Original Research Article

The Egyptian Journal of Immunology

The Egyptian Journal of Immunology
Volume 30 (1), 2023: 57–72.
www.Ejimmunology.org

Bioactivity of *Trigonella foenum* (Fenugreek) oil on immunological and biochemical response of *Schistosoma mansoni* infected mice

Elham M. Hassan[1], Gamalat Y. Osman[1], Azza H. Mohamed[1] and Tarek A. Salem[2,3]

[1]Department of Zoology, Faculty of Science, Menoufia University, Shebin El-Kom, Egypt.
[2]Department of Molecular Biology, Genetic Engineering & Biotechnology Institute, Sadat City University, Sadat city, Egypt.
[3]Department of Biochemistry, College of Medicine, Qassim University, Al-Qassim 51452, Saudi Arabia.

Corresponding author: Elham Hassan, Department of Zoology, Faculty of Science, Menoufia University, Shebin El-Kom, Egypt.
Email: Elhammahmoud11@yahoo.com.

Abstract

The effect of fenugreek oil (FO) on some parasitological, immunological, and biochemical parameters in mice infected with *Schistosoma mansoni* were investigated. Chromatography mass spectrometry (GC/MS) analysis of FO revealed that linoleic acid, (E,E)-4-decadienal, and isopropyl myristate are the major constituents of FO. The results showed that treatment of *S. mansoni*-infected mice with 0.15 ml of FO daily for 10 successive days exhibited a significant reduction in the number of *S. mansoni* male worms, and coupled worms as compared to an infected control group (p<0.05). Regarding total egg counts and oogram patterns, FO effectively reduced the percentage of hepatic and intestinal egg counts, and elevated immature and dead eggs in ratios closely to praziquantel (PZQ) treated mice. Meanwhile, FO significantly elevated the levels of glutathione and co-enzyme Q-10 (COQ-10) up to 0.33±0.02 ng/ml and 0.28±0.02 ng/ml, respectively. However, when accompanied with PZQ, COQ-10 level was closer to that of the normal control group (0.37 ± 0.021 ng/ml). The result also showed that FO significantly reduced levels of lipid per-oxidation (0.165±0.01 ng/ml) and vascular endothelial growth factor (0.25±0.02 pg/ml) as compared to the PZQ-treated group (0.234±0.02 ng/ml and 0.31±0.008 pg/ml, respectively). Moreover, FO recovered normal values of caspase-7, and when accompanied with PZQ, annexin-V was also significantly reduced. However, treatment of S*. mansoni*-infected mice with PZQ led to a significant increase in the level of annexin-V as compared to *S. mansoni*-infected mice group (p<0.05). It can be concluded that FO may have a potential anti-schistosomal, antioxidant and anti-inflammatory activities. Also, it may have a recovering effect on apoptotic parameters toward the normal values.

Keywords: Anti-inflammatory; Antioxidants; Apoptosis; 4-Decadienal; Fenugreek oil; Granuloma; Linoleic acid; *Schistosoma mansoni; Trigonella foenum*

Date received: 30 August 2022; **accepted:** 21 November 2022

Introduction

Fenugreek (*Trigonella foenum-graecum*) is a plant belongs to *Fabaceae* family and cultivated worldwide as a semiarid crop.[1] In ancient Egypt and up till now, it is used for nutritional and medical purposes.[2] The chemical constitution of fenugreek seeds includes variety of active

substances like alkaloids, flavanoids, phenols, fatty acids, amino acids, minerals and vitamins.[3] Fenugreek seeds-extracted oil (FO) is rich with aromatic compounds and fatty acids such as linoleic acid which is the most constituent of fatty acids.[4] Linoleic fatty acid is an essential poly-unsaturated fatty acid, soluble in many organic solvents. It is also found in the oils of sunflower, corn and almonds.[5,6] Previous studies revealed that linoleic acid has anti-inflammatory and anti-parasitic activities.[7] E,E-2,4-decadienal was also reported as a constituents of FO, which is an aromatic compound that found in butter, beef, fish and peanut. It is known to have anti-parasitic activity.[8,9] The extract of fenugreek seeds was reported to exhibit anti-oxidant activity in rats.[10,11] Fenugreek seeds were found to suppress colon and breast cancer by inducing apoptosis.[12] An *in vitro* study of fenugreek extract reported that it has a selective cytotoxic effects towards T-cell lymphoma.[13] Another study revealed that fenugreek seeds extract somewhat prevented the proliferation of *Acanthamoeba* cyst.[14] In diabetic rats, it was shown that regular consumption of fenugreek seeds elevated antioxidants levels and prevented retinal degeneration exerted by the oxidative stress.[15]

Schistosomiasis is a tropical chronic disease caused by blood flukes (trematode worms) of genus *Schistosoma*. It is highly prevalent in Africa and responsible for more than 280,000 deaths annually.[16,17] Schistosomiasis affects either intestine or urinary tract causing pathogenic symptoms, ranging from abdominal pain, diarrhea, bloody stool, or urine to more complicated cases of kidney failure, infertility, or bladder cancer.[18] Many drugs were developed for treating schistosomiasis, for example, praziquantel (PZQ) is the most effective drug with less side effects.[19,20]

Recent research studies were directed towards the application of natural products instead or in-combination with chemical drugs to treat various diseases, to achieve more safe treatment with less side effects. The natural products with biological effects are attributed to different active constituents including flavonoids, alkaloids, saponins, essential fatty acids, and vitamins. Many studies revealed anti-parasitic activities of various natural products including black cumin oil, bee propolis, pumpkin seeds oil, olive oil, saffron extract, Alpinia and fenugreek seeds.[21,15,22,23,24,25]

This study was designed to test the hypothesis that fenugreek seeds oil either alone or in combination with PZQ could effectively treat *S. mansoni*-infected mice. Therefore, we assessed parasitological, immunological, and biochemical effects of fenugreek seeds oil as a natural product candidate for treatment of *S. mansoni*-infected mice.

Materials and Methods

Fenugreek seeds oil

The Fenugreek oil was purchased from a natural product company (Captain's company, Cairo). This oil was cold extracted from the Fenugreek seeds and applied in a dose of 0.15 ml / mouse daily for 10 successive days.[26]

Anti-schistosomal drug

The praziquantel (PZQ) drug was purchased from the International Pharmaceutical Industries Company (EIPICO, Alexandria, Egypt). Each tablet was grinded and freshly suspended in distilled water and administered orally to mice in 3 doses each of which 250 mg/kg for three alternative days.[27]

Experimental animals

Swiss male albino mice (CD-I strain, weighing 20±2 g) were caged, maintained under standard laboratory conditions, and fed at the Parasitology Research Lab, Zoology Department, Faculty of Science, Menoufia University.

Schistosoma mansoni cercariae

Biomphalaria alexandrina snails infected with *S. mansoni* miracidia were obtained from the Theodor Bilharz Research Institute (TBRI) Giza, Egypt. Egyptian strain of *S. mansoni* cercariae were shed from laboratory bred infected *B. alexandrina* snails.

Ethical consideration

The protocol of the study was reviewed and approved by the Institutional Animal Care and Use Committee, Faculty of Science, Menoufia

University (approval number: MUFS /S /Pa /2/20).

Gas chromatography mass spectrometric (GC/MS) analysis of Fenugreek seeds oil

The gas chromatography analysis of FO was performed by using a Trace GC Ultra / ISQ Single Quadrupole MS, TG-5MS apparatus (Thermo Scientific Inc., USA) with fused silica capillary column (30m, 0.251mm, 0.1 mm film thickness), according to the method described by Adams, 1995.[28] It was performed in the GC/MS laboratory; National Research Center, Cairo.

Mice infection with S. mansoni cercariae

Infected snails were washed with dechlorinated tap water and left uncovered under a white fluorescent light for one hour in a tube for cercarial shedding as described by Pellegrino et al., 1962.[29] Infection of mice was performed by subcutaneous infection of 70 ± 5 *S. mansoni* cercariae for each mouse.[30]

Study groups

A total of 35 Albino mice were divided into 5 groups. Group 1 included seven normal healthy mice, served as the normal control group. Group 2 included seven mice, infected with 70±5 *S. mansoni* cercariae and served as the infected control group. Group 3 included seven mice, infected with 70±5 *S. mansoni* cercariae and treated with 250 mg/kg of PZQ for three alternative days starting from the 8th week post infection.[27] Group 4 included seven mice, infected with 70±5 *S. mansoni* cercariae and treated with 0.15 ml/mouse FO for 10 successive days,[26] staring from the 8th week post infection. Group 5 included seven mice, infected with 70±5 *S. mansoni* cercariae and treated with a combination of 250 mg/kg of PZQ and 0.15 ml/mouse FO, staring from the 8th week post infection.

S. mansoni worm load

At the end of the treatment period, mice were sacrificed by decapitation. Hepatic and portomesenteric vessels were perfused to recover worms for subsequent counting.[31,32]

S. mansoni egg count

Three samples were collected from liver and intestine of each mouse and prepared for *S. mansoni* eggs count according to the method of Cheever and Anderson, 1971.[33] Briefly, 0.5 g of the tissue sample was treated with 5 ml of 5% KOH and examined under the low power of the light microscope.

Oogram pattern

Three tissue specimens were collected from each liver and intestine of each mouse. The intestine fragment was washed by 0.9% saline from depresses. A piece of one cubic cm of the liver and the intestine was placed between a microscope slide and a coverslip. One hundred eggs were counted microscopically and classified into immature, mature and dead eggs according to the method of Pellegrino et al., 1962.[29]

Liver histopathological examination and granuloma measurement

Representative samples from liver tissues were taken from each study mouse and fixed in neutral formalin (10%). Paraffin-embedded sections (5µm thick) were stained using Hematoxylin and Eosin (H&E).[34] 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 according to the method described by Mahmoud and Warren, 1974.[35]

Preparation of liver homogenate

During mouse dissection, a part of the liver was removed and frozen at -80^{o}C. One gram of liver tissue was placed in 10 ml of distilled water and homogenized by using mechanical homogenizer in ice pack. The mixture then was centrifuged at 12000×g for 30 minutes as described by Adams and Burgess, 1957.[36] The supernatant was collected and frozen at -80^{o}C for subsequent assays.

Determination of hepatic Glutathione (GSH)

GSH level was determined in liver homogenate by using an enzyme linked immunosorbent assay (ELISA) commercial kit (Cat. No. CSB-E12144r, Cusabio Technology, USA). [37] Briefly, a sample of 100 µl of liver homogenate was titrated in wells of ELISA plates and incubated for 2 hours at 37°C. Hundred microliters of Biotin-antibody working solution were added to each well and incubated for 1 hour at 37°C. Each well was aspirated and washed for three times. The remaining wash buffer was removed by aspirating or decanting. Hundred microliters of HRP-avidin working solution were added to each well and incubated for 1 hour at 37°C. Ninety microliter of TMB substrate (provided in the kit) were added to each well and incubated for 30 minutes at 37°C. Fifty microliters of a stop solution were added to each well and the optical density of each well was determined using an ELISA micro-plate reader (BK-EL 10c, Shandong, China) at wavelength 450 nm.

Determination of hepatic Coenzyme Q-10 (CoQ-10)

CoQ-10 was determined in liver homogenate by using a commercial competitive ELISA Kit (Cat. No: MBS7241152, Cusabio Technology, USA).[38] Briefly, a sample of 100 µl of liver homogenate was titrated in the ELISA wells and incubated for 1 hours at 37°C. Each well was aspirated and washed for five times. Substrates A and B (50 µl each), provided in the kit, were added to each well including the blank control well, subsequently. The plate was incubated for 10-15 minutes at 37°C and then, 50 microliters of a stop solution were added to each well including the blank control well. The optical density was determined at 450 nm wavelength by using a microplate reader.

Determination of hepatic malondialdehyde (MDA)

The level of MDA, as an end product of lipid peroxidation (LPO), was measured by reacting with the thiobarbituric acid (TBA).[39] Briefly, 0.2 ml of 10% (w/v) liver homogenate was added to 0.2 ml of 8.1% SDS, 1.5 ml of 20% acetic acid solution and 1.5 ml of 0.8% aqueous solution of TBA. The mixture was brought up to 4.0 ml with distilled water and heated at 95°C for 60 minutes. After cooling, 1.0 ml of distilled water and 5.0 ml of mixture of n-butanol and pyridine were added and shaken vigorously. After centrifugation at 1300 ×g for 10 min, the organic layer was taken, and its absorbance was measured at wavelength 535 nm by using a microplate reader.

Determination of hepatic vascular endothelial growth factor (*VEGF)*

VEGF level was determined in liver homogenate by a commercial ELISA kit (Cat. No: EK0540, Boster company, USA).[40] Briefly, 100 µl of the liver homogenate were titrated in the wells and incubated at 37°C for 120 minutes. Hundred microliters of the prepared 1x biotinylated anti-rat VEGF antibody, were added to each well and incubated for 60 minutes at 37°C. The plate was washed with the 300 µl wash buffer. The remaining wash solution was discarded, and then 100 µl of 1x avidin-biotin-peroxidase were added into each well and incubated for or 30 minutes at 37°C. The plate was washed 5 times with the 1x wash buffer and 100 µl of stop solution were added. The optical density was read at wavelength 450 nm by using a microplate reader.

Determination of hepatic Caspase 7

The expression of Caspase 7 is determined in liver homogenate by a commercial ELISA kit (Cat. No. DEIA-FN231, Creative Diagnostic Company, USA).[41] Briefly, 100 µl of liver homogenate were dispensed into the wells and incubated for 90 minutes at 37°C. The plate was washed two times with 400 µl washing solution. The remaining wash solution was discarded and then, 100 µl of Biotin-labelled antibody working solution were added into wells and incubated at 37°C for 60 min. After washing for three times, 100 µl of working solution were added into each well and incubated for 30 min at 37°C. The wells were washed with washing solution five times and then 90 µl of TMB substrate were added into each well. The plate was incubated for 15 minutes at room temperature. Fifty microliters of stop solution were added into each well and mixed thoroughly. The optical density was read

at wavelength 450 nm by using a microplate reader.

Expression of hepatic Annexin V

As an apoptotic index, annexin V was measured by applying a staining method using a commercial kit (Cat. No. 556547, Booster company, USA).[42] One ml of liver homogenate was placed into 5ml tube and then resuspended in 2 ml binding buffer. An aliquot of 100 µl of cells suspension was placed into another 5 ml test tube. Then 5µl of annexin V fluorescein isothiocyanate (FITC) labeled was add and 5 µl propidium iodide (PI), incubated for 15 minutes in dark at room temperature. After the incubation time the cells were resuspended in 200 µl 1x binding buffer. The labeled cells were detected by flow cytometry apparatus (Beckman Inc., USA). Cells that stain positive for FITC Annexin V and negative for PI are undergoing apoptosis.

Statistical analysis

Data are presented as Mean ± Standard deviation (M±SD); they were collected, tabulated, and statistically analyzed by using the statistical package for the social sciences program, version 18 (SPSS; SPSS Inc. Chicago, Illinois, USA). Student t-test, for normally distributed data, was used to calculate the significance of differences observed between mean values of experimental and control groups. The level of significance was set at $p < 0.05$.[43]

Results

Chemical analysis of FO components

A fenugreek oil (FO) sample was analyzed by chromatography mass spectrometry (GC/MS) technique. As shown in Table 1 and Figure 1, the FO sample consisted of 38 compounds. The total peak areas of the detected compounds reached 99.5 %, the probabilities of the structures of the detected compounds are listed in Table 1. The major compounds were 9,12-octa-decadienoic acid (Z,Z) or as named linoleic acid (43.7%), E,E-2,4-decadienal (22.5%), isopropyl myristate (6.1%) and 1-methyl dodecyl propionate (5.3%), representing 77.7% of the total peak areas.

Table 1. Components of Fenugreek oil (FO) as determined by gas chromatography mass spectrometry (GC/MS) analysis.

Peak No.	Rate/ min.	MW	Molecular formula	Area %	Identified compounds
1	10.10	142	C10H22	0.40	3-Ethyl-3-methylheptane
2	11.39	170	C12H26	0.25	Dodecane
3	15.99	154	C10H18O	0.32	2-Decenal, (E)
4	16.33	212	C15H32	0.86	Pentadecane
5	17.40	152	C10H16O	22.52	2,4-Decadienal, (E,E)-
6	17.54	198	C14H30	0.65	Tetradecane
7	18.65	182	C12H22O	0.39	2-Dodecenal
8	19.23	196	C14H28	0.59	3-Tetradecene, (Z)-
9	19.42	226	C16H34	0.22	Hexadecane
10	21.76	212	C15H32	0.96	Dodecane- 2,6,11-trimethyl-
11	22.46	206	C14H22O	0.24	2,4-bis(1,1-dimethylethyl), Phenol
12	22.82	254	C18H38	0.51	Octadecane
13	24.01	242	C16H34O	0.79	1-Hexadecanol
14	24.16	198	C14H30	0.25	Tetradecane
15	26.58	296	C21H44	0.86	2,6,10,15-tetramethyl heptadecane
16	27.49	282	C20H42	0.56	Eicosane
17	28.31	252	C18H36	0.55	1-Octadecene
18	29.03	270	C17H34O2	6.14	Isopropyl myristate
19	29.42	268	C18H36O	0.23	6,10,14 Trimethyl-2-pentadecanone

Table 1. Continued.

Peak No.	Rate/ min.	MW	Molecular formula	Area %	Identified compounds
20	30.42	310	C22H46	0.24	Docosane
21	30.89	450	C32H66	0.68	Docosane-11-decyl
22	31.02	270	C17H34O2	1.00	Pentadecanoic acid-14-methyl ester
23	31.70	422	C30H62	0.39	Triacontane
24	32.22	330	C19H38O4	2.01	Hexadecanoic acid-2,3-dihydroxy propyl ester
25	32.32	256	C16H32O2	5.34	1- Methyl dodecyl propionate
26	32.87	326	C20H26N2O2	0.34	Dasycarpidan-1-methanol acetate
27	33.27	272	C20H32	0.66	Kaur-16-ene
28	33.91	292	C19H32O2	0.26	9-Octadecen-12-ynoicacid methyl ester
29	34.17	294	C19H34O2	2.03	9,12-Octadecadienoicacid methyl ester
30	34.26	296	C19H36O2	1.94	9-Octadecenoic acid (Z) methyl ester
31	34.80	408	C29H60	0.42	Nonacosane
32	35.76	280	C18H32O2	43.74	9,12-Octadecadienoic acid (Z,Z) (linoleic acid)
33	36.55	170	C9H14O3	0.27	Methyl 2,2-dimethyl-3-oxocyclobutaneacetate
34	38.92	366	C24H46O2	0.50	9-Octadecenoic acid (Z) hexyl ester
35	40.14	282	C18H34O2	0.57	9-Octadecenoic acid (Oleic acid)
36	41.61	478	C32H62O2	1.20	9-Octadecenoic acid (Z) tetradecyl ester
37	50.91	252	C17H32O	0.23	13-Heptadecyn-1-ol
38	51.92	414	C29H50O	0.27	ç-Sitosterol [Stigmast-5-en-3-ol-(3á)]

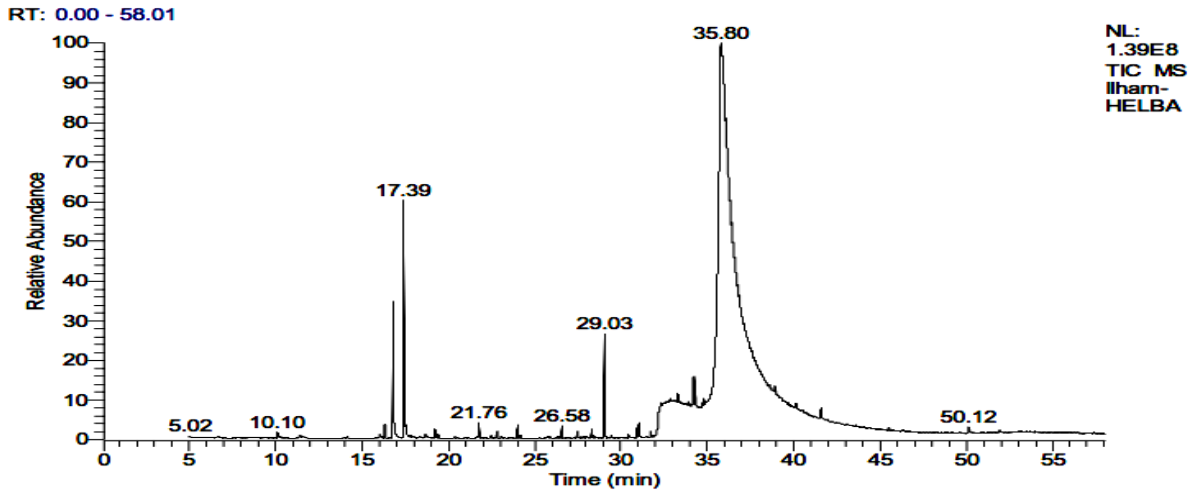

Figure 1. Gas chromatography mass spectrometry (GC/MS) for FO analysis. The peak 35.80 represents [9,12-octa-decadienoic acid (Z,Z)] or linoleic acid (43.74%). The peak 17.38 represents E,E-2,4-decadienal, (22.52%). The peak 29.03 represents isopropyl myristate (6.14%).

Effect of FO and /or PZQ on S. mansoni worms

The effect of FO and/or PZQ treatment on worm burden is illustrated in Table 2A. *S. mansoni* infected mice treated with FO showed significant reduction in total, coupled and male worms by 59.5%, 70.0% and 65.1%, respectively, as compared with the infected control group (p<0.05). No worms were observed in PZQ treated animals either alone or combined with FO.

Effect of FO and /or PZQ on total S. mansoni eggs

Table 2 B shows hepatic and intestinal *S. mansoni* eggs in the studied groups. The results indicated that treatments of *S. mansoni*-infected mice with either PZQ or FO resulted in significant reductions in total egg counts by 64.6% and 66.2 %, respectively, as compared to the infected control group (p<0.05). The maximum reduction (80.8%) in egg counts was observed in the *S. mansoni*-infected group treated with FO combined with PZQ when compared with the infected control group. In the intestinal tissue, treatment of *S. mansoni*-infected mice with FO significantly reduced the egg count by 67.7% as compared to the infected control group (p<0.05). Meanwhile, treatment of *S. mansoni*-infected mice either with PZQ or PZQ combined with FO resulted in the highest reduction of total egg counts by 98.9% and 95.3% respectively, as compared to the infected control group (p<0.05).

Effect of FO and /or PZQ on oogram pattern

Oogram pattern in liver tissue is illustrated in Table 2 C. Immature egg counts showed significantly increased levels in *S. mansoni*-infected groups treated with either PZQ, FO alone or combined by 68.8%, 64.9% and 62.3%, respectively (p<0.05), as compared to the infected control group. However, treatment of *S. mansoni*-infected mice with FO significantly increased the dead egg ratio to 140% as compared to the infected control group (p<0.05). Furthermore, treatment of *S. mansoni*-infected mice with a combination of FO with PZQ showed the maximum ratio of dead eggs by 490.9% as compared to the infected control group. While treatment of *S. mansoni*-infected mice with PZQ showed a significant reduction in mature egg counts by 54.2% compared with the infected control group (p< 0.05). Similarly, FO treatment significantly reduced the number of mature egg count, but to a lesser extent by 35% as compared to *S. mansoni*-infected control group. The maximum reduction in mature egg count (86.5 %) was observed in the infected mice treated with FO combined with PZQ as compared with the infected control group.

Intestinal oogram pattern is shown in Table 2 D. The highest ratio of dead eggs (126.3%) was noted in the *S. mansoni*-infected group treated with a combination of FO with PZQ as compared to the infected control group (p<0.05). Meanwhile, the ratio of mature eggs was significantly reduced by 83.8% (p<0.05) in the *S. mansoni*-infected group after treatment with a combination of PZQ with FO.

Table 2. Effect of FO and/or PZQ on some parasitological parameters of *S. mansoni* infected mice.

	(A) mean worm number				(B) mean egg count/g tissue	
	Mean ± SD				Mean ± SD	
	Male	Female	Couple	Total	Liver	Intestine
Ic	4.3± 0.9	3 ± 0.5	5 ± 0.8	17. ±1.1	15333 ± 1009	25567 ± 1559
I+PZQ	---	---	---	---	5433 ± 978*	266.7 ± 41*
Percentage (%) Compared to IC	---	---	---	---	64.6%	98.9%
I+FO	1.5 ± 0.3*	2.5± o.57	1.5 ± 0.3*	7 ± 0.7*	5175 ± 999*	8250 ± 1250*
Percentage (%) Compared to IC	48.8%	16.6 %	40.0 %	32.4%	66.2%	67.7%
I+FO+PZQ	---	---	---	---	2940 ± 571*	1200 ± 320*
Percentage (%) Compared to IC	---	---	---	---	80.8%	95.3 %

Table 2. Continued.

	(C) Oogram pattern in liver tissue			(D) Oogram pattern in intestine tissue		
	Mean ± SD			Mean ± SD		
	Immature	Mature	Dead	Immature	Mature	Dead
Ic	15.4 ± 3.6	74.2 ± 4.3	11 ± 1.6	17.6 ± 1.5	50.6 ± 4.0	32 ± 3.1
I+PZQ	26 ± 1.9*	34 ± 2.2*	40 ± 3.4*	29 ± 3.0*	20.8 ± 2.0*	50.2± 2.6*
Percentage (%) Compared to IC	68.8%	54.2%	263.6%	64.8 %	58.9 %	56.9 %
I+FO	25.4 ± 2.9*	48.2 ± 4.2*	26.4 ± 3.1*	20.2 ± 2.0	32.2 ±1.6*	47.6 ± 4.0*
Percentage (%) Compared to IC	68.8%	54.2%	263.6%	64.8 %	58.9 %	56.9 %
I+FO+PZQ	25 ± 3.7*	10 ± 1.9*	65 ± 4.4*	19.4 ± 2.0	8.2 ± 1.0 *	72.4 ± 3.0*
Percentage (%) Compared to IC	62.3%	86.5%	490.9%	10.2 %	83.8%	126.3 %

NC: normal mice control group; IC: mice infected with *S. manoni* (infected group); I+PZQ: mice infected with *S. mansoni* and treated with PZQ; I+FO: mice infected with *S. mansoni* and treated with FO; I+PZQ+FO: mice infected with *S. mansoni* and treated with PZQ combined with FO. Data are expressed as mean ± SD (standard deviation). (*) Significant difference as compared to *S. mansoni*-infected group ($p< 0.05$).

Effect of FO and /or PZQ on hepatic granuloma diameter

Granuloma diameter (µm) is illustrated in Table 3. As compared with *S. mansoni*-infected mice, the treatment of infected mice with PZQ showed a moderate reduction in granuloma diameter by 15.7%. While the maximum reduction (25%) in the granuloma diameter was noted after the treatment of *S. mansoni*-infected mice with FO ($p<0.05$). Figure 2 shows light micrograph of liver sections obtained from mice of the different study groups, H&E stained at 400 x.

Effect of FO and /or PZQ on hepatic glutathione (GSH) level

Results shown in Table 3 illustrate that the GSH level was significantly reduced in mice infected with *S. mansoni* by 37.3% when compared with the normal control group ($p<0.05$). In comparison with the *S. mansoni*-infected control group, treatment of infected mice with PZQ showed small reduction (5.8%) in GSH level. While a significant elevation in GSH level (54.6%) was observed in *S. mansoni*-infected group treated with FO ($p<0.05$). The maximum ratio of elevation in GSH level (95%) was noted after treatment of *S. mansoni*-infected mice with a combination of FO with PZQ when compared with the *S. mansoni*-infected group ($p<0.05$).

Effect of FO and /or PZQ on hepatic CoQ-10 level

Table 3 illustrates CoQ-10 levels in the different groups of the study. Results showed that the level of hepatic CoQ-10 was significantly reduced by 54% in mice infected with *S. mansoni* reduction as compared to that of normal control mice ($p<0.05$). While CoQ-10 levels were significantly elevated by 39% and 56% in *S. mansoni*-infected mice treated with PZQ and FO, respectively as compared to *S. mansoni*-infected mice ($p<0.05$). Treatment of *S. mansoni*-infected mice with a combination of PZQ with FO resulted in a maximum elevation of CoQ-10 (105%) as compared to the *S. mansoni*-infected group ($p<0.05$).

Effect of FO and /or PZQ on hepatic MDA

Results of hepatic MDA are shown in Table 3. Infection of mice with *S. mansoni* resulted in significant elevation in MDA level by 38.4% as compared to the normal control group ($p<0.05$). Treatment of *S. mansoni*-infected mice with PZQ resulted in small reduction in the MDA level by 4.5% as compared to the *S. mansoni*-infected mice. Moreover, a significant reduction in MDA level by 32.7% and 38.0% was observed in *S. mansoni*-infected mice treated with either FO or FO combined with PZQ, respectively as compared to the *S. mansoni*-infected group ($p<0.05$).

Effect of FO and /or PZQ on hepatic VEGF

The results showed that levels of VEGF in mice infected with *S. mansoni* exhibited a significant elevation (71%) compared to the normal control group (p< 0.05; Table 3). Treatment of *S. mansoni*-infected mice with PZQ led to a small elevation (7%) in VEGF levels as compared to infected mice. However, treatment of *S. mansoni*-infected mice with FO resulted in a small reduction in VEGF level (14%) as compared to the *S. mansoni*-infected mice. While treatment of *S. mansoni*-infected mice with a combination of FO with PZQ resulted in the lowest VEGF level (28%) as compared to the *S. mansoni*-infected control group.

Effect of FO and /or PZQ on hepatic caspase 7

Levels of caspase 7 in the study groups are shown in Table 3. The results illustrated that infection of mice with *S. mansoni* resulted in a significant elevation in the level of Caspase 7 (158 %) as compared to the normal control group (p< 0.05). No significant change was observed in the level of caspase 7 (4%) after treatment of *S. mansoni*-infected mice with PZQ as compared with the infected control group. However, levels of caspase 7 decreased by 28% in the *S. mansoni*-infected mice treated with a combination of FO with PZQ as compared to the *S. mansoni*-infected mice. Furthermore, treatment of *S. mansoni*-infected mice with FO resulted in the maximum caspase 7 level reduction (68%) as compared to the infected control group (p<0.05).

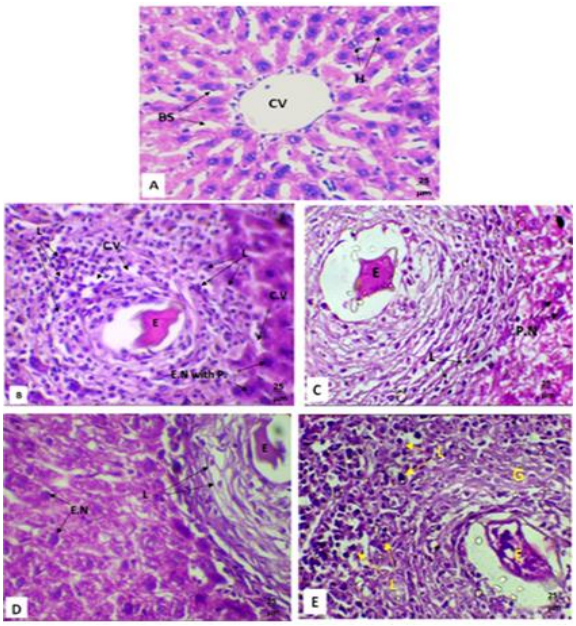

Figure 2 (A-E). Light micrograph of liver sections stained with H & E (400 x magnification). (A): Normal control liver mouse showing normal recognizable hepatocytes (H), blood sinusoids (BS) surrounding central vein (CV), (B): Liver of *S. mansoni*-infected mice eight-week post infection showing abnormal hepatocytes with enlarged nucleus (EN), pyknotic nuclei (P) and high prevalence of lymphocytes (L), (C): Liver of *S. mansoni*-infected mice treated with PZQ drug, showing abnormal hepatocytes with enlarged (EN), pyknotic nuclei (P) and numerous lymphocytes (L) surrounding egg (E), (D): Liver of *S. mansoni*-infected mice treated with FO showing reduced lymphatic infiltration (L) surrounding granuloma egg (E) hepatocytes appear to have enlarged nucleus (EN). (E): Liver of *S. mansoni*-infected mice treated with PZQ combined with FO, showing numerous lymphocytes (L) surrounding granuloma (G).

Table 3. Effect of FO and/or PZQ on some immunological and biochemical parameters of *S. mansoni* infected mice.

Parameters	Granuloma Diameter (µm	GSH (ng/ml)	CoQ 10 (ng/ml)	LPO (MDA) (ng/ml)	VEGF (pg/ml)	Caspase 7 (ng/ml)	% Annexin expression
Nc		0.33 ± 0.02	0.39 ± 0.02	0.177 ± 0.01	0.17 ± 0.01	0.097 ± 0.01	3.6 ± 0.36
Ic	34.4 ± 0.9$^{\#}$	0.20 ± 0.01$^{\#}$	0.18 ± 0.01$^{\#}$	0.245 ± 0.02$^{\#}$	0.29 ± 0.03$^{\#}$	0.25 ± 0.02$^{\#}$	8.5 ± 0.45$^{\#}$
Percentage (%) compared to NC		37.3%	54%	38.4%	71%	158%	160%
I+PZQ	29 ± 1.9 $^{\#*}$	0.19 ± 0.03	0.25 ± 0.02	0.234 ± 0.02	0.31 ±0.01	0.26 ± 0.01$^{\#}$	10.8 ± 1.87$^{\#}$
Percentage (%) compared to IC	15.7%	5.8%	39%	4.5%	7%	4%	27%

Table 3. Continued.

Parameters	Granuloma Diameter (µm	GSH (ng/ml)	CoQ 10 (ng/ml)	LPO (MDA) (ng/ml)	VEGF (pg/ml)	Caspase 7 (ng/ml)	% Annexin expression
I+FO	25.8 ± 2.8*	0.32 ± 0.09*	0.28 ± 0.02$^{\#*}$	0.165 ± 0.01*	0.25 ± 0.02	0.08 ± 0.02*	8.7 ± 1.1$^{\#}$
Percentage (%) compared to IC	25%	54.6 %	56%	32.7%	14%	68%	2.3%
I+FO+PZQ	26.8 ± 1.9*	0.39 ± 0.02*	0.37 ± 0.02	0.152 ± 0.01*	0.21 ± 0.01	0.18 ± 0.01$^{\#}$	3.8 ± 1.02$^{\#}$
Percentage (%) compared to IC	22%	95%	105%	38%	28%	28%	55.%

NC: normal mice control group; IC: mice infected with *S. manoni* (infected group); I+PZQ: mice infected with *S. mansoni* and treated with PZQ; I+FO: mice infected with *S. mansoni* and treated with FO; I+PZQ+FO: mice infected with *S. mansoni* and treated with PZQ combined with FO. Data are expressed as mean ± standard deviation. ($^{\#}$) Significant change as compared to normal control group ($p< 0.05$); (*) Significant change as compared to *S. mansoni*-infected group ($p< 0.05$).

Effect of *FO and /or PZQ on hepatic expression of Annexin V*

The expression of hepatic annexin V as an apoptotic marker was determined by flow cytometric analysis and illustrated in Table 3 and Figure 3. The expression of annexin V was significantly increased in *S. mansoni*-infected mice by 160 % as compared to that of the normal control group ($p<0.05$). Treatment of *S. mansoni*-infected mice with PZQ or FO resulted in small elevation in annexin V expression (27 % and 2.3 %, respectively), which was not different than the infected control group (8.5%). On the other hand, treatment of *S. mansoni*-infected mice with a combination of PZQ and FO resulted in a significant decrease (55.3 %) in the expression of annexin V as compared to the infected control group ($p<0.05$).

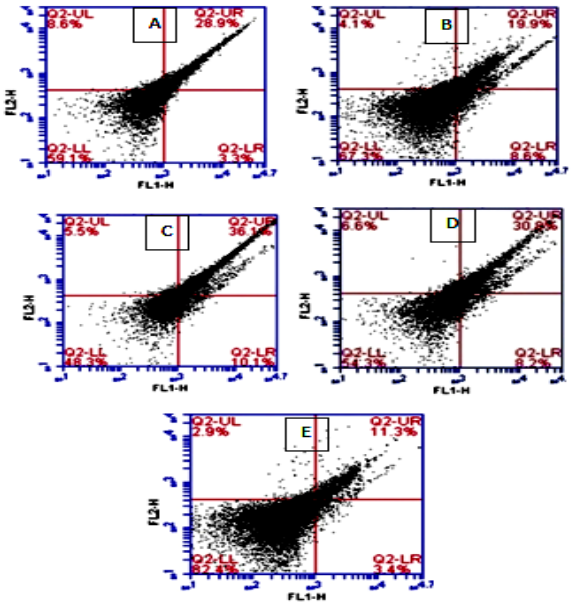

Figure 3. Flow cytometer analysis of hepatic Annexin V apoptotic marker. (A): Normal control group, (B): *S. mansoni*-infected group, (C): *S. mansoni*-infected mice treated with PZQ, (D): *S. mansoni*-infected mice treated with FO, (E): *S. mansoni*-infected mice treated with combined PZQ and FO

Discussion

Some parasitological and biochemical parameters were investigated to evaluate the effect of Fenugreek oil (FO) on *S. mansoni*-infected mice. The antiparasitic activities of FO were compared with that of standard anti-schistosomal drug, PZQ. Our results showed that treatment of *S. mansoni*-infected mice with FO significantly reduced the total and coupled worm numbers when compared to the infected control group. Meanwhile, treatment of *S. mansoni*-infected mice with the standard drug PZQ alone or in combination with FO resulted in complete absence of *S. mansoni* worms.

Also, our results showed that treatment of *S. mansoni*-infected mice with FO or PZQ caused a significant reduction in hepatic and intestinal total eggs of *S. mansoni* by 66% and 64%, respectively, as compared to *S. mansoni*-infected group. In addition, the reduction of total eggs count reached 80% by treating *S. mansoni*-infected mice with a combination of PZQ and FO.

Moreover, results of the current study showed that treatment of *S. mansoni*-infected mice with FO recorded a significant reduction in ratio of mature and dead eggs. Also, the mean granuloma diameter was significantly reduced as compared to either PZQ-treated group or *S. mansoni*-infected control group.

Such findings revealed the anti-schistosomal activity of FO, as it significantly reduced the burden of schistosomiasis comparable to the standard anti-schistosomal drug. This effect may be due to the anti-inflammatory effect of fenugreek which was previously reported.[44] The ability of FO to inhibit the granuloma formation may be another evidence for this anti-inflammatory activity.

In order to explain this anti-inflammatory action of FO, analysis of FO constituents was conducted by chromatography mass spectrophotometric method. Data showed that linoleic acid is one of the most abundant fatty acids in the oil extract of fenugreek seeds. A previous study of Pauls et al., 2018 reported the anti-inflammatory effect of linoleic acid through dampening human activated macrophages.[45] Also, this essential fatty acid was found to have anti-parasitic activity against *Giardia lamblia* and *leishmania donovani*.[7] Also, previous reports revealed the anti-schistosomal effect of pumpkin seeds oil, the authors attributed this action to the presence of linoleic acid as a major constituent of pumpkin seeds oil.[23,46]

Another major constituent, the aromatic compound (E,E)-2,4-decadienal, was detected in the fenugreek seeds oil. A previous study revealed the antiparasitic activity of *Ailanthus altissima* plant extract is due to the presence of this compound. The inhibitory effect of (E,E)-2,4-decadienal against *Meloidogyne javanica* which is a member of nematode species was reported.[8] Accordingly, it can be assumed that the action of FO against *S. mansoni* worm might be due to the presence of (E,E)-2,4-decadienal.

In order to interpret the antischostosomal action of FO, some biochemical and immunological parameters were investigated. Oxidative stress in schistosomiasis is accompanied by mediated site-specific tissue damage such as in the liver, especially at sites of granulomatous inflammation, owing to the production of highly reactive chemicals, reactive oxygen species (ROS), by macrophages and eosinophils. Results of the current study showed a significant elevation in the level of MDA, as a marker for LPO, in mice infected with *S. mansoni* when compared to the normal control group. Meanwhile, treatment of *S. mansoni*-infected mice with FO significantly reduced the level of LPO as compared to either *S. mansoni*-infected mice or PZQ treated group. As compared to PZQ, treatment of S. *mansoni*-infected mice with a combination of PZQ and FO resulted in a significant reduction in the level of LPO. Despite that PZQ exhibits a harmful effect against *S. mansoni* worms, it seems to have less protective effect against ROS.[47]

GSH is an important antioxidant that protects cells from oxidative damage, also it is one of the most effective antioxidants against *Schistosoma* worm.[48] Also, CoQ-10 is a member of electron transport chain, but during oxidative stress, it acts as a strong free radical scavenger.[49] The current study showed that infection of mice with *S. mansoni* significantly reduced the levels of GSH and CoQ-10 when compared to normal control group. This result agreed with a previous study, reported that

schistosomiasis inhibits the antioxidant components of the host such as GSH.[50]

On the other hand, treatment of *S. mansoni*-infected mice with FO led to significant increase in the levels of hepatic GSH and CoQ-10 as compared to those of either *S. mansoni*-infected group or PZQ-treated mice group. A previous study reported that PZQ administration caused short-term decline of GSH levels in healthy mice.[51] In contrast to PZQ, treatment of *S. mansoni*-infected mice with a combination of PZQ and FO resulted in a significant elevation in the levels of GSH and CoQ-10. These findings indicate the ability of FO in triggering the antioxidant potential activity against the generation of ROS accompanied with schistosomiasis. The antioxidant capacity of FO can be attributed to the presence of potent antioxidants constituents such as linoleic acid, caffiec acid and phenolic compounds. Also, linoleic acid has a strong free radicals scavenging activity and consequently reducing LPO level.[52, 53] This finding is in agreement with a previous study, reported that fenugreek seeds elevated GSH level in response to oxidative stress caused by induced hyperglycemia in rabbits.[54] Also, it was reported that fenugreek seeds reduced the accumulation of intracellular reactive oxygen species and exhibited the ability in protecting mitochondrial DNA from oxidative damage.[55] The current study indicated that FO exhibited a protective effect against ROS where it induced GSH and CoQ-10 activities while it reduced the LPO as compared to PZQ.

VEGF is angiogenic factor that is involved in creating new blood vessels after injury and in the hypoxic cases to compensate blood deficiency at cells.[56] In the current study, VEGF was significantly increased in *S. mansoni*-infected mice as compared to the normal controls. Elevated VEGF help increasing blood supply and consequently help the immune system functioning and tissue recovery.[57] This result goes in harmony with that of Loeffler et al., 2002[58] who found that *S. mansoni* soluble egg antigens (SEA) promote angiogenesis by up-regulating VEGF. On the other hand, VEGF levels were not different between *S. mansoni*-infected mice treated with FO and *S. mansoni*-infected mice. On the other hand, PZQ treatment did not change the VEGF level when compared with *S. mansoni*-infected controls. As compared to PZQ-treated group, treatment of *S. mansoni*-infected mice with a combination of PZQ and FO resulted in a significant decrease in VEGF level.

The inhibitory action of Fenugreek extract on the generation of new blood vessels has been reported.[59] The anti-angiogenic activity of FO was postulated due to the presence of linoleic acid, reported to have an anti-angiogenic effect in the carcinogenic mammary glands through inhibiting VEGF level.[60] In contrary to this finding, Al Hadidi, 2015, reported that fenugreek extract can enhance the VEGF expression.[61] This disagreement could be due to the difference in the model used, where, his study was conducted on human differentiated monocytes. As regards to the effect of PZQ on the expression of VEGF, a previous study reported no effect in the level of VEGF in a schistosomiasis model.[62]

According to our results, the ability of FO to reduce the diameter of granuloma can be associated with its inhibitory effect on VEGF. It can be assumed that FO acts as a natural anti-inflammatory product due to its ability to reduce the blood supply around the granuloma through inhibiting VEGF.

Apoptosis plays an important role during schistosomiasis life cycle in human.[63] In the current study, the effect of FO on the levels of apoptotic markers, caspase 7 and annexin V, were assessed. The results showed an elevation in the levels of caspase 7 and annexin V in mice infected with *S. mansoni* when compared to those of the normal mice. As shown in Tables 3, treatment of *S. mansoni*-infected mice with FO resulted in significant reduction in the levels of caspase 7 and annexin V when compared with those of *S. mansoni*-infected mice. On the other hand, treatment of mice infected with *S. mansoni* with PZQ did not change levels of caspase 7 and annexin V as compared to those of *S. mansoni*-infected mice. Treatment of mice infected with *S. mansoni* with a combination of PZQ and FO significantly reduced levels of caspase 7 and annexin V as compared to PZQ-treated mice ($p<0.05$).

The anti-apoptotic action of FO was not previously studied. According to our findings, FO

exhibited an inhibitory action on apoptotic markers that were associated with schistosomiasis. Meanwhile, PZQ treatment has no effect on the levels of apoptotic markers that were induced by schistosomiasis. Furthermore, levels of apoptotic markers were ameliorated when PZQ was combined with FO in treating *S. mansoni*-infected mice. However, a previous study revealed the anti-apoptotic effect of linolic acid on tachyzoites of *Toxoplasma gondii*.[64] Also, saponin which is a component of the fenugreek seeds, was found to have a strong anticancer activity by enhancing mitochondria-mediated apoptosis.[65] This disagreement might be based on the ability of FO to improve the immunological and biochemical features of the liver leading to downregulation of apoptotic markers.

Finally, we can conclude that fenugreek oil exhibited an anti-schistosomal activity by reducing the egg count, oogram and granuloma diameter. Its action could be based on improvement of antioxidant status of the host, inhibiting the angiogenesis which led to reduction of granuloma formation. Therefore, FO could be considered a promising candidate in resistance of schistosomiasis complications. It is proposed to combine the natural product FO oil with PZQ, the chemical antischistosomal drug. Such combination may lead to reducing the PZQ therapeutic dose and the drug inconvenient side effects.

Author Contributions

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

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The author(s) denies receipt of any financial support for the research, authorship, and/or publication of this article.

Ethical approval

The protocol of the study was reviewed and approved by the Institutional Animal Care and Use Committee, Faculty of Science, Menoufia University (approval number: MUFS /S /Pa /2/20).

References

1. Wani S, Kumar P (2018). Fenugreek: A review on its nutraceutical properties and utilization in various food products. *Saudi Society and Agricultural Sciences;*17(2): 97-106.
2. Kumar R, Patil S, Patil M, et al. (2009). Isolation and evaluation of disintegrant properties of fenugreek seed mucilage. *International Journal of Pharm Tech Research;* 1(4): 982-996.
3. Kan Y, Kan A, Ceyhan T, et al., (2005). Atomic absorption spectrometric analysis of *Trigonella foenum-graecum* L. seeds cultivated in turkey. *Turkish Journal of Pharmaceutical Sciences;* 2 (3): 187-191.
4. Beyzi E. (2020). Chemometric methods for fatty acid compositions of fenugreek (*Trigonella foenum-graecum L.*) and black cumin (*Nigella sativa L.*) seeds at different maturity stages. *Industrial Crops and Products;* 151- 112488.
5. Narinder K, Vishal C, Anil G. (2014). Essential fatty acids as functional components of foods- a review. *Food Science and Technology;* 51 (10): 2289–2303.
6. Haynes w. (2016). CRC Handbook of Chemistry and Physics. 97 th ed. Boca Raton, *CRC Press;* 3 : 338.
7. Saini, S, Rai A. (2020). Linoleic acid inhibits the release of *Leishmania donovani* derived microvesicles and decreases its survival in macrophages. *Affiliations Expand;* 10: 406. DOI.10.3389/fcimb.2020.00406.
8. Caboni P, Ntalli N, Aissani N, et al. (2012). Nematicidal activity of (E,E)-2,4-decadienal and (E)-2-decenal from *Ailanthus altissima* against *Meloidogyne javanica*. Agricultural and Food Chemistry; 60 (4):1146-51. doi:10.1021/jf2044586
9. Keskes H, Belhadj S, Jlail L et al., (2018). LC–MS–MS and GC–MS analyses of biologically active extracts of Tunisian Fenugreek (*Trigonella foenum-graecum* L.) Seeds. *Food Measurement and Characterization;*12: 209-220.
10. Acharya S, Acharya K, Paul S. (2011). Antioxidant and antileukemic properties of selected fenugreek (*Trigonella foenum*-graecum L.)

genotypes grown in western Canada, Canadian *Plant Science;* 91 (1): 99-105.

11. Suresh P, Kavitha C, Babu S et al., (2012). Effect of ethanol extract of *Trigonella foenum* graecum (Fenugreek) seeds on Freund's adjuvant-induced arthritis in albino rats. *Affiliations Expand*; 35 (4): 21.
12. Khoja K, Shafi G, Hasan T, et al., (2011). Fenugreek, a naturally occurring edible spice, kills MCF-7 human breast cancer cells via an apoptotic pathway. *Asian Pacific Journal of Cancer Prevention;* (12): 3299-3304.
13. Alsemari A, Alkhodairy F, Aldakan A, et al., (2014). The selective cytotoxic anti-cancer properties and proteomic analysis of *Trigonella foenum-graecum*. *BMC Complementary and Alternative Medicine*; 14(1): 114. DOI:10.1186/1472-6882-14-114
14. Kaya Y, Baldemir A, Karaman U, et al*.,* (2019). Amebicidal effects of fenugreek (*Trigonella foenum-graecum*) against *Acanthamoeba* cysts. *Food Science and Nutrition*; 7(2): 563–571.
15. Gupta S, Kumar B, Nag T, et al., (2014). Effects of *Trigonella foenum-graecum* (L.) on retinal oxidative stress, and proinflammatory and angiogenic molecular biomarkers in streptozotocin-induced diabetic rats. *Molecular and Cellular Biochemistry;* 388 (1-2): 1- 9.
16. De-Oliveira C, Frezza T, Garcia V, et al*.,* (2017). *Schistosoma mansoni*: In vivo evaluation of *Phyllanthus amarus* hexanic and ethanolic extracts. *Experimental Parasitology;* 183:56-63.
17. Abdel-Gawa S, Abu-Sarea E, Saleh L. (2018). Epidemiological determinants correlating hepatitis C and Schistosomiasis *mansoni* in one of Upper Egypt governorates. *Basic and Applied Sciences;* 7(2): 171-177.
18. World Health Organization (2021). Schistosomiasis
19. Andrews P. (1985). Praziquantel: mechanisms of anti-schistosomal activity. *Pharmacology and Therapeutics;* 29(1):129 -156.
20. Almeida A, Mendes T, de Oliveira R, et al., (2016). Morphological characteristics of *Schistosoma mansoni* PZQ-Resistant and -susceptible strains are different in presence of praziquantel. *Front microbiol;* (7): 594.
21. Mahmoud M, El-Abhar H, Saleh S. (2002). The effect of *Nigella sativa* oil against the liver damage induced by *Schistosoma mansoni* infection in mice. *Ethnopharmacol*; 79(1):1-11.
22. Maghrabi O, Mohamed A, Osman G, *et al.,* (2015). Antischistosomal activities of propolis in *Schistosoma mansoni* infected mice. *Biological and pharmaceutical research,* 6(2). 110-119.
23. Beshay E, Rady A, Afifi F, *et al.,* (2018). Schistosomicidal, antifibrotic and antioxidant effects of *Cucurbita pepo* L. seed oil and praziquantel combined treatment for *Schistosoma mansoni* infection in a mouse model. *Helminthology,* 93(3):1-9.
24. Shaaban A, Ibrahim H, Mohamed A. (2019). Effect of *Crocus sativus* aqueous extract (saffron) on *Schistosoma mansoni* worms in experimentally infected mice. *Egyptian journal of aquatic biology and fisheries;* 23(4): 391-408.
25. Zahra M, Salem T, El-Aarag B. (2019). *Alpinia zerumbet* (Pers.): Food and medicinal plant with potential *in vitro* and *in vivo* anti-cancer activities. *Molecules;* 24(13): 2495.
26. Hassan A, Khalil W, Ahmed K. (2006). Genetic and histopathology studies on mice: effect of fenugreek oil on the efficiency of ovarian and liver tissues. *African Journal of Biotechnology;* 5 (5): 477 - 483.
27. Utzinger J, Keiser j, Shuhua X, *et al.,* (2003). Combination chemotherapy of schistosomiasis in laboratory studies and clinical trials. *Antimicrob Agents Chemother;* 47(5):1487-1495.
28. Adams R. (1995). Identification of Essential Oil Components by Gas Chromatography/Mass Spectrometry. 3rd ed. Carol Stream, Allured Publishing Corp; 325.
29. Pellegrino J, Oliveria C, Faria J, *et al.,* (1962). New approach to the screening of drugs in experimental *schistosomiasis mansoni* in mice. The American journal of tropical medicine and hygiene; 11: 201-215.
30. Peters P. and Warren K. (1969). A rapid method of infecting mice and other laboratory animals with *Schistosoma mansoni*: subcutaneous injection. *Parasitology*; 1969 (55): 558.
31. Cheever A. (1969). Quantitative comparison of the intensity as *Schistosoma mansoni* infection in man and experimental animals. *Transactions of The Royal Society of Tropical Medicine and Hygiene*63 (6): 781-795.
32. Duvall R, Dewitt B. (1967). An improved perfusion technique for recovering adult Schistosomes from laboratory animals. *The American journal of tropical medicine and hygiene;* 16:438 - 447.
33. Cheever A, Anderson L. (1971). Rate of destruction of *Schistosoma mansoni* eggs in tissues of mice. *The American journal of tropical medicine and hygiene*; 20 (1): 62-68.

34. Von L. (1962). Host response to eggs of *S. mansoni*. I. Granuloma formation in the unsensitized laboratory mouse. *The American journal of parasitology*; 41:711–731.
35. Mahmoud A, Warren K. (1974). Anti-inflammatory effects of tartar emetic and niridazole suppression of Schistosome egg granuloma. *Immunology;* 112: 222-229.
36. Adams, D. and Burgess, E. (1957): The effect of the degree of homogenization on the atalase activity of liver homogenates. *British Journal of Cancer*, 11(2): 310 - 325.
37. Akerboom T, Sies H. (1981). Assay of glutathione, glutathione disulfide, and glutathione mixed disulfides in biological samples. *Methods in enzymology;* (77): 373 -382.
38. Akerboom T, Sies H. (1981). Assay of glutathione, glutathione disulfide, and glutathione mixed disulfides in biological samples. *Methods in enzymology;* (77): 373 -382.
39. Ohkawa H, Wakatsuki A, Kaneada C. (1979). Assay for lipid peroxides in animal tissue by thiobarbituric acid reaction. *Analytical Biochemistry;* 95: 351-358.
40. Stewart M, Rosenfeld P, Penha F, *et al.,* (2012). Pharmacokinetic rationale for dosing every 2 weeks versus 4 weeks with intravitreal ranibizumab, bevacizumab, and aflibercept (vascular endothelial growth factor Trap-eye). *Retina*; 32: 434 - 457.
41. Saunders P, Cooper J, Roodell M, *et al*., (2000). Quantification of active caspase 3 in apoptotic cells. *Analytical Biochemistry*; 284 (1):114-24.
42. Kabakov A, Kudryavtsev V, Gabai V. (2011). Determination of cell survival or death. *Methods in Molecular Biology*; 787 (231): 44.
43. *Sokal R, Rohlf F. (1981). Biometry; 241–242.*
44. Kawabata T, Cui M, Hasegawa T, *et al.,* (2011). Anti-inflammatory and anti-melanogenic steroidal saponin glycosides from fenugreek (*Trigonella foenum*-graecum) seeds. *Planta Medica*; 77 (7): 705-710.
45. Pauls S, Rodway L, Winter T, *et al.,* (2018). Anti-inflammatory effects of α-linolenic acid in M1-like macrophages are associated with enhanced production of oxylipins from α-linolenic and linoleic acid. *Nutritional Biochemistry*; 57:121-129.
46. Bardaa S, Nihed B, Aloui F, *et al*., (2016). Oil from pumpkin (*Cucurbita pepo* L.) seeds: evaluation of its functional properties on wound healing in rats. *Lipids in Health and Disease;* 15:73.
47. Doenhoff M, Cioli D, Utzinger J. (2008). Praziquantel: mechanisms of action, resistance and new derivatives for schistosomiasis. *Current Opinion of Infectious Diseases*; 21(6): 659 - 67.
48. Pompella A. Visvikis A. Paolicchi A. et al. (2003). The changing faces of glutathione, a cellular protagonist. *Biochemical Pharmacology*; 66 (8): 1499 - 1503.
49. Hemmi N, Raj K. (2006). *Coenzyme Q_{10}: Absorption, tissue uptake, metabolism and pharmacokinetics. Free Radical Research;* 40 (5): 445–453.
50. Wilson R, Coulson P. (2009). Immune effector mechanisms against schistosomiasis: looking for a chink in the parasite's armour. *Trends in Parasitology;* 25 (9): 423-431.
51. Velebny, S.; Hrckova, G. and Konigova, A. (2010). Reduction of oxidative stress and liver injury following silymarin and praziquantel treatment in mice with Mesocestoides vogae (Cestoda) infection. *Parasitology International*; 59 (4):524 - 531.
52. Peyrat M, Cuvelier M, Berset C. (2003). Antioxidant activity of phenolic compounds in 2,2-azobis (2-amidinopropane) dihydrochloride (AAPH)-induced oxidation: Synergistic and antagonistic effects. *American Oil Chemists' Society; 80 (10): 1007-1012.*
53. Mattes R. (2009). Is there a fatty acid taste? *Annual Revew of Nutrition;* 29: 305-327.
54. Hamadi S. (2012). Effect of trigonelline and ethanol extract of Iraqi Fenugreek seeds on oxidative stress in alloxan diabetic rabbits. *Basic and Applied Sci*ence; 12 (1): 23 - 26.
55. Luan G, Wang Y, Wang Z, *et al.,* (2018). Flavonoid Glycosides from Fenugreek Seeds Regulate Glycolipid Metabolism by Improving Mitochondrial Function in 3T3-L1 Adipocytes in Vitro. *Agricultural and Food Chemistry*; 66(12): 3169-3178.
56. Senger D, Galli S, Dvorak A, *et al.,* (1983). Tumor cells secrete a vascular permeability factor that promotes accumulation of ascites fluid. *Science*; 219 (4587): 983-1005.
57. Amo Y, Masuzawa M, Hamada Y, *et al.,* (2004). Serum concentrations of vascular endothelial growth factor-D in angiosarcoma patients. *British Journal of Dermatology*; 150 (1): 160-161.
58. Loeffler D, Lundy S, Singh K, *et al.,* (2002). Soluble egg antigens from *Schistosoma mansoni* induce angiogenesis-related processes by up-regulating vascular endothelial growth factor in human

endothelial cells. *Infectious Diseases*; 185 (11):1650.

59. Habib Z, Hammad H, Afifi F, *et al.,* (2017). *in vitro* and *in vivo* evaluation of the antiangiogenic activities of *Trigonella foenum-graecum* extracts. *Asian Pacific Journal of Tropical Biomedicine*; 7 (8): 732 - 738.
60. Masso, P.; Zangani, D. and Vaughan, M. (2002): Inhibition of angiogenesis by the cancer chemopreventive agent conjugated linoleic acid. Cancer Research, 62 (15): 4383-4389.
61. Al Hadidi Y. (2015). The role of fenugreek extract (FE) on VEGF-A and HIF-1α expression in human differentiated monocytes (dTHP-1). *The future of vaccine development;* DOI: 10.13140/RG.2.1. 3100.2326.
62. Abd El-Aal N, Hamza R, Magdy M. (2017). Anti-angiogenic and anti-lymphangiogenic role of praziquantel and artemether in experimental mansoniasis. *Acta Parasitologica*; 62 (4): 708 - 716.
63. Lee E, Young N, Lim N, *et al.,* (2014). Apoptosis in schistosomes: toward novel targets for the treatment of schistosomiasis. *Trends in Parasitology*; 30(2):75-84.
64. Shamseddin J. Akhlaghi L. Razmjou E. *et al.,* (2015). Conjugated linoleic acid stimulates apoptosis in RH and Tehran strains of *Toxoplasma gondii*, *in vitro. Iranian Journal of Parasitol*ogy; 10(2): 238-244.
65. Meng X. Dong H. Pan Y. (2019). Diosgenyl saponin inducing endoplasmic reticulum stress and mitochondria-mediated apoptotic pathways in liver cancer cells. *Journal of agricultural and food chemistry*; 67(41): 11428-11435.