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Artichoke leaf extract protects liver of *Schistosoma mansoni* infected mice through modulation of hepatic stellate cells recruitment

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HIGHLIGHTS

- The antiparasitic and hepatoprotective properties of artichoke leaf extract were studied in *S. mansoni* infected mice.
- Artichoke reduced granuloma size, protected liver tissue in between granulomas and improved liver enzymes.
- Protection was mediated by modulation of HSCs recruitment increasing them inside granulomas and decreasing them outside.

GRAPHICAL ABSTRACT

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ABSTRACT

Schistosomiasis is the second most common human parasitic disease worldwide. It is responsible for 300000 deaths per year. Liver fibrosis is the main pathology of schistosomiasis and its complications are the major cause of death in infected cases. Unfortunately, the therapeutic dose of praziquantel (PZQ) – the main drug treatment – doesn't markedly affect fibrosis. In the present study, antiparasitic and hepatoprotective properties of artichoke leaf extract (ALE) were tested on mice experimentally infected with *Schistosoma mansoni* (*S. mansoni*) and were compared to PZQ. Four mice groups were infected with *S. mansoni*. The first three groups received ALE, ALE + PZQ and PZQ respectively. The 4th was the positive control and the 5th was the negative control group. Worm load, egg count, granuloma numbers and diameters were measured to assess ALE anti-schistoaomal properties. Masson's trichrome staining of fibrosis, immune staining of hepatic stellate cells (HSCs) and estimation of liver enzymes were done to assess its hepato-protective action. Although it had no significant effects on worm or tissue egg load and granuloma number, ALE caused significant reduction of granuloma diameter, improvement of liver functions and liver fibrosis. ALE caused statistically significant changes in HSCs distribution. It reduced granuloma size by increasing HSCs recruitment inside granuloma and limited liver fibrosis by their inhibition in the peri- and inter-granuloma liver tissue. It was concluded that despite failure of ALE to treat *S. mansoni* infection, it can limit liver damage caused by this parasite by modulating HSCs recruitment.

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Abbreviations

ALT	alanine aminotransferase
ALE	artichoke leaf extract
AST	aspartate aminotransferase
CCl4	carbon tetra chloride
HSCs	hepatic stellate cells
H-score	histo-score
LDL	low density lipoprotein
NASH	nonalcoholic steatohepatitis
PI	post infection
PZQ	praziquantel
S. mansoni	Schistosoma mansoni
SMA-α	smooth muscle actin alpha
Th2	T helper 2
TGF-β	transforming growth factor beta.

1. Introduction

Schistosomiasis is the second most common human parasitic disease ranking immediately after malaria. In addition, it is the second most common neglected tropical disease with more than 200 million reported cases worldwide 90% of them live in sub-Saharan Africa (Johnston et al., 2015; Liu et al., 2016). According to the World Health Organization (WHO, 2014), more than 61.6 million patients were treated for schistosomiasis and at least 258 million individuals required preventive treatment in 2014. Over 300,000 deaths are reported annually mainly due to liver fibrosis and hematemesis. Even the living patients usually suffer many disabilities (Adenowo et al., 2015).

Human infection starts by skin penetration of schistosome cercariae which are transformed into migrating schistosomula that travel through the veins passing by the lungs and lodge into the venous plexuses of liver and either mesenteric blood vessels -in *Schistosoma mansoni, S. japonicum, S. mekongi, S. guineensis and S. intercalatum*- or urogenital venous plexus – in *S. haematobium* -. Two months later, they mature into adults, mate and start laying ova. They evade the immune system and manage to survive for years in their host blood vessels laying thousands of ova that are excreted with either stool or urine -according to the species-. Some of these ova fail to be excreted and become trapped in liver, gastrointestinal or genitourinary tissues (Andrade, 2009; Barsoum et al., 2013).

Tissue trapped ova are the main cause of morbidity. Their released miracidial antigens are capable of initiating T helper 2 (Th2) mediated immune reactions that lead to granulomatous inflammation and inter-granulomatous fibrosis of liver and other affected organs (Burke et al., 2009; Pearce and MacDonald, 2002; Fairfax et al., 2012). Liver fibrosis is mainly mediated through an actin containing pericytes with high plasticity called hepatic stellate cells (HSCs) (Andrade, 2009; Chen et al., 2016). These cells are present in a quiescent form in the space of Disse acting as storage place for vitamin A and regulator of collagen homeostasis (Moreira, 2007). Upon liver injury, these cells are activated and undergo numerous phenotypic changes. They are transdifferentiated into myo-fibroblasts expressing smooth muscle actin alpha (SMA-α) on their surface to increase their contractile potential. They lose their retinoid-storing capacity, increase their rough endoplasmic reticulum and change their cytoskeletal organization (Hellerbrand, 2013). These myo-fibroblasts deposit enormous amounts of extracellular matrix components, including fibronectin and collagen, and secrete large amounts of pro-inflammatory, chemotactic and pro-fibrogenic cytokines (Moreira, 2007; Chen et al., 2016; Tacke and Weiskirchen, 2012). In schistosomiasis, HSCs are recruited to the periphery of egg granulomas and surrounding liver tissue causing liver fibrosis (Meng et al., 2016).

Fibrosed granulomatous liver parenchyma compresses and destructs the portal vasculature leading to portal hypertension and its disabling sequelae. Unfortunately, the therapeutic dose of the main anti-schistosomal drug, praziquantel (PZQ) cannot markedly affect this damage of theses disastrous ova. This major defect encouraged research to develop drugs that could provide hepatic protection where plant therapy played an important role (El Ridi and Tallima, 2013).

Artichoke (*Cynara scolymus*) is a medicinal plant that proved wide range of benefits. Artichoke leaf extract (ALE) has shown antioxidant (Pagano et al., 2016), anti-inflammatory (Ben Salem et al., 2015), antibacterial (Gaafar and Salama, 2013; Ionescu et al., 2013), antiviral (Elsebai et al., 2016), choleretic (Kraft, 1997) and even anti-cancer activities (Pulito et al., 2015). It can also inhibit cholesterol biosynthesis and low density lipoprotein (LDL) oxidation. Above this all, it proved hepatoprotective effect against many hepatotoxic agents (El Morsy and Kamel, 2014; Colak et al., 2016). In view of the foregoing, this study was undertaken to determine the possible antiparasitic and/or hepatoprotective properties of ALE on *S. mansoni* experimentally infected mice.

2. Materials and methods

2.1. Study design

Mice were divided into five groups each consisted of 10 mice. Mice of group I (GI) were infected with *S. mansoni* then treated by ALE. Mice of group II (GII) were infected with *S. mansoni* then treated by ALE followed by PZQ. Mice of group III (GIII) were infected with *S. mansoni* then treated by PZQ. Mice of group IV (GIV) were infected with *S. mansoni* and did not receive any treatment (control positive). Mice of group V (GV) were non-infected non-treated group (control negative).

2.2. Procedures

2.2.1. Experimental animals and S. mansoni infection

Male pathogen free BALB/c mice (6–8-week-old, 18–20 gm) were obtained from the Schistosome Biological Supply Program, Theodor Bilharz Research Institute, TBRI (Giza, Egypt). They were kept under standard housing conditions in the animal house of TBRI. Mice were maintained on a standard commercial pelleted diet (El-Kahira company for oils and soap) in an air-conditioned animal house at 20–22 °C. The animal experiments were conducted at the TBRI animal unit in accordance with international, ethical guidelines after approval of the institutional ethical committee of TBRI. Mice were infected by subcutaneous injection of 80–100 cercariae per mouse of an Egyptian strain of *S. mansoni* (Peters and Warren, 1969).

2.2.2. Plant extract

Leaves of artichoke *(Cynara scolymus)* were obtained by plant taxonomist at the Faculty of Agriculture, Menoufia university. They were washed with tap water and then exposed to air to be dried. Dried leaves were ground into fine particles. Methanol was added in a percentage of 5:1. The mixture was kept at room temperature for 72 h and frequently shaken. The total extracts were filtered, then concentrated to dry in a rotary evaporator at 40 ± 5°C and finally the yielded crude total extract of the plants was preserved at 4°C until use (El-Sherbini et al., 2009). ALE was given in a dose of 1.5 g/kg/day (El Morsy and Kamel, 2014) from the day 35 post infection