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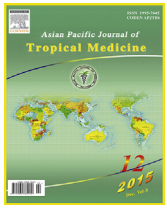

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Immunomodulatory effect of garlic oil extract on *Schistosoma mansoni* infected mice

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ABSTRACT

Objective: To assess the effect potency, and the immunomodulatory response of garlic oil extract in enhancing the host's immune system against the disorders caused by *Schistosoma mansoni* (*S. mansoni*) in mice at different stages of worm maturation.
Methods: A total of 70 male CD-1 Swiss albino mice were divided into 7 groups. Group I: healthy control. Group II: garlic oil group orally administrating 100 mg garlic oil extract/kg b.wt. 3 d a week for 6 weeks. Group III: infected with *S. mansoni* cercariae and left untreated for 42 d. Group IV: treated with garlic oil extract from day 1 to day 7 post infection (PI). Group V: treated with garlic oil extract from day 14 till day 21 PI. Group VI: administrating garlic oil extract from day 35 until day 42 PI. Group VII received oil extract from the first day of infection for 42 d.
Results: Garlic oil extract showed changes in the parasite tegument with a significant decrease in worm burden, hepatic and intestinal ova count with a decline in granuloma number and diameter. These alterations were accompanied with a reduction in serum TNF α, ICAM-1, IgG and IgM after 7 and 42 d post *S. mansoni* cercarial infection.
Conclusions: Results obtained confirmed the effect of garlic oil extract on the larval and mature stage of the parasite and in enhancing the host's immune system against the disorders caused by *S. mansoni* in mice.

1. Introduction

Parasitic helminths of genus *Schistosoma* are the causative agents of schistosomiasis, an infectious disease affecting humans and animals. Schistosomiasis has attracted increased focus and funding for control [1]. Schistosomiasis tops all the endemic parasitic diseases world-wide particularly in Egypt [2].

Studies on the relationship of the disease and immune response in schistosomiasis during the acute phase of the infection demonstrated that the pathology caused by the blood fluke *Schistosoma mansoni* (*S. mansoni*) is induced by the host's granulomatous response to eggs deposited in the liver and the intestines [3] which is maximal by the 8th week of infection. The toxic egg material destroys the host tissue cells and the antigenic material stimulates the development of large inflammatory reactions (granuloma) around the egg [2].

Cytokines have a major role in the development of pathology and resistance to infection. There are different outcomes that are determined by a balance between different immune responses-modulated by certain cytokines-which are directed both against larval and adult stages of the parasite, as well as parasite eggs trapped in the tissues [4].

Eggs trapped in the pre-sinusoidal portal venules secrete soluble egg antigens which are taken up by macrophages. Subsequently, macrophages stimulates T helper cells to secrete tumor necrosis factor α (TNF α), which in turn drive a cell-mediated response and attract more immune cells around the ova. As the granuloma becomes more organized, the T helper cells, produce different interleukins completing granuloma maturation towards the late stage of granuloma formation [4].

Besides, Intracellular adhesion molecule-1 (ICAM-1) which is present on endothelial cells, antigen presenting cells and fibroblasts plays an important role in inflammatory and immunological responses. It has been focused on the interplay between ICAM-1 expression and *S. mansoni* induced granulomogenesis [5].

TNF α triggers the release of ICAM-1 which plays key role in early granuloma formation and aggravation of hepatic fibrosis [6]. In addition, ICAM-1 expression is induced by products of

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deposited *S. mansoni* eggs [7]. Also, immunoglobulins IgG and IgM have been shown to have a pivotal role in the humoral response to schistosomal infection. High levels of IgG have been associated with periportal fibrosis and portal hypertension in patients with advanced schistosomiasis mansoni [8].

Due to the lack of a vaccine, patient therapy is heavily reliant on chemotherapy with praziquantel [9], but concerns over drug resistance and possible reoccurrence of infection encouraged the search for new drug from natural resources [10]. Nowadays, there is an increased demand for using plants in therapy "back to nature" instead of using synthetic drugs which may have adverse effects that may be more dangerous than the disease itself [11].

Additionally, a comprehensive knowledge of tegumental components would be helpful in the development of new drugs. Thus, the importance of studying the tegument of schistosomes arises because it acts as an interface between the parasite and its environment in the host. This interface is used to evade the immune responses of the host [12].

Ancient Egyptians realized the benefits of garlic as a folk remedy for a variety of ailments. Garlic has been shown to have plentiful medicinal effects [13]. Lately, the anthelmintic effect of garlic has been a matter of interest for researchers [14]. Moreover, garlic (*Allium sativum*) has been described by having some immunomodulatory activity against parasites. The therapeutic effect of garlic extract may be maintained by increasing phagocytosis along with killing the parasite by macrophages *in vivo* [15].

It is [16] also reported that treatment with garlic greatly improved the antioxidant status in *S. mansoni* infection with a noticeable reduction in worm burden and egg load by acting upon improving the immunological host system. One of the main immunological responses of garlic administration is the decrease of lipopolysaccharide induced proinflammatory cytokines, such as TNF α [17].

Considering the promising activities of garlic oil extract as anti parasite, this work was carried out to assess the effect potency, and the immunomodulatory response of garlic oil extract in enhancing the host's immune system against the disorders caused by *S. mansoni* in mice at different stages of worm maturation.

2. Materials and methods

2.1. Experimental animals

Male CD-1 Swiss albino mice [weight, (20 ± 2) g], were bred and maintained under conventional conditions at the experimental animal research unit of the Schistosome Biological Supply Program at Theodor Bilharz Research Institute (Giza, Egypt). They were fed a standard commercial pellet diet. They were given carrot, lettuce and milk as source of vitamins and water and were monitored daily for health status. The animal experiments were carried out according to the internationally valid guidelines in an institution responsible for animal ethics (Theodor Bilharz Research Institute).

2.2. Infection of animals

S. mansoni cercariae were provided by the Malacology Laboratory of Schistosome Biological Supply Program, Theodor Bilharz Research Institute, where laboratory-bred *Biomphalaria alexandria* were maintained. Infection was subcutaneously injected using freshly shed (60 ± 10) cercariae to each mouse [18].

2.3. Drugs

Each capsule contains 10 mg/kg of concentrate pure garlic oil which equivalent to 1 000 mg of fresh garlic bulb. Manufactured by The Vitamin Shoppe Co. U.S.A. Garlic oil extract was given to mice in a dose of 100 mg/kg b.wt. by the method described before [19] using an esophageal tube 3 d a week for 6 weeks (42 d) according to the following experimental design.

2.4. Experimental design

Animals were divided into seven groups, each group of 10 mice. Group I: healthy control. Group II: garlic oil group orally administrating 100 mg garlic oil extract/kg b.wt. 3 d a week for 6 weeks. Group III: infected with *S. mansoni* cercariae and left untreated until the end of the experiment (42 d). Group IV: treated with garlic oil extract from day 1 to day 7 post infection (PI). Group V: orally treated with garlic oil extract from day 14 till day 21 PI. Group VI: administrating garlic oil extract from day 35 until day 42 PI. While the last group (VII) received oil extract from the first day of infection till the end of the experiment (42 d). All mice were necropsied 42 d after cercarial exposure and worms were recovered from the portal system and mesenteric veins by perfusion technique as explained earlier [20]. The worms were classified according to sex and counted. Adult male worms were prepared for scanning electron microscopic examination. The number of eggs/g tissues (liver and intestine) were assessed following digestion with 4% KOH as expressed before [21]. The percentage of egg developmental stages (Oogram pattern) was examined earlier [22].

2.5. Histopathology

Sampling slices from the liver tissue were taken from mice and fixed in 10% formalin. Paraffin sections (4 μm thickness) were stained with Ehrlich's haematoxylin and eosin (H & E) [23]. The associated histopathological changes were observed. Granuloma number and diameter were measured using an ocular micrometer [24].

2.6. Scanning electron microscope

Adult worms of *S. mansoni* were washed several times with normal saline and then fixed in 2.5% gluteraldehyde and dehydrated by serial dilution of ethanol using automatic tissue processor (Leica EM TP). Then the samples was dried using CO_2 critical point drier (Tousimis Audosamdri-815). Specimens coated by gold sputter coater (SPI-Module) and examined with scanning electron microscope (JEOL-JSM–5500 LV) by using high vacuum mode at the Regional Center of Mycology and Biotechnology, El Azhar University, Cairo, Egypt.

2.7. Serum sample preparation for immunological studies

Blood was collected from mice in different groups by heart acupuncture. The sera were separated, centrifuged at 2 000 rpm