

Dept. of Parasitology,
Faculty of Medicine, Assiut University

**SCANNING ELECTRON MICROSCOPY OF ADULT
SCHISTOSOMA MANSONI AFTER TREATMENT
WITH PRAZIQUANTEL, FLUBENDAZOL
AND COMBINATION OF BOTH
(With 4 Plates)**

By

**LAILA A. OMRAN; FATMA G. SAYED;
SALMA M. ABDEL RAHMAN and AMAL M. ABDO**

(Received at 25/12/2005)

الميكروسكوب الإلكتروني الماسح لديدان الشistosوزوما مانسوناي بعد العلاج
بالبرازيكوانتل والفلوبندازول وخليط من العقارين

ليلى علي موسى عمران ، فاطمة جلال سيد ، سلمى محمد عبد الرحمن ،
أمل محمد عبده

من المعروف أن العقاقير المضادة للشistosوزوما تسبب تغيرات في التركيب الدقيق للديدان. تم إجراء هذه الدراسة على ٦٠ من فئران التجارب البيضاء المعدية بسركاريا الشistosوزوما مانسوناي ، وقد قسمت الفئران إلى ٤ مجموعات (مجموعة ضابطة، مجموعة معالجة بعقار الفلوبندازول، مجموعة معالجة بعقار البرازيكوانتل، مجموعة معالجة بالعقارين معا). وتم تقييم تأثير العلاج على كل من ديدان الذكر والأنثى بعد أسبوع وثلاثة أسابيع من العلاج. وقد تم تجميع الديدان عن طريق الضخ من خلال الوريد البابي والمعوى ثم تم الفحص بالميكروسكوب الإلكتروني الماسح.

SUMMARY

Many antischistosomal drugs are known to cause changes in the ultrastructure of adult worms. This study was done using 60 albino mice infected with cercariae of *Schistosoma mansoni*. Mice were divided into 4 groups (Control, Flubendazole-treated, Praziquantel-treated and the last group treated by combination of both drugs). The effect of treatment was evaluated on male and female worms after one and three weeks post treatment. Worms were collected by perfusion of portal vein and

mesenteric veins separately then examined by scanning electron microscopy.

Key words: *Schistosoma mansoni*, SEM, Flubendazole, Praziquantel.

INTRODUCTION

The tegument of *Schistosoma mansoni* worms has protective, absorptive and secretory function (Podesta, 1982). Scanning electron microscopic observations on tegumental development of *S. mansoni* worms were reported by Sneft *et al.* (1978) and Zaman (1992). Ultrastructure observations of the tegument and reproductive organs of *S. mansoni* after treatment with different antischistosomal drugs were reported by Irie *et al.* (1989), Leitch and Probert (1990) and Shalaby *et al.* (1991).

Praziquantel is efficacious against adult stages of all human schistosome parasites and has become the drug of choice for morbidity control of schistosomiasis. However, drug resistance to Praziquantel was discussed recently by Ismail *et al.* (2002) and Cioli and Pica-Mattocchia (2003).

The effect of Flubendazole on *S. mansoni* infection in mice was evaluated by Nessim *et al.* (2000). The synergistic activity of combined drugs on adult *S. mansoni* was reported by Shaw and Brommer (1983) and Botros *et al.* (1989).

This work aimed to study the effect of Praziquantel and Flubendazole either alone or in combination with each other as treatment for *S. mansoni* in experimentally infected mice using scanning electron microscopy.

MATERIALS and METHODS

I. Experimental infection of mice: (Standen, 1963)

This study was done by using 60 albino mice weighing 25-30 gms. 8-9 weeks old. They were divided into 4 groups (15 mice each):

Group (1): Each mouse was infected with 60 cercariae of *S. mansoni* and not received treatment (control).

Group (2): Each mouse was infected with 60 cercariae of *S. mansoni* and after 8 weeks the mice were treated with Flubendazole as a single oral dose (100 mg/kg body weight).

Group (3): Each mouse was infected with 60 cercariae of *S. mansoni* and after 8 weeks the mice were treated with Praziquantel in a single oral dose (300 mg/kg body weight).

Group (4): Each mouse was infected with 60 cercariae of *S. mansoni* and after 8 weeks the mice were treated with combination of both drugs (Praziquantel 150 mg/kg and Flubendazole 50 mg/kg). Both drugs were administered orally using stomach tube.

Five mice were sacrificed from each group at 9 and 11 weeks post infection (1 and 3 weeks post treatment).

II. After 8 weeks post infection mice were subjected to the following:

- 1- Faecal examination for *S. mansoni* eggs by using the modified Kato technique (Katz *et al.*, 1970)
- 2- Worm load was determined by perfusion of liver portal vein, mesenteric veins separately (Smithers and Terry, 1965). Worms were examined and sex was identified.
- 3- Worms were fixed in 3.5% gluteraldehyde for 2 hours and sent to the EM unit of Assiut University to be processed and examined by JEOL- JSM-5400 LV Scanning Electron Microscope.

RESULTS

SEM of *S. mansoni* adult (male):

Group (1) control group:

1. **Suckers region:** The oral sucker is oval, covered with sharp spines varying in size, its ventral border showed sensorial papillae beside the spines. The ventral sucker is bigger, more prominent appeared tire-like with sensory papillae (Plate I, Fig. 1).
2. **Ventral region:** Just behind the ventral sucker, the body increases in width and folded ventrally to form gynaecophoric canal. Along the canal more slender spines were observed and other short pointless spines present in the middle regions together with sensorial papillae (Plate I, Fig. 2).
3. **Dorsal region:** The dorsal tegument situated near the gynaecophoric canal, presented tubercles with numerous spines. The surface of the worm is strongly wrinkled leading to numerous grooves between the folds and tubercles. Between the grooves there are spines and sensorial papillae (Plate I, Fig. 3).

Group (2. a): Effect of Flubendazole (100 mg/kg) on adult male *S. mansoni* after one week post treatment:

- 1. Suckers region:** The oral sucker appeared flabby, the ventral sucker showed apparent loss of tire- like appearance (Plate II, Fig. 1).
- 2. Ventral region:** The gynaecophoric canal showed a decrease in the interwrinkler spines with the presence of multiple pores (Plate II, Fig. 2).
- 3. Dorsal region:** The tubercles of tegument are heavily affected. There is partial or complete loss of spines from some tubercles with nipple like apices. Intertubercular spaces are heavily wrinkled (Plate II, Figs. 3).

Group (2. b): Effect of Flubendazole (100 mg/kg) on adult male *S. mansoni* after 3 week post treatment:

- 1. Suckers region:** The effect was the same as previous group.
- 2. Ventral region:** The effect on the gynaecophoric canal was the same as previous group.
- 3. Dorsal region:** The effect on this group was more prominent and appeared as partial or complete loss of spines on the tubercles; some of these spines were distracted. The intertubercular space seems to be more wrinkled (Plate II, Figs. 4). The tubercles are disrupted with blunt or damaged spines (Plate II, Fig. 5).

Group (3.a): Effect of Praziquantel (300 mg) on the adult male *S. mansoni* after one week post treatment:

- 1. Suckers region:** In this group the ventral and oral suckers were not affected (Plate III Fig. 1).
- 2. Ventral region:** The gynaecophoric canal was not affected (Plate III Fig. 2).
- 3. Dorsal region:** The tubercles of the tegument were affected in the form of partial or complete disappearance of spines from some tubercles and the intertubercular spaces showing multiple swellings (Plate III Fig. 3).

Group (3.b): Effect of Praziquantel (300 mg) on the adult male *S. mansoni* after 3 week post treatment:

- 1. Suckers region:** The oral and ventral suckers were slightly affected.
- 2. Ventral region:** The effect on the gynaecophoric canal was the same as previous group.

3. Dorsal region: Showing complete disappearance of the tubercles and spines with the appearance of multiple pores all over the surface of the worm (Plate III Fig. 4).

Group (4.a): Effect of combination of Flubendazole (50 mg/kg) and Praziquantel (150 mg/kg) on adult male *S. mansoni* after one week post treatment:

1. Suckers region: The oral and ventral suckers are distorted in shape (Plate IV, Fig. 1).

2. Ventral region: There is a decrease in number of spines inside the canal with hairy-like appearance (Plate IV, Fig. 2).

3. Dorsal region: There is marked shrinkage of tegument with multiple pores; the tubercles have lost their shape with absence of spines (Plate IV, Fig. 3).

Group (4.b): Effect of combination of Flubendazole (50 mg/kg) and Praziquantel (150 mg/kg) on adult male *S. mansoni* after 3 week post treatment:

1. Suckers region: The effect on the oral and ventral suckers was the same as previous group.

2. Ventral region: The effect on the gynaecophoric canal was the same as previous group.

3. Dorsal region: Complete absence of spines on tubercles with multiple pores and nipple like appearance on the dorsal region (Plate IV, Fig. 4).

SEM of *S. mansoni* adult (female):

Group (1) control:

1. Suckers region: The tegumental surface between the oral and ventral sucker is smooth and free of spines but has sensory bulbs with short knob-like cilia. The region behind the ventral sucker showed transverse ridges, uniformly marked with numerous pits (Plate I, Fig. 4).

2. The middle region: The tegument is full of wrinkles and is devoid of papillae, spicules or spines. The surface furrows and ridges tend to branch and anastomose in complex pattern (Plate I, Fig. 5).

Group (2): Effect of Flubendazole (100 mg/kg) on adult female *S. mansoni* after one week post treatment:

1. Suckers region: Damage of the ventral sucker is observed.

2. The middle region: The tegument was severely affected showing multiple pores, heavy wrinkles with decrease in number of sensory

bulb. The surface furrows and ridges tend to branch and anastomose in complex pattern (Plate II, Fig. 6).

Group (3): Effect of Praziquantel (300 mg) on the adult female *S. mansoni* after one week post treatment:

- 1. Suckers region:** Showed no changes.
 - 2. The middle region:** The tegument of female *S. mansoni* showed the presence of heavy wrinkles and surface swellings (Plate III, Fig. 5).
- Group (4):** Effect of combination of Flubendazole (50 mg/kg) and Praziquantel (150 mg/kg) on adult female *S. mansoni* after one week post treatment:

- 1. Suckers region:** Showed no changes.
- 2. The middle region:** The tegument of the female was affected by the increase in number of pores and focal swellings (Plate IV, Fig. 5).

LEGENDS OF FIGURES

Plate I:

- Fig. 1:** SEM of anterior end of *S. mansoni* (control group).
- Fig. 2:** SEM of gynaecophoric canal of male *S. mansoni* showing the spines.
- Fig. 3:** SEM of dorsal surface of male *S. mansoni* showing the tubercles with spinous processes on their surface.
- Fig. 4:** SEM of control female *S. mansoni* showing oral and ventral suckers.
- Fig. 5:** SEM of female *S. mansoni* showing normal tegument.

Plate II:

- Fig. 1:** SEM of Flubendazole treated male (1 W.P.T) showing damaged oral and ventral suckers.
- Fig. 2:** SEM of Flubendazole treated male (1 W.P.T) showing multiple pores in the gynaecophoric canal with loss of its spines.
- Fig. 3:** SEM of Flubendazole treated male (1 W.P.T) showing imposition of tubercles with nipple like apices.
- Fig. 4:** SEM of Flubendazole treated male (3 W.P.T) showing partial or complete loss of spines on the tubercles.
- Fig. 5:** SEM of Flubendazole treated male (3W.P.T) showing tubercles with deformed or damaged spines.

Fig. 6: SEM of Flubendazole treated female (1 W.P.T) showing complex pattern of the ridges on the dorsal surface.

Plate III:

Fig. 1: SEM of Praziquantel treated male (1 W.P.T) showing normal oral and ventral suckers.

Fig. 2: SEM of Praziquantel treated male (1 W.P.T.) showing normal appearance of gynaecophoric canal.

Fig. 3: SEM of Praziquantel treated male (1 W.P.T.) showing decrease number of the spines.

Fig. 4: SEM of Praziquantel treated male (3W.P.T.) showing absence of tubercles and spines from dorsal surface.

Fig. 5: SEM of Praziquantel treated female (1 W.P.T) showing the middle surface of the tegument with heavy wrinkles, sensory bulbs and focal swellings.

Plate IV:

Fig. 1: SEM of male treated by combination of Flubendazole and Praziquantel (1 W.P.T) showing suckers distorted in shape.

Fig. 2: SEM of male treated by combination of Flubendazole and Praziquantel (1 W.P.T) showing the gynaecophoric canal with decrease number of spines with hairy like appearance.

Fig. 3: SEM of male treated by combination of the Flubendazole and Praziquantel (1 W.P.T) showing the dorsal surface with marked shrinkage of the tegument, absence of the spines and multiple pores.

Fig. 4: SEM of male treated by combination of Flubendazole and Praziquantel (3 W.P.T) showing the dorsal surface with complete absence of spines.

Fig. 5: SEM of female treated by combination of Praziquantel and Flubendazole (1 W.P.T) showing increase number of pores.

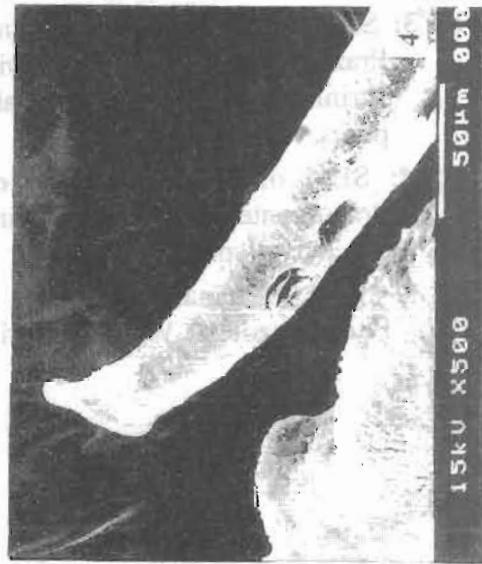
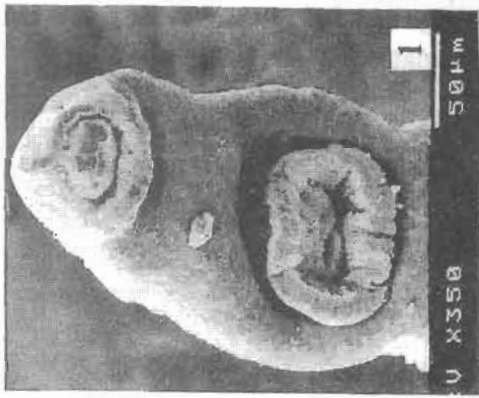


Plate I

Plate III

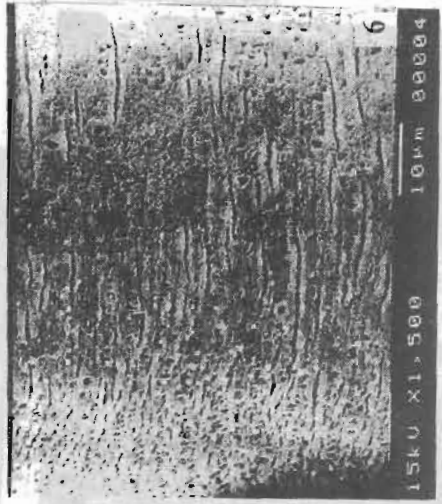


Plate II

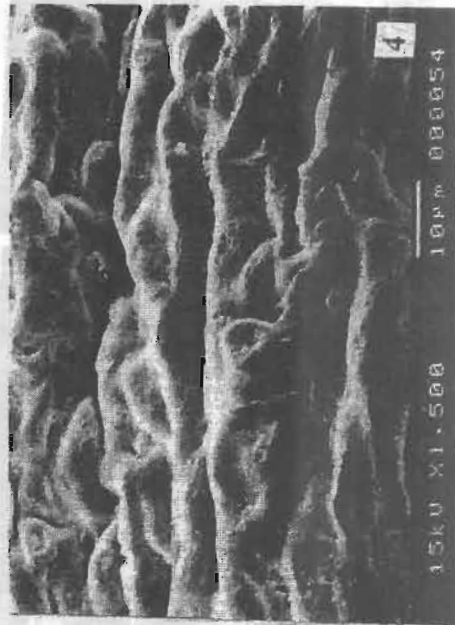
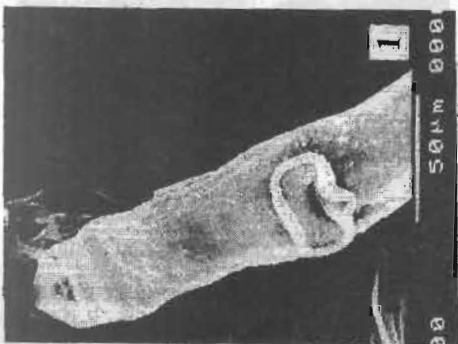


Plate III

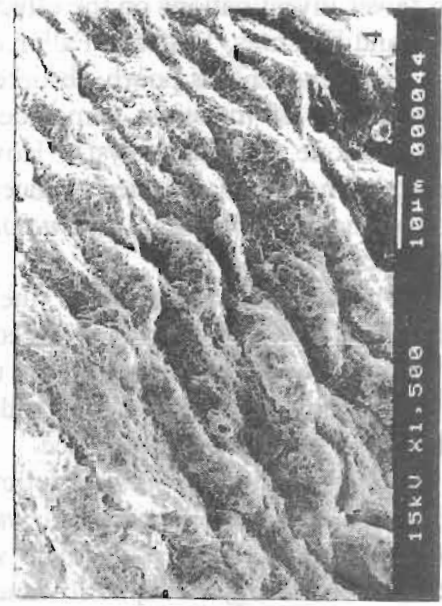
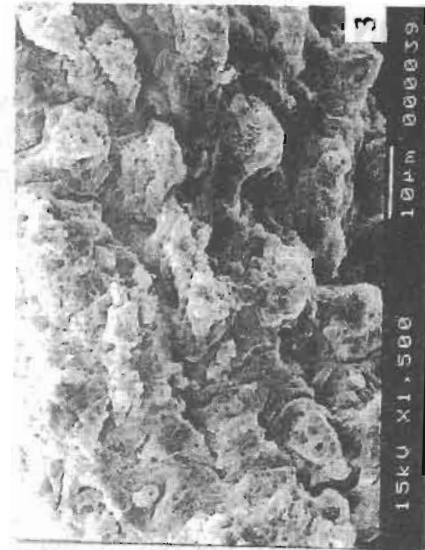


Plate IV

DISCUSSION

Praziquantel was discovered to be effective in treatment of many cestodes and trematodes. It is the drug of choice for treatment of all forms of schistosomiasis (Hardman *et al.*, 1996). It is more effective in destruction of spined papillae, wrinkling and disturbing interpapillar space (Shalaby *et al.*, 1991). Male worms exhibit more pronounced and extensive surface alteration, which include surface blebbing, swelling, wrinkling, constriction and surface lesions, particularly on the spined tubercle (Becker *et al.*, 1980; Shaw and Erasmus, 1988 and Fallon *et al.*, 1996).

In the present study exposure of *S. mansoni* to Praziquantel resulted in quick reactions, mainly on the tegument. Loss of spines occurred rapidly after (1W.P.T.) and became more severe at (3W.P.T.). The anterior region was almost not affected, which indicates different sensitivity of the tegument, as also reported by Mansoury (1997).

The effect of Praziquantel on adult female *S. mansoni* was also marked on the tegument. Mortality of the female worms as a result of Praziquantel treatment was manifested by their absence after the second week. This is of interest as the majority of previous studies on the effect of Praziquantel treatment on the worms revealed striking effects on male worms only (Mehlhorn *et al.*, 1981 and Modha *et al.*, 1990). There have been fewer investigations on the effect of Praziquantel on female worms (Shaw and Erasmus, 1988 and Fallon *et al.*, 1994).

Flubendazole is poorly absorbed from intestine and is effective in the treatment of intestinal nematodes. It is also effective against the larval cestode (Hydatid cyst). However no proven effect against trematodes has been reported (Pancera *et al.*, 1979). Al-Waili (1987) reported the cure of two *S. haematobium* patients after treatment with Flubendazole.

As can be seen from the present results, the effect of Flubendazole on suckers and gynaecophoric canal is more pronounced. On the other hand, the effect on the tegument was more pronounced in the group treated by Praziquantel and for these reasons the combination of the two drugs has its importance.

SEM of adult *S. mansoni* following treatment with a combination of Flubendazole and Praziquantel was described for the first time. At (1W.P.T.) the effect on adult male was marked on the ventral sucker together with the gynaecophoric canal and the tegument. The effect at (3W.P.T.) showed more extensive destruction on the tegument. This

destruction was more severe and pronounced in comparison to the other groups treated by Flubendazole and Praziquantel alone. Shaw and Brommer (1983) and Botros *et al.*, (1989) reported synergistic activity on adult *S. mansoni* treated by combined low doses of Praziquantel and Oxamniquine. As regards of female worms detected in this study, the tegument was affected at (1W.P.T.) and completely disappeared after the second week. This result was worst and more convenient than using the full doses of Flubendazole and Praziquantel for treatment where the females *S. mansoni* were severely affected.

Conclusively, the effect of combination of Praziquantel and Flubendazole on *S. mansoni* is marked and was illustrated best by SEM in the present work. This emphasizes the necessity of using this combination especially in cases which show resistance to Praziquantel treatment alone.

REFERENCES

- Al-Waili, N.S. (1987):* Mebendazole in treatment of *S. haematobium*. Trans. Roy. Soc. Trop. Med. Hyg. 81, 781.
- Becker, B.; Mehlhorn, H. and Echert, J. (1980):* Light and electron microscopic studies on the effect of praziquantel on *S. mansoni*, *D. dendriticum* and *F. hepatica* (Trematoda). Zeitschrift Fur. Parasitenkunde, 63: 113 – 128.
- Botros, S.; Soliman, A.; El-gawhar, N.; Selim, M. and Guirguis, N. (1989):* Effect of combined low dose praziquantel and oxamniquine on different stages of schistosome maturity. Trans. Roy. Soc. Trop. Med. and Hygi. 83, 86 – 89.
- Cioli, D.M. and Pica-Mattocchia, L. (2003):* Praziquantel. Parasitol Res, 90 Supp. 1: S 3-9.
- Fallon, P.G.; Foobees, R.E. and Wharton, G.A. (1996):* Temporal difference in praziquantel and oxamniquine induced tegumental damage to adult *S. mansoni* implications for drug-antibody synergy. Parasitology, 112: 47 – 58.
- Fallon, P.G.; Smith, P.; Nicholls, T.; Mobha, J. and Doenhoff, M.J. (1994):* Praziquantel-induced exposure of *S. mansoni* alkaline phosphatase drug-antibody synergy, which acts preferentially against female worms. Parasite Immunol. 16: 529 – 35.
- Hardman, J.G.; Limbird, L.E.; Molinoff, P.B.; Ruddon, R.W. and Gilman, A.G. (1996):* Goodman and Gilman's the pharmacological basis of therapeutics. 9th. Ed. McGraw Hill.

- Irie, Y.; Utsunomiya, H.; Tanaka, M.; Ohmae, H. and Yasuraoka, K. (1989): *S. japonicum* and *S. mansoni*: Ultrastructural damage in the tegument and reproductive organs after treatment with levo-and dextro praziquantel. Am. J. Trop. Med. Hyg., 41(2): 204 – 211.
- Ismail, M.M.; Farghaly, A.M.; Dyab, A.K.; Afify, H.A. and El-Shafei, M.A. (2002): Resistance to praziquantel, Effect on drug pressure and stability test. J. Egypt. Soc. Parasitol. 32(2): 589 – 600.
- Katz, N.; Coelho, P.M. and Pellegrino, J. (1970): Evaluation of kato quantitative method through the recovery of *Schistosoma mansoni* eggs added to human faeces. J. Parasitol., 56: 1032 – 1036.
- Leitch, B. and Probert, A.J. (1990): *S. haematobium*: The effect of Astiban on the cell composition and ultrastructure of the vitelline gland and the ultrastructure of the tegument and gastrodermis. J. Helminthol., 64(1): 65 – 69.
- Mansoury, S.T. (1997): Effect of two trematocidal drugs on the morphology and tegumentary ultrastructure of *S. mansoni*. J. Egypt. Soc. Parasitol., 27(1): 233 – 241.
- Mehlhorn, H.; Becker, B.; Andrews, P.; Thomas, H. and Frenkel, J.K. (1981): In vivo and in vitro experiments on the effects of praziquantel on *S. mansoni*. A light and electron microscopic study. Arzneimittelforschung. 31: 344 – 54.
- Modha, J.; Lambertucci, J.R.; Doenhoff, M.J. and McLaren, D.J. (1990): Immune dependance of schistosomicidal chemotherapy, on ultrastructural study of *S. mansoni* infected adult worms exposed to praziquantel and immune serum in vivo. Parasite Immunol. 12: 321 – 34.
- Nessim, N.G.; Hassan, S.I.; William, S. and El-Baz, H. (2000): Effect of broad spectrum anthelmintic drug flubendazole on *S.mansoni* in experimentally infected mice. Arzneim-forsch., Drug Res. 50 (II), (1129 – 1133).
- Pancera, C.F.; Alves, A.L. and Paschoalotti, M.A. (1979): Effect of wide spectrum drugs on *S.mansoni* experimentally infected mice. Rev. Inst. Med. Trop. S. Paulo. 39: 159.
- Podesta, R.B. (1982): Adaptive features of the surface epithelial syncytium favouring survival in an immunologically hostile environment. Proc. 4th. Int. Parasitol., Elsevier Biochemical Amsterdam.

- Shalaby, I.M.; Banaja, A.A. and Ghandour, A.M. (1991):* Scanning electron microscopy of the tegumental surface in vivo treated *S. mansoni* Saudi-Arabian geographical strain with Oxamniquine and Praziquantel. J. Egypt. Soc. Parasitol., 21(3): 797 – 810.
- Shaw, M.K. and Erasmus, P.A. (1988):* *S.mansoni* praziquantel-induced changes to the female reproductive system. Exp. Parasitol., 65: 31 – 42.
- Shaw, J.R. and Brommer, K.W. (1983):* The treatment of experimental schistosomiasis with a combination of oxamniquine and praziquantel. Trans. Roy. Soc.Trop. Med. Hyg. 77, 39 – 40.
- Smithers, S.R. and Terry, R.J. (1965):* The infection of laboratory hosts with cercariae of *S. mansoni* and the recovery of adult worms. J. Parasitol., 55: 695 – 700.
- Sneft, A.W.; Gilber, W.B. and Knopf, P.M. (1978):* Scanning electron microscope observations on the tegument maturation in *S. mansoni* grown in permissive and non-permissive hosts. Am. J. Trop. Med. Hyg., 27(4): 258-66.
- Standen, O.D. (1963):* Experimental infection of mice. The role of snail antigens in the immunity of schistosomiasis of albino mice. Ms thesis, Med. Lab. National Res. Center, Cairo.
- Zaman, V. (1992):* Atlas of Medical Parasitology (Third Edition). Singapore University Press. p. 194.