

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

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Received: November 15, 2020; Accepted: December 5, 2020 ; Available online December 20, 2020

### 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<sub>2</sub>Cl<sub>2</sub>, C<sub>4</sub>H<sub>8</sub>O<sub>2</sub> and CHCl<sub>3</sub> 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<sub>50</sub> 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). G<sub>7</sub> includes infected mice treated with 200 mg/kg body weight praziquantel for 2 days; G<sub>8</sub> 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 a 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 (Sepcic *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  $-20^{\circ}\text{C}$ . Identification of specimen details were carried out, and the two voucher specimens were transferred 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  $37^{\circ}\text{C}$  and finely powdered. About 20g of sponge specimens were extracted by drenching in 100 ml of three different absolute organic solvents ( $\text{CH}_2\text{Cl}_2$ ,  $\text{C}_4\text{H}_8\text{O}_2$ , and  $\text{CHCl}_3$ ) for one day then filtered at  $37^{\circ}\text{C}$ . Then the solvent was removed by evaporation and remains were dried at  $40^{\circ}\text{C}$  using a rotatory evaporator and then stored at  $-20^{\circ}\text{C}$  until use.

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### 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  $22 \pm 2$ g and aged of 6–7 weeks old were infected with  $100 \pm 10$  *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 LD<sub>50</sub> 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 (G<sub>1</sub>): administrated orally LD<sub>50</sub> CH<sub>2</sub>Cl<sub>2</sub> extract of *Negombata magnifica* (*Nm*) (7.85 mg/kg body weight) for 2 consecutive days;

Group 2 (G<sub>2</sub>): administrated orally *Nm*-C<sub>4</sub>H<sub>8</sub>O<sub>2</sub> extract (11.25 mg/kg body weight) for 2 days.

Group 3 (G<sub>3</sub>): administrated orally *Nm*-CHCl<sub>3</sub> extract (10 mg/kg body weight) for 2 days.

for two consecutive days;

Group 4 (G<sub>4</sub>): administrated orally LD<sub>50</sub> CH<sub>2</sub>Cl<sub>2</sub> extract of *Callyspongia siphonella* (*Cs*) (12.32 mg/kg body weight) for 2 days;

Group 5 (G<sub>5</sub>): administrated orally *Cs* - C<sub>4</sub>H<sub>8</sub>O<sub>2</sub> extract (13.11 mg/kg body weight) for 2 days;

Group 6 (G<sub>6</sub>): administrated orally *Cs*-CHCl<sub>3</sub> extract (14.25mg/kg body weight) for 2 days;

Group 7 (G<sub>7</sub>): administrated orally praziquantel treated group (200 mg/kg body weight) for 2 days;

Group 8 (G<sub>8</sub>): 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 7<sup>th</sup>, 8<sup>th</sup> and the 9<sup>th</sup> 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).

### 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 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):

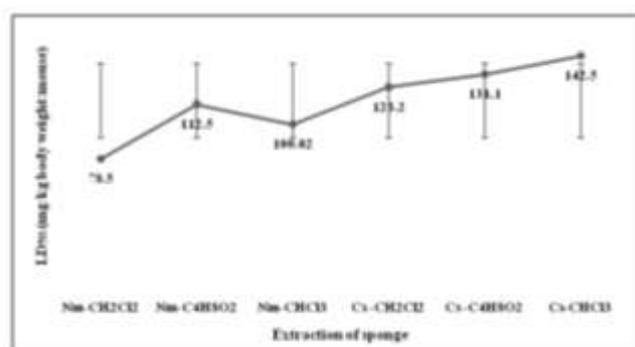
These were examined in three samples/mouse (Tendler *et al.*, 1986). Each piece was compressed between two clean glass slides and studied under a microscope (Mati and Melo, 2013). The mean of each stage/animal was obtained (Helmy *et al.*, 2009).

### Statistical analysis of data:

Results were expressed as means  $\pm$  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).



**Fig. 1: Lethal dose (LD<sub>50</sub>) values for infected mice after exposure time (one day) to Nm and Cs sponge extractions.**

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

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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	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
	8 weeks	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
	9 weeks	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**	0.73±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

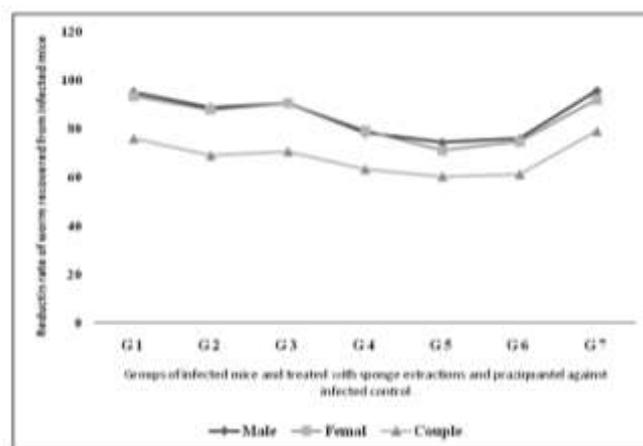


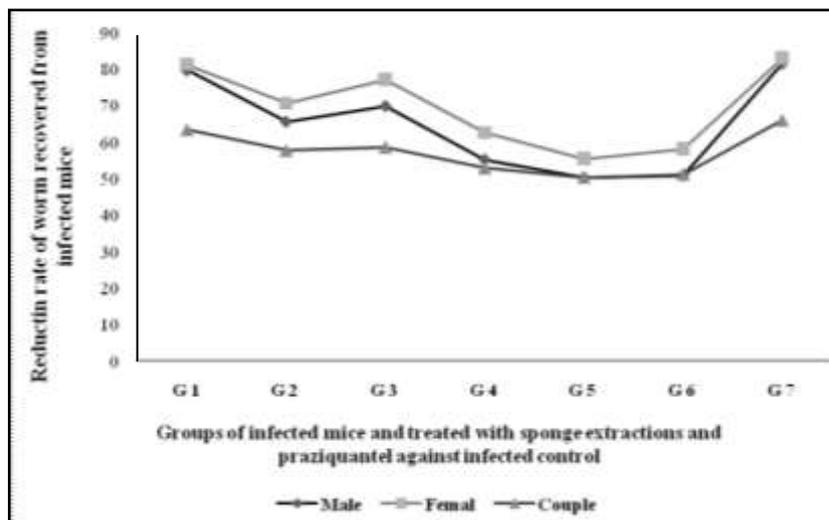
Fig. 2. Reduction rate of worms recovered from tissues of infected mice and treated groups post scarified at 9 weeks

Oral administration of infected mice to *Nm*-CH<sub>2</sub>Cl<sub>2</sub> extract (G<sub>1</sub>) and Praziquantel (G<sub>7</sub>) 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 (G<sub>8</sub>).

Also (G<sub>1</sub>), (G<sub>2</sub>) (G<sub>3</sub>) and (G<sub>7</sub>) 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 (G<sub>8</sub>) (Table 1).

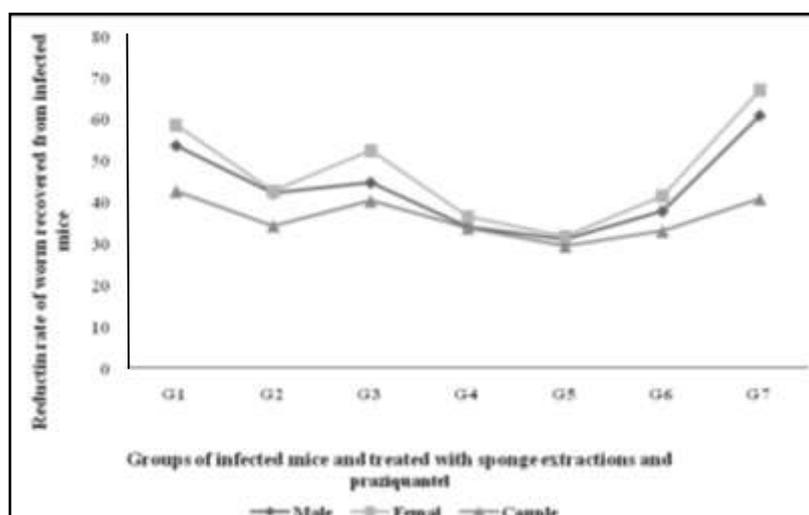
No significant reduction in the mean numbers of separate male and female or couple worms induced in treated infected mice of (G<sub>4</sub>), (G<sub>5</sub>) and (G<sub>6</sub>) at 8 WPT compared to the infected control group (G<sub>8</sub>) (Table 1 and Fig. 3). However, there was a reduction in number 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 (G<sub>1</sub>-G<sub>6</sub>), as well as (G<sub>7</sub>) compared

with the Non-treated infected mice group (G<sub>8</sub>) (Table 1 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 (G<sub>1</sub>-G<sub>6</sub>) and (G<sub>7</sub>) compared with the (G<sub>8</sub>) (Table 2).

Also sponge extractions from G<sub>1</sub> to G<sub>6</sub> resulted in no significant reduction in the number of eggs in the tissues liver and

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intestine of the treated group at 7 WPT compared with the non-treated infected group (G<sub>8</sub>). On the other hand, only (G<sub>7</sub>) 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 (G<sub>8</sub>) (Table 2).

Only (G<sub>1</sub>), (G<sub>2</sub>) and (G<sub>3</sub>), as well as (G<sub>7</sub>) 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).

Reduction rate in the ova count/g liver was a highly significant at 9 WPT (94 %), (83%), (89%), and (96%) in the case of group *Nm* extractions (G<sub>1</sub>), (G<sub>2</sub>), (G<sub>3</sub>) and (G<sub>7</sub>) respectively. The egg load in the intestine was a highly significant (92%), (81%), (90%) and (95%) in the same groups, respectively. Groups of *Cs*-extraction (G<sub>4</sub>), (G<sub>5</sub>), (G<sub>6</sub>) were significant (79%), (72%) and (77%) in the liver at 9 WPT. in the (G<sub>4</sub>) and (G<sub>6</sub>) only produced significant decrease (72%) and (71%), respectively in intestine eggs count at 9 WPT (Table 2).

Table 2. Effect of sponge extractions and PZQ on fecal egg counts and eggs recovered from tissues in infected treated mice compared with non-treated infected mice according to weeks post-treatment

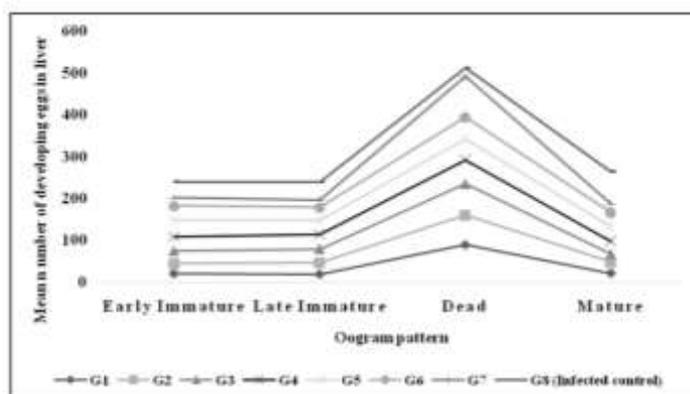
Variable		Number of eggs after scarified						Number of eggs before scarified (Mean±SE)
		Periods post infection (Mean±SE)						
		7 Weeks		8 Weeks		9 Weeks		
		L	I	L	I	L	I	
Number of mice groups	1	5.75±0.43	7.01±0.51	3.92±0.31	4.35±0.25	0.78±0.18	1.09±0.17	15.65±0.053
	2	7.56±0.64	8.54±0.53	5.23±0.27	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±0.203

Numbers of eggs ×10<sup>3</sup> 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)

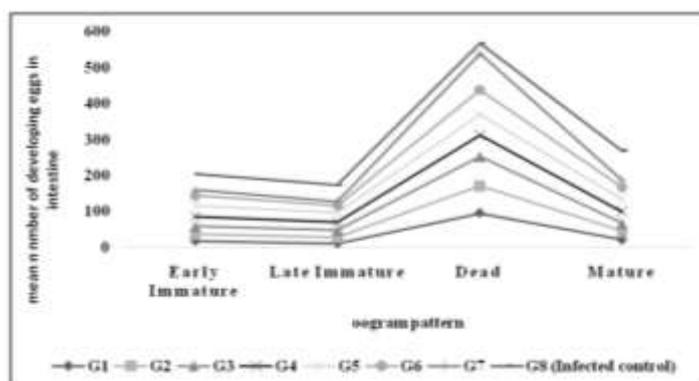
### Oogram recorded:

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*-extractions treated group (G<sub>1</sub>), (G<sub>2</sub>), and (G<sub>3</sub>) showed a significant in the mean number of dead eggs, immature and mature in the liver tissues at 9 WPT. The

other groups (G<sub>4</sub>), (G<sub>5</sub>) and (G<sub>6</sub>) had a non-significant correlation. The treated G<sub>7</sub> had recorded a highly significant in the mean number of dead eggs immature and mature compared with the non-treated infected group (G<sub>8</sub>). 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	
Oogram pattern	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±2.3	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

## 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-Mattocchia 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.

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## عوامل مضادة لداء البهارسيا من مستخلصات الإسفنج المستخرجة من خليج العقبة، البحر الأحمر في مصر

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## المستخلص

مرض البهارسيا هو مرض طفيلي ناجم عن الإصابة بـ *الديدان المفلطحة*، يصيب ملايين على مستوى العالم. اللافقاريات البحرية عامة والإسفنجيات البحرية خاصة كائنات واعدة لإنتاج مركبات نشطة بيولوجيا. والهدف من الدراسة الحالية محاولة تطوير وإستبدال مضادات لمرض البهارسيا جديده بدلا من إستخدام الأدوية الإصطناعية. وقد تم تجميع نوعين من الإسفنج البحرى هما *نيجومياتا ماجنيفيكا* (ن م) و *كاليسيونجيا سيفونيللا* (ث س) وتم استخراج مستخلص منها للتحقيق فى تأثيرها كمضادات لمرض البهارسيا. تم إذابة عينات الإسفنج فى درجة حرارة الغرفة لمدة يوم واحد مع كلوريد الميثيلين و خلاص الإيثيل والكلوروفورم بشكل منفصل ثم تصفية العينات وتجفيفها عند درجة حرارة 40 درجة مئوية بـ استخدام مبخر روتارى. وتم حفظ الكتل التى تم تجفيفها عند 2 درجة مئوية حتى يتم إستخدامها. تم إصابة الفئران بواسطة السركاريا من خلال معهد تيودور بلهارس للأبحاث. تم إعطاء واحد على عشرة من الجرعة من متوسط التركيز المميت للفئران عن طريق الفم. تم تسجيل إثار كل مستخلص على كل من الديدان وعدد البيض الكلى. أوضحت النتائج ان الجرعة التى تم إعطائها عن طريق الفم لكل من المستخلصات بداية من المجموعة الأولى والثانية والثالثة والرابعة حتى المجموعة السادسة كانت كما يلى (7085)، (11.25)، (10)، (12.32)، (13.11)، (14.25) ملي جرام لكل كيلوجرام من الجسم من الفئران على التوالي. الجرعة التى تم اعطائها عن طريق الفم للمجموعة الاولى والثانية والثالثة للفئران المصابة خلال 9 اسابيع من العلاج تسببت فى حدوث انخفاض كبير للغاية فى متوسط عدد الذكور والإناث للديدان. وكانت نسبة تأثر ذكور الديدان أكبر من الإناث فى الأسبوع التاسع بعد العلاج. أيضا تسببت المجموعة الأولى والثانية والثالثة فى انخفاض كبير فى عدد ديدان الإناث مقارنة بمجموعة الفئران الغير معالجة (المجموعة الثامنة). وكانت الإناث أكثر تأثرا من الذكور بعد 8 اسابيع من العلاج. المجموعة الأولى والثانية والثالثة وكذلك المجموعة السابعة أدوا الى إنخفاض كبيرا فى أعداد البيض الموجود فى كل من الكبد والأمعاء فى الأسبوع الثامن بعد العلاج.

الخلاصة: توصلت النتائج الى كفاءة إستخدام المستخلص من الإسفنج *نيجومياتا ماجنيفيكا* و *كاليسيونجيا سيفونيللا* كمضادات لمرض البهارسيا وأنه من الممكن أن يكون من العوامل الحيوية النشطة.

الكلمات الدالة: مضادات البهارسيا ، مسخلصات الإسفنج ، خليج العقبة ، البحر الاحمر، مصر.