Parasitological evaluation of Ro 15-9268, a 9-acridanone-hydrazone derivative against *Schistosoma mansoni* in mice, and observations on changes in serum enzyme levels

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Abstract *Schistosoma mansoni* is one of the major causes of schistosomiasis prevalent in tropical and subtropical areas, especially in poor communities. It is estimated that at least 90 % of those requiring treatment for schistosomiasis live in Africa. The primary control strategy employed for schistosomiasis is mass drug administration (MDA). The aim is to reduce disease through treatments with a single lower dose of Ro 15-9268 as a new antischistosomal drug. In the present search, the efficacy of Ro 15-9268 was studied in mice using a dose of 12.5 mg/kg of body weight (b.wt.) against an Egyptian strain of *S. mansoni*. This was carried out at 2 days and 3, 4, and 6 weeks post–cercarial exposure of mice. The criteria used were the worm load, oogram pattern and number of ova in the liver and intestine, hepatic enzyme activity, and liver histopathology. The tested agent has led to a significant reduction in worm burden (89.80 %) in liver and portomesenteric veins concurrent with a hepatic shift at the second week posttreatment followed by a complete disappearance of worms, 4 weeks postmedication. The oogram of infected animals treated revealed an increased number of dead ova 2 days posttreatment and complete absence of immature and mature ova 2 weeks later. The hepatic and intestinal egg counts significantly declined by about 96 and 98 %, respectively, 6 weeks after treatment, and the fecal egg count completely disappeared from stool 4 weeks after medication. The hepatic histopathological changes were improved, ova were markedly degenerated, and worms showed fragmentation and degeneration after drug administration. In conclusion, when Ro 15-9268 was administered to mice infected with the Egyptian strain of *S. mansoni*, at a low dose level (12.5 mg/kg b.wt.), encouraging results were obtained. The drug showed high efficacy against schistosomal worms as well as histopathological inflammatory changes.

Introduction

Schistosomiasis is an important parasitic disease in many parts of the world and has affected the course of human history many times over (Gonnert and Andrews 1977; Becker et al. 1980). It has significant economic and public health consequences in many developing countries and affects the improvement of living standards (Nour 2010). Globally, an estimated 700 million people are at risk of infection with schistosomiasis in 76 countries (WHO 2010), and it causes more than 200,000 deaths per year (Van der Werf et al. 2003). Furthermore, the impact of the severe and debilitating effects of schistosomiasis accounts for the loss of 4.5 million disability-adjusted life years annually (Steinmann et al. 2006).

Chronic complications with schistosomiasis are generally developed in individuals harboring a high parasite load (van de Vijver et al. 2004). In general, complications usually involve organ damage including cardiopulmonary and gastrointestinal organs, urinary tracts, liver, and spleen (Nour 2010; Sripa et al. 2010; Negrão-Corrêa et al. 2012). Furthermore, a study by Oakeshott et al. (2010) also found urinary schistosomiasis to be a significant contributor to bladder cancer. Other frequent complaints are neurological effects as recorded by Li et al. (2011). Moreover, epidemiological studies reported a

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high prevalence and incidence of HCV, particularly within families in rural areas endemic for schistosomiasis (Shadan 2008; Wilson et al. 2011). Although enormous progress has been made in the control of schistosomiasis by international, national, and private agencies, effective absolute permanent results have not yet been achieved. Till now, the treatment and prevention of schistosomiasis is through either sanitation, snail eradication, or chemotherapy. The latter is predominantly considered as the most effective and safe (Staudt et al. 1992; Harder 2002) starting from the early periods, in spite of the unquestionable value of various modern drugs against *Schistosoma mansoni*. Nowadays, the most used drugs against different species of the genus *Schistosoma* are tartar emetic (Christopherson 1918), hycanthone (Pellegrino et al. 1967), niridazole (Lee et al. 1971), oxamniquine (Foster 1973), metrifonate (Omer and Teesdale 1978), oltipraz (Abaza et al. 1984), artemether (Mahmoud and Botros 2005), and praziquantel (Mehlhorn et al. 1983; Idowu et al. 2007; Silveira-Lemos et al. 2013). However, different problems were frequently encountered after the treatment with these drugs, like toxic effect (Shadan 2008), carcinogenicity (Dissous and Grevelding 2011), and strain resistance (Xu et al. 2005). These comments strengthen the need for increasing the researches on experimental chemotherapy, aiming at finding more efficacious new antischistosomal drugs like 9-acridanone hydrazine derivatives.

Hydrazones have been demonstrated to possess, among other, antimicrobial, anticonvulsant, analgesic, anti-inflammatory, antiplatelet, antitubercular, and antitumoral activities (Zulkepli et al. 2011). Moreover, 9-acridanone-hydrazones proved to be active against *Schistosoma* as reported by Sulaiman et al. (1989) and Guirguis (2003). In addition, the derivative Ro 15-8843 was tested against the Puerto Rican strain of *S. mansoni* in baboons (Andrews et al. 1983; Sturrock et al. 1987). Yet no data are found concerning the activity of such compounds against the Egyptian strain of *S. mansoni*.

The present study was suggested to assess the efficacy of the derivative Ro 15-8843, at a lower dose level (12.5 mg/kg), against the Egyptian strain of *S. mansoni* in mice by the use of parasitological, hematological, biochemical, and histopathological criteria.

Materials and methods

Experimental animals

Swiss male albino mice (20–25 g) were used in the present study. The animals were housed in plastic cages in a temperature-controlled room and provided a free access to a standard basal diet and water.

Infection

Infection of mice with cercariae of *S. mansoni* (Egyptian strain) was carried out using a tail immersion technique (120 ±cercariae/mouse) according to Oliver and Stirewalt (1952). Seven weeks postexposure to cercariae, the stools of mice were examined for the presence of ova. The infected animals are then separated and allowed for the experimental studies.

Drug

The drug chosen for the present study is Ro 15-8843. It was supplied by Hoffmann-La Roche Co. Ltd. (Basel, Switzerland), as a yellow water-insoluble powder. It was kept in darkness at 5 °C until use. A stock of the drug was prepared as a suspension in 1 % cremophor EL glycerol. It was orally administered to the infected animals as a single dose (12.5 mg/kg of body weight (b.wt.)) using esophageal tube. This dose was chosen to be lower than those doses evaluated by Sturrock et al. (1987) against the Puerto Rican strain of *S. mansoni* in baboons.

Animal grouping

Mice were randomly divided into four main groups: (I) control (neither treated nor infected, (II) infected, (III) infected–treated (once 7 weeks after infection), and (IV) normal–treated (orally administered Ro 15-9268 once). Each group was subdivided into four subgroups to be sacrificed 2 days and 2, 4, and 6 weeks posttreatment (or 7, 9, 11, and 13 weeks postinfection).

Parasitological studies

To determine the worm burden and distribution, worms were recovered from the liver and portomesenteric veins of sacrificed mice using the perfusion technique adopted by Pellegrino et al. (1967). To construct the oogram, 100 eggs were examined in each of three fragments cut from the distal part of the small intestine after opening and cleaning. For the determination of egg count per gram tissue, weighed pieces of liver and intestine were digested in 4 % KOH solution and incubated at 37 °C for 24 h and then examined under the microscope using counting chambers. The Kato thick-smear technique was applied for the estimation of ova count per gram of stool of infected mice (Foster 1973).

To count the schistosomal ova in the stool of mice, the Kato thick-smear technique was applied using the method depicted by Martin and Beaver (1968). Intestinal oogram was determined according to Pellegrino and Siqueira (1956).

Histological studies

For the histopathological study, pieces of liver samples were immersed in 10 % formalin solution and then allowed for histological preparation with hematoxylin-and-eosin stain and Masson trichrome stain for demonstration of collagen fibers.

Statistical analysis

Statistical analysis was carried out using t-test and analysis of variance (ANOVA) according to Bailey (1995).

Results

Treatment of infected mice with the tested drug, at the dose level 12.5 mg/kg b.wt., caused a significant reduction in the number of ova excreted per gram of stool ($P < 0.001$; Table 1). The mean number of schistosomes in both liver and portomesenteric veins ($P < 0.01$), at the second day posttreatment, was also reduced. At the same testing period, the number of coupled schistosomes, in the liver and portomesenteric veins, dwindled from 21.67 to 14.33 ($P < 0.05$) and from 21.00 to 1.67 pair/mouse ($P < 0.001$) at the second day and second week posttreatment, respectively. Eventually, no schistosomes were recovered from the liver or the portomesenteric veins by the perfusion technique starting from the fourth week posttreatment (Figs. 11, 12, and 13).

Due to the treatment with the tested drug, the oogram of the small intestine of *S. mansoni*-infected mice was remarkably altered (Table 3). Just 2 days after treatment, the relative number of dead ova was significantly ($P < 0.001$) increased from 2.00 to 11.17 %. Starting from the second week posttreatment, neither the immature nor mature ova appeared, nor was the relative number of dead ova reduced to 100 % (Table 2). On the other hand, at the same testing period, the average count of ova per gram of liver or intestinal tissue was

reduced to reach, respectively, 95.79 and 98.34 % at the sixth week posttreatment (Table 3).

Seven weeks after infection of mice with *S. mansoni*, liver sections revealed multiple periovular granulomas, most of them present in the lobular parenchyma rather than in the portal tract. The majority of granulomas were of cellular type, consisting of densely packed inflammatory cells. At the ninth week postinfection, both hepatic lobules and portal vessels were distended by variable-sized cellular and fibrocellular granulomas. More degenerative changes were seen in ova, and Kupffer cells were hyperplastic and pigmented. Portal vessels were much distended by inflammatory cells, granulomas, and collagen fibers (Figs. 1–4).

Two days after treatment, no changes were seen in the infected livers. Yet, 2 weeks posttreatment, the mean value of hepatic granuloma count was significantly ($P < 0.05$) reduced (Table 4), of small size (Figs. 5–7). More degenerative changes were seen in the ova, and the worms were convoluted around themselves, with some worms possessing a thin caliber and others showing multiple subcuticular vacuoles. After 4 weeks posttreatment, all granulomas were of the fibrocellular type, all ova showed marked degenerative changes, and some were attacked by multinucleated giant cells (Figs. 5–7). At the sixth week after treatment (Figs. 8–10), the size of granulomas was noticeably diminished, circumscribed, and fibrocellular with less inflammatory cells and more fibrous collagenous tissue. Ova were either markedly degenerated or completely absent.

As regards the effect of the tested drug on some physiological parameters of *S. mansoni*-infected mice (Fig. 14), the elevations in the activities of serum ASAT, ALAT, and AP were significantly reduced markedly throughout the testing periods. After 6 weeks, the recorded value was greatly similar to that of the normal value. According to ANOVA analysis, very highly significant variations ($P < 0.001$) were found in the activity of the tested enzymes due to schistosomal infection and postinfection treatment with the tested drug. No significant differences were recorded due to treatment of normal animals.

Discussion

For the assessment of an antischistosomal drug, it is important to study several criteria related to parasitic intensity, stages, and distribution through tissues of the host. Among these criteria are the worm burden and distribution within the hepatic portal system, the percentages of ova stages in the small intestine (oogram), and ova counts in the liver, small intestine, and stool. As regards the worm burden and distribution, the data of the present work revealed that the percentage of worms recovered from the livers of *S. mansoni*-infected mice ranged between 20.92 and

Table 1 Effect of 9-acridanone hydrazine (Ro 15-9268) on the stool ova count of experimental *Schistosoma mansoni*-infected mice.

Before treatment		After treatment	
Time (weeks)	No./g stool	Time	No./g stool
7	244.50±12.42	2 days	102.83±7.0*
9	306.50±8.17	2 weeks	9.33±2.42*
11	457.67±16.45	4 weeks	0*
13	641.83±35.46	6 weeks	0*

Data are tabulated as mean±SE of six animals

*$P < 0.001$, very highly significant

Table 2 Chemotherapy with 9-acridanone hydrazine (Ro 15-9268) drug (P.O., 12.5 mg/kg single dose) on the oogram in the experimental *Schistosoma mansoni*-infected mice

	Time	Immature stages (eggs/g of rectal tissue)				Eggs/g of rectal tissue		
		I	II	III	IV	immature	mature	dead
Before treatment	7 weeks	9.0±1.32	22.0±2.34	25.0±1.34	7.5±1.09	63.5±2.26	34.5±2.64	2.00±0.68
	9 weeks	7.50±1.34	13.67±1.73	35.83±3.64	14.67±1.17	71.67±2.08	27.0±2.39	1.33±0.42
	11 weeks	5.83±2.98	7.5±1.73	14.17±1.7	5.17±1.35	32.67±2.53	62.00±2.29	5.33±3.36
	13 weeks	4.33±0.8	6.17±1.08	7.5±1.52	10.17±1.01	28.17±2.12	65.67±1.94	6.17±0.95
After treatment	2 days	8.67±1.80	15.67±1.73	25.83±1.68	9.0±1.51	59.17±2.63	29.67±2.50	11.17±0.6
	2 weeks	—	—	—	—	—	—	—
	4 weeks	—	—	—	—	—	—	—
	6 weeks	—	—	—	—	—	—	—

Data are tabulated as mean±SE of six animals. Oograms performed did not detect viable eggs

25.92 % while those recovered from portomesenteric veins reached 74.08–79.08 %. In the same sense, some other authors recorded 84 % (Foster 1973) and 80 % (Ghaleb et al. 1979) worms recovered from the portomesenteric veins and the remaining from the liver. The administration of Ro 15-9268, at the dose level 12.5 mg/kg b.wt., to the infected mice led to a marked reduction in liver and portomesenteric veins worms at the second week posttreatment. Starting from the fourth week postadministration of drug, no worms were recovered from the liver or portomesenteric veins by the perfusion technique (Figs. 11, 12, and 13). The hepatic shift is commonly used to detect and evaluate the antischistosomal properties as it indicates the influence of the extraneous chemical compound on the worms, releasing their hold on the walls of the host's vessels, followed by their sweeping by blood flow and causing an abnormally high proportion of hepatic worms (Foster 1968). The onset of antischistosomal action of R0 15-9268 seems to start within 2 days and 2 weeks posttreatment. In this context, Utzinger et al. (2003) found a pronounced hepatic shift 4 days after treatment of *S. mansoni*-infected mice with Ro 15-5458 at a dose level of 15 mg/kg b.wt.

By studying the intestinal oogram of *S. mansoni*-infected mice, it was apparent that the immature ova predominated 7 and 9 weeks after infection (64–72 %) while the mature ones predominated after 11 and 13 weeks of infection (67–72 %). Changes in the oogram are considered significant when one or more developmental stages of immature ova disappeared (Pellegrino et al. 1967). The most remarkable changes observed in the present work in the oogram of infected mice treated with Ro 15-9268 were an increase in the number of dead ova 2 days posttreatment and the complete disappearance of live mature and immature ova 2 weeks after medication. This indicates that the antischistosomal action of Ro 15-9268 is superior to that of praziquantel (PZQ), which was efficacious only against the mature ova while the immature ones survived (Matsuda et al. 1983). The latter author pointed out that the *Schistosoma*-infected mice treated with PZQ should be retreated with the same agent 9 days after the first treatment. In the present study, the number of schistosomal ova per gram of liver reached 18.41×10^3–49.07×10^3 while that of the intestine was 37.99×10^3–77.81×10^3 throughout the follow-up period. The higher proportion of ova in the intestinal tissue than in liver may be due to an increased proportion of pairing among schistosomes in the

Table 3 Chemotherapy with 9-acridanone hydrazine (Ro 15-9268; P.O., 12.5 mg/kg single dose) drug on hepatic and intestinal ova counts in the experimental *Schistosoma mansoni*-infected mice.

Hepatic (×10³/g)				Intestinal (X10³/g)			
Time	Infected	Time	Infected–treated	Time	Infected	Time	Infected–treated
7 weeks	18.41±0.93	2 days	15.63±1.48	7 weeks	31.79±1.22	2 days	9.91±0.63[a]
9 weeks	19.61±1.64	2wk	9.89±1.09[a]	9 weeks	30.06±2.73	2 weeks	5.76±0.77[a]
11 weeks	29.15±1.79	4 wk	3.13±0.51[a]	11 weeks	43.12±4.48	4 weeks	2.37±0.48[a]
13 weeks	49.07±2.75	6 wk	2.07±0.32[a]	13 weeks	77.81±3.82	6 weeks	1.29±0.20[a]

Data are presented as mean±SE of six animals

[a] Highly significant

Fig. 1–4 A liver sections of mice after 9, 11, and 13 weeks postinfection with *Schistosoma mansoni* showing: *1* large lobular fibrocellular granuloma formed of chronic inflammatory cellular infiltrate and collagen fibers encircling ova (arrow). *2* Large fibrocellular granuloma with marked ova degeneration, pigmented Kupffer cells, and congested sinusoids (arrow). *3* Lobular fibrocellular granuloma formed of chronic inflammatory cellular infiltrate and collagen fibers encircling ova (arrow). *4* Large irregularly outlined fibrocellular granuloma with more inflammatory cells at the periphery (arrow). ×200
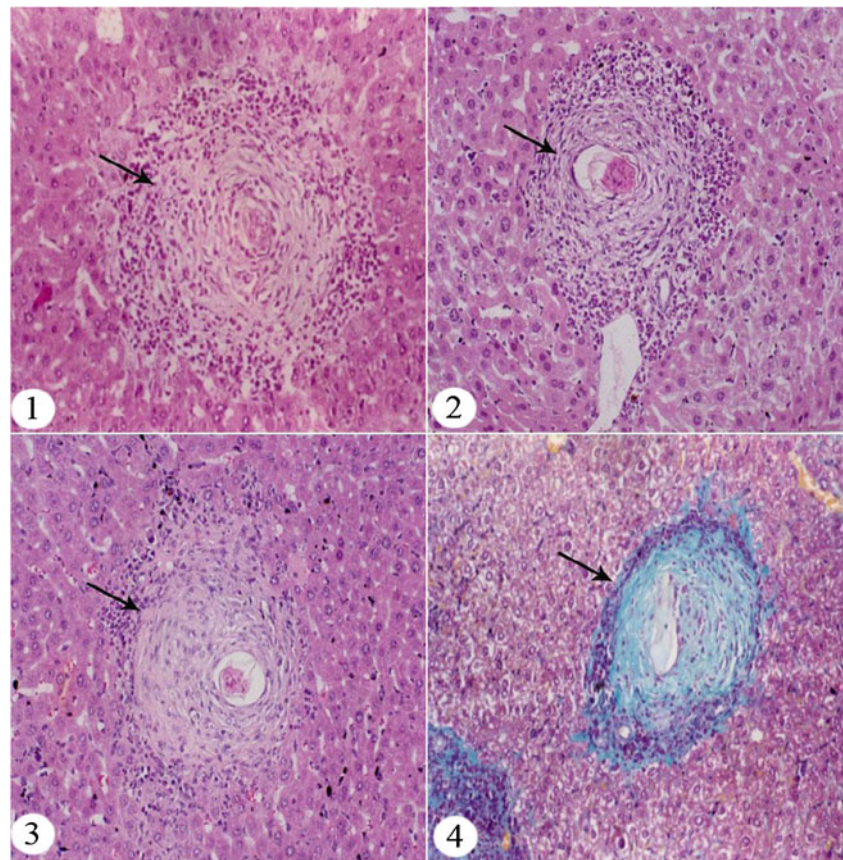
portomesenteric tributaries (Ghaleb et al. 1979; Mehlhorn et al. 1981, 1982).

When the infected mice were treated with Ro 15-9268, at the dose level of 12.5 mg/kg b.wt., tissue ova counts significantly declined where the reduction percentage reached about 96 and 98 % in the liver and intestine, respectively, 6 weeks after treatment. This finding may be corroborated by the results of Sturrock et al. (1987) who found that treatment of *S. mansoni*-infected baboons with three different derivatives of the 9-acridanone hydrazone compound, namely Ro 15-8728/00, Ro 15-9263/00, and Ro 16-9097/001, at a dose level

of 50 mg/kg b.wt., reduced the egg load substantially over 95 %. Also, tissue egg counts were greatly diminished in *S. mansoni*-infected vervet monkeys when treated with two dosage levels of Ro 15-54589.

As regards the fecal egg count, the results of the present study revealed a progressive increase in the numbers of schistosomal ova per gram of stool of infected mice from the 7th till 13th weeks postinfection. However, in the infected animals treated with the selected drug, the fecal egg count was remarkably reduced at the second day (≈58 %) and second week (≈97 %) posttreatment. Moreover, complete disappearance of fecal eggs was established starting from the fourth week posttreatment. Coinciding with these findings, the findings of Sturrock et al. (1987) recorded complete elimination of fecal eggs in *S. mansoni*-infected monkeys treated with three different 9-acridanone hydrazine derivatives, but at a dose level of 50 mg/kg b.wt., which is four times the dose chosen in the present work. In addition, Sulaiman et al. (1989) reported that fecal eggs in *S. mansoni*-infected vervet monkeys were terminated after treatment with Ro 15-5458 at dose levels of 25 and 15 mg b.wt. Consequently, Ro 15-5458 proved to be more efficient drug as compared with PZQ in the treatment of schistosomiasis as reported by Guirguis (2003).

Seven weeks after infection with *S. mansoni*, multiple periovular granulomas were observed in sections of infected

Table 4 Chemotherapy with 9-acridanone hydrazine (Ro 15-9268) drug (P.O., 12.5 mg/kg single dose) on the number of granulomas per five successive microscopic fields (10×10) of experimental schistosomiasis in mice

Time	Infected	time	Infected–treated
7 weeks	13.33±3.93	2 days	13.83±0.39
9 weeks	16.01±0.07	2 weeks	13.73±0.70*
11 weeks	21.25±2.29	4 weeks	11.00±1.67**
13 weeks	23.83±1.68	6 weeks	9.12±1.23**

Data are presented as means±SE of six animals

*$P<0.05$ significant, **$P<0.001$ highly significant

Fig. 4–7 Liver sections of *Schistosoma mansoni*-infected mouse after 2 and 4 weeks of treatment with Ro 15-9268 (12.5 mg/kg b.wt.) showing: *4* a thin delicately folded dead worm in the portal blood vessel (arrow). *5* Lobular fibrocellular granuloma with marked degenerative changes in ova (arrow). *6* Multiple large subcuticular vacuoles in dead worms distending the portal blood vessel (arrow). *7* Portal tract with two granulomas containing ova with marked degeneration and attacked with giant cells (arrows). (*4* and *7*: ×400; *5* and *6*: ×200)
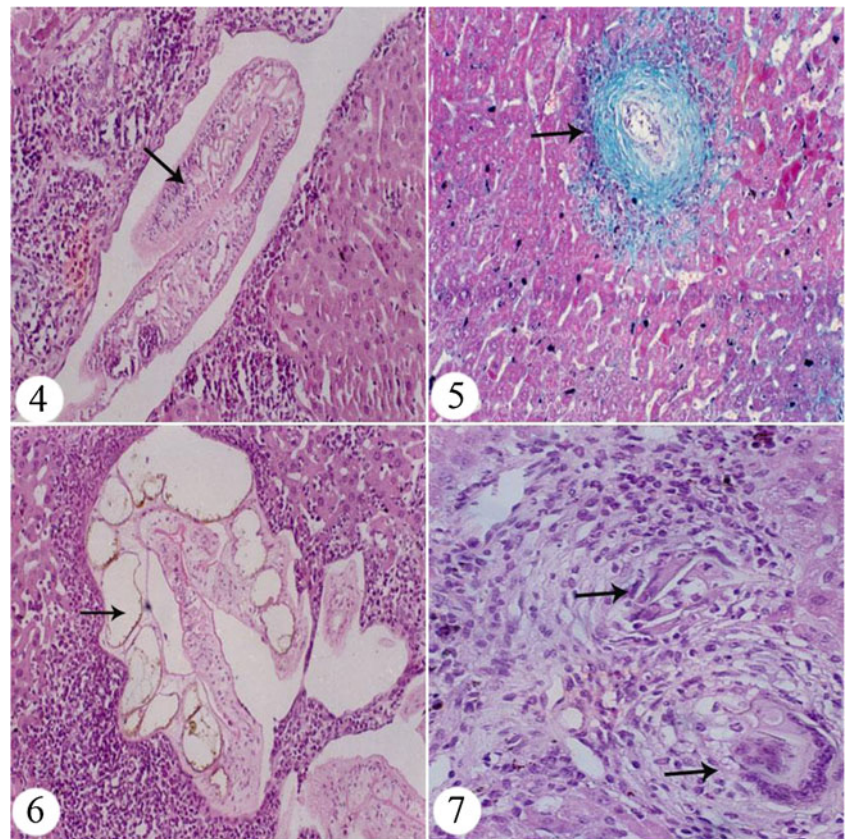
livers. Most of them were of cellular type and present in the lobular parenchyma rather than in the portal tracts. Nine weeks after infection, hepatic lobules and portal tracts were distended by both cellular and fibrocellular granulomas, where ova showed degenerative changes. At the 11th and 13th weeks postinfection, portal granulomas were more than lobular ones and ova exhibited more degenerative changes. At all-time intervals, inflammatory cells were found in association with granulomas. Microvasculation studies in murine liver showed that granulomas formed around schistosomal eggs and lodged in the presinusoidal capillaries impede hepatic blood flow (Warren 1972), including portal hypertension
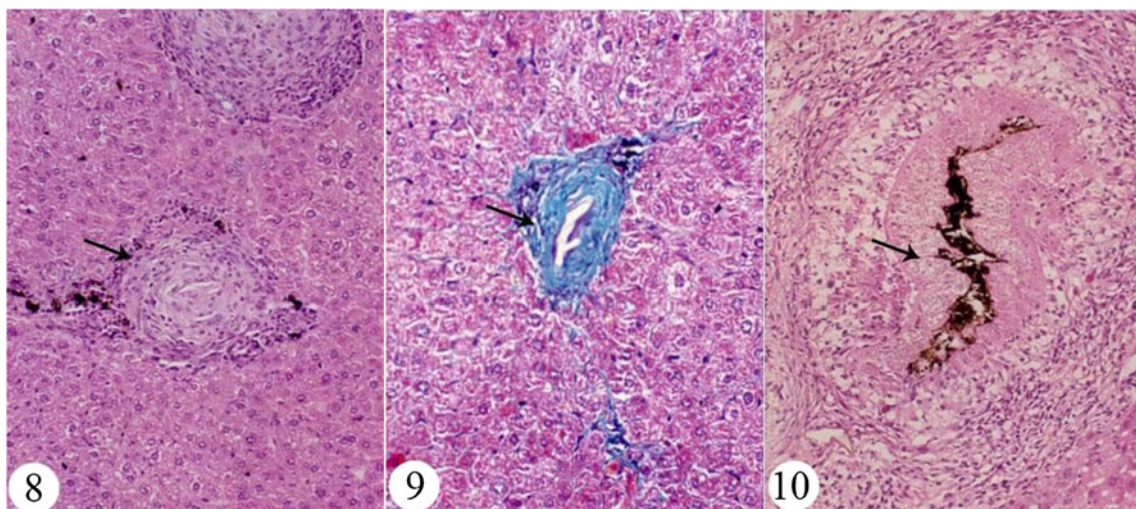

Fig. 8–10 Liver sections of *Schistosoma mansoni*-infected mouse after 4 and 6 (*9* and *10*) weeks of treatment with Ro 15-9268 (12.5 mg/kg b.wt.) showing: *8* Small well-circumscribed fibrocellular granuloma with less peripheral inflammatory cells and more pigmented macrophage cells (arrow). *9* Small fibrous granuloma and ova as folded egg shell surrounded with densely packed and contacted collagen fibers (arrow). *10* Degenerated worm surrounded by chronic inflammatory cells and fibrous connective tissue (arrow). ×200

Fig. 11 Effect of Ro 15-9268 treatment on worm distribution of mice infected with *S. mansoni*
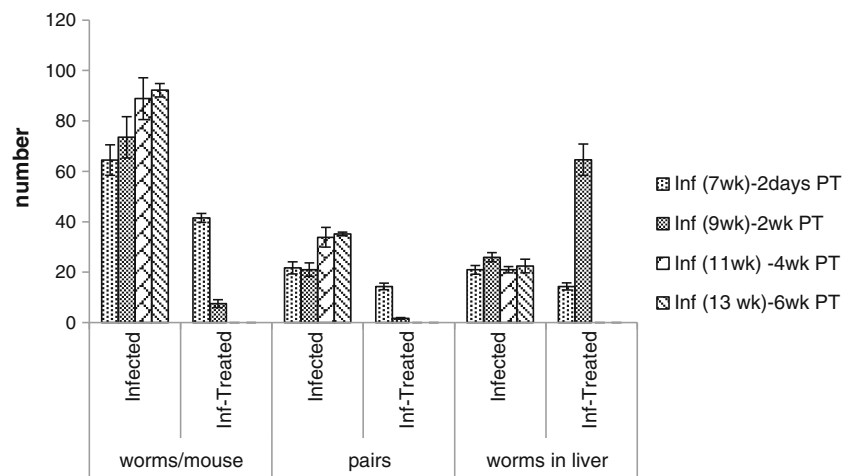
(Cheever et al. 1992). The chronic granulomatous host response around disseminated parasite eggs, aggravated by fibrosis, is the major contributor to the pathology of the disease (Abaza et al. 1984).

Granuloma formation is initiated by antigens secreted by the miracidium through microscopic pores within the rigid egg shell (Van der Werf et al. 2003; Cioli and Pica-Mattoccia 2003; van de Vijver et al. 2004; Barduagni et al. 2008). The continuous egg depositions with subsequent chronic inflammatory host response are responsible for liver fibrosis. In addition, the schistosomes secrete or excrete antigenic materials which circulate in the blood and cause pathological changes that could be seen in the acute phase of infection (Warren 1972). In this respect, Badawy et al. (1991) found certain histopathological changes in the liver of *S. mansoni*-infected mice before oviposition, which is indicative of the effect of living worms.

Liver sections of mice after infection with *S. mansoni* revealed multiple periovular cellular granulomas, consisting of densely packed inflammatory cells. At the 11th and 13th weeks postinfection, both hepatic lobules and portal vessels were distended by variable-sized cellular and fibrocellular granulomas.

Improvement of the histopathological changes, 2 weeks after treatment, to some extent, was evidenced by the decrease in the number of hepatic cellular granulomas compared with that in the infected untreated group. Such improvement might be related to the decrease in worm burden (89.8 % reduction) and marked suppression of egg deposition (≈50 % reduction). The marked improvement of the histopathological picture was noticed 4 and 6 weeks after treatment expressed by the significant decrease in the number and size of the granulomas with the reduction of intact ova, which was related to cessation of egg deposition after parasite eradication. The healing process of the granulomatous reaction was accelerated with more degenerative changes in the ova. Treatment with successful schistosomicidal drugs eradicates the worms, stopping their egg production. At the same time, these eggs may modulate the host reaction to these eggs (Strenger et al. 1967). When another derivative of 9-acridanone hydrazine compound, namely Ro 15-5458, was used to treat vervet monkeys infected with *S. mansoni*, it likewise showed a marked improvement of the histopathological changes in the liver examined

Fig. 12 Effect of Ro 15-9268 (P.O., 12.5 mg/kg single dose) on worm distribution in liver of mice infected with *S. mansoni*
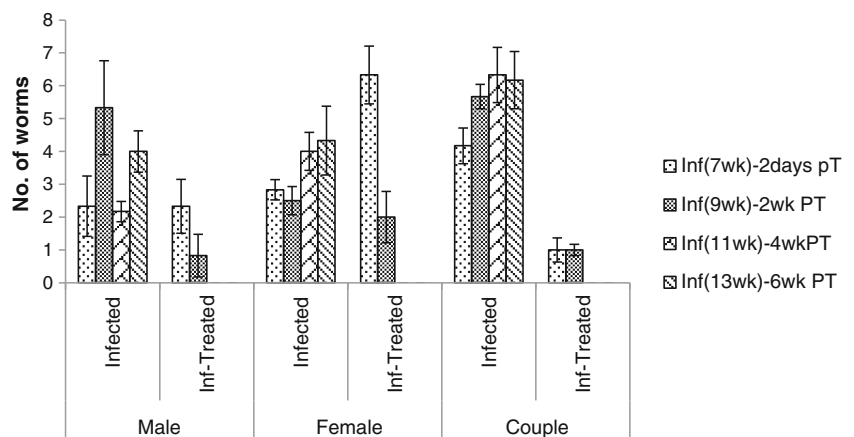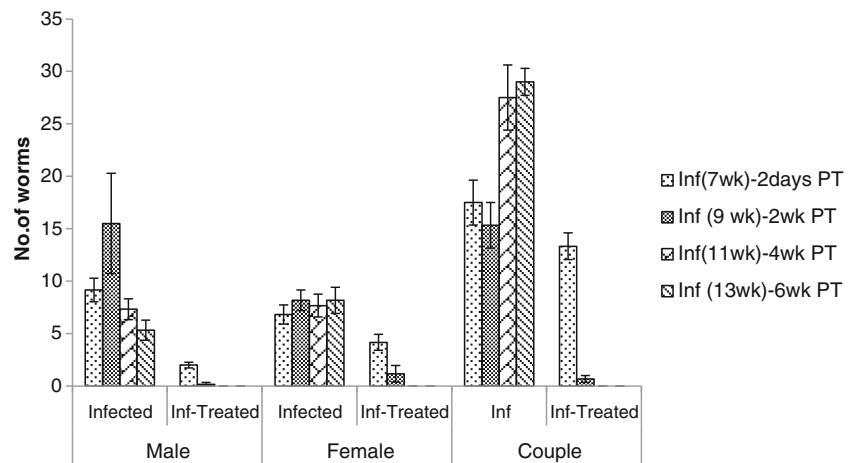
Fig. 13 Effect of Ro 15-9268 (P.O., 12.5 mg/kg single dose) on worm distribution in portomesenteric veins of mice infected with *S. mansoni*


7 weeks after treatment. In the same sense, Sabry (1994) obtained similar results when mice infected with *S. mansoni* (Egyptian strain) were treated with Ro 15-5458. The author attributed such improvement to the decreased worm burden (98.5 %) and suppression or cessation of egg deposition in the hepatic tissue (91.3 %).

The effect of Ro 15-9268 upon the schistosomal worm was evidenced in the present work depending on the histopathological point of view (Fig. 14). Dead worms were seen lodged in the portal blood vessels, 2 weeks posttreatment. It seems likely that the tegmental changes of the worm may be an important aspect of drug activity leading to death and elimination of worms. Worms were characteristically of short length and thin caliber, and subcuticular vacuoles were seen in some of them. Later on, some worms showed fragmentation and degeneration while others induced granuloma formation. Drug-induced tegmental damage has been described in *S. mansoni* worms after treatment with a variety of schistosomicidal drugs (Otubanjo 1981). Reversibility of hepatic fibrosis after Ro 15-9268 therapy occurred as it reduced hepatic granuloma diameter and cellularity. In addition, the

worms disintegrated completely in the centers of the typical granulomas that were replaced by small fibrotic scars. Reversal of hepatic fibrosis was also recorded after treatment with specific schistosomal agents (Thomas and Gonnert 1978; Emonard and Grimaud 1989).

In the present work, the elevation in the activities of AST, ALT, and ALP of *S. mansoni*-infected mice seems consequent to the damage of hepatic cells and/or impaired permeability of cell membrane due to heavy schistosome egg deposition (Cheever et al. 1992; Mahmoud et al. 2002). The return to the normal values after therapy could be due to the stopping of egg production and eradication of the parasite, which were evidenced in the parasitological part of this study. The main possible explanations for these results are that Ro 15-9268 reduces the *S. mansoni* worm and/or regulates the adhesion molecules playing a key role in egg trapping and increases the mobilization of eggs from the liver and intestine to the exterior of the body in the feces. These data may suggest an antifibrotic effect of Ro 15-9268 in the early stages of liver fibrosis. Ro 15-9268 might able to block liver fibrosis through killing parasite to alleviate liver inflammation.

Fig. 14 Chemotherapy with 9-acridanone hydrazine drug (P.O., 12.5 mg/kg single dose) on the serum enzyme activity of experimental schistosomiasis in mice
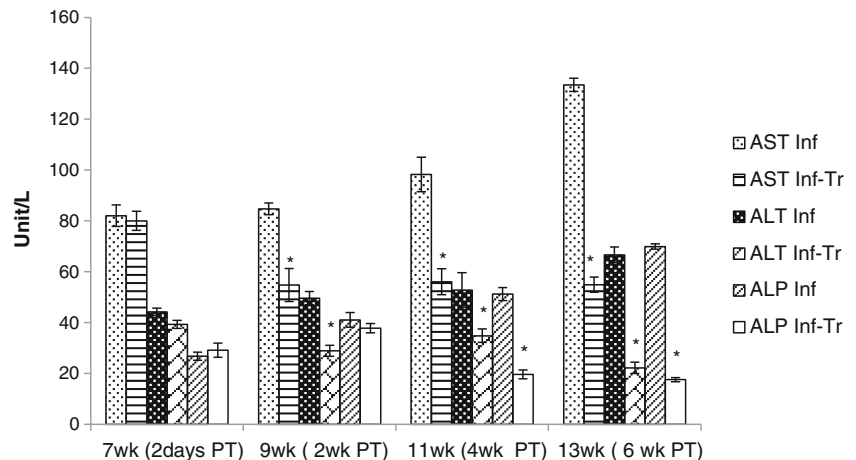
In conclusion, when Ro 15-9268 was administered to mice infected with the Egyptian strain of *S. mansoni*, at a low dose level (12.5 mg/kg b.wt.), encouraging results were obtained. The drug showed a high efficacy against schistosomal worms as well as histopathological inflammatory changes.

Guidelines for ethical publications Authors declare that this manuscript was consistent with the guidelines and principles of the Research Ethics Committees in Theodor Bilharz Research Institute, Egypt, and are in accordance with the guidelines of the Canadian Council on Animal Care (CCAC).

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