

POTENTIAL THERAPEUTIC AND PROPHYLACTIC EFFECTS OF PURSLANE (*PORTULACA OLERACEA*) OIL EXTRACT IN MURINE SCHISTOSOMIASIS MANSONI

By

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Abstract

Schistosomiasis is a neglected tropical illness, relying on a single drug to treat, its efficacy decreased as drug resistant strains emerged due to widespread usage. The study evaluated the therapeutic and prophylactic effects of Purslane (*Portulaca oleracea*) oil extract in comparison to praziquantel (PZQ) on experimental *S. mansoni*-infected mice by measuring parasitological, antioxidant activity, immunological, and histopathological parameters. Mice were infected followed by a single oral dose of PZQ or daily Purslane oil extract for 15 days, six weeks post-infection. Purslane treated group induced significant reduction in mean worm burden, hepatic, and intestinal egg loads, serum levels of ALT, AST along with significant rise in CAT, SOD, GSH-R, GPx activities, TNF- α , IL-4, IL-10, dead eggs percentage, and moderate decrease in granuloma diameter. PZQ was more effective than purslane. Purslane's methanol extract has promising therapeutic but little prophylactic effects against *S. mansoni*.

Keywords: *Schistosoma mansoni*, Purslane oil extract, Praziquantel, Assessment

Introduction

Schistosomiasis is a neglected tropical illness affecting about 250 million people in 78 countries (WHO, 2023). *Schistosoma mansoni*, *S. haematobium* and *S. japonicum*, are three main species that cause disease and have a major negative impact on human health as well as social and economic development (Lo Verde, 2019). The egg deposition starts the schistosomiasis pathology. In response to continuous stimulation by egg antigens, immunological and inflammatory cells are progressively recruited to the infection site, causing development of egg granuloma and, ultimately, fibrosis (Chuah *et al*, 2014).

Diverse cytokines are intimately linked to this process and have a significant role in tissue fibrosis and can either stimulate or inhibit particular immune responses (Zhou *et al*, 2021). Unfortunately, the damage caused by eggs was not significantly affected by the therapeutic dose of praziquantel (PZQ). There was strong evidence about emergence of resistant strains to Praziquantel that hinders to develop novel antischistosomal, inexpensive treatments giving hepatic protection. The plant therapy comes first (Al-Olayan *et al*, 2016). De Almeida *et al*. (2016) reported

that anti-parasitic chemicals were from natural sources, medicinal plants and herbs.

Purslane (*Portulaca oleracea*) is an annual grassy used extensively as a therapeutic herb in countries throughout Asia, the Mediterranean and Central Europe (Rakhshandeh *et al*, 2022). It belongs to family Portulacaceae and grows wild in field crops and lawns in a range of climates and geographical regions globally (Petropoulos *et al*, 2016). In Egypt, it's referred to as Rejlal (Shehata and Soltan, 2012). The World Health Organization designated purslane as a global panacea and listed it among the most often used medicinal plants (Katalinic *et al*, 2006).

The pharmacological efficacy and biosafety of purslane were proved (Abou Zid and Mohamed, 2011; Ghasemian *et al*, 2019). Many active ingredients are found in purslane, including terpenoids, flavonoids, phenolics, alkaloids, organic acids, minerals, and vitamins (Jaafari *et al*, 2021). Due to its abundance of antioxidants such as glutathione, ascorbic acid, α -tocopherol, and omega-3 fatty acids, purslane seed extract was explored as the potential dietary supply (Uddin *et al*, 2014).

The current study aimed to assess therape-

utic and prophylactic effect of Purslane oil extract against *S. mansoni* in Albino mice, by parasitological, antioxidant activity, immunological, and histopathological parameters.

Material and Methods

Animal and infection: The study was conducted on 48 pathogen-free male mice of CDI-strain, aged 6-8 weeks, & 20-24g weight. Mice and experiments were obtained and performed in Biological Unit of Theodor Bilharz Research Institute, Giza, Egypt. They were maintained on sterile water and a balanced dry food containing protein 14%, and in conditioned room at 28°C.

Ethical rules in using animals in research were in line with the Helsinki Declarations (2013) and with the TBRI Guidelines.

Infected *Biomphalaria alexandrina* with Egyptian strain *S. mansoni* were kept in 200 ml of distilled water and exposed to artificial light for 2hrs for cercariae shedding (Tekwu *et al*, 2017). Mice were infected subcutaneously with 0.2ml aliquots of a cercarial suspension contained $\sim 80 \pm 10$ into their loose skin of the back (Olivier and Stirewalt, 1952).

Grouping: Mice (48) were divided into six equal groups: GI: Normal control, GII: Positive control, infected with *S. mansoni*, GIII: Normal purslane control, orally given methanolic extract of purslane seed (200mg/kg), once daily for 15 consecutive days, GIV: Prophylaxis before infection, given methanolic extract of purslane seed (200mg/kg), once daily for 15 consecutive days, GV: *S. mansoni*-infected, given methanolic extract of purslane seed (200mg/kg), once daily for 15 consecutive days six weeks post infection, and GVI: *S. mansoni*-infected, given oral praziquantel (single 500mg/kg) six weeks post infection.

Seed extracts: 1. Methanolic extracts: Seeds were purchased from local markets, and underwent authentication and preparation at the Unit of Genetic Engineering and Biotechnology, Faculty of Science, Mansoura University. Seeds were subjected to a drying process at a temperature of 40°C within an

oven for one day until totally dried. Dried seeds were crushed and submerged in 70% (v/v) methanol at 45°C for 48hr., evaporated and liquefied in distilled water. The solutions were thoroughly agitated until the extract dry weight was completely dissolved.

2- Phytochemical analysis: Investigation of phenolic content of purslane seed extracts was detected using Folin-Ciocalteu (F-C) test, and the flavonoids content was detected using aluminum chloride method as described by Abdel Moneim (2013). Total phenolic and flavonoid contents were expressed as milligram gallic acid and milligram quercetin equivalents per gram dried weight of the extract using the calibration curves of gallic acid and quercetin. The purslane extract was administered orally to mice daily for 15 days, with a dosage of 200mg/kg body weight (Farag *et al*, 2021).

Drugs: Praziquantel (Distocide[®], EIPICO) 600mg tablets were crushed into powder and dissolved in 60ml distilled water (1ml/mouse), and given orally by gastric gavage in a single dose of 500mg/kg BW.

Sample collection: Forty-eight hours after last dose administration, animals were euthanized under isoflurane inhalation and decapitated. Blood samples were taken and centrifuged at 2000 rpm for 10 minutes to separate serum and kept in a freezer at -20°C until used to measure parameters. Livers were dissected and rinsed in ice-cold 50mM Tris \pm HCl, pH 7.4, twice, weighed and directly homogenized again in the ice-cold to give a 10% (w/v) homogenate which was centrifuged at 2000g for 5 min at 4°C and supernatants were used for biochemical analysis.

Parasitological parameters: A. For worm burden perfusion of the portal and mesenteric veins were employed as 0.9% NaCl (w/v) (Delgado *et al*, 1992). The worms were allowed to settle in a 15-cm Petri dish for 20 min, their number and sex were identified. The worm burdens were determined by counting number of males, females, and coupled worms recovered using a stereo microscope. This formula was used to determine the red-

uction percentage in parasite load. % reduction = value of infected control - value of treated mice/value of infected control $\times 100$.

B. Oogram pattern: Liver and intestinal tissue samples were incubated in 5% KOH at 37°C overnight, and the recovered eggs were counted in 50 μ l aliquots, calculated at $\times 40$, and the average eggs/g of hepatic and intestinal tissues were determined. Eggs retained in intestinal wall was classified as immature, mature, dead, and percentage of different developmental stages was recorded.

Liver enzymes: AST and ALT activities were determined calorimetrically (Reitman and Frankel, 1957).

Antioxidant parameters: Activities of antioxidant enzymes in liver tissues homogenates were assayed, superoxide dismutase (SOD) by Nishikimi *et al.* (1982), catalase by Aebi, (1984), glutathione peroxidase (GPx) by Beutler *et al.* (1963), and glutathione reductase (GSH-R) by Koracevic *et al.*, (2001), Biodiagnostic Co., Giza, Egypt.

Immunological parameters: Serum levels of TNF- α , IL-4, & IL-10 were measured by ELISA (BioSource Co., San Diego, Catalog Number: MBS175904 and Quantikine Co. McKinley Place, Minneapolis, USA, Catalog Number: R6000B), the results were expressed in picograms per milliliter (pg/ml).

Histopathological examination: Liver specimens were fixed in 10% phosphate buffered formalin, embedded in paraffin, sectioned at 5 μ m thickness, and stained with hematoxylin and eosin, or Masson's Trichrome stains. Mean diameter of granulomas was measured by an ocular micrometer. Reduction % in granuloma diameter relative to positive control mice (GII) was calculated as follows: % Reduction of granuloma diameter = Mean diameter of controls - mean diameter of treated groups / Mean diameter of controls in five microscopic fields in serial tissue sections (Lichtenberg *et al.*, 1962).

Statistical analysis: Data were coded, computerized and analyzed by IBM-SPSS 24.0 (IBM-SPSS Inc., Chicago, IL, USA). Descriptive data: Means, standard deviations,

medians, ranges, frequency and percentages. Shapiro-Wilk tested the normality of continuous variables, and ANOVA tested variables with more than two categories for mean differences. A P-value < 0.05 was significant.

Results

The mean phenolic content of purslane seed extracts was 43.7mg gallic acid/1g dry extract, and that of flavonoid content was 27.4mg quercetin/1g dry extract. *S. mansoni*-infected mice showed significant elevations in levels of ALT, AST, TNF- α , IL-4, & IL-10 (P < 0.05), but significant reduction in activities of CAT, SOD, GSH-R, & GPx (P < 0.05) as compared to normal control. Purslane treated mice showed significant reduction in mean worm burden (89.8%), hepatic egg load (80.9%) and intestinal egg load (90.4%), levels of ALT, & AST along with significant increase in CAT, SOD, GSH-R, & GPx activities, TNF- α , IL-4, IL-10, dead eggs percentage (86.2%), and moderate decrease in granuloma diameter compared to infected control. Infected mice given prophylactic purslane showed non-significant decrease in mean worm burden (22.5%), hepatic egg load (30.1%) and intestinal egg load (27.1%) serum levels of ALT, & AST, along with a non-significant increase in CAT, SOD, GSH-R, GPx activities, TNF- α , IL-4, IL-10, dead eggs (17.6%), and minimal decrease in granuloma diameter compared to infected control (GII). There was significant (p < 0.001) effect of different treatment regimens on all parasitological parameters with highest effect with oral praziquantel (GVI) followed by methanolic seed extract of purslane post infection (GV). There was significant (p < 0.001) effect of treatment regimens on ALT & AST with the highest effect in non-infected mice taking purslane oil extract (GIII) followed by methanolic seed extract of purslane post infection (GV), oral praziquantel (GVI) and then prophylactic methanolic seed extract of purslane (GIV). As to antioxidant activities on murine schistosomiasis, there was a significant (p < 0.001) treatment effect on all parameters with the hig-

hest one with methanolic seed extract of purslane post infection (GV) followed by oral praziquantel (GVI) then prophylactic methanolic seed extract of purslane (GIV) in contrast to infected (GII). There was a significant ($p < 0.001$) treatment effect on all immunological parameters. TNF- α , showed the highest effect was with (GV) and (GVI) followed by (GIII), then (GIV). For IL-4, the highest effect was with (GIII) followed by oral praziquantel (GVI), (GIV) then (GV). For IL-10, the highest effect was with (GV) and (GVI) followed by (GIII) then (GIV).

Histopathological examination showed. A-

Table 1: Effect of purslane seed extract and PZQ on murine schistosomiasis

Variations	GSH-R ($\mu\text{mol/mg}$)	SOD (U/L)	CAT (U/mL)	GPx (mU/mg)
Normal control	42.7 \pm 1.2	96.0 \pm 1.3	3.6 \pm 1.3	3.68 \pm 0.41
Infected control	24.0 \pm 1.0	54.5 \pm 3.4	0.12 \pm 0.06	1.07 \pm 0.18
Normal & purslane oil extract	35.1 \pm 1.4	85.3 \pm 0.5	0.76 \pm 0.01	3.01 \pm 0.13
Purslane prophylactic	26.2 \pm 0.6	68.1 \pm 1.5	0.43 \pm 0.04	1.94 \pm 0.66
Infected & purslane oil extract	38.5 \pm 1.3	94.5 \pm 1.5	0.98 \pm 0.02	2.98 \pm 0.42
Infected & PZQ	30.7 \pm 1.4	85.6 \pm 3.0	0.78 \pm 0.05	2.29 \pm 0.59
* F-ratio	289.13	467.18	44.71	33.72
* P value	< 0.001	< 0.001	< 0.001	< 0.001

Table 2: Immunological parameters of groups

Items	GI	GII	GIII	GIV	GV	GVI	F-Ratio	P-value
TNF- α	43.4 \pm 5.8	572.5 \pm 43.6	342.2 \pm 33.9	403.5 \pm 19.6	263.6 \pm 12.7	257.5 \pm 39.1	287.75	< 0.001
IL-4	18.3 \pm 1.8	78.4 \pm 3.3	27.5 \pm 1.4	53.0 \pm 5.6	66.4 \pm 14.9	40.3 \pm 9.8	69.51	< 0.001
IL-10	85.3 \pm 9.8	442.0 \pm 59.9	287.5 \pm 39.1	367.2 \pm 53.9	105.3 \pm 19.3	97.4 \pm 15.2	133.34	< 0.001

Discussion

Schistosomiasis ranks second importance among the tropical diseases after malaria (WHO, 2023). There is a strong evidence about the development of PZQ resistance (Utzing *et al*, 2007). Research on novel therapies for schistosomiasis is therefore strongly encouraged. Herbal medicines contain phytoconstituents with various pharmacological activities, which inhibit the production of reactive oxygen species (Aborehab and Boshra, 2019).

According to our research, infected mice treated with purslane showed significant reductions in worm count, hepatic, and intestinal egg load along with a significant increase in percentage of dead eggs. PZQ was more effective than purslane. Purslane seed oil extract may have antischistosomal agents such as flavonoids, polyphenols, and tannins that reduced infection-related the liver dam-

Normal control showed normal hepatic architecture. B- Positive control showed large granuloma of fibrocellular type. C- Purslane prophylaxis showed large granuloma of cellular types with minimal decrease in granuloma diameter. D- Purslane treated showed fibrocellular granuloma with moderate decrease in granuloma diameter. E- Praziquantel treated showed small fibrous granuloma with a marked decrease in their diameter (H&E and Masson's Trichrome-stained at x 200).

Details were given in tables (1 & 2) and figures (1, 2, 3, 4, 5 & 6).

age and oxidative stress (Mohammed *et al*, 2023).

The degree of hepatocellular injury was evaluated by measuring AST, and ALT. The two enzymes' activities were significantly upregulated in infected, untreated mice, indicating that *S. mansoni* causes liver damage. It was also detected in the PZQ-treated mice. Comparably, the purslane-treated group's ALT and AST levels significantly decreased, indicating reduced liver injury compared with those in the infected untreated mice. These agreed with the outcomes of Abdel-Hafeez *et al*. (2012), Mohamed *et al*. (2014), and Elmalawany *et al*. (2023).

Given that granuloma and immune-related cell types are known to be associated with reactive oxygen species generation and oxidative damage, the immunological response of inflammatory cells is most likely the primary cause of the increase in oxidative stre-

ss in the tissues (Al-Olayan *et al.*, 2016).

The antioxidant enzymes were analyzed, SOD, CAT, GSH-R, and GPx. *S. mansoni*, infected control showed statistically significant decrease ($p < 0.001$) in the antioxidant enzyme activity in contrast to normal controls. Purslane and PZQ treatment considerably ($p < 0.05$) alleviated this inhibition. Mohamed *et al.* (2014), Beshay *et al.* (2019), and Elmalawany *et al.* (2023) have found comparable results in mice and human schistosomiasis, which supports this.

The current study showed that eight weeks post infection, cytokines TNF- α , IL-4, and IL-10 were peaked. Owing to the inflammatory responses generated by *S. mansoni* eggs suggesting that Th2 immune responses are usually elicited in persistently infected mice in response to schistosomes chemokine, fibroblast, collagen, and matrix protein, are all upregulated by IL-4, indicating that this cytokine is essential for the formation of granulomas (Liu *et al.*, 2002). This agreed with Franchimont *et al.* (1999), who showed that granuloma size was decreased upon exogenous IL-10 treatment. Besides, mice lacking IL-10 showed the opposite result (Sanin *et al.*, 2015).

Mice infected and treated with praziquantel, or purslane seed extract showed a notable decrease in these proinflammatory cytokine levels. These results agreed with Azevedo *et al.* (2012), Mohamed *et al.* (2014), and Femoe *et al.* (2022). According to Pradere *et al.* (2013), TNF- α is an acute-response cytokine that helps HSCs initiate the NF- κ B pathway. Femoe *et al.* (2022) reported that the murine schistosomiasis *mansoni*, treated with PZQ led to the significant reductions in the serum levels of TNF- α .

In the present study, livers in Purslane and PZQ treated mice showed a significant reduction in diameter of eggs granulomas as compared with infected untreated ones. This agreed with El-Lakany and Noseir (2007), who found that PZQ significantly regulated cellular immunological responses by decreasing the CD4 T cells number and increasing

CD8 number, in turn reduced hepatic granuloma size.

In the present study, prophylactic purslane showed non-significant decrease in mean worm burden, hepatic egg load, and intestinal egg load, levels of ALT, &AST, with a non-significant increase in CAT, SOD, GSH-R, & GPx activities, TNF- α , IL-4, IL-10, dead eggs percentage, and minimal decrease in granuloma diameter as compared to infected control.

Many authors reported that Purslane's tannins, flavonoids, and alkaloids have antimicrobial and anti-parasitic properties (Dhole *et al.*, 2011; Abdel-Hady *et al.*, 2014; Eskandari *et al.*, 2016; Fomum and Nsahlai, 2017; Othman *et al.*, 2019; Metwaly *et al.*, 2021). Because of their anti-parasitic property, these chemicals could be responsible for the schistosomicidal effect, over and above their antioxidative and anti-inflammatory properties (Lee *et al.*, 2014).

Conclusion

Purslane methanol extract is cheap, available and has promising therapeutic effects without any marked side effects, but weak prophylactic effects on murine *S. mansoni*.

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Explanation of figures

- Fig. 1: Phytochemical constituents of purslane seed extracts
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 Fig. 4: Mean oogram pattern.
 Fig. 5: Mean granuloma size
 Fig. 6: ALT & AST serum levels of different treatments
 Fig. 7: Hematoxylin-eosin and Masson's trichrome-stained liver sections (x200).

