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THE THERAPEUTIC EFFICACY OF DORAMECTIN, IVERMECTIN AND LEVAMISOLE AGAINST DIFFERENT STAGES OF *TRICHINELLA SPIRALIS* INFECTION IN RATS

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SUMMARY

In this study, 3 commonly used anthelmintics are tried in experimental *Trichinella spiralis* infection in an attempt to reach a successful treatment. Doramectin (0.2 mg/kg), ivermectin (0.2 mg/kg) and levamisole (7.5 mg/kg) were tested for their anthelmintic efficacies against experimental *T. spiralis* infection in rats. Animals were infected with 500 larvae and the drugs were tested against adult worms at 4th day, migrating larvae at 10th day and encysted larvae at 35th day post-infection. Rats were sacrificed, 5 days post treatment. Mature worms and migrating larvae counts were significantly reduced in doramectin and ivermectin medicated rats compared with non-treated controls. Levamisole was the least effective drug with 4.83 and 3.57 % efficacies against mature worms and migrating larvae, respectively. Doramectin showed the highest efficacy in eliminating both mature worms and migrating larvae of *T. spiralis* (97.75 and 86.23 %, respectively) followed by ivermectin (94.99 and 83.85 %, respectively). The tested drugs failed to attack the encysted larvae in the diaphragms of infected rats.

Moreover, experimental infection of rats with *T. spiralis*, significantly reduced the values of total proteins and albumin while globulin, urea and creatinine values and the activities of AST and ALT were significantly increased. Doramectin and ivermectin induced an improvement in the previous parameters when given at 4th and 10th days post-infection but failed when injected at 35th day post-infection.

INTRODUCTION

The nematode *Trichinella spiralis* is found world wide in numerous species of carnivores and omnivores and is the etilogic agent of trichinellosis (Gamble et al., 1997). Infection with *T. spiralis* in human beings has a longstanding association with ingestion of infected pork, although in recent years, important infection sources have included bears, walruses, and horses (Bailey and Schantz, 1990 and Dupouy-Camet et al., 1994). The adult

worms inhabit the upper part of the small intestine, while the larvae inhabit the skeletal muscles. In rats, development of *T. spiralis* larvae to the adult stage is rapid, being complete in 4 days post infection and the time of onset of capsule formation in diaphragm starts on day 13 and completed at 5 weeks post infection (Teppema et al., 1973). Diagnosis of trichinellosis is achieved by detection of the adult worms in stool during the intestinal phase and by finding the encapsulated larvae in muscle biopsies.

Data from the literature are scarce and focussed mainly on the efficacy of some anthelmintics against encysted larvae only (Omar et al., 1995). The need for an effective compound for the prevention and treatment of trichinellosis in humans and animals has led to the testing of other drugs which are clinically effective against most other nematodes. Consequently, the present objective aimed to evaluate the efficacy of doramectin, ivermectin and levamisole on different stages of *T. spiralis* infection in rats with special reference to biochemical changes in their serum.

MATERIALS AND METHODS

Drugs:

1- Doramectin (Dectomax)® was available as 1 % injectable solution, Pfizer, Egypt.

2- Ivermectin (Ivomec)® was provided by MSD AGVET Divison of Merk Sharp & Dohme Ltd, Holland as 1.0 % injectable solution.

3- Levamisole (Pamisol-L),® was obtained as 10 % injectable solution, Ozzano Emilia- (Bologna) Italy.

Doramectin, ivermectin and levamisole were injected subcutaneously in a single therapeutic doses of 0.2, 0.2 and 7.5 mg/kg b.wt, respectively.

Parasite:

T. spiralis larvae were isolated from diaphragms of infected pigs obtained from Cairo Abbatoir.

Preparation of the inoculum:

(Gamble et al., 1997). Heavily infected pig diaphragms were cuted into small pieces and mannually mixed. Pieces were digested with artificial digestion fluid (1 % pepsin, W/V & 1 % Hcl, V/V). At the end of digestion process, larvae were collected using Baermann technique in phosphate buffer saline according to Pritchard and Kruse (1982). The sedimented larvae were separated in clean petri-dishes, washed in phosphate buffer saline and the number of larvae per ml solution was calculated.

Animals :

One hundred and fifty mature albino rats of 150-180 g b.wt. were obtained from the Laboratory Animal Colonies, Helwan, Egypt. The animals were parasitic free and kept on dry ration and controlled water source. One hundred and twenty rats were infected orally with 500 larvae/rat.

Experimental design:

Infected rats were divided into 4 groups of 30 animals, each. The 1st group was left as infected non-treated control while the 2nd, 3rd and 4th groups were administered a single subcutaneous dose of doramectin, ivermectin and levamisole

(0.2, 0.2 and 7.5 mg/kg b.wt, respectively). The other, 30 rats were kept as non-infected non-treated group.

The experiment was designed into 3 phases:

Phase (1): 10 rats from groups II, III & IV were medicated at 4th day post-infection. Five rats from all groups (I, II, III, IV & V) were sacrificed, 5 days post-treatment to detect the effect of drugs on mature worms in the intestine.

Phase (2): 10 rats from groups II, III & IV were treated at 10th day post-infection. Five rats from all groups were sacrificed 5 days post-treatment to explore the efficacy of drugs against migrating larvae.

Phase (3): 10 rats from groups II, III & IV were treated at 35th day post-infection. Five rats from all groups were sacrificed 5 days post-treatment to detect the efficacy of anthelmintics against encysted larvae.

The another 5 rats from each group were kept for 30 days after medication where blood samples were taken to explore the effect of infection and medications on serum enzymatic activities and some serum constituents.

Parasitological examination:

For counting the number of adult worms, small intestine of each rat was excised and transferred to a large petri dish containing normal saline solution and divided into 3 parts. Each part was evacuated by the aid of an water current then opened longitudinally with a fine scissors. The intestinal wall was scratched to collect worms that embedded in the mucosa. Encysted larvae were diagnosed primarily by trichinoscope then, diaphragms were artificially digested according to Gamble et al. (1997) and the recovered motile larvae were counted using a stereo microscope at a low power.

Anthelmintic effect of each drug was calculated in term of mean number of living worms per rat. The total number of larvae in each rat digest was counted, from which, the mean number of motile larvae per rat was calculated. Moreover, efficacy of each drug was calculated according to the equation:

$$\text{Efficacy \%} = \frac{A - B}{A} \times 100$$ (Hosking et al., 1996).

Where

A = Number of worms or larvae extracted from control animals

B = Number of worms or larvae extracted from treated animals.

Biochemical examination:

Blood samples were collected from the orbital plexus of controls and infected rats, 30 days after each medication. Samples were left to clot at room temperature for 20 min, then centrifuged at 1500 r.p.m. for 15 min. The obtained sera were collected and used to determine the values of total proteins (Reinhold, 1953), albumin (Doumas et al., 1971) and globulin (Coles, 1974). The activi-

ties of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) (Reitman and Frankel, 1957) and levels of urea (Fawcett and Scott, 1960) and creatinine (Husdan and Rapoport, 1968) were estimated in serum.

Differences between means were compared using the student's t-test (Snedecor and Cochran, 1967).

RESULTS

1- Effect on mature worms:

The mean number of living *T. spiralis* worms per rat (wpr) and the efficacy percentage of the tested anthelmintics are shown in Table 1. Mean worm count for control group (Fig. 1, A & B) was greater than 115 wpr. The mean number of living worms in the intestine of rats medicated with doramectin (2.6 wpr) and ivermectin (5.8 wpr) was significantly lower than in non-medicated control rats (115.8 wpr), suggesting an 97.75, 94.99 and 4.83 % efficacy against the parasite respectively. All doramectin and ivermectin-treated rats harbored dead worms or fragments of dead worms. In the doramectin-treated group, 2 of 5 rats were free from worms and one had an abnormal living male worm. There was no significant difference between the control and levamisole-treated rats as their mean wpr are 115.8 and 110.2, respectibvely.

2- Effect on migrating larvae:

The effect of doramectin, ivermectin and levamisole on the number of migrating *T. spiralis* larvae in diaphragms of rats when injected at 10th day post-infection was shown in Table 2. At that time, the number of motile larvae recovered at necropsy of each animal reflected the number of migrating larvae per rat. The number of larvae found in diaphragms of the non-treated rats (Fig. 1 C) was generally high and all were infected. Compared to control group, a marked reductions in the number of larvae that success to reach into diaphragms were observed after the administration of doramectin and ivermectin at 10th day post-infection (Effecacies were 86.23 and 83.85 %, respectively). No significant reduction in the number of motile larvae was observed after levamisole medication, as its efficacy was 3.57 %.

3- Effect on encysted larvae:

The number of encysted larvae found in diaphragms of control (Fig. 1 D) and all medicated groups were generally high (Table 3). Injection of doramectin, ivermectin and levamisole at 35th day post-infection failed to attack the already encysted larvae in diaphragms of infected rats as their efficacies were 12.43, 11.95 and 4.88 %, respectively.

4- Effect on serum constituents and enzymatic activities:

The mean values of serum biochemical data for infected and non-infected groups evaluated 30 days post-treatment, were shown in Tables 4-6. The results revealed a highly significant increase in the activities of AST and ALT in the infect-

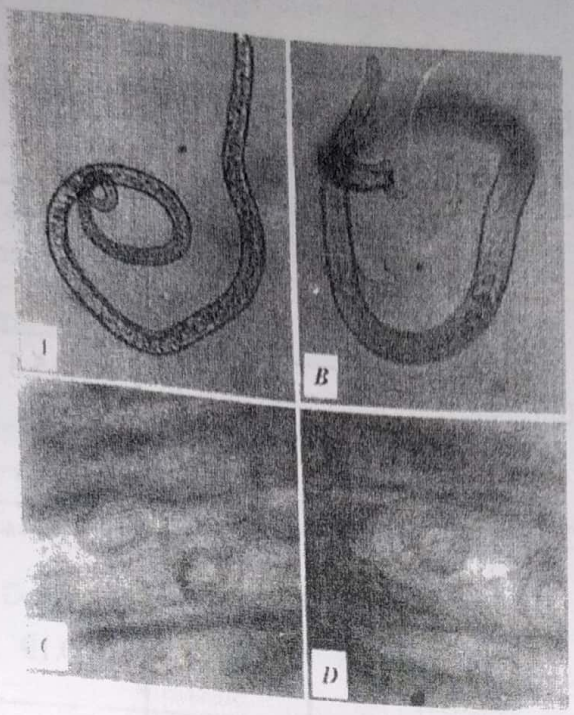

Fig. 1:

A- Adult female (X 40) and B- Adult male (X 100) *T. spiralis* extracted from the small intestine of control group at 9th day post-infection (Fresh specimen, notice the characteristic lateral flaps on either side of cloacal opening).

C- Early arrival of *T. spiralis* larvae to diaphragms, starting to develop cyst wall at 15th day post-infection (Fresh specimen, X 100).

D- Complete *T. spiralis* cyst showing a marked cyst wall at 40th day post-infection (Fresh specimen, X 100).

Table (1): Effect of the tested drugs on *T. spiralis* worms when injected at 4th day post-infection (n = 5).

Groups	Treatments	Dose (mg/kg b.wt)	Average no. per rat	Mean no. per rat	Efficacy %
I	Control (Infected non-treated)	0.0	96-134	115.8 ± 3.39	-
II	Doramectin	0.2	0-8	2.6*** ± 0.19	97.75
III	Ivermectin	0.2	2-11	5.8*** ± 0.25	94.99
IV	Levamisole	7.5	91-136	110.2 ± 3.97	4.83
V	Control (Non infected-non treated)	0.0	0	0	-

- Data are obtained 5 days post-treatment
- Data of groups II, III and IV are compared with those of group I

*** Significant at $P < 0.001$

Table (2): Effect of the tested drugs on migrating larvae of T. spiralis when injected at 10th day

(post-infection "n = 5").

Groups	Treatments	Dose (mg/kg b.wt)	Average no. per rat	Mean no. per rat	Efficacy %
I	Control (Infected non-treated)	0.0	1106-1578	1326.25 ± 49.46	-
II	Doramectin	0.2	124-213	182.61*** ± 8.90	86.23
III	Ivermectin	0.2	161-293	214.13*** ± 10.68	83.85
IV	Levamisole	7.5	983-1518	1278.80 ± 41.36	3.57
V	Control (Non infected-non treated)	0.0	0	0	-

- Data are obtained 5 days post-treatment
- Data of groups II, III and IV are compared with those of group I

*** Significant at $P < 0.001$

Table (3): Effect of the tested drugs on encysted larvae of *T. spiralis* when injected at 35th day post-infection (n = 5).

Groups	Treatments	Dose (mg/kg b.wt)	Average no. per rat	Mean no. per rat	Efficacy %
I	Control (Infected non-treated)	0.0	1212-1492	1366.14 ± 63.85	-
II	Doramectin	0.2	1018-1266	1196.20 ± 48.57	12.43
III	Ivermectin	0.2	1096-1285	1202.76 ± 46.72	11.95
IV	Levamisole	7.5	1101-1388	1299.38 ± 57.32	4.88
V	Control (Non infected-non treated)	0.0	0	0	-

- Data are obtained 5 days post-treatment
- Data of groups II, III and IV are compared with those of group I

Table (4): Effect of the tested drugs when injected on the 4th day post-infection on the impaired serum parameters induced *T. spiralis* in rats. (n = 5).

Group	Treatments	AST u/ml	ALT u/ml	Urea mg/dl	Creatinine mg/dl	Total proteins gm%	Albumin gm %	Globulin gm %
I	Control (Infected non-treated)	192.26*** ± 4.52	58.56*** ± 2.38	77.32*** ± 3.60	2.61*** ± 0.15	3.43*** ± 0.22	1.06*** ± 0.11	3.66*** ± 0.11
II	Doramectin	127.26*** ± 4.60	39.21*** ± 1.36	55.21** ± 2.81	1.99** ± 0.18	5.48*** ± 0.26	3.12*** ± 0.16	2.60*** ± 0.15
III	Ivermectin	132.18*** ± 3.85	40.05*** ± 1.51	55.46** ± 2.55	2.02** ± 0.16	5.45*** ± 0.20	3.10*** ± 0.19	2.61*** ± 0.12
IV	Levamisole	184.31 ± 4.12	56.61 ± 2.27	74.58 ± 3.42	2.48 ± 0.19	3.44 ± 0.26	1.07 ± 0.12	3.63 ± 0.11
V	Control (Infected non-treated)	124.16 ± 4.52	35.82 ± 1.16	52.16 ± 2.09	1.87 ± 0.10	5.62 ± 0.21	3.28 ± 0.14	2.59 ± 0.10

- Data are obtained 30 days post-treatment
- Data of group I is compared with those of group O .
- Data of groups II, III and IV are compared with those of group I.

* Significant at P < 0.01
** Significant at P < 0.05
*** Significant at P < 0.001

Table (5): Effect of the tested drugs when injected on the10th day post-infection on the impaired serum parameters induced by *T. spiralis* in rats. (n = 5).

Group	Treatments	AST u/ml	ALT u/ml	Urea mg/dl	Creatinine mg/dl	Total proteins gm%	Albumin gm %	Globulin gm %
I	Control (Infected non-treated)	214.81*** ± 6.19	73.26*** ± 2.84	91.18*** ± 4.33	3.26*** ± 0.16	3.02*** ± 0.28	1.08*** ± 0.11	3.70** ± 4.52
II	Doramectin	171.21*** ± 4.63	49.81*** ± 1.80	66.59** ± 2.50	2.24** ± 0.14	5.44*** ± 0.26	2.94*** ± 0.16	2.99* ± 0.16
III	Ivermectin	179.66** ± 4.38	51.82*** ± 1.69	69.73** ± 2.82	2.50** ± 0.13	5.21*** ± 0.24	2.91*** ± 0.10	3.04* ± 0.14
IV	Levamisole	201.13 ± 5.66	69.48 ± 2.27	90.26 ± 2.27	3.18 ± 0.19	3.10 ± 0.21	1.02 ± 0.11	3.56 ± 0.19
V	Control (Infected non-treated)	126.08 ± 4.15	35.11 ± 1.46	54.10 ± 2.08	1.79 ± 0.13	5.86 ± 0.22	3.47 ± 0.14	2.52 ± 0.18

- Data are obtained 30 days post-treatment
- Data of group I is compared with those of group O .
- Data of groups II, III and IV are compared with those of group I.

* Significant at $P < 0.01$
** Significant at $P < 0.05$

Table (6): Effect of the tested drugs when injected on the 35th day post-infection on the impaired serum parameters induced by *T. spiralis* in rats. (n = 5).

Group	Treatments	AST u/ml	ALT u/ml	Urea mg/dl	Creatinine mg/dl	Total proteins gm%	Albumin gm %	Globulin gm %
I	Control (Infected non-treated)	201.94*** ± 6.18	.2.326*** ± 2.81	83.22*** ± 4.18	2.96*** ± 0.18	2.80*** ± 0.19	1.12*** ± 0.11	3.96*** ± 0.15
II	Doramectin	198.62 ± 4.95	60.37 ± 2.25	80.97 ± 4.05	2.88 ± 0.16	2.86 ± 0.21	1.45 ± 0.13	3.83 ± 0.11
III	Ivermectin	191.98 ± 4.82	62.29 ± 2.48	81.51 ± 5.16	2.63 ± 0.14	2.82 ± 0.20	1.40 ± 0.10	3.91 ± 0.12
IV	Levamisole	195.47 ± 4.72	61.51 ± 2.59	83.12 ± 5.80	2.90 ± 0.18	2.69 ± 0.18	1.18 ± 0.11	3.86 ± 0.12
V	Control (Infected non-treated)	124.02 ± 4.69	36.02 ± 1.26	53.98 ± 2.16	1.84 ± 0.11	5.81 ± 0.26	3.38 ± 0.17	2.61 ± 0.18

- Data are obtained 30 days post-treatment
- Data of group I is compared with those of group
- Data of groups II, III and IV are compared with those of group I.

*** Significant at P < 0.001

ed non-treated rats. In addition, urea and creatinine levels showed a highly significant increase allover the experimental periods. When injected at 4th and 10th days post-infection, doramectin and ivermectin (but not levamisole) reduced the abnormal activities of AST and ALT in serum of infected rats. Moreover, they significantly decreased the abnormal levels of urea and creatinine in their serum. Doramectin, ivermectin and levamisole did not improve the altered parameter in rats injected at 35th day post-infection.

Experimental infection of rats with *T. spiralis* produced a highly significant decrease in the levels of total proteins and albumin in serum, while globulin value was increased. The obtained results showed that the tested drugs did not improve the altered parameters when injected at 35th day post-infection.

DISCUSSION

Trichinellosis is a zoonotic disease capable of infecting all mammals including man. In fact, the infection has been diagnosed in most parts of the world including Egypt (Antonios and Salem, 1989). The severity of the disease and the lack of an established treatment for that parasite directed the attention toward investigating the antiparasitic effect of doramectin, ivermectin and levamisole on experimental trichinellosis in rats.

The obtained results indicated that doramectin and ivermectin were effective in removing mature worm burdens of *T. spiralis* while levamisole remained ineffective. In similar studies, Barton et al. (1995) and Pradhan et al. (1993) found that cattle and calves are completely cured from nematodal infection after treatment with a single dose of doramectin and ivermectin, respectively. The anthelmintic activity of avermectins could be attributed to modulating GABA-gated chloride channels which are more accessible in nematodes than in vertebrates (Arena et al., 1992). However numerous reports have documented the high efficacies expected from levamisole against nematodes in different animals (Patil et al., 1996 and Sharma and Siddiqui, 1996), the results obtained from this study confirm the presence of levamisole resistance occuring simultaneously in some species of round worms. A growing number of reports describing anthelmintic resistance by nematodes have appeared in the literature (Borgsteede, 1991). Resistance to levamisole has only been reported in *Ostertagia ostertagi* (Williams and Broussard, 1995).

The obtained results showed that the numbers of migrating larvae that success to reach into the diaphragms of untreated rats were high. This finding agrees with that obtained by Mikhail (1979) who reported that migrating *T. spiralis* larvae were found in liver prior to arrival into diaphragm, while no larvae were found in the heart. Moreover, new born larvae were isolated also from their kidneys.

The application of doramectin and ivermectin at 10th day post-infection revealed a reduction in the

number of larvae that succeeded to encyst in the diaphragms of infected rats in comparison with control non-treated animals. Other studies showed that avermectins are recommended for field use because of their ease administraion and significant larvicidal activity (Sharma and Siddiqui, 1996). There was no significant difference in the number of migrating larvae between the control and levamisole-medicated rats.

Concerning the effect on the already encysted larvae, doramectin, ivermectin and levamisole failed to reduce their numbers in the diaphragms of infected rats when injected at 35th day post-infection. This observation is inconsistent with that recorded by Omar et al. (1995). They found that ivermectin efficacy against encysted *T. spiralis* larvae was 73.5 % when injected 6 weeks post-infection. This disagreement might be due to the differences in the time of medication.

T. spiralis infection, significantly increased the activities of AST and ALT and levels of urea and creatinine as compared with the non-infected rats. These changes may attributed to the liver and kidney damages which induced during migration of larvae. Hyperactivity of AST and ALT is indicative of hepatic damage (Boyd, 1962), whereas the elevated levels of urea and creatinine in serum is known to reflect the state of glomerular filteration and indicate kidney disease (Coles, 1974). The present findings are supported by those previously recorded by Mikhail and Milad (1975) who isolated the larvae of *T. spiralis* from liver during migration. In addition, Mikhail et al. (1978) observed lesions in the livers of rats infected with 500 *T. spiralis* larvae. Moreover, new born larvae were isolated from the kidney of rats (Mikhail, 1979).

A marked improvement towards the normal in the values of the above mentioned parameters was observed after treatment with doramectin and ivermectin. The reduction in the activities of AST and ALT, and the decrease in the levels of urea and creatinine in serum of medicated rats may be due to their larvicidal activity. Since all animals under invstigation were experimentally infected with *T. spiralis* only and free from any other parasites, the improvement of the altered parameters after doramectin and ivermectin-medications could be satisfactory ascribed as due to the effect of both drugs on *T. spiralis* only.

In rats treated at 35th day post-infection, the drastic reduction in total proteins and albumin could be attributed to liver involvement by metabolic products of nematodes and damage of the liver parenchyma during the migration of larvae (Reinhold, 1953). The increased amount of globulin is a compensatory reaction to restore osmotic pressure in serum which is reduced as a result of low albumin content and also may be due to increased formation of antibodies against parasite or its metabolic products (Reinhold, 1953).

In conclusion, the present study suggest that avermectins gave the most promising results and at dosage levels and formulations. Consequently doramectin and ivermectin can have continued practical application in controlling *T. spiralis* infection in animals.

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