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THE POSSIBLE ANTISCHISTOSOMAL EFFECT OF ALLICIN IN *SCHISTOSOMA MANSONI* INFECTED ALBINO MICE

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Abstract

Schistosomiasis is endemic mainly in Africa and the Middle East Countries, causing acute and chronic clinical pathogenicity. This study evaluated the potential antischistosomal efficiency of allicin against *S. mansoni* infected mice. Twenty CD1 male Albino mice were divided into two groups (10 mice/group). G1 (infected and treated with 0.9% NaCl), and G2 (infected and treated with 20mL/kg of allicin). Seven weeks post-infection, each group was intraperitoneally injected with allicin (3 times/week), sacrificed at the end of the 8th-week. Liver was harvested for studing worm burden, egg load, and oogram pattern. The recovered worms from both groups were subject to SEM and DNA fragmentation tests to monitor the differences.

The results showed a significant reduction in worm burden and changes in oogram pattern of G2 mice as compared to G1 (control). Allicin gave marked deformation of oral, ventral suckers and atrophy of the tubercles as well as marked fragmentation of the worms DNA.

Keywords: Antischistosomal, allicin, ultrastructure, reduction rates, DNA.

Introduction

Schistosomiasis is considered as a parasitic disorder resulted from infection with blood flukes (trematode worms) of the genus *Schistosoma* (WHO, 2019). An estimated 240 million people are affected in 78 countries, and close to 800 million are at risk (Butrous, 2019).

Schistosomiasis cause acute and chronic clinical syndromes (Bonnefond *et al*, 2019). Its deaths was difficult to estimate due to hidden pathology as liver and kidney failure, bladder cancer and ecto-pic pregnancies by genital infection (WHO, 2019).

Before the availablility commercial anthelmintics drugs, worms were managed by certain plants based mainly on belief pain rather than knowledge (Hrckova and Velebeny, 2013). Scientists searched for antiparasitic drugs better than the risky praziquantel (Stelma *et al*, 1995) from medicinal plants for development of new safe treatment (Abdel Hady *et al,* 2008; Sadref-ozalayi *et al*, 2018). *Allium sativum* (garlic) has a high concentration of different sulfur compounds that are responsible for its flavor and health therapeutic effects as anti-inflammatory, antitumor, and antioxidant (Moutia *et al*, 2018). Allicin [S-(2-propenyl) 2-propene-1-sulfinothioate] is one of the most greatly common organosulfur compounds derived from *A. sativum* and responsible for many beneficial effects associated with this plant (Rahman, 2007; Borlinghaus *et al*, 2014). The different beneficial effects of allicin as antitumor, and antioxidant have been reported (Gruhlke *et al*, 2017; Huang *et al,* 2017).

Recent studies depended on different aspects in evaluation of antihelminthic efficacy as drugs. The worm burden, egg density, and physiological parameters, the drug ability to affect the worms on the molecular levels in different ways increased (Metwally *et al*, 2018; Morais *et al*, 2018; El-khadragy *et al*, 2019).

This study aimed to evaluate the anti-schistosomal efficiency of allicin in experimentally infected mice based on parasitologic examination, ultra-structural changes, and DNA fragmentation assay.

Material and Methods

Experimental animal and design: Twenty CD1 white male Albino mice with an average weight of 20±2g were obtained from

Theodore Bilharz Research Institute (TBRI, Giza, Egypt). Albino mice were kept in two plastic cages (10mice/cage) in the animal house, Zoology Department, Faculty of Science Port-Said University. They were divided into two groups (10mice/group): G1 (infected treated with saline/control), and G2 (infected and treated with allicin).

Infection: Mice were incubated with 60± 10 *S. mansoni* cercariae for one hr using a partial immersion technique (Olivier and Stirewatt, 1952). Seven weeks post-infection, stool samples were collected from all mice and examined by light microscope for *S. mansoni* eggs.

Treatment: Allicin liquid form was obtained pure from Science-Med, Egypt. Distilled water was used to proper concentration (20 mL/kg of body weight), according to Zhang *et al*. (2013). The concentration was prepared immediately before injection. At the 7th week post-infection, mice were injected intraperitoneal with 0.1 ml of NaCl (0.9%) for G1, and 0.1 ml of allicin (20 ml/kg body wt) for G 2. The injections were applied for two weeks (three days/week). Mice of G1 & G2 were sacrificed after 8th week post-infection.

Worm burden: Worms were recovered from tissues following Wang *et al.* (2004) methodology. Immediately, after dissection of the animals, each tissue was placed into a plastic folder and then compressed between two clean glass plates until the parenchyma was evenly strewn into a transparent layer. The intestines were removed and located in a petri dish to examine the mesenteric veins (Kloetzel, 1967). The worms were counted under a stereomicroscope and classified into male, female and copulated. Reduction rate of recovered worms from treated mice as compared to untreated ones was represented in formula: $P = (C-V) / C \times 100$ (Tendler *et al*, 1986). As (P) means the percentage reduction, C represented the number of recovered worms from the infected-untreated group, and V means number of parasites recovered from the infected-treated animals.

Egg load/density: Post scarification 0.5gm of livers and intestines were put in 5% KOH solution tube and incubated for 24hr at 37 ºC for (Cheever, 1968). Total egg count represented the mean number of eggs/mg of each tissue (Helmy *et al*, 2009). The percentage of reduction of the total eggs removed from each tissue of allicin treated mice was applied after equation of Tendler *et al*. (1986) with a minor modification

Oogram pattern: Equal fragments of liver and intestine from both groups were washed with 0.09% NaCl and left to dry on filter paper. Each tissue was compressed between 2 clean glass slides. Developmental stage of 100 eggs maturity was classified into dead, immature (early immature, and late immature), and mature (Pellegrino *et al*, 1962) to detect each stage. Reduction percentage of each stage of maturity was calculated as mentioned before.

SEM: Recovered worms from both groups were fixed in equal amount of 4% glutaraldehyde and cacodylate 0.2 for 2hr., washed in sucrose 0.4 and cacodylate 0.2 for 2hr and then in osmium 2% and cacodylate 0.3 for 1hr for post-fixation. Worms were washed with distilled water and dehydrated in ascending series of ethanol for 5min each (30, 50, 70, & 90%) and in 100% ethanol for 10 min. Samples were then were let to dry and mounted on copper stubs using double-sided adhesive tape, coated with gold using an S150A sputter coater (Edwards, UK). Images were captured using a Philips XL30 SEM (Philips, Eindhoven, Netherlands) operated at 10-30 kV, at the Electron Microscopy Unit, Theodor Bilharz Research Institute.

Detection of DNA fragmentation by agarose gel analysis: Worms from both groups were subject to DNA extraction "DNeasy Plant Mini Kit (Qiagen)" after (Miller *et al*, 1988). DNA separation was done using gel electrophoresis on a 2% agarose gel contained 1% GelRed (1:500). BIO-RAD Gel DOC TM XR+ to visualize DNA fragmentation.

Statistical analysis: Data were subjected to Student's t-test version 20 of SPSS program to determine significance. $P < 0.05$ was interpreted as statistically significant.

Results

The average of total worms recovered from infected saline mice was 47±5.10 (Tab.1). Allicin showed a significant efficiency in elimination of total number of recovered worms with a reduction of 51.06% as compared with control. The highest significant reduction rates were recorded for male worms by 78.57%, but non-significant reduction rates in egg density in liver (0.21%) or intestine (24.68%).

Oogram pattern of eggs: Dead worms were noticed by deformations in external shape and internal contents of eggs (Fig. 1). Percentage of each stage and rate of changes in oogram patterns in liver and intestine was given (Tab.2). The percentage of dead eggs in liver of allicin-treated mice was 24% with a significant reduction rate P < 0.01 as compared with control. Dead eggs percentage from the treated mice is higher than that of saline animals by 15-folds, with a significant change in total immature eggs in allicin-treated mice as compared to control. Oogram pattern in intestine showed significant change in dead eggs higher than in liver. Reduction rate of deed eggs in allicin-treated animals as compared with control was 30-folds. Total immature eggs recorded a highly significant change as compared to control.

The ultrastructural differences in the tegument of the male worms recovered from the infected saline mice (control/saline worms) and allicin-treated groups (allicin worms). Oral sucker of the control worms was smaller, and less protuberant, and edged than the ventral sucker (Fig. 2A). Oral sucker of allicin worms was larger and more dilated than the normal one (Fig. 2B), with tegument denaturation and internal swelling which was more obvious at higher magnification (Fig. 2C). Ventral sucker in treated mice showed a less prominent outer edge as compared with control oral sucker, internal swelling and tegument denaturation (Fig. 2B). Tegument of control male worms showed several tubercles arranged in regular form and separated by the intertubular ridges (Fig. 2D). Tegument in allicin male worms showed great changed as compared with control. The intertubular ridges were not observed, the tubercles suffered from atrophy, this disappearance of tubercles was complete in some regions and partial in the others (Fig. 2E), tubercles at higher magnification were highly deformed (Fig. 2F).

Gel electrophoresis of recovered worms from both groups (Fig. 3), in control worms (lane A), no fragmentation in DNA pattern. Reversely, the allicin-worms were suffering from the laddering DNA (Lane B).

Table 1: Worm burden and egg load per gram of liver and intestine in mice treated with allicin.

Variants	Worm burden				Egg load	
	Male	Female	Couple	Total	Liver	Intestine
Average	3.6±1.69	11.2±2.42	8.20±2.35	23±3.31	6500±1683.25	14240.25±2010.48
Reduction rate	78.57	43.43	21.15	51.06	0.21	24.68
P value	0.02	-	-	0.03	-	-

P value= reduction rates to control

Table 2: Oogram pattern of eggs at different stages of maturity in liver and intestine of mice treated with allicin.

Variations	Oogram in liver					Oogram in intestine				
	Dead	Early Immature	Late Immature	Total	Mature	Dead	Early Immature	Late Immature	Total	Mature
Percentage	24	5	11.75	16.75	59.25	24	19.50	16	35.50	40.50
Change rate	-1400	70.59	47.54	57.49	-0.42	-2900	-33.56	50.62	24.47	22.41
P-value	0.01	0.04	0.04	0.03	-	0.001	-	0.02	0.003	-

Discussion

Worms diminished in treated animal showed of anti-schistosomal efficacy (Selem *et al*, 2018; Sadrefozalayi *et al,* 2018; El-Khadragy *et al,* 2019). In the present study, allicin gave (51%) high reduction rate of recovered worms than (21.7%) by *A. sativum* crude (Metwalley, 2015). Allicin gave non-significant reduction in egg density in liver (0.21%) and intestine (24.68%) as compared to control. The result disagreed with Metwally *et al.* (2018). Dead eggs were 24% in liver and intestine of allicin-treated mice, respectively, with a significant change rate as compared to control. The changes in oogram pattern and increased percentage of dead eggs was due to allicin as garlic crude or its oil proved to have significant change in oogram pattern (El-Shenawy *et al*, 2008; Mantawy *et al*, 2011; Metwally *et al*, 2018).

Tegument of adult *Schistosoma* is a protective sheath that improves the defense and also essential in biological functions as in the uptake of nutrients, osmoregulation, and excretion. Hence, the importance of topographical studies clarified aspects of drug-induced damage (Amin and Mikhail, 1989). The normal tegument also plays the fundamentally the main role to link the parasite with the intravascular environment in its host (El-Shabasy *et al*, 2015).

In the present study, both suckers suffered from tegumental denaturation with internal swelling due to the intravascular importance of suckers. This change agreed with Fakahany *et al*. (2014); Hassan *et al*. (2016) and Matos-Rocha *et al*. (2017).

The characters of male tubercles tegument were greatly affected and became atrophied with some regions completely were devoid of these tubercles. In harmony with the present result, Lima *et al*. (2011) tested allicin efficiency *in vitro* at different doses, which resulted in integumental deformation as wrinkling, tegumental drilling, deformation of tubercles, ulceration, and formation of vesicles.

Gel electrophoresis reflected that allicin is a genotoxic compound against *S. mansoni* worms caused laddering and fragmentation of DNA. Osman *et al.* (2016) found that garlic water extract is an antihelminthic drug causing genomic instability and DNA variation in *Capillaria* species.

Conclusion

Allicin has antischistosomal effect caused significant elimination of adults and increased dead eggs in the oogram pattern. It also caused severe ultra-structural tegmental lesions and acting as a genotoxic compound against *S. manosoni* resulted in fragmentation of their DNA.

Ethical standards: Ethical considerations were confirmed by the Zoology Department and the Ethical Committee of Faculty of Science, Port Said University.

Statement of conflict: The authors neither have conflict of interest nor received fund.

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Explanation of Figures

Fig. 1: Oogram pattern of *S. mansoni* egg at different stages of maturity.

Fig. 2: SEM of *S. mansoni* male: A, D (control), B-C, E-F (allicin treated); Oral sucker (OS), ventral sucker (VS); internal swelling, tubercles, intertubular ridges (arrow).

Fig. 3: Electrophoretic separated genomic DNA from *S. mansoni* M (ladder marker), A (control), B (allicin treated).