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The hepatoprotective activity of blue green algae in *Schistosoma mansoni* infected mice

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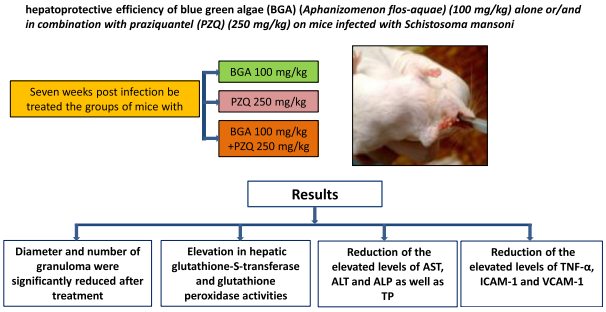
HIGHLIGHTS

- The effect of blue green algae (BGA) on *S. mansoni* infected mice was studied.
- BGA ameliorated the biochemical function of liver of *S. mansoni* infected mice.
- BGA improved the antioxidant status of *S. mansoni* infected mice.
- BGA reduced the elevation of TNF-α, ICAM-1 and VCAM-1.
- BGA reduced the harmful side effect of antischistosomal drug Praziquantel.

GRAPHICAL ABSTRACT



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ABSTRACT

This study aims to evaluate the immunomodulatory effects of a natural product, blue green algae (BGA) (100 mg/kg BW), alone or combined with praziquantel PZQ (250 mg/kg BW) on granulomatous inflammation, liver histopathology, some biochemical and immunological parameters in mice infected with *Schistosoma mansoni*. Results showed that the diameter and number of egg granuloma were significantly reduced after treatment of *S. mansoni*-infected mice with BGA, PZQ and their combination. The histopathological alterations observed in the liver of *S. mansoni*-infected mice were remarkably inhibited after BGA treatments. BGA decreased the activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) as well as the level of total protein (TP) while the level of albumin was increased. Treatment of infected mice with BGA, PZQ as well as their combination led to significant elevation in the activities of hepatic antioxidant enzymes glutathione peroxidase (GPX) and glutathione-S-transferase (GST) as compared with control group. Combination of BGA and PZQ resulted in significant reduction in the level of intercellular adhesion molecules-1 (ICAM-1), vascular adhesion molecules-1 (VCAM-1) and tumor necrosis factor-alpha (TNF-α) when compared to those of the *S. mansoni*-infected group. Overall, BGA significantly inhibited the liver damage accompanied with schistosomiasis, exhibited a potent antioxidant and immunoprotective activities. This study suggests that BGA can be considered as promising for development a complementary and/or alternative medicine against schistosomiasis.

1. Introduction

Hepatic schistosomiasis is one of the most prevalent forms of chronic liver diseases in the world, often leading to the liver

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fibrosis (El-Lakkany et al., 2012 and Dias et al., 2013). The immunopathology of schistosomiasis is generally attributed to the granulomatous reaction around the tissue deposited eggs and is considered to be a T cell mediated immune response (Alves-Oliveira et al., 2006). Granuloma formation requires the recruitment of inflammatory cells from blood stream, via up-regulation of adhesion molecules on activated vascular endothelial cells, induced by cytokines and chemokines released at the site of inflammation (Pearce and MacDonald, 2002). In addition, infection with *Schistosoma mansoni* alters enzymatic and non-enzymatic antioxidant status (de Oliveira et al., 2013).

Schistosomiasis control represents the target of many research programs all over the world (Murray, 2005). 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 (Mekonnen et al., 2013). Ismail et al. (1999) 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. Concern over the development of such resistance has also drawn the attention of many investigators to alternative drugs.

Blue–green algae (BGA) have attracted attention as health beneficial foods and as source materials for drug development (Schaap et al., 2012). *Aphanizomenon flos-aquae* (AFA) is a fresh water unicellular blue–green algae that spontaneously grows in Upper Klamath Lake (Germany) and that is consumed as a nutrient-dense food source and for its health-enhancing properties (Pugh and Pasco, 2001). AFA is an important source of the blue photosynthetic pigment phycocyanin (PC), which has been described as a strong antioxidant (Bhat and Madyastha, 2001) and anti-inflammatory (Reddy et al., 2000). Also, Vadiraja et al. (1998) and Romay and Gonzalez (2000) reported that the C-phycocyanin, a constituent of BGA, is shown to be hepatoprotective, antiarthritic, and most importantly anti-inflammatory in nature.

This study aims to evaluate the immunomodulatory effects of BGA alone or combined with PZQ on granulomatous inflammation, liver histopathology, some biochemical and immunological markers in mice infected with *S. mansoni*. Meanwhile, the role of the BGA in reducing the harmful side effects generated by PZQ treatment was assessed.

2. Materials and methods

2.1. 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.

2.2. Animals and study design

Seventy adult male Swiss albino mice (25.00 ± 2.00 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 *S. 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 (70.00 ± 5.00 cercariae/mouse) (Peters and Warren, 1969).

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 (10th 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. (2003). 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 (2010).

2.3. Blood and tissue sampling

At the end of experiment, all animals were sacrificed by decapitation and peripheral blood was collected and serum was separated by centrifugation at 3000 rpm for 5 min and kept at −20 °C until use. Livers were removed and rinsed with physiological saline. A measure of 0.5 grams of liver was weighed and mechanically homogenized by using electrical homogenizer (Potter-Elvehjem) in a 10-fold volume of ice-cold 20.00 mM tris–HCl buffer (pH 7.4). The homogenate was divided into aliquoted and kept at −70 °C for biochemical studies, and small pieces of liver were separated immediately fixed in 10% formalin for histological detection.

2.4. Liver function tests

The activities of serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were estimated according to the method of Reitman and Frankel (1957). The activity of serum alkaline phosphatase (ALP) was measured colorimetrically according to the method of Kind and King (1954). Serum total protein (TP) content was determined by a colorimetric method using bovine serum albumin as standard as described by Jacobs et al. (1964). The level of serum albumin (Alb) was determined by using the method of Baure (1982).

2.5. Assessment of hepatic GPX and GST activities

The activity of hepatic glutathione peroxidase (GPX) was measured by spectrophotometrically assayed by using 1-chloro-2-4-dinitrobenzene (CDNB) and glutathione as described by Habig et al. (1974). While, the activity of hepatic and glutathione-S-transferase (GST) was measured according to the method described by Paglia and Valentine (1967).

2.6. Measurement of hepatic granuloma diameter, number and histopathological investigations

For determination of granuloma diameter, small pieces of liver were separated and immediately fixed in 10% formalin and the