Efficacy of alcoholic extract of *Thymus vulgaris* on experimental Schistosomiasis: Histopathological and Histochemical study

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Abstract: The repeated chemotherapy of schistosomiasis has resulted in emergence of resistant schistosome strains and so there is an intensive need to search for alternative drugs. The present work aimed to evaluate anti-schistosomal activity of *Thymus vulgaris* ethanolic extract (TVE)against *Schistosoma mansoni* infected mice. **50** mice were divided into 5 groups, **G1**: control negative ,**G2**: infected untreated group (infected by ±80 *S. mansoni* cercariae by subcutaneous route),**G3** infected then treated group with **500mg/kg** of TVE at 7th week post infection (pi) and continued for 4 weaks , **G4**:infected then treated group with **500mg/kg** of praziquantel **(PZQ)**for 2 successive days at 7thweek pi and **G5**: combined group infected then treated with TVE and **PZQ** . All mice were sacrificed after the end of therapy. Parasitological assessment regarding worm burden showed that a highly significant reduction (**P < 0.0001**) in the mean total worms in all infected treated groups when compared with infected control group. The highest statistically significant reduction rate (**81.8%)** was obtained in combined treatment group **(G5)** followed by **PZQ** treated group **(G4) (77.6%),** while insignificant statistical reduction rate was reported in TVE treated group **(G3) (16.4%).** Regarding the liver tissue egg load there was a high significant reduction rate (**P < 0.0001**) in the mean number of counted ova per gram liver tissue in all treated groups when compared with infected control group, the highest statistically significant reduction rate **(88.1%)** was obtained in combined group **(G5)** followed by **PZQ** treated group **(G4) (76.1%)**, then TVE treated group (**G3) (31.8%).** Histopathological results indicated a reduction in the number and size of granulomatous inflammatory infiltrations in the liver of treated mice compared to non-treated one. The histochemical examination of the different treated mice groups showed that combined **PZQ** and TVE treatment led to marked improvement of the apoptotic changes (mild apoptosis) occurring in hepatocytes nuclei following *S. mansoni* infection, while TVE alone was less effective than **PZQ** producing slight improvement of apoptosis (moderate apoptosis). Concerning liver reduced glutathione level, both TVE and **PZQ** when given alone or in combination produced a significant elevation of the lowered liver GSH of infected group above the normal levels. The results of the present work suggested that TVE has anti-schistosomal activities that are more prominent when combined with PZQ.

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1. Introduction:

Schistosomiasis is one of neglected tropical diseases (NTDs) caused by blood flukes (trematode worms) of the genus *Schistosoma*. This parasite has many species reported by the WHO and are named intestinal or urinary schistosomiasis according to its habitat and clinical manifestation in human host. Schistosomiasis transmission has been reported from 78 countries most of them are developing countries located in tropics and sub-tropic areas where people are routinely involved in daily agricultural, domestic, occupational, and recreational activities with cercaria infested water **(WHO, 2018).** In *Schistosoma mansoni*, the natural infection pathway starts with exposure to cercaria in infected water which penetrate the skin of human host then develop to adult worm within months residing in mesenteric venous plexus laying eggs that finds its way to outside in stool **(Bergquist, 2002).** In chronic infection, a strong host CD4 response is elicited by the abundant antigenic fraction of the miracidial secretion which called soluble egg antigen (SEA). This response is targeting the eggs of *Schistosoma*, especially those failed to find its way to outside the body and swept by the circulation to hepatic tributaries of portal vein, in the form of cellular aggregates of inflammatory cells forming periovular granuloma which develop later in life to hepatic fibrosis **(Andrade, 2009).** Hepatic fibrosis is a chronic inflammatory response to *Schistosoma* egg antigens starting with formation of periovular granuloma which is a normal strategy of the liver, important to protect the host tissue from harmful

lytic effect of egg secretions, rather than a reaction to destroy the miracidium **(Reis and Andrade, 1987).** Granuloma formation is a helper T cell-mediated reaction with predominance of TH1 response at first then switch to TH2 response. This phenomenon is exclusive to hepatic granuloma not seen in other body granuloma **(Kamdem et al., 2018).** This reaction is driven by cytokines that play a crucial role in the inflammatory response involved in *Schistosoma* hepatic fibrosis. Among these, Interleukin (IL)-1β, IL-6, IL-10, and Tumor Necrosis Factor-α (TNF-α) are recorded **(Pearce and MacDonald, 2002 & Burke et al., 2009).** The conventional treatment of all types of schistosomiasis including *S. mansoni* involves Praziquantel (PZQ) as the drug of choice. However, the effect of this drug in treatment of hepatic fibrosis is questioning since its therapeutic effect is largely affected by the stage of the disease, parasite worm load, individual age, rate of reinfection and even the site of lesion **(Vennervald and Dunne, 2004).** Furthermore, some individuals exhibit persistent inflammatory response toward the egg secretions post treatment which prone them to develop advanced stages of liver disease in absence of adult worm **(Cavalcanti and Peralta 2015).**In addition, some individuals need to repeat the drug dose many times to get the aimed effect making them vulnerable to the harmful adverse effect of the anti-schistosomal treatment. When it comes to chronic diseases like hepatic fibrosis, a cheap, effective, affordable drug with no or minimal side effects is welcomed and valued by the patients and health authorities. With emergence of resistant strains of *Schistosoma* parasite to different drugs including PZQ in the last few decades, the urgent need to find an alternative treatment with fewer side effects has shifted the interest of researchers toward herbal plants.

Thymus (*Thymus vulgaris L., Lamiaceae*) is a herb abundantly grows in the Mediterranean basin including Egypt. Since decades, this plant has been described as antispasmodic, antimicrobial, antihelminthic, expectorant, antiseptic, carminative, sedative, and anti-oxidative herb **(Guidi and Landi, 2014 & Rustaiyan et al, 2000).**The noteworthy anti-inflammatory effects of *thymus* stand out among its therapeutic effects and are largely attributed to its main bioactive compounds thymol and carvacrol **(Amiri ,2012 & Nickavar et al., 2005).** *Thymus vulgaris* anti-inflammatory effect is mainly exhibited through many mechanisms recorded in vivo and in vitro. Thymol was found to inhibit release of inflammatory cytokines TNF-α and IL-6 and suppress inflammatory enzymes production like cyclooxygenase-2 (COX-2), inducible nitric oxide synthase (iNOS) **(Liang et al., 2014).**Also, its inhibitory effect on leukocyte migration contributes to its anti-inflammatory action **(Fernanda Carolina et al., 2012).** Borneol, another anti-inflammatory compound present in thyme, has been reported to suppress the production of the pro-inflammatory cytokines IL-1*β* and IL-6 in mice **(Juh´as et al., 2008).**

Glutathione (GSH) plays an important role in host antioxidant defence directly through the scavenging of reactive oxygen species, and indirectly through its function as a cofactor of antioxidant enzymes **(Franco et al., 2007).** The oxidative processes that occur during infection by *S. mansoni* suppress the host enzymatic detoxification activities and so impairment of the liver GSH content of tested mice, thus producing reduction of the antioxidant capacity of the liver and leading to the generation of lipid peroxides that may play a central role in the pathology associated with schistosomiasis (**Bessa et al., 2012)**. Also, the decrease in glutathione levels may be attributed to inhibition of glutathione reductase enzyme that maintains glutathione in its reduced form (GSH) **(Gharib et al., 1999).** *Thymus vulgaris* due to its anti-oxidative activity, it has the ability to decrease GSH depletion strongly **(Gramza-Michalowska et al., 2011).**

Aim of the study:

Our study aimed to investigate the effect of *Thymus vulgaris* alcoholic extract on *Schistosoma mansoni* infected mice by parasitological, histopathological, histochemical and biochemical methods.

2. Materials and methods:

***Thymus vulgaris* alcoholic extract preparation (Attia et al, 2015)**:

Thymus vulgaris aerial parts were obtained from experimental farm of Faculty of Agriculture, Zagazig University in April 2018. It was kindly confirmed by Ass. Prof. Dr. Mohamed Ahmed Abdulkader, Faculty of Agriculture, University of Zagazig, Egypt. Voucher specimens are kept in the Pharmacognosy Department, Faculty of Pharmacy, University of Zagazig, Egypt.150 g of fresh aerial parts of *T. vulgaris* were macerated in to 90% ethyl alcohol for 5 days. After that, the extract was filtrated through Whatman No 1 filter paper and then the ethanol was removed via rotary evaporator to obtain viscous residue of crude plant extract which were subjected to lyophilization to afford 2 grams of powdered extracts of the plant. The suspensions of the alcoholic extract of *T. vulgaris* prepared for oral administration by using 0.5% Tween-80 (ADWIC, Egypt) in normal saline.

Mice, experimental infection and treatment:

This study was carried out on male laboratory bred and parasite free Swiss albino mice, eight weeks old and 18-20 grams in weight. Mice were obtained

from Schistosome Biological Supply Center at Theodor Bilharz Research Institute (TBRI). They were housed in a suitable environment in the Scientific and Medical Research Centre, Zagazig University (ZSMRC). The mice were maintained on a standard commercial pelleted diet with free accessible water and in an air-condition animal house at 20-22°C all over the time of study. Mice were divided to 5 groups: (10 mice/group), G1: non-infected, non-treated (control negative). G2: infected control group (infected by ±80 *S. mansoni* cercariae by subcutaneous route). G3: infected then treated with oral TVE 500 mg/kg daily **(Attia et al., 2015)** for 4 weeks starting at 7th week post infection (**Mahmoud et al., 2014).** G4: infected then treated by Praziquantel (PZQ) obtained from Egyptian International Pharmaceutical Industries Company (E.I.P.I.Co.), at an oral dose of (1000 mg/kg) divided into two successive days **(Piper et al., 1990 and Shaheen et al., 1994)** at 7th week post infection. G5: combined group (infected then treated with TVE and PZQ) with same doses descried before**.**

Animal ethics protocol:

Mice were reared and sacrificed according to the protocol of The Institutional Animal Care and Use Committee according to Zagazig University (ZU-IACUC) for animal Use in Research and Teaching. Efficacy of the herbal extract and PZQ treatment was assessed by parasitological, histopathological, histochemical and biochemical examination.

I. Parasitological examination:

a) Worm burden:

The animals were anesthetized using Sodium pentobarbital 80 mg/kg, euthanized and *S. mansoni* worms were recovered by portal perfusion (**Smithers and Terry 1965**), with perfusion buffer [phosphate buffered saline, 0.02 µl/ml heparin (monoparin; CP Pharmaceuticals Ltd., Wrexham, UK)]. The worms were washed free of erythrocytes and counted using a dissecting microscope. The adult worms were then counted as described by (**Wang et al., 2004)** and percentage worm reduction was calculated following the formula described below (**Melman et al., 2009**).

Worm burden reduction percentage = Mean no. of worms from control group - Mean no. of worms from treated group x 100%/ Mean no. of worms from control group.

b) Liver tissue egg load: A piece of the liver tissues obtained after mice scarification was stored at −20°C for counting of deposited eggs. The eggs were counted and expressed as eggs/gram liver according to the method of **Cheever (1968).**

II. Histopathological studies of liver tissues: Liver specimens were fixed in 10% formalin and kept as paraffin blocks for the following :

a) Estimation of number of liver egg granulomas: According **to Ali and Hamed (2006),** the stained sections from infected animals were examined in comparison to their controls. For each mouse, the number of hepatic granulomas was determined in five randomly selected microscopic fields / liver section at high power magnification. Three liver sections were examined for each mouse. The mean number of hepatic granulomas / liver section was calculated. The percentage reduction in the mean granuloma number (MGN) =

[(MGN of control group) - (MGN of treated group)] x 100% / [MGN of control group].

b) Estimation of diameter of liver granulomas (Romeih et al., 2008): Was performed using graduated eyepiece lenses. Two perpendicular maximum diameters were measured to get the mean diameter for each granuloma. Five randomly selected microscopic fields / liver section were examined at high power magnification. Three liver sections were examined for each mouse. The mean diameter of hepatic granuloma was determined, while the percentage reduction in the mean granuloma diameter (MGD)= [(MGD of control group) - (MGD of treated group)] x 100% / [MGD of control group].

c) Type of the granuloma, cellular, fibrocellular, or fibrous, was determined according to the cellular to fibrous ratio by microscopic examination on different magnifications (100x and 400x) **(Romeih et al., 2008).**

III- Histochemical evaluation of the liver using the Feulgen reaction:

In Feulgen reaction the hepatocytes DNA is stained depending on the reaction of (Schiff or Schiff-like reagents) with the aldehyde groups present in the deoxyribose molecules via HCl acid hydrolysis. Normal DNA stained bright red against green background. The degree of staining is proportional to the concentration of DNA. Apoptosis is graded according to color changes of DNA as follow: marked apoptosis (faint staining of nuclear DNA), moderate apoptosis (pale red staining of nuclear DNA) and mild apoptosis (less than bright red staining of nuclear DNA) **(Chieco and Derenzini 1999).**

IV- Hepatic Glutathione Assay:

Hepatic GSH levels were measured as described by **Ellman (1959).** Based on the reduction of Ellman's reagent (5,5'–dithiobis-2- nitrobenzoic acid (DTNB) with GSH to produce a yellow compound. The chromogen is directly proportional to the GSH concentration and its absorbance was measured at 405 nm. 0.2 g of liver tissues were mixed with 4 ml of 0.02 M EDTA and homogenized well. Then 2.5 ml aliquots were obtained and mixed with 2.0 ml of distilled water and 0.2 ml of 50% Trichloroacetic acid (TCA) and all

tubes were shaken intermittently for 10-15 mins then centrifuged at 3000 x g for 15 mins. 2.0 ml of liver tissue supernatant was collected and put in a fresh tube into it 2.0 ml of 0.4 M Tris buffer (pH 8.9) and 0.01 M DTNB were added then shaken slowly. Within 5 mins of DTNB addition, absorbance was measured.

Statistical analysis:

All data in the present work were analyzed using SPSS version 17.0. Comparing the values of the different treated groups with the values of the control group was performed using One-way analysis of variance (ANOVA) test and post hoc test. The results were expressed as Mean ± Standard deviation. The minimal level of significance was at P<0.05.

3. Results:

Parasitological parameters:

(A)Worm burden:

The results of the tested drugs on worm burden in different treated groups showed a highly significant reduction (P < 0.0001) in the mean total worms in all infected treated groups when compared with infected control group (**Table-1).** The highest statistically significant reduction rate (**81.8%**) was obtained in combined group (G5) followed by PZQ treated group (G4) (**77.6%**), while insignificant statistical reduction rate was reported in TVE treated group (G3) (**16.4%**).

Table (1): Effect of *Thymus Vulgaris* extract and praziquantel treatment on total *Schistosoma* worm burden:

Groups	Total worm burden		F- test	P- value	Post-hoc test
	Mean± SD	% reduction			
Control infected group (G2)	33±4.9		62.2	<0.0001*	P1>0.05 P2<0.001* P3<0.001*
TVE treated group(G3)	27.6±4.2	16.4%			
PZQ treated group(G4)	7.4±2.3	77.6%			
Combined group (G5)	6±2.5	81.8%			

*Significant differences at P-value < 0.05.**P1** (G2 versus G3), **P2** (G2 versus G4), **P3** (G2 versus G5).

(B) Liver tissue egg load:

There was a high significant reduction rate (P < 0.0001) in the mean number of counted ova per gram liver tissue in all treated groups compared with infected control group (**Table-2**). The highest statistically significant reduction rate (**88.1%**) was obtained in combined group (G5) followed by PZQ treated group (G4) (**76.1%**), then TVE treated group (G3) (**31.8%**).

Table (2): Effect of *Thymus Vulgaris* extract and praziquantel treatment on liver tissue egg load:

Groups	Liver tissue egg load		F- test	P- value	Post-hoc test
	Mean±SD	%Reduction			
Control infected group (G2)	2495.8±362.3		95.4	<0.0001*	P1<0.01* P2<0.001* P3<0.001*
TVE treated group(G3)	1703±126.4	31.8%			
PZQ treated group(G4)	597.6±62.7	76.1%			
Combined group (G5)	295.8±31.5	88.1%			

*Significant differences at P-value < 0.05.P1 (G2 versus G3), P2 (G2 versus G4), P3 (G2 versus G5).

Determination of the level of liver reduced glutathione (GSH):

Results shown in table (3) showed that the infected mice had suffered from a significant reduction in liver GSH levels in comparison with the normal mice. Both TVE and PZQ when given alone or in combination produced a significant elevation of the lowered liver GSH of infected group above the normal levels.

Table (3): Effect of *Thymus Vulgaris* extract and praziquantel treatment on level of liver GSH:

Groups	GSH levels (mg/g) (Mean±SD)	F- test	P- value	Post-hoc test
Control normal group (G1)	2.74 ± 0.4	14.9	<0.0001*	P1<0.001* P2<0.001* P3<0.001* P4<0.001*
Control infected group (G2)	1.9 ± 0.3			
TVE treated group (G3)	4.2 ± 0.6			
PZQ treated group (G4)	3.4 ± 0.5			
Combined group (G5)	4.38 ± 0.8			

*Significant differences at P-value < 0.05. P1 (G2 versus G1), P2 (G2 versus G3), P3 (G2 versus G4), P4 (G2 versus G5).

Histopathological parameters:

-Effect of *Thymus Vulgaris* extract and praziquantel on the liver granulomas number and diameter in different groups: The results showed a high statistically significant reduction (P< 0.001) in the liver granuloma numbers and diameters in the different treated groups when compared with the infected control group (Table-4). Group 5 (combined group) showed the maximum reduction of the mean of the granuloma number and diameter, which gave a mean of (**2.1±0.7)** and (**32.6±9.3)** respectively with reduction percentages of **83%** and **89%** respectively.

Table (4): Effect of *Thymus Vulgaris* and praziquantel treatment on granuloma no. and granuloma diameter:

		G2	G3	G4	G5
Granuloma no.	**(Mean± SD)** **% reduction**	**12.6±3.1**	**5.2±0.9** **59%**	**7.1±1.4** **44%**	**2.1±0.7** **83%**
	F- test **P- value** **Post-hoc test**	**43.6** **<0.001*** **P1<0.001*P2<0.001*P3<0.001***			
Granuloma diameter	**(Mean± SD)** **% reduction**	**287.3±39.5**	**103.1±25.7** **64%**	**148.4±27.6** **48%**	**32.6±9.3** **89%**
	F- test **P- value** **Post-hoc test**	**68.2** **<0.001*** **P1<0.001*P2<0.001*P3<0.001***			

*Significant differences at P-value < 0.05.P1 (**G2** versus **G1**), P2 (**G2** versus **G3**), P3 (**G2** versus **G4**), P4 (**G2** versus **G5**).

The histopathological examination of the different treated mice groups showed that combined PZQ and TVE treatment led to marked decrease in hepatic granuloma number and size and changed the cellular content with increase in fibroblasts, fibrocytes and deposition of collagen fibers. Most of granulomas were fibrocellular and fibrous. The surrounding hepatic tissue showed marked improvement of configuration. These positive changes were also showed on using PZQ but less than that with combined treatment, while TVE alone was less effective than PZQ producing large fibrocellular and cellular granulomas with central intact *Schistosoma* eggs and excess infiltration of inflammatory cells in the surrounding liver tissue (**Fig. 2,3,4 and 5).**

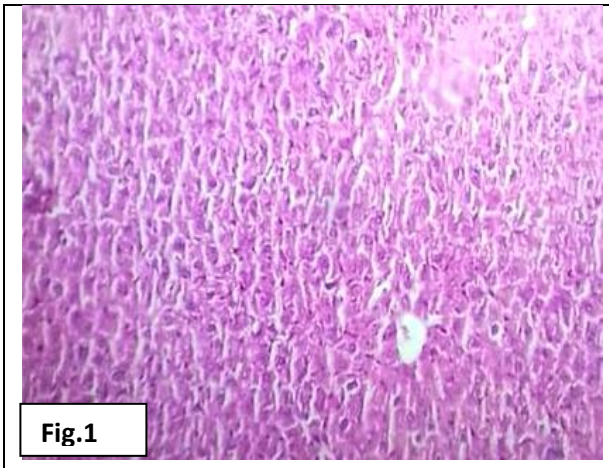


Fig.1 Liver section of non-infected group (G1) showing normal hepatic architecture, normal liver lobules with normal central vein surrounded by cords of the hepatocytes (**hematoxylin and eosin, ×400**).

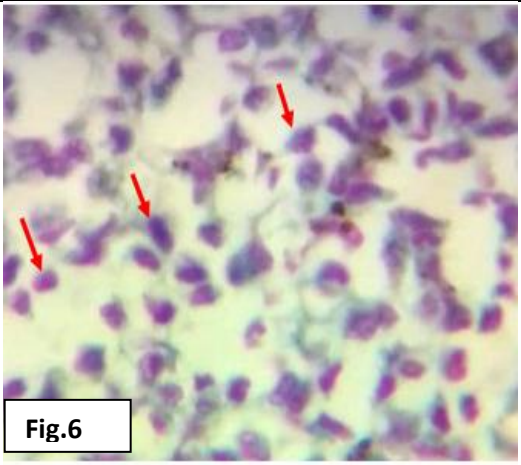


Fig.6: Liver section of non-infected group (G1) showing normal bright red stained hepatocytes nuclei with intact nuclear membrane and rounded contour (red arrows) (**Feulgen X 1000**).

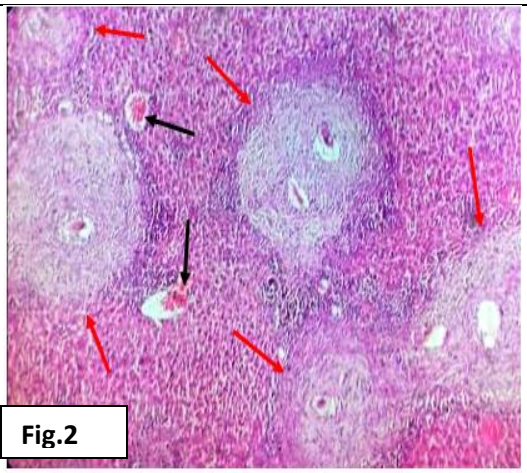


Fig.2 Liver section of infected group (G2) exhibiting multiple large fibrocellular granulomas with excess inflammatory cells and central intact *Schistosoma* eggs (red arrows) with areas of haemorrhage within the hepatic lobules (black arrows) (**hematoxylin and eosin, ×100**).

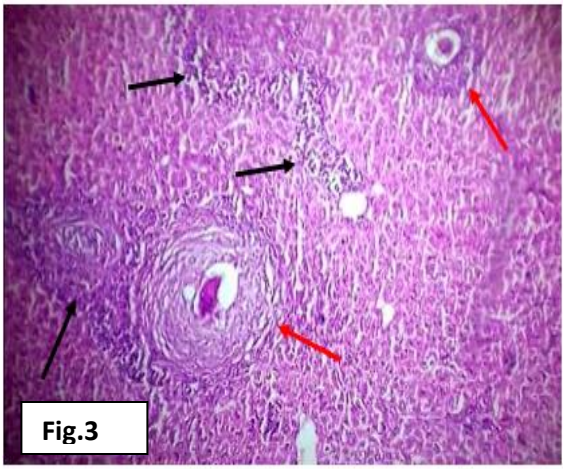


Fig.3 Liver section of infected group treated with TVE (G3) exhibiting large fibrocellular and cellular granulomas with central intact *Schistosoma* eggs (red arrows), excess inflammatory cells infiltrate the surrounding liver tissue (black arrows) (**hematoxylin and eosin, ×100**.

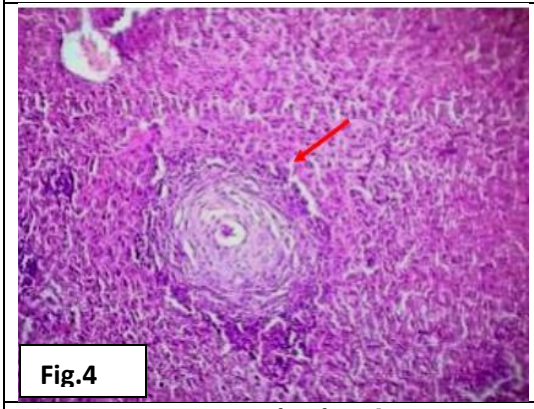


Fig.4: Liver section of infected group treated with PZQ (G4) showing medium sized fibrocellular granuloma with central degenerated *Schistosoma* egg (red arrow) (**hematoxylin and eosin, ×100).**

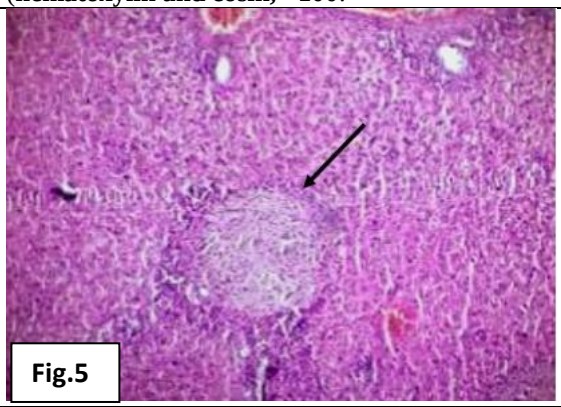
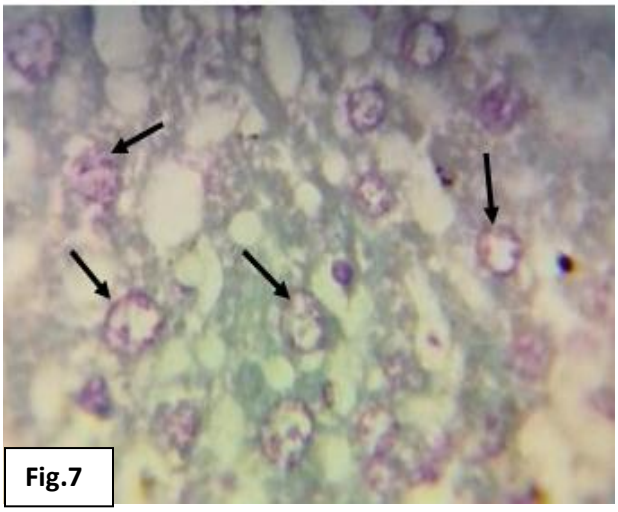
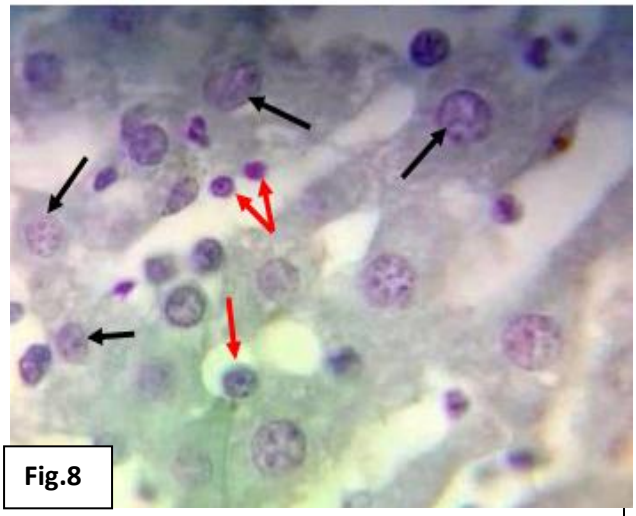


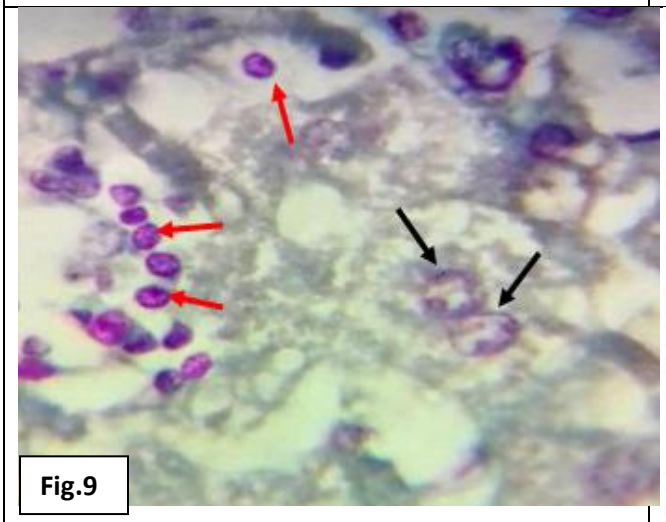
Fig.5: Liver section of infected group treated with PZQ and TVE (G5) showing small sized fibrocellular granuloma (black arrow) with absent *Schistosoma* egg in the center and improvement of the surrounding hepatic tissue architecture (**hematoxylin and eosin, ×100**).

Histochemical parameters:

The Feulgen histochemical stain was used to demonstrate DNA in tissue sections and for assessment of apoptotic changes. The histochemical examination of the different treated mice groups showed that combined PZQ and TVE treatment led to marked improvement of hepatocytes nuclear architecture with preservation of the vesicular appearance and the regular distribution of their chromatin with intact nuclear membrane .These protective changes were also recorded on using PZQ but less than that with combined treatment as still present few degenerated hepatocytes nuclei with fragmented pale stained chromatin, while TVE alone was less effective than praziquantel producing slight improvement of nuclear architecture (moderated apoptosis) but still present some degenerated pale stained hepatocytes nuclei with fragmented chromatin(**Fig. 7,8,9 and 10).**







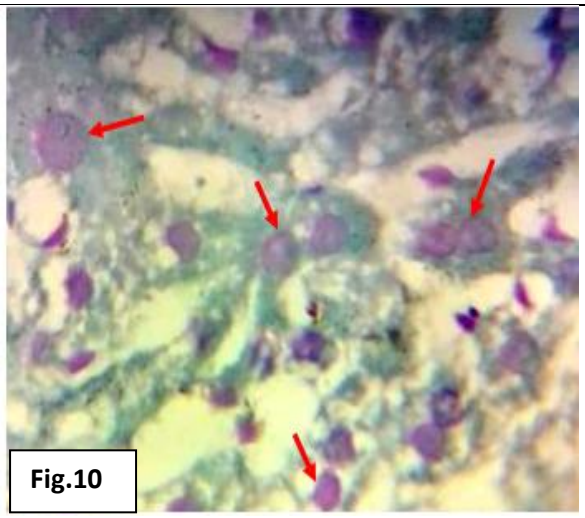


Fig.7: Liver section of infected group (G2) showing degenerated ballooned hepatocytes nuclei with partially damaged nuclear membrane and fragmented pale red stained chromatin (sever apoptosis) (black arrows)(Feulgen X 1000).**Fig.9:** Liver section of infected group treated with PZQ(G4) showing marked improvement of hepatocytes nuclear architecture regarding the vesicular appearance, regular bright red stained chromatin and intact nuclear membrane (red arrows), however still present few degenerated hepatocytes nuclei with fragmented pale red stained chromatin (mild apoptosis) (black arrows) (**Feulgen X 1000**).

Fig.8 Liver section of infected group treated with *TVE* (G3) showing slight improvement of nuclear architecture (red arrows), but still present some degenerated pale red stained hepatocytes nuclei with fragmented chromatin (moderate apoptosis) (black arrows) (Feulgen X 1000).**Fig.10:** Liver section of infected group treated with PZQ and TVE (G5) showing marked improvement of hepatocytes nuclear architecture regarding the average size, vesicular appearance, regular distributed bright red stained chromatin and intact nuclear membrane (red arrows) (**Feulgen X 1000).**

4. Discussion:

The use of PZQ in treatment of schistosomiasis and other helminthes infections showed low cure rates in Africa including Egypt and a tolerance to higher dose (**Doenhoff et al., 2008).** This encouraged the search for new therapeutic agents especially from natural resources. Many natural products have been used long time ago for prevention and treatment of

diseases. The most known species of thyme is *Thymus vulgaris.* Thyme has several biological effects including antispasmodic **(Van Den Broucke and Lemli, 1983),** antimicrobial **(Marino et al., 1999**), antioxidant **(Miura et al., 2002)** and antifungal activities **(Soliman and Badeaa, 2002).** The primary constituents of *Thymus vulgaris* (Al-Zaatar) are the volatile oils, including phenols, thymol and carvacol. Thyme oil was reported be directed for topical use, because it may lead to dizziness, vomiting and breathing difficulties (**Newall et al., 1996)**, so, its hydroalcoholic extract was effective in reduction of ulcer size in cutaneous leishmaniasis (**Nilforoushzadeh et al., 2008**). The volatile oil of *Thymus vulgaris* was effective against *Lucilia sericata* 3rd stage larvae (**Morsy et al., 1998**) and *Pediculus humanus* (**Veal**, **1996**). The different pharmacological activities of *Thymus vulgaris* extract may be due to its constituents, thymol and carvacrol. *Thymus vulgaris* extract has been shown to increase the immune responses and exerts potent antioxidant effects (**Rota et al., 2007**). In addition, TVE causes parasite destruction by alteration of permeability of the cell membrane and modulation of membrane organization and surface electrostatics, resulting in extracellular release of membrane-associated materials (**Sanchez et al., 2004**).The toxicity of *Thymus Vulgaris* aquatic plant extracts compared with copper sulfates (CuSO4) against *Bulinus truncatus*, the vector of urinary schistosomiasis was investigated by **Al-Obaidi et al.,(2018**) and the study showed that the *T. vulgaris* extracts were less effective than CuSO4 and its toxicity to the snails was dependent on the concentration and the time of exposure. They concluded the possibility to use the target extracts in control of *Bulinus truncates* snails.

The current study was done to evaluate anti schistosomal effects of the *Thymus vulgaris* ethanolic extract (TVE) compared with PZQ in experimentally infected mice. Medicinal plants have been used traditionally for treatment of schistosomiasis in Eastern and Southern Africa (**Sheir et al., 2001 and Al-Sayed et al., 2014**) through their curative and bioactive compounds.

In our study, the results of the tested drugs on worm burden in different treated groups showed a highly significant reduction ($P < 0.0001$) in the mean total worms in all infected treated groups when compared with infected control group. The highest statistically significant reduction rate (**81.8%**) was obtained in combination group (**G5**) followed by PZQ treated group (**G4**) (**77.6%**), while insignificant statistical reduction rate was reported in *TVE* treated group (**G3**) (**16.4%**). Nearly similar results were reported by **Attia et al. (2015)** who tested the effects of albendazole, thyme, and myrrh against mice infected with *Trichinella spiralis* and found a significant reduction in the number of adult worms in treated groups ($p < 0.01$) and the least reduction was detected in thyme-treated group with efficacy of 79.4%.On the other hand ,thyme oil caused significant reduction in *Toxocara vitulorum* (*T. vitulorum*) larval count in liver, kidney and heart of infected rats treated with thyme oil post infection (**38.2%, 45.9%** and **56%**) at the first week and (**48.7%, 58%** and **69.3%**) at second week respectively **(Amin and El-Kabany**, **2013).**Our results showed that there was a high significant reduction rate ($P < 0.0001$) in the mean number of counted *S. mansoni* ova per gram liver tissue in all treated groups compared with infected control group, the highest statistically significant reduction (**88.1%**) was obtained in combined group (**G5**) followed by PZQ treated group (G4) (**76.1%**), then TVE treated group (**G3**) (**31.8%**). This reduction in the tissue egg load can be attributed to the reduction occurred in the worm load. In agreement with these findings, **Pensel et al. (2014**) evaluated *Thymus vulgaris* and *O*riganum. *vulgare* (*O. vulgare)* essential oils efficacy against protoscoleces and cysts of *E. granulosus* in vitro and found that they provoked the loss of protoscoleces viability in addition protoscoleces treated with *O. vulgare* and thymol lost their infectivity, since following inoculation of treated parasites in mice no cysts were recovered. Moreover, **Albani et al. (2015)** found that in vivo application of albendazole in combination with thymol against *Echinococcus multilocularis* resulted in a significant reduction of the cysts weight compared to untreated mice, also both albendazole and thymol had a scolicidal effect**.** Similar results of **Farag et al. (2019)** showed that mean number of brain cysts of mice infected with *Toxoplasma gondi* Me-49 strain was significantly reduced in the group treated with TVE which was near to Spiramycin in cyst reduction (**47.5%**&**48.89%**) respectively also they added that the brain and liver pathological lesions in *Thymus* treated group showed considerable improvement. A study by **(El-kersh et al., 2018)** showed that the combined **PZQ** and *Echinacea purpurea* (**EP**) which is a perennial medicinal herb caused significant reduction in *S. mansoni* tissue egg load (**87%**) for intestine and (**81%**) for liver of infected mice .In work by **Santoro et al. (2007**) who investigated the effect of essential oils obtained from *Origanum vulgare L.* (oregano) and *Thymus vulgaris L.* (thyme) on growth and ultrastructure of diverse evolutive forms of *Trypanosoma cruzi* indicated that oregano and thyme essential oils are effective against *T. cruzi*, with higher activity of thyme.

Regarding the level of liver reduced glutathione (GSH) the results shown in table 3 illustrated that the infected mice exhibited a significant reduction in liver

GSH levels compared with the normal control group. TVE and PZQ when given alone or in combination produced a significant elevation of the lowered liver GSH of infected group above the normal level. This effect of *Thymus Vulgaris* can be explained by its antioxidant actions **(Guidi and Landi, 2014 & Rustaiyan et al., 2000).**Similar results were recorded by **Mahmoud et al. (2014)** who tested *Propolis* (Pps) and PZQ as anti-schistosomal drugs and found that PZQ succeeded in restoring liver GSH level and added that this action could be due to anti-schistosomal activity of PZQ and a subsequent decrease in eggs deposition and granuloma number in liver tissue. In agreement with these finding, **Muema et al. (2015)** tested the anti-schistosomal effects of methanolic extracts of Apple (*Malus domestica*), Lemon (*Citrus limon*) and Onion (*Allium cepa*) on mice infected with *Schistosoma mansoni* and reported that *S. mansoni* induced a significant reduction in liver GSH levels and they explained this as the parasite liberates its own free radicals which leads to consumption of the host reduced GSH. Following administration of plant extracts, augmentation of liver GSH activity and reduction of its depletion occurred. The findings of our study also, agree with **Bin Dajem et al. (2011)** that tested the effect of green tea (*Camellia sinensis*) extracts on *S. mansoni* infected mice and found highly significant increase in liver GSH levels compared to control group.

The results of histopathological examination in our study showed a high statistically significant reduction ($P< 0.001$) in the liver granuloma numbers and diameters in the different treated groups when compared with the infected control group. Group 5 (combined group) showed the maximum reduction of the mean of the granuloma number and diameter, with reduction percentages of **83%** and **89%** respectively. Similar results were obtained with **Osama et al. (2011)** who evaluated anti-schistosomal activity of crude aqueous extract of ginger in *S. mansoni* infected mice and found that ginger-treated mice showed fewer and smaller granulomas in liver and intestine than in non-treated group. Moreover, **Mahmoud et al. (2014)** found that separate treatment with ethanolic extract of propolis (Pps) or PZQ caused reduction in the number of liver granuloma while the combination of both resulted in reducing granuloma diameter as well. **El-Sayed and Ramadan (2017)** found that treatment with *T. vulgaris* in mice groups improved *T. gondii* induced liver pathology. **Nilforoushzadeh et al. (2008)** found that hydroalcoholic extracts of *Thymus vulgaris, Achillea millefolium* and *propolis* were significantly more effective than systemic glucantime or alcohol for the treatment of leishmaniais in Balb/c mice, the highest efficacy was observed for *propolis*, followed by *Achillea millefolium* and then *Thymus vulgaris*. They explained that these effects may be due to the ability of TVE to stimulate natural killer cell activity and release of nitric oxide and tumor necrosis factor from macrophages.

Schistosoma mansoni infection induces hepatocytes apoptosis through soluble egg antigen (SEA) which mediate the TNF-related apoptosis **(Duan et al., 2014).** Concerning the histochemical examination of the different treated mice groups in the current study, it showed that combined PZQ and TVE treatment led to marked improvement of hepatocytes nuclear architecture with preservation of the vesicular appearance and the regular distribution of their chromatin with intact nuclear membrane. These changes were also recorded on using PZQ but less than that with combined treatment, while TVE alone was less effective than PZQ producing slight improvement of nuclear architecture but still present some degenerated pale stained hepatocytes nuclei (moderate apoptosis) with fragmented chromatin. This effect can be explained by the antioxidant action of TVE that give protection against reactive oxygen species protecting the host cells from damage and preserve the normal cellular DNA **(Rota et al. 2007).**Similar results were obtained by **Eraky et al. (2016**) who found that mice infected with *T. gondi* and treated with TVE or a combination of sulfadiazine and pyrimethamine showed less than bright red staining of brain DNA that indicates mild apoptosis.

Conclusion:

TVE treatment had anti-schistosomal actions that were more significant when combined with PZQ through reduction of worm burden and liver egg load. Moreover, it reduced schistosomal induced hepatic pathology, reduced the liver granuloma size and to some extent caused improvement in the apoptotic changes of the liver. Also, it succeeded in elevation of the lowered liver reduced GSH.

Recommendation:

Based on our results, we recommend that further studies should be performed to examine the exact mechanism of action of *Thymus vulgaris* on *S. mansoni* and to study the anti schistosomal effect of essential oil of thyme to determine which is more effective as anti-schistosomal agent with best concentration for this medicinal plant.

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