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Cisplatin augments the anti-schistosomal effect of praziquantel in a schistosoma-infected cancer model

Mohamed Labib Salem[1], Afrah Salama[2], Afnan Hamdy El-Gowily[2], Mohammed A Mansour[2]* & Mohammed Mahmud Ali El-Said[3]

[1]Zoology Department & [2]Biochemistry Division, Department of Chemistry, Faculty of Science, Tanta University, Egypt

[3]Biochemistry Department, Faculty of Science, Alexandria University, Egypt

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Schistosomiasis is the third most devastating tropical disease worldwide caused by blood flukes of the genus *Schistosoma*. Praziquantel (PZQ) is the drug of choice for treating all species of schistosomes. However, PZQ kills only adult *Schistosoma* worms, not immature stages. The inability of PZQ to abort early infection or prevent re-infection, and the lack of prophylactic effect prompt the need for novel drugs and strategies for the prevention of schistosomiasis. Tumor burden can be developed in *Schistosoma*-infected patients. The present study aimed to determine the host responses to mutual interaction between cancer, represented by Ehrlich ascites, and infection, represented by Schistosomiasis. Mice infected with *Schistosoma* and challenged with tumor 4-5 weeks later showed the same anti-schistosomal (worm and egg burden) and antitumor (total tumor cell count and mouse survival) parameters when compared to mice infected with *Schistosoma* alone or challenged with tumor cells alone. As expected, combinatorial treatment with PZQ and cisplatin of Schistosoma-infected mice that were challenged with tumor cell line decreased the tumor burden as well as the worm and egg burden after treatment as compared to the non-treated controls; while the worm burden and egg counts were significantly decreased (P <0.001) in treated group (VI) treated with cisplatin (0.5 mg/kg), group (VII) treated with cisplatin (2 mg/kg), group (VIII) treated with PZQ/ cisplatin (0.5 mg/kg) and group (IX) treated with PZQ / cisplatin (2 mg/kg) by 44.55% , 74%, 100% and 97.8% in worm burden, and by 47%, 78.7%, 96% and 97% in liver egg count , respectively than that of group (II) non treated *S. mansoni* infected alone and (IV) non treated *S. mansoni*/EAC alone. Also, Group IX caused a significant reduction (P <0.05) in worm burden than that of group VI. Also, total ascetic volume and the tumor cell counts in Ehrlich's ascites carcinoma (EAC)-cells were significantly decreased (P <0.001) in groups VIII and IX than that of the group (III) non-treated (EAC) inoculated alone. There was no mutual interaction between schistosomiasis infection and tumor burden. Also, whereas, PZQ did not affect on the antitumor parameters, cisplatin even at low doses had anti-schistosomal effects.

Keywords: Cisplatin, Ehrlich ascites, Praziquantel, Schistosomiasis, Worm burden

Schistosomiasis is the widespread tropical disease caused by infection with parasitic blood flukes. *Schistosoma mansoni*, *Schistosoma haematobium*, and *Schistosoma japonicum* are the most widely distributed species and cause the highest burden of the disease, particularly in sub-Saharan Africa[1]. Praziquantel (PZQ) is the only drug available for treatment of this disease, and it is active against adult schistosomes, but has little activity against the juvenile schistosomula, the young developmental stages of the parasite[2]. Should serious praziquantel drug resistance arise, there are no viable alternatives to this drug. Even so, drug discovery for schistosomiasis has languished. Cancers and parasites have many properties in common, particularly those that relate to their respective capacities to evade host defense mechanisms. Hence, Tumor can be developed in Schistosoma-infected patients. Alternatively, patients with tumor can be subjected to *Schistosoma* infection. It is not clear, however, whether there is a mutual effect of these diseases with or without treatment with the choice drugs. This clarified by the previous study of Edington who found that a possible association between lymphoreticular tumors and *Schistosoma mansoni*[3]. Besides, other study showed that *S. mansoni* soluble egg antigen-induced angiogenesis-related processes by up-regulating vascular endothelial growth factor in human

*Correspondence:
Phone: 00201021236313
E-mail: biomansour@hotmail.com

endothelial cells[4]. Also, José exhibited that the brain tumor as a clinical presentation of schistosomiasis, *mansoni* infection[5] and another study resulted from that lymph node involvement by *S. mansoni* in a patient with a malignant neoplasm of the colon. Despite the topography of the lymph node in the territory of organ drainage, the lesion in the lymph node was represented only by the granulomatous component, without neoplastic involvement, these previous studies observed the association of the ectopic form of schistosomiasis with a benign neoplasia in two cases (uterine leiomyoma and ovarian cystic teratoma). The association with malignant neoplasia was observed in several cases. Some of these cases as (squamous cell carcinoma of the penis, carcinoma of the uterine cervix and renal cell carcinoma) there was a tightly intimate association between neoplastic cells and *S. mansoni* eggs. It is possible that local hyper vascularization related to tumor growth facilitates the migration of the eggs resulting in the simultaneous association[6.]

On the otherwise, the ectopic sites and forms of *S. mansoni* affecting the peritoneum are usually associated with the hyperplastic manifestations of the disease (pseudo-tumoral form) that seems to be directly related to the egg of the parasite. Other possible manifestations of immunity under these certain circumstances, the egg acts as an antigenic complex, which would provoke an exaggerated granulomatous inflammatory response in the organism[7].

Schistosomal pathology is a direct consequence of the immunological response to oviposition in host tissue especially the liver. Liver injury is typically associated with infiltration of inflammatory cells leading to fibrosis. It is reported that the primary cause of mortality and morbidity in schistosomiasis is hepatic fibrosis at chronic and advanced stages, which develops because of inflammatory granulomas around deposited parasite eggs[3,8]. Furthermore, inflammatory cells, through the formation and release of hydroxyl radicals, are responsible for various mutations[4], sister chromatid exchanges, and breaks of the DNA strands[5,9].

The previously studies estimate more developed and extensive microvascular pre-ovulatory follicles as compared to that of the other follicles, that producing some substrates, nutrients, and trophic hormones that permit follicular development and growth. Therefore, vascularization in the female pelvis was distinctive, also the changes that occur in angiogenesis during reproductive life and pregnancy may facilitate the implantation of ectopic schistosomiasis[6,10]. In addition, the neoplasms supposedly serve as a migratory route for the adult parasites and the embolization of eggs. Implying, the presence of *S. mansoni* eggs caused inflammation and oxidative stress, which may lead to a poorer prognosis in the case of ovarian tumors. Nevertheless, there was insufficient evidence to confirm the malignization of a benign lesion due to the presence of *S. mansoni*[4,11].

Praziquantel (PZQ) is the drug of choice for all species of *Schistosoma* as an effective anti-schistosomal drug[12]. Although treatment with this drug is effective, frequent schistosome reinfection occurs later. Moreover, PZQ alone failed to improve hepatic pathological alterations induced by schistosomiasis[13]. As the most severely affected organ during *Schistosoma mansoni* infection is the liver, treatment targeting schistosomiasis-associated hepatotoxicity remains a promising approach which worth investigation[14.]

Cis-diamminedichloroplatinum (II), commonly known as cisplatin (CP), is a highly effective antineoplastic drug used for the treatment of a diverse spectrum of malignancies[15]. Interestingly, the pathogenesis of cisplatin toxicity is attributed to the formation of ROS, caspase activation, DNA damage and mitochondrial damage[16]. In this report, we examined the host responses to the mutual interaction between cancer, represented by Ehrlich ascites, and infection, represented by Schistosomiasis. In addition, the anti-schistosomal effect of cisplatin was investigated.

We examine how the toxicity of anticancer agents is just a matter of dose or ‘only dose makes the poison’. Thus, the opportunity exists to discover new anti-schistosomal using the anticancer pharmacopeia. If these drugs were active *in vivo* at low and tolerable doses, they could potentially become anti-schistosomal. This is more possible in view of the fact that many anticancer drugs have already been used in the treatment of non-neoplastic diseases at a low dose.

Materials and Methods

Mice

One hundred and eight adult female Swiss albino mice (CD1 strain) weighing 20±2 g used in this study were obtained from National Research Center (NRC, Cairo, Egypt). Animals were housed (12 animals per

cage) at the animal facility at Zoology Department, Faculty of Science, Tanta University, in 12 h/12 h dark/light cycle under laboratory condition of temperature and humidity. The mice were fed with rodent pellets and tap water *ad libitum*. The study was performed in accordance with the guidelines issued by the faculty of Science, Tanta University, Egypt. The animals were euthanized according to the method approved by the Canadian Council on Animal Care (CCAC).

Infection with *S. mansoni*

Schistosoma mansoni cercariae used for the infection were obtained from the *Biomphlaria alexandrina* snails supplied by the Schistosome Biological Supply Program, Theodore Bilharz Research Institute at Warrak El-Hadar, Imbaba, Cairo, Egypt within one hour after shedding. The mice were first transferred to suitable size container contained warm freshwater to bath the animals to half-way up the tail. The animals were then exposed to a known number of fresh water suspended cercariae (40-50 cercariae), through partial immersion of their tails into the cercarial suspension. The animals were let in this suspension for 2 h[17]. Briefly, the cercariae of *S. mansoni* were shed from the infected snails. Three representative samples each of 0.5 mL were drawn out using serological pipette into clean glass watch. Two drops of Lugol's iodine solution were added to each sample to kill and stain the cercariae. The number of cercariae was counted under a dissecting microscope, and the average number of cercariae/mL of cercarial suspension was counted.

All the animals were trapped in plastic falcon tube. After that, the animals have been transferred individually into a glass tube containing fresh dechlorinated water. Known numbers of fresh-suspended cercariae (40-50 cercariae) have been added to each tube using a serological pipette. The animals have left in the falcon tubes for 60 min then transferred into special cages, finally, the cercariae in the glass tubes have been counted after the infection.

Inoculation of the tumor cells

Ehrlich ascites carcinoma (EAC) cells were originally obtained from the National Cancer Institute (Cairo University, Egypt). EAC cells were collected from donor mouse on the eight days of tumor growth and were suspended in sterile isotonic saline, the viable EAC cells were counted by using trypan blue method and were adjusted for intraperitoneal (*i.p.*) inoculation of about 1×10^6 cells/mouse.

Preparation of drug solutions

Cisplatin was obtained from Sigma-Aldrich (St. Louis, MO) and kept at 4°C until used. Biltricide tablets (Alexandria Co., Egypt) containing praziquantel were prepared freshly before the treatment. Mice were treated with either 2 mg/kg of cisplatin (*i.p.*) or 0.5 mg/kg of cisplatin (*i.p.*) for six consecutive days. PZQ was injected orally (600 mg/kg) for three consecutive days alone or in combination with cisplatin. While PZQ drug was suspended in DMSO and was given orally using stomach gavages' and cisplatin drug doses were prepared in sterile saline

Experimental design

Swiss albino mice were infected with (40-50) cercaria at day "0" and injected (*i.p.*) with 300 µL of 1×10^6 EAC cells/mouse at day "31" post infection, then divided into 9 groups of 12 animals each, as follows: Group I (naive mice; Control); Group II: non treated *S. mansoni*-infected mice (Inf. alone); Group III: non treated EAC-bearing mice (EAC); Group IV: non treated *S.mansoni* and EAC infected mice (Inf.EAC); Group V: *S. mansoni* and EAC infected mice treated with PZQ (PZQ); Group VI: *S. mansoni* and EAC infected mice treated with cisplatin (0.5 mg/kg) *i.p.*; Group VII: *S. mansoni* and EAC infected mice treated with cisplatin (2 mg/kg, *i.p.*); Group VIII: *S. mansoni* and EAC infected mice treated with cisplatin (0.5 mg/kg) *i.p.* and PZQ orally administered; Group IX: *S. mansoni* and EAC infected mice treated with cisplatin (2 mg/kg) *i.p.* and PZQ orally administered. After 24 h of inoculation, the mice were divided equally into these groups as shown in (Fig. 1).

At the end of the experiment, 1 mL of blood samples were collected by eye bleeding with EDTA filled tubes from all groups and the blood was

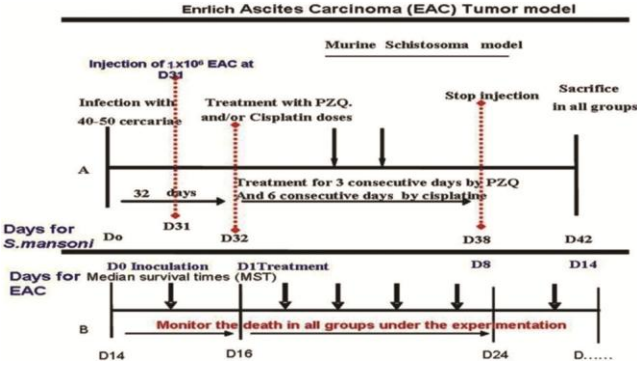

Fig. 1 —Experimental plan for different mice groups

centrifuged at 2000-3000 × g for 15 min at 15-24°C, the plasma quickly separated and frozen at −20°C until used. After collection of the blood samples, mice were euthanized, and their liver tissues were excised, immediately removed and washed using chilled saline solution then weighed. The tissues were minced and homogenized separately (10% W/V) in ice-cold sodium phosphate buffer (0.01 M, pH 7.4) containing 1.15% KCl in a potter El Vehjem type homogenizer. The homogenate was centrifuged at 10.000 xg for 15 min at 4°C and the resultant supernatant was used for determination of the biochemical parameters.

The anti-tumor activity was measured in EAC is bearing mice with respect to the following parameters: the changes in the total body weight, tumor volume, and total tumor cell counts. Also, parasitologically parameters were measured in *S. mansoni* infected mice after liver perfusion. The percentage of worm reduction after treatment was calculated and the anti-schistosomal effect of the drugs was parasitological assessed by determining the *S. mansoni* worm load and the tissue egg loads.

Worm burden recovery

Perfusion of adult worms from the liver and Porto-mesenteric system was performed after infection[18]. The percent of change (% reduction of worm) was calculated as follows:

$$(\%\ \text{Change}) = C - T\ /C \times 100$$

Where C is the mean number of worms recovered from infected-untreated mice and T is the mean number of worms recovered from infected-treated mice.

Tissue egg load

The liver and the intestine which was previously cleaned up and weighed were digested separately in 4% KOH solution at 37°C for 6 h. After digestion, tissue suspensions were centrifuged at 1500 rpm for 5 min and supernatants removed. After three cycles of washing and centrifugation, the number of eggs was determined in two aliquots of 100 µL each using light microscope. Results were expressed regarding the mean number of eggs per gram of tissue for intestine and liver[19].

Measurement of granuloma volume

Lesions containing eggs in their centers were selected for measurement, and the diameter of each liver granuloma was obtained by measuring two diameters of the lesion at right angles to each other using an ocular micrometre. The mean diameter of all slide lesions from each mouse of each group was determined and the volume of each lesion was calculated, assuming a spherical shape, from its mean diameter using the following formula:

$$\text{Volume} = R^3 \times 22/7 \times 4/3$$

Liver function tests

Plasma Aminotransferase (AST and ALT) was assayed by using available commercial kits (*Randox*, Egypt)[20], Alkaline phosphatase activity was assayed calorimetrically by using commercial kit (*Bio diagnostic*, Egypt)[20], Total protein concentration was assayed calorimetrically by using commercial kit (*Bio diagnostic*, Egypt)[21] and Total bilirubin was assayed calorimetrically by using commercial kit (*Diamond Diagnostic*, Egypt)[22].

Tissue homogenate

The right lobe of the liver was homogenized in *Tris*–HCl 50 mm buffer. Homogenates were centrifuged at 3500 rpm for 25 min at 4°C and supernatants were stored at −70°C for the determination of some oxidative stress biomarkers.

Biochemical analysis

Liver homogenate (10%) was prepared and used to estimate reduced glutathione (GSH) was estimated[23], The method is based on the oxidation of GSH by 5,5'-dithiobis-2-nitrobenzoic acid, [DTNB], and the resultant yellow coloured ion, is measured at 412 nm. Total malondialdehyde (MDA), the extent of lipid peroxidation was measured in the term of thiobarbituric acid reactive substance (TBARS) formation was measured. CAT activity was measured spectrophotometrically at 240 nm by calculating the rate of regarding to H_2O_2 the substrate of the enzyme. The glutathione peroxidase activity (GSH-Px), Tissue total antioxidants capacity, Total thiol content and non-protein thiol (GSH), The protein content of tissues was measured[24]. Thioredoxin reductase was assayed[25]. ASA activity and ASB activity were assayed[26]. The reaction was followed up by measuring the absorption due to enzyme activity at 515 nm.

Statistical analysis

In the present study, all results were expressed as Mean ± S.E of the mean. Data were analyzed by one-way analysis of variance (ANOVA) and POSTHOCTUKEY ALPHA test using the Statistical Package for the Social Science (SPSS® Inc., USA) program, version 20 followed by least significant

difference (LSD) to compare significance between groups[27]. The criterion for statistical significance was net at $P \leq 0.05$.

Results

Treatment with cisplatin and PZQ reduced the gain of body weight due to tumor

As shown in (Fig. 2), the treatment with cisplatin alone showed a significant decrease in the total body weight as compared to the Inf. EAC group. The combinatorial treatment with cisplatin (0.5 mg/kg) and PZQ showed a marginal increase in the total body weight when compared to the group treated with cisplatin (0.5 mg/kg) alone (Fig. 2A). In addition, the treatment with PZQ showed no changes in the body weight as compared to the Inf. EAC group. Both of untreated and PZQ treated groups showed an increase in the total body weight at the day 14 after tumor inoculation due to the increase of the total ascetic volume.

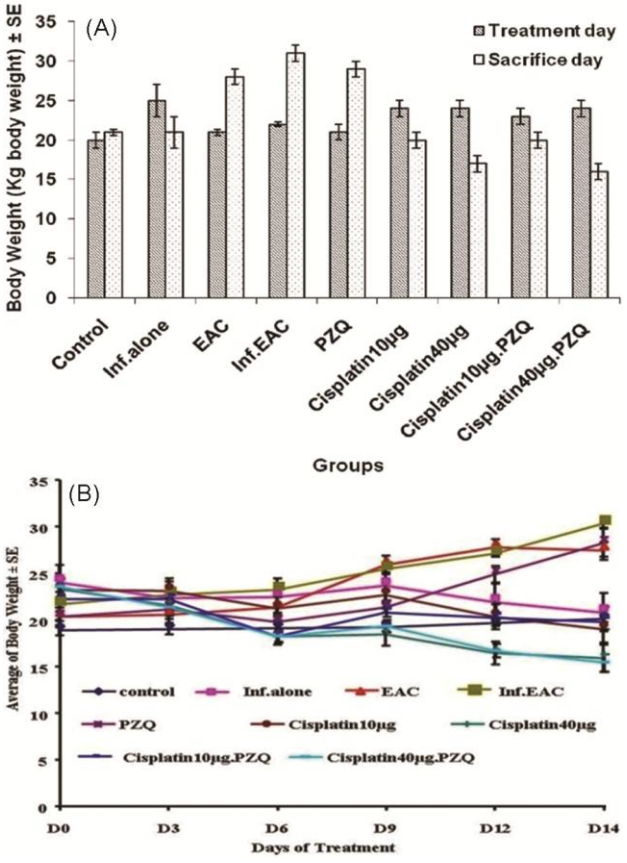

Fig. 2 — Evaluation of body weight in the different groups under study. (A) The body weight of adult female mice at the start of treatment and the end of experiment in the different groups; (B) The kinetic changes in the total body weight of mice after inoculation with EAC-tumor cells and treatment with praziquantel or with cisplatin at different doses alternatively. Different groups of mice (8 per group) were inoculated with 1×10^6 EAC-cells, 24 h later, mice were treated according to the experimental plan. Mice were weighted on day 0, 2, 4, 6, 8 and 14 after inoculation with tumor. The inoculated tumor groups were sacrificed on day 14 and the changes in the total body weight were calculated

Primary evaluation of the tolerance of cisplatin and PZQ by *S. mansoni*/EAC infected mice

To test the tolerable doses of cisplatin by group IV (*S. mansoni*/EAC non-treated mice). Female albino mice were infected with (40-50) *S. mansoni* cercareia at day zero of experiment then at day 31 from infection (day zero for inoculating with EAC cell) by 1×10^6 EAC cells after 7 weeks post-infection (first day of EAC inoculation); the cisplatin was given intraperitoneal with a dose 2 mg/kg in comparison with the curative dose of the reference anti-schistosomal drug PZQ. To this end, the number of death in animal groups was recorded as compared with that of control treated with vehicle over a period of 24 h. After 24 h if these compounds didn't show any objective toxicity as monitored by the observation of the death rate at that dose level, another dose of 0.5 mg/kg body weight was given to mice (Table 1).

Preliminary investigation of the tolerance of cisplatin doses under investigation to *S. mansoni*/ EAC -infected mice at a dose level of 2 mg/kg body weight showed apparent toxicity in praziquantel (600 mg/kg), group IX (Cisplatin (2 mg/kg) with PZQ) the toxicity reaches to 12.5 % as monitored by the observation of the death rate at that dose level. Based on these results, all groups were inoculated at PZQ 600 mg/kg body weight for three times daily, but groups were inoculated at cisplatin given for six times daily at a dose level of 2 mg/kg and 0.5 mg/kg body weight, respectively.

Combinatorial effect of PZQ and cisplatin on worm burden and egg count

The total number of the worms and the percent reduction of the worm burden did not exhibit

Table 1 — Shows the rate of death of mice given PZQ orally at three dose of 12 mg/kg of body weight and/or cisplatin 0.5 mg/kg and 2 mg/kg of body weight *i.p.* at six consecutive days

Groups	Dead/Total	% of Death
Inf. alone	1/8	12.5
EAC	2/8	25
Inf. EAC	2/8	25
PZQ	1/8	12.5
Cisplatin (0.5 mg/kg)	0/8	0
Cisplatin (2 mg/kg)	2/8	25
Cisplatin (0.5 mg/kg) PZQ	0/8	0
Cisplatin (2 mg/kg) PZQ	0/8	0

significant difference between Inf. alone and Inf. EAC groups. The groups treated with PZQ alone or combined with cisplatin (2 mg/kg) exhibited high significant decrease (P <0.05) in the worm burden as compared to infected controls (Fig. 3).The mean egg count in the intestine and liver exhibited significant reduction (P <0.05) in the groups treated with PZQ alone or PZQ combined with cisplatin (0.5 mg/kg) or (2 mg/kg) as compared to Inf. alone and Inf. EAC groups (Fig. 4). There was no significant change between the infected controls and Inf. EAC groups in the egg count. On the other hand, highly significant decrease was exhibited only in the groups treated with PZQ alone or combined with cisplatin (0.5 mg/kg) or (2 mg/kg).

Granuloma diameter exhibited slight decrease in PZQ treated group as compared to Inf. alone group (P <0.05), while in all treated groups, it exhibited a significant decrease (P <0.05) except the group treated with PZQ alone which showed no significant change as compared to immunized infected control (Fig. 5).

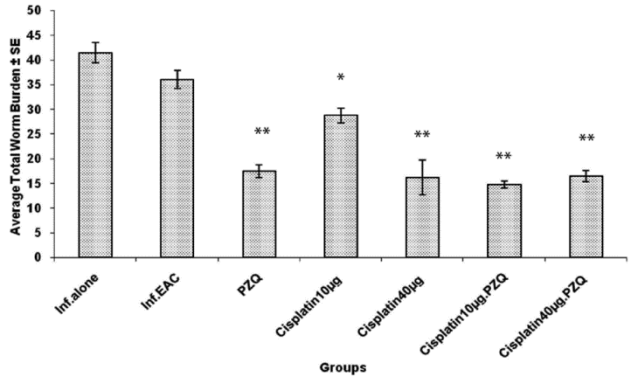

Fig. 3 — Worm burden in all treated groups under study in the different groups (*P value is <0.05, **P value is <0.01)

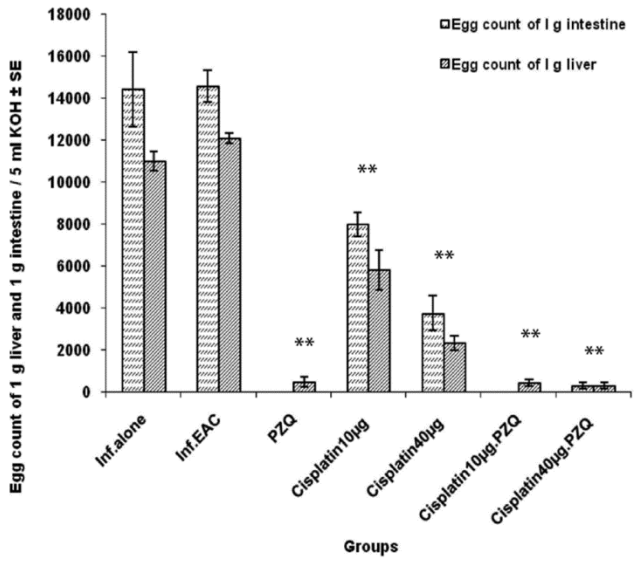

Fig. 4 — Egg count in 1 g liver and 1 gram intestine in the different groups under study (*P value is <0.05, **P value is <0.01)

Treatment with PZQ had no antitumor effect against EAC *in vivo*

Figure 6 exhibited that while both of the Inf. EAC and PZQ groups had tumor after 14 days of inoculation, the cisplatin and cisplatin. PZQ groups had no tumor. Interestingly, the co-treatment of PZQ and cisplatin exhibited a toxic effect on the tumor bearing mice, whereas 2 mice of 12 were dead starting from day 4 until day 12 (data not shown). The results exhibited that although the total ascetic volume in the group treated with PZQ was slightly higher than the volume of the untreated group, the total number of live cells was less than it in the Inf. EAC group.

Differential white blood cells count in swiss albino mice

As shown in (Tables 2 & 3) lymphocyte count was significantly decreased in EAC, Inf. EAC and cisplatin (2 mg/kg) with or without PZQ groups as compared with that in normal mice. On the other hand, lymphocyte count was slightly decreased in cisplatin (0.5 mg/kg). PZQ group as compared with that in Inf. group. In contrast, PZQ and cisplatin

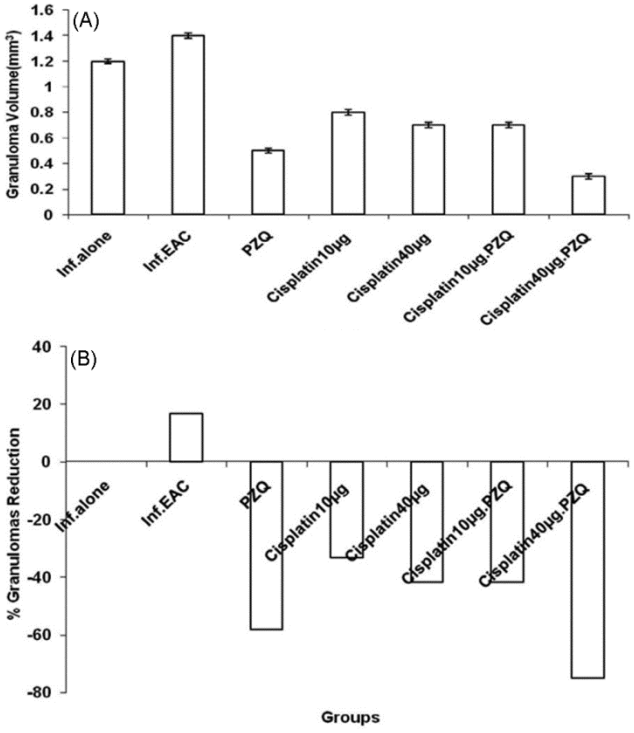

Fig. 5 — Changes in the granuloma volume (mm^3) in liver sections of all groups under study

(0.5 mg/kg) groups exhibited a significant increase in lymphocyte count as compared with that in Inf. EAC group. Also, cisplatin (2 mg/kg). PZQ group exhibited a significant decrease in lymphocyte count as compared with that in PZQ group. The count was significantly decreased in cisplatin (2 mg/kg) with or without PZQ groups as compared with that in cisplatin (0.5 mg/kg) group. Additionally, cisplatin (0.5 mg/kg). PZQ group exhibited a significant increase in lymphocyte count as compared with that in cisplatin (2 mg/kg) group. In contrast, it was slightly decreased in cisplatin (2 mg/kg).

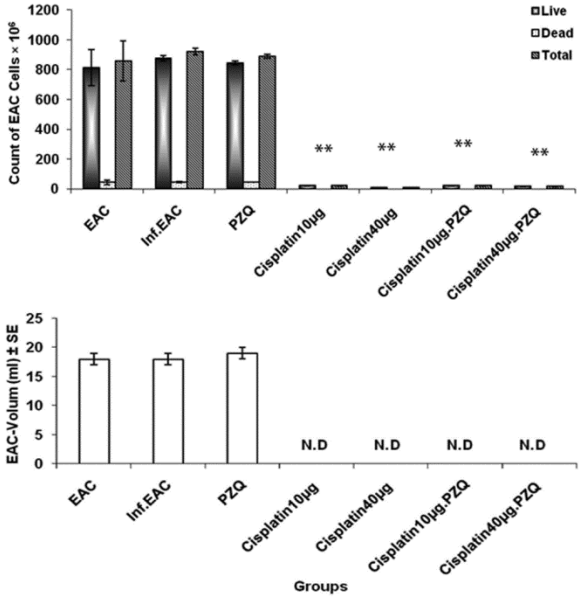

Fig. 6 — The effect of cisplatin on tumor cell number and ascetic fuid volume. (A) Antitumor effect of cisplatin at doses of 0.5 mg/kg and 2 mg/kg and/or PZQ at the dose of 600 mg/kg body weight combined with each other; (B) Effect of the treatment with cisplatin at doses of 0.5 mg/kg and 2 mg/kg and/or PZQ at the dose of 600 mg/kg body weight on Ascetic volume (**P* value is <0.05, ***P* value is <0.01)

PZQ group as compared with that in cisplatin (0.5 mg/kg) PZQ group.

Effect of PZQ and/or cisplatin on some biochemical parameters of *S. mansoni* /EAC-bearing mice

As shown in (Table 4) ALT and AST enzyme activities in serum were significantly increased in all untreated mice groups as compared to naive mice. Nevertheless, ALT and AST enzyme activities in serum were significantly decreased in PZQ treated mice as compared to Inf. EAC group. Meanwhile, ALT activity had a significant increase in cisplatin 10 µg and cisplatin 40 µg groups as compared to Inf. EAC group. On the other hand, AST activity was significantly increased in cisplatin (0.5 mg/kg) or cisplatin (2 mg/kg) groups and in cisplatin (2 mg/kg). PZQ group as compared to Inf. EAC group. Also, PZQ group exhibited a significant increase in ALP activity as compared to Inf. EAC group. However, cisplatin (0.5 mg/kg) or (2 mg/kg) with or without PZQ groups exhibited a significant decrease in GGT activity as compared to Inf. EAC group. Interestingly, treated group with cisplatin (2 mg/kg) with PZQ showed a significant decrease in GGT activity as either compared with that in cisplatin (0.5 mg/kg) or (2 mg/kg) groups.

The total protein (TP) concentration was significantly increased in all treated groups as compared with any of the untreated groups. Whereas TP was significantly increased in cisplatin (2 mg/kg) group, it was significantly decreased in cisplatin (0.5mg/kg). PZQ group as compared to cisplatin (2 mg/kg) alone group. On the other hand, PZQ with or without cisplatin (0.5 mg/kg) treated groups showed a significant decrease in total bilirubin concentration as compared to Inf. EAC group. However, total bilirubin concentration was

Table 2 — Assessment of the changes in the differential leucocytes' counts in different groups under study

	Collection results of CBC				
	platelet $\times 10^3$/cmm	WBCs$\times 10^3$/cmm	segmented %	lymphocyte %	Monocyte %
Control	772±58	10±1	3±1	84±1	13±1
Inf. alone	542±48	6±0.4[a]	6±2	87±4	5±1[A]
EAC	481±60	6±1[a]	7±0.4	74±8[b]	9±2
Inf. EAC	536±43	6±1[A]	7±1	85±2	7±2
PZQ	439±91[a]	7±1	7±2	86±5[c]	7±1[a]
Cisplatin (0.5 mg/kg)	706±86	8±1[d]	5±1	82±2	9±2
Cisplatin (2 mg/kg)	456±110	4±1[A, e. F]	6±1	77±2	6±1[A]
Cisplatin (0.5 mg/kg) PZQ	513±49	7±1[a, g]	7±2	86±5	7±1[a]
Cisplatin (2 mg/kg) PZQ	508±123	3±1[A, b, c, e, F, h]	6±1	84±2	8±1[a]
P -value	0.028*	0.000**	0.252	0.008*	0.000**

Table 3 — Assessment the changes Absolute count of differential leucocytes counts in different groups under study

	Collection results of absolute counts		
	Absolute segmented	Absolute Lymphocyte	Absolute Monocyte
Control	0.2±0.04	8±0.4	1±0.09
Inf. alone	0.4±0.1	5±0.1 [a]	0.3±0.03 [A]
EAC	0.4±0.05	4±0.0 [A]	1±0.1 [A]
Inf. EAC	0.4±0.09	5±0.2 [A]	0.4±0.07 [A]
PZQ	0.5±0.09	6±0.6 [d]	0.4±0.04 [A]
Cisplatin (0.5 mg/kg)	0.5±0.07	7±0.4 [c, d]	1±0.2 [b]
Cisplatin (2 mg/kg)	0.2±0.04	3±0.4 [A, b, E, F]	0.2±0.04 [A, F]
Cisplatin (0.5 mg/kg) PZQ	0.5±0.1	6±0.5 [a, g]	0.4±0.04 [A, f]
Cisplatin (2 mg/kg) PZQ	0.2±0.04	3±0.3 [A,E, F, h]	0.3±0.04 [A, f]
P -value	0.120	0.000**	0.000**

Table 4 — Effect the administration of cisplatin and praziquantel on plasma parameters in the mice model.

Groups Mean ±SE	ALT (U/L)	AST (U/L)	ALP (U/L)	γ-GT (U/L)	Protein (g/dL)	Total Bilirubin (mg/dL)
Control	22±0.3	34.42±0.4	126.4±0.8	9.44±0.1	7.28±0.04	0.543±0.08
Inf. alone	62±2 [A]	73.91±0.8 [A]	157.0±6 [A]	20.60±0.1 [A]	4.85±0.03 [A]	0.721±0.02
EAC	25±1 [B]	64.34±0.9 [A,B]	193±2 [A,B]	28.78±0.3 [A,B]	4.97±0.03 [A]	8.890±0.2 [A,B]
Inf. EAC	43±0.5 [A,B,C]	69.12±0.9 [A,B,C]	174.1±1 [A, b, C]	25.3±0.9 [A,B,C]	4.91±0.07 [A]	8.143±0.02 [A,B,C]
PZQ	30±2 [A,B,D]	62.8±0.6 [A,B,D]	227±1 [A,B, C,D]	24.24±0.1 [A,B,C]	5.65±0.03 [A,B ,C,D]	5.722±0.07 [A,B,C,D]
Cisplatin (0.5 mg/kg)	49±0.9 [A, B, C ,d ,E]	81.65±0.9 [A,B,C,D,E]	71.7±1 [A,B, C,D,E]	20.07±0.2 [A,C,D]	6.79±0.05 [A,B,C,D,E]	8.926±0.03 [A,B,D,E]
Cisplatin (2 mg/kg)	52±0.5 [A,B,C,D,E]	90.85±0.7 [A,B,C,D,E,F]	68.6±1 [A,B,C,D,E]	15.4±0.3 [A,B,C,D,E]	8.19±0.1 [A,B,C,D,E,F]	10.425±0.04 [A,B,C,D,E,F]
Cisplatin (0.5 mg/kg). PZQ	39±0.5 [A,B,C,E,F,G]	69.6±0.5 [A,B,C,E,F,G]	76±0.6 [A, B,C,D,E]	16.03±0.3 [A,B,C,D,E]	6.32±0.04 [A,B,C,D,E,F,G]	7.259±0.04 [A,B,C,D,E,F,G]
Cisplatin (2 mg/kg). PZQ	41±0.8 [A,B,C,E,F, G]	74.3±0.4 [A,C,D,E,F,G,H]	70.3±1 [A, B,C,D,E]	41±0.8 [A,B,C,E,F, G]	2.64±0.005 [A,C, D,E, f ,h]	70.3±1 [A, B, C,D,E]
P-Value	0.000**	0.000**	0.000**	0.000**	0.000**	0.000**

significantly increased in cisplatin (0.5 mg/kg) or cisplatin (2 mg/kg) groups as compared to Inf. EAC group (Table 5).

Hepatic Catalase activity was significantly decreased in Inf. alone, cisplatin (2 mg/kg) and cisplatin (2 mg/kg). PZQ groups as compared to control naïve mice (Table 5). The levels of MDA were significantly increased in Inf. alone, Inf. EAC groups and in all treated groups as compared with that in naive mice. MDA level was significantly increased in cisplatin (2 mg/kg) and cisplatin (2 mg/kg). PZQ groups as compared to Inf. EAC group. PZQ group showed a significant increase in hepatic MDA level as compared to cisplatin (0.5 mg/kg) alone group (Table 5). Hepatic GSH level was significantly decreased in cisplatin (0.5 mg/kg) or (2 mg/kg) with or without PZQ treated mice as compared either with that in EAC or PZQ groups. As compared with GSH level in Inf. EAC group, cisplatin (0.5 mg/kg) and cisplatin (2 mg/kg) with or without PZQ groups showed a significant decrease. However, GSH level was significantly increased in cisplatin (0.5 mg/kg). PZQ group as compared with that in cisplatin (0.5 mg/kg) alone group. Additionally, cisplatin (0.5 mg/kg) or (2 mg/kg) with PZQ groups induced a significant increase in GSH level as compared to cisplatin (2 mg/kg) group (Table 5).

Hepatic GPx activity was significantly decreased in all untreated groups and all treated groups as compared with that in normal mice. In addition, cisplatin (2 mg/kg) group induced a significant decrease in GPx activity as either compared with that in inoculated EAC alone group or in Inf. EAC group (Table 5). As compared to the normal mice, Hepatic

Table 5 — Effect the administration of cisplatin and praziquantelliver homogenate parameters in the mice model.

Groups	Catalase (M/min/g liver)	MDA content (nM/g liver)	Thioredoxinreductase (TNB µM/min/mL	GPx (µM of NADDPH oxidized /min/g liver)	GSH (µM/g liver)	ASA (nM/h/mg protein)	ASB (nM/h/mg protein)
Control	226±35	169±10	0.749±0.01	2.63±0.1	19±1	0.562±0	73 ± 4
Inf.alone	147±6 [a]	310±16 [A]	0.0913±0.03 [A]	1.13±0.1 [A]	18±0.2	0.665±0.015 [a]	80.4± 0.2 [a]
EAC	159±4	191±4 [B]	0.864±0.01 [a,B]	1.60±0.05 [A, b]	22.7±1 [B]	0.353±0.019 [A ,B]	48.9± 0.1 [A, B]
Inf.EAC	158±4	250±9 [A]	0.893±0.02 [A,B]	1.50±0.03 [A]	21.5±1	0.365±0 [A, B]	49.9±0.8 [A, B]
PZQ	186±16	311±7 [A,C]	0.482±0.02 [A,B,C,D]	1.45±0.04 [A]	21.85±0.7	0.309±0.02 [A,B]	37.4±1 [A, B, C, D]
Cisplatin (0.5 mg/kg)	150±18	283±31 [A,C]	0.549±0.03 [A,B,C,D]	1.14±0.08 [A, c]	12.4±1 [A, B, C,D, E]	0.384±0.01 [A, B]	40.1±0.4 [A, B, c, D]
Cisplatin (2 mg/kg)	105±17 [A]	317±10 [A,C, d]	0.462±0.03 [A,B,C,D]	0.96±0.07 [A, c, d,e]	9.52±0.7 [A, B, C,D, E]	0.634 ±0.03 [C, D, E, F]	44.9±0.3 [A, B, e]
Cisplatin (0.5 mg/kg).PZQ	162±14	289±6 [A,C]	0.583±0.01 [a,B,C,D,e,G]	1.32±0.1 [A]	17±1 [C, d, E, F,G]	0.400 ±0.01 [A, B, G]	38.1 ±0.9 [A, B, C, D, g]
Cisplatin (2 mg/kg).PZQ	127±9 [a]	347±7 [A,C,D ,f]	0.489±0.02 [A,B,C,D,h]	1.16±0.07 [A]	16±0.5 [C, D, E, G]	0.508±0.03 [B, C, D, E, f,G,h]	43.4 ±0.6 [A,B, d]
P -Value	0.001*	0.000**	0.000**	0.000**	0.000**	0.000**	0.000**

thioredoxin reductase activity was significantly decreased in Inf. alone group and all treated groups. In contrast, the activity was significantly increased in inoculated EAC alone and Inf. alone groups as compared with that in normal mice. Additionally, thioredoxin reductase activity was significantly decreased in all treated groups as either compared with that in inoculated EAC alone or Inf. Alone groups (Table 5).

Hepatic Aryl sulphatase A (ASA) activity was significantly increased in cisplatin (2 mg/kg) with or without PZQ groups as either compared with Inf. alone group or Inf. EAC groups. Additionally, cisplatin (0.5 mg/kg) or (2 mg/kg) with PZQ groups exhibited a significant decrease in ASA activity as compared with that in cisplatin (2 mg/kg) group. In contrast, cisplatin (2 mg/kg) with PZQ group exhibited a significant increase in ASA activity as compared with that in cisplatin (0.5 mg/kg). PZQ group. Hepatic Aryl sulphatase B (ASB) activity was significantly increased in all untreated groups and all treated groups as compared with that in normal mice. In contrast, PZQ with or without cisplatin (0.5 mg/kg) or (2 mg/kg) groups exhibited a significant decrease as compared with that in Inf. EAC group (Table 5).

Discussion

We showed in the current study that inoculation of EAC cells in Inf. EAC group did not affect the total worm burden and egg counts in the intestine and liver. Nevertheless, cisplatin with or without PZQ treatment showed a significant toxic effect on the total worm burden and egg counts in the intestine and liver as compared with either Inf. or Inf. EAC mice. Also, it is revealed that the treatment of PZQ combined with cisplatin (0.5 mg/kg) or (2 mg/kg) in infected animals caused almost similar high percentage of eradication of worms and tissue egg load. The death of the worms due to the treatment with anti-schistosomal drugs was attributed to metabolic disorders, mechanical destruction and muscular contraction of the treated worms, suggested the direct effects on the eggs, due to the enhancement of the immunological reactions[28].

In addition, the administration of cisplatin induced a significant reduction in the size of this granulomatous inflammation compared to the livers of infected non-treated animals. While the diameter of the granulomas reached approximately 1.2 mm^3 in infected liver of Inf. alone mice and 1.4 mm^3 in infected liver of Inf/EAC bearing mice, treatment with cisplatin (2 mg/kg) significantly reduced their diameter to 0.4 mm^3 as compared to Inf. alone mice, 0.7 mm^3 as compared to Inf. EAC bearing mice. This reduction in granuloma size was approximately similar to that in the PZQ treated group. In the view of this study, it can be concluded that the decrease in the number of the eggs in cisplatin treated mice and the stability of number of worms in the same mice may be explained on the basis that cisplatin (0.5 mg/kg) or (2 mg/kg) may act on the reproductive organs of the worm and the interruption of oviposition in the intestinal wall and by the maturation of viable eggs already there. Moreover, mice treated with PZQ and cisplatin (2 mg/kg) displayed a highly significant

increase in the percentage of egg reduction in the liver, that in agreement with Tavares who reported that cisplatin may affect the female reproductive organs of the parasites leading to a significant decrease in egg counts and worm burden[29].

Parasitologically, the addition of cisplatin, increased the potency of PZQ in reducing the worm burden and ova count/gram liver, which correlate with Pitta who found that drugs that were and are being used in human chemotherapy looking to the past to improve rational design drugs in the future[30]. Not only clinical used compounds will be shown but also synthesized and tested compounds *in vitro* and *in vivo* in animal models. Also, the provided patents filed of compounds presenting schistosomicidal activity between 1988 and 2012 that included synthetic platinum complexes.

Several studies have revealed that there is an association between Schistosomiasis infection and developing of cancer disease[31]. Infections with *Schistosoma* in general can initiate the chronic carcinogenesis through the inflammation due to the persistence of infectious agents. The long persistence of the worms in the body causes the activation of the macrophages which located at the sites of inflammation. Due to this activation, the macrophages release the reactive oxygen radicals and reactive nitrogen radicals[32]. The potential power of these radicals can lead into DNA damage and subsequently to further events such as mutations, DNA strand breaks, and sister chromatid exchanges[33]. The use of metal containing drugs as anti-parasitic agents has been very little explored.

As mean, some metal complexes as gold complexes that previously developed exhibited anti-parasitic agents using the metal–drug synergism approach as a powerful tool which could provide effective treatment for parasitic diseases[34]. This activity enhancement is possibly related to the stabilization of the drug by coordination to the metal ion, which leads to a longer residence time of the drug in the organism and more efficient biological targeting[35]. Intriguingly, Metal containing drugs act as novel insights into fighting the infection of parasite.

Consistently, Song reported that cisplatin has cytotoxic and anti-schistosomal effects on *Schistosoma japonicum* adult worms *in vitro* and their inhibition on thioredoxin glutathione reductase (TGR)[36]. Indeed, Mammals have two distinct detoxification enzymes, thioredoxin reductase and glutathione reductase, while in schistosomes these catalytic activities are performed by one molecule, thioredoxin-glutathione reductase. Our results confirm earlier observations that praziquantel and cisplatin, two currently available anti-schistosomal drugs[37]. A promising recent success in the identification of new anti-schistosomal drug candidates as inhibitors of the schistosome enzymes.

On the other hand, the activities of plasma ALP and GGT were an extremely significant decreased in PZQ either co treated with cisplatin (0.5 mg/kg) or cisplatin (2 mg/kg) groups, but AST activity was extremely significant increased, also total lipid and total protein contents were extremely significant increased as compared with that in non-treated Inf. EAC mice. Whereas, PZQ with cisplatin (0.5 mg/kg) or cisplatin (2 mg/kg) treated groups showed extremely significant increase hepatic ALT and AST levels.

Reduction in total protein level in serum and tissues of animals during EAC/infection have been reported on numerous occasions. The current study, showed a significant decrease in serum total protein of Inf. EAC bearing non-treated mice as compared to control mice. These data are in consistent with the previous study that obtained decrease in total proteins may be attributed to that Inf. EAC bearing has the usual nutritional requirement for essential amino acids, suggesting that amino acids may provide an alternative energy source to carbohydrate for host metabolism or contribute to glucose synthesis and carbohydrate repletion through gluconeogenesis in infected animals[38]. This, in turn, indicates a high level of deamination or transamination. In addition, such decrease may also occur because of tissue damage and the action of hydrolytic enzymes released by developing parasites or host lysosomes. The present results recorded amelioration of liver function enzymes and variably improvement of other parameters after treatment of Inf. EAC bearing with either PZQ with or without cisplatin. In the present study, hepatotoxicity by cisplatin treatment is characterized by a significant increase in total bilirubin. In agreement with Cavelli, who found that liver toxicity of cisplatin was characterized by mild to moderate elevation of serum bilirubin[39].

On an contrary, treated group of PZQ with cisplatin (2 mg/kg) showed extremely significant decrease in TrxR activity, GSH content. But, the same treated group showed a slightly significant decrease in hepatic total protein content and ASB level but

showed an extremely significant increase in MDA, ASA levels. Meanwhile, PZQ with cisplatin (0.5 mg/kg) treated group showed extremely significant decrease in TrxR activity and ASB level, also a slightly significant decrease in GSH content.

In addition, TrxR decreased in the Infected alone group due to Schistosomes eggs possess significant amounts of TrRx that utilize H_2O_2require reduced Trx *via* TrxR and NADPH. While, Schistosomes have a unique redox pathway controlling the multifunctional enzyme TrGR maintains high levels of reduced glutathione either by direct reduction of glutathione disulphide by thioredoxin system plays a significant role in redox balance and in the antioxidant defense of *S. mansoni*. On the other hand, TrxR increased in EAC alone group due to tumor proliferation is dependent on a constant deoxyribose nucleotide supply which in turn depends on an active Trx/TrxR system, enhanced DNA synthesis and defense against the host's immune system. Whereas, Cisplatin with PZQ treated groups act as potential inhibitors of the parasite enzyme TrGR which is a sole enzyme of TxR and GR in adult *S. mansoni*, also the glutathione-cisplatin adduct (GS-Pt) was able to inhibit both TrxR1 and GRX. Furthermore, results may suggest that the inhibition of hepatic TrxR by cisplatin, greatly reduced the GSH level and resulted in rapid worm death. This in agreement with the previous study that obtained that TrxR was considered a potential target for the development of novel drugs against tumours and infectious pathogens; we report that cisplatin can inhibit parasite load.

On the other hand, the inhibition or stripping of the enzyme by cisplatin would lead to indirect inhibition of DNA synthesis through non-availability of various nucleosides, thus interfering with cell metabolism and replication. Alterations of membrane-associated enzymes are also involved in various multidrug-resistance mechanisms[40].

Arylsulphatases A and B are lysosomal and hydrolytic enzymes that occur in various tissues and fluids and important parameters of drug toxicity and hepatotoxicity, While Arylsulfatases increases in inflammation, intoxication, and malignancy. Present study showed the specific activity of hepatic ASB was progressively increased with the progression of the infection[41], but PZQ treated group with or without cisplatin doses showed decreased in ASB, so that toxification decreased than that in cisplatin alone treated groups which showed increase hepatic arylsulphatases in accordance with the previous study that exhibited that the increase in the number of large lysosomes after platinum drug use, and platinum has been shown to accumulate in the lysosomes and peroxisomes in the liver[42]. Besides, overproduction of the reactive oxygen species which affects the integrity of the lysosomal membrane affecting lysosomal enzymes due to the increased ROS, increased amounts of H_2O_2 can penetrate into lysosome. It suggested that the elevation in the hepatic ASA and ASB activities may be related to lysosomal membrane permeabilization mediated by oxidative stress, which is one of the predominating features in *S. mansoni* infection, cisplatin toxicity.

The strategy of combination therapy has the additional advantage that it might significantly delay the possible development of drug-resistant parasites. This is of pivotal importance and has been reviewed in great detail in the case of malaria, where a combination therapy has been proposed as an approach to delay or reverse resistance in the *Plasmodia* parasites. The pathogenesis of cisplatin toxicity is attributed to the formation of ROS[43], DNA damage and mitochondrial damage. Hence, The DNA is thought to be the critical target for this type of compounds has clearly demonstrated that cisplatin can induce nucleus independent apoptotic signalling. The formation of platinum adducts has been shown to block DNA replication[44].

The main cause of mortality and morbidity in human schistosomiasis is hepatic fibrosis which is essentially dependent on granulomas. While Granulomatous inflammation in schistosomiasis is a cell-mediated hypersensitivity to parasitic egg antigens that are lodged in hepatic tissue[45,46]. Administration of PZQ and/or cisplatin in the present study resulted in a reduction in egg deposition, hence improved liver architecture and prevented or attenuated the decrease in tissue antioxidant enzymes. Hence, these combinatorial therapies may provide cellular protection against reactive oxygen species arising due to infection. It is still puzzling whether the mutual action of antiparasitic and anticancer drugs may be enormously related to similar target molecules for these drugs in cancers and parasites.

Conclusion

The oxidative processes that occur upon infection with *Schistosoma* seem to go uncontrolled; such events may be, at least, in part, responsible for the

pathology associated with schistosomiasis. At the same time, treatment with both cisplatin and PZQ may be beneficial due to their immuno-modulating and anti-oxidative actions. We recommend increasing the treatment time of both drugs as a novel insight to complete eradication of worm and ova. Intriguingly, further analysis of the mutual interaction between PZQ and cisplatin is still required.

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