Research Article

# Antischistosomal Activity of *Trigonella foenum* (Fenugreek) Crude Seeds Water Extract on Infected Mice

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**Abstract** | Anti-parasitic activity of the fenugreek crude seeds water extract (FWE) on *Schistosoma mansoni* infected mice was studied in the present work and Praziquantel (PZQ) was applied as a standard drug. FWE was chemically analyzed by the high-performance liquid chromatography (HPLC) technique. The main recorded constituents were rutin, P-coumaric, hydroxyl-benzoic acid, ferulic acid, and sinapic acid. Results of the present study indicated that FWE (0.5 ml/mouse for 10 successive days) exhibited a significant reduction (P<0.05) in worm burden. A scan electron microscope examination of male worms showed tegument corrosions with reduced spines density and abnormal spine tubular shape. Treatments of infected mice with FEW or PZQ significantly reduced hepatic and intestinal total egg count, while showed a significant increase in dead egg percentages and reduced mature egg count in oogram pattern as compared to infected control mice. Hepatic granuloma diameters in FWE treated infected mice were significantly reduced when compared to the infected control group. FEW also reduced the lymphocytic infiltration around the granulomatous tissue. It can be concluded that FWE exhibited anti-schistosomal and anti-inflammatory effects on infected mice.

**Keywords** | *Schistosoma mansoni, Trigonella foenum*, Fenugreek water extract, Praziquantel, Rutin, P-cumaric

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## INTRODUCTION

*Trigonella foenum* (fenugreek) is a plant that belongs to the Fabaceae family and is cultivated worldwide as a semiarid crop (Wani and Kumar, 2018). It's found in northern Africa and India and famous as a human food with a distinctive flavor, it can also be drunken as a tea. It's used as whole seeds or powder and was applied firstly in the pharmaceutical fields in ancient Egypt. The majority of the common active constituents of fenugreek seeds are phenolic compounds, in addition to amino acids, protein, vitamin A, starch and minerals such as Calcium, Phosphorus, Magnesium, Iron, Zinc and Manganese (Kumar et al., 2009; Wani and Kumar, 2018). One of the main phenolic constituents of fenugreek seeds is rutin, a bioflavonoid phenolic compound found in a variety of plants including citrus, which is characterized by its distinctive pigment (Patel and Patel, 2019). Coumarin derivatives are also important constitutions that are commonly found in fenugreek seeds (He et al., 2015). They are colorless crystalline solids with a sweet odor found in many plants like cinnamon and cherry (Kruger et al., 2018). Other fenugreek phenolic constituents are ferulic and sinapic acids. They are organic hydroxy-cinnamic acids found naturally in the plant cell wall and represent the precursor of aromatic compounds manufacturing (Bunzel et al., 2003; Pajak et al., 2018). Fenugreek seeds have considerable medical importance. For example, it

had been reported to improve body health and digestion functions in rabbits (Abdel-Wareth et al., 2021). Moreover, administration of ethanolic or ether extracts of fenugreek seeds exhibited anti-inflammatory, anti-arthritic activities, and antioxidant effects in arthritic albino rats (Suresh et al., 2012; Pundarikakshudu et al., 2016). Also, water extract of fenugreek was reported to exert an anti-helminthic effect (Swarnakar et al., 2014). The antiparasitic and anti-inflammatory effects of fenugreek seeds are attributed to its active constituents like rutin, coumarin, sinapic and ferulic acids (Chauhan et al., 2018; Yin et al., 2019; Lopes et al., 2020; Quinn et al., 2020; Liseth et al., 2021).

Schistosomiasis is a chronic disease that is caused by the trematode blood fluke *Schistosoma* and is responsible for the infection of at least 236.6 million people who required preventive treatment, it is second to malaria in tropical countries and is listed as a neglected tropical disease (WHO, 2021) and has a high prevalence in Africa. Schistosomiasis can strike either intestine or urinary tract resulting in pathogenic symptoms like diarrhea, bloody stool or urine, abdominal pain, or even more hazardous cases of hepatic and kidney failure (Abdel Gawad et al., 2018; WHO, 2021).

Different drugs were developed for schistosomiasis treatment and the most efficient one is PZQ. Though it's the most effective drug with fewer side effects, recent researches still provide proof about *Schistosoma*-drug resistance. Pinto et al. (2021) found that the worm of *S. mansoni* that was previously exposed to PZQ developed new protein content which was less sensitive to the drug.

This study aims to estimate the anti-schistosomal effect of fenugreek seeds water extract on *Schistosoma mansoni* infected mice.

## MATERIALS AND METHODS

### Fenugreek plant seeds (*Trigonella foenum*)

Fenugreek dry seeds were purchased from a local market, Shebin El-Kom, Menoufia. Fenugreek water extract (FWE) was prepared according to Sakr and Abo-El-Yazid (2012) in the laboratory of Faculty of Science, Menoufia University and followed the standards.

### Standard drug (Praziquantel, PZQ)

PZQ drug was purchased from Egyptian International Pharmaceutical Industries Company (EIPICO). Each tablet was grinded and freshly suspended in distilled water, administered orally to mice in 3 doses each of 250 mg/kg for three alternative days (Utzinger et al., 2003).

### Experimental animals

Swiss male albino mice CD-I strains (weighing 20±2 g) and *Biomphalaria alexandrina* snails infected with *Schistosoma mansoni* miracidia were obtained from Theodor Bilharz Research Institute (TBRI) Giza, Egypt. Mice were maintained under standard laboratory conditions and feeding at Parasitological Research Lab in Zoology Department, Faculty of Science, Menoufia University. Egyptian strain of *Schistosomamansoni* cercariae were shed from laboratory-bred infected *B.alexandrina*snails (Ethical number: MUFS /S /Pa /1 /20).

### Chemical analysis of FWE using high-performance liquid chromatography (HPLC)

HPLC analysis was carried out using Agilent Technologies 1100 series liquid chromatography according to Kim et al. (2005).

### Mice infection

Infected snails were washed with de-chlorinated tap water and left uncovered under white fluorescent light for one hour in a tube for cercarial shedding according to Pellegrino et al. (1962). Infection of mice was performed by subcutaneous injection of 70 ± 5 *S. mansoni* cercariae to each mouse according to Moore et al. (1977).

### Experimental design

Thirty-five mice were divided into 5 groups (7mice/each) as follow:

Group 1: served as normal control; Group 2: infected with 70±5 *S. mansoni* cercariae and served as an infected group; Group 3: infected and treated with 3 doses of PZQ each of hich 250 mg/kg for three alternative days up to 8 weeks post-infection; Group 4: infected and treated with FEW in a dose of 0.5 ml/mouse for 10 successive days up to 8 weeks post-infection; Group 5: infected and treated with PZQ accompanied to FWE up to 8 weeks post-infection.

### Parasitological parameters

#### Worm load

At the end of the treatment, mice were killed by decapitation. Hepatic and porto-mesenteric vessels were perfused to recover worms for subsequent counting (Cheever, 1969; Duvall and De Witt, 1967).

### Scanning electron microscope (SEM)

Adult *S. mansoni* worms from the experimental groups were fixed in glutaraldehyde buffer solution with 0.1 M sodium cacodylate buffer (pH 7.2) at room temperature for subsequent examination (Matos-Rocha et al., 2016). Joel JSM-5300, Japan scanning electron microscope was applied at Faculty of Science, Alexandria University, Egypt.

### Egg count

According to the method of Cheever and Anderson (1971), three samples were collected from the liver and intestine of each mouse and were prepared (0.5 g tissue / 5ml 5% KOH for each sample) and examined under the lower power of the microscope.

### Oogram pattern

Three tissue fragments (each of 1 cm) were collected from the liver and intestine of each mouseand placed between a slide and a cover-slip. One hundred eggs were counted microscopically and classified into immature, mature, and dead eggs according to the method of Pellegrino et al. (1962).

### Granuloma diameter and liver histopathology

Representative samples from liver tissues were taken from the animal groups and fixed in neutral formalin (10%). Paraffin-embedded sections (5µm thick) were stained using Hematoxylin and Eosin (Romeis, 1989). Granuloma containing a single egg was measured using an ocular micrometer. For each mouse, 40-50 granulomas were measured and the percent reduction in granuloma diameter relative to the infected controls was calculated (Mahmoud and Warren, 1974).

### Statistical analysis

Data are presented as Mean ± Standard deviation (M±SD). Student t-test, for normally-distributed data, was used to calculate the significance of differences observed between mean values of experimental and control groups in each experiment at a level of significance of $P < 0.05$ (Sokal and Rohlf, 1981).

## RESULTS

### Chemical analysis of FWE applying high-performance liquid chromatography (HPLC)

As shown in Table 1 and Figure 1, FWE was analyzed by the High-performance liquid chromatography (HPLC) technique into different phenolic compounds. Rutin represented the highest concentration (2705.96 mg/g) among phenolic constituents and followed by p-coumaric which recorded 768.59 mg/g. Then, p-hydroxybenzoic recorded 513.36 mg/g, while other phenolic constituents showed lower concentrations (Ferulic, Sinapic, and Apigenin-7-glucoside).

**Table 1:** High-performance liquid chromatography (HPLC) analysis of FWE (Phenolic compounds).

| Compounds | µg/g |
|---|---|
| Rutin | 2705.96 |
| p-coumaric | 768.59 |
| p-hydroxybenzoic | 513.36 |
| Ferulic | 355.29 |
| Sinapic | 346.85 |
| Apigenin-7-glucoside | 214.00 |

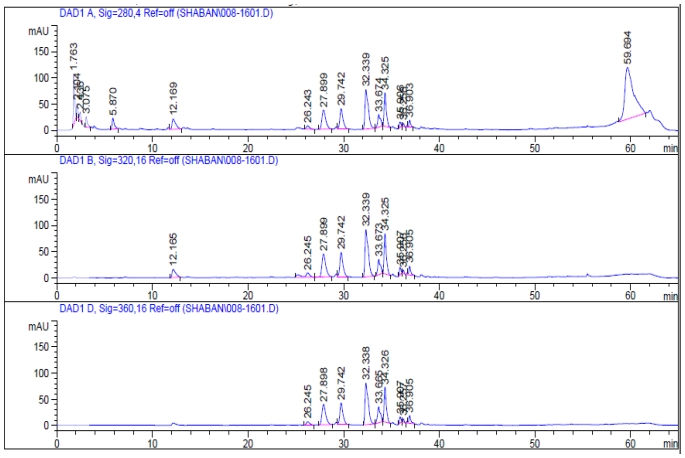


**Figure 1:** High performance liquid chromatography (HPLC) analysis of FWE analysis.

### Effect of FWE and/or PZQ on some parasitological parameters of *S. mansoni* infected mice

#### Worm burden

Worm burden was illustrated in Table 2A, *S. mansoni*

**Table 2:** Effect of FWE and /or PZQ on worm burden and total egg count of *S. mansoni* infected mice.

| **Effect of FWE and /or PZQ on worm burden in *S. mansoni* infected mice** | | | | |
|---|---|---|---|---|
| **Treatment/ Worm** | **Ic ± Standard deviation** | **I+FEW ± Standard deviation** | **Change % (Compared to infected control group)** | |
| Male | 4.3± 0.9 | 2.2 ± 0.3 | 48.8 ↓ | |
| Female | 3 ± 0.5 | 3.5± 0.4 | 16.6 ↑ | |
| Couple | 5 ± 0.8 | 3 ± 0.3 | 40 ↓* | |
| Total | 17. ±1.1 | 11.7± 0.9 | 32.4↓* | |
| **Effect of FWE and /or PZQ on total egg count in *S. mansoni* infected mice** | | | | |
| Egg/ Treatment | Egg count/gm (Liver tissue) ±Standard deviation | Reduction↓% (Compared to infected control group) | Egg count/gm (Intest. tissue) ±Standard deviation | Reduction↓% (Compared to infected control group) |
| Ic | 15333 ±1009 | --- | 25567 ± 1559 | --- |
| I+PZQ | 5433 ±978 | 64.6 ↓* | 266.7 ± 41 | 98.9 ↓* |
| I+FWE | 7467 ± 965 | 51.3 ↓* | 4233 ± 609 | 83.4 ↓* |
| I+FWE+PZQ | 3500 ± 500 | 77.2 ↓* | 1867 ± 340 | 92.7 ↓* |

* Significant change compared to infected control group. ($P < 0.05$). Ic (infected control), I+PZQ (infected and treated with PZQ), I+FWE (infected and treated with fenugreek water extract), I+FWE+PZQ (infected and treated with fenugreek water extract accompanied with PZQ).

infected mice treated with FEW (I+WE) showed a clear reduction in total worm number and number of couples by 32.4 % and 40%, respectively, as compared to the infected control group (IC). There is a significant (P > 0.05) reduction in male worm number in infected mice treated with FWE by 48.8%, while no worms were observed in praziquantel treatments (PZQ) either alone or accompanied with fenugreek as compared to the infected control group.

Scanning electron microscope (SEM) of S. *mansoni* worms (Figures 2, 3).

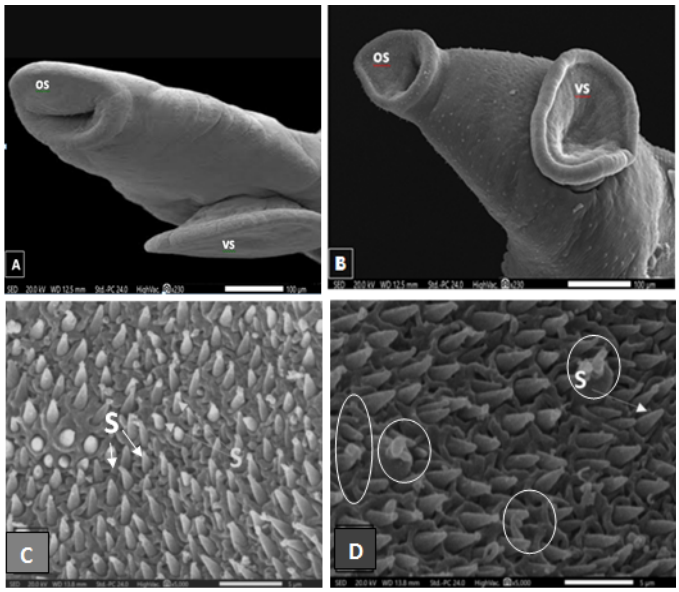


**Figure 2:** Scanning electron micrographs of *S. mansoni* worm collected from (A) Infected control mouse showing an anterior end with normal oral (os) and ventral suckers (vs). (B) Infected mouse treated with FWE showing no clear change in the oral and ventral suckers. (C) Enlarged portion of (A) showing oral sucker region covered with sharp spines. (D) Enlarged portion of (B) showing some corrosions in oral sucker spines after FEW treatments (circled).

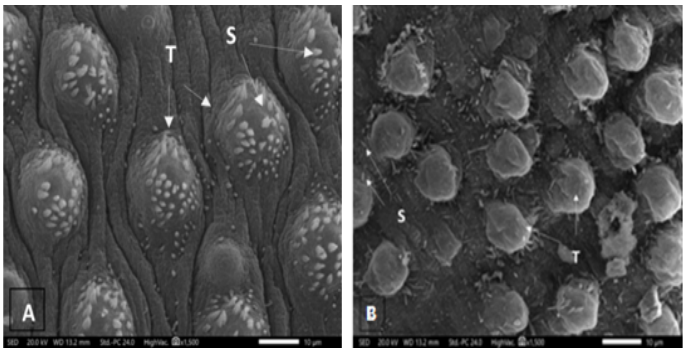


**Figure 3:** Scanning electron micrographs of *S. mansoni* worm collected from **(A)** Infected control mouse showing normal dorsal tegument tubercles (T) with spines (S). **(B)** Infected mouse treated with FWE showing mostly reduced spines (S) density and abnormal tubercular shape (T).

### EGG COUNT/G TISSUE

As shown in Table 2B, treatments of infected mice either with PZQ or FWE led to a significant (P > 0.05) reduction in the total hepatic egg count by 64.6% and 51.3%, respectively. While egg percentage was reduced by 77.2% in the infected mice were treated with PZQ accompanied to FWE as compared to that of the infected control group. In the intestinal tissue, treatment of infected mice with FEW significantly (P > 0.05) reduced eggs count by 83.4%, while, treatment of infected mice with PZQ showed the highest reduction in total intestinal egg count by 98.9%, as compared to the infected control group (Table 2).

### OOGRAM PATTERN

Oogram pattern in liver tissue was illustrated in Table 3A. Immature egg showed a significantly (P > 0.05) increased percentage in the infected group treated with PZQ by (68.8%), while FWE showed extra elevated immature egg percentage by 87%, as compared to the infected control group. The highest increase in the immature percentage was recorded in the infected mice treated with FEW accompanied with PZQ (109.1%) as compared to that of the infected control group. Treatment of infected mice with PZQ showed a significant (P > 0.05) reduction in mature egg percentage (54.2%), and also, but to less extent, FWE reduced mature egg count significantly (P > 0.05) by 33.9%. The highest reduction rate in mature egg percentage was recorded in the infected mice treated with FWE accompanied to PZQ by 73.6 %, as compared to the infected control group.

Oogram pattern in intestine tissue was illustrated in Table 3B. The highest immature count was recorded in the infected group treated with FWE accompanied with PZQ (144.3 %). While, the dead egg percentage was reduced closely in either PZQ or FWE by 56.9 % or 50%, respectively. The most reduction in mature egg count was recorded in the infected group treated with PZQ accompanied with FWE by 70.4%, as compared to the infected control group.

### HEPATIC GRANULOMA DIAMETER (GD)

Granuloma diameter was illustrated in Table 3C. In comparison with infected control mice, all treated groups showed a significant (P > 0.05) reduction in granuloma diameter in closed rates, and the most reduced diameter was exhibited in the infected group was treated with FEW accompanied to PZQ by 18.6% (Figure 4).

**Table 3:** Effect of FWE and /or PZQ on oogram patern and hepatic granuloma diameter of *S. mansoni* infected mice.

| **Effect of FWE and /or PZQ on oogram pattern (liver tissue) in *S. mansoni* infected mice.** | | | | | | |
|---|---|---|---|---|---|---|
| Egg stage/ Treatment | Immature % ±Standard deviation | Increase ↑ % (Compared to infected control group) | Mature % ±Standard deviation | Reduction↓% (Compared to infected control group) | Dead % ±Standard deviation | Increase↑% (Compared to infected control group) |
| Ic | 15.4 ± 3.6 | --- | 74.2 ± 4.3 | --- | **11** ± 1.6 | --- |
| I+PZQ | 26 ± 1.9 | 68.8 ↑* | 34 ± 2.2 | 54.2 ↓* | 40 ± 3.4 | 263.6 ↑* |
| I+FWE | 28.8 ± 4 | 87 ↑* | 49 ± 4.4 | 33.9 ↓* | 22.2 ± 3 | 101.8 ↑* |
| I+FWE+PZ | 32.2 ± 4.2 | 109.1 ↑* | 19.6 ± 2 | 73.6 ↓* | 48.2 ± 3.7 | 338↑* |
| **Effect of FWE and /or PZQ on oogram pattern (intestine tissue) of *S. mansoni* infected mice.** | | | | | | |
| Egg stage/ Treatment | Immature % ±Standard deviation | Increase ↑ % (Compared to infected control group) | Mature % ±Standard deviation | Reduction ↓ % (Compared to infected control group) | Dead % ±Standard deviation | Increase ↑ % (Compared to infected control group) |
| I+c | 17.6 ± 1.5 | --- | 50.6 ± 4 | --- | **32 ±** 3.1 | --- |
| I+PZQ | 29 ± 3 | 64.8 ↑* | 20.8 ± 2 | 58.9 ↓* | 50.2± 2.6 | 56.9 ↑* |
| I+FWE | 24.8 ± 3 | 40.9 ↑ | **27 ±** 2 | 46.2 ↓* | 48 ± 3 | 50 ↑ |
| I+FWE+PZQ | 40 ± 4 | 144.3 ↑* | 15 ± 2 | 70.4 ↓* | 45 ± 3 | 40.6↑* |
| **Effect of FWE and /or PZQ on hepatic granuloma diameter in *S. mansoni* infected mice.** | | | | | | |
| Treatment | Ic | I+PZQ | I+FWE | I+FWE+PZQ | | |
| Granuloma diameter ±Standard deviation | 34.4 ± 0.9 | 29 ± 1.9 | 29.3 ± 0.8 | 28 ± 2 | | |
| Reduction ↓ % (Compared to infected control group) | ---- | 15.7 ↓* | 14.9↓* | 18.6 ↓* | | |

* Significant change compared to infected control group. ($P < 0.05$). Ic (infected control), I+PZQ (infected and treated with PZQ), I+FWE (infected and treated with fenugreek water extract), I+FWE+PZQ (infected and treated with fenugreek water extract accompanied with PZQ).

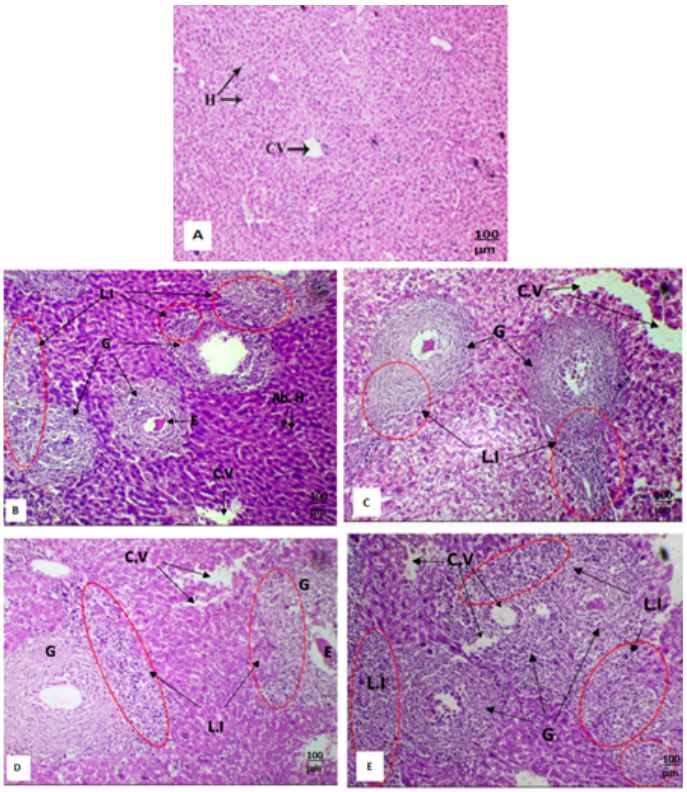


**Figure 4:** (A-E): Light micrograph of liver section of (A) Normal control mouse showing normal hepatocytes (H) and central vein. (B) *S. mansoni* infected mouse eight-week post infection showing abnormal hepatocytes (Ab. H), multiple *Schistosoma* eggs (E) provoke granuloma formation (G) of large diameters containing extended regions of lymphocytic infiltration (L.I). Cytoplasmic vacuolization is clear (C.V). (C) *S. mansoni infected* mice treated with PZQ drug showing granuloma (G) with high lymphocytic infiltration (L.I), clear cytoplasmic vacuolization (C.V). (D) *S. mansoni infected* mice treated with FWE showing granuloma (G) with clear lymphocytic infiltration (L.I) and mild cytoplasmic vacuolization (C.V). (E) *S. mansoni infected* mice treated with PZQ accompanied with FWE showing multiple granuloma (G) with high lymphocytic infiltration (L.I), multiple cytoplasmic vacuolization (C.V). (A-E: H and E X100).

## DISCUSSION

In the current study, the effect of FWE treatment on some parasitological parameters of mice infected with *S. mansoni* was investigated. Treatment of *S. mansoni* infected mice with the standard drug praziquantel (PZQ) alone or combined with (FWE) showed a complete absence of worms. The treatment of infected mice with FWE significantly reduced total and coupled worm numbers, as compared to the infected control group. Meanwhile, the analysis of fenugreek water extract by using HPLC revealed that rutin and P-cumaric phenolic compound were the main constituents of FWE, they were found in high concentrations. Such constituents were reported to have anti-parasitic activities. Chauhan et al. (2018) reported that rutin exhibited a strong anti-leishmanial effect against both sensitive and resistant strains by enhancing cell-mediated immune response. Therefore, in the present work, FEW can be speculated to have a similar attitude against *S. mansoni* worms. An *in vitro* study on fenugreek water extract by Kor et al. (2013) found that it was effective against resistant species of *Plasmodium*. They added that fenugreek seeds exhibited an anti-microbial activity against *Helicobacter pylori* and restricted the growth of *staphylococcus* and *pseudomonas in vitro.* Also, water extract of fenugreek seeds was reported to have a direct anti-helminthic effect on *Gastrolthylax carmenifer* in cattle (Swarnakar et al., 2014). That was by causing rupture of tegument parenchymal cells, detachment of the tegument surface lead finally to disability of the parasite to be connected to the host.

In the present study, treatment of *S. mansoni*-infected mice either with PZQ or FEW caused significant reductions in total egg count in liver and intestine, somewhat PZQ reduction rate exceeded that of fenugreek, while accompanied treatment of them got the highest reduction rate of egg count in the liver. Generally, the reduction of egg capacity in each treatment can be in part attributed to their reducing action on total worm burden and also the number of coupled worms, so consequently, egg-laying was clearly reduced. A study by Lopes et al. (2020) found that cumaric acid showed anti-parasitic activity against *Leishmania braziliensis* and *Plasmodium falciparum* by blocking enzymes that are responsible for the parasite's development. A previous study revealed that cumaric acid prevented the growth of *Entamoeba histolytica* (Muruato et al., 2021). Aqua's extract of the cinnamon plant was reported to have an inhibitory effect on *Plasmodium falciparum* development *in vitro* (Parvazi et al., 2016). Since, cumaric acid is one of the main constituents of cinnamon (Kruger et al., 2018), therefore, FWE also can be assumed to have a resembling effect on *Schistosoma*. Thus, it can be deduced that cumaric acid in FEW can reduce the total egg count by restricting the worm development and thus preventing egg-laying.

The obtained results of oogram pattern of the hepatic and intestinal tissue, PZQ, and FWE significantly reduced mature egg and elevated dead egg percentages, while combined treatment of them caused better effect as compared to that of the infected control group. The water extract of fenugreek seeds was reported to have selective lethal effects on malignant cells *in vitro* by restricting abnormal cell growth provoking apoptosis (Alsemari et al., 2014), it may have a similar effect on the parasite by restricting egg growth.

Granuloma is an immune-inflammatory response towards *S. mansoni* egg; it protects the hepatic tissues from egg secreted toxins. But if the immune response around granuloma was not regulated, it will cause a hazardous effect on tissues (Guimaraes et al., 2015). In the current work, PZQ and FWE showed a significant reduction in granuloma diameter, while accompanied treatment got the smallest diameter, as compared to the *S. mansoni*-infected group. The reducing effect of FWE on granuloma diameter could be due to its active phenolic compounds ferulic and sinapic acids that were confirmed by HPLC. According to Quinn et al. (2020), Sinapic acid-containing extracts were found to have anti-inflammatory properties by reducing pro-inflammatory cytokines in human blood *in vitro*. Ferulic acid was reported to inhibit the inflammation induced by lipo-polysaccharide by inhibiting reactive oxygen species generation, inhibiting the intracellular malon-dialdehyde formation, and switching the intracellular antioxidant enzyme activities to normal levels (Yin et al., 2019). in addition, fenugreek seeds were reported to have a strong antioxidant activity by raising glutathione (Patil and Jain, 2014). So it can deduce that FWE can neutralize the egg secreted products, restricting extra inflammatory reactions and consequently reducing the granuloma diameter.

## CONCLUSIONS AND RECOMMENDATIONS

Fenugreek water extract (FWE) exhibited marked anti-parasitic activities against *S. mansoni* adult worms and eggs; it also reduced granuloma diameter and tissue damage. Furthermore, the combination of FWE with PZQ ameliorated the antiparasitic efficacy of PZQ against *S. mansoni*-infected mice as compared to that of the PZQ alone.

## NOVELTY STATEMENT

FWE were used for the treatment of *Schistosoma mansoni* and showed a direct antihelminthic activity toward

the worms and eggs and also anti-inflammatory effect on granuloma. Chemical analysis of FWE was performed to deduce its active properties and support the current data.

## AUTHOR'S CONTRIBUTION

GYO, AHM and TAS conceived the study and designed the experiments. EMH performed the experiments. GYO, AHM, TAS and EMH analyzed results. EMH wrote the maniscript. All authors reviewed and approved the final version of the maniscript.

### Conflict of interest

The authors have declared no conflict of interest.

## REFERENCES


Abdel-Gawad S, Abu-Sarea E, Saleh L (2018). Epidemiological determinants correlating hepatitis C and Schistosomiasi s*mansoni* in one of Upper Egypt governorates. Basic Appl. Sci., 7(2): 171-177. https://doi.org/10.1016/j.bjbas.2017.10.008

Abdel-Wareth A, Elkhateeb F, Ismail Z, Lohakare J (2021). Combined effects of fenugreek seeds and probiotics on growth performance, nutrient digestibility, carcass criteria, and serum hormones in growing rabbits. Livest. Sci., 215: 104616. https://doi.org/10.1016/j.livsci.2021.104616

Alsemari A, Alkhodairy F, Aldakan A, Al-Mohanna M, Bahoush E, Shinwari Z, Alaiya A (2014). The selective cytotoxic anti-cancer properties and proteomic analysis of *Trigonella foenum*-Graecum. BMC Comp. Altern. Med., 14: 114. https://doi.org/10.1186/1472-6882-14-114

Bunzel M, Ralph J, Kim H, Lu F, Ralph S, Marita J, Hatfield R, Steinhart H (2003). Sinapate-dehydro-dimers and sinapate-ferulate hetero-dimers in cereal dietary fiber. J. Agric. Food Chem., 51(5): 1427–1434. https://doi.org/10.1021/jf020910v

Chauhan K, Kaur G, Kaur S (2018). Activity of rutin, a potent flavonoid against SSG-sensitive and resistant *Leishmania donovani* parasites in experimental leishmaniasis. Int. Immunopharmacol., 64: 372-385. https://doi.org/10.1016/j.intimp.2018.09.026

Cheever A, Anderson L (1971). Rate of destruction of *Schistosoma mansoni* eggs in tissues of mice. Am. J. Trop. Med. Hyg., 20(1): 62-68. https://doi.org/10.4269/ajtmh.1971.20.62

Cheever A (1969). Quantitative comparison of the intensity as *Schistosoma mansoni* infection in man and experimental animals. Trans. R. Soc. Trop. Med. Hyg., 63(6): 781-795. https://doi.org/10.1016/0035-9203(69)90122-9

Duvall R, Dewitt B (1967). An improved perfusion technique for recovering adult Schistosomes from laboratory animals. Am. J. Trop. Med. Hyg., 16: 438-447. https://doi.org/10.4269/ajtmh.1967.16.483

Guimaraes C, Marcello J, Peralta J (2015). Schistosomiasis: Clinical management of liver disease. Clin. Liver Dis., 6(3): 59–62. https://doi.org/10.1002/cld.495

He Y, Ding C, Wang X, Wang H, Suo Y (2015). Using response surface methodology to optimize countercurrent chromatographic separation of polyphenol compounds from fenugreek (*Trigonella foenum-graecum* L.) seeds. Jliq Chromat., 38(1): 29-35. https://doi.org/10.1080/10826076.2013.864981

Kim I, Darwin W, Huestis M (2005). Simultaneous determination of nicotine, cotinine, norcotinine, and trans-30-hydroxycotinine in human oral fluid using solid-phase extraction and gas chromatography-mass spectrometry. J. Chromatogr. Anal. Technol. Biomed. Life Sci., 814: 233–240. https://doi.org/10.1016/j.jchromb.2004.10.034

Kor N, Didarshetaban M, Pour H (2013). Fenugreek (*Trigonella foenum*-graecum L.) as a valuable medicinal plant. Int. J. Adv. Biol. Biomed. Res., 1(8): 922-993.

Kruger S, Winheim L, Morlock G (2018). Planar chromatographic screening and quantification of coumarin in food, confirmed by mass spectrometry. Food Chem., 239: 1182-1191. https://doi.org/10.1016/j.foodchem.2017.07.058

Kumar R, Patil S, Patil M, Patil S, Paschapur M (2009). Isolation and evaluation of disintegrant properties of fenugreek seed mucilage. Int. J. Pharm. Tech. Res., 1(4): 982-996.

Liseth R, Muruato J, Juárez A, Alejandro H, Morales M, Ortega S, Hernández B, José R (2021). Therapeutic perspectives of *p*-coumaric acid: Anti-necrotic, anti-cholestatic and anti-amoebic activities. World Acad. Sci. J., 3: 47. https://doi.org/10.3892/wasj.2021.118

Lopes S,Yepes L, CastilloY, Robledo S (2020). Alkyl and aryl derivatives based on p-coumaric acid modification and inhibitory action against *Leishmania braziliensis*and *Plasmodium falciparum.* Molecules, 25(14). https://doi.org/10.3390/molecules25143178

Mahmoud A, Warren K (1974). Anti-inflammatory effects of tartar emetic and niridazole suppression of *Schistosome* egg granuloma. J. Immunol., 112: 222-229.

Matos-Rocha T, Cavalcanti M, Veras D, Feitosa A, Gonçalves G, Portela-Junior N, Lúcio A, Brayner F (2016). Ultrastructural changes in *Schistosoma mansoni* male worms after in vitro incubation with the essential oil of *Menthavillosa* huds. Rev. Inst. Med. Trop. Sao Paulo, 58: 4. https://doi.org/10.1590/S1678-9946201658004

Mendes C, Rocha J, Direito R, Fernandes A, Sepodes B, Figueira M, Ribeiro M (2019). Anti-inflammatory activity of grapefruit juice in an *in vivo* model of ulcerative colitis: Comparability studies of unprocessed and bioprocessed juices. J. Funct. Foods, 63: 103564. https://doi.org/10.1016/j.jff.2019.103564

Moore D, Denniston K, Furth M, Williams B, Blattner F (1977). Construction of chimeric phages and plasmids containing the origin of replication of bacteriophage lambda. Science, 198(4321): 1041-1046. https://doi.org/10.1126/science.929185

Muruato L, Juárez J, Hernandez H, Morales A, Ortega M (2021). Therapeutic perspectives of *p*-coumaric acid: Anti-necrotic, anti-cholestatic and anti-amoebic activities. World Acad. Sci. J., 3: 47. https://doi.org/10.3892/wasj.2021.118

Pajak P, Socha R, Broniek J, Królikowska K, Fortuna T (2018). Antioxidant properties, phenolic and mineral composition of germinated chia, golden flax, evening primrose, phacelia, and fenugreek. Food Chem., 2019 (275): 69–76. https://doi.org/10.1016/j.foodchem.2018.09.081

Parvazi S, Sadeghi S, Azadi M, Mohammadi M, Arjmand M, Vahabi F, Sadeghzadeh S, Zamani Z (2016). The Effect of Aqueous Extract of cinnamon on the metabolome of *Plasmodium falciparum* using HNMR spectroscopy. J. Trop. Med., pp. 3174841. https://doi.org/10.1155/2016/3174841

Patel P, Patel D (2019). Beneficial role of rutin, a naturally

occurring flavonoid in health promotion and disease prevention: A systematic review and update. Bioact. Food Diet. Intervent. Arthr. Relat. Inflammmat. Dis., 2: 457-479. https://doi.org/10.1016/B978-0-12-813820-5.00026-X

Patil S, Jain G (2014). Holistic approach of *Trigonella foenum-graecum* in phytochemistry and pharmacology. A review. Curr. Trends Technol. Sci., 3: 1.

Pellegrino J, Oliveria C, Faria J, Cunhan A (1962). New approach to the screening of drugs in experimental schistosomiasis*mansoni* in mice. Am. J. Trop. Med. Hyg., 11: 201-215. https://doi.org/10.4269/ajtmh.1962.11.201

Pinto-Almeida A, Mendes T, Ferreira P, Abecasis A, Belo S, Afonso A (2021). A comparative proteomic analysis of praziquantel-susceptible and praziquantel-resistant *schistosoma mansoni* reveals distinct response between male and female animals. Front. Trop. Dis., 10: 3389. https://doi.org/10.3389/fitd.2021.664642

Pundarikakshudu K, Shah D, Panchal A, Bhavsar G (2016). Anti-inflammatory activity of fenugreek (*Trigonella foenum-graecum Linn*) seed petroleum ether extract. Ind. J. Pharmacol., 48(4): 441-444. https://doi.org/10.4103/0253-7613.186195

Quinn L, Scott K, Finn S, Hayes M, Gray S (2020). An *in vitro* study determining the anti-inflammatory activities of sinapinic acid-containing extracts generated from Irish rapeseed meal. Eur. Soc. Med., 8(10): 2225. https://doi.org/10.18103/mra.v8i10.2225

Romeis B (1989). Mikroskopische Technik. Auflage, Urab and Schwarzenb-erg, Munchen-Wien-Baltimore. 17: 235-236.

Sakr S, Abo-El-Yazid S (2012). Effect of fenugreek seed extract on adriamycin-induced hepatotoxicity and oxidative stress in albino rats. Toxicol. Ind. Health, 28(10): 876-885. https://doi.org/10.1177/0748233711425076

Sokal R, Rohlf F (1981). Biometry. Freeman and Co. pp. 241–242.

Suresh P, Kavitha C, Babu S, Reddy V, Latha K (2012). Effect of ethanol extract of *Trigonella foenumgraecum* (Fenugreek) seeds on Freund's adjuvant-induced arthritis in albino rats. Affiliations Expand, 35(4): 21. https://doi.org/10.1007/s10753-012-9444-7

Swarnakar G, Roat K, Sanger B, Kumawat A (2014). Anthelminthic effect of *Trigonella foenum*-graecum on tegument of *Gastrothylax crumenifer*in cattle of Udaipur. Int. J. Curr. Microbiol. App. Sci., 3(5): 599-606.

Utzinger J, Keiser j, Shuhua X, Tanner M, Singer B (2003). Combination chemotherapy of schistosomiasis in laboratory studies and clinical trials. Antimicrob. Agents Chemother., 47(5): 1487-1495. https://doi.org/10.1128/AAC.47.5.1487-1495.2003

Wani S, Kumar P (2018). Fenugreek: A review on its nutraceutical properties and utilization in various food products. J. Saudi Soc. Agric. Sci., 17(2): 97-106. https://doi.org/10.1016/j.jssas.2016.01.007

World Health Organization (2021). Schistosomiasis.

Yin Z, Wu W, Sun C, Liu H, Chen W, Zhan Q, Lei Z, Xin X, Ma J (2019). Antioxidant and anti-inflammatory capacity of ferulic acid released from wheat bran by solid-state fermentation of *Aspergillus niger*. Biomed. Environ. Sci., 32(1): 11-21.