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Effects of garlic on *Schistosoma mansoni* harbored in albino mice: Molecular characterization of the host and parasite

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ABSTRACT

Garlic has been used for its health benefits for thousands of years. Modern research confirmed many of the healing properties of garlic, including its antiparasitic activity. This study was designed to evaluate the antischistosomal action of garlic through detecting the changes in DNA profile of *Schistosoma mansoni* worms and the infected mouse. Forty mice were subcutaneously infected with ~200 *Schistosoma mansoni* cercariae/mouse. Infected mice were divided into four equal groups: non-treated, prophylactic, therapeutic, and continuously-treated. Non-infected control and garlic-treated groups were assigned for the sake of comparison. Garlic extract (50 mg/kg bw/mouse) was given orally, day after day, at a fixed daytime. Seven weeks post-infection, adult schistosomes were recovered by perfusion and the livers of the mice were excised out and were processed for DNA extraction and Random Amplification of Polymorphic DNA-Polymerase Chain Reaction (RAPD-PCR). The results showed that garlic exerted no major changes in the genome of schistosomes. Nevertheless, that schistosomal infection induced genetic alterations in the DNA of mice, and garlic was able to ameliorate such alterations to a great extent.

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1. Introduction

Schistosomiasis is a tropical disease in man caused by trematode worms of the genus *Schistosoma* and currently affecting over 200 million people worldwide (Simeonov et al., 2008). Due to the lack of a vaccine, patient therapy is heavily reliant on chemotherapy with praziquantel as the World Health Organization-recommended drug, but concerns over drug resistance and possible reoccurrence of infection encouraged the search for new drug leads, possibly from natural resources (Abdualla et al., 2007; Abebe, 2008).

Garlic has been used as a folk remedy for a variety of ailments since ancient times. In recent times, garlic has been shown to have plentiful medicinal effects such as antimicrobial, antithrombotic, hypolipidemic, hypoglycemic and antitumor activities (Thomson and Ali, 2003). Lately, the anthelmintic effect of garlic has been a matter of interest of researchers (Abdel-Rahman et al., 1998; Ayaz et al., 2008; Streliaeva et al., 2000; Sutton and Haik, 1999). Recently, garlic and its derivatives have been proved to have a prominent antibilharzial effect by Metwally (2006), Riad et al. (2008, 2009), El-Shenawy et al. (2008), and Mantawy et al. (2011).

In our previous study on the antischistosomal effect of garlic, garlic crude juice evoked a highly significant reduction in the mean worm count as compared to the infected non-treated mice. This reduction was 67.2%, 56.3%, and 77.5% in the prophylactic, therapeutic, and the group treated before and after infection, respectively. Moreover, the mean egg load in hepatic and ileal tissues of all garlic-treated groups dropped extremely beyond the control levels (Riad et al., 2009). Additionally, treatment with garlic resulted in a variety of changes in the ultrastructure of the tegument of the surviving worms as previously described by Riad et al. (2009), where the most prominent damage observed in the tegument of garlic-treated worms was in the form of oedema and blebbing. At the same time, garlic treatment resulted in noticeable suppression in granulomatous lesions, plus the recovery of most of the histopathological and ultrastructural changes in the livers of infected mice (Unpublished results).

In the present study, an attempt has been carried out using RAPD-PCR assay to determine whether the ultrastructural impacts observed in schistosomes as a result of garlic treatment are accompanied by alterations in their genome. As for the host, the technique was used to detect genetic alterations in mice infected with *Schistosoma mansoni*, and the ability of garlic treatment to repair these alterations.

Generally, the RAPD-PCR method has been initially used to detect polymorphism in genetic mapping, taxonomy and phylogenetic studies and later in genotoxicity and carcinogenesis studies; but complete

Abbreviations: CTAB, Cetyltrimethylammonium bromide; dNTPs, Deoxynucleotide triphosphates; RAPD-PCR, Random amplification of polymorphic DNA-polymerase chain reaction; SBSC, Schistosome biology supply center.

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potential of this technique to assess genetic damage has not been explored in the fields of drug development (Atienzar and Jha, 2006).

2. Materials and methods

2.1. Chemicals

All chemicals used in the current study were analar quality, products of Merck, Germany; Sigma-Aldrich, USA, and El-Nasr Pharmaceutical Chemical Company, Egypt.

2.2. Garlic extract

Fresh Egyptian garlic was purchased from local suppliers in Cairo, Egypt. Garlic cloves were peeled and weighed. The crude extract was prepared as previously described by Senapati et al. (2001). Briefly, garlic bulbs were separated, peeled, and washed with distilled water. After drying in a shed, about 500 g of clean garlic bulbs were crushed in a blender until a uniform consistency was achieved. The resulting paste was stored at −20 °C and the extract freshly prepared day after day. Garlic extract working solution (50 mg/kg bw/mouse) was given orally with an oesophageal tube attached to a syringe, day after day, at a fixed daytime. The dose selected for the present work is equivalent to the amount of garlic recommended for an average human (~4 g).

2.3. Animals

Sixty naïve adult male Swiss albino mice (*Mus muscullus*, CD_1 strain) of similar age and weight (18–20 g) were purchased from the Schistosome Biology Supply centre (SBSC) at Theodor Bilharz Research Institute, Imbaba, Giza, Egypt. Mice were transported to the animal care facility of the Zoology Department at the Faculty of Science, Ain Shams University, two weeks prior to the initiation of the experiments for acclimatization to the laboratory conditions. A temperature of 25 ± 2 °C and 12 h light/dark cycle were maintained. Mice were reared in hygienic polypropylene cages, bedded on autoclaved wood shavings, given free access to water and standard pelleted diet, and were monitored daily for health status. All aspects of the present study agreed strictly with the regulations and guidelines set by the University of Ain Shams Ethics Committee for care and use of experimental animals.

2.4. Infection of animals

Forty mice were subcutaneously infected with ~200 *Schistosoma mansoni* cercariae/mouse, according to Peters and Warren (1969).

2.5. Experimental protocol and treatments

Infected mice were divided randomly into four equal groups and served as follows: Infected control group: infected and given distilled water, day after day, from the 1st day of infection till the end of the 7th week post-infection. Prophylactic: treated with garlic for a week before infection. Therapeutic: treated with garlic from the 1st week of infection till the end of the 7th week post-infection. Continuously-treated: treated with garlic one week prior to infection till the end of the 7th week post-infection. Negative control (normal) group and garlic-treated control group were assigned for the sake of comparison. Garlic administered was orally at a dose of 50 mg/kg body weight, day after day, for 7 weeks. Normal and infected control mice were orally administered the vehicle (distilled water) in a similar manner. Twenty-four hours after the last drug exposure, mice were autopsied, worms were recovered from the portal system and mesenteric veins by perfusion technique according to Smithers and Terry (1965), then the livers were excised out.

2.6. DNA isolation

DNA extraction of the mice livers and the recovered adult schistosomes were carried out by cetyltrimethylammonium bromide (CTAB) precipitation method described by Yap and Thompson (1987) and modified by Mostafa et al. (2003).

2.7. RAPD-PCR

Random Amplification of Polymorphic DNA-Polymerase Chain Reaction (RAPD-PCR) technique was processed according to the method described by Williams et al. (1990) with some modifications.

Arbitrary primers (Kit-H, Operon Technologies Inc., Alameda CA, USA; and Metabion, Planegg-Martinsried, Germany) were used for screening genomic DNA from liver and from schistosomes (Table 1).

For each caste of genomic DNA, the reaction was made in a sterile 0.25 mL microfuge tube containing the following reagents: 1.0 μL (10 mM) dNTPs (Deoxynucleotide Triphosphates) mixture, 2.5 μL 10× PCR Buffer, 2.5 μL (0.75 mM), Bovine serum albumin (Sigma-Aldrich, USA), 0.4 μL (2.5 U/μL) *Taq* DNA polymerase, 2.5 μL (50 pM) Primer (pM/μL), 1.0 μL (~50 ng) Template Genomic DNA. The mixture volume was completed to 25 μL with double distilled water.

Amplification was carried out in Biometra PCR thermocycler (Germany). The cycler was programmed for 5 min initial denaturation step at 94 °C, followed by 35 cycles of 1 min at 94 °C of denaturation, 1 min at 36 °C of primer annealing, and 2 min at 72 °C of elongation. The program ends with 10 min at 72 °C for extra extension.

The reaction products were resolved by 2% agarose gel (stained with ethidium bromide) electrophoresis. The observed bands were scored as presence or absence for each group, then the commonality percentages between all groups were calculated based on pairwise comparison between them for a particular primer, using the formula: Commonality Percentage $= Nxy/(Nx + Ny) \times 100$.

Where Nxy: the number of shared bands between x and y; and Nx and Ny: the number of bands in individuals x and y (Lynch, 1990).

3. Results

3.1. RAPD-PCR Profiles for Schistosomes

Collectively, the primers used with schistosomal genomic DNA of all groups (untreated and treated with different garlic regimes) produced 31 reproducible bands, distributed as 12, 6, 3, 3, 4 and 3 fragments with primers AA-1, AC-3, AD-4, OPH-8, OPH-12 and OPH-13, respectively (Figs. 1 and 2). Out of those 31 bands, fourteen were monomorphic (shared among all schistosome groups). On the other hand, 11 bands were polymorphic, eight of them were exclusively found in DNA profiles of normal schistosomes. Moreover, 5 bands were exclusively found in the DNA profiles of worms recovered from all garlic-treated groups.

The comparison between the different RAPD-PCR patterns showed that primers AA-1 and AC-3 yielded a characteristic pattern for

Table 1
The nucleotide sequence of the selected arbitrary primers.

Primer code	Sequence	GC, %	Molecular weight
AA-1	5′-AGACGGCTCC-3′	70%	3013
AB-2	5′-GGAAACCCCT-3′	60%	2997
AC-3	5′-CACTGGCCCA-3′	70%	2973
AD-4	5′-GTAGGCCTCA-3′	60%	3028
OPH-2	5′-TCGGACGTGA-3′	60%	3068
OPH-4	5′-GGAAGTCGCC-3′	70%	3053
OPH-8	5′-GAAACACCCC-3′	60%	2966
OPH-12	5′-ACGCGCATGT-3′	60%	3028
OPH-13	5′-GACGCCACA C-3′	70%	2982
OPH-18	5′-GAATCGGCCA-3′	60%	3037

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