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Assessment of Antibilharzial and Biochemical Effects of Triclabendazol in Experimental Schistomiasis

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Abstract: Schistomiasis is one of the major public health problems, and for more than two decades Praziquntel (PZQ) has remained the drug of choice for its treatment. However, studies proved that reliance on one drug raised the concern of development of tolerance or even resistance. The present work aimed at studying the effect of different schedules of the flukicidal drug triclabendazole (TCBZ), in a dose of 120mg/kg body weight, on *Schistoma mansoni* worm load, female fecundity and egg deposition in liver and intestine of infected mice, and at studying its biochemical toxic effects on some liver enzymes activities.The present findings indicated that the administration of TCBZ to mice infected with Egyptian *S.mansoni* strain was not effective except when the drug was given after the start of egg shedding in the stools. However, the antischitosomal effect was moderate as the results showed 50% reduction in worm burden and around 40% reduction in liver and intestinal egg loads. On the other hand, the impact on oogram pattern was not clear except regarding the percentage of dead ova which was much higher than the corresponding control. As regards the biochemical parameters studied, no change in the activity of all tested enzymes was observed. However, animals which received two doses of the drug after the start of egg shedding exhibited 24% reduction in alanine transaminase (ALT) activity. In conclusion, our results indicated that, still, there is no alternative to prazquantel and there is an urgent need for discovery of new antischistosomal drugs. In addition studying different combinations and schedules of the already available drugs as artemether and TCBZ is recommended.

INTRODUCTION

Schistosomiasis is one of the major public health threats. More than 600 million people worldwide are at risk of infection, and close to 200 million are actually infected.(1)

Chronic morbidity is the major impact of schistosomiasis on ill health. This is due to repeated infection and development of non-fatal but debilitating sequelae, ranging from subtle to severe morbidity specific to

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schistosomiasis as hepatic fibrosis and urinary obstructions[2] .

Since morbidity is caused by eggs of Schistosoma deposited in tissues, a reduction or elimination of the adult worms will reduce the risk of morbidity development. Thus the main tool for the recent strategy of schistosomiasis control is chemotherapy as currently recommended by WHO [3]. For more than two decades, praziquantel (PZQ) has remained the drug of choice for the treatment of the three common Schistosoma species, *Schistosoma mansoni, Schistosoma haematobium* and *Schistosoma japonicum.* Usually PZQ chemotherapy of *S.mansoni* using the standard dose of 40 mg/kg body weight[3,4]. results in cure rates of more than 70% and mean egg count reduction rates of more than 95%, after six to twelve weeks post treatment.

However, interestingly and unexpectedly, low cure rates have been reported from Northern Senegal. Initial studies there demonstrated a cure rate of only 18%. Also, in Egypt cure rates lower than normal were recorded from *S.mansoni* infected patients who were treated with the recommended single oral dose of PZQ.[5,6] Thus, reliance on a single drug has raised considerable concern that tolerance or even resistance to the drug might develop and there are a continuing need for new antischistosomal drugs[5,7].

Triclabendazol (TCBZ) is a benzimidazole antihelminthic reported to have a specific flukicidal effect against mature and immature stages of *Fasciola hepatica (F. hepatica)* and *F. gigantica* in small and large domestic ruminants[8]. TCBZ was tested in vitro and *in vivo* against a range of helminths. Although *in vitro* activity was found against *Hymenolepis diminuta* (0.5 mg/ml), *Fasciola hepatica* (2.5 mg/ml), *Taenia crassiceps,* and *Schistosoma mansoni* (61 mg/ml), yet , *in vivo* activity was only found

against *F.hepatica.* A single oral dose of 40 mg / kg killed 99% of adult flukes in the rat. This spectrum of activity suggests a mechanism of action unlike that of other benzimidazole anti-helminthics[8-10].

When TCBZ was tested against *S.mansoni* in mice, controversial results were reported. Khalil (2000) and El-Sayad *et al.,* (2001) recorded that with oral administration of single dose of 120 mg/kg , worm reduction ranged between 84% and 87%. Male worms were more susceptible to the drug than females. TCBZ induced cessation of egg laying. Also, the drug had no prolonged toxic effect on hepatocytes and was expected to be useful in treatment of human schistosomiasis[11,12].

In contrast, Keiser *et al.,* (2006) reported that with the administration of TCBZ in a single oral dose of 120 mg/kg, 3 days before infection of mice with S.mansoni or double doses of 120 mg/kg 7 weeks after infection, the drug failed to significantly reduce intestinal tissue egg loads and eggs of all developmental stages were observed. They concluded that TCBZ displays weak and inconsistent antischistosomal activity[13].

In view of the facts that there is an urgent need for schistosomicidal drugs rather than praziquantel and that TCBZ in the tested schedules gave inconsistent results, while it proved to be effective against both mature and immature Fasciola hepatica. Thus, this study was designed to test the efficacy of TCBZ on different stages of maturation of *S.mansoni* worms. Also, to study the biochemical toxic effects of TCBZ on some serum enzymes activities in mice infected with *S.mansoni* .

MATERIAL AND METHODS

Female CD –1 Swiss albino mice, 8 weeks in age, from 18-22 g in weight, infected percutaneously by *S.mansoni* cercaria (100 cercariae/mouse) , were supplied by the Schistosome Biology Supply Center, Theodore Biliharz Research Institute (Giza, Egypt) . The mice

were divided into 8 groups , each one consisted of 10 mice as follows :

Triclabendazole groups (TCBZ): TCBZ was administered orally in a dose of 120 mg/kg to 3 subgroups in the following schedules:

Group I: Received 3 doses on days 7 , 21, and 35 post infection (PI)

Group II: Received 3 doses on days 21 , 35, and 49 PI .

Group III: Received 2 doses on days 49 and 50 PI .

Praziquantel groups (PZQ): PZQ was administered orally in a dose of 600 mg/kg to 3 subgroups in the following schedules :

Group IV: Received a single dose on day 35 PI .

Group V: Received a single dose on day 49 PI .

Group VI: Received two doses on days 49 & 50 PI .

Control groups: 2 subgroubs (VII and VIII) infected untreated mice matching infection schedules of both TCBZ and PZQ.

Control uninfected, untreated (IX): This group consisted of 5 mice which were not infected and did not receive any treatment. It was used as control for biochemical tests.

Drugs :

Triclabendazole: a benzimidazole derivative (Fasinex suspension) was obtained from a local pharmacy.

Dose: 120 mg/kg body weight[11].

Praziquantel (Distocide suspension) was provided by Ministry of Health and Population (MOH & P) .

Dose: 600 mg/kg body weight[14].

Drugs were administered orally using a stomach tube .

I. Parasitological study

* Stool examination Started 35 days post infection and continued daily until mice were sacrificed. Number of eggs per gram stool was calculated for each group of

mice[15].

* Perfusion of mice. Mice were killed by cervical dislocation after two weeks from the last treatment. Worms were recovered from the hepatic and portomesenteric vessels using the perfusion technique[16]. Worms were counted and sex was identified.

* Oogram pattern. After perfusion, the small intestine was separated. Three pieces (each of 1 cm in length) of the small intestine were cut, opened longitudinally, rinsed in saline, slightly dried on filter paper and then placed between two slides .The fragments were examined by low power microscopy. The stages of egg development were recorded and the mean number of various stages was calculated for each mouse.[17-19]

* Tissue egg load the number of eggs/g tissue was determined by weighing 0.3 g of the liver and intestine from each mouse and digesting it overnight in 5 ml KOH (5%). After complete digestion, the samples were vortexed and 3 aliquotes of 100 µl each were examined microscopically and ova were counted [20].

II Biochemical study

Serum alkaline phosphatase activity (ALP), aspartate transaminase (AST), alanine transaminase (ALT), and total protein content from all groups of mice were determined.[21-23]

III Statistical analysis

Drug efficacy was assessed by comparing the mean number of total worms in any treatment with that of the respective control group. Differences were tested for significance using an unpaired two tailed student t–test, allowing for unequal variance. The data were considered significant if $p<0.05$. Statistical analysis was done with version 11.5 of the Statistical Package for Social Sciences (SPSS).

RESULTS

The results obtained in different groups were compared with those of the

corresponding control groups as shown in table 1; group I was compared with control group VII and groups II & III were compared with group VIII. The total worm burden in groups II & III showed reduction when compared with the control (35.4% & 49.3%, respectively). On the contrary, there was an increase of 33.8 % in group I when compared with its corresponding control.

On the other hand, when the groups which received PZQ were compared with the control, group IV exhibited 45.9% reduction while groups V & VI showed 89.9% & 100% reduction, respectively.

As regards male to female ratio of collected worms, it was 1.6 : 1 & 2.3 : 1 in groups II & III, respectively compared to 1: 1 in their control group and 2.8:1 in the corresponding PZQ group (V). In the group which received TCBZ when harboring either immature worms or mature worms before egg laying (group I), the male to female ratio was exactly equal to its control group [1.3:1].

The distribution of *S.mansoni* worms indicated that the majority were collected from the portomesenteric vessels of mice in groups I& II (79% & 70.9%, respectively) which is almost the same as the control group (68%). In group III, 53% only of the collected worms were from the portomesenteric vessels which are lower than its control group (76%).

As regards the groups which received PZQ, the percentage of portomesenteric worms was much lower than the control especially when PZQ was administered 49 days post infection. When PZQ was given in two doses 49 and 50 days PI, no worms were detected in the dissected mice whether in the liver or the portomesenteric vessels .

Table 2 shows the mean number of eggs per gram of liver and intestine of the dissected mice in addition to the percentage of the developmental stages of *S.mansoni* ova deposited in the wall of the

intestine. When the results of the groups which received TCBZ were investigated, it was found that the mean number of ova/gram liver in group I was equal to the corresponding control or even more than its control (group III).

On the other hand, in group III, the mean number of ova/gram liver and intestine (4.4 $\times 10^3$ & 6.8 $\times$ 10^3, respectively) were much lower than the corresponding control group (7.8 $\times$ 10^3 & 11.8 $\times$ 10^3 respectively). All groups treated with PZQ showed significantly lower mean number of ova/gram tissue than its control (1.7, 2.3 & 2.4 $\times$ 10^3 in the liver & 0.6, 0.9 & 1.5 x 10^3 in the intestine in groups IV & V & VI, respectively).

As regards the oogram pattern of ova deposited in the wall of the intestine of the infected mice, the percentage of dead ova in group I (which received TCBZ while harboring immature *S.mansoni* worms & mature worms before egg laying) was lower (16.8%) than the corresponding control (27.5%). On the contrary, when TCBZ was administered to mice harboring mature worms after the start of egg laying (groups II & III), the percentage of dead ova was higher than that in their control (13.2 %, 16.3 % compared to 7.7 %, respectively). The mice treated with PZQ showed significantly higher percentage of dead ova than their controls whatever the stage of development of *S.mansoni* worms was and the day the drug was administered .

Table 3 shows the effect of TCBZ administration on some biochemical parameters. When the results of both groups VII and VIII (infected untreated control) were compared to that of uninfected untreated mice (group IX), an observed increase in all liver function tests (ALT, AST, and ALP) and total protein content were found in the infected groups.

As regards group I, which received TCBZ while worms were immature or mature before egg laying; generally, no

change in the level of all tested biochemical parameters was observed when compared with corresponding controls (group VII). The same results were observed when another dose of TCBZ was administered 49 days PI after egg shedding (group II), when compared to the control mice (group VIII). On the other hand, in group III which received two doses of TCBZ on days 49 and 50 PI after the start of egg shedding, there was no change in the level of liver enzymes except that a reduction of 24% was detected in the activity of ALT (13.6 ±31 μ/L) when compared to its control group VIII (18.0 ± 2.1 μ/L).

As regards the groups which received PZQ (groups IV, V, and VI), a clear reduction was observed in the activity of all tested enzymes (ALT, AST, and ALP) and total protein content. In addition, the level of the tested parameters reached the normal value obtained in mice of group IX (uninfected untreated group).

DISCUSSION

In this study, we used the fasciocidal drug triclabendazole at the dose of 120 mg/kg body weight at different schedules to study its effect on *Schistosoma mansoni* worm load, fecundity of female worms, and egg deposition in the liver and intestine of infected mice.

Concerning the effect of TCBZ administered at days 7, 21, and 35 post infection (Group I), there was no reduction in worm burden (-33.8%). The male to female ratio was equal to that in the control group (1.3:1). Both the hepatic and intestinal tissue egg loads were not affected. Moreover, there was no notable effect of TCBZ on the percentage of different stages of egg maturation. On the contrary PZQ (group IV) showed clear reduction in total worm burden (45.9%). The male to female worm ratio was higher (2.5:1) than the control group. PZQ showed a clear reduction in both liver and intestinal tissue egg loads. Also, the percentage of

immature eggs decreased while that of mature and dead eggs increased.

From these results, it could be concluded that 7, 21, and 35 days old *Schistosoma mansoni* worms were not susceptible to TCBZ. This time span exactly covers the period of worm development from the lung form to the time before maturation and starting oviposition at days 34-35 post infection.

As regards the effect of TCBZ administered to mice harboring mature *S.mansoni* worms (groups II and III), the drug induced worm burden reduction in both groups that amounted to 35.4% and 49.3%, respectively. Both groups showed a higher male to female ratio especially group III which received TCBZ at two doses, 49 and 50 days PI (2.3:1) as compared to the control group (1:1). Hepatic shift was notable in group III as 53% of worms were collected from the portomesenteric region compared to 76% in the control group. The administration of TCBZ, 49 days post infection showed an inconsistent effect on tissue egg load when compared with its control, an increase of 29.5% in liver and a reduction of 17% in the intestine, was obtained in this group. Surprisingly, the administration of another TCBZ dose on day 50 PI induced clear reduction in both liver (40.5%) and intestinal egg loads (42 %).

Furthermore, the changes in oogram pattern were consistent with the previous findings. In group I which received TCBZ while worms were immature or mature before egg laying, the percentage of immature ova was 40% higher than the control and that of dead ova was lower by 35% than the control denoting that the administration of TCBZ before worms start egg laying has no antischistosomal effect.

When another dose of TCBZ was administered 49 days PI after egg shedding (group II) a 20% reduction in the percentage of immature ova was observed when compared to the control; while the

percentage of dead ova increased by 70%

The administration of two doses of TCBZ on days 49 and 50 PI (group III), induced a marked effect on the oogram pattern as treated mice exhibited a two times increase the percentage of dead ova.

Regarding PZQ administration 49 days PI (group V), it showed a marked effect, as expected, on worm load reduction , (89.9%) which increased to reach 100% reduction when a second dose was given on the second day at 50 days PI. The drug was more lethal to female *S.monsoni* worms. Moreover, groups V & VI exhibited a highly significant reduction in the number of ova/gram liver and intestine. The oogram pattern in both PZQ groups (V&VI) indicated that the mean percentage of dead ova was significantly higher (73.0±28.2 & 97.1±8.2) than the control group VIII (7.7±2.0) while mature and immature ova were significantly lower.

The findings of the present study were different from the results obtained by Keiser et al., (2006), who assessed the effect of TCBZ and its two main metabolites against two different strains of *S. monsoni* harbored in mice. They reported that oral administration of TCBZ caused limited reduction in total male and female worm burdens, and no significant reductions in either the hepatic or intestinal tissue egg loads were observed. In addition, eggs of all developmental stages were present in mice treated with TCBZ. Furthermore, eggs collected from fecal samples of treated mice were able to hatch and develop active miracidia.[13]

The present findings indicate that the administration of TCBZ to mice infected with Egyptian *S.mansoni* strain was not effective except when the drug was given after the start of egg shedding in the stools. However, the antibilharzial effect was moderate as the results showed 50% reduction in worm burden and around 40% reduction in liver and intestinal egg load.

On the other hand, the impact on oogram pattern was not clear except regarding the percentage of dead ova which showed clear reduction when compared to the corresponding control.

In contrast to our findings Khalil (2000) who used the same *S*. *mansoni* strain reported excellent antischistosomal effect. She reported a total worm reduction of 84% after administration of a single oral dose of 120 mg/kg TCBZ to mice harboring an Egyptian strain of *S.mansoni.* She also, reported that the schistosomicidal effect of TCBZ was more observed on male than on female worms in contrast to our findings. Four weeks after TCBZ treatment she noted a clear tissue egg load reduction which amounted to 68% in the liver and 74% in the intestine. The oogram of TCBZ treated mice showed progressive disappearance of immature eggs that by the end of the fourth week PI, no immature eggs were detected indicating complete cessation of egg laying.[11]

As regards the biochemical parameters studied, infected untreated control groups showed a clear increase in all liver function tests (ALT, AST, and AP) and total protein content as compared to uninfected untreated control mice (group IX) reflecting a degree of liver damage due to schistosomiasis. This was also documented by Ghanem *et al.,* (1970) and Mahmoud *et al.,* (2002)[24,25]. In general, all groups treated with TCBZ showed no change in all biochemical tests when compared with corresponding controls. However, group III (animals receiving two doses of the drug after the start of egg shedding) exhibited 24% reduction in ALT which reached a level almost equal to that of the control group (IX). The drug prevented liver damage by eliminating the parasite burden from the liver, thus avoiding liver cell damage

These results were in agreement with Moreno *et al.,* (1997) who reported that treatment of fascioliasis in goats by TCBZ

at fourth week of infection induced reduction in lactate dehydrogenase (LDH) and AST levels, as well as reduction of Gamma-glutathione transferase (GGT) level. At 8 weeks PI, treatment only prevented bile duct lesions, and thus avoided increased GGT levels. When, treatment was delayed 16 weeks PI, no effect on serum GGT, LDH, and AST levels was observed, since hepatic regeneration had by then already taken place, and serum enzyme levels had thus returned to normal.[26]. El-Morshedy *et al.,* (1999) observed that liver function tests (ALT, AST, and serum bilirubin) were within normal among human fascioliasis patients before and after treatment with TCBZ.[27]

The group which received PZQ exhibited a reduction in the levels of ALT, Alkaline phosphatase and total proteins which almost reached normal levels obtained in normal mice (control uninfected untreated group). This indicates that the death of *S.mansoni* worms and the cure of mice from infection resulted in regeneration of the liver and that most biochemical reactions returned to their normal values.

In view of our results regarding triclabendazole, still, there is no alternatives to praziquantel and there is an urgent need for discovery of new antischistosomal drugs in addition to studying different combinations and schedules of the already available drugs as artemether and TCBZ.

Table 1 : The Effect of TCBZ administered at different schedules on mice harboring immature and mature *S.mansoni* stages, on worm burden , distribution and sex:

Groups	Schedule of treatment (days post infection)	Number of mice	Mean number of worms + SD							Total worm burden-reduction (%)
			Hepatic worms	Porto-mesenteric worms	% Porto-mesenteric worms	Total males	Total females	Male to female ratio	Total worms	
TCBZ120 mg/kg										
I	7,21,35	9	4.0 ±1.5	15.8 ± 9.7	79	11.3 ± 5.2	8.4 ±5.3	1.3 :1	19.8 ± 9.6	-33.8
II	21,35,49	5	5.4± 3.8	13.2± 5.1[b]	70.9	11.6± 3.6	7.5±1.9[b]	1.6 :1	18.6± 5.1[b]	35.4
III	49,50	9	6.8± 2.6	7.8± 3.4[b]	53	10.2± 4.6[a]	4.4±1.3[b]	2.3 :1	14.6± 5.7[b]	49.3
PZQ 600 mg /kg										
IV[1]	35	4	3.8± 2.2	4.3± 1.3	53	5.8± 2.2	2.3± 1.3	2.5 :1	8.0± 3.5	45.9
V[2]	49	8	2.3±2.5[b]	0.63± 1.2[b]	21	2.1± 2.**5**[b]	0.75±1.1[b]	2.8:1	2.9± 3.**3**[b]	89.9
VI[3]	49,50	10	0.0±0.0[b]	0.0± 0.0[b]	0	0.0± 0.0[b]	0.0±0.0[b]	0:0	0.0± 0.**0**[b]	100
Control infected untreated	-									
VII[1]		8	4.6± 5.2	10.1± 5.0	68	8.5± 4.3	6.3± 4.1	1.3:1	14.8± 7.5	
VIII[2,3]		8	6.8± 3.1	22.5± 3.6	76	14.6± 2.8	14.13±2.7	1:1	28.8± 3.7	

a: P < 0.05 b: P < 0.001

[1]Controls for group I
[2]Controls for group II
[3]Controls for group III

Table 2 :The effect of TCBZ administered at different schedules on mice harboring immature and mature *S.mansoni* stages , on tissue egg load and oogram pattern:

Groups	Schedule of treatment (Days post infection)	Mean eggs per gram of tissue x 10^3 (±SD) Hepatic	Intestinal	Mean % egg developmental stages Immature 1st	2nd	3rd	4th	Total % immature	Mature	Dead
TCBZ 120 mg/kg										
I	7,21,35	6.9 ± 4	11.0 ± 8.9	3.7±.3.7	8.5± 4.1	9.3±8.1	28.7± 12.9	50.1± 20.7	33.0± 7.5	16.8±21.1
II	21,35,49	10.1 ±6.7	9.8 ±.7.6	6.0± 4.8	8.3± 5.3	9.7± 10.2	22.8± 3.2	46.3± 11.3	40.5± 4.5	13.2± 9.5
III	49,50	4.4 ±1.6	6.8 ±.3.6	6.4± 4.1	12.1±2.2	11.7± 6.9	26.9± 9.8	57.6± 14.5	26.7± 9.8	16.3±11.6
PZQ 600 mg /kg										
IV[1]	35	1.7± 0.6	0.6± 0.4[a]	0.0± 0.0	0.0± 0.0	0.0±0.0	17.4±12.1	17.4±12.0	39.0±17.4	43.6±24.2
V[2]	49	2.3± 2.2[b]	0.9± 1.0[b]	0.0± 0.0	2.1± 5.9[a]	1.4+2.9[b]	5.0±13.0[b]	3.3± 8.8[b]	18.1±21.2	73.0±28.2b
VI[3]	49,50	2.4± 2.1[b]	1.5± 1.6[b]	0.0±0.0[b]	0.0± 0.0[b]	0.0+0.0[b]	0.0±0.0[b]	0.0± 0.0[b]	2.9±8.2b	97.1±.8.2b
Control infected untreated										
VII[1]	-	7.4± 2.9	10.7± 7.8	1.5± 2.1	2.4± 6.0	5.0± 5.9	23.8± 17.7	35.8± 20.2	36.7±13.4	27.5± 1.1
VIII[2,3]		7.8± 3.6	11.8± 6.6	7.1± 4.3	10.8± 7.5	19.5± 2.1	23.8± 5.2	58.6± 4.2	33.7± 3.8	7.7± 2.0

a : P < 0.05 b: P < 0.01

[1]Controls for group I
[2]Controls for group II
[3]Controls for group III

Table 3: The effect of TCBZ administered at different schedules on mice harboring immature and mature *S.mansoni* worms , on some biochemical parameters

Groups	Schedule of treatment (days post infection)	Number of mice	Glutamtic-pyruvic transaminase (ALT) Test (U/L)	Glutamtic-oxaloacetic transaminase (AST)Test (U/L)	Alkaline- phosphatase test (U/L)	Total protein test (g/dl)
uninfected untreated Control **IX**	-	5	12.6±2.2	13.0±3.5	83.0±5.5	7.6±1.1
TCBZ **120 mg/kg**						
I	7,21,35	3	17.0±4.0	18.3± 2.1	88.2± 4.3	9.2± 1.5
II	21,35,49	3	17.3±5.2	18.7± 2.5	91.2± 11.5	9.6± 2.3
III	49,50	3	13.6±3.1	17.3± 3.8	91.3± 13.2	8.9±1.7
PZQ **600 mg /kg**						
IV[1]	35	3	12.3 + 4.0	17.7 + 2.1	79.1± 8.4	6.9 ± 0.9
V[2]	49	3	12.6 + 3.1	15.3 + 2.1	74.5 ± 9.6	8.1 ± 0.9
VI[3]	49,50	3	15.4 + 3.5	17.7+ 4.2	81.7 ± 10.4	7.5 ± 1.4
infected untreated Control						
VII[1]	-	3	18.0 ± 2.6	16.7± 2.1	93.0 ± 6.4	9.6 ± 0.9
VIII[2,3]		3	18.0 ± 2.6	16.7± 2.1	93.0 ± 6.4	9.6 ± 0.9

[1]Controls for group I
[2]Controls for group II [3]Controls for group III

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