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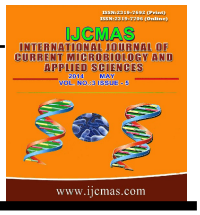


Original Research Article

Hepatotoxicity and Femoral Chondrodystrophy of Pregnant Mice Infested with *Scistosoma mansoni* and treated with Praziquantel

Hassan I. El-Sayyad[1]*, Osama A. Abbas[2], Maha F. Soliman[3], Zahraa A. Greash[2]

Mansoura University[1], Port-Said University[2], Suez Canal Univeristy[3], Department of Zoology, Faculty of Science, Egypt
Corresponding author

Keywords

Praziquantel *Scistosoma mansoni* Pregnant Mice; Hepato-toxicity and Femoral Chondro-dystrophy

A B S T R A C T

Praziquantel (PZQ) is widely and effectively used in the control of bilharziasis which constitutes a major endemic health problem in Egypt. There is no available work concerned hepatotoxicity and chondrodystrophy of praziquantel with or without bilharzial infestation on pregnant women. The present study aimed to evaluate the hepatotoxicity and chondrodystrophy of PZQ in infested mother mice. Eighty virgin and fertile male albino mice(one male/three females) with an average body weight of 25 to 28 g were used. Mating was carried out, and pregnancy was determined by examining sperm in vaginal smears. Pregnant mice were arranged into four groups; control, praziquantel-treatment ((600 mg/kg) divided into 2 equal doses of 300 mg/kg given 8 hours at 8th day of gestation) and bilharzial infested mother with or without praziquantel-treatment. Pregnant mice were sacrificed at 16 days of gestation and both livers and femoral bone were incised and subjected to histological investigations. PZQ-treatment revealed coagulative necrosis of hepatocytes, increase mitotic figures, leukocytic infiltration around blood vessel and reduction of cartilage cells and thinning of trabecular bone. Also, bilharzial infested maternal liver treated with PZQ showing dissolution of granulomatous lesions, congested blood vessels and restoration of newly-developed chondrocytes and restoration of almost normal epiphyseal cartilage and bone trabeculae. The authors concluded that PZQ-treatment of schistosomal infested mice during gestation showed hazardous impacts on liver bone of mothers which may influence on growth of their fetuses.

Introduction

Schistosomiasis is the second most common parasitic infection of humans after *malaria* in terms of public health importance (Abdulla *et al*., 2007) that infect 200 million people worldwide and are responsible for hundreds of thousands of deaths annually (Tran *et al.*, 2006). It is a man-made disease related to watering contact in the agricultural fields and affecting millions of people in developing

countries in the tropical and subtropical parts of Africa, Asia and South America. It is a bisexual trematode living in the portal blood and the venous plexus. Its life cycle necessitates the presence of an intermediate host - fresh water mollusc - that differs according to place. The pathogenetic stage is the ova that initiate an immunologically delayed hypersensitivity cell-mediated reaction in the organs where they are deposited (El-Garem, 1998). Schistosomiasis, known as bilharziasis, a parasitic disease that led to liver cirrhosis and hepato-splenomegaly and is caused by either *Schistosoma mansoni* or *haematobium* (Wiwanitkit, 2005).

Schistosomiasis is complicated by specific end organ pathology including hepatic fibrosis and malfunction, urinary obstruction and bladder cancer (Ross *et al*., 2002). The main cause of mortality and morbidity in human Schistosomiasis is hepatic fibrosis. Chronic infections with all *Schistosoma* species with the exception of *S. haematobiurn* can cause significant morbidity and mortality as a result of granuloma formation in the intestine and liver. The resulting hepatic fibrosis can lead to portal hypertension that eventually can be complicated by splenomegaly, esophageal varices, hematemesis, and death (El-Garem, 1998; Wynn *et al.,* 1998; Ross *et al.,* 2002).

S. mansoni is a widespread parasitic infection that may lead to serious complications, such as hepatic periportal fibrosis and portal hypertension, due to deposition of schistosome eggs in the tissues (Othman *et al*., 2010). The chronic egg- induced granulomatous response in the liver and intestine may eventually cause extensive tissue scarring and development of portal hypertension (Wynn *et al.,* 2004). During infection of vertebrate hosts with *S. mansoni*, trapped eggs induce tissue damage and granulomatous lesions that interfere with normal organ functions. Macroscopic and histopathological studies showed multiple liver abscesses around granulomas of *S. mansoni* in the acute and chronic phases of Schistosomiasis (Muller *et al.,* 2001; Wilson *et al*., 2007; Anthony *et al.,* 2012).

Concerning bone, its defect is common in patients with cholestatic liver disease. The importance of vitamin D status and calcium malabsorption in the pathogenesis of bone disease in these patients remains undefined (Bengoa *et al.,* 1984). Osteoporosis resulting in a high risk for fracture is a common complication in patients with liver disease, particularly in those with chronic cholestasis and with end-stage cirrhosis.

The pathogenesis of bone loss in liver patients is poorly understood but it mainly results from low bone formation as a consequence of cholestasis or the harmful effects of alcohol or iron on osteoblasts. Increased bone resorption has also been described in cholestatic women with advanced disease. The management of bone disease in liver patients is addressed to reduce or avoid the risk factors for osteoporosis and fracture (Guañabens and Parés, 2011). The etiology of osteoporosis is multifactorial and only partially understood. Various factors linked to the pathogenesis of bone loss are vitamin D, calcium, insulin growth factor–1, receptor activation of nuclear factor–κB ligand (RANKL), bilirubin, fibronectin, leptin, proinflammatory cytokines, and genetic polymorphisms (Yadav and Carey, 2013). The elevated serum calcium level might be an independent risk factor for liver cirrhosis (Yin *et al.,* 2013).

Many studies reported increased average of decreased birth weight reaching 4% to 18% for babies of infected mothers (Amano *et al.*, 1990; Siegrist and Siegrist-Obimpeh, 1992; El-Nahal *et al.,* 1998; Qunhua *et al.,* 2000). Schistosomiasis causes both anemia, malnutrition (McGarvey *et al.*, 1996; Friedman *et al.*, 2005; Kanzaria *et al.*, 2005; Ajanga *et al*., 2006; Leenstra *et al.*, 2006) and maternal iron deficiency (Roche and Layrisse, 1966) which increased incidence of prenatal mortality and morbidity (Bialek and Knobloch, 1999) as well as associated with adverse pregnancy outcomes including still birth, prematurity, low birth weight and possibly maternal mortality (Allen, 2000; WHO, 2002). Schistosomiasis was found to be associated with elevated proinflammatory cytokines in human maternal, placental, and cord blood, as well as an increased risk for the development of acute subchorionitis at the maternal-fetal interface (Kurtis *et al.,* 2011).

Praziquantel, the drug of choice for treatment of Schistosomiasis shown excellent safety and therapeutic effect against Schistosomiasis morbidity. The drug is produced by the pharmaceutical Bayer A.G. and E. Merck (Reich and Govindaraj, 1998) used for treatment both types of bilharziasis in Brazil, Cambodia, China, Egypt, Morocco and Saudi Arabia. Subsequently, some countries have succeeded in preventing Schistosomiasis transmission (WHO, 2012). It has been documented that PZQ had mild side-effect and very low toxicity in animal studies (Stelma *et al.,* 1995).

However, recent studies recommended that the drug must be re-evaluated because of its potential carcinogenicity and genotoxicity (Omar *et al*., 2005). Women of childbearing age (including pregnant women) are at considerable risk of morbidity in areas endemic for Schistosomiasis (WHO, 2002). Later studies, including monitoring in humans and pigs have shown that PZQ induces a greater frequency of hyperploid lymphocytes as well as structural chromosomal aberrations, but not in all the individuals treated (Montero and Ostrosky, 1997). In vitro studies have demonstrated that Praziquantel can induce micronuclei in syrian hamster embryonic cells and in lymphocytes of some individuals. The same was found about structural chromosomal aberrations. Fetal death and fetal resorption were found when PZQ was administered in high doses to pregnant rats between the 6^{th} and 10^{th} day of gestation (Frohberg, 1989; Montero and Ostrosky, 1997). The study aimed to evaluate the toxicity of PZQ in liver and femoral bone of infested mother mice.

Materials and Methods

Sixty female fertile virgin females and twenty fertile males of albino mice (*Mus musculus*) were purchased from Theodor Bilharz Research Institute, Cairo, with an average body weight of 25-30 grams. Thirty females albino mice were infected subcutaneously with 60 ± 10 *S. mansoni* cercariae and thirty females stilled pure, then all females albino mice marked and housed in cages in the animal House of Department of Zoology, Faculty of Science, Port-Said University. They are housed under standard colony condition maintained in a room temperature of 20-25^{o}C, exposed to 12 hour light/dark cycle and stayed for acclimatization for two months before starting the experiment. They are fed on standard diet composed of 50% grinding barley, 10% grinding yellow maize, 20% milk and 10% vegetables was

supplied. Water and food were available for consumption *ad libitum* throughout the experimental period.

Mice were observed daily and only healthy animals for control groups were used in this experiment. Mating was carried out by housing the female albino mice with fertile males in separate cages at a ratio of three females with one male for overnight. At early morning, vaginal smear for all mated females were examined and the presence of vaginal plug or sperm in the vaginal smear determined the zero day of gestation.

Female albino mice were divided into four main groups (15 in each group) scarified at 14^{th} and 16^{th} day of gestation as well as at parturition as following: Group (I): Control pregnant, Group (II): Praziquantel-treatment pregnant, Group (III): Schistosomal infestation pregnant, Group (IV): Praziquantel treatment and Schistosomal infestation pregnant.

Praziquantel-treatment (PZQ)

Praziquantel (600 mg tablet) was supplied from Alexandria Pharmaceuticals and Chemical Company, Alexandria, Egypt. The drug was suspended in distilled water at a concentration of 30 mg/ml and dose for each mouse as a curative dosage (600 mg/kg) divided into 2 equal doses of 300 mg/kg given 8 hours apart at 8^{th} day of gestation.

Light microscopic investigations

Maternal liver and femur bone of mother mice were removed and immediately fixed in formal saline for 24 hours. The femur bones were decalcified in 5% nitric acid for overnight followed by careful washing in water and returned to 10% formal saline. Both liver and decalcified femur were dehydrated in an ascending grades of ethyl alcohol, cleared in terpineol and embedded in molten paraplast 58-62 C. Serial 6 µm thick histological sections were cut and mounted on clean glass slides and stained in haematoxylin and eosin (Harris, *et al.*, 1980), cleared in xylene and mounted in canada balsam, then examined under bright field light microscopy and photographed.

Results and Discussion

Liver

In control, the hepatic tissue is composed of polygonal hepatocytes joined to one another in anastomosing plates, with borders that face either the sinusoids or adjacent hepatocytes. The hepatocytes are arranged in trabecules running radiantly from the central vein and are separated by sinusoids containing Kupffer cells. They are regular and contain a large spheroidal nucleus with a distinctly marked nucleolus and peripheral chromatin distribution. Some cells have two nuclei each. Few spaced hepatic sinusoids were arranged in between the hepatic cords and contained fine arrangement of Kupffer cells (Fig 1A-B).

Experimental PZQ-treated mother exhibited hepatotoxicity associated with disruption of the normal integrity of hepatic lobules. Focal necrotic foci of hepatocytes were detected characterized by dissolutions of hepatic cords and lysis of hepatocytes and marked hypertrophy of Kupffer cells. Many of the mitotic figures and leukocytic infiltrations were localized around the central vein (Fig 2A-D).

In bilharzial infested mother, there were well defined large fibrocellular

granulomatous lesions centred around living ova, including living miracidium and surrounded by lymphocytes, epithelioid cells, eosinophils, polymorphonuclear cells and fibrous tissue. Multiple granulomatous lesions, focal areas of hepatic necrosis, cloudy swelling as well as hydropic degeneration of hepatocytes were seen in some parts. Many focal area of leukocytic infiltaration with karyomegaly of hepatocytes and hypertrophied Kupffer cells having dark-brown bilharzial pigment become clearly evident (Fig 3A-C).

Femoral Epiphyseal cartilage

The femur of control mother showed a marked differentiation of the epiphyseal cartilage into five regions; including, resting, proliferating, hypertrophied, calcified and ossified zone. In the proliferative zone, the chondrocytes are arranged in cartilage column parallel with each other. The calcified zone characterized by traversed trabecular bone and enclosed in between the bone marrow (Fig 5A-B).

In experimental PZQ-treated mother, there was a marked resorption of the epiphyseal cartilage with the deranged epiphyseal line. The cartilage column cells lacked regular arrangement associated with moderate loss of most of them. The cartilage stromata become widened and separated the cartilage cells. Bone trabeculae attained considerably thinning (Fig 6A).

On the other hand, experimental bilharzial infested mothers exhibited massive resorption of the epiphyseal cartilage. The chondrocytes sparsely distributed within matrix and lacked columnar arrangement of cartilage cells. The cartilage stomata was increased, and regenerated cartilage cells are detected (Fig 6B). In experimental bilharzial infested mother treated with PZQ, there was a marked amelioration of the epiphyseal cartilage but lacked regular cartilage column arrangement. The cartilage stromata become reduced. Bone trabeculae retained to the almost normal pattern (Fig 6C).

Bilharziasis represents the major important health especially affecting the maternal tissues which intern influenced on fetuses growth and differentiation. Praziquantel represent one of the more affecting drug of treatment causing cytotoxic effect in both maternal and fetal tissues. Bilharzial infestation world-wide and share in increased incidence of mortality among patients as a result of bilharzial complication. 250,000,000 persons infected worldwide, including 20% of pregnant women living in hyperendemic areas (Moore and Smith, 1989; Adebamowo *et al*., 1991). Significant morbidity, mortality and severe disease are associated with defective regulation in the human population that it affects (Wynn *et al.*, 1998). The morbidity due to schistosomiasis has been shown to be greater than was previously thought (Fenwick and Webster, 2006), where more than 200 000 deaths per year are due to schistosomiasis (WHO, 2012).

Schistosomiasis causes both anemia, under nutrition (McGarvey *et al.*, 1996; Friedman *et al.*, 2005; Leenstra *et al.*, 2006) and maternal iron deficiency (Roche and Layrisse, 1966) causing increased prenatal mortality and morbidity worldwide (Bialek and Knobloch, 1999) and associated with adverse pregnancy outcomes including still birth, prematurity, low birth weight and possibly maternal mortality (Allen, 2000) as well as decreased work capacity, and perhaps to impaired fetal growth (WHO, 2002).

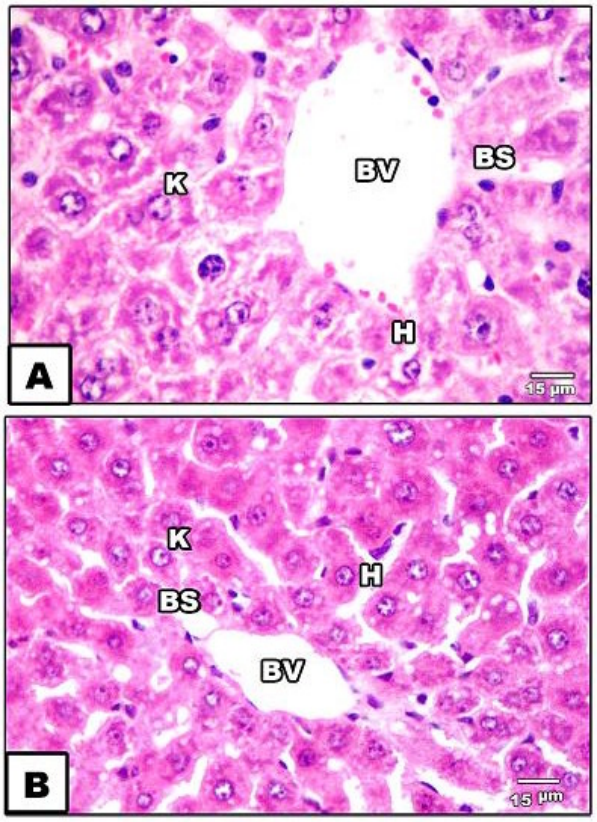
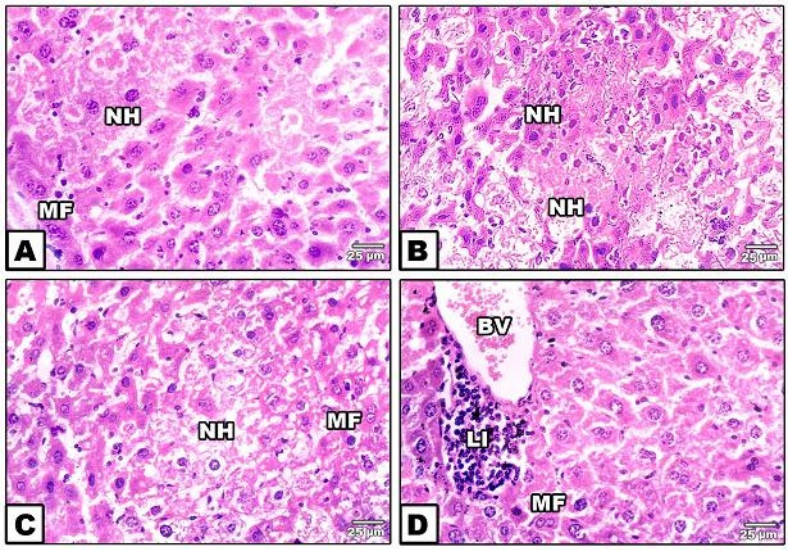
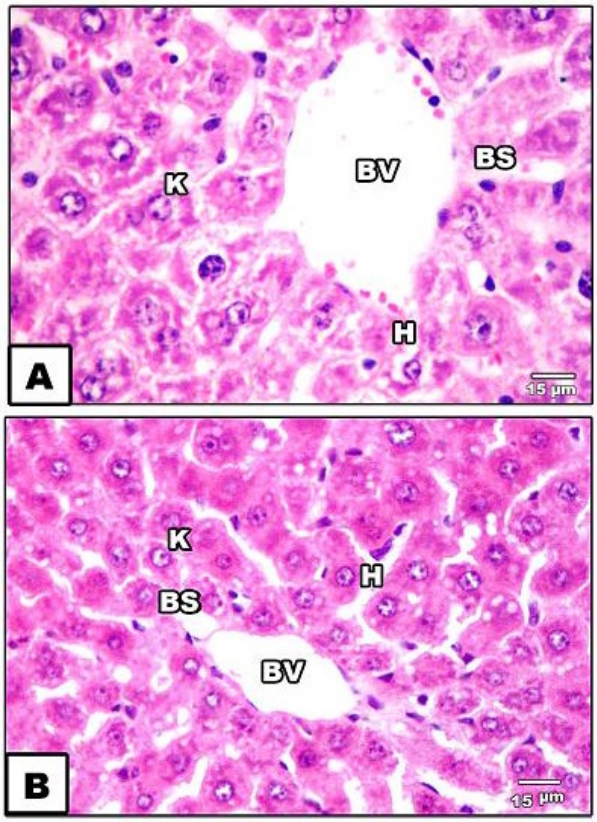


Fig.1 (A-B) Photomicrographs of histological sections of control maternal liver showing normal radially arranged cords of hepatocytes around the central vein. (Abbreviation: CV, central vein; BS, blood sinusoid; H, hepatocyte ; K, Kupffer cell) . **HX.-E.**





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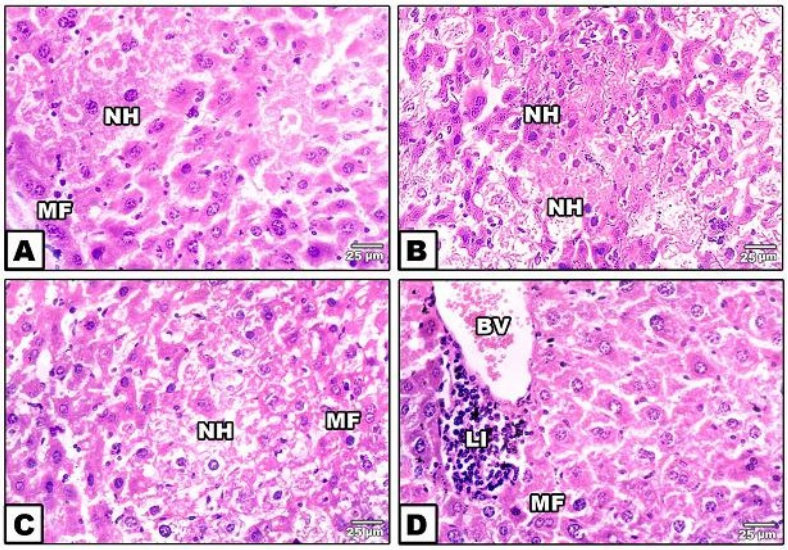

Fig 2 (A-D): Photomicrographs of histological sections of maternal liver treated with PZQ showing coagulative necrosis of hepatocytes (Fig. A-C), increase of mitotic figures and leukocytic infiltration around the blood vessel (Fig. D). (Abbreviation; BV, blood vessel; NH, necrotic hepatocytes; MF, mitotic figure; LI, leukocytic infiltration). **HX.-E.**

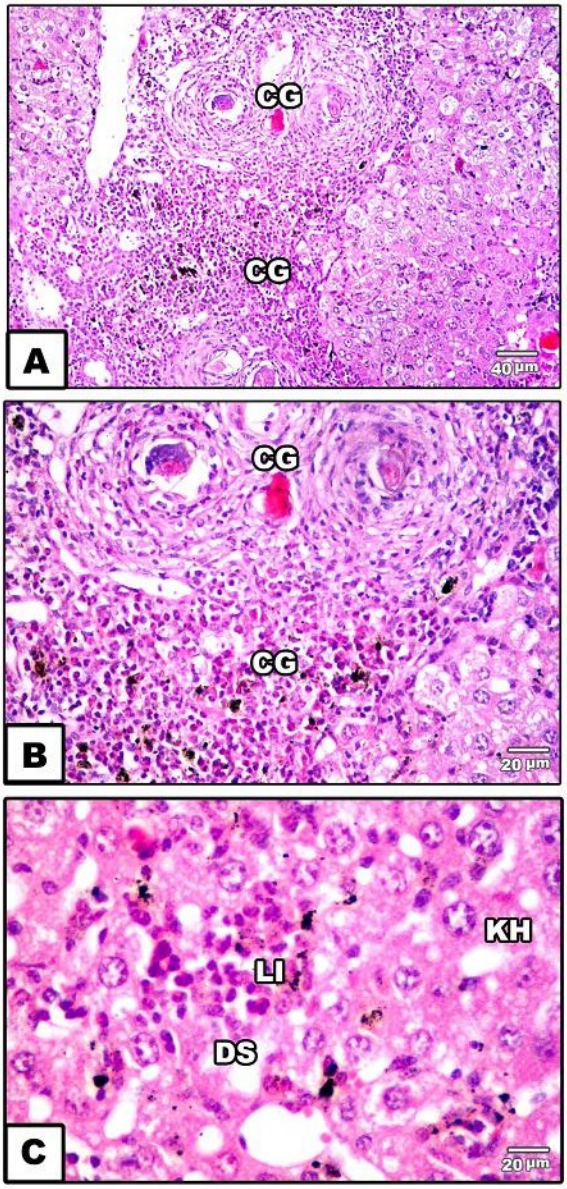

Fig.3 (A-C): Photomicrographs of histological sections of bilharzial infested maternal liver showing fibrotic granulomatous lesions , massive damage of hepatocytes, hypertrophied Kupffer cells and abundant distribution of bilharzial pigments. (Abbreviation: DH: degenerated hepatocytes, LI: Leucocytic infiltration, CG: cellular granuloma,). **HX.-E.**

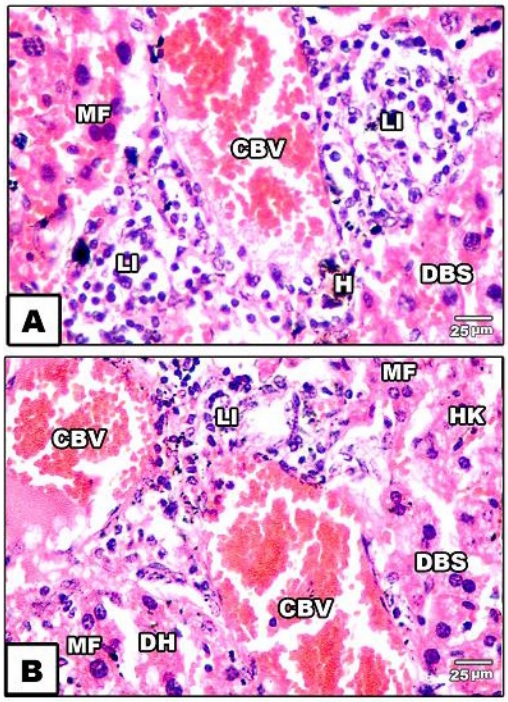

Fig (4A-B): Photomicrographs of histological sections of bilharzial infested maternal liver treated with PZQ showing dissolution of granulomatous lesions, pyknotic hepatocytes and congested blood vessels. (Abbreviation: DH: degenerated hepatocytes, LI: Leucocytic infiltration, CG: cellular granuloma,). **HX.-E.**

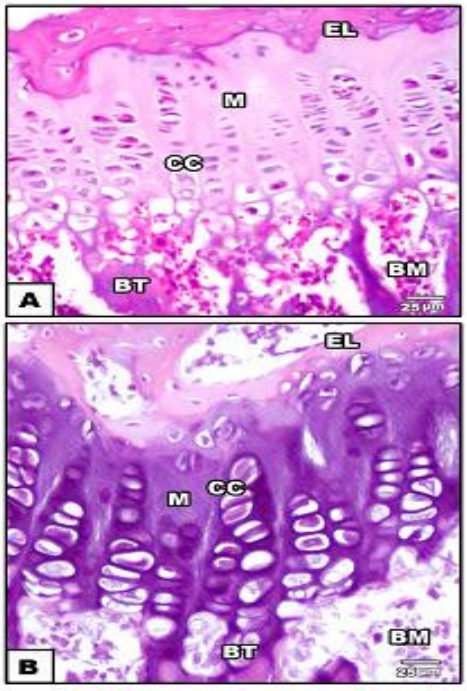

Fig.5 (A-B): Photomicrographs of histological section of control maternal epiphysis showing normal arranged cartilage column and thick trabeculae bone. (Abbreviation: EL: epiphysial layer, CC: cartilage column, BM: bone marrow, M: matrix). **HX.-E.**

From the present study, Praziquantel-treatment developed hepatotoxicity involved scattered foci of coagulative necrosis, leukocytic infiltration and increased average of mitotic figures. Similar findings of hepatotoxicity were recorded by Montero and Ostrosky, (1997) in albino rats. It possesses reversible and less toxic effects on the liver (El-Sharkawy *et al.*, 1993). Moderate hepatotoxic effect was detected in two cases treated with praziquantel (Abad *et al*., 1988).

Recent studies recommended that Praziquantel drug must be re-evaluated because of its potential carcinogenicity and genotoxicity. It induced a significant hepatotoxic, genotoxic and carcinogenic effects. It induced a significant increase in the mean values of AST, ALT and bilirubin with areas of hyaline degeneration, fatty changes, dysplasia and necrosis in the liver sections. It also induced a significant increase in the incidence of chromosomal aberrations as polyploidy, fragment, deletion and ring chromosome as compared with control group (Omar *et al*., 2005) and mild increases in liver enzymes have also been reported in some patients (Bayer Inc., 2007).

According to previous work reported by

several authors, bilharzial infestation developed fibrotic lesions throughout liver lobules which were association with hypertrophy of kupffer cells and accumulation of bilharzial pigment. However praziquantel-treated infested pregnant mice revealed marked dissolution of granulomatous lesions and increase of congested blood vessels. The stricken finding of reducing collagen deposition and formation of cellular fibrosis is of interest. Although some authors such as Behrman *et al*., (2008) and Abdel-Hafeez *et al.,* (2012) reported that praziquantel did not affect the fibrotic lesions, others reported similar finding of reducing granulomatous lesions and liver collagen deposition. As, Morcos *et al*., (1985) found that fibrosis was arrested and liver collagen content had diminished to normal levels by 20 weeks after PZQ treatment. It reduced the number, diameter and cellularity granulomata (Da Silva and Noël, 1995). As well as, It improved liver function parameters in mice infected with *S. mansoni* and treated with PZQ (500mg/kg for 2 successive days) (Badawy *et al.,* 1996).

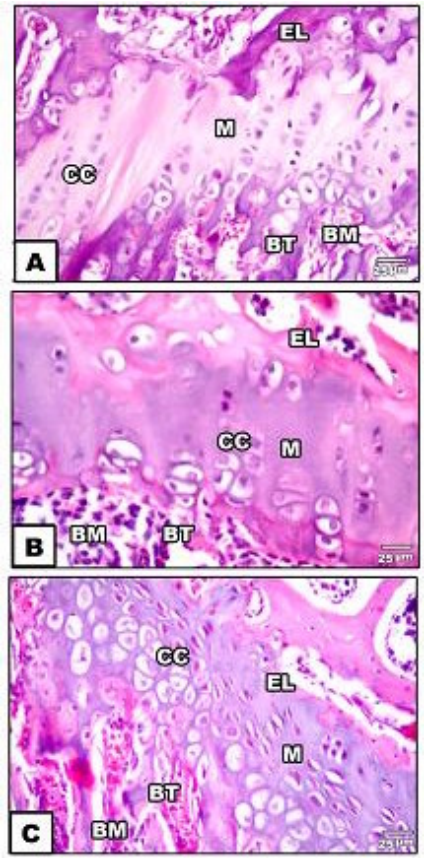

Fig.6 (A-C): Photomicrographs of histological section of maternal epiphysis. A. Praziquantel-treatment showing reduction of cartilage cells and thinning of trabecular bone. B. Schistosomal infestation showing sparse distribution of cartilage cells and increase of cartilage matrix. C. Praziquantel of infested mice showing restoration of newly-developed chondrocytes and restoration of almost normal epiphyseal cartilage and bone trabeculae. (Abbreviation: EL; epiphysial layer, CC; cartilage column, BM; bone marrow, BT; bone trabeculae). **HX.-E.**

In addition, experimental induction of Bilharzial infestation alone and that associated with praziquantel, as well as praziquantel-treatment led to alterations in femoral bone involving disorganization of cartilage column and banishments of many of the chondrocytes and increase cartilage stroma. The trabecular bone attained a considerable thinning. Infested pregnant mother showed the least cartilage column damage, however, established thinning of bone trabecula. The observed finding seemed to be related to increase liver damage by either bilharzial infestation or praziquantel-treatment, which interfere with vitamin D synthesis in hepatocytes and reproduce bone defects.

Schistosomal infection may suppress estradiol secretion (Wang *et al*., 2001), that plays a fundamental role in skeletal growth and bone homeostasis and any deficiency in its level cause bone loss (Weitzmann and Pacifici, 2006). Also, Hardy and Cooper (2009) stated that the inflammatory disease can increase bone resorption, decrease bone formation but most commonly impacts on both of these processes resulting in an uncoupling of bone formation from resorption in favour of excess resorption.

Osteoporosis resulting in a high risk for fracture is a common complication in patients with liver disease, particularly in those with chronic cholestasis and with end-stage cirrhosis (Guañabens and Parés, 2011).

Various factors linked to the pathogenesis of bone loss are vitamin D, calcium, insulin growth factor–1, receptor activation of nuclear factor–κB ligand (RANKL), bilirubin, fibronectin, leptin, proinflammatory cytokines, and genetic polymorphisms (Yadav and Carey, 2013).

Finally, the present study concluded that Praziquantel- treatment of bilharzial infested pregnant mice showed non-desirable results in both liver and femoral bone tissues of infested mother mice, which suggested delaying conception until cure from bilharziasis.

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