Parasitological and Biochemical parameters in *Schistosoma mansoni* infected mice and treated with aqueous thymus leaves and *Citrus maxima* (pomelo) peels extracts

Wafaa F.A. Ahmed[1], Rasha M.Bahnasy[2] and Amina, M. G. Zedan[3]

[1.] Department of Biological and Environmental sciences (Parasitoloy), Faculty of Home Economic, Al Azhar University –Tanta
[2.] Nutrition and food science Department, Faculty of Home Economic, Al Azhar University –Tanta
[3.] Department of Biological and Environmental sciences (Genetics), Faculty of Home Economic, Al Azhar University –Tanta
Corresponding author E- mail: memo_modern2010@yahoo.com

Abstract : The aim of this study was to assess protection level of aqueous thymus leaves300,600mg/kg mice and *Citrus maxima* (pomelo) peels 600mg/kg mice extracts on experimentally infected mice with *Schistosoma mansoni* cercaria for two weeks of the first day post infection (50 cercariae/mice). This extract was administrated orally by stomach tube. All mice were sacrificed at the 7th week post infection. The possible effect of aqueous thymus leaves and *Citrus maxima* peels extracts against *S. mansoni* infected mice was evaluated by recording percentage of the recovered worms, tissue eggs and viability of ova(oogram pattern),mortality rate among mice and biochemical parameters including liver enzymes(Got and Gpt) level, serum total protein level, albumin and cholesterol were also determined. IgM and IgG antibody responses were also determined. Result showed that protection with thyme leaves and *Citrus maxima* peels extracts prevented most biochemical changes, also markedly improved IgM and IgG antibody, in *Schistosoma* infected treated mice, compared with the infected- untreated ones. In addition, remarkable reduction in worms, tissue eggs and alteration in oogram pattern were recorded in all the treated groups. The antioxidant and antischistosomal action of pomelo and the effects of thyme were greatly diverse according to treatments groups.

[Wafaa F.A. Ahmed, Rasha M.Bahnasy and Amina, M. G. Zedan. **Parasitological and Biochemical parameters in *Schistosoma mansoni* infected mice and treated with aqueous thymus leaves and *Citrus maxima* (pomelo) peels extracts.** *J Am Sci* 2015;11(10):95-103]. (ISSN: 1545-1003). http://www.jofamericanscience.org. 11

Keywords: *Schistosomia mansoni; antioxidants;* IgM and IgG antibody, antihelmintic *medicinal plant.***(** pomelo and thyme)

1. Introduction

Parasitic helminthes of genus *Schistosoma* are the causative agents of Schistosomiasis, an infectious disease affecting humans and animals **(Li *et al.,* 2011)**. For humans, it is one of the most prevalent parasitism in the world, second behind malaria **(Skelly & Shoemaker 2000)**. The world health organization (WHO) indicated that more than 200 million people are infected worldwide**.** Schistosomiasis tops all the endemic parasitic diseases world-wide particularly in Egypt **(EL Baz, *et al.,* 2003).** because of the unavailability of a Schistosomiasis vaccine, control of the disease depends mainly on chemotherapy praziquantel (pzq), which is active against all Schistosoma species and the recommended drug by the world health organization (WHO) for schistosomiasis treatment. There are emerging problems praziquantel treatment, which include the appearance of drug resistance in the treatment of *S. mansoni* and possibly *S. japonicum*, along with allergic or hypersensitivity reactions against praziquentel treatment. Histopathological examination of some patients showed the presence of viable eggs and granuloma in the productive phase post –treatment with (pzq). So there is an increased demand for using plants in therapy ``back to nature`` instead of using synthetic drugs which may have adverse effects that may be more dangerous than the disease itself **Mady *et al.,* 2001 and Jackson et al., 2015**. The plant, *citrus maxima* (j. burm) merr. (rutaceae), is commonly known as shaddock or pomelo. The plant is indigenous to tropical parts of Asia. The plant is cited as antitoxic, appetizer, cardiac stimulant and stomach tonic in ancient and medieval literature Arias, B.A. and Ramon-Laca, 2005. *Citrus maxima* (family: Rutaceae) *c. maxima* (j. Burman) Merrill is correct under the international code of Botanical Nomenclature (ICBN) **(Scora, and Nicolson, 1986)**.

The major flavanones of pomelo are neohesperidin and naringin, which are high in the seed case of unripe citrus fruits **(Chung *et al.,* 2000)** and its extract showed antioxidant activity though free radical- scavenging *in vitro* and to reduce reactive oxygen species in H_2O_2-treated HepG2 cells **(Lim, *et al.,* 2006)**. *C. maxima* essential oil is composed of a-pinene, sabinine, 3-B-pinine, 4 methyl- 1 –hexane-3,3- dimethyl, geranylformate,z-

citral, geranylformate, E-citral, geranyl acetate, B-farnesene **(Singh *et al.*,2012)**. Hespiridin, naringin, caffeic, p-coumaric, ferulic and radical scavenging activity of MECM was explored and the extract was found to be having significant free radical scavenging activity when tested against different free radicals **(Kundu Sen *et al.*,2010)**.Anti-inflammatory activity of MECM was explored against different acute models of inflammation **(Kundu Sen *et al.*, 2011)**. The antitumor activity of MECM was investigated against Ehrlichs-ascites carcinoma in Swiss albino mice **(KunduSen *et al.*, 2011)**. thyme is commonly used as culinary herb and thyme oil is used in food flavouring in the USA, thyme is listed as GRAS (generally recognised as safel). Thyme is stated possess carminative, antispasmodic actions, antitussive, expectorant, secretomotor, bactericidal, anti-helminticand astringent properties. Traditionally, it has been used for dyspepsia, chronic gastritis, asthma, diarrhea in children, enuresis in children, laryngitis, tonsillitis (as agargle), and specifically for pertussis and bronchitis.

Pharmacological Actions of thyme *In vitro* and in vivo animal studies

Antitussive, expectorant and ant-ispasinodicnctions are considered to be the major pharmacological properties of thyme **(van Den and Broucke, 1983)**, and have been associated with the volatile oils, e.g. (thymol,carvacrol) and flavonoid constituents. Thyme oil has produced hypotensive and respiratory stimulant effects in Rabbits following oral or intramuscular administration, and in rats following intravenous injection G41, an increase in rhythmic heart contraction was also observed in rabbits. Hypotensive activity in rats has been reported for thymus orospedanus, this action was attributed to adrenaline (epinephrine) antagonism. Thymol possesses antihelmintic (especially hook- worms, antibacterial, and antifungal properties) G4. There are no reports about pomelo and thyme as anti-schistosomal effects. Accordingly, in this study. We investigated the aqueous extract of dried pomelo(400 mg/kg) and thyme (300 and 600 m9 /kg mice) on *Schistosoma mansoni* infected mice to determine the protection level for two extracts using biochemical and parasitological parameters.

2. Material and methods

Preparation of extracts:

Both thyme leaves powder(7.5g) and pomelo peels powder (5g) were soaked in 100 ml of warm distilled water then allowed staying in refrigerator for 24 hours at 4^{o}C. The mixtures were filtered with Whattman No 1 filter paper (24cm). Thyme doses 300 mg and 600 mg, Pomelo dose 400 mg

Animals:

Thirty male Swiss albino mice weighing 17- 22 g were obtained from experimental research center of Theodor bilharz Institute (SBSP/TBRI), Giza, Egypt. They housed in polypropylene cages at 25+ 2^{0}C with l2h / l2h light /dark cycle, and had free access to pelleted food with tap water *ad libitum*.

The experimental design:

The animals were randomly divided into five groups with six in each,

G1 normal healthy animals

G2 *S. mansoni* infected mice

G3 *S. mansoni* infected mice treated with pomelo peels extract 400 m g/kg mice/dialy.

G4 *S. mansoni* infected mice treated with thyme extract 300 mg/kg mice/ dialy.

G5 *S. mansoni* infected mice treated with thyme extract 600 m g/kg mice/ dialy.

Parasites and infection:

Cercariae of *S. mansoni* Egyptian strain were obtained from SBSP/TBRI and used for infection immediately after shedding from *Biomphalaria Alexandrina* snails. Infection was carried out with 50 *S. mansoni* Cercariae / mouse by subcutaneous injection **(Holland *et al.*, 1974)**

Parasitological study:

After seven weeks post infection, mice of all groups were weighted and killed by decapitation according the ethical rules and Animal Experimentation committee of our institution.

Recovery of adult worms of infected mice:

The adult worms were recovered from the hepatic system and the liver by perfusion with citrate saline (8.5 gm of sodium chloride, 15 gm of sodium citrate and 1000 ml of Dist. water) **Smithers and Terry 1965**. When the liver, kidney and gut become pale, the perfusion process was stopped. The perfusate was collected in a container attached to the perfusion plate. The coils of the intestine were lifted from the tray and washed down in order to dislodge any worms adhering to them.

Worm counting **(Tendler *et al.*,1986)**– the degree of protection can calculated as follows P= c-v/c x 100

where p=% protection, c= mean number of parasite recover from infected mice, and V= mean number of parasite of recover from treated mice.

Tissue egg burden:

The number of eggs/g tissue (liver and intestine) was assessed following digestion with 4% KOH **(kamel *et al.*,1977)**.

Egg count:

Egg was assessed **(Brunet *et al.*,1997).**

Serum biochemical analyses:

Blood samples were collected and left at room temperature to clot. Sera were separated by

centrifugation at 3000 rpm for 5 minutes and kept at -20 ºC until use.

Serum albumin level was determined to indicate the tissue damage and exudation using commercial kit supplied by Diamond, RA50, Ireland. **(Doumas *et al.*,1971)** were estimated in different experimental groups.

Serum total protein contents were determined by colorimetric method using bovine serum albumin as standard (Stanbio Laboratory, USA). Serum cholesterol was determined using a kit from Stanbio Laboratory, USA.

Serum aspartate aminotransferase (AST) were determined to assess liver function **(Reitman and Frankel, 1957)** using commercial kits (Roche Diagnostics, GmbH, D-68298, Mannheim, Germany), Alanine aminotransferase (ALT) was measured **(Reitman and Frankel, 1957)** using kitpurchased from Greiner Diagnostic GmbH (Germany). Both enzyme activities were determined photo metrically.

Immunological studies:

Determination of IgG in serum:

The IgM reactivity pattern was studied by immunofluorescence test. The level of serum IgG was determined according to the method of **Wilson *et al.* (2006)** using a commercial ELIZA kit (Mouse IgG GenWay Biotech, Cat. No. CA92121). Absorbance was measured on ELIZA platereader at 450 nm. The serum Ig levels were assayed by the single radial immunodiffusion method* of (**Kreutzer, 1963**)

Statistical Analysis:

The results were presented as mean standard deviation(S.D.) in each group. Results were analyzed statistically by one way analysis of variance (ANOVA) using SPSS (Statistical Package for the Social Sciences, version 9) software followed by post-hoc test at least significance difference between groups at $p \leq 0.05$.

3. Results:

1- Mortality rate

The percentage of mortality was 16.7% in infected control group, mortality percentage was 50% in group which received the aqueous pomelo peels extract(600mg/kg mice) for two weeks of the first day post infection, where three mice had died during the experiment and the mortality rate was 33.3%, 0%, respectively in groups which received aqueous thyme leaves extract 300, 600 mg/kg mice weight, for two weeks of the first day post infection where two, zero mice were died during the experiment, the results are shown in table 1.

Table (1):Mortality rate among different groups.

Groups	No. at the beginning of the exp.	No. of died animals	Percentage
Control –	6	0	0%
Control +	6	1	16.7%
Pome1o	6	3	50%
Thyme1	6	2	33.3%
Thyme2	6	0	0%

2-Total body weight:

Mean body weight of animals of different groups was illustrated in table (2).Correlation between negative control group, infected control group and between groups which received aqueous thyme leaves extract 300, 600mg/kg mice weight, for two weeks of the first day post infection was significant ($p \leq 0.05$) while correlation between negative control group and group which received the aqueous pomelo peels extract for two weeks of the first day post infection was non-significant ($p \geq 0.05$).

Table 2:Total body weight of animals of different groups

Groups	Weight before	Weight after
Control –	30.50 ± 1.64^{a}	38.66 ± 2.50^{a}
Control +	19.66 ± 2.65^{d}	22.40 ± 6.94^{b}
Pome1o	26.83 ± 3.76^{ab}	34.00 ± 2.82^{a}
Thym1	24.50 ± 3.01^{bc}	24.50 ± 3.87^{b}
Thym2	22.83 ± 4.26^{cd}	22.16 ± 5.87^{b}
Sig.	0.000	0.000

3-Effect of aqueous pomelo peels and thyme leaves extract on parasitological study:

In the present investigation, aqueous pomelo peels and thyme leaves extract was effective in reducing warm burden, ova count and oogram in

S.mansoni infected treated mice compared to infected control positive group. The result of the present study showing that aqueous pomelo peels 600 mg/kg mice and thyme leaves extract 300 mg/kg mice weight which given for two weeks of the first day post infection with *S.mansoni*, gave positive significant correlation in total worm load and in copula count ($p \leq 0.05$), The percentage of protection were 71.43% and 64.29% respectively, while aqueous thyme leaves extract 600 mg/kg mice weight which given for two weeks of the first day post infection with *S.mansoni*, gave negative significant correlation ($p \geq 0.05$) in total worm load and in copula count, the percentage of protection was 38.57%, also all infected treated groups gave negative significant correlation in male and female count ($p \geq 0.05$) compared infected control positive group (table 3).

Table (3): Effect of aqueous pomelo peels and thyme leaves extract on worms load.

Groups	Total	male	female	copula
Control +	14.0±3.6[a]	2.0±1.0	2.0±1.0	5.0±1.0[a]
Pomelo	4.0±1.0[b]	1.3±0.6	0.7±0.5	1.0±1.0[b]
Thy1	5.0±1.7[b]	1.0±1.0	2.0±1.7	1.0±1.0[b]
Thy2	8.6±6.3[ab]	1.0±1.2	2.4±3.7	2.6±2.4[ab]
Sig.	0.071	0.58	0.82	0.051

Oogram:

The obtained data was presented in (table 4) showing increase in dead ova in infected treated groups mice compared with untreated group, mature eggs had positive significant correlation in all infected treated groups mice compared with infected untreated group, While immature eggs had negative significant correlation in all infected treated groups mice compared with infected untreated group ($p \geq 0.05$).

Tissue bound ova:

The treated infected mice groups significantly affected the tissue bound ova patterns as compared to the untreated infected mice group ($p \leq 0.05$) in the liver and intestine (table 5).

Table (4): Effect of aqueous pomelo peels and thyme leaves extract on Oogram.

Treatments	Immature	Mature	Dead
Control +	32±9.8	7.09±5.0a	23.00±5.1
Pomelo	33±12.6	2.51± 7.6b	40.00±5.0
Thy1	41±1.7	5.77± 7.5b	32.66±11
Thy2	51±18.7	1.52± 12.5b	34.66±15
Sig.	0.29	0.008	0.28

Table(5):Effect of aqueous pomelo peels and thyme leaves extract on tissue bound ova.

Treatments	Intestine	Liver
Control +	916.67±175.5a	510.00±225.2a
Pomelo	83.67±3.2b	98.3±2.8b
Thy1	194.67±2.5b	37.33±2.5b
Thy2	161.00±22.9b	155.50±59.5b
Sig.	0.000	0.002

The present investigation revealed that aqueous pomelo 600 mg/kg mice peels and thyme leaves extract (300,600) mg/kg mice on albumin, GpT and GoT was significantly effective in all groups of the treatment infected mice as compared to the untreated infected group ($p \leq 0.05$),But there is no any correlation of protein between the treatment infected groups as compared to the untreated infected group($p \geq 0.05$) table 6 and fig 6(a, b).

Table (6): Effect of aqueous pomelo peels and thyme leaves extract on albumin, total protein and liver enzymes(Gpt, Got).

Treatments	Albumin g/dL	Total protein g/dL	GPT U/L	GOT U/L
Control-	5.28±0.1ab	6.8±0.3a	95.8±17.0d	307.2±13.3cd
Control +	5.72±0.3a	6.4±1.1ab	247.0±7.5a	822.7±50.0a
Pomelo	5.03±0.3b	5.3±0.4b	175.0±25b	257.3±2.5d
Thy1	5.16±0.2b	5.9±0.7ab	171.8±18b	403.3±18.3bc
Thy2	5.18±0.3b	6.0±0.6ab	144.3±5.5c	474.8±145.2b
Sig.	0.07	0.19	0.00	0.00

There is no any correlation between the level of Cholesterol and the type of treatment in infected groups compared to the untreated infected group ($p \geq 0.05$). But there is positive correlation of IgM only in the treatment infected group with aqueous thyme extract (300) mg/kg mice ($p \leq 0.05$) as compared to the untreated infected mice. The positive correlation of IgG was only in the treatment infected mice with pomelo 600 mg/kg mice and thyme 600 mg/kg mice ($p \leq 0.05$) as compared to the untreated infected mice.

Table (7): Effect of aqueous pomelo peels and thyme leaves extract on Cholesterol and IgM and IgG.

Treatments	IgMmg/dL	IgGmg/dL	Cholesterol mg/dL
Control-	175.00±4.4a	96.33±1.2a	155.0±15.7 a
Control +	38.33±1.5cd	70.00±3c	74.6±5.9b
Pomelo	44.00±2c	81.00±3.6b	80.0±5b
Thy1	93.00±6.1b	73.67±3.7c	84.5±5.3b
Thy2	29.33±12.9d	40.33±3.5d	83.8±4.7b
Sig.	0.000	0.000	0.00

4. Discussion:

Previous studies have shown that the interaction between schistosomal parasites and the mammalian host is extremely complex many parasitologists have focused their studies on the epidemiology of the schistosomiasis.

The efficacy of praziquantel is restricted to the adult stages of the parasite and the mechanism of action of this drug is still not completely understood. Praziquantel is administered to 100 million people every year and less sensitive strains have already been isolated from those peoples. This phenomenon leads to use of large amount of drug administration which become a serious problem **(Doenhoff *et al.*, 2008).**Searching of new drug against schistosomiasis is become the need of time and also recommended by the World Health Organization **(Stothard, 2009).**

Therefore, The current study dealt with the possible antioxidant properties of aqueous citrus maxima peels (600mg/kg mice) and compared with the effect of two concentration (300- 600mg/kg mice) of antioxidant and anti helminthes properties of thyme leaves extract and the effect of aqueos citrus maxima peels and thyme leaves extract on the *S.mansoni* infected mice. Liver enzyme level estimate (Got and Gpt), IgG, IgM, some biochemical parameters (estimation of cholesterol, albumin and total protein levels) and the parasite load markers were used to evaluate their effects.

During the present work, mortality rate reached its lower level (0%) in infected treated group which received (600mg/kg mice) of antioxidant and anti helminthes of thyme leaves extract compared to (2%) in treated infected group which received (300mg/kg mice) of antioxidant and anti helminthes of thyme leaves extract. while mortality rate reached (3%) in infected treated group which received (600mg/kg mice) of antioxidant aqueous citrus maxima peels extract. Positive control group had a mortality rate (1%).

No significant improvement of animal weight in infected treated groups and untreated infected control.

The present investigation indicated that different concentrations (300, 600mg/kg mice) of aqueous thyme leaves extract and the effect of aqueous citrus maxima peels (600mg/kg mice) extract on the infected mice were highly effective in inhibiting egg-laying by adult female worms in comparison to control untreated female worms. This may be due to separation of adult worm pairs under the effect of the two used plants (aqueous pomelo peels and thyme leaves) extracts indicating that this drug affects the ability of both male and female worms to couple and consequently inhibit egg output by female adult worms.

Gerges *et al.*,1994 reported that worm burden and egg count were significantly reduced in pzq- treated animal when compared to infected untreated mice.

The effect of two plant extracts on infected mice was in reducing worm burden and egg count when compared with infected untreated mice, indicating their effective antischistosomal action. The current study revealed the importance of antioxidant aqueous pomelo peels and antioxidant and antihelminthes thyme leaves extract in the treatment of schistosomal infection and reduction of worm load and worms in copula which lead to reduction in viable eggs in tissues as well as ova count in liver and intestine, also increasing of dead eggs. Also, **Ali, 2007** proved the importance of antioxidant in the treatment of schistosomal infection and reduction of worm load as well as ova count. Also **Mahmoud *et al.*(2002)** declared that treatment of mice infected with *S. mansoni* parasite, by using black seed oil, was effective in reducing egg count in both liver and intestine. In addition, goyazensolide, a natural compound isolated from the plant *Eremanthus goyazensis*, showed a significant inhibitory effect on egg laying of *S. mansoni* female worms during *in vitro* cultivation (**Barth *et al.*, 1997).**

In addition, **Mahmoud *et al.*(2002)** stated that administration of the black seed oil to *S. mansoni* infected mice showed high activity against adult worms. In addition, similar results have been recorded on the effect of other plants against schistosome at different stages cercariae, schistosomula and adult worms **(Naples *et al.*, 1992, Ahmed & Ramzy 1997, Molgaard *et al.*, 2001 and Lyddiard *et al.*,2002).**

Some authors demonstrated that the death of the worms due to the treatment with antischistosomal drugs was attributed to metabolic disorders, mechanical destruction and muscular contraction of treated worms **(Doenhoff*et al.*,2002 and Ibrahim *et al.*, 2010).** The antioxidant effect of aqueous citrus maxima peels extract is in accordance with **Farrag *et al.* (2002, 2005)** who attributed the effects on *S. mansoni* due to the antioxidant characteristics of the vit.E and Se as antioxidant elements.

Antioxidant supplementations are thought to enhance the immunity of the host to attack the parasite and reduce infectious morbidity and protect the mice from pathogens to a certain level **(Farrag *et al.*, 2005).**

Oral administration of the studied supplements in combination with PZQ, effectively ameliorated the above serum marker of infected mice. This positive response maybe attributed to their ability to protect and stabilize cellular membranes permeability and integrity. This protective action of the used micronutrients is supported by **(Khalifa *et al.*, 2002)** and **(Soudani *et al.*, 2011).**

The possible mechanism which may explain the antischistosomal effect of *C. maxima* extract is that it contains active constituents (triterpenes and falvonoidalaglycons) which may have a direct effect on the vitality of schistosome's different stages as well as the fecundity of the remaining female adult worms **(El-Naggar, 2007).**

Some authors also documented that the plant extract strongly influence some antioxidant biomarkers (glutathione concentration, glutathione reductasectivity and lipid peroxidation) of adult worms. These parameters have an important role in the protection of the parasite against host oxidant killing **(Mohamed *et al.*,2008)**. On the other hand **El-Naggar, 2007** also proved that the plant extract affect cholinesterase activity of adult worms leading to its inhabitation and the paralysis of the worm.

The reduction of ova count by the studied supplementation is possibly due to a positive linear relationship between the egg output and the worm burden, where the reduction of the number of worms is correlated with the reduction in the ova count. However, several other factors may also explain such reduction in schistosomal egg count. These factors are a probable diminished fecundity of the worm pairs and an increased rate of egg excretion due to the egg death **(Riad *et al.*,2009).**

As albumin is the most abundant protein in serum and contains multiple lysine residues, measurement of fructosamine is mainly referred to glycated albumin **(Lapolla *et al.*,2005).**

In the present work, serum cholesterol and total protein levels were not elevated in the infected-treated groups as compared to infected control group, while AST, ALT and albumin levels were elevated in the infected-untreated group as compared to control group.This increase was significantly decreased by aqueous pomelo peels and thyme leaves extract treatment.

These enzymes are commonly employed as biological markers for hepatic cell damage and impaired cell membrane permeability or due to heavy Schistosoma egg deposition **EL-Shenawy & Soliman, 2003.**

In consistent with several studies, the present study showed that the inflammatory reactions induced in livers of *S.mansoni* infected mice are ensured by marked increase in serum ALT levels and a decrease in albumin level **(Mohamed *et al.*, 2008 and Allam, 2007).** The increase of this enzymes in serum may be due to the destruction of hepatocytes by the action of toxins of the parasite eggs leading to their release into the circulation **(Cheever and Anderson 1971)** the decrease in serum albumin may be due to its glycation by glucose forming fructosamine together with reduction in its synthesis by damaged liver. This is emphasized by the elevated serum fructosamine level **(Mahmoud *et al.*,2002 and**

Mohamed *et al.*,2008). Hypoalbuminemia is one of the factors responsible for the onset of ascites related to liver fibrosis **(Horie *et al.*,1998)**.

Increasing serum level of total IgG was observed in the treated infected mice with aqueous pomelo peels 600mg/kg mice compared to infected untreated mice while decreasing serum level of total IgG was observed in the treated infected mice with aqueous thyme leaves 600mg/kg mice extract compared to infected untreated mice. IgM had increase only in the treated infected mice with aqueous thyme 300mg/kg mice compared to infected untreated mice.

The humoral response (IgG, IgM and IgE) in acute patients to egg and worm antigens does not differ from the chronic phase. However, a high level of IgG and IgM antibodies to KLH were detected in acute patients (Iramaya Rodrigues Caldas et al., 2008)

References

1. Ahmed AH, Ramzy RMR 1997. Laboratory assessment of the molluscicidal and cecaricidal activities of the Egyptian weed, *Solanumnigrum* L. Ann Trop Med Parasitol 91: 931-937.
2. Ali HF. (2007). Evaluation of antioxidants effect of *Citrus reticulate* in *Schistosomamansoni* infected mice. Trends Med. Res. 2(1):37-43.
3. Allam G. Vasoactive intestinal peptide inhibits liver pathology in acute murine *Schistosomiasi smansoni* and modulates IL-10,IL-12 and TNF-α production. Immunobiol 212; 2007: 603–612.
4. Arias, B.A. and Ramon-Laca L (2005) Pharmacological properties of citrus and their ancient and medieval uses in the Mediterranean region. J. Ethnopharmacol., 2005, 97, 89 – 95.
5. Appiah AD, *DeVlas SJ. 2002. Interpreting low praziquantel cure rates of Schistosoma mansoni* infections in Senegal. Trends Parasitol. 18: 125-129.
6. Barth LR, Fernandes APM, Riberio-Paes JT, Rodrigues V. 1997.Effects of goyazensolide during *in vitro* cultivation of *Schistosoma mansoni*.MemInstOswaldo Cruz 92: 427-429.
7. Brunet, L. R., Finkelman, A. W. Cheever, M. A. Kopf, F. D.and Pearce, E. J. 1997. IL-4 protects against TNF-_-mediated cachexia and death during acute schistosomiasis. J. Immunol. 159:777.
8. Capron M, Capron A. Immunoglobulin E and effector cells inschistosomiasis. Science 1994;264:1876±7.].
9. Cheever AW, Anderson LA (1971).Rate of destruction of *Schistosoma mansoni* eggs in tissues of mice. Am. J. Trop. Med. Hyg. 20:62-68.
10. Chung, S.K. Kim, S.H. Choi, Y.H. E.Y. Song, E.Y. Kim. S.H. Food Ind. Nutr., 2000, 5, 42–52.
11. Doenhoff M, Cioli D, Utzinger J: Praziquantel: mechanisms of action, resistance and new derivatives for schistosomiasis. CurrOpin Infect Dis 21; 2008: 659–667.
12. Doenhoff MJ, Kusel JR, Coles GC, Cloll, D. (2002). Resistance of *Schistosoma mansoni* to praziquantel: is there a problem? Trans. R. Soc. Trop. Med. Hyg. 96:465- 469.
13. Doumas BT, Watson WR, Biggs HG. (1971). Albumin standard and measurement of serum albumin with bromocresol green.Clinica Chimica Acta. 31:87-96.
14. EL Baz, M.A; Morsy, T.A.; EL Bandary, M.M. &Motawea, S.M. - Clinical and parasitological studies on the efficacy of Mirazid in treatment of schistosomiasis haematobium in Tatoon, Etsa Center, El-Fayoum Governorate. J. Egypt. Soc. Parasit., 33: 761-776, 2003.
15. El-Naggar DM. (2007). Antibilharzial study of some extracts from Citharexylum quadrangular Jacq. Ph. D. Thesis, Fac. Pharm. Girls, Al-Azhar Univ.
16. EL-Shenawy, N.S. &Soliman, M.F.M. - Evaluation of the protective effect of two antioxidative agents in mice experimentally induced with *Schistosoma mansoni*: Biochemical and Parasitological aspects. J. Egypt. Ger. Zool., 40(A): 201-216, 2003.
17. Farrag E, Mantawy M, Foda DS, Abdel-Ghaffar FA.(2002). Role of selenium in protection of mice against *Schistosoma mansoni*in fection. J. Egypt Ger. Soc. Zool. 39(A):71-87.
18. Farrag E, Maghraby A, Foda DS (2005). Chemoprophylactic effect of selenium and vitamin E against Schistosoma mansoni infected mice. Egypt Pharm. J. 4:487-497.
19. Gerges F.R., Haroun N. H., El-Nahal H. M. S. & kamel E. G. Effect of praziquantel administration on infected albino mice with *S. mansoni,* Egypt J. Bilh, 16(1994)157.
20. Gruber BL (2003). Mast cells in the pathogenesis of fibrosis.Curr.Rheumatol.Rep. 5:147–53.
21. 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. Soc. 16:132-134.
22. Holmgren A, Björsnstedt M. (1995). Thioredoxin and thioredoxineductase.Methods Enzymol. 252:199–208.
23. Horie S, Nagai H, Yuuki T, Hanada S, Nakamura N (1998). Effectiveness of recombinant human serum albumin in the treatment of ascites in liver cirrhosis: evidence

from animal model. Gen Pharmacol. 31:811-815.
24. Ibrahim R, Nagy F, Aly E, Mohamed A, El-Assal F, El-Amir F (2010).Effect of Treatment with Antifibrotic Drugs in Combination with PZQ in Immunized *Schistosoma mansoni* Infected Murine Model. J. Am. Sci. 6(5):208-216.
25. Iramaya R C., Ana C.C.A., Lucia, F.A.V., Alda, M. S.S., Rodrigo C.O., Giovanni G.(2008) a high level of IgG and IgM antibodies to *schistosomiasi smansoni* were detected in acute patients.
26. Jackson M. Muema, Meshack A. Obonyo, Sospeter N. Njeru and Joseph K. Mwatha (2015): Antischistosomal Effects of Selected Methanolic Plant Extracts in Swiss Albino Mice Infected with *Schistosoma mansoni*. European Journal of Medicinal Plants 9(1): 1-11.
27. Jayapal M, Tay HK, Reghunathan R, Zhi L, Chow KK, Rauff M, Melendez AJ (2006). Genome-wide gene expression profiling of human mast cells stimulated by IgE or FcεRI-aggregation reveals a complex network of genes involved in inflammatory responses. BMC Genomics 7:210-227.
28. Kamel IA, Cheever AW, Elwi A, Mosimann JE, Danner R (1977). *S. mansoni* and *S. haematobium* infection in Egyption technique for recovery of worms at necropsy. Am. J. Trop. Med. Hyg. 26:696-701.
29. Khalifa TI, El-Gendi OD, Ammar HA, El-Naggar DM: IridoidGlycosides from Citharexylum quadrangular. Asian J Chem14; 2002: 197-202.
30. KunduSen, S. Saha, P. Bhattacharya, S. Bala, A. Mazumder, A.U.K. Gupta, M. Haldar. P.K. Evaluation of *in vitro* antioxidant activity of *Citrus Limetta* and *Citrus maxima* on reactive oxygen and nitrogen species. Pharmacologyonline, 2010, 6, 850-857.
31. KunduSen, S. Gupta, M. Mazumder, U.K. Haldar, P.K. Saha. P. Bhattacharya, S. Naskar, S. Panda. S.P. Exploration of *in vivo* antioxidant potential of *Citrus maxima* leaves
32. Against paracetamol induced hepatotoxicity in rats Pharmacologyonline, 2011, 7, 702-709.
33. Lapolla A, Traldi P, Fedele C. (2005). Importance of measuring product of non enzymatic cglycation of proteins. Clin. Biochem. 38:103-115.
34. Li, Y., D. Chen, A.G. Ross, M.L. Burke and X. Yu 2011. Severe hepatospleicschistosomiasis: Clinicopathologic study of 102 cases undergoing splenectomy. Hum. Pathol., 42: 111-119.
35. Lim, H.K Yoo, E.S. Moon, J.Y Jeon, Y.J. Cho. S.K. Food Sci. Biotech., 2006, 15, 312– 316.
36. Lyddiard JR, Whitfield PJ, Bartlett A. 2002. Antischistosomal bioactivity of isoflavonoids from *Millettiathonningii* (Leguminosae). J Parasitol 88: 163-170.
37. Mady, N.I.; Allam, A.F. & Salem, A.I. - Evaluation of the addition of Nigellasativa oil triclabendazole therapy in the treatment of human fascioliasis. J. Egypt.Pharmacol. exp. Ther., 20: 807-827, 2001.
38. Mohamed AA, Abo-Amou DE, Shehata MA, El-Ashery NE (2001). Glutathione peroxidase and nitric oxide in patients with chronic liver diseases. Egypt. J Schistosomiasis Infect Endem. Dis. 23:27-46.
39. Mahmoud MR, El-Abhar HS, Saleh S. 2002.The effect of Nigella sativa oil against the liver damage induced by *Schistosoma mansoni* infection in mice. J Ethnopharmacol 79: 1-11.
40. Mohamed AM, Mahmoud SS, Farrag AA (2008). Influence of Sativa seeds against liver fibrosis and consequence complications in murine schistosomiasis. Int.J. Biotechnol. Biochem. 4:325–346.
41. Molgaard P, Nielsen SB, Rasmussen DE, Drummond RB, Makaza N, Andreassen J 2001. Anthelmintic screening of Zimbabwean plants traditionally used against schistosomiasis. J Ethnopharmacol 74: 257-264.
42. Morais CN, Carvalho BM, Melo WG, Melo FL, Lopes EP, Domingues AL, Jucá NT, Martins JR, Diniz GT, Montenegro SM. (2010). Correlation of biological serum markers with the degree of hepatic fibrosis and necroinflammatory activity in hepatitis C and schistosomiasis patients.MemInstOswaldo Cruz Rio de Janeiro. 105:460-466.
43. Naples JM, Shiff CJJ, Rosler KH 1992.*Schistosoma mansoni*: Cercaricidal effects of cedar wood oil and various of its components. Am J Trop Med Hyg 95: 390-396.
44. Ndamba J, Nyazema N, Makaza N, Anderson C, Kaondera KC, 1994. Traditional herbal remedies used for the treatment of urinary schistosomiasis in Zimbabwe. J Ethnopharmacol 42: 125-132. [Links]
45. Reitman, S,.and Frankel, S. (1957). Colorimeteric method of estimation of serum aspartate aminotransferase (ASAT) activity Amer. J. Clin. Path., 28: 56.
46. Reitman, S,.and Frankel, S. (1957). Colorimeteric method of estimation of serum alanine aminotransferase (ALAT) activity Amer. J. Clin. Path., 28: 56.

47. Riad NHA, Taha HA, Mahmoud YI. Effects of garlic on albino mice experimentally infected with *Schistosoma mansoni*: A parasitological and ultrastructural study. Trop Biomed 26(1); 2009: 40–50.
48. Scora, R.W Nicolson. D.H. Taxon, 1986, 35, 592-595. Shengliang Y, Coles GC, Doenhoff MJ, Southgate VR 2001.In vitro responses of praziquantel-resistant and-susceptible *Schisotosma mansoni* to praziquantel.Int J Parasitol 31: 1227-1235.
49. Shen DZ. (2008). A target role for mast cell in the prevention and therapy of hepatic fibrosis. Med. Hypotheses 70:760–764.
50. Silva LM, Oliveira AS, dos-Santos RR, Andrade ZA, Soares MBP (2004).Comparison of immune responses of *Schistosoma mansoni*-infected mice with distinct chronic forms of the disease. Acta Tropica 91:189–196.
51. Singh, P. Shukla, R. Prakash, B. Kumar, A. Singh, S. Mishra, P.K. Dubey.N.K. Food Chem. Toxicol., 2010, 48, 1734–1740.
52. Skelly P J & Shoemaker CP. Induction cues tegument formation during the transformation of *S. mansoni*cercariae, Int J parasitol, 30(2000)625.
53. Smithers, S.R., and terry, R.J.(1965). The infection of laboratory hosts with cercariae of *S. mansoni* J. Parasitology 55,695-700.
54. Soudani N, Ben Amara I, Sefi M, Boudawara T, Zeghal N:Effects of selenium on chromium (VI)-induced hepatotoxicityin adult rats. Exp Toxicol Pathol Exp Toxicol Pathol 63; 2011:541-548.
55. Stothard JR, Chitsulo L, Kristensen TK Utzinger J. Control of schistosomiasis in sub-Saharan Africa: progress made, new opportunities and remaining challenges. Parasitol 136 (13);2009:1665-75.
56. Tendler, M. R.,Pinto, M.A., Lima,O., Gebara, G.,and Katz, N. *S.mansoni* vaccinatin with adult worm antigens, Int. J.Parasitol., 16(1986)347.
57. Tuna B, Yorukoglu K, Unlu M, Mungan MU, Kirkali Z (2006). Association of Mast Cells with Microvessel Density in Renal Cell Carcinomas. Eur. urol. 50:530–534.
58. van Den and Broucke CO. The therapeutic value of Thymus species Fitoterapia 1983; 4: 171-174.
59. Wilson, M., Schantz, PM. and Nutman, T. (2006). Molecular and immunological approaches for diagnosis of parasitic infection. (7thedi.). American Society for Microbiology. Washington, DC, USA.

9/17/2015