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Immunohistochemical Activities and Anti-Helminthic of Blue Green Algae in Schistosoma mansoni Infection in Albino Mice

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Research Article

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ABSTRACT

This study aims to evaluate the anthelminthic effect of Blue Green Algae (BGA) against Schistosoma mansoni *in vivo*. Immunohistochemical and parasitological markers were determined to evaluate the antischistosomal activity of BGA. In addition, the morphological changes of *S. mansoni* adult worms were detected by scanning electron microscopic examination after treatment of *S. mansoni* infected mice with 100 mg/kg BW BGA alone or combined with 250 mg/kg BW PZQ. Results showed that treatment of *S. mansoni*-infected mice with BGA, praziquantel (PZQ) or in combination led to a significant decrease in the weight of liver and spleen as compared with those of *S. mansoni*-infected mice. Meanwhile, administration of the BGA, PZQ or in combination significantly reduced the total worm burden a significant decrease in ova count/g of liver and intestine. Both BGA and/or PZQ showed a remarkable effect on the oogram pattern in liver and intestine. The decrease in angiogenesis was most evident in the group receiving the combination of BGA and PZQ where differences in vascular endothelial growth factor (VEGF) expression were significantly less in the sinusoids. The electron microscopy showed a swelling, vacuolization, fusion of the tegumental ridges and loss or shortening of the spines on the tubercles, erosion, cracks and peeling of *S. mansoni* adult worm after treatment with BGA. This study revealed that BGA has a remarkable antischistosomal activity through inhibition of angiogenesis that required to the schistosomiasis and by deteriorating action on the adult worm evidenced by scanning electron microscopy.

INTRODUCTION

Schistosomiasis remains a major public health problem in endemic countries and is caused by infection with any one of three primary schistosome species [1]. In chronic schistosomiasis, the etiology of disease is linked to an accumulative process of eggs deposition in host tissue where the immune response to *S. mansoni* eggs is cell-mediated and is regulated both positively and negatively by cytokines, cellular and humoral responses, resulting in recruitment of the cellular elements that organize in the form of granulomas around the eggs [2,3]. Subsequent to the granulomatous responses, fibrosis set in, resulting in more permanent disease sequelae [4].

Schistosomiasis control represents the mean target of many research programs all over the world [4]. The effect of praziquantel (PZQ) on schistosomes has been studied in many countries. Most of the studies made are on its efficacy in the treatment of *S. mansoni* and have reported different cure and egg excretion reduction rates [5]. Ismail et al. reported that PZQ does not prevent re-infection, is inactive against juvenile schistosomes and only has a limited effect on already developed liver and spleen lesions [6]. The development of such resistance has drawn the attention of many investigators to alternative drugs.

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Blue-green Algae (BGA) have attracted attention as health beneficial foods and as source materials for drug development [7]. Aphanizomenon Flos-Aquae (AFA) are a fresh water unicellular blue-green alga that spontaneously grows in Upper Klamath Lake (Germany) and that is consumed as a nutrient-dense food source and for its health-enhancing properties [8]. AFA is an important source of the blue photosynthetic pigment phycocyanin (PC) which has been described as a strong antioxidant and anti-inflammatory [9,10]. Also, Vadiraja et al. and Romay and Gonzalez, reported that the C-phycocyanin, a constituent of BGA, is shown to be hepatoprotective, an arthritic, and most importantly anti-inflammatory in nature [11,12]. BGA also improves the antischistosomal efficacy of PZQ by scavenging the ROS associated with schistosomiasis. In addition, it exhibited a hepatoprotective activity [13]. The BGA supplement ameliorated the antioxidant capacity without exhibiting harmful side effects on liver or kidney function. The hepato- and nephroprotective effect of BGA in mice might be attributed to its ROS scavenging properties. Also, BGA exhibited the ability to induce the hematopoietic progenitor to produce blood cells which have roles in enhancement of immunologic response and in maintaining the healthy condition of the individuals [14].

The tegument of the *S. mansoni* is an important target for the action of drugs, since it makes direct contact with the compounds. The tegument is a barrier that isolates it from the external environment and ensures maintenance of vital functions. It is responsible for absorbing nutrients, metabolizing lipids and cholesterol, tissue proliferation and repair and the selective absorption of drugs, so the tegument is considered as an important target for antischistosomal drugs [15]. Through this specialized tegument, adult worms perform the basic activities for their survival: assimilate blood nutrients from the host, are able to escape from the immune response of the host against their presence [16].

Angiogenesis, the formation of new endothelial vessels from pre-existing post-capillary venule, is a characteristic feature of inflammatory diseases, wound repair and cancer [17]. Also, the relationship of angiogenesis and fibrosis has recently been demonstrated to play a role in the pathogenesis of fibrosis during some pathological conditions including hepatic schistosomiasis [18]. Angiogenesis plays a complex and extraordinary role in schistosomiasis, which is illustrated by several focal areas of vascular proliferation associated with the presence of a positive staining for the vascular endothelial growth factor (VEGF) [19].

This study aims to evaluate the effects of BGA alone or combined with PZQ on some parasitological and immunohistochemical parameters, as well as the topography of adult worm was investigated by SEM in mice infected with *S. mansoni*.

MATERIALS AND METHODS

Materials

Praziquantel (PZQ) tablets (600 mg/tablet) were obtained from SEDICO Pharmaceutical Company (6th October City - Egypt). Blue green algae (BGA) tablet (250 mg) (Aphanizomenon flos aquae) was obtained from German Pharmaceutical Industries, (Life Blau-Green Alge, Hergestellt, Deutschland). Tablets of BGA or PZQ were ground and suspended in distilled water for oral administration by stainless steel bent feed needle (length metric 50.8 mm and gage 18) from A Harvard Bioscience Company.

Animals and Experimental Design

Seventy adult male Swiss albino mice (25 ± 2 g) were purchased from Schistosome Biological Supply Program (SBSP) unit at Theodor Bilharz Research Institute (TBRI), Giza, Egypt. Animals were quarantined and allowed to acclimate for a week prior to experimentation at the animal room of Zoology Department, Faculty of Science, Menufiya University. Animals were handled under standard laboratory conditions with a 12-h light/dark cycle at a temperature of 25 ± 2˚C. They had free access to standard food and water; all experiments were done in compliance with the guide lines for the care and use of laboratory animals. Cercariae of *Schistosoma mansoni* Egyptian strain were obtained from infected Biomphalaria alexandrina snails purchased from the Schistosome Biological Supply Center at Theodor Bilharz Research Institute (TBRI), Giza, Egypt. Forty mice for infected groups were subcutaneously infected with (70 ± 5 cercariae/mouse) [20].

Animals were randomly divided into seven groups, 10 mice each as follows: Group I (N): Non-infected control mice. Group II (BGA): Non-infected mice treated with BGA daily for 15 consecutive days. Group III (PZQ): Non-infected mice treated with PZQ daily for 3 consecutive days. Group IV (*S. mansoni*-infected): infected control mice. Group V (Infected+BGA): infected mice treated with BGA, for 15 consecutive days, 7th weeks post infection. Group VI (Infected+PZQ): infected mice treated with PZQ, for 3 consecutive days, 7th weeks post infection. Group VII (Infected+PZQ and BGA): infected mice treated with a combination of BGA for 15 consecutive days and PZQ for 3 consecutive days, 7th weeks post infection. All animals were sacrificed after the end of treatment (9th weeks post infection) by decapitation.

For the groups receiving PZQ, it was administrated orally to mice in 3 doses each of 250 mg/kg/mouse for three consecutive days according to Utzinger et al. [21]. For the groups receiving BGA, it was administrated orally to mice in dose of 100 mg/kg/mouse for 15 consecutive days according to Kuriakose and Kurup [22].

Parasitological Parameters

S. mansoni worm's burden

Animals were sacrificed and perfusion of hepatic vascular vessels was performed according to the method reported by Duvall and Dewitt [23].

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Ova count

Number of ova/g tissue was calculated according to Cheever and Anderson [24].

Oogram method

The method was described to detect the percentage of different developmental stages of *Schistosoma* eggs according to Pellegrino et al. [25].

Immunohistochemical Staining for Determination of Hepatic Vascular Endothelial Growth Factor (VEGF)

Liver tissues from mice were fixed immediately in 10% neutral buffered formalin solution and embedded in paraffin. Immunohistochemical staining was performed using a labeled streptavidin-biotin method (Hus and Raine). Briefly, the dewaxed, rehydrated sections (5 µm thick) were treated with 0.3% hydrogen peroxide in methanol for 30 min to block endogenous peroxidase activity and then washed three times with phosphate buffered saline (PBS, pH 7.2). The sections were boiled with ethylene diamine tetra acetic acid (EDTA) buffer solution (pH 8.0) for 20 min. with microwave, followed by cooling at room temperature for 20 min. Nonspecific binding was blocked with 2% normal goat serum at room temperature for 15 min. followed by treatment with primary antibody at 37˚C for (VEGF: a monoclonal mouse antibody (Labvision catalog No. MS-146-R7) supplied as 1 ml of ready to use antibody for 1 hour at room temperature. After washed by PBS, sections were then incubated with biotin labeled secondary antibody for 10 min and washed with PBS. Peroxidase conjugated streptavidin was added for 20 min and then washed with PBS. Finally, sections were developed with 3, 3-diaminobenzidine and hydrogen peroxide as achromogen and counterstained with hematoxylin. For the negative control, PBS was used instead of primary antibody. The sections were examined using a Carl-Zeiss light microscopy (Oberkochen, Germany) [26].

Scanning Electron Microscopical Examination

Adult *S. mansoni* worms from the infected treated mice and from the corresponding control group were prepared for electron microscopic examination. The worms were fixed in 2.5% glutaraldhyde and 2% paraformaldehyde in 0.1 M cacodyl ate buffer (pH 7.4). Then, the samples were washed with deionized water; they were stepwise dehydrated in 50%, 80% and absolute ethanol. Samples were dried in carbon dioxide critical point drying apparatus. The samples were mounted on aluminum specimen stubs using colloidal silver adhesive paint. Mounted samples were coated with a thin layer of gold vapor [27]. SEM images were taken using a Jeol JSM 5300 scanning electron microscope and a Jeol TFC-1100 Ion sputtering device at Faculty of Science, Tanta University, Egypt.

Statistical Analysis

The data were presented as mean ± SD. The significance of the difference between the means was compared using the Student's t- test [28]. The level of significance was accepted when $p<0.05$.

RESULTS

Effect of BGA and PZQ on Body Weight and Relative Organ Weight Percentage

In order to investigate the effect of BGA and PZQ on the body weight gain of both normal and *S. mansoni* infected mice, the body weight was invested before and after the experimental **(Table 1)**. At the beginning of the experiment mice weights are between 25 to 27 g. After 9th weeks post infection the mean body weight of mice in normal control group was 30.8 ± 1.5 g, also, no significant difference between normal treated groups. In contrast, showed a remarkable significant decrease ($P<0.05$) in body weight of mice after infection with *S. mansoni* as compared with normal control group. Meanwhile, a significant increase ($P<0.05$) was observed in the body weight of *S. mansoni*-infected mice after treatment with the combination of BGA and PZQ as compared with those of *S. mansoni*-infected mice. Results showed a remarkable significant increase in the weight of both liver and spleen ($P<0.05$) after infection with *S. mansoni* as compared to those of non-infected control group. On the other hand, a significant decrease was observed in the weight of liver and spleen of *S. mansoni*-infected mice after treatment with BGA or PZQ as well as the combination of BGA and PZQ as compared with those of *S. mansoni*-infected mice.

Table 1. Effect of BGA and PZQ on the weight of liver and spleen of mice infected with *S. mansoni*.

Group	Body weight	Liver	Spleen
Normal	30.8 ± 1.5	1.8 ± 0.28	0.25 ± 0.05
Normal+BGA	29.9 ± 1.4	1.8 ± 0.26	0.27 ± 0.11
Normal+PZQ	29 ± 0.6	1.7 ± 0.06	0.2 ± 0.03
Infected control	24.9 ± 1.4$^{\$}$	4.8 ± 1.9$^{\$}$	0.72 ± 0.08$^{\$}$
Infected +BGA	25.8 ± 1.5*	3.2 ± 0.2*	0.3 ± 0.08*
Infected+PZQ	24.3 ± 1.3	2.7 ± 0.2*	0.35 ± 0.11*
Infected+BGA/PZQ	27.42 ± 0.8*,♣	1.9 ± 0.2*	**0.29±0.03***,♣

Data are expressed as Mean ± SD

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Mice were orally administrated with 100 mg/kg of BGA for 15 consecutive days.

Mice were orally administrated with 250 mg/kg for 3 days, 7th weeks post infection.

(*) Significant difference when compared to control infected group at (P<0.05)

($) Significant difference when compared to normal control group at (P<0.05)

(♣) Significant difference between Infected+PZQ group and Infected+BGA/PZQ group at (P<0.05)

Effect of BGA and PZQ on Some Parasitological Parameters in S. mansoni-infected Mice

Worm burden

Table 2 illustrates the effect of BGA and PZQ on the *S. mansoni* worm burden in animals of different groups of the study. Results showed that treatment of *S. mansoni*-infected mice with either BGA or PZQ as well as the combination of BGA and PZQ resulted in significant reductions (P<0.05) in total worm burden and in the mean numbers of male, female and couple of *S. mansoni* as compared to those in *S. mansoni* infected mice. Also, results illustrated a significant reduction (P<0.05) in total worm burden in *S. mansoni*-infected mice treated with BGA or PZQ as well as the combination of BGA and PZQ as compared to that of *S. mansoni*-infected mice.

Ova count

Results illustrated in **Table 2** showed a significant reduction (P<0.05) in ova count/g of liver and intestinal tissue after treatment of *S. mansoni*-infected mice with either BGA or PZQ as well as the combination of BGA and PZQ as compared with that of *S. mansoni* infected mice control group. Results indicated that the treatment of *S. mansoni*-infected mice with combination of BGA and PZQ caused a significant (P<0.05) reduction in ova count in hepatic and intestinal tissue as compared to those of *S. mansoni*-infected mice treated with either BGA or PZQ alone.

Table 2. Effect of S mansoni infection and treatment with PZQ, BGA or their combination on worm burden and ova count in mice.

Group	Worm burden				Ova count No. of ova/g tissue	
	Total	Couple	Male	Female	Liver	Intestine
Infected control	48.6 ± 1.5	12.5 ± 0.7	16.9 ± 0.9	19.2 ± 1.3	2070 ± 114.2	3579 ± 58
Infected +BGA	21.5 ± 1.2*	7.5 ± 0.9*	9.7 ± 0.6*	7.5 ± 0.9*	1268 ± 73.3*	2292 ± 81*
Infected+PZQ	0.0	0.0	0.0	0.0	848 ± 65.8*	1394 ± 88.6*
Infected+BGA/PZQ	0.0	0.0	0.0	0.0	526 ± 41.2*,♣	997 ± 72.6*,♣

Data are expressed as Mean ± SD

Mice were orally administrated with 100 mg/kg of BGA for 15 consecutive days.

Mice were orally administrated with 250 mg/kg for 3 days, 7th weeks post infection.

(*) Significant difference when compared to control infected group at (P<0.05)

($) Significant difference when compared to normal control group at (P<0.05)

(♣) Significant difference between Infected+PZQ group and Infected+BGA/PZQ group at (P<0.05)

Oogram pattern

As shown in **Table 3**, the oogram pattern in hepatic and intestinal tissues showed remarkable significant reduction (P<0.05) in *S. mansoni* infected mice treated with BGA or PZQ as well as their combination of BGA and PZQ as compared to those of *S. mansoni* infected group. Results also illustrated remarkable significant reductions in both immature and mature ova count of liver and intestine; while, dead ova was remarkably increased. Results indicated that the treatment of *S. mansoni*-infected mice with combination of BGA and PZQ caused a significant (P<0.05) reduction in mature and immature eggs in hepatic and intestinal tissues as compared to those of *S. mansoni*-infected mice treated with either BGA or PZQ alone. In contrast, dead ova were remarkably significantly (P<0.05) increased in the treated group with combination of BGA and PZQ as compared to those of *S. mansoni*-infected mice treated with either BGA or PZQ alone.

Table 3. Effect of *S. mansoni* infection and treatment with PZQ, BGA or their combination on oogram pattern in mice infected with *S. mansoni*.

Groups	Hepatic tissues			Intestinal tissues		
	Immature	Mature	Dead	Immature	Mature	Dead
Infected control (IC)	87.8 ± 1.8	58.5 ± 1.8	20.0 ± 1.2	100 ± 5.5	69.4 ± 7.2	18 ± 1.2
Infected+BGA	51.7 ± 1.7*	35.5 ± 1.7*	32.2 ± 2.0*	43.8 ± 1.5*	32.0 ± 3.4*	43.2 ±1.7*
Infected+PZQ	27.3 ± 1*	20.4 ± 0.8*	40.5 ± 0.7*	36.7 ± 0.4*	23.4 ± 0.9*	84.8 ± 3.8*
Infected+BGA and PZQ	20.3 ± 0.2*,♣	16.2 ± 1.8*,♣	53 ± 7*,♣	13.0 ± 0.48*,♣	12.7 ± 0.8*,♣	96.7 ± 1.8*,♣

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Data are expressed as Mean ± SD

Mice were orally administrated with 100 mg/kg of BGA for 15 consecutive days.

Mice were orally administrated with 250 mg/kg for 3 days, 7th weeks post infection.

(*) Significant difference when compared to control infected group at (P<0.05)

($) Significant difference when compared to normal control group at (P<0.05)

(♣) Significant difference between Infected+PZQ group and Infected+BGA/PZQ group at (P<0.05)

Detection of hepatic Vascular Endothelial Growth Factor (VEGF) expression

The periovular granuloma in schistosomiasis exhibits strong positivity for vascular endothelial growth factor (VEGF). Results, shown in **Figure 1b**, illustrate a high expression on proliferating blood vessels around the granulomas in *S. mansoni* infected control group. On the other hand, the immunohistochemical staining for VEGF in *S. mansoni*-infected mice treated either with BGA or PZQ as well as the combination of BGA and PZQ showed a moderate expression in few vascular endothelial cells around granuloma (vascular collar) **(Figure 1c, 1d and 1e)**.

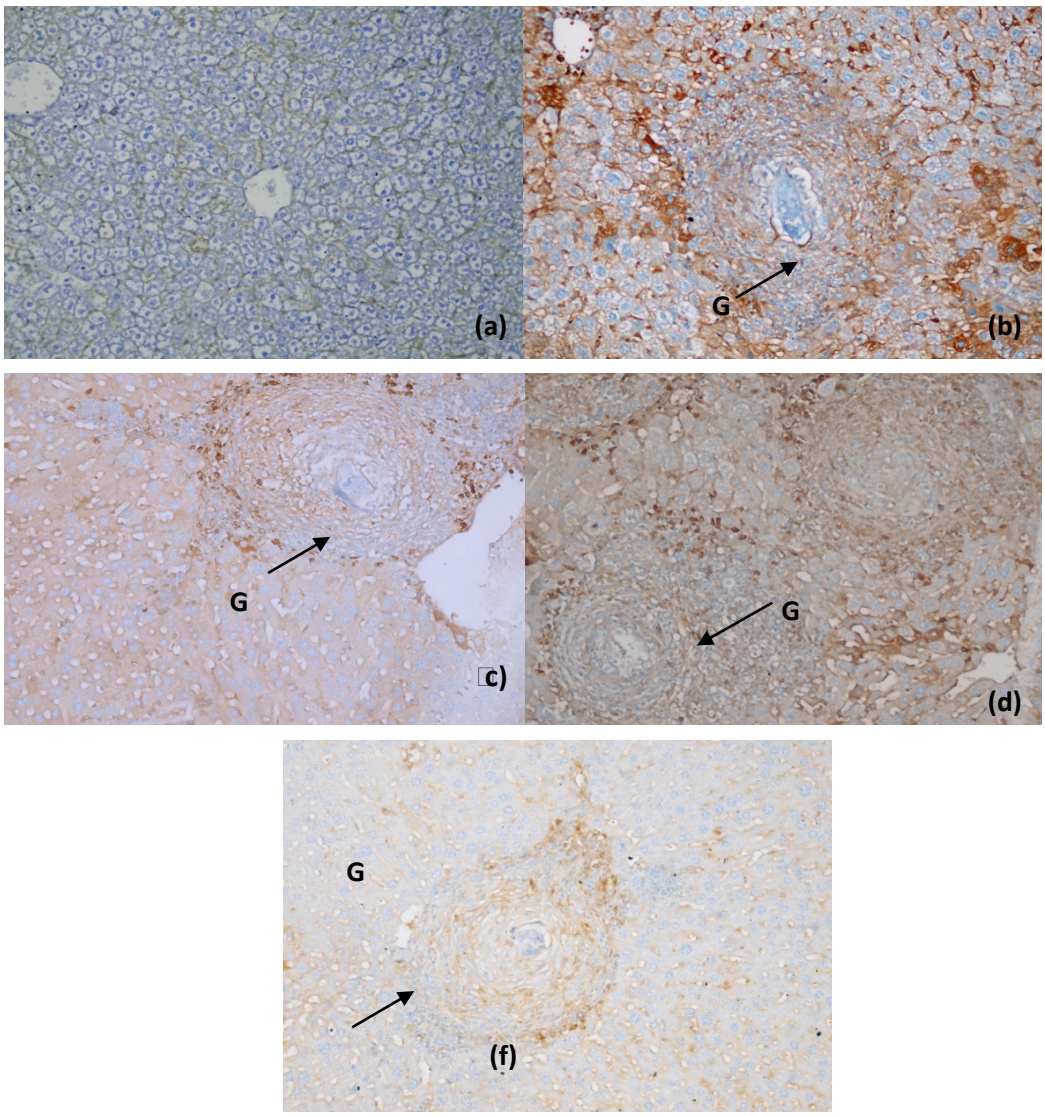

Figure 1. Immunohistochemical staining for vascular endothelial growth factor (VEGF) (X200) a) normal control mouse showing negative expression of hepatic VEGF (X200). b) *S. mansoni*-infected mouse, showing high expression on proliferating blood vessels. c) Immunohistochemical staining for VEGF in *S. mansoni*-infected mouse treated with BGA, showing a moderate expression of vascular (collar"C") endothelial cells around granuloma. d) *S. mansoni*-infected mouse treated with PZQ, showing a moderate staining in vascular endothelial cells around granuloma (vascular collar). e) *S. mansoni*-infected mouse treated with a combination of BGA and PZQ, showing staining in few vascular endothelial cells around granuloma.

Effect of BGA on the topography of adult worm

Figure 2 shows that the tegumental surface of *S. mansoni* worm recovered from untreated infected mice 9th weeks' post-

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infection was examined by SEM. It was provided with numerous large tubercles bearing spines the areas between the tubercles (intertubercular matrix) were devoid from spines **Figure 2A and 2C**.

Administration of the 100 mg/kg of blue green algae to the group of infected mice 7 week post-infection for 15 days consecutive. Worms of treated group revealed a variety of changes in the tegumental surface. The tubercles on the dorsal surface showed extensive loss of spines, the papillary pores show abnormal widening, numerous blebs and some inflammatory **(Figure 2B and 2D)**. The ventral sucker showed abnormal structural alternations included lobulation, retraction, lost some spines and aggregation of host inflammatory cells **(Figure 2F)**.

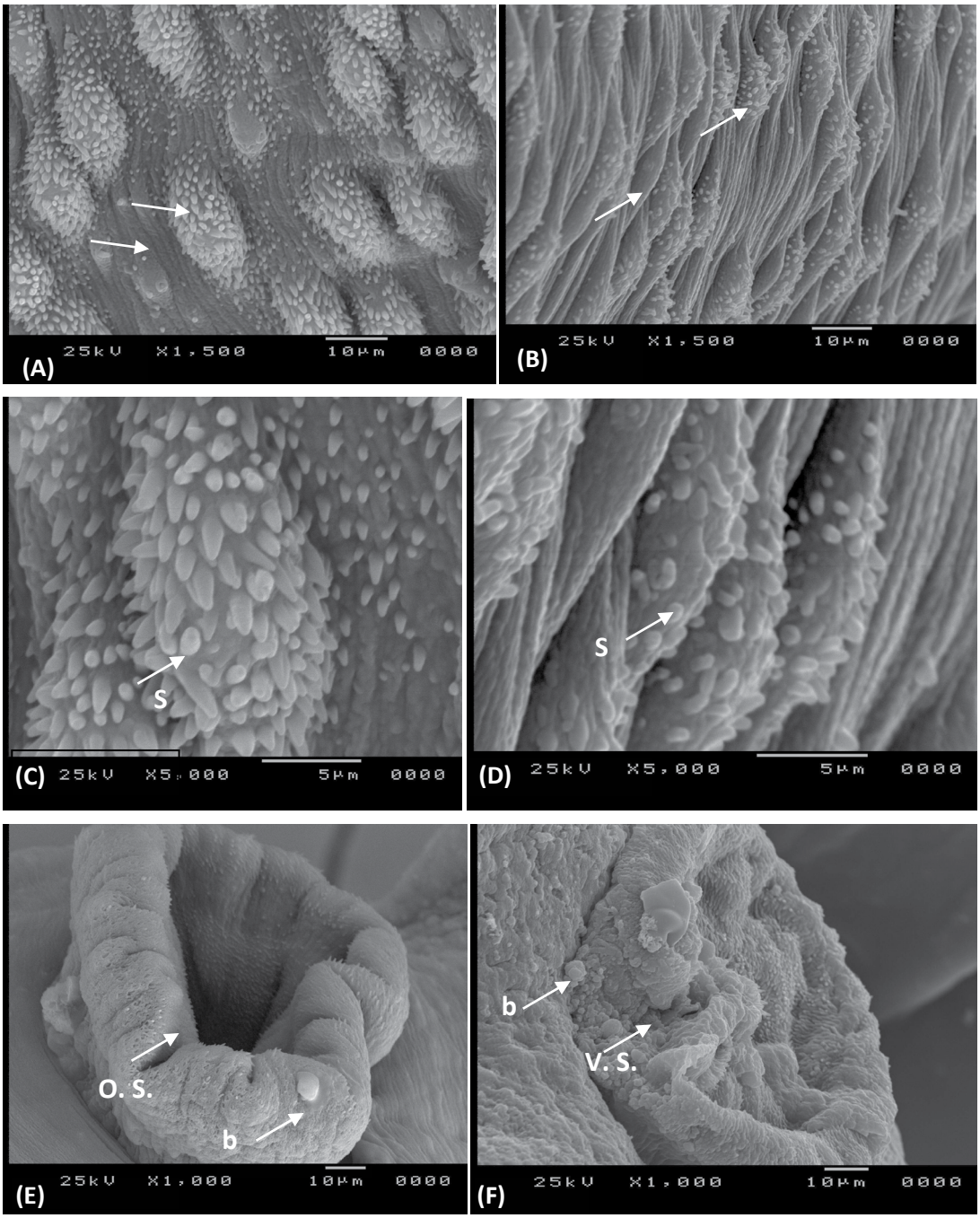

Figure 2. SEM micrograph of surface of adult worm *S. mansoni*-infected mice treated with BGA.

Morphological observation by scanning electron microscopy of adult worm was collected 9th weeks post infection. It was provided with numerous large tubercles bearing spines the areas between the tubercles (intertubercular matrix) were devoid from spines **(Figure 2A and 2C)**. Morphological damage observed by scanning electron microscopy worm were collected 9 weeks after infection from mice treated with BGA (100 mg/kg) on 49 days post infection. The tubercles on the dorsal surface showed extensive loss of spines, the papillary pores showing abnormal widening, numerous blebs (b) and some inflammatory (B, D). The ventral sucker show abnormal structural alternations included lobulation, retraction, lost some spines and aggregation of host inflammatory cells **(Figure 2E and 2F)**.

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DISCUSSION

Results of the current study revealed that the infection of mice with *S. mansoni* resulted in a remarkable increase in the weight of liver and spleen as compared to those of the normal control group. While, treatment of *S. mansoni*-infected mice with either BGA or PZQ as well as the combination of blue green algae and PZQ resulted in a significant reduction in the weight of liver and spleen as compared to those of the *S. mansoni* infected group.

Results of the present study showed a significant reduction in worm burden after treatment of *S. mansoni*-infected mice with BGA or PZQ as well as the combination of BGA and PZQ for 15 days consecutive. Data indicated that PZQ and its combination with BGA have the most potent inhibitory action on worm burden rather than BGA alone. In addition, a remarkable decrease in eggs load was observed after treatment of *S. mansoni*-infected mice with BGA or PZQ as well as the combination of BGA and PZQ. These findings indicated that both PZQ and BGA and their combination showed inhibitory effects on total worm burden leading to a remarkable reduction in eggs count. Consequently, this inhibitory action resulted in a decrease in liver and spleen weight.

These data are in agreement with the results of Massoud et al. have used myrrh extract as a treatment of schistosomiasis and attributed also the significant reduction in hepatic and intestinal egg loads to the reduction that occurred in the worm load or might be the reduction in the eggs production capacity of the female worms [29]. The same observations were reported by Mahmoud et al. reported that worm's burden and eggs load were reduced after treatment of *S. mansoni*-infected mice with *Nigella sativa* oil [30]. In addition, results of the current study go in harmony with the report of Coutinho et al. that liver weight was increased in mice infected with *S. mansoni* and this hepatomegaly may be due to the formation of collagen which associated with schistosomiasis [31]. Moreover, Nessim and Mohamed recorded that the anti-inflammatory drug, diclofenac, exhibited a significant reduction in the worms and in the tissue eggs load compared to the untreated controls [32]. Also our results are in agreement with the results of Jatsa et al. reported that treatment of *S. mansoni*-infected mice with PZQ or/and *Clerodendrum umbellatum*, caused a reduction in weight of liver and spleen [33]. Also, Allam who reported that curcumin was effective in reducing worm and tissue-egg burden in *S. mansoni*-infected mice [34]. He also stated that hepatosplenomegaly and eosinophilia induced by *S. mansoni* infection were largely improved with curcumin treatment. Meanwhile, Mohamed et al. revealed that oral administration of *Lupinus termis* (100 mg/kg) caused a reduction in worm burden and egg lying in *S. mansoni*-infected mice [35].

In addition, results of the current study go in parallelism with the report of Kamel et al. reported that the reduction rates of the number of *S. mansoni* worms recovered from infected mice and the number of ova/g tissue in liver and intestine of infected treated mice after treatment with methanol extract of the plants *Chenopodium ambrosioides, Conyza dioscorides* and *Sesbania sesban* [36]. Furthermore, the same observations were reported by Reda et al. that considered the praziquantel or/and radiation-attenuated vaccine were as a strong evidence of efficiency of the anti-schistosomal drugs, causing a significant reduction in the percentage of worm burden, oogram and ova count [37]. Also, El-Lakkany et al.; Kasinathan et al. and Costa-Silva et al. recorded that treatment of schistosomiasis with PZQ yields a significant reduction in hepatic and intestinal egg loads as well as the capacity of the female worms production [38-40]. The same finding was observed by Liu et al. proved the activity of PZQ in killing *S. mansoni* adult worms in mice. It is reported that PZQ accelerates the calcium uptake of the parasite leading to damage in the adult schistosome tegument exposing antigens on the worm surface to trigger cellular and humoral immuno-responses [41,42]. However, this leads to the detachment of worms to the endothelium of the vessels wall [43]. BGA exhibited antischistosomal activity where the treatment with BGA resulted in a significant reduction in worm burden and the ova count/gm liver or intestine.

The current study demonstrates the angiogenesis during schistosomiasis by immunohistochemical staining using vascular endothelial growth factor (VEGF) in the livers of different groups of mice. The result showed high expression of hepatic VEGF in *S. mansoni*-infected mice, this finding run in parallel with the results of Lenzi et al. and Botros et al. reported that the VEGF immunoexpression in the liver section from *S. mansoni*- infected mice showed a high expression of proliferating blood vessels [44,45].

The decrease in angiogenesis was observed in *S. mansoni*-infected mice after treatment with BGA or PZQ as well as a combination of BGA and PZQ. The decrease in angiogenesis was most evident in the infected animals that treated with a combination of BGA with PZQ where differences in VEGF expression were significantly less in the sinusoids when compared with those of mice treated with BGA or PZQ. These data are in consistence with the study of Botros et al. showed that treatment of *S. mansoni*-infected mice with PZQ in combination with artemether led to moderate expression in few angiogenesis and less severe histopathological. Similar observations have also been reported in mice with schistosomiasis following treatment with praziquantel [46]. Also, Tawfeek et al. reported significantly higher VEGF level in the sera of schistosomiasis patients with high infection intensity. In addition, Nagle et al. showed that BGA induced the liver protection by reducing the expression of VEGF [47-49]. Also, Kim et al. recorded that sea blue algae act as hepatoprotective agent which reduce angiogenesis and apoptosis that induced during gastrointestinal cancer. In addition, Dobolyi et al. found that β-carotene was described as a potential anti-inflammatory and angiogenesis action, due to less expression of proliferative blood vessels. Moreover, results of the current study go in harmony with the report of Mondul et al. revealed that treatment the cigarette smokers with β-carotene and vitamin E led to decrease the serum level of VEGF leading to prevention of lymph angiogenesis [50-52].

The present study is the first to document tegumental alternation in *S. mansoni* induced by blue green algae as a treatment of *S. mansoni* infected mice were investigated. In this study revealed the dose of 100 mg/kg of Blue green algae alone induced

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exrensive alternations in the tegument surface, with swelling, vacuolization or blebbing, fusion of tegumental ridges. The observed morphological alternations could be a mechanism for the killing of the worms by Blue green algae. The alternation in ventral sucker must result in a loss of ability to adhere to blood vessels rendering ingestion of nutrients from blood more difficult. This result was agreement with Xiao et al. reported that the treatment of *S. mansoni* infected mice with artemether induced extensive alternations in the tegument surface, with swelling, vacuolization of *Schistosoma*. The ultrastructure alterations in the surface of *Schistosoma* worms were investigated by several authors for the evaluation of antischistosomal drugs [53,54].

CONCLUSION

In conclusion, this study provides information for raising the interesting possibility that BGA may eventually have therapeutic potential in the treatment or prevention of disorders involving *S. mansoni*-infection. The combination of BGA with PZQ may be the aim for the future work in this Filed to evaluate the therapeutic activity of such combination.

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