Therapeutic Efficacy of Dormectin, Ivermectin and Levamisole Against Different Stages of *Trichinella spiralis* in Rats

Sıçanlarda *Trichinella spiralis* Farklı Aşamaları Karşı Dormectin, Ivermectin ve Levamizol Terapötik Etkinliği

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ABSTRACT

Objective: Three commonly used Antihelmenthic drugs including Dormectin (0.2 mg/kg), Ivermectin (0.2 mg/kg) and Levamisole (7.5 mg/kg) were examined for their antihelmenthic efficacy against experimental *Trichinella spiralis* infection in rats.
Methods: One hundred and twenty rats were orally infected with 500 *T. spiralis* larvae. Drugs were tested against adult worms at 4th day, against migrating larvae at 10th days and against encysted larvae at 35th day post infection (dpi). Rats were sacrificed five days post treatment. Mature worms and migrating larvae counts were detected.
Results: Significant effect was detected in rats treated with Dormectin and Ivermectin compared to non-treated controls. Dormectin showed an efficacy of 97.75% and 86.23% in eliminating both mature worms and migrating larvae respectively. Ivermectin showed an efficacy of 94.99% and 83.85% respectively. However, Levamisole was the least effective drug; its efficacy was 4.83% and 3.57% against mature worms and migrating larvae respectively.
Conclusion: All of the tested drugs failed to inhibit the encysted larvae in the diaphragms. Moreover, *T. spiralis* infection in rats reduced significantly the values of total proteins, and albumin while globulin, urea and creatinine values were significantly increased together with AST and ALT activities. *(Turkiye Parazitol Derg 2011; 35: 86-91)*

Key Words: *Trichinella spiralis*, treatment, avermectins

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ÖZET

Amaç: Antihelmentik etkili olan ve sıklıkla kullanılan Dormectin (0.2 mg/kg), Ivermectin (0.2mg/kg) ve Levamisole'un (7.5mg/kg) antihelmentik etkisi deneysel olarak *Trichinella spiralis* ile enfekte edilmiş sıçanlarda incelenmiştir.
Yöntemler: Toplamda 120 sıçan 500 *T. spiralis* larvası ile enfekte edilmiştir. İlaçlar, enfeksiyondan sonraki (esg) 4. günde erişkin kurtlara karşı, 10. günde göç eden larvalara karşı ve 35. günde enkiste larvalara karşı test edilmiştir. Tedavi sonrası beşinci günde sıçanlar kesilerel erişkin kurtlar ve göç eden larvaların sayısı belirlenmiştir.
Bulgular: Tedavi edilmemiş kontrollar ile kıyaslandığında Dormectin ve Ivermectin ile tedavi edilmiş sıçanlarda ilaçların önemli etkisi olduğu görülmüştür. Dormectin'in erişkin kurtları ve göç eden larvaları ortadan kaldırmada sırasıyla %97.75 ve %86.23 oranında; Ivermectin'in ise %94.99 ve %83.85 oranında etkili olduğu, ancak Levamisole'un %4.83 ve %3.57 oranlarıyla en az etkili ilaç olduğu belirlenmiştir.
Sonuç: Test edilen bütün ilaçların diyaframdaki enkiste larvalara karşı başarısız olduğu görülmüştür. Bundan başka T. spiralis enfeksiyonunun, sıçanlarda globulin, üre ve kreatinin değerlerinde AST ve ALT aktiviteleri ile birlikte önemli oranda artışa yol açtığı, ancak total protein ve albumin seviyelerinde ise önemli oranda azalmaya yol açtığı belirlenmiştir. *(Turkiye Parazitol Derg 2011; 35: 86-91)*

Anahtar Sözcükler: *Trichinella spiralis*, tedavi, avermectins

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INTRODUCTION

Trichinellosis is an important zoonotic disease world-wide caused by ingestion of meat infected with nematodes of genus *Trichinella* (1). *Trichinella spiralis* is the most pathogenic and prevalent species causing disease in man. Eight other species were recorded causing disease in different countries world-wide (2). Human infection with *T. spiralis* is due to ingestion of infected pork. Recently; bears, walruses and h*orses have been reported as i*mportant sources of infection (3). Moreover, some flesh flies (such as *Sarcophaga* species) (4), and cockroach (6), play a role as paratenic? hosts in accidentally transmission of this parasite. Adult worms inhabit the upper part of small intestine, while larvae inhabit skeletal muscles. In rats, development of *T. spiralis* larvae to the adult stage is rapid (4-5 days post infection). Capsule formation in the diaphragm starts 13 days post infection and completed within 5 weeks post infection (7). Diagnosis of trichinellosis is achieved by detection of adult worms in stool during the intestinal phase and by finding the encapsulated larvae in muscle biopsies. Recently, by using PCR technology, great improvement was recorded concerning diagnosis of infection (1, 5). However; only few studies were developed on treatment protocols of infected cases. In this respect, data from the literature are scarce and focused mainly on the efficacy of some antihelminthic drugs against encysted larvae only (8). The need for an effective compounds for the prevention and treatment of trichinellosis in animals and human has led to the testing of other drugs which are clinically effective against most other nematodes. Consequently, this study aimed to evaluate the efficacy of Dormectin, 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:

- Dormectin (Dectomax) was available as 1% injectable solution, Pfizer, Egypt.
- Ivermectin (Ivomee) was provided by MSD AGVET Division of Merk Sharp & Dohme Ltd, Holland as 1.0% injectable solution.
- Levamisole (Pamisol-L) was obtained as 10% injectable solution, Ozzano Emilia-(Bologna) Italy.

Dormectin, Ivermectin and Levamisole were injected subcutaneously in single therapeutic doses of (0.2, 0.2 and 7.5 mg/kg b.wt.) respectively.

Parasite: *T. spiralis* larvae were isolated from the diaphragms of infected pigs obtained from Cairo Abattoir.

Preparation of the inoculums (5): Heavily infected pig diaphragms were cut into small pieces and manually mixed. These pieces were digested with artificial digestion fluid (1%pepsin, W/V & 1% HCL, V/V). By the end of the digestion process, larvae were collected using Baermann technique in phosphate buffer saline (3). Sediment of larvae was 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 albino rats of (150-180 gm.b.wt.) were obtained from The Laboratory Animal Colony, Helwan, Egypt. The animals were parasite free and kept on dry rations 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 with 30 rats in each. The first group was infected but left without treatment as a control. The second, third and fourth groups had single subcutaneous doses of Dormectin, Ivermectin and Levamisole (0.2, 0.2 and 7.5 mg/kg b.wt, respectively). The other 30 rats were kept as the non-infected non treated group.

Experiment was divided into three phases:

- Phase One: Ten rats from groups II,III & IV were given medication at the 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 Two: Ten rats from groups II, III & IV accepted medication at the 10th 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 against migrating larvae.
- Phase Three: Ten rats from groups II, III & IV were treated at the 35th day post-infection. Five rats from all groups were sacrificed 5 days post treatment to detect the effect of anti-helminthic drugs against encysted larvae.

The remaining 5 rats from each group were kept for 30 days after medication. Blood samples were taken from them 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, the 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 water current then opened longitudinally with fine scissors. The intestinal wall was scratched to collect embedded worms in the mucosa. Encysted larvae were diagnosed primarily by trichinoscopy. Diaphragms were then artificially digested (9). Recovered motile larvae were counted using a stereo microscope at low power.

The antihelminthic effect of each drug was evaluated by calculating the mean number of living worms per rat. The total number of larvae in each rat digest was also counted. From these, the mean number of motile larvae per rat was calculated. Moreover, the efficacy of each drug was calculated according to the equation (2):

$$\text{Efficacy \%} = \frac{\text{A-B}}{\text{A}} \times 100$$

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 30 days after each medication from the orbital plexus of the control group and infected rats. Samples were left to clot at room temperature for 20 minutes, then centrifuged at 1500 r.p.m. Obtained sera were collected and used to determine the values of total proteins, albumin and globulin (10). The activities of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) and levels of urea and creatinine were estimated in serum (11).

Differences between means were compared using the student's t- test (12).

RESULTS

1. Effect on mature worms: The mean number of living *T. spiralis* worms per rat (w.p.r) and the efficacy percentage of the tested antihelminthic drugs are shown in Table 1. Mean worm count for control group was greater than 115 (w.p.r) The mean number of living worms in the intestine of rats treated with Dormectin (2.6 w.p.r) and Ivermectin (5.8 w.p.r) was significantly lower than in non-treated control rats (115.8 w.p.r), suggesting a 97.75%, 94.99% and 4.83% efficacy respectively against the parasite . All Dormectin and Ivermectin treated rats harbored dead worms or fragments of dead worms. In the Dormectin-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 w.p.r were 115.8% and 110.2% respectively.

2. Effect on Migrating Larvae: The effect of Dormectin, Ivermectin and Levamisole on the number of migrating *T. spiralis* larvae in diaphragms of rats when injected at the 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 was generally high and all were infected. Compared to the control group, marked reductions in the number of larvae that succeeded in reaching the diaphragms were observed after administration of Dormectin and Ivermectin at the 10th day post-infection (Efficacies were 86.23% and 83.85% respectively). No significant reduction in the number of motile larvae was observed after Levamisole medication, its efficacy was 3.57%

3. Effect on Encysted Larvae: The number of encysted larvae found in diaphragms of control and all medicated groups were generally high (Table 3). Injection of Dormectin, Ivermectin and Levamisole at the 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.

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

Groups	Treatment	Dose (mg/kg b.wt.)	Average No. / rat	Mean No. / rat	Efficacy %
I	Control (Infected Non-treated)	0.0	96-134	115.8 ± 3.39	-
II	Dormectin,	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 were obtained 5 days post treatment, Data of groups II, III and IV were 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	Treatment	Dose (mg/kg b.wt.)	Average No. / rat	Mean No. /rat	Efficacy %
I	Control (Infected Non-treated)	0.0	1106-1578	1326.25 ± 49.46	-
II	Dormectin,	0.2	124-213	182.61 ± 8.9 ***	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 were obtained 5 days post treatment, Data of groups II,III and IV were compared with those of group I, *** = Significant at p< 0.001

4. Effect on Serum Constituents and Enzymatic Activities: The mean value of serum biochemical data of infected and non-infected groups evaluated 30 days post-treatment was shown in (Tables 4-6). The results revealed a highly significant increase in the activities of AST and ALT in the infected non-treated rats. Urea and creatinine levels also showed a highly significant increase over all the experimental periods.

When injected at 4th and 10th days post infection, Dormectin, 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. Dormectin, Ivermectin and Levamisole did not improve the altered parameter in rats injected at the 35th day post infection day.

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

DISCUSSION

Trichinellosis is a zoonotic disease capable of infecting all mammals, including man. Infection has been diagnosed in most parts of the world (1).The severity of the disease, together with lack of an established treatment for that parasite, directed attention towards investigating the antiparasitic effect of Dormectin, Ivermectin and Levamisole on experimental trichinellosis in rats.

Table 3. Effect of the tested Drugs on encysted larvae of *T. spiralis* when injected at 35th day post-infection ('n=5')

Groups	Treatment	Dose (mg/kg b.wt.)	Average No. / rat	Mean No. / rat	Efficacy %
I	Control (Infected Non-treated)	0.0	1212-1492	1366.14 ± 63.85	-
II	Dormectin	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 were obtained 5 days post treatment, Data of groups II, III and IV were 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 by *T. spiralis* in rats ('n=5')

Groups	Treatment	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	Dormectin	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** ± 2.35	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 (Non-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 were obtained 30 days post treatment
Data of groups II, III and IV were compared with those of group I
Data of group I is compared with those of group O
Data of groups II, III and IV were compared with those of group I

* = Significant at p < 0.0,
** = Significant at p < 0.05
*** = Significant at p < 0.001

Table 5. Effect of the tested Drugs when injected on the 10th day post-infection on the impaired serum parameters induced by *T. spiralis* in rats ('n=5')

Groups	Treatment	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 ***	3.62 ± 0.16	***3.02 ± 0.28 ***	1.08 ± 0.11 ***	3.70 ± 4.52 **
II	Dormectin	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 (Non-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 were obtained 30 days post treatment, Data of group I is compared with those of group O, Data of groups II,III and IV were compared with those of group I

* = Significant at p <0.01,
** = Significant at p <0.05,
*** = Significant at p <0.001

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')

Groups	Treatment	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	Dormectin	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	2.09 ± 0.18	2.69 ± 0.18	1.18 ± 0.11	3.86 ± 0.12
V	Control (Non-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 were obtained 30 days post treatment
Data of group I is compared with those of group O
Data of groups II, III and IV were compared with those of group I

* = Significant at p < 0.001

The obtained results indicated that Dormectin and Ivermectin were effective in removing the mature worm burden of *T. spiralis,* while Levamisole remained ineffective. Similar studies reported that cattle and calves were completely cured from nematode infection after treatment with a single dose of Dormectin and Ivermectin respectively (13). The antihelminthic activity of Avermectin could be attributed to modulating GABA-gated chloride channels which are more accessible in nematodes than in vertebrates (14). However, numerous studies reported high efficacy of Levamisole against nematodes in different animals (15). Results obtained from this study confirmed the presence of Levamisole resistance occurring simultaneously in some species of round worms. Many reports described the antihelminthic resistance by nematodes (16). Resistance to Levamisole has only been reported in *Ostertagia ostertagi* (17). This study revealed a high number of migrating larvae that succeeded in reaching the diaphragms of untreated rats. This finding agreed with other studies which reported that the migrating *T. spiralis* larvae were found in the liver prior to arrival into diaphragm (18). However, no larvae were detected in the heart. Moreover, newborn larvae were also isolated from their kidneys. Administration of Dormectin and Ivermectin at the 10th day post-infection revealed

a reduction in the number of larvae that succeeded in encysting 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 significant larvicidal activity (15). No significant difference was detected in the number of migrating larvae between the control and Levamisole-medicated rats. Concerning the effect of drugs on the already encysted larvae; Dormectin Ivermectin and Levamisole failed to reduce their numbers in the diaphragms of infected rats when injected at the 35thday post-infection. This observation is inconsistent with that recorded by other studies (8). 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, ALT, levels of urea and creatinine as compared with the non-infected rats. These changes may be attributed to liver and kidney damage induced during migration of larvae. Hyperactivity of AST and ALT is indicative of hepatic damage, whereas the elevated levels of urea and creatinine in serum reflected the state of glomerular filtration and indicate kidney disease (10). The present findings were supported by those previously recorded by others (19) where they observed lesions in the liver of rats infected with 500 *T. spiralis* larvae. They isolated the larvae from the liver during their migration. Moreover, newborn larvae were isolated from the kidney of rats (18). Marked improvement in the values of the above mentioned parameters towards normal was observed after treatment with Dormectin 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 investigation were experimentally infected with *T. spiralis* only and free from any other parasites, the improvement of the altered parameters after Dormectin and Ivermectin-medications could be satisfactorily ascribed as due to the effect of both drugs on *T. spiralis* only. In rats treated at the 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 (20). Increased amount of globulin is a compensatory reaction to restore osmotic pressure in serum which is reduced as a result of low albumin content. This also may be due to increased formation of antibodies against the parasite or its metabolic products (21). In conclusion, the present study suggested that Avermectins gave the most promising results at dosage levels and formulations. Consequently, Dormectin and Ivermectin can have continued practical application in controlling *T. spiralis* infection in animals.

Conflict of Interest

Authors declare that there is no conflict of interest.

REFERENCES

1. Atterby H, Learmount J, Conyers C, Zimmer I, Boonham N and Taylor M. Development of a real-time PCR assay for the detection of *T. spiralis* in situ. Veterinary Parasitology 2009; 161: 92-8. [CrossRef]
2. Hosking B, Watson T and Leathwick D. Multigeneric resistance to oxfendazole by nematodes in cattle. Vet Ree 1996; 138: 67-8. [CrossRef]
3. Dupouy Camel J, Soule C, Ancelle T. Recent news on trichinellosis: another outbreak due to horsemeat consumption in France in 1993. Parasite 1994; 2: 99-103.
4. Maroll M, and Pozio E. Influence of Temperature on the survival and infectivity of *T. spiralis* larvae in Sarcophaga argyrostoma (Diptera, Sarcophagidae) Maggots. J Parasitol 2000; 86: 633-4.
5. Guenther S, Nöckler K, von Nickisch-Rosenegk M, Landgraf M, Ewers C, Wieler LH et al. Detection of *T. spiralis*, *T. britovi* and *T. pseudospiralis* in muscle tissue with real-time PCR. J Microbiological Methods 2008; 75: 287-92. [CrossRef]
6. Chan OT, Lee E K, Hardman J M and Navin J J, 2004. The cockroach as a host for *Trichinella* and *Enterobius vermicularis*: implications for public health. Hawaii Med. J 63 March 2004; 74-7.
7. Teppema JS, Robinson JE, Ruitenberg E. Ultra structural aspects of capsule formation in *Trichinella spiralis* infection in the rat. Parasitol 1973; 66: 291-6. [CrossRef]
8. El-Azzouni M Z. Effect of Ivermectin on experimental trichinosis. J Egypt Soc Parasitol 1997; 27: 331-40.
9. Melvin DM, Brooke MM. Laboratory Procedures for the Diagnosis of Intestinal Parasites. 3rd. ed. Centre for Diseases Control 1982; 8282.
10. Gamble H, Wisnewski N and Wasson L. Diagnosis of trichinellosis in swine by enzyme immunoassay using a synthetic glycan antigen. A J V R 1997; 58: 1417-21.
11. Coles E. Veterinary Clinical Pathology. 2nd Ed. Philadelphia, London.
12. Toshiaki O, Keiji M, Fumiko M and Kazuhiro L. Evaluation of fluorimetrically estimated serum bile acid in liver disease. Clinica Chimica Acta 1977; 75: 81-90. [CrossRef]
13. De Muth JE. Overview of biostatistics used in clinical research. Am J Health Syst Pharm 2009; 66: 70-81.
14. Barton N, Mitchell P, Hook F and Reynolds J. The therapeutic efficacy and prophylactic activity of Dormectin against *Dictyocaulus vivparus* in cattle. Aust Vet J 1995; 72: 349-51. [CrossRef]
15. Arena JP, Liu K, Paress P and Cully D. Expression of a glutamate-activated chloride current in Xenopus oocyst injected with *Caenorhabditis elegans* RNA: evidence for modulation by Avermectin. Mol Brain Res 1992; 15: 339-48. [CrossRef]
16. Anderson N, Martin PJ, Jarrett RG. The efficacy of mixtures of albendazole sulphoxide and levamisole against sheep nematodes resistant to benzimidazole and levamisole. Aust Vet J 1991; 68: 127-32. [CrossRef]
17. Borgsteede F. Further studies with a strain of *Ostertagia ostertagi* resistant to morantel tartarate. Int J Parasitol 1991; 21: 867-70. [CrossRef]
18. Osuga T, Mitamura K, Mashige F, Imai D. Evaluation of fluorimetrically estimated serum bile acid in liver disease. Clin Chim Acta 1977; 15; 75: 81-90.
19. Mikhail E. The occurrence of *T. spiralis* larvae in tissues other than skeletal muscles. J Egypt Soc Parasitol 1979; 9: 269-72.
20. Mikhail E, Milad M and Sabet S, 1978. Experimental trichinosis: A pathological study of hepatic, renal and gonadal involvement. J Egypt Public Health Assoc 1978; 53: 327-31.
21. Reinhold JG. Chemical Evaluation of the functions of the liver. Clin Chem 1955; 1: 351-421.