Does Urtica Pilulifera Have Anti-Schistosomiasis Mansoni Activity?

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***Abstract*—**Schistosomiasis tops all the endemic parasitic diseases world-wide particularly in Egypt. Application of plant extract is common ethnobotanical practice for treatment of different diseases. The present study was undertaken to assess the effect of the crude extract of Urtica pilulifera aerial part on experimentally infected mice with Schistosoma mansoni. The schistosomicidal activity of the U.P extract was evaluated, six weeks post-infection, on some parasitological and biological aspects including worm load, liver function and IL6. Mice were divided into nonimmunized non infected group, nonimmunized infected group and the other groups were immunized with petroleum ether and methanol extractions of Urtica pilulifera (whole plant & herb). After immunization, the immunized groups were infected with 150 Schistosoma mansoni cercariae. From the results, the worm load of mice immunized with petroleum ether and methanol extractions of Urtica pilulifera (whole plant & herb) was decreased significantly comparing with the control group, also SGOT & SGPT were decreased in the immunized groups compared to control one. On the other hand, the values of IL-6 were decreased in mice groups immunized with petroleum ether and methanol extractions of Urtica pilulifera comparing with the control group (infected non immunized group). Whole plant extraction of Urtica pilulifera gave best results more than herb extraction. Morever, the petroleum ether extract of Urtica pilulifera decreased the number of worms of Schistosoma mansoni, values of SGOT,SGPT and IL-6 more than the methanol extraction of Urtica pilulifera. These results indicate that Urtica pilulifera extracts may represent a new inexpensive and effective natural antigen against Schistosoma mansoni infection.

***Keywords*—** Schistosomiasis- Worm load- GPT- GOT- IL6.

I. Introduction

SCHISTOSOMIASIS is a widespread helminthic disease. Schistosomiasis tops all the endemic parasitic diseases world-wide particularly in Egypt[1] and over 200 million people worldwide suffer from it and kills more than 20000 people every year[2].Chronic egg-induced inflammation in the liver can lead to fibrosis,portal hypertension,bleeding and eventual death [3]. There is yet no vaccine available and the current mainstay of control is chemotherapy with praziquantel. In view of concern about the development of tolerance and/or resistance to praziquantel, there is a need for research and development of novel drugs for the prevention and cure of schistosomiasis[4] and the tolerance to PZQ has been reported in Egypt[5].

Most pathology in schistosome-infected animals is attributed to the host's reaction to the eggs, which is maximal by the 8th week of infection. The toxic egg material destroys the host tissue cells and the antigenic material stimulates the development of large inflammatory reactions (granuloma) around the egg material [6]. Schistosoma mansoni infection develops unique severe granulomatous inflammatory reactions in the liver that lead to certain dysfunction, which represents the start of various fatal consequences, associated with this disease [7].

High rate of oxidative processes and formation of hepatic malonaldehyde are due to the peroxidative damage to the liver microsomal membrane lipid and impairment of the antioxidant defense characterize schistosomiasis[8],[9]. The antioxidant defense mechanisms remove reactive oxygen species once formed [10] .

The role of plants extract in this regard has been recently investigated[11],[12]. Therefore, the present study was undertaken to assess the efficacy of immunized mice with crude extract to resist Schistosoma mansoni through measuring the parasite load as well as biochemical parameters of experimentally infected mice.

II. Materials And Methods

A. Infection and experimental groups

Forty eight female Swiss albino mice, weighing 18-20 grams were obtained from the animal house (National Research Center; Dokki, Cairo-Egypt). Mice were divided into 6 groups (8 mice/group). Group I was given saline and used as normal control mice(nonimmunized non infected group). Group II was infected and served as infected control(nonimmunized infected group). From group III : VI immunized with (14 mg/kg) petroleum ether and methanol extracts of Urtica pilulifera . After 2 weeks from first immunization all immunized mice take poster dose from extracts(hafl the first dose). Groups III and IV immunized by PE extract of whole parts and herb (14 mg/ kg) . Group V and VI immunized by methanol extract of whole parts and herb(14 mg/ kg). Immunized mice groups were infected by tail immersion method with 150 Schistosoma mansoni cercariae per each mouse (Oliver and Stire Walt,1956).

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Parasite load: 42 days after cercarial exposure, all animals were sacrificed. Adult worms were recovered from the portal and mesenteric veins by perfusion[13].

B. . Biochemical Estimation

Blood samples collection: After 48 days of cercarial exposure, blood samples were retro-orbitally collected from the inner canthus of the eye under light ether anesthesia using capillary tubes (Micro Hematocrit Capillaries, Mucaps). Blood was collected in clean vials and serum was separated in an electric centrifuge at 3000 rpm for 10 min.

Serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were measured according to Bergmeyer et al., (1986)[14].

Serum IL-6 levels were measured by using a polyclonal ELISA kits (RapidBio Lab., Calabasas, California, USA)

Statistical analysis: All data were conducted with the software packages Microsoft SPSS version 11.0, for statistical evaluation. Results are expressed as mean ± S.E. The results were analysed for statistical significance by one way ANOVA.

III. Results And Discussion

Application of Urtica pilulifera. extract is common ethnobotanical practice for treatment of different diseases[15],[16],[17].

Schistosomiasis causes a reduction in the levels of protective endogenous antioxidants and increases generation of free radicals[8],[9]. Most investigations of the response of schistosomiasis to antioxidant substance have concerned on single compound and little is known of possible interactive effects of different antioxidants. As these latter seldom occur in nature, it is important to understand any interaction (synergistic or addative) which may occur. Therefore, the present investigation dealt with possible anti-schistosomiasis properties of U.P petroleum ether and methanol extract for whole plant(herb, root and seeds) or only herb extract.

TABLE I
THE WORM LOAD COLLECTED FROM MICE IMMUNIZED WITH CRUDE AND HERB OF PETROLEUM ETHER AND METHANOLIC EXTRACTIONS AND INFECTED WITH 150 *S. MANSOMI* CERCARIAE

Mice groups		Mean number of worms ±S.E
1- Infected control (n = 8)		40.63 ± 1.61
LSD		(2,3,4,5)
Petroleum ether	2- Whole plant	10.0 ± 2.42
	LSD	(1,2,4)
	3- Herb	26.25 ± 1.66
	LSD	(1,3,5)
Methanol extract	4- Whole plant	15.5 ±2.02
		(1,2,3,4)
	5- Herb	28.38 ±1.6.
	LSD	(1,3, 5)

LSD: Least Significant Difference

Serum AST and ALT levels were elevated in the infected-nonimmunized group as compared to control group, while this increase was significantly decreased in immunized mice groups with both petroleum ether and methanol extract. These enzymes are commonly employed as biological markers for hepatic cell damage and impaired cell membrane permeability or due to heavy Schistosoma egg deposition[8]. There was least significant difference between the effect of immunized infected mice with both extracts and infected control on AST and ALT . Moreover, there were significant differences between the immunization with whole plant and herb extract. This difference may be due to the different compound separated by each solvent and /or different parts.

Expulsion of helminth parasites requires a coordination of both immune and functional host responses [18] There are a number of factors that can influence host resistance to infection including nutritional status and anti-oxidant activity[19].

Previous study reported that methanol and petroleum ether extract of all parts of U. pilulifera extracts were found to exhibit the best antioxidant activity against various oxidative stress in vivo and this activity has been attributed to the reduction of lipid peroxidation and elevation of antioxidant enzyme activities [20] . Therefore, the presence of antioxidant compounds such as vitamins, flavonoids and minerals in this plant provides further evidence for the beneficial chronic effects of U. pilulifera extract on hazardous effect produce through infection.

The previous work exhibited remarkable increments in total leukocytes and imunoglobin G and M in mice treated with U.P. extract as compared with control animals which could be attributed to the powerful defense reaction and allergic manifestation against the schistosomes and/or their egg(unpublished paper). On the other hand, increase in total protein in mice treated with U.P. may increase the globulin which represent responsive mechanism enhancing the immunity of the host as described before [21].

TABLE II
SERUM LEVEL OF INTERLEUKIN- 6 (IL-6) IN MICE IMMUNIZED WITH CRUDE AND 1HERB OF PETROLEUM ETHER AND METHANOLIC EXTRACTIONS AND INFECTED WITH 150 SCHISTOSOMA MANSONI CERCARIAE.

Mice groups		Mean of IL-6±S.E
1- Normal control		22.43 ± 1.19
		(2,3,5,6)
2- Infected control		43.25 ± 1.13
		(1,3,4,5,6)
Petroleum ether	3-Whole plant	24.17 ± 1.64
	LSD	(2,4,5,6)
	4- Herb	33.27 ± 1.21
	LSD	(1,2,4,6)
Methanol extract	5- Whole plant	27.01 ± 1.74
	LSD	(1,2,3,5)
	6- Herb	36.47 ± 0.91
	LSD	(1,2,4,6)

LSD: Least Significant Difference.

TABLE III
SERUM LEVEL OF SGOT AND SGPT IN MICE IMMUNIZED WITH CRUDE AND HERB OF PETROLEUM ETHER AND METHANOLIC EXTRACTIONS AND INFECTED WITH 150 SCHISTOSOMA MANSONI CERCARIAE.

Mice groups		Mean of SGOT±S.E	Mean of SGPT±S.E
1- Normal control		24.75 ± 2.07	28.63 ± 1.55
LSD		(2,3,5,7, 9)	(2,3,4,5,6)
2- Infected control		93.13 ± 3.60	98.25 ± 3.33
LSD		(1,3,5,7,9)	(1,3,4,5,6)
Petroleum ether	3- Whole plant	36.00 ± 1.25	41.38 ± 1.74
	LSD	(1,2,4,5,6)	(1,2,4,5,6)
	4- Herb	47.88 ± 1.52	56.50 ± 2.77
	LSD	(1,2,3,6)	(1,2,3,6)
Methanol extract	5- Whole plant	49.38 ± 2.48	62.25 ± 4.43
	LSD	(1,2,3,6)	(1,2,3,6)
	6- Herb	60.88 ± 3.55	74.75 ± 6.56
	LSD	(1,2,3,4,5)	(1, 2,3,4,5)

LSD: Least Significant Difference.

IL6 production is well-established and reliable marker of macrophage activasion [22] . The extract of *U.P* ameliorate the effect of infection on the IL6.

However, the effect of *UP* extract in increasing total protein, thyroid ,humeral immune response(IgG and IgM) in previous study , decrease in IL6 in this experiment and decrease liver enzymes may reflect its capability to improve liver damage as a result of infection.

The phytochemicals study in *U.P*. showed that this plant extract contains genistein. Genistein seem to affect the Ca2+ homeostasis (by way of altering the Ca2+ flux into or through the parasite's tegumental interface) that, in turn, leads to detrimental changes in the parasite [23]. Perhaps the changes in the Ca2+ homeostasis in the parasite subsequently also lead to changes in the activities of several enzymes/metabolic processes[24],[25] in the parasite under the high energy demand because of the anthelmintic stress caused by the plant-derived components.

It is concluded that immunized mice with *Urtica pilulifera*. whole plant petroleum ether extract markedly reduced worm load/mouse which may have been reflected on a moderate improvement of the tested biochemical parameters expressing liver function.

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