Research Journal of Parasitology

ISSN 1816-4943

Academic Journals Inc.

www.academicjournals.com

Research Journal of Parasitology 8 (2): 55-63, 2013
ISSN 1816-4943 / DOI: 10.3923/jp.2013.55.63

Efficacy of Combined Therapy of Artemether and Somatostatin in Heptic Hydroxyproline in Experimental Murine Schistosomiasis Mansoni

[1]Mai A. Hegazi and [2]Madiha Mahmoud
[1]Department of Parasitology, Faculty of Medicine for Girls, Al-Azhar University, Cairo, Egypt
[2]Department of Pharmacology, Theodor Bilharz Research Institute Giza, Egypt

Corresponding Author: Mai A. Hegazi, Department of Parasitology, Faculty of Medicine for Girls, Al-Azhar University, Cairo, Egypt Tel: +0201112477314

ABSTRACT

The study aimed to examine the effect of combined therapy of artemether (ART) and somatostatin (SOM) on liver fibrosis in experimentally infected mice with *Schistosoma mansoni*. Infected mice were investigated following administration of either SOM, ART or their combination. Each regimen was administered at 2, 5 and 13 weeks post-infection (PI) to separate groups which were respectively sacrificed at 5, 8 and 16 weeks PI. Liver fibrosis was assessed by chemical measurement of heptic hydroxyproline as a marker of collagen content in liver. The extent of infection was monitored by liver egg load. The results were interpreted in comparison with corresponding ones in untreated *S. mansoni*-infected mice and age-matched normal control mice. Combined treated group showed the highest significant lower mean of hepatic hydroxyproline content than the infected control in 2, 5 and 13 weeks PI (793.41±64.91, 1010.87±75.67 and 1021.42±135.60) respectively in comparison to control group. ART administration group attained nearby significant lower measures as that of combined therapy group in the three successive periods of the experiment (893.57±31.83, 1035.91±42.24, 1228.34±26.52) in comparison to control group. SOM treated group attained significant lower hydroxyproline content in only 5 weeks PI (1145.30±80.91) and 13 weeks PI (1154.53±60.84). The study concluded that the combined therapy seemed not to be more effective than ART alone. By implication SOM therapy does not appear to have an additive effect on ART treatment.

Key words: *Schistosoma mansoni*, somatostatin, artemether

INTRODUCTION

The main inflammatory reaction against *Schistosome* eggs dislodged in the hepatic portal venules is the granulomatous formation with subsequent fibrosis (Alves Oliveir *et al*., 2006). Hepatic fibrosis precipitates presinusoidal portal hypertension and bleeding oesophageal varices (Mahmoud, 1984; Mansy *et al*., 1990, 1992). The degree of hepatic fibrosis depends on the viability of *Schistosome* eggs and their number. Viable eggs trigger the host granulomatous reaction as miracidium in mature eggs secrete antigens while no response is observed around dead eggs (Pellegrino and Katz, 1969; Warren and Boros, 1975; Cheever *et al*., 1992). There is also a positive correlation between the severity of hepatic fibrosis and the number of eggs in the tissue (Yue *et al*., 1984). The main feature of Liver fibrosis is the accumulation of extracellular matrix

proteins including collagen (Chatterjee *et al.*, 2005). Livers contain 20 times more collagen than normal in experimental hepatosplenic schistosomiasis (Dunn *et al.*, 1977). Among protein collagen hydroxyproline is a major component (Szpak, 2011). Hydroxyproline is a reflection of liver collagen synthesis during liver fibrosis (Ala-Kokko *et al.*, 1987). Hepatic egg granulomas contains the majority of proline incorporated into hydroxyproline (Olds *et al.*, 1985). It is suggested that there are factors in *S. mansoni* egg granulomas which elevate the free L-proline content in the fibrotic liver (Tanabe *et al.*, 1991). Inhibition of the deposition of extracellular matrix proteins is the aim of any antifibrotic therapy (Bataller and Brenner, 2005). Early administration of antischistosomal drugs might lead to resorption of collagen, while late treatment at chronic stages leads only to little diminution in fibrosis (Mehlhorn *et al.*, 1982).

Artemether is already being widely used against malaria (McIntosh and Olliaro, 2001) has been shown to have antischistosomal properties against all human Schistosome species (Utzinger *et al.*, 2002, 2003). Its effect is directed towards reduction of adult worms especially females (Xiao *et al.*, 2000, 2001). It reduces number of excreted ova (Mahmoud *et al.*, 2006) with increase number of dead ova (Seif el-Din *et al.*, 2011; Sayed El-Ahl *et al.*, 2013). Significant reduction in eggs prevents granuloma formation with subsequent morbidity in liver (Botros *et al.*, 2010). ART anti-pathologic activities is apparent especially in early treatment after infection (Hamza *et al.*, 2012). Even granulomata after ART treatment show lower collagen content than control (Mahmoud *et al.*, 2006).

Somatostatin is a peptide hormone with regulatory effect on the endocrine system. It affects neurotransmission and cell proliferation. Somatostatin inhibits the release of numerous secondary hormones through interacting with G-protein-coupled somatostatin receptors (Chatterjee and Van Marck, 2001). Clinically, Somatostatin, is reported to decrease portal pressure, control variceal bleeding and reduce hepatic fibrosis (Fevery and Raptis, 1997). In schistosomiasis that cause clinical morbidity in the rodent model SOM has been associated with significant decrease in liver ova count and a significant increase in dead ova (Mansy *et al.*, 1998). Also significant decreased size of cellular and fibrocellular granulomas (Mansy *et al.*, 1998). SOM administration has its direct morphological morbid effect on the adult worm of Schistosoma mansoni (Sayed El-Ahl *et al.*, 2013). Significant reduction of hepatic hydroxyproline levels is accompanied by treatment of acute and chronic infected animals with somatostatin (Chatterjee *et al.*, 2005). SOM can immunomodulate schistosomiasis-induced inflammatory responses in the liver and intestines and so it has a direct effects on schistosomiasis-caused morbidity (Mansy *et al.*, 1998). It has possible effects on the *Schistosoma mansoni* parasite stages (Sayed El-Ahl *et al.*, 2013).

Based on these reports, the hypothesis that combined treatment of ART and SOM could potentiate alleviation of hepatic fibrosis caused by schistosomiasis is presented. To prove this ART and SOM are administrated as combined and single therapies for *Schistosoma mansoni* infected mice. Heptic hydroxyproline as a marker of collagen deposition in infected mice was monitored compared to uninfected or untreated groups.

MATERIALS AND METHODS

Experimental animals and infection: Laboratory bred male Swiss albino mice weighing about 20-25 gm 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 Staden (1949). The experiment started with 200 mice but actually carried out on 90 survived mice.

Mice grouping: Group I (18 mice): Infected non treated control group. Group II (18 mice): non Infected non treated control group. Group III (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 daily 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, 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: tissue bounding ova (oogram) pattern was done to determine the percentage of immature, mature and dead *S. mansoni* eggs according to Pellegrino *et al.* (1962), ova count per gram tissue (liver and intestine) according to Cheever (1968).

Determination of hepatic content of hydroxyproline: One gm of liver tissue was taken and total liver collagen was determined as hydroxyproline (Trans-4 hydroxy-L-proline) according to the method of Woessner (1961).

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. The p-value of 0.05 or less was taken to signify statistical significance.

RESULTS AND DISCUSSION

On reviewing the literature, trial of combined regimen of ART and SOM administration in treatment of schistosomiasis is the first one to general knowledge. In the present study, combined treatment group attained the lowest significant mean hepatic hydroxyproline content over ART and SOM single therapy groups. At 2,5 and 13 weeks PI it was 793.41±64.91, 1010.87±75.67 and 1021.42±135.60 in comparison to normal control (660.30±51.48, 660.30±51.48, 660.30±51.48) and the infected control (1209.88±80.12, 1436.57±89.11, 1696.21±90.41), respectively (Table 1). This significant low hepatic hydroxyproline content in treated group could be correlated with the significant low mean egg load in the same group (Table 2) in the corresponding periods. It was 2.22±1.13, 2.47±0.37, 11.40±1.42 in comparison to infected control (11.32±1.41, 10.39±0.53, 17.01±1.68). At 2 weeks PI (Table 2) there was complete absence of immature and mature ova (0±0) and higher significant mean level of dead ova (100±0) in comparison to infected control (40.33±2.42, 47.67±2.29, 12.00±1.36). The other two periods (5 and 13 weeks PI) also showed highest mean dead ova (91.71±3.91, 84.78±3.21) in comparison to control (11.00±1.57, 29.25±6.35).

Table 1: Hepatic Hydroxyproline content and egg load in mice liver following drug treatment at 2, 5 and 13 weeks post infection with *S. mansoni* cercariae and sacrificed at 5, 8 and 16 weeks post infection respectively. Values given are Means±SE

	Time of Start treatment/Time Of sacrifice					
	Hydroxyproline (µg/g liver)			Egg load in liver		
Animal groups	2/5 weeks	5/8 weeks	13/16 weeks	2/5 weeks	5/8 weeks	13/16 weeks
Somatostatin	1174.30±61.85	1145.30±80.91*#	1154.53±60.84*#	10.26±1.12	9.23±0.64	16.01±2.17
Artemether	893.57±31.83*#	1035.91±42.24*#	1228.34±26.52*#	2.65±0.46*	3.28±0.39*	13.94±1.17
Combined (SOM+ART)	793.41±64.91*#	1010.87±75.67*#	1021.42±135.60*#	2.22±1.13*	2.47±0.37*	11.40±1.42
Normal Control	660.30±51.48	660.30±51.48	660.30±51.48	-------	-------	-------
Infected control	1209.88±80.12	1436.57±89.11	1696.21±90.41	11.32±1.41	10.39±0.53	17.01±1.680

SOM: Somatostatin, ART: Artemether, PI: Post infection. *Significant difference from infected control at $p<0.05$. #Significant difference from normal control at $p<0.05$

Table 2: Oogram pattern in liver and intestine following drug treatment at 2, 5 and 13 weeks post infection with *S. mansoni* cercariae and sacrificed at 5, 8 and 16 weeks post infection respectively. Values given are Means±SE

		Oogram pattern (Mean±SE)		
Periods in weeks (PI treatment/sacrifice)	Animal group	Immature	Mature	Dead
2/5	SOM	41.00±1.81	40.00±2.88	19.00±1.18*
	ART	0±0*	0±0*	100±0*
	Combined (SOM+ART)	0±0*	0±0*	100±0*
	Infected control	40.33±2.42	47.67±2.29	12.00±1.36
5/8	SOM	18.88±2.78*	19.75±1.94*	61.38±4.21*
	ART	0±0*	18.78±4.11*	81.22±4.11*
	Combined (SOMART)	0±0*	8.29±3.91*	91.71±3.91*
	Infected control	43.17±2.10	45.83±1.79	11.00±1.57
13/16	SOM	21.28±1.83	37.58±1.86*	41.14±2.73*
	ART	2.5±1.71*	22.6±3.89*	74.90±3.95*
	Combined (SOM+ART)	0±0*	15.22±3.21*	84.78±3.21*
	Infected contro	20.62±4.74	50.13±4.47	29.25±6.35

SOM: Somatostatin, ART: Artemether, PI: Post infection . *Significant difference from infected control at $p<0.05$

The present results coincide with Pellegrino and Katz (1969), Warren and Boros (1975), Cheever *et al.* (1992) and Yue *et al.* (1984) where granulomatous response and subsequently hydroxyproline level is affected by reduction on egg load and increased number of dead ova.

In the present study, mice group under ART single administration recorded nearby results of combined treatment group. ART single therapy group followed combined treatment group in attaining lower significant mean Hydroxyproline level in the 2, 5 and 13 weeks period PI (893.57±31.83, 1035.91±42.24, 1228.34±26.52) than normal control and infected control. This also could be correlated with significant lower mean egg load by ART therapy (Table 2) in the corresponding 3 periods (2.65±0.46, 3.28±0.39. 13.94±1.17) in comparison to control. As combined therapy at 2 weeks PI ART single therapy group showed complete absence of immature and mature ova (0±0) and higher significant mean level of dead ova (100±0) in comparison to infected control (Table 2). The other two periods (5 and 13 weeks PI) also showed higher mean dead ova (81.22±4.11, 74.90±3.95) in comparison to control group (11.00±1.57, 29.25±6.35) which was reflected on the hydroxyproline level. In agreement of the present study is Mahmoud *et al.* (2006) where complete absence of all egg developmental stages and the high reduction of egg load in

treated mice with ART (200 mg k^{-1}) at 5,6,7 weeks PI (95.5%) are detected. There are also minimal number of portal cellular granuloma with lower diameter and moderate collagen content in comparison to control. Dead ova have no granulomatous reaction around (Pellegrino and Katz, 1969; Warren and Boros, 1975; Cheever *et al*., 1992). There is also a positive correlation between the severity of hepatic fibrosis and the number of eggs in the tissue (Yue *et al*., 1984). ART reduces no of excreted ova (Mahmoud *et al*., 2006) with increase no of dead ova (Seif el-Din *et al*., 2011; Sayed El-Ahl *et al*., 2013). Significant reduction in eggs prevents granuloma formation with subsequent morbidity in liver (Botros *et al*., 2010). Anti-pathologic activities of ART is apparent especially in early treatment after infection (Hamza *et al*., 2012). Even granulomata after ART treatment shows lower collagen content than control (Mahmoud *et al*., 2006). In the present study, at 13 weeks PI none of combined therapy or single therapy with ART showed lower mean egg load this could be attributed to the late period of ART administration to infected mice groups. Early use of ART on schistosomiasis infection is more effective (Mahmoud *et al*., 2006) as juvenile stages of schistosomes are more susceptible to artemether than adult worms (Xiao *et al*., 2000; Hamza *et al*., 2012). Diminution or even no egg granulomas in liver sections of mice treated with artemether, at early administration after infection could be detected (Mahmoud and Botros, 2005; Botros *et al*., 2010).

In the present study (Table 2), SOM treated group attained non-significant lower mean egg load (10.26±1.12, 9.23±0.64, 16.01±2.1) at the three periods in comparison to infected control group. However, SOM followed ART therapy in attaining significant lower mean hydroxyproline content in 5 and 13 weeks PI (1145.30±80.91, 1154.53±60.84), respectively in comparison to infected control group. This could be attributed to the significant higher dead ova after SOM administration in 2, 5 and 13 weeks PI (19.00±1.18, 61.38±4.21, 41.14±2.73) in comparison to control. There was also significant reduction of mean number of mature ova 5 and 13 weeks PI periods (19.75±1.94, 37.58±1.86) in comparison to control group. So the decreased liver ova count and the increased dead eggs detected in these subgroups may participate in part in decreasing hepatic fibrogenesis which is reflected on hydroxyproline level. These findings are supported by Chatterjee *et al*. (2005) where treatment of acute and chronic infected animals with somatostatin significantly reduces hepatic hydroxyproline levels. Associated significant decrease in liver ova count and a significant increase in dead ova after SOM treatment has been domcumented by Mansy *et al*. (1998). Lower mean hydroxyproline of the present study coincide with significant decreased size of cellular and fibrocellular granulomas after SOM administration (Mansy *et al*., 1998). 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* has strong affinity for the SOM receptor subtypes 2, 3 and 5 and can interact with them on *S. mansoni* egg and worm stages preventing the pathological morbidity of the parasite in the body (Chatterjee *et al*., 2005). Sayed El-Ahl *et al*. (2013) reveals that SOM therapy has its direct morphological morbid effect on the adult worm of *Schistosoma mansoni* including male and female worms in experimentally infected mice. An observation of the present study is that hepatic hydroxyproline content (Table 1) reached its lowest level at 5 weeks PI in SOM treated group. This is in agreement with Chatterjee *et al*. (2005). Somatostatin therapy is effective after two days of treatment with no further reduction in pathology after five days of therapy (Chatterjee *et al*., 2007). It is worthy to note that exogenous administration of SOM in experimental animals could alleviate the pathology caused by schistosomiasis through reducing either the number of parasite eggs or the secretion of fibrosis inducing-mediators (Chatterjee *et al*., 2005). There is also direct association between *S. mansoni* induced fibrosis and low endogenous SOM

levels in human subjects (Chatterjee *et al*., 2004). Octreotid as SOM analogue, has been shown to modulate fibroblast activation *in vitro* (Priestley *et al*., 1994; Pasquali *et al*., 2000). It exerts antifibrotic properties in hepatic, esophageal, or digestive fibrosis of rat models of (Fort *et al*., 1998; Wang *et al*., 2001; Turkcapar *et al*., 2003) and decreases the accumulation of connective tissue in mice (Mansy *et al*., 1998). Some data also indicate that octreotid inhibits the *in vivo* expression of transforming growth factor-b, a key fibrogenic mediator in rat models of intestinal and peritoneal fibrosis (Gunal *et al*., 2001). SOM is shown to significantly reduce growth hormone secretion which can lead to a decline in circulating insulin-like growth factor-1 (IGF-1). In turn this may affect collagen production as insulin-like growth factor and growth hormone stimulate growth and collagen deposition (Patel, 1990; Allen and Goldberg, 1992; Cotran *et al*., 1994). The main feature of the presented data is the nearby results of ART single administration to that of combined treatment while that of SOM is somewhat away even the significant results.

CONCLUSION

All over the study periods combined therapy showed the upper hand in lowering hydroxyproline in liver of infected mince. Mostly the combined treatment was followed by ART single therapy with nearby results then SOM single therapy. So, it is concluded that the use of SOM plus ART in alleviating hepatic fibrosis in experimental infection of *Schistosomia mansoni* is not powerfully recommended since the major effect is always referred to ART alone.

REFERENCES

Ala-Kokko, L., F. Stenback and L. Ryhanen, 1987. Preventive effect of malotilate on carbon tetrachloride-induced liver damage and collagen accumulation in the rat. Biochem. J., 246: 503-509.

Allen, D.B. and B.D. Goldberg, 1992. Stimulation of collagen synthesis and linear growth by growth hormone in glucocorticoid treated children. Pediatrics, 89: 416-421.

Alves Oliveir, L.F., E.C. Moreno, G. Gazzinelli, O.A. Martins filho and A.M. Silveira *et al*., 2006. Cytokine production associated with periportal fibrosis during chronic schistosomiasis mansoni in humans. Infect. Immun., 74: 1215-1221.

Bataller, R. and D.A. Brenner, 2005. Liver fibrosis. J. Clin. Invest., 115: 209-218.

Botros, S.S., O. Hammam, M. Mahmoud and R. Bergquist, 2010. Praziquantel efficacy in mice infected with PZQ non-susceptible S. mansoni isolate treated with artemether: Parasitological, biochemical and immunohistochemical assessment. APMIS, 118: 692-702.

Campbell, R.C., 1989. Statistics for Biologists. 3rd Edn., Cambridge University Press, Cambridge, UK., Pages: 446.

Chatterjee, S. and E. Van Marck, 2001. The role of somatostatin in schistosomiasis: A basis for immunomodulation in host-parasite interactions? Trop. Med. Int. Health, 6: 578-581.

Chatterjee, S., A. Mbaye, A.T. Alfidja, J. Weyler and J.T. Scott *et al*., 2004. Circulating levels of the neuropeptide hormone somatostatin may determine hepatic fibrosis in *Schistosoma mansoni* infections. Acta Trop., 90: 191-203.

Chatterjee, S., G. Vrolix, I. Depoortere, T. Peeters and E. Van Marck, 2005. The therapeutic effect of the neuropeptide hormone somatostatin on Schistosoma mansoni caused liver fibrosis. BMC Infect Dis., Vol. 5 10.1186/1471-2334-5-45.

Chatterjee, S., J.O. De Beeck, A.V. Rao, D.V. Desai and G. Vrolix *et al.*, 2007. Prolonged somatostatin therapy may cause down-regulation of SSTR-like GPCRs on Schistosoma mansoni. J. Vect. Borne. Dis., 44: 164-180.

Cheever, A.W., 1968. Condition affecting the accuracy of potassium hydroxide digestion techniques for counting *Schistosoma mansoni* eggs in tissues. Bull. World Health Org., 39: 328-331.

Cheever, A.W., J.G. Maccedoni, S. Deb, E.A. Cheever and J.E. Mosimann, 1992. Persistence of eggs and hepatic fibrosis after treatment of infected mice. J. Trop. Med. Hyg., 46: 752-758.

Cotran, R.S., V. Kumar and S.L. Robbins, 1994. Inflammation and Repair. In: Pathologic Basis of Disease. Schoen, F.J. (Ed.). WB Saunders Company Philadelphia, pp: 51-92.

Dunn, M.A., M. Rojkind, K.S. Warren, P.K. Hait, L. Rifas and S. Seifter, 1977. Liver collagen synthesis in murine schistosomiasis. J. Clin. Invest., 59: 666-674.

Fevery, J. and S.A. Raptis, 1997. Optimal Management of Upper Gastrointestinal Bleeding. Vol. 19, Wells Medical, England, pp: 7-64.

Fort, J., F. Oberti, C. Pilette, N. Veal and Y. Galloi *et al.*, 1998. Antifibrotic and hemodynamic effects of the early and chronic administration of octreotide in two models of liver fibrosis in rats. Hepatology, 28: 1525-1531.

Gunal, A.I., S. Duman, S. Sen, A. Unsal, E. Terzioglu, F. 'Akcicek and A. Basci, 2001. By reducing TGF beta 1, octreotid lessens the peritoneal derangements induced by a high glucose solution. J. Nephrol., 14: 184-189.

Hamza, R.S., A.S. Metwaly and D.A. Abo El-Maaty, 2012. Effects of artemether treatment on prepatent and patent schistosoma mansoni infection in experimentally infected mice. PUJ, 5: 147-154.

Lemaire, M., M. Azira, R. Dannecker, P. Marbach, A. Schweitzer and G. Maurer, 1989. Disposition of Sandostatin, a new synthetic somatostatin analog, in rats. Drug. Metab. Dispos., 17: 699-703.

Mahmoud, A.A.F. and K.S. Warren, 1974. Anti-inflammatory effect of tartaremetic and niridazole suppression of schistosoma egg granuloma. J. Immunol., 112: 222-228.

Mahmoud, A.A.F., 1984. Granuloma Formation Around Schistosome Eggs as a Manifestation of Delayed Hypersensitivity. In: Tropical and Geographical Medicine, Warren, K.S. and A.A.F. Mahmoud (Eds.). McGraw Hill, New York.

Mahmoud, M., F. Ebeid and M. Nosseir, 2006. Enhanced role of grapefruit juice on the antischistosomal activity of artemether on the liver of *Schistosoma haematobium* infected hamsters. Sci. Pharm., 74: 59-75.

Mahmoud, M.R. and S.S. Botros, 2005. Artemether as adjuvant therapy to praziquantel in murine Egyptian *Schistosomiasis mansoni*. J. Parasitol., 91: 175-178.

Mansy, S.S., E.L. Badrawy, N. Nada, G. El Garm and A. Akl *et al.*, 1990. Immunolocalization of type III procollagen on hepatosplenic schistosomiasis. N. Egypt. J. Med., 4: 609-618.

Mansy, S.S., N. Edward and M. Tawfik, 1992. Intrahepatic portal vein changes and haemostatic behaviour of blood platelets in p atients with hepatosplenic schistosomiasis. Egypt. J. Bilh., 14: 35-42.

Mansy, S.S., H.A. Yehia, M.M. Hassan, E.A. Hassan, M.M. Youssef, A.A. Hadi and C.D. Mackenzie, 1998. Effect of octreotide on the pathology of hepatic schistosomiasis. Arzneimittelforschung, 48: 855-861.

McIntosh, H.M. and P. Olliaro, 2001. Artemisinin Derivatives for Treating Severe Malaria (Cochrane Review). In: The Conchrane Library: Updte Software, Cochrane Infectious Diseases Group (Eds.). John Wiley and Sons, Ltd., Oxford.

Mehlhorn, H., J.K. Frenkle, P. Andrews and H. Thomas, 1982. Light and electron microscope studies on *Schistosoma mansoni* granulomas of mouse liver following treatment with praziquantel. Treponmed. Parasit., 33: 229-239.

Moore, D.L., D.I. Grove and K.S. Warren, 1977. The *Schistosoma mansoni* egg granuloma: Quantitation of cell populations. J. Pathol., 121: 41-50.

Olds, G.R., A. Griffin and T.F. Kresina, 1985. Dynamics of collagen accumulation and polymorphism in murine *Schistosoma japonicum*. Gastroenterology, 89: 617-624.

Pasquali, D., P. Vassallo, D. Esposito, G. Bonavolonta, A. Bellastella and A.A. Sinisi, 2000. Somatostatin receptor gene expression and inhibitory effects of octreotide on primary cultures of orbital fibroblasts from *Graves ophthalmopathy*. J. Mol. Endocrinol., 25: 63-71.

Patel, J.C., 1990. Somatostatin. In: Principles and Practice of Endocrinology and Metabolism. Becker, K.L. (Ed.). J.B. Lippincott Company, Philadelphia, pp: 1297.

Pellegrino, J. and N. Katz, 1969. Laboratory evaluation of antischistosomal agents. Ann. N. Y. Acad. Sci., 160: 429-460.

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.

Priestley, G.C., R.D. Aldridge, P.J. Sime and D. Wilson, 1994. Skin fibroblast activity in pretibial myxoedema and the effect of octreotid (Sandostatin) *in vitro*. Br. J. Dermatol., 131: 52-56.

Sayed El-Ahl, S.A., M.A. Hegazi, M. Mahmoud, F. El-Zahraa, M. Awadallah and M.A. Abd Rabo, 2013. Parasitological changes within experimentally murine schistosomiasis mansoni upon treatment by somatostatin, artemether and their combination. Res. J. Parasitol., 8: 1-13.

Seif el-Din, S.H., A.M. Al-Hroob and F.A. Ebeid, 2011. *Schistosoma mansoni*: N-acetylcysteine downregulates oxidative stress and enhances the antischistosomal activity of artemether in mice. Exp. Parasitol., 128: 230-235.

Staden, O.D., 1949. Experimental schistosomiasis: Maintenance of schistosoma mansoni in the laboratory with some notes on experimental infection with *S. haematobium*. Ann. Trop. Med. Parasit., 43: 268-283.

Szpak, P., 2011. Fish bone chemistry and ultrastructure: Implications for taphonomy and stable isotope analysis. J. Archaeol. Sci., 38: 3358-3372.

Tanabe, M., N. Kaneko and T. Takeuchi, 1991. *Schistosoma mansoni*: Higher free proline levels in the livers of infected mice. Exp. Parasitol., 72: 134-144.

Turkcapar, N., S. Bayar, A. Koyuncu and K. Ceyhan, 2003. Octreotid inhibits hepatic fibrosis, bile duct proliferation and bacterial translocation in obstructive jaundice. Hepatogastroenterology, 50: 680-683.

Utzinger, J., J. Chollet, Z. Tu, S. Xiao and M. Tanner, 2002. Comparative study of the effects of artemether and artesunate on juvenile and adult *Schistosoma mansoni* in experimentally infected mice. Trans. R. Soc. Trop. Med. Hyg., 96: 318-323.

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.

Wang, J., H. Zheng and M. Hauer-Jensen, 2001. Influence of short-term octreotid administration on chronic tissue injury, transforming growth factor beta (TGFbeta) overexpression and collagen accumulation in irradiated rat intestine. J. Pharmacol. Exp. Ther., 297: 35-42.

Warren, K.S. and D.L. Boros, 1975. The Schistosome Egg Granuloma a Form of Cell Mediated Immunity. In: Mononuclear Phagocytes in Immunity, Infection and Pathology, Van Furth, R. (Ed.). Blackwell Scientific Publications, ltd., London.

Woessner, Jr. J.F., 1961. The determination of hydroxyproline in tissue and protein samples. Arch. Biochem. Biophys., 93: 440-447.

Xiao, S.H., J. Chollet, J. Utzinger, H Matile, J.Y. Mel and M. Tanner, 2001. Artemether administered together with haemin damages shcistosmes *in vitro*. Trans. Royal Soc. Trop Med. Hyg., 95: 67-71.

Xiao, S.H., M. Booth and M. Tanner, 2000. The prophylactic effects of artemether against *Schistosoma japonicum* infections. Trends Parasitol., 16: 122-126.

Yue, W.J., J.Q. You and J.Y. Mei, 1984. Effects of artemether on *S. japonicum* adult worms and ova. Acta Pharmacol. Sin., 5: 60-63.