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Research Article
Possible Ameliorative Effects of Nitazoxanide Against *Schistosoma mansoni*-induced Biochemical Insults in Mice

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Abstract

Background and Objective: *Schistosoma mansoni* (*S. mansoni*) worms inhabit the portal triad which may affect the blood elements. The present study investigated the ameliorative effects of Nitazoxanide (NTZ) on some biochemical parameters in *S. mansoni*-infected mice. **Materials and Methods:** Swiss albino mice (n = 42) were used in this study and were divided into three groups. G1was normal (n = 6), 36 mice were infected with 100 *S. mansoni* cercariae were classified into two groups, G2 infected control group given only the vehicle (n = 18) and G3 was infected and treated with NTZ at a dose of 100 mg kg^{-1} for 7 days (n = 18). Treatment started 7 weeks' post-infection by oral route. Blood samples were collected at 1, 2 and 4 weeks post treatment to obtain serum for liver functions (ALT, AST and ALP), kidney functions tests (blood urea and serum creatinine) and cholinergic function (serum cholinesterase level). **Results:** NTZ decreased significantly the elevated levels of liver enzymes, urea and creatinine in treated mice at 1, 2 and 4 weeks post treatment (WPT). NTZ caused significant elevation in the level of AChE at 2 and 4 WPT. **Conclusion:** Nitazoxanide was not only drastic but also showed some corrective action in biochemical parameters in *S. mansoni*-infected mice.

Key words: Acetylcholinesterase, biochemical, nitazoxanide, *Schistosoma mansoni*, liver function

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Nitazoxanide (NTZ) is a nitrothiazolide derivative structurally related to the anthelmintic and molluscicidal agent, niclosamide. It was developed in Romark Laboratories and marketed in USA as Alinia[1]. NTZ was approved by the USA-FDA as an anti-protozoal drug for the treatment of infectious diarrhea caused by *Giardia* and *Cryptosporidium* in children in 22/11/2002 and for the adults in 21/7/2004[2]. The recommended dose of NTZ is 500 mg in adults and adolescents, 200 mg in children aged 4-11 years and 100 mg in children aged 1-3 years; the dose was given twice daily for three days[3]. The efficacy of this drug was documented as an anthelmintic against cestodes (*Taenia*, *Hymenolepis*, *Echinococcus*)[4,5], for trematodes (*Fasciola*)[6,7] and for nematodes (*Ascaris*, *Trichuris* and *Strongyloides*)[8,9] with promising results. These advances prompted some researchers to put NTZ as a broad-spectrum anthelmintic drug and eventually a broad-spectrum anti-parasitic drug[2] or even, may be added to "the WHO model list of essential medicines"[10].

The anti schistosomal activity of NTZ showed controversy as four studies demonstrated that administration of the drug in a murine model of *Schistosoma mansoni* showed varied degrees of efficacy against the adult worms and eggs[1,11-13]. As, Abdel-Rahman *et al.*[11] for the first-time, proved that the drug succeeded to reduce about 60% of worm load in *S. mansoni*-infected mice treated with a dose of 65 mg kg^{-1} twice daily for 7 days[11]. Abdulla *et al.*[1] examined the effect of NTZ in dose of 100 mg kg^{-1} once or twice daily for 4 days and reported 34% reduction in the hepatic egg count in *S. mansoni*-infected mice and significantly improved hepatic and spleen pathology without effect on worm burden[1]. El-Enain *et al.*[12] studied the effect of single oral dose of NTZ (140 mg kg^{-1}) in *S. mansoni*-infected mice (by tail immersion with 100 cercariae) previously immunized by soluble egg antigen (SEA) six weeks before infection. The drug showed 42.7% reduction in worm-load after 12 weeks post infection[12]. El-Taweel *et al.*[13] reported 27, 45.1 and 64.9% reduction of total worm load at 1, 2 and 4 WPT, respectively in NTZ-treated *S. mansoni*-infected mice (by tail immersion with 100 cercariae) whereas, treatment commenced 7 weeks post infection with a dose of 100 mg kg^{-1} orally for 7 days[13].

Data regarding safety of NTZ with special attempts to the biochemical effects is scarce. This prompted authors to carry out this study aimed to explore the effects of this drug on some biochemical parameters in mice infected with *S. mansoni*.

MATERIALS AND METHODS

Forty-two female Swiss albino mice of the CD-1 strain weighing 20±2 g was obtained from the animal facility of the TBRI, Giza; Egypt. NTZ (Sigma pharmaceutical company for Al-Andalus Medical Company, Batch No: 21581). Mice were divided into three groups. The first group was non-infected negative control group (n = 6). Mice of the second group were infected with *S. mansoni* cercariae (100 cercariae/mouse) by tail immersion and left as positive control group (n = 18). The third group was infected as the second group (n = 18) and orally treated with NTZ at 50 days' post infection with a dose of 100 mg kg^{-1}/day for 7 days[13]. The drug was administered after overnight fasting and eating was allowed after 1 h. Blood samples were withdrawn from normal, infected non-treated and treated mice for serum biochemical parameters of liver functions (ALT, ALP and AST), kidney functions (urea and creatinine) as well as serum cholinesterase (ChE) level to assess the neurotoxic potential as discussed before[14].

Ethics: Ethical approval for the study protocol was obtained from the Institutional Review Board (IRB) for the use of experimental animals of the Medical Research Institute (MRI), University of Alexandria, Egypt.

Statistical analysis: Results of the studied variables were expressed as means±standard deviations. The significance of the differences between two groups (normal vs control or NTZ-treated vs control) was assessed using student's t-test through Minitab version-14 statistical software (Minitab Ltb, State College, Pennsylvania, USA). Numbers in parentheses [ ] show the percentage of change in infected group in relation to normal mice (normal-infected/normal) and numbers in parentheses ( ) show the percentage of change in treated mice in relation to infected mice (infected-treated/infected) as follow:

a : Statistically significant at $p<0.05$ compared to non-infected
A : Highly significant at $p<0.01$ compared to non-infected
b : Statistically significant at $p<0.05$ compared to non-treated
B : Highly significant at $p<0.01$ compared to non-treated

RESULTS AND DISCUSSION

It is the first time to record the effect of the anti-protozoal drug (nitazoxanide) on some biochemical parameters in *S. mansoni*-infected mice as no one of the four studies[1,11-13] examined the antischistosomal effects of this drug studied the

Table 1: Liver function tests in non-infected and *S. mansoni*-infected mice under NTZ treatment at different times of follow up

		Experimental groups		
Parameters (U L^{-1})	WPT	Normal	Infected	NTZ-treated
ALT	1	41.37±6.21	64.40±3.90A [+55.6%]	49.25±4.66B (-23.5%)
	2	51.20±7.96	92.50±3.54A [+80.6%]	68.50±8.61B (-25.94%)
	4	33.20±5.89	100.33±6.51A [+202.2%]	60.00±6.79B (-40.1%)
AST	1	82.00±6.96	119.60±11.00A [+45.8%]	109.67±4.93 (-8.30%)
	2	96.50±7.92	144.00±7.13A [+49.2%]	130.00±12.7b (-9.7%)
	4	88.57±9.24	159.00±8.17A [+79.5%]	95.00±9.08B (-40.2%)
ALP	1	50.25±17.71	112.20±37.60A [+123.2%]	89.00±6.24 (-20.6%)
	2	76.77±2.01	175.00±9.40A [+127.9%]	118.50±8.33B (-32.2%)
	4	49.75±2.41	155.33±6.01A [+212.2%]	78.67±3.32B (-49.3%)

ALT: Alanine aminotransferase, AST: Aspartate aminotransferase, ALP: Alkaline phosphatase, WPT: Week post treatment, NTZ: Nitazoxanide, +: Increase, -: Decrease

Table 2: Kidney function tests in *S. mansoni*-infected mice under the effect of NTZ treatments at different follow up periods

		Experimental groups		
Parameters (mg dL^{-1})	WPT	Normal	Infected	NTZ-treated
Blood urea	1	24.80±1.74	40.00±2.92A (+61.2%)	43.00±5.47 (+7.5%)
	2	29.20±2.66	55.00±4.14A (+88.3%)	40.50±6.36B (-26.3%)
	4	20.73±3.84	73.67±7.75A (+255.3%)	49.00±9.90B (-33.4%)
Serum creatinine	1	0.90±0.44	1.06±0.30 (+17.7%)	1.30±0.06 (+22.6%)
	2	0.70±0.43	1.25±0.09a (+78.5%)	1.25±0.05 (0%)
	4	0.58±0.17	1.45±0.54A (+150%)	1.10±0.02 (-24.1%)

WPT: Week post treatment, NTZ: Nitazoxanide, +: Increase, -: Decrease

Table 3: Blood acetylcholinesterase level and activity in *S. mansoni*-infected mice under the effect of NTZ treatments at different periods of follow up

		Experimental groups		
Parameter	WPT	Normal	Infected	NTZ-treated
AChE	1	10.15±0.65	9.00±0.8a (-11.3%)	8.60±0.40 (-4.4%)
	2	9.98±0.48	8.00±0.17A (-19.8%)	9.05±0.49B (+13.1%)
	4	9.90±0.40	7.57±0.66A (-23.5%)	8.80±0.28B (+16.2%)

AChE: Acetylcholinesterase, WPT: Week post treatment, NTZ: Nitazoxanide, +: Increase, -: Decrease

selected biochemical parameters in this recent study. In this study, mice in the infected non-treated group showed highly significant elevation of serum ALT (55.6, 80.6 and 202.2%), AST (45.8, 49.2 and 79.5%) and ALP levels (123.2, 127.9 and 212.2%) compared to non-infected normal mice at 8, 9 and 11 WPT. NTZ treatment resulted in highly significant reduction in ALT activity at 1, 2 and 4 weeks after treatment (23.5, 25.9 and 40.1%) as compared to the non-treated mice. The serum AST level decreased insignificantly (8.3 and 9.7%) at 1 or 2 WPT but decreased significantly at 4 WPT (40.2%). The serum ALP level decreased insignificantly at 1 WPT (20.6%) but was significantly reduced at 2 and 4 WPT (32.2 and 49.3%) (Table 1). It was noticed that no alteration in the liver enzymes in NTZ-treated mice more than in the control group. But, the amelioration in liver functions occurred was not more than 50%.

Regards the kidney functions in *S. mansoni*-infected mice, the blood urea and serum creatinine were increased in response to the period of infection and were progressively raised above the normal level (urea level 61.2, 88.3, 255.3% and creatinine level 17.7, 78.5 and 150%) at 8, 9 and 11 weeks post-infection, in Table 2, respectively which indicated the damaging effects of schistosomiasis on the kidney of infected hosts. NTZ-treated mice after one week of treatment with 100 mg kg^{-1} showed insignificant increase in blood urea (7.5%) but highly significant decreased at 2 and 4 WPT (26.3 and 33.4%). Serum creatinine changed insignificantly at 1, 2 and 4 WPT (+22.6, 0 and -24.1%). The amelioration in kidney functions especially in blood urea after NTZ treatment was not more than 33% especially at the 4th week of treatment.

Blood AChE activity in *S. mansoni*-infected mice showed in Table 3 shows progressive decrease with the progress of infection as there was significant decrease in its level about 11.3, 19.8 and 23.5% at 8, 9 and 11 WPT, respectively. NTZ treatment declined the blood AChE activity insignificantly only about 4% at 1 WPT compared to non-treated group. But, the drug raised the enzyme levels (13.1 and 16.2%) at 2 and 4 WPT, respectively. The drug showed no cholinergic damage in treated mice. Studies related to safety of nitazoxanide in

laboratory animals were scarce as only one study carried out by Murphy and Friedmann[15], examined the toxicological profile of this drug in rat and mice. Oral LD_{50} in mice was 1365 mg kg^{-1} but in rats was greater than 10 g kg^{-1}, the only recorded signs were weakness and decreased activity in both rats and mice. Rats were given single oral dose of 50, 150 and 450 mg kg^{-1} for 14 weeks for sub-chronic toxicity studies which indicated that there are neither systemic effects nor blood-related anomalies at the selected doses in rats[15]. Also, it was found that one study declared that NTZ treatment of calves infected with cryptosporidiosis resulted in significant decrease in some biochemical parameters as total protein, albumin and globulin in comparison with the non-treated one[16].

To this date, no research papers could be accessed in the literature in cases of NTZ-treated infections dealing with the selected parameters in this study in spite of the huge use of NTZ in more than 150 million people with intestinal parasitic infections have been treated worldwide which insures its high safety profile[17].

CONCLUSION AND FUTURE RECOMMENDATIONS

This study declared nitazoxanide was safe drug without adverse biochemical insults with the advantage of mild ameliorative in *S. mansoni*-infected mice within the limits of the studied doses. Further studies either experimentally or clinically on healthy or diseased conditions are required to ascertain such biochemical effects.

SIGNIFICANCE STATEMENT

In spite of the tremendous researches and use of nitazoxanide, this study discovered for the first time that the drug was not only unhazardous to the *Schistosoma*-infected mice hosts but also it had some ameliorative effects on some biochemical parameters of liver, kidney and cholinergic functions. This study will help the researchers to uncover the critical area of *S. mansoni*-infected mice that many researchers were not able to explore. Thus, a new theory on nitazoxanide biochemical effects and possibly other benefits, may be arrived at.

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