

## Research Article

# Antischistosomal Activity of *Zingiber officinale*, *Piper nigrum*, and *Coriandrum sativum* Aqueous Plant Extracts on Hamster Infected with *Schistosoma mansoni*

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Schistosomiasis continues to affect the health and quality of life of millions of people around the world. Schistosomiasis has been ranked the second disease after malaria in terms of importance as a targeted tropical disease. Praziquantel (PZQ) is the only drug approved by the World Health Organization (WHO) for the treatment of schistosomiasis. Being the only drug, parasite resistance to this drug has developed. Therefore, the search for new alternatives has been the goal of many researchers. In this study, the effects of aqueous extracts of *Zingiber officinale*, *Piper nigrum*, and *Coriandrum sativum* on *Schistosoma mansoni* infected golden hamsters (Egyptian strain) were evaluated *in vitro* and *in vivo* at different doses of 500, 250, 125, 62.5, and 31.25  $\mu\text{g/ml}$ . *In vitro*, adult worms of *S. mansoni* were tested in RPMI-1640 medium for 48 hrs. The results showed that the concentrations 500, 250, and 125  $\mu\text{g/ml}$  of *Zingiber officinale* and *Piper nigrum* caused dead of 100% of adult worms within 6 and 12 hrs of incubation, respectively. Although, aqueous extract of *Coriandrum sativum* at concentrations 500, 250, and 125  $\mu\text{g/ml}$  resulted dead of 100% parasites after 12 to 24 hrs of incubation. In conclusion, *Zingiber officinale* and *Piper nigrum* showed efficacy against schistosomiasis in both *in vitro* and biological experiments of Egyptian schistosome strain, while *Coriandrum sativum* gave less effective results than the previous ones. Therefore, *Zingiber officinale* and *Piper nigrum* may become an innovative treatment for schistosomiasis.

## 1. Introduction

The World Health Organization (WHO) considers the infection as an ignored tropical disease, with an evaluated 732 million people being defenseless to disease around the world recognized transmission regions [1]. Schistosomiasis has a disease classification Category II alongside malaria in significance as a target tropical disease by WHO. Steinmann and his colleagues reported that more than 200 million people from Asia, South America, and Africa are infected with schistosomiasis; also, nearly 800 million people around the world are at risk from schistosomiasis [2, 3]. The World Health Organization furthermore evaluated that schistosome contaminations and geo-helminths accounted for more than 40% of the burden of tropical disease in the world, excluding malaria [4]. Peoples get contaminated with schistosomiasis

when they make communication with fresh water contaminated with infective stage cercariae. Predominance of schistosomiasis remains high at present in sub-Saharan Africa. 17.5 million individuals worldwide were treated for schistosomiasis; only in sub-Saharan Africa that 11.7 million people of those were treated, in 2008 [1]. In sub-Saharan Africa, approximately 120 million people have symptoms related to schistosomiasis, whereas around 20 million tolerate suffering as result of chronic symptoms of the disease [5].

Schistosomiasis was discovered for the first time by Ruffer [6], when *S. haematobium* infection was diagnosed in mummies. It is obvious that the schistosomiasis is clearly a chronic disease of the Ancient Egypt. In 20th Dynasty, Ruffer found in two Egyptian mummies' schistosome eggs were calcified. By radiological investigation too emphatically proposed that *S. haematobium* illness was the main reason of

bladder calcification in two other mummies [7, 8]. Also, in 1937, the first epidemiological surveys of schistosomiasis were conducted throughout Egypt. In year 2000, the Schistosomiasis Research Project succeeded in achieving its goals where the cooperation between the Egyptian Ministry of Health and the United States Agency for International Development (USAID): in nine governorates from different parts of Upper and Lower Egypt random samples were taken from the inhabitants of those governorates to study the different epidemiological parts of schistosomiasis as well as the determinants of contamination and morbidity. The study showed that in Upper Egypt, the predominance of the *S. mansoni* infection was uncommon, where it was important only in Fayoum with a predominance of 4.3%, and its prevalence ranged from 17.5% to 42.9% in Lower Egypt as it is considered an endemic disease with age stratified prevalence of contamination peaking in the 15–19 years old age group. On the opposite, the ratio from 4.8 to 13.7% was prevalence of *S. haematobium* in Upper Egypt; the spread of schistosomiasis reached a peak in the 10–14 age groups. The prevalence of *S. haematobium* was rare in Lower Egypt where the highest rate of infection was 1.8% and recorded in the Suez Canal city and the Ismailia [9]. Wei et al. [10] said that the history of schistosomiasis prevalence in China dates back more than 2100 years and that by examining two ancient corpses in China from Hunan and Hope provinces, *S. japonicum* eggs were found.

Despite the importance of schistosomiasis on public health and the risk of further spread of the disease and in endemic countries, the schistosomiasis control programs have been intensified; it fundamentally relies on chemotherapy. Trypanosomiasis, leishmaniasis, and schistosomiasis are among the most neglected tropical diseases, until the 1970s when praziquantel (PZQ) was discovered treatment for schistosomiasis was difficult and almost toxic [11, 12]. PZQ was introduced to the market in 1988 [11], and until now, it is the only drug available to control and treatment of schistosomiasis recommended by the World Health Organization [12]. For example, treatment of schistosomiasis with a single drug for many decades intensively and exclusive may sooner or later raise logical concerns that schistosomiasis may resistant to PZQ [13]. Moreover, PZQ works against adult schistosomiasis, but it is ineffective against smaller stages of schistosomiasis, such as schistosomula, preadults, and juvenile adults. As a result, it is sometimes necessary to repeat treatment to kill those worms that have matured since then. Furthermore, having sole drug recommended by WHO to treat millions of people who are infected in different geographical regions is a real concern. Subsequently, new antischistosomal drugs must be developed that are effective and safe. In recent years, the increasing need to produce and develop new drugs against schistosomiasis fundamentally from natural sources has led the scientific society to concentrate research on the possibility of producing a drug for schistosomiasis. Also, there were attempts to examine a treatment of nanoparticles from calcium silicate containing 5% copper oxide [14].

Natural sources, especially plants, have been for thousands of years the primary source of medicine [15]. Folk treatment has been used by medicinal plants, and they have

been used as effective drugs to treat many diseases that have provided modern medicine, especially parasites. As a consequence, there are many plant extracts or natural compounds from plants that have a strong influence on schistosomiasis [16–22]. Natural products have many advantage to use as a controlling factor, in underdeveloped countries you find it difficult to purchase expensive Western medicines and find medicinal plants a haven because they are within their reach to cheapen their prices; also, the natural extract is more effective and has fewer side effects because it does not contain manufactured chemical products; finally, it is more secure for the environment. So, these natural plant extracts are possible and effective to be used as antimicrobial as well as anti-parasitic agents [23].

*Zingiber officinale* has many medicinal properties, including anti-inflammatory [24, 25], anticoagulant [24, 26], hypocholesterolaemic [27, 28], antimigraine [29, 30], antidiabetic [31], antiarthritis [32, 33], antinausea properties [34, 35], antithrombotic [24, 26], and hypolipidaemic [24, 26, 28, 31]. Iqbal et al. [36] in sheep results showed the use of aqueous extract of crude powder from dried ginger, which showed anthelmintic activity. Lin et al. [37] studied some compounds derived from ginger as larvicidal agents for *Angiostrongylus cantonensis* worms. The same researcher Lin et al. [38] used the previous compound to study the effects on some nematodes (*Anisakis simplex*) and showed that the used compounds kill or at least reduce the movement of the larvae in *Anisakis simplex*. With regard to schistosomiasis, Sanderson et al. [39] studied the effect of ethyl acetate extract from ginger (*Z. officinale*) *in vitro* and also *in vivo* the live body in laboratory mice against the adult worms of *Schistosoma mansoni*. Note that the results *in vitro* were promising, but *in vivo*, no visible difference was seen between treated and nontreated mice.

Genus Piper belongs to the Piperaceae family, and this genus contains about 2000 species of evergreen plants and a climber. Some species are especially used in traditional medicine of China, India, Southwest Asia, and Latin America. *P. nigrum* is used in many Asian countries for the treatment of indigestion, asthma, pain, respiratory infections, and rheumatoid arthritis [40]. Also, it is stimulating, digestive, tonic, and antiseptic [41]. *P. nigrum* oil showed antioxidant, carminative, and larvicidal, as well as antibacterial and antifungal activities [42–45]. Insecticides are against to *Sitophilus zeamais* [46]. Furthermore, inhaling *P. nigrum* oil was effective in reducing symptoms of smoking withdrawal including craving for cigarettes and anxiety [47].

*Coriandrum sativum* L. (coriander) belongs to the Umbelliferae family also known as Apiaceae; it is a medicinal and culinary plant, and in China, it is called Chinese parsley and is a popular spice and is widely used in cooking due to its pleasant and delicate aroma [48]. In addition to its traditional use in food, in folk medicine, it has also been widely used in carminative, digestive, spasmolytic, and galactagogue [49, 50]. The extract of coriander seed, in addition to coriander seed oil, possesses antimutagenic activities, anticancer, antioxidant, antibacterial [51–54]. In cosmetics, shampoo, soaps, emulsions, lotions, and creams, coriander seed oil is one of the main ingredients [49, 55–58].

TABLE 1: Identified components in aqueous extract of *Zingiber officinale*.

No.	RT	M. wt	M. formula	Prediction	Area	Area %
1	8.41	290	C19H30O2	10,13-Octadecadiynoic acid	435707.41	0.31
2	10.25	170	C10H18O2	4-Nonenoic acid	836484.93	0.59
3	13.87	185	C9H15NO3	Ecgonine	754614.45	0.53
4	14.23	178	C6H10S3	Trisulfide, di-2-propenyl	920209.04	0.64
5	19.76	206	C13H18O2	5-Methyltricyclo [6,2,1.0(2, 7)] undeca-4,9,dien-3,6,-diol	1146674.12	0.80
6	21.27	302	C20H30O2	cis-5,8,11,14,17-Eicosapentaenoic acid	469991.74	0.33
7	21.27	286	C20H30O	Retinol	469991.74	0.33
8	23.07	238	C15H26O2	2-Furanmethanol, tetrahydro-à,à,5-trimethyl-5-(4-met hyl-3-cyclohexen-1-yl)-, [2S-[2à,5á(R*)]]-	2796232.19	1.96
9	23.70	236	C15H24O2	Bisabolone oxide	1534365.84	1.07
10	23.70	264	C17H28O2	7,10,13-Hexadecatrienoic acid	1534365.84	1.07
11	24.67-	312	C19H36O3	Oxiraneundecanoic acid, 3-pentyl-,methyl ester, trans-	809996.68	0.57
12	24.67	366	C24H46O2	Cyclopropanedodecanoic acid, 2-octyl-	809996.68	0.57
13	32.07	294	C19H34O2	9-cis,11-trans-Octadecadienoate	4830120.01	3.38
14	32.70	298	C19H38O2	Methyl stearate	5227679.62	3.66
15	33.31	308	C20H36O2	Linoleic acid ethyl ester	2386891.33	1.67
16	33.42	310	C20H38O2	Ethyl oleate	3734744.82	2.61
17	33.91	284	C18H36O2	Hexadecanoic acid, ethyl ester	2416524.37	1.69
18	33.91	312	C20H40O2	Octadecanoic acid, ethyl ester	2416524.37	1.69
19	39.73	644	C27H44O3	Tristrimethylsilyl ester	486374.48	0.34
20	39.73	416	C27H44O3	9,10-Secocholesta-5,7,10(19)-triene-3,24,25-triol, (3á,5Z,7E)-	486374.48	0.34
21	39.73	496	C27H52O4Si2	2,3-BIS[(Trimethylsilyl)oxy] propyl ester	486374.48	0.34

This study is aimed at assessing the antischistosomal activity of three crude aqueous extracts: *Zingiber officinale* (ginger), *Piper nigrum* (black pepper), and *Coriandrum sativum* (coriander) against *S. mansoni* in golden hamster by evaluating three parameters, *in vitro* (worm recovery) and surface topography of worms, finally *in vivo* by examination of histopathology for the kidney, liver, and spleen tissues after the dose.

## 2. Materials and Methods

**2.1. Plant Materials.** *Zingiber officinale* (stem), *Piper nigrum* (seed), and *Coriandrum sativum* (seed) were obtained and identified in the Horticulture Department, Faculty of Agriculture, Ain Shams University. The part of the collected plant was cleaned, washed, and dried in the shade, and to avoid the losing the active ingredients, they should not be exposed to sunlight.

**2.2. Aqueous Extract Preparation.** By electric grinder, the dried materials were ground to fine powders. A quantity of crushed leaves weighing 200 g was dissolved with two liters of distilled water at a ratio (1:10 *w/t*) by method of cool extraction and evaporated of the extract *in vacuo*. The extracts were concentrated *in vacuo* by using a rotary evaporator at 40°C. Finally, the extracts were placed in porcelain dishes in temperature-controlled oven to remove the remain-

ing water in the extracts to give a residue of 8.5 g. At 4°C, the residues were stored for further use [59].

**2.3. Toxicological Study.** The maximum nontoxic concentration (MNTC) (the maximum concentration without toxic effect and expressed in  $\mu\text{l/ml}$ ) of the plant extract on Vero cells had been carried out by serial dilutions of 10-300  $\mu\text{l/ml}$ . Briefly,  $2 \times 10^5$  cells/ml of Vero cells were treated with the serial dilutions of the extract in microtiter plates and had been incubated at 37°C in a carbon dioxide (5% humidity) for 72 hrs. Furthermore, the microscopic plates were examined in order to determine the toxic concentration of the extract through its ability to induce cell death. Cytotoxicity of the extract concentration at 50% was not exceeded to maintain the cell viability and their ability to cleave the tetrazolium salt MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (Sigma, Chem, St. Louis, MO), producing formazan blue product [60]. Briefly, supernatants were removed from the wells, and 25  $\mu\text{l}$  of an MTT (Sigma, St. Louis, MO) solution (2 mg/ml in PBS) had been added to each well, and the plates were incubated at 37°C for 90 min. Then, dimethyl sulfoxide (DMSO) (25  $\mu\text{l}$ ) was added to each well to get rid of crystallized formazan. The plates were kept for 15 min on a shaker to be ready for the determination of the optical density at 492 nm (OD492). The MNTC value is the extract dilution which maintains normal cells morphologically and density in comparison with the untreated control cells with at least 95% of the optical density.

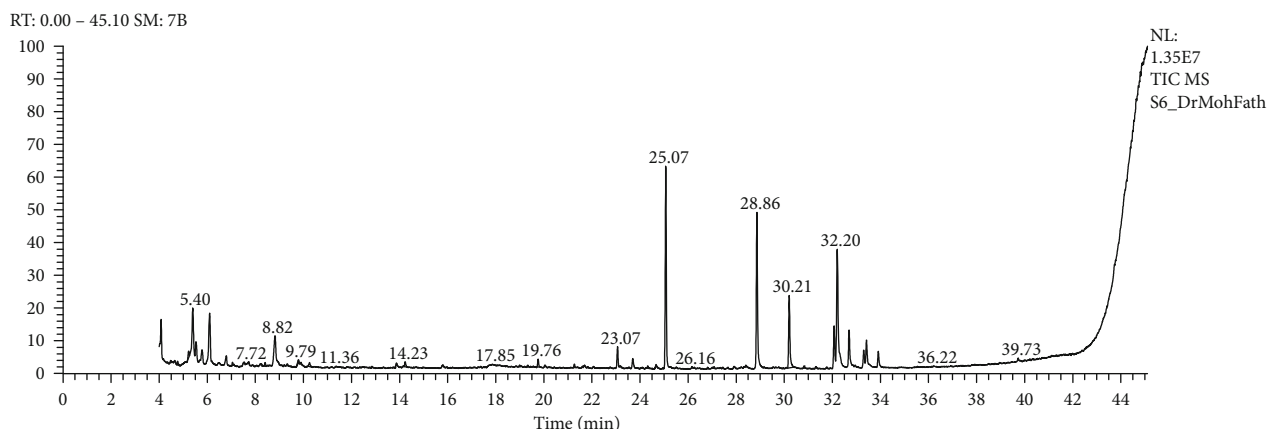


FIGURE 1: GC-MS chromatogram of aqueous plant extract of *Zingiber officinale*.

## 2.4. Experimental Design

**2.4.1. Infection of Hamster with *Schistosoma Cercariae*.** At the start of bioassay (week 0), general anaesthesia was administered to the hamster to produce loss of consciousness and suppression of reflex activity and muscle relaxation. A ratio based on volume of 3:1 Ketamine and Rompun and (Agar, Holland) was used to provide a combined effect of anaesthesia. The anaesthesia dose of 0.02 ml/30 g hamster body weight was injected intraperitoneally. The anaesthetised hamsters were shaved on the stomach area, and on a wooden rack, it was arranged. To keep the shaving area moist, cotton wool has been sloped in water to allow easy penetration of cercariae. A 1 cm ring of metal was placed on the shaven area of each hamster; then, a suspension constituting approximately 250 live cercariae was dispensed in the metal ring using a micropipette, and a period of 30 minutes was allowed for cercariae to penetrate into the hamster [61].

**2.4.2. Parasite (Liver Perfusion).** For *in vitro* bioassay, *S. mansoni* adult worms of Egyptian strains were brought from victimized infected golden hamsters following the technique of Stirewalt and Dorsey [62] from the livers of the hamster after eight weeks of postinfection. Another infected animal group was used for *in vivo* assay.

**2.4.3. In Vitro Study.** To culture the parasites, three times via Roswell Park Memorial Institute (RPMI) 1646 culture medium (Biowhittaker, Lonza, B-4800 verriers, Belgium), the adult worms were washed. The medium was augmented with L-glutamine, 2090 fetal calf serum, and antibiotics (300 g streptomycin, 300 penicillin, and 160 g gentamycin per ml). Using 24 well culture plates (TPP, St. Louis, Mo), each of which contained one (ml) of the same medium, seven pairs of worms were transported in each well. Then, one (ml) of tested material with concentrations 500, 250, 125, 62.5, and 31.25  $\mu\text{g/ml}$  was added in each well excluding the negative control wells (media with dimethyl sulfoxide (DMSO)) while, PZQ (10  $\mu\text{g/ml}$ ) was used as a positive control. The final size in each well was 2 ml. The plates were incubated at 37°C in a moist atmosphere containing 5% CO<sub>2</sub> (Thermo Fisher Scientific, Marietta, OH, USA) for 48 hrs and monitored at different

time intervals (2, 4, 6, 12, 24, and 48 hrs). In a sterile laminar flow chamber, all steps were performed. The experiment was done in triplicate and repeated three times. After each incubation time, treated worms were examined for their mating (pairing), motility (worms motor activity changes), and death rate using an inverted optical Olympus microscope.

Worms showing no motility were counted as dead. Changes in worm's motor activity of schistosomes were assessed qualitatively, and their motor activity reduction was named as "slight" or "significant" overseeing the adult schistosomes in the *in vitro* experiment at all-time intervals.

**2.4.4. Electron Microscope.** Samples of the adult worms (male and female) with the occurrence of morphological changes in their integument were treated and processed immediately according to methods of Glauert [63]; then, the samples were examined by Environmental Scanning Electron Microscope (Inspect S; FEI, Holland) at Electron Microscopy unit of Theodor Bilharz Research Institute (TBRI).

## 2.4.5. In Vivo Study

**(1) Golden Hamsters.** A total of thirty adult hamsters 105–130 g infected *Schistosoma mansoni* were maintained at the Schistosome Biological Supply Center (SBSC) at Theodor Bilharz Research Institute (TBRI), Embaba, Giza, Egypt. Golden hamsters were kept separately in a cage prepared for the examination of fecal materials for obtaining parasites in the study. Randomly, infected gold hamsters were divided into 6 groups, each containing 5 gold hamsters at the time of the experiment:

Group (1): healthy control contained the following:

(i) Uninfected hamster (healthy control)

Group (2): negative control contained the following:

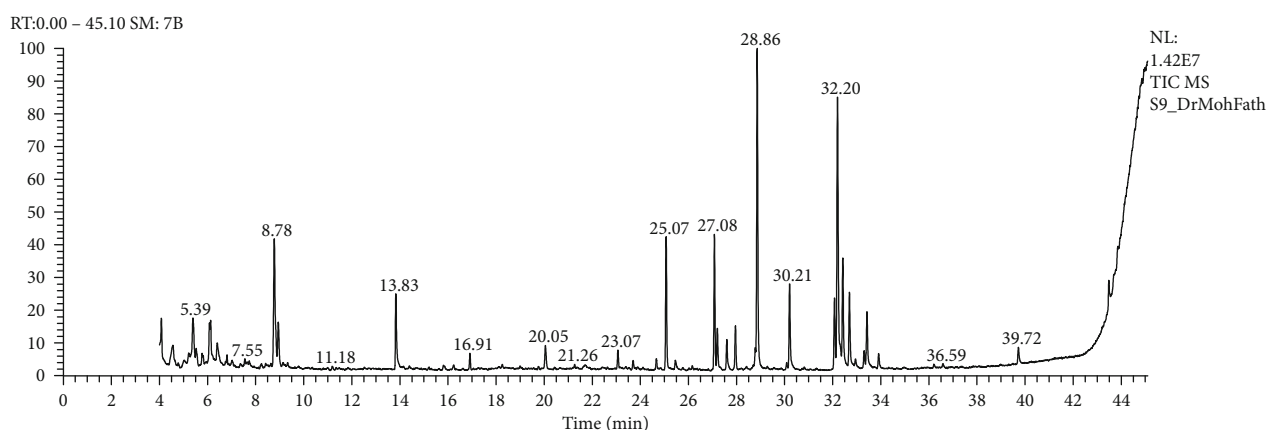
(ii) Infected hamster with adult *Schistosoma mansoni* given distill water (negative control)

Group (3): positive control contained the following:



TABLE 2: Identified compounds in aqueous extract of *Piper nigrum*.

No.	RT	M. wt	M. formula	Prediction	Area	Area %
1	8.41	290	C19H30O2	10,13-Octadecadiynoic acid	435707.41	0.31
2	10.25	170	C10H18O2	4-Nonenoic acid	836484.93	0.59
3	13.87	185	C9H15NO3	Ecgonine	754614.45	0.53
4	14.23	178	C6H10S3	Trisulfide, di-2-propenyl	920209.04	0.64
5	19.76	206	C13H18O2	5-Methyltricyclo [6,2,1.0(2, 7)] undeca-4,9,dien-3,6,-diol	1146674.12	0.80
6	21.27	302	C20H30O2	cis-5,8,11,14,17-Eicosapentaenoic acid	469991.74	0.33
7	21.27	286	C20H30O	Retinol	469991.74	0.33
8	23.07	238	C15H26O2	2-Furanmethanol, tetrahydro-à,à,5-trimethyl-5-(4-met hyl-3-cyclohexen-1-yl)-, [2S-[2à,5á(R*)]]-	2796232.19	1.96
9	23.70	236	C15H24O2	Bisabolone oxide	1534365.84	1.07
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11	24.67-	312	C19H36O3	Oxiraneundecanoic acid, 3-pentyl-,methyl ester, trans-	809996.68	0.57
12	24.67	366	C24H46O2	Cyclopropanedodecanoic acid, 2-octyl-	809996.68	0.57
13	32.07	294	C19H34O2	9-cis,11-trans-Octadecadienoate	4830120.01	3.38
14	32.70	298	C19H38O2	Methyl stearate	5227679.62	3.66
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18	33.91	312	C20H40O2	Octadecanoic acid, ethyl ester	2416524.37	1.69
19	39.73	644	C27H44O3	Tristrimethylsilyl ester	486374.48	0.34
20	39.73	416	C27H44O3	9,10-Secocholesta-5,7,10(19)-triene-3,24,25-triol, (3á,5Z,7E)-	486374.48	0.34
21	39.73	496	C27H52O4Si2	2,3-BIS[(Trimethylsilyl)oxy] propyl ester	486374.48	0.34

FIGURE 2: GC-MS chromatogram of aqueous plant extract of *Piper nigrum*.

(iii) Infected hamster with *Schistosoma mansoni* treated with 200 mg/kg PZQ

Group (4): contained the following:

(iv) Infected hamster with adult *Schistosoma mansoni* treated with 400 mg/kg *Zingiber officinale*

Group (5): contained the following:

(v) Infected hamster with adult *Schistosoma mansoni* treated with 400 mg/kg *Piper nigrum*

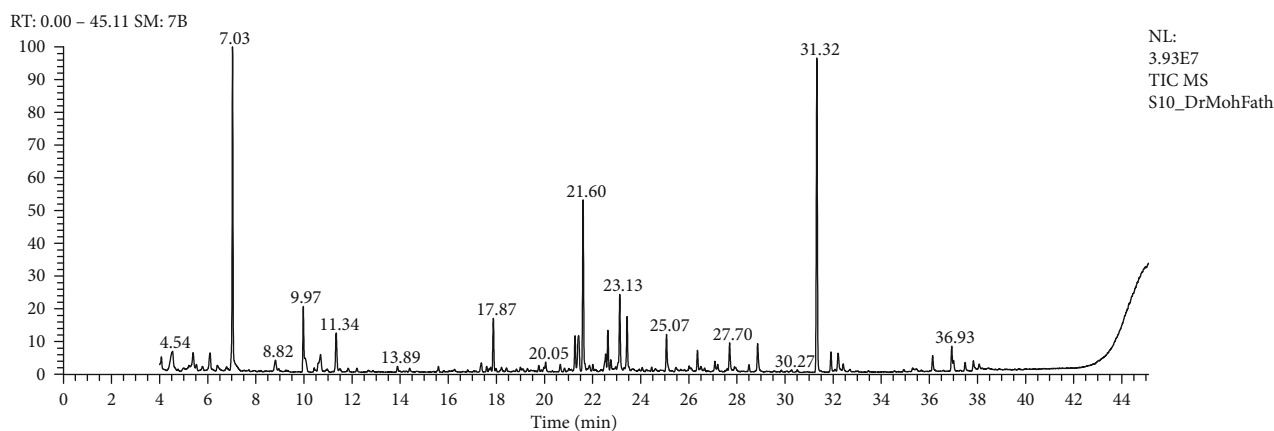
Group (6): contained the following:

(vi) Infected hamster with adult *Schistosoma mansoni* treated 600 mg/kg *Coriandrum sativum*

**2.4.6. Histopathological Assessment.** After perfusion and retrieval of the specimens, the liver, spleen, and kidney were removed from infected treated and nontreated animals as well as the healthy organs from the control animals. All organ samples were preserved in 10% formalin for at least 2 weeks. An illustrative portion was brought and washed overnight to

TABLE 3: Identified compositions in aqueous extract of *Coriandrum sativum*.

No.	RT	M. wt	M. formula	Prediction	Area	Area %
1	7.03	154	C10H18O	Eucalyptol	96269827.62	15.83
2	8.82	156	C11H24	Undecane	5794870.38	0.95
3	8.82	198	C14H30	Tetradecane	5794870.38	0.95
4	10.69	154	C10H18O	Isoborneol	12540682.49	2.06
5	11.34	154	C10H18O	Terpineol	17243978.97	2.84
6	17.37	204	C15H24	Caryophyllene	4468317.88	0.73
7	20.66	302	C20H30O2	Eicosapentaenoic acid	3115712.88	0.51
8	21.27	220	C15H24O	Spathulenol	11419424.94	1.88
9	22.54	220	C15H24O	Caryophyllene oxide	8352660.04	1.37
10	22.76	222	C15H26O	Cubanol	3522130.03	0.58
11	27.08	278	C20H38	Neophytadiene	3709243.58	0.61
12	27.70	220	C15H24O	Calarene epoxide	10741169.28	1.77
13	31.32	290	C20H34O	Kolavelool	115105074.02	18.93
14	32.21	296	C19H36O2	6-Octadecenoic acid	8163860.89	1.34
15	32.42	296	C20H40O	Phytol	2779103.81	0.46
16	36.13	286	C20H30O	Phenanthrenol	6181615.02	1.02
17	36.13	286	C20H30O	Ferruginol	6181615.02	1.02
18	37.49	314	C21H30O2	Dronabinol	3498351.03	0.58
19	37.49	332	C21H32O3	Pregnan-20-one	3498351.03	0.58
20	37.83	340	C19H20N2O4	Benzenamine	4175296.67	0.69

FIGURE 3: GC-MS chromatogram of aqueous plant extract of *Coriandrum sativum*.

get rid of excess formalin. Sequentially, the tissues were dehydrated in increasing of alcohol concentrations 50%, 80%, 90%, and 96% at hourly stepped intervals.

Excess alcohol was removed from the tissues twice in xylene. Infiltration with paraffin wax was accomplished for 3 hrs in the paraffin wax oven set at 2°C below the melting point of wax [64]. In a fresh molten paraffin wax, the tissues were embedded and left to dry. Using a microtome, the tissues were sectioned at 0.5 mm thickness and placed in a hot oven for 15 min. The tissue sections were dewaxed in xylene, rehydrated, and stained with haematoxylin and eosin (H&E) dyes. The stained tissues were slipped with Distyrene Plasticizer Xylene (DPX), dried, and tested microscopically for granulomas [65].

**2.4.7. Identification of Most Potent Plant Extract.** Identification of antischistosomal activity of three plants, *Zingiber officinale*, *Piper nigrum*, and *Coriandrum sativum*, was assessed at the Regional Center for Mycology and Biotechnology (RCMB) at Al-Azhar University Egypt by extraction method, and absorbance of oil solutions in methanol was measured with the UV-240 spectrophotometer (Shimadzu Corporation, Kyoto, Japan). The preparation of the extract was done by maceration method. Maceration was done using the appropriate solvent with several times shaking or stirring at room temperature [66].

**2.4.8. (GC-MS) Gas Chromatography-Mass Spectrometry Analysis.** The samples were performed using Trace

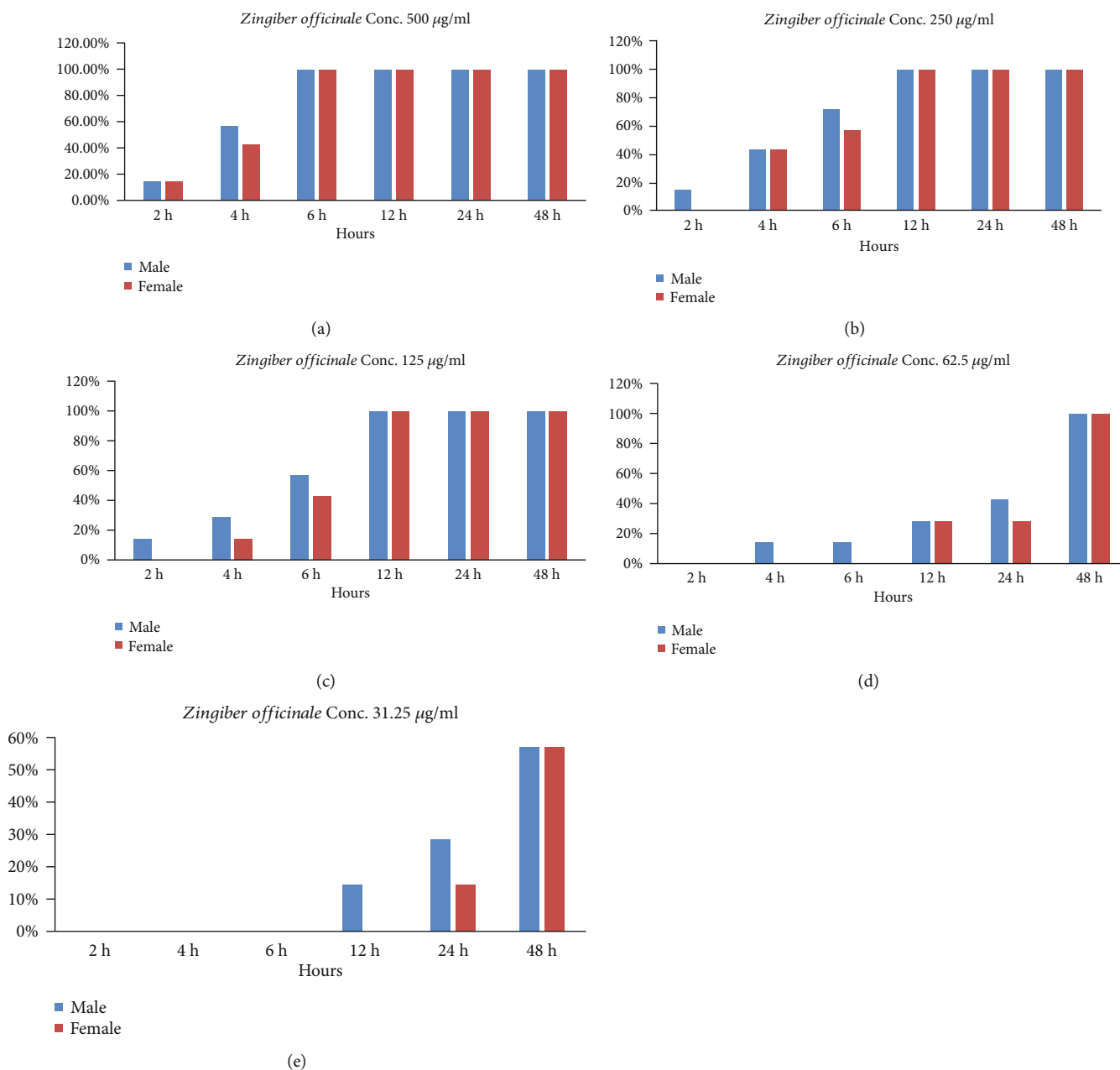


FIGURE 4

GC1310-ISQ mass spectrometer (Thermo Scientific, Austin, TX, USA) with a direct capillary column TG-5MS (30 m × 0.25 mm × 0.25 µm film thickness). The column oven temperature was initially held at 50°C and then increased by 5°C/min to 230°C hold for 2 min. increased to the final temperature 290°C by 30°C/min and hold for 2 min. The injector and MS transfer line temperatures were kept at 250 and 260°C, respectively. Helium was used as a carrier gas at a constant flow rate of 1 ml/min. The solvent delay was 3 min, and diluted samples of 1 µl were injected automatically using Autosampler AS1300 coupled with GC in the split mode. EI mass spectra were collected at 70 eV ionization voltages over the range of m/z 40–1000 in full scan

mode. The ion source temperature was set at 200°C. The components were identified by comparison of their retention times and mass spectra with those of WILEY 09 and NIST 11 mass spectral database.

**2.5. Statistical Analysis.** The collected data were analysed using the Statistical Package for Social Science (SPSS) for windows version 25.0. Normality tests were used to determine whether a given set of data was normally distributed. The Shapiro Wilk test was used to check for normal distribution of data [67]. Moreover, all data were quantitatively presented. The groups were compared using one way ANOVA for comparison of quantitative data of more than two groups

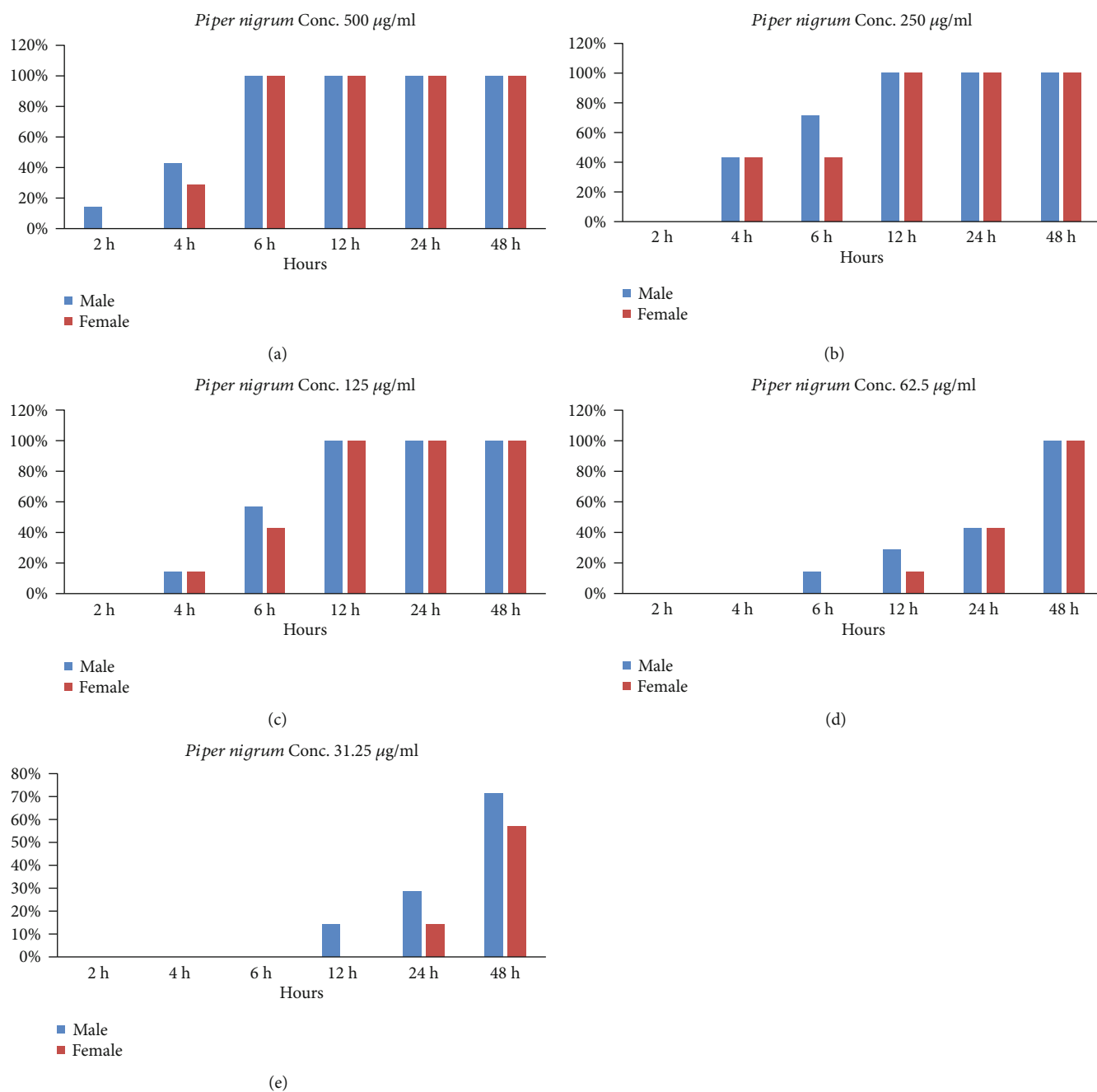


FIGURE 5

for parametric data. The  $p$  value  $<0.05$  was considered statistically significant.

### 3. Results

**3.1. Cytotoxicity.** The cytotoxicity assay of tested plant extracts (*Zingiber officinale*, *Piper nigrum*, and *Coriandrum sativum*) indicated that at maximum nontoxic dose (MNTD) of each extract treated Vero cells did not show any morphological differences in comparison with control, at the value of 250, 350, and 300 µl/ml, respectively.

**3.2. Phytochemistry.** *Zingiber officinale* has a distinctive aroma and flavors that is pungency and a spicy sweet and is caused by volatile oils that make up 1-3% of the weight of fresh ginger. The chemical constituents of the significant oils gained from *Z. officinale* are recorded in Table 1 (Figure 1).

Furthermore, the chemical structures of the essential oils obtained from *Piper nigrum* are registered in Table 2 (Figure 2).

Also, the chemical compounds of the important oils acquired from *Coriandrum sativum* are listed in Table 3 (Figure 3).



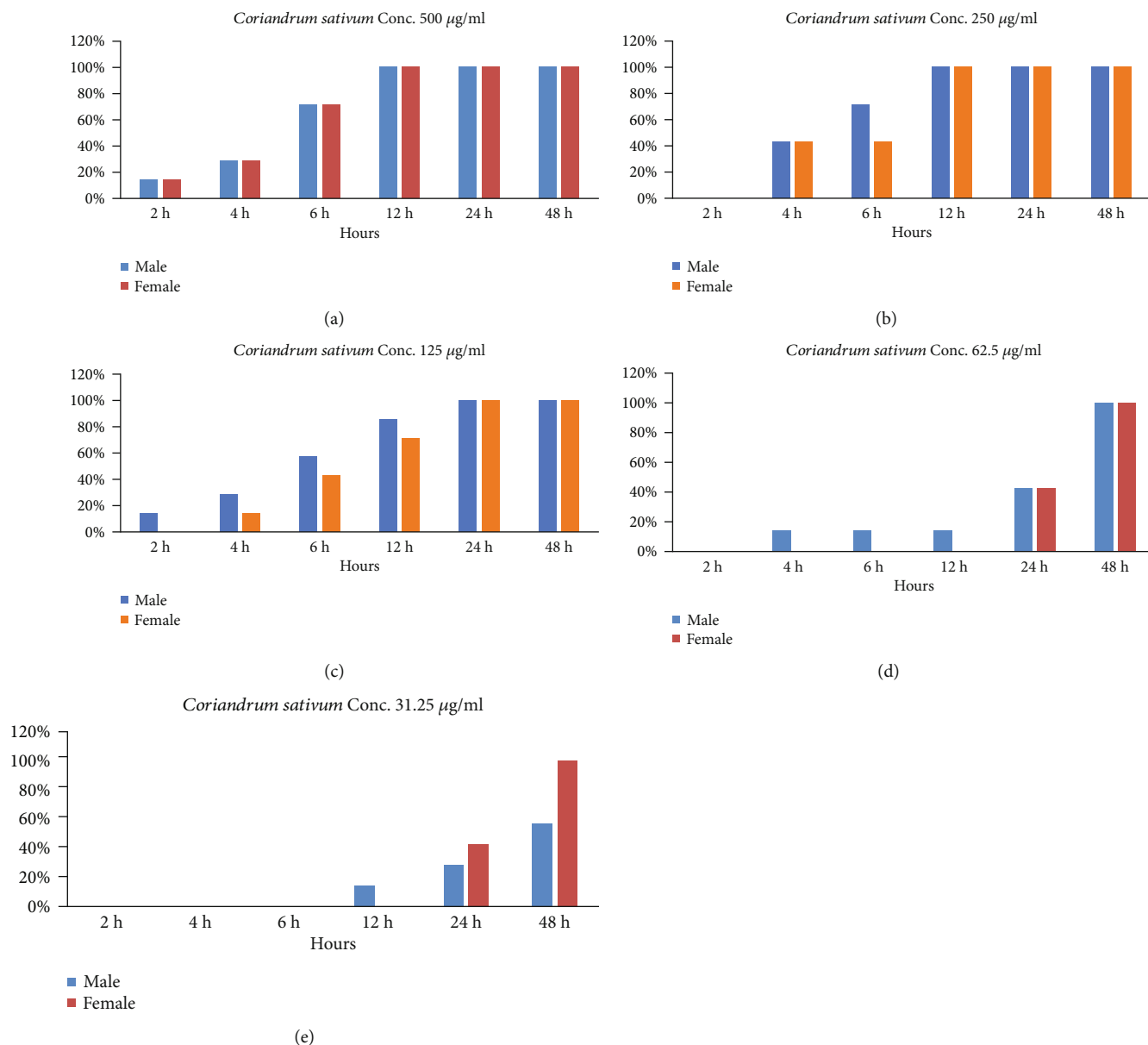


FIGURE 6

**3.3. Antischistosomal Activity.** The effectiveness of *in vitro* treatment of *Zingiber officinale*, *Piper nigrum*, and *Coriandrum sativum* aqueous extracts on adult worms of *S. mansoni* at various concentrations had been studied. The movement, a marked decrease in adult worm motility, was observed at most concentrations. The proportion of adult worms that has their movement decreased was directly related to incubation time and concentration. The motility activity of all adult worms was monitored after 2 hrs of incubation, which were exposed to concentrations of 500, 250, and 125  $\mu\text{g/ml}$  of both *Zingiber officinale* and *Piper nigrum* slightly decreased. The concentrations of 62.5 and 31.25  $\mu\text{g/ml}$  began to reduce the motility of the worms after 12 hrs without obvious loss of movement. Whereas the effect of *Coriandrum sativum* was less than that of *Zingiber officinale* and *Piper nigrum*, it showed a reduction in the appearance of movement after

12 hrs at high concentration. In the negative control groups, it was observed that the movement of the worms remained unchanged for 24 hrs, while the movement decreased in the 48 hrs period, but no complete lack of movement occurred. Moreover, the effect of 10  $\mu\text{g/ml}$  of PZQ (positive control group) led to a decrease in the movement activity of adult worms from the first 2 hrs of incubation, and a complete loss of activity occurred in all worms at 4 hrs period.

Concerning natural mating, the effect of all aqueous extracts on the natural mating process was efficient, and this mainly depends on the dose used and time of exposure. The aqueous extracts cause the mating separation between the adult couple schistosome. About 87% of the parasites were separated in the first 2 hrs using 500 and 250  $\mu\text{g/ml}$ ; 79% of the worms were separated within 4 hrs with 125 and 62.5  $\mu\text{g/ml}$ , and at a lower concentration of 31.25  $\mu\text{g/ml}$

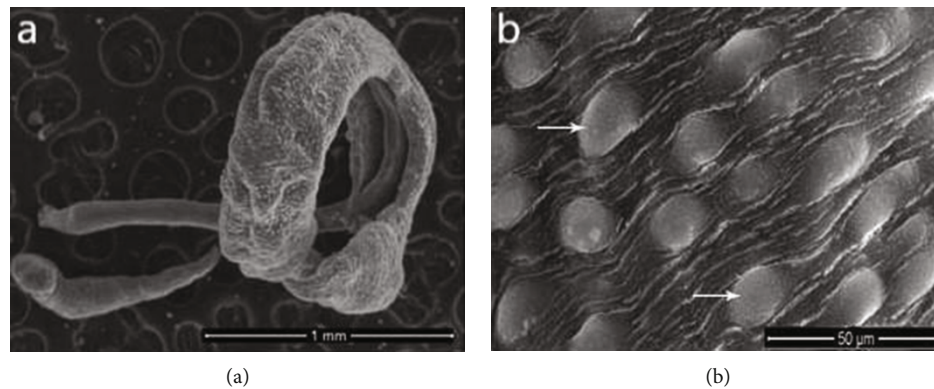


FIGURE 7: Normal tegument of *S. mansoni* from the rat tissue.

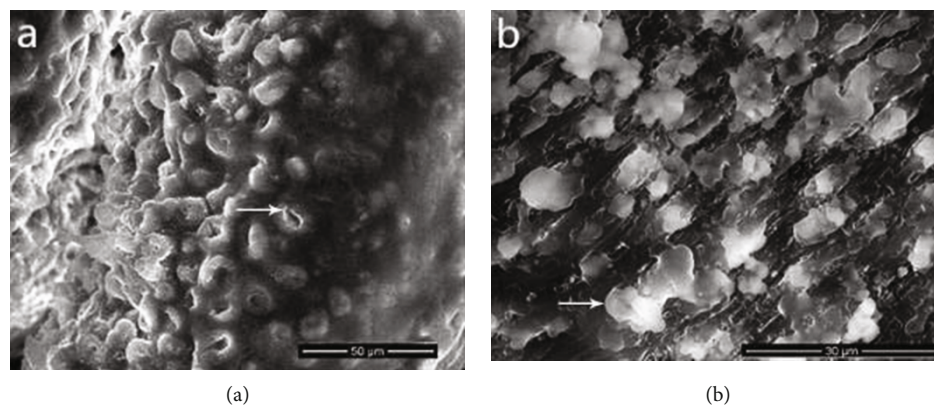


FIGURE 8: Effect of traditional treatment by PZQ drug.

separation was 55% after 12 hrs of *Zingiber officinale* and *Piper nigrum*. However, *Coriandrum sativum* showed less influenced than *Zingiber officinale* and *Piper nigrum* which the separation appeared after 6 hrs (60%) at high concentration. Praziquantel (10  $\mu\text{g}/\text{ml}$ ) as a positive control group caused separation in a couple adult worms after the first 2 hrs of incubation. On the other hand, pair disconnection was observed after about 12 hrs after incubation in negative control groups. Besides, it was confirmed that concentrations that were not 99% deadly to parasites were effective couple inhibitor; in all samples, they were unattached from pairs.

Present result delineated that the existence of *S. mansoni* adult worms exposed to aqueous extracts of *Zingiber officinale*, *Piper nigrum*, and *Coriandrum sativum* depended immediately on both concentration and the incubation time. The concentrations 500, 250, and 125  $\mu\text{g}/\text{ml}$  of *Zingiber officinale* and *Piper nigrum* caused dead of 100% of adult worms within 6 and 12 hrs of incubation, respectively (Figures 4 and 5). Although, aqueous extract of *Coriandrum sativum* at concentrations 500, 250, and 125  $\mu\text{g}/\text{ml}$  resulted dead of 100% parasites after 12 to 24 hrs of incubation (Figure 6). *Zingiber officinale* and *Piper nigrum* (500  $\mu\text{g}/\text{ml}$ ) lead to critical death rate ( $p < 0.001$ ) between schistosome worms after 6 hrs of incubation, whereas 250 and 125  $\mu\text{g}/\text{ml}$  concentration of the *Zingiber officinale* and *Piper nigrum* extracts expressed their death rate impact on adults *S. mansoni* after 12 hrs of incubation, respectively (Figures 4 and 5). The present result

showed a variation was watched among female and male adult parasites in reaction to diverse concentrations of extracts used in either existence rates or mortality influence, where male worms were more harmful.

On the other side, (PZQ) treated group appeared the full passing of worms (100%) after 4 hrs of incubation. Conversely, untreated groups were still living at 48 hrs of incubation; the experiment had ended at this point.

**3.4. Tegumental Changes of *S. mansoni* Adult Worms Exposed to Plant Extracts Visualized by Scanning Electron Microscope (SEM).** When studying the normal tegument of adult *Schistosoma mansoni* worms obtained from untreated golden hamsters by scanning electron microscopy, it was found that the oral sucking in *S. mansoni* was oval shape and covered with different sizes of sharp spines, but the ventral sucker was round and also covered with spines. Ventrally, the gynaecophoric canal was found. There are many large, spiny tubercles on the dorsal surface of worms, while the areas between the tubercles were disappear spines. Also, there are small spines in lines that support the ventral surface of the worm. The tegument around the tubercle was wrinkled (Figure 7).

After treatment with *Zingiber officinale*, *Piper nigrum*, and *Coriandrum sativum*, clear tegumental changes of *S. mansoni* adult worms in the golden hamster appeared. Likewise, ultramorphological variations were observed in both

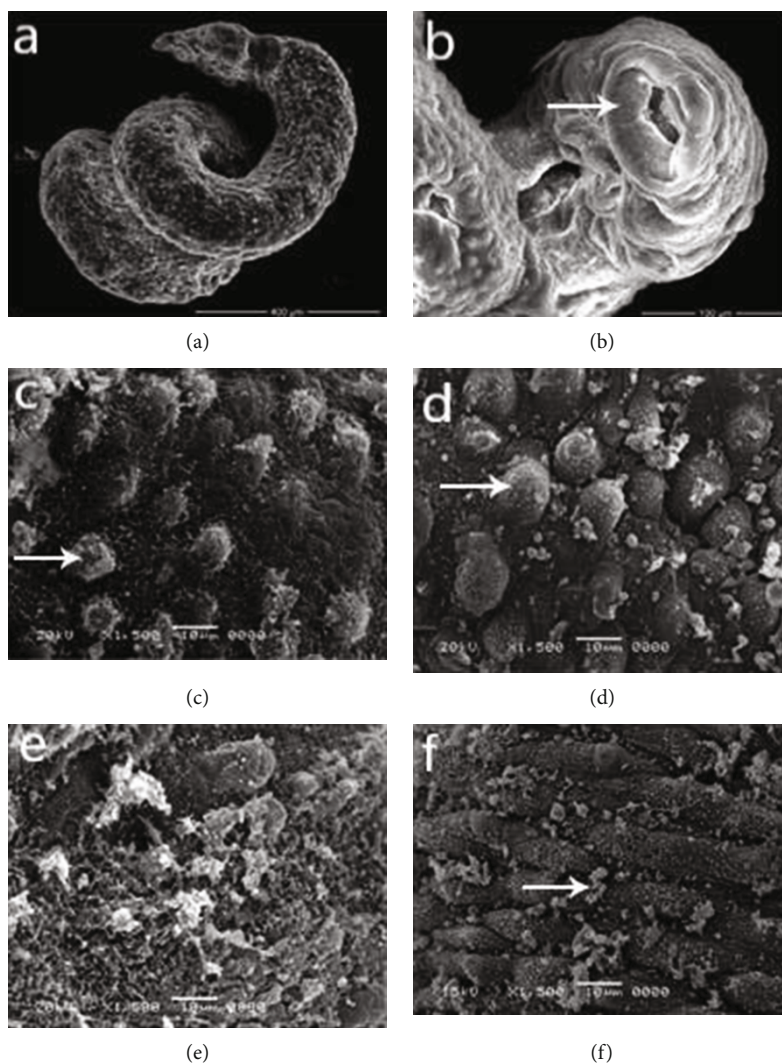


FIGURE 9: Scanning electron microscopy of *S. mansoni* (adult worms) after exposure to different concentration of *Zingiber officinale*: (a) destroyed sucker; (b–d) male dorsal surface showing tegumental peeling with damage and peeling of tubercles and spines.

male and female adult *Schistosoma* after 48 hrs of incubation *in vitro* with concentrations 500, 250, 125, 62.5, and 31.25  $\mu\text{g}/\text{ml}$  from plant extracts. The adult worms exposed to *Zingiber officinale* and *Piper nigrum* appeared morphological changes of tegument and were variable in terms of dose dependent, when compared to untreated group ones. On the other side, the known treatment by PQZ revealed a similar tegumental modification in 100% of schistosome worms (Figure 8).

The morphological changes were clear in the male of *S. mansoni* since it appeared abnormalities in the tubercles and spine harm (devastation, peeling of tubercles, spines, and tegument sloughing or peeling), particularly on its dorsal surface. The appearance of bubbles surrounding the morphologically modified tubercles was notice in addition to sucker change or devastation. The oral sucker of few worms was warped. Whereas in females, the tegument wrinkling, scaling, and corrosion (peeling and contraction of the dorsal area) and sucker modifications or demolition were notice (Figures 9 and 10).

Concerning with *Coriandrum sativum* extract, the worms appeared similar morphological tegumental changes but of lower degree to *Zingiber officinale* and *Piper nigrum* stimulate morphological changes (Figure 11).

**3.5. Histopathological Assessment.** By light microscopy, histological examinations of the kidney, liver, and spleen sectors of the control group (healthy group) have been established. It revealed that the cortical parenchyma of the kidney contains a number of renal corpuscles together with proximal and distal renal tubule ( $\times 200$ ) (Figure 12). In addition, liver hepatocytes extended from the central vein to the periphery of the hepatic lobules at which the portal tracts appear ( $\times 100$ ) (Figure 8). However, the spleen with a normal architecture that was composed of white and red pulps encompassed by a capsule of thick connective tissue. The white pulp was collected of a central, T cell wealthy area, and a periarterial lymphoid sheath encompassed by B cell-rich essential follicles. The white pulp was isolated from the red pulp by the peripheral sinus implanted in a layer of peripheral area lymphocytes ( $\times 100$ ) (Figure 12).



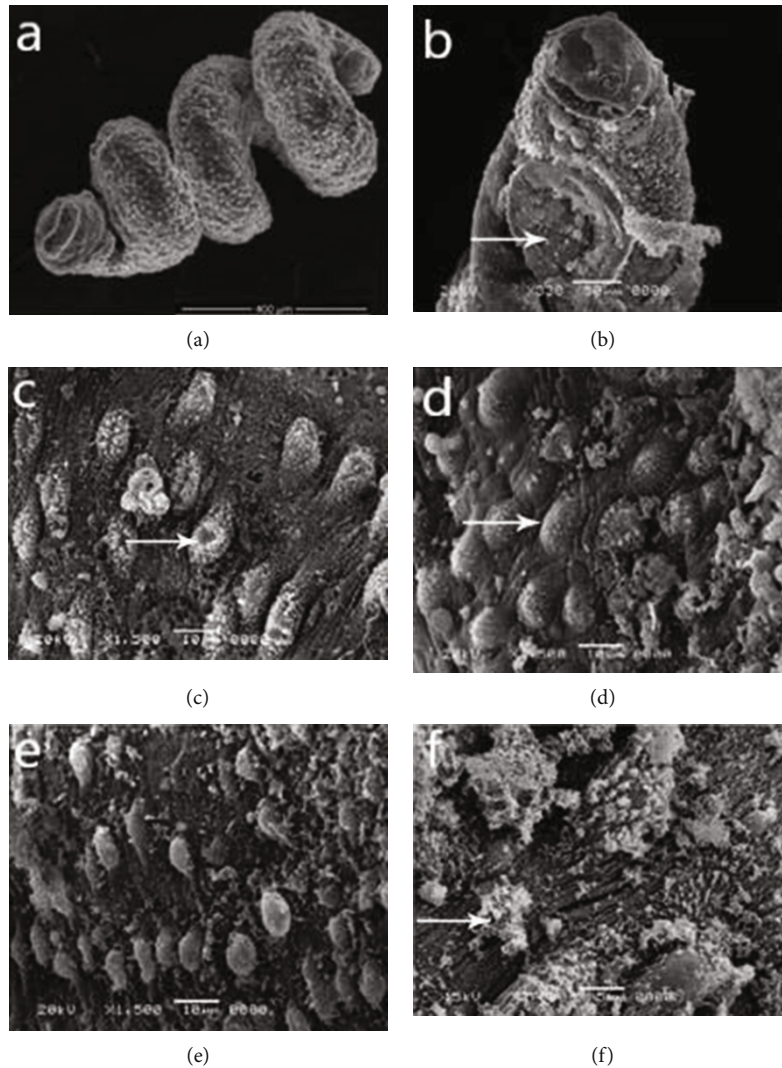


FIGURE 10: Scanning electron microscopy of *S. mansoni* (adult worms) after exposure to different concentration of *Piper nigrum*: (a) destroyed sucker; (b–d) male dorsal surface showing tegumental peeling with damage and peeling of tubercles and spines.

However, the effect of the parasite on the infected untreated control group showed no obvious effect on the kidney; nevertheless, there was an effect on both the liver and the spleen that revealed pathological chronic granulomatous injuries in the hepatic parenchyma. These injuries were created of many bilharzial eggs containing miracidia, encompassed by a lot of chronic inflammatory cells in the shape of epithelioid cells, lymphocytes, plasma cells, macrophages, and eosinophils forming granuloma with extreme zone of fibrosis (Figure 13). Also, the spleen appeared ova encompassed by inflammatory cellular response, and the borders between white and red pulp begun to vanish. A few splenic cells were assessed. Most of the cells were darkly stained, and the sinusoidal spaces were big.

Histological liver sections treated with *Zingiber officinale*, *Piper nigrum*, and *Coriandrum sativum* appeared temperate diffuse infiltration of liver parenchyma by chronic inflammatory cells without watched eggs or zone fibrosis (Figures 14–16). They appeared the nonattendance of bilharzial eggs and fibrosis with importance decrease of liver parenchyma infil-

tration by the chronic inflammatory cells. On the other hand, spleen sections exhibited more or less degeneration of ova, surrounded by lympho-epithelioid cellular inflammatory cellular infiltration.

#### 4. Discussion

Schistosomiasis is a global health problem, and due to it being a neglected disease, the search is still ongoing for a medicine to treat this disease. Praziquantel is the only treatment from WHO, and it is a safe drug when used for the elderly, adults, and children as well as pregnant women, and also low toxicity and with few side effects, the use of the drug has increased and despite the number of patients that has resistance to praziquantel. Therefore, it has become one of the main research goals to develop new compounds that can treat schistosomiasis alongside praziquantel [68, 69].

Because of the diversity in biological activities and molecules provided by natural products, they are considered a good alternative to conventional or chemical therapeutic

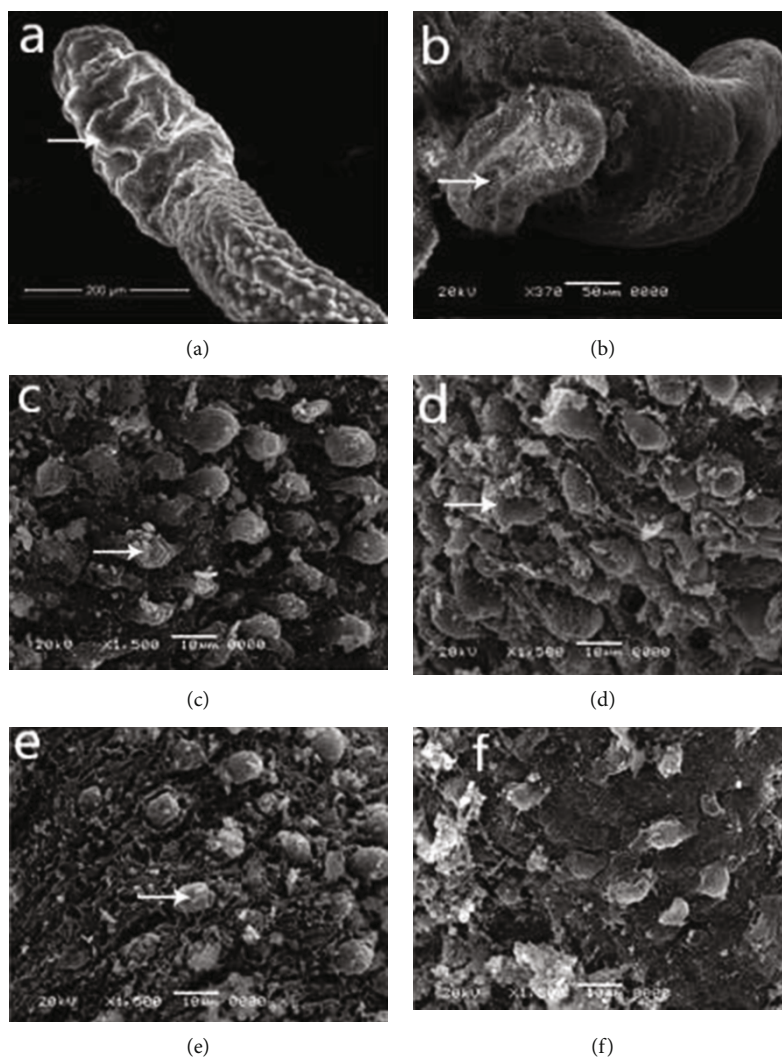


FIGURE 11: Scanning electron microscopy of *S. mansoni* (adult worms) after exposure to different concentration of *Coriandrum sativum*: (a) destroyed sucker; (b–d) male dorsal surface showing tegumental peeling with damage and peeling of tubercles and spines.

compounds. This biological diversity is the result of a large number of active compounds unknown to date. Despite development in medical chemistry, biotechnology, and genomics, discovering new drugs to treat schistosomiasis remains defy [69, 70]. In this context, the effectiveness of the compounds to be tested on schistosomiasis is determined on various strategies such as killing adult worms, preadult worms, and schistosomula and killing life-cycle phases such as cercariae and miracidia, as well as getting rid of the intermediate host and also worm egg-laying inhibitor, were often assessed as biological activity indicator and toxicity in studies interested to schistosomiasis [71].

Aqueous extract of *Zingiber officinale*, *Piper nigrum*, and *Coriandrum sativum* was chosen because water is a safe, non-toxic widespread dissolvent, and avoid the high toxicity of organic dissolvent (such as methanol, acetone, chloroform, and dichloromethane) to living cells [72].

It is well documented that the composition of volatile components of herbs is known to vary with several factors. For example, *Zingiber officinale* has many types of essential

oils that can be classified by main volatile compounds, i.e., ecgonine, retinol, bisabolone oxide, linoleic acid ethyl ester, hexadecanoic acid, ethyl ester octadecanoic acid, ethyl ester, and tris(trimethylsilyl) ester; moreover, *Piper nigrum* has proceroside, 2-nonenal, methanamine, benzofuranone, acetic acid, bisabolol oxide neophytadiene, and diisooctyl phthalate, while *Coriandrum sativum* has eucalyptol, terpineol, eicosapentaenoic acid, kolavelool, phytol, and ferruginol. The essential oil compositions of aromatic plants rely on genetics [73] and agronomical practice and climatic factors [74] like fertilizer [75], light condition [76], species [77], drying method [78], regions [79], and isolation technique [80].

In current study, *in vitro* and in experimentally infected hamster was evaluated the influence of different dose of *Zingiber officinale*, *Piper nigrum*, and *Coriandrum sativum* aqueous extracts against *Schistosoma mansoni* (Egyptian strain). By check out all obtainable literature, no work prior to the use of any *Coriandrum sativum* extract against *S. mansoni* was shown. So, this was the first study to examine the efficacy of *Coriandrum sativum* against *S. mansoni*. Furthermore,



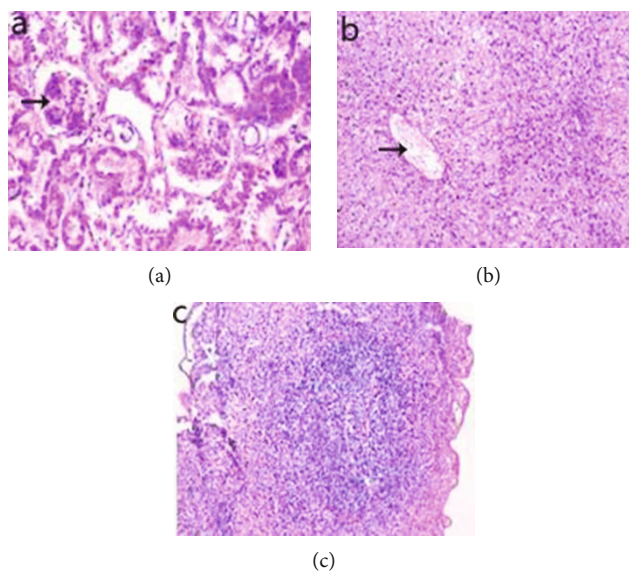


FIGURE 12: Histological section of negative control (healthy control) staining with H&E: (a) control kidney showing normal cortical structure ( $\times 200$ ); (b) control liver showing normal hepatic lobular architecture ( $\times 100$ ); (c) control spleen showing normal architecture ( $\times 100$ ).

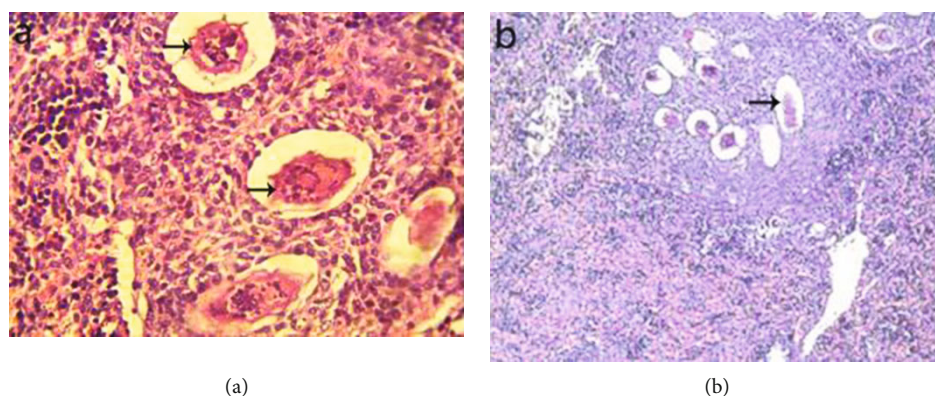


FIGURE 13: Histological section of infected untreated control staining with H&E: (a) spleen revealed aggregate of deposited bilharzia ova, surrounded by lympho-epithelioid tissue reaction ( $\times 200$ ); (b) liver showing multiple egg granulomas and a worm impacted inside a portal vein ( $\times 100$ ).

*in vitro* antischistosomal studies were carried out on adult male and female worms, as an initial step.

In the current study, medicinal plant extracts of *Zingiber officinale*, *Piper nigrum*, and *Coriandrum sativum* at different doses (500, 250, 125, 62.5, and 31.25  $\mu\text{g}/\text{ml}$ ) demonstrated antischistosomal activity in *S. mansoni in vitro* in relation to motility, mating, survival time, and tegumental change in male and female adult worms. Concentrations of 500 and 250  $\mu\text{g}/\text{ml}$  in the plant extracts were most effective in the shortest incubation period, as the observed effects were dose dependent. Three medicinal plant extracts were investigated that caused disconnect between natural mating schistosomes, decreased motility, muscular contractions, mechanical destruction, and paralysis that caused the worms to die most of the time.

Several previous studies have been reported of differences in drug sensitivity between males and females of *S. mansoni*; female worms *S. mansoni* are often less sensitive than male

worms in studies of praziquantel resistance [81] and in studies that estimated bioactivity of ginger extract [39]. In contrast, compounds other than praziquantel showed higher survival rates for males than for females, such as alkanethiosulfuric amino acids [82] and 2-(butylamino)-1-phenyl-1-ethanethiosulfuric acid [83]. Some other studies' data found no differential sensitivity between male and female worms [21]. Mostafa et al. [84] found an effect of a crude aqueous extract of ginger against *S. mansoni in vivo*; also, Hassan et al. [85] evaluated the effectiveness of aqueous ginger in improve of the effects of harm in mice infected with *S. mansoni*. Tonkal and Morsy [86] estimated the myrrh as the most successful product, the stem of *Commiphora molmol*, was extracted to oleo-gum resin, believed to influence of muscles of schistosomes, resulting in separated of male and female couples. On the contrary, Ramzy [87] obtained *Commiphora molmol* had no effect on couple of the worms, the spines, tubercles, and sensory bulbs which showed remain intact

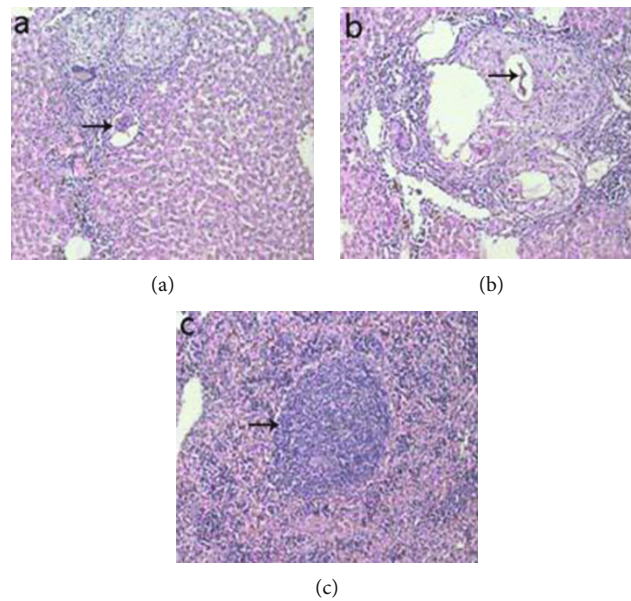


FIGURE 14: Histological section of infected treated with 400 mg/kg *Zingiber officinale* group staining with H&E: (a, b) liver showing multiple epithelioid granulomas around deposited bilharzia ova  $\times 200$ ; (c) spleen revealed congested red pulp and hypertrophic red pulp  $\times 200$ .

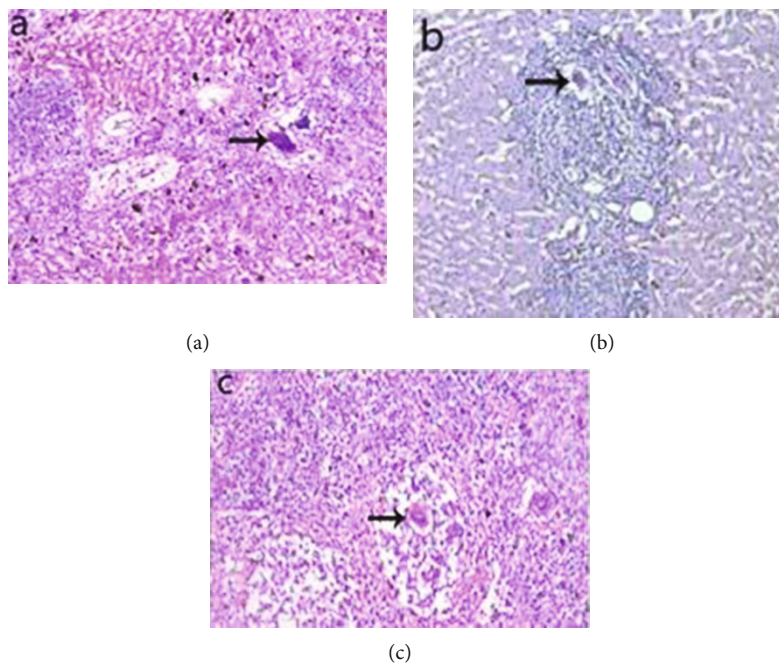


FIGURE 15: Histological section of infected treated with 400 mg/kg *Piper nigrum* group staining with H&E: (a, b) liver revealed deposited bilharzia ova, surrounded by lympho-epithelioid cellular reaction  $\times 200$ ; (c) spleen showing many fresh deposited and degenerated ova  $\times 200$ .

add to the ventral side of the *schistosoma*. Campelo et al. [68] studied the effect of some structure activity similar of pipar-tine on *S. mansoni*; they found the ability of the compounds to separate the worms and reduce their movement, and they attributed that to the ability of the compounds to combine with the enzymes and ion channel on the tegument of the adult worms. Parreira et al. [88] studied the effect of some derivatives from *Piper cubeba* on the activity of *S. mansoni*; their results showed the ability of the lignans to separate pairs

of adult worms, motor activity decreased, and reduce the number of eggs within 24 hrs of incubation. Noel [89] explained in his study that paralysis is related with essential neuromodulators and neurotransmitters like serotonin, acetylcholine, and dopamine.

*Zingiber officinale* and *Piper nigrum* extracts are more effective couple inhibitors than *Coriandrum sativum* extract. While, 500 mg/ml of *Zingiber officinale* extract was as efficient as PZQ *in vitro*. Xiao et al. [90] explained in his study

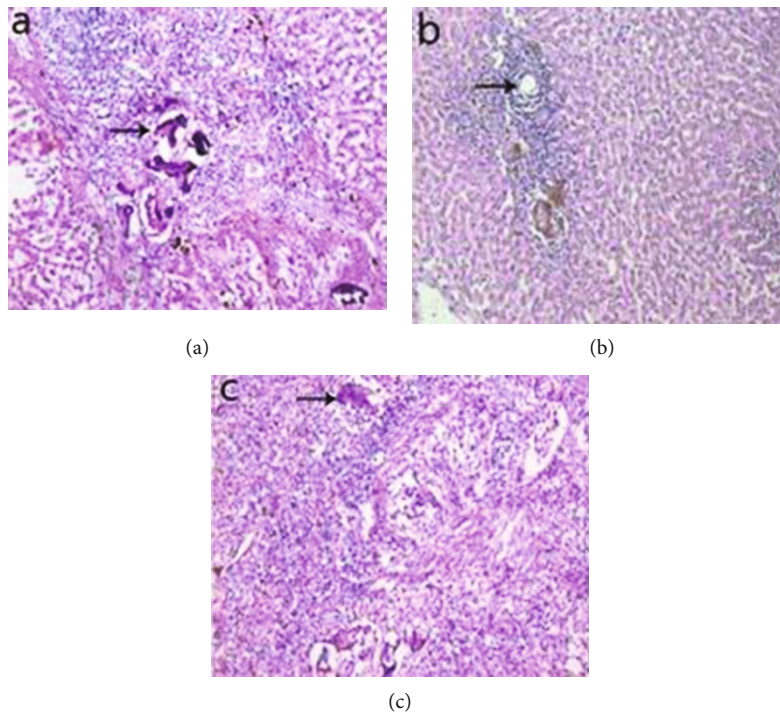


FIGURE 16: Histological section of infected treated with 600 mg/kg *Coriandrum sativum* group staining with H&E: (a, b) liver revealed multiple epithelioid granulomas around deposited bilharzia ova  $\times 200$ ; (c) spleen showing some degenerated ova, surrounded by lympho-epithelioid cellular inflammatory cellular infiltration ( $\times 200$ ).

the effect of praziquantel on *S. mansoni*, when adult worms of schistosomes exposed to 1–3  $\mu\text{g/ml}$  of PZQ *in vitro* undergo nearly quick spastic paralysis. Equal, the parts of the tegument were vacuolization, and blebbing occurs on the surface, particularly in adult male worms. Whereas, in 2004, Pica-Mattoccia and Cioli [81] recorded the same effect of PZQ on schistosome worms but with different concentrations 0.1 and 1  $\mu\text{g/ml}$ . Thereafter, all worms were death. These results were very similar to what was obtained in this study.

In spite of the first effects of the drug contained a speedy influx of calcium into parasite and the muscle contraction and paralysis dependent on calcium [91–93]. The hypothesis to explain how PZQ works is that this drug embedded itself to the membrane resulting lipid phase transition and following destabilizing effect on the membrane. It is well documented that praziquantel reacts with the outer membrane of tegument and cuts it dramatically. The plant extracts used gave the same results as PZQ, so it is expected that the method of its work will be the same of PZQ.

The outer surface or tegument is the area of contact between the environment in the host and *S. mansoni*, so the tegument has many functions and features, which have made the study of great importance, because the outer surface of *Schistosoma* is the main target of antischistosomal drugs [94–102]. The ultrastructural assessment is often focused on male worms because female worms are not consistently associated with the host's microenvironment, because most of the female body is trapped inside the male's gynecological duct. On the other hand, previous studies have shown that soft tissue changes are more pronounced in males than in

females due to the presence of many tubercles on the surface [103–105]. In contrast, the results by de Moraes et al. [15] that when treating *Schistosoma mansoni* with piplartine extracts, there is no difference in effect between male and female worms.

In this study, scanning electron microscopy was used to determine the effects of the tegument of the worms after incubating the parasite with the aqueous extracts of *Zingiber officinale*, *Piper nigrum*, and *Coriandrum sativum*. The results showed the destruction of the oral sucker and the ventral sucker of both the male and female worms, and also by examining the dorsal surface of the male worms, changes in the tubercles appeared collapsed, wrinkled, and shrinking in size and reduced in number, as well as most of the tubercles without spines and some of them carry spines with some bubbles around it.

It is clear that changes to the tegument will lead to the imbalance or disappearance of the immunological camouflage of the worms, and thus, it will be easy to attack by the host's immune system [106]. In addition, the changes caused by the plant extract have a profound effect on metabolism, which leads to the death of the worm [107]. The changes of tegumental structure are proportional to exposure time and concentration of the drug [97]. These results were consistent with many previous research that Mostafa et al. [84] evaluated the activity of crude aqueous extract of ginger plant on *Schistosoma mansoni* on mice strain C57. Also, de Oliveira et al. [108] established the activity of aqueous fraction extract and crude dichloromethane of *Baccharis trimera* against *S. mansoni*. The changes of the morphology in adult worms are caused by a compound in medical plant extracts with



antischistosomal activity through sarcoplasmic membrane, and the parasite tegument may be associated with an increasing in exposure of the surface of the adult worms to antigens. Yones et al. [109] assessed the influence of ornamental and edible pomegranate ethanolic extracts, and Kang et al. [110] studied the effect of hederacolchiside A1 extracted from *Pulsatilla chinensis* on *Schistosoma japonicum* and *Schistosoma mansoni*. Their results showed killing worms at the age of 11 days in mice and also improving pathological parameters in mice carrying *Schistosoma japonicum* age one day.

One of the most dangerous pathological changes that can affect the liver and the spleen, including damage to them, leading to loss of their function as well as liver cancer, is the fibrosis caused by infection with *Schistosoma mansoni* [111]. *S. mansoni* characterized by early symptoms, aggregate, and huge egg lying, the symptoms of liver fibrosis caused by *S. mansoni* are the most dangerous among the symptoms of schistosomiasis prevalent. Thus, determining effective measures to even reverse or prevent liver fibrosis is crucial in controlling *S. mansoni* infection [112].

Lenzi et al. [113] described the granuloma as a muscular, hybrid, and dynamic structure formed by lymphocytes, neutrophils, eosinophils, giant cells, epithelioid cells, macrophages, reticular fibres, fibroblasts, and mast cells surrounding schistosome eggs enclosed in different organs; these granuloma was generally occurring in the liver, the spleen, the lung, and the intestine of hamster infected with *S. mansoni* [114, 115].

The histopathological examination of the liver and spleen in hamsters treated with *Zingiber officinale*, *Piper nigrum*, and *Coriandrum sativum* revealed granulomas less in number and smaller in size compared to the untreated group. Supporting results were obtained by the previous authors but using other therapeutic extracts [84, 109, 112]. Additionally, Mehlhorn et al. [96] added that after treatment with praziquantel, liver lesions regress more rapidly than other antischistosomal drugs. Badawy et al. [116] stated that the decrease in granuloma diameter could be due to the decrease of the third type procollagen responsible for the formation of granulomas. Decrease of granuloma diameter after treatment with extracts may be due to the suppression of Th1 and Th2 lymphocytes and their cytokines that have an important and effective role in the formation and development of the granuloma [117]. Verma and Asnani [118] recorded that the restoration of liver cells (hepatocyte) to their natural appearance after treatment with plant extracts is due to the restoration and improvement of the level of neutral, basic, and acidic proteins in addition to the carbohydrate content. Whereas Sakr [119] said that plant extracts, such as ginger, help to return the normal hepatic strand organization and also restoring normal appearance of hepatocytes by free radicals scavenging and its strong effect as an antioxidant.

Mathew and Boros [120] showed that the decrease in the development of the granuloma resulting from the treatment with extracts was a suitable decrease with the production of soluble egg antigens or/and deactivation or decrease of delayed hypersensitivity T cells (TDH). Also, on the surface of hypersensitivity T cells, part of the antigen molecule is

bound to an antibody called the L3T4 epitope which is affected by monoclonal antibodies that greatly suppressed the production of antigen-induced interleukin 2 (IL-2) and also granulomatous inflammation.

## Data Availability

The (the effect of different medicinal plants on limiting the spread of schistosomiasis as well as an attempt to find an alternative drug to praziquantel (PZQ) from natural materials) data used to support the findings of this study have been deposited in Yones et al. [109] (<http://doi.org/10.1155/2016/2872708>), Augusto et al. (2020) (DOI:10.1186/s13071-020-04367-w), and Alves et al. (2020) (doi:10.1017/S003118202000181X. Epub 2020 Sep 22.).

## Conflicts of Interest

The authors declare that they have no conflicts of interest.

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