سورة البقرة الآية: ٣٢

Acknowledgments

First, and foremost, thanks are due to Allah,

the most beneficent and merciful of all.

I am greatly indebted to late Prof. Dr. **Nahed H.A. Riad,** professor of Histology and Histochemistry, Zoology Department, Faculty of Science, Ain Shams University for her precious advice, continuous encouragement and for her critical reading of the manuscript. It is a great honor to work under her supervision.

My profound appreciation is due to Prof. Dr. **Nagui H**. **Fares**, Professor of Cell Biology and Histology, Zoology Department, Faculty of Science, Ain Shams University for his supervision, valuable guidance and careful reading of the manuscript. His research experience has added a lot to the integrity of this thesis.

I also owe a dept of gratitude to Dr. **Samia M. Fawzy**, Assistant professor of Parasitology, Zoology Department, Faculty of Science, Ain Shams University for her supervision, and careful reading of the manuscript.

In fact, I owe much to Dr. **Yomna I. Mahmoud,** Assistant professor of Cell Biology and Histology, Zoology Department, Faculty of Science, Ain Shams University for her unlimited assistance, instructive guidance and kind support. No word of thanks can equal her contribution.

Finally, and most importantly, I must express my deepest gratitude to my husband for the encouragement, patience and forbearance he has shown throughout the time this work has taken to be finished.

كلمة شكر

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تأثير بعض المواد المضادة للبلهارسيا على كبد الفئران البيضاء المعداة ببلهارسيا المستقيم: دراسات هستولوجية وتركيبية دقيقة

رسالة مقدمة للحصول على درجة الماجستير فى العلوم كجزء مكمل لمتطلبات
رسالة الماجستير بكلية العلوم
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2014

EFFECT OF SOME ANTISCHISTOSOMAL AGENTS ON THE LIVER OF *SCHISTOSOMA MANSONI*-INFECTED ALBINO MICE: HISTOLOGICAL AND ULTRASTURCTURAL STUDIES

A Thesis Submitted for the Degree of Master of Science as a partial fulfillment for requirements of Master in Science (Zoology)

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Introduction

Schistosomiasis is a parasitic disease that affects 200 million people worldwide, and being endemic in 74 countries in tropical regions of Africa, Asia and America **(Chitsulo *et al*., 2000; Simeonov *et al*., 2008).** Since the first clinical trials, PZQ has been a tremendous success as a single dose therapy is effective, non-toxic, and relatively cheap. Therefore, there is very high bar for any new anti-schistosomal compound to compete with PZQ as a cornerstone of bilharzial eradication **(Nwaka and Hudson, 2006).** Due to the lack of a vaccine, schistosomiasis control is heavily reliant on chemotherapy with PZQ as the World Health Organization-recommended drug, but concerns over resistance and possible reoccurrence of infection encouraged the search for new drug leads, possibly from natural resources **(Abdulla *et al*. 2007; Abebe, 2008).**

Garlic is one of the earliest documented plants used for its plentiful medicinal effects **(Rivlin, 2001).** The antihelminthic effect of garlic has been verified by many investigators **(Hamdy *et al*., 1983; Mansy, 1998; Abdel-Rahman *et al*., 1998; Sutton and Haik, 1999; Streliaeva *et al*., 2000).** Recently, garlic and its derivatives have been proved to have a prominent antibilharzial effect **(Riad *et al.* 2007, 2008, and 2009, El-Shenawy *et al*. 2008, Lima *et al*. 2011 and Mantawy *et al*. 2011 and 2012).** On

the other hand, the herb myrrh and its new commercial extract in the Egyptian medicine market (Mirazid) have been investigated, experimentally and clinically, against trematode infections, particularly schistosomiasis and fascioliasis, with stories of success and disagreement regarding their efficacy against schistosomes **(Abdul-Ghani *et al.*, 2010).**

Three decades ago, a new therapeutic strategy for schistosomiasis control adopting combined therapy approach launched using PZQ and oxamniquine combinations **(Pugh and Teesdale, 1983; Shaw and Brammer, 1983; Creasey *et al*., 1986).** Later, other drugs combinations were used **(Kamel *et al.*, 2000; Utzinger *et al*., 2001; El-Shenawy *et al*., 2008; Mostafa and Soliman, 2010; Keiser *et al*., 2011; Mantawy *et al*., 2011; Xiao *et al*., 2011; Abdel-Hafeez *et al.*, 2012).** Based on these previous studies, it could be hypothesized that combining Mirazid, PZQ and garlic may increase worm burden reductions, as these compounds display broad-spectrum antischistosomal activities and the susceptibilities of the different stages to the three drugs are distinctively different. PZQ is known to kill adult worms effectively, with no effect on younger stages **(Xiao *et al*., 1993).** The inventor of Mirazid, Massoud and his collaborators (2004), also stated that the therapeutic effect of Mirazid is more evident when the drug is given on day 21 and day 45 post-infection, after the worms have became mature. Quite the

opposite, garlic exhibits high levels of activity against adult and juvenile stages of schistosomes **(Riad *et al.*, 2007 and 2009).** Thus, the current work also investigates whether the combination between Mirazid and other agents well-known for their antibilharzial activity will make more effective use of these agents.

Aim of the Work

The present work was designed to estimate the validity of the herbal drug "Mirazid" and its combination with PZQ and/or garlic against *Schistosoma mansoni* infection. In the current study, since the liver is considered the major target organ for pathogens and consequently, it acts chiefly as a metabolizing and detoxifying organ. Therefore, it was chosen for both parasitological and histological studies.

To achieve this goal, the plan of the present investigation was constructed to inspect:

- Parasitological parameters:

  1. Worm burden.

  2. Tissue egg load (egg count/g of liver and ileum).

  3. Hepatic granuloma count and diameter measurement.

- Histological parameters:

  1. Liver Paraffin section.

- Histochemistry parameters:

  1. Periodic Acid Schiff's (PAS) for carbohydrates.

  2. Mercuric-Bromophenol Blue for total protein.

3. Gomori's Trichrome teqnique for collagen fibers.

Ultrastructural parameters:

1. Ultrathin sections of liver tissues.

Hopefully, the accomplished results would be of some value in curing, or at least minimizing the injurious responses of schistosomiasis.

Review of Literature

Schistosomiasis

Schistosomiasis is caused by the platyhelminth worms of the genus *Schistosoma*, trematodes that live in the bloodstream of humans and animals. Among human parasitic diseases, schistosomiasis ranks second behind malaria in terms of socio-economic and public health importance in tropical and subtropical areas **(El Ridi and Tallima, 2009).**

History

Schistosomiasis has been known since time immemorial. The ancient Egyptians contracted the disease more than 4,000 years ago (1900 B.C.) and recorded it in the Kahun Papyrus **(El-Zayadi, 2004)**. It is as old as civilization. Pharonic temple murals of the 19th dynasty in ancient Egypt (1500 B.C.) depict men with ascites and scrotal edema, generally accepted as evidence of hepatic schistosomiasis **(Rubin and Farber, 1994).** In 1910, Marc Armand Ruffer found *Schistosoma haematobium* eggs in two Egyptian mummies dating from the 20th dynasty, 1250 to 1000 B.C. **(Ruffer, 1910).** In 1851, The German parasitologist Theodor Bilharz discovered *Schistosoma* from a human autopsy material in Cairo and described the relationship of the parasite to pathologic lesions.

Epidemiology and Geographical Distribution

Human schistosomiasis is principally caused by one of the following 6 species of parasitic worms, *Schistosoma haematobium, S intercalatum, S. japonicum, S. malayensis, S. mansoni,* and *S. mekongi*. Other species of animal schistosomes cause human infection, including schistosomes of birds and small mammals that cannot mature in the human host but die in the skin where they cause dermatitis **(Dhawan *et al*., 2008)**.

The disease is endemic in 74 developing countries in the Middle East, Africa, and South America, infecting more than 200 million people in rural and peri-urban areas. Close to 800 million people worldwide, especially children under 14 are at risk of infection. The prevalence is particularly related to large-scale increases in man-made water development projects, like dams, which have augmented the number of snail habitats leading to the spread of schistosomiasis **(Engels *et al*., 2002; Steinmann *et al*., 2006; El Ridi and Tallima, 2009).**

Life Cycle

Schistosomes have complex life cycles involving invertebrate and vertebrate hosts as well as free-swimming stages **(Aragon *et al*., 2009).** All *schistosoma* infections follow direct contact with fresh water that harbors free-swimming larval forms of the

parasite known as cercariae. Cercariae penetrate the skin of humans. The cercariae shed their bifurcated tails, and the resulting schistosomulae enter capillaries and lymphatic vessels en route to the lungs. After several days, the worms migrate to the portal venous system, where they mature and unite. Pairs of worms then migrate to the superior mesenteric veins. Egg production commences four to six weeks after infection and continues for the life of the worm usually three to five years. Eggs pass from the lumen of blood vessels into adjacent tissues, and many then pass through the intestine and are shed in the feces. The life cycle is completed when the eggs hatch; releasing miracidia that, in turn, infect specific freshwater snails (*Biomphalaria species*). After two generation within the snail primary and then daughter sporocysts, cercariae are released **(Ross *et al*., 2002).**

Pathogenesis

Symptoms of schistosomiasis depend on the species of the schistosome and the stage of disease. Most infected individuals are asymptomatic or have only mild nonspecific symptoms. Only 5-10% of infected individuals develop severe clinical symptoms, which are usually associated with heavy infestations **(Dhawan *et al*., 2008). Garcia *et al*. (2007)** mentioned that the syndromes associated with schistosomiasis are related to the stage of

infection, previous host exposure, worm burden, and host response. They cited those syndromes as follows:

- **Schistosome Cercarial Dermatitis (Swimmers' itch):** Cercarial dermatitis follows skin penetration by cercaria. After the penetration, petechial hemorrhage with edema and pruritus occur. The subsequent maculopapular rash, which may become vesicular, may last 36 h or more. Cercarial dermatitis is common with *S. mansoni* infections and a constant feature of human infection with avian schistosomes, with cercarial death occurring at the subcutaneous tissues and immediate hypersensitivity reactions occurring at the invasion sites.

- **Acute Schistosomiasis (Katayma Fever):** Acute schistosomiasis (Katayma fever) is associated with heavy primary infections and the initiation of egg production throughout areas of high transmission risk. Characteristic symptoms include high fever, hepatosplenomegaly, lymphadenopathy, eosinophilia, and dysentery.

- **Chronic Schistosomiasis:** After the production of eggs by the adult worms, the eggs become trapped in the fine venules and become able to pass through the tissues, escaping into the intestine (*S. mansoni*) or, less commonly, the bladder *(S. haematobium).* The eggs liberate a number of soluble antigens, evoking minute abscesses, which facilitate their

passage into the lumen. The passage of eggs through the wall of intestine or bladder leads to symptoms that correlate with the worm burden of the host, including fever, abdominal pain, liver tenderness, urticaria, and general malaise. In *S. mansoni* infection, blood and mucus are detected in the stool and the patient may have diarrhea or dysentery.

Intestinal disease, As eggs are deposited in the tissues, the antigenic substances released by the eggs invoke a host immune response that includes the formation of granulomas around the eggs trapped in the tissues. Many eggs are retained in the intestinal tissues. Cellular infiltrates include lymphocytes, eosinophils, macrophages, and fibroblasts. Granuloma aggregation in the intestinal tract wall is the major cause of pathologic changes. The intestinal wall becomes inflamed, thickened, and fibrotic, leading to mechanical obstruction. Intestinal schistosomiasis is common in *S. mansoni* infections **(El-Garem, 1998).** Among the spectrum of intestinal lesions, polyps are the commonest **(Ismail *et al*., 1994).** The presence of schistosomal colonic polyposis has been associated with increased morbidity and mortality **(Abaza *et al*., 1986).** The mechanism of polyp formation starts by deposition of schistosomal eggs in the superficial layers of submucosa where the connective tissue is loose, delicate, and not bounded superficially by firmer tissue. This allows the accumulation of