Antibilharzial agents from marine sponge extracts from Gulf of Aqaba, Red Sea, Egypt

Ibraheem M. M. Gobaara
Zoology Department, Faculty of Science, Al-Azhar University, Cairo11884, Egypt
ibraheemgobaara@azhar.edu.eg

ABSTRACT

Bilharziasis is a parasitic disease caused by platyhelminthes, affecting millions worldwide. Marine invertebrates generally and marine sponges especially are promising organisms for the synthesis of novel bioactive compounds. There is an urgent need to investigate and develop a new and potential antibilharzial agent instead of using synthetic drugs. The extracts of two marine sponges: Negombata magnifica (Nm) and Callyspongia siphonella (Cs) collected from Gulf of Aqaba have been investigated for their effect as anthelmintic agents. Extracts from both types of sponges were obtained by using CH$_2$Cl$_2$, C$_4$H$_8$O$_2$ and CHCl$_3$ solvent. Mice were divided into 6 groups; (G1-G3) include infected mice with cercaria and administrated orally for 2 days a dose of one-tenth of LC$_{50}$ of each extract from Nm (7.85, 11.25 and 10, mg/kg body weight/mouse, respectively); (G4-G7) administrated each extract from Cs (12.32, 13.11 and 14.25mg/kg body weight/mouse, respectively). G7 includes infected mice treated with 200 mg/kg body weight praziquantel for 2 days; G8 is infected mice and not treated (control group). The effects of each extract on the worm recovery and total egg count were determined.

Oral administration of extract to infected mice (G1, G2 and G3) at 9 weeks post treated (WPT), induced a highly significant reduction, in the mean numbers of male and female worms. The males being more affected than females at 9 WPT. Also, treated mice in (G1), (G2) and (G3) showed a significant reduction in the mean number of female worms compared to the infected untreated mice group (G8). The females being more affected than males at 8 WPT. Only (G1), (G2) and (G3), as well as (G7) produced significant decline in the tissues liver and intestine egg counts at 8 WPT.

In conclusion the current data indicated that the investigated sponge extracts can be applied as potential agent to treat bilharziasis. Also, the results provide a basis for exploring extracts from marine sponge as sources for new bioactive agents.

Keywords: Antibilharzial, Extracted, Marine sponge, Gulf of Aqaba, Red Sea, Egypt

INTRODUCTION

Bilharziasis is one of the most common diseases in the world, which caused by Platyhelminthes called Schistosoma (WHO, 2019) which is endemic in Africa and the middle east countries mainly Egypt, causing acute and chronic clinical pathogenicity to man (Saad et al.,2019; Bonnefond et al., 2019).

Its deaths was difficult to estimate due to hidden pathology as hepatic cancer and colorectal cancer (Osada et al.,2005; Neghina et al., 2009;WHO,2019). There are more than 240 million people infected and around 800 million are at risk to infected with this helminthes (Butrous, 2019; WHO, 2018, 2019). The strategy for controlling the Bilharziasis, only just one
drug is a praziquantel (Doenhoff et al., 2008; Riad et al., 2009). However, after many years of praziquantel usage, a decreased susceptibility to the drug and the emergence of drug-resistant strains was reported (Doenhoff et al., 2008; Riad et al., 2009; Campelo et al., 2018). Besides, praziquantel has poor efficacy against juvenile forms, also genotoxicity and mutagenic effect (Vale et al., 2017). It is necessary to invest in novel biological pathways for development of new safe treatment (Sadref-ozalayi et al., 2018; Abu Almaaty et al., 2020) to circumvent challenges linked to drug resistant parasites and better than the risky praziquantel (Stelma et al., 1995; Keiser, 2010; Zhang et al., 2016; Abou El Dahab et al., 2019).

The marine environment represents a rich resource of novel chemical defenses against various types of parasites and/or competitors in the habitat space (Gomes et al., 2016; Herath et al., 2019; Abu Almaaty et al., 2020). Marine animals in general and marine invertebrates in particular are promising organisms for synthesis of novel bioactive compounds (Scepic et al., 1997; Van Soest et al., 2008). A lot of recent studies suggested that some bioactive compounds isolated from marine organisms are shown to exhibit antimicrobial, antifungal, anti-inflammatory, anticancer, antiparasites and other pharmacological activities (Herath et al., 2019; Rady and Bashar, 2020). Bioactive compounds obtained from marine fauna, 70% of which comes from sponges (Purushottama, et al., 2009; Mehbub et al., 2014). Although the marine ecosystem features a rich biodiversity, most of them are not yet been explored (Jiménez, 2018; Herath et al., 2019). Therefore, there's a desire to explore and develop a new anthelmintic candidates to bypass the widespread drug resistance problem that exists in populations of parasites around the world (Kaplan, and Vidyashankar, 2012 and Herath et al., 2019).

The present work aims to investigate the effect of different extracts from two sponges; Negombata magnifica (Nm) and Callyspongia siphonella (Cs) from Gulf of Aqaba, Red Sea, Egypt as potential antibilharzial agents instead of using synthetic drugs to treat bilharzias.

MATERIALS AND METHODS

Preparation of Sponge Samples:
Collection, identification, extraction, and fractionation:
Specimens of marine sponges were collected during June 2019 from various areas of the Gulf of Aqaba at different depths between 6 and 20 meters. Immediately the collected samples were cleaned with 0.9% saline and preserved in a freezer at −20°C. Identification of specimen details were carried out, and the two voucher specimens were transfered at Marine laboratory, Department of Zoology, Faculty of Science, Al-Azhar University, Cairo, Egypt. The identification of the specimens has been carefully checked based on Rady and Bashar (2020). The taxonomy is directed according to Ruggiero et al. (2015).

Samples were defrosted, macerated in distilled water and then air dried at 37°C and finely powdered. About 20g of sponge specimens were extracted by drenching in 100 ml of three different absolute organic solvents (CH₂Cl₂, C₆H₅O₂, and CHCl₃) for one day then filtered at 37°C. Then the solvent was removed by evaporation and remains were dried at 40°C using a rotatory evaporator and then stored at −20°C until use.
Antibilharzial agents from marine sponge extracts from Gulf of Aqaba, Red Sea, Egypt

Praziquantel (PZQ):
This drug is a chemotherapeutic treatment of schistosome infection. PZQ was given orally (for treatment at first day of 4 weeks p.i) in a dose of 200 mg/kg body weight for 2 consecutive days. The tablet was dissolved in 10% of dimethyl sulphoxide. PZQ was purchased from Alexandria Company for drugs and chemicals (Alexandria, Egypt).

Experimental animal:
White albino mice with an average weight of 22±2g and aged of 6–7 weeks old were infected with 100±10 Schistosoma mansoni cercariae using a partial immersion technique (Olivier and Stirewatt, 1952). Cercaria was obtained from Theodore Bilharz Research Institute Giza, Egypt. The mice were given a week to adjust to a normal diet and unlimited water at the Animal House in the Department of Zoology, Faculty of Science, Al-Azhar University, prior to the experiment. The local ethics panel and animal science committee accepted the experimental protocol.

Determination of LD50 and the percentage of yield of different crude extracts were calculated according to Mona et al. (2012).

Experimental design:
This study was conducted between May 2019 and September 2019 using 8 main groups for 9 weeks. Mice were infected with cercaria and divided into 8 groups (G1-G8);
Group 1 (G1): administrated orally LD50 CH2Cl2 extract of Negombata magnifica (Nm) (7.85 mg/kg body weight) for 2 consecutive days;
Group 2 (G2): administrated orally Nm-C4H8O2 extract (11.25 mg/kg body weight) for 2 days.
Group 3 (G3): administrated orally Nm-CHCl3 extract (10 mg/kg body weight) for 2 days.
for two consecutive days;
Group 4 (G4): administrated orally LD50 CH2Cl2 extract of Callyspongia siphonella (Cs) (12.32 mg/kg body weight) for 2 days;
Group 5 (G5): administrated orally Cs - C4H8O2 extract (13.11 mg/kg body weight) for 2 days;
Group 6 (G6): administrated orally Cs-CHCl3 extract (14.25mg/kg body weight) for 2 days;
Group 7 (G7): administrated orally praziquantel treated group (200 mg/kg body weight) for 2 days;
Group 8 (G8): infected and not treated (control group)

Fecal samples:
These were collected from all mice and examined after 4 weeks post-infection, by light microscope for S. mansoni eggs. Mice were sacrificed at the 7th, 8th and the 9th weeks post infection.

Worm burden:
Worms were recovered from tissues of control and treated mice by Wang et al. (2004) methodology. Immediately, after dissection of the animals, the worms were counted under a dissecting microscope and classified into males, females and copulated, each tissue of worms were added to glass watch in doses the same as used in vivo to show whether they was affected by endogenous factors of the mice which may decrease their effectiveness. Then the worms were compressed between two clean glass plates until the parenchyma was evenly strewn into a transparent layer (Kloetzel, 1967).
Ibraheem M. M. Gobaara

Egg load count:

Eggs were counted from stool after cercarial infection to determine the presence of *S. mansoni* eggs. Liver and intestine were removed and located in a petri-dish after the mice were scarified (Lago *et al.*, 2019). Eggs were counted from tissues under a microscope using 10X magnification. The egg count was multiplied after Smithers and Terry (1965).

Percentage of egg developmental stages (Oogram pattern):

These were examined in three samples/mouse (Tendler *et al.*, 1986). Each piece was compressed between two clean glass slides and studied under a microscope (Mati and Melo, 2013). The mean of each stage/animal was obtained (Helmy *et al.*, 2009).

Statistical analysis of data:

Results were expressed as means ± standard error of the means (SE). Differences between groups were analyzed by using one way analysis of variance (ANOVA). The mean number of worms and eggs recovered from the different groups were subjected to Student’s t-test using Microsoft office 16 to determine their statistical significance in comparison with the control groups. The data were considered significant if *P* <0.05.

RESULTS

The LD<sub>50</sub> values for all extracts of *Negombata magnifica*(Nm): Nm-CH<sub>2</sub>Cl<sub>2</sub> (G<sub>1</sub>), Nm-C<sub>4</sub>H<sub>8</sub>O<sub>2</sub> (G<sub>2</sub>), Nm-CHCl<sub>3</sub> (G<sub>3</sub>) and *Callyspongia siphonella* (Cs): Cs - CH<sub>2</sub>Cl<sub>2</sub> (G<sub>4</sub>), Cs - C<sub>4</sub>H<sub>8</sub>O<sub>2</sub> (G<sub>5</sub>), Cs-CHCl<sub>3</sub> (G<sub>6</sub>) were 78.5, 112.5, 100.02 and 123.2, 131.1 and 142.5 mg/kg body weight/mouse, respectively. The Cs extracts showed the highest yield than the Nm extracts as shown in Figure (1).

In the current study, the results obtained showed that treatment of the infected mice by orally administrated extracts induced a highly significant reduction in the mean numbers of male worms (94.8%), (90.3%), (88.4%) and (95.3%) in (G<sub>1</sub>), (G<sub>2</sub>) and (G<sub>3</sub>) as well as Praziquantel (G<sub>7</sub>), respectively after 9 weeks post treatment (WPT). Also, the mean numbers of female worms showed a highly significant reduction, (91.6%) (90.2%), (87.5%) and (93.1%) in the same groups, respectively compared to the infected untreated mice group (G<sub>8</sub>). Also, it was obvious that the reduction number of male is higher than that of female worms at 9 WPT. Moreover, sponge extractions of (G<sub>1</sub>),(G<sub>3</sub>) and (G<sub>7</sub>) caused a significant reduction in the mean numbers of coupled worms (75.7%), (70.4%) and (78.7%), respectively compared to the infected untreated mice group (G<sub>8</sub>) (Table 1). Administration dose of (G<sub>4</sub>), (G<sub>5</sub>) and (G<sub>6</sub>) to infected mice at 9 WPT, caused a significant reduction in the
Antibilharzial agents from marine sponge extracts from Gulf of Aqaba, Red Sea, Egypt

mean numbers of male worms (78.06%), (74.1%) and (75.5%), respectively and females (79.0%), (70.8%) and (74.5%), respectively neither no coupled worms burden compared to the infected untreated group (Table 1 and Fig. 2).

Table 1. The mean number of worms recovered from tissues after treatment with groups and scarified 7, 8 and 9 weeks post treat.

| Variable | G1 | G2 | G3 | G4 | G5 | G6 | G7 | G8 |
|----------|----|----|----|----|----|----|----|----|
| Periods post infection | 7 weeks | 8 weeks | 9 weeks |
| Number of Worms (M±SE) | | | |
| M | 6.8±1.9 | 8.5±2.6 | 8.1±3.2 | 9.7±3.8 | 10.0±4.2 | 9.2±3.5 | 5.7±3.3 | 14.7±3.9 |
| F | 3.4±1.0 | 4.7±2.1 | 3.9±1.9 | 5.2±2.3 | 5.6±3.4 | 4.8±1.5 | 2.7±2.3 | 8.2±1.7 |
| C | 11.5±2.8 | 13.2±5.2 | 12.0±8.2 | 13.3±6.7 | 14.2±7.5 | 13.4±7.3 | 11.9±7 | 20.1±6.8 |
| M | 3.3±0.7** | 5.6±2.4 | 4.9±1.3 | 7.3±2.9 | 8.1±3.1 | 8.0±1.8 | 3.0±2.6** | 16.3±4.6 |
| F | 2.1±0.3** | 3.3±1.1*** | 2.5±0.7*** | 4.2±1.8 | 5.0±1.2 | 4.7±2.8 | 1.9±1.0** | 11.2±2.7 |
| C | 8.4±2.9 | 9.7±4.7 | 9.5±3.5 | 10.8±6.2 | 11.4±5.4 | 11.2±3.2 | 7.8±5.5 | 22.9±7.1 |
| M | 0.8±0.7*** | 1.8±0.2 *** | 1.5±1.0*** | 3.4±0.8** | 4.0±1.0** | 3.8±1.2** | 0.7±0.5*** | 15.5±3.0 |
| F | 0.5±0.3*** | 0.9±0.2*** | 0.7±0.7*** | 1.5±0.9** | 2.1±1.3** | 1.8±0.0** | 0.6±0.2*** | 7.2±2.8 |
| C | 5.6±1.3** | 7.2±1.7 | 6.8±1.7 | 8.5±0.4 | 9.2±0.9 | 9.0±0.0 | 4.9±0.9** | 23±4.5 |

Fig. 2. Reduction rate of worms recovered from tissues of infected mice and treated groups post scarified at 9 weeks

Oral administration of infected mice to Nm-CH2Cl2 extract (G1) and Praziquantel (G7) at 8 WPT, showed a significant reduction efficiency (79.7%) and (81%), respectively in the mean number of male worms burden compared to the infected untreated mice group (G8).

Also (G1) (G2) (G3) and (G7) caused a significant reduction (81.2%), (70.5%), (77%) and (83 %), respectively in the mean number of female worms compared to the infected untreated mice group (G8) (Table 1).

No significant reduction in the mean numbers of separate male and female or couple worms induced in treated infected mice of (G4), (G5) and (G6) at 8 WPT compared to the infected control group (G8) (Table1 and Fig. 3). However, there was a reduction in number of male and female worms with females being more affected than males at 8 WPT.
Fig. 3. Reduction rate of worms recovered from tissues of infected mice and treated groups post scarified at 8 weeks.

The results obtained showed no significant reductions in mean number of worms burden infected mice 7 WPT in groups (G₁, G₆), as well as (G₇) compared with the Non-treated infected mice group (G₈) (Table1 and Fig. 4). However, females being more affected than males at 7 WPT.

Fig. 4: Reduction rate of worms recovered from tissues of infected mice and treated groups post scarified at 7 weeks.

Treatment of infected mice by the investigated sponge extractions as well as by Praziquantel resulted in no significant reduction in the number of eggs in the fecal of mice in (G₁,G₆) and (G₇) compared with the (G₈) (Table 2).

Also sponge extractions from G₁ to G₆ resulted in no significant reduction in the number of eggs in the tissues liver and
Antibilharzial agents from marine sponge extracts from Gulf of Aqaba, Red Sea, Egypt

...of the treated group at 7 WPT compared with the non-treated infected group (G8). On the other hand, only (G7) produced significant reduction (70%) in egg count in the tissue liver and without significant in the intestine (66%) at 7 WPT compared with the non-treated infected group (G8) (Table 2).

Only (G1), (G2) and (G3), as well as (G7) produced significant decline in the tissues liver egg counts (81%), (75%), (79%) and (85%), respectively. The same groups produced significant reduction in the intestine egg counts (78%), (71%), (74%) and (82%), respectively at 8 WPT (Table 2).

| Variable | Number of eggs after scarified |
|----------|--------------------------------|
|          | Periods post infection (Mean±SE) |                          |
|          | 7 Weeks       | 8 Weeks       | 9 Weeks       |                          |
|          | L | I       | L | I       | L | I       |                          |
| Number of mice groups |
| 1 | 5.75±0.43 | 7.01±0.51 | 3.92±0.31 | 4.35±0.25 | 0.78±0.18 | 1.09±0.17 | 15.65±0.053 |
| 2 | 7.56±0.64 | 8.54±0.53 | 5.23±0.27 | 5.61±0.41 | 2.42±0.20 | 2.82±0.31 | 18.76±0.087 |
| 3 | 5.93±0.38 | 7.87±0.32 | 4.52±0.31 | 5.02±0.52 | 1.57±0.32 | 1.42±0.28 | 16.25±0.090 |
| 4 | 8.84±0.42 | 10.38±0.5 | 7.89±0.51 | 7.82±0.42 | 3.31±0.42 | 4.18±0.40 | 22.18±0.014 |
| 5 | 10.28±0.5 | 11.1±0.45 | 8.54±0.54 | 9.17±0.75 | 3.92±0.62 | 5.10±0.71 | 22.95±0.102 |
| 6 | 9.05±0.50 | 10.2±0.62 | 7.43±0.64 | 7.53±0.43 | 3.24±0.45 | 4.30±0.62 | 20.98±0.070 |
| 7 | 5.42±0.34 | 6.51±0.24 | 3.11±0.32 | 3.46±0.52 | 0.49±0.21 | 0.67±0.14 | 14.95±0.063 |
| 8 | 18.6±0.95 | 19.3±0.89 | 21.2±0.78 | 19.5±0.72 | 14.4±0.85 | 15.1±0.71 | 38.56±2.03 |

Numbers of eggs x10^3 Values were expressed as mean ± SD; numbers in parentheses indicate the percentage of reduction compared with the infected non-treated group. 7, 8 and 9 W, weeks post infection. Liver (L), Intestine (I)

Oogram recorded:
The reduction rate of each stage changes in Oogram patterns in the liver and intestine were given Table (3) and Figures (5 & 6). In the liver tissues, the *Nm*-extraction treated group (G1), (G2), and (G3) showed a significant in the mean number of dead eggs, immature and mature in the liver tissues at 9 WPT. The other groups (G4), (G5) and (G6) had a non-significant correlation. The treated G7 had recorded a highly significant in the mean number of dead eggs immature and mature compared with the non-treated infected group (G8). Oogram pattern in liver showed significant change in dead eggs higher than in intestine (Table 3 and Fig. 6).
Fig. 5. Oogram pattern of eggs at different stages of maturity in liver of infected mice and treated with sponge extractions and praziquantel compared with non-treated infected mice.

Fig. 6. Oogram pattern of eggs at different stages of maturity in intestine of infected mice and treated with sponge extractions and praziquantel compared with non-treated infected mice.

Table 3. The mean number of developing egg, and worm stages recovered from tissues of treated mice in different groups after 9 weeks post treatment compared with untreated group.

| Groups | G1 | G2 | G3 | G4 | G5 | G6 | G7 | G8 |
|--------|----|----|----|----|----|----|----|----|
| Liver  |     |    |    |    |    |    |    |    |
| Early Immature | 20.2±3.6 | 23.4±2.2 | 30.5±2.7 | 33.6±4.1 | 39.3±3.2 | 35.2±2.8 | 18.9±4.2 | 38.6±2.5 |
| Late Immature | 18.6±2.0 | 27.1±3.5 | 33.2±2.6 | 35.3±1.9 | 33.5±4.2 | 30.4±2.7 | 17.5±3.4 | 43.5±3.8 |
| Dead    | 89.2±3.9 | 70.5±2.3 | 75.1±4.3 | 57.7±3.2 | 50.4±3.7 | 51.2±2.8 | 98.5±3.5 | 20.5±4.1 |
| Mature  | 21.2±5.3 | 25.4±0.9 | 20.2±2.5 | 30.1±1.5 | 35.4±3.8 | 33.8±1.3 | 20.6±2.3 | 78.2±3.4 |
| Intestine |     |    |    |    |    |    |    |    |
| Early Immature | 17.2±3.4 | 19.6±1.8 | 21.5±3.5 | 27.4±2.4 | 30.2±2.5 | 28.4±3.6 | 14.5±4.2 | 45.7±3.3 |
| Late Immature | 10.2±1.7 | 17.4±2.1 | 19.5±3.4 | 23.7±1.1 | 25.4±3.9 | 20.5±3.5 | 9.8±2.4 | 47.9±1.8 |
| Dead    | 95.2±1.8 | 75.3±3.2 | 82.2±3.4 | 60.2±2.5 | 57.3±1.9 | 68.2±3.4 | 100±2.4 | 29.5±3.2 |
| Mature  | 20.4±3.6 | 24.2±1.8 | 22.1±3.4 | 33.5±2.5 | 34.2±2.9 | 32.2±3.2 | 19.3±3.1 | 83.7±2.8 |
Antibilharzial agents from marine sponge extracts from Gulf of Aqaba, Red Sea, Egypt

DISCUSSION

Bilharziasis has a serious impacts on public health (deBrito et al., 2017; Lago et al., 2018) and there is one obtainable drug (Praziquantel, PZQ) used to treat it. PZQ has low efficacy against schistosomula and juveniles (Ammar et al., 2020) and immature stages (Doenhoff et al., 2008). However, it is efficient against all Schistosoma spp. with the recommended dose and its efficacy is low in heavily infective individuals (Raso et al., 2004; Utzinger et al., 2003). Therefore, efforts are dedicated to developing different approaches to treat bilharziasis alongside praziquantel (Silva et al., 2017; Lago et al., 2018). The hard part of new drug discovery is to seek out a substance that could expeditiously wreck the parasite whilst no longer harming the host (de-Moraes et al., 2012; Musili et al., 2015). The emergence of drug-resistant bilharzia strains has brought great attention toward natural bioactive compounds. In a recent publication, Barbosa deCastro et al., (2013) compiled various therapeutic from naturalist sources, which include saponins, flavonoids, alkaloids, phenolic compounds, and terpenoids. Other studies have been done to assess natural substances of plant origin as antibilharzial agents like Neem (Taher, et al., 2016), Curcuma longa (Rizk et al., 2000), garlic (Riad et al., 2009), and ginger (Al-Sharkawi et al., 2007; Mostafa et al., 2011).

With regard to marine sources, some compounds have shown unique biological properties, in part due to their singular chemical identity, and are currently employed as new therapeutic agents, either unmodified or as prototypes for the designing and synthesis of new drugs (Eustáquio et al., 2011; Cherigo et al., 2015). The present study represents an initial trial to use the extraction of marine sponge as associate antibilharzial agent.

Researchers have demonstrated that marine organisms are a rich source of structurally bioactive compounds and novel and biologically active metabolites (Blunt et al., 2005; Ibrahim et al., 2017; El-Damhougy et al., 2017).

The effectiveness of Negombata magnifica (Nm) and Callyspongia siphonella (Cs) were tested in vivo in Schistosoma mansoni infected mice at various dose/day, on various strategies such as killing the worms, and also worm egg-laying inhibitor starting in the fourth week post infection and ended by ninth week post treated. The results showed a significant reduction in worm burden, liver and intestinal egg load, with a significant increase in the percentage of dead eggs in the oogram pattern. These results are in agreement with previous studies that showed a significant reduction of both the worm burden and egg count in when treated with chemical drugs as PZQ-treated settings compared to the untreated group (Tansatit et al., 2012; Tekwu et al., 2017; Beshay et al., 2019). Both ethanolic and crude extracts of Negombata magnifica are extremely potential for anti bilharzial properties although ethanolic extract remains to require more information about its effects on host

In the current study the sensitivity of (Nm) in male worms s. mansoni more sensitive than female as well as PZQ at 9 weeks post treatment this is agree with several previous studies have been reported of differences in drug sensitiveness between males and females of S. mansoni; male worms S. mansoni are often more sensitive than female worms in studies of praziquantel resistance (Pica-Mattoccia and Cioli 2004) and in studies that estimated bioactivity of ginger extract (Sanderson et al., 2002). But in contrast to our result, study demonstrated showed higher survival rates for males than for females, such as alkane thiosulfuric amino acids (de Oliveira et al., 2008). A previous study reported that the treatment with curcumin might, significantly, reduce the worm burden, but after three months of infection, compared to the results of
untreated infected groups (El-Ansary et al., 2007), which may be due to used long duration of treatment. Compared with the present study which used short duration treatment.

The present study showed that treatment of S. mansoni infected mice with sponge extracts induced significant reductions in the liver egg count, or the intestinal egg count. On the contrary to our results, a previous study reported that the treatment with curcumin might, significantly, reduce the egg count, compared to the results of untreated infected groups (El-Ansary et al., 2007).

The current result revealed that at four weeks post infection there are some worms still alive which agree with a previous investigation revealed that at the same time of infection and treated with PZQ, there were some male worms still alive (Melkus, 2020; Yang, 2009).

In the current result, we reported the reduction of the number of eggs per gram of the S. mansoni tissue, beside, the abnormal development of stages of developing egg (oogram pattern) in the intestinal wall of the treated mice groups this is an agreement with Aly, (2017). the present study used treatment protocol of sponge with soluble of methylene chloride, ethyl acetate and chloroform, In another study, a treatment protocol with methanol extract and soluble glycoprotein fraction of *Allium sativum* (*A. sativum*) was applied to target the inhibition of ova production in the hosts exposed to infection by which the further damages of host tissues and organs have been avoided (Kamel, and El-Shinnawy, 2015). Another study used water as extract of *Zingiber officinale*, *Piper nigrum*, and *Coriandrum sativum* was chosen in order to water is a safe, nontoxic widespread dissolvent, and avoid the high toxicity of organic dissolvent (such as methanol, acetone, chloroform, and dichloromethane) to living cells (Kinuthia et al., 2015).

Finally, we concluded that sponge extracts is a remarkable non-toxic extraction with many medical properties, and efficacy as an anti-schistosomal drug is much as it might improve the pathogenic changes induced in S. mansoni infected mice, and induced regimens in the treatment of the S. mansoni infections.

**Conclusion:**

The results of the present study elucidated that, oral ingestion of (Nm) and (Cs) extracts to infected mice was effective in reducing worm and egg count when compared with infected untreated mice, indicating their effective anti-bilharzial action. Reduction of worm burden and egg count with Nm-extract was the highest percentage compared with Cs-extract as also PZQ.

**REFERENCES**

Abou El Dahab, M.M.; Shahat, S.M.; Mahmoud, S.S.M. and Mahana, N.A. (2019). In vitro effect of curcumin on Schistosoma species viability, tegument ultrastructure and egg hatchability. Exp. Parasitol., 199: 1–8.

Abu Almaaty, A.H.; Rashed H.A.; EL-Shenawy N.S.; and Soliman, M.F.M. (2020). The possible antischistosomal effect of Allicin in Schistosoma mansoni Infected albino mice. J. Egypt. Soc. Parasitol., 50: 135–140.

Al-Sharkawi, I.M.; El-Shaikh, K.A.; Tabl, G.A. and Ali, J.A. (2007). The effect of ginger on Schistosoma mansoni infected mice. Delta J. Sci., 31: 1–10.

Aly, I. (2017). Efficacy of soluble glycoprotein fraction from *Allium sativum* purified by size exclusion chromatography on murine Schistosomiasis mansoni. Microbial Pathogl., 107: 243-248.
Antibilharzial agents from marine sponge extracts from Gulf of Aqaba, Red Sea, Egypt

Ammar, A.I.; Afifi, A.F.; Essa, A.; Galal-Khallaf, A.; Mokhtar, M.M.; Shehab-Eldeen, S. and Rady, A.A. (2020). Cucurbita pepo Seed oil induces microsatellite instability and tegumental damage to Schistosoma mansoni immature and adult worms. In vitro infection and drug resistance, 13: 3469–3484

Barbosa, de Castro, C.C.; Dias, M.M.; Pessoa, de Rezende, T.; Magalhães, L.G. and Da Silva, Filho, A.A. (2013). Chapter 8 - Natural Products with Activity against Schistosoma species. In: Mahendra, R., Kateryna, K.(Eds.), Fighting Multidrug Resistance with Herbal Extracts, Essential Oils and their Components. Academic Press, San Diego., 109–134.

Beshay, E.; Rady, A.; Afifi, A. and Mohamed, A. (2019). Schistosomicidal, antifibrotic and antioxidant effects of Cucurbita pepo L. seed oil and praziquantel combined treatment for Schistosoma mansoni infection in a mouse model. J. Helminthol., 3: 286–294.

Blunt, J.W.; Copp, B.R.; Munro, M.H.G.; Northcote, P.T. and Prinsep, M.R. (2005). Marine natural products. Nat. Prod. Rep., 22: 16-61.

Bonnefond, S.; Cnopsb, L.; Duvignauda, A.; Bottieaub, E. and Pistone, T. (2019). Early complicated schistosomiasis in a returning traveler: Key contribution of new molecular diagnostic methods. Int. J. Infect. Dis., 79:72-4.

Butrous, G. (2019). Schistosome infection and its effect on pulmonary circulation. Glob. Cardiol. Sci. Pract., 31: 1-5.

Campelo, Y.; Ombredane, A. and Vasconcelos A.G. (2018). Structure–activity relationship of piplartine and synthetic analogues against Schistosoma mansoni and cytotoxicity to mammalian cells. Int. J. Molecular Sci., 19: 1802

Cherigo, L.; Lopez, D. and Martinez-Luis, S. (2015). Marine natural products as breast cancer resistance protein inhibitors. Mar. Drugs, 13: 2010–2029.

deBrito, M.R.M.; Peláez, W.J.; Faillace, M.S.; Militão, G.C.G; Almeida, J.R.G.S.; Argüello, G.A.; Szakonyi, Z.; Fülpö, F.; Salvadori, M.C.; Teixeira, F.S. Freitas, R.M.; Pinto, P.L.S.; Mengada, A.C.; Silva, M.P.N.; Da Silva Filho, A.A. and de Moraes J. (2017). Cyclohexene-fused 1,3-oxazines with selective antibacterial and antiparasitic action and low cytotoxic effects. Toxicol. Vitr., 44: 273-279

de Oliveira Penido, M.L.; Zech Coelho, P.M. and de Mello, R.T. (2008). Antischistosomal activity of aminooalkanethiols, aminooalkanesulfuric acids and the corresponding disulfides. Acta Tropica, 108: 249–255

Doenhoff, M.J.; Cioli D. and Utzinger J. (2008). Praziquantel: mechanisms of action, resistance and new derivatives for schistosomiasis. Curr. Opin Infect. Dis., 6:659–667.

de Moraes, J.; Nascimento, C.; Yamaguchi, L.F.; Kato, M.J. and Nakano, E. (2012). Schistosoma mansoni: in vitro schistosomicidal activity and tegumental alterations induced by piplartine on schistosomula. Exp. Parasitol., 2:222–227
El-Ansary, A.K.; Ahmed, S.A. and Aly, S.A. (2007). Antischistosomal and liver protective effects of Curcuma longa extract in Schistosoma mansoni infected mice. Indian. J. Exp. Biol. 45: 791–801

El-Damhougy, K.A.; El-Naggar, H.A.; Ibrahim, H.A.H.; Bashar, M.A.E. and Abou-Senna F.M. (2017). Biological activities of some marine sponge extracts from Aqaba Gulf, Red Sea, Egypt. Int. J. Fish. and Aquat. Stud., 5(2): 652-659.

Eustáquio, A.S.; Nam, S.-J.; Penn, K.; Lechner, A.; Wilson, M.C.; Fenical, W.; Jensen, P.R. and Moore, B.S. (2011). The discovery of salinosporamide K from the marine bacterium Salinispora pacifica by genome mining gives insight into pathway evolution. Chem. BioChem., 12: 61–64.

Gomes, N.G.M.; Dasari, R.; Chandra, S.; Kiss, R. and Kornienko, A. (2016). Marine invertebrate metabolites with anticancer activities: solutions to the “supply problem”. Mar. Drugs, 14: 98.109

Helmy, M.M.F.; Mahmoud, S. and Fahmy, Z. (2009). Schistosoma mansoni: Effect of dietary zinc supplement on egg granuloma on Swiss mice treated with Praziquantel. Exp. Parasitol., 122:310-7.

Herath, D.H.M.P.; Preston S.; Abdul Jabbar Garcia-Bustos, J.; Taki C.A.; Addison, R.S.; Hayes, S.; Beattie, K.D.; McGee, S.L.; Martin, S.D.; Ekins, M. G.; Hooper, J.N.A.; Chang, B.C.H.; Hofmann, A.; Davis, R.A. and Gasser, R.B. (2019). Identification of Fromiamycalin and Halaminol A from Australian. Mar. Drugs J, 17:598-613.

Herbert, D.; Orekov, T.; Roloson, A.; Ilies, M. and Perkins, C. (2010). Arginase I suppresses IL-12/IL-23p40-driven intestinal inflammation during acute schistosomiasis. J. Immunol., 11:6438–46.

Ibrahim, H.A.; El-Naggar, H.A.; El-Damhougy, K.A.; Bashar, M.A.E. and Abou-Senna, F.M. (2017). Callyspongia crassa and C. siphonella (Porifera, Callyspongiidae) as a potential source for medical bioactive substances-Aqaba Gulf, Red Sea, Egypt. J. Bas. App. Zool., 78:7-10.

Jiménez, C. (2018). Marin natural products in medicinal chemistry. ACS Med. Chem. Lett., 9: 959–961

Kamel, R.O.A. and El-Shinnawy, N.A. (2015). Immunomodulatory effect of garlic oil extract on Schistosoma mansoni infected mice. Asian Pac. J. Trop. Med., 8: 999–1005.

Kaplan, R.M. and Vidyashankar, A.N. (2012). An inconvenient truth: global warming and anthelmintic resistance. Vet. Parasitol., 186: 70–78.

Keiser, J. (2010). In vitro and in vivo trematode models for chemotherapeutic studies. Parasitol., 137:589-603.

Kinuthia, K.G.; Anjili, O.C.; Kabiru, W.E.; Kigondu, M.E.; Ingonga, M.J. and Gikonyo, K. N. (2015). Toxicity and efficacy of aqueous crude extracts from Allium sativum, Callistemon citrinus and Moringa stenopetala against L. major. Kabarak J. Res. Innovation, 3: 9–20.

Kloetzl, K. (1967). Egg and pigment production in Schistosoma mansoni infections of the white mou-se. Am. J. Trop. Med. Hyg., 16:293-9.

Lago, E.M.; Xavier, R.P.; Teixeira, T.R. Silva, L.M.; da Silva, F.A.A. and de Moraes, J. (2018).
Antischistosomal agents: state of art and perspectives. Future Medical Chemistry, 10: 89–120
Lago, E.M.; Silva, M.P.; Queiroz, T.G.; Mazloum, S.F.; Rodrigues, V.C.; Carneúba, P.U.; Pinto, P.L.; Rocha, J.A.; Ferreira, L.L.G.; Andricopulo, A.D. and de Moraes J. (2019). Phenotypic screening of nonsteroidal anti-inflammatory drugs identified mefenamic acid as a drug for the treatment of schistosomiasis. EBioMedicine, 43: 370-379
Mati, V.L. and Melo, A.L. (2013). Current applications of oogram methodology in experimental schistosomiasis; fecundity of female Schistosoma mansoni and egg release in the intestine of AKR/J mice following immunomodulatory treatment with pentoxifylline. J. Helminthol., 1:115-24.
Mehbub, M.F.; Lei, J. and Zhang, W. (2014). Marine sponge derived natural products between 2001 and 2010: trends and opportunities for discovery of bioactives. Mar. Drugs., 12: 4539–4577.
Melmus, M.W. (2020). Elucidation of cellular responses in non-human primates with chronic schistosomiasis followed by praziquantel treatment. Front. Cell Infect. Microbiol., 10:57, doi.org/10.3389/fcimb.2020.00057.
Mona, M.H.; Omran, N.E.E.; Mansoor, M.A. and El-Fakharany Z.M. (2012). Antischistosomal effect of holothurin extracted from some Egyptian sea cucumbers, Pharma. Biol., 9: 1144–1150
Mostafa, O.M.; Eid, R.A. and Adly, M.A. (2011). Antischistosomal activity of ginger (Zingiber officinale) against Schistosoma mansoni harbored in C57 mice. Parasitol. Res., 109: 395-403.
Musili, R.; Muregi, F.; Mwatha, J.; Murru, D.; Rewa, L.M.; Kamau, T.; Menaine, A.; Chege, S.; Thiong’o, J.; Ng’ang’a Z. and Kimani, G. (2015). Antischistosomal Activity of Azadirachta indica and Ekebergia capensis in Mice Infected with Schistosoma mansoni. Eur. J. Medicinal Plants, 6: 92-102.
Neghina, A.; Merkler, C.; MarincuI, M.R. and Iacobiciu, I. (2009). Intestinal schistosomiasis, importation of a neglected tropical disease in Romania: Case report of a traveler to endemic regions. Travel Med. Infect. Dis., 7:49-51.
Olivier, L. and Stirewalt, M.A. (1952). An efficient method for exposure of mice to cercariae of S. mansoni. J. Parasitol., 38:19-23.
Osada, Y.; Kumagai, T.; Masuda, K.; Suzuki, T. and Kanazawa, T. (2005). Mutagenicity evaluation of Schistosoma spp. extracts by the umu-test and V79/HGPRT gene mutation assay. Parasit. Int., 1:29-34
Pica-Mattoccia, L. and Cioli, D. (2004). Sex- and stage-related sensitivity of Schistosoma mansoni to in vivo and in vitro praziquantel treatment. Int. J. Parasitol., 34: 527–533.
Purushottama, G.B.; Venkateshvaran, K.; Pani, P.K. and Nalini, P. (2009). Bioactivities of extracts from the marine sponge Halichondria panicea. J. Venom Anim. Toxins incl. Trop. Dis., 3: 444-459

Ibraheem M. M. Gobaara

Rady, I. and Bashar, M.A.E. (2020). Novel extracts from Callyspongia siphonella and Negombata magnifica sponges from the Red Sea, induced antiproliferative and proapoptotic activity in HepG-2, MCF-7, and Caco-2 cancer cell lines. Egypt. J. Aquat. Biol. Fish., 7:319-347.

Raso, G.; N’goran, E.K.; Toty, A.; Luginbühl, A.; Adjoua, C.A. and Tian-bi, N.T. (2004). Efficacy and side effects of praziquantel against Schistosoma mansoni in a community of Western Côte d’Ivoire. Trans R. Soc. Trop. Med. Hyg., 98: 18-27.

Riad, N.H.A.; Taha, H.A. and Mahmoud Y.I. (2009). Effects of garlic on albino mice experimentally infected with Schistosoma mansoni: A parasitological and ultrastructural study. Trop Biomed., 26: 40–50.

Rizk, M.; Hafez, S. and Farouk, H. (2000). Measurement of urea cycle enzyme activities in mice under the influence of different stages of Schistosoma mansoni infection and Curcuma longa treatment. J. Egypt. Ger. Soc. Zool., 32: 319–333

Ruggiero, M.A.; Gordon, D.P.; Orrell, T.M.; Bailly, N.; Bourgoin, T.; Brusca, R.C.; Cavalier-Smith, T.; Guiry, M.D. and Kirk P.M. (2015). A higher-level classification of all living organisms. Plos one, 4: 10-15

Saad, A.A.; Azzam, A.M.; Mostafa, B.B.; El-Said, Kh.M. and Hanafy, R.A. (2019). Improvement molluscidal activity of Anagalis arvensis extracted by copper oxide nanoparticles against Biomphalaria alexandrina snails. Egypt. J. Aquat. Biol. Fish., 23: 27–41.

Sadrefozalayi, S.; Aslanipour, B.; Alan, M. and Calan, M. (2018). Determination and comparison of in vitro radical scavenging activity of both garlic oil and aqueous garlic extracts and their in vivo antioxidant effect on schistosomiasis disease in mice. Turk. J.A.F. Sci. Tech., 6: 820-7

Sanderson, L.; Bartlett, A. and Whitfield, P. J. (2002). In vitro and in vivo studies on the bioactivity of a ginger (Zingiber officinale) extract towards adult schistosomes and their egg production. J. Helminthol., 76: 241–247

Sepcic, K.; Batista, U.; Vacelet, J.; Macek, P. and Turk, T. (1997). Biological activities of aqueous extracts from marine sponges and cytotoxic effects of 3 alkylpyridinium polymers from Reniera sarai. Comp Biochem. Physiol., 1 :47-53.

Silva, M.P.; de Oliveira, R.N. and Mengarda A.C. (2017). Antiparasitic activity of nerolidol in a mouse model of schistosomiasis. Int. J. Antimicrobial Agents, 0: 467–472

Smithers, S.R. and Terry, R.J. (1965). The infection of laboratory hosts with cercariae of Schistosoma mansoni and recovery of the adult worms. Parasitol., 55: 695-18.

Stelma, F.; Tall, I.; Sow, S.; Kongs, A. and Niang, M. (1995). Efficacy and side effects of praziquantel in an epidemic focus of Schistosoma mansoni. Am. J. Trop. Med. Hyg., 2:167-70.

Taher H.; Shaldoum F. and Fayez W. (2016). Complementary effect of Neem and Mirazid on mice
Antibilharzial agents from marine sponge extracts from Gulf of Aqaba, Red Sea, Egypt

experimentally infected with *S. mansoni*. Current Sci. Int., 5: 286-298.

Tansatit, T.; Sahaphong, S.; Riengrojpitak, S.; Viyanant V. and Sobhon P. (2012). *Fasciola gigantica*: The in vitro effects of artesunate as compared to triclabendazole on the 3-weeks-old juvenile. Exp. Parasitol., 131: 8-19.

Tendler, M.; Pinto, R.M.; Oliveira, L.A.; Gebara, G. and Katz, N. (1986). *Schistosoma mansoni* vaccination with adult worm antigens. Int. J. Parasitol., 16: 347-52.

Tekwu, E.M.; Bosompem, K.M. and Anyan, W.K. (2017). *In vitro* assessment of anthelmintic activities of *Rauwolfia vomitoria* (Apocynaceae) stem bark and roots against parasitic stages of *Schistosoma mansoni* and cytotoxic study. J. Parasitol. Res., 2017: 11 pages

Utzinger, J.; Chollet, J.; Tu, Z.W.; Xiao S.H. and Tanner, M. (2003). Comparative study of the effects of artemether and artesunate on juvenile and adult *Schistosoma mansoni* in experimentally infected mice. Trans. R. Soc. Trop. Med. Hyg., 96: 318-323.

Vale, N.; Gouveia, M.J.; Rinaldi, G.; Brindley, P.J. and Gärtner, F. (2017). Praziquantel for schistosomiasis: single-drug action, and resistance. Antimicrobial. Agents Chemother., 61: 2-16.

Van Soest, R.W.M.; Boury-Esnault, N.; Hooper, J.N. A.; Rützler, K.; De-Voogd, N.J.; Alvarez-De-Glasby, B.; Hajdu, E.; Pisera, A.B.; Vacelet, J.; Manconi, R; Schoenberg, C.; Janussen, D.; Tabachnick, K.R.; Klautau, M.; Picton, B. and Kelly, M. (2008). World Porifera database. http://www.marinespecies.org

Wang, Y.; Holmes, E.; Nicholson, J.K.; Cloarec, O. and Chollet, J. (2004). Metabonomic investigations in mice infected with *Schistosoma mansoni*: an approach for biomarker identification. Proc. Natl. Acad. Sci., 101:12676-81

WHO (2018). Schistosomiasis Fact Sheet, Geneva, Switzerland.

WHO (2019). Soil-transmitted helminth infections. www.who.int/news room/factsheets/detail/soil transmitted helminth infections.

Yang, L. (2009). Enhancement the oral bioavailability of praziquantel by incorporation into solid lipid nanoparticles. Pharmazie, 64: 86–89.

Zhang, G.; Li, J.; Zhu, T.; Gu, Q. and Li, D. (2016). Advanced tools in marine natural drug discovery. Curr. Opin. Biotechnol., 42: 13–23.
عوامل مضادة لداء البلهارسيا من مستخلصات الأسفلنج المستخرج من خليج العقبة، البحر الأحمر في مصر

إبراهيم مصطفى إبراهيم محمد جبارة
قسم علم الحيوان كلية العلوم جامعة الأزهر القاهرة مصر

المستخلص
مرض البلهارسيا هو مرض طفيلي ناجم عن إصابة بلحد الديدان المفلحة. يصيب ملايين على مستوى العالم. اللافقاريات البحرية عامة والأسفلنجات البحرية خاصة كائنات واعدة لإنتاج مركبات نشطة بيولوجيا. والهدف من الدراسة الحالية محولة تطوير واستبدال مضادات لمرض البلهارسيا جديدة بدلاً من استخدام الأدوية الأصلية. وقد تجمع نوعين من الأسفلنج البحرية ما تيجيوميتكا ماجنيفيكا (ن م) وكالليسيومنيا سيفيتيلا (ث س) وتم استخراج مستخلص منها للتحقق في تأثيرها لمضادات لمرض البلهارسيا. تم إعداد عينات الأسفلنج في درجة حرارة الغرفة لمدة يوم واحد مع كلوريد الميثيلين وخلايا البلهارسيا والكلورورفون بشكل منفصل ثم تصنيف العينات وتخفيقها عند درجة حرارة 40 درجة منوية باستخدام مبرد روتاري وتم حفظ الكلن التي تم تخفيقها عند 2 درجة منوية حتى يتم استخدامها. تم إصابة الفئران بواسطة السرطان من خلال معهد تيودور بلهارس للأبحاث. تم إعطاء واحد على عشرة من الجرعة من متوسط التركيز المميت للفئران عن طريق الفم. ثم تسجيل إثر كل مستخلص على كل من الفئران وعدد البيض الكلي. أوضحت النتائج أن الجرعة التي تم إعطاؤها عن طريق الفم لكل من المجموعة الأولى والثانية والثالثة والرابعة حتى المجموعة السادسة كانت كما يلي (7085), (12.25), (11.25), (13.11), (12.32), (10.25), (14.25) ملي جرام لكل كيلوجرام من الجسم من الفئران على التوالي. الجرعة التي تم إعطاؤها عن طريق الفم للمجموعة الأولى والثانية والثالثة للفئران المصابة خلال 9 أسابيع من العلاج تسببت في حدوث انخفاض كبير للغاية في متوسط عدد الذكور والإناث للديدان. وكانت نسبة تأثر ذكور الفئران أكبر من الإناث في الأسبوع التاسع بعد العلاج. أما تأثير المجموعة الأولى والثانية والثالثة في انخفاض كبير في عدد دينان الإناث مقارنة بالمجموعة الفئران الغير معالجة (المجموعة الثانية). وكانت الإناث أكثر تأثيراً من الذكور بعد 8 أسابيع من العلاج. المجموعة الأولى والثانية والثالثة وكذلك المجموعة السابعة أدوا إلى انخفاض كبير في أعداد البيض الموجود في كل من الكبد والأمعاء في الأسبوع الثامن بعد العلاج.

الخلاصة: توصلت النتائج إلى فائدة استخدام المستخلصات من الأسفلنج تييجوميتكا ماجنيفيكا وكالليسيومنيا سيفيتيلا كمضادات لمرض البلهارسيا وأنه من الممكن أن يكون من العوامل الحيوية النشطة.

الكلمات الدالة: مضادات البلهارسيا، مسحوقات الأسفلنج، خليج العقبة، البحر الأحمر، مصر.