Role of berberine on schistosomiasis-inducted oxidative stress and damage in spleen of mice

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Abstract: Schistosomiasis is of great public health and socio-economic importance in the developing world. The current study aimed to investigate the effect of berberine on schistosomiasis-induced splenic injury. Mice were divided into three groups. The first group acted as a control non-infected group. The second and the third groups were infected with *Schistosoma mansoni* cercaria. The third group received berberine chloride on day 46 postinfection for 10 days. Infection induced severe splenic tissue damage as well as an alteration of the oxidative stress biomarkers. Berberine was able to improve the splenic histology and the change in glutathione, malondialdehyde, nitric oxide, catalase and super oxide dismutase. These findings suggest that berberine exerts its beneficial effects on *S. mansoni*-induced oxidative stress may be attributed to its antioxidant activity. This could help in protecting host tissue from injuries induced by parasites.
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1. Introduction

Schistosomiasis is a chronic, parasitic disease caused by blood flukes (trematode worms) of the genus *Schistosoma*. At least 243 million people required treatment which should be repeated over a number of years (WHO, 2013). Schistosomiasis transmission has been documented in 78 countries. However those requiring treatment targeted at most at-risk population groups live in 52 countries (Amer et al., 2013; WHO, 2013).

In the light of the absence of a vaccine and the probability of emerging resistance, a search for alternative treatments is a commonly accepted need for further research (McManus and Loukas, 2008). Natural products, mainly plants, have been the source of medicines for thousands of years. In recent decades, there has been a growing interest in the scientific community to search for extracts and pure compounds, especially those derived from plants that exhibit potential schistosomicidal properties, as one alternative method to the conventional chemical control (Ndamba et al., 1994).

Berberine is an isoquinoline alkaloid, present in roots and stem-bark of Berberis species (Vuddanda et al., 2010). Berberine based formulations, are widely used in traditional systems of medicine including, Ayurveda and Traditional Chinese Medicine. Berberine has demonstrated wide range of pharmacological activities including; antihypertensive, anti-inflammatory, antioxidant, antidepressant, anticancer, anti-diarrhoeal, antimicrobial and antiparasitic (Singh and Mahajan, 2013). Based on these pharmacological functions, we designed the current work to study the role of berberine chloride on schistosomiasis-inducted oxidative stress and damage in spleen of mice.

2. Material and Methods

2.1. Animals

Thirty male Swiss **albino mice** were bred under specified pathogen-free conditions and fed a standard diet and water *ad libitum*. The experiments were performed only with mice at an age of 10-12 weeks and were approved by state authorities and followed Saudi Arabian rules for animal protection.

2.2. Infection of Mice

S. mansoni cercariae were from Schistosome Biological Supply Center at Theodor Bilharz Research Institute, Imbaba, Giza, Egypt. Mice were exposed to *S. mansoni* (100 cercariae/mouse) using tail immersion method, modified by Oliver and Stirewalt (Olivier and Stirewalt 1952).

2.3. Experimental design

Animals were allocated to three groups of ten mice each. Group I, served as vehicle control (Non-infected) and received water (100 µl water/mouse) by oral administration for 10 days. Group II (Infected) and III (Infected + BER) were infected with *S. mansoni*. Each mouse was subjected to intraperitoneal injection with 100 cercariae (Peters and Warren 1969). On day 46 p.i. with *S. mansoni*, the animals of Group III received 100 µl BER (12 mg/Kg) (one-third of the 50% lethal dose) (Sigma, St. Louis, MO, USA)(Jahnke et al., 2006) for 10 days. On day 55 p.i with *S. mansoni*, the animals of all groups were cervically dislocated.

2.4. Spleen histology

Tissue samples of the spleen of all groups

were immediately fixed after animal dissection in 10% neutral buffered formalin, dehydrated and processed for paraffin sectioning. Sections were then deparaffinized, stained with hematoxylin and eosin stains.

2.5. Biochemical analysis

Spleen samples were homogenized immediately to give 50% (w/v) homogenate in ice-cold medium containing 50 mM Tris–HCl, pH 7.4. The homogenate was centrifuged at 500×g for 10 min at 4 ◦C. The supernatant (10%) was used for the various biochemical determinations.

Glutathione (GSH) was determined chemically in liver homogenate using Ellman's reagent (Ellman 1959). The method is based on the reduction of Ellman's reagent (5,5` dithiobis (2-nitrobenzoic acid) with GSH to produce a yellow compound. The chromogen is directly proportional to GSH concentration, and its absorbance was measured at 405 nm.

Lipid peroxidation in liver homogenate were determined according to the method of Ohkawa et al. (1979) by using 1 ml of trichloroacetic acid 10% and 1 ml of thiobarbituric acid 0.67%, followed by heating in a boiling water bath for 30 min. Thiobarbituric acid reactive substances were determined by the absorbance at 535 nm and expressed as malondialdehyde (MDA) equivalents formed.

The assay of nitric oxide (NO) in spleen homogenate was done according to the method of Green et al. (1982). In acid medium and in the presence of nitrite the formed nitrous acid diazotises sulphanilamide, this is coupled with N-(1–naphthyl) ethylenediamine. The resulting azo dye has a bright reddish–purple color which was measured at 540 nm.

Catalase (CAT) activity was assayed by the method of Aebi (1984). CAT reacts with a known quantity of H_2O_2. The reaction is stopped after exactly one minute with catalase inhibitor. In the presence of peroxidase (HRP), remaining H_2O_2 reacts with DHBS and AAP to form a chromophore with color intensity inversely proportional to the amount of CAT in the original sample.

Superoxide dismutase (SOD) activity was assayed by the method of Nishikimi et al., (1972). This assay relies on the ability of the enzyme to inhibit the phenazine methosulphate-mediated reduction of nitroblue tetrazolium dye.

2.6. Statistical analysis

One-way ANOVA was carried out, and the statistical comparisons among the groups were performed with Duncan's test using a statistical package program (SPSS version 17.0). All p values are two-tailed and $p < 0.05$ was considered as significant for all statistical analysis in this study.

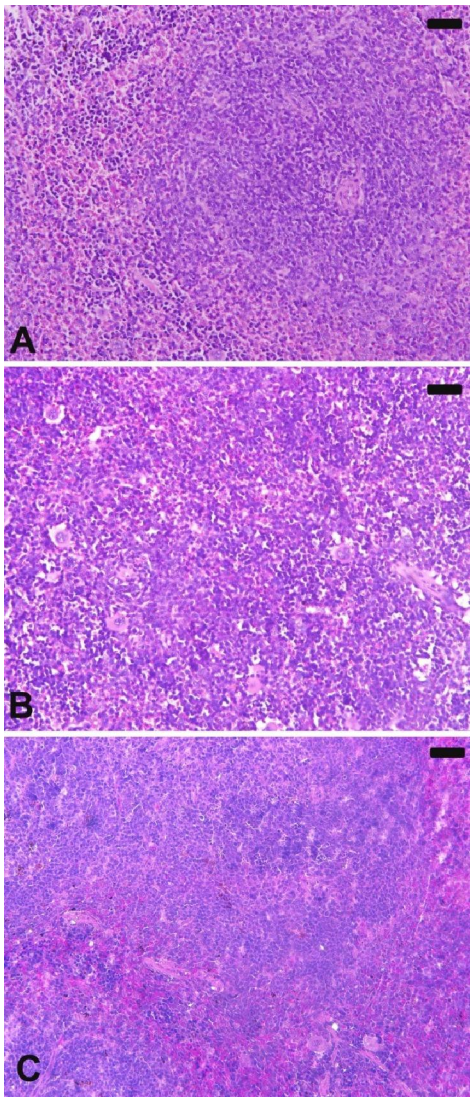

Fig.1. Sections of mouse spleen infected with *S. mansoni* on day 46 p.i. (A) Non-infected spleen with normal architecture. (B) Infected spleen with disorganized pulps. (C) Infected-treated mice with BER. Spleen appeared with less lesion and improved tissue damage. Sections are stained with hematoxylin and eosin. Bar = 50 μm.

3. Results and Discussion

The normal spleen archeticture is showen in Fig.1A. The spleen is composed of white and red pulps surrounded by a capsule of dense connective tissue. The white pulp was composed of a central, T-cell rich zone, and a peri-arterial lymphoid sheath surrounded by B-cell-rich primary follicles. The white pulp was separated from the red pulp by the marginal sinus embedded in a layer of marginal zone lymphocytes. On day 55 post-infection with *S. mansoni*, the white pulp enlarged due to cellular

proliferation. The limit between white and red pulp started to disappear (Fig. 1B), and the spleen increased in size. The splenomegaly due to infection with *S. mansoni* has been reported by Brand et al. (2012). Infection induced a splenic vacuolation of some cells. Also, most of the cells were darkly stained and the sinusoidal spaces were large. The histological lesion is still present with some improvements after treatment of mice with berberine (Fig.1C).

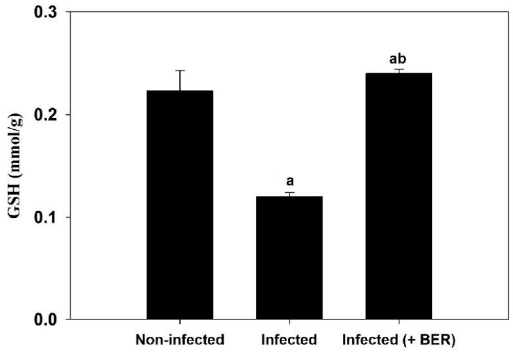

Fig. 2. Effect of berberine (BER) on glutathione (GSH) level in the splenic homogenate of *S. mansoni* infected mice. Values are means ± SE. a: Significant against vehicle (non-infected) control group at $P \leq 0.05$, b: Significant against infected control group at $P \leq 0.05$.

Schistosomiasis is associated with liberation of free radicals and disturbance in the cellular antioxidant system. It has been revealed that there is an important role of antioxidant processes in mediating liver injury in schistosomiasis due to an increased production of reactive oxygen intermediates (La Flamme et al. 2001). Hence, the suppressive effect of berberine is due, in part, to the fact that berberine has antioxidants effect (Zhang et al., 2013.). The generation of oxygen-derived free radicals may be an initial, nonspecific defense reaction of the host toward parasitic infection. Due to their participation in the metabolic processes, antioxidants may protect the host against oxidant-mediated damage and against the harmful effects of substances produced as a result of the host's defense response (Abdallahi et al., 1999).

S. mansoni induced a highly significant reduction in GSH level in the spleen (Fig. 2), which indicates that schistosomiasis causes more liberation of free radicals. On the other hand, BER inoculated to *S. mansoni* infected mice resulted in highly significant increment in GSH level of spleen. Our results are in agreement with the observation of El-Sokkary et al. (2002) and de Oliveira et al. (2013).

El-Sokkary et al. (2002) concluded that there were reductions in glutathione, superoxide dismutase, and vitamin E in the spleen of *S. mansoni* infected mice. In addition, the level of GSH was increased as a result of treatment of infected mice with an antioxidant.

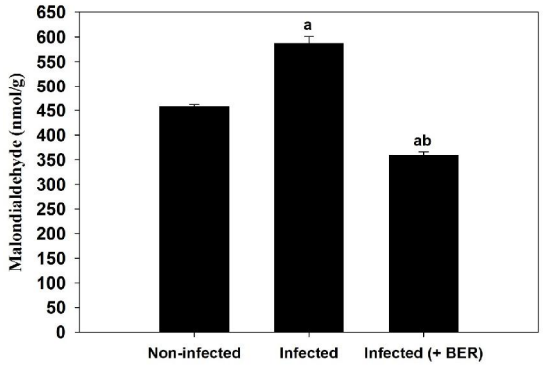

Fig.3. Effect of berberine (BER) on malondialdehyde (MDA) level in the splenic homogenate of *S. mansoni* infected mice. Values are means ± SE. a: Significant against vehicle (non-infected) control group at $P \leq 0.05$, b: Significant against infected control group at $P \leq 0.05$.

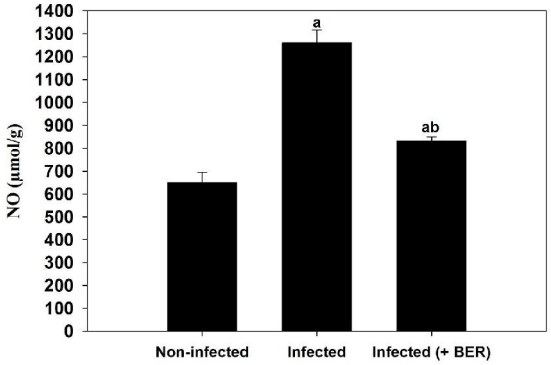

Fig.4. Effect of berberine (BER) on nitric oxide (NO) level in the splenic homogenate of *S. mansoni* infected mice. Values are means ± SE. a: Significant against vehicle (non-infected) control group at $P \leq 0.05$, b: Significant against infected control group at $P \leq 0.05$.

In the current investigation, evidence of increased levels of MDA (Fig. 3) and NO (Fig. 4) in spleen and of *S. mansoni* infected mice was seen. On contrary, the treated *S. mansoni* infected mice with oral adminstration of BER caused a highly significant decrease in NO and MDA levels of the spleen (El-Sokkary et al., 2002).

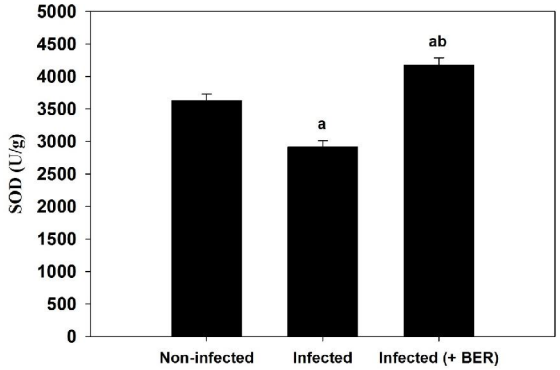

Fig.5. Effect of berberine (BER) on super oxide dismutase (SOD) level in the splenic homogenate of *S. mansoni* infected mice. Values are means ± SE. a: Significant against vehicle (non-infected) control group at $P \leq 0.05$, b: Significant against infected control group at $P \leq 0.05$.

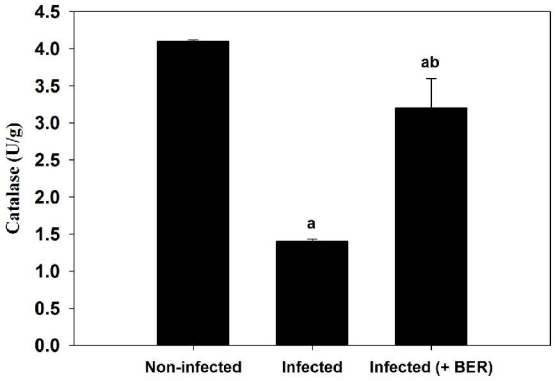

Fig.6. Effect of berberine (BER) on catalase level in the splenic homogenate of *S. mansoni* infected mice. Values are means ± SE. a: Significant against vehicle (non-infected) control group at $P \leq 0.05$, b: Significant against infected control group at $P \leq 0.05$.

In the present study, the activity of superoxide dismutase greatly declined six weeks post infection. The decrease in SOD may result from production of H_2O_2 during oxidative metabolism as indicated by Pinteaux et al. (1996). The reduced antioxidant production was due to increased oxygen metabolites causing a decrease in the activity of antioxidant defense system. SOD is an important defense enzyme which catalyzes the dismutation of superoxide radicals (McCord et al., 1971). In our data and other reports (Pinteaux et al., 1996), relatively low content of antioxidant enzymes in kidney and testes may cause it more vulnerable to oxidative stress. However, berberine almost restored the splenic SOD (Fig. 5) activities to near control levels. Namely, our results indicate that the preventive effects of berberine may be due to scavenging of free radicals by its antioxidant nature.

Furthermore, the present data reveals a highly significant and progressive reduction in catalase activity (Fig. 6) which started four weeks post *S. mansoni* infection. In agreement with this, Gharib et al. (Gharib et al., 1999) showed that peroxide dismutation yields H_2O_2 which is detoxified by catalase resulting in decrease in its activity.

Collectively, the findings of the present investigation suggest that berberine exerts its beneficial effects on *S. mansoni*-induced oxidative stress may be attributed to its antioxidant activity. This could help in protecting host tissue from injuries induced by parasites.

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