Original Article

Effect of wheat germ oil and propolis on murine schistosomiasis *mansoni*: Evaluation of their impact on induced liver fibrosis by targeting the TGF-β1/Smad3 pathway

Tahany A AL-Attar[1], Mona F Faheem[1], Huda I Abd El-Hafiz[2], Hend A Elshahat[3], Fatma H Shalaan[1]

Departments of Medical Parasitology[1], Clinical Pharmacology[2], and Pathology[3], Faculty of Medicine, Menoufia University, Menoufia, Egypt

ABSTRACT

Background: Praziquantel (PZQ), the sole drug for schistosomiasis, was repeatedly linked to reinfections, resistance, and low cure rates. This necessitates an urgent need to search for alternative natural products and identify novel drug targets.

Objective: To investigate the role of wheat germ oil (WGO) and propolis in mice infected by *S. mansoni* compared to PZQ.

Material and Methods: Fifty mice were randomly divided into five equal groups: negative, and positive controls, propolis treated, WGO treated in comparison to PZQ treated group. Worm burden, oogram pattern, intestinal and hepatic egg counts, number and diameter of hepatic granulomas, were assessed to evaluate their efficacy. To assess the degree of fibrosis, an immunohistochemical study was performed by examining hepatic tissue stained by Masson trichome. In addition, biochemical and immunological parameters (ALT, AST, IL-4, IL-10, and TGF-β1) were determined. To identify the mechanism of action of the propolis and WGO on hepatic fibrosis, a real time quantitative PCR (RT-qPCR) was performed to assess the genetic expression of TGFβ1/Smad3 pathway.

Results: In WGO and propolis-treated groups, there were significant reductions in all parasitological parameters (worm load, intestinal and hepatic egg counts) with an increased percentage of dead eggs in the oogram pattern. Besides, there was a substantial decline in granuloma number and diameter. Similarly, mice of both groups had substantial hepatoprotective effects demonstrated by improvement of liver enzymes, reduction of IL-4, and elevation of IL-10. Furthermore, the regulation of genetic expression of TGFβ1/Smad3 showed a notable decrease that coincided with a reduction of fibrous tissue in hepatic granulomas. Additionally, there was a decrease in the expression of α-smooth muscle actin (α-SMA) in the groups treated with propolis, WGO, and PZQ, compared to the untreated group. The WGO group demonstrated significant improvements compared to the other groups.

Conclusion: The current study highlighted the potential role of propolis and WGO as beneficial natural herbal medicine for schistosomiasis treatment; this may also assist in mitigating the adverse effects of PZQ.

Keywords: α-SMA; IL-4; IL-10; propolis; schistosomiasis *mansoni*; TGF-β1/Smad3 pathway; wheat germ oil.

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Corresponding Author: Mona F. Faheem; **Tel.:** +20 1110091561; **Email:** mona.fahem.12@med.menofia.edu.eg

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INTRODUCTION

Schistosomiasis is a serious helminthic disease caused by parasitic trematodes of the genus *Schistosoma*, resulting in patient morbidity and possible mortality. Three *Schistosoma* spp. (*mansoni*, *haematobium*, and *japonicum*) currently infect about 200 million people, most of whom live in the world's poorest areas[1]. A substantial complication of schistosomiasis *mansoni* is hepatic peri-portal fibrosis. The host's immune reaction to *Schistosoma* eggs causes the development of granulomas and fibrosis[2]. During the initial phases of schistosomiasis, the release of antigenic substances prompts a robust Th1 immune reaction. This response is primarily characterized by elevated production of Th1-type pro-inflammatory cytokines, comprising IFN-γ, TNF-α, and IL-12[3]. However, the hosts' inability to eliminate schistosomes stimulates the secretion of Th2-type inflammatory cytokines, driving the polarization of macrophages toward the alternatively activated M2 phenotype. Meanwhile, M2-type macrophages and Th2 cells sustain an elevated type II immune response within the liver's microenvironment through Th2 accompanying cytokines such as IL-4, IL-5, IL-10, and IL-13[4].

Furthermore, type II immune responses exhibit potent profibrotic effects via release of profibrotic agents (TGF-β1, and IL-13)[5]. Both factors promote collagen accumulation around the edges of the egg granuloma. As liver fibrosis progresses, hepatic

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stellate cells (HSCs) transform into fibroblasts and release α-SMA protein[6]. Moreover, the secretion of TGF-β1 through hepatic macrophages, HSCs, and hepatic cells promotes the phosphorylation of the Smad protein complex. Of note, TGF-β1/Smad pathway that plays a crucial role in liver fibrosis, is initiated by phosphorylation of Smad2 and Smad3 *via* TGF-β. This leads to their combination with Smad4 within the nucleus, thus promoting fibrosis. Research highlighted the importance of Smad3 when it was observed that mice without Smad3 were resistant to liver fibrosis induced by dimethyl-nitrosamine[7]. Cytokines, particularly TGF-β1, are primarily responsible for activating HSCs and extracellular matrix generation. In fact, TGF-β serves as a central element in the pathogenesis of liver conditions, *e.g.*, hepatitis and cirrhosis. It is recognized as the most fibrogenic hepatic cytokine since it triggers fibrosis by activating hematopoietic stem cells and excessive extracellular matrix (ECM) production. A disrupted balance in Smad signaling is critical to the development of hepatic fibrosis, with Smad3 and Smad4 promoting fibrosis. Besides, Smad2 and Smad3 are strongly activated during liver fibrosis, with Smad3 proving essential for fibrosis progression. Moreover, Smad4 works with Smad2/3 to transcribe genes that further drive fibrosis[8]. Accordingly, this complex enhances the transcription of target genes, including the connective tissue growth factor that plays a major role in endorsing HSC activation besides ECM installation[9].

Chronic liver inflammation due to schistosomiasis causes a persistent overexpression of reactive oxygen species (ROS) and other oxidative stress byproducts. These elements perform a critical role in the progression of liver fibrosis associated with schistosomiasis[10]. Unfortunately, PZQ is the sole effective and safe medication against schistosomiasis; however, it is ineffective on juvenile schistosomes, leading to reinfection[11]. Accordingly, searching for novel drug targets and developing efficient anti-schistosomal drugs is urgent and of paramount importance.

Propolis, a natural substance produced by bees, consists of wax, amino acids, essential oils, key flavonoids, phenolic compounds, and various acids, including caffeic acid. It is known for its broad-spectrum antioxidant and anti-inflammatory assets[12]. Additionally, propolis demonstrated antiparasitic activity against giardiasis[13], *Plasmodium* spp., *Trypanosoma* spp., *Leishmania* spp., *T. vaginalis*[14], and *S. mansoni*[15]. On the other hand, WGO is widely regarded as a natural, nutrient-rich feed improver and is frequently utilized as a dietary enhancement for humans[16]. It offers several health benefits, including regulation of serum lipid levels, reduction of cholesterol absorption, improvement of fertility, and slowing down the aging process[17]. Additionally, WGO has anti-inflammatory, antioxidant[18], and antiparasitic properties[19,20].

Our experimental study aimed to investigate the potential efficacy of propolis and WGO in comparison to PZQ. Assessment of their inhibitory activity on TGF-β1/Smad3 pathway was our secondary objective.

MATERIAL AND METHODS

This experimental randomized controlled study was conducted at Theodor Bilharz Research Institute and Parasitology Department, Menoufia University, from February 2024 to March 2025.

Study design: The study was conducted on Swiss albino mice that were confirmed to be pathogen-free by stool microscopic examination. Mice were randomly categorized into five groups. Except for the negative non infected non treated control group I, mice were subcutaneously injected with *S. mansoni* cercariae. After infection, group II mice received no treatment, while those in groups III, IV, and V were treated with propolis, WGO, and PZQ respectively. Establishment of infection was confirmed by the presence of eggs in the stools of mice 42 d post-infection (dpi), while mice were decapitated at 60 dpi (end of experiment). Parasitological, biochemical, and histopathological studies were conducted to evaluate the treatment potential efficacy. The RT-qPCR method was utilized to evaluate the impact of propolis and WGO on liver fibrosis induced by schistosomiasis through TGF-β1/Smad3 signaling pathway.

Sample size calculation: To find the appropriate sample size, the resource equation approach was employed, ensuring the maximum sample size while accounting for potential dropout rates. The following formula was applied: n = 20/K + 1, where "n" indicates the number of mice per group and "K" represents the number of groups[21]. The calculation was based on a study power of 80% and a 95% confidence interval. Accordingly, 10 mice were assigned to each group.

Experimental animals: Fifty Swiss albino mice (6-8 w, 20-25 g) were maintained in a controlled housing setting at the Theodor Bilharz Research Institute (TBRI) animal facility in Giza, Egypt. During the study, five animals per cage were housed under pathogen-free conditions at a consistent temperature of 24±3°C, with a 12-hour light/dark cycle. Food and water were provided *ad libitum* throughout the trial.

Mice infection: Except for the control group, mice were subcutaneously injected with freshly shed *S. mansoni* cercariae. Cercaria were ready for use in a stock solution comprising approximately 70 cercariae per 0.5 ml of refined water[22].

Drug therapy: Praziquantel, obtained from the Egyptian International Pharmaceutical Industries Company (EIPCO, Egypt), was prepared as a 2%

cremophor solution (Sigma Aldrich, USA) and administered by oral gavage 45 dpi at a dose of 500 mg/kg BW for two succeeding days[23]. An Egyptian company (El-Captain) produces WGO at a facility in 6th October City, Egypt. The WGO was dissolved in corn oil and administered orally using gavage. Its dosage was set at 0.2 mg/kg per day. The treatment began on the 45th dpi and lasted for 15 d[24]. A 10-gram sample of dry propolis powdered extract, obtained locally from an Imtenan shop in Cairo, Egypt, was mixed with 1 liter of 100% ethanol and left to incubate overnight at 100 rpm. The mixture was filtered and incubated in a rotary evaporator set at 40°C and 100 rpm. The dried mixture (8.3 g) was extracted, and 2.5 g were dissolved in 250 ml of saline[25]. Each mouse received a daily dose of 0.1 ml. Treatment commenced on the 45th dpi and continued for 15 d[26].

Authentication of mice and sampling: Mice were decapitated 60 dpi. Collected blood was centrifuged, and separated serum was stored at -20°C until use. Adult worms were dissected from the liver first, and then the liver was split into three sections for 1) ova per gm tissue, 2) immunological and molecular assays, and 3) histopathological and immunohistochemical studies. Blood samples were withdrawn, and serum levels of ALT, and AST were determined.

Parasitological studies included determination of worm load, oogram pattern, and hepatic and intestinal ova counts/gm tissue.

Worm load: Adult worms were obtained by liver perfusion and differentially counted[27] as males, females, and attached pairs.

Ova per gram tissue: To determine the egg count, weighed sections of hepatic and intestinal tissues were incubated with 5 ml of 5% KOH at 37^{0}C for 18 h. Sedimented eggs were counted per gram of hepatic and intestinal tissue by multiplying the average egg count found in each 1 ml sample by the total KOH volume and dividing this value by the sample's weight[28].

Oogram pattern: Microscopic examination of three pressed intestinal fragments measuring 1 cm from every mouse was performed. One hundred eggs were counted for each fragment, and categorized as immature, mature, or dead. The average percentage for each type was then calculated[29].

Immunological and biochemical assays: Liver tissue homogenate (100 mg) in phosphate-buffered saline (0.1 mol/l, pH 7.4) was centrifuged at 1500×g for 10 min, followed by another centrifugation at 10,000×g for 15 min. The resulting supernatant was subsequently used to determine IL-4, IL-10, and TGF-β1. Serum levels of ALT and AST were determined using colorimetric kit systems (Biodiagnostic Co., Egypt). All biochemical and immunological assays were kindly performed at the central laboratory in the Faculty of Medicine, Menofia University.

Molecular study of TGF-β1 and Smad3: After preparation of liver tissue homogenates, total RNA was separated using the QIAamp RNA Blood Mini Kit (Qiagen, USA). Following manufacturer instructions, total RNA was isolated from the supernatant of the liver tissue homogenate employing the TRIzol® Plus RNA Purification Kit (Cat. #12183-555, Life Technologies, Abcam®, Cambridge, MA, USA). The semi-quantitative real-time PCR analysis began with 1.5 µg of RNA from each specimen being reverse-transcribed into cDNA. As previously described[30], RT-qPCR was carried out using the Applied Biosystems 7500 RT-PCR System (Applied Biosystems, Carlsbad, CA, USA). For measuring mice TGF-β1 and Smad3 cDNA, the TaqMan® Gene Expression Assays Rn01484924_m1 (Cat. #4331182) and Rn01441749_m1 (Cat. #4331182) were used, respectively. We used GAPDH as the reference gene, and the following table shows forward and reverse primers[31].

Gene	Forward primer	Reverse primer
TGF-β1	5'TACGGCAGTGGCTGAACCAA3')	5'CGGTTCATGTCATGGATGGTG-3'
Smad3	5'5CTCCAAACCTCTCCCCGAAT3'	5'GAGTTGGAGGGGTCAGT GAA3'
GAPDH	5'CAGATCCACAACGGATATATTGGG3'	5'CATGACAACTTTGGCA TTGTGG3'

Histopathological studies: Liver tissue samples were excised from each mouse and immediately fixed in 10% buffered formalin solution for 24 h. Thin sections (4 µm) were deparaffinized and stained with H&E and Masson's trichrome. Stained sections were examined using an Olympus light microscope to establish the extent of granuloma development, the severity of hepatic fibrosis, and other histopathological changes. The number and size of granulomas in each group were quantified at 100× using a digital image analysis system (Leica Qwin 500; Leica Microsystems, Wetzlar, Germany). Granulomas were quantified in five different fields (100×), separated at least 250 µm apart[32].

Immunohistochemical studies: A mouse anti-α SMA antibody was acquired from Abcam, USA, and used to detect activated HSCs by the avidin-biotin immuno-peroxidase method[33]. The immunohistochemical staining was carried out on 4 µm slices of tissue blocks in paraffin. Sections were dewaxed and rehydrated in descending grades of ethanol concluded with distilled water. For 7 min, the sections were exposed to 6% hydrogen peroxide to inhibit endogenous peroxidase activity. The slices were microwaved in a citrate solution (pH 6.0) for 12 min to retrieve the epitopes. For immunostaining, primary and secondary staining kits were obtained from Thermo Fisher Scientific, Waltham, MA, USA. According to the manufacturer's

instructions, sections were covered by the primary antibody diluted 1:100 against α-SMA (clone 17H19L35) for 1 h at room temperature. A secondary antibody, biotinylated goat anti-polyvalent, was added, followed by streptavidin peroxidase enzyme for 10 min. After washing in PBS, diaminobenzidine chromogen was added for 5 min to visualize peroxidase activity. Hematoxylin was used as a counterstain before the sections were examined under a light microscope. Brown staining of either cell membrane or cytoplasm indicated positivity[33].

Statistical analysis: Results were recorded and evaluated statistically using the SPSS Statistical Platform for Social Sciences, version 26 (SPSS Inc., Chicago, IL, USA). Two categories of statistics were used: Descriptive statistics, *e.g.*, number (No.) and percent (%) for qualitative data, and mean±SD for quantitative data. Analytic statistics: To assess records normality, Kolmogorov-Smirnov test was used to select the suitable statistical analysis, *i.e.*, parametric or non-parametric test. A one-way analysis of variance (ANOVA) test was used to compare quantitative normally scattered variables. In contrast, a Post Hoc Tukey HSD test detected group differences. The Kruskal-Wallis test compared quantitative non-normally distributed variables, whereas the post-hoc Dunn's test detected differences among the groups. A $P<0.05$ was considered statistically significant.

Ethical consideration: The Faculty of Medicine, Menoufia University, Egypt's ethics committee authorized the experimental procedures (IRB number: 12/2024 PARA 16). A standard procedure was implemented in line with the World Health Organization's Good Laboratory Practice guidelines. Animal experiments adhered to the ethical rules and regulations set by the animal care committee of Menoufia University's Faculty of Medicine, along with internationally accepted standards for the use and care of experimental animals.

RESULTS

Parasitological results: Treated groups displayed a statistically significant ($P<0.001$) decline in worm load relative to the positive control group. The most effective treatment was PZQ (2.0±0.89), with the highest reduction% (88.69%±3.80%), followed by WGO (5.0±1.41, 70.22%±9.81%) and propolis (8.83±2.32, 48.98%±5.92%) (Table 1).

Table 1. Effects of propolis, WGO, and PZQ on total worm load, reduction in total worm%, and differential reduction%.

Variable		GII	GIII	GIV	GV	Statistical analysis
Total worm load		17.17±2.99	8.83±2.32	5.0±1.41	2.0±0.89	
Statistical analysis	***P*1**		**<0.001***	**<0.001***	**<0.001***	***F* = 60.45, *P* < 0.001***
	***P*2**			0.026*	**<0.001***	
	***P*3**				0.125	
Total warm reduction (%)			48.98±5.92	70.22±9.81	88.69±3.80	
Statistical analysis	***P*2**			**<0.001***	**<0.001***	***F* = 48.76, *P* < 0.001***
	***P*3**				**<0.001***	
Males No.		6.0±0.89	3.33±0.82	1.67±0.82	1.17±1.17	
Statistical analysis	***P*1**		**<0.001***	**<0.001***	**<0.001***	***K* = 18.21, *P* < 0.001***
	***P*2**			0.035*	**0.004***	
	***P*3**				1	
Reduction% of males No			44.84±9.17	71.59±13.80	81.75±18.97	
Statistical analysis	***P*2**			**0.018***	**0.002***	***F* = 10.31, *P* = 0.002***
	***P*3**				0.735	
Females No.		1.83±1.17	0.33±0.82	0.50±0.55	0.17±0.41	
Statistical analysis	***P*1**		**0.022***	0.051	**0.010***	***K* = 9.17, *P* = 0.027***
	***P*2**			1	1	
	***P*3**				1	
Reduction% of females No.			80.0±44.72	73.33±25.28	80.0±44.72	
Statistical analysis	***P*2**			1	1	***K* = 1.05, *P* = 0.592**
	***P*3**				1	
Couples No.		9.33±2.25	5.17±1.17	2.83±0.75	0.67±0.82	
Statistical analysis	***P*1**		**<0.001***	**<0.001***	**<0.001***	***K* = 21.19, *P* < 0.001***
	***P*2**			0.051	**<0.001***	
	***P*3**				0.081	
Reduction% of couples No.			44.40±4.14	67.93±12.35	93.50±7.25	
Statistical analysis	***P*2**			**<0.001***	**<0.001***	***F* = 48.82, *P* < 0.001***
	***P*3**				**<0.001***	

Data were expressed as mean±SD; **GII:** Control infected; **GIII:** Propolis treated; **GIV:** WGO treated; **GV:** PZQ treated; ***F:*** One-way ANOVA test, ***K:*** Kruskal-Wallis test, ***P:*** Comparing the four groups. ***P*1:** Compared to the corresponding value of GII; ***P*2:** Compared to the corresponding value of GIII, ***P*3:** Compared to the corresponding value of GIV; ***:** Significant ($P<0.05$).

Changes in the oogram pattern of egg viability showed a significant (P<0.001) reduction in the fraction of mature and immature ova and an increased fraction of dead eggs in all treated groups. The PZQ treated group showed the best results with a reduced number of immature eggs (4.17±2.64), and 92.23% reduction%; number of mature eggs (5.33±1.03) and 87.51% reduction%. The WGO treated group followed with reduced number, and percentage of immature eggs (31.0±3.29, 41.19%); and mature eggs, (21.83±2.79, 48.83%). Propolis treated group followed, with number and percentage of immature eggs (39.67±3.14, 24.73%), and mature eggs (29.33±1.75, 31.16%). Moreover, PZQ induced the highest egg mortality of 90.50±2.43 dead eggs, followed by WGO (47.17±4.26 dead eggs), then Propolis (31.0±3.74 dead eggs) (Table 2).

Table 2. Effects of propolis, WGO, and PZQ on oogram pattern, and reduction%.

Variable		GII	GIII	GIV	GV	Statistical analysis
Immature eggs		52.83±2.14	39.67±3.14	31.0±3.29	4.17±2.64	
Statistical analysis	***P*1**		**<0.001***	**<0.001***	**<0.001***	***K* = 21.43, *P* < 0.001***
	***P*2**			**<0.001***	**<0.001***	
	***P*3**				**<0.001***	
Reduction% of immature eggs			24.73±7.67	41.19±7.15	92.23±4.76	
Statistical analysis	***P*2**			**0.002***	**<0.001***	***F* = 168.06, *P* < 0.001***
	***P*3**				**<0.001***	
Mature eggs		42.67±2.50	29.33±1.75	21.83±2.79	5.33±1.03	
Statistical analysis	***P*1**		**<0.001***	**<0.001***	**<0.001***	***F* = 320.37, *P* < 0.001***
	***P*2**			**<0.001***	**<0.001***	
	***P*3**				**<0.001***	
Reduction% of mature eggs			31.16±4.04	48.83±5.68	87.51±2.26	
Statistical analysis	***P*2**			**<0.001***	**<0.001***	***F* = 278.29, *P* < 0.001***
	***P*3**				**<0.001***	
Dead eggs		4.50±1.05	31.0±3.74	47.17±4.26	90.50±2.43	
Statistical analysis	***P*1**		**<0.001***	**<0.001***	**<0.001***	***F* = 796.49, *P* < 0.001***
	***P*2**			**<0.001***	**<0.001***	
	***P*3**				**<0.001***	

Data were expressed as mean±SD; **GII:** Control infected; **GIII:** Propolis treated; **GIV:** WGO treated; **GV:** PZQ treated; ***F:*** One-way ANOVA test, ***K:*** Kruskal-Wallis test, ***P:*** Comparing the four groups. ***P*1:** Compared to the corresponding value of GII; ***P*2:** Compared to the corresponding value of GIII, ***P*3:** Compared to the corresponding value of GIV; ***:** Significant (P<0.05).

Regarding hepatic and intestinal eggs, all treated groups displayed a substantial reduction compared to the positive normal control group. The highest reduction was recorded in the PZQ-treated group (89.85%, and 92.32%, respectively), followed by the WGO-treated group (70.19%, and 64.33%, respectively), then the propolis-treated group (56.0%, and 53.04%, respectively) (Table 3).

Table 3. Effects of propolis, WGO, and PZQ on hepatic and intestinal ova/g, reduction%

Variable		GII	GIII	GIV	GV	Statistical analysis
Hepatic ova/g		4648.5±798.64	2033.17±704.08	1391.67±584.19	461.67±59.08	
Statistical analysis	***P*1**		**<0.001***	**<0.001***	**<0.001***	***F* = 20.25, *P* < 0.001***
	***P*2**			0.495	**<0.001***	
	***P*3**				0.092	
Reduction% of hepatic ova/g			56.0±14.01	70.19±11.19	89.85±2.09	
Statistical analysis	***P*2**			0.097	**<0.001***	***F* = 15.96, *P* < 0.001***
	***P*3**				**0.016***	
Intestinal ova/g		6968.67±726.07	3277.33±483.78	2432.50±655.40	529.67±53.66	
Statistical analysis	***P*1**		**<0.001***	**<0.001***	**<0.001***	***F* = 146.69, *P* < 0.001***
	***P*2**			0.087	**<0.001***	
	***P*3**				**<0.001***	
Reduction% of intestinal ova/g			53.04±3.71	64.33±12.25	92.32±1.16	
Statistical analysis	***P*2**			0.056	**<0.001***	***F* = 44.59, *P* < 0.001***
	***P*3**				**<0.001***	

Data were expressed as mean±SD; **GII:** Control infected; **GIII:** Propolis treated; **GIV:** WGO treated; **GV:** PZQ treated; ***F:*** One-way ANOVA test, ***K:*** Kruskal-Wallis test, ***P:*** Comparing the four groups. ***P*1:** Compared to the corresponding value of GII; ***P*2:** Compared to the corresponding value of GIII, ***P*3:** Compared to the corresponding value of GIV; ***:** Significant (P<0.05).

Biochemical results: Liver enzymes (ALT, and AST) exhibited a substantial increase in GII compared to GI (150.33±2.42 versus 44.05±0.38 U/L in ALT, and 192.17±5.19 versus 61.00±2.37 U/L in AST). After administration of the propolis, WGO, and PZQ to mice of groups III, IV, and V, ALT exhibited a substantial decline (79.17±0.75, 67.33±1.37, 106.17±5.04, respectively) when compared to GII. Similarly, AST significantly (P<0.001) decreased to 87.50±1.05, 79.0±1.41, and 137.50±2.35, respectively, compared to GII. There was a significant (P<0.001) difference between all treated groups (Fig. 1).

Immunological results: Results of cytokine inflammatory markers revealed that IL-4 exhibited a significant (P<0.001) increase in GII compared to GI (19.20±0.46 versus 11.26±0.40 Pg/ml). After administration of propolis, WGO, and PZQ to mice of groups GIII, GIV, and GV, a significant (P<0.001) decrease was recorded (15.12±0.46, 12.85±0.39 and 16.98±0.62 versus 19.20±0.46 Pg/ml) compared with GII. In all treated groups, IL-4 significantly (P<0.001) declined, particularly WGO (GIV). Furthermore, IL-10 significantly (P<0.001) declined in GII compared with GI (23.32±0.57 versus 41.95±0.89 Pg/ml). Administration of propolis, WGO, and PZQ to mice of groups GIII, GIV, and GV exhibited a significant (P<0.001) increase of 30.7±0.93, 39.33±0.75, and 29.63±0.71 versus 23.32±0.57 Pg/ml, respectively, compared with GII. Similarly, IL-10 significantly (P<0.001) increased in all treated groups, particularly WGO (GIV) (Figs. 2, 3).

Assessment of hepatic fibrosis development showed that TGF-β1 exhibited a significant (P<0.001) increase in GII compared to GI (98.80±0.47 versus 46.07±0.66 ng/ml). Administration of propolis, WGO, and PZQ to mice of groups III, IV, and V revealed a significant (P<0.001) decline in TGF-β1 levels compared to GII (67.18±0.49, 63.90±0.52, and 75.95±0.59 versus 98.80±0.47 ng/ml, respectively). Mice treated with WGO (GIV) showed a significant decrease (P<0.001) in comparison to mice of groups III and V (Fig. 4).

Molecular results: Gene replication of TGF-β1 and Smad3 in the hepatic tissue homogenate showed a significant (P<0.001) rise in the TGF-β1 gene expression in GII when compared to GI (1.87±0.05 versus 1±0). All treated groups (III, IV, and V) exhibited a significant decrease (P<0.001) in gene expression of TGF-β1 compared with GII, particularly WGO (GIV) (1.58±0.04, 1.37±0.05, and 1.68±0.04 versus 1.87±0.05, respectively). Gene expression of Smad3 showed a significant (P<0.001) increase in GII compared to GI (1.77±0.05 versus 1±0). Moreover, gene expression of the Smad3 significantly (P<0.001) decreased in GIII, GIV, and GV compared to GII (1.52±0.04, 1.43±0.05, and 1.63±0.05, respectively versus 1.77±0.05). There was a significant (P<0.001) decline in both inflammatory and fibrotic genes in WGO (GIV) compared to all the other treated groups (Fig. 5).

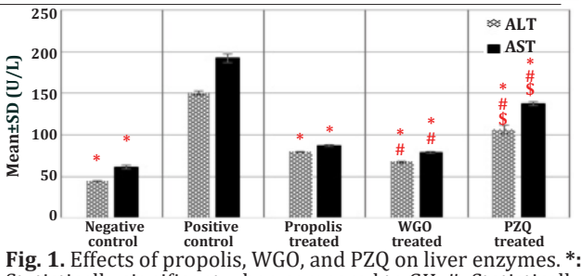

Fig. 1. Effects of propolis, WGO, and PZQ on liver enzymes. ***:** Statistically significant when compared to GII. **#:** Statistically significant compared to the propolis-treated group. **$:** Statistically significant compared to the WGO-treated group.

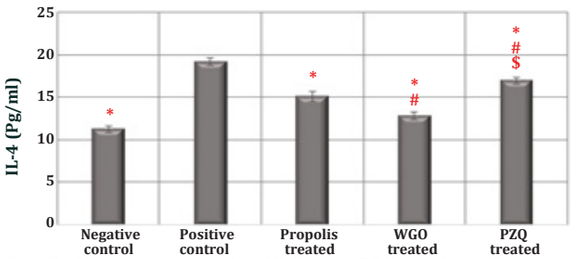

Fig. 2. Effects of propolis, WGO, and PZQ on IL-4 (Pg/ml). ***:** Statistically significant when compared to GII. **#:** Statistically significant compared to the propolis-treated group. **$:** Statistically significant compared to the WGO-treated group.

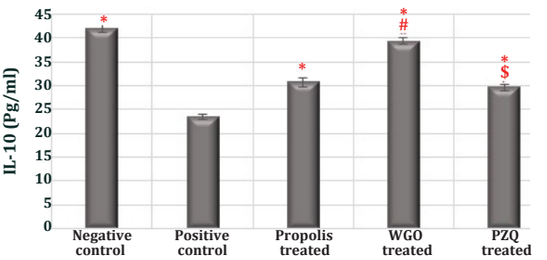

Fig. 3. Effects of propolis, WGO, and PZQ on IL-10 (Pg/ml). ***:** Statistically significant when compared to GII. **#:** Statistically significant compared to the propolis-treated group. **$:** Statistically significant compared to the WGO-treated group.

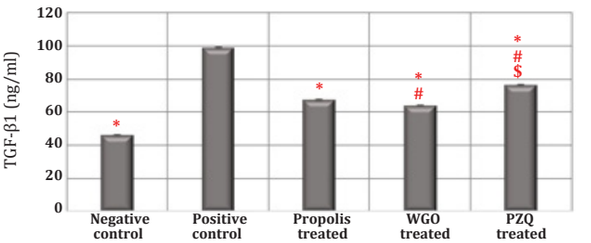

Fig. 4. Effects of propolis, WGO, and PZQ on TGF-β1 (ng/ml). ***:** Statistically significant when compared to GII. **#:** Statistically significant compared to the propolis-treated group. **$:** Statistically significant compared to the WGO-treated group.

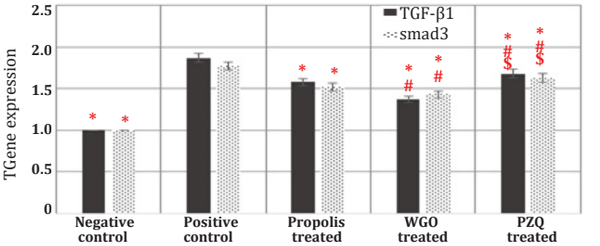

Fig. 5. Effects of propolis, WGO, and PZQ on TGF-β1 and smad3 gene expression. ***:** Statistically significant when compared to GII. **#:** Statistically significant compared to the propolis-treated group. **$:** Statistically significant compared to the WGO-treated group.

Histopathological results: Liver sections, stained with H&E, showed that the mean number of hepatic cell granulomas in all treated groups significantly decreased, compared to GII. The reduction% of granuloma number in the liver reached 78.89% in the PZQ-treated group, followed by the WGO-treated group, which reduced to 64.53%, then the propolis-treated group, which reduced to 53.08%. The granuloma size also decreased in all treated groups, with lowering percentages of granuloma diameter reaching 77.71% in the PZQ-treated group, 58.03% in the WGO-treated group, and 43.86 % in the Propolis-treated group (Table 4).

Table 4. Effects of propolis, WGO, and PZQ on the hepatic granuloma number, and diameter, and reduction%.

Variable		GII	GIII	GIV	GV	Statistical analysis
Hepatic granuloma No.		19.33±4.76	9.0±2.19	6.5±1.87	4.0±1.55	***K*** = 18.79, ***P*** < **0.001***
Statistical analysis	***P1***		**<0.001***	**<0.001***	**<0.001***	
	P2			0.897	0.043	
	P3				0.897	
Reduction% of hepatic granuloma No.			53.08±7.53	64.53±13.81	78.89±8.31	***F*** = 9.51, ***P*** = **0.002***
Statistical analysis	***P2***			0.218	**0.002***	
	P3				0.086*	
Hepatic granuloma diameter (µm)		371.0±48.26	204.3±49.3	154.8±16.13	81.33±14.13	***F*** = 69.51, ***P*** < **0.001***
Statistical analysis	***P1***		**<0.001***	**<0.001***	**<0.001***	
	P2			0.166	**<0.001***	
	P3				**0.013***	
Reduction% of hepatic granuloma diameter (µm)			43.86±17.47	58.03±3.55	77.71±5.0	***K*** = 14.00, ***P*** < **0.001***
Statistical analysis	***P2***			0.109	**<0.001***	
	P3				**0.018***	

Data were expressed as mean±SD; **GII:** Control infected; **GIII:** Propolis treated; **GIV:** WGO treated; **GV:** PZQ treated; ***F:*** One-way ANOVA test, ***K:*** Kruskal-Wallis test, ***P:*** Comparing the four groups. ***P*1:** Compared to the corresponding value of GII; ***P*2:** Compared to the corresponding value of GIII, ***P*3:** Compared to the corresponding value of GIV; ***:** Significant (*P*<0.05).

The normal control group displayed normal hepatocytes with central veins. Mice of GII displayed ova and many granulomas with numerous chronic inflammatory cells. Magnification displayed granuloma with ova containing miracidium, chronic inflammatory infiltrate, and adult worms in the portal tract. The propolis-treated group showed ova degeneration. The WGO-treated group showed reduced size and numbers of granuloma with hydropic change of liver cells. Mice treated with PZQ showed reduced inflammatory infiltration around viable ova (Fig. 6).

Hepatic fibrosis was detected with Masson's trichrome stain of liver sections from the positive control group, revealing condensed fibrous connective tissue in the granulomas and portal tracts. The propolis-treated group showed a milder fibrous tissue reaction in the granulomas. The WGO-treated group also showed a moderate reduction of fibrous tissue. The PZQ-treated group showed a marked reduction of fibrous tissue in the granulomas (Fig. 7).

Immunohistochemical results: Immunohistochemical staining of liver sections showed high expression of α-SMA. Propolis-treated and WGO-treated groups showed low expression of α-SMA. The PZQ-treated group showed negative expression of α-SMA (Fig. 8).

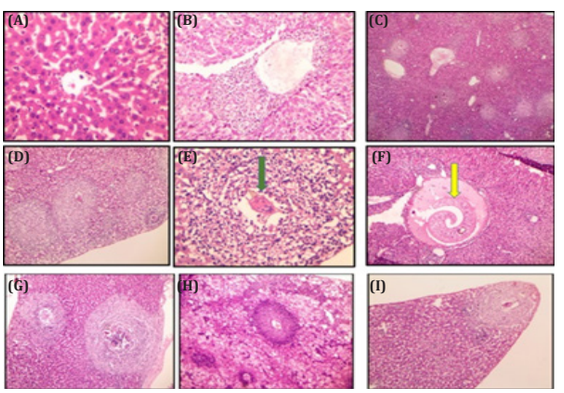

Fig. 6. Photomicrographs of the liver stained with H&E. **(A)** The control group showed normal hepatocytes and central vein (×400). **(B)** Dilated portal tract with mild inflammatory infiltrate (x100). **(C, D)** GII showed numerous granulomas with bilharzial ova surrounded by numerous chronic inflammatory cells (×40 and x100). **(E)** Magnification (x400) showed bilharzial granuloma with bilharzial ova containing miracidium and chronic inflammatory infiltrate (green arrow). **(F)** Adult schistosome worm in the portal tract (yellow arrow, x100). **(G)** Propolis-treated group (GIII) showing ova degeneration (x 100). **(H)** WGO-treated group (GIV) showed reduced size and numbers of granuloma with hydropic change of liver cells (x 100). **(I)** PZQ-treated group (GV) showed reduced inflammatory infiltrate around viable *Schistosoma* ova (x 100).

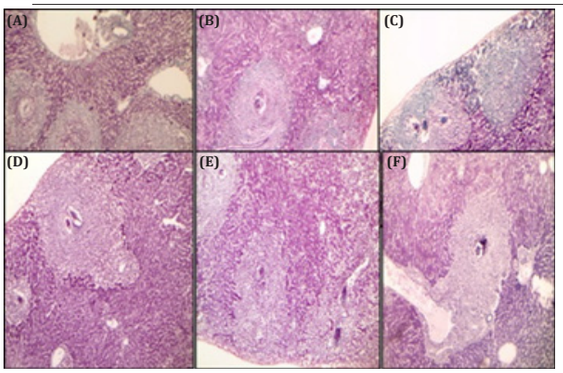

Fig. 7. Photomicrographs of the liver stained with Masson trichrome stain. **(A, B, C)** The infected untreated group (GII) showed granulomatous lesions with condensed fibrous connective tissue in the granuloma and portal tracts (×100). **(D)** Propolis treated group (GIII) showed reduced fibrous tissue in the granuloma (x 100). **(E)** WGO-treated group (GIV) showed reduced fibrous tissue in the granuloma (x 100). **(F)** PZQ-treated group (GV) showed reduced fibrous tissue in the granuloma (x 100).

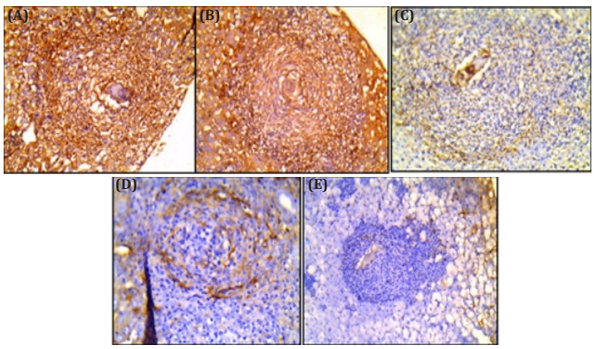

Fig. 8. Photomicrographs of the liver stained with α-SMA. **(A, B)** Schistosome granuloma showing high expression of α-SMA (x 200). **(C)** Propolis-treated group showing low expression of α-SMA (x 200). **(D)** WGO stained group showing low expression of α-SMA (x 400). **(E)** PZQ-treated group showing negative expression of α-SMA (x 200).

DISCUSSION

Chronic schistosomiasis-induced liver fibrosis is a significant complication that can result in mortality. The current study showed parasitological, biochemical, molecular, and histological findings for the potential benefits of propolis and WGO compared to PZQ against murine schistosomiasis *mansoni*. Propolis possesses immunomodulatory capabilities that enhance nonspecific host defense mechanisms through macrophage activation, elevating macrophages' antiparasitic efficacy by encouraging the expression of ROS. Immunization with propolis potentially protects against murine *T. saginata* infection[34]. Additionally, propolis treatment caused disruption of the *Toxocara* body musculature and cuticle[35]. Soufy *et al.*[36] proposed that the antiparasitic effect of Egyptian propolis may be due to its phenolic compounds responsible for its anti-protozoal effect through enhancement of oxidative defense mechanisms. Therefore, the anti-cryptosporidial effects of propolis could be attributed to the phytochemical constituents, particularly phenolic compounds. Deghbar *et al.*[37] highlighted the immunomodulatory and anti-hydatidic properties of the ethanolic extract of propolis in treating hydatid disease. Recently, its antiparasitic outcome was attributed to the antioxidant flavonoid activity of caffeic acid, a biologically active component that causes alterations of the morphological features, disrupts mitochondrial integrity, and affects the cellular plasma membrane, thereby facilitating apoptosis[38].

Conversely, the administration of WGO to infected mice demonstrated both antioxidant and anti-inflammatory properties. It showed promising anti-inflammatory effects in liver toxicity in rats, which is likely attributed to its high content of unsaturated fatty acids (81%). These fatty acids not only possess anti-inflammatory properties, but also are able to reduce oxygen-free radicals[39]. Furthermore, WGO downregulated the expression of NF-kB, a critical regulator of pro-inflammatory cytokines, which may be tied to its ROS-scavenging capabilities[40]. *In vitro* studies indicated the inhibitory effects of WGO on the motility and growth of *T. vaginalis* trophozoites[41].

Regarding parasitological parameters, our results conformed with de L Paula *et al.*[15] who reported the effect of Brazilian green propolis with different doses at different periods in reducing total worm burden in experimental murine schistosomiasis *mansoni*. However, PZQ resulted in a 91% reduction in worm burden, and there were noteworthy decreases in egg load per gram of ileum tissue in the groups treated with Brazilian green propolis for different studied groups; egg reductions percent in the ileum and liver significantly decreased. Additional experimental studies by Silva *et al.*[42] claimed that Brazilian propolis extract decreased the worm burden of *S. mansoni* by 53.77%. Similarly, Sarhan *et al.*[38] reported reduced *T. spiralis* adult and larval counts treated with Egyptian propolis by 75.1% and 77%, respectively. Furthermore, Barakat *et al.*[43] displayed a decline in the parasitic burden in the liver

of treated mice with WGO and propolis compared to the positive control group of *T. gondii*-infected mice. In addition, Elmahallawy *et al*.[41] indicated that treating *T. gondii*-infected mice with WGO and propolis is effective against tissue cysts, destroying and decreasing parasite load in the brains compared with the positive control group. Furthermore, Abdelmaksoud *et al*.[19] reported that WGO had a noticeable antiparasitic effect against cryptosporidiosis, recording the lowest *Cryptosporidium* oocyst number.

Regarding immunological and histopathological results, our results agreed with Huang *et al*.[44], who demonstrated the key role played by egg granulomas on progression of liver fibrosis induced by schistosomiasis by stimulating the production of various cytokines from macrophages and lymphocytes. These include platelet-derived growth factor (PDGF) and TGF-β1 which induce the proliferation and transformation of HSCs into myofibroblasts. The latter actively produce collagen, leading to liver fibrosis. Besides, several studies[9,45,46] proved that upon liver injury or exposure to inflammatory stimuli, HSCs transform into myofibroblasts, which express α-SMA and generate hydroxyproline-rich collagen, resulting in excessive ECM deposition.

The TGF-β1 signaling pathway plays a significant role in maintaining hepatocyte apoptosis, and normal liver homeostasis. Since TGF-β1 controls the parenchymal cells, inflammatory cells, and HSCs, immune cells release the inflammatory mediators[47,48]. Our current outcomes recorded a substantial increase in the levels of TGF-β1 in the infected group compared with the normal control group, and there was a considerable improvement in this profibrotic mediator after propolis and WGO administration. Our results align with two recent studies who proposed that propolis and WGO inhibited fibrotic alterations in liver caused by toxoplasmosis[43,49]. Because PZQ exhibited a significant decrease in TGF-β1, it correspondingly displayed an antifibrotic effect. Alongside its antiparasitic activity, Liu *et al*.[7] demonstrated that continued treatment with PZQ inhibited the progression of liver fibrosis induced by schistosomiasis *mansoni*.

Our histopathological results agreed with Barakat *et al*.[43], who investigated the potential efficacy of WGO, propolis, or both on chronic toxoplasmosis. The investigators recorded a substantial rise in pathological lesions in infected mice compared with non-infected group. Decreased pathologic changes were detected in the WGO, propolis, and combined treatment in lung and liver sections. De L Paula *et al*.[15] reported a substantial decline in the number and diameter of the granulomas in liver sections of Brazilian green propolis-treated *S. mansoni*. Moreover, they postulated that this diameter reduction was strictly concomitant with propolis' anti-inflammatory and hepatoprotective effects. This granulomatous significant change in the current study could be attributed to the molecular genetic significant decrease in TGFβ1/Smad3 in all treated groups due to control of HSCs activity through TGF-β1 /Smads pathway. Results of our study agreed with Liu *et al*.[7] who proposed that PZQ suppressed the activation of HSCs by up-regulating Smad7. This up-regulation of Smad7 blocked the TGF-β1-Smads pathway in carbon tetrachloride (CCl4)-induced liver tissue fibrosis.

In conclusion, administration of propolis and WGO, displayed a potential antiparasitic activity and reduced liver fibrosis induced in murine schistosomiasis *mansoni*. Both significantly decreased schistosomal parasitic burden, and restored the molecular and histopathological alterations triggered by schistosomiasis. The antifibrotic hepatoprotective effects attained through the novel strategy of inhibiting the TGF-β1/Smad3 pathway offer new insights into the potential advance of innovative therapeutic approaches for liver fibrosis tempted by *Schistosoma* spp., without adverse consequences. We recommended further investigation on the *in vitro* effects of propolis and WGO on juvenile schistosomes. Consider testing propolis and WGO with PZQ, as they could be used as supplementary treatment in early cases with PZQ treatment to modify the susceptible liver fibrosis.

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Author contribution: AL-Attar TA, Faheem MF, Shalaan FH contributed equally in performing parasitological studies. Abd El-Hafiz HI was responsible for pharmacological dosage methodology and data analysis of the biochemical, and molecular results. Elshahat HA examined the liver sections and interpreted the histopathological results. AL-Attar TA, Faheem MF, Abd El-Hafiz HI, and Shalaan FH shared the original draft preparation, writing, review, and editing of the manuscript. All authors revised and approved the final version before publication.

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