RESEARCH ARTICLE

Antiparasitic and physiological evaluation of *Curcuma longa* extract and/or PZQ on *Schistosoma mansoni* infected mice.

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Manuscript Info	*Abstract*
	Praziquantel (PZQ) is the drug of choice against schistosomiasis. However, PZQ was found not fully effective. A new trend from natural plant sources was used to compete schistosomiasis. In the present study, the antischistosomal activities of Curcumin extract and /or PZQ were evaluated. Parasitological and histopathological examination of liver has been explored. Moreover, Surface topography of the recovered worms was inspected by SEM. On the other hand, the total protein and DNA fragmentation percentage were estimated in infected and infected treated mice as compared to control group. Treatment of infected CD1 mice with curcumin extract alone didn't show any detectable changes in the parasitological or histological parameters. The infected mice treated with PZQ only, showed a significant decrease in the total worm burden, egg load (52%), with increase in degenerated eggs (58%) in the oogram pattern. However, treating infected CD1 mice with combined therapy (curcumin extract + PZQ) showed extremely significant reduction in female and male worm count (93% & 84%) respectively. Liver tissue egg load decreased by 88%, intestinal egg load decreased by 97%. Combined therapy decreased granuloma count and diameter with significant increase in degenerated egg in the oogram count. Moreover, the studied biomarkers, related to liver function and DNA damage were significantly improved in infected mice treated with curcumin combined with PZQ as compared to either drug alone. . In conclusion, curcumin extract in combination with PZQ has a synergetic effect and could be more promising in controlling schistosomiasis.

Introduction:-
Schistosomiasis is one of the major communicable diseases affecting human and animals either domestic or wild. It is considered the second important parasitic infection after malaria in terms of public health and economic important (Brooker et al., 2009). They reported that 207 million people in the developing countries and with 779 million, mostly children, are at risk of the infection in 76 tropical and subtropical countries where the disease is considered endemic, especially in Africa, Asia and Latin America.

One of the causative agents of the disease is a trematode worm, Schistosoma mansoni (Bos *et al.*, 2009). Schistosoma mansoni infects over 83 million people in Africa and the Middle East (Criscione *et al.*, 2009). Recent articles documented the infection of 391–597 million people, with 800 million, mostly children, at risk of the infection (King 2010, and Siddiqui *et al.*, 2011). Hotez *et al.*, in 2012 was reported that Egypt is the MENA (Middle East North African) country with the highest prevalence (7.2 out of 80.4 million people, about 9%) of schistosomiasis, in spite of the many efforts to control this parasitic infection over many years ago (El-Khoby et al., 2000).

Schistosomiasis has been suspected as a risk factor for various types of cancers e.g., bladder cancer, colorectal cancer and hepatic cancer. However, the mechanisms of the carcinogenesis are still unclear (Osada et al., 2005). The fact that Schistosomiasis has a mutagenic (Aboul-Ela, 2002) and a co mutagenic effect (El-Sharkawy et al., 2003) may be one of those mechanisms.

Schistosomiasis mostly affects the liver and intestine causing granuloma formation, fibrosis and certain necrotic changes in the hepatic tissues (Elbanhawey *et al.*, 2007).

The drug of choice for treating all schistosome species is Praziquantel (PZQ).It was developed in the late 1970s (Seubert *et al.*, 1977) and has become the only available drug used in control programs (Doenhoff et al., 2008).However, this drug is not effective in the treatment of immature forms or preventing re-infection (Magnussen, 2003). Furthermore, the extensive usage of PZQ, in non-infected and non-diagnosed individuals for prevention, lead to emergence of resistant strains of *Schistosoma mansoni* (Melman *et al.*, 2009, Van der Werf, 2003 and Zhang & Coultas 2013).

The resistance of PZQ has already been documented in Egypt and Senegal, in conjunction with new findings about its metabolism and genotoxic properties. This necessitates further evaluation of the genotoxic and mutagenic effects of this drug. As well as, there is a pressing need to develop new safe and effective drugs acting alone, or in combination with PZQ to combat the growing threat of drug-resistant parasites (El Ridi and Tallima, 2013).

The main cause of mortality and morbidity in schistosomiasis is hepatic fibrosis at chronic and advanced stages (Friedman, 2003), which develops as a result of inflammatory granuloma around deposited parasite eggs. However, PZQ alone failed to improve hepatic pathological alterations induced by schistosomiasis (El-Lakkany et al., 2011). As the most severely affected organ during *S. mansoni* infection is the liver, treatment targeting schistosomiasis-associated hepato-toxicity remains a promising approach worth investigation (Abdel-Hafeez et al., 2012).

In several countries with major endemic infections, PZQ is not only widely available for treatment but is also being actively distributed to prevent or control disease ("morbidity control"). In high-prevalence areas, treatment is now given indiscriminately to the entire population (El Khoby et al., 1998).

There are increasing efforts in the last few years to search for anti-parasitic drugs from natural sources, especially from plants, which are the main source of biologically active compounds for the development of new treatments (Magalhães *et al.,* 2009).

Traditional medicinal plants were applied by some authors for the treatment of schistosomiasis (Ndamba *et al.*, 1994, Sparg *et al.*, 2000, Molgaard *et al.*, 2001). One of these compounds is curcumin. Curcumin is a yellow pigment from rhizomatous plant turmeric (*Curcuma longa*) widely cultivated in tropical and subtropical regions throughout the world, (Cerny *et al.*, 2011). Curcumin is commonly used as a spice and coloring agent in several foods such as curry, mustard and potato chips as well as cosmetics and drugs (Okada *et al.,* 2001 and Joe *et al.,* 2004). Extensive in vitro and in vivo studies indicated that curcumin has potent antitumor, anti-viral, anti-oxidant and anti-inflammatory properties (Aggarwal and Harikumar, 2009 and Tu *et al.*, 2011). Moreover, several recent reports showed that curcumin exerts beneficial effects in animal models of liver toxicity, inflammation and cirrhosis (Chen and Zheng, 2008 and Fu *et al.,* 2008) as well as parasiticidal agents. It has an activity against *Leishmania* (Koide *et al.*, 2002 and Das *et al.,* 2008), *Giardia lambia* (Perez-Arriaga *et al.,* 2006) and *Trypanosoma* (Nagajyothi *et al.,* 2012). The first studies about the curcumin effects on *S. mansoni* showed the schistosomicidal effect of the oil extract of *C. longa* against *S. mansoni* infected mice (El-Ansary *et al.,* 2007). Allam (2009) and Morias *et al.,* (2013) described *in vivo* and *in vitro* Schistosomicidal activity of curcumin, respectively, against *S. mansoni* adult worms. Recently, El-Agamy *et al.,* (2011) showed that curcumin has a potent anti-fibrotic activity in suppressing and reversing *S. mansoni*-induced liver fibrosis.

The aim of the present work was to evaluate the antishistosomal activity of curcumin against *S. mansoni* infected mice*,* from the parasitological and histopathological point of view. As well as, some physiological parameters were estimated in PZQ and/or curcumin treated groups in comparison to the control one.

Materials and methods:-
Treatment Regimens/Experiment design:-
Forty femaleCD1 mice (20-30 gm) were maintained at Schistosoma Biological Supply Program (SBSP) at Theodor Bilharz Research Institute (TBRI), Giza, Egypt. Experiments were also performed at TBRI.All procedures contributing to this work comply with legal ethical guidelines of the Medical Ethics Committee of the TBRI on the care and use of laboratory animals. The mice were divided into eight groups (10 mice in each) as the following:

Group 1: healthy control (non-infected non-treated).

Group 2: healthy control treated orally with curcumin extract as a yellow water insoluble powder (300 mg/kg) twice/week (non-infected treated) continue from5[th] week to 8th week (4 weeks).

Group 3: healthy control treated orally with PZQ to mice in half of its curative dose
(250 mg/kg), once in the fifth week post-infection (Gonnert and Andrews, 1977) (non-infected treated)

Group4: healthy control treated orally with curcumin (300 mg/kg) for 4 weeks in addition to single half dose of PZQ (250 mg/kg) administered as combination therapy(non-infected treated).

Group 5: infected and received normal diet (infected non-treated).

Group 6: infected and treated orally with a single half dose of PZQ (250 mg/kg) after 6 weeks post infection (infected treated)

Group 7: infected and treated orally with curcumin (300 mg/kg) twice/week after 5 weeks post infection over a period of 4 weeks (infected treated).

Group 8: infected and treated orally as combined therapy with curcumin (300 mg/kg) twice/week after 5 weeks post infection over a period of 4 weeks and single half dose of PZQ (250 mg/kg) after 6 weeks post infection (infected treated).

At the end of the experiment, all mice were sacrificed. Samples of liver tissue and serum were collected for further analysis.

Drugs:-
Praziquantel drug (suspension), a product of Egyptian international pharmaceutical industries company (E.I.P.I. Co) was purchased locally. Curcumin obtained from (XI'AN LUKEE BIO-TECH CO., LTD).

Chemicals:-
All common chemicals used were purchased from one of the following suppliers Sigma Co. (St. Loius, MO, USA). All other reagents were of the highest grade commercially available.

Mice infection:-
S. mansoni cercariae and clean CD1 female albino mice were supplied by the Schistosome Biological Supply Program (SBSP) at Theodor Bilharz Research Institute, Imbaba, Giza, Egypt. Male CD1 albino mice were infected with (60 ± 10) *S. mansoni* cercariae via subcutaneous route suspended in 0.1 mL solution. The ethical obligations to experimental animals were followed. The experiments were carried out at Zoology Department, Faculty of Science, Ain Shams University, Cairo, Egypt.

Ethics Statement:-
The animal experiments were conducted in accordance with the ethical guidelines for animal handling and care as established and approved by the Ethical Research Committee of Theodor Bilharz Research Institute (TBRI), Giza, Egypt.

Worm recovery:-
Worms were recovered from the hepatic portal system and mesenteric blood vessels by perfusion technique according to the method of Clegg and Smithers (1972).

The worms were examined and counted (total number of male and female worms) under a binocular microscope.

Egg count:-
Eggs in the liver and intestine were counted after scarification of mice, a piece of the liver tissues and the small intestine were stored at −20°C for counting of deposited eggs, where tissues were digested with 4 % potassium hydroxide (KOH) as described by Cheever (1970). The total liver eggs, total intestinal eggs and the fecundity of female worms were determined.

An oogram study:-
An oogram was performed by studying alteration in the percentages of the various stages of viable eggs as well as of the increase in the percentage of dead eggs in the mucosa of the small intestine as described by Pellegrino et al. (1962).

Histopathological investigations:-
A small piece of liver was removed from the left lateral lobe from each mouse after perfusion, and preserved in 10% formalin. Liver sections were prepared and stained with Haematoxylin and Eosin. Reductions in mean counts and diameters of hepatic granuloma, in the treated groups, were then determined and compared to those in the infected untreated control groups. Granuloma structural configurations, including cellular components and associated hepatic histopathological alterations were also examined.

Electron Microscopy:-
Adult worms of *S. mansoni* (7weeks old) were fixed in 3% gluteraldehyde on ice as a fixative for electron microscopy examination for 2h then post-fixed in 1% osmium tetra oxide. Worms were then dehydrated with ethanol and at critical point dryer. Specimens were mounted on metal stubs, coated with carbon and gold then examined with a JOEL JEM-1200 EXII electron microscope at Central Lab., Faculty of Science, Ain Shams University, Cairo, Egypt.

Blood Samples preparation:-
Blood was collected   from sacrificed mice in vacuum container and centrifuged 3000 g for 10 min. Plasma samples were collected and stored at -80 until used. The liver was dissected out, and washed in ice-cold saline, blotted dry, then stored at -80 until used for the DNA fragmentation assay.

Biochemical Analysis:-
The alanine amino transferase (ALT), aspartate amino transferase (AST) and alkaline phosphatase (ALP) levels in plasma, markers of hepato-cellular damage, were established by colorimetric assay using a commercial kit (spectrum diagnostics Egyptian company of biotechnology, Egypt).Total protein and albumin was determined to indicate the tissue damage and exudation were measured according to Doumas *et al.,* (1981) using commercial kit that mentioned above

DNA fragmentation% assay:-
A distinctive feature of apoptosis at the biochemical level is DNA fragmentation. This method was used as a semi-quantitative method for measuring apoptosis.DNA fragmentation% assay was conducted using the procedure of Perandones *et al* (1993) with some modifications.

The liver tissue (40 mg) was mechanically dissociated in 400 µl hypotonic lyses buffer to obtain cell lysate. The lysate was incubated at 50 °C for 2.5 hrs. The cell lysate was centrifuged at 13.800xg for 15 minutes. The supernatant (SN) containing small fragments of DNA were separated immediately. The pellet was re-dissolved in TE to be homogenous prior to centrifuge to ensure adequate separation of fragmented DNA from intact DNA. Equal volume of 10% TCA was added to both supernatant and pellet, and then incubated for 15 min at room temperature. The samples were centrifuged at 2000rpm for 10 min. The periceptate was re-suspended in equal volume of 5%TCA, then incubated at 100 °C (boiling water bath) for 10 min. the samples were centrifuged at 1500 rpm for 15 min. The supernatant containing the extracted DNA was left to cool at room temperature. Two volumes of colorimetric reagent were added to one volume of the extracted DNA (small and large fragments), kept at 30°C overnight till blue color is developed. The developed blue color was quantified spectrophotometrically at 600 nm (Spectrophotometer). The results were expressed as amount of % fragmented DNA by the following formula;
% Fragmented DNA = T x 100/T + B, where T(supernatant   and B (pellet)

Statistical analysis:-
Statistical evaluation was conducted with Instate Program Graph Pad. Software Inc, San Diego, USA, version3.6, Copyright©1992-2003 Results were expressed as mean ± S.E. The results were analyzed for statistical significance by one way ANOVA followed by Tukey-Kramer multiple comparison test. Values of p < 0.05 were regarded as significant. All values were tested for normality. Student's unpaired t-test and the Mann-Whitney test were used to analyze the statistical significance of differences between mean experimental and control values, and a *P* value of ≤0.05 was considered significant.

Results:-
Worm reduction:-
Reduction in total worm burden in Curcumin treated group was statically non-significant ($P \geq 0.05$) with 35% reduction compared to the control group. On the other hand, *S. mansoni* infected and treated groups with multiple doses of Curcumin extract accompanied with PZQ or PZQ only resulted in a highly significant (P<0.0001) reduction in the total worm burden, with reduction ranging between 74 % and 81% compared to the infected control group. Reduction in Female and male worm burdens were statistically non-significant ($P >0.05$) ranging between 14% and 24% in Curcumin treated groups compared to the control ones (Table 1).

On the other hand, PZQ treated groups showed highly significant reduction in male and female worm burden (*P<* 0.0001). Also the groups of combined therapy (Curcumin and PZQ) showed extremely significant reduction *(P<* 0.0003) in male and female worm burden that reached 84% in male and 93% in female worm burden.

Tissue egg load:-
Liver tissue egg load showed non-significant ($P \geq 0.05$) reductions of 7% followed by28% in curcumin and PZQ treated groups respectively, and reached 52% in combined treated group (Curcumin and PZQ). A negligible, non-significant ($P \geq 0.05$) reduction in eggs per gram tissue of small intestine was seen in Curcumin treated group. In contrast, an extremely significant reduction *(P<* 0.0002) reduction in eggs per gram tissue of small intestine that reached 88% and 97% in PZQ and combined treated groups respectively (Table 2).

Hepatic granuloma measurements:-
Table 3 shows the effects of Curcumin or \and PZQ in reducing the mean granuloma counts/low power field and their diameters in treated groups compared to those in the control ones. Only groups treated with PZQ or \and curcumin showed statistically significant ($P \leq0.05$) reductions in the mean count of hepatic granulomas of 30.6% and 34.4%, respectively, whereas a statistically non-significant reduction of only13.8% ($P \geq 0.05$) was observed in curcumin treated groups. Whereas, PZQ or \and Curcumin treated groups showed a statistically significant ($P \leq0.05$) reductions in the mean diameter of the hepatic granulomas of 35.2% and 41.5%, respectively, in contrast, a statistically non-significant reduction of only12.6% ($P \geq 0.05$) was observed in curcumin treated groups. We could say that, Curcumin and PZQ enhance the mode of action of each other in the combined treatment.

Oogram pattern:-
The percentage of intact eggs in the treated group with Curcumin was 78.8%, while this percent slightly decreased to 42% in PZQ treated group, on the other hand the percentages of intact eggs is significantly decreased (P ≥0.05) and reached 24% in combined treated group (Curcumin and PZQ) as compared to a percentage of 95% in the control group. Likewise, there were no statistically significant (P ≤0.05) changes in the percentages of dead eggs, in Curcumin treated group 21%, and the percentage of the dead eggs increased 58% in PZQ treated group, while the dead eggs percentages highly increased 76% in combined treatment group (Curcumin and PZQ) compared to a percentage of 5% in the control group (Table 3).

Histopathological changes:-
Fibro-cellular granulomas, a chronic granulomatous inflammation constituted around 75% of the estimated granulomas in the infected untreated control group with mean diameter of 336.24 μm ±15.83. They are marked by concentric fibrosis with many fibroblasts encircled the trapped eggs with large peripheral zone of chronic inflammatory cells, resulted in disorganization of hepatic strands and lobular structure with vacuolar degeneration. Central veins and portal venules were dilated and portal spaces were infiltrated with inflammatory cells. In addition, presence of active newly formed cellular granulomas constitutes about 20% of the estimated granulomas in the

infected untreated control group (Fig.1a). With the progression of infection, granulomas were reduced in size and started to heal, with fewer inflammatory cells and more fibrous tissue content and this fibrous granuloma constituted around 5% in the infected untreated control group.

Treatment with Curcumin alone has a negligible effect where a large granuloma with a trapped egg, accumulation of inflammatory cells surrounding the granuloma accompanied by vacuolated hepatocytes and disorganization of the hepatic strands accompanied by decrease in the active cellular granuloma by 20% and increase in the healed fibrous granuloma by 15% of the estimated granuloma compared to their corresponding infected untreated groups (Fig.1b).

Administration of PZQ alone showed medium sized fibro-cellular granuloma with starting ova degeneration but with presence of a peripheral zone of chronic inflammatory cells, resulted in disorganization of hepatic strands and lobular structure with vacuolar degeneration (Fig.1c).

A combined treatment with Curcumin and PZQ resulted in a remarkably reduced size of granuloma, increase in degenerated ova and less inflammatory cells within the hepatic granuloma, in addition to a marked decrease in inflammatory cells found in the portal tracts and return to the normal architecture of the hepatic strands and lobular structure. These results accompanied by a decrease in the active cellular granuloma to reach 0% and increase in the healed fibrous granuloma by 30% of the estimated granuloma compared to their corresponding infected untreated groups (Fig.1d).

Ultrastructure examination:-
Scanning electron microscopy revealed that the dorsal surface of *S. mansoni* male worms recovered from infected control group had intact tegument bearing large numerous tubercles with evenly distributed spines. Intertubercular surface composed of circular folding with minute sensory papillae (Fig. 2a). Oral and ventral suckers are obviously rounded to oval in shape (Fig. 2b).

In the majority of male worms obtained from mice treated with Curcumin extract, there is a slight change in the aspect and form of the tubercles that appeared reduced in some instances accompanied with loss of some of their spines, and, as light shrinkage in the inter-tubercular areas (Fig. 2c).In addition to distortion oforal and ventral suckers of some worms (Fig. 2d)

The tubercles of the male worms obtained from PZQ-treated mice, were heavily wrinkled and collapsed with loss of nearly all of the spines, in addition of being short and blunt. Also, swellings and pronounced oedema were noted in the integumental areas (Fig. 2e). Sever dilation and oedema in the oral sucker with lose of its spines was also observed (Fig. 2f)

While combined treatment with both Curcumin extract and PZQ cannot be showed due to the very limited number of perfused worms, but we could expect that the effect will be extremely significant.

Evaluation of plasma total protein and albumin:-
Total protein and albumin levels in plasma of control and infected mice are presented in (Fig.3). As illustrated in Fig.3 (a&b), plasma levels of total protein was elevated significantly in infected non-treated mice ($P<0.05$), while a significant decrease of albumin was observed as compared to control group. Supplementation of Curcumin or PZQ to non-infected mice showed significant change in total protein ($P<0.01$). On the other hand, the infected treated mice with Curcumin showed significant increase in albumin level as compared to control group ($P< 0.05$) (Fig.3b).

Liver function assessments:-
Infection of *Schistosoma mansoni* damages the hepatic cells leading to a significant increase in serum levels of ALT ($p<0.01$, fig.4a) while, no significant change was observed in serum AST and ALP as compared to control non-infected group (fig 4b&c). On the other hand, Treatment of the infected mice with curcumin plus PZQ decreased the levels of serum AST ($p<0.05$), ALT ($p<0.01$) levels significantly as compared to the infected untreated group (Fig.4a&b). administration of Curcumin and PZQ in combination decrease the AST activity as compared to their administration separately in infected group ($p < 0.01$ and $p < 0.05$, respectively)(fig4b).

Supplementation of PZQ or curcumin plus PZQ to infected mice showed significant increase in serum ALP level ($P<0.01$, $P<0.001$, respectively) as compared to infected non-treated mice (Fig.4c).

Hepatic DNA fragmentation:-

The degree of DNA fragmentation was determined by separating the cleaved DNA from the intact chromatin by centrifugation and measuring the amount of DNA present in the supernatant and pellet using the diphenylamine method (Sellins& Cohen, 1987). The degree of DNA fragmentation refers to the ratio of DNA in the supernatant to the total DNA in the supernatant and pellet.

Compared to untreated control and infected mice, the changes in hepatic nuclear DNA fragmentation seen following different treatment of infected mice with curcumin and/or PZQ are represented. (Fig.5). *S. mansoni* infection caused significant (*P*<0.05) degree of hepatic DNA fragmentation compared to the control mice. In addition, treatment with PZQ or curcumin alone led to a significant increase (P < 0.01) in hepatic DNA fragmentation, compared to the infected untreated mice. However, combined treatment with PZQ and curcumin resulted in a remarkable significant (P < 0.01) decrease in % DNA fragmentation reaching a level close to that of control (Fig.5).

Discussion:-

In the present study, treating *S. mansoni* infected mice with *Curcuma longa* extract alone, could not induce significant reductions in total worm burdens, liver egg count and intestinal egg count. The total worm burden was non-significantly reduced and doesn't exceed 35% in curcumin treated group. Female worm reduction was also minimal and statistically non-significant (14%), in addition to the male worm reduction was also non-significant but reached 24%. Meanwhile, the effect of curcumin extract alone on tissue egg loads was nearly negligible. These results were in the contrary of El-Ansary *et al*. (2007) who reported that treatment with *C. longa* showed significant reduction in worm burden and ova count compared to three months infection duration. This could be attributed to the short term of treatment duration in our study.

Treatment of *S. mansoni* infected mice with PZQ showed a significant reduction in total worm burden that reached 78% in male worms and not exceed 68% in female worms. In contrast, PZQ treated group does not show any significant reduction in liver egg count that was only 28% reduction but this reduction increased to 88% in intestinal egg count compared to the infected control group. These results coincide with El-Ansary *et al*. (2007) and Farah *et al*. (2000) who reported that worm burden and egg count were significantly reduced in PZQ-treated animals when compared to the untreated groups. On the other hand, we could say that after PZQ treatment male and female worms were still alive and PZQ does not show complete eradication of worms, it is in agreement with Yang *et al.* (2009) and El-Ansary *et al.* (2007) who revealed that after a month post infection in mice treated with PZQ there was some male worms still alive.

Although *C. longa* extract when used in treatment of Schistosomiasis alone was less effective in reducing worm burden (34 %) when compared to PZQ (74%), On the contrary, using combined treatment of curcumin and PZQ with its half dose caused highly significant reduction in worm burden that reached 84% in male worms and 93% in female worms. In addition to decrease in liver egg count by 52% that is highly increased to 97% reduction in intestinal egg count. There is obvious worm shift to the liver and this could be due to the inability of the worm to go to its normal path in the portal vein.

The combined treatment of curcumin and PZQ showed extremely reduction of worm burden and reduction of deposited eggs. These observations were also reported by Utzinger *et al*. (2002), Suleiman *et al*. (2004) and Mati *et al*. (2010) as they revealed that reduction in the worm recovery and egg density in treated mice was considered as a strong indication of the effectiveness of antischistosomal drugs.

Histopathological investigations of liver sections in group treated with Curcumin only caused a non-significant reduction of only 13.8% and 12.6 in granuloma count and diameter respectively.

Compared to the control group, this disagreed with the report of Allam (2009) who showed that treatment of *S. mansoni* by Curcumin was effective in reducing worm, tissue-egg burdens and hepatic granuloma. It is worth to mention that although Praziquantel is reported to be highly curative, but it has a non-significant effect on granuloma count and diameters as well as mice still suffer the progressive disease pathology with chronic inflammatory cells. However, using a combined therapy showed a decrease in granuloma count and diameter that does not exceed 41% reduction with decrease in inflammatory cells and complete degeneration of ova. These agreed with El-Ansary *et al*. (2007) and El-Fakahany *et al*. (1993) who revealed medium sized fibro-cellular granuloma with starting ova

degeneration and a peripheral zone of chronic inflammatory cells accompanied by disorganization of hepatic strands architecture in PZQ treated groups.

Although Curcumin did not cause a significant reductions in mean granuloma diameters or count, it appeared to be highly efficient in inducing heal of hepatic granulomas that reached 30% with disappearance of active cellular granuloma compared to those in the control. These results were similar to that discussed by Abdul-Ghani *et al.*(2010) on Myrth, revealing highly efficacious in inducing healing of hepatic granulomas compared to those in the control.

On the other hand, treatment of *S. mansoni* infected mice with curcumin induces a slight alteration in the oogram pattern of eggs in the mucosa of the small intestine. These alterations were marked by non-significant reduction in intact eggs 78% and slight increase in dead eggs 21% as compared to the infected control group which possesses 95% intact cells and 5% dead cells. These reductions increased in PZQ treated groups and highly increased in combined treatment group.

Changes in the number and character of eggs in the oogram provide a simple, sensitive, and reliable criterion for the screening of drugs active against *S. mansoni* that represents the effects of the combined therapy on oviposition, as well as on the maturation and survival of eggs trapped in the intestinal mucosa.

As these results, alterations in the oogram reveal the success of the combined therapy to interrupt the processes of oviposition and egg development. Generally, it could be said that this combined therapy exhibit an ovicidal activity on eggs already laid in the intestinal wall, these findings were inconsistent with the results done by Abdul-Ghani *et al*.(2010) on Myrth that shown the failure of myrrh to interrupt the processes of oviposition and egg development in schistosome infected mice.

Combined therapy causes nearly complete eradication of worms; significant decrease in the number of intact mature eggs accompanied by increase in the number of dead eggs with decrease in granuloma number and diameter that accompanied by disappearance of active cellular granuloma and increase in healed fibrous granuloma with return to the normal architecture of the hepatic strands and lobular structure.

According to our results we conclude that Curcumin is a remarkable non-toxic plant with many medical properties, but it could not be used alone as an anti-schistosomal drug whereas it only improves the alterations of hematological, biochemical ,antioxidants parameters previously induced in *S. mansoni* infected mice. These results coincide with Mahmoud and Elbessoumy (2013); who reported that this could be more promising in controlling the pathology of this disease which is mostly due to the toxins released by the eggs.

On the other hand, concomitant administration of curcumin and PZQ exceeded the effect of the PZQ alone. Most measurements were markedly improved in the bi-therapy intervention group, resulting in a very high healing level of hepatic granulomatous lesions, as evidenced by the ameliorative effects for all parameters studied suggesting a synergistic effect of curcumin with PZQ.

Hepatic damage can affect the metabolic processes in the body due to the role of liver in general metabolism. Enzymes are necessary for normal cellular metabolism including that of the liver (Rajamanickam and Muthuswamy, 2008). Hepato protective activity of curcumin separately or in combination with PZQ was evaluated on *Schistosoma mansoni* infected mice by estimation of serum hepatic enzymes. Hepatic cells appear to participate in a variety of enzymatic metabolic activities.

Plasma ALT levels were elevated (p<0.01) in the infected-untreated group as compared to control group, while this increase was significantly decreased (p<0.01) by Curcumin/PZQ combination treatment. These results agree with that obtained by Nahla *et al*, (2008) ; Naglaa *et al*, (2012) and Mahmoud and Elbessoumy, (2013) who observed that Infection of mice with *S. mansoni* showed a significant increase in serum levels of ALT. On the other hand, no significant change was observed in serum AST and ALP levels in the infected-untreated group as compared to control group. In contrast, Nahla *et al,* 2008 and Mahmoud and Elbessoumy, (2013) reported a significant elevation in serum AST and ALP levels in *Shistosoma mansoni* infected mice. 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 and Soliman 2003). There was significant difference between the effect of Curcumin and PZQ separately or in combination on AST, ALT and ALP.

On the other hand, Mahmoud and Elbessoumy, (2013) found that the infected treated mice treated with Curcumin showed reduction in the serum ALT and AST levels as compared to infected non-treated mice.

Due to the aforementioned, it is clear that the combination of Curcumin and PZQ had more potent effect on ALT, AST of the infected mice than the treatment with each compound separately.

Allam (2009) reported that, infected mice treated with Curcumin restore the hepatic ALT and AST activities that were decreased by *S. mansoni* infection. This amelioration in the activities of liver enzymes could be attributed to the reduction in hepatic granuloma size and fibrosis as well as absence of necrotic hepatic tissue in infected treated mice (Allam, 2009).

Schistosoma mansoni causes a rather silent infection in humans until the parasite has accomplished oviposition, by 6 to 8 weeks after infection, when it becomes symptomatic (Neva and Brown 1994). Some eggs laid in the mesenteric vessels are carried by the blood flow and become trapped in the liver. Once the eggs have reached the liver, they can no longer be eliminated, and they promote granulomatous reactions that isolate the eggs from the hepatic parenchyma. Collagen is deposited around the eggs by myofibroblasts. However, the fibrosis and vascular damage alter the blood flow in the liver, producing portal hypertension (Cheever AW, Andrade ZA. 1967, El Scheich *et al*, 2012). An inflamed, enlarged, and fibrotic liver was associated with increased AST and ALT levels in serum, which are markers of hepatic injury. Here, we have showed that the administration of curcumin in addition to PZQ at the chronic phase of infection is capable of reducing ALT and AST levels in serum, hepatomegaly, and hepatic granuloma size. These results indicate that combined treatment with curcumin and PZQ in mice infected with *S. mansoni* interferes with the overall hepatic disease, reducing hepatic injury and fibrosis and ameliorating morbidity (Mata-Santos *et al*, 2014).

In addition we found significant abnormalities in plasma total protein and albumin in infected mice as compared to control group. Plasma levels of total protein (P<0.05) was increased significantly in infected non-treated group as compared to control group, while albumin level was significantly decreased (P<0.05). These results supported through the work of Mohammed *et al*., (2006) who recorded a marked increase of protein content in *S. haematobium* infected patients. In addition Naglaa *et al*., (2012) reported a significance reduction in albumin levels in mice infected with *Schistosoma mansoni*. These results are in accordance with that obtained by Smithers and Walker (1961) while it opposited to that reported by Mahmoud and Elbessoumy, (2013); Pope *et al.,* (1980) El-Heig *et al.,* (1977) who recorded a marked decrease of protein content in *S. mansoni* infected mice. The increase of serum total proteins and the decrease of plasma albumin may have been due to liver dysfunction. The increased levels of plasma total proteins, associated with the intensity of infection, could also have been due to immunological reactions and the production of immunoglobulin, which occurred before treatment. The low level of serum albumin could also be explained by high protein loss due to heavy infection. El-Hawry recorded that the decrease in albumin fraction may be due to decrease anabolism or increased catabolism, malnutrition and/or mal-absorption may contribute to the decreased biosynthesis of albumin.

It was obvious, following oral co-administration of curcumin with PZQ in infected rat, that there was a significant improvement in the levels of these indices, which indicates recovery of some liver functions. These results agree with that obtained by El-Ansary *et al*., (2007) who observed that infected mice treated with curcumin revealed low serum level of protein. At contrast, Curcumin was reported to cause elevation of total protein and albumin levels compared with infected non treated mice (Mahmoud and Elbessoumy, 2013; Pope *et al*.,1980 and El-Emam *et al*., 2011), although our study agree with them that treatment with curcumin significantly elevate the albumin level as compared to infected non treated mice.

EL-Ansary *et al*., (2007); Kaiser *et al*., (1989) found normal level of serum proteins before and after treatment.
It is likely that oxidative stress is a key factor in the process of liver fibrosis. It was evidenced previously that oxygen free radical damage leads to liver fibrosis in murine models of schistosomiasis (Muriel, 2009; Ohnishi *et al.*, 2013). It was suggested that generation of reactive oxygen (ROS) and nitrogen species (RNS) likely contribute to both onset and progression of S. mansoni-induced liver fibrosis. Thus, new schistosomicidal drugs that ameliorate the activity of the oxidative stress system may effectively alleviate liver injury.

It was recently reported that elevated oxidative/nitrosative stress leads to fragmentation of nuclear DNA in liver, which contribute to hepatocellular apoptosis as well as necrosis (Mukhopadhyay *et al*., 2011).Furthermore, DNA fragmentation is considered as a hallmark event in cell apoptosis. In our study, we evaluated the level of DNA damage through quantification of fragmented DNA. We reported high DNA fragmentation level quantified in hepatic tissues of infected mice potentially concurrent with inflammatory granulomatous reactions.

Recent studies reported oxidative/nitrosative DNA damage in *S. haematobium*-associated bladder cancer supports our results of a strong correlation between *S. mansoni* infection and increased levels of DNA damage in liver (Ma *et al*., 2011).

In our study, we evaluated the level of DNA damage through quantification of fragmented DNA in PZQ-treated mice. In contrast to our results, in a previous report (Eidetal., 2014) showed a reduced level of DNA damage in infected PZQ-treated animals compared to control ones.

Administration of Curcumin, to non-infected animal group showed a significant increase in liver DNA fragmentation % as compared to control group which also exposed by DNA ladder assay banding pattern. This result was supported with Jun Cao *et al*. (2006) who reported that curcumin can apparently act as a prooxidant. Curcumin itself resulted in ROS that damage in human peripheral blood lymphocytes DNA (Kelly *et al.*, 2001).

Jun Cao *et al*. (2006) inferred that ROS and lipid peroxidation generated directly or indirectly by Curcumin underlies the mechanism of curcumin-induced DNA damage. So doses of Curcumin imposed oxidative stress and damaged DNA.

On the other hand, Curcumin treatment combined with PZQ for infected animals decreased remarkably the level of fragmented DNA level compared to infected untreated mice. The reduced level of DNA damage in infected curcumin + PZQ-treated animals as compared to infected ones, may be attributed to the elimination of the parasite and the reestablished immunological responsiveness of the host.
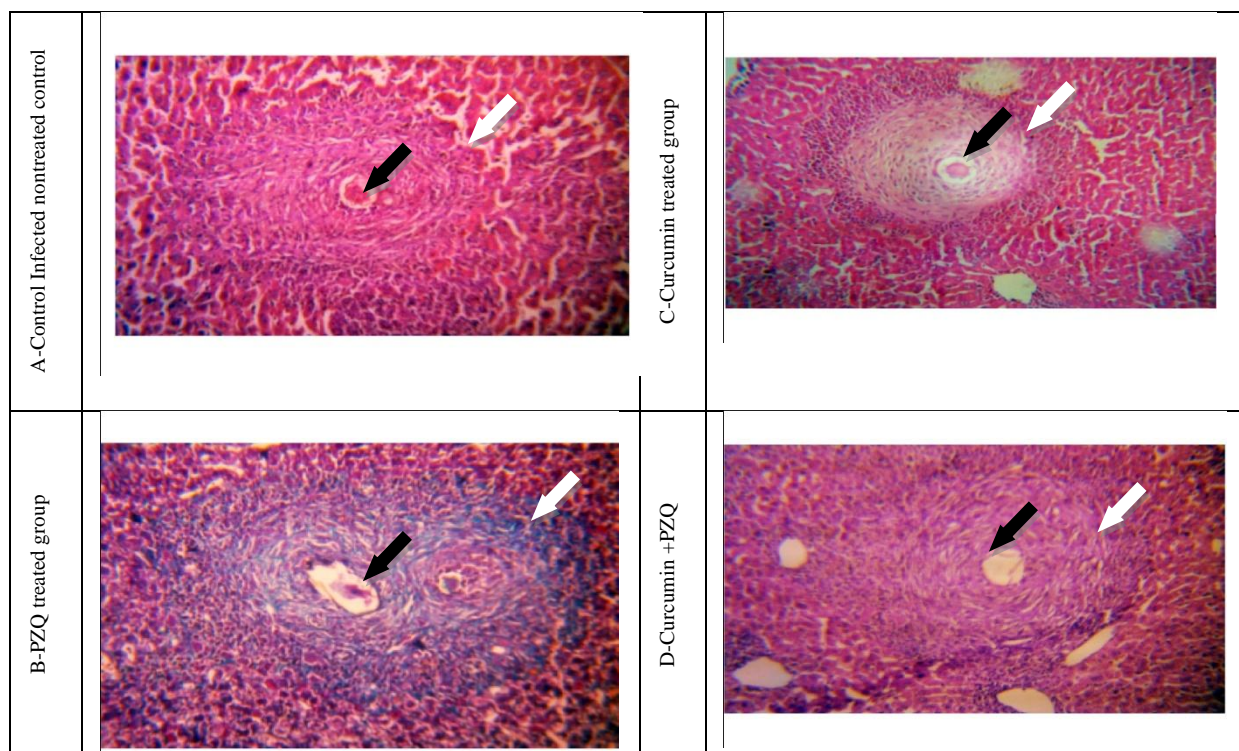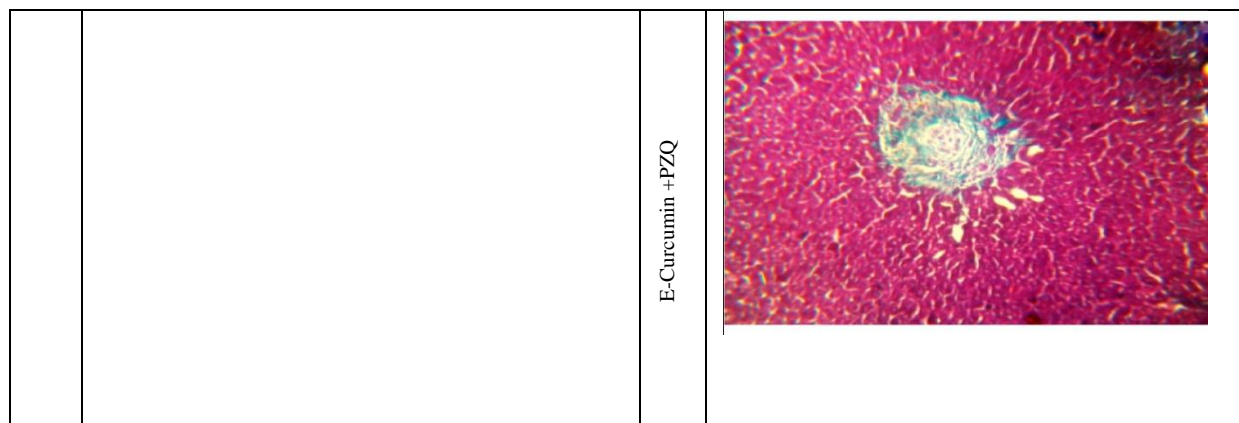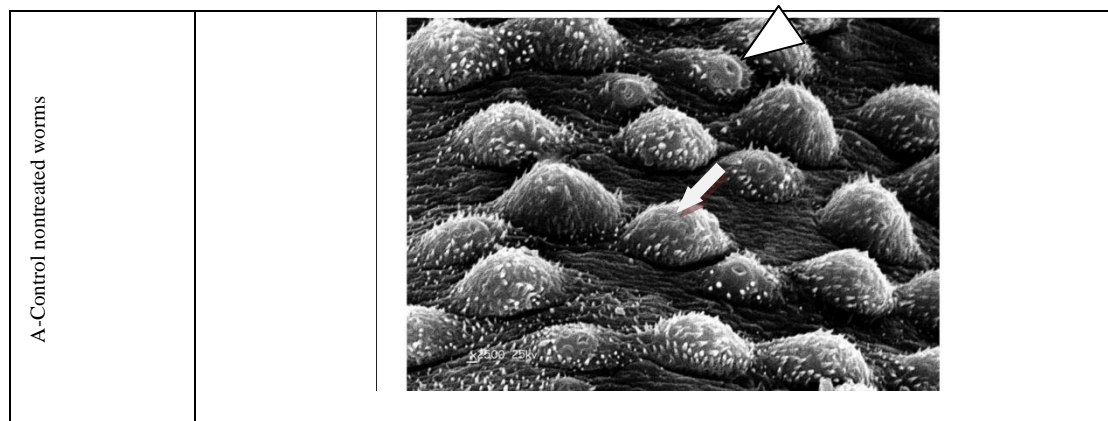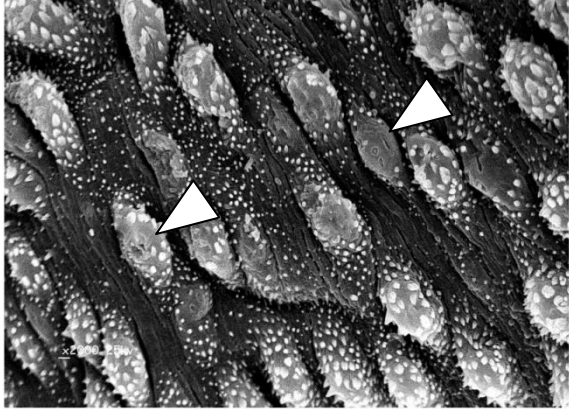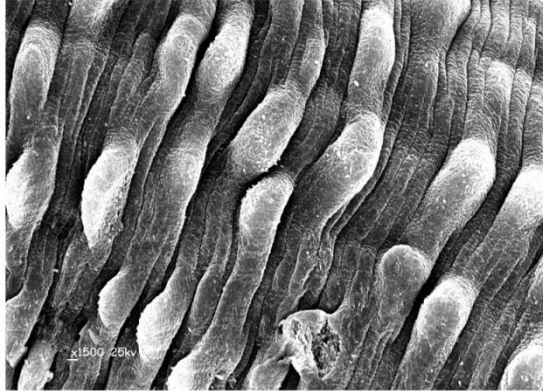
Fig. 1.A.Photomicrograph of a liver section of *S. mansoni*-infected,non-treated mouse showing a large fibrocellular granuloma with a trapped egg(black arrow), accumulation of inflammatory cells surrounding thegranuloma with large peripheral zone of chronic inflammatorry cells (white arrow) and disorganization of the hepatic strands (Sections from the liver of infectedmice killed 7 weeks, examined by light microscopy (×400).B.Photomicrograph of a liver section of PZQ treated S. mansoni mice alone showing medium sizedfibrocellular granuloma with starting ova degeneration(black arrow) and a peripheral zone of chronic inflammatorry cells (white arrow), with disorganization in the hepatic strands.C-Photomicrograph of a liver of Curcumine treated mice showing a large granuloma with a trapped egg (black arrow) , accumulation of inflammatory cells surrounding theGranuloma (white arrow), with disorganization in the hepatic strands.D-hotomicrograph of a liver of combined treated S. mansoni- infected mice showing a remarkably reduced sized fibrocellular granuloma with complete ova degeneration (black arrow)and less inflammatory cells (white arrow).E-Photomicrograph of a liver of combined treated S. mansoni- infected mice showing a marked decrease in inflammatory cells in portal tracts and return to the normal architecture of the liver lobule.

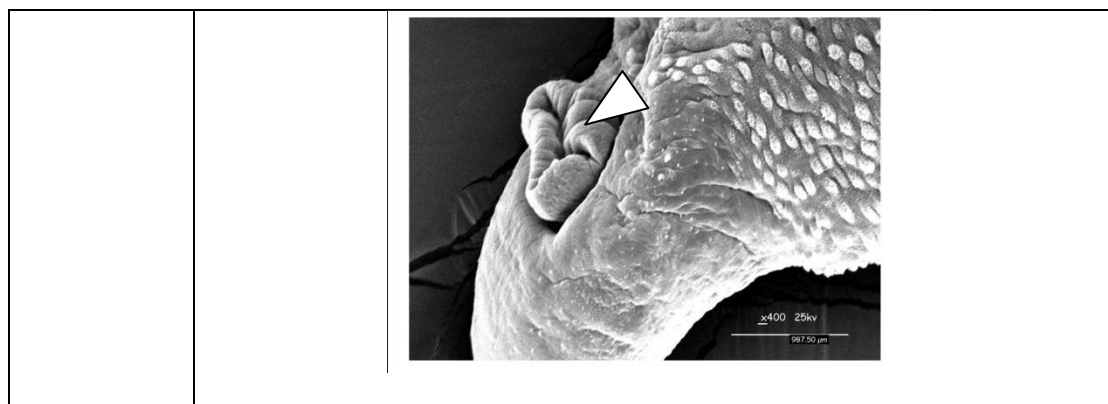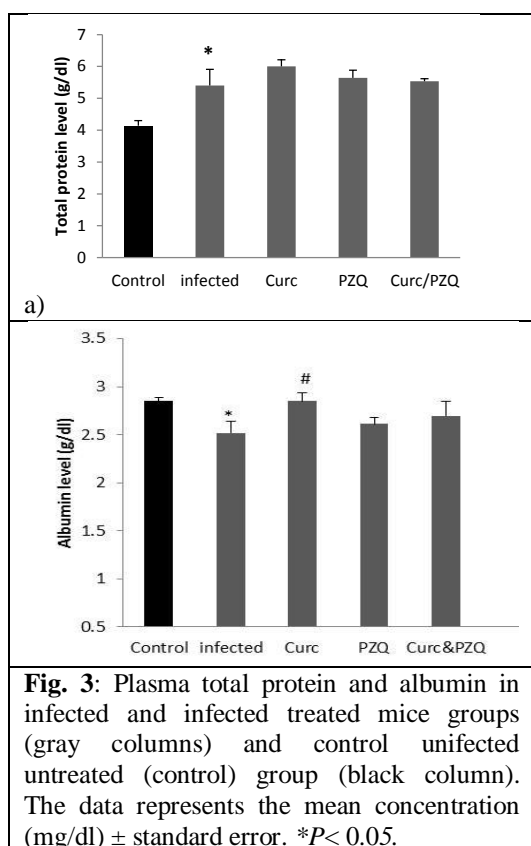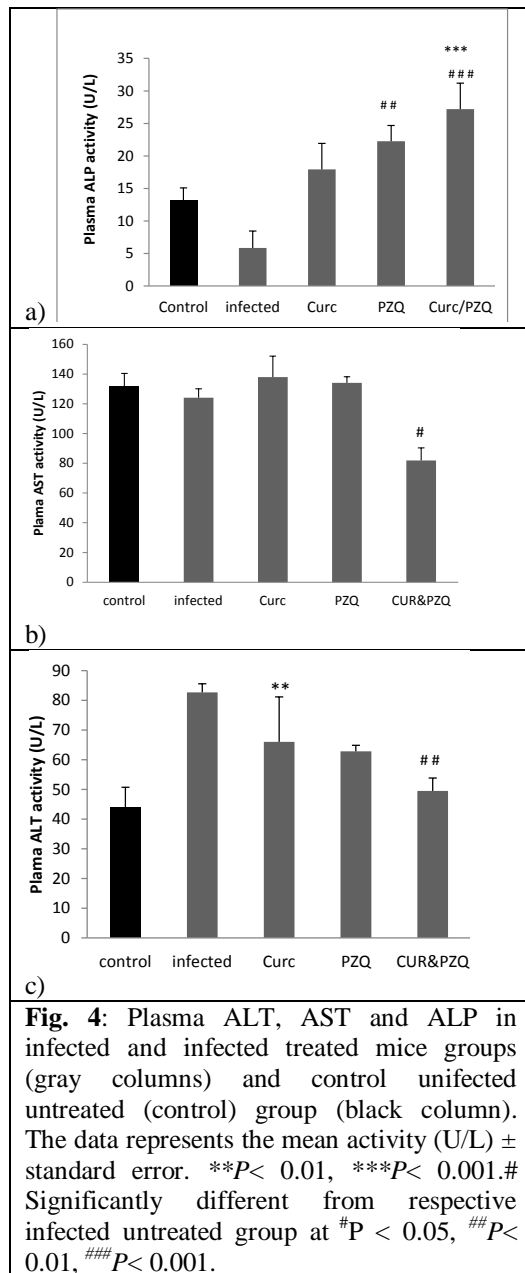|	
B-Control nontreated worms	
C-Curcumin treated worms	
D-PZQ treated worms	

Fig. 2.Electromicrographs showing the tegument of Schistosoma mansoni ; (A) Tegument of untreated worms has numerous tubercles (arrow) with evenly dispersed spines. Intertugumental areas have circular folding with minute sensory papillae (arrow head). (x600); (B) Ventral sucker of untreated worms with normal architecture; it was rounded to oval in shape.(x150); (C)Tegumental surface of worms treated with curcumin extract, some tubercles appeared small (arrows), however lost some of their spines (arrow heads). Shrinkage and wrinkling in the areas between the tubercles was observed. (x1200); (D)Intertegumental areas of worms treated with PZQ have swellings and pronounced oedema, which appeared thickened with raised knobs. Sever dilation and oedema in the oral sucker (arrow head) was also observed. (x300).

Fig. 3: Plasma total protein and albumin in infected and infected treated mice groups (gray columns) and control unifected untreated (control) group (black column). The data represents the mean concentration (mg/dl) ± standard error. *$P < 0.05$.

Fig. 4: Plasma ALT, AST and ALP in infected and infected treated mice groups (gray columns) and control unifected untreated (control) group (black column). The data represents the mean activity (U/L) ± standard error. **$P< 0.01$, ***$P< 0.001$.# Significantly different from respective infected untreated group at #$P < 0.05$, ##$P< 0.01$, ###$P< 0.001$.

Fig.5: effect of Curcumin with/ without PZQ on hepatic DNA fragmentation in S.mansoni-infected mice.* Significantly different from respective unifected untreated (control) group at **$P< 0.01$. # Significantly different from respective infected untreated group at #$P< 0.05$, ###$P< 0.001$.
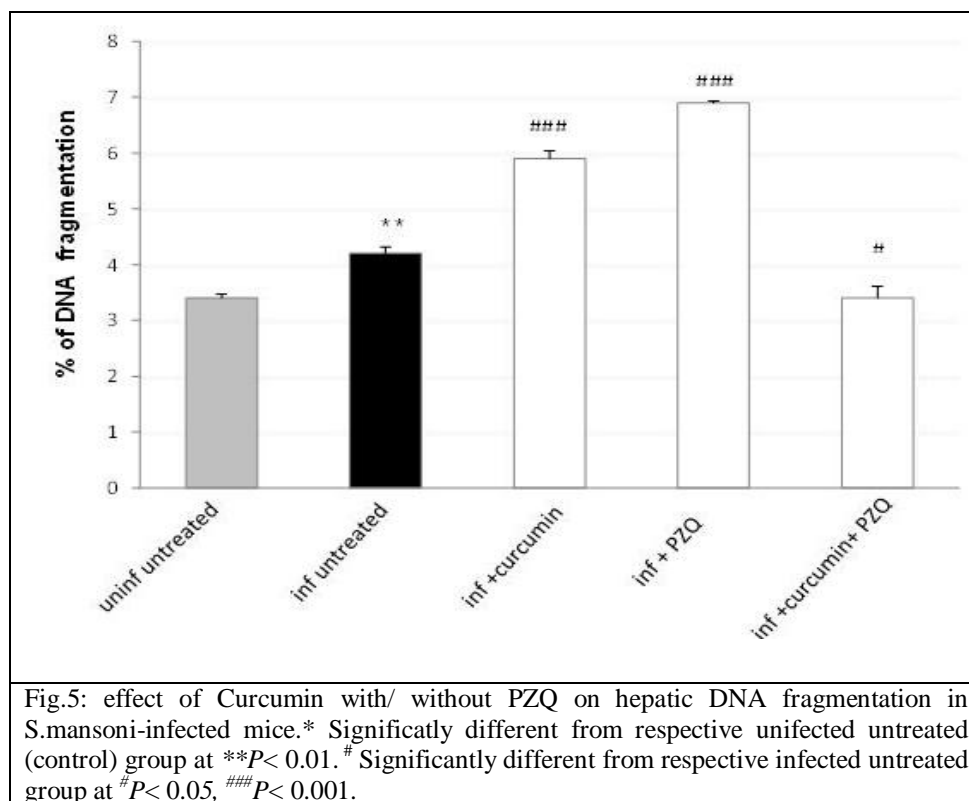
Table 1: Worm reduction in infected CD1 mice treated with multiple doses of Curcumin or/and PZQ.

Mice groups	Total worms	Red. (%)	P	Female worms	Red. (%)	P	Male worms	Red. (%)	P
Infected control	8.40±1.95	----	----	3.80±0.84	----	----	4.60±1.14	----	----
Infected treated with curcumin	5.50±2.38	35%	0.08	3.25±0.96	14%	0.38	3.50±1.29	24%	0.21
Infected treated with PZQ	2.20±0.45	74%	0.0001***	1.20±0.45	68%	0.0001***	1.00±0.00	78%	0.0001***
Infected treated with curcumin + PZQ	1.60±0.50	81%	0.0002***	0.25±0.50	93%	0.0003***	0.75±0.50	84%	0.0004***

Red. (%): percent of reduction and *P*: P-value.

Table 2: Tissue egg load in CD1 mice treated with multiple doses of Curcumin or/and PZQ.

Mice groups	Liver	Red.	P	Intestine	Red. (%)	P
Infected control	1607±67	----	----	1678±51	----	----
Infected treated with curcumin	1489±118	7%	0.85	1672±1647	0.3%	0.99
Infected treated with PZQ	1158±395	28%	0.23	206±110	88%	0.0002***
Infected treated with curcumin + PZQ	772±351	52%	0.06	57±71	97%	0.0005***

Red. (%): percent of egg reduction

Table 3Liver Granuloma diameter (µm) in CD1 mice treated with multiple doses of Curcumin or/and PZQ and an oogram pattern

Animal groups	number of granuloma in 10 successive power fields (10x10)	% reduction in number of granuloma	mean granuloma diameter in µm	% reduction of mean granuloma diameter	Types of granuloma (%)			State of eggs (%)	
					Cellular	fibro cellular	Fibrous	Intact	Degenerated
Infected control	10.33± 2.19		336.24± 15.83		20%	75%	5%	95%	5%
CURCUMINE	8.9±2.24	13.8%	294± 29.62	12.6%	10%	75%	15%	78.8%	21.2%
Infected treated with PZQ	7.14±1.21	30.6*%	217.86± 33.25	35.2*%	0%	80%	20%	42%	58%
CURCUMINE+PZQ	6.78±1.79	34.4*%	196.57± 47.34	41.5*%	0%	70%	30%	24%	76%

Conclusion:-
Curcumin treatment combined with PZQ appears to reduce fibrosis following schistosome infection, suggesting a synergistic effect of Curcumin combined with PZQ that may slow progression of liver fibrosis in individuals affected by schistosomiasis.

It was suggested that generation of reactive oxygen (ROS) and nitrogen species (RNS) likely contribute to both onset and progression of *S. mansoni*-induced liver fibrosis. On the cellular level, generated free radicals can have deleterious effects on macromolecules causing peroxidation of cell lipids and DNA and protein oxidation (Muriel, 2009). Elevated nitric oxide (NO) generation during inflammation has been found to mediate disease processes by inducing cell apoptosis in tissues, and causing damage to DNA by oxidation (Ohnishi *et al.,* 2013). Accumulation of DNA damage with time can lead to cellular gene modifications that may be mutagenic or carcinogenic.

These results inspire more hope for further study on *C. longa*. The results of the antischistosomal efficacy of Curcumin were different among studies. This could be due to the not well defined chemical entity, where simply a plant extract could vary greatly in its active ingredients among different batches of preparation, in addition to the relatively low bioavailability, poor absorption and rapid metabolism that results in low serum levels (Anand *et al.*, 2007). As a result we could need more investigations' and trials related to the dose and the period of treatment. In fact, all published studies that claimed antischistosomal activity for Curcumin did not refer to a specific chemical constituent responsible for such activity. Moreover, the mechanism of action of Curcumin on schistosomes is not fully understood.

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