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Research Article

Anatomical and histological alternations of the spleen in rat, *Rattus norvegicus* exposed to mercury

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Abstract

This study aimed to investigate the histological, morphological and histochemical structures of the spleen of rats exposed to mercury chloride. Sixteen adult rats, *Rattus norvegicus* were obtained and divided into two groups as a control group that orally administered distilled water daily for ten weeks, and the second group administrated $Hgcl_2$ at a dose of 3mg\kg B.W\IP three times weekly. The results showed a significant difference as a decrease in the weight of the spleen of treated rats and an increase in the mean length of the spleen. There were significant differences in capsule thickness and the diameter of lymphoid follicles of the spleen compared with the control group. The mean capsule thickness significantly decreased, while the diameter of lymphoid follicles was increased. The histological examinations revealed lymphoid hyperplasia and proliferation of the red pulp (PALS) macrophage with vacuolated sub capsular cells, haemosidrosis with fibrous tissue, and aggregation of inflammatory cells with giant cells, aggregation of necrotic foci and minerals in granuloma center. In addition, in the PAS staining, alternation and necrosis of splenic cord, accumulations of hemosiderin in the red pulp, cellular degeneration and necrosis with an accumulation of adipose tissue, and degeneration of lymphocytes in the cortical area of the splenic corpuscle were observed. The Mallory trichrome staining revealed necrosis and degeneration of endothelial cells with loss of nuclei, degenerations of germinal center and necrosis of medullary sinuses in addition to hemosiderin accumulations.

Keywords: Parasitic contamination, Vegetable, Worm, Protozoan.

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Introduction

Mercuric chloride ($HgCl_2$) is used in a broad group of disinfectants because it has a strong sterilization ability. This feature makes it used in ointments to treat skin infections and sores (Jaya et al. 2009). Mercury is a hazardous chemical that can form many toxic organic compounds (Ostrovskii et al. 2000). Environmentally, mercury's physical and chemical states are complex and rely on several environmental factors, such as sediment in soil, the percentage of organic and trace substances in the water, pH, sunlight, and the adsorption to solid particles (Benes & Havlik 1979).

Mercury absorbs from the skin and the intestinal tract. It does difficulty cross the blood-brain barrier but can accumulate in the placenta (Berlin 1986). Hg compounds affect specifically the central nervous system (CNS), while the kidney is the target organ

Table 1. Body weight, spleen weight &length of spleen in $Hgcl_2$ treated male rats.

Groups	Body weight (g)	Spleen weight(g)	Spleen length
Control Group	194.20 ±6.390	0.913 ± 0.025	31.5833 ±1.04195
Mercuric chloride Group ($Hgcl_2$)	154.90 ±4.872	1.370 ±0.034	43.1667±1.51217

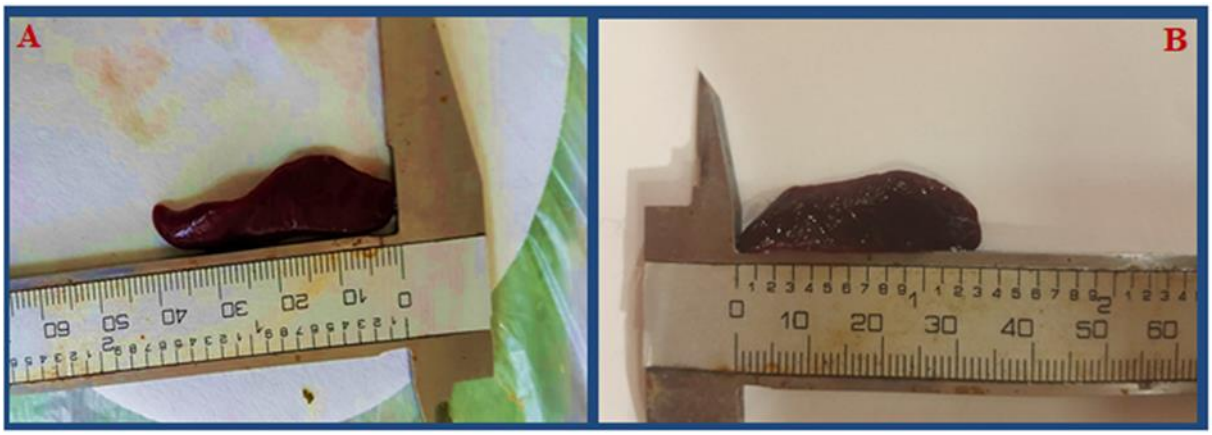

Fig.1. Showing spleen of untreated animals: A) visceral surface B) parietal surface.

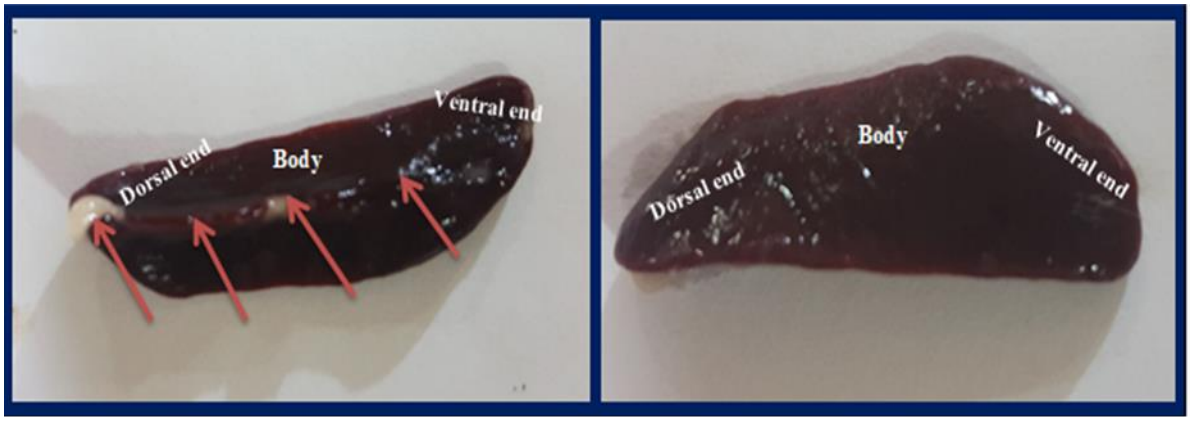

Fig.2. Showing spleen of treated animals: Left) visceral surface Right) parietal surface.

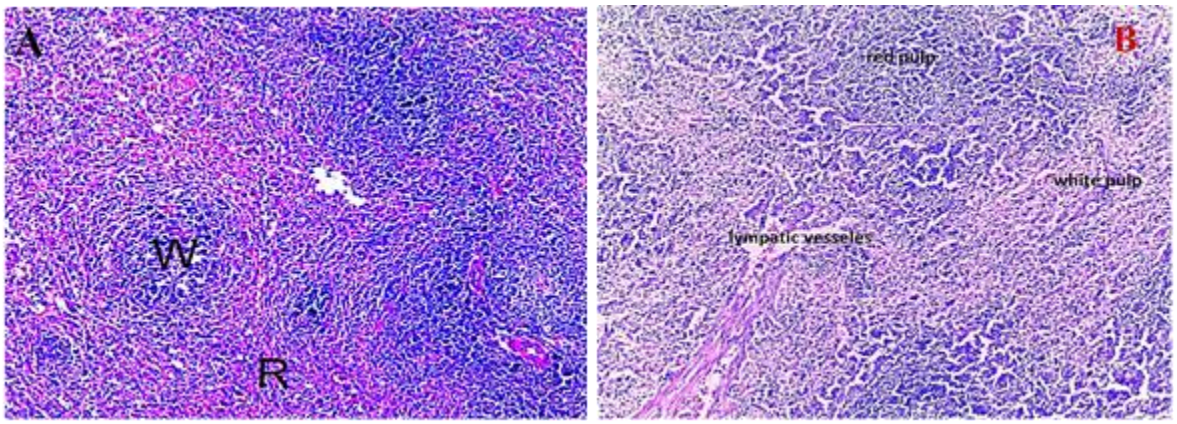

Fig.3. Histological section of rat spleen showing A) normal white pulp (W) and red pulp (R) B) normal white pulp and red pulp with lymphatic vessels (H&E 200 X).

for inorganic Hg. Therefore, exposure to various mercury compounds causes many pathological effects in the central nervous system. The signs of Hg poisoning include allergic manifestations, neurodegenerative and autoimmune disorders (Clarkson et al. 2003; Tchounwou et al. 2003;

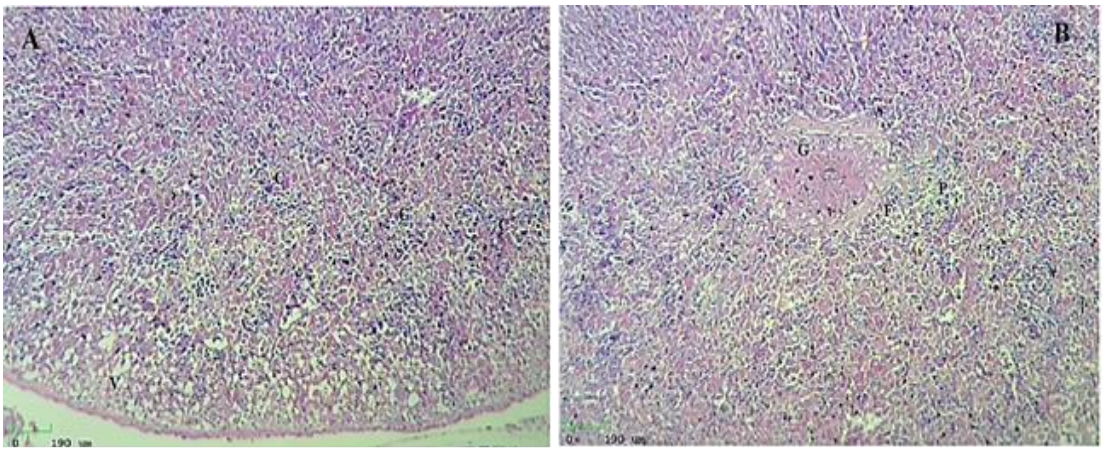

Fig.4. Section of right spleen: A) show vacuolated of sub capsular cell (V), cellularity lymphocyte(C) B) show lymphoid hyperplasia(p), red pulp are rich in cellularity. Granuloma (G) with fibrous capsule (F) (H&E 40x).

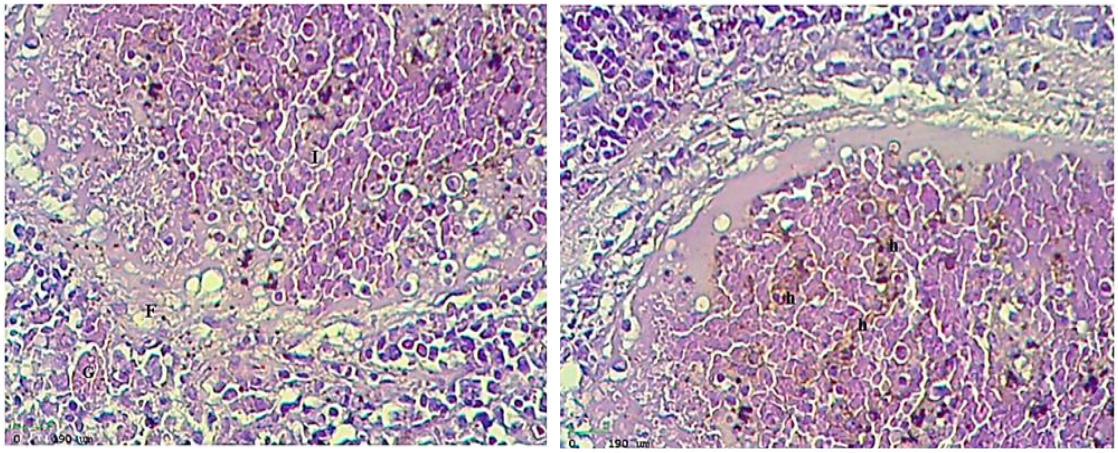

Fig.5. Section of right spleen Left) show Giant cell (G), fibrous connective tissue (F) aggregation of inflammatory cells (I) Right) Show hemosiderin (h) (H&E 40x).

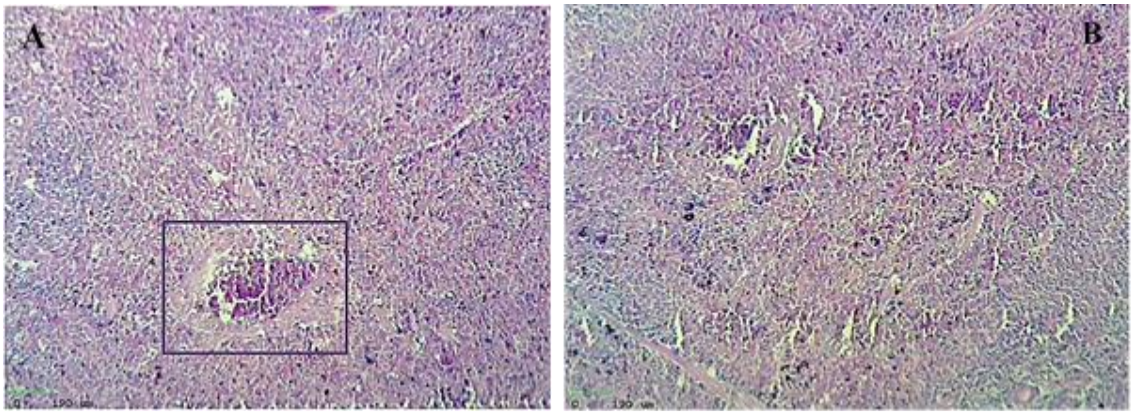

Fig.6. Section of right spleen A) show aggregation of minerals in granuloma center B) show necrotic foci (N) (H&E, 40X).

Havarinasab & Hultman 2005; Hultman et al. 2006).

The spleen is a secondary lymphoid organ in vertebrates (Aughey & Frye 2001). It is the largest filter for blood in the body, enclosed by a fibrous connective tissue capsule (Steiniger & Barth 2000). The fibrous and elastic tissue capsule sent trabeculae into parenchyma divided into lobules (Shier et al. 1995; Nance & Sanders 2007). These lobules are lined with a red pulp containing arterial capillaries with small venous vessels and white pulp consisting

Table 2. Estimation of capsule thickness and lymphoid follicles diameter of spleen in experimental groups.

Groups	Capsule thickness (µm)	Diameter of lymphoid follicles (µm)
Control Group	18.44±1.246	2.882±1.788
Mercuric chloride Group ($Hgcl_2$)	24.43±2.160	2.510±1.593

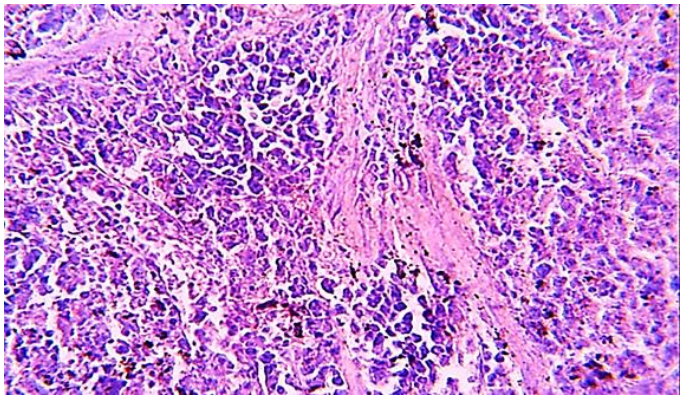

Fig.7. Section of spleen show damage of splenic cord hyperplasia of trabecula necrosis of splenic cord tissue hemosiderin accumulation (PAS, 40X).

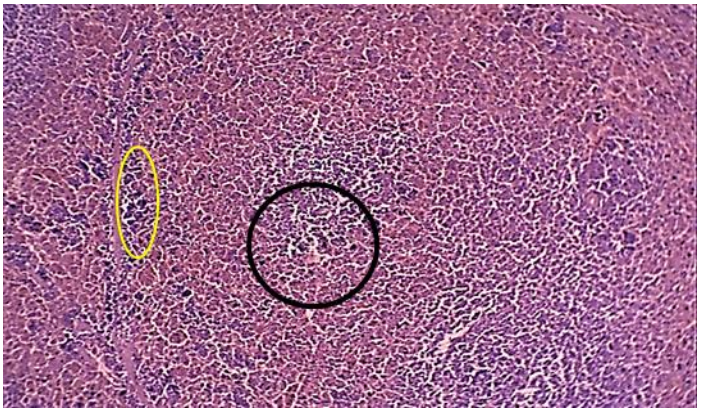

Fig.8. Section of spleen show (yellow) hemosiderin accumulation, and (black) necrosis of germinal center (PAS, 40X).

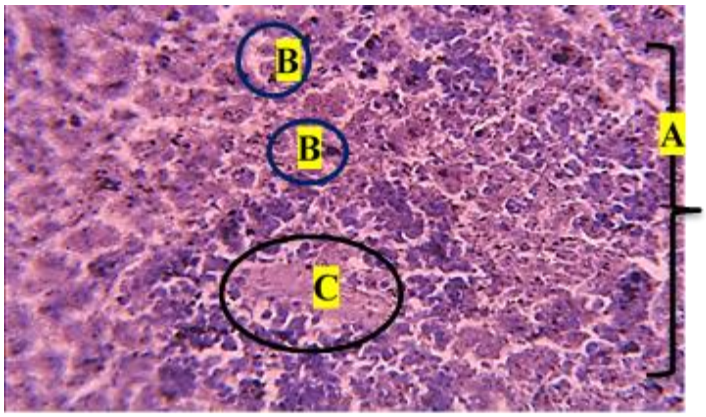

Fig.9. Section of spleen show (A) damage of spenic cord (B) hemosiderin stain and (C) necrosis and damage of trabeculae and splenic cord (PAS, 40X).

mainly of T and B lymphocytes (Shier et al. 1995; Miller et al. 1979). This study aimed to investigate the histological, morphological, and histochemical structures in the spleen of rats, *Rattus norvegicus* exposed to mercury as $Hgcl_2$.

Materials and Methods

A total of sixteen adult rats weighing 180-200g and

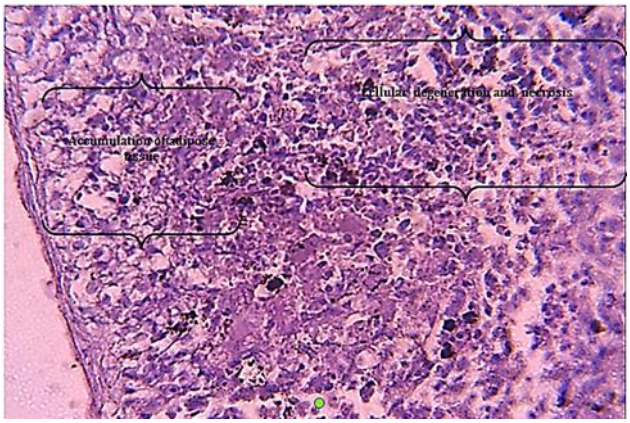

Fig.10. Section of spleen show cellular degeneration and necrosis with accumulation of adipose tissue (PAS, 40X).

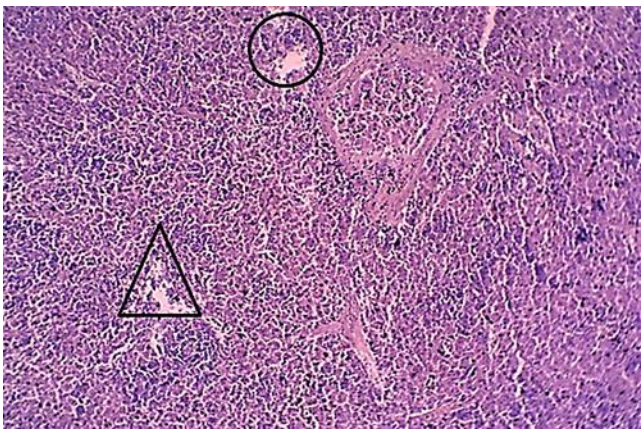

Fig.11. Section of spleen (Circle) show damage and degeneration of lymphocytes in the cortical area of the splenic corpuscle (Triangle) necrosis of splenic cord (PAS, 40X).

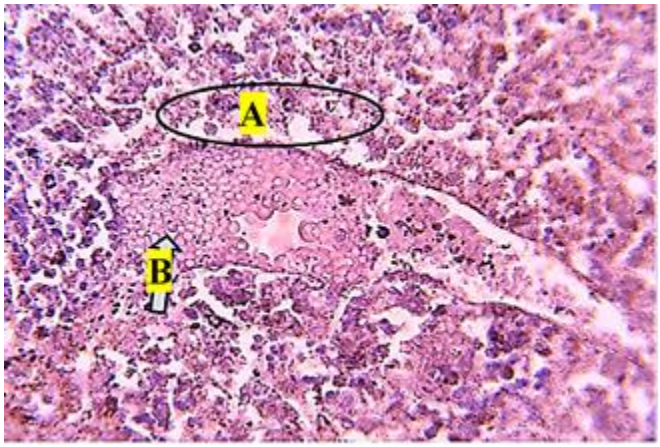

Fig.12. Section of spleen show general damage of spenic tissue, (A) hemosiderin stain and (B) congestion vein (PAS, 40X).

ages of 8-10 weeks were obtained from the animal house of Basrah University. They were divided into 2 groups (N = 8) for the experiment in the animal house with 20-25°C temperature and 5% humidity.

Preparation of chemicals: The chemicals were acquired from Merck India Ltd. The chemical was administered three times per week as intraperitoneal injection at a dose of 3mg/kg body.wt for 10 weeks. LD50 of $Hgcl_2$ had been calculated 40mg/kg body.wt (Uma et al. 2012).

Experimental protocol: The animals were divided into 2 groups, including (I): as control, orally given

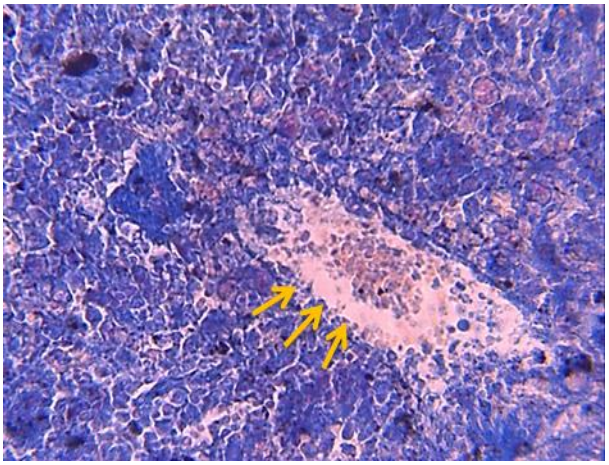

Fig.13. Section of spleen show necrosis and degeneration of endothelial cells with lots of nuclei (40x) Mallory's trichrome stain.

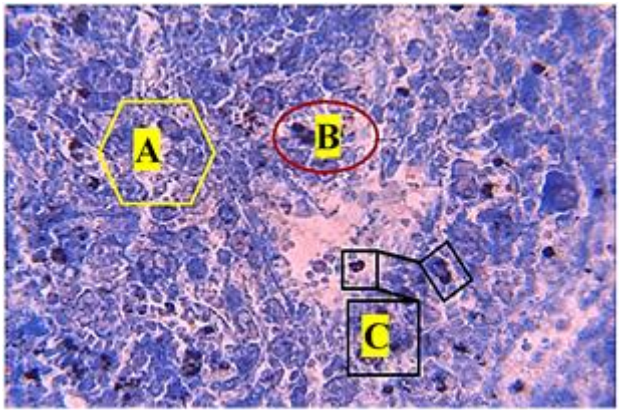

Fig.14. Section of spleen (A) damage of sinusoids and splenic cord (B) show necrosis and degeneration of the medullary sinuses (C) hemosiderin stain (40x) Mallory's trichrome stain.

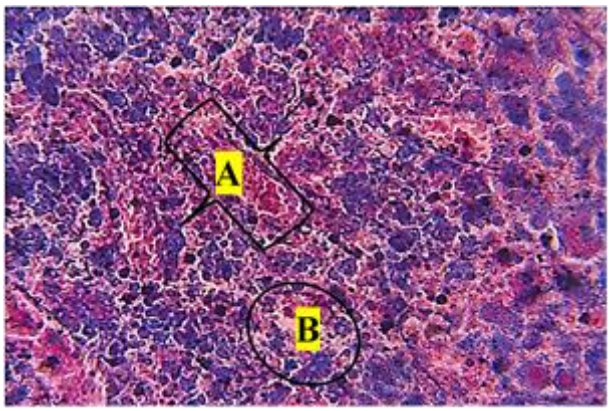

Fig.15. Section of spleen (A) show accumulation of blood cells between of splenic cord (B) show necrosis and degeneration of germinal center (40x) Mallory's trichrome stain.

distilled water daily for 10 weeks and (II): male rats received $Hgcl_2$ at a dose of 3mg\kg B.W\IP three times weekly for 10 weeks.

Morphological study: For morphological examination, ventral midline skin near the xiphoid process was cut up to the pubis to remove the spleen. The length and diameter of the spleen were measured after dissection, measuring was done using a digital Vernier calliper.

Histological study: The samples of the spleen were immediately fixed in 10% neutral buffered formalin for 24 hours then dehydrated with the series

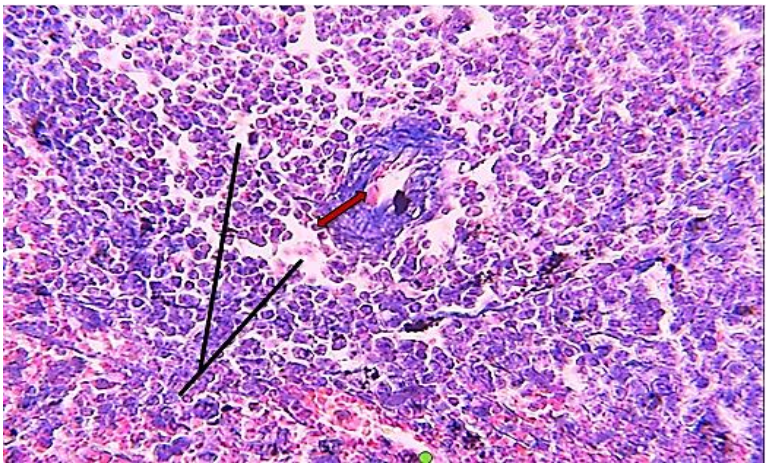

Fig.16. Section of spleen show degeneration of germinal center (40x) Mallory's trichrome stain.

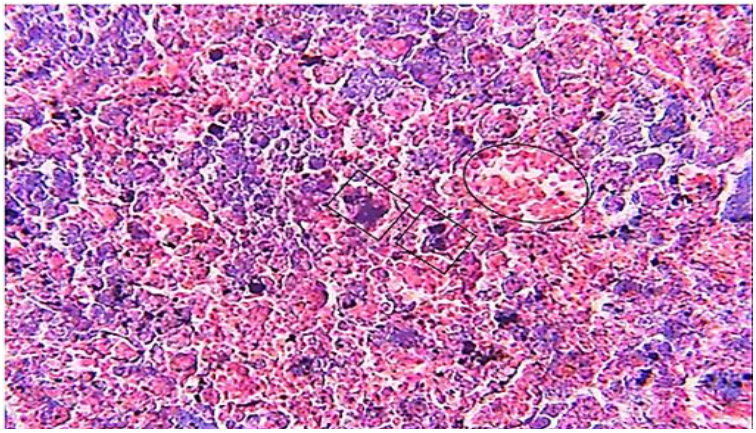

Fig.17. Section of spleen (A) show hemosiderin stain (B) show expansion of medullary sinus (40x) Mallory's trichrome stain.

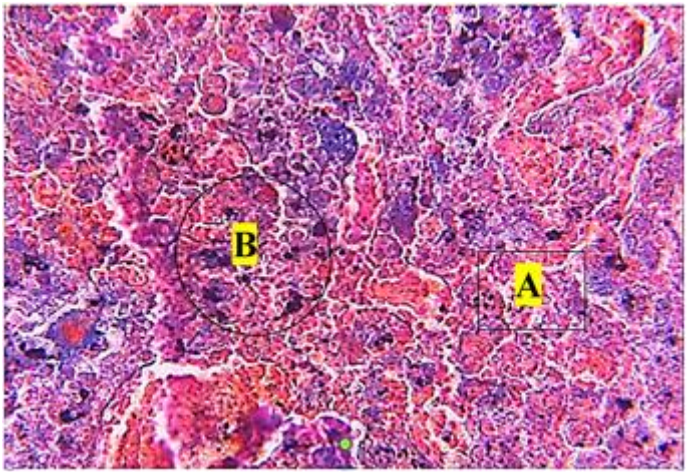

Fig.18. Section of spleen (A) show hemosiderin stain (B) show necrosis of medullary sinus (40x) Mallory's trichrome stain.

concentration of ethyl alcohol and embedded in paraffin wax then cut by rotary microtome to 4-6µm. Then the histological sections were stained with hematoxylin and eosin, periodic acid Schiff (PAS) and Mallory trichrome (Luna 1968).

Results and Discussion

The result of the macroscopic examination of the spleen in the control group showed an elongated tongue-like i.e. ribbon-like spleen with dark red colour located anteriorly in the left abdomen. It had a

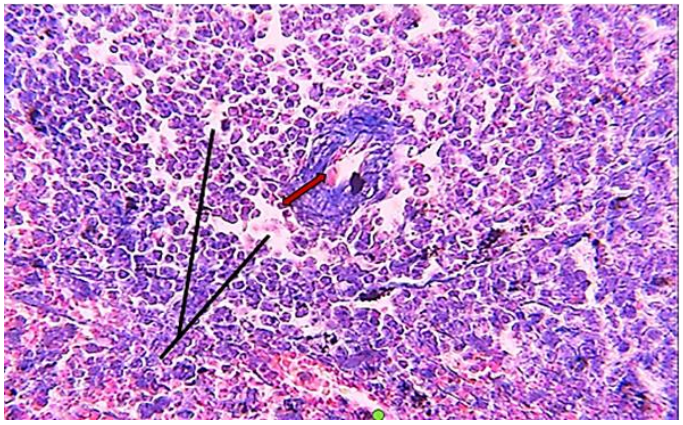

Fig.19. Section of spleen **(**Red**)** show hyperplasia of general artery wall (Black) damage in venous sinus (40x) Mallory's trichrome stain.

rectangle shape in treated animals with increased weight and size (Figs. 1-4). A significant decrease was observed in the body weight of the treated rats and a significant increase in the mean length of the spleen (P<0.05) (Table 1). These findings agreed with Changming et al. (2019) that revealed mercury reduced the body weight and increased the cecal size of chickens. In addition, Altunkaynak et al. (2016) showed a decreased ovary volume and increased tretic follicles in the rats exposed to mercury oxide.

Based on the histological examinations, the spleen is enclosed in a capsule composed of fibrous and elastic tissue with smooth muscle. Furthermore, several trabeculae are entered into the spleen's parenchyma divided into lobules. The two major functional spleen lobules are to produce the hematogenous red pulp, which contains arterial capillaries with small venous vessels and the lymphoid white pulp containing mainly T and B lymphocytes (Figs. 5 and 6).

In the treated groups, the mean capsule thickness was 24.43±2.160mm with the mean diameter of lymphoid follicles as 2.510±1.593mm. Significant differences in the capsule thickness and the diameter of lymphoid follicles compared to the control group (18.44±1.246) were found (P<0.05), revealing a decrease in the capsule thickness. The diameter of the lymphoid follicles was 2.882±1.788 showing an increase (Table 2). The histopathological results displayed chronic diseases as an earlier cause of the lymphoid hyperplasia and proliferation of the macrophage of the red pulp (PALS) with vacuolated subcapsular cells (Figs. 7 and 8) occurring focally or regionally. There are haemosidrosis with fibrous tissue and aggregation of inflammatory cells with large cells (Figs. 9 and 10). Moreover, the results of histopathological examinations showed aggregation of the necrotic foci and minerals in the granuloma centre (Figs. 11 and 12). These results were similar to Ansar & AlGhosoon (2016) findings that showed changes in the spleen of rats exposed to mercuric oxide as vacuolization, swelling, and monocular infiltration. Similar results have been reported by Oriquat et al. (2012) in rats exposed to mercury in all tested organs causing their abnormal functions duo the disturbance in calcium and phosphate homeostasis.

The periodic acid Schiff (PAS) staining of the spleen tissue revealed damage and necrosis of splenic cord, accumulations of hemosiderin in the red pulp, cellular degeneration and necrosis along with the accumulation of adipose, and alternation and degeneration of the lymphocytes in the cortical area of the splenic corpuscle (Figs. 13, 14, 15, 16, 17 and 18). In addition, Mallory trichrome staining revealed necrosis and degeneration of endothelial cells that had lost their nuclei, degenerations of germinal centre, necrosis of medullary sinuses and hemosiderin accumulations (Fig. 19). These histological alternations may be related to the formation of reactive oxygen species (ROS), leading to cell death (Jahan et

al. 2019). The accumulations of hemosiderin in the red pulp may be due to the destruction of the erythrocytes that usually occur in the spleen's red pulp, which increases iron stores (Cullen & Stalker 2016).

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