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Research paper

Comparative parasitological and electron microscopic studies on the effects of Nitazoxanide and Praziquantel in *Schistosoma mansoni*-infected mice

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Abstract

This study was designed to evaluate antischistosomal activity of Nitazoxanide (NTZ) in *Schistosoma mansoni*-infected mice compared to Praziquantel (PZQ). Fifty four infected mice were recruited into 3 groups, each of 18 mice. Group 1 was infected non-treated control. Group 2 was infected and then treated with PZQ 500 mg for two days, and group 3 was infected and treated with NTZ 100 mg/kg for seven days. Efficacy of drugs was assessed by Parasitological, and scanning electron microscopic studies. PZQ reduced (4.9%, 22.5% and 50.7%) of faecal eggs, (22%, 22.6% and 55.1%) of intestinal eggs, (20.4%, 44.3% and 46.7%) of hepatic egg counts and (27%, 45.1% and 64.9%) of total worm load whereas, NTZ reduced (4.9%, 22.5% and 50.7%),of faecal eggs, (22%, 22.6% and 55.1%) of intestinal eggs ,(20.4%, 44.3% and 46.7%) of hepatic egg counts and (27%, 45.1% and 64.9%) of total worm load at 1, 2 and 4 WPT, respectively. The percentages of dead eggs were more than 80% after PZQ treatment and only 30% after NTZ at 4 WPT. PZQ showed extensive tegumental damages in male and female worms more than NTZ at 2 WPT. Our findings concluded that Nitazoxanide showed weaker antischistosomal activity in animal models than praziquantel.

Keywords: *Antischistosomal; Electronmicroscopy; Nitazoxanide; Parasitological; Praziquantel;Schistosoma.*

1. Introduction

Schistosomiasis represents a major health problematic issue in tropical and subtropical areas, especially those with inadequate access to healthy drinking water and sanitation. It is described as a never-ending disease as it still causes nearly 300,000 deaths annually. (Othman AA and Soliman RH 2015, Tchuenté LAT et al. 2013) Infection with different Schistosoma spp. causes high morbidity and occasional mortality in the affected individuals. Progressive damage occurs in several organs, resulting in gastrointestinal bleeding, haematuria, sepsis, severe anemia, and carcinoma to the liver and bladder. (Elbaz T and Esmat G 2013)

Thanks to successful chemotherapy campaigns, many countries have recently achieved marked progress towards the control of schistosomiasis. (Inobaya M T et al.2014) In Egypt, for example, none of the 20 villages had prevalence above 10% in 2010 compared to more than one thousand villages in 1996 with chemotherapy being the mainstay of such reduction. (Barakat RMR 2014) The current chemotherapy of this devastating disease relies upon PZQ making it the cornerstone in morbidity control programs. (Utzinger J et al. 2003) The number of schistosomiasis -infected person treated with PZQ in 2013 was 39 485 376. (WHO 2015) PZQ effectively kills adult schistosomes at the very young stages, yet its efficacy against immature worms is minimal, and thus it cannot abort early infections. (De Oliveira RN et al. 2014) This might explain the lower cure rates in the endemic regions where individuals are likely to be simultaneously infected with the immature and adult stages of the parasite. (N'Goran EK et al. 2003) Although; no clinical relevant resistance ascribed to PZQ to these days was reported, the massive use of a single chemotherapeutic agent makes development of drug resistance a potential threat. (Wang W et al. 2012, Pinto-Almeida A et al. 2016) So the development of new schistosomicidal drugs remains a vital challenge as, therefore, investigators have been searching for alternative medications by screening natural and chemical substances for their potential activity as antischistosomal agents. (El Ridi RAF and Tallima HAM 2013)

Several synthetic or natural drugs combined with PZQ, or PZQ derivatives or natural extracts and/or naturally derived molecules, along with other chemicals, have been proposed as a basis for alternative antischistosomal drugs. (Utzinger J et al. 2003, Thibaut JPB et al. 2009, Xiao SH et al. 2011, Sadhu PS et al. 2012, Aires AL et al. 2014) The identification of the most active ingredients in plant extracts can be very difficult. In addition, most of the proposed antischistosomal agents do not achieve the complete demolition of the musculature, tegument and suckers of the *Schistosma mansoni* worms. (Thibaut JPB et al. 2009, Aires AL et al. 2014) A recent trend in the discovery of a new antischistosomal agent is drug repositioning of the already available drugs for known and approved indication are assayed *in vitro* as well as *in vivo* against different Schistosoma spp. (Abdulla MH et al. 2009, Cowan N and Keiser J 2015, Panic G et al. 2015) to save the time and costs of preclinical toxicological evaluation and phase I clinical studies.

Nitazoxanide (NTZ) is a nitrothiazolide derivative structurally related to the anthelmintic and molluscicidal agent, niclosamide. It was developed in Romark Laboratories,USA. (Abdulla MH et al. 2009) and FDA approval as an antiprotozoal drug was accessed in 22 /11/2002 for the treatment of infectious diarrhea caused by Giardia and Cryptosporidium in children, and in 21/7/ 2004 for the adults. (Fox LM and Saravolatz LD 2005) The recommended dose of NTZ is 500 mg in adults and adolescents, 200 mg in children

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aged 4–11 years and 100 mg in children aged 1–3 years; the dose was given twice daily for three days. (Stockis A et al. 2002) The anthelmintic activity was tested against cestodes (Rossignol JF and Maisonneuve H 1984, Stettler M et al. 2003) as Taenia, Hymenolepis, Echinococcus, for trematodes as in Fasciola (Rossignol JF et al. 1998, Favennec L et al. 2003) and for nematodes (Abaza H et al. 1998, Diaz E et al. 2003) as in Ascaris, Trichuris and Strongyloides with promising results. These advances prompted NTZ to be ranked as a broad-spectrum anthelmintic drug and eventually a broad-spectrum anti-parasitic drug (Fox LM and Saravolatz LD 2005) or even, may be added to "the WHO model list of essential drugs". (Hotez PJ 2014) Data about the antischistosomal action are not clear. One study demonstrated that NTZ administration in a schistosomiasis mansoni murine model decreased hepatic egg count by 34% and significantly improved liver and spleen pathology, although, no effect upon the worm burden could be observed. (Abdulla MH et al. 2009)

2. Materials and methods

2.1. Infected snails, mice and drugs:
Laboratory-bred *Biomophalaria alexandrina* snails infected with *S. mansoni* miracidia [Egyptian CD strain] were purchased from "the Schistosome Biologic Supply Center (SBSC), Theodore Bilharz Research Institute, Cairo; Egypt". Fifty four female Swiss albino mice (CD-1 strain) weighing 20±2 g were brought from the animal house facility of medical research institute, Alexandria; Egypt. NTZ and PZQ were bought from a local market in tablet form and dissolved in Cremophor EL 2%(from Safepharma pharmaceutical company, Alexandria; Egypt).

2.2. Mice infection with *S.mansoni*:
Infected *B. alexandrina* snails (25-30 days after miracidial exposure) were handled in an aquarium in an aerated dark place for 48 hours. The snails were then washed with dechlorination tap water and exposed to white fluorescent electric light for a period of 30-60 min. to stimulate shedding of cercariae. (Liang YS et al. 1987) The number of cercariae was counted by the use of clean dry glass slide and iodine drops for staining and killing of the available cercariae, and the average number per ml was calculated. "Each mouse was infected with 100 cercariae" (Hamza RS et al. 2012) by body immersion technique. (Smithers SR and Terry RJ 1965) Infected animals were then segregated into groups of six in separate stainless steel wire-mesh cages and received a standard well-balanced diet as standard commercial diet pellets and water was provided *ad libitum*. Housing conditions were selected as controlled temperature (25±3 0C) with a relative humidity of 50±15% and lighting conditions (12 h light/12 h dark cycle). The mice were housed in accordance to the WHO and NRC guidelines.(WHO 1998, NRC 2011) Fecal samples of infected animals were collected 49 days after cercarial infection. (Khalil SS 2000) The fecal pellets were suspended in saline and examined microscopically for identification of *S.mansoni* eggs. (Katz N et al. 1972)

2.3. Experimental design and treatment schedule:
S.mansoni-infected mice were acclimatized for a week from the start of the experiment with only healthy animals. These mice were divided into three groups of 18 each. The first group was left untreated and received only the vehicle as a control group. At 50 days post-infection, mice of the second and the third groups were orally treated either with PZQ 500 mg/kg (Ebeid FA et al. 1994) but as a single dose which is the recommended therapeutic dose or with NTZ 100 mg/kg (Abdulla MH et al. 2009) but for seven days, which is lower than the recommended therapeutic dose (205 mg/kg).(FDA 2005) Both drugs were administered after overnight fasting. Eating was allowed one hour after drug administration.

2.4. Efficacy Evaluation:

2.4.1. Parasitological Parameters:

The following parameters were evaluated 1, 2 and 4 WPT:

2.4.1.1. Fecal egg counts: Stool pellets of treated and non-treated mice were collected, weighted, comminuted in saline and examined microscopically. The number of "eggs per gram (EPG)" of stool was recorded. (Katz N et al. 1972)

2.4.1.2. Worm load: Adult worms were collected from mice by perfusion of the Porto-mesenteric and hepatic vessels (Smithers SR and Terry RJ 1965) then identifying their sex.

2.4.1.3. Tissue egg counts: The egg counts in a small quantity of the liver and intestine were determined per gram following its digestion in Potassium hydroxide solution 5% according to the method of (Cheever AW 1968) and its modification by (Chaiworaporn R et al. 2005).

2.4.1.4. Oogram pattern: The percentage of different developmental stages of *S. mansoni* eggs was determined in 1 cm of the intestine (Pellegrino et al., 1962).

2.4.2. Scanning Electron Microscopy (SEM):
Worms recovered from the treated and control mice at two WPT then "fixed in glutaraldehyde" 2.5% buffered with "0.1M phosphate buffer" (pH 7.2) at room temperature then dehydrated through ascending ethanol concentrations (30-100%). This was followed by critical-point drying using carbon dioxide liquid. Specimens were mounted on aluminum stubs then coated with gold. The specimens were examined by SEM using a Jeol-JSM-5300 model. (Bricker CS et al., 1983)

2.5. Ethical considerations:
The study protocol was revised and approved from the "institutional review board (IRB)" of the medical research institute (MRI), University of Alexandria.

2.6. Statistical analysis:
Data were expressed as mean ± standard deviation and analyzed by Minitab version-14 statistical software (Minitab Ltd, State College, Pennsylvania, USA). Student's t-test was used for comparison of means of treated and non-treated groups. A p value > 0.05 was considered statistically non-significant while $p<0.05$ was significant. Fisher's exact test was used to compare the difference in proportions of reduction in male and female worms of each treatment and between PZQ and NTZ.

3. Results

Treatment of *S. mansoni* infection in mice with the reference drug, PZQ as a single dose 500 mg/kg orally resulted in a sharp decline in the faecal eggs after one week of treatment (63%, $p < 0.01$) as compared to an infected non-treated group. Eggs were not found in the feces after the 2nd or the 4th week of treatment (100% reduction, $p < 0.01$). But, NTZ caused non-significant reduction (only about 5%) in faecal egg burden after one week of treatment .The drug elicited significant fecal egg reduction (22.5%, $P>0.05$) at 2 WPT and (50.6 %, $P<0.01$) at 4th week of treatment (Table 1).

As regards to worm load reductions, this study noticed that PZQ caused pronounced curative effects on infected mice, where the mean number of total worm load was significantly reduced (83%,$P<0.05$) at 1 week post-treatment as compared to non-treated group (Table 2). At 2 and 4 WPT, PZQ killed 93.9 % and 97.3% of the total worm load .Whereas, the total worm burden achieved by NTZ at 1 WPT was (26.2%, $P<0.05$). The drug showed significant reduction (45% and 61.6%,$P<0.05$) at 2 and 4 WPT. Both drugs showed non- significant difference in proportions of reductions between male and female worms ($P>0.05$) "at the different time intervals" (Table 2).

In the current work, the infected non-treated mice were loaded with higher eggs in the intestinal tissue more than in the hepatic tissue at different intervals of follow up. PZQ treatment reduce d the intestinal more than the hepatic tissue egg load as there was

(70.3%, 79.3% and 88.1%, *P*<0.01) reduction rates in the intestinal tissue and (63.8%, 69% and 85.7%, *P*<0.01) reduction rates in the hepatic tissue at 1,2 and 4 WPT, respectively. NTZ reduced the intestinal egg count in rates (22%, 22.6% and 55.1%) at 1, 2 and 4 WPT when compared to non-treated animals. Also, the drug was able to reduce the hepatic tissue egg load in rates (20.4%, 44.3% and 46.7%) in high statistical significance (Table 3).

Oogram pattern in the infected non-treated group showed that about 60% of eggs were immature whereas dead eggs constituted only 7-12%, and the mature eggs formed 24-32% of the total eggs at different follow up periods. PZQ induced marked changes in comparison to the non-treated infected group, as it produced a highly significant increase in the percentage of dead eggs to 79.87 % at 1 WPT, 83.2% at 2 WPT and 85.75% at 4 WPT as well as highly significant reduction in immature eggs to 3.67% at 1 WPT, 2.6% at 2 WPT, and 1.5% at 4 WPT, respectively. As regards mature eggs, they were reduced to 13 % at 4 WPT. Mild changes were obtained in the oogram pattern after treatments with NTZ as only 16-30.5% of eggs were dead different intervals of follow up and mature eggs reached to 51% at 4 WPT and not more than 18.5% of eggs were immature at final cut off value (Table 4).

By using scanning electron microscopy of recovered worms at 2 WPT, PZQ showed a pronounced tegumental damage of *S.mansoni* male and female worms in the form of extensive damage with rupture of the tubercles and loss of spines in wide areas. Moreover, a marked ulceration in the tegument was detected in the outer surface of the feminine worms. Some teguments showed severe erosion or even shedding of tegumental membranes exposing the underlying muscle layers. The feminine tegument was more affected than male one. But, NTZ showed a minor damaging effect on the tegument of both male and feminine worms in the form of focal lesions in the inter-tubercular ridges (Fig.1).

Table 1: Independent Samples t-test comparing the mean difference of egg counts in Nitazoxanide and Praziquantel -treated and Non-treated groups

Parameters	WPT	Infected Non-treated	PZQ-treated	NTZ treated
Egg count (EPG)	1	566.00 ± 55.05	204.00±15.7	538.25 ± 39.47
% change			-63%	-4.9%
P value			P<0.01	P>0.05
Egg count (EPG)	2	765.00 ±70.83	0.00±0.00	592.50 ± 90.69
% change			-100%	-22.5%
P value			P<0.01	P<0.05
Egg count (EPG)	4	720.00 ± 62.11	0.00±0.00	355.00 ± 28.07
% change			-100%	-50.6%
P value			P<0.01	P<0.01

Abbreviations: PZQ :Praziquantel ,NTZ: nitazoxanide ,EPG: egg per gram

Table 2: The Worm Distribution in *S.mansoni*-Infected mice 1, 2 and 4 Weeks Post-Treatment with Nitazoxanide Compared with Praziquantel and Non-Treated Mice.

			Worm recovery (mean± SD)	
Group	WPT	Total worms	Total males	Total females
G1: non-treated	1	19.80±2.86	13±2.24	6.8±2.59
	2	28.67±5.51	18.67±3.21	9.67±3.06
	4	28.67±2.65	18.67±2.08	10.00±2.65
G2: PZQ-treated	1	3.33±1.58a (83%)	2.23±0.10A (82.7%)	1.07±0.08A (84%)
	2	1.70±0.10A (93.9%)	1.16±0.5A (93.7%)*	0.50±0.01A (94.8%)*
	4	1.00±0.10A (97.3%)	0.70±0.01A (96.2%)*	0.30±0.58A (97%)*
G3: NTZ-treated	1	14.6±1.81a (-26.2%)	9.4±1.14a (-27.6%) *	5.2±0.83 (-23.5%)*
	2	15.75±0.95a (-45%)	9.75±0.95a (47.7%)*	6±0.81 (-37.9%)*
	4	11.00±1.15a (-61.6%)	6.20±1.41a (67.8%)*	5±0.81 (-50%)*

a:Statistically significant at P<0.05 compared to the control. A: Statistically significant at P<0.05 compared to the control. * Non-significant difference in proportions (P>0.05).Abbreviations, PZQ: Praziquantel,NTZ: nitazoxanide ,% R = reduction

Table 3: The Mean Tissue Egg Counts (Epg) $X10^3$ in the Intestine and Liver of *S. mansoni*-Infected Mice Treated with Nitazoxanide or Praziquantel at1, 2 and 4 Weeks Post-Treatment Compared to non-treated Mice.

Tissue	WPT	Infected Non-treated	PZQ-treated	NTZ-treated
Intestine	1	10.28±0.4	3.05±0.16 a (-70.3%)	8.00±1.1a (-22.1%)
	2	14.6±0.3	3.02±0.89A (-79.3%)	8.4±1.6a (-45.2%)
	4	16.5±0.6	1.96±0.15A (-88.1%)	8.8±0.7a (-46.6 %)
Liver	1	4.5±0.7	1.65±0.13A (-63.8%)	3.6±1.4 (-20%)
	2	6.3±0.6	1.95±0.14A (-69%)	4.8±0.8a (-23.8%)
	4	7.8±1.3	1.11±0.14A (-85.7%)	5.4±0.5a (-30.7%)

a:Statistically significant at P<0.05 compared to the control,*non-significant difference in proportions (P>0.05.
Abbreviations , PZQ: Praziquantel, NTZ: nitazoxanide ,%R = reduction

Table 4: Changes in the Oogram Patterns in the Submucosa of the Small Intestine of *S. mansoni*-Infected Mice treated with Nitazoxanide or Praziquantel at 1, 2 and 4 Weeks Post-Treatment.

Oogram pattern	WPT	Infected Non-treated	PZQ- treated	NTZ-treated
Dead	1	7.80±0.59	79.87±5.20A	16.75±0.89b
	2	11.50±0.38	83.20±5.54A	22.25±0.99b
	4	13.00±0.41	85.75±3.30A	30.50±0.71b
Mature	1	32.60±0.70	16.67±0.84A	27.25±0.50b
	2	27.00±0.16	14.20±0.76A	34.25±1.02b
	4	24.50±0.12	13.00±1.16A	51.00±1.41b
Immature	1	59.60±2.28	3.67±0.03A	56.00±0.35a
	2	61.50±3.00	2.60±0.05A	43.50±2.50b
	4	62.50±0.71	1.50±0.09A	18.50±0.71b

b: Statistically significant at P<0.05. Abbreviations, PZQ: Praziquantel, NTZ: nitazoxanide

4. Discussion

It is the first record of full parasitological parameters besides the electron microscopic ultastructural findings in the assessment of the antischistosomal property of Nitazoxanide in an animal model in comparison with the reference drug, Praziquantel. Firstly, the drug administered in single oral doses of 100 mg/kg for seven successive days at 7 WPI and it resulted in statistically significant reduction in faecal egg counts by a rate of 22%, at the 2nd week after treatment and this value reached only to 50 % at the 4th week. But, PZQ in a single dose of 500 mg/kg orally reduced faecal eggs to 63% at 1 WPT. These results were nearly similar with the results of Khalil SS 2000 who investigated PZQ in a dose of 600 mg/kg for 2 days in murine infection with 120 *S. mansoni* cercariae 8 WPI, the drug elicited 69.2% fecal egg reduction, But, Sweify MM 2009 reported only about 23% of fecal egg reduction after one week of treatment with a single dose of PZQ 600mg/kg at 7 WPI. The reference drug showed complete eradication of feacal eggs at 2 WPT similarily as reported by Khalil SS 2000 or nearly as noticed by Issa RM. 2006 who administered infected mice with a PZQ at dose of 500mg/kg at two consecutive days after 45 days of infection with 100 cercariae), and it could reduce (98.3%) of faecal egg counts in at 2 WPT. PZQ continued in success to destroy all eggs at 4 WPT. These results agreed with the findings of Khalil SS 2000 and Aly AMN 2010. However, Abou-El-Maatti DM et al. 2006 found only 50.6 % reduction in egg count of stool of infected mice treated orally with PZQ 500 mg/kg as a single dose at 4 WPT.

Secondly, NTZ reduced the whole number of recovered schistosomal worms in treated mice not more than 61% at 4 WPT .These results were contrary to the findings of Abdulla MH et al. 2009 who reported no effect of NTZ on the worm burden in infected mice treated orally with the same dose (100 mg/kg) once or twice a day for four times (42-day days post-infection with 140 cercariae by S/C injection). This may be due to a difference in methodology in the number of infested cercariae, method of infestation, course of medication as well as the interval between the drug administration and animal sacrifice. But, treatment with PZQ showed a highly significant reduction in (83%) at 1WPT which is nearly similar to the finding of (Botros SS et al. 2007) that used PZQ 500 mg/kg for two successive days in infected mice (at 6 WPI with 80 cercariae by tail immersion technique) caused 93% reduction in the whole worms at 1 WPT. Khalil SS 2000 found a 69.2% reduction in PZQ-treated mice (600 mg/kg as single oral dose at seven-week post-infection with 100 cercariae by paddling technique). At 2 weeks post-treatment, PZQ killed 93.9 % the total worm load as compared with the infected control group. But, Botros SS et al. 2007 and Emam MH et al.2009 separately reported that PZQ produced 90.8% and 100% reduction in the whole worm load by using the same dose for two days. At 4 WPT, PZQ reduced the entire worm load (96.4%) with high statistical significance. These results were agreed with Helmy MMF et al.2009 and Bakr M et al. 2009 who reported 97.2 % and 91.47% reduction in worms in PZQ-treated mice with the same dose.

Equal sensitivity of female and male worms was noticed under the effect of PZQ treatment as there was significant reduction (84%,94.8% and 97%) of the female worms and (82.7%, 93.7% and 96.2 %) in male worms with non-significant difference at different intervals. "Similar results were reported" *in vivo* by Nessim & demerdash, 2000; Botros et al., 2004. Bakr et al., 2009; Soliman et al., 2012 recorded that female worms being more sensitive to PZQ than male worms .Xiao et al., 1985 *in vitro*, Gonnert & Andrews, 1977 and Seif-El-Din et al., 2013 *in vivo* demonstrated that females worms were "shown to be more susceptible'' to PZQ than males.

The number of schistosome eggs found throughout the tissues of infected animals is affected by the number of eggs laid by the worm (about 300 eggs /day), the number escaped to the stool (about 50%), and the number destroyed in the host tissues. It was noticed that the eggs per worm pair increases in the tissues with time factor in a nearly linear pattern, while the number of faecal eggs decreases to about 50% between 8 and 20 weeks of infection.(Cheever AW et al.1994) The use of drugs in the management of infection seemed to account for the apparent destruction of eggs into the tissues. (Cheever AW and Anderson LA 1971, Cheever AW et al. 1992)

In the current work, the infected non-treated mice were loaded with higher eggs in the enteric tissue more than in the hepatic one as reported previously by (Botros S et al. 2004, Sweify MM 2009, Seif el-Din SH et al.2010) but against that reported by Khalil SS 2000. PZQ reduced (56.7%, 77% and 89.3%) of the enteric egg load was more than in the liver tissue (39.1%, 62.6 % and 80%) at 1,2 and 4 WPT, respectively. These findings were in contrary to El-Shafei MMA et al. 2002 who used the drug with a same dose and recorded that the rate of egg reduction in the hepatic tissues was 98.9% more than that in the intestinal one (87.9%).NTZ resulted in a highly statistical significant reduction (22%-55%) and (20.4%-46.7%) in the intestine and in the liver egg load at different intervals' post treatment.

Changes in "the characteristic feature and the number of eggs (oogram pattern)" provide a simple, sensitive, and reliable factor for the screening of active drugs against *S. mansoni*. It assesses the drug effects on the oviposition, developement and survival of trapped eggs in the intestinal mucosa. (Pellegrino J & Faria J 1965, Araújo N et al. 1991) This was achieved by studying of the alteration in the percentages of the various stages of viable eggs (mature or immature) and the increase in the percentage of dead eggs. In mice infected with *S. mansoni*, egg-laying begins in about day 30 post-infection with the unripe eggs require about six days for the miracidium to develop. A substance is considered with an antischistosomal activity when 50% or more mature or dead eggs and absence of undeveloped eggs of one or more developmental stages occurs. (Pellegrino J & Faria J 1965)

The changes in the oogram after drug administration may be related to three factors, loss of muscle tone due to the hepatic shift of the worm being removed from the egg-laying site, specific degenerative changes on the reproductive organs of worms leading to inhibition or cessation of egg-laying and death of the worms. (Pellegrino J et al. 1962; Farag HF et al. 1978) Viable eggs are important in immunologically mediated pathogenesis of schistosomiasis as miracidia secrete antigens, which induce the host granulomatous reaction. So, killing of eggs may reduce the host reaction. (El-Shafei MMA et al. 2002) Furthermore, an increase of lifeless eggs can be considered as a hallmark sign for the effective antischistosomal treatment. (Botros S et al. 2004)

In this work, PZQ produced a very important effect on the mean % of total undeveloped Ova as it caused nearly complete disappearance of these Ova (3.67, 2.6 and 1.5) at 1,2 and 4 weeks after treatment, respectively with reduction rate of 93.8 %, 95.7% and 97.6% compared to non-treated infected mice. Furthermore, PZQ produced a greatly consequential reduction of full-grown Ova (16.67%,14.2% and 13%) at 1, 2 and 4 weeks after drug treatment, respectively. Also, it produced a highly significant increase in the deceased Ova to 85.75 % at 4 WPT. These are similar to that reported by Khalil SS 2000) who found that after PZQ therapy, the immature Ova were present in a ratio of 18% at 1WPT and complete disappearance occurred in the 2nd and 4th week after treatment with progressive reduction of mature Ova (28%, 12% and 2 %) and marked increase in dead Ova (54%,88% and 98%) at 1, 2 and 4 four after stoppage of treatment. These results indicated that PZQ is lethal to immature and mature eggs as it caused impairment of egg production which may be due to degenerative changes of the reproductive organs of female worms confirmed by many investigators. (Sarvel AK et al. 2006, Conceiço MJ et al. 2008, Holtfreter MC et al. 2011) NTZ resulted in significant reduction in immature eggs, but not more than 18.5% compared to non-treated control. It could rise the percentage of dead eggs to 30.5% and mature eggs to 51% at different intervals of follow up. This denotes that NTZ has mild degenerative effects on the reproductive system.

Scanning electron microscopy has become a useful tool in the study of the ultrastructural changes on the surface of the *Schistosoma* worms in response to chemotherapy by showing the damaging effects on the tegumental structures (tubercles, spines, and inter-tubercular ridges), oral and ventral suckers. (Shaohong L et al., 2006) These ultrastructural damages are directly proportional with the potency of these treatments (Voge M et al. 1980) and may clarify the procedure of killing of these worms. (Hassan M et al. 2003) As it was thought that focal tegumental changes induced by an these drugs might be repaired in a period of 7-14 days after cessation of treatment while in case of severe damage, the host immune response might impact this repair process effectively. (Popiel I et al., 1985) These morphological damages are associated with an increased exposure of the worm antigens (epitopes) at its surface (Harnett W & Kusel JR 1986), leading to the disappearance of the immunological "disguise" and inability to engulf food by oral and ventral suckers. This is believed to cause death of the worms. (Shaw MK and Erasmus DA 1987, Tran MH et al. 2006)

PZQ in this study at 2 WPT showed a noticeable tegumental damage in *S.mansoni* male and female worms in the form of rupture of the tubercles and loss of spines in wide areas in male worms and marked ulceration in the outer surface of the female worms. Some teguments showed severe erosion or even shedding exposing the "underlying muscle layers". These results were in agreement with several studies *in vitro* or *in vivo*. (Bricker CS et al.1983, Harnett W and Kusel JR 1986, Shaw MK and Erasmus DA 1987, Bakr M et al. 2009, Pinto-Almeida A et al. 2016) PZQ appears to damage the tegumental membrane that disrupts the active immune evasion at the level of the tegument and exposes surface antigens that were previously masked. (Fitzsimmons CM et al., 2004) Making it more susceptible to the host humoral immunity pathways leading to decay of worms by host immunoglobulin mediated mechanisms. (Siddiqui AA et al.2003) NTZ showed minor damaging effects on the tegument of both male and female worms with various localized lesions in the inter-tubercular ridges at 2 WPT. Nevertheless, NTZ may act by interfere with the ferredoxin reductase enzyme which is crucial to anaerobic energy metabolism leading to death of the worms. (Hoffman PS et al.2007)

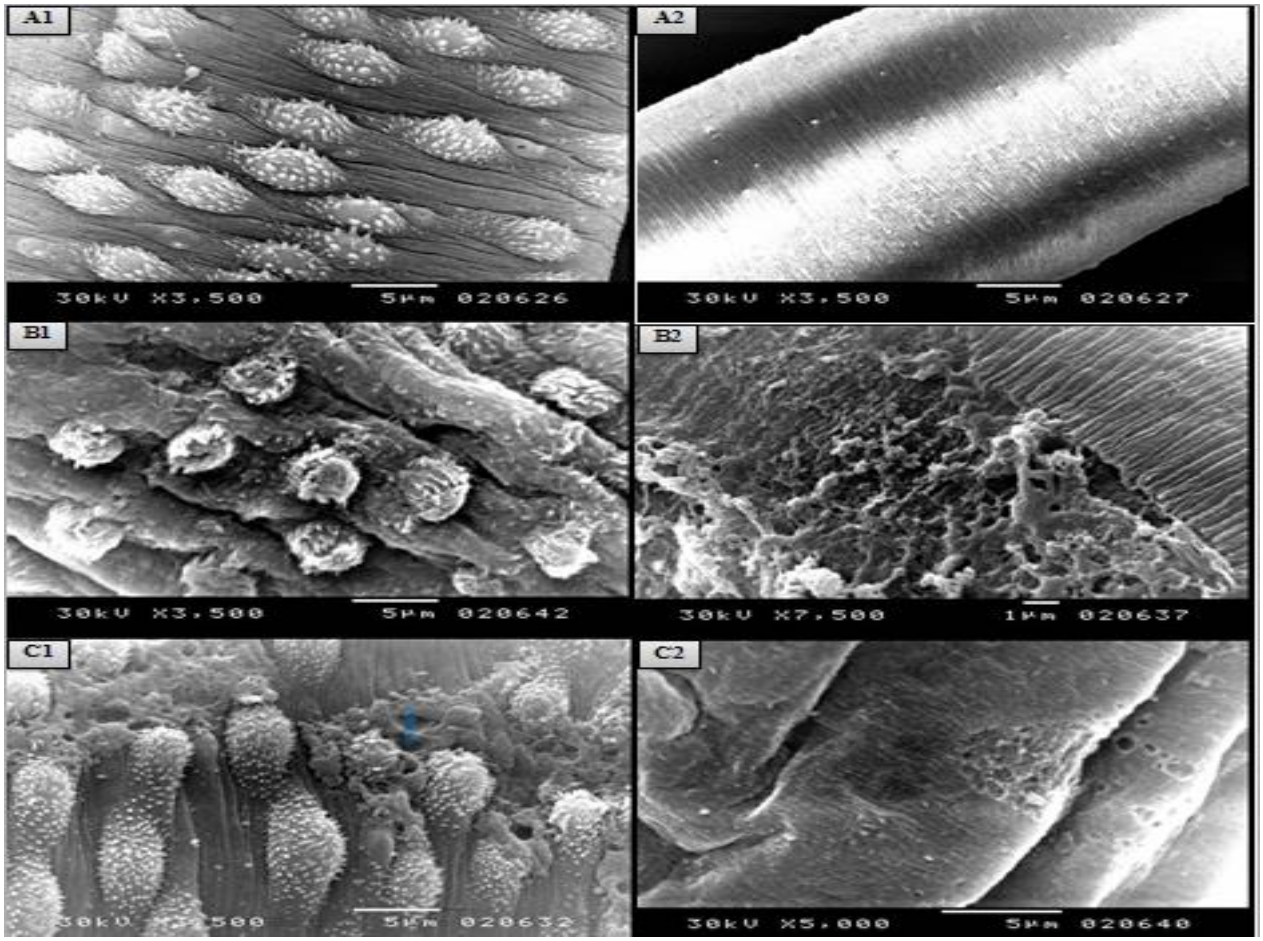


Fig. 1: Electromicrographs of the Dorsal Surface of *S .mansoni* worms under the Effects of Nitazoxanide or Praziquantel Compared to Control at Two Weeks Post-Treatment. (A1: Male Control, A2: Female Control), (B1: Male PZQ-Treated, B2: Female PZQ-Treated), (C1: Male NTZ-Treated, C2: Female NTZ-Treated).

5. Conclusion

Nitazoxanide showed weak activity in animal models of schistosomiasis compared with the reference drug, Praziquantel and much attention should be paid for the use of NTZ in therapeutic doses with or without PZQ in further studies.

Conflict of interest

The authors declare that there is no conflict of interests.

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