III</td>
<td>32.2±9.93</td>
<td>87.56%</td>
<td>12.08</td>
<td>&lt;0.001**</td>
</tr>
<tr>
<td>G IV</td>
<td>9.5±4.20</td>
<td>85.34%</td>
<td>11.4</td>
<td>&lt;0.001**</td>
</tr>
<tr>
<td>G V</td>
<td>11.2±6.16</td>
<td>57.83%</td>
<td>3.55</td>
<td>&lt;0.001**</td>
</tr>
<tr>
<td>G VI</td>
<td>4.2±2.15</td>
<td>94.50%</td>
<td></td>
<td>&lt;0.001**</td>
</tr>
</tbody>
</table>

* Compared to GI  
 b Compared to GII  
 c Compared to GIV  
 *P<0.05: Significant  
 ** P<0.001: Highly significant  
 # P>0.05: non-significant

Table (2): The means of larval counts in eyes of treated and control groups of animals.

<table>
<thead>
<tr>
<th>Experimental Groups (No.=10)</th>
<th>Mean number of larvae± SD</th>
<th>% of Reduction</th>
<th>T</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>GI</td>
<td>0±0</td>
<td>-</td>
<td></td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>GII</td>
<td>9.7±4.63</td>
<td>38.71%</td>
<td>3.24</td>
<td>0.005**</td>
</tr>
<tr>
<td>G III</td>
<td>8±1.21</td>
<td>83.22%</td>
<td>6.88</td>
<td>&lt;0.001**</td>
</tr>
<tr>
<td>G IV</td>
<td>3.1±2.4</td>
<td>70.51%</td>
<td>5.58</td>
<td>&lt;0.001**</td>
</tr>
<tr>
<td>G V</td>
<td>3.1±2.4</td>
<td>57.83%</td>
<td>1.29</td>
<td>0.21</td>
</tr>
<tr>
<td>G VI</td>
<td>0.2±0.77</td>
<td>96.34%</td>
<td>9.21</td>
<td>&lt;0.001**</td>
</tr>
</tbody>
</table>

* Compared to GI  
 b Compared to GII  
 c Compared to GIV  
 *P<0.05: Significant  
 ** P<0.001: Highly significant  
 # P>0.05: non-significant
Figure (1): Photograph of brain sections from mice of different study groups stained with H & E (x 400).

a) Brain of mice from GI showing no histopathological changes.
b) Brain of mice from GII showing numerous cross sections of *Toxocara* larvae deposited with the cerebral parenchyma.
c) Brain of mice from GIII showing cross section of *Toxocara* larvae deposited with the cerebral parenchyma.
d) GIV showing slight necrosis of neurons.
e) GV showing necrosis of neurons and neuronophagia.
f) GVI showing necrosis of sporadic neurons.
DISCUSSION

The current study was conducted on 60 albino mice that were classified into six experimental groups: the control group GI (negative control), GII that was infected with embryonated *T. canis* eggs (infected control), GIII that was infected with ZOE treated embryonated *T. canis* eggs, GIV that was infected with embryonated *T. canis* eggs and treated with albendazole, GV that was infected with embryonated *T. canis* eggs and treated with Z. officinal extract (ZOE). GVI that was infected with embryonated *T. canis* eggs and treated with a combination of albendazole and ZOE.

The present study showed that the experimental infection of mice with *T. canis* embryonated eggs shows extra-intestinal migration of larvae in the brain and eyes. It was found that larvae were recovered from the brain of infected mice (GII) after 4 weeks. It seems that the transmission of larvae was from the intestine to the liver and lungs, then the larvae migrate throughout the body and accumulate mainly in the brain in accordance with the previous studies (Abo-Shehada and Herbert, 1989). Analyzing *T. canis* larval migration, Lescano et al. (2004) observed a higher concentration of larvae in the liver and lungs within the first five days after infection, while in the brain the highest values were obtained between the 15th and 60th days after infection. It was observed that larval recovery showed progressive in-
crease over the course of infection, with special predilection for the central nervous system (Othman et al., 2010; Taira et al., 2011; Caldera et al., 2013). Othman et al. (2010) recorded that progressive increase in the number of brain larvae was accompanied by increased expression of pro-inflammatory cytokines (TNF-α and IL-6) in a chronic stage of the infection (5–16 weeks PI).

The current study demonstrated that, the mean number of larvae in the brains was significantly decreased in GIII that was infected by T. canis eggs incubated with 1 ml of ZOE for 24 hours before animal inoculation (32.2±9.93) with a percentage of reduction of 57.83%. The obtained results suggested that the ZOE reduced the infectivity of T. canis eggs by direct effect on Toxocara larvae in embryonated T. canis eggs. At the same time all treated groups showed a highly significantly decreased in the mean number of larvae in the brains with a higher percentage of reduction of 87.56% in GIV that was treated by Albendazole, 85.34% in GV that was treated by ZOE and 94.50% in GVI that was treated by both Albendazole and ZOE. These results indicated that the administration of ZOE with Albz increased the effect of treatment (there was marked significant decrease in the mean larval count in the group VI in relation to group GIV, (P <0.002).

In this study, the anthelmintic action of Z. officinal on T. canis observed might occur through alterations in the metabolic activity of this larval stage after exposure to the drug. Another parameter for investigating the curative effect of ZOE on toxocariasis in the present study was histopathological studies of brain and the eyes of treated mice versus the untreated infected controls in which no abnormality was observed in the histopathologic sections of the examined tissues.

The histological section of brain for animals infected with 500 egg /ml after 4 weeks of infection showed normal retina (GI), retinal necrosis associated with inflammatory infiltrate (GII), moderate retinal necrosis (GIII). Retina of infected mice group treated by a combination of ZOE and albendazole showing no histopathological. The noticed pathological changes in the organs could be attributed to the mechanical irritation caused by the presence of viable larvae and to what is called nematode polyprotein allergen which is an antigen abundant in somatic and excretory-secretory products of the parasite and stimulates the host’s immune system (Christi et al., 1993; Yahiro et al., 1998). It is possible that the excretory-secretory products, the cuticle and the products of disintegration of the parasites’ body represent the principal stimuli affecting the pathological picture.

The picture of the pathology regressed to near normal picture after treatment especially in groups IV, V and VI. There were significant effects of treatments with both ZOE and albendazole, exhibiting significant in vivo anti-T. canis activity. ZOE was recorded to have similar effect as albendazole against T. canis regarding both larval counts and histopathologically when compared to infected control group. However, a greater effect was obtained in their combinations.
The improvement of the pathological picture may be due to marked reduction of the larval number in tissues after administration of the extract. The sluggish movements of the remaining larvae in the tissues as it decrease the damage which caused by the mobile living larvae (Antonios et al., 1990). The obtained anti- *T. canis* effect of *Z. officinal* extract in this study may be attributed to its active constituents. Ginger is composed of five constituents: [10]-shogaol, [6]-shogaol, [10]-gingerol, [6]-gingerol, and hexahydrocurcumin. Lin et al. (2010 a , b) found that these constituents have larvicidal activity against *Angiostrongyluscantonensis* and *Aniskis simplex* larvae by their killing or reducing their spontaneous movement larvaes.

This extract was found to have significant antiparasitic activities against *Trichinellaspis*, *Giardialamblia*, *Dirofilariaimmitis* and hydatid cyst (El-Melegy et al., 2006;Mahmoud et al., 2014; Merawin et al., 2010; Moazeni and Nazer , 2011). In addition, it was found that *Z. officinale* displayed some degree of antischistosomal activity through reducing the *S. mansoni* eggs output and the liver granuloma size (Al-Sharkawi et al., 2007). It has also antischistosomal effect against *S. mansonimiracidia* and cercariae(Adewunmi et al., 1990). Moreover, *Z. officinale* strongly inhibited the growth of *T. vaginalis*(Harborne and Baxter, 1993).

Abo-Shehada and Herbert (1989) claimed that the larvae that reached the brain are no longer susceptible to antihelminthic agents. In other studies however, where larvicidal efficacy of antihelminthic treatment was studied in mice during the first few days to 4 weeks of larval infection, the recorded partial efficacy did not seem to be dependent on the age of larvae.

Ginger is used widely in a variety of foods because of its nutritional composition and flavouring compounds. Fresh ginger is reported to contains protein, fat, minerals, fibers, carbohydrates, lipids (including glycerides, phosphatidic acid, lecithins, and fatty acids), protease, iron, calcium, magnesium, potassium, and phosphorous. It also contains vitamins such as thiamine, riboflavin, niacin and vitamin C (Ibrahim et al., 2010).

**CONCLUSION**

In conclusion, *Z. officinale* ethanol extracts have a potent anti-helminthic activity against *T. canis*. It is considered as new, cheap and safe pharmaceutical natural product with good protective and curative effects against *T. canis* infection. Further in vivo and in vitro studies will be needed to standardize the doses of these natural products and to determine the exact mechanisms of their action as anti-*T. canis* effects.

**REFERENCES**

Treatment of Toxocara Canis


الاتجاهات الجديدة في علاج السهمية الكلبية التي تصيب أماكن غير متعددة

ا.د. منى نصر 1-أ.م.د. عزة الغريب 1-أ.م.د. نجوى السيد 2-أ.م.د. الخولي 1-د. دينا هادي 1

قسم الطفيليات كلية الطب، جامعة بنها 1، معهد بحوث أمراض العيون 2

في هذه الدراسة تم التحقق من فاعلية مستخرج الزنجبيل في علاج داء السهميات الكلبية في فئران التجربة وتمت مقارنة النتائج التي حصلنا عليها بتلك التي تنتج من العلاج بدواء الألبندازول فقط والتي تنتج باستخدامهما معاً.

وقد تم اختيار فئران من فئران التجربة البيضاء البالغة 10 أسابيع من العمر وتم تصنيفهم إلى 6 مجموعات كل مجموعة تشمل 10 فئران.

المجموعة الأولى: (الضابطة) غير مصابة وتتناول 0.8 مل من الماء المقطر المعقم.
المجموعة الثانية: (الضابطة المصاببة بالعدوى) الفئران المصاببة ببويضات السهمية الكلبية.
المجموعة الثالثة: الفئران المصاببة بعطر بويضات السهمية الكلبية المعالجة بالزنجبيل قبل إعطائها للفئران.
المجموعة الرابعة: الفئران المصاببة بعطر بويضات السهمية الكلبية وتعالج بالألبندازول (الضابطة المصاببة بالعدوى).
المجموعة الخامسة: الفئران المصاببة بعطر مستخرج الزنجبيل.
المجموعة السادسة: الفئران المصاببة وتعالج بالألبندازول ومستخرج الزنجبيل معاً.

وقد أظهرت الدراسة أن مستخرج الزنجبيل (المجموعة الخامسة) كان له تأثير مثبط على معدل استرداد البريقات بشكل كبير في المخ والعين بالمقارنة بالمجموعة الثانية (الضابطة المصاببة بالعدوى) وال مجموعة الرابعة (الضابطة الدوائية).

كما أظهرت الدراسة أن استخدام مستخرج الزنجبيل والألبندازول معاً في العلاج له تأثير فعال في تقليل عدد البريقات بنسبة كبيرةً محققةً أفضل النتائج وقد لوحظ أن الاختلاف كان أكثر وضوحاً في المجموعة الخامسة حيث كانت النسبة في المخ والعين 96.34% و96.40% على التوالي.

ولوحظ أن البريقات في جميع المجموعات المعالجة أظهرت حركة بطيئة كما وجد أن استخدام مستخرج الزنجبيل يقلل بشكل كبير التغيرات المرضية الناجمة عن السهمية ويسبب تراجعها إلى قرب وضعها الطبيعي ويبدو أن فاعليته ضع السهمية الكلبية مثل فاعلية الألبندازول ومع ذلك، تم الحصول على تأثير أكبر عند الجمع بينهما.