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Histological study for detection impact of plastic materials through drinking water on local male rabbits

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Abstract--The aim of this research was conducted to evaluating the effect of plastic containers in depicting hot weather on animal health through the histological techniques for liver and kidney organs. Laboratory animal used for this purpose, in animal house of agriculture college university of karbala and the experiment appear from 4/6/2020 to 4/8/2020. Thirty local male rabbits, age 4 months and average weight 1100-1400gm were divided into equal three groups ;First group T1 served as controls negative (10 animals) were received water in metallic container at room temperature. Second group T2: Control positive (10 animals) were received water in plastic container at room temperature, while third group T3: also (10 animals) and received water exposed to 45°C temperature in plastic container. Feeding and water offered ad-libitum. The result of histological examination revealed that T3 received water in plastic container with exposed temperature (45°C), recorded alteration in histology lesion in liver and kidney, also showed infiltration and accumulation of inflammatory cell, exudates, swelling and necrosis and all the lesion in nephrocyte after 45 days was observed, while the first and second groups the liver organ show no pathological lesion on each lobule of liver and normal central vein with normal hepatic cord while kidney looked normal histology structure for kidney cortex, plus, showing normal glomeruli that contain the glomerular tuft surround by bowman space as well as normal urinary tubules with all details.

Keywords---plastic container, liver, kidney, necrosis.

Introduction

Water is an inorganic, clear, tasteless, odorless, and almost colorless chemical substance that is the primary constituent of Earth's hydrosphere and all known living species' fluids (in which it works as a solvent) (Sharma, 2021). Even though it contains no calories or organic nutrients, it is necessary for all known forms of life. Its chemical formula is H₂O, which means that each of its molecules is made up of one oxygen and two hydrogen atoms that are joined together by covalent bonds. At a 104.45° angle, two hydrogen atoms are linked to one oxygen atom (Saqlain, *et al.*, 2018).

Plastics (or polymers) is given by macromolecule chains, formulated from monomer units by chemical reactions. Typical reactions for chain assembling are poly addition (continuous or step wise) and condensation polymerization (poly condensation) (Heet *al.*, 2021).

Bottled water is a consumer food product that is made out of spring, filtered, mineral, effervescent, artesian, or well water that has been meticulously processed to fulfill regulatory requirements (Daffi, *et al.*, 2021). According to historical records, the sale of bottled water originated in the United Kingdom (Holy Well bottling business) in the 17th century, when water from mineral springs was supposed to have therapeutic and curative powers and was sold as a remedy for diseases (Walker, 2020). When Johann Jacob Schwappe learned how to carbonate water in 1783, plain water's effervescent character provided mineral water a lot of competition (Daffi *et al.*, 2021). In the nineteenth century, the popularity of bottled water and carbonated drinks in the United States grew due to decreasing glass costs and innovative bottling procedures. Sales were prevalent until the early twentieth century, when chlorination was developed to make tap water safer, causing bottled water sales to fall (Sawangjang, 2019).

Microplastics (MPs) are currently a global environmental pollutants and health hazards that caused by MPs cannot be ignored. However, studies on MP toxicity in mammals are scare. some investigation prove the effects of polystyrene microplastic (PS-MP) particles on the hematological system of mice through traditional [toxicology](#). The toxicological examinations showed that the polystyrene microplastics significantly decreased white blood cell count, increased platelets count, and inhibited the growth of colony-forming unit CFU-G, CFU-M and CFU-GM (Sun and *et al.*, 2021). Some studies used some vitamin to ameliorating the deficiency in white blood cells like vitamin c and zinc. (Al-Azawi and Alkenany, 2017).

Material and Methods

A total of 30 male rabbit divided into 3 groups G1 consider as control negative group, G2 used as control positive group (which consume water with plastic container) and G3 is treated group (which consume pre-treated water in plastic container at 45°C temperature). After the end of the experiment, the animals were killed and samples were taken from the liver and kidneys, where the method recommended by (Lee and Luna 1968) was followed to prepare the tissue sections, as the liver and kidneys were fixed with the fixed solution for 24 hours and then

washed with ethyl alcohol at gradual concentrations and the routine tissue methods were adopted to obtain On the histological sections, the sections were stained using hematoxylin and eosin dye. Then the slides were examined with a light microscope to observe the histological changes.

Histopathology of liver and kidney study

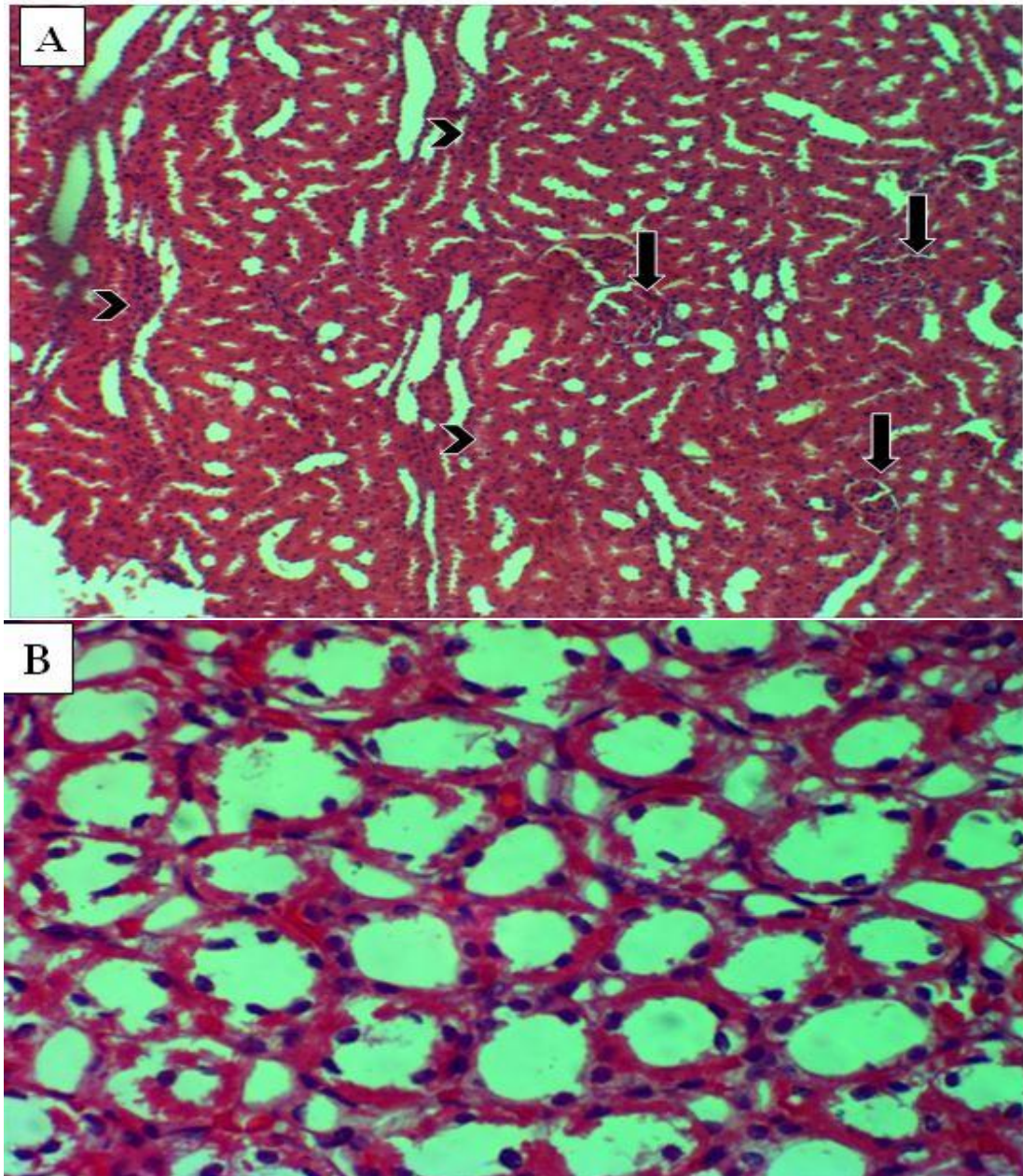


Figure (1): Photomicrograph of kidney of T1 group rabbits.

Normal kidney histology. **AandB/** Cortex area of kidney, glomerulus (arrows), proximal and distal renal convoluted tubules (arrowheads). Medulla area of kidney, loop of Henltubules.H&E. **A:** x100, **B:** x400. H&E.

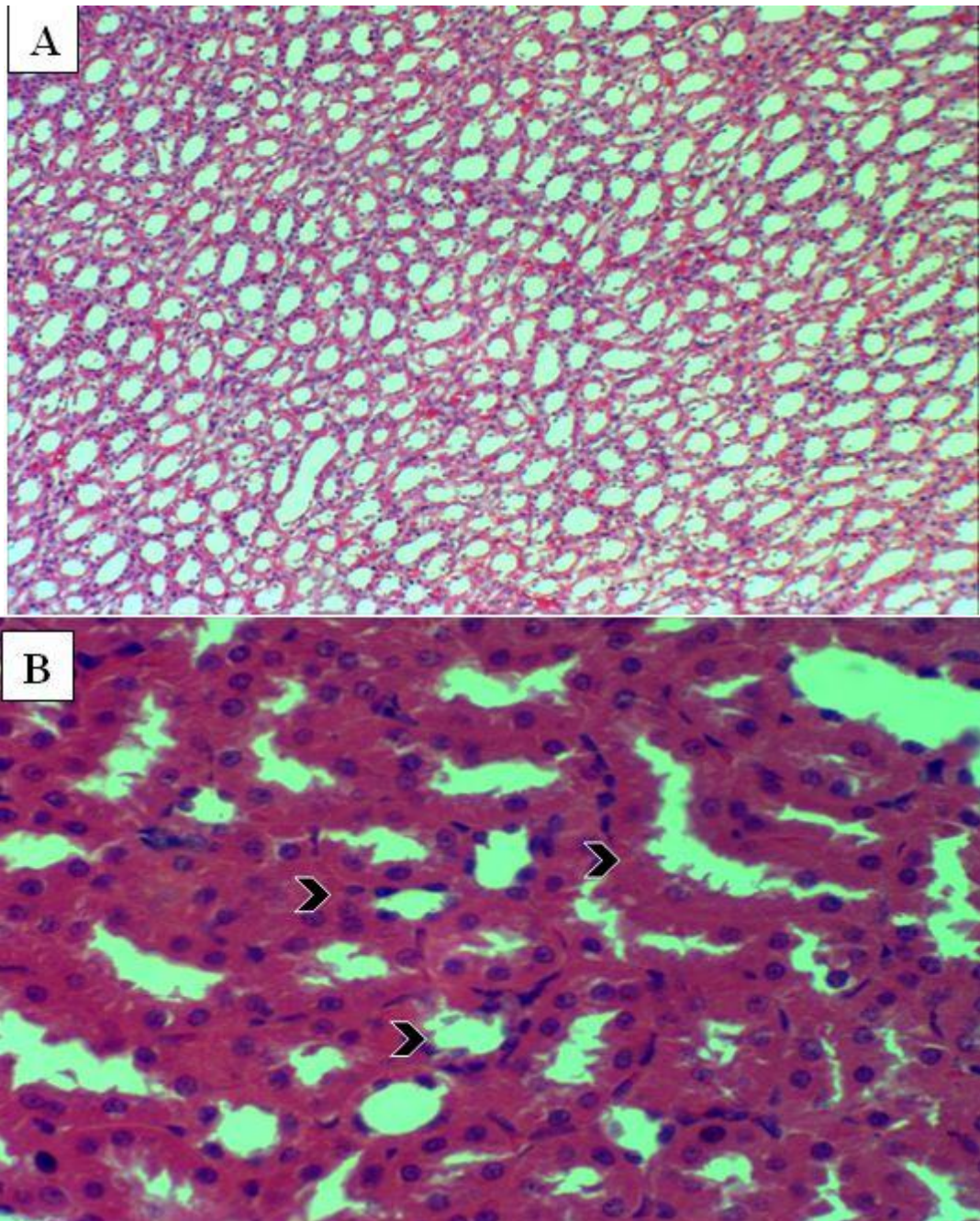


Figure (2): Photomicrograph of kidney of T2 group rabbits.

Normal kidney histology. **A and B**/ Cortex area of kidney, glomerulus (arrows), proximal and distal renal convoluted tubules (arrowheads). Medulla area of kidney, loop of Henle tubules. H&E. **A**: x100, **B**: x400.

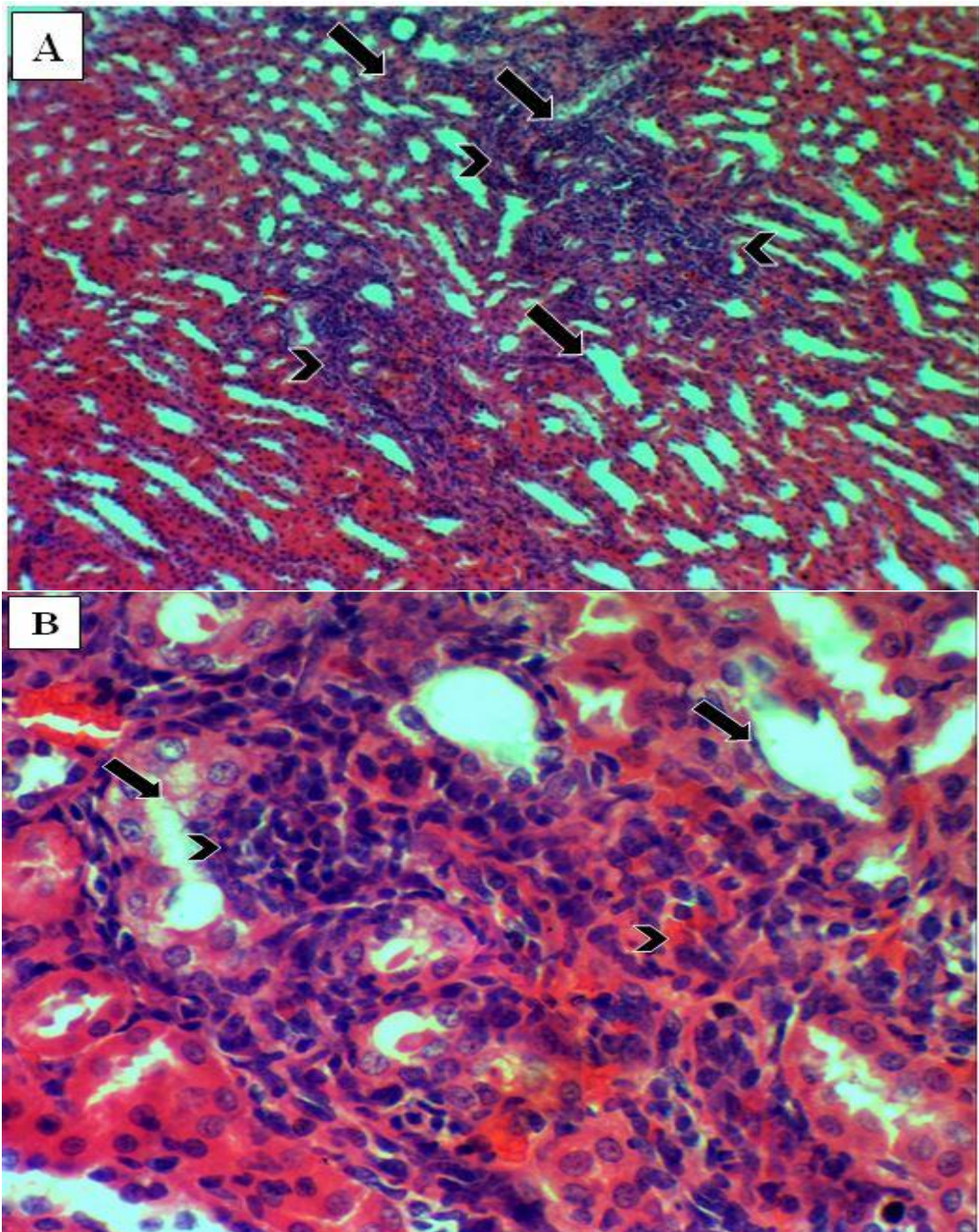


Figure (3): Photomicrograph of kidney of T3 group rabbits.

A/Necrosis of epithelial cells of renal tubules (arrows) with infiltration of inflammatory cells (arrowheads) forming a cluster occupied the cortex parenchyma**B/**infiltration of inflammatory cells in cortex areas, where the aggregation of inflammatory cells as clusters led to absence of renal tubules in affected areas.H&E. **A:** x100, **B:** x400.

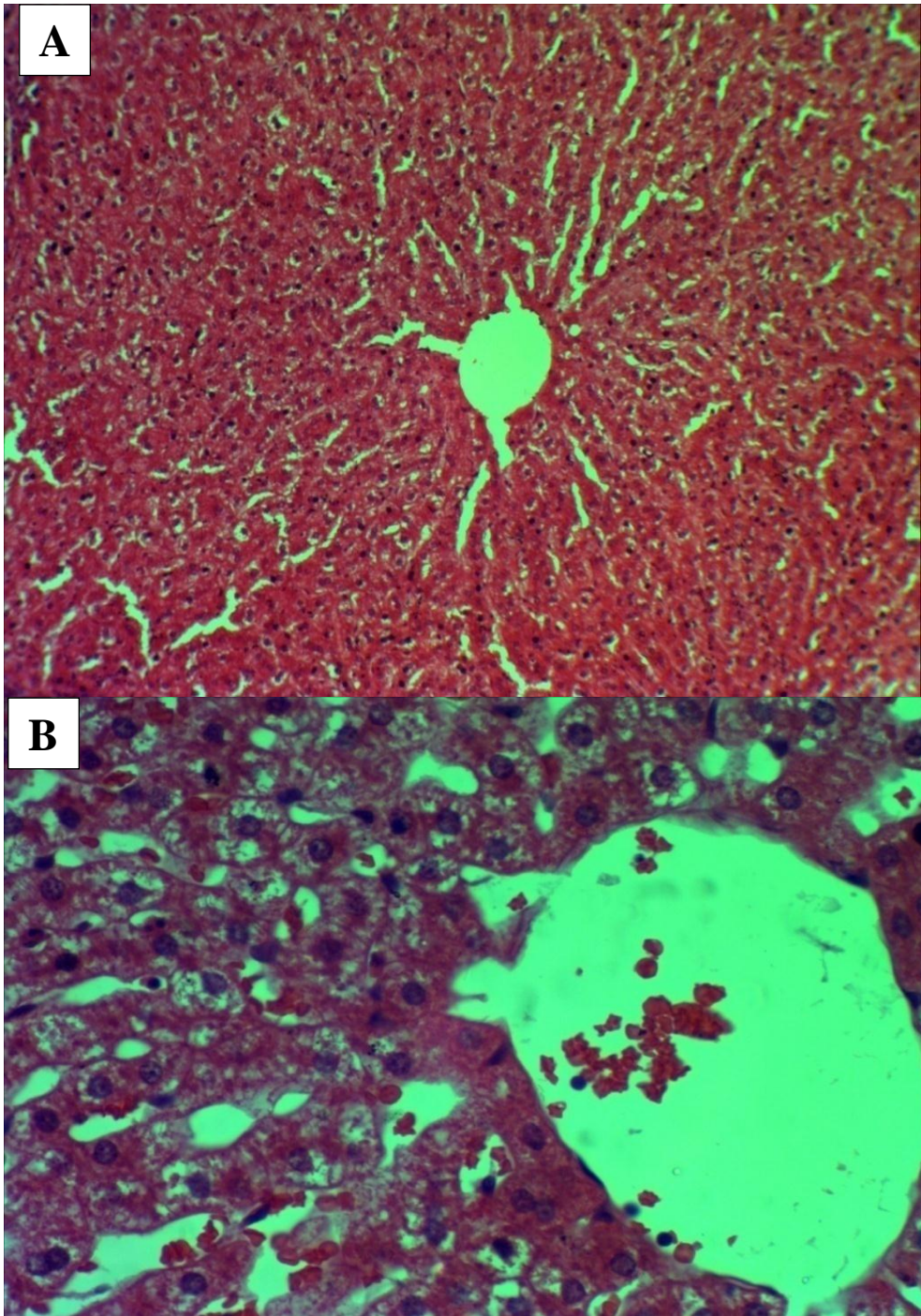


Figure (4): Photomicrograph of liver of T1 group rabbits.

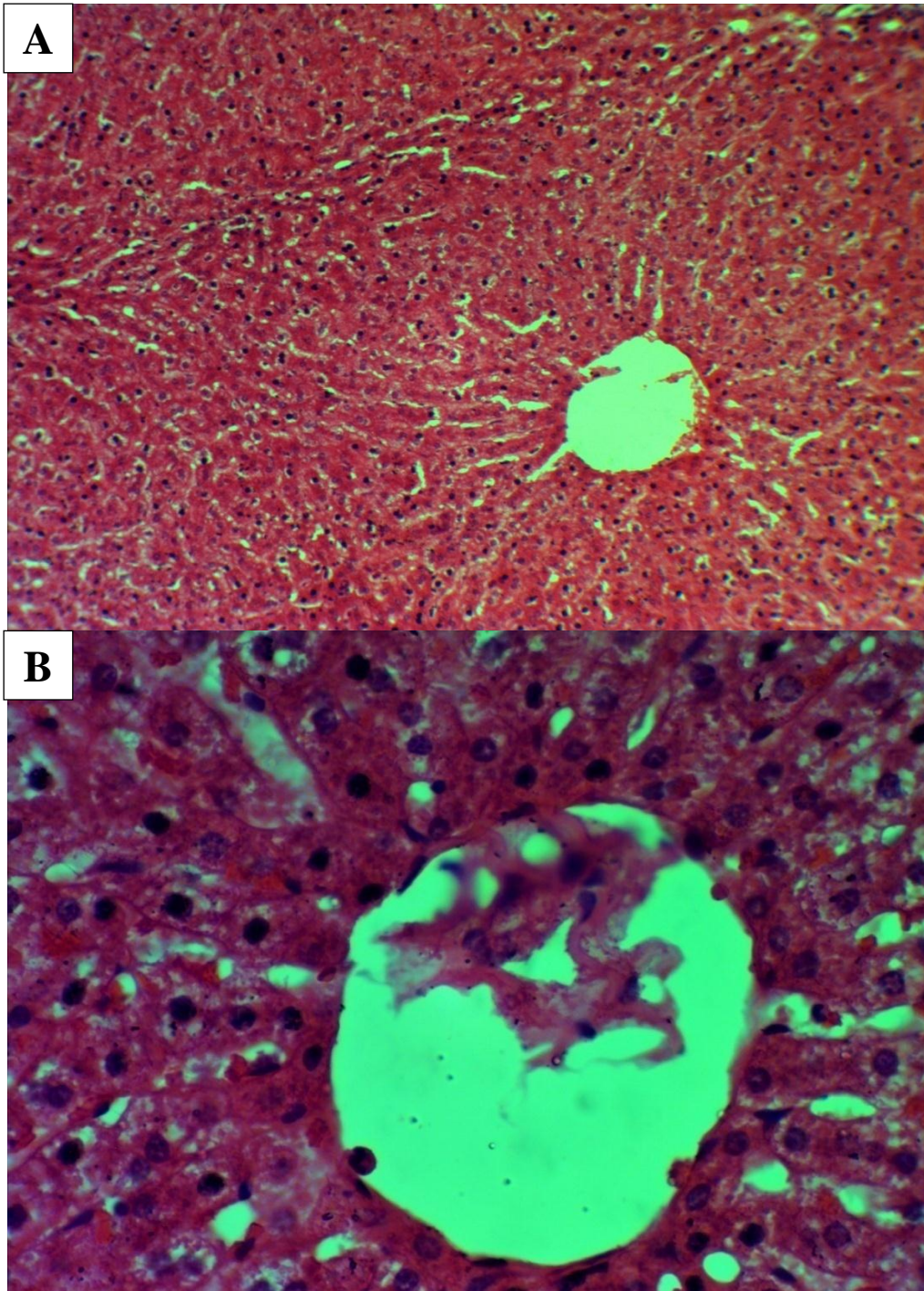


Figure (5): Photomicrograph of liver of T2 group rabbits.

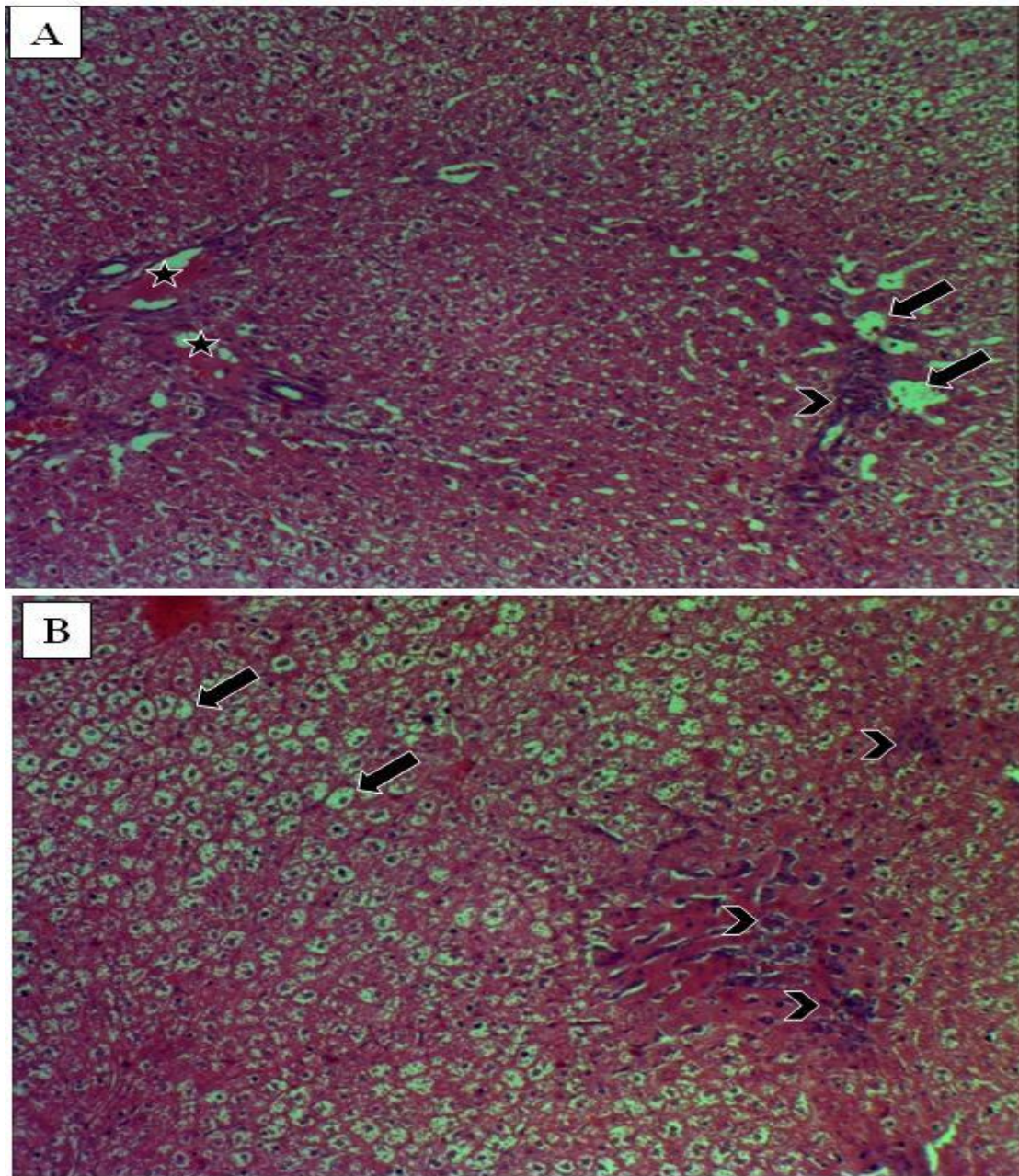


Figure (6): Photomicrograph of liver of T3 group rabbits.(AandB)

A-Necrosis of hepatocytes of liver forming a spaces (arrows) in liver parenchyma with presence of exudate in affected areas. Infiltration of inflammatory cells (arrowhead) in affected areas. Swallowing of hepatocytes (stars) led to obstruction of hepatic sinusoid. H&E. x100.**B**-Necrosis of hepatocytes of liver forming a spaces in liver parenchyma filled with inflammatory cells (arrowheads) in affected areas. Swallowing of hepatocytes (arrows) led to obstruction of hepatic sinusoid. H&E. x100.

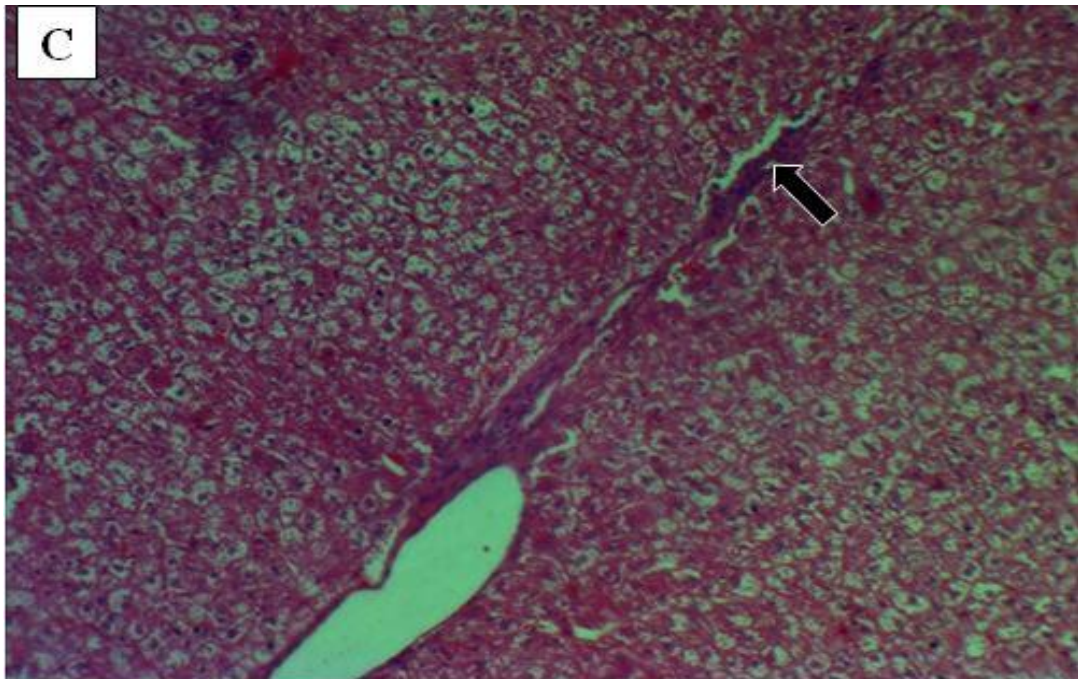


Figure (7): Photomicrograph of liver of T3 group rabbits (C)
 Liver fibrosis (arrows) was observed in parenchyma of affected liver. H&E, **C:** x100.

Discussion

The liver is the first organ to encounter ingested nutrients, drugs and environmental toxicants that enter hepatic portal vein from the digestive tract and level of liver function can be detrimentally altered by injury resulting from acute or chronic exposure to toxicants effect of toxic substance with low level on different organ lead to deleterious effects on kidney and liver (Tawfiq, 2012). Figures of control negative and control positive of liver show no pathological lesion on each lobule of liver and normal central vein with normal hepatic cord .there was no tissue lining the hepatic lobule border, Figures of control negative and control positive of kidney show normal histology structure for kidney cortex, plus, showing normal glomeruli that contain the glomerular tuft surround by bowman space as well as normal urinary tubules with all details. While the treated group show alteration in histology lesion As the plastic material show toxic effect that cause cell injury by production of free radical(ROS) which stimulating oxidative stress and causes necrosis and aggregation of inflammatory cells in nephrocyte and that deals with (Cahyono, et al ,2019.Guerrera,et al,2021) in their study on the rats and fish. Show there is epithelial sloughing, congested and necrosis in kidney. While the effect on liver show infiltration of inflammatory cell accumulation ,exudates in hepatocyte, swelling and necrosis all the lesion due to effect of toxic micro plastic material that inter the hepatocyte and destroyed the function of their organelles. The Dilated sinusoids and change in liver normal structure may be due to the loss of structural proteins after the toxicant

exposure. The result deal with Similar changes were recorded in the liver of Catlacatla exposed to 1-4 ppm BPA (Faheem, et al, 2016). One of the nonspecific responses of fish in toxic conditions is hepatocyte vacuolation this vacuolization is typical of lipid accumulation in liver. Similar changes in liver were reported by (Peixoto et al. 2013) in *Barbus bocagei* caught from Vizela River, Portugal. (Ameur, et al. 2012) found similar histopathologic changes in liver of *Mugil cephalus* and *Dicentrarchus labrax* from Bizerte Lagoon, Tunisia. Kidney sections of fish exposed to BPA showed necrosis, degeneration of renal tubules, inflammation in hematopoietic tissue, shrinkage of tube lumen, and hemorrhage. Similar results were reported in *Cyprinus carpio* exposed to deltamethrin (Cengiz, et al, 2006) and in *Oreochromis niloticus* exposed to heavy metals (Khidr, et al, 2008). Other researcher show that's micro plastic toxicity can cause inflammation (xu, B, et a.2016), oxidative damage and necrosis (Zhang, et al .2021). in the study of oxidative effect of BPA on histology of liver and kidney show there is ruptured central vein, lipid like vacuolization, macrophage and lymphocytes infiltration, ruptured and degenerated hepatocytes, In kidney there is damaged renal tubules, shrinkage of tubules and tubule lumen and degeneration of tubules and hematopoietic tissue (Faheem and Khalid Parvez Lone, 2018). As the micro plastic material consider toxic substances such as Polyvinyl chloride (PVC) in study the effect of PVC on histology of liver howed mild to moderate hepatocytic glycogen depletion and fatty vacuolation, \ fatty degeneration infiltration of hepatic cells into the central vein and single-cell necrosis (coagulative hepatocellular Necrosis) were also observed in the liver (Iheanacho, et al ,2020). The result of histopathology agreement with (akram, et al, 2021) in them study on effect of bisphenol A on fish that showing the kidneys, pyknosis, glomerulosclerosis, expanded Bowman's space, and ceroid development were found, while in the liver, ceroid formation, hemorrhages, pyknosis, karyorrhexis, karyolysis, nuclear hypertrophy, and eccentric nuclei were observed. Degeneration of neurons in the cerebellum, lipofuscin accumulation, microgliosis, necrotic neurons, inflammatory cells, and bleeding were all observed histologically in different regions of the brain of treated fish. Necrosis, inflammatory reaction, neutrophilic myocarditis, and hemorrhages were discovered during light microscopic examination of different parts of the heart of bighead carp. Finally, it is hypothesized that BPA has negative impacts on physical, blood-biochemical, and histopathological parameters in multiple visceral tissues of exposed fish.

Conclusion

Plastic container used in drinking water through heat exposure has a big impact on the health by affected on liver and kidney parenchyma.

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