Effect of Allicin on *Schistosoma mansoni* mature and immature worms

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Abstract

Background: Garlic is this ancient medicinal plant which has a wide range of uses, including fighting against microbes, viruses, fungi, protozoa, and helminths. Allicin is the active ingredient in garlic and responsible for its antiparasitic effects. The current research was conducted to demonstrate the possible curative and preventative benefits of allicin on both adult and juvenile *S. mansoni* worms. Methods: For 96 hours, immature and adult *S. mansoni* worms were cultured with varying doses of allicin (25, 50, 100, and 200 µg/ml). Their motor activity and death rate were evaluated every 24 hours using light microscopy. The ultrastructural effects of allicin on worms, were assessed by scanning electron microscopy. The researchers used 40 male Swiss albino mice strain CD1, divided into four groups with 10 animals in each group. Allicin was administered to three groups at varied intervals after they were infected with *S. mansoni* cercariae. Parasitological studies, including worm burden, oogram pattern, and tissue egg load, were used to assess the efficacy of allicin on day 54 after infection. The results revealed a high significant difference in *S. mansoni* immature worms when incubated with allicin at doses of 50, 100, and 200 µg/ml. Also incubating adult worms of *S. mansoni* with allicin at concentrations of 100 µg/ml and 200 µg/ml were significant. In vivo, there was non-significant impact on worm load with high significant rise in dead egg percentage. Conclusion: These data suggest that allicin is effective against *S. mansoni* worms in vitro, but with little impact in vivo.

Keywords: Topics covered include allicin, *S. mansoni*, schistosomiasis, and worms (both adult and juvenile).

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1. Introduction

Human schistosomiasis is among the most ignored tropical parasitic infections. Worldwide, schistosomiasis is endemic in 77 nations in the tropics and subtropics with estimated 250 million infected people [1]. The prevalence of schistosoma infection is considerable in North Africa and the Middle East, with around 7.2 million affected people in Egypt alone [2]. After the Aswan High Dam was built, *S. mansoni* became much more common in the Nile Delta of Egypt than *S. haematobium* [3]. The health and financial costs associated with schistosomiasis infection are substantial.

Praziquantel (PZQ) is the only drug used globally for the control of schistosomiasis. Despite the drug's efficacy and safety in treating the disease there is growing evidence in researches suggests that PZQ cannot eradicate the early stages of schistosomes [4]. Meanwhile, in endemic regions, resistance is becoming more apparent [5]. This has piqued the interest of several researchers to look for potential alternative medicinal plants for schistosomiasis therapy. One plant-based medicinal compound that shows promising effects is garlic. Allicin, garlic's active ingredient, is responsible for the herb's sulfhydryl modifying activity, which makes it an effective antiparasitic agent [6]. The effects of allicin on parasites were investigated in few studies. Consequently, the purpose of this research is to investigate the possible therapeutic and/or prophylactic benefits of allicin against schistosoma.

2. Materials and Methods

The fieldwork was carried out in the Schistosome Biological Supply Center (SBSC), Theodor Bilharz Research Institute (TBRI), Giza, Egypt.

Drug: Allicin was bought from www.iherb.com in the form of liquid (Allimax liquid).

(1) In vitro effect of allicin on *S. mansoni*: Immature and adult *S. mansoni* worms were incubated in petri dishes for 96 hours with variety of allicin concentrations (ranging from 25 to 200 µg/ml). Their motor activity and death rate were evaluated

every 24 hours using light microscopy. In order to study the ultra-structural effects of allicin on worms, scanning electron microscopy was used.

(2) The impact of allicin on *S. mansoni* in experimental mice:

Mice infected with *S. mansoni* were classified as following

(Ten mice per group):

Group 1: Infected non-treated (control).

Group 2: Infected and treated with allicin (8mg/ Kg by intravenous route) 24 hrs. before infection, the same day of infection and 24 hrs. post infection (prophylactic group).

Group 3: Infected and treated with allicin (8mg/ Kg by intravenous route) one week post infection for 3 days (therapeutic effect on schistosomules).

Group 4: Infected and treated with allicin (8mg/ Kg by intravenous route) 6 weeks post infection for 4 days (therapeutic effect on adult worms).

After 54 days of infection, all of the mice were scarified.

Parasitological studies, including worm burden, oogram pattern, and tissue egg load (liver and intestine), were used to assess the impact of allicin on *S. mansoni* infected mice.

Data analysis using statistical methods

The data was coded, tabulated, and analyzed using SPSS version 20. The Student's t-test is a statistical tool for comparing the means of two sets of parametric data. The Fisher exact test (FET) was used for inter-group comparison of categorical data. In all analyses, a P-value less than 0.05 was considered statistically significant, a P-value less than 0.0001 was considered highly significant, and a P-value more than 0.05 was considered insignificant.

3. Results

1) In vitro effect of allicin on *S. mansoni* :

The results of incubating adult worms of *S. mansoni* with allicin at concentrations of 100 µg/ml and 200 µg/ml were significantly different from the control non-treated group. At an allicin concentration of 200 µg/ml, all worms were killed completely after 72 hours, but at a concentration of 100 µg/ml, it took 96 hours to get the same result. At allicin dosages of 25 and 50 µg/ml, no fatal impact was seen until the end of the experiment (Table 1).

There was a high significant difference when comparing the control group that was not treated with allicin with the group incubated with *S. mansoni* immature worms at doses of 50, 100, and 200 µg/ml. At an allicin dosage of 200 µg/ml, all worms were killed in about 72 hours, but at a concentration of 100 µl/ml, it took 96 hours to kill half of the worms. No worms died at a dose of 50 µl/ml; however their movement was slow throughout the trial. Up to the end of the experiment, allicin at concentration of 25 µg/ml had no effect (Table 2).

(2) The impact of allicin on *S. mansoni* in experimental mice:

1- Worm burden (54 days post infection): all treated groups showed a slight but statistically insignificant drop in the mean number of worms, male and female. Statistically, there was no significant increase in the mean number of couple worms and total worm burden across all treated groups (p value >0.05), as shown in Table 3.

2- Oogram pattern, (54 days after infection): it was observed that the group treated with allicin six weeks post infection had a significant increase of dead eggs and a significantly lower percentage of immature and mature eggs (P value <0.01). A high significant rise in the percentage of dead eggs was seen in the prophylactic group (P value <0.01), although there was no significant drop in the number of mature and immature eggs (P value >0.05). The percentage of immature eggs decreased significantly in the group treated with allicin one week after infection (p value <0.01), but the percentages of mature and dead eggs increased non-significantly (p value >0.05), as shown in Table 4.

The results showed the changes in the four stages of immature ova in oogram pattern in intestinal tissue. Prophylactic group had significant reduction on 1st stage (p value <0.05) and non-significant effect on 2nd, 3rd and 4th stages (p value >0.05) compared with the control. Allicin treated group 1 week post infection group had significant reduction on 3rd stage (p value <0.05) and non-significant reduction on 1st, 2nd and 4th stages compared with the control (p value >0.05). Allicin treated group 6 weeks post infection group had highly significant reduction on 1st stage (p value <0.01) and significant reduction on 2nd, 3rd and 4th stages compared with the control (p value <0.05) (Table 5).

3-Tissue egg load in liver and intestine (54 days post infection): In the prophylactic group, there was a high significant decrease in the ova count per gram of intestinal tissue (p value <0.01), whereas in the allicin treatment group one week post infection, there was a non-significant decrease (p value >0.05). A non-significant increase in ova count/gm. of intestinal tissue was observed in the allicin-treated group six weeks after infection (p value >0.05) when compared to the control group. But this group showed significant difference when compared with prophylactic group and allicin treated group 1 week post infection (P value <0.05).

In comparison to the control group, the group treated with allicin 6 weeks post infection had a significant increase in ova count/gm. of hepatic tissue (p value <0.05), whereas the groups treated with allicin 1 week post infection and the prophylactic group showed non-significant increases (p value >0.05). Allicin treated group six weeks post infection showed significant difference when compared with prophylactic group and allicin treated group one week post infection (P value <0.05). (Table 6).

4.Discussion

Praziquantel according to the WHO is the exclusive anti-schistosomal treatment option. Although it works, there are major concerns about using a single pharmacological treatment in the long run, and one of those problems is the evolution of drug resistance [13]. Another big drawback of this therapy aside from resistance,

the partial cure rate with juvenile stages of schistosomes compared to adult worms [14].

Several medicinal plants have emerged as anti-parasitic medications and played a major role in recent years. Although some plant species used in traditional medicine have shown effectiveness in treating helminthes, very few have been tested for their ability to combat S. mansoni. The primary chemical ingredient in garlic is allicin, which is an organosulfur molecule [17].

Allicin is the active ingredient in garlic that has antibacterial properties. Garlic processing variations result in unreliable processed garlic standards, and even standardized brands might differ in allicin concentration and bioavailability [18].

In this study, allicin was examined in vitro and in vivo against both adult and juvenile S. mansoni worms. The effectiveness was assessed in an in vitro experiment by monitoring the worms' motor activity and death rate. The effectiveness was assessed in vivo experiment by determining the worm burden. Based on our findings, when adult S. mansoni worms were incubated with 200µg/ml of allicin in a petri dish, the motility of the worms decreased within 24 hours, and they died out entirely after 72 hours. In contrast, when the concentration was 100µl/ml, the motility of the worms decreased within 48 hours, and they died out entirely after 96 hours. Adult S. mansoni incubated in vitro at doses of 50µg/ml of allicin did not show any impact. Only one study that dealt with the in vitro effects of allicin on adult S. mansoni. In this study worms was incubated with allicin at doses of 5, 10, 15, and 20 µg /ml, no worm mortality was seen within 2 hours, according to this work[19]. Their short period of observation (only 2 hours after treatment) may explain these findings. According to the results of this study the in vitro incubation of S. mansoni immature worms at a concentration of 200 µg/ml of allicin, reduced motility within 24 hours and all of the worms died in 72 hours. At a concentration of 100 µg/ml, the motility decreased within 48 hours, and half of the worms died in 96 hours. At doses of 50 µg/ml, worm motility reduced after 72 hours without worm death for up to 96 hours, while lower concentrations did not show any impact.

The efficacy of allicin is thought to be due to a disulfide exchange-like interaction that it has with the sulfhydryl-group of cysteine [20]. Using scanning electron microscopy

(SEM) in the current research demonstrated that allicin had a noticeable impact on the tegument of juvenile S. mansoni worms. The worms' oral suckers swelled, their ventral suckers shrank, and there was significant tegumental degradation along with the appearance of vesicles. The current research also showed that allicin affected the tegument of adult worms. Scanning electron microscopy (SEM) revealed that the worms' teguments were extensively damaged, with edema of the oral sucker, vesicles formation, many erosions, and visible peeling. These results are in agreement with those of [19], who found that allicin treatment caused alterations to the tegument of adult S. mansoni at all doses, including tubercle destruction, vesicle development, and ulcers that exposed the worm's muscles.

In this work treatment of experimental mice with allicin resulted in decrease in the number of female and male worms and increase in the number of couples and total worms, but none of these effects were statistically significant, suggesting that allicin did not significantly affect worm burden. Only one study that we are aware of addressed the topic of allicin's impact on mice infected with S. mansoni. In contradiction with our result, [21] reported that allicin significantly reduced the mean worm count (20.33%) compared to the control. Different doses, timings, and routes of allicin administration might account for this disparity. The oogram pattern revealed, that allicin-treated group had a much higher proportion of dead ova and a significantly lower percentage of immature and mature ova in comparison to the infected untreated group. These effects are more pronounced in the allicin-treated group six weeks after infection. According to these findings we suggest that allicin may affect adult worm's fertility.

Consistent with previous findings, which reported that the percentage of mature ova increased, whereas the percentage of immature ova decreased and the percentage of dead ova increased. Results of this work also demonstrated that ova count in intestinal tissue was significantly lower in the prophylactic group compared to the control group, while no significant impact was seen in the other groups. According to these results allicin may be beneficial if administered early as a prophylactic treatment. These findings are in line with the previous work by [21], which stated that the intestinal tissue ova count was decreased after allicin administration.

In contrast, the group that received allicin six weeks after infection showed a statistically significant rise in ova count in hepatic tissue. The cause may be presence of high number of dead ova in this group, which were unable to pass outside. This finding disagrees with [21], who found reduction in hepatic tissue ova count after allicin administration.

Allicin is quickly metabolized to allyl-mercaptoglutathion, diallyl disulfide, diallyl trisulfide, and other thiosulfate metabolites in the blood circulation; this may be the cause of mild impact of allicin on S. mansoni infection in mice [22]. It is possible that these metabolites do not have any effect on S. mansoni. Besides allicin may be interacting with other serum proteins that contain free sulfhydryl groups [22]. On the other hand, allicin may be influencing the host immune system in some way, decreasing release of TNFα which has a significant impact on the control of the immunological response. In a study conducted by [23], it was shown that allicin blocks the production of pro-inflammatory cytokines in intestinal epithelia decreasing intestinal inflammation.

In conclusion, our work showed that allicin kills adult S. mansoni worms effectively in vitro, but has mild effect in vivo. To identify allicin activity against S. mansoni, more in vivo studies are required.

5. References

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Table (1): In vitro effect of allicin on *S.mansoni* adult worms

Groups	No. of worms	Incubation period	Adult worm activity No. (%)				P value with control group	P value of different times in the same group
			Normal	Slow	Sluggish	Dead		
Healthy control	14 7"	24 hours 48 hours 72 hours 96 hours	14(100%) 14(100%) 14(100%) 7(100%)	0(0%) 0(0%) 0(0%) 0(0%)	0(0%) 0(0%) 0(0%) 0(0%)	0(0%) 0(0%) 0(0%) 0(0%)		
200 µg Allicin /ml media	14 0"	24 hours 48 hours 72 hours 96 hours	0(0%) 0(0%) 0(0%) 0(0%)	0(0%) 0(0%) 0(0%) 0(0%)	14(100%) 2(14.3%) 0(0%) 0(0%)	0(0%) 12(85.7%) 14(100%) 0(0%)	<0.001** <0.001** <0.001** -	<0.001**
100 µg Allicin /ml media	12 7"	24 hours 48 hours 72 hours 96 hours	12(100%) 0(0%) 0(0%) 0(0%)	0(0%) 12(100%) 0(0%) 0(0%)	0(0%) 0(0%) 12(100%) 0(0%)	0(0%) 0(0%) 0(0%) 7(100%)	- <0.001** <0.001** <0.001**	<0.001**
50 µg Allicin /ml media	11	24 hours 48 hours 72 hours 96 hours	11(100%) 11(100%) 11(100%) 11(100%)	0(0%) 0(0%) 0(0%) 0(0%)	0(0%) 0(0%) 0(0%) 0(0%)	0(0%) 0(0%) 0(0%) 0(0%)		
25 µg Allicin /ml media	12	24 hours 48 hours 72 hours 96 hours	12(100%) 12(100%) 12(100%) 12(100%)	0(0%) 0(0%) 0(0%) 0(0%)	0(0%) 0(0%) 0(0%) 0(0%)	0(0%) 0(0%) 0(0%) 0(0%)		

Media: RPMI 1640 with L-Glutamine.

** P value <0.001: highly significant difference.

"Remaining number of worms after taking some worms for scanning electron microscopy examination.

Table (2): In vitro effect of allicin on *S. mansoni* immature worms (21 days old).

Groups	No. of worms	Incubation period	Immature worm activity No. (%)				P value with control group	P value of different times in the same group
			Normal	Slow	Sluggish	Dead		
Healthy control	17 8"	24 hours 48 hours 72 hours 96 hours	17(100%) 17(100%) 17(100%) 8(100%)	0(0%) 0(0%) 0(0%) 0(0%)	0(0%) 0(0%) 0(0%) 0(0%)	0(0%) 0(0%) 0(0%) 0(0%)		
200 µg Allicin /ml media	17 0"	24 hours 48 hours 72 hours 96 hours	0(0%) 0(0%) 0(0%) 0(0%)	17(100%) 0(0%) 0(0%) 0(0%)	0(0%) 17(100%) 0(0%) 0(0%)	0(0%) 0(0%) 17(100%) 0(0%)	<0.001** <0.001** <0.001** -	<0.001**
100 µg Allicin /ml media	15 4"	24 hours 48 hours 72 hours 96 hours	15(100%) 0(0%) 0(0%) 0(0%)	0(0%) 15(100%) 0(0%) 0(0%)	0(0%) 0(0%) 15(100%) 2(50%)	0(0%) 0(0%) 0(0%) 2(50%)	- <0.001** <0.001** <0.001**	<0.001**
50 µg Allicin /ml media	14	24 hours 48 hours 72 hours 96 hours	14(100%) 14(100%) 0(0%) 0(0%)	0(0%) 0(0%) 14(100%) 0(0%)	0(0%) 0(0%) 0(0%) 14(100%)	0(0%) 0(0%) 0(0%) 0(0%)	- - <0.001** <0.001**	<0.001**
25 µg Allicin /ml media	5	24 hours 48 hours 72 hours 96 hours	5(100%) 5(100%) 5(100%) 5(100%)	0(0%) 0(0%) 0(0%) 0(0%)	0(0%) 0(0%) 0(0%) 0(0%)	0(0%) 0(0%) 0(0%) 0(0%)		

Media: RPMI 1640 with L-Glutamine.

** P value <0.001: highly significant difference.

" Remaining number of worms after taking some worms for scanning electron microscopy examination.

Table (3): Effect of allicin on *S. mansoni* mature worm burden in infected mice (54 days post infection).

Groups	No of couples mean± SD	No of male worms mean± SD	No of female worms mean± SD	Total worm burden mean± SD
Control group	5.63± 2.13	2.38± 0.92	0.75± 0.46	14.38± 4.31
Prophylactic group	6.86± 2.27	1.57± 1.27	0.43± 0.54	15.71± 3.55
Allicin treated group 6 weeks post infection	8.29± 3.45	1.71± 1.25	0.43± 0.54	18.71± 6.18

Control group: infected not treated.
Prophylactic group: treated with allicin (8mg/ Kg by intravenous route) 24 hours before infection, the same day of infection and 24 hours post infection.
Allicin treated group 6 weeks post infection: treated with allicin (8mg/ Kg by intravenous route) 6 weeks post infection (therapeutic effect on adult worms).

Table (4): Effect of allicin on oogram pattern of *S. mansoni* infected mice (54 days post infection).

Groups	Mature egg % mean± SD P value	Dead egg % mean± SD P value	Immature egg % mean± SD P value
Control group	51.41± 11.1	13.04± 5.88	34.48±8.53
Prophylactic group	40.99± 15.11	26.73± 11.07 0.009**	33.09±7.9
Allicin treated group 1 week post infection	60.51± 11.21 **b**	19.16± 11.02	20.3±10.35 **b** 0.003**
Allicin treated group 6 weeks post infection	24.66± 8.54 **bc** <0.001**	65.35± 8.11 <0.001**	9.96±5.19 **bc** <0.001**

Control group: infected not treated.
Prophylactic group: treated with allicin (8mg/ Kg by intravenous route) 24 hours before infection, the same day of infection and 24 hours post infection.
Allicin treated group 1 week post infection: treated with allicin (8mg/ Kg by intravenous route) one week post infection (therapeutic effect on schistosomules).
Allicin treated group 6 weeks post infection: treated with allicin (8mg/ Kg by intravenous route) 6 weeks post infection (therapeutic effect on adult worms).
** p value <0.01: High Significant difference between treated groups versus control group.
b: Significance with prophylactic group.
c: Significance with allicin treated group 1 week post infection.

Table (5): Effect of allicin on developmental immature egg stages in oogram pattern of *S. mansoni* infected mice (54 days post infection).

Groups	Immature egg % of different stages (mean± SD) P value			
	1st stage	2nd stage	3rd stage	4th stage
Control group	9.04±5.15	7.06±5.36	9.07±5.43	9.32±6.39
Prophylactic group	3.87±1.96 0.027*	6.81±3.9	10.59±7.11	11.82±4.49
Allicin treated group 1 week post infection	7.79±3.2 **b**	3.20±1.92	3.88±3.3 **b** 0.047*	5.43±5.74 **b**
Allicin treated group 6 weeks post infection	1.26±0.92 **c** 0.002**	2.37±1.82 **b** 0.046*	4.12±2.84 **b** 0.05*	2.21±1.69 **b** 0.014*

Control group: infected not treated.
Prophylactic group: treated with allicin (8mg/ Kg by intravenous route) 24 hours before infection, the same day of infection and 24 hours post infection.
Allicin treated group 1 week after infection: treated with allicin (8mg/ Kg by intravenous route) one week post infection (therapeutic effect on schistosomules).
Allicin treated group 6 weeks after infection: treated with allicin (8mg/ Kg by intravenous route) 6 weeks apost infection (therapeutic effect on adult worms).
* p value <0.05: Significant difference between treated groups versus control group.
** p value <0.01: High significant difference between treated groups versus control group.
b : Significance with prophylactic group.
c : Significance with allicin treated group 1 week post infection.

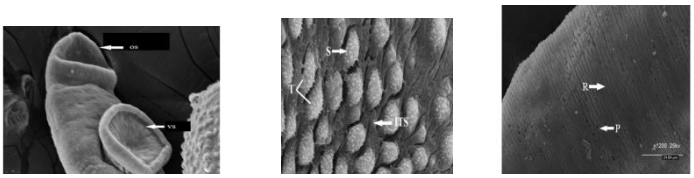
Table (6): Effect of allicin on *S. mansoni* tissue egg load (intestine and liver) of infected mice (54 days post infection).

Groups	No of ova/gram intestinal tissue Mean ±SD P value	No of ova/gram hepatic tissue Mean ±SD P value
Control group	35268.75 ± 10446.36	17050 ± 8945.07
Prophylactic group	19685.71 ± 8354.97 (0.008**)	17342.86 ± 7027.06
Allicin treated group 1 week post infection	27142.86 ± 20608.97	24514.29 ± 12390.6
Allicin treated group 6 weeks post infection	47750.0 ± 27714.17 **bc**	31842.86 ± 13368.6 **bc** (0.024*)

Control group: infected not treated.
Prophylactic group: treated with allicin (8mg/ Kg by intravenous route) 24 hours before infection, the same day of infection and 24 hours post infection.
Allicin treated group 1 week post infection: treated with allicin (8mg/ Kg by intravenous route) one week post infection (therapeutic effect on schistosomules).
Allicin treated group 6 weeks post infection: treated with allicin (8mg/ Kg by intravenous route) 6 weeks post infection (therapeutic effect on adult worms).
* p value <0.05: Significant difference between treated groups versus control group.
** p value <0.01: High significant difference between treated groups versus control group.
b : Significance with prophylactic group.
c : Significance with allicin treated group 1 week post infection.

Ultrastructural analysis of allicin-induced morphological changes in adult *S. mansoni* worms

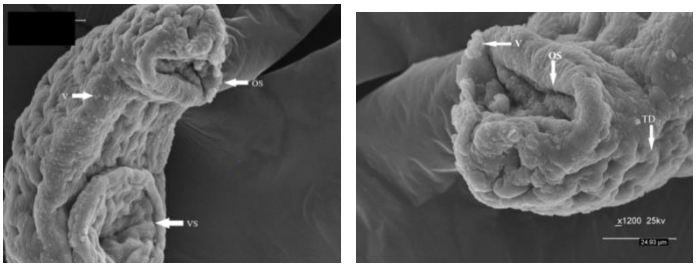
1) SEM of *S. mansoni* adult worms incubated in RPMI 1640 medium (control) for 72 hours.



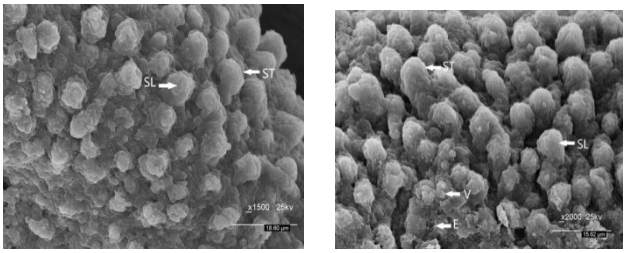
(A) **(B)** **(C)**

Fig. (1): SEM of adult *S. mansoni* worms incubated in RPMI 1640 medium (control) for 72 hrs showing: **(A)** Adult male worm with patent and intact oral sucker (OS) and ventral sucker (VS). The tegument area between the oral and ventral suckers does not have any tubercles or spines (×400). **(B)** Dorsal surface of adult male with tubercles (T) covered with numerous apically directed spines (S) with intact ridged intertubercular spaces (ITS) in between (×2000). **(C)** Adult female worm showing ridged (R) and porous (P) tegument, but no tubercles or spines (×1200)

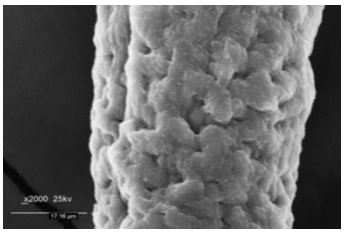
2) SEM of adult *S. mansoni* worms incubated in RPMI 1640 with allicin (200 µg/ml) for 72 hours.



(A) **(B)**



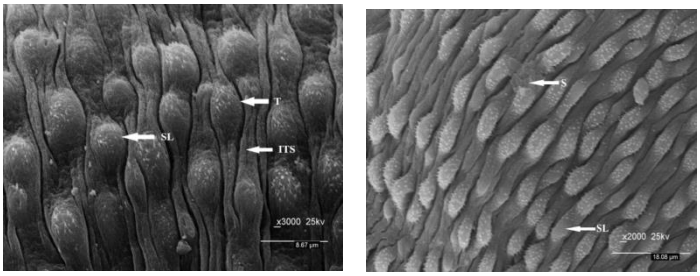
(C) **(D)**



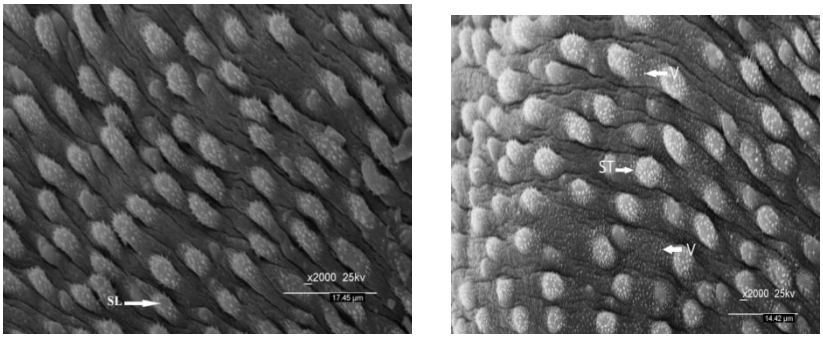
(E)

Fig. (2): SEM of adult *S. mansoni* worms incubated in RPMI 1640 with allicin (200 µg/ml) for 72 hrs showing male worm with: **(A&B)** Swollen oral sucker (OS) with disrupted architecture of the tegument (TD) and appearance of vesicles (V) (x500 and x1200 respectively). **(C&D)** Dorsal surface showing disrupted and swollen tubercles (ST), with complete loss of spines (SL), erosions (E) in the tegument and appearance of vesicles (V) (x1500 and x2000 respectively). **(E)** Massive destruction of the tegumental layer with loss of tubercles (x2000).

3) SEM of adult *S. mansoni* worms incubated in RPMI 1640 with allicin (100 µg/ml) for 72 hours.



(A) **(B)**

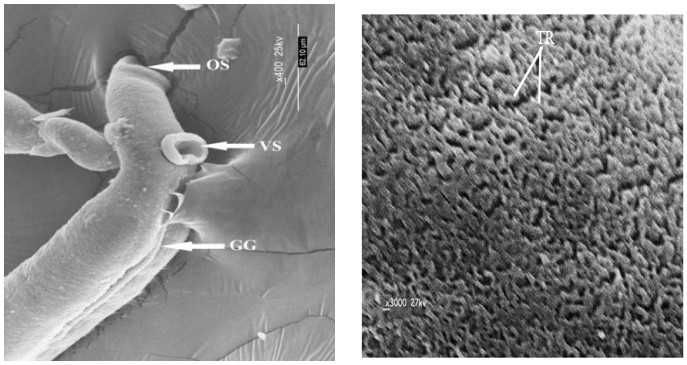


(C) **(D)**

Fig. (3): SEM of adult *S. mansoni* incubated in RPMI 1640 with allicin (100 µg/ml) for 72 hrs showing dorsal surface of male worm with: **(A, B& C)** Loss of spines (SL) of some tubercles (T) and disruption and sloughing of other tubercles (x3000, x2000 and x2000 respectively). **(D)** Swollen tubercles (ST) with appearance of multiple vesicles (V) (x2000).

Ultrastructural analysis of allicin-induced morphological changes in immature *S. mansoni* worms

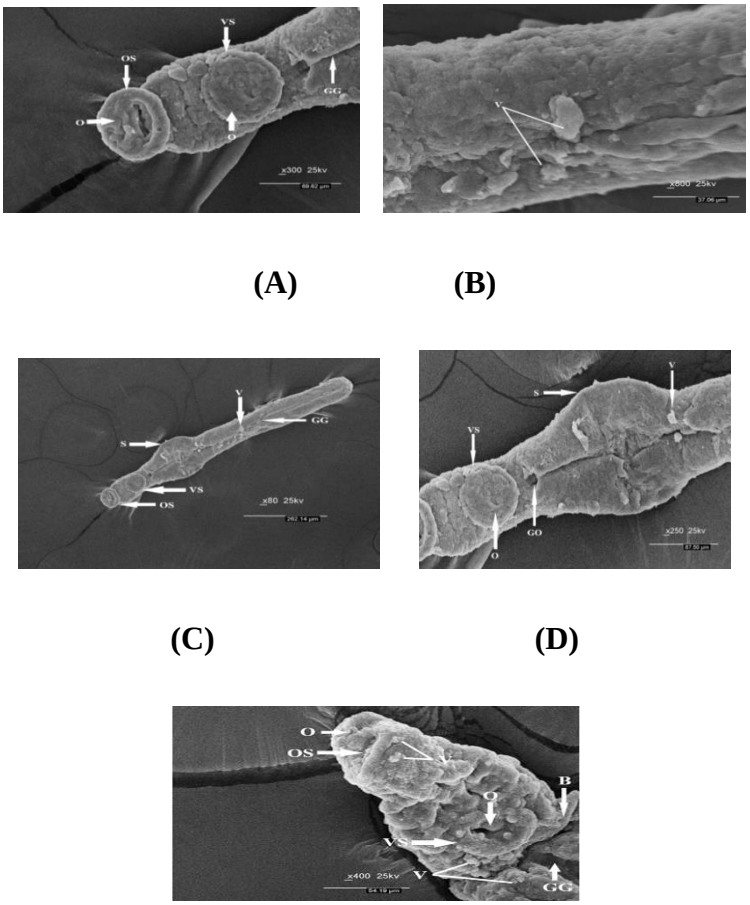
1) SEM of immature *S. mansoni* worms incubated in RPMI 1640 medium (control) for 72 hours.



(A) **(B)**

Fig. (4): SEM of immature *S. mansoni* worms incubated in RPMI 1640 medium (control) for 72 hrs showing: **(A)** Patent oral sucker (OS), patent ventral sucker (VS), gynaecophoric groove and the dorsal surface showing rows of tegumental ridges with absence of tubercles and spines (×400). **(B)** The dorsal surface showing rows of tegumental ridges (TR) (x 3000).

2) SEM of immature *S. mansoni* worms incubated in RPMI 1640 with allicin (200 µg/ml) for 72 hours.



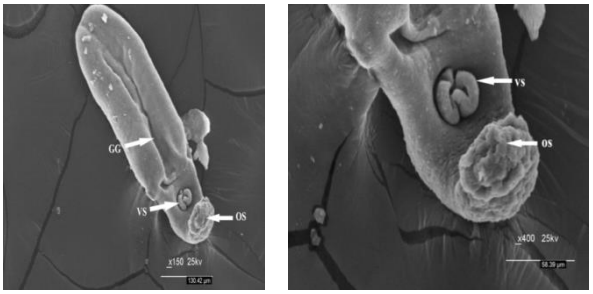
(A) **(B)**

(C) **(D)**

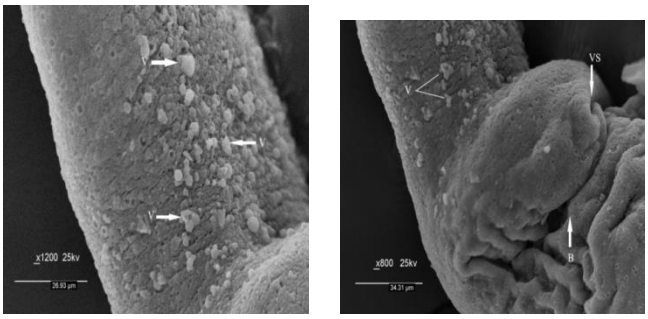
(E)

Fig. (5): SEM of immature *S. mansoni* worms incubated in RPMI 1640 with allicin (200 µg/ml) for 72 hrs showing: **(A)** Oedema (O) and swelling of the oral sucker (OS) and ventral sucker (VS) with swollen area in between (×300). **(B)** Ventral surface of the worm, showing presence of multiple vesicles (V) (x 800). **(C)** Oedema (O) and swelling of the oral sucker (OS) and ventral sucker (VS) with presence of large swelling at the middle of the body and presence of some vesicles (V) (×80). **(D)** Oedema (O) of the ventral sucker (VS) and swelling of the body (S) below gynecophoric opening (GO) with presence of some vesicles (V) (×250). **(E)** Oedema (O) of the oral sucker (OS) and ventral sucker (VS) with bending of the body (B) and presence of multiple vesicles (×400).

3) SEM of immature *S. mansoni* worms incubated in RPMI 1640 with allicin (100 µg/ml) for 72 hours.



(A) **(B)**



(C) **(D)**

Fig. (6): SEM of immature *S. mansoni* worms incubated in RPMI 1640 with allicin (100 µg/ml) for 72 hrs showing: **(A&B)** Swollen oral sucker (OS) and shrunken ventral sucker (VS) (x400 and x150 respectively). **(C&D)** Multiple vesicles and bending of the body at the ventral sucker (x1200 and x800 respectively).