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A STUDY OF THE POTENTIAL THERAPEUTIC EFFECT OF GINGER (*ZINGIBER OFFICINALE*) LOADED NANOPARTICLES ON MURINE SCHISTOSOMIASIS *MANSONI*

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Abstract

Chemotherapy is the most widely advocated method of antischistosomal control. Repeated chemotherapy has resulted in the emergence of drug-resistant schistosome strains. In the last few years, such resistance has drawn the attention to alternative drugs especially from natural sources (ginger). Nanoparticles have received special attention because they act as potent drug delivery systems. This study evaluated the antischistosomal effect of ginger extract loaded on chitosan nanoparticles on *Schistosoma mansoni* experimentally infected mice. The present study was conducted on sixty eight female BALB/C mice. Mice were exposed to 80±10 cercariae per mouse and divided into 3 main groups;(G1) negative control, (G2) positive control, G3) infected /treated, either by ginger extract (G3a), chitosan nanoparticles (G3b), praziquantel (G3c) or ginger extract loaded on chitosan nanoparticles (G3d). All groups were evaluated by parasitological and biochemical parameters. The results showed that worm burden and the egg density in liver were significantly reduced with P value<0.001 in G3d. The alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities were significantly decreased in group G3d with p value<0.001 which indicated recovery of the liver tissue.

Key words: Ginger, Praziquantel, Chitosan nanoparticles, *Schistosoma mansoni*

Introduction

Schistosomiasis control strategy is based on the treatment of infected patients with selected drug as praziquantel (Mostafa *et al*, 2011). For centuries, ginger has been used in traditional medicine for respiratory disorders, stroke, hypercholesterolaemia, and schistosomiasis (Ali *et al*, 2008; Islam *et al*, 2008). It has antibacterial, antifungal, antioxidant, and anti-inflammatory effects and it increases the phagocytic activity and disease resistance against pathogens (Imtiyaz *et al*, 2013; El-Sayed *et al*, 2015). Unfortunately, the long term worldwide application of praziquantel coupled with the discovery of praziquantel-tolerant schistosome has triggered concern over the development of drug-resistant *Schistosoma* strains (Appiah *et al*, 2000).

Few investigations were done upon the antihelminthic activity of ginger and its constituents and showed that both crude powder and aqueous extract of dried ginger showed anti-helminthic activity in sheep (Iqbal *et al*, 2006). The nanotechnology embraces promise for medication and nutrition, as materials at the nanometer dimension exhibit novel properties different from those of isolated atoms and bulk material (Isaac *et al*, 2013).

Given the previous layout, the current work studied the anti-schistosomal effect of ginger extract loaded on chitosan nanoparticles on *Schistosoma mansoni* experimentally infected mice.

Material and Methods

Experimental design: The study was carried out on sixty eight female BALB/ C mice, 6-8 weeks old (18-20g),divided into 3 main groups;(G1) 8 mice uninfected untreated, (G2) 12 infected untreated mice, (G3) infected/treated groups subdivided into, (G3a) 12 mice treated by ginger extract, (G3b) 12 mice treated by chitosan nanoparticles, (G3c) 12 mice treated by praziquantel and (G3d) 12 mice treated by ginger extract loaded on chitosan nanoparticles.

Mice infection: Mice infected with 80±10 *S. mansoni* cercaria via subcutaneous route (Peters and Warren, 1969). Mice and cercariae were purchased from the Schistosome

Biological Supply Program Unit, Theodor Bilharz Research Institute (TBRI), Imbaba.

Treatment of mice: Aqueous extract of ginger was prepared by dissolving thirty gram of ginger powder in sixty ml of distilled water then it was squeezed out through piece of cloth. The extract was stored at-20°C, and freshly prepared every three days. Aqueous ginger extract was orally administered (500mg/kg/day with an esophageal tube for five weeks starting from the 5th week post-infection three days per week (Mostafa *et al,* 2011). PZQ tablets (Distocide®) was supplied by Egyptian International Pharmaceutical Industries Company, EIPICO. It was given orally to mice in a dose of 500mg /kg body weight for 2 consecutive days from 5th week post-infection (Muchirah *et al,* 2012). Chitosan (with degree of deacetylation 85-93%) and sodium tripolyphosphate (TPP) were purchased from Sigma Aldrich, St. Louis, USA. Chitosan nanoparticles were synthesized via the ionotropic gelation of the chitosan with TPP anions.

Parasitological studies: *S. mansoni* worms from the hepatic portal system and mesenteric veins of sacrificed mice were done by the perfusion technique (Pellegrino *et al,* 1977). Egg counting in hepatic tissues after scarification of mice, a piece of the liver tissues was stored at -20°C for counting of the deposited eggs (Cheever, 1968).

Biochemical measurements: Activities of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in the liver homogenate were assayed according to the method (Bergmeyer *et al,* 1986) using Sigma Diagnostic kits (USA). Serum total protein concentrations (TP), documenting the extent of liver dysfunction, were determined calorimetrically using a Sigma Diagnostic kit (Sigma Chemical Co., St. Louis, MO) following the instructions provided by the manufacturer. Reduced glutathione (GSH) was measured (Schmidt and Schmidt, 1981). Lipid peroxidation was estimated by measuring liver thiobarbituric acid reactive substances (TBARS) as malon-dialdehyde (MDA), which was estimated (Ohkawa *et al,* 1979).

Results

In comparison to G2 (Tab. 1) showed mean total worm burden 25.75±3.15, the mean total worm burden in G3a, G3b, G3c & G3d were (8.57±98; 6.13±2.59; 4.57±1.51; 1.75± 1.04), respectively with significant difference (P- value <0.001).

Table 1: Effect of different drugs on worm burden in groups:

Groups	Mean ± SD	P value
G2	25.75 ± 3.15	
G3a	8.57 ± .98	<0.001
G3b	6.13 ± 2.59	<0.001
G3c	4.57 ± 1.51	<0.001
G3d	1.75 ± 1.04	<0.001

Concerning oogram pattern (Tab. 2), in G2, immature egg was 62.79±6.66 but, mature one was 34.53±6.54 & dead egg was 2.69±1.22. In G3a, immature egg was 71.79±2.05 but, mature one was 26.71±2.31& dead egg was 1.50±1.14. In G3b, immature egg was 69.72±5.16 but mature one was 28.42±4.71 & dead egg was 1.87±1.23. In G3c, immature egg was 65.56±8.52, but, mature egg was 23.87±6.36 and dead egg was 10.57±4.87. In G3d, immature egg was 56.83±6.81 but mature egg was 31.44± 4.67 and dead egg was 12.99±1.63, significant as compared to positive control (P <0.001).

Table 2: Oogram pattern in different study groups.

	Immature eggs %		Mature eggs%		Dead eggs%	
Group	Mean ± SD	P value	Mean ± SD	P value	Mean ± SD	P value
G2	62.79 ±6.66		34.53± 6.54		2.69 ± 1.22	
G3a	71.79± 2.05	<0.006	26.71± 2.31	<0.012	1.50 ± 1.14	<0.074
G3b	69.72± 5.16	<0.035	28.42± 4.71	<0.05	1.87 ± 1.23	<0.20
G3c	65.56± 8.52	<0.49	23.87± 6.36	<0.007	10.57 ± 4.87	<0.005
G3d	56.83± 6.81	<0.098	31.44± 4.67	<0.29	12.99 ± 1.63	<0.001

Egg count per gram of liver or intestinal tissue compared to positive control (G2) where t mean hepatic and intestinal egg load/gm tissue were (5876.13± 1111.55 & 17584.38±1176.73) respectively, with a decrease in mean hepatic & intestinal egg load in G3a (3885.43±802.24 & 9523.71± 701.97), G3b (5100.13±646.11 & 11957.00± 754.12), G3c (2627.71± 497.19 & 3944.86± 492.28) and in G3d (2941.13±472.31& 11957.00±754.12) respectively (Tab. 3).

Table 3: Egg count per gram of liver & intestinal tissue

Group	Intestine		Liver	
	Mean ± SD	P value	Mean ± SD	P value
G2	17584.38 ± 1176.73		5876.13 ± 1111.55	
G3a	9523.71 ± 701.97	<0.001	3885.43 ± 802.24	<0.002
G3b	11957.00 ± 754.12	<0.001	5100.13 ± 646.11	<0.11
G3c	3944.86 ± 492.28	<0.001	2627.71 ± 497.19	<0.001
G3d	4555.63 ± 574.85	<0.001	2941.13 ± 472.31	<0.001

In G3a: compared to positive control there was a significant change in ALT, AST, TP, GSH & MDA. In G3b: compared to positive control there was a significant change in ALT, AST, TP, GSH & MDA. In G3c: compared to positive control there was a significant change in ALT, AST, GSH & MDA but, TP changes were insignificant. In G3d: compared to positive control there was a highly significant change in ALT, AST, TP, GSH & MDA (Tab. 4).

Table 4: ALT, AST, TP, GSH & MDA measurements in different groups.

Group	AST		ALT		TP		GSH		MDA	
G1	28.29 ± 1.50		23.43 ± 2.57		7.01 ± .07		3.87 ± .11		29.99 ± .27	
G2	73.88 ± 4.49		66.63 ± 3.70		7.69 ± .10		1.83 ± .10		40.29 ± .38	
G3a	64.57 ± 3	<0.001	61.29 ± 1.	<0.05	7.64 ± .	0.4	3.11 ± .	<0.001	37.14 ± .1	<0.001
G3b	52.25 ± 2	<0.001	44.75 ± 1	<0.001	7.18 ± .	<0.001	2.74 ± .	<0.001	39.79 ± .	<0.05
G3c	57.86 ± 1	<0.001	42.71 ± .	<0.001	7.14 ± .	<0.001	2.99 ± .		38.09 ± .	<0.001
G3d	45.13 ± 3	<0.001	46.25 ± 2	<0.001	6.84 ± .	<0.001	2.05 ± .	<0.001	33.44 ± .	<0.001

P value <0.05, <0.01, &<0.001=significant, highly significant and very highly significant respectively.

Statistical analysis: Data were expressed as M±SD. Differences between groups were determined using an ANOVA followed by the student-Newman-Keuls t-test. The level of significance was accepted with p value < 0.05.

Discussion

In the present study, the usage of the crude aqueous extract of ginger loaded on chitosan nanoparticles at a dose of 500 mg/kg body weight showed antischistosomal activity as indicated by reduction in worm recovery and egg density in the hepatic tissue of treated mice. Ginger was reported by various authors to have some degrees of anti-schistosomal effects through increasing the rates of schistosome worm reduction or reducing *S. mansoni* egg output and liver granuloma size (Al-Sharkawi *et al*, 2007; Mostafa *et al*, 2011). This agreed with anti-helmintic Lin *et al.* (2010a, b) of ginger against some nematode larvae whom used 6-gingerol, 10- shogaol, 10-gingerol, 6-shogaol and hexahydrocurcumin, a constituent isolated from ginger as larvicidal agents against *Angiostrongylus cantonensis* and *Anisakis simplex* larvae, respectively. They found that the constituents killed *A. cantonensis* and *A. simplex* larvae or reduced spontaneous movements in a time- and do-se-dependent way. Reduction in worm recovery and egg density in treated mice was considered as a potent evidence of effectiveness of anti-schistosomal drugs (Utzinger *et al*, 2002; Suleiman *et al*, 2004; Mati *et al*, 2010).

The decrease in levels of cytoplasmic enzymes AST, ALT & TP contents in the liver of G3d, might reflect necrosis in hepatocytes with increased leakage of these enzymes to blood and replacement of normal liver tissue by granuloma lesions around entrapped schistosome eggs. Elevations of AST and ALT in sera of *S. mansoni* infected mice and patients with hepatosplenomegaly or decompensatation of liver cirrhosis (Fahim *et al,* 2000; Mahmoud *et al,* 2002). Awadalla *et al.* (1975), Hanna *et al.* (2003); Ahmed and Mostafa (2003); and Ahmed and Allam (2009) found a decrease of AST & ALT contents in liver homogenate of *S. mansoni* infected mice. El-Badrawy *et al.* (1991) and Hanna and Fayez (1996) found increases in serum transaminases in *S. mansoni* infected mice that reflect increased leakage of cytoplasmic enzymes from liver cells to blood stream.

In the present study, treatment of *S. mansoni* infected mice with aqueous ginger extract loaded on chitosan nanoparticles produced a potential decrease of elevated liver peroxidation and increased liver function that reflect the antioxidant defense system represented by GSH. This agreed with Ahmed ***et al.* (2000a, b)** reported that feeding of ginger in diet (1% w/w) lowered lipid peroxidation by maintaining the activities of antioxidant enzymes-superoxide dismutase, catalase and glutathione peroxidase in experimentally induced oxidative stress in rats. Besides, Ahmed *et al.* (2008) reported that feeding of ginger attenuated lindane-induced lipid peroxidation in rats accompanied by modulation of oxygen free radical scavenging enzymes as well as reduced glutathione (GSH) and the GSH dependent enzymes glutathione peroxidase, glutathione reduct ase and glutathione-S-transferase.

Sakr (2007) showed that ginger exhibited potent free radicals scavenging activity as it reduced serum level of malndialdehyde and increased the catalytic activity of serum superoxide dismutase.

Conclusion

The present study showed that ginger loaded on chitosan nanoparticles have anti-schistosomal activities and decrease the produced oxidative damage of liver cells. Consequently, helps in liver tissue recovery and provides a basis for subsequent experimental and clinical trials.

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Explanation of figures

Fig. 1: Effect of different drugs treatment on worm burden in different study groups.

Fig. 2: Oogram pattern in different study groups.

Fig. 3: Egg count per gram of liver & intestinal tissue.