ANTISCHISTOSOMAL EFFICACY STUDY ON PRAZIQUANTEL, ITS ALKALINE HYDROLYSIS AND THE SUN-DECOMPOSED PRODUCTS

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إ دواء برازيكوانتل (PZQ) المعطى بجرعة مقدارها 40 مغ/كغ من وزن الجسم للفئران البيضاء المصابة بداء شستوسوما مانسوناي (البلهارسيا) كان غير فعال بما يكفي لإحداث انخفاض معنوي في متوسط الشفاء من الإصابة بديدان البلهارسيا 16.6%. ويدل الانخفاض الذي سببه المركب الناتج من الحلمأة القلوية (PZQ-HP) 28.6% ، والمركب الناتج من التحلل الشمسي (PZQ-SDP) 47.6% للدواء على أنهما أكثر فاعلية من الدواء نفسه. ولا توجد فروق معنوية في تعداد البيوض في الكبد وفي أنسجة الأمعاء الذي تم أجراؤه على مجموعة الفئران المصابة والتي تم علاجها ، وعلى مجموعة الفئران المصابة والتي لم يتم علاجها. إن الأورام الحبيبية الملحوظة في أكباد الفئران المصابة معملياً بداء شستوسوما مانسوناي غير المعالجة كانت خلوية وموجودة بصورة رئيسية في القناة البابية في النسيج الحشوي (البرنشيمة) تحت المحفظة Subcapsular . كما لوحظ وجود خضاب شستوسوما في خلايا كوفر مع تغلظ في القناة البابية. وقد تمثل الضرر الوعائي في انسدادات الأورام الحبيبية ، والتهاب الوريد ، وقيود وعائيةperivascular cuffing حول الوريد المركزي واتساع جيبي. كما تبين أن التغيرات الهستوباثولوجيه الملحوظة في المجموعات المعالجة بكل من دواء برازيكوانتل والمركب الناتج منه بالحلمأة القلوية كانت متماثلة ، ومتميزه بتناثر الأورام الحبيبية في القناة البابية وفي الأنسجة الحشوية داخل الفصيصات. وتمثل الضرر الوعائي في هاتين المجموعتين(PZQ) و (PZQ-HP) في التهاب أوردة بعض الجذيرات البابية ، وانسدادات الأورام الحبيبية ، وقيود وعائية نادرة حول الوريد المركزي. وقد دلت هذه النتائج على أن فاعلية هذين المركبين كانت متماثلة. أما الأورام الحبيبية في المركب الناتج من التحلل الشمسي فقد تبين أنها موزعة بين الجذيرات البابية والأنسجة الحشوية بالتساوي مع البيوض المركزية و/أو القشور أو النخر. وكان خضاب شستوسوما أقل حدة عن مثيله في الفئران غير المعالجة مع بؤر نادرة من كريات البيضاء ، وقيود وعائية حول الوريد المركزي والتهاب في الوريد البابي. وكان عدد الأورام الحبيبية في مجموعة الفئران المعالجة بالمركب الناتج من التحلل الشمسي لدواء برازيكوانتل أقل من عدد الأورام الحبيبية في مجموعة الفئران المصابة وغير المعالجة ولكنها كانت أكبر حجماً ، بينما كان متماثلاً في الفئران المعالجة بدواء برازيكوانتل وبالمركب الناتج من حلمأته القلوية في الحجم ولكنها كانت مختلفة بصورة معنوية في العدد ($p=0.01$) بالمقارنة مع الفئران المصابة وغير المعالجة. وقد تم تفسير وتبرير هذه التباينات. وبالرغم من موت نصف المجموعة المعالجة بالمركب الناتج بالتحلل الشمسي لدواء برازيكوانتل خلال الأسبوعين الأول والثاني للمعالجة ، فإن الدراسة الهستوباثولوجية لم تكشف عن أي دلائل على وجود أي آثار كبدية سامة.

mice, was not active enough to cause a significant reduction in the mean worm recovery 16.6 %. The reduction caused by the alkaline hydrolysis product, (PZQ-HP) 28.6 %, and the sun decomposed product, (PZQ-SDP) 47.6 % of PZQ suggested higher antischistosomal activity of these two products compared to PZQ. There were no significant differences in liver and intestine tissue egg count carried on the infected-treated groups, and the infected-untreated group. The granulomas observed in the livers of the experimentally *S. mansoni* infected-untreated mice were mainly cellular existing within the portal tract and subcapsular parenchyma. Schistosome pigments were observed in the Kuffer cell with thickening of the portal tract. The vascular lesions comprised granulomatous occlusions, periphelebitis, perivascular cuffing of the central vein and sinusoidal dilatation. The hepatic

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histopathological changes observed in the PZQ, and the PZQ-HP-treated groups were similar, characterized by the scattering of the granulomas in the portal tract and intralobular parenchyma. The vascular lesions in these groups (PZQ and (PZQ-HP)) comprised periphelebitis of some portal radicles, granulomatous occlusions and rarely the perivascular cuffing of the central vein. These results indicated comparable efficacy of the two compounds. The granulomas of the PZQ-SDP treated group were found distributed between the portal radicles and parenchyma in equal manner with central egg and/or shell or necrosis. Schistosome pigments were less intense than in the infected-untreated mice with infrequent leucocytic foci, perivascular cuffing of the central vein and periphlebitis of the portal vein. The number of granulomas in the SP-treated group was less than in the infected-untreated mice with greater size whereas that of PZQ- and PZQ-HP-treated groups were comparable in number and size but significantly different in number ($p= 0.01$) in comparison with the infected-untreated mice. These variations were interpreted and justified. Despite of the death of half the group treated with PZQ-SDP compound within the first and second week post treatment; there were no signs of hepatotoxic effects as revealed by the histopathological study conducted at our laboratories.

Key words: Schistosomiasis, praziquantel (PZQ), histopathology, photodecomposition, thermal-decomposition, efficacy.

Introduction

Worldwide, six hundred million people are subject to the risk of infection by schistosomiasis and actually 200 million people are infected (1-3). It has been reported that, an estimation for infection rate throughout the world could not be obtained and the population being infected by schistosomiasis is highly increasing in the developing countries due to displacement, construction of irrigation schemes, and water barriers which has created a suitable habitat for fresh water snails (2, 4-6). Human Schistosomiasis and its control became a major health problem in tropical and subtropical countries including Sudan (7, 8).

The United States Food and Drug Administration (US/FDA) has approved Praziquantel (PZQ) for use against all forms of schistosomiasis (9,10).

The development of PZQ for the treatment of schistosomiasis as well as other trematode (fluke) and cestode (tape worm) infections is an important advance in anthelmintic chemotherapy as the drug is highly effective against a broad range of helminths, where other drugs have failed. PZQ can be orally administered in a single dose or several doses in one day, with minor apparent toxicity (10,11).

Under the conditions of the climatic zone 111 prevailing in Khartoum/Sudan, with a mean kinetic temperature of 32.5 ^{o}C, 40 days per annum with temperature above 30 ^{o}C, and 15 days above 40 ^{o}C (12), PZQ was observed to change in color. The stability studies conducted in our laboratories confirmed that, the drug was subject to photo-degradation, and/or hydrolysis via the lactam ring, leading to formation of the corresponding acid derivatives (13,14).

The analyses of the unreacted PZQ and the correspondingly resulting products were carried out by HPLC (13, 15, 16).

Clinicopathological findings in the experimentally *S. mansoni* infected mice treated with PZQ, PZQ-HP, and the PZQ-SDP indicated that, the three compounds have induced partial suppression of worm fecundity as judged by the significant reduction in egg per gram of faeces relative to the infected- untreated control, which approximately reached 75% for all the treated groups (17, 18).

The efficacy of the HP compound was found to be comparable to that of PZQ in terms of percent reduction in worm recovery (17-20). Thus, it became a prime objective to conduct a histopathological study on experimentally *S. mansoni* infected mice, treated with PZQ, PZQ-HP and PZQ-SDP against infected untreated mice and uninfected mice to further assess the efficacy and/or toxicity of these compounds and/or their relevant metabolites.

Material and methods

Animals:

Sixty male and female locally bred Albino mice, aging between 8 and 12 weeks, weighing 20 to 25 grams were used in the experiment. Eight mice were kept as uninfected control. The rest of the mice were each infected with 100 cercariae of *S. mansoni*. All the mice were kept at the same laboratory conditions and were freely allowed food and water.

Drugs:

PZQ, which is chemically 2-cyclohexyl-carbonyl-4-oxo-1, 2, 3, 6, 7, 11b-hexahydro-4H-pyrazino [2.1a]

isoquinoline, was kindly donated by the General Medicine Co. LTD, Khartoum, Sudan.

The PZQ-HP compound was obtained by refluxing PZQ in sodium hydroxide (1:1 molar ratio) for three hours at 90 °C with continuous stirring. The resultant solution was adjusted to pH 4.5 by adding 0.5 M hydrochloric acid, and was left to precipitate overnight. The resultant solid product was recrystallized from warm methanol (13, 14, 21).

The PZQ-SDP product was obtained by direct exposure of PZQ powder to sun rays over a period of 4 months, when it lost about 50% of its initial content as determined by HPLC (13, 15, 17).

The final suspensions of the three compounds which were orally administered to the infected mice, were prepared by separately weighing 40 mg of each compound followed by suspending and sonicating the compound in 10 ml of 15 % solution of propylene glycol in water.

Parasitological parameters:

The miracidia were collected from pooled stool sample of bilharzia patients at Abu Ushar, Gezira Province, Sudan. Snails were collected and individually placed in glass tubes, each containing 5 ml of distilled water. Two to three miracidia were added to the water and allowed one hour to penetrate the snail. Collection of cercariae commenced on the fourth week, post infecting the snails. A hundred cercariae were used to infect each mouse (22, 23).

Histopathological techniques:

Mice were sacrificed on the tenth week post infection. Adult worm recovery and tissue egg count on liver and intestine were performed on the sacrificed mice (17, 18, 23).

Pieces of the livers of the uninfected animals, infected-untreated animals and the infected animals treated with PZQ, PZQ-HP and PZQ-SDP were fixed in 10 % neutral buffered formalin, processed, sectioned and stained with haemotoxylin and eosin (H & E).

The hepatic lesions were microscopically viewed and investigated. The number of granulomas per field was counted under the microscope with its eyepiece fitted with a micrometer to allow determination of granuloma size.

Experimental design:

The mice, each of which was infected with 100 cercariae of *S. mansoni,* were divided into 4 groups, each consisting of 13 mice following appearance of the eggs in faecal samples on the seventh week post infection. Three groups were separately treated with 40 mg/kg body weight of PZQ, PZQ-HP and PZQ-SDP compounds respectively, whereas the fourth group was reserved as infected- untreated control.

The different groups were designated as follows:

Group (A): Infected, untreated control.
Group (B): Infected, treated with PZQ.
Group (C): Infected, treated with PZQ-HP .
Group (D): Infected, treated with PZQ-SDP.

All the experimentally *S. mansoni* infected-untreated, infected-treated, and the uninfected control mice were sacrificed on the third week post treatment (Tenth week post infection).

Adult worms were recovered by using the perfusion technique and were counted under the microscope. Tissue egg count was performed on liver and intestine of infected-untreated and infected-treated groups by adopting the potassium hydroxide digestion method (17, 18, 23).

Sections of livers of all the groups were microscopically examined.

Statistical analysis:

Statistical analysis was carried by using SPSS package for windows, version 7.5, and applying the student t-test.

Results

The number of worms recovered from the infected untreated animals, and the animals infected and treated with PZQ, PZQ-HP and PZQ-SDP is shown in table 1. The lowest number of recovered worms relative to infected-untreated control group is that of PZQ-SDP group (47.6%), PZQ-HP group (28.6%), and the PZQ group (16.6%), respectively. These results were significant for all the infected and treated groups ($p= 0.01$). If the results are described in worm/pair, the difference in worm recovery would not be that significant, and could mainly be attributed to variations in sex ratio (male: female) between infected- untreated control group (11:10), and the infected PZQ, PZQ-HP and PZQ-SDP-treated groups (5:2, 7:3, and 7:4), respectively. Free *S. mansoni* male worms were counted, and they were observed to have a reduced length, ranging from half to two-thirds of the coupled parasites, length in all the treated groups. The number

of free parasites was 15, 11, and 1 for PZQ, PZQ-HP, and the PZQ-SDP -infected and treated groups, respectively. It is worth mentioning that, half the population of the SP-treated group died within the first and the second week post treatment.

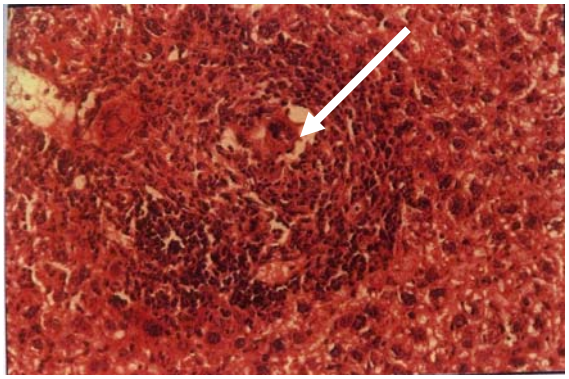

Figure 1. Liver of the infected untreated mouse showing a typical cellular granuloma with the ova centrally situated. Schistosome pigment is also apparent in Kuffer cells. H&E x 250.

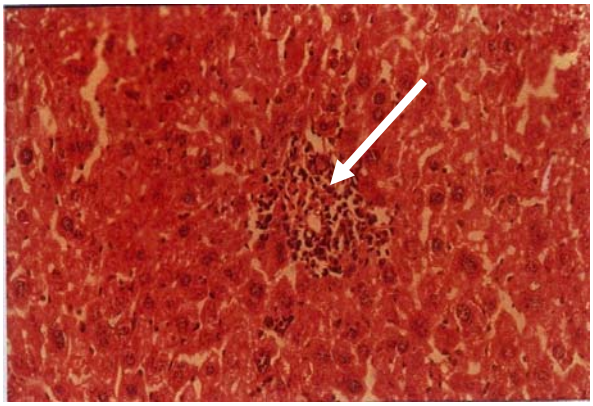

Figure 2. Liver of infected and PZQ-HP-treated mouse showing leucocytic foci. Note: Schistosome pigment of the Kuffer cells. H&E x 250.

All the infected and treated groups showed lower liver and intestine tissue egg count compared to the infected-untreated group. The liver egg load was found to be in the order of PZQ-HP- treated group>PZQ-treated group> PZQ-SDP-treated group. On the other hand, the intestine egg load was in the order of PZQ-SDP-treated group> PZQ-treated group> PZQ-HP-treated group.

The most striking lesion observed in the liver of the infected-untreated control group was the presence of the granulomas within the portal tract and subcapsular parenchyma. The granulomas were predominantly cellular with or without centrally situated eggs or their shells (Figure1).

The cells were mainly lymphocytes with epithelioid and other mononuclear cells. A small number of granulomas showed necrosis encircled by lymphocytes and mononuclear cells (Figure1).

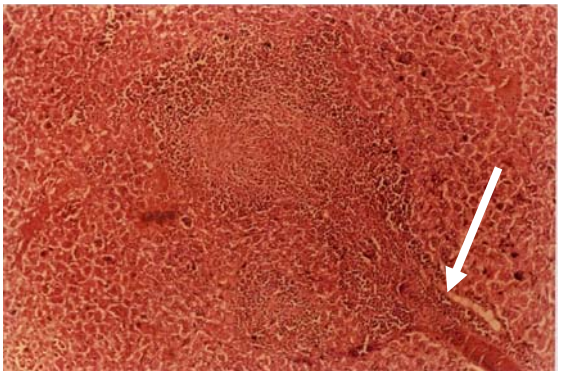

Figure 3. Liver of infected and PZQ-HP-treated mouse showing thickening of the portal tract, granulomatous occlusion and periphlebitis of the portal radicles. H&E x 250.

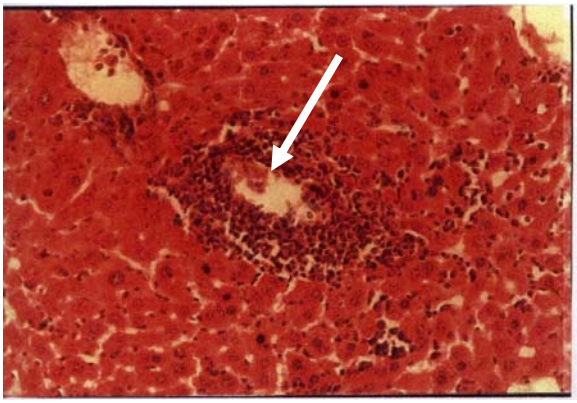

Figure 4. Liver of infected-untreated mouse showing perivascular cuffing of the central vein. Note Schistosome pigment in the surrounding Kuffer cells.H&E x 250

The newly formed intraportal granulomas contained reticular tissue between the central eggs, or the shells and the enveloping cellular substance composed mainly of lymphocytes.

The average number of granulomas in the liver of the infected-untreated mice ranged between 18 and 41 with an average of 25 ± 9 (Table 2). The size of the granulomas varied from 139 to 216 μm with an average of 177 μm ± 39 (Table 3).

Plenty of schistosomal pigment was observed especially in Kuffer cells, which were swollen and proliferated (Figures 1, 2 and 4).

Thickening of the portal tract was evident due to infiltration with mononuclear cells (Figure 3).

Multiple foci of leucocytic infiltration were seen between the hepatic cells in almost all the examined sections, suggesting active hepatitis (Figure 2).

Table 1: Adult worm recovery and liver & intestine tissue egg count.

Group	No. of mice	Average Number of worms / mouse ($\bar{x}$ ± SD)	% worm recovery	Sex ratio (M:F)	Eggs / gram ($\bar{x}$ ± SD)		Observations
					Liver	Intestine	
Infected, untreated (A)	13	42±4	------	11:10	5207 ± 3150	4176 ± 2512	----------
Infected, PZQ treated (B)	13	35±5	16.6	5:2	3690 ± 1920	3524 ± 1971	15 single parasites with shrunked length
Infected, PZQ-HP treated (C)	13	30±5	28.6	7:3	4392 ± 1487	2768 ± 2315	11 single parasites with shrunked length
Infected, PZQ-SDP treated (D)	13	22±4	47.6	7:4	3393 ± 848	3854 ± 1755	1 single parasite with shrunked length

Table 2: Minimum, maximum and average number of granulomas in the liver of the infected-untreated control group, the PZQ, PZQ-HP and PZQ-SDP treated groups**.**

Group	Minimum No. of granulomas	Maximum No. of granulomas	Average No. ($\bar{x}$ ± SD)
Infected, untreated control	18	41	25 ± 9
Infected, PZQ treated	10	24	15 ± 8
Infected, PZQ-HP treated	11	13	12 ± 1
Infected, PZQ-SDP treated	15	24	20 ± 4

Table 3: Minimum, maximum and average size of granulomas in the livers of the Infected-untreated control group, PZQ, PZQ-HP and PZQ-SDP-treated groups.

Group descrip-tions	Minimum size of granuloma (µm)	Maximum size of granuloma (µm)	Average size of granuloma ($\bar{x}$ ± SD) µm
Infected, untreated control	139	216	174 ± 39
Infected, PZQ treated	121	165	155 ± 31
Infected, PZQ-HP treated	146	191	174 ± 25
Infected, PZQ-SDP treated	182	241	214 ± 30

Vascular lesions were constant features which comprised granulomatous occlusion, periphlebitis of the portal radicles (Figure 3), as well as perivascular cuffing of the central vein and sinusoidal dilatation (Figure 4).

Histopathological examinations carried out on the livers of the infected, PZQ and PZQ-HP treated groups, revealed that the number of their granulomas was comparable, whereas the difference was significant when compared with the infected-untreated group ($p = 0.01$).

The hepatic lesions in the two groups (Infected, PZQ and PZQ-HP-treated mice) were similar to a great extent, characterized by the scattering of the granulomas in the portal tracts and the intralobular parenchyma.

The granulomas were cellular, having a number ranging between 10 and 24 with an average of 15 ± 8 for the PZQ-treated group, where it was 11 -13 with an average of 12 ± 1 granulomas for the PZQ-HP-treated group (Table 2). The size of granulomas varied from 121 - 165 µm with an average of 155 µm ± 31 and 146 - 191 µm with an average of 174 µm ± 25 for the PZQ and the PZQ-HP treated groups, respectively (Table 3).

Schistosome pigment was found in a moderate amount in the swollen kuffer cells (Figure 2).

The vascular lesions were evident in some sections of the livers of PZQ and PZQ-HP treated groups that comprised periphlebitis of some portal radicles and granulomatous occlusion. Central vein perivascular cuffing was rarely seen (Figure 4).

The examined livers of the PZQ-SDP-treated group revealed that the granulomas were found intraportal and intraparenchymal in almost equal

manner. They were cellular with the typical granuloma structure i.e. central eggs and/or shells or central necrosis enveloped by lymphocytes and other mononuclear cells. The number of granulomas ranged between 15 and 24 with an average of 20 ± 4 (Table 2).

The size of the granulomas in the PZQ-SDP-treated group was relatively large compared to that of the infected-untreated control, PZQ-HP and PZQ treated groups as it ranged between 182 and 241 μm with an average of 214 μm ± 30 (Table 3).

Schistosomal pigment was observed in the Kuffer cells but not as intense as in the infected- untreated control group. Infrequent multiple leucocytic foci and perivascular cuffing was observed. Periphlebitis of the portal vein was also detected in some examined sections of the PZQ-SDP treated group.

Discussion

The toxicity and efficacy of the PZQ hydrolyzed product and the PZQ sun decomposed product were compared to those of PZQ by administrating a low dose of 40 mg/kg body weight (common for human) to albino mice infected with *S. mansoni*. The PZQ dose was not active enough to cause a significant reduction in the mean worm recovery (only 16.6%), whereas the reduction caused by PZQ-HP and PZQ-SDP compounds was 28.6% and 47.6%, respectively. This indicated that, the two had greater antischistosomal activity than that of PZQ.

Shrinkage in the length of the recovered uncoupled male worms was observed and found to be 42.9%, 36.7%, and 4.5% for PZQ, PZQ-HP and PZQ-SDP- treated groups, respectively.

Although the reduction in worm recovery is significant for PZQ, PZQ-HP and PZQ-SDP-treated groups compared to infected-untreated mice ($p = 0.01$), the differences were slight when the worm reduction was expressed in worm/pair i.e. 10, 9 and 8 pairs for PZQ, PZQ-HP and PZQ-SDP, respectively. This could be an explanation for the trivial differences in egg deposition in the liver and intestine of the infected-untreated, and the infected-treated groups (Table 1).

The variations in hepatic pathological lesions were used as a tool to further assess the antischistosomal activity of PZQ, its PZQ-HP and PZQ-SDP products in line with the clinicopathological findings (16, 17).

The common feature in the livers of the infected-untreated group, PZQ, PZQ-HP and PZQ-SDP treated groups is the presence of cellular granulomas However, the number, size and location of these granulomas differ in the various groups and the number was found to be 25 for the infected-untreated group, where it was 15, 12, and 20 for the PZQ, PZQ-HP and the PZQ-SDP-treated groups, respectively. This indicated that PZQ and PZQ-HP had caused partial worm fecundity suppression.

A similar finding was reported in mice experimentally infected with *S.mansoni* and *S. japonicum* treated with PZQ, which resulted in reduction of egg production capacity and the phenomenon was attributed to injuries induced by PZQ in the sexual apparatus of the two worms' species (20).

Although no gross and microscopic examinations were carried out in the worms we have recovered, shrinkage and reduction in size was observed in some of those recovered from PZQ, PZQ-HP, and PZQ-SDP- treated groups.

The size of the hepatic granulomas seen in the group treated with PZQ (155 μm) was less than in PZQ-HP (174 μm), PZQ-SDP (214 μm) and the infected-untreated group (174 μm), reflecting a diminishing cellular response and tissue reaction. This might be attributed to reduction in egg viability induced by PZQ at this early stage of infection. Presence of newly formed small granulomas at later stages of experimental *S. bovis* infection in calves was reported by some researchers (24), and this was attributed to reduced egg viability and spontaneous modulation phenomenon as that reported by some researchers in *S. mansoni* infections (25). The latter is an immunologically mediated response to the eggs characterized by suppression of cellular response to the eggs at chronic stages of the infection. As this experiment was terminated at an early stage (Tenth weeks post infection), viability reduction could be the only possible explanation. On the other hand, Tanaka *et al.* (20) had examined the egg viability in *S. mansoni* experimentally infected mice, and treated with different doses of PZQ ranging from 50-500 mg/kg body weight. The results obtained revealed that the ratio of immature to mature eggs was proportional to the dose increase as judged by the hatchability test carried out.

It is evident from the present results that PZQ, it's PZQ-HP and PZQ-SDP products were not highly effective at a dose of 40 mg/kg against *S. mansoni* infection in mice (26). Tanaka *et al.* (20) reported similar results and they showed that PZQ at a very

high dose of 500 mg/kg caused only 38.9% reduction in worm number. On the other hand, PZQ proved to possess a strong antischistosomal activity against *S. japonicum* in experimentally infected mice as evident by the 80-100% destruction of worms (20). The cidal activity of PZQ against *S.japonicum* was strongly supported by many authors (27-30).

The granulomas observed in the livers of the PZQ-SDP-treated group were characterized by their biggest size and that they were present intraparenchymal in a large number as compared to the other groups. The severe tissue reaction exemplified by the large size of the granulomas might be explained on the grounds that, PZQ-SDP had no effect on egg viability besides, this compound which is metabolized in the liver, might have insulted the hepatic tissues provoking more cellular reactions and hence bigger size of the granulomas.

The death of half the number of the mice treated with the PZQ-SDP compound between the first and the second week post treatment is worth mentioning. In this regard, no signs of hepatoxic effects due to the administration of this compound and/or its metabolites were evident during the histopatho-logical examination carried out in the livers of the PZQ-SDP treated group. The lesions observed and described in our study, were specifically induced by the worms and their eggs. Whatever the explanation might be, this compound (PZQ-SDP) deserves further investigation regarding its chemical composition, structure, mode of action and toxicity.

Vascular lesions comprising granulomatous occlusion, periphelebitis of the portal radicles and central vein, and sinusoidal dilatation were markedly pronounced in the infected untreated control. The PZQ, PZQ-HP and PZQ-SDP treated groups showed moderate changes which indicated that variations in vascular lesions were proportional to the number of worms and their viable eggs.

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