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Pathological and biochemical alterations induced by synchronize lead intoxication and bilharzial Infection in mice

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ABSTRACT

Pollution with lead and exposure to parasitic infection are very widespread health problems faced developing countries. In Egypt, schistosomiasis is not only a prime health problem, but is also an economic one as it affects millions of farmers at an early age, thus diminishing their productivity and creating a serious socioeconomic problems. The present study aimed to examine the combined effect of exposure to lead and infection with *Schistosoma mansoni* on progression of hematological and hepatic dysfunction by measuring the hematological parameters in peripheral blood, liver function tests and histopathological changes in hepatic tissues. Mice were used as experimental animal, these animals were divided into four groups, each one consisted of 10 animals. The first group is a control group, the second group was intoxicated with lead, this group were kept under the same condition of control of feeding for 4 weeks and intoxicate with 250 ppm of lead acetate in drinking water for another 4 weeks and sacrificed at the end of the 8th week. The third group was infected with *S. mansoni* by subcutaneous injection with 100 cercaria for each mouse and sacrificed at the end of the 8th week after infection. The fourth group was intoxicated with lead and infected with *S. mansoni* and intoxicated with lead. These animals were fed a balanced diets and infected subcutaneously with 100 cercariae, after 4 weeks post-infection, animals were treated with 250 ppm of lead acetate in drinking water for another 4 weeks and sacrificed at the end of 8th week. Statistical analysis of the results revealed that mice infected with *S. mansoni* and exposed to lead acetate in comparsion with those uninfected but exposed to lead or mice only suffered from schistosomal infection showed significant effects on hematological and biochemical parameters, compared with controls as decreasing serum in albumin content and A/G ratio. Hematological data as hemoglobin, RBCs count, and hematocrit, were also reduced. On the other hand, serum total proteins and globulins, total leucocytes count and neutrophils, sALT and sAST activities, were elevated under the same experimental conditions. Serious histopathological changes in hepatic tissues were also observed. It has, therefore, been concluded that the effect of each factor (lead intoxication or schistosomal infection) exacerbates the effect of the other which finally lead to bad additive effects on the organisms.

KEY WORDS: Liver, Lead, Schistosomiasis, Toxicity, combined effect of lead and *S. mansoni*.

1. INTRODUCTION

Pollution with heavy metals, insecticides, polycyclic aromatic hydrocarbons, in addition to exposure to bacterial, viral and parasitic infection are very widespread health problems faced developing countries. In Egypt, schistosomiasis is not only a prime health problem, but is also an economic one as it affects millions of farmers at an early age, thus diminishing their productivity and creating a serious socioeconomic problem (Elkhfif, 2001).

Lead exposure can occur from many sources such as air, water, industrial pollutants and soil. There are six sources of lead exposure in Egypt and other developing countries as gasoline additives, food cans soldering, paints, ceramic glazes, drinking water conducting channels, and folk remedies. Health hazards from increased lead exposure as a result of industrial and environmental pollution are recognized. Lead exposure has been found to cause a wide range of biochemical and physiological dysfunctions by generating reactive oxygen species and different free radicals and inhibiting antioxidant enzymes activities, such as catalase and superoxide dismutase, it also causes increase in lipid peroxidation and decreases the level of glutathione. Lead is a highly poisonous environmental pollutant and is known to affect organs like liver, kidney, blood, and central nervous system of mammals (Abdou and Hassan, 2014)

The safe range of daily intake of lead according to recommendation of US Environmental Protection Agency (US EPA) is 25 µg/dL, while in children it was recommended that the blood lead level should not rise above 10 µg/dL. The toxic effects lead exposure on the blood and blood forming organs, gastrointestinal, nervous, renal, reproductive and cardiovascular systems have been reported (Mayer-Baron, 2000). On the other hand studies on the effect of lead toxicity on the course of shistosomiasis are still insufficient. El khafif (2001), have shown the toxic effects of combined lead exposure and *Shistosoma mansoni* infection on bone marrow cells. However, the toxic effect of chronic lead toxicity on liver in *S. mansoni* and *S. haematobium* infection has not yet been fully investigated.

Morbidity and mortality due to schistosomiasis is very high among developing countries in Africa, South America and Asia. The main agent of human schistosomiasis is *Schistosoma mansoni (*dos Santos, 2007). Predominantly,

eggs of *Schistosoma mansoni* are deposited in the liver and intestines which resulting in parasitic disease (Allam, 2007). The clinical symptoms of schistsomiasis include hepatosplenomegaly, liver fibrosis, portal hypertension, and liver cirrhosis. Acute symptoms of schistosomiasis include fever, diarrhea, abdominal pain, weight loss, and eosinophilia (David; 1980; Mahmoud, 2002).

Eggs of Schistosoma mansoni are transported to the liver by portal circulation and acute schistosomiasis is associated with heavy primary infections and with the initiation of egg production (Chang, 2006). The eggs are deposited in the portal venous system while eggs are trapped in the peri-sinusoidal spaces of the liver, thus leads to periportal granulomatous inflammation and the deposition of scar tissue around the eggs trapped inside the liver (Andrade, 2009). Granulomas are formed of inflammatory cells as macrophages, eosinophils and lymphocytes (Brito, 1997). The present study aimed to examine the combined effect of exposure to the heavy metal (lead) and infection with *Schistosoma mansoni* on progression of hematological and hepatic dysfunction by measuring the hematological parameters in peripheral blood liver function tests and histopathological changes in hepatic tissues.

2. MATERIALS AND METHODS

Experimental animals: Male albino mice *(Mus musculus)*, Weighing about 18g (16 - 20g), obtained from Schistosoma Biological Supply Program (SBSP), Theodor Bilharz Research Institute, Egypt, were used as experimental animals throughout the present research work. Animals were housed in groups in plastic cages and were maintained under standard controlled condition. Cercariae of *schistosoma mansoni* were obtained from the schistosome biological supply program (SBSP), Theodor Bilharz Research Institute Egypt and used within ½ hr of being shed.

Chemicals: Lead acetate was used as the source of lead in this study. Lead acetate is a substance with small white transparent crystals with an acetous odour and a sweet taste. This substance becomes anhydrous at 40°C and rendered basic when heated. Lead acetate has been used at a dose 250 ppm in drinking water.

Experimental design: Animals were randomly divided into four groups, 10 animals in each as follow:

Control group: These mice were fed on a balanced diet and received water *ad libitum.* Animals were sacrificed at the end of 8th weeks of the experiment.

Intoxicated group with lead: These mice were kept under the same condition of control of feeding for 4 weeks and intoxicate with 250 ppm of lead actate in drinking water for another 4 weeks and sacrificed at the end of the 8th week.

Infected group with *S. mansoni:* A group of this animals were fed with the same diet as a control group and infected by subcutaneous injection with 100 cercariae of *S. mansoni* for each mouse. Animals were sacrificed at the end of the 8th week after infection.

Infected with *S. mansoni* and intoxicated with lead group: These animals was fed with balanced diets and infected subcutaneously with 100 cercariae of *S. mansoni* by the former method. After 4 weeks post-infection, animals were treated with 250 ppm of lead acetate in drinking water for another 4 weeks and sacrificed at the end of 8th week.

Collection of blood samples: Blood samples were received in clean and dry glass tubes contained EDTA as anticoagulant for the haematological examinations and the Hematological parameters were determined using an automated hematology analyzer sysmex (K-1000). Leucocyte differential counted was preceded according to Turgeon (2005), by using Giemsa stain (Atlas Medical Company, UK).

Collection of serum samples: Blood Samples were received in conical glass centrifuge tubes and put for 1 hr in the refrigerator for clotting after which sera were separated using cooling centrifuge at 1000 g for 5 min. and Kept at -20°C till analysis within one week.

Biochemical analyses: Serum aminotransferases (sALT and sAST), total proteins, albumin and globulins were determined using an automatic multiparameter apparatus, ASTRA 8-Synchron.

Quantitative estimation of lead in various tissues and serum:

The lead content in tissues and serum were estimated by the method described by Allen (1989), as follows:

- 1ml of serum or 1gm of tissue was added to 10 ml conc. H_2SO_4
- The specimens were heated gradually (70-280°C) in sand path with addition of drops of H_2O_2 every while.
- The clear solution obtained was made up to 10 mL with distilled water.
- The solution was furthermore diluted to give a suitable reading compared to standard solutions.
- Convenient standard solutions (0.00 - 1.00 ppm) was prepared.
- Lead metal analysis was carried out using atomic absorption spectrophotometer (Perkin-Elmer model 2389).

Histological Preparation and measurement of granuloma diameter: Experimental animals were sacrificed by cervical decapitation. They were immediately dissected, and small pieces of the liver were quickly removed and fixed in Bouin's fluid. After fixation specimens were dehydrated in an ascending series of alcohol, then kept in terpineol for three days to ensure complete dehydration and clearing purposes. Cleared specimens were rinsed in three changes of xylol before embedding in paraffin wax (m.p.56-58°C). Three sections of 5 microns thick were taken from each liver sample, each being at a distance of at least 500 microns from the preceeding one and mounted

on clean slides without adhesive medium. For histological examination, sections were stained with Ehrlich's haematoxyline and eosin.

Individual granulomas were measured, only granulomas containing single egg were selected for measurement. Each section was examined for schistosome eggs, and the diameter of each egg, including the reaction around it, was determined by measuring two diameter at right angles to each other with a calibrated ocular micrometer (Boros & Warren, 1970). The mean of periovular granuloma diameter of each group was calculated for about 40 lesions. The percent change between any two groups was calculated according to the formula: percentage of change = (A-B) /A × 100

Where, A: Mean granuloma diameter of infected group, B: Mean granuloma diameter of infected-treated group.

Statistical Analysis: Data are expressed as mean±SD. The level of statistical significance was taken at $P < 0.05$, using one way analysis of variance (ANOVA) test followed by Dunnett test to detect the significance of differences between each group and control. All analysis were performed by using, GraphPad Prism software version 5.

3. RESULTS

The data recorded in table (1) indicate a marked elevation in serum lead concentration in mice treated with lead acetate for four weeks, the results showed highly significant increase ($P<0.01$). The percentage increase was found to be 140% in serum Lead compared to control group. *S.mansoni* infection didn't induce any observable changes in serum lead concentration. Lead intoxication of mice pre-infected with *S.mansoni* induced highly significant ($P<0.01$) elevation in serum lead concentration. The percentage increase was 112% on comparing with control animals.

Table.1. Lead concentration in serum and body organs after chronic lead acetate administration and/or *S.mansoni* infection in albino mice

Lead (μg/dl)	Control group Mean ± SD	Intoxicated group with lead Mean ± SD	Infected group with *S. mansoni* Mean ± SD	Infected with *S. mansoni* and intoxicated with lead Mean ± SD
Serum	0.432 ± 0.032	$0.974 \pm 0.044^{**}$	0.42 ± 0.023	$0.914 \pm 0.06^{**}$
Liver	0.48 ± 0.022	$1.03 \pm 0.029^{**}$	0.441 ± 0.028	$0.862 \pm 0.047^{**}$
Kidney	0.47 ± 0.033	$1.115 \pm 0.025^{**}$	0.432 ± 0.028	$1.05 \pm 0.016^{**}$
Testis	0.41 ± 0.013	$0.695 \pm 0.04^{**}$	0.39 ± 0.019	$0.72.2 \pm 0.028^{**}$
Brain	0.38 ± 0.012	$0.462 \pm 0.028^{*}$	0.363 ± 0.021	$0.473 \pm 0.04^{*}$

* : Significant ($P < 0.05$); ** : Highly significant ($P< 0.01$).

Hepatic lead concentration showed highly significant ($P<0.01$) elevation by 124% and 80% in Lead intoxicated animals and combined infected and intoxicated groups respectively. The data present in table (1) showed that administration of lead acetate to normal and/or infected mice induced highly significant ($P<0.01$) increases in kidney lead concentration. The percentage increases were 155% and 152% respectively. Administration of lead acetate to normal and/or infected animals induced highly significant ($P<0.01$) increases in testicular lead concentration. The percentage increases were 76% and 80% respectively compared to control group. Intoxication with lead acetate and combination of intoxication and infection with *S. mansoni* induced highly significant ($P<0.01$) elevation in brain lead concentration with percentages of 23% and 25% respectively as illustrated in table (1).

The hematological data obtained are presented in table (2) which appeared that the administration of lead acetate or infection with *S. mansoni* to mice induced marked depression ($P<0.01$) in RBCs count, hemoglobin concentration, hematocrit, MCV, MCH, and MCHC in all experimental groups, when compared with controls. The data recorded in table (2) indicate a marked increase in WBCs count in mice treated with lead acetate ($P<0.01$), the percentage of increase was found to be 16%. Treatment with lead acetate of mice pre-infected with *S.mansoni* induced highly significant ($P<0.01$) increase in WBCs count. The percentag of such elevation was 75% compared with control. Differential count for leucocytes which illustrated in in table (2) showed a highly significant reduction ($P<0.01$) of lymphocytes percentage by 38 and 35% in (lymphopenia) in case of treatment with lead or infected and treated with lead groups respectively. Neutrophil percentage was reduced in mice infected with *S.mansoni* ($P<0.05$) at the 8^{th} week of infection with percentages of -15% compared with controls (Table 2). On the other hand, there was significant increase ($P<0.05$) in neutrophil percentage and with highly significant increases ($P<0.01$) mice treated with lead acetate only and in treated animals which were pre-infected with *S. mansoni*, by percentages increase were 77 and 56% respectively. Monocyte percentage was significantly elevated ($P<0.05$) 4 weeks post-treatment of mice with lead acetate and 4 weeks post-treatment of mice pre-infected with *S.mansoni* with percentages 33&21% compared with control, respectively (Table 2). On the other hand, monocyte percentage was highly significantly decreased ($P<0.01$) 8 weeks infection of mice with *S.mansoni*. The percentage of reduction was 46% compared with

control. The data recorded in table (2) indicated a marked elevation in eosinophil percentage in all experimental animals, thereafter results showed highly significant (P<0.01) increase compared with control. The data obtained showed non-significant changes in basophil percentage in all experimental animals.

Table.2. Hematological parameters after chronic lead acetate administration and/or *S.mansoni* infection albino mice

Animal groups / Hematological parameters	Control group Mean ± SD	Intoxicated group with lead Mean ± SD	Infected group with *S. mansoni* Mean ± SD	Infected with *S. mansoni* and intoxicated with lead Mean ± SD
RBCs Count ($x10^6/\mu L$)	8.4±0.18	6.21±0.4**	6.94±0.2**	5.78±0.6**
Hb (g/dL)	15.34±0.23	11.46±1.2**	12.9±0.6**	10.4±1.6**
Ht (%)	43.22±1.4	33.86±3.2**	36.7±1.4**	31.38±4.6**
MCV (μ^3)	45±1.2	50.64±1.5**	52.98±0.5	50.48±1.8**
MCH (Pg)	19.86±0.31	17.32±1.2**	18.3±0.3**	17.1±0.8**
MCHC (g/dL)	36.69±0.35	33.5±0.5**	35±0.21**	32.86±0.3**
WBCs count ($10^3/\mu L$)	8±0.6	8.9±0.5**	7.5±0.8	14±0.7**
Lymphocytes %	65±2	40±4.1**	67±2	42±4**
Neutrphils %	27±1.9	48.5±2.7**	23±2.5*	40±2.6**
Monocytes %	6.3±1.2	8±0.6*	3.4±1.5**	8±0.6*
Eosinophils %	1.2±0.8	3.2±0.3**	6±0.5**	9±0.8**
Basophils %	0.5±0.4	0.3±0.2	0.5±0.4	1±0.7

* : Significant (P < 0.05); ** : Highly significant (P< 0.01).

Total proteins showed highly significant elevations (p<0.01) in all experimental groups (Table.3). The percentages of elevations were 13, 9 and 15% treated with lead, infected and infected and treated with lead acetate groups respectively. The data shown in table (3) indicated highly significant decrease (p<0.01) in serum albumin in all experimental groups while it was found to be significantly decreased (p<0.05) only at 4-week post-treatment in mice only treated with lead acetate. The percentages of reductions were found to be -3, -7, and -13 for intoxicated, infected, infected and intoxicated groups respectively. Serum globulin content was found to be highly significantly elevated (p<0.01) in all experimental groups. The percentages elevations were 33, 27 and 48%for intoxicated, infected, intoxicated and infected groups respectively.

Table.3. Liver function tests after chronic lead acetate administration and/or *S.mansoni* infection in albino mice

Animal groups / Liver function tests	Control group Mean ± SD	Intoxicated group with lead Mean ± SD	Infected group with *S. mansoni* Mean ± SD	Infected with *S. mansoni* and intoxicated with lead Mean ± SD
sAST (IU/L)	143.6±10.3	203.2±6.1**	234±11.1**	256.4±9.2**
sALT (IU/L)	53.8±4.8	90.2±3.7**	90±2.9**	100±5.15**
Total proteins (g/dl)	5.47±0.07	6.16±0.1**	5.98±0.1**	6.3±0.1**
Albumin (g/dl)	3.04±0.05	2.92±0.09*	2.82±0.1**	2.64±0.06**
Globulins (g/dl)	2.48±0.03	3.24±0.06**	3.16±0.1**	3.66±0.06**
A/G ratio	1.23±0.04	0.9±0.07**	0.89±0.1**	0.72±0.06**

* : Significant (P < 0.05); ** : Highly significant (P< 0.01).

Results compiled in table (3) showed sAST, statistical analysis of this data indicated highly significant (P<0.01) increases in sAST activities in all experimental groups as compared with control values. The level of sAST was found to exhibit increase in its activity in animals treated with lead acetate as well as in infected groups. In mice infected with *S.mansoni* and treated with lead acetate, sAST activity was highly significantly changed (P<0.01),

when compared with infected mice. Result compiled in table (3) also showed sALT in treated animals with lead, 8-week infected animals, animals infected for 8 weeks and treated with lead, Statistical analyses of the this data indicated highly significant increases ($P<0.01$) in sALT activities in all experimental groups as compared with control values. In mice infected with *S.mansoni* and treated with lead acetate, sALT activity was found to be highly significantly ($P<0.01$) changed, when compared with infected mice.

Liver histology of control animals showed as in other mammalian animals, the structural unit of the mouse liver is the hepatic lobule which is made up of radiating plates of cells forming a network around a central vein (Fig. 1). The hepatic cells are polyhedral in shape with large, centrally located nuclei and granular cytoplasm. The sinusoids are narrow blood spaces with irregular boundaries composed of endothelial cells in addition to large kupffer cells which are known to be actively phagocyte cells. The bile ductile appears rounded and is bounded by a layer of cuboidal cells encircled by a thin sheath of connective tissue. The portal veins are comparatively large in size being either empty or containing a few blood cells.

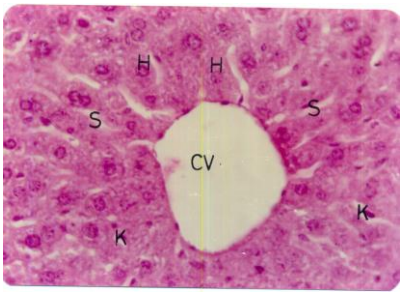

Figure.1. Section of the liver a Control mouse (x450) H: Hepatocyte, S: Sinusoid space, K: Kupffer cell, CV: Central vein,

Examination of liver sections obtained from mice treated with 250 ppm of lead acetate for 4 weeks showed that there were apparent signs of degenerative changes. The normal structural organization of the hepatic lobules was impaired and the hepatocytes showed severe cytoplasmic vacuolation which is so extensive in some cells to the extent that only slight remnants of the cytoplasmic mass were left (Fig.2). The central veins were found congested and engorged with blood. Sinusoidal spaces were filled with blood indicating hemorrhage. Most of the portal veins were found congested and inflammatory leucocytic cells comprised mainly of lymphocytes were spread over several liver areas and bile ducts were enlarged and seemed hyperplastic (Fig.2 and 3)

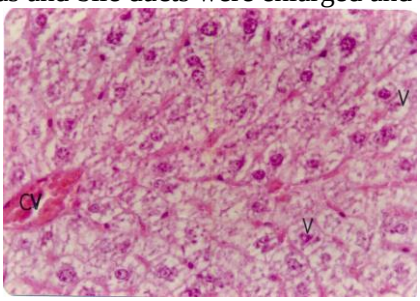

Figure.2. Section of the liver intoxicated mouse with lead (x450) CV: Congested central vein, V: Cytoplasmic vaculation

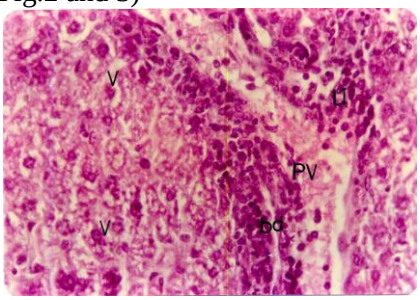

Figure (3) Section of the liver intoxicated mouse with lead (x450), PV: Congested portal vein, V: Cytoplasmic vaculation, bd: Hyperplastic bile duct, LI: Inflammatory leucocytes infiltration

Animals examined at 8 weeks post-infection with *S.mansoni* showed granulomas with different sizes and the hepatic tissue around the granulomas showed some pathological changes such as congestion of central vein, dilation of sinusoid spaces and leucocytic infiltration (Fig. 4). These granulomas were characterized by epithelioid macrophages and huge population of fibroblasts. The periphery of the granuloma showed numerous lymphocytes and plasma cells while the center, in which the damaged egg is present, there is clusters of eosinophils (Fig. 4, 5 and 6). The mean diameter of these granulomas was 397±59.9 µm (Table.4).

Table.4. Effect of Lead Exposure on Granuloma Diameter in the Liver of mice Infected with *S. mansoni*

Animal groups	**Infected group with *S. mansoni* Mean ± SD**	**Infected with *S. mansoni* and intoxicated with lead Mean ± SD**
Granuloma diameter	397±59.9	306.8±22.5**

n : 40 Granuloma diameter per group; ** : Highly significant ($P < 0.01$).

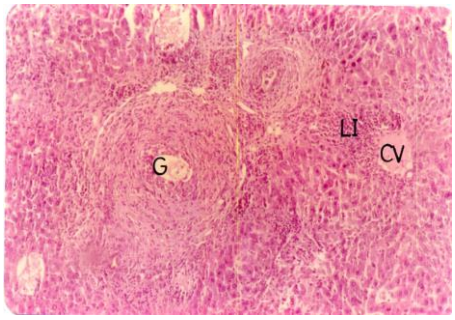

Figure (4) Section of the liver in infected mouse with *S. mansoni* (x110)
G: Periovular granuloma, CV: Congested central vein, LI: Inflammatory leucocytes infiltration

Examination of liver sections obtained from mice infected with *S. mansoni* for 4 weeks and then given 250 ppm of lead acetate for 4 weeks showed small and large periovular granulomas. The main cellular constituents in periovular granuloma were fibroblasts in addition to few lymphocytes, macrophages, and giant cells (Fig.8). The liver parenchyma adjacent to or at variable distance from the granulomas showing cytoplasmic vacuolation, inflammatory leucocytic infiltration, blood haemorrhage in sinusoidal spaces and congested portal veins (Figs. 7 and 9). The mean diameter of these granulomas appeared significantly reduced (306.8±22.5µm) when compared to those of untreated controls (397±59.9µm (Table 4). The percentage of suppression in the mean granuloma diameter as a result of lead acetate administration was 22.77%.

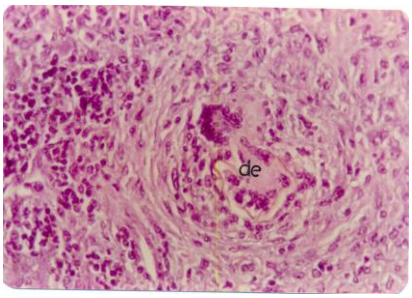

Figure (5) Section of the liver in infected mouse with *S. mansoni* (x450), de: Dead egg invaded by macrophage

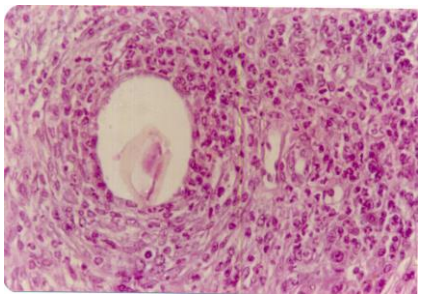

Figure (6) Section of the liver in infected mouse with *S. mansoni* (x450)

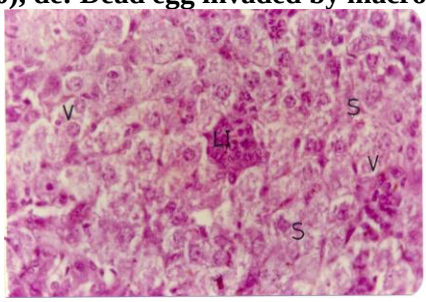

Figure (7) Section of the liver in mouse intoxicated with Lead and infected with *S. mansoni* (x450)
V: Cytoplasmic vaculation, S: Hemorrhage in sinusoidal space, LI: Inflammatory leucocytes infiltration

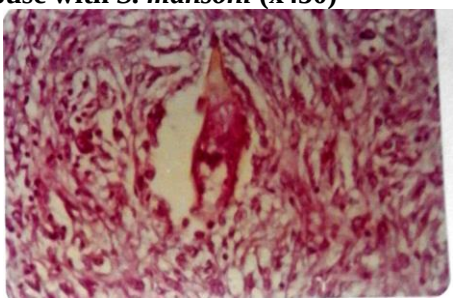

Figure (8) Section of the liver in mouse intoxicated with Lead and infected with *S. mansoni* (x450)

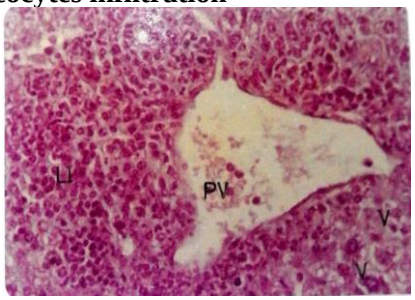

Figure (9) Section of the liver in mouse intoxicated with Lead and infected with *S. mansoni* (x450)
PV: Congested portal vein, V: Cytoplasmic vaculation, LI: Inflammatory leucocytes infiltration

DISCUSSION

Results of the present study have shown that chronic intoxication with lead acetate induced highly significant elevations in blood, kidney, liver, testis and brain lead. Lead concentrations were higher in the kidneys, liver and blood serum but slightly increased in testis and brain. The brain, which showed the lowest level of lead concentration,

most probably due to blood-brain-barrier (Bayomy, 1994). These results run in agreement with those reported by Speich, (1983) who recorded that lead concentrations were higher in the kidney and spleen but slightly increased in livers of rabbits treated with 9.66μmol/L of lead acetate in drinking water for 25 weeks. Also, these results are in agreement with those of Nehru and Kausal (1993), who reported that liver less accumulates lesser amount of lead compared with kidneys of rats treated with 5, 10 and 50 mg/kg body weight for 60 and 90 days on every alternated day.

The liver is the essential body organ responsible for detoxification and withdrawal of many injurious substances in the body. Thus, it is quite reasonable to detect many pathological consequences in this organ in animals subjected to different chemicals. On the other hand the infection with *S.mansoni* was found to induce the formation of hepatic periovular granuloma (Bloch, 1972; Akl, 192). The interaction of *Schistosoma mansoni* infection and lead intoxication brought about more drastic complications in such organ. Data shown in Table. 3, demonstrated that treatment with lead acetate caused a significant elevation in the activities of liver enzymes AST and ALT in serum confirming the histological damage shown in the liver. The present results revealed a significant increase in ALT and AST in serum of lead acetate treated rats compared with negative control group. These results are in agreement with the results of Herman (2009), who reported that releasing of AST, ALT, and LDH from the cell cytosol can occur as secondary changes to cellular necrosis. In addition, significant decrease in serum albumin of lead acetate treated group compared to control group. Decrease in albumin level in plasma could be attributed to changes in protein and free amino acids metabolism and their synthesis in the liver (Gaskill, 2005). This decrease effect of albumin might be caused by the interference of lead with protein synthesis or by the binding of lead to some metal-binding proteins and their removal through detoxification processes (Yousef, 2004). The mechanism involved in effect of lead on albumin synthesis has been reported that lead caused a disruption in protein and RNA synthesis. Also, the observed decrease in albumin could be attributed to the damaging effect of lead acetate on liver cells as confirmed by increasing in the activities of serum AST and ALT after treatment with lead acetate (Abdou and Newairy, 2006). On the other hand it could be that the oxidative damages may be the primary cause of lead toxicity leading to lipid peroxidation and cellular damage. It has been shown that lead acetate undergoes metabolism in liver via esoteric and oxidative pathways generating elevated MDA levels that lead to hepatic necrosis (Gaskill, 2005). Similarly, *S.mansoni* infection induced elevations in serum enzyme activities, however, sALT activity was highly affected in response to infection than sAST, which also reflects the effect of *S. mansoni* on the liver. Here again, it is relevant to emphasize that the elevations were correlated with the progress of the disease (i.e. depended on the stage of infection). Similar elevated values in aminotransferase activities both in early as well as in late stages were also recorded by other investigators (Sadun and Williams, 1966; Abdel Ghaffar, 1987) in albino mice infected with *S. mansoni*. They also attributed this change to persistent active hepatic cell damage and/or increased permeability of the cells due to toxic or anoxic effects.

S. mansoni infection did markedly elevate serum total proteins; such manifestations were correlated with the progress of the disease. Additive elevation in serum total proteins were produced when the bilharzial animals were exposed to lead compared to infected and control groups. These results are in agreement with those of El-Husseini (1986), who reported that *S. mansoni* infection induced a gradual increase in serum total proteins with increasing the infection period in mice exposed to 100 cercariae. Also, (Metwally, 1990; El-Fandy, 1992) were recorded an elevation in serum total proteins.

It is evident from the present findings that lead acetate treatment induced decrease in serum albumin content. These results are in agreement with those reported by Emara (1985), who recorded a decrease in serum albumin in workers exposed to Pb, Hg and TNT in explosive industry. Under the present experimental conditions might be due to mild damage of hepatic cells that are responsible for the synthesis of albumin with urea, failure in its manufacture and consumed of albumin in metabolism of Haem (Haem + Albumin → Metheamalbumin). *S.mansoni* infection induced a reduction at the 8th week of infection. These results are in agreement with those of Bolch (1972), who recorded a transient elevation in serum albumin content which reached its maximum at the 6th week of infection and started to fall markedly 8 weeks post-infection in mice infected with 20 cercariae of *S. mansoni*. Similarly, El-Husseini (1986) found a gradual non-significant decrease in serum albumin content from the 2nd to the 10th weeks post-infection. The combined effect of chronic lead intoxication and *S. mansoni* infection induced additive reduction in serum albumin content. This reduction might be due to an excessive loss following haemorrhage from intestine and also during the catabolism of haeme (Walter, 1987).

Serum globulins showed increase in all experimental animal groups. These results are in agreement with those of Eissa (1993), who recorded elevation of serum globulins in workers exposed to lead in battery industry. Similarly, serum globulins were found to be gradually elevated in mice infected with *S. mansoni* (Bloch, 1972 and El- Husseini, 1986). Albumin/globulins ratio was reduced in all experimental animal groups. These results are in concortant with those of Eissa (1993) who, indicated that A/G ratio decreased in workers exposed to lead in battery industry. Similarly, Emara (1985), found decreases in A/G ratio in workers exposed to Pb, Hg and TNT in explosive

industry. Likewise, El-Husseini (1986), found that A/G ratio showed a gradual decline with the lapse of time of infection but slight increase occurred in the 10th week in mice infected with 100 cercariae of *S.mansoni.*

The results of the present study demonstrated that lead acetate administration to male mice resulted in significant decrease of Hb, hematocrit and RBCs count of mice treated with lead acetate in contrast to those in the negative control mice (Table.2). On the other hand, WBCs count of lead acetate treated rats was elevated compared to the negative control group, and these results are in agreement with those described by Simsek (2009). However, Topashka-Ancheva (2003), showed that lead intoxication could lead to damage of erythrocytes membrane resulting in hemolysis or decrease of blood iron level which may be the reason of decreasing the concentration of Hb and PCV. These hematological alterations might be also due to the effect of lead on the activity of δ-aminolevulinic acid dehydrogenase which acts as key enzyme of heme synthesis. Previous study reported that lead inhibits the conversion of coproporphyrinogen III to protoporphyrin IX leading to reduction in Hb production and shortening of life span of Erythrocytes (2001).

WBCs count was found to be markedly increased in mice treated with lead acetate. These results are in agreement with those of Mazhar (1987), who found that WBCs count was increased in the Nile catfish (Clarias lazera) caused by mercury as an environmental pollutant. This increase in the total leucocyte count was anticipated to a state of stress after exposure to mercury. Leukocytosis observed in chronic lead poisoning was probably due to an increase in the number of neutrophils (Walter, 1987). Similarly, leucocyte counts were increased in mice and rats treated with $1/_{10}$ LD_{50} of water hyacinth extract for 30 days, (Hegazi, 1985; Abu-El-Zahab, 1992). On the other hand, *S.mansoni* infection induced a high significant decrease in WBCs count at the 4th week of infection but it was returned to its normal count 8 weeks after initiation of infection.

Lymphopenia, neutropilia, eosinophilia and moncytosis were recorded after chronic intoxication with lead acetate in mice. These results are in agreement with those of Rosenblatt and Marcus (1985), who recorded an increase in eosinophils in lead intoxicated patients. Likewise, Abu-El-Zahab (1992), recorded a decrease in lymphocytes percenntage and an increase in neutrophils, and monocytes percentage in mice and rats treated with $1/_{10}$ and 1/5 LD_{50} of water hyacinth extract as a result of severe acute stimuli to the haemopoietic system if exposed to toxic materials. But monocytosis occurred for phagocytic the damaged cells resulted from the toxic effect of water-hyacinth extract on the rats organs. S.mansoni infection induced neutropenia, eosinophilia and decrease in monocyte percentage. These results are in agreement with those of El-Rafie (1988), who found that eosinphilia was characteristic of *S.mansoni* infection in early and late stages of infection. Likewise, (El-Rafie, 1988; Omran, 1992) found that neutropenia, lymphopenia and reduction in concentration of peripheral blood monocytes were detected in late stages of hepatosplenic schistosomiasis. Excessive recuitment of monocytes due to bilharzial granulomata around eggs might led to a depletion of circulating monocytes. Exposure of mice infected with S.mansoni to lead acetate induced, lymphopenia, neutrophilia, monocytosis and eosinophilia which might be due to removal of cell damage by break down of *S. mansoni* eggs and phagocytosis.

Long term lead exposure generates reactive oxygen species and different free radicals. Also, it inhibits antioxidant enzymes activities, such as superoxide dismutase (SOD) and catalase (CAT), while it decreases the level of glutathione (Rahman, 2006). Lead induced oxidative damage in the kidneys and liver as evidenced by enhancement of lipid peroxidation (Farrag, 2007; El-Nekeety, 2009).

Chang (2006), reported that eggs are transported to the liver by portal circulation. Acute schistosomiasis is associated with heavy primary infections and with the initiation of egg production. In the portal venous system eggs are deposited while eggs are trapped in the peri-sinusoidal spaces of the liver, thus causing periportal granulomatous inflammation and the deposition of scar tissue around the eggs trapped inside the liver (Andrade, 2009). Granulomas are formed of inflammatory cells "eosinophils, macrophages, and lymphocytes" (Brito and Borojevic, 1997).

Granulomas were remarkable by concentric fibrosis and many fibroblasts encircled the trapped eggs (Amer, 2013; Kadry, 2013; Moreover & Dkhil, 2014), reported that infection with *S. mansoni* caused a severe hepatic granulomatous inflammatory response which appears in form of inflammatory cellular infiltration, cytoplasmic vacuolation and degeneration of hepatocytes. The presence of huge number of granulomas resulted in disorganization of the hepatic strands and lobular structure where granulomas are surrounded by a cuff of aggregated lymphocytes, epitheloid cells, eosinophils and collagenous fibres. Also, the hepatic sinusoids were dilated and apparently contained more Kupffer cells. From the previous studies; parasite induced a marked hepatic oxidative stress in schistoseme-infected mice. (Amer, 2013; Dkhil, 2014) deduced that *S. mansoni* altered the levels of free radicals and enzymatic/non-enzymatic antioxidants significantly. In the same manner, Fahmy (2014), reported that schistosome infected mice elevated the level of MDA, while decreased the GSH level and catalase activity significantly in hepatic tissue. A significant elevation was noticed in MDA and nitrite/nitrate levels, meanwhile; a significant reduction was tabulated in an antioxidant markers (GSH, glutathione reductase, catalase, thioredoxine reductase) of infected liver.

The present histological examination for the liver of lead intoxicated mice, showed that chronic lead intoxication has induced histopathological changes in the liver of these animals. The present work clearly revealed that the histopathological effects of lead intoxication were cytoplasmic vaculation of hepatocytes accompanied with the congestion of central and portal veins and hemorrhage in sinusoidal spaces, leucocytic infiltration and hyperplastic bile ductules. These results were also observed by Saker (1994), during their studies of the toxic effects of feeding of water-hyacinth to chicken. They have found that many histopathological changes were induced in the liver of chicken fed on diets containing 50% or 75% water hyacinth. The hepatocytes showed cytoplasmic vacuolation which was so extensive in some cells to the extent that only slight remnants of the cytoplasm were left and the central veins were congested and filled with blood in addition to leucocytic infirltrations. High concentrations of the heavy metals in the used water hyacinth might be responsible for the induction of the histopathological changes recorded in the liver of the chicken. Similarly, Saleh and Hamza (1984) found increase of the size of the liver of *Tilapia zillii* inhabiting the polluted water which might be due to the high accumulation of pollutants in the liver. The histopathological changes in the liver were congested blood vessels, intracytoplasmic vacuoles of hepatocytes and the lobular liver structure has largely disappeared. Such pathological effects were modulated by the presence of lead and cadmium.

S. mansoni infection induced formation of periovular granulomas at the 8th week of infection which, at the cellular level, composed of eosinophils, macrophages, lympbocytes and fibyoblasts and the mean diameter of these granulomas was 397 ± 59.9 μm. These results were in agreement with those of (Bolch, 1972; Akl, 1992) who reported that S.mansoni infection induced formation of periovular hepatic granulomas which were composed of prominent eosinnophils, macrophages, lymphocytes, epitheloid cells, plasma cells, mast cells and fibroblasts at the 8th week of infection. Likewise, Knauft and Warren (1969), found that the maximum diameter of heptic granuloma was observed at the 8th week of infection and decreased markedly at the 16th weeks of infection. Also, Bolch (1972), found that the hepatic tissue intimately adjacent to granulomas was abnormal. The abnormality was exhibited by distortion of liver cells and sinusoids. It is to be mentioned in this respect that Byram (1977), recoded that the schistosome eggs produced various materials including soluble lipids, enzymes and antigens, some of which diffuse into host tissues throught minute pores in the egg shell. These substances in addition to spines of eggs were responsible for granuloma formation.

Mice infected with S.mansoni and meanwhile, treated with lead acetate showed additive histopathological changes in the liver with reduced diameter of granuloma which may be due to a more development of the disease and/or increase of WBCs & globulins. The smallest sizes of granulomas were noticed in mice firstly treated with lead acetate and then infected with S. mansoin. Although granulomas had small size they showed the same cellular structure as those appeared after infection with *S. mansoni* only. The decrease in granuloma's diameter might be due to the toxicity of lead which increased the serum globlins (and hence immunoglobulins) and white blood cells which are considered as lines of defense in the body.

4. CONCLUSION

The results of this study revealed that mice exposed to lead acetate and infected with *S. mansoni* in comparison with those uninfected but exposed to lead or mice only suffered from schistosomal infection showed significant effects on hematological and biochemical parameters, compared with controls as decreasing serum in albumin content and A/G ratio. Hematological data as hemoglobin, RBCs count, and hematocrit, were also reduced. On the other hand, serum total proteins and globulins, total leucocytes count and neutrophils, sALT and sAST activities, were elevated under the same experimental conditions. Serious histopathological changes in hepatic tissues were also observed. It has, therefore, been concluded that the effect of each factor (lead intoxication or schistosomal infection) exacerbates the effect of the other which finally lead to bad additive effects on the organisms.

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