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# AMELIORATIVE EFFECT OF *CALOTROPIS PROCERA* AND/OR PRAZIQUANTEL ON *SCHISTOSOMA MANSONI* INFECTED MICE

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**Abstract**

Seventy albino mice were infected with~ 80 *S. mansoni* cercariae and were classified into: GI: infected non-treated group, GII: treated with *C. procera* alcoholic extract after infection (therapeutic), GIII: treated with *C. procera* aqueous extract after infection (therapeutic), GIV: treated with *C. procera* alcoholic extract before infection (prophylactic alcoholic), GV: treated with *C. procera* aqueous extract before infection (prophylactic aqueous), GVI: treated with praziquantel, treated group with praziquantel combined with aqueous extract of *C. procera* and G.VII: infected and treated with half dose of Praziquantel combined with aqueous plant extract. Seven weeks post infection, all mice were autopsied, and livers and ilea were parasitological examined (Tissue egg load) and histological assessments (Number & size of hepatic granulomas). Schistosomes recovered from all groups were processed to calculate total worm burdens.

The results showed that mice treated with *C. procera* alcoholic and aqueous extracts showed more significant reduction in total worm burden (43.7% & 46.4%), total egg tissue load (60% & 50.7%) and number of hepatic granuloma (45.9% & 55.5%) than mice in prophylactic aqueous or alcoholic groups (38.3% & 36%, 27.2% & 44%, 25.6% & 39%, respectively). Female worms recovered from mice of aqueous or alcoholic treated groups showed less fecundity than those recovered from mice of prophylactic groups (1475±181 & 2821±200, corresponding to 3674±1447 & 3023±709, respectively). Mice treated with praziquantel or treated with praziquantel combined with *C. procera* aqueous extract showed the highest reduction in total tissue egg load (86.4% & 97.4%, respectively). Mice treated with praziquantel combined with *C. procera* aqueous extract showed higher reduction in total egg tissue (97.4%), number and size of hepatic granuloma (72.3% & 31.4%) than those treated with praziquantel (86.4%, 55.5% &10.8%, respectively).

**Key words:** *Schistosoma mansoni,* Albino mice**,** *Calotropis procera*, Praziquantel.

## Introduction

The only antibilharzial drug Praziquantel (PZQ) is used for schistosomes pathogenic to Egyptian patients (Doenhoff *et al*, 2008; Farrag *et al*, 2015). The widespread usage of PZQ, in undiagnosed and non-infected individuals for prevention, developed resistant strains of *S. mansoni* (Melman *et al*, 2009; Zhang and Coultas, 2013). Expensive chemotherapeutic treatments caused drug resistance, toxicity and side effects (Omar *et al*, 2005), besides, difficulty of reservoir control, all these, emphasize the requirement for a safe and active natural compounds derived from herbal extracts, for treatment of *Schistosoma*. The searches for anti-parasitic compounds from natural sources increased over many of the last decades.

Till now, the herbs and medicinal plants are the major phytochemical source of biologically active compounds for new drugs (Pontin *et al*, 2008; Magalhae *et al*, 2010).

*Calotropis procera* (Oshar or Apple of Sodom) is a flowering plant of family Apocynaceae widespread to Africa, Western Asia, South Asia, and Indochina, with large amount of latex and various medicinal properties (Kirtikar, 1935; Jain *et al*, 1996; Iqbal *et al*, 2005).

The study aimed to evaluate *in vivo* the schistosomacidal activity of *Calotropis procera* aqueous and alcoholic extracts with and without praziquantel.

## Materials and Methods

Animals: Native adult male Swiss Albino mice of CD1 strain, 7-8 weeks old & weigh ing ~20g, were purchased from Schistosome Biological Supply Program (SBAP), Theodore Bilharz Research Institute (TBRI), Giza. Mice were raised under natural light-dark cycle. Sawdust was used as a bedding material and changed twice a week to keep them dry and clean. Mice were given access to water and standard rodent food pellets, obtained from Agricultural-Industrial Integrated Company, *ad libitum*. All experiments were conducted during the winter and approved by the TBRI Ethical Committee.

Experimental Design: A total of 70 adult mice were randomly divided into seven groups of 10 mice each. Each mouse was infected with 80±10 *S. mansoni* cercariae: GI (control): infected and non-treated. GII & GIII (curative alcoholic and aqueous): infected and treated with alcoholic and aqueous extract for 5 consecutive days after $5^{th}$ week of infection. GV & GV (prophylactic alcohol and aqueous): treated with *C. procera* alcoholic and aqueous extract before infection for 5 consecutive days. GVI (drug control): infected and treated with Praziquantel (1000mg/kg body weight/two days) after $5^{th}$ week of infection. GVII: infected and treated with half dose of Praziquantel (500mg/kg body weight) combine with aqueous plant extract after $5^{th}$ week of infection, all mice were autopsied after $7^{th}$ week of infection.

Mice were infected with ~80 *S. mansoni* cercariae/mouse (Peters and Warren, 1969). After autopsying and perfusing the mice. Livers were removed, examined for gross pathology, rinsed with phosphate buffered saline, and then weighed. Each liver was divided into two portions and put in the following solutions: 10% Formalin (for histological study) and 5% of NaOH for egg count. The intestine of each mouse was removed, weighed and put in 5% sodium hydroxide for egg counting (Cheever, 1968). Recovered worms from each infected mouse were counted separately, and their sex was determined. The fecundity of the female worms was calculated. The granuloma count was calculated as the number of granulomas in 10 successive fields using the low power of the light microscope. Measurements were done only for solitary granulomas having a single egg in their centers; by an ocular micrometer.

## Results

Reduction of *S. mansoni* total worm burden**:** Administration of *C. procera* stem extracts in infected mice showed a significant decrease in total worm burden in all groups. Treated mice (300mg/kg body weight) of *C. procera* alcoholic and aqueous extracts for 5 successive days after infection showed more significant reduction in total worm burden than those treated before infection, (43.7% & 46.4%) corresponding to (38.6% & 36%), respectively. Male worm burden reduction in curative alcoholic group and female worm burden reduction in curative aqueous group were the highest (55.2% & 48%, respectively) among infected mice treated with plant extracts only. Mice from curative alcoholic group gave more reduction in male worm burden than female in same group (55.2% corresponding to 18.5%), while the reverse was observed in curative aqueous group, 29.6 % corresponding to 48%.

**T**he oral administration after five weeks of infection resulted in extremely significant difference between control group and Praziquantel treated group (G.VI), as well as, the group treated with 500mg/kg body weight of Praziquantel combined with aqueous extract. No worms were detected in these two groups (Tab. 1).

Table 1: Effect of *C. procera* on worm burden in S. mansoni infected mice.

| Groups | Male | Female | Total | Reduction% | | |
|---|---|---|---|---|---|---|
| | | | | Male | Female | Total |
| Control(G.I) | 12.50±3.464 | 13.5 ±3.507 | 29.50±4.561 | - | - | - |
| Treated-alcoholic(G.II) | 5.600±1.643 | 11 ±0.5477 | 16.600±1.483[a] | 55.2% | 18.5% | 43.7% |
| Treated-aqueous(G.III) | 8.800±0.8367 | 7 ±1.342 | 15.80±1.673[a] | 29.6% | 48 % | 46.4% |
| Pro- alcohol(G.IV) | 8.800±3.834 | 7.600±2.191 | 16.40±6.025[a] | 29.9% | 28.3% | 38.3% |
| Pro-aqueous(G.V) | 9.400±2.302 | 7.700±1.817 | 17.00±3.808[a] | 24.8% | 28.3% | 36% |
| Treated with PZQ(G.VI) | - | - | - | 100% | 100 % | 100% |
| Treated with PZQ+ aqueous(G.VII) | - | - | - | 100% | 100 % | 100% |

a: versus control $p < 0.05$

Reduction of tissue egg load: Mice treated with both *C. procera* stem extracts (alcoholic or aqueous, before or after infection) showed decrease in total egg count. Mice treated with alcoholic or aqueous stem extracts after infection showed a significant reduction in total number of tissue eggs compared with those treated with alcoholic or aqueous stem extracts before infection (60% & 50.7% corresponding to 27.2% & 44%). Mice treated with aqueous stem extract before infection showed a significant reduction in total number of tissue eggs (44%) than those treated with alcoholic stem extract (27.2%). Mice treated with alcoholic stem extract after infection showed a significant reduction in total number of tissue eggs (60%) than those treated with aqueous stem extract (50.7%). Mice treated with PZQ with aqueous extract showed the highest reduction in number of eggs in small intestine and liver tissues (97.4%). Least female fecundity was recorded in mice treated with alcoholic stem extract (1475±181), as compared with other groups (Tab. 2).

Oogram: showed that the infected mice treated with alcoholic or aqueous extracts gave intestine loaded with more immature eggs (45.5% & 44.5%, respectively) than all other groups. Mice treated with alcoholic or aqueous extracts before infection gave more mature eggs in intestinal tissues (79.5% & 70.5%, respectively) than control one. PZQ treated group gave (70.6%).

Mice treated with PZQ only or with PZQ combined with aqueous stem extract gave no immature eggs in intestinal tissues. Infected and treated with half dose of PZQ combined with aqueous plant extract showed the lowest number of mature eggs (17.3%), and highest number of dead eggs, 82.7% (Tab. 3).

Table 2: Effect of *C. procera* on tissue egg load in *S. mansoni* in infected mice.

| Mice | egg in liver/g | Reduction | egg in int./g | Reduction | Total egg/ g tissue | Reduction | Fecundity. No egg/worm |
|---|---|---|---|---|---|---|---|
| G.I | 20884±2380 | - | 21489±2976 | - | 40120±4031 | - | 2971 ±780 |
| G.II | 5021±1534 [a] | 75.9% | 10550±1096 [a] | 51% | 16232±1405 [a] | 60% | 1475 ±181 |
| G.III | 8133±851 [a] | 61% | 11617±1706 [a] | 46% | 19750±2556 [a] | 50.7% | 2821±200 |
| G.IV | 19025±3739 | 8.9% | 10150±351 [a] | 53% | 29175±4085 [a] | 27.2% | 3674±1447 |
| G.V | 11367±1798 [a] | 45.5% | 11021±1041 [a] | 49% | 22387±1770 [a] | 44% | 3023 ±709 |
| G.VI | 5450±424 [a] | 75% | 1182±96 [a] | 94.2% | 6623±315 [a] | 86.4% | ND |
| G.VII | 733±104 [a] | 81% | 400±50 [a] | 98% | 1133±58 [a] | 97.4% | ND |

a: versus control $p < 0.05$

Table 3: Mean of ova pattern in *S. mansoni* infected mice groups.

| Groups of mice | Immature % | Mature % | Dead % |
|---|---|---|---|
| Control (G.I) | 26.2 | 66.5 | 8.5 |
| Treated- alcohol (G.II) | 45.5 | 32 | 22.5 |
| Treated- aqueous (G.III) | 44.5 | 34.2 | 20.5 |
| Pro- alcohol (G.IV) | 17 | 79.5 | 3.5 |
| Pro-aqueous (G.V) | 23.5 | 70.5 | 7.3 |
| PZQ (G.VI) | 0 | 70.6 | 30.6 |
| PZQ+ aqueous (G.VII) | 0 | 17.3 | 82.7 |

Hepatic granuloma number and diameter: In plant extract-treated mice showed that the mice in curative groups exhibited higher reductions in granuloma numbers (45.95 & 55.5%, respectively) than these in prophylactic groups (25.6% & 39%, respectively). Besides, administration of *C. procera* aqueous extract in mice of both curative and prophylactic groups showed higher reductions in granuloma numbers (55.5% & 39%, respectively) than these treated with alcoholic extract (45.9% & 25.6%, respectively). The highest percentage of reduction in granuloma number and size were observed in mice treated with both PZQ combined with aqueous extract of *C. procera* (72.3% & 31.4, respectively)

The infected mice treated with stem extracts showed significant reductions in the granuloma sizes than the control group differences between these reduction values were nearly minor, except in curative aqueous group (22.1%, 20%, 20.4% corresponding to 11.2%,Tab. 4)).

Table 4: Effect of *C. procera* on number and size of hepatic granulomas in mice infected with *S. mansoni.*

| Groups of mice | Granuloma No. | Reduction | Granuloma Size (µm) | Reduction |
|---|---|---|---|---|
| Control (G.I) | 12.64 ±1.128 | - | 388.2 ±11.63 | - |
| Treated-alcoholic (G.II) | 6.840 ±0.2191[a] | 45.9% | 302.1 ±28.86[a] | 22.1% |
| Treated- aqueous (G.III) | 5.620 ±0.4087[a] | 55.5% | 344.5 ±28.76[a] | 11.2% |
| Prophylactic-alcoholic (G.IV) | 9.400 ±0.5477[a] | 25.6% | 310.6 ± 1.972[a] | 20% |
| Prophylactic-aqueous (G.V) | 7.720 ± 1.627[a] | 39% | 308.7 ±32.64[a] | 20.4% |
| PZQ (G. VI) | 5.620 ±1.539[a] | 55.5% | 346.3 ±18.89[a] | 10.8% |
| PZQ+ aqueous (G. VII) | 3.500 ±1.643[a] | 72.3% | 266.4 ±11.62[a] | 31.4% |

a: versus control $p < 0.05$

Ameliorative effect of *C. procera* on liver of *S. mansoni* infected mice: Infected Control Group: Macroscopically, the results demonstrated that the mice infected with *S. mansoni* had displayed massive hepatomegaly. Most of all livers taken from this group were firm, dark, congested, and mottled. Multiple granulomas were visible as grey speckling covering the entire organ. Pigments and fats were constantly detected. In the histological sections, the severe disruptions of the ordinary hepatic lobular arrangement were the most marked observations. Neither of the liver cord could be followed, nor could the limits of the lobules be perceived (Fig. 3). Besides, the liver exhibited fibrocellular granuloma with irregular outlines and excess inflammatory cells (mononuclear cells, eosinophils and polymorphs) admixture with collagenous fibrous tissue (Fig. 4).

Most of the hepatocytes have clear signs of vacuolation. Other cells had pale, shrunken nuclei, foamy cytoplasm and disrupted plasma membranes. The nuclei were markedly pyknotic or have clear changes comprising pleomorphism and karyolysis deformations. Kupffer cells were increased, not only in number but also in size and degree of stainability; many phagocytic cells were distended with schistosomal pigments, which appeared as yellow brown granules (Fig. 5).

Alcoholic Treated Group: Liver sections showed improved architecture where the hepatocytes were seen organized and looked almost normal. The central veins were nearly almost normal (Fig. 6). The nuclei were uninjured and having intact nuclear membrane with perceptible nucleoli. Some exceptions appeared as vacuolation within hepatocytes at peripheries near plasma membrane (Fig.7). Granuloma decreased in number and had very small size, 6.8 & 302.1 µm, respectively, compared to control group (Tab. 4).

Aqueous treated group: Comparing with (G.II), the histological sections of liver of the group (G.III) showed lacking in normal architecture of the lobular structure despite of the presence of smaller number of cellular granulomas, 5.6 ones corresponding to 6.8 granulomas. The diameters of granulomas in group were larger than diameters of ones in control group, 344.5µm & 302µm, respectively **(**Fig. 8). Hepatocytes were slightly va-

cuolated at their peripheries near the plasma membrane. In addition, the cell nuclei were unharmed, having intact nuclear membrane and apparent nucleoli. (Fig. 9)

The efficacy of plant extracts (either alcoholic or aquatic) on prophylactic groups (G.IV & V) were less evident in relation to number of granulomas than curative groups (G.II & III), 9.4 & 7.7 compared with 6.8 & 5.6, respectively. On contrast, reductions in sizes of granulomas in groups were as similar as those in group. No major histological differences in hepatic tissues of mice related to both groups. Generally, hepatic tissues were more healthy than control group including relatively restored lobular architecture, some central veins were appeared to be hypertrophied (Fig. 10). Prominent Kupffer cells and infiltrative cells were noticed in the associated sinusoids, many hepatocytes were slightly vacuolated at their peripheries near to the plasma membrane with pronounced bilharzial pigments among. Hepatocytic nuclei were unharmed having intact nuclear membrane with apparent nucleoli (Fig. 11).

Mice treated by praziquantel with or without aqueous extract: Livers, which picked up from mice related to these groups revealed the best histological recovery along the present study. Main histological observations include normal hepatic lobules, well organized hepatic strand, normal central veins and pericentral areas, intact hepatic nuclei with healthy nuclear membranes and nucleoli were plain. Besides, in (G. VI) granulomas were with marked concentric fibers compared to those in the curative or prophylactic groups (Fig.12). On contrast, in (G.VII) granulomas were very infrequent to be observed if compared to those of control group (3.5 granulomas with mean diameter 266.4 µm corresponding to 12.64 ones with mean diameter 388.2 µm, respectively); also, the granulomas were composed of inflammatory cells with almost no fibers (Fig.13).

## Discussion

In the present study, effects of *C. procera* stem extracts on *S. mansoni* were dependent on the extraction method and time used to apply after or before infection, besides, mutual effect of aqueous stem extract with another drug; Praziquantel. The present data showed that treating the infected mice with the stem extracts of *C. procera* evoked a significant reduction in the total worm burden; post-infection treatment with *C. procera* alcoholic stem extract decreased worm burden by 71%. Treatment with *C. procera* aqueous extract showed a 42% reduction in the total worm count as compared to the infected non-treated mice. Meanwhile, the pre-infection groups treated with *C. procera* alcoholic and aqueous stem extract, caused a 38% and 36% reduction in the total worm burden of infected mice, respectively. Botros *et al.* (2013) examined the activity of aqueous stem latex and flowers of *C. procera* given orally (500mg/kg body weight for 3 consecutive days) and showed significant worm reductions by 45.31% & 53.7%, respectively. Their results had indicated that both plant extracts have some efficacy in the treatment of *Schistosoma*. There were differences in the apparent susceptibility of the worms to the influence of the extracts that might be attributed to different concentrations of active constituents in the *C. procera* extracts.

In the present study worms recovered from mice in the group treated with Praziquantel were observed to have 100% reduction in relation to the control. In addition, these recovered from mice in the group treated by PZQ and *C. procera* aqueous stem extract also had 100% reduction worm burden. Being a standard drug, its activity usually ranges between 80-100%, which agreed with Cheever *et al.* (1992) and Bonesso-Sabadini and Dias (2002) who reported 99% & 97.5% reduction of worm by Praziquantel treatment. However, Mahmoud *et al.* (2007) tested the action of garlic on *S. mansoni* mice infected treated with (50mg/kg/b.w.) evoked a significant reduction in the total worm burden. But, Abdul-Ghani *et al.* (2010) studied effects of 500mg/kg/day from myrrh for five days, without significant efficacy in red-

ucing parasite burdens and tissue egg loads or in changing oogram patterns.

In relation to egg count, the data obtained in present work may be comparable with corresponding results recorded by some authors. Iqbal *et al.* (2005) found that *C. procera* flowers crude powder and aqueous extracts had exhibited 88.4 & 77.8% reduction in egg count of gastrointestinal nematodes infecting sheep. In present study, *C. procera* aqueous extracts exhibited 73.1% & 51% reduction in egg count of hepatic tissue and small intestine, respectively. Botros *et al.* (2013) had recorded that the percentage of eggs of *S. mansoni* lodged in tissues of the infected mice and treated with the *C. procera* stem latex and flower latex extracts were ≈34% and 38.5% of liver and intestine, respectively. These results were more or less lower than those recorded in present study.

In the present study, the prophylactic groups were not effective on *Schistosoma* eggs whether the number of eggs on the percentage of dead (non-viable) eggs where there is no significant difference between these groups and control infected ones. On the other hand, the infected group and treated with aqueous or alcoholic extracts caused a markedly reduced mature eggs and elevation in dead eggs which reflect a moderate improvement of this extract on the resistance of mice against *S. mansoni* infection. The most effective group is PZQ combined with aqueous extract, which produced no immature eggs and high elevation in dead eggs. The present data agreed with Ferrari *et al.* (2003) who reported that oxamniquine and praziquantel reduced the average number of eggs per gram of tissue. In addition, Mohamed *et al.* (2016) revealed that propolis caused a reduction in percentage of immature and mature eggs and increase in percentage of dead eggs in intestinal tissue of *S. mansoni* infected and treated mice compared with infected control group. Al-Olayan *et al.* (2016) recorded a complete disappearance of all immature and mature ova in mice infected and treated with *Ceratonia siliqua* pod extract and administration of PZQ caused the complete death of ova.

Hepatic fibrosis caused by *S. mansoni* infection, is of great importance among chronic liver diseases worldwide. In the present results, kupffer cells were evidenced in the control group to be suffering from hyperplasia and hypertrophy. Sindram *et al.* (2001) depicted kupffer cells as resident macrophages of the liver, which have the potential to be activated in response to any toxic effect that result inflammatory cells. Kupffer cell eminence and pigmentation was observed in the present study and was reported by several authors (El-Badrawy *et al*, 1990; Nosseir *et al*, 2000).

However, the histological examination of the hepatic tissues of the infected and treated mice with *C. procera* stem extract showed that the all infected and treated mice before and after infection showed a significant decrease in size and in number of granuloma in hepatic tissue compared with the control group. Low number of granuloma could be referred to the drop in the number of worms, worm fecundity and egg count in tissue. The reduced size of granulomas may depend on the fact that *C. procera* has notable anti-inflammatory property. Moreover, latex of *C. procera* was studied for its inflammatory reactions using pedal oedema and air pouch models of inflammation in rats (Iqbal *et al*, 2005). Also, these results agreed with De Oliveira *et al.* (2014) who evaluated the effect of the crude dichloromethane extract of *Baccharis trimera* plant and aqueous fraction (40 & 200mg/kg) caused decreasing in granuloma sizes with minimal degenerative changes in the liver tissue were observed.

The present work showed disturbed lobular organization in liver tissues in the mice treated with *C. procera* alcoholic stem extract fewer and smaller size of granuloma compared with the control group. The fibers of granuloma were less in the treated group with pronounced bilharzial pigments. Inspecting liver sections showed that the liver lobular architecture was relatively restored.

Central veins and pericentral areas were normal, without major morphological changes in the hepatic cells except for mild hepatocyte vacuolation.

Nosseir *et al.* (2000) and Hogan *et al.* (2002) reported that the granuloma response around the disseminated schistosomal eggs aggravated by fibrosis was major contributor to the pathology of the disease and granuloma formation is a protective host response that insulates host tissue from damaging influences the pathogen and the inflammatory response. This result agreed with Waiganjo and Ochanda (2014) who found that *Ocimum americanum* hexane, *Bridelia micrantha* water and *O. americanum* water plant extracts without or few granulomas shownby worm reduction and less pathogenesis.

Praziquantel combined with *C. procera* aqueous extract was more effective against hepatocytes damage. Also, it reduced the ova count in liver and intestine as well as the granuloma size. Livers obtained from mice from this group showed fewer number and smaller volume of granulomas. These results proved that the plant could have an impact on the granuloma where the plant possesses anti-inflammatory properties. This agreed with Soliman *et al.* (2017) who found that hostacortin significantly reduced granuloma size by 22.5% & 31.6% for doses 100 & 200 mg/kg, respectively. Also, hostacortin combined with PZQ improved liver status by significant reduction in number of worms, egg count and size of granuloma.

## Conclusion

Applying of *C. procera* aqueous extract during the treatment of *Schistosoma mansoni* infected mice by Praziquantel (half recommended dose) gave maximum improvement to hepatic profile, and minimizes its side effects. Moreover the combined treatment reduced the ova count in liver and intestine as well as the granuloma size.

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**Explanation of Figures**

Fig. 1: Ova pattern in *S. mansoni* infected mice groups.

Fig. 2: Reduction percentage of number and size of hepatic granulomas in mice infected with *S. mansoni*.

Fig. 3: Hepatic granulomas of mice liver in non-treated group (G.I) showing multiple large fibrocellular bilharzial granuloma (arrow). Note irregular outlines and excess inflammatory cells with collagenous fibrous tissue (arrowhead).

Fig. 4: Hepatic granulomas of mice liver in non- treated group (G.I) showing a granuloma is composed of a necrotic center, ovum of Schistosoma (OV) surrounded with inflammatory cells (IC) and concentric fibers (F) fibrocellular stage.

Fig. 5: Hepatic tissue of mice liver in non- treated group (G.I) showing different degrees of vacuolation (V). Focal necrosis is noticed (white arrowhead), Kupffer cells are distinctly hypertrophied (arrow), some *Schistosoma* pigment (black arrowhead).

Fig. 6: Hepatic granulomas of mice liver in treated curative group (G.II) revealing reduced size of fibrocellular granuloma (arrow), normal central vein (CV).

Fig. 7: Hepatic tissue of mice liver in treated curative group (G.II) showing mild vacuolation in cytoplasm of hepatic cells (black arrowheads), slightly hypertrophied nuclei (white arrowhead), *Schistosoma* pigments are observed (arrow).

Fig. 8: Hepatic granulomas of mice liver in treated group (G.III) showing reduction in size of granuloma (arrow).

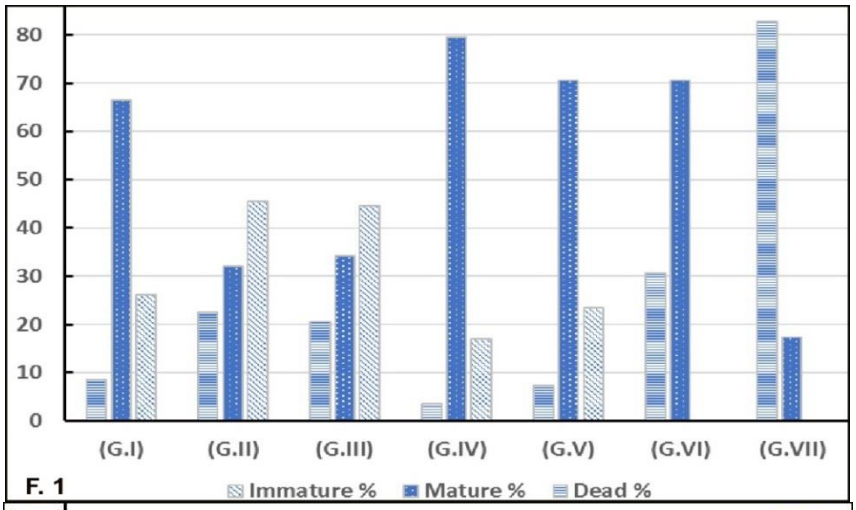
Fig. 9: Hepatic tissue of mice liver in (G.III) showing improvement of hepatocytes (arrows).

Fig. 10: Hepatic tissue of mice liver in treated group (G.V) showing a slightly hypertrophied central vein (CV). Great reduction of size of cellular granulomas (arrows).

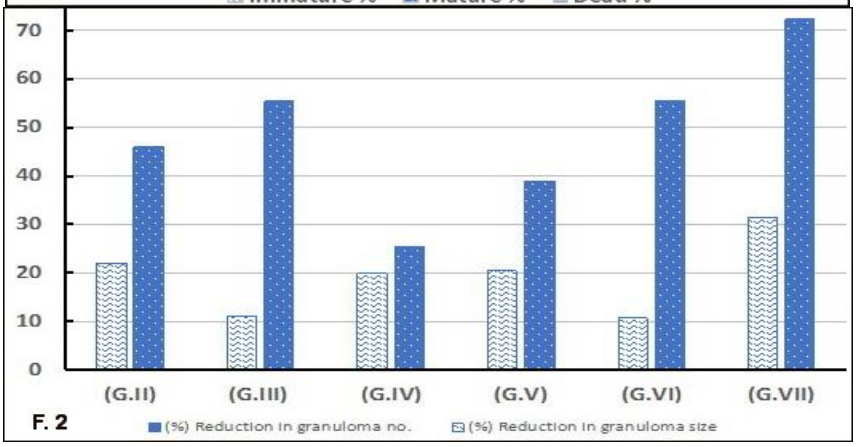
Fig. 11: Hepatic tissue of mice liver in treated group (G.V) showing many healthy recovered hepatocytes (arrowhead). Normal Kupffer cells (arrow).

Fig. 12: Hepatic granuloma of mice liver in treated group (G.VI) showing regular and normal hepatocytes (arrow). Granuloma is composed of a necrotic center, ova of *Schistosoma* surrounded with inflammatory exudates and concentric fibers (arrowhead).

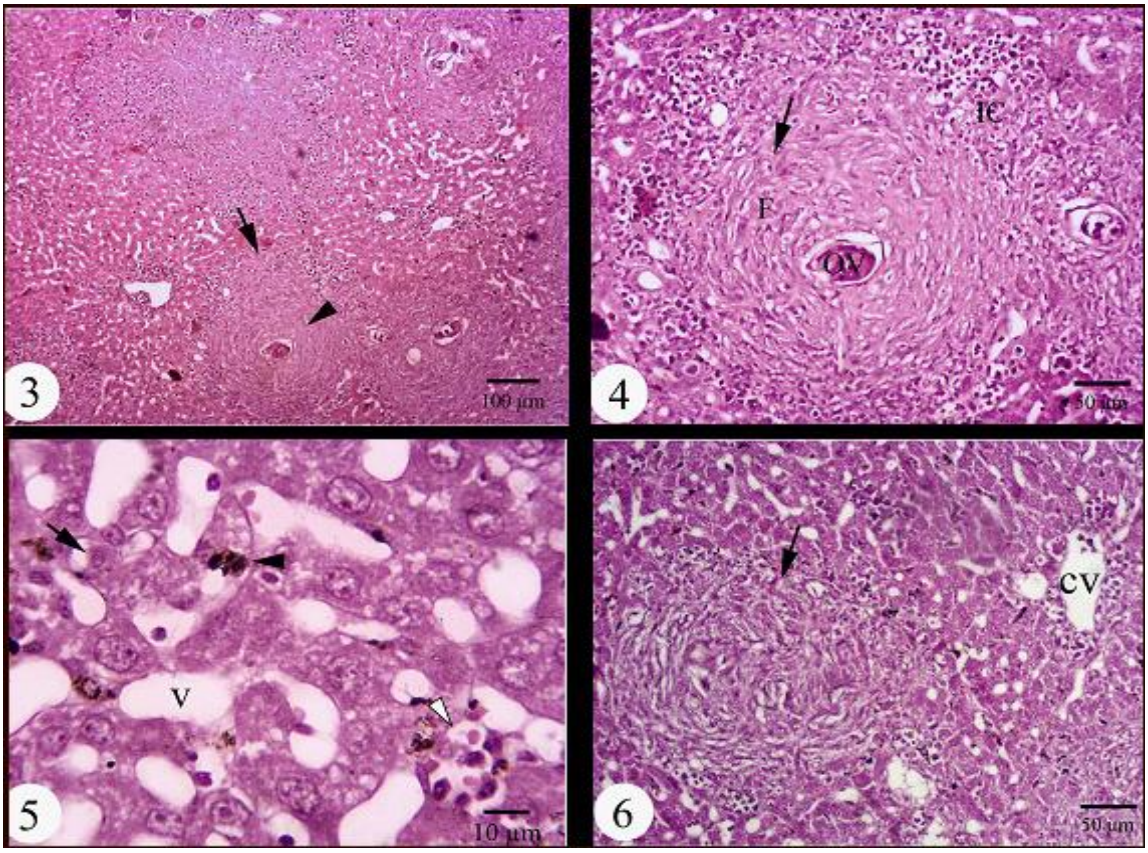
Fig. 13: Hepatic tissue of mice liver in treated group (G.VII) showing smaller size and less number of granulomas (arrows).

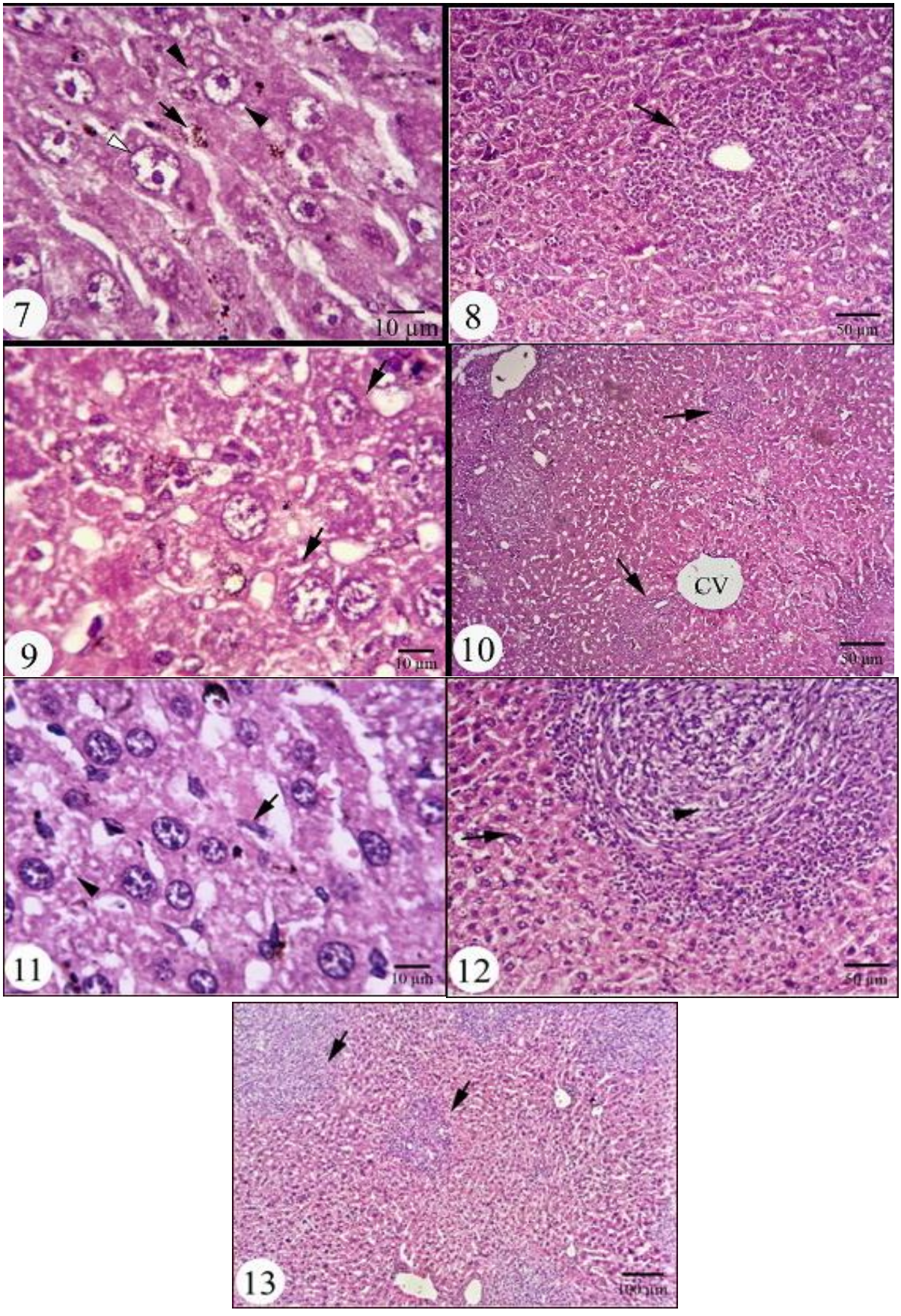

80
70
60
50
40
30
20
10
0
(G.I)
(G.II)
(G.III)
(G.IV)
(G.V)
(G.VI)
(G.VII)
Immature %
Mature %
Dead %

F. 1


70
60
50
40
30
20
10
0
(G.II)
(G.III)
(G.IV)
(G.V)
(G.VI)
(G.VII)
(%) Reduction in granuloma no.
(%) Reduction in granuloma size

F. 2


3
100 µm
4
IC
F
GV
50 µm
5
V
10 µm
6
CV
50 µm


7
10 µm
8
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9
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CV
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13