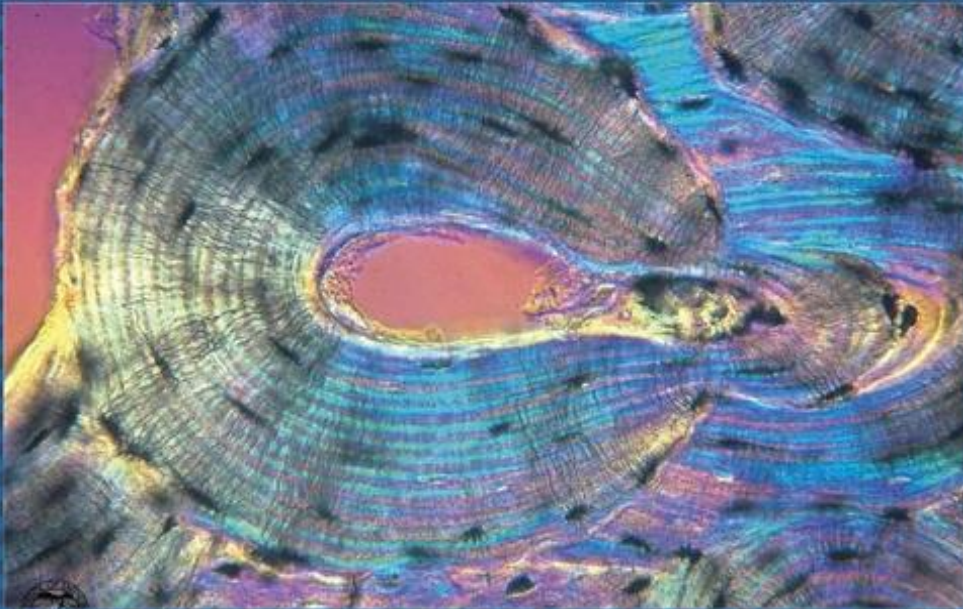




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A Histological Study on The Effects of Bisphenol A Administration on The Liver, Spleen and Pancreas of Adult Male Albino Rats and The Possible Protective Role of Lycopene

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ABSTRACT

Bisphenol A (BPA) is an endocrine disturbing element liberated through the environment and broadly used all over the world. The purpose of this work was to explore the impacts of BPA on liver, spleen and pancreas of adult male albino rats and the probable protecting role of Lycopene. Thirty adult male albino rats were distributed into three groups; group I (control group) , group II administrated BPA orally (50 mg/kg) for 30 days and group III (BPA+ Lycopene treated group) administered Lycopene (LYC) concomitantly with BPA at a dose of 10 mg/kg. At the end of the experiment, rats of all groups were sacrificed. Liver, spleen and pancreas were extracted, fixed and processed for histologic study. The area % of the collagen fibers in liver, spleen and pancreas was measured in the different groups and statistically analyzed. Structural alterations were discovered in liver, spleen and pancreas of BPA-treated rats including dilated congested blood vessels and vacuolar degeneration of the cells. When Lycopene (LYC) was concomitantly administered with BPA, It produced marked improvement at structural and ultrastructural levels. The morphometric results declared that the area % of the collagen fibers in the liver, spleen and pancreases was considerably increased in the BPA-treated group in comparison to the control group. Meanwhile the area % of the collagen fibers of BPA+ Lycopene treated group was more or less comparable to the control group with no significant difference. It was concluded that LYC has noticeable protective effects versus the damaging effects of BPA.

INTRODUCTION

Bisphenol A (BPA) is an environmental substance consumed in the manufacture of epoxy resins like food wrapping and can- covering, and plastic products (Fleisch *et al.*, 2010). BPA could be liberated from the walls of containers [Geens *et al.*, 2012], spreads to the circulation, and triggers cytotoxic outcomes [Dobrzyńska and Radzikowska, 2013]. Even small doses of BPA influence growth and reproduction (Welshons *et al.*, 2006).

BPA is absorbed from the alimentary canal into blood (Fisher *et al.*, 2011). It is highly conjugated in the liver to create a major metabolite, bisphenol glucuronide, which in turn is eradicated in urine (Pottenger *et al.*, 2000). BPA works as an endocrine disturbing compound [Korkmaz *et al.*, 2010]. It also acts as a xenoestrogen regulating the endocrine pathways through a receptor-mediated method (Hatef *et al.*, 2012).

BPA was discovered in more than 90% of the human tissue samples inspected and there is increased interest regarding its higher bioaccumulation in fetal and child tissues (Calafat *et al.*, 2008).

Interest has been provoked in using natural compounds isolated from plants versus the damaging outcomes of chemical compounds (Szymanska *et al.*, 2017).

Lycopene (LYC) is a red pigment present in tomatoes, guava, watermelon and grapefruit (Kong *et al.*, 2010). Among the public dietary carotenoids, LYC is considered the most predominant carotenoid (Pirayesh Islamian and Mehrali, 2015). Over 85% of LYC intake is derived from tomato and its products (Canene *et al.*, 2005).

This study investigated the effects of BPA on the liver, spleen and pancreas of adult male albino rats and the probable defensive role of LYC.

MATERIALS AND METHODS

Preparation of Materials:

BPA was attained from Sigma-Aldrich, St. Louis, MO, USA in the form of powder and melted in corn oil. Lycopene was obtained from North China Pharmaceutical Co., Ltd. (Shijiazhuang, China) in the form of powder and dissolved in corn oil.

Animals and Experimental Design:

A total number of 30 adult male albino rats (3 months) weighing about 250-300 gm. were used in this study. The animals were obtained from the animal house of the Faculty of

Medicine, Assiut University. They were retained under standard laboratory circumstances, nourished with commercial food and provided with water ad libitum. Rats were segregated into three groups (10 rats each):

- Group I (control): This group took corn oil by mouth (vehicle) daily throughout the experimental period.
- Group II (BPA-treated group): Rats of this group received BPA (50 mg/kg body weight) through a gastric tube for 30 days (Richter *et al.*, 2007).
- Group III (BPA+ LYC treated group): Rats in this group received BPA in the same way as group II with concurrent administration of LYC daily (10 mg/kg body weight) orally by gastric tube (Tokaç *et al.*, 2015).

At the end of the experimental period, rats from all three groups were anaesthetized by ether inhalation and sacrificed. The liver, spleen and pancreas were removed and exposed to the following:

1-Light Microscopic Study:

The specimens were fixed in 10% formalin and managed for H&E, PAS and Masson's trichrome staining. The slides were then inspected by a light microscope and photographed (Kiernan, 2001).

2-Electron Microscopic Study:

Instantly after sacrificing, small parts of liver, spleen and pancreas were fixed in 5% cold glutaraldehyde for 24 hours. Then the samples were passed through 3-4 changes of cacodylate buffer washing (pH 7.2) for 20 minutes in each change and postfixed in cold osmium tetroxide for 2 hours. Dehydration was then done by utilizing ascending grades of ethanol. Embedding was performed in Epon using gelatin capsules for the polymerization. Semithin sections were made using the LKB ultramicrotome.

The sections were stained by toluidine blue.

Ultrathin sections (50-80nm) from designated parts were gathered on copper grids. Ultrathin sections were distinguished with uranyl acetate and lead citrate (Hayat, 1986). They were inspected by Transmission electron microscopy (Jeol 100x) and photographed at 80kv at Assiut University Electron Microscopy Unit.

The study was following the international ethics and regulations for animal research in laboratory applications.

3-Morphometric and Statistical Analysis:

In the sections stained by Masson's trichrome, the area % of the collagen fibers within the liver, spleen and pancreas was calculated in the control and treated groups.

The selected sections were scanned by the mean of CX41 optical microscope (Olympus, Center Valley, PA, USA) that was provided with the Olympus U-CMAD3 digital camera that connected to a computer. The data attained was showed as mean \pm standard deviation (SD). This data is more analyzed by using Social Sciences (SPSS, 16.0). The level of the statistical significance was then analyzed by ANOVA test.

RESULTS

1-Histological Results:

A-Liver:

By light microscopic examination, the liver of the control group showed cords of hepatocytes radiating from the central vein and separated by blood sinusoids. The hepatocytes were polygonal with vesicular nuclei and prominent nucleoli (Figs. 1A& 4A). By PAS stain the glycogen was present within the hepatocytes as purple-magenta-colored granules (Fig. 2A). Masson's trichrome stain showed the normal distribution of collagen around blood vessels (Fig. 3A). By electron microscope, the hepatocytes were polygonal with a rounded nucleus. The cytoplasm

contains mitochondria, endoplasmic reticulum, glycogen granules and lysosomes (Fig. 5A).

Regarding the BPA-treated group, the light microscopic examination of the liver showed dilated congested central vein, vacuolar degeneration of the hepatocytes and congestion of the hepatic sinusoids (Figs. 1B& 4B). By PAS stain there was a marked decrease in the glycogen within the hepatocytes (Fig. 2B). By Masson's trichrome stain there was an increase in the amount of collagen around the vessels in the portal tract area (fig. 3B). By electron microscope, the hepatocytes were irregular with irregular nuclei. The cytoplasm was vacuolated and contained damaged mitochondria and glycogen granules (Fig. 5B).

Light microscopic examination of the liver of LYC group showed cords of hepatocytes separated by hepatic sinusoids and radiating from the central vein (Figs. 1C& 4C). By PAS stain the amount of glycogen was increased in comparison with the treated group (Fig. 2C). By Masson's trichrome stain there was a more or less normal distribution of collage within the liver (Fig. 3C). By electron microscopic examination the hepatocytes were more or less similar to the control group (Fig. 5C).

B-Spleen:

Light microscopic examination of the spleen of the control group revealed the white pulp with periarterial lymphatic sheath and the red pulp that contained splenic cords and sinuses (Figs. 6 A and B & 8A). Masson's trichrome stain showed the normal distribution of collagen fibers within the spleen (Fig. 7A). By electron microscope, the spleen showed aggregations of lymphocytes of different sizes with heterochromatic nuclei and irregular rim of dense chromatin. The cytoplasm of lymphocytes contained mitochondria and rough endoplasmic reticulum (Fig. 9A).

The light microscopic examination of the spleen of BPA treated group showed disorganization of the white pulp with thickening of the periarterial lymphatic sheath. The lymphocytes were densely packed with pyknotic nuclei and the red pulp was congested (Figs. 6 C and D & 8B). By Masson's trichrome the collagen fibers increased within red and white pulps (fig. 7B). By electron microscope, the lymphocytes were deformed with irregular nuclei and vacuolated cytoplasm (fig. 9B). Light microscopic examination of spleen of LYC group was more or less similar to control group but some congestion of the red pulp was still present (Figs. 6 E and F & 8C). By Masson's trichrome, the amount of collagen fibers within the spleen decreased in comparison with BPA treated group (Fig. 7C). On electron microscopic examination there was a marked improvement in comparison with BPA treated group but some lymphocytes had irregular nuclei and some congestion of the red pulp was still present (Fig. 9C).

C-Pancreas:

By light microscopic examination, the pancreas of the control group showed pancreatic acini lined with pyramidal cells with basal nuclei. Islet cells appeared as lightly stained cells (Figs. 10A & 13A). Acinar cells showed a strong PAS-positive reaction (Fig. 11A). Masson's trichrome staining showed very thin connective tissue between pancreatic lobules (Fig. 12A). By electron microscopy, pancreatic acinar cells showed a rounded basal nucleus, apical dense zymogenic granules and densely backed rough endoplasmic reticulum (Fig. 14A).

Regarding the BPA treated group, light microscopic examination of the pancreas revealed dilated congested blood vessels, destroyed acinar cells and the islets showed areas of focal degeneration (Figs. 10B & 13 B & C). Acinar cells showed a reduction in the intensity of PAS-positive reaction (Fig. 11B). By Masson's trichrome staining there was an increase in the thickness of the connective tissue layer in the walls of blood vessels and between the pancreatic acini (Fig. 12B). On electron microscopic examination there was congestion of blood vessels, irregular dense nucleus of acinar cells and focal areas of haemorrhage (Fig. 14B).

Light microscopic examination of the pancreas of LYC treated group showed pancreatic acini with rounded basal nuclei and dark zymogen granules (Figs. 10C & 13D). Acinar cells showed a strong PAS-positive reaction (Fig. 11C). By Masson's trichrome there was a normal distribution of collagen fibers within the pancreas (Fig. 12C). By electron microscopy, acinar cells were more or less similar to the control group (Fig. 14C).

2-Morphometric Results:

The morphometric results declared that the area % of the collagen fibers in the liver, spleen and pancreases were significantly increased in group II (BPA-treated group) as compared to group I (control group). Meanwhile, the area % of the collagen fibers of group III (BPA+ Lycopene treated group) was more or less comparable to the control group with no significant difference (Table 1 & Histogram 1).

Table 1: Showing the mean values of area % of the collagen fibers in the different studied groups.

Site	Group I	Group II	Group III	P-value ¹	P-value ²	P-value ³
	Mean \pm SD	Mean \pm SD	Mean \pm SD			
Liver	151.21 \pm 9.26	194.59 \pm 14.56	153.91 \pm 26.05	0.000*	0.832	0.016*
Spleen	149.34 \pm 3.31	205.15 \pm 4.10	152.38 \pm 2.14	0.000*	0.174	0.000*
Pancreas	139.48 \pm 4.35	192.43 \pm 11.66	148.08 \pm 10.72	0.000*	0.188	0.001*

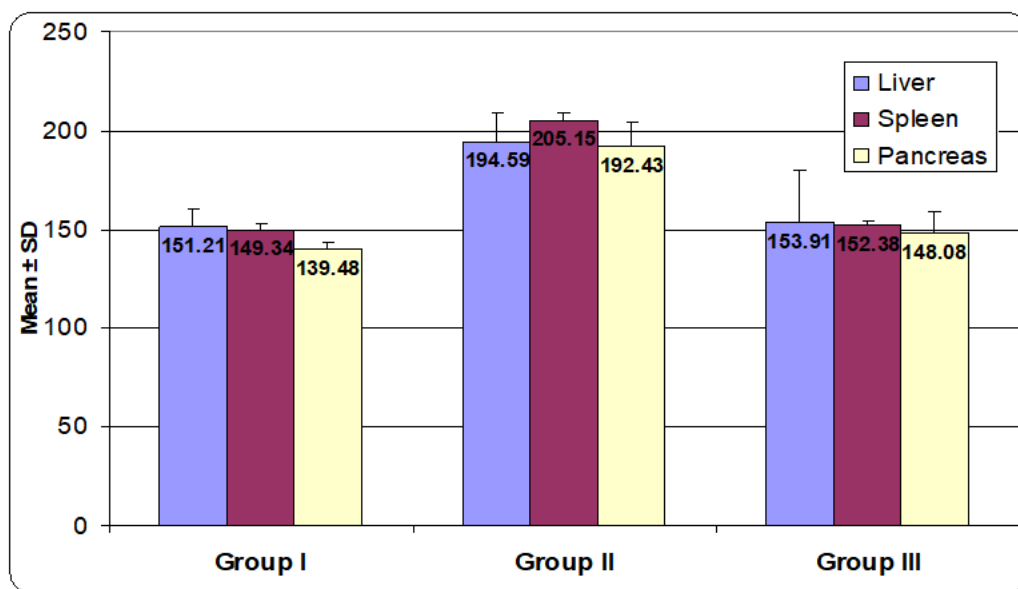
SD: standard deviation

* Statistically significant difference (P < 0.05)

P-value¹: Comparison between groups 1&2

P-value²: Comparison between groups 1&3

P-value³: Comparison between group 2&3

**Histogram 1:** Showing the mean values of area % of the collagen fibers in the different studied groups.

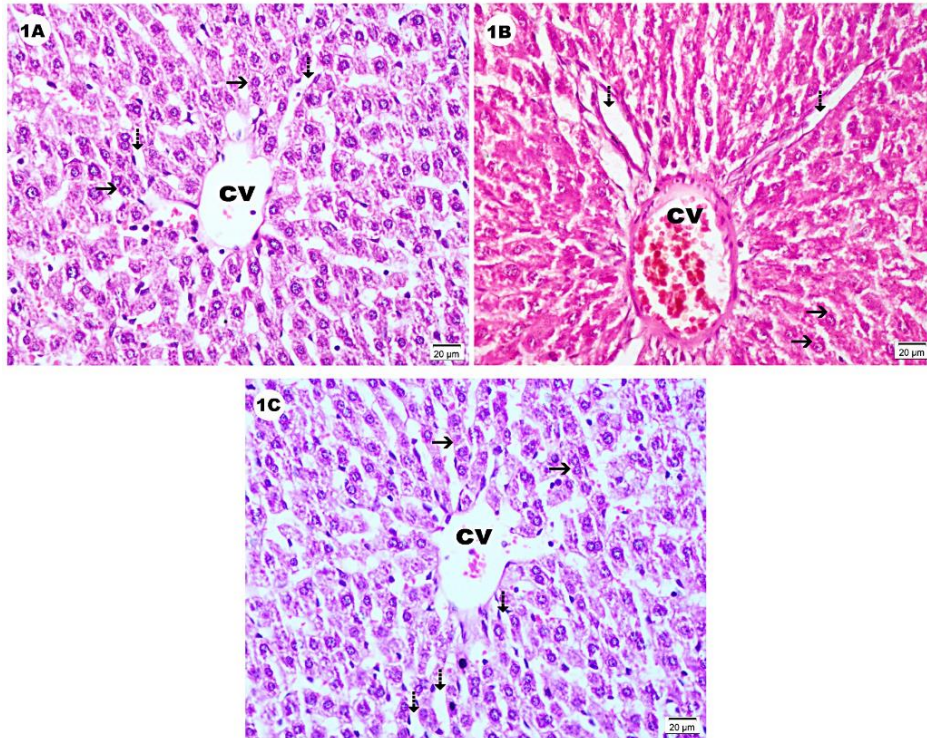


Fig (1): A photomicrograph of the liver of adult albino rat stained with H&E:

- A- Group I: has cords of hepatocytes (arrow) radiating from the central vein (CV) and separated by hepatic sinusoids (interrupted arrow).
- B- Group II: has dilated congested (CV), vacuolar degeneration of hepatocytes (arrows) and dilated hepatic sinusoids (interrupted arrow).
- C- Group III: has cords of hepatocytes (arrow) radiating from (CV) and separated by somewhat dilated hepatic sinusoids (interrupted arrow). (x 400)

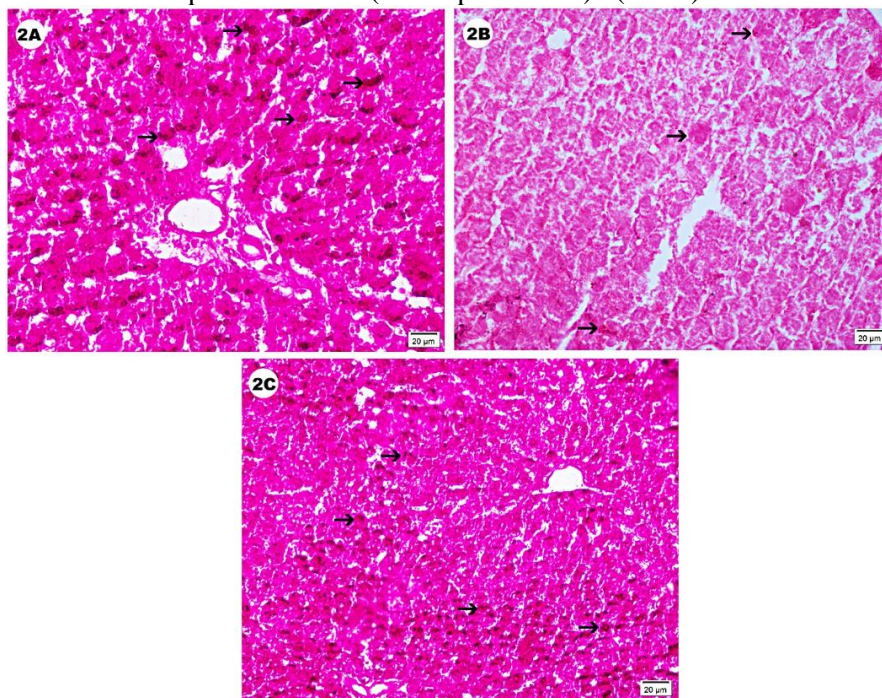


Fig (2): A photomicrograph of the liver of adult albino rat stained with PAS:

- A- Group I: has intense purple-magenta coloration demonstrating the glycogen within the hepatocytes (arrow).
- B- Group II: has marked decrease in the intensity of the purple-magenta color within hepatocytes (arrow).
- C- Group III: has strong PAS positive reaction (arrow). (x 400).

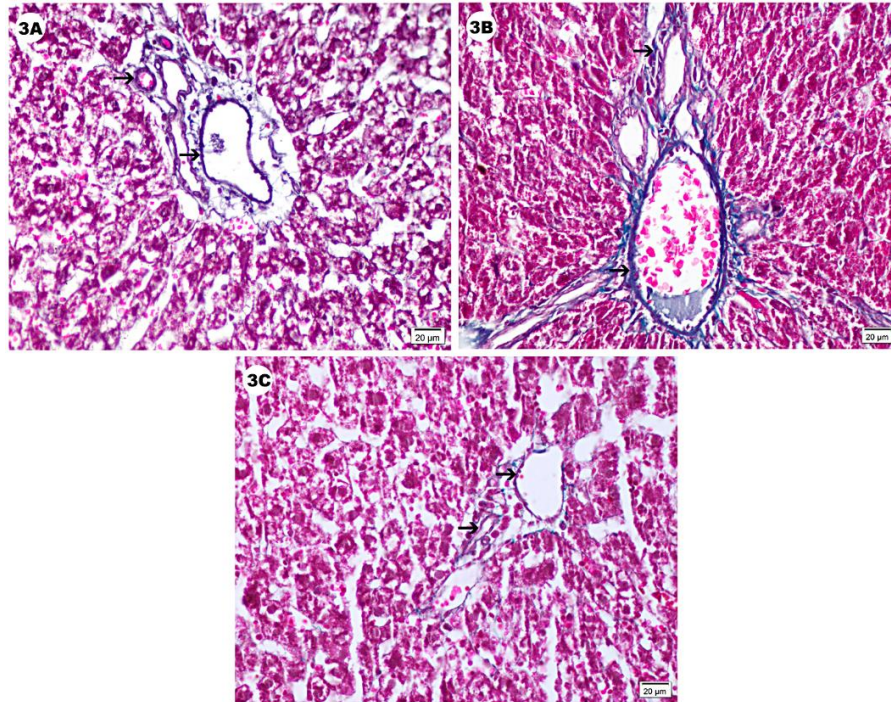


Fig 3: A photomicrograph of the liver of adult albino rat stained with Masson's trichrome:

- A- Group I: has normal distribution of collagen in the portal tract area indicated by the blue color (arrow).
- B- Group II: has great increase in the amount of collagen around the vessels in the portal tract area (arrow).
- C- Group III: has apparently normal distribution of collagen fibers (arrow).(x400)

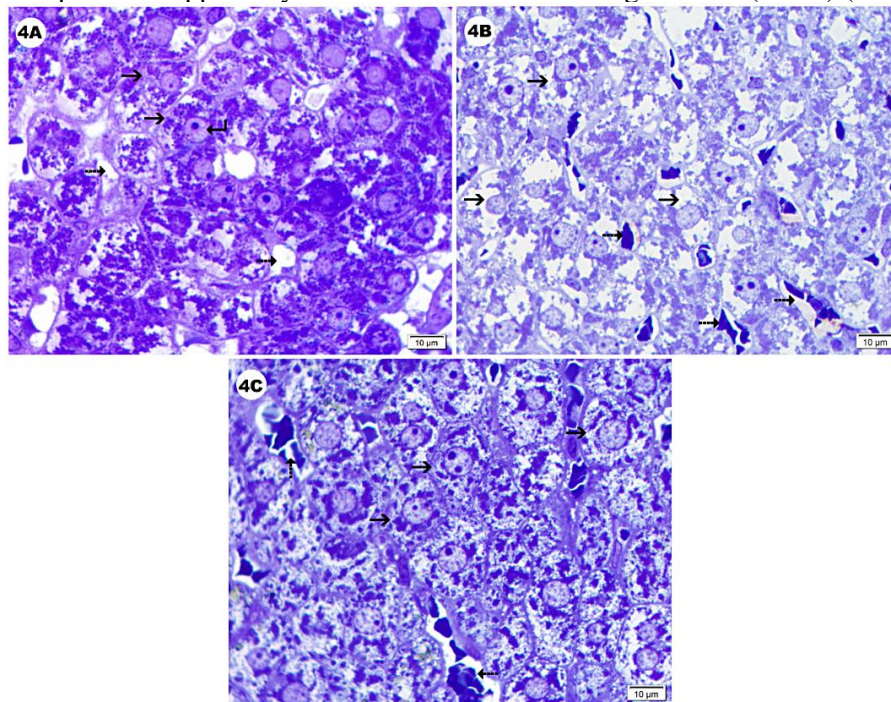


Fig 4: A photomicrograph of the liver of adult albino rat stained with Toluidine blue:

- A- Group I: has polygonal hepatocytes (arrow) with vesicular nuclei and prominent nucleoli (indented arrow). Hepatocytes are separated by hepatic sinusoids (interrupted arrow).
- B- Group II: has congestion of the hepatic sinusoids (interrupted arrow) and vacuolated cytoplasm of the hepatocytes (arrow).
- C- Group III: has apparently healthy hepatocytes (arrow) separated by dilated somewhat congested hepatic sinusoids (interrupted arrow).(X1000).

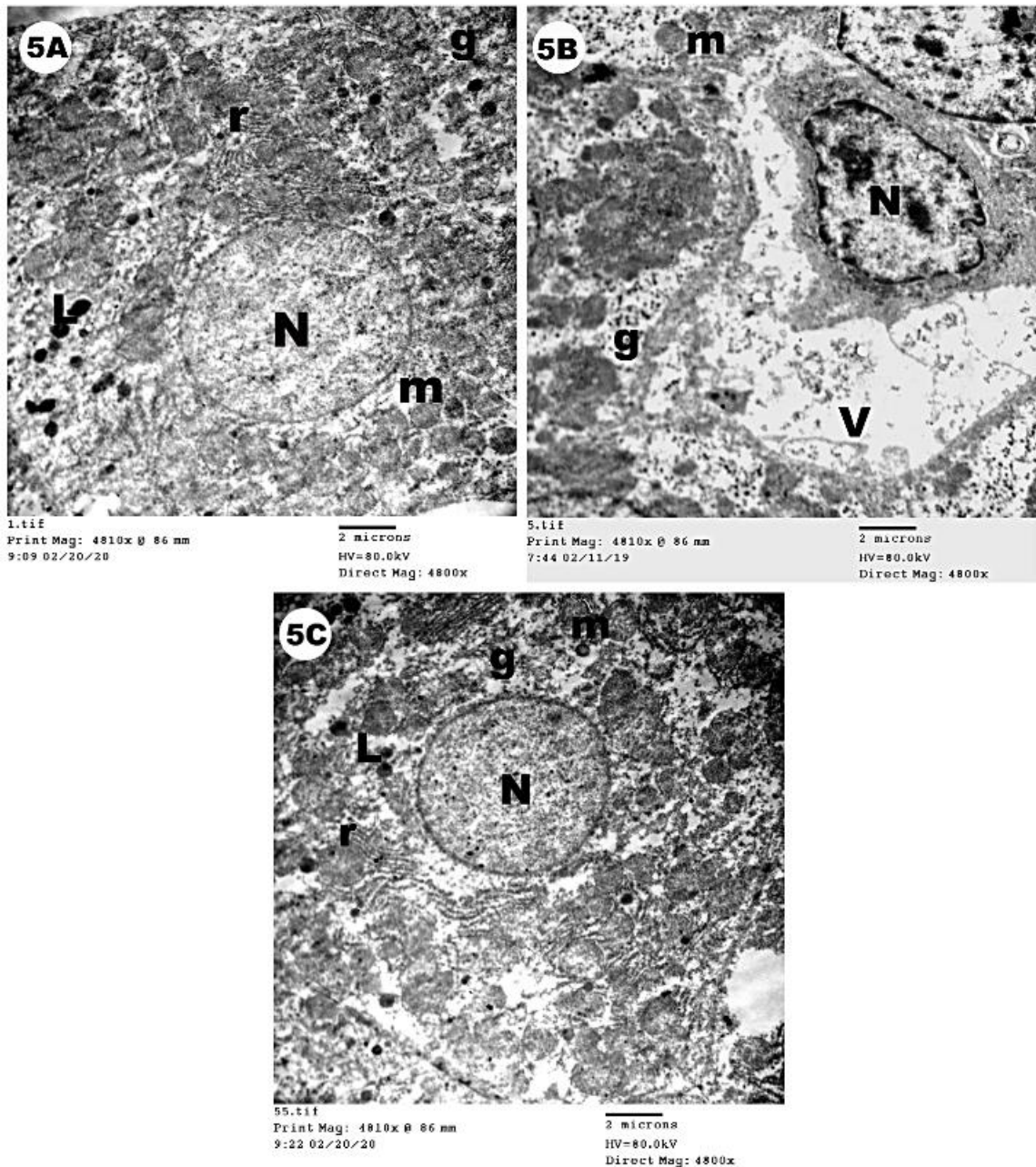


Fig (5): An electron micrograph of the liver of adult albino rat:

- A- Group I: has hepatocyte with rounded nucleus (N). The cytoplasm contains mitochondria (m), endoplasmic reticulum (r), glycogen granules (g) and lysosomes (L).
- B- Group II: has hepatocyte with irregular nucleus (N). The cytoplasm is vacuolated (V) with some damage mitochondria (m) and glycogen granules (g).
- C- Group III: has hepatocyte with rounded nucleus (N). The cytoplasm contains mitochondria (m), endoplasmic reticulum (r), glycogen granules (g) and some lysosomes (L).(x 4800)

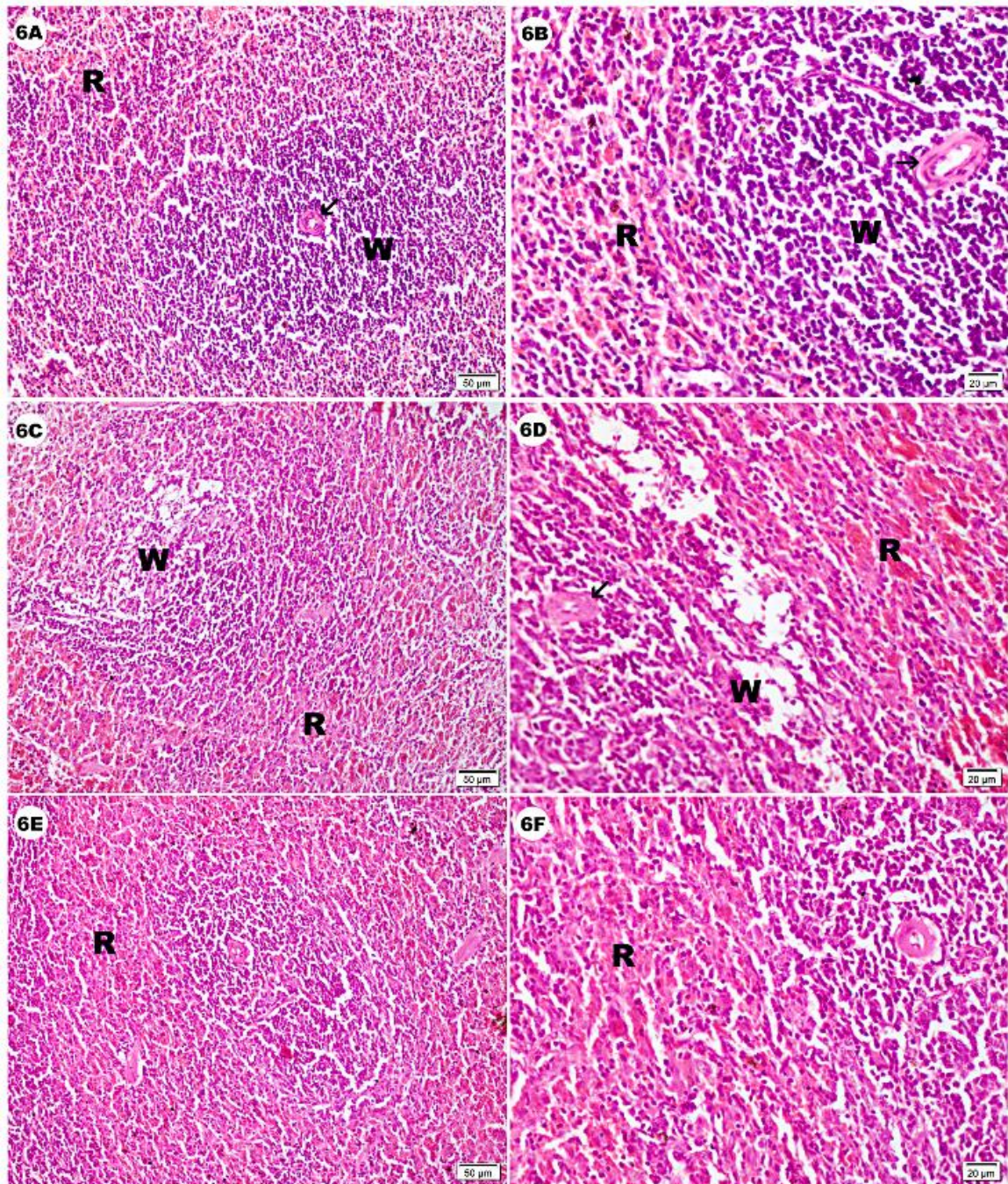


Fig (6): A photomicrograph of the spleen of adult albino rat stained with H&E:

A & B - Group I: has the white pulp (W) with periarterial lymphatic sheath (arrow) and the red pulp (R) that contains splenic cords and sinuses.

C & D- Group II: has disorganization of the white pulp (W) with thickening of periarterial lymphatic sheath (arrow) and congested red pulp (R).

E & F- Group III: has a picture more or less similar to control group with some congestion of the red pulp (R). (A, C, E x 200. B, D, F x 400)

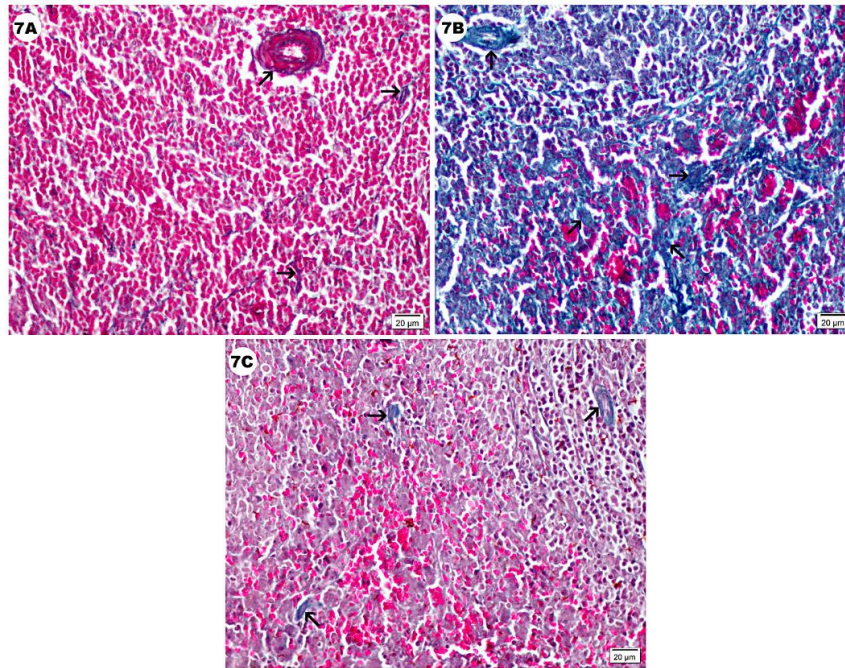


Fig (7): A photomicrograph of the spleen of adult albino rat stained with Masson's trichrome:

- A- Group I: has normal distribution of collagen fibers within the spleen (arrow).
- B- Group II: has apparent increase of collagen fibers within red and white pulp (arrow).
- C- Group III: has the normal distribution of collagen fibers in the spleen (arrow). (x400)

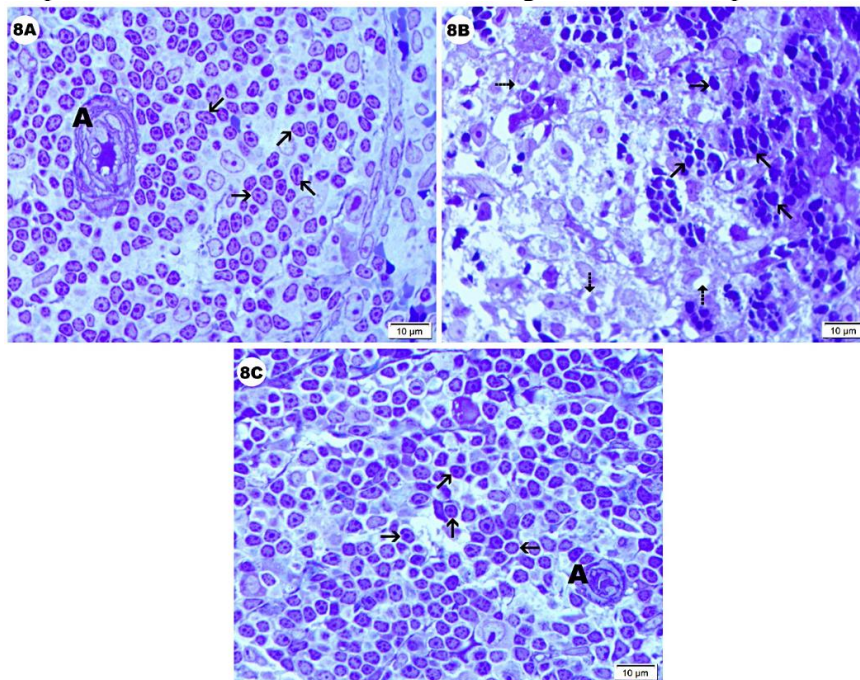


Fig. 8: A photomicrograph of the spleen of adult albino rat stained with Toluidine blue:

- A- Group I: has normal healthy lymphocytes of different size (arrow) and normal splenic arteriole (A).
- B- Group II: has many densely packed lymphocytes with picknotic nuclei (arrow). The red pulp contains many large vacuolated cells (interrupted arrow).
- C- Group III: has picture similar to control group many lymphocytes of different sizes (arrow) and splenic arterioles (A).(x 1000).

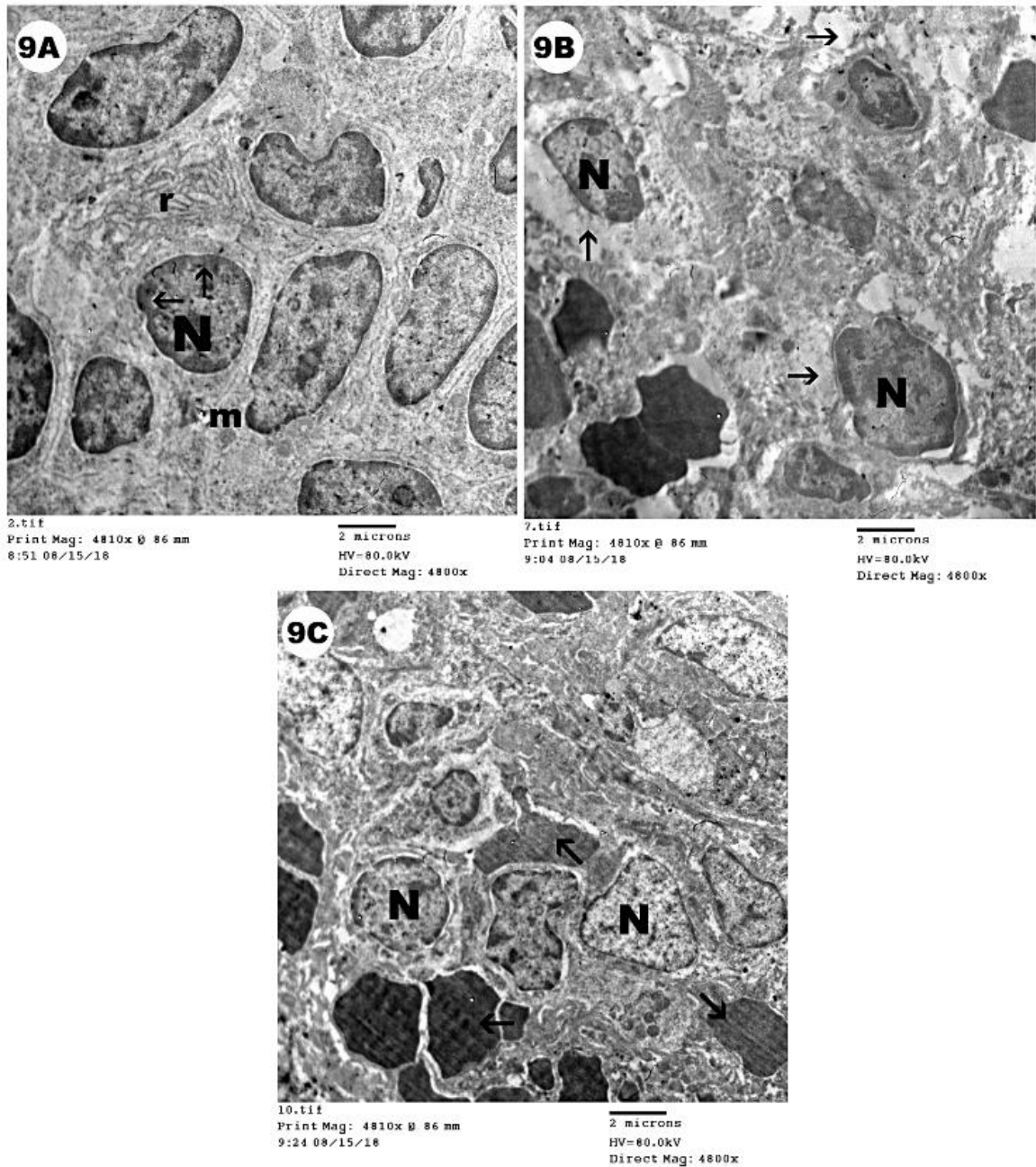


Fig (9): An electron micrograph of the spleen of adult albino rat:

- A- Group I: has lymphocytes of different size with heterochromatic nuclei (N) and irregular rim of dense chromatin (arrow). The cytoplasm contains mitochondria (m) and rough endoplasmic reticulum (r).
- B- Group II: has deformed lymphocytes with irregular nuclei (N) some of them are electron dense and vacuolated cytoplasm (arrow).
- C- Group III: has many lymphocytes of different size with irregular nuclei (N) and congested blood sinusoids (arrow). (x 4800)

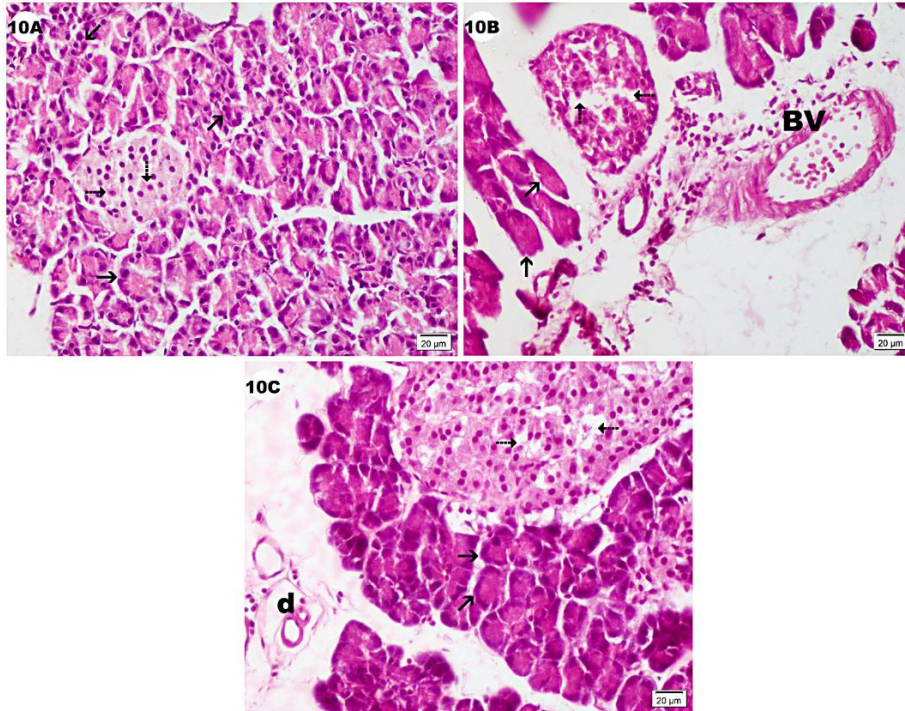


Fig (10): A photomicrograph of the pancreas of adult albino rat stained with H&E:

- A- Group I: has pancreatic acini line with pyramidal cells (arrow) with basal nuclei. Islet cells appear as lightly stained cells (interrupted arrow).
- B- Group II: has dilated congested blood vessel (BV). The acini appear irregular and destroyed (arrow). The islets show areas of focal degeneration (interrupted arrow).
- C- Group III: has pancreatic acini with rounded basal nuclei (arrow) and interlobular duct (d). Areas of vacuolation in the islet of Langerhans (interrupted arrow) are noticed. (x 400)

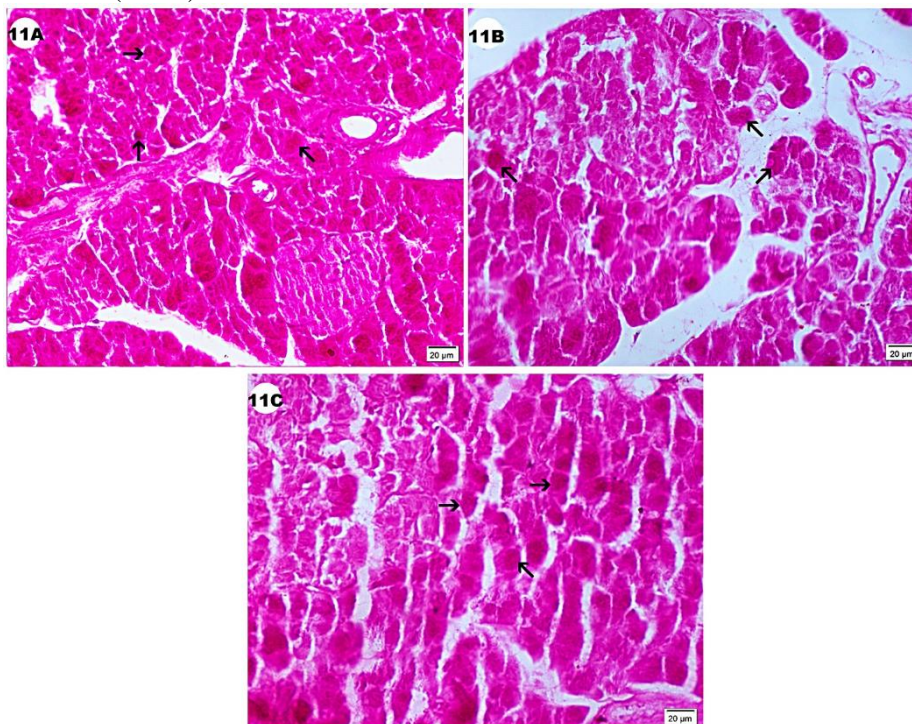


Fig (11): A photomicrograph of the pancreas of adult albino rat stained with PAS:

- A- Group I: has strong PAS positive reaction (arrow).
- B- Group II: has decrease in the intensity of PAS positive reaction (arrow).
- C- Group III: has strong PAS positive reaction (arrow).(x 400)

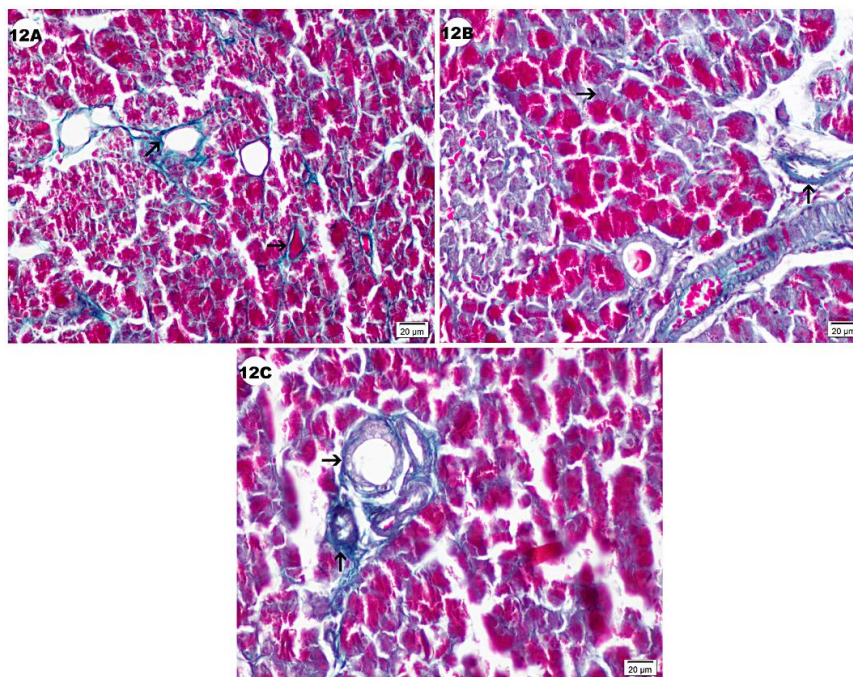


Fig.12: A photomicrograph of the pancreas of adult albino rat stained with Masson's trichrome:

- A- Group I: has very thin connective tissue between the pancreatic lobules (arrow).
- B- Group II: has increase in the thickness of connective tissue layer in the walls of blood vessels and between pancreatic acini (arrow).
- C- Group III: has normal distribution of collagen fibers within the pancreas (arrow).(x400)

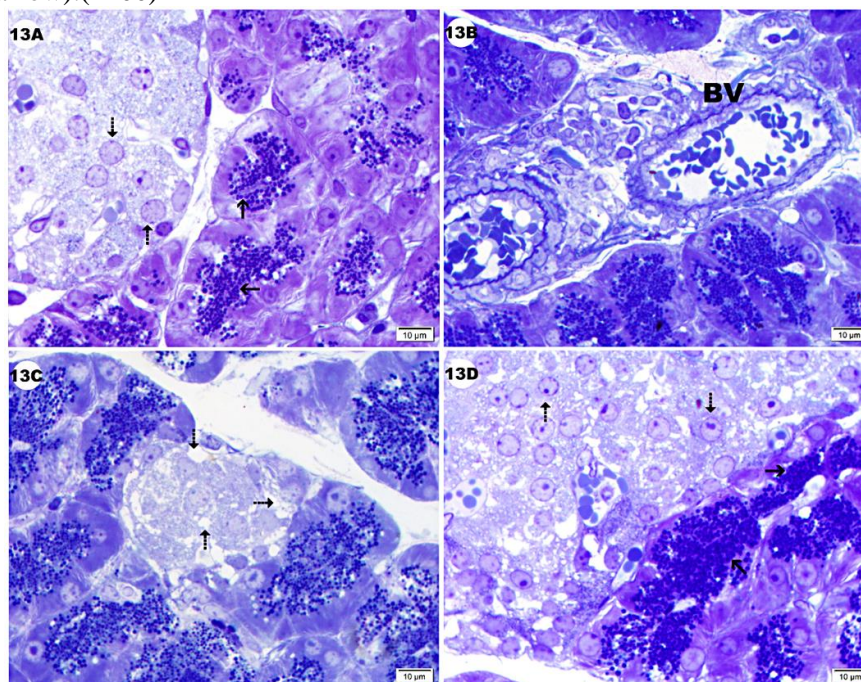


Fig.13: A photomicrograph of the pancreas of adult albino rat stained with Toluidine blue:

- A- Group I: has dark blue zymogen granules in the apical part of cells lining the pancreatic acini (arrow). Pale staining cells of islets (interrupted arrow) are present .
- B & C- Group II: has dilated and congested blood vessels (BV) with focal degeneration in islets (interrupted arrow).
- D- Group III: has picture that similar to the control group with dark zymogen granules in pancreatic acinar cells (arrow) and healthy appearance of the islet cells (interrupted arrows).(x 1000)

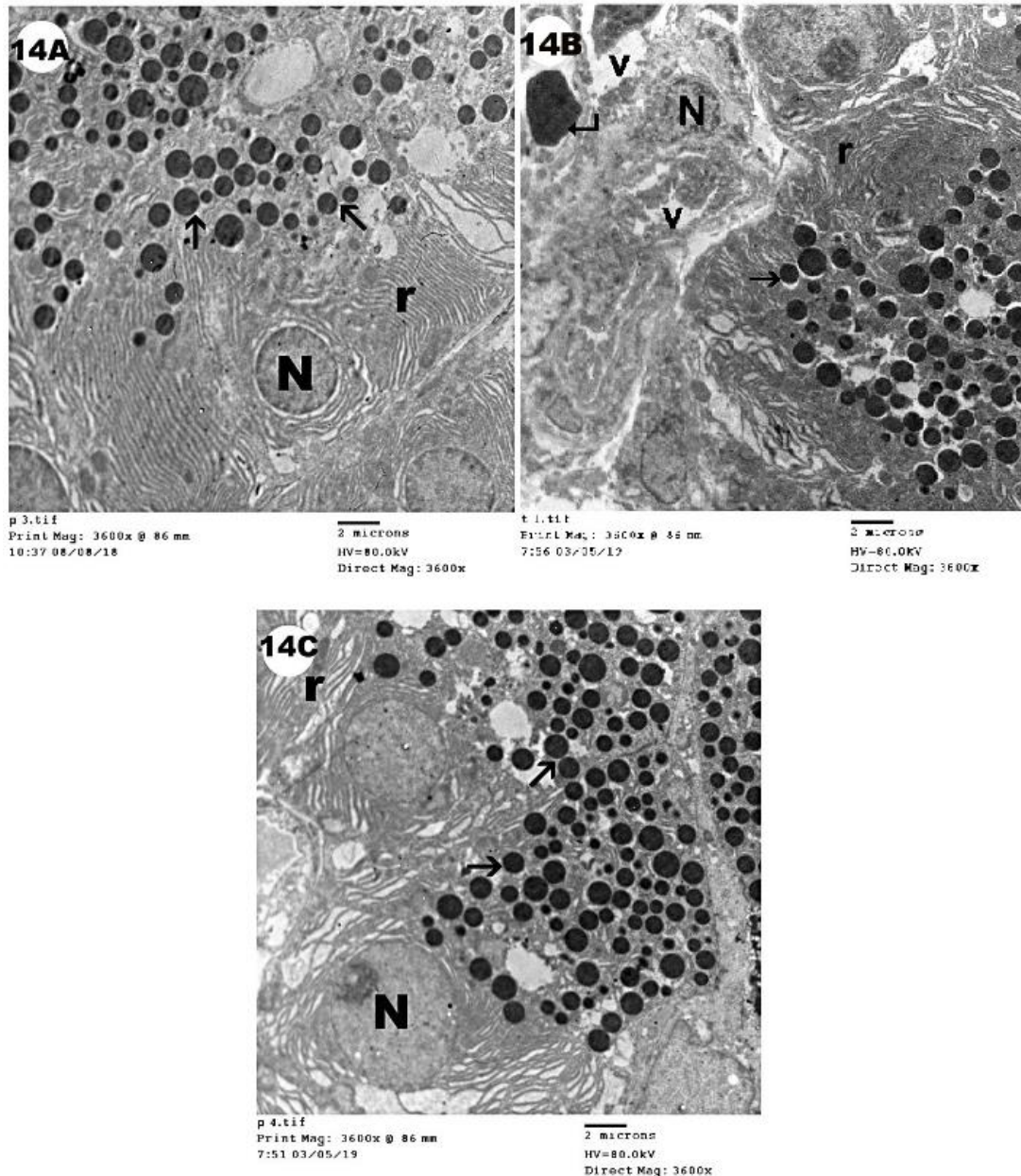


Fig (14): An electron micrograph of the pancreas of adult albino rat:

- A- Group I: has rounded basal nucleus (N), apical dense zymogenic granules (arrow) and densely backed rough edoplasmic reticulum (r).
- B- Group II: has irregular dense nucleus of acinar cell (N), apical dense zymogenic granules (arrow), backed rough edoplasmic reticulum (r), some vacuolation (V) and focal areas of haemorrhage (curved arrow).
- C- Group III: has rounded basal nuclei (N), many dense zymogenic granules (arrow) and densely backed edoplasmic reticulum (r).(x 3600)

DISCUSSION

BPA is a chemical compound that penetrates our environment owing to its constant release. Its release can happen through discharge from public wastewater treatment plants, burning of domestic waste and ordinary breakdown of plastics (Flint *et al.*, 2012). The

current study showed that BPA administration resulted in dilatation and congestion of hepatic sinusoids and central vein, degenerative alterations in the hepatocytes. These consequences agreed with those documented by Popa *et al.*, (2014).

The present results were in harmony with prior studies that noticed degenerative changes in liver cells linked to BPA administration (Jehane *et al.*, 2015).

Kamel *et al.*, (2018) attributed liver damage provoked by BPA to the accumulation of BPA poisonous metabolites and the capability of production of reactive oxygen species in the liver. An additional mechanism by which BPA exerted its harmful effects on the liver was declared by Marmugi *et al.*, (2012) who observed hepatic transcriptional effects in the BPA-treated mice.

Asahi *et al.*, (2010) stated that BPA encouraged mitochondrial dysfunction and rough endoplasmic injury which in turn essential for the protein pathway. Furthermore, BPA treatment reduced glycogen content by increasing glycolysis and diminishing glycogen phosphorylation (Jayashree *et al.*, 2013). An increase of pro-inflammatory cytokines disrupts the homeostasis between oxidants and antioxidants and also disturbs enzymes responsible for DNA repair, all of which demonstrate inflammatory processes associated with BPA (Yongvanit *et al.*, 2012).

The present study showed that BPA resulted in disorganization of the white pulp of the spleen with thickening of the periarterial lymphatic sheath. The lymphocytes were closely crowded with deeply stained nuclei and vacuolated cytoplasm. The red pulp was markedly congested. These effects were in agreement with Dawoud *et al.*, (2009) who registered lymphocytic reduction and numerous focal necroses in mice during BPA treatment. Youn *et al.* (2002) detected an increase in splenocytic proliferation in male mice treated with BPA. Also, BPA provoked a decrease in the number of macrophages, T-cells and B-cells in the spleen (Sugita *et al.*, 2003). The present study revealed that administration of BPA resulted in dilated congested blood vessels, damaged pancreatic acinar cells

and zones of focal degeneration in the islets of Langerhans. These results were in agreement with preceding studies, which found that BPA treatment in rats resulted in congested blood vessels, atrophy and hemorrhages in Islets of Langerhans and vacuolar disintegration in the acinar epithelium (Amaravathi, 2011). Mitochondrial impairment, and changed expression levels of Key mitochondrial genes were formerly reported in the pancreas of offspring of BPA-exposed rat dams (Wei *et al.*, 2011) and in separated rat islets (Song *et al.*, 2012). The mitochondrial injury was related to the creation of reactive oxygen species which resulted in rises in the inflammatory cytokine production and ultimately increased b-cell death and dysfunction (Kepp *et al.*, 2011).

LYC is a powerful antioxidant and has many other bioactive capacities; alteration of intercellular gap junction contact, hormonal and immune systems, and adjustment of gene function (Wang, 2012). LYC is the strongest singlet oxygen quencher amongst ordinary carotenoids, because of its high amount of conjugated dienes (Imran *et al.*, 2020) and is believed to be the most powerful hunter of ROS between other main dietary carotenoids (Zhao *et al.*, 2018). LYC enhanced the proapoptotic gene expression including caspase 3, 9 and p53 (Gupta *et al.*, 2013), adjusted cellular proliferation, glycolysis and diminished the oxidation stress (Bhatia *et al.*, 2018). Experimental reports delivered proof that LYC may work as an anti-inflammatory agent versus particular diseases including those of liver, prostate, lung, and colon (Jiang *et al.*, 2018).

The antioxidant action of Lycopene is attained by numerous mechanisms. The best-verified mechanism is via a strong singlet oxygen quencher (Ojha *et al.*, 2013). Another mechanism for the antioxidant capacity of lycopene is interaction with free radicals (Campos *et al.*, 2017).

The anti-inflammatory influence of lycopene has been noted in several previous studies (Hazewindus *et al.*, 2012). Probable mechanisms responsible for its anti-inflammatory response may include the inhibition of release and production of pro-inflammatory cytokines and alteration of signal transduction pathways (Palozza *et al.*, 2010).

Also, lycopene had a defensive function versus oxidative harm to cell membranes and DNA strand, and that it considerably relieved structural changes caused by free radicals in the rat's liver and in various tissues (Sheik Abdulazeez and Thiruvengadam, 2013).

In addition, preceding studies stated that there was noticeable inhibition in BAX protein expression levels in the liver (Hassan *et al.*, 2018) and kidney (Bayomy *et al.*, 2017) during lycopene treatment in rats. This signified a prominent anti-apoptotic role for lycopene.

Furthermore, many in vitro, in vivo, clinical studies signifying the possible protective effects of lycopene versus mycotoxins, pesticides, bacterial toxins, metals, fluoride, and many toxic chemical agents. The protective influence of lycopene was chiefly attributed to its free-radical scavenging, anti-oxidative, chelating and antiapoptotic properties, each of which has the ability to change the inflammatory response (Hedayati *et al.*, 2019).

In the current work, morphometric results came in harmony with histological results. Area % of the collagen fibers in the liver, spleen and pancreases was considerably increased in the BPA-treated group in comparison to the control group. Meanwhile, area % of the collagen fibers of the BPA+ Lycopene treated group was more or less similar to the control group with no significant difference.

Conclusion: It was decided that LYC had obvious protective impacts versus BPA elicited destructive effects on the liver, spleen and pancreas.

Recommendation:

More studies are needed to be done as regards this issue, using a different protocol to clarify the possible protective effects of LYC against the hazards of BPA.

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