http://www.pjbs.org

PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences 16 (24): 1849-1861, 2013
ISSN 1028-8880 / DOI: 10.3923/pjbs.2013.1849.1861

Overview on Cysteine Protease Inhibitors as Chemotherapy for *Schistosomiasis mansoni* in Mice and also its Effect on the Parasitological and Immunological Profile

[1]A. Farid, [2]A. Abdel Malek, [2]I. Rabie, [2]A. Helmy and [1]A.M. El Amir
[1]Department of Zoology, Faculty of Science, Cairo University, Egypt
[2]Theodore Bilharz Research Institute, Giza, Egypt

Abstract: The present study evaluated the use of 3 types of Cysteine Protease Inhibitors (CPIs) with praziquantel (PZQ) as chemotherapy against *schistosomiasis mansoni* in mice. All groups were going to assessment of flurometh ylketone (FMK), Vinyl Sulfone (VS) and Sodium Nitro Prussid (SNP) by measurement of parasitological, immunological and histological parameters. In our study, The ova count/gm liver or intestine on with PZQ treatment showed 99.1 and 95.2% Percent Reduction (PR), respectively compared to control group. The most effective CPI was FMK when combined with PZQ recording 99.8 and 99.6% PR for liver and intestine, respectively. Regarding to the oogram pattern, FMK, VS and SNP treatment either at 3 or 5 wk PI revealed marked decrease in the immature and mature ova counts and an increase of the dead ova percentages. The effect of CPIs was studied on the PR of Mean Granuloma Diameter (MGD) and Mean Granuloma Number (MGN) of infected treated groups compared to infected control and PZQ treated groups. FMK treatment proved to be highly was effective against *S. mansoni* in mice disintegrating ova and reduction in granulomatous size and numbers. The microscopic examination of liver sections of infected mice showed a large cellular granuloma with living central ova. sections of Infected mice liver treated with FMK or VS alone or combined with PZQ showed a great reduction in granuloma size as small cellular granuloma with central degenerated ova. We observed that these CPIs alone or combined with PZQ could effectively block schistosomal activity and prevented its growth and differentiation. Briefly, the best schistosomicidal effect of CPIs, that gained by drug administration orally in a dose of 50 mg kg^{-1} mouse, was observed with FMK. This was followed by VS and lastly with SNP. These results gave evidence that CPIs can selectively arrest parasite replication without untoward toxicity to the host.

Key words: Praziquantel, flurometh ylketone, vinyl sulfone, sodium nitro prussid, egg profile, granuloma

INTRODUCTION

Schistosomiasis is considered as the second endemic disease after malaria in terms of the extent of endemic areas and the number of infected people (Utzinger and Keiser, 2004). The majority of those infected and at risk live in Africa (Steinmann *et al*., 2006). The most usual effect of schistosomiasis is to depress the general health, growth and physical abilities, leading to the reduction of the income of individual and family and the national productivity in general (Mohey El-Dine, 1978). Although some promising results of schistosomiasis vaccines have been reported in animal models, the potential feasibility, measurability and applicability of current candidates are simply not sufficient to justify human trials, at least, at this stage. Due to the unavailability of a schistosomiasis vaccine (Gryseels *et al*., 2006), the control of schistosomiasis depends on the reduction of morbidity at both individual and community levels using schistosomicidal drugs (WHO, 2002). Praziquantel (PZQ) is the drug of choice for treatment of schistosomasis (WHO, 2010) because of its safety, broad-spectrum activity and reasonable cost (Cioli, 2000).

In view of concern about the emergence of resistance to PZQ, there is a need for developing novel antischistosomal drugs. Among the targets being explored for chemotherapy development is a family of cysteine proteases enzymes (CPs) that play key roles in the life cycle of protozoan and helminth parasites (McKerrow, 1999).

A wide range of parasitic protozoa (including *Leishmania*, *Plasmodium* and *Giardia*) metazoan (including *Fasciola* and *Schistosoma*) depends on CPs for their survival in host (Klemba and Goldberg, 2002). There are specific irreversible inhibitors of CPs inhibit the degradation of hemoglobin (Hb) in developing schistosomula, eventually resulting in worm death and reduce egg production.

Corresponding Author: A.M. El Amir, El Ashgar District, P 109. F 9, 6th of October City, Cairo, Egypt Tel: +201001649556

This study aims to test the action of three different compounds acting as CP inhibitors (CPIs) namely fluromethylketone (FMK), vinyl sulfone (VS) and Sodium Nitro Prussid (SNP). The study includes parasitological, immunological and histological parameters.

MATERIALS AND METHODS

Animals: Male C57BL/6 strain, albino mice, 6 to 8 wk old (18±20 g), clean from parasitic infection, they were obtained from Schistosome Biological Supply Centre (SBSC), Theodor Bilharz Research Institute (TBRI) and maintained under standard laboratory care. Mice were infected with 100 cercariae of the Egyptian strain of *S. mansoni* supplied from SBSC, TBRI by tail immersion method (Ismail *et al.*, 1996).

Drugs: The chosen dose of PZQ was 500 mg kg^{-1} and optimak working dose of 3 CPIs (FMK, VS and SNP) was determined by 50 mg kg^{-1} for each one.

Experimental design: A total number of 450 infected Albino mice were divided into 15 Groups (30 mice/gp), the 15 groups of mice were treated as the following design:

Group A: Mice were infected and left untreated as infected control
Group B: Mice were treated with PZQ for 2 consecutive days at 3 and 5 wk PI
Group C: Mice were treated with FMK for 14 consecutive days at 3 and 5 wk PI
Group D: Mice were treated with VS for 14 consecutive days at 3 and 5 wk PI
Group E: Mice were treated with SNP for 14 consecutive days at 3 and 5 wk PI
Group F: Mice were treated with FMK for 14 consecutive days and PZQ for 2 consecutive days at 3 and 5 wk PI
Group G: Mice were treated with VS for 14 consecutive days and PZQ for 2 consecutive days at 3 and 5 wk PI
Group H: Mice were treated with SNP for 14 consecutive days and PZQ for 2 consecutive days at 3 and 5 wk PI

Tissue egg load (Number of ova/g tissue): At the end of perfusion, the small intestine was removed according to Pellegrino *et al.* (1962); three fragments were taken for oogram studies. The rest of the intestine and a portion of the liver were weighed for ova counting (Cheever, 1970; Cheever and Anderson, 1971).

The number of ova in each tissue was counted in the 3 slides and the average number was calculated as follows:

$$\text{Average number of ova in 0.1 mL} = \frac{\text{(No. of eggs in the three samples)}}{3}$$

$$\text{Then the number of ova in 5 mL} = \frac{\text{(Avg. No. of ova in 0.1 mL)}}{0.1} \times 5$$

The number of ova in 5 mL represents the number of ova in the weight of the liver or intestine previously digested, so the number of ova in 1 g tissue was calculated as follows:

Number of ova in 1 g (liver or intestine) = (Number of ova in 5 mL) x1 g /Weight of liver or intestine in grams recorded before digestion

Percent egg developmental stages (oogram pattern): From each animal three intestinal fragments were obtained, examined under microscope and the mean number of each stage in a total of 100 ova was calculated.

Liver histopathological studies: The liver specimens were obtained from mice of different groups and examined histologically using Heamatoxylin and Eosin (H and E) (Drury and Wallington, 1967).

Measurement of the granuloma diameter and number: Five slides/animal and three sections/slide in each group were prepared. The Mean Granuloma Diameter (MGD) and the Mean Granuloma Number (MGN)/group was calculated for about 140 lesions. The percent suppression of MGD/treated group was calculated according to the formula:

$$\text{Suppression (\%)} = \frac{\text{M.G.D. of control group-M.G.D of treated group}}{\text{M.G.D of control group}} \times 100$$

RESULTS

Tissue egg load (ova count)

The number of ova/gm hepatic tissues: On treatment of infected mice with PZQ at 3 and 5 wk PI, the obtained results showing 94.9 and 99.1% PR, compared to control group, respectively. Mice gps (B, C and D) treated with CPIs individually showed a significant ($p<0.001$) low reduction in ova count/gm liver either when treatment was at 3 or 5 wk PI compared to those treated with PZQ alone,

where it was reduced by 16.5, 14.9 and 11.6% on using FMK, VS and SNP alone 3 wk PI and 43.1, 39.2, 36.2% at 5 wk PI. Treatment of mice at 3 wk PI using combination of the tested CPIs with PZQ, illustrated a reduction in ova count/gm liver as 75.6, 70.5 and 59.1% on using FMK, VS and SNP, respectively. While when mice were treated, at 5 wk PI, ova count/gm liver recorded 88.2 and 66.4% on using FMK, VS and SNP. However, all the recorded reductions, as a result of CPIs treatment with PZQ, were significantly ($p<0.001$) higher in comparison to infected untreated control (Table 1).

As seen, the reduction of ova count/g liver is related to treatment time at 3 or 5 wk PI and the most effective combination was between FMK and PZQ administrated at 5 wk PI, where, ova count/g liver was 98.2±71 with PR = 99.6%.

The number of ova/gm intestinal tissue: The PR of ova count/gm intestine is directly proportional with treatment time at 3 or 5 wk PI and PZQ is the most effective compound.

All the recorded reductions were significant ($p<0.001$) compared to control. On the other hand, an additive effect was obtained in most cases of combination between the tested inhibitors with PZQ (gp F and H). The most effective treatment was FMK with PZQ (gp F) at 5 wk PI, where the PR was 99.8% (Table 2).

Percent egg developmental stages (Oogram pattern): Oogram pattern was assayed at 8 wk PI in all infected mice groups (Table 3). It was concerned the ovicidal activity and maturity of the *S. mansoni* eggs.

In comparison to infected control mice (group A), PZQ treatment (group B) recorded a high significant mean no. of dead ova (74±6.2) when administrated at 3 wk PI ($p<0.001$) and 74±7.9 at 5 wk PI.

The treatment of infected mice with 3 different CPIs alone at 3 and 5 wk PI recorded a significant ($p<0.001$) increase in dead ova mean number and decrease in immature ova and no change in mature ova count.

Dead ova numbers were provoked significantly ($p<0.001$) in group (F) that orally treated at 3 and 5 wk PI with FMK combined with PZQ (89.6±5.9 and 99.1±3.7, respectively).

Measurement of hepatic granuloma diameter (MGD): All the data of CPIs treated groups were compared to the data of PZQ treated gp (gp B) (Table 4). The PZQ group

Table 1: Ova count g^{-1} liver in *S. mansoni* infected mice groups treated with FMK, VS and SNP alone or in combination with PZQ at 3 and 5 wk PI

		Ova count g^{-1} tissue			
		3 wk PI		5 wk PI	
Groups		M±SD	PR (%)	M±SD	PR (%)
A	Infected control	23980±190		24250.0±170	
B	PZQ	1215.8±236***	94.9	215.8±120***	99.1
C	FMK	20023.3±212$^{***\#\#\#}$	16.5	13788.5±291$^{***\#\#\#}$	43.1
D	VS	20407.0±187$^{***\#\#\#}$	14.9	14747.7±300$^{***\#\#\#}$	39.2
E	SNP	21198.3±180$^{***\#\#\#}$	11.6	15467.1±210$^{***\#\#\#}$	36.2
F	FMK and PZQ	5858.6±217$^{***\#\#\#}$	75.6	98.2±71***	99.6
G	VS and PZQ	7076.3±321$^{***\#\#\#}$	70.5	2854.4±199$^{***\#}$	88.2
H	SNP and PZQ	9810.3±274$^{***\#\#\#}$	59.1	8157.3±228$^{***\#\#\#}$	66.4

*Significance referred to control (T-test), #Significance referred to PZQ (Chi^2-test), N = 30) FMK: fluromethyl keton, VS: Vinyl sulfone, SNP: Sodium nitro prussid, PZA: Praziquantel, wk: week, PI: post infection, M±SD: mean±standered deviation, PR: percent reduction

Table 2: Ova count g^{-1} intestine in *S. mansoni* infected mice groups treated with FMK, VS and SNP alone or in combination with PZQ at 3 and 5 wk PI

		Ova count g^{-1} tissue			
		3 wk PI		5 wk PI	
Groups		M±SD	PR (%)	M±SD	PR (%)
A	Infected control	36506.0±749		37710±540	
B	PZQ	1825.3±236***	95.0	1813.3±120***	95.2
C	FMK	27197.0±317$^{***\#\#\#}$	25.5	19822.8±410$^{***\#\#\#}$	47.4
D	VS	29679.4±399$^{***\#\#\#}$	18.7	24897.1±274$^{***\#\#\#}$	34.0
E	SNP	30847.6±240$^{***\#\#\#}$	15.5	26211.3±360$^{***\#\#\#}$	30.5
F	FMK & PZQ	7861.2±370$^{***\#\#\#}$	78.5	59.3±114$^{***\#}$	99.8
G	VS & PZQ	8292.4±411$^{***\#\#\#}$	77.3	1125.8±216***	97.0
H	SNP & PZQ	12173.2±396$^{***\#\#\#}$	66.7	2500.4±194***	93.4

*Significance referred to control (T-test), #Significance referred to PZQ (Chi^2-test), N = 30 FMK: fluromethyl keton, VS: Vinyl sulfone, SNP: Sodium nitro prussid, PZA: Praziquantel, wk: week, PI: post infection, M±SD: mean±standered deviation, PR: percent reduction

Table 3: Percent egg development stages (Oogram pattern) in infected mice groups treated with FMK, VS and SNP alone or in combination with PZQ at 3 and 5 wk PI

		Oogram pattern					
		3 wk PI (M±SD)			5 wk PI (M±SD)		
Groups		Immature	mature	Dead	Immature	mature	Dead
A	Infected control	63.3±5.4	32.4±3.4	04.3±0.4	60.4±4.7	33.3±2.9	06.3±0.5
B	PZQ	14.0±1.9***	12.0±2.8***	74.0±6.2***	18.0±2.6***	08.0±1.7***	74.0±7.9***
C	FMK	25.3±5.6***###	38.7±5.2***###	36.0±2.3***###	32.6±4.7***###	20.4±4.6***###	47.0±3.9***###
D	VS	27.3±2.2***###	42.7±5.1***###	30.0±2.8***###	38.2±2.9***###	25.8±6.7***###	35.0±3.1***###
E	SNP	34.6±3.3***###	42.4±3.2***###	23.0±3.5***###	41.3±5.2***###	19.7±4.8***###	29.0±2.4***###
F	FMK and PZQ	7.7±5.2***###	3.1±5.6***###	89.6±5.9***###	0.9±5.1***###	0.3±4.9***###	99.1±3.7***###
G	VS and PZQ	7.9±0.4***###	5.9±4.3***###	86.2±2.7***###	2.1±4.6***###	1.2±4.5***###	96.7±8.2***###
H	SNP and PZQ	25.6±4.7***###	8.6±4.6***##	65.8±7.1***###	6.3±3.2***###	3.6±2.7***###	90.1±5.8***###

*Significance referred to control (T-test), #Significance referred to PZQ (T-test), N = 30 FMK: fluromethyl keton, VS: Vinyl sulfone, SNP: Sodium nitro prussid, PZA: Praziquantel, wk: week, PI: post infection, M±SD: mean±standered deviation

Table 4: Mean Granuloma Diameter (MGD) in *S. mansoni* infected mice groups treated with FMK, VS and SNP alone and combined with PZQ at 3 and 5 wk PI

		MGD um			
		3 wk PI		5 wk PI	
Groups		(M±SD)	PR (%)	(M±SD)	PR (%)
A	Infected control	305.0±39.91		299.0±34.61	
B	PZQ	105.0±4.22***	65.60	121.4±6.23***	59.40
C	FMK	142.0±55.20***	53.44	161.0±31.25***	46.20
D	VS	163.0±32.21***#	46.56	186.7±53.50***##	37.60
E	SNP	250.0±33.00***###	18.03	261.0±72.90*###	12.70
F	FMK and PZQ	131.0±51.80***	57.10	105.0±15.30***	64.90
G	VS and PZQ	155.6±55.70***#	49.00	186.7±53.50***##	39.60
H	SNP and PZQ	189.0±46.96***###	38.00	261.0±72.90*###	12.70

*Significance referred to control (T-test), # Significance referred to PZQ (Chi^2-test), N = 30 FMK: fluromethyl keton, VS: Vinyl sulfone, SNP: Sodium nitro prussid, PZA: Praziquantel, wk: week, PI: post infection, M±SD: mean±standered deviation, PR: percent reduction, MGD: mean granuloma diameter

Table 5: MGN in infected mice groups treated with FMK, VS and SNP alone and combined with PZQ at 3 and 5 wk PI

		MGN			
		3 wk PI		5 wk PI	
Groups		(M±SD)	PR (%)	(M±SD)	PR (%)
A	Infected Control	5.30±1.19		6.40±1.17	
B	PZQ	3.20±0.26	39.6	3.90±1.60***	34.4
C	FMK	0.50±0.73***###	90.6	3.00±1.58***##	53.1
D	VS	2.80±1.60***	47.2	2.80±0.84***##	56.3
E	SNP	4.60±1.40***###	11.3	0.67±0.82###	89.5
F	FMK and PZQ	0.33±0.52***###	93.8	0.22±0.27***###	94.8
G	VS and PZQ	4.60±1.03***	13.2	2.80±0.84***##	56.3
H	SNP and PZQ	3.40±1.14***	35.9	0.67±0.82***###	89.5

*Significance referred to control (T-test), #Significance referred to PZQ (Chi^2-test), N = 30 FMK: Fluromethyl keton, VS: Vinyl sulfone, SNP: Sodium nitro prussid, PZA: Praziquantel, wk: week, PI: post infection, M±SD: mean±standered deviation, PR: Percent reduction, MGN: mean granuloma number

recorded a noticeable significant PR ($p<0.001$), as 65.6 and 59.4% when treatment was at 3 or 5 wk PI.

Mice groups that treated with VS and SNP alone (gp D and E) either at 3 or 5 wk PI had lower PR than PZQ treated group, but FMK treatment alone (gp C) recorded 53.4 and 46.2% when given at 3 and 5 wk PI, respectively, with nonsignificant difference compared to the PZQ treated group.

The same action was done in case of treatment with FMK combined to PZQ at 3 and 5 wk PI treatment (gp F) which recorded PR 57.1 and 64.9%, respectively, with nonsignificant difference compared to the PZQ group.

Measurement of hepatic granuloma number (MGN): It was noticed t hat the PR of MGN in the FMK-treated groups either alone (gp C) PR = 53.1% or combined with PZQ (gp F) PR = 94.8% was significantly ($p<0.001$) higher than PZQ-treated group PR= 34.4% (Table 5).

When mice were treated with VS earlier at 3 wk PI (gp D), it recorded a significant ($p<0.001$) high PR = 47.2%, not higher than PR recorded by FMK but higher than PR after PZQ treatment, which decreased to only 13.2% (gp G) when combined with PZQ at treatment. But when treatment was at 5 wk PI, VS had a significant,

($p<0.001$) increase in PR (56.3 and 56.3%) alone and in combination with PZQ, which was higher than the PZQ treatment PR.

The SNP-treated group alone (gp E) or in combination with PZQ (gp H) at 3 wk PI recorded a significant low PR of MGN (11.3 and 35.9%, respectively), while PR showed a highly significant increase in PR when treatment was at 5 wk PI (89.5 and 89.5%, respectively) in comparison to infected control gp (gp A) and PZQ-treated gp (gp B).

Measurement of different immunoglobulin isotypes: The previous literature indicated that the parasitic infection stimulates an increase in the level of the Igs (total and isotypes). ELIZA experiment was assessed and obviously confirmed these results. Experimental groups of this study are normal uninfected control, infected control (group A), PZQ-treated (group B), CPIs-treated groups (FMK, VS and SNP) as groups (C, D and E, respectively) and CPIs and PZQ-treated as groups (F, G and H, respectively). Treatment given was done at 3 and 5 wk PI, while Igs assessment (IgM, total IgG, IgG2 and IgG4) was performed at 8 wk PI.

Table (6 and 7) summarize the data of all measured Igs to mice groups as M±SD and difference percentage was calculated referring to the results of infected control (group A).

Total IgG level: Infection causes a significant ($p<0.001$) slight increase in IgM level (51.5 and 57.7%), remarkable increase in IgG2 level (100 and 90%) and IgG4 level (96.8 and 106%). While there is a vigorous increase only in total IgG level (380 and 283.9%) in both infected untreated groups of mice (only administrated saline at 3 or 5 wk PI, respectively).

IgM level: It was clear that VS treatment stimulated an enhancement of primary immunization isotype (IgM) at 3 wk PI recording the highest significant ($p<0.001$) difference percentage alone or combined with PZQ (62 and 101%, respectively).

Also, when the mice treated at 5 wk PI, VS was the effective immune enhancer recording 90.2 and 141.5% at combination with PZQ which recorded only 88.8% when administered alone as a nonsignificant elevation of IgM level, while FMK recorded a significant ($p<0.001$) increase when combined with PZQ (114.6%).

Table 6: Determination of different serum Immunoglobulin Isotypes of *S. mansoni* infected mice treated with FMK, VS and SNP alone or in combination with PZQ at 3 wk PI

		Immunoglobulin isotypes							
		IgM		Total IgG		IgG2		IgG4	
Groups		M±SD	Diff (%)	M±SD	Diff (%)	M±SD	Diff (%)	M±SD	Diff (%)
Control (Uninfected)		0.33±0.23		0.30±0.09		0.28±0.18		0.31±0.03	
A	Infected control	0.50±0.20$^{\#\#\#}$	51.5	1.44±0.22$^{\#\#\#}$	380	0.56±0.13$^{\#\#\#}$	100.0	0.61±0.23$^{\#\#\#}$	96.8
B	PZQ	0.682±0.23	36.4	1.844±0.14	28.0	0.987±0.19	76.3	1.23±0.24	101.6
C	FMK	0.72±0.19***	44.0	1.73±0.27***	20.1	0.90±0.23***	60.7	1.00±0.18***	63.9
D	VS	0.81±0.27***	62.0	1.62±0.13***	12.5	0.97±0.13***	73.2	1.51±0.29***	147.5
E	SNP	0.54±0.19^{-}	8.0	1.52±0.17***	5.5	0.66±0.30^{-}	17.9	1.35±0.22***	121.3
F	FMK and PZQ	0.73±0.20***	46	1.90±0.12***	31.9	1.10±0.23***	80.4	1.45±0.21***	137.7
G	VS and PZQ	1.01±0.21***	101	1.82±0.23***	26.4	1.49±0.24***	166.1	1.71±0.24***	180.3
H	SNP and PZQ	0.70±0.25***	40.0	1.51±0.10^{-}	4.9	0.80±0.30**	42.9	1.23±0.22***	101.6

*Significance referred to control (T-test), $^{\#}$Significance referred to PZQ (Chi^2-test), N = 30 FMK: fluromethyl keton, VS: Vinyl sulfone, SNP: Sodium nitro prussid, PZA: Praziquantel, wk: week, PI: post infection, M±SD: mean±standered deviation, % diff: difference percentage

Table 7: Determination of different serum Immunoglobulin Isotypes of *S. mansoni* infected mice treated with FMK, VS and SNP alone or in combination with PZQ at 5wk PI

		Immunoglobulin isotypes							
		IgM		Total IgG		IgG2		IgG4	
Groups		M±SD	Diff (%)	M±SD	Diff (%)	M±SD	Diff (%)	M±SD	Diff (%)
Control (uninfected)		0.26±0.22		0.31±0.12		0.20±0.18		0.29±0.23	
A	Infected control	0.41±0.19$^{\#\#\#}$	57.7	1.19±0.13$^{\#\#\#}$	283.9	0.38±0.12$^{\#\#\#}$	90.0	0.6±0.21$^{\#\#\#}$	106.9
B	PZQ	0.774±0.12	88.8	1.598±0.23	34.3	0.88±0.11	131.6	1.035±0.21	72.5
C	FMK	0.60±0.2***	46.3	1.42±0.22***	19.3	0.87±0.22***	128.9	0.91±0.19***	51.7
D	VS	0.78±0.21***	90.2	1.55±0.15***	30.3	0.70±0.19***	84.2	1.33±0.25***	121.7
E	SNP	0.45±0.19	9.8	1.35±0.18***	13.4	0.55±0.21***	44.7	1.11±0.19***	85.0
F	FMK and PZQ	0.88±0.23***	114.6	1.66±0.14***	39.5	0.90±0.19***	136.8	1.28±0.22***	113.3
G	VS and PZQ	0.99±0.21***	141.5	1.78±0.27***	49.6	1.37±0.24***	260.5	1.51±0.25***	151.7
H	SNP and PZQ	0.68±0.30***	65.9	1.49±0.10***	25.2	0.99±0.32***	160.5	1.05±0.232***	75.0

*Significance referred to control (T-test), $^{\#}$Significance referred to PZQ (Chi^2-test), N = 30 FMK: fluromethyl keton, VS: Vinyl sulfone, SNP: Sodium nitro prussid, PZA: Praziquantel, wk: week, PI: post infection, M±SD: mean±standered deviation, % diff: difference percentage

IgG2 level: Again, PZQ treatment caused a nonsignificant noticeable elevation of IgG2 level when it was used as treatment for *S. mansoni* infected groups of mice at 3 (6.3%) and 5 wk PI (131.6%). On the other hand, the effect of CPIs was clear earlier at 3 wk PI on the IgG2 level. VS treatment recorded nearly the same but significant ($p<0.001$) increase of Igs as that stimulated by PZQ (73.2%). The combination between VS and PZQ recorded the best result, was stimulated the IgG2 secretion recording a highly significant data ($p<0.001$), 166.1% at 3 wk PI and 260.5% at 5 wk PI.

When FMK treatment was given individually, it causes a significant ($p<0.001$) increase (60.7%) and 80.4% when combined with PZQ. At 5 wk PI, FMK treatment caused a high significant ($p<0.001$) IgG2 level recording 128.9% as individual treatment and 136.8% when mice were treated with FMK in combination with PZQ.

IgG4 level: Earlier, at 3 wk PI treatment of PZQ recorded a moderate nonsignificant elevation of IgG4 level (101.6%) as a differential percentage referring to the data of infected untreated group.

On the other hand, VS again recorded a good indication as an immune-enhancer treatment increasing significantly ($p<0.001$) the IgG4 level when administered individually (147.5%) or combined with PZQ (180.3%).

FMK was played a significant role when given to mice at 3 wk PI ($p<0.001$) on elevating IgG4 level only when combined with PZQ recording 137.7%. When mice were treated at 5 wk PI, FMK had a little effect on the IgG4 level when administered alone (51.7%), but it was recorded a significant ($p<0.001$) high effect when given in combination with PZQ (113.3%).

It was found that the only detectable effect of SNP treatment on the stimulation of IgG4 secretion. Its effect was a significant increase of IgG4 when administrated individually earlier when administered individually or combined with PZQ at 3 wk PI (121.3 and 101.6%, respectively). But, it recorded a moderate significant ($p<0.001$) increase of IgG4 when treatment was given at 5 wk PI individually and in combination with PZQ, (85 and 75% respectively).

Histopathological studies: Histopathological sections were prepared and examined to evaluate the efficacy of three CPIs treatment (FMK, VS and SNP) alone or combined with PZQ at 3 and 5 wk PI on the *Schistosomiasis mansoni* in infected gps of mice.

Liver sections from *S. mansoni* infected mice at 8 wk PI (gp A), showed loss of hepatic lobular architecture, hydropic degeneration in the hepatocytes, interstitial fibrosis, interlobular fibrosis and large area of necrosis

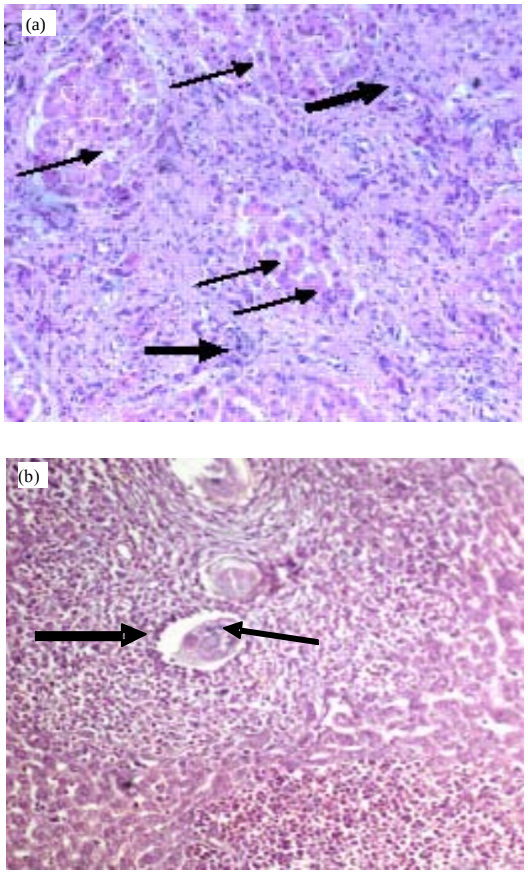

Fig. 1(a-b): Light micrographic liver section of 8 wk PI *S. mansoni* infected mice (untreated control) (gp A., (a) Loss of hepatic architecture, thickened portal tracts with fibrous tissue (thin arrows), heavy lymphocytes infiltration (thick arrows), (HE, X40) and (b) Cellular granuloma with central living ova (thin arrow) surrounded by chronic inflammatory cells and fibrous tissue (thick arrow) (HE, X20)

(Fig. 1a). Granuloma formation with calcification and abcess formation was also found (Fig. 1b). Thickened portal tracts with fibrous tissue and heavy infiltration with mononuclear cells were observed along hepatocytes and within the portal area of the liver. A sign of early fibroplasias was evident.

Liver sections from infected mice treated with FMK (gp C), at 3 or 5 wk PI, showed a great reduction in granuloma size as small cellular granuloma with central degenerated ova (Fig. 2a and b). Also, VS treatment showed a mild effect on immune system activation, infected mice treated with VS at 3 or 5 wk PI (gp D)

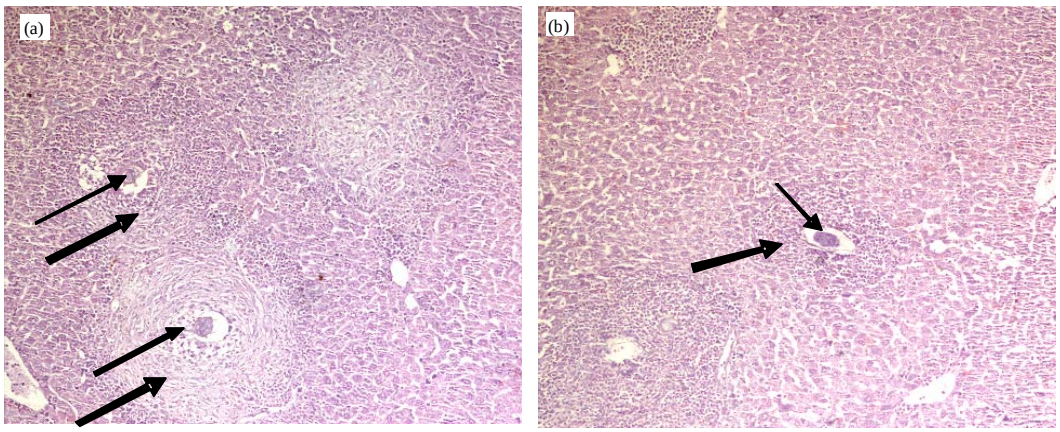

Fig. 2 (a-b): Light micrographic liver sections of *S. mansoni* infected mice treated with FMK (gp C), (a) Treatment at 3 wk PI, showing a small fibrocellular granuloma (thick arrows) with central degenerated ova (thin arrows) (HE, X10) and (b) Treatment at 5 wk PI, showing a small cellular granuloma (thick arrow) with central degenerated ova (thin arrow) (HE, X10)

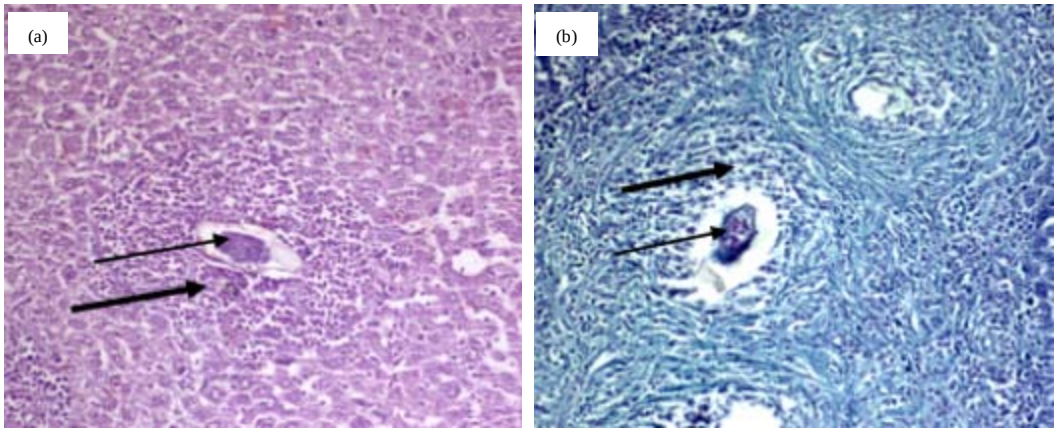

Fig. 3(a-b): Light micrographic liver sections of *S. mansoni* infected mice treated with VS (gp D), (a) Treatment at 3 wk PI showing moderate size cellular granuloma (thick arrow) with central living miracidium (thin arrow) (HE, X20) and (b) Treatment at 5 wk PI showing moderate size fibrocellular granuloma (thick arrow) with central degenerated ova (thin arrow) (Masson Trichrome, X20)

showed a moderate size cellular granuloma with central living miracidium (Fig. 3a, b). On the other hand, SNP treatment at 3 or 5 wk PI (gp B) has no immunoprotective effect. So, the microscopic examination of liver sections of infected mice showed a large cellular granuloma with living central ova (Fig. 4a, b).

When PZQ combined with FMK or VS in the treatment of infected mice, granuloma was absent in some liver sections where mice were treated with FMK at both 3 and 5 wk PI (gp F) (Fig. 5a, b) and only at 3 wk PI when mice were treated with VS (gp G) (Fig. 6a), while treatment of VS at 5 wk PI showed degenerated granuloma with dead central ova was observed in others (Fig. 6b). Large sized fibrocellular granuloma with central living ova was observed in liver sections of mice treated with SNP combined with PZQ at 3 wk PI (gp H) (Fig. 7a). But at 5 wk PI, there were small fibrocellular granuloma with dead central ova (Fig. 7b).

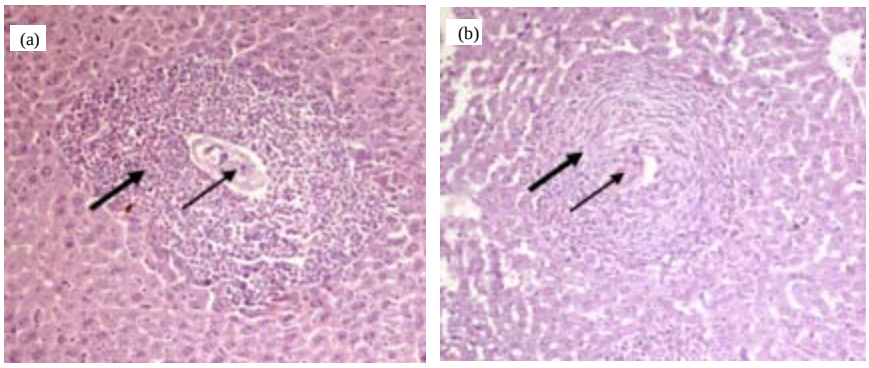

Fig. 4(a-b): Light micrographic liver sections of *S. mansoni* infected mice treated with SNP (gp E), (a) Treatment at 3 wk PI showing large cellular granuloma (thick arrow) with living central ova (thin arrow) (HE, X40) and (b) Treatment at 5 wk PI showing moderate size fibrocellular granuloma (thick arrow) with degenerated central ova (thin arrow) (HE, X20)

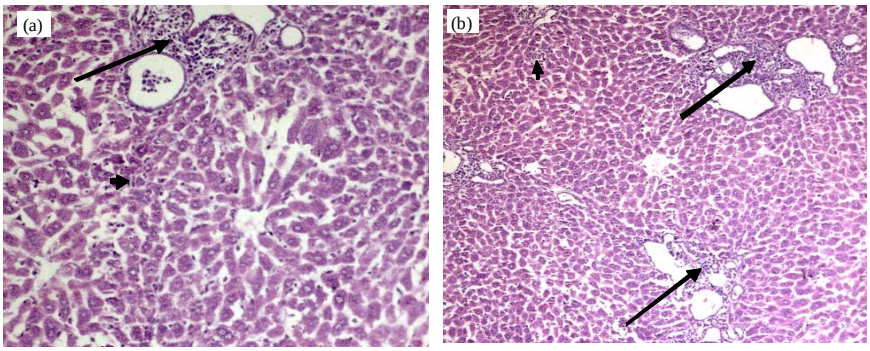

Fig. 5(a-b): Light micrographic liver sections of *S. mansoni* infected mice treated with FMK combined with PZQ (gp F), (a) Treatment at 3 wk PI showing no granuloma formation with scattered chronic inflammatory cells in portal tracts (thin arrow) and in between hepatocysts (arrow head) (HE, X20) and (b) Treatment at 5 wk PI revealed absence of granuloma with focal collection of chronic inflammatory cells in portal tracts (thin arrow) and in between hepatocysts (arrow head) (HE, X 10)

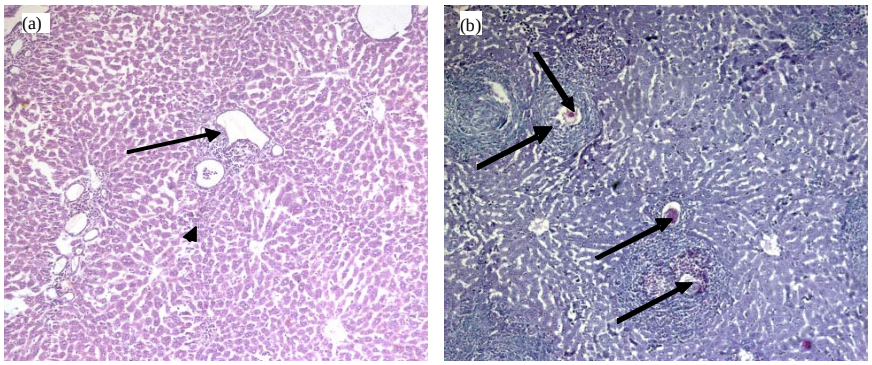

Fig. 6(a-b): Light micrographic liver sections of *S. mansoni* infected mice treated with VS combined with PZQ (gp G), (a) Treatment at 3 wk PI revealed the absence of granuloma with focal collection of chronic inflammatory cells in portal tracts (thin arrow) and in between hepatocysts (arrow head) (HE, X10) and (b) Treatment at 5 wk PI showing small fibrous granuloma (thick arrow) with dead central ova (thin arrows) (Masson Trichrome, X 20)

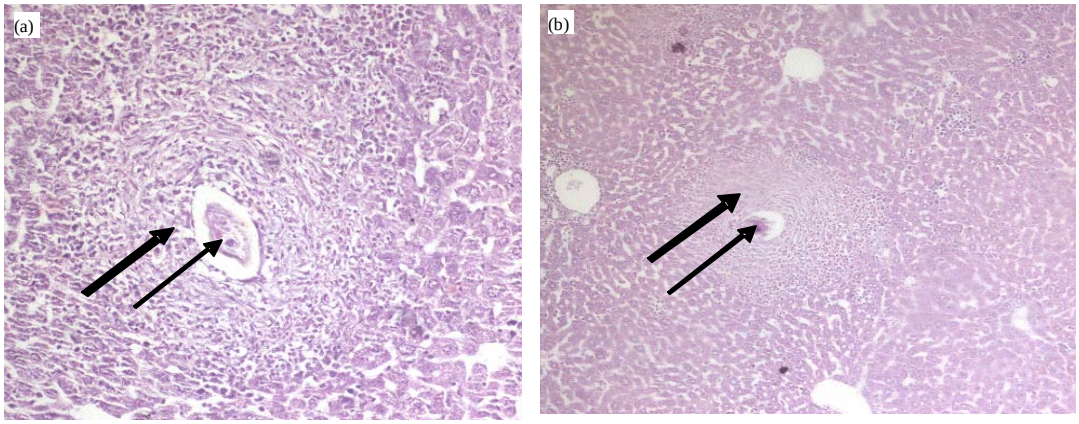

Fig. 7(a-b): Light micrographic liver sections of *S. mansoni* infected mice treated with SNP combined with PZQ (gp H) (a) Treatment at 3 wk PI showing large sized fibrocellular granuloma with central living ova (HE, X20) and (b) Treatment at 5 wk PI showing small fibrocellular granuloma (thick arrow) with dead central ova (thin arrow) (HE, X20)

DISCUSSION

Up to 1975, chemotherapy of schistosomiasis had relied on the use of antimonials and a variety of other drugs targeting DNA synthesis, carbohydrate and protein metabolism (Harder, 2002). Since then, three drugs have been used for treatment: metrifonate, oxamniquine and PZQ; however, it is the latter that is now universally employed, as recommended by the WHO for either individual or mass treatment (Raso *et al*., 2004). PZQ is effective against all five species of schistosomes infecting humans; on the other hand juvenile parasites (between 7 and 28 days-old) are less susceptible to PZQ than adults (Sabah *et al.,* 1986; Utzinger *et al,* 2003). So individuals continuously exposed to new infections must be retreated (Utzinger *et al.,* 2003). PZQ is well tolerated, easily administered in tablet form and inexpensive (Raso *et al*., 2004; Savioli *et al.,* 2004).

Despite the success of PZQ in treatment of schistosoma, the prospect of relying on a single drug to treat 200 million people and the potential for drug resistance, particularly in areas of high transmission, must be considered (Cioli, 2000). Suspicions were raised that low cure rates for PZQ in a *S. mansoni* outbreak in Senegal in the early 1990s were partly due to drug resistance, although alternative interpretations discussed included the high pretreatment levels of infection and intense disease transmission that resulted in individuals harboring large numbers of immature parasites inherently less sensitive to the drug (Danso-Appiah and De-Vlas, 2002).

Also, Utzinger *et al*. (2003) reported that the reliance on one single anti-schistosomal drug is alarming and the scientific community had called for research and development of novel and inexpensive drugs against schistosomiasis.

Protease inhibitors are key components of human immune-deficiency virus therapy (Van Den Berg *et al*., 2004). Also, CPIs have a demonstrated activity in a variety of parasitic models, for example, FMK had been shown to cure murine malaria (Mckerrow, 1999), VS worked in animal models of Leishmaniasis and Chagas disease (Engel *et al.* (1998) and CPIs are reported to arrest or cure murine schistosomiasis (Padma *et al.,* 1995). This suggests that CPIs may provide an alternative to traditional therapy in drug-resistant organisms (Mckerrow, 1999). So, this study was designed for assessment of 3 types of CPIs, to define which of them is the best choice.

Earlier, Pellgerino and Katz (1968) revealed that, for the assessment of an anti-schistosomal agent, it is important to study several criteria related to the parasitic intensity, stages and distribution through the tissues of the host. Among these criteria, the worm burden and distribution within hepatic portal system, the percentages of ova pattern in parts of large intestine and liver. Recently, these criteria were used for the assessment of drug efficacy in *S. mansoni* mice (Abdel-Ghaffar, 2004; Abdel-Ghaffar *et al*., 2005).

As regard to combination chemotherapy for schistosomiasis, the partner drugs should have different mechanisms of action to reduce the resistance development and/or target different developmental stage

of parasite to enhance cure and egg reduction rates in case the two drugs exhibit synergism. It might be possible to achieve the same or even higher levels of efficacy by using smaller doses of either agent, which might also result in fewer or milder side effects (Utzinger and Keiser, 2004).

Utzinger *et al.* (2003) reported that the PZQ and artesunate combination resulted in cure and egg reduction rates of 69 and 89%, respectively which are significantly higher than the ones observed after PZQ and artesunit monotherapies.

The number of ova per gram tissue is also an important criterion for the evaluation of both magnitude of infection and efficacy of antischistosomal agents. Initially, the highest numbers of eggs are deposited in the liver, but the percentage of ova in this organ decreases with increasing age of infection and the percentage of ova in colon correspondingly increases (Friedman, 2004). Webbe and James (1977) added that the observed distribution of eggs in these organs reflects the distribution of adult worms in relation to the age of the infection. The number of ova/g tissue is also an important criterian for the evaluation of the effect of antischistosomal agent on the infection (Mostafa, 2005). Mostafa (2001) used black seed oil in treatment of *S. mansoni* infected albino mice. He reported that the decrease in eggs found in tissues is not only attributed to reduction in worm burden but also, to the decline in worm fecundity and female productivity.

Shalaby *et al.* (2003) reported that the PR in the intestinal ova count was higher than that of the hepatic ova count in infected mice, 2 wk post treatment. He attributed this variation to the excretion of some ova from the intestine prior to digestion and to the hepatic shift of worms after treatment.

The ova count test on treatment with LPSF - PT05 to *S. mansoni* infected mice, showed that the number of immature eggs was lower with a significant reduction at a dose of 100 mg Kg^{-1} (Pitta *et al.,* 2006; Neves *et al.*, 2011; Da Silva *et al.*, 2012).

In our study, the ova count g^{-1} liver on treatment with PZQ at 3 and 5 wk PI, showing 94.9 and 99.1% PR. While, ova count g^{-1} intestine recorded 95 and 95.2% PR respectively, compared to control gp. The most effective PR of ova count g^{-1} intestine was 99.8% obtained from treatment with FMK combined with PZQ at 5 wk PI and the PR of ova count g^{-1} liver was 99.6 % on treatment by the same combination of drugs.

At the beginning the changes in the oogram of intestinal fragments were considered significant when one or more developmental stages of immature as well as mature eggs disappear (Pellegrino *et al.,* 1962).

In the present work, PZQ gp recorded the highest significant PR ($p<0.001$) in dead ova when treatment was at 3 and 5 wk PI. The administration of CPIs, either alone or combined with PZQ, to the *S. mansoni* infected mice resulted in significant changes in the oogram at both time intervals. FMK, VS and SNP treatment either at 3 or 5 wk PI revealed marked decrease in the immature and mature ova counts and an increase of the dead ova percentages. Live immature and mature ova nearly disappeared recording very low numbers, while 89.6 ±5.9 and 99.1±3.7 of dead ova were detected in gps orally treated with FMK combined with PZQ in comparison to 74±6.2 and 74±7.9 on using PZQ and 36±2.3 and 47±3.9 on using FMK alone at 3 and 5 wk PI, respectively. Again the combination between FMK and PZQ showed a maximum efficient results between all CPIs used in this study.

This was agreed with El-Missiry *et al.* (1996), Wasilewski *et al.* (1996) and Metwally *et al.* (1997). They showed that there were a reduction in the number of mature ova, an increase in the dead ova counts and a disappearance of mature stages in mice infected with *S. mansoni* and treated with PZQ.

Matsuda *et al.* (1983), El-Garem (1991) and Giboda and Smith (1994) indicated that antischistosomal action of PZQ was effective only against mature ova while the immature ones survived and they pointed out that the infected mice treated with PZQ should be retreated with the same agent 9 days after the first treatment.

From the above, we observed that these CPI could effectively block schistosomal activity and prevented its growth and differentiation. Briefly, the best schistosomicidal effect of CPIs, that gained by drug administration orally in a dose of 50 mg kg^{-1} mouse, was observed with FMK. This was followed by VS and lastly with SNP. While their combination with PZQ, either treatment was performed at 3 or 5 wk PI gave significant results. The best combination was also that with FMK inhibitor.

These results were in agreement with Engel *et al.* (1998). They reported that, treatment with FMK-derivarized pseudopeptides rescued mice from lethal infection. The optimal pseudopeptide scaffold was phenylalanine-homophenylalamine. They provided a proof of concept that CPIs can be given at therapeutic doses to animals to selectively arrest a parasitic infection.

It was obvious that liver sections from *S. mansoni* infected mice at 8 week PI, showed loss of hepatic lobular architecture, hydropic degeneration in the hepatocytes, interstitial fibrosis, interlobular fibrosis and large area of necrosis. Granuloma formation with calcification and abcess formation was also found. Thickened portal tracts with fibrous tissue and heavy infiltration with

mononuclear cells were observed along hepatocytes and within the portal area of the liver. A sign of early fibroplasias was evident.

The microscopic examination of liver sections of infected mice showed a large cellular granuloma with living central ova. While, liver sections from infected mice treated with FMK or VS, at 3 or 5 wk PI showed a great reduction in granuloma size as small cellular granuloma with central degenerated ova. The same effect had been noticed when FMK or VS combined with PZQ. On contrast, SNP treatment at both intervals has no immunoprotective effect alone or combined with PZQ.

The granulomas formation is initiated by antigens secreted by miracidium through microscopic pores within the rigid egg shell (McKerrow and Davies, 2001). The continuous egg deposition with a subsequent chronic inflammatory host response is responsible for the liver fibrosis (Boros, 1989). This fibrosis blocks normal blood flow from the portal venous system to sinusoids resulting in portal hypertension and its complications (McKerrow and Davies, 2001).

Granuloma is a nodule of inflammatory tissue composed of clusters of activated macrophages and T lymphocytes, often with associated necrosis and fibrosis (Merchant and Amonkar, 2002). This inflammation is a form of chronic delayed-type hypersensitivity, often in response to persistent microbes, or in -response to particulate antigens that are not readily phagocytized (Afdhal and Nunes, 2004).

The effect of CPIs was studied in this thesis alone or in combination with PZQ on the PR in MGD and MGN of infected treated gps compared to infected control and PZQ treated gps. In the case of MGD, FMK treatment alone or combined with PZQ at 3 and 5 wk PI recorded nonsignificant difference compared to the PZQ treated gp. On the other hand, it was noticed that the PR of MGN in the FMK-treated gps either alone or combined with PZQ was significantly ($p<0.001$) higher than PZQ-treated gp. FMK treatment proved to be highly effective against *S. mansoni* in mice showing complete disintegrating ova and reduction in granulomatous size and numbers.

The treatment with a successful drug is reflected by a significant decrease in the number and size of the granulomas (Neves *et al*., 2011).

Lescano *et al.* (2004) recorded that, a significant decrease in liver and spleen weights was seen on the 20th day among animals treated with 50 or 100 mg kg^{-1} of artemether and also among those that received the drug of 50 mg kg^{-1} 60 days after infection. These results were referred to the decrease in MGD and MGN in the animals tissue.

(Pitta *et al.*, 2006); Neves *et al.* (2011) and Da Silva *et al.* (2012) reported that, the histological effect of the LPSF–PT05, on granulomatous inflammation showed that at 10, 30 or 100 mg Kg^{-1} per day had a positive effect in reducing the liver damage caused by *S. mansoni* infection as shown by the reduced number of worms and the down modulation of granulomatous response and this avoiding the development of host pathology.

El-Banhawey *et al.* (2007) demonstrated that, the histopathological examination of liver sections revealed moderate to small sized hepatocellular granulomas when PZQ chemotherapy is administered. PZQ reduced the number, diameter and cellularity granulomata. The effect of PZQ treatment on mice at 8 wk PI showed well defined granuloma. During the next 4 wk of infection (13-16th), fibrosis appeared as, perioral thickened sheath. PZQ treatment caused a complete disintegrating of schistosome ova beside schistosomal pigments.

Aly *et al.* (2010) used Diphenyl Dimethyl Bicarboxylate (DDB) and dexamethasone in treatment of *S. mansoni* infected mice. It was obvious that both drugs had no effect on worm burden, but altered tissue egg distribution. This indicates that neither drugs interfered with the development of adult worm or oviposition. Thus, both drugs can modulate liver pathology. These data suggested that dexamethasone was a convenient and promising co-adjuvant agent that decreased morbidity in murine schistosomiasis.

These results gave us evidence that CPIs can selectively arrest parasite replication without untoward toxicity to the host. Furthermore, this can be achieved with reasonable dosing schedules and oral administration of the drug.

It is obvious that treatment with FMK alone or in combination with PZQ recorded the best results regarding all included measured parameters, parasitological, immunological and histological.

So, FMK with PZQ is considered as the antischistosomal drug of choice between three CPIs in this study. It indicates its safety and powerful efficacy to eradicate the *S. mansoni* infection with a promising percentage.

REFERENCES

Abdel-Ghaffar, O., 2004. Assessment of the efficacy of Ro 16-2308 against the Egyptian strain of S. *mansoni* in mice: Parasitological, hematological and biochemical criteria. Egypt. J. Zool., 42: 173-203.

Abdel-Ghaffar, O., S.M. Rawi and A.I. Ishaq, 2005. Evaluation of the curative efficacy of RO 15-8843 against mansonian *schistosomiasis* in albino mice. J. Egypt. Ger. Soc. Zool., 47: 15-22.

Afdhal, N.H. and D. Nunes, 2004. Evaluation of liver fibrosis: A concise review. Am. J. Gastroenterol., 99: 1160-1174.

Aly, I.R.B., M.A. Hendawy, E. Ali, E. Hassan and M.M.F. Nosseir, 2010. Immunological and parasitological paramemeters after treatment with dexamethasone in murine *Schistosoma mansoni*. Mem. Inst. Oswaldo. Cruz., 105: 729-735.

Boros, D.L., 1989. Immunopathology of *Schistosoma* mansoni infection. Clin. Microbiol. Rev., 2: 250-269.

Cheever, A.W., 1970. Quantitative comparison of the intensity of *S. mansoni* infection in man and experimental animals. Trans. R. Soc. Trop. Med. Hyg., 63: 781-795.

Cheever, A.W. and L.A. Anderson, 1971. Rate of destruction of *Schistosoma mansoni* eggs in tissues of mice. Am. J. Trop. Med. Hyg., 20: 62-68.

Cioli, D., 2000. Praziquantel: Is there real resistance and are there alternatives? Curr. Opin. Infect. Dis., 13: 659-663.

Da Silva, A.C.A., J.K.A.L. Neves, J.I. Irmao, V.M.A. Costa and V.M.O. Souza *et al*., 2012. Study of the activity of 3-benzyl-5-(4-chloro-arylazo)-4-thioxo-imidazolidin-2-one against *Schistosomiasis mansoni* in mice. Scient. World J., 10.1100/2012/520524

Danso-Appiah, A. and S.J. De-Vlas, 2002. Interpreting low praziquantel cure rates of *Schistosoma mansoni* infections in Senegal. Trends Parasitol., 18: 125-129.

Drury, R.A. and E.A. Wallington, 1967. Carleton Histopathological Techniques. 4th Edn., Oxford University Press, New York.

El-Banhawey, M.A., M.A. Ashry, A.K. El-Ansary and S.A. Aly, 2007. Effect of *Curcuma longa* or praziquantel on *S. mansoni* infected mice: Liver-histological and histochemical study. Indian J. Exp. Biol., 45: 877-889.

El-Garem, A.A., 1991. Chemotherapy of schistosomiasis. Egypt J. Bilh., 13: 1-6.

El-Missiry, A.G., H .S. El-Nahal, A.A. Metwally, M.Y. Abdel-Hamid and N.M.A. Amer, 1996. Experimental *Schistosomiasis mansoni*: Efficacy of Ro 15-5458 hoffmann-la roache on different intensities of infections. New Egypt. J. Med., 15: 257-261.

Engel, J.C., P.S. Doyle, I. Hsieh and J.H. Meckerrow, 1998. Cysteine protease inhibitors cure an experimental *Tryponosoma cruzi* infection. J. Exp. Med., 188: 725-734.

Friedman, S.L., 2004. Mechanisms of disease: Mechanisms of hepatic fibrosis and therapeutic implications. Nat. Clin. Pract. Gastroenterol. Hepatol., 1: 98-105.

Giboda, M. and J.M. Smith, 1994. *Schistosoma mansoni* eggs as a target for praziquantel efficacy of oral application in mice. J. Trop. Med. Hyg., 97: 98-102.

Gryseels, B., K. Polman, J. Clerinx and L. Kestens, 2006. Human schistosomiasis. Lancet, 368: 1106-1118.

Harder, A., 2002. Chemotherapeutic approaches to schistosomes: Current knowledge and outlook. Parasitol. Res., 88: 395-397.

Ismail, M.M., S.A. Taha, A. Farghally and A.S. El-Zony, 1996. Laboratory induced resistance to praziquantel in experimental *Schistosomiasis*. J. Egypt. Soc. Parasitol., 24: 685-695.

Klemba, M. and D.E. Goldberg, 2002. Biological roles of proteases in parasitic protozoa. Ann. Rev. Biochem., 71: 275-305.

Lescano, S.Z., P.P. Chieffi, R.R. Canhassi, M. Boulos and V.A. Neto, 2004. Antischistosomal activity of artemether in experimental *Schitosomiasis mansoni* Rev. Sauad Public., 38: 71-75 [Article in Portuguese].

Matsuda, H., H. Tanaka, S. Nogami and M. Muto, 1983. Mechanism of action of praziquatel agains *S. mansoni* infection. J. trop. Med. Hyg., 87: 119-121.

McKerrow, J.H., 1999. Development of cysteine protease inhibitors as chemotherapy for parasitic diseases: Insights on safety, target validation and mechanism of action. Int. J. Parasitol., 29: 833-837.

McKerrow, J.H. and J.C. Davies, 2001. Parasitic Diseases. In: Medical Immunology, Parslow, T.G., D.P. Stites, A.I. Terr and J.B. Imboden (Eds.). 9th Edn., Long Medical Book/McGraw-Hill Medical Publishing Div., New York, pp: 673-687.

Merchant, S.A. and P.P. Amonkar, 2002. Genitourinary bilharziasis: A review. Ind. J. Radiol., 12: 239-244.

Metwally, A., A. Abdel-Hadi, G.E. Mikail, O. Abou Shadi, H. Sabry and H. El-Nahal, 1997. Study of efficacy of the new antischistosomal drug 10-12 (Diethylamino) ethyl 7-9-acridanone-(thiazolidine) hydrazine against an Egyptian strain of *S. mansoni* in mice. Arzeim-Forsch. Drug Res., 47: 975-979.

Mohey El-Dine, M.F., 1978. The problem of *Schistosoma* in Egypt. Proceedings of the International Conference Schist, (ICS'78), Ministry of Health, Egypt, pp: 2-5.

Mostafa, O.M.S., 2001. Experimental use of black seed oil against *S. mansoni* in albino mice: I. some parasitological and biochemical parameters. Egypt. J. Med. Lab. Sci., 10: 99-113.

Mostafa, A.F.M., 2005. Assessment of novel chemotherapeutic agent against the Egyptian strain of *S. Haematobium* in hamster. Ph.D. Thesis, Faculty of Science, Cairo University.

Neves, J.K.A.L., M.D.C.A. de Lima, V.R.A. Pereira, C.M.L. de Melo and C.A. Peixotoc *et al*., 2011. Antischistosomal action of thiox-oimidazolidine compounds: An ultrastructural and cytotoxicity study. Exp. Parasitol., 128: 82-90.

Padma, M.V., M. Behari, N.K. Misra and G. Ahuja, 1995. Albendazole in neurocysticercosis. Natl. Med. J. India, 8: 255-258.

Pellegrino, J., C.A. Oliveira, J. Faria and A.S. Cunha, 1962. New approach to the screening of drugs in experimental *Schistosoma mansoni* in mice. Am. J. Trop. Med. Hyg., 11: 201-215.

Pellgerino, J. and N. Katz, 1968. Experimental chemotherapy of *Schistosomiasis*. Adv. Parasit., 6: 233-251.

Pitta, M.G.R., A.C.A. Silva and J.K.A.L. Neves, 2006. New imidazolidinic biososters: Potential candidates for antischistosomal drugs. Memorias do Instituto Oswaldo Cruz, 101: 313-316.

Raso, G., E.K. N'Goran, A. Toty, A. Luginbuhl and C.A. Adjoua *et al*., 2004. Efficacy and side-effects of praziquantel against *S. mansoni* in a community of Western cote d'lvoireo. Trans. Roy. Soc. Trop. Med. Hyg., 89: 18-27.

Sabah, A.A., C. Fletecher, G. Webbe and M.J. Doenhoff, 1986. *Schistosoma mansoni*: Chemotherapy of infections of different ages. Exp. Parasitol., 61: 294-303.

Savioli, L., M. Albonico, D. Engels and A. Montresor, 2004. Progress in the prevention and control of schistosomiasis and soil-transmitted helminthiasis. Parasitol. Int., 53: 103-113.

Shalaby, K.A., L. Yin, A. Thakur, L. Christen, E.G. Niles and P.T. LoVerde, 2003. Protection against *S. mansoni* utilizing DNA vaccination with genes encoding Cu/Zn cytosolic superoxide dismutase, signal peptide-containing superoxide dismutase and glutathione peroxidase enzymes. Vaccine, 22: 130-136.

Steinmann, P., J. Keiser, R. Bos, M. Tanner and J. Utzinger, 2006. Schistosomiasis and water resources development: systematic review, meta-analysis and estimates of people at risk. Lancet Infect. Dis., 6: 411-425.

Utzinger, J., J. Keiser, X. Shuhua, M. Tanner and B.H. Singer, 2003. Combination chemotherapy of schistosomiasis in laboratory studies and clinical trials. Antimicrob. Agents Chemother., 47: 1487-1495.

Utzinger, J. and J. Keiser, 2004. Schistosomiasis and soil transmitted helminthiasis: Common drugs for treatment and control. Expert Opin. Pharm., 5: 263-285.

Van Den Berg, T.K., H. Honing, N. Frank, Van Remoortere and W.E. Schiphorst *et al*., 2004. Lacdi NAc-glycans constitute a parasite pattern for galictin-3- mediated immune recognition. J. Immunol., 173: 1902-1907.

WHO, 2002. Prevention and control of schistosomiasis and soil transmitted helminthiasis. World Health Organization, Geneva, Technical Report Series No. 912.

WHO, 2010. Schistosomiasis. Media Centre Fact Sheets No. 115. http://www.who.int/ mediacentre/f actsheets /fs115/en/index.html

Wasilewski, M.M., K.C. Lim, J. Philips and J.H. Mckerrow, 1996. Cysteine protease inhibitors block schistosome hemoglobin degradation *in vitro* and decrease worm burden an egg production *in vivo*. Mol. Biochem. Parasitol., 81: 179-189.

Webbe, G. and C. James, 1977. A comparison of the susceptibility to praziquantel of *Schistosoma haematobium*, *S. japonicum*, *S. mansoni*, *S. intercalatum* and *S. mattheei* in hamsters. Parasitol. Res., 52: 169-177.