Effect Of Chemotherapy And Ultra-Violet Irradiation On Embryonation And Infectivity of *Toxocara canis* Eggs By Using Murine Model

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Abstract

The present study aimed to investigate the in vivo effect of albendazole, ivermectin and UV irradiation on subsequent embryonation and infectivity of *Toxocara canis* eggs. For this propose, nine naturally infected puppies with *Toxocara canis* of two months old from the same environmental condition were allocated into three equal groups in separate cages. GroupI was received albendazole orally at a dose of 100 mg/kg b.w.; groupII was s/c injected with ivermectin at a dose of 0.3mg/kg. Group III was kept without treatment as control. Faecal samples were collected 3 times daily for 7 days post-treatment and the expelled *T. canis* worms were collected. At 7th day post-treatment, necropsy of all puppies was carried out and the adult *T. canis* worms were collected from their intestine, coecum and colon. The collected female worms from puppies groups I and II were dissected for preparation of eggs for embryonation. Eggs from worms of the third group (control group) were divided into 3 groups; one group (subgroup III a) was exposed to UV irradiation for 30 minutes before incubation, while the other two subgroups (subgroup IIIb & subgroup IIIc) were incubated till embery-onation. After embryonation, (subgroup III b) was exposed to irradiation for 30 minutes while the other group (subgroup IIIc) was kept without irradiation as a control non treated and non irradiated group. Embryonation was evaluated micro-scopically at 20th day. The infectivity of embryonated *T. canis* eggs was tested by mouse. The present data showed a rapid expulsion of worms in ivermectin treated puppies (48-120 hr. post-treatment) compared to (48-160 hr post- treatment) in puppies treated by albendazole. The lowest percentage of embryonation (35%) was recorded in albendazole treated group followed by (42%) in irradiated fertilized eggs (subgroup IIIa) as 65% and 58% of eggs cultures respectively were arrested at one cell stage, irregular cell division and a typical blastomeres and

gastrulae. Normal embryonation was recorded in (subgroups III b, c) and ivermectin treated groups. Comparatively lower mortalities were recorded in mice group inoculated with irradiated eggs containing second larval stage (subgroup III b). Lower number of larvae was detected from the liver of mice groups inoculated with irradiated larvae. Stained tissue section slides revealed that 20- 25 cross section of encapsulated *T. canis* larvae in mice belonged to group IV at 80th day post inoculation compared to 2-3 cross section of *T. canis* larvae in mice belonged to other mice groups. It was concluded that albendazole and UV irradiation showed an ovicidal effect against *T. canis* eggs and consequently reduce the occurrence of infective eggs in the environment. UV irradiation of embryonated eggs resulted in lower infectivity and mortality in inoculated mice compared to other groups.

Key words : *Toxocara canis*, emberyonation, infectivity, treatment, irradiation

Introduction

Toxocara canis is a parasite of canine carnivores and probably causes human toxocariasis. The bitches gradually accumulates *T. canis* larvae from the soil and tissues of prey and leak the larvae into its puppies both during the latter part of pregnancy (parentally) and during the nursing period through mammary gland (30). After birth, these larvae mature 25-40 days post partum and the puppies then contaminate the environment with eggs as every puppy can be expected to shad *T.canis* eggs (7).

(29) reported a syndrome of persistent eosinophilia in 2 children by administration of embryonated eggs of *T.canis*. (22) established the role of *T. canis* as an agent of human disease. (2) mentioned that, man being an aberrant host where *T. canis* can not complete its life cycle in man but cause a spectrum of diseases ranging from no symptoms to eosinophilia covert toxocariasis visceral and ocular larva migrans.

Most of the anthelmintic drugs are generally directed against larval and adult stages of helminthes.(10). However (27) reported

that, anthelmintics may also express ovicidal activity as in benzimidazoles. (25, 21) supposed other methods of treatments by using irradiated larvae of *Dictyocaulus viviparous* and *Anclystoma caninum* and found that , most larvae were died in the tissues of the host after few weeks. However little is known about the influence of anthelmintic treatment or effect of irradiation on the development and infectivity of *T. canis* eggs.

So, the present study aimed to investigate the in vivo effect of albendazole and ivermectin as two anthelmintics or UV irradiation on subsequent embryonation and infectivity of T. canis eggs by using murine model.

Materials and Methods

Experiment I : Effect of albendazole and ivermectin on survival and expulsion of *T.canis*:

Animals: Nine naturally infected puppies with *T. canis* of two months old were divided into three equal groups in separate cages and treated as follow:

Group I: Three puppies were orally treated with albendazole 2.5% (Product of Pharma Swede Egypt) at a dose of 100 mg/kg b.w according to (19).

Group II: Three puppies were injected s/c with ivermectin 1%(Nasromectin, product of El- Nasr Pharmaceutical Chemical Co.) at a dose of 0.3 mg/kg according to (24).

Group III: Three puppies were kept without treatment as a control group.

All puppy groups were kept on clean source of water and food.

Parasitological examination:

Faecal samples of all puppies were collected 3 times daily for 7 days post-treatment for collection of the expelled *T. canis* worms according to (5). At 7th day post- treatment, necropsy of all puppies was carried out and the adult *T. canis* worms were collected from the intestine, coecum and colon. The collected worms from each puppy were kept separately in saline and the female worms were dissected for preparation of eggs for emberyonation according to (12, 26).

Eggs from worms of group III (control group) were divided into 3 groups; one group (subgroup III a) was exposed to UV irradiation for 30 minutes before incubation and the other two groups (subgroup IIIb & subgroup IIIc) were incubated at 26C° in 2.5% potassium-dichromate till emberyonation. After embryonation, (subgroup III b) was exposed to irradiation for 30 minutes according to (1) while (subgroup IIIc) was kept without irradiation as control non treated non irradiated group. The percentage of embryonation and stage of development of eggs were evaluated microscopically at 20th day of incubation according to (5). The embryonated eggs from all female groups were used experiment II.

Experiment II : Infectivity of embryonated *T.canis* eggs after UV irradiation and the in vivo treatment:

1- Mice inoculation

The infectivity of embryonated *T. canis* eggs was tested by mouse inoculation as described by (11, 6, 9). Forty -five mice were used and allocated into five equal groups as in table (1).

Table (1): Mice groups inoculated by different types of treated eggs

Group	Number of mice	Type of embryonated egg inoculum
Group I	9	embryonated eggs from pups treated with albendazole
Group II	9	embryonated eggs from pups treated with ivermectin
Group III	9	Irradiated fertilized eggs
Group IV	9	Irradiated embryonated . eggs
Group V	9	Non treated Eggs

The mice were inoculated with approximately 1500 embryonated eggs/ mouse by stomach tube according to (13). Mortalities among inoculated mice groups were recorded. For counting migratory larvae in different tissues, three mice from each group were scarified at 4 and 8th day post-inoculation and their intestine , liver and lung were minced separately for detection of larvae according to (18).

2-Histopathological examination:

The remaining mice were scarified at 80th day post inoculation and specimens were taken from their intestine and immediately fixed in 10% neutral buffered formalin. After proper fixation a thin paraffin section of about 5-7 microns were routinely prepared and stained with haematoxylin and eosin stain for microscopical examination according to (8) . Each stained tissue section was examined with light microscope (100X) and 15 random fields from each group were counted according to (11).

Results

Experiment I:

Table (2) showed that ivermectin cause rapid expulsion of alive adult ***T.canis*** worms (48-120 hrs post treatment) in treated puppies, while albendazole led to delayed expulsion of died macerated worms from treated puppies after (48-160 hr p. t) , The lowest percentage of embryonation (35%) was recorded in albendazole treated group as 65% of eggs cultures

Table (3): Mortalities and mean number of detected *T. canis* larvae from internal organs of mice at 4th and 8th days post infection (pi)

	Mortality		Intestine		liver		lung	
	No	%	4th day	8th day	4th day	8th day	4th day	8th day
Group I	2	22.22	196.33	105.6	68.7	97.6	0.0	0.0
Group II	2	22.22	207.67	134.3	82.0	104.67	0.0	0.0
Group III	2	22.22	192.3	123.0	78.5	88.75	0.0	0.0
Group IV	-	0.0	186.25	169.75	12.25	15.25	0.0	0.0
Group V	3	33.33	214.5	132.8	94.5	122.75	0.0	0.0

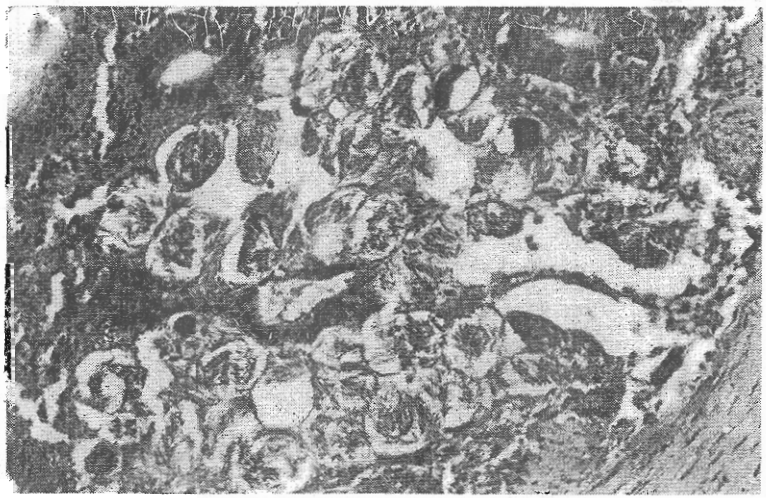

Fig (1) :C.S in intestinal showed 20- 25 cross section of encapsulated *T. canis* larvae in mice inoculated with irradiated embryonated eggs at 80th day post inoculation (arrow). H&E (X100)

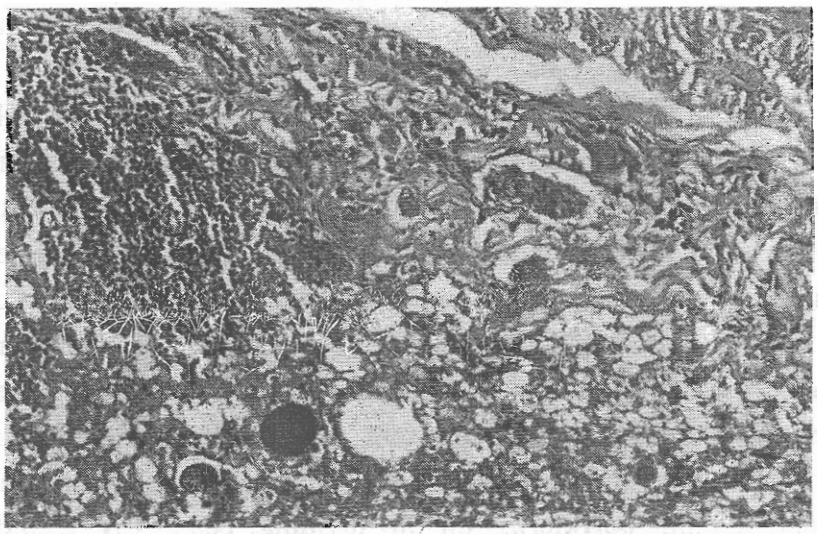

Fig (2): C.S in intestinal showed 2-3 cross section of encapsulated T. canis larvae in mice belonged to group I at 80th day post inoculation (arrow) H&E (X100)

Discussion

Although several studies were carried out on the biology, embryonation, and infectivity of *T. canis* in canine and paratenic hosts, the ovicidal effect of ivermectin or albendazole against its eggs have not been reported yet . Furthermore, most studies investigating ovicidal activity of anthelmintics are preformed the in vitro, while it had been suggested that in vitro effect of anthelmintics compounds may not necessarily reflect in the vivo activity (20). So the present study investigated the in vivo ovicidal effect of 2 different anthelmintic compounds ; ivermectin , albendazole as well as UV irradiation on subsequent embryonation and infectivity of *T. canis* eggs in mice

The present data showed that the two used drugs had good anthelmintic effect on alive adult *T.canis* worms. The alive worms were rapidly expelled alive from puppies treated with ivermectin. Such rapid expulsion may attributed to the paralytic effect of ivermectin on helminthes (

5). Meanwhile albendazole led to delayed expulsion of died macerated worms from treated puppies.

The present study showed that albendazole had an ovicidal activity against *T. canis* eggs isolated from expelled females by inhibiting the subsequent egg development. Incubated eggs were largely arrested at one cell stage or irregular cell division followed by abnormal blastomeres and gastrulae and consequently low percentage of eggs reach complete development (eggs containing L2). In this respect, (15,16) reported that treatment with anthelmintic affect the eggs in the oviduct of the female worms that might impair subsequent egg development in vitro. (17) demonstrated the mode of action of albendazole and he reported that it inhibited tubulin polymerization in dividing cells. (5) recorded that albendazole had in vivo ovicidal activity against *A.suum* eggs that were isolated from deworming pigs including the arrestment of egg development at one cell stage followed by irregular cell division. On the other hand, the ovicidal effect of ivermectin was not clear as the incubated eggs were normally developed as the control group. These results were met with study results of (5) who declared that non benzimidazole compounds such as ivermectin have only shown larvicidal effect in vitro.

Exposure of *T.canis* fertilized eggs to UV rays for 30 min. led to reduction in the number of embryonated eggs. Such reduction may be due to interference with the protein synthesis and the enzymes function of in UV irradiated eggs (23).

In the present study, the murine model had been used due to the similarity between the progression of *T. canis* in mouse and in human (28). The present study revealed that comparatively higher mortalities in all groups except in group IV (infected with irradiated embryonated eggs). This result was agreed with (4) that there was no mortality among mice infected by *T.canis* irradiated eggs. The low mortalities in mice belonged to group IV

may be attributed to the encapsulation of larvae in intestinal tissues and little number of larvae migrate from the intestine to liver and other tissues in such mice as mentioned by (3).

There were few differences between the infectivity of eggs of ivermectin, albendazole treated groups as well as between irradiated fertilized eggs and control groups. These results were agreed by (5) who mentioned that the small proportion of eggs that became embryonated followed albendazole treatment appears fully infective for mice.

The mean number of recovered larvae from intestine, liver and lung was comparatively low when compared to the inoculum. Similar results were obtained by (14). The mean numbers of recovered larvae from all mice groups after 8 days post infection were nearly similar, while there were reduction in its numbers specially in group IV. This may be due to the low ability of migration. This result was agreed with (3) who mentioned that irradiation of infective *T.canis* larvae reduced their migration.

Concerning the infectivity of UV irradiated embryonated *T. canis* eggs, the present study revealed that there was no mortalities among mice and showed comparatively low mean number of recovered larvae. The majority of hatched larvae did not migrate from the intestine and became encapsulated as shown in stained tissue section slides. This result was in line with that of (4) who recorded no mortality among mice infected with irradiated *T. canis* eggs and also was met with that of (1) who recorded 27% reduction in the total larval recovery in mice infected by UV irradiated eggs compared to control. This was attributed by (3) who mentioned that irradiation of infective *T.canis* larvae reduced their pathogenesity, inhibited their migration from liver and such larvae died faster than non-irradiated larvae for the first 20 days of infection.

It was concluded that:

1- Albendazole showed an ovicidal effect against *T. canis* eggs and consequently reduced the occurrence of infective eggs in the environment after treatment with a single dose.

2- Ivermectin resulted in a rapid expulsion of alive *T. canis* female worms without an ovicidal effect against its eggs .

3- UV irradiation of fertilized *T. canis* eggs reduced its embryonation but not its infectivity

4- There were few differences between the infectivity of eggs of ivermectin, albendazole treated , irradiated fertilized and control groups.

5- UV irradiation of embryonated eggs decreased infectivity and subsequently mortality among inoculated mice.

Further studies are needed to determine the antigenisty and immunization of the paratenic hosts and puppies against *T. canis* infection.

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تأثير العلاج بالأدوية والأشعة فوق البنفسجية على النمو الجنينى والقدرة على العدوى لبويضات إسكارس الكلاب باستخدام نماذج من الفئران

الباحثان

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استهدفت الدراسة معرفة تأثير عقارى البندازول و ايفرمكتين فى داخل الجسم الحى وكذلك الأشعة فوق البنفسجية على النمو الجنينى والقدرة على العدوى لبويضات إسكارس الكلاب باستخدام نماذج من الفئران. تم اختيار ٩ كلاب صغيرة عمر شهرين من نفس الظروف البيئية ومصابة إصابة طبيعية بالإسكارس وقسمت الى ثلاث مجموعات متساوية عولجت كالاتى: المجموعة الاولى تم تجريعها بعقار البندازول٥و٢% بمعدل ١٠٠ ملج/كجم مرة واحدة, و المجموعة الثانية تم حقنها بعقار أيفرمكتين تحت الجلد بمعدل ٣ملج/ كجم , المجموعة الثالثة تركت بدون علاج كمجموعة ضابطة وتم تجميع البراز ثلاث مرات يوميا ولمدة أسبوع بعد العلاج بهدف تجميع ديدان الاسكارس التى تم طردها من الجسم نتيجة تأثير العلاجات وفى اليوم السابع تم تشريح الكلاب وتجميع ديدان الاسكارس من امعائها وتم تحديد إناث ديدان الإسكارس وأخذ البويضات المخصبة من الأ جزاء العلوية من الأرحام للمجموعتين الاولى والثانية وتحضينها فى ثانى كرومات البوتاسيوم٥و٢% عند درجة حرارة٢٦ م لمدة ٢٠ يوم وتم حساب نسبة التكوين الجنينى ومراحله المختلفة أما بويضات المجموعة الثالثة فقسمت الى ثلاث مجموعات عرضت إحداهن(تحت المجموعة الثالثة أ) الى الأشعة فوق البنفسجية لمدة نصف ساعة وتحضينها مع المجموعتين(تحت المجموعة الثالثة ب وتحت المجموعة الثالثة ج) فى ثانى كرومات البوتاسيوم٥و٢ عند درجة حرارة٢٦ م لمدة ٢٠ يوم وتم حساب نسبة التكوين الجنينى ومراحله المختلفة وبعد اكتمال النمو الجنينى تم تعريض (تحت المجموعة الثالثة ب) للاشعة فوق البنفسجية لمدة نصف ساعة أما (تحت المجموعة الثالثةج) فتركت بدون تعرض للأشعة وقد تم تجريع الخمس مجموعات المختلفة من البويضات لخمس لمجموعات متساوية من الفئران عن طريق أنبوبة اللي المعدي. أثبتت الدراسة أن عقار أيفرمكتين أدى إلى طرد سريع لديدان حية من إسكارس الكلاب وعند تحضينها كانت نسبة التكوين الجنينى بها مرتفعة (٧٦%) أما عقار البندازول٥و٢% فقد أدى إلى طرد ديدان ميتة من إسكارس الكلاب وعند تحضينها كانت نسبة التكوين الجنينى بها منخفضة(٣٥%) أما بالنسبة لتعريض البويضات المخصبة (تحت المجموعة الثالثة أ) للأشعة فوق البنفسجية لمدة نصف ساعة أظهرت النتائج ان نسبة التكوين الجنينى بها كانت منخفضة أيضا (٤٢%) .وقد أوضحت الدراسة أن القدرة على العدوى كانت مرتفعة كما كانت نسبة الوفيات مرتفعة أيضا فى كل المجموعات ماعدا (تحت المجموعة الثالثة ب) التي تم تعريضها للأشعة فوق البنفسجية لمدة نصف ساعة بعد اكتمال نموها الجنينى. وقد بينت الدراسات الهستوباثولوجية التي أجريت بعد ٨٠ يوما على الفئران بعد تجريعها بالأنواع المختلفة من البويضات ان البويضات الناضجة والتى تعرضت للأشعة فوق البنفسجية لمدة نصف ساعة لم يهاجر الكثير من يرقاتها الى الكبد ولكن تحوصلت في أنسجة الأمعاء .