Delta J. Sci. 2007, 31: 1 - 10 ZOOLOGY

THE EFFECT OF GINGER ON SCHISTOSOMA MANSONI INFECTED MICE

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(Received: 11June 2005)

Abstract: The present study was performed to evaluate the antischistosomal activity of the medicinal plant ginger *Zingiber officinale*. Mice in groups of five animals were individually infected with 100 Schistosoma mansoni cercariae. Four weeks post infection, mice were orally treated with 1200 mg / kg of ginger for ten consecutive days. After the last dose, all animals were sacrificed to evaluate the efficacy of ginger in treatment of the infection. The results obtained showed moderate reduction of 16.5 % in the worm burden compared with control infected animals. The liver egg count showed a marked reduction of 53.8 %. Ginger treatment showed a significant reduction in the size of liver granuloma where a percentage reduction of 66.35 was observed. Ginger treatment was slightly reflected on the liver function at such rate of infection, where an improvement in serum arginase activity was recorded While no appreciable improvement in hepatic ALT and AST activities, albumin and creatinine contents. In conclusion, *Z. officinale* displayed some degree of anti-schistosomal activity through reducing of the *S. mansoni* eggs output and the liver granuloma size

Key words: *Schistosoma mansoni, ginger and granuloma.*

INTRODUCTION

Bilharziasis is considered as one of the most important diseases in the world, caused by parasitic worms called schistosomes. More than 300 million people are infected and over one billion people live at risk. The disease is widely prevalent in the most parts of Africa, South America and Asia. Indeed, the highest mortality in human Schistosoma mansoni occurs in the minority of people who develop hepatosplenic schistosomiasis characterized by periportal fibrosis, portosystemic shunts and hematemesis (Cheever and Andrade, 1967 and Hoffmann *et al.*, 2002).

Praziquantel (PZQ) (Gonnert and Andrews, 1977) is currently the drug of choice in the treatment of both schistosomiasis and several clinically important helminth diseases in human (Archer, 1985). Praziquantel is the drug of choice in treatment of schistosomiasis. The potential for the development of resistance to PZQ was highlighted in 1995 by its apparently low efficacy, when used to treat a newly established focus of *S. mansoni* in Senegal (Stelma *et al.*, 1995). In Egypt, some patients received three doses of PZQ failed to be completely cured (Ismail *et al.*, 1996).

Many plant species have been used throughout the world in traditional medicine for the treatment of both veterinary and human helminthes (Hammond *et al.*, 1997), but few plants have been screened for activity against adult *Schistosoma sp.* Ginger is a rhizome of *Zingiber officinale* (Family Zingiberaceae), cultivated extensively in almost all tropical and subtropical countries (Blumenthal *et al.*, 2000). Today, ginger is official in the national pharmacopeias of Austria, China, Egypt, Germany, Great Britain, Japan and Switzerland (BP, 1988; Bradley, 1992; DAB, 1997; JP IXX, 1993; Newall *et al.*, 1996; ÖAB, 1981; Ph. Helv. VII, 1987 and Tu, 1992). Ginger rhizome contains oleoresin (4.0 – 7.5 %); volatile oil (1.0 – 3.3 %); carbohydrates, mainly starch (40 – 60 %); proteins (9 – 10%); lipids (6 - 10 %); vitamins niacin and A; minerals; and amino acids (Bradley, 1992; Bruneton, 1995; Budavari, 1996; ESCOP, 1997; Leung and Foster, 1996; Newall *et al.*, 1996 and Witchl and Bisset, 1994).

The British Herbal Compendium indicates ginger for action dyspepsia, colic, prophylaxis of travel sickness, vomiting of pregnancy and anti – inflammatory agent (Bradley, 1992). Extracts of rhizomes of ginger (Z. officinale) showed activity against S. mansoni miracidia and cercariae (Adewunmi *et al.*, 1990). Many authors reported that the pungent phenotlic constituents of Z. officinale rhizome prevent the hatching of S. haematobium eggs (Kucera, 1975 and Kucera and Kucerova, 1975). Kucera *et al.* (1975) found that aqueous extract and powdered rhizomes of ginger have shown to stop terminal haematuria in school children suffering from urinary schistosomiasis as well as to reduce the egg count in the urine.

Sanderson et al. (2002) found that all male and 99.6 % of female S. mansoni were killed after an in vitro exposure to 200 mg l^{-1} for 24 hours of the ginger extract. No significant differences wereobserved in the worm burden of host mice following five consecutive daily treatments of 150 mg/kg of the ginger extracted in 5 % ethanol administered either orally or subcutaneously compared to infected control mice (Sanderson *et al.*, 2002). Ginger extract at concentration of 150 mg/kg has no demonstrable antischistosomal activity in infected mice, while in vitro studies showed that the ginger extract at concentration of 200 mg l^{-1} caused a significant effect against adult S. mansoni (Sanderson *et al.*, 2002). Therefore, the current study was conducted to investigate the antischistosomal activity of the ginger extract at a high concentration of 1200 mg / kg, which is much higher than used in the pervious studies.

MATERIALS AND METHODS

Animals, parasite and Drug:

Ginger is supplied from the Arab Company for Pharmaceuticals and Medicinal Plants (MEPACO), Enshas El - Raml, Sharkeiya Governorate, Egypt. Ginger was suspended in distilled water and was given to animal orally using stomach gavage. Female Swiss albino mice weighing 22 ± 2 g were obtained from the Experimental Animal Research Unit of the Schistosome Biological Supply Program at Theodor Bilharz Research Institute, Al-Giza, Egypt.

Egyptian strain of Schistosoma mansoni cercariae of Egyptian strain maintained in *Biomphalaria alexandrina* were used in this study.

Preliminary investigation of the toxicity of ginger: To determine the acute toxicity of ginger eight groups each of five animals were given ginger at increasing oral doses ranged from 400 mg/kg to 3200 mg/kg with 400 mg/kg increments. The ninth group was injected orally distilled water and kept as a control group. Animal groups were closely observed after dosing for more than 24hours post - administration. The death rate was used to judge on toxicity.

Determination of the antischistosomal activity of the ginger: In this experiment a group of mice were infected with S. mansoni at a rate of infection of 100 cercariae/mouse using body immersion technique according to the method described by Christensen et al. (1984). After 30 days of infection, the animals were divided into two subgroups of 10 mice each. One group served as control, while the other group was dosed by 1200 mg / kg of ginger extract for ten consecutive days. All animals were sacrificed after the last dose of treatement to evaluate the efficacy of ginger in management of S. mansoni infection.

Parasitological studies:

For evaluation of the antischistosomal activity of giniger, the following criteria were considered:

A. Worm recovery: The worm burden and sex were determined after perfusion of the hepatoportomesentric vessels according the technique of Christensen et al. (1984) .

B. Egg count: The number of eggs per gram of liver tissue was determined by weighing a piece of liver (0.1 g) and divided it into four fragments, each fragment crashed between a slide and cover slip. The fragments were examined by light microscope to determine live and dead ova according to method described by Pellegrino et al. (1962).

Hepatic Inflammation Measurements: A portion of left lobe of liver of each animal was cut off, fixed in 10 % buffered formalin for 24 h and dehydrated in ethyl alcohol 70 %. Sections of 4 - 6 µm thickness were prepared and stained with hematoxylin and Eosin (Harris, 1900). The diameter of granuloma surrounding eggs were measured using an ocular micrometer,

and the volume of each granuloma was calculated assuming a spherical shape (volume = π (diameter)³ / 6 and the mean volume for each lesion was calculated from these according to method described by Cheever *et al.* (1987).

Biochemical Assays: The enzymatic activities of ALT, AST, ALP and arginase in serum and liver homogenate were measured by commercial kits according to Reitman and Frankel, 1957; King and Armstrong, 1934; King *et al.*, 1937 and Brown and Cohen 1959, respectively. Albumin, creatinine and urea contents were measured in serum according to Doumas, 1971; Henry, 1974 and Patton and Crouch, 1997., respectively.

Statistical analysis: Data are presented as mean ± SD and/or SEM. Student's t – test was used to calculate the significance of differences observed between mean values of experimental and control groups in each experiment at a level of significance of $P < 0.05$.

RESULTS

Acute toxicity of the drug: The study of acute toxicity of ginger showed neither death nor other behavioral or toxicological changes in all mice groups at a dose up to 3200 mg / kg of ginger suspended in distilled water.

Warm load: Table (1) shows a moderate, but statistically insignificant reduction in the worm burden and sex for mice infected with 100 Egyptian strain of S. mansoni cercariae, following ten consecutive daily oral treatments of 1200 mg / kg of ginger compared to infected control mice. A slight (16.5 %) but insignificant worm reduction occurred in ginger treated group.

Ova count: Table (2) shows that ginger extracted caused a highly significant reduction on liver egg load, but the reduction (53.8 %) was observed only on live ova. Whereas, the dead ova count were quite same as the infected untreated control.

Granuloma Measurements: Table (3) shows a statistically significant difference between ginger treated group and infected untreated control with respect to the size of liver granuloma. It is clear that ginger caused a high reduction percentage of granuloma size 66.35 %, compared to control group.

Biological studies: Table (4) displays the effect of 1200 mg / kg of ginger extract for ten consecutive days on liver and kidney function in S. mansoni infected mice. Ginger caused a highly significant decrease in the liver homogenate of AST activity, also a significant increase was observed on serum ALP activity. Although, no significant changes occurred on ALT and arginase activities and albumin, creatinine and urea contents.

Table 1: Effect of ginger on the worm burden and sex in mice infected with *S. mansoni*.

Animal groups	Dose	Number of worms / mouse (Mean ± SD)		
		Total males	Total females	Total worms
Untreated control	0	34.4 ± 7.1	17.6 ± 3.6	52 ± 10.1
Positive Control		33.3 ± 6.8	16.8 ± 4.4	50 ± 12.0
Treated ginger	1200 x 10	28.0 ± 2.3 (18.6)	15.4 ± 4.5 (12.5)	43.4 ± 5.1 (16.5)

The numbers between parentheses are the percentage change (%) recorded comparing to control. * Dose = mg ginger/kg/day X number of doses.

Table 2: Effect of ginger on liver tissue egg load of mice infected with S. mansoni.

Animal groups	Dose	Mean ± SD eggs / 0.1 g liver tissue		
		Live ova	Dead ova	Total ova
Untreated control	-	356 ± 53	290 ± 47	647 ± 87
Treated ginger	1200 x 10	165* ± 47 (54)	293 ± 633 (-0 96)	458* ± 85 (29.2)

*Significant at P < 0.05. The numbers between parentheses are the percentage change (%) recorded comparing to control. **Dose = mg ginger/kg/day X number of doses.

Table 3: Changes in granuloma size in liver of S. mansoni- infected mice after treatment with ginger.

Animal groups	Dose**	Granuloma volume (μm³)		
		No. of granuloma	Average ± SEM	% change
Untreated control	0	31	129.9 ± 19.83	--
Treated ginger	1200 x 10	71	43.7* ± 4.84	66.35

* significant at P < 0.05. ** Dose = mg ginger/kg/day X number of doses. The percentage change (%) recorded comparing to control.

Table 4: The effect of ginger on liver ALT and AST activities, and serum ALP and arginase activities, and albumin, creatinine and urea contents in S. mansoni infected mice compared to normal control mice.

	Normal	Infected untreated		Ginger 1200 mg/kg	
	Average ± SD	Average ± SD	% diff	Average ± SD	% diff
ALT	55.23 ± 12.88	38.6* ± 6	30.1	48.16 ± 11.08	12.8
AST	46.79 ± 7.82	37.48* ± 6.01	19.9	27.06* ± 1,53	42.17
ALP	302.3 ± 56.92	340.4 ± 6.87	-12.6	375.1* ± 28.59	-24.08
Arginase	94.21 ± 23.29	117.9 ± 33.33	-25.15	97.62 ± 25.42	-3.62
Albumin	3.59 ± 0.25	3.86 ± 0.25	-7.52	3.75 ± 0.3	-4.46
Creatinine	1.89 ± 0.42	1.39 ± 0.4	-26.46	1.43 ± 0.44	24.34
Urea	6.94 ± 0.61	3.53* ± 0.29	49.13	6.15 ± 0.59	11.38

* significant at P < 0.05

The percentage change (%) recorded are compared to normal uninfected control. ALT and AST activities are expressed as (μ mol pyruvate min^{-1} g^{-1} wet liver), ALP activity is expressed as (U /l), and arginase activity is expressed as (μ mol urea min^{-1} g^{-1} wet liver). Albumin unit is expressed as (g / 100 ml), urea and creatinine contents are expressed as (mmol /l).

DISCUSSION

Ginger extract showed no appreciable anti-schistosomal activity in infected mice after administration at 1200 mg / kg for ten consecutive days, but in vitro study, 200 mg l^{-1} of ginger produced a significant effect on worm survival (Sanderson *et al.*, 2003). So it seems that the individual compound(s) responsible for the activity observed in vitro does not reached to a level that could achieve a sufficient curative serum concentration of active compound(s) in the mesenteric and portal vessels. However, for some drugs with proven antischistosomal activity, their effect depends on dose regimen and the period of treatment. With oltipraz for example, a slow acting drug, approximately two months are required before its full schistosomicidal effects becomes evident (Bueding *et al.*, 1982). So, probably, a better curative effect of ginger could be improved by changing the dose regimen and / or period of treatment.

However, the results obtained showed that ginger administration caused a highly significant reduction on live egg load in liver tissue. The effect of ginger may not on ovum itself, because no drugs acts on the eggs themselves, In fact effective antischistosomal drugs usually display no effect on the ova themselves. And ova deposition by worms continue their development in the tissue up to maturation. The mature ova remain alive in the tissues for a period of 12 days till their death and elimination in the stools (Standen, 1953; Bang and Hairston, 1946 and Vogel and Minning, 1947). Accordingly, the significant reduction of live ova might be due the effect of ginger administration on worm fecundity. Some drugs seem to act initially on the reproductive organs of the worms (Bang and Hairston, 1946; Vogel and Minning, 1947 and Kikuth and Gönnert, 1948). However, the level of treatment may have a partial activity during treatment. The drug may cause cessation of egg laying in most of the females or may affect in some way the function of oviposition. The cumulative egg output of individual worm pairs was highly variable, wide variations in vitro (Schirazian and Schiller, 1983 and El-Ridi *et al.* 1997) and in vivo (Cheever *et al.* 1994). Despite this variability, the mean number of eggs present in the tissues could be used as an indicator of schistosome fecundity and that a direct comparison of this variable could be made between treated and untreated host mice, irrespective of any difference in their infection intensities (Sanderson *et al.*, 2003).

The results obtained in the present study is in agreement with those of Sanderson *et al.* (2003) who reported in vitro bioassay a significant reduction in the mean egg output of surviving females following exposure to sublethal concentrations of ginger extract. It is not known if these anti-fecundity effects were the result of one or more of the compounds present.

The present study showed also that ginger administration showed marked aniinflammatory activity where it significantly reduced the volume of

granuloma size by 66.35 %. That is not oddity for ginger effectivity, because the remedy *Zingiber officinale* (ginger root) has been used for thousands of years in the Far East to treat inflammatory diseases. Shen et al. (2003) suggested that the inhibitory effects of ginger root extract on nitric oxide and prostaglandin E (2) production by sow osteoarthrotic cartilage explants is an important role for ginger root extract as an antiarthritic agent in osteoarthrosis in the sow. Tumeric is a spice that comes from the root of Curcuma longa, a member of the ginger family, Zingaberaceae. Curcuminoids are components of tumeric, which include mainly curcumin (diferuloyl methane), demethoxy curcumin and bisdemethoxy curcumin. Also Chainani (2003) demonstrated that curcumin is safe and has anti-inflammatory activity by inhibition of a number of different molecules that play a role in inflammation.

S. mansoni infection results in a hepato cellular injury, which in turn, leads to the release of the enzymes from the injured hepatic cells into blood circulation (Hanna *et al.*, 2003). In the present study, the significant lower liver homogenate AST and ALT level in the infected groups may due to the existence of the inflammatory hepatic granuloma reported to be present as a result of egg deposition and the presence of worms as well as its toxins. In this respect, significant diminutions of ALT activities in liver tissues of infection with S. mansoni are reported by El-Elaimy *et al.* (1988) and El-Assar *et al.* (1989). Other investigators found increases in serum transaminases in S. mansoni infected animals (El-Badrowy *et al.*, 1991 and Hanna and Fayez, 1996). The results obtained in this study showed that the anitinflammatory activity of ginger was slightly reflected on improvement of the status of the bilharzial liver. S. mansoni infection caused marked decrease in the hepatic level of AST and ALT activities and significant increase of the serum ALP activity. Arginase was markedly elevated by infection accompanied by reduction of urea level. Treatment of infected mice treated with 1200 mg/ kg of ginger for ten days did not ameliorate the hepatic tissue activity of ALT, AST and ALP as well as albumin and creatinine contents. However, significant amelioration was observed in serum arginase activity and urea level. This may mean that ginger administration showed some degree of improvement in the liver status.

In conclusion, *Z. officinale* has a moderate anti-schistosomal activity in mice. It significantly reduced the production of eggs outup in S. mansoni infected mice a observation that is supported both in vitro (Sanderson et al. 2003) and in vivo (Adewunmi and Furu, 1989). In addition this study showed that Z. officinale clearly reduce the liver granuloma size and this modulation of the liver granulomas was relected on some degree of improvement in the status of the liver.

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تأثير نبات الزنجبيل على الفئران المصابة بطفيل شيستوسوما مانسونى

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*** قسم علم الحيوان – كلية العلوم- جامعة حلوان.**

تهدف الدراسة إلى تقدير الكفاءة الطبية لنبات الزنجبيل كمضاد للبلهارسيا المعوية وذلك بعد عدوى الفئران بمائة سركاريا لكل فأر. وبعد أربع أسابيع من العدوى. وقد قدم العلاج عن طريق اعطائه بالفم للفئران بجرعة ١٢٠٠ مج/كجم من نبات الزنجبيل لمدة١٠ أيام متتابعة. وبعد الجرعة الأخيرة، قدرت كفاءة الزنجبيل وذلك من خلال النتائج التالية.

- انخفاض متوسط فى عدد الديدان المستردة بنسبة ١٦,٥%.
- انخفاض فى عدد البيض فى الكبد بنسبة ٥٣,٨% وذلك بالمقارنة بالمجموعة الضابطة.
- كما أظهرت النتائج أيضاً انخفاض معنوى فى حجم الالتهاب الحبيبى فى الكبد، وكان بنسبة ٦٦,٣٥%. كما لوحظ تغير بسيط فى وظائف الكبد مثل التحسن فى أنزيم الارجينيز ولكن لم يحدث أى تغير فى الأنزيمات الأخرى كأنزيم AST, ALT وأيضاً لم يحدث تحسن فى محتوى الزلال والكرياتينين. والنتائج تقترح أن نبات الزنجبيل له نشاط ضد طفيل الشيستوسوما مانسونى المسبب للبلهارسيا المعوية وهذا يظهر من خلال انخفاض عدد البيض وانخفاض الالتهاب الحبيبى فى الكبد.