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Parasitological Changes Within Experimentally Murine Schistosomiasis Mansoni upon Treatment by Somatostatin, Artemether and Their Combination

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ABSTRACT

The study explores the direct effect of somatostatin (SOM) on some stages of *Schistosoma mansoni*. Experimentally infected mice with *Schistosoma mansoni* were investigated following administration of either SOM, artemether (ART) or their combination. Each regimen was administered at 2, 5 and 13 weeks Post-infection (PI) to separate groups. The most presented period of worm reduction was in mice group received SOM at 5 weeks PI (44.28%), 2 weeks PI in ART administration (79.90%) and 2 weeks PI in combined treated group (84.16%). A significant reduction in mature ova ($p < 0.05$) was recorded in ART and combined treated groups at administration 2 weeks PI in addition to an increase in immature and dead ova in all treated groups than controls. The highest percentage reduction of total egg load was recorded in combined treated groups at 2, 5 and 13 weeks PI (88.4, 85.74 and 56.93%), respectively compared to other regimens. The morphological changes of the adult worms in SOM treated groups were elongation, erraticism, agitation and fragility. Male worms showed poorly developed suckers and tuberculations. Both male and female worms showed empty intestines. In ART treated group the male worms showed stunted growth and deformity of suckers. In female worms beside stuntedness, they showed moderately developed ovary, empty intestine and absence of vitelline glands. These observations were highly pronounced in worms recovered from combined treated groups. The study concluded that SOM has an inhibitory role on the parasite of *Schistosoma mansoni* itself regarding physiological development, worm reduction, egg production and maturity.

Key words: *Schistosoma mansoni*, somatostatin, artemether, egg production, maturity

INTRODUCTION

Somatostatin is a peptide hormone with an inhibitory action on the release of numerous secondary hormones. Somatostatin has a role in neurotransmission and cell proliferation. It acts via interaction with G-protein-coupled somatostatin receptors (Chatterjee *et al.*, 2001). Five cell surface somatostatin receptors have been characterized, termed SSTR-1 to SSTR-5 (Chatterjee *et al.*, 2001). In the study of host-parasite interactions caused by human schistosomiasis, neuropeptides play an important role (Weinstock and Elliott, 1998, 2000). To fight parasitic diseases, neuropeptides communicate and/or reciprocally modulate between the endocrine,

nervous and immune system (Ten Bokum *et al.*, 2000). Upon Clinical use of Somatostatin for two decades, it is reported to decrease portal pressure, control variceal bleeding and hepatic fibrosis (Mansy *et al.*, 1998; Chatterjee *et al.*, 2002). Somatostatin was found to reduce schistosomiasis clinical morbidity in the rodent model (Mansy *et al.*, 1998; Chatterjee *et al.*, 2002, 2005, 2007). In schistosomiasis the reaction to *Schistosoma* eggs can be minimized by stopping ova production by living worms, killing of female worms or by suppressing immunological reactions around *S. mansoni* eggs (Mahmoud and Warren, 1974). Somatostatin could play an important role in schistosomiasis mansoni. It has interactions via their influence on intersystem signaling. Therapeutically it has direct effects on schistosomiasis-caused morbidity by their immunomodulation of schistosomiasis-induced inflammatory responses in the liver and intestines. In addition, Somatostatin has possible effects on the *Schistosoma mansoni* parasite stages (Mansy *et al.*, 1998; Chatterjee *et al.*, 2001; 2005; 2006; De Man *et al.*, 2002; De Jonge *et al.*, 2003).

Praziquantel in treatment of schistosomiasis lacks efficacy against schistosomulae and develops resistance. A considerable number of researchers try to find an alternative (Lar and Oyerinde, 2007; Abdel Aziz *et al.*, 2011; El-Kott *et al.*, 2011). Artemether is already being widely used against malaria (McIntosh and Olliaro, 2001), has been shown to have antischistosomal properties against all human *Schistosome* species (Xiao *et al.*, 2001; Utzinger *et al.*, 2002, 2003) and to act on the juvenile stages of the parasite. So ART has a prophylactic effect in schistosomiasis as reducing the worm burden and hence the morbidity of disease (Utzinger *et al.*, 2001; Xiao *et al.*, 2002; Engels *et al.*, 2002) with a good safety profile for human (Gordi and Lepist, 2004).

Combination of different chemotherapeutics had better therapeutic response than each drug alone (Burger, 1970; Morsy, 2009). It is logically to assume that the use of the combination of SOM and ART may be beneficial for the treatment of *S. mansoni* infection.

The present study tried to explore the direct potential effect of SOM on some different stages of *Schistosoma mansoni* in experimentally infected mice. A proved antischistosome drug (ART) and combined administration of SOM and ART were applied to the corresponding groups of mice treated by SOM to realize the magnitude of SOM efficacy.

MATERIALS AND METHODS

Experimental animals and infection: Laboratory bred male Swiss albino mice weighing about 20-25 g and *Schistosoma mansoni* cercariae (Egyptian strain) were obtained from *Schistosoma* Biological Supply Program (SBSP) Unit at Theodor Bilharz Research Institute (TBRI), Giza, Egypt. Cercariae were used immediately after shedding from *Biomphalaria alexandrina* snails. Cercarial count was done according to Moore *et al.* (1977). Infection was done in a dose of 60±10 cercariae/mouse by body immersion technique according to Liang *et al.* (1987).

Mice grouping: Group I (18 mice): Infected non treated control group. Group II (54 mice): Infected group subdivided into three subgroups each of 18 mice: 1st subgroup: treated at 2 weeks PI and sacrificed at 5 weeks, 2nd subgroup treated at 5 weeks PI and sacrificed at 8 weeks, 3rd subgroup: Treated at 13 weeks PI and sacrificed at 16 weeks. The infected treated mice were equally divided between three regimens of treatment: SOM alone, ART alone and combined SOM plus ART.

Drug regimen: SOM was administered as Octreotid (SOM analogue) in a dose of 0.006 mg subcutaneously in 2 equal divided doses for two weeks, ART as single dose of 300 mg kg^{-1}

intramuscularly and combined treatment with both SOM and ART by the same mentioned doses. Octreotid is a somatostatin analog having the same pharmacological actions as the native SOM. It was used due to its higher specificity, better potency and longer duration of action and different routes of administration (Lemaire *et al.*, 1989). It was supplied as watery ampoules each contain 0.1 mg mL^{-1}.

Sacrificing mice was done by decapitation without anesthesia. Each mouse of the studied groups was processed for assessment of the following:

- *Schistosoma mansoni* worm burden was carried out through Hepatic and Mesenteric perfusion techniques according to Pellegrino and Coelh (1978)
- Morphological examination of the recovered worms using acetoacid alum carmin stain was done according to Gray (1954)
- Tissue bounding ova (oogram) pattern to determine the percentage of immature, mature and dead *S. mansoni* eggs was done according to Pellegrino *et al.* (1962)
- Ova count per gram tissue (liver and intestine) was done according to Cheever (1968)

Statistical analysis: Results were collected, tabulated, statistically analyzed using one-way Analysis of Variance (ANOVA) according to Campbell (1989). Comparison between two groups was done by the Student's t-test. p-value of 0.05 or less was taken to signify statistical significance.

RESULTS AND DISCUSSION

The presented morphological changes of recovered worms of the treated mice groups by SOM are considered the first records to our knowledge. In comparison to worms of infected control group (Fig. 1, 2), the majority of the worms were erratic, agitated and fragile. By ordinary microscope male worms (Fig. 3) showed poorly developed tuberculation, poorly formed irregular suckers and
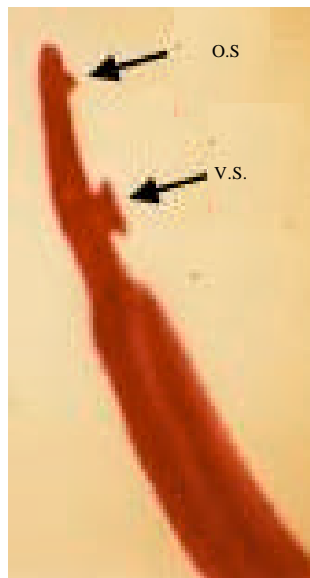

Fig. 1: Anterior part of adult *S. mansoni* male worm showing well developed oral and ventral suckers recovered from infected non treated mice group (x 40)

Fig. 2: Normal *S. mansoni* female worm recovered from infected non treated mice group showing average length, with well developed ovary. It shows pigmented intestine and well developed vitelline follicles (x40)

Fig. 3: *S. mansoni* male worm showing poorly developed suckers, testes and empty intestinal caeca with obviously widening of gynaecophoric canal recovered at 8 weeks PI after treatment with SOM at 5 weeks PI (x 40)

slightly affected testes. The majority of the female recovered worms (Fig. 4, 5) treated with SOM showed elongation, moderately developed suckers, ovaries and intestine. Empty intestines both in female and male worms are also observed, which may be due to the effect of the drug on intestinal musculature, which prevents the worms from feeding. These abnormal morphological findings indicate that SOM used may play a dominant role in the affection of female gonads which coincided with Sher *et al.* (1986). Our detected morphological changes may be mostly attributed to the interference of the drug with the biochemistry or/and physiology of the worms. They could be also attributed to the inhibitory role of SOM on growth hormone and another inhibitory role on tissue

Fig. 4: *S. mansoni* male worm showing curled or coiled appearance, poorly developed suckers and irregular tegument with absence of tuberculations. It shows absence of testes and empty intestine, recovered at 16 weeks PI after treatment with SOM at 13 weeks PI (x 40)
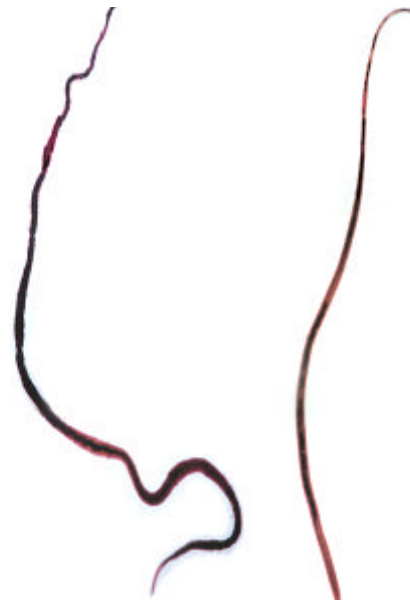

Fig. 5: *S. mansoni* female worm showing abnormal elongation of the worms, abnormal suckers and fragile appearance. Worms recovered at 8 weeks PI after treatment with SOM at 5 weeks PI (x 10)

growth and cell proliferation (Chatterjee *et al.* 2002). Susceptibility of the parasite to direct effect of SOM might be explained by Chatterjee *et al.* (2005, 2006) who localized somatostatin receptors SSTR5 on *S.mansoni* egg and worm stages.

In the present study, it is worthy to report that the highest worm reduction percentage (44.28%) was in mice group received SOM at 5 weeks PI (Table 1). No available recorded studies about the effect of SOM on number of worm load or their tissue distribution could be reviewed. This

Table 1: Total worm burden in hepatic and porto-mesenteric vessels, percent worm reduction and percent hepatic shift following drug regimen administration in different periods of post-infection

Periods in weeks (PI treatment/sacrificing)	Animal group	Total worm burden (Mean±SE)	Worm reduction (%)	Hepatic shift (%)
2/5	SOM	20.17±1.47	23.39	0.84
	ART	5.29±0.99*	79.90	29.68
	Combined			
	(SOM+ART)	4.17±0.70*	84.16	40.05
	Infected control	26.33±1.86	-	13.06
5/8	SOM	12.63±1.08*	44.28	13.86
	ART	7.44±2.01*	67.18	47.85
	Combined			
	(SOM+ART)	7.00±1.23*	69.12	20.43
	Infected control	22.67±1.23	-	18.39
13/16	SOM	17.29±2.82	37.05	11.57
	ART	12.2±1.200*	10.78	42.62
	Combined			
	(SOM+ART)	11.78±1.61*	39.21	42.44
	Infected control	19.38±2.35	-	15.48

*Significant difference from infected control at $p<0.05$. SOM administration at 5 weeks PI records the least worm burden (12.63±1.08), the highest percent reduction of worms (44.28%) and the maximum hepatic shift (13.86%) in comparison to other periods of administration. ART and combined treatment administration at two weeks PI show the least worm burden (5.29±0.99, 4.17±0.7), the highest percent reduction of worms (79.90%, 84.16%), respectively comparison to other periods of administration. ART shows the highest hepatic shift (47.85%) while that of combined treatment administration(42.44%) is at 5 weeks PI

effect could be attributed to the presence of SOM receptors on adult worm (Chatterjee *et al.*, 2005). The authors reported that somatostatin receptors SSTR2 and SSTR3 are present on both egg and worms. They suggested that therapeutic doses of somatostatin might affect the production of the adult parasite stage, by binding to SOM receptors on the parasite surface.

Hepatic shift was minimal in the infected mice treated with SOM at 2 weeks (acute stage) (0.84%), reached its maximum at 5 weeks (acute stage) (13.84%) with slight reduction to 11.57% at 13 weeks (chronic stage) in comparison to their infected control groups (13.06, 18.39 and 15.48%), respectively (Table 1). This is could be explained by the abnormal affection of male suckers which made the worm unable to move against the blood stream, since they hold the female worms against the blood flow by being embedded within the wall of the vessels followed by shifting of the worms to the liver after losing their attachment from mesenteric vessels.

Regarding oogram pattern (Table 2) in *S. mansoni* infected mice treated with somatostatin, showed significant reduction ($p<0.05$) in immature and mature stages of eggs in infected mice treated with SOM at 5 weeks (acute stage) (18.88±2.78 and 19.75±1.94) when compared with the infected control group (43.17±2.10 and 45.83±1.79), respectively while the mice treated at 13 weeks (chronic stage) showed a significant reduction in mature eggs (37.58±1.86) in comparison to infected control group (50.13±4.47). Absence of immature eggs at oogram pattern indicates an early interruption of egg laying process. This is in concern with Chatterjee *et al.* (2005) who suggested the probability of the direct action of the drug on the female genital system or through its receptors on the eggs. A significant increase in the number of dead ova (61.38±4.21) was recorded in infected treated mice with SOM at 5 weeks (acute stage) and (41.14±2.73) at 13 weeks (chronic stage) in comparison to (11.00±1.57 and 29.25±6.35) in infected control groups, respectively. These findings

Table 2: Oogram pattern and Egg load in liver and intestine following drug regimen administration in different periods of post-infection

Periods in weeks (PI treatment/ sacrificing)	Animal group	Oogram pattern (Mean±SE)			Egg load (Mean±SE)		
		Immature	Mature	Dead	Liver	Intestine	Total
2/5	SOM	41.00±1.81	40.00±2.88	19.00±1.18*	10.26±1.12	18.09±1.49*	28.35±2.52*
	ART	0±0*	0±0*	100±0*	2.65±0.46*	2.11±0.44*	4.76±0.71*
	Combined (SOM+ART)	0±0*	0±0*	100±0*	2.22±1.13*	1.92±1.02*	4.15±1.94*
	Infected control	40.33±2.42	47.67±2.29	12.00±1.36	11.32±1.41	26.13±2.9	35.79±3.69
5/8	SOM	18.88±2.78*	19.75±1.94*	61.38±4.21*	9.23±0.64	23.60±1.69	31.58±2.31
	ART	0±0*	18.78±4.11*	81.22±4.11*	3.28±0.39*	2.59±0.16*	5.86±0.42*
	Combined (SOM+ART)	0±0*	8.29±3.91*	91.71±3.91*	2.47±0.37*	2.76±0.37*	5.23±0.35
	Infected control	43.17±2.10	45.83±1.79	11.00±1.57	10.39±0.53	26.33±3.48	36.72±3.47
13/16	SOM	21.28±1.83	37.58±1.86*	41.14±2.73*	16.01±2.17	24.58±1.43	40.59±2.48
	ART	2.5±1.71*	22.6±3.89*	74.90±3.95*	13.94±1.17	9.49±0.96*	22.94±1.09*
	Combined (SOM+ART)	0±0*	15.22±3.21*	84.78±3.21*	11.40±1.42	7.08±0.79*	18.49±1.74*
	Infected control	20.62±4.74	50.13±4.47	29.25±6.35	17.01±1.68	25.91±1.91	42.93±3.34

*Significant difference from infected control at p<0.05. A significant reduction in mature ova (p<0.05) is recorded in ART and combined treated groups at administration 2 weeks PI in addition to an increase in immature and dead ova in all treated groups than controls. The highest percentage reduction of total egg load was recorded in combined treated groups at 2, 5 and 13 weeks PI (88.4, 85.74 and 56.93%), respectively compared to other regimens. Significant effect of SOM administration in comparison to control group is presented in lower mean of egg load (28.35±2.52) at 2 weeks PI and higher mean of dead ova in 2 and 13 weeks PI (19.00±1.18, 41.14±2.73)

were in agreement with the study carried out by Mansy *et al.* (1998). Tissue bound ova pattern in the small intestine recovered from infected control mouse showed living ova of different developmental stages (Fig. 9) in comparison to large number of dead eggs and broken shells recovered from SOM treated mouse at 5 weeks PI showing (Fig. 10).

In the present study, concerning the tissue egg load (Table 2), SOM administration at 2 weeks recorded a significant reduction (p<0.05) in the total mean number of eggs/gm liver and intestine tissues (28.35±2.25) in comparison to infected control group (35.79±3.69) with no further significant reduction in later periods of treatment (5 and 13 weeks), such results are in agreement of Mansy *et al.* (1998) and Chatterjee *et al.* (2005) who suggested that the therapeutic effect of SOM may inhibit the production of the parasite stage with consecutive decrease in total worm burden. They also suggested the affection of parasite genitalia and decrease number of couples by binding of SOM to its receptors on the parasite surface. But later on, Chatterjee *et al.* (2007) discovered no significant reduction in the total egg count after SOM treatment for five days in acute and chronic infected animals. The authors explained their finding by the down-regulation of SSTR-like G-protein Coupled Receptors (GPCRs) on adult *S. mansoni* worms after prolonged SOM treatment.

After ART administration, female schistosome worms were hardely recovered and were not available for mounting. This point of sex susceptibility is to be discussed in a separate paper. But recovered schistosome males (Fig. 6, 7) showed stuntedness with absence of suckers and testes. Similar observations were noticed by Xiao and Catto (1989), who found about 30-50% reduction in the lengths of male and female *S. mansoni* worms, respectively at 14 days after administration of ART with a daily dose of 300 mg kg^{-1} per day for 2 days. Utzinger *et al.* (2001) declared

Fig. 6: *S. mansoni* male worm, showing severe stuntedness with absence of suckers and testes recovered at 16 weeks PI and treated with ART at 13 weeks PI (x 40)

Fig. 7: *S. mansoni* male worm showing marked stuntedness and poorly developed suckers, recovered at 16 weeks PI (chronic stage) after treatment with ART at 13 weeks PI (x 40)

Fig. 8: *S.mansoni* male worms showing severe stuntedness, absence of suckers and testes, empty intestine and loss of tuberculations recovered at 16 weeks PI (chronic stage) after combined treatment at 13 weeks PI (x 40)

Fig. 9: Tissue bound ova pattern in the small intestine recovered from infected control mouse showing living ova of different developmental stages

Fig. 10: Tissue bound ova pattern in the small intestine recovered from SOM treated mouse at 5 weeks PI showing large number of dead eggs and broken shells

reductions of the glycogen content of schistosome worms after an intramuscular injection of ART as a single dose (300 mg kg^{-1}) for 1 day. The reduction was 50%, increased to 73% after 3 days and was higher in female schistosomes than males suggesting sex-specific drug susceptibility. Upon oral single dose of artemether administration, Utzinger *et al.* (2003) reported severe and extensive tegumental damage in *S. mansoni* worms recovered from experimentally infected mice.

Regarding ART, the drug induced a significant reduction in total number of worms (Table 1) in *S. mansoni* infected and treated mice compared with the infected control untreated ones (p<0.05). The reduction percentage of total worms was 79.90% in acute stage (2 weeks), 67.18% (5 weeks) and 10.78% in chronic stage (13 weeks) treated mice PI. The highest worm reduction percentage (79.90%) in mice group received ART at 2 weeks post infection was recorded by many studies. Utzinger *et al.* (2001), *in vivo* study with *S. japonicum,* demonstrated that, the juvenile stages of the parasite were more susceptible to artemether than the adult worms. Xiao *et al.* 2002) reported that the highest susceptibility is confined to the larval migratory stages. Another explanation by Xiao and Catto (1989) was that perhaps young liver stages have non efficient antioxidant protective mechanisms.

Concerning the hepatic shift (Table 1), it was increased in the infected mice treated with ART at 2 weeks (29.68%) reached its maximum at 5 weeks as acute stage (47.85%) and reduced to

42.62% at 13 weeks (chronic stage) in comparison to their infected control groups 13.06, 18.39 and 15.48%, respectively. This is commences within Xiao and Catto (1989) who observed hepatic shift of the worms towards the liver during the first 8 h after oral treatment with a single dose of ART (300 mg kg^{-1}). Although, the highest susceptibility to ART is confined to the larval migratory stages (Xiao *et al.*, 1995, 2000a), the drug also possesses a degeneration effect of reproductive organs and hence inhibiting egg formation of adult schistosomes besides a decrease of worm body size and hepatic shift (Wu *et al.*, 1983; Xiao and Catto, 1989; Xiao *et al.*, 1995).

Concerning the oogram pattern (Table 2) in *S. mansoni* infected mice treated with ART, it showed a significant reduction (p<0.05) in immature and mature stages at all periods of treatment 2, 5 and 13 weeks in comparison to infected control group. Complete absence of eggs was mainly in infected mice treated with ART at 2 weeks in comparison to infected control group 40.33±2.42 and 47.67±2.29, respectively, while at 5 weeks they were 0±0, 18.78±4.11 in comparison to infected control group 43.17±2.10 and 45.83±1.79, respectively. This can be contributed to the effect of the drug on mainly 2-5 weeks worms (acute stage) which supported by Xiao *et al.* (1998, 2000a) and Utzinger *et al.* (2001). At 13 weeks they were 2.5±1.71 and 22.6±3.89 in comparison to infected control group 20.62±4.74 and 50.13±4.47. The highest significant increase in the number of dead ova in infected treated mice with ART at 2 weeks was 100±0, 81.22±4.11 at 5 weeks (acute stage) and 74.90±3.95 at 13 weeks (chronic stage) corresponding to 12.00±1.36, 11.00±1.57 and 29.25±6.35 in infected control groups, respectively. Xiao *et al.* (2000b) attributed that to the effect of ART on reproductive organs of the female worms which were difficult to locate. This finding is also in agreement with Mahmoud *et al.* (2006) who found that a complete absence of all egg developmental stages and the highest reduction in egg load were observed in groups treated with ART (200 mg k^{-1}) at 5, 6 and 7 weeks PI.

Regarding the tissue egg load (Table 2), represented by egg/gm tissues of liver and intestines, ART administration at 2, 5 and 13 weeks post infection recorded a significant reduction (p<0.05) in the percentage of the total egg load, it was 86.70, 84.04 and 46.56%, respectively. The highest reduction in the acute stages of infection can be attributed to the effect of ART on early stages of *S. mansoni* (schistosomulae) leading to reduction in the total number of worms. This is in agreement with Xiao and Catto (1989) in a study of effect of ART in infected mice with *S. mansoni* treated on day 14 or 21 after infection, worm reduction rates of 83 to 98% were obtained. Also due to ART effect on reproductive organs of the female worms (Xiao *et al.*, 2000b).

Regarding combined therapy of both SOM and ART seemed to be more effective than the SOM alone. The morphological changes of recovered worms of the different treated mice groups by both SOM and ART (Fig. 8) shows *S.mansoni* male worms with severe stuntedness, absence of suckers and testes, empty intestine and loss of tuberculations. Apparently, the morphological changes are worse than that recorded for each drug administration alone.

The highest hepatic shift (Table 1) was recorded for the mice group received combined therapy at 13 weeks PI (42.44%) where the maximum mean total egg load in liver and intestine was 18.49±1.74 also for the same period with significant lower difference (p<0.05) than the infected control group (42.93±3.34).

In context of the total mean worm burden (Table 1) in mice group received combined treatment at different periods (2,5 and 13 weeks) PI, they were 4.17±0.71, 7±1.23 and 11.78±1.61 with percentage of worm reduction 84.16, 69.12 and 39.21%, respectively. All combined doses and their periods recorded significant reduction which was highly pronounced at 2 weeks treatment post infection.

Oogram pattern (Table 2) showed complete disappearance of immature and mature egg stages with remarkable increased in dead ova stage (100%) especially at 2 weeks. In mice groups received combined therapy at 2, 5 and 13 weeks PI, a highly pronounced effect mainly on immature stage was observed as none of immature ova could be seen along the examined periods. A significant increase in the mean numbers of dead ova have been recorded as 100, 91.71±3.91 and 84.7±3.21 compared with 12±1.36, 11±1.57 and 29.25±6.35 in the corresponding controls. These data was accompanied by significant decrease in all mean numbers of mature ova as 0±0, 8.29±3.91 and 15.22±3.21 compared with 47.67±2.29, 45.83±1.79 and 50.13±4.47 in the corresponding control groups. On reviewing the literatures no available data was found concerning the use of combined therapy of SOM and ART in treatment of schistosomiasis mansoni.

CONCLUSION

The study concluded that SOM has an inhibitory role on the parasite of *Schistosoma mansoni* itself regarding physiological development, worm reduction, egg production and maturity. This effect does not rise to being nominated to schistosomicidal drug but just as an adjuvant therapy in treatment of schistosomiasis.

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