

Impact of Plant Extracts on Parasitological and Histological Parameters of Albino Mice Infected with *Schistosoma mansoni*

Fayez A. Bakry¹, Somaya M Ismail^{2,3}

¹Theodor Bilharz Research Institute, Giza, Egypt

²Zoology Department- Faculty of Science, Cairo University, Giza, Egypt

³Faculty of Science and Home Economics, Bisha University, Bisha, Saudi Arabia

Email address:

Fayezbakery@yahoo.com (F. A. Bakry)

To cite this article:

Fayez A. Bakry, Somaya M Ismail. Impact of Plant Extracts on Parasitological and Histological Parameters of Albino Mice Infected with *Schistosoma mansoni*. *Journal of Biomaterials*. Vol. 1, No. 1, 2017, pp. 10-18. doi: 10.11648/j.jb.20170101.12

Received: March 7, 2017; Accepted: March 29, 2017; Published: May 3, 2017

Abstract: This study elucidates the potential of the plant extract of *Phoenix dactylifera* and *Zingiber officinal* plants in treating *Schistosoma mansoni* infected mice. The parasitological parameters, worm burden/mouse, number of ova/g tissue in the liver and intestine and the developmental stages of ova in infected treated mice were determined. In addition, glycolytic enzymes, liver function enzymes and histological changes in liver tissues of infected treated mice were studied. The present results showed a significant reduction in the number of worms in groups treated with plant extracts as well as in the number of mature and live ova in the treated group compared to the infected control group. In addition, the present results showed a significant reduction in Lactate dehydrogenase, Aspartate aminotransferase and alanine aminotransferase enzyme activity in *Schistosoma mansoni* infected group as compared to the normal, healthy control, while a significant increase was noticed in acid phosphatase, alkaline phosphatase level and other glycolytic enzymes as compared to the normal healthy control. Moreover, treatment of the infected mice with plant extracts recorded no significant difference in the level of liver function enzymes and all glycolytic enzymes as compared to the normal healthy control. A noticeable remark to the effect of *Z. officinal* and *P. Dactylifera* pointed out to that there is no side effects on liver function and all glycolytic enzymes and as compared to the normal, healthy control group. In conclusion, plant extracts can be applied clinically as a prophylactic treatment against schistosomiasis together with the ideal antischistosomal drug praziquantel.

Keywords: *Phoenix dactylifera*, *Zingiber officinal*, *Schistosoma mansoni*, Male Mice, Glycolytic Enzymes, Histological Changes

1. Introduction

Schistosomiasis is one of the most prevalent tropical diseases whose burden is mainly concentrated in sub-Saharan Africa and affects approximately 207 million people [1-4]. Schistosomiasis is one of the most prevalent tropical diseases whose burden is mainly concentrated in the sub therapy of established infections relies almost exclusively upon Praziquantel (PZQ), which affects schistosome voltage-gated calcium channels [5], that make treatment with PZQ is the core component of schistosomiasis control programs [6-8]. However, while PZQ effectively kills adult schistosomes and the very young stages shortly after skin penetration, its

efficacy against schistosomula is minimal with only a 25-30% reduction in worm burdens [9]. So there is a pressing need to develop new antischistosomal drugs, given the possibility of resistance emerging against PZQ [10]. Medicinal plants have been used virtually in all cultures as a source of medicine on a natural basis for the maintenance of a good health, e.g., *Zingiberofficinale*, *Nigella sativa* and *Asparagus officinalis* [11-14]. *Phoenix dactylifera* L is a monocotyledonous woody perennial belonging to Family Arecaceae which comprises 200 genera and 3000 species [15]. The beneficial health and nutritional values of date palm (*Phoenix dactylifera* L.) for human and animal consumption have been claimed for centuries [16]. The date pits, also called pips, stones, kernels or seeds, form part of

the integral date fruit. Depending on variety and grade quality, the pits represent about 6–12% of the total weight of the mature date [16]. The seed powder is also used in some traditional medicine and has been investigated for potential human health benefits [17], and in addition to animal feed to enhance growth [18–21], the latter is an action that has been ascribed to an increase in the plasma level of estrogens [22] or testosterone [23]. *Phoenix dactylifera* palm extract (*P. dactylifera* L.) is used in traditional medicine for its pharmacological properties. *Phoenix dactylifera* palms (*P. dactylifera* L. *Arecaceae*) have been cultivated in the Middle East over at least 6000 years ago [24]. The fruit of *P. dactylifera* is regarded as high energy food due to their contains different chemical compounds such as, sugar (44–88%), protein (2.3–5.6%), saturated and unsaturated fatty acids (0.2–0.4%), and trace elements including boron, cobalt, copper, fluorine, magnesium, manganese, selenium, and zinc [25]. Studies indicate that the extracts of *P. dactylifera* have potent antioxidant activity [26].

Ginger (*Z. officinalis* L., Zingiberaceae) is widely used in traditional Chinese medicine [27]. The medicines are purported to be effective treatment for inflammation, oxidant stress, helminthiasis and schistosomiasis [28]. It has also an antischistosomal effect against *S. mansoni* miracidia and cercariae [29]. Phytochemical reports have shown that the main constituents of ginger are zingerone, paradol, gingerols and shogaols. These agents are known to have the ability to suppress the inflammatory and transformative processes of carcinogenesis. Some agents have been found to have antibacterial and antiprotozoal activities [30]. Another study has suggested that ginger free radical scavenging activity may reduce larvae survival [31].

The present work aims to evaluate the effect of plant extracts of *P. dactylifera* L and *Z. officinalis* on infection of mice with *S. mansoni* cercariae and some liver enzymes in *S. mansoni* infected mice representing the glycolytic pathway and liver function enzymes. In addition, parasitological, histopathological parameters associated with changes in hepatic pathogenesis and granuloma diameter were assessed in an attempt to study the effect of treatment with plant extract on the infected mouse model.

2. Materials and Methods

2.1. Experimental Animals

Male CD-1 Swiss albino mice (weight, 20 ± 2 g), bred and maintained at Schistosomiasis Biological Supply Centre (SBSC) Theodor Bilharz Research Institute (Giza, Egypt), were used. They were fed a standard commercial pellet diet and were kept in an air-conditioned room at 21°C. All animal experiments were conducted in accordance with valid guidelines for the animal ethics committee.

2.2. Mice Infection

Schistosoma mansoni cercariae were provided by SBSC,

Theodor Bilharz Research Institute (TBRI). Infection was subcutaneously performed using freshly shed 60 ± 10 *S. mansoni* cercariae per mouse [32].

2.3. Zingiber Officinal (Ginger) Plants

The rhizome of ginger (gingerol) was obtained from the Cultivation and Production of Medicinal and Aromatic Plants Department at the National Research Centre, Egypt. In order to prepare the ethanolic (EthOH) total extract, it was ground into a fine powder using a pestle and mortar, and the powder of ginger (30 g) was refluxed in ethanol (600 ml) in a Soxhlet apparatus for 2 days. EthOH was evaporated under reduced pressure to give a brown extract (yield: 11%). The material was subsequently reconstituted in a known volume of sunflower oil [33].

2.4. Date Palm (Phoenix Dactylifera L.) Pit

Phoenix Dactylifera belongs to the Kingdom Plantae, Division Magnoliophyta, Class Liliopsida, Order Arecales, Family Arecaceae, Genus *Phoenix*, Species *dactylifera* and the binomial name *Phoenix dactylifera* Linnaeus. Date fruits were obtained from Fayoum governorate, Egypt. To prepare the extract of *P. dactylifera* fruit, the flesh was washed by Tap water, then with distilled water, the flesh was manually separated from the pits and cut into small pieces, then dried at room temperature and ground into powder using a blender. The dried powder soaked in cold distilled water (1:3 ratio, weight to volume) the solution was filtered and kept for 48 hours at a temperature of 4°C, the methods were described previously by Al-Qarawi et al., [34]. The water extract was prepared freshly and given to the animals. *P. dactylifera* extract was given orally in a dose of 50ml of extract of date fruit (20 mg/kg b.wt.) for 21 consecutive days three times a day. The dose and the route of inoculation were selected on the basis of the previous studies [35].

2.5. Toxicity of the Tested Plant Extracts In Albino Mice

The acute toxic effect of the plant extract to albino mice (20–25 g) was previously recorded by Kamel et al. [13]. In the present study, four groups, each of 6 mice were used. First group (Control groups) uninfected untreated mice, which received only the vehicle, second group uninfected mice was orally and administered *P. dactylifera* extract in a dose of 50ml of aqueous extract of date fruit (20 mg/kg b.wt.) for 21 consecutive days three times a day. Third group uninfected mice were orally and administered in a dose of 50ml of extract of ginger extract (20 mg/kg b.wt.) for 21 consecutive days three times a day. A fourth group was exposed to 120 *S. mansoni* cercariae/animal subcutaneously in abdominal skin, according to the method of Xue et al. [36] and divided into 3 subgroups, each of 6 mice were used. First subgroup infected mice daily administered with an equivalent amount of drinking the vehicle (distilled water), second subgroup infected mice were orally and administered *P. dactylifera* extract in a dose of 50ml of extract of date fruit (20 mg/kg b.wt.) and third subgroup infected mice were

orally and administered in a dose of 50ml of extract of ginger extract (20 mg/kgb.wt.) for 21 consecutive days three times a day.

Perfusion of uninfected and infected mice, four weeks post treatment; mice were euthanized by decapitation and perfused. The mean number of worms/mouse was determined in each experiment [37].

2.6. Egg Developmental Stages (Oogram)

The percentages of immature, mature and dead eggs from the small intestinal wall of infected mice were computed from a total of hundred eggs per intestinal segment. Immature eggs are characterized by partially developed embryos with clear transparent parts within the eggshell. The mature ones contain fully developed meracidium. Dead eggs exhibited dark, retraction and irregular outline of dead embryos. Three segments per animal were examined. [38].

2.7. Tissue Egg Load

The number of eggs per gram tissue (liver and intestine) of infected mice was determined [39].

2.8. Biochemical Parameters

Each liver was then taken and is divided into 0.25g portions. The liver portion was taken and covered with aluminum foil and stored at -20°C until used for homogenization and biochemical assays. To prepare liver tissue homogenates, one portion weighing 0.25g from each liver aluminium package was taken and homogenizes in 2.5 ml of the specific recorded solution to give 10% concentration and then used for the assay. Similar periods elapsed between homogenization and enzyme was assayed in two livers from each group on the same days. All physiological parameters determined in this study were determined spectrophotometrically, using reagent kits purchased from BioMerieux Company, France. Hexokinase (HK) was assayed according to the method of Uyeda and Raker [40]. Pyruvatekinase (PK) was assayed according to the method of McManus and James, [41]. Lactate dehydrogenase (LDH) activity was measured spectrophotometrically according to the method of Cabaud & Wroblewski [42]. Glucose phosphate isomerase (GPI) was measured using the method of King [43]. Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT) activities were determined according to the method of Reitman and Frankel [44]. Acid phosphatase (ADP) and alkaline phosphatase (ALKP) activities were determined according to Fishman and Ferner (1953) and Kink and Kink (1954) respectively.

2.9. Histopathology and Granuloma Measurement

Livers from previous groups were harvested from the mice, fixed at 10% buffered formalin and processed to paraffin blocks. Sections (4µm thick) were cut every 250 µm to avoid measuring the same granuloma. Five liver sections were prepared from each animal and stained with the haematoxylin and eosin and Masson trichrome stains. Measurements of the granulomas were conducted on non-contiguous granulomas, each containing a single egg (with intact or degenerated miracidia), using an ocular micrometer. The mean diameter of each granuloma was calculated by measuring two diameters of the lesion at right angles to each other. Granuloma structural configurations, including cellular components and associated hepatic histopathological changes, were studied.

2.10. Statistical Analysis

The data are presented as mean ± standard deviation. The main groups were compared by analysis of variance. Comparison of means was done by 2-tailed unpaired t-test. SPSS computer program (version 13.0 windows) was used. The lethal dose (LD₁₀₀) of the tested plants and its 95% confidence limits were calculated [45].

3. Results

3.1. Parasitological Studies

Administering *Z. officinal* and *P. dactylifera* extract of *S. mansoni* infected mice in oral dose 50 ml of aqueous extract are shown in table (1) & (2). The lowest number of worms was obtained from mice infected with *Schistosoma* cercariae administration with *Z. officinal* and *P. dactylifera* with a reduction of 50.6% and 74%, respectively, than that of control. Treatment of infected mice with oral dose 50ml of an extract of *Z. officinal* showed that (Table 2) the number of ova/ g tissue in the liver and intestine of infected treated mice was reduced by 978.1 ± 121.4 and 1121.2 ± 122 , respectively ($p < 0.01$). For *P. dactylifera* extract, the number of ova/g tissue in the liver and intestine showed a considerable reduction of 77.5 and 77% respectively ($p < 0.001$). The percentage of the immature stages of *Schistosoma* ova were higher in the experimental group with *P. Dactylifera* L. (45.9 ± 1.2) than that of the control (16 ± 1.4). Meanwhile, the percentage of mature ova was significantly lower in the experimental group with *P. Dactylifera* L. (12.4 ± 4.8) than that of the control (78.6 ± 1.8). The percentage of dead ova was significantly higher in the experimental group with *P. Dactylifera* L. (41.2 ± 1.3) than that of the control (4.3 ± 1.2).

Table 1. Number of worms in mouse infected with *Schistosoma mansoni* cercariae and exposed to *Zingiber officinal* and *Phoenix Dactylifera* L. extract.

	Mean number of worms/mice ± SD			Total mean number of worms /mice ± SD	Percent worm of reduction %
	Male	Female	Pairs		
Control infected	2.5 ± 0.9	1.7 ± 1.3	3.3 ± 0.9	8.5 ± 0.4	
<i>Zingiber officinal</i>	0.4 ± 0.6**	0***	3 ± 0.8*	4.2 ± 1.5**	50.6%
<i>Phoenix Dactylifera</i> L.	0.2 ± 0.5***	0***	2.1 ± 0.5**	2.2 ± 0.1***	74%

*P<0.05, **P< 0.01 & ***P< 0.001

Table 2. Development stages of ova (oogram) of infected mouse with *Schistosoma mansoni* cercariae and exposed to *Zingiber officinal* and *Phoenix Dactylifera*L.extract.

	% of different developed stages of ova			Number of ova / g tissue (% reduction)	
	Mature	Immature	Dead	Intestine (%reduction)	Liver (%reduction)
Control infected	78.6 ± 1.8	16 ± 1.4	4.3 ± 1.2	2211.92 ± 213.92	1844.3 ± 233.4
<i>Zingiber officinal</i>	25.5 ± 1.3	41.2 ± 1.7	32 ± 2.4	1121.2 ± 122.1** (49.3)	978.1 ± 121.4** (47)
<i>PhoenixDactylifera</i> L.	12.4 ± 4.8	45.9 ± 1.2	41.2 ± 1.3	499.2 ± 47.1*** (77.5)	388.2 ± 83.2*** (77)

*P<0.05, **P<0.01 & ***P<0.001

3.2. Biochemical Studies

The present results in the table (3) showed a significant reduction in Lactate dehydrogenase (LDH) enzyme activity in *S. mansoni* infected group as compared to the normal, healthy control, while a significant increase was noticed in other glycolytic enzymes Hexokinase (HK), Pyruvatekinase (PK) and Glucose phosphate isomerase (GPI) as compared to the normal healthy control. Moreover, treatment of the infected mice with *Z. officinal* and *P. Dactylifera* recorded no significant difference in all glycolytic enzymes as compared to the normal healthy control. A noticeable remark to the effect of *Z. officinal* and *P. Dactylifera* pointed out to that there is no side effects on all glycolytic enzymes (LDH, HK,

PK & GPI) as compared to the normal, healthy control group. Table (4) shows significant reduction in Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) enzymes in infected group. While significant increase was observed in Acid phosphatase and alkaline phosphatase (ALKP) level as compared to the normal healthy control group. Moreover, treatment of the infected mice with *Z.officinal* and *P. Dactylifera*L recorded no significant difference in the level of liver function enzymes as compared to *S. mansoni* infected group. Treatment of the normal, healthy mice with *Z. officinal* and *P. Dactylifera*L showed no side effects on the level of enzymes.

Table 3. Effect of extract of *Zingiber officinal* and *Phoenix Dactylifera*L.on some glycolytic enzymes in mouse liver.

Groups	Enzyme activity $\mu\text{mol}/\text{min}/\text{mg}$ protein			
	LDH	HK	GPI	PK
Control	36.3 ± 1.5	0.08 ± 0.12	84.4 ± 2.5	8.2 ± 1.4
Infected mice control	22.6 ± 1.2***	0.12 ± 0.2***	124.5 ± 2.2***	11.3 ± 1.2**
Uninfected mice treated with <i>Zingiber officinal</i>	35.2 ± 1.7	0.083 ± 0.52	85.2 ± 1.3	8.9 ± 0.56
Infected mice treated with <i>Zingiber officinal</i>	31.3 ± 1.5*	0.09 ± 0.5*	109.5 ± 1.8*	10.1 ± 1.6*
Uninfected mice treated with <i>Phoenix Dactylifera</i> L.	36.2 ± 1.8	0.076 ± 0.23	84.7 ± 1.6	8.5 ± 0.43
Infected mice treated with <i>Phoenix Dactylifera</i> L.	33.5 ± 1.2*	0.092 ± 0.5*	98.4 ± 1.2*	9.4 ± 1.2*

*P< 0.05, & ***P< 0.001

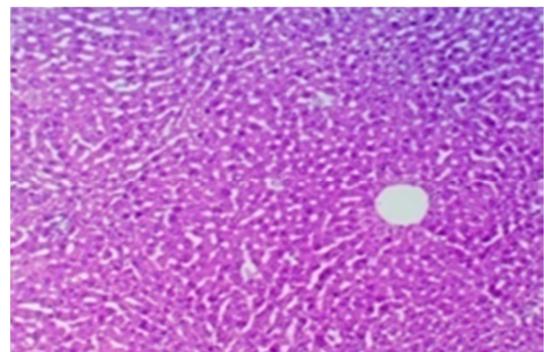
Table 4. Effect of *Zingiber officinal* and *Phoenix Dactylifera* L. extract on liver function enzymes in mouse liver.

Groups	Enzyme activity $\mu\text{mol}/\text{min}/\text{mg}$ protein			
	Aspartate amino transferase (AST)	Alanine amino transferase (ALT)	Acid phosphatase (ADP)	Alkaline phosphatase (ALKP)
Normal control	35.52 ± 2.2	24.2 ± 2.26	7.1 ± 1.15	8.2 ± 0.22
Infected mice control	21.3 ± 1.2***	12.6 ± 0.8***	10.5 ± 0.4***	10.2 ± 0.23**9
Uninfected mice treated with <i>Zingiber officinal</i>	33.1 ± 0.24*	24.5 ± 0.4	7.2 ± 0.5	8.5 ± 0.66
Infected mice treated with <i>Zingiber officinal</i>	30.32 ± 1.4*	20.5 ± 1.4*	9.2 ± 0.17*	9.1 ± 0.44*
Uninfected mice treated with <i>Phoenix Dactylifera</i> L	33.4 ± 1.5	24.2 ± 1.2	7.4 ± 0.4	8.5 ± 0.32
Infected mice treated with <i>Zingiber officinal</i>	32.12 ± 1.2	23.2 ± 1.2	8.4 ± 0.13(8.6 ± 0.23

*P< 0.05, **P< 0.01 & ***P< 0.001

3.3. Histopathological Studies

Liver sections of untreated uninfected mice showed normal hepatic lobular architecture with hepatocytes arranged in thin plates. The portal tracts were within normal limits and contained arteries, veins and bile ducts. The hepatocytes contained rounded, regular nuclei with lymphocytes scattered between hepatocytes and the sinusoids. Moreover, no hydropic, steatotic, feathery or ballooning changes, degeneration or apoptosis were observed (Figure 1).

**Figure 1.** Liver section from uninfected untreated mouse showing normal hepatic architecture and normal hepatocytes (100X).

Liver sections from *S. mansoni* infected mice sacrificed four weeks after infection showed a typical large fibrocellular and cellular granuloma centered on living ova, including living miracidium and surrounded by lymphocytes, eosinophils, polymorphonuclear cells (Figure 2). Liver sections of the infected group treated with *Z. officinal* showed a reduction in granuloma size and marked fragmentation of the ovum inside the granuloma were represented (Figure 3).

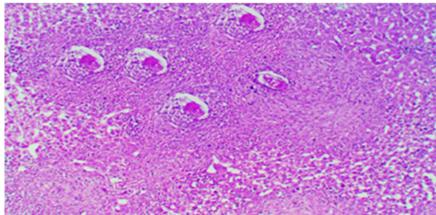


Figure 2. Liver sections of infected untreated mice (four weeks post-infection) showing irregular outlined large fibrocellular granuloma consisting of collagenous fibrous tissue surrounding two living intact ova and peripheral zone of chronic inflammatory cells (100X).

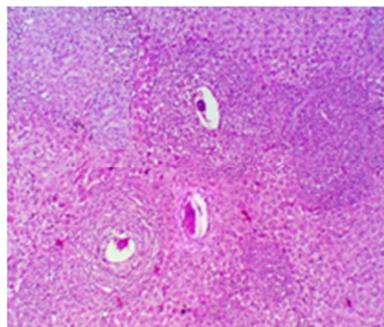


Figure 3. Liver sections from the infected group treated with *Zingiber officinal* showed a reduction in granuloma size and marked fragmentation of the ovum inside the granuloma were represented (100X).

Liver sections of the infected group treated with *P. Dactylifera* L showing small sized fibrocellular granuloma with degenerated ova and less inflammatory cells (Figure 4). Compared to the infected treated group, treatment with *Zingiber officinal* and *Phoenix Dactylifera* L after 4 weeks of infection reduced the diameters of the granulomas by 16% and 33.3%, respectively.

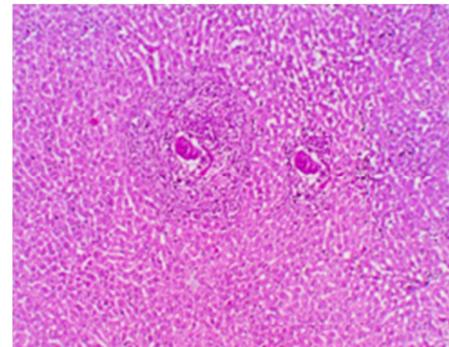


Figure 4. Liver sections from the infected group treated with *Phoenix Dactylifera* L showing small sized fibrocellular granuloma with degenerated ova and less inflammatory cells.

Granuloma diameter and associated histopathological changes in mice infected with *S. mansoni* treated with *Z. officinal* and *P. Dactylifera* extract after 4 weeks. Moreover, there was a significant reduction in the granuloma diameters in groups treated with *Zingiber officinal* by 16%. ($P < 0.001$) (4C), in comparison with an untreated infected group. Treated groups with *Z. officinal* showed size-variable, circumscribed fibrocellular granulomas with central ova encircling living or dead miracidia, surrounded by lymphocytes, epithelioid cells, neutrophils, eosinophils and collagen bundles (Table 5).

Table 5. Number and diameter of Granuloma and associated histopathological changes in mice infected with *S. mansoni* treated with *Zingiber officinal* and *Phoenix Dactylifera* L after 4 weeks.

	No of granuloma in successive power fields (10x10) mean± SD	%reduction no of granuloma	Granuloma diameter in µm (mean ± SD)	% reduction of granuloma diameter
Infected mice control	7.4± 1.2		231.1±31.2	
Infected mice treated with <i>Zingiber officinal</i>	4.1±1.4	44.6%	194.3±22.4	16%
Infected mice treated with <i>Phoenix Dactylifera</i>	2.1±1.7	71.6%	154.2± 15.1***	33.3%

*** $P < 0.001$, ** $P < 0.01$ and * $P < 0.05$

Treated groups post *P. Dactylifera* L extract administration, no degenerated and dead worm sections were seen, and most worm granulomas developed to the late stage, which was characteristic of the emergence of the large amount of fibrocellular around the granuloma. Also, all treatment regimens significantly increased the percentage of

dead ova in the examined liver sections compared to the infected untreated group. Furthermore, the percentage of ova degeneration was increased in the liver sections from groups treated with *Z. officinal* (65%) and in the group treated with *P. Dactylifera* L 75%. (Table 6).

Table 6. Types and state of Granuloma and associated histopathological changes in mice infected with *S. mansoni* treated with *Zingiber officinal* and *Phoenix Dactylifera* L after 4 weeks.

	Types of granuloma cellular %	Types of granuloma fibro cellular %	State of eggs intact %	State of eggs degenerated %
Infected mice control	45	55	85	15
Infected mice treated with <i>Zingiber officinal</i>	15	95	35	65
Infected mice treated with <i>Phoenix Dactylifera</i>	10	90	25	75

*** $P < 0.001$, ** $P < 0.01$ and * $P < 0.05$

4. Discussion

In the present study, treatment of *S. mansoni* infected mice with dose 50ml of the extract of *Z. officinal* and *P. dactylifera* extract highly reduced the rates of worm burden/mouse by 50.6% & 74%, respectively. This agrees with the observations of Ritchie et al. [36] that showed infectivity of *S. mansoni* cercariae was inhibited after treatment with 100 ppm of bis tri-nbutyltin oxide for 5 min. The same finding was observed by Viyanant et al. [37] who used sublethal concentrations of copper sulphate and tributyltin fluoride and Gawish, [48] Who used sublethal concentrations of Niclosamide. Also, Kamel et al. [13] recorded 54.4%, 53.7% and 76.9% reduction in worm load/mouse post treatment with these plants. In the same year, Mostafa et al. [49] reported that worm burden and egg density in liver and faces of *S. mansoni* on the antischistosomal activity of *Calotropis procera*, *Ficus elastic* and *Z. officinale* in mice infected with *S. mansoni* were found by Seif- el-Din et al. [50].

The same observation was recorded in the number of ova in the intestine and liver of infected mice treated with with oral dose 50ml of an extract of *Zingiber officinal* and *P. dactylifera* extract. The reduction in the number of ova explained by WHO [51] that, the few specimens of the cercariae that no survived post treatments with different molluscicides, could infect the exposed mice and developed to adult worms laid low number of ova. This may be due to disturbance in their physiological activities as Bayluscide affects the respiratory enzymes, which are essential factors in physiological processes of the cercariae and adult worms [52-44].

In the present study, significant increases in glycolytic enzymes PK, GPI and HK were observed in infected groups, while LDH enzyme activity showed significant reduction. The enhancement in the activities of glycolytic enzymes in infected mice could be attributed to increase metabolic actives of infected liver tissue to compensate the inhibition of host Krebs's cycle caused by the parasitic infection [55, 56]. The reduction in the activity of LDH enzyme as an important glycolytic enzyme may be attributed to the change that occurred in the permeability of the plasma membrane as a result of egg and worm toxins in necrotic which lead to changes in the integrity of the cell membranes and discharge of the enzyme [57]. LDH inhibition revealed the aerobic – anaerobic switch induced by the developing parasite and Lower activity in LDH in the direction of lactate oxidation direction could be easily correlated to the crabtree effect of schistosomes [57]. Through which lactate is accumulated and glycogen depleted confirming inhibition of aerobic respiration and stimulation of anaerobic glycolysis through Hexokinase, a rate limiting enzymes of glycolysis [58]. Concerning AST and ALT enzyme activities, significant reduction was observed in both infected mice groups. The decrease observed in AST and ALT attributed to the hepatocellular damage resulted from egg deposition where the transaminases level showed an intimate relationship to cell necrosis and/or increased cell membrane permeability

led to the discharge of the enzyme to the blood stream [59, 60]. The decrease in transaminases level, providing additional support for the side effect of the *S. mansoni* infection on the mitochondria of the hepatic cells as it is the supcellular localization of transaminases [61]. In the present study, Acid phosphatase and alkaline phosphatase (ALKP) show significant elevation in both infected and treated infected mice groups. Higher levels of Acid phosphatase and alkaline phosphatase (ALKP) in tissue were observed by El-Aasar et al. [59] which was attributed to the irritation of liver cells from toxins or metabolic products of growing schistosomoules of adult worms and eggs or due to increase loss of intracellular enzyme by diffusion through cell membrane which appear to act as a stimulus to the synthesis of more enzyme. *There are no side effects on all glycolytic and liver bunction enzymes of inbected and uninbected mice treated with Zingiber officinal and P. dactylifera extract as compared to the normal, healthy control group. The results of the present study indicated that the exogenously administered Zingiber officinal and P. dactylifera extract may prevent toxicity in mice. The protective effect of Zingiber officinal and P. dactylifera extract is probably due to a counter action of free radicals by its antioxidant nature. Zingiber officinal and P. dactylifera extract may be recommended as an adjuvant therapy with certain anticancer [62, 63].*

The manifestations of schistosomiasis are mainly attributed to granulomatous inflammation around the parasite eggs [64]. The formation of granulomas depends predominantly on CD4+ T cell specific for egg antigen and represents a delayed-type hypersensitivity [61]. At the same time, hepatic stellate cells (HSCs) comprise 10-15% of all hepatic cells and they are recruited to areas of hepatic injury and become activated [65]. They adopted a myofibroblast-like phenotype, secreting extracellular matrix components [66].

In this study, although all treated groups revealed significant diminution of granuloma diameter, at the same time, the groups treated with *Zingiber officinal* and *Phoenix Dactylifera* revealed lower pattern than the control treated with and this may be due to the effect of previous immunization of the infected animals before treatment. The present data showed that size-variable, circumscribed fibrocellular granulomas with central ova encircling living or dead miracidia, surrounded by lymphocytes, epithelioid cells, neutrophils, eosinophils and collagen bundles with an untreated infected group. Treated groups with *Z. officinal*. While treating groups post *P. Dactylifera* extract administration, no degenerated and dead worm sections were seen, and most worm granulomas developed to the late stage, which was characterized by the emergence of the large amount of fibrocellular around the granuloma. Also, all treatment regimens significantly increased the percentage of dead ova in the examined liver sections compared to the infected untreated group. This agrees with Rollino et al.,

[67] who claimed that egg production of schistosome

starts 4-6 weeks after infection and continues for the life of the worm. Eggs pass from the lumen of blood vessels into the adjacent tissues. Spicher et al., [68] mentioned that eggs of schistosome can either succeed in reaching the lumen of the organ (intestine) and leave the body with urine or feces or remain trapped in the body tissues, where they die and induce granuloma formation or even be transported in the blood flow to the other organs (including peritoneum) where they determine granulomas.

These observations agree with Rollino et al. [67] who observed that in chronic schistosomiasis, tissue injury is mediated by egg-induced granulomas and there is a subsequent appearance of fibrosis. Also, Michael and Anthony, [69] mentioned that in granulomatous reactions, the eggs induction leads to fibrosis. This in turn may lead to portal hypertension or urinogenital dysfunction, depending on the parasite species. The disease symptoms are therefore attributable to immunopathology.

5. Conclusion

Treatment of *S. mansoni* infected mice with plant extract (*Zingiber officinal* and *Phoenix Dactylifera*L) significantly reduced both worm burden and egg production and ameliorate liver histology and function to seminormal levels. The concomitant use of *Zingiber officinal* and *Phoenix Dactylifera*L enhanced therapeutic efficacy. This was evidenced by the disappearance of mature worms and eggs, especially in the group treated with *Zingiber officinal* and *Phoenix Dactylifera*L with remarkable healing of hepatic granulomatous lesions and approximately normalization of liver enzyme levels. In addition, the present results showed a significant reduction in Lactate dehydrogenase, Aspartate aminotransferase and alanine aminotransferase enzyme activity in *Schistosoma mansoni* infected group as compared to the normal, healthy control, while a significant increase was noticed in acid phosphatase, alkaline phosphatase level and other glycolytic enzymes as compared to the normal healthy control. Moreover, treatment of the infected mice with plant extracts recorded no significant difference in the level of liver function enzymes and all glycolytic enzymes as compared to the normal healthy control. A noticeable remark to the effect of *Z. officinal* and *P. Dactylifera* pointed out to that there is no side effects on liver function and all glycolytic enzymes and as compared to the normal, healthy control group. Treatment with plant extract with immunization resulted in significant reduction of parasitological parameters and rise of specific immunoglobulins. Plant extract can be applied clinically as a prophylactic treatment against schistosomiasis together with the ideal anti-schistosomal drug praziquantel.

References

- [1] King CH, Dickman K, Tisch DJ (2005) Reassessment of the cost of chronic helminthic infection: a meta-analysis of disability-related outcomes in endemic schistosomiasis. *Lancet* 365: 1561-1569.
- [2] Gryseels B, Polman K, Clerinx J, Kestens L (2006) Human schistosomiasis. *Lancet* 368: 1106-18.
- [3] Steinmann P, Keiser J, Bos R, Tanner M, Utzinger J. (2006) Schistosomiasis and water resources development: Systematic review, meta-analysis and estimates of people at risk. *Lancet Infect Dis* 6:411-25.
- [4] Sayed AA, Simeonov A, Thomas CJ, Inglese J, Austin CP, Williams DL (2008) Identification of oxadiazoles as new drug leads for the control of schistosomiasis. *Nat Med*, 4: 407-412.
- [5] Greenberg RM (2005). Are Ca²⁺ channels target of praziquantel action? *Int J Parasitol*, 35: 1-9.
- [6] Utzinger J, Keiser J. (2004) Schistosomiasis and soil-transmitted helminthiasis: common drugs for treatment and control. *Expert Opin Pharmacother* 5, 263-285.
- [7] World Health Organization (2006) Preventive chemotherapy in human helminthiasis. World Health Organization, Geneva, Switzerland.
- [8] World Health Organization (2011) Schistosomiasis: number of people treated in 2009. *Wkly. Epidemiol. Rec.* 86: 73-80.
- [9] Xiao SH, Yue WJ, Yang YO, You JQ (1987): Susceptibility of *Schistosoma japonicum* to different developmental stages to praziquantel. *Chin. Med J* 100: 759-768.
- [10] Caffrey CR. (2007) Chemotherapy of schistosomiasis: present and future. *Curr Opin Chem Biol* 11: 433-99. influences of IL-1, IL-18 and HMGB1, *Cytokine* 69(1): 136-145.
- [11] Hoareau L, Dasilva EJ (1996), Medicinal plant: a re-emerging health aid. *Electron J Biotechnol* 2: 60-5.
- [12] Tanwer BS, Vijayvergia R (2010): Phytochemical evaluation and quantification of primary metabolites of *Alangium salviifolium*. *Int J Pharm Biosci* 1: 1-6.
- [13] Kamel EG, EL-Emam MA, Mahmoud SS, Fouda FM, Bayoumy FE (2011): Parasitological and biochemical parameters in *Schistosoma mansoni* infected mice treated with methanol extract from the plant *Chenopodium ambrosioides*, *Conyza dioscorides* and *Sesbania sesban*. *Parasitol. Int J* 60: 388-392.
- [14] Aly HF, Mantawy MM (2013): Efficiency of ginger (*Zingiber officinale*) against *Schistosoma mansoni* infection during host-parasite association. *Parasitol. Int J* 62: 380-389.
- [15] McClintock E. (2007) *Arecaceae* palm family. The Jepson Manual: http://ucjeps.berkeley.edu/cgi-bin/get_JM_treatment.pl?Phoenix+dactylifera (Accessed on October 18, 2007).
- [16] Barreveld WH. (2007) Date Palm Products. *FAO Agricultural Services Bulletin* No. 101. (<http://www.fao.org/docrep/t0681e/t0681e00.htm#con>). (Accessed on October 18, 2007).
- [17] Hamada JS, Hashim IB, Sharif FA (2002) Preliminary analysis and potential uses of date pits in foods. *Food Chem* 76: 135-7.
- [18] Afifi M, Abdou F, El-Sayed M. (1966). Date stone meal as a substitute for barley in chick rations. *Trop. Agric*; 43: 12-7.
- [19] Jumah HF, Al-Azzawi II, Al-Hashimi SA (1973). Some nutritional aspects of feeding ground date pits for broilers. *Mesopotamia J Agric* 8: 139-45.

- [20] Kamel BS, Diab MF, Ilian MA, Salman AJ. Nutritional value of whole dates and date pits in broiler rations. *Poult Sci* 1981; 60: 1005–11.
- [21] Hussein AS, Alhadrami GA (2003) Effect of enzyme supplementation and diets containing date pits on growth and feed utilization of broiler chicks. *Agric Mar Sci* 8: 67–71.
- [22] Elgasim EA, Alyousif YA, Homeida AM(1992) Possible hormonal activity of date pits and flesh fed to meat animals. *Food Chem* 52: 149–50.
- [23] Ali BH, Bashir AK, Al Hadrami G. (1999) Reproductive hormonal status of rats treated with date pits. *Food Chem*; 66: 437–41.
- [24] Marzouk HA, Kassem HA (2011). Improving fruit quality, nutritional value and yield of Zaghoul dates by the application of organic and/or mineral fertilizers. *Scientia Horticulturae*.127: 249–54.
- [25] Al-Farsi MA, Lee CY. (2008). Nutritional and functional properties of dates: a review. *Crit Rev Food Sci Nutr*. 48:877–87.
- [26] Al-Humaid AI, Mousa HM, El-Mergawi RA, Abdel-Salam AM. (2010) Chemical composition and antioxidant activity of dates and datecamel- milk mixtures as a protective meal against lipid peroxidation in rats. *Am J Food Tech*.5:22–30.
- [27] Goto C, Kasuya S, Koga K, Ohtomo H, Kagei N (1990). ethal efficacy of extract from *Zingiberofficinale*(traditional Chinese medicine)or [6]-shogaol and [6]-gingerol in Anisakis larvae in vitro. *Parasitol. Res*. 76:653–656.
- [28] Islam MS, Choi H (2008). Comparative effects of dietary ginger (*Zingiber officinale*) and garlic (*Allium sativum*) investigated in a type diabetes model of rats. *J. Med. Food*. 11:152–159.
- [29] Adewunmi CO, Oguntimein BO, Furu P (1990). Molluscicidal and 1492 J. Med. Plants Res. antischistosomal activities of *Zingiber officinale*. *Planta. Med*. 56: 374– 37.
- [30] Ali B H, Blunden G, Tanira M O, Nemmar A (2008). Some phytochemical, pharmacological and toxicological properties of ginger (*Zingiber officinale Roscoe*): a review of recent research. *Food Chem. Toxicol*. 46:409–420.
- [31] Hierro I, Valero A, Perez P, Gonzalez P, Cabo MM, Montilla MP, Navarro MC (2004). Action of different monoterpenic compounds against *Anisakis simplex* s.l,L3 larvae. *Phytomedicine* 11:77–82.
- [32] Holland JC, Pellegrino J, Cozinelli F (1974): Infection of mice with cercariae, schistosomula of *S. mansoni*by intravenous and subcutaneous routes. *Rev Inst Med Trop Sao Paulo* 16: 132-4.
- [33] Ahui MLB, Champy P, Ramadan A, Pham Van L, Araujo L, André K, Diem S, Damotte D, Kati-Coulibaly S, Michel Atté Offoumou M A, Dy M, Thieblemont N, Herbelin A (2008). Ginger prevents Th2-mediated immune responses in a mouse model of airway inflammation. *Int. Immunopharmacol*. 8:1626–1632.
- [34] Al-Qarawi AA, Mousa HM, Ali BEH, Abdel-Rahman H, El-Mougy AA (2004) Protective effect of extracts from dates (*Phoenix dactylifera* L.) on carbon tetrachloride–induced hepatotoxicity in rats. *Int J Appl Res Vet Med* 2: 176–80.
- [35] Saddiq A A, Bawazir A E. (2010) Antimicrobial Activity of Date Palm (*Phoenix dactylifera*) Pits extracts its role in reducing side effect of Methyl prednisolone on the someNeurotransmitter content in the Brain, Hormone Testosterone in adulthood. *International Society for Horticultural Science (ISHS)* 882: 665-690.
- [36] Xiao SH, Mei JY, Jiao PY (2011) Effect of mefloquine administrated orally at single, multiple or combined with artmetheter, artesunate or praziquantel in treatment of mice infected with *Schistosomajaponicum*. *Parasitol Res* 108: 399-406.
- [37] Smithers SE, Terry RJ (1965)The infection of laboratory hosts with cercariae of *S. mansoni* and recovery of worms. *J Parasitol* 55: 695- 700.
- [38] Secor WE (1996)Pattern of oogram and ovacount in infected mice with *S. mansoni*. *J Infect Dis* 174: 1131- 5.
- [39] Kamel IA, Cheever AW, Elwi A, Mosimann JE, Danner R (1977): *S. mansoni* and *S. haematobium* infection in Egyptian technique for recovery of worms at necropsy. *Am J Trop Med Hyg* 26: 696- 701.
- [40] Uyeda, K. & Racker, E. (1965):Regulatory mechanisms in carbohydrate metabolism. VII. Hexokinase and phosphofructokinase. *J. Biol. Chem*. 240: 4682-4688.
- [41] McManus, D. P. & James, B. L. (1975): Anaerobic glucose metabolism in the digestive gland of *Littorina saxatilis rudis* (Maton) and the daughter sporocysts *Microphallus similis* (jag). *Comp. Biochem. Physiol*. 51(13): 293-297.
- [42] Cabaud, P. & Wroblewski, F. (1958): Colorimetric measurement of lactic dehydrogenase activity of body fluids. *Am. J. Clin. Pathol*. 30,234.
- [43] King, Y. S. (1974): Cultivation of *Bulinus physopsis globosus* (Morelt) and *Biomphalaria pfeifferi* (Krauss) snail hosts of schistosomiasis. *Sterkiana*, (7): 52-54.
- [44] Reitman, S. and Frankel, S.(1957): A colorimetric method for the determination of serum glutamic oxaloacetic and glutamic pyruvic transaminases. *Amer. J. Clin.Pathol.*,28:56.
- [45] Litchfield JT, Willcoxon F. (1949): A simplified method of evaluating dose-effect experiments. *J PharmacolExpTher* 96: 99- 113.
- [46] Ritchie, L. S., Lopez, V. A., Cora, J. M. (1974): prolonged applications of an organotin against *Biomphalariaalexandrina* and *Schistosoma mansoni* in molluscicides in Schistosomiasis control: TC. Change Ed Academic press New York and London: ppts: 77 - 88.
- [47] Viyanant,V., Thirachantra,S. and Sornmani,S (1982): The effect of controlled release copper sulphate and tributyltin fluoride on the mortality and infectivity of *Schistosoma manson miracidia*. *Southeast Asian J.Trop .Med. Pub.Hilth.*, 13(2),225.
- [48] Gawish, F.M. (1997) Evaluation of combination of certain molluscicide against *Biomphalaria alexandrina* and the free living stages of *Schistosoma mansoni*. Ph.D. Thesis, Zoology Dep. Girls Coll. For Arts, Sci. & Education, Ain Shams University.
- [49] Mostafa OM, Eid RA, Adly MA (2011): Antischistosomal activity of ginger (*Zingiberofficinale*) against *Schistosomamansoni* harbored in C57 mice. *Parasitol Res* 109: 395- 403.

- [50] Seif el-Din SH, EL-Lakkany NM, Mohamed MA, Hamed MM, Sterner O, Botros SS (2013): Potential effect of the medicinal plants *Calotropis procera*, *Ficus elastic* and *Zingiber officinale* against *Schistosoma mansoni* in mice. *Pharm Biol*, Online: 1-7.
- [51] WHO (1963) Data sheet on pesticides. No.63, Niclosamide. (1963) WHONBC/DC/88.63.
- [52] Bakry F. A., Eleiwa E. M., Taha S. A., and Ismil S. M. (2016). Comparative toxicity of Paraquat herbicide and some plant extracts in *Lymnaea natalensis* snails *Toxicology and Industrial Health* 2016, Vol. 32(1) 143-153.
- [53] Fahmy S. R., Abdel-Ghaffar F., Bakry F. A. , Sayed D. A. (2014). Ecotoxicological Effect of Sublethal Exposure to Zinc Oxide Nanoparticles on Freshwater Snail *Biomphalaria alexandrina*. *Arch Environ Contam Toxicol* (2014) 67:192–202.
- [54] Bakry F. A., El-Homossany K., Ismil S. M. (2016). Alterations in the fatty acid profile, antioxidant enzymes and protein pattern of *Biomphalaria alexandrina* snails exposed to the pesticides Basudin and Selecron *Toxicology and Industrial Health*, 2016, Vol. 32(4) 666–676.
- [55] Ahmed, S. A. and Gad, M. Z. (1995) Effect of schistosomal infection and its treatment on some key enzymes of glucose metabolism in mice livers *Arzneim. Forsch*, 45, 1324-1330.
- [56] Tielens, A. G. (1997) *Biochemistry of Trematode*. In: Fried, B. and Graczyk, T.K. Eds., *Advances in Trematode Biology*, CRC Press, Boca Raton, 309-343.
- [57] Tielens, A. G. (1997) *Biochemistry of Trematode*. In: *Advances in Trematode Biology* (Fried, B and Graczyk, T. K. Ed). pp 309-343.
- [58] Kuser, P. R; Krauchrenco, S; Antunes, O. A., Polikarpov, I (2000) The high resolution crystal structure of yeast Hexokinase with the correct primary sequence provides new insights into its mechanism of action. *J. Biol. Chem.* 275, 20814.
- [59] ElAasar A. A; ElMerzabani, M. M; Zakhary, N. I; Farag H. I; Abdeen A. M; Abd El-Salam, I. and Mokhtar, N. M (1989) Biochemical and biophysical studies on schistosomal liver of mice. *Egypt. J. Bilh.* 11,19.
- [60] El-Shazly, A. M; Soliman, M; ElKalla, M. R; Rezk, H; ElNemr, H. E; Handousa, A. E. and Helmy, M. M Studies on patients with *Schistosoma mansoni*; HCV and/or typhoid fever. *J. Egypt. Soc. Parasitol.* 2001, 31, 583.
- [61] Mansour, M. M.; Farid Z, Bassily, S, Salah LH., and Watten, R. H (1982) Serum enzyme tests in Hepatosplenic schistosomiasis. *Trans. R. Soc. Trop. Med. Hyg.* 76,109.
- [62] Zaidi, S. M. and Banu, N. (2004) Antioxidant potential vitamin A, E and C in modulating oxidative stress in rat brain. *Clinica Chimica Acta*, 33, 229-340.
- [63] Ajith, T. A., Usha, S. and Nivitha, V. (2007) Ascorbic acid and α -Tocopherol protect renal damage. *Clinical and Experimental Nephrology*, 9, 24-30.
- [64] Abath FG, Morais CN, Montenegro CE, Wynn TA (2006) Montenegro SM Immunopathogenic mechanisms in schistosomiasis: what can be learnt from human studies? *Trends Parasitol.* 22: 85-91.
- [65] Cassiman D, Lib brecht L, Desnet V, Deneff C, Roskams T (2002). Hepatic Stellate cell/myofibroblast subpopulations in fibrotic human and rat livers. *J. Hepatol.* 36: 200-209.
- [66] Mann J, Mann DA (2009). Transcriptional regulation of hepatic stellate cells. *Advanced drug delivery reviews* 61: 497-512.
- [67] Rollino C, Guzman H, Beltrame G, Ferro M, Quattrocchio G, Bellis D, Quarello F (2008) Retroperitoneal fibrosis and schistosomiasis: A causal relationship? *Europ. J. Inter. Med.*, 19: 297 - 299.
- [68] Spicher VM, Genin B, Jordan AR, Brandt LR, Coulter CL (2004) Peritoneal schistosomiasis: An unusual Laparoscopic Finding. *J. pediat. Surg.*, 39: 631-633.
- [69] Michael JD, Anthony EB (1998) *Schistosomiasis*. School Biol. Sc., Univ. Wales, Bangor, Gwynedd, UK., pps: 2139-2140.