

CHROMIUM (VI) INDUCED HISTOLOGICAL CHANGES OF PANCREATIC ISLETS AND LIVER: A PRELIMINARY STUDY OF METAL INDUCED DIABETES MELLITUS

ABSTRACT

Diabetes mellitus is growing in prevalence worldwide. Uncontrolled industrialization has released heavy metal pollutants and toxic agents that may play a role in the islet function and development of diabetes mellitus. Among the toxic metals, chromium (Cr) is widely used in industries and is considered a major cause of environmental pollution. Cr (III) constitutes an essential nutrient, while Cr (VI) is highly toxic. Occupational exposure to Cr (VI) compounds is associated with several adverse effects on human health. Cr (VI) is associated with the production of reactive oxygen species (ROS) that affect different types of tissues in our body. Our aims and objectives of this study were to analyse the chromium (VI) induced diabetic condition by examining histology of liver and pancreas. The pancreatic sections of the treated group showed marked morphological changes. Photomicrograph of histology of treated pancreas exhibited the disruption of islets, disorientation of cells and disrupted connective tissue septa. Chromium (VI) treated liver revealed periportal fatty infiltration (PFI), central vein congestion and dilatation of sinusoidal spaces. Treated liver showed lower PAS response and mild depletion of hepatic glycogen. The changes of pancreatic islets may reflect an inhibition in insulin synthesis. In this study, changes in the architecture of pancreatic islets as well as liver may be the reason behind diabetes, but further experiments needs to be performed.

Keywords: Liver, Pancreas, Islets of Langerhans, Diabetes Mellitus

1. INTRODUCTION

Diabetes mellitus is growing in prevalence worldwide. Diabetic population now is about 150 million globally and the World Health Organization (WHO) predicts it will double by 2025¹. Uncontrolled industrialization has resulted in human population being exposed to metals that have the potential to cause or exacerbate diseases. Thus, more attention is needed to investigate and prevent the possible factors which may induce diabetes. Uncontrolled industrialization has released heavy metal pollution. Several studies have indicated that the deficiency and efficiency of some essential trace metals may play a role in the islet function and development of diabetes mellitus. Some toxic metals have also been shown to be elevated in biological samples of diabetes mellitus patients. Widespread pollution by heavy metals has important consequences for human health². Among the toxic metals, chromium (Cr) is widely used in industries such as electroplating, steel manufacturing, leather tanning, metal finishing and pigment. Chromium commonly enters the environment in the effluents from these industries. Once released into soil and water, it is considered a major cause of environmental pollution. Chromium is listed among the 126 priority pollutants by the US EPA. It is also listed among the 25 most hazardous substances posing the greatest risk to human and ecosystem fitness³. Chromium exists in several oxidation states, though Cr (III) and Cr (VI) species are the most common forms. Cr (III) constitutes an essential nutrient, while Cr (VI) is highly toxic⁴. Occupational exposure to Cr (VI) compounds is associated with several adverse effects on human health such as lung toxicity and bronchial asthma, and it also causes nephro and hepatotoxicity^{5,6}. Squamous cell carcinoma is the most frequent type of cancer among Cr (VI)-exposed workers⁷. The generation of free radicals occurs when chromium undergoes redox-cycling reaction⁸. Cr (VI) enters cells rapidly, and once inside the cell it is reduced by intracellular reductants to short-lived chromium intermediates such as Cr (V), Cr (IV) and ultimately kinetically stable form of Cr (III). This reduction process generates reactive oxygen species (ROS), such as superoxide (O⁻), hydroxyl (OH⁻) and (H₂O₂), which serve as sources of hydroxyl radicals. Cr (VI) exposure generates oxidative stress in many systems^{9,10}. Oxidative stress results from an imbalance between the antioxidant defence systems and ROS generation that affect different types of tissues in our body. Trivalent form of Cr has high biological activity

which is required for the optimal glucose uptake by cells^{11,12,13}. Cr (III) regulates insulin and blood glucose levels by stimulating insulin signaling pathway and metabolism by up-regulating glucose transporter (GLUT4) translocation in muscle cells^{13,14}. Cr (III) deficiency results in the elevation of blood glucose levels and if it is persisted for a prolonged period, it may lead to the development of diabetes^{13,15}. Some reports show that Cr supplements decrease the blood sugar level in diabetes^{13,16}. Briefly, cumulative evidences indicate the essential nature of these metals for the maintenance of human normal physiology. While their imbalance predisposes to glucose intolerance which subsequently converts to diabetes related complications^{13,17}. Our aims and objectives of this study were to analyse the chromium (VI) induced diabetic condition by examining histology of liver and pancreas.

2. MATERIALS AND METHODS

The study was carried out on healthy mice weighing between 80 to 100 g. The animals were housed in clean plastic cages under natural light and dark cycles at room temperature. Animals in all groups were fed *ad libitum* and allowed free access to water. All animals received human care. Mice were divided into control and their respective treated group. After 5 days of acclimation, the animals were divided into two equal groups (n=5/group) as follow:

- Group I (Control group): Untreated animals.
- Group II (Treated group): mice were injected with single dose of Cr (VI).

Group II animals were treated with chromium (VI) oxide (purified, CrO₃, M=99.99g/mol, MERCK, B.N. MHOM602462) 24 mg/kg body weight in 1 ml water / day for 21 days¹⁸. The i.p administration of Cr (VI) was selected for being the least aggressive¹⁹.

The mice were sacrificed by cervical dislocation. Pancreatic tissues and liver were dissected out and fixed in Bouin's fluid for 24 hours and processed via paraffin wax embedding method²⁰. Paraffin-embedded sections were cut at 5 µm and stained with haematoxylin and eosin (H&E) for light microscopic examination. Liver was also stained with periodic acid Schiff (PAS) method.

3. RESULTS AND DISCUSSION

3.1. Histopathological findings and analysis of pancreas

Histopathology of islets of Langerhan's of pancreas of control animal revealed normal architecture with compact arrangement of cells throughout the study. The islets appeared lightly stained than the surrounding acinar cells, with intact interlobular connective tissue and interlobular duct (picture not shown).

The pancreatic sections of the treated group showed marked morphological changes. Blood vessels were seen congested and dilated. Some islets cells showed pyknosis. A significant number of islets cells were found to be reduced in number. The architecture of connective tissue septa in treated group was found to be altered. Photomicrograph of histology of treated pancreas exhibited the disruption of islets, disorientation of cells and disrupted connective tissue septa (Fig 1). Islets showed cells with condensed nuclear bodies. Reduction in the amount of cells under treated conditions was noticed. Cr treated islet showed extensive vacuolation and atrophied cells. Mild degrees of fibrosis with infiltration of a few inflammatory cells were also observed (Fig 2).

3.2. Histopathological findings and analysis of liver

Treated mice exhibited marked changes in the general cytomorphology of liver as evident by the enlargement of central hepatic venule and disorientation of hepatocytes. The histological examination of the H-E stained control liver tissues showed normal cytoarchitecture of liver with visible central veins with radiating cords of hepatocytes (Fig 3A). Treated liver revealed few uniform sized cell bodies stained in eosin were found in the central hepatic venule. These may be periportal fatty infiltration (PFI) (Fig 3B). Treatment with chromium caused central vein congestion with significant dilatation of sinusoidal spaces (Fig 3C).

3.3. PAS analysis of liver

Liver section of treated group showed vacuolisation in the liver parenchyma, with significant dilatation of sinusoidal spaces, congestion of blood vessel and increasing amounts of connective tissue in the portal area (Fig 4). Treated liver showed lower PAS response. Chromium induced alteration resulted in approximately mild depletion of hepatic glycogen in comparison to the control group (Fig 5B).

It is worthy to mention that chromium (VI) had deleterious effect on the ultrastructure of pancreas as well as liver. Treated islet cells with ill-defined edges indicated necrosis. The histopathology of islets in treated mice indicated progressive destruction of cells (Fig 1 and 2). The changes of pancreatic islets reflect an inhibition in insulin synthesis and secretion as reported in diabetic animals^{21,22}. In rat, the absorbed chromium is transferred to the liver where the liver tissue retains 10.9% of chromium oxide and 51.1% of sodium chromate²³. In this work, the Cr was transferred to the liver and exerted its effects on liver cells. Mammals need Cr³⁺ to maintain balanced glucose²⁴, and thus chromium may facilitate insulin action^{25, 26}. In this work marked histopathological changes of liver like sinusoids, centrilobular vein and portal vessels congestion were seen (Fig 3B and C). The fibrosis observed in portal area can be explained by the fibrogenic effects of chromium. These results indicate that the chromium has a direct participation in liver structures alterations.

Liver and pancreas tissues both act as glucose sensor and damage to these tissues plays an important role in the onset of diabetes mellitus (DM). The pancreatic β -cell possesses the ability to respond to a minor increase in the blood glucose level, thereby maintaining that level. The liver plays a major role in maintaining glucose homeostasis by regulating glucose absorption, accumulation and catabolism through mediation of various metabolic signals. Thus, the effect of protective agents on tissues such as the liver and pancreas that regulate glucose metabolism is an interesting area to explore. In our study, histopathological examination of treated pancreas showed the morphological alteration of islet cells. Histological examination of liver from treated mice revealed morphological changes. Since glycogen deposition from glucose in the hepatocytes of treated animals is detected by PAS, glycogen depletion became the main criterion for histopathological evaluation in the current study. In our study chromium (VI) induced state was associated with low hepatic glycogen levels in liver determined by PAS (Fig 5B).

4. CONCLUSION

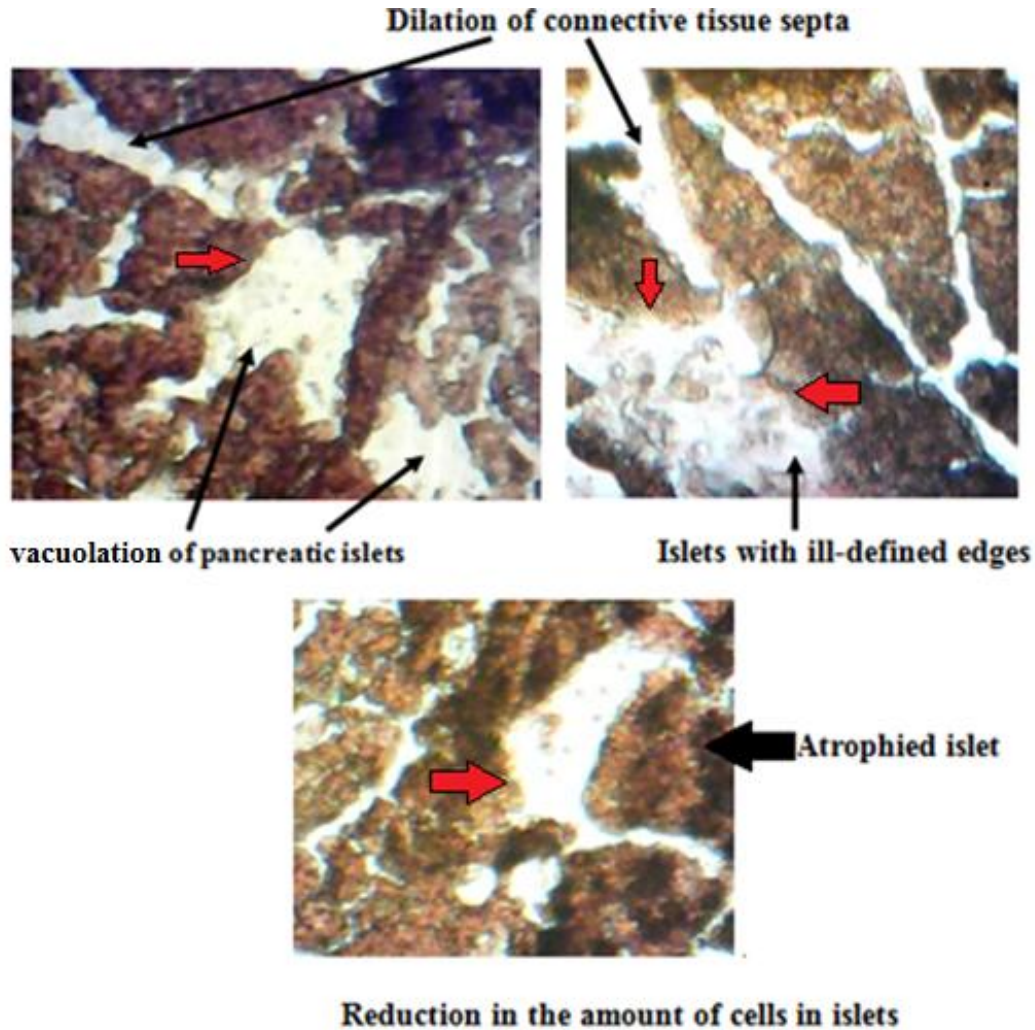
Our result may affect glucose homeostasis. Therefore metal induced alteration of pancreas and liver may persuade the condition of diabetes mellitus, but further experiments needs to be performed.

5. CONFLICT OF INTERESTS

The authors declare that there is no conflict of interests regarding the publication of this paper.

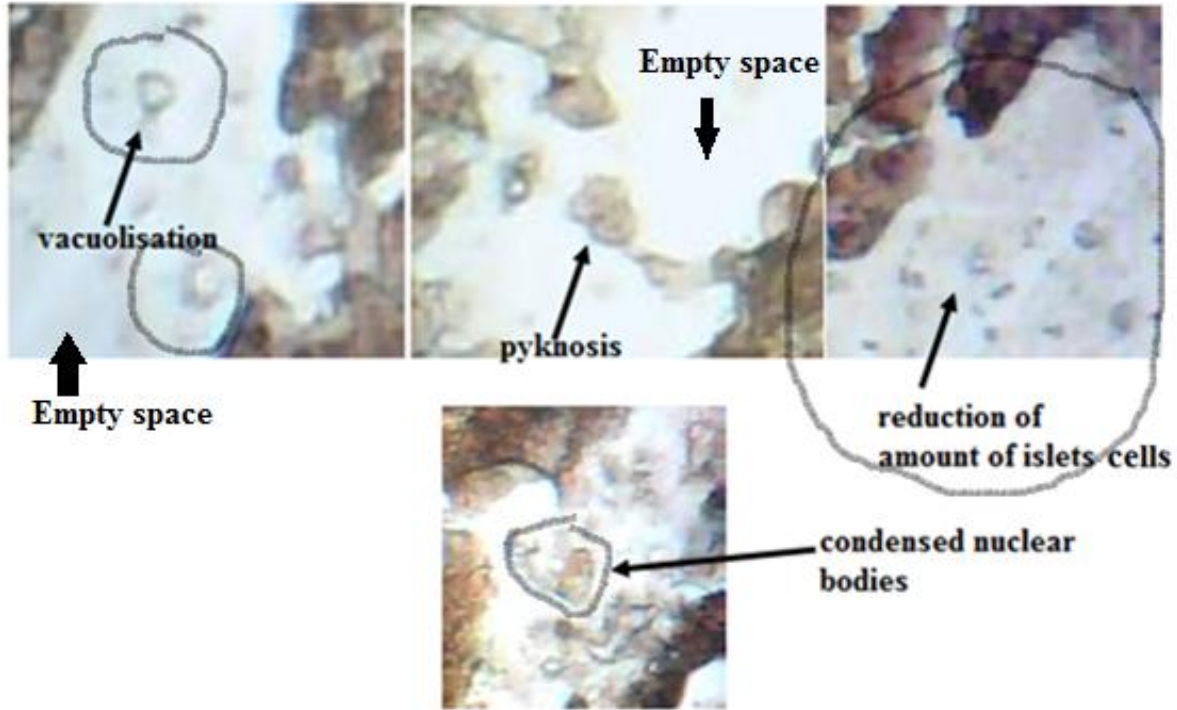
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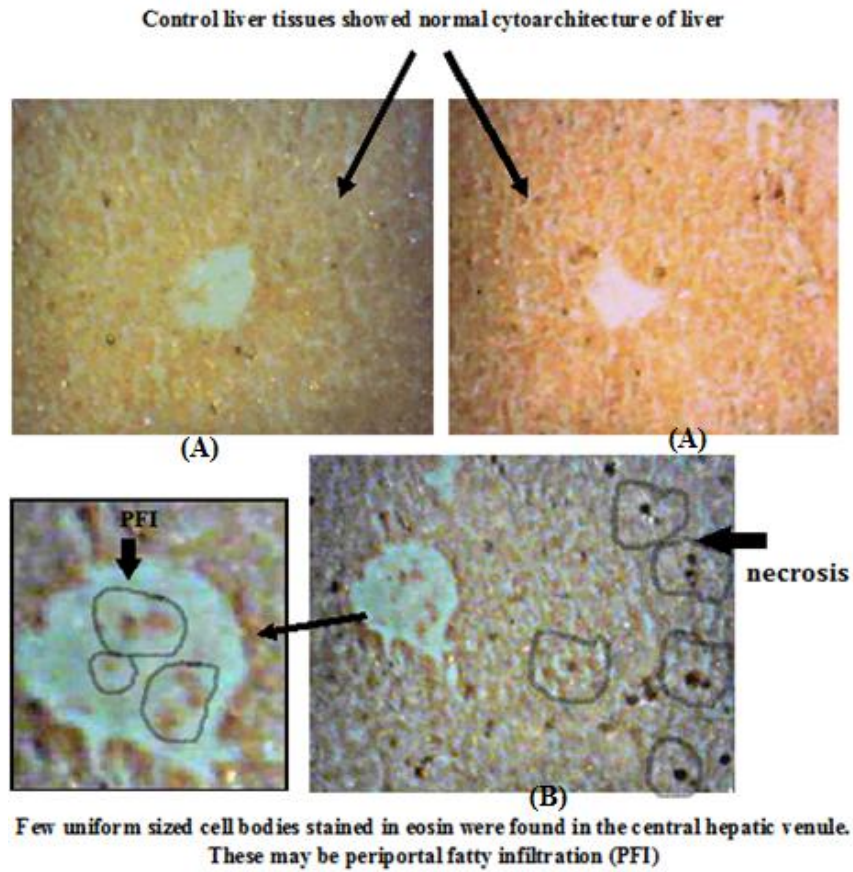
(Fig 1)

Figure 1: H-E stained section of treated mice pancreatic islets (x 400). Notice the dilated interlobular connective tissue (CT) septa. Photomicrograph of pancreatic tissue of the treated group showed the indistinct boundary between the endocrine and exocrine part (red arrow).



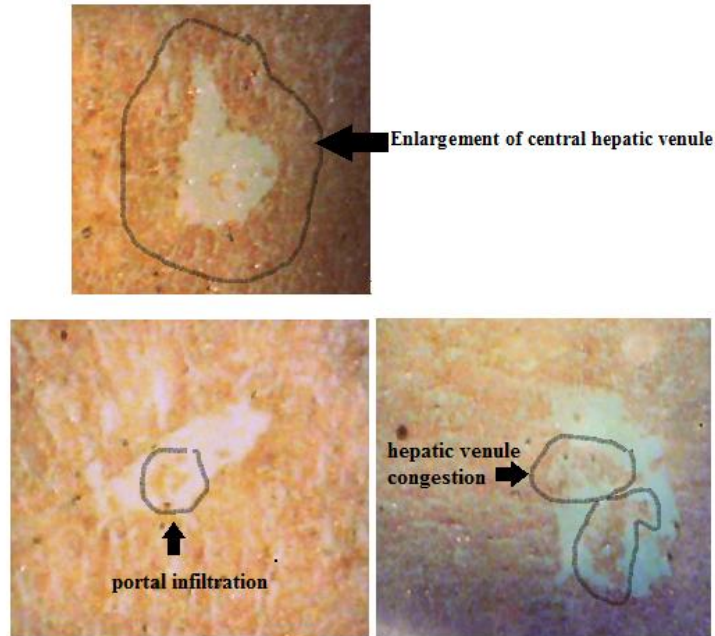
(Fig 2)

Figure 2: A photomicrograph of murine pancreatic tissue of the treated group showing degenerated islet's cells with nuclear pyknosis and nuclear fragmentation. Notice the empty spaces left after cell degeneration. Cells were altered with much reduction in the number and disorderly arranged.



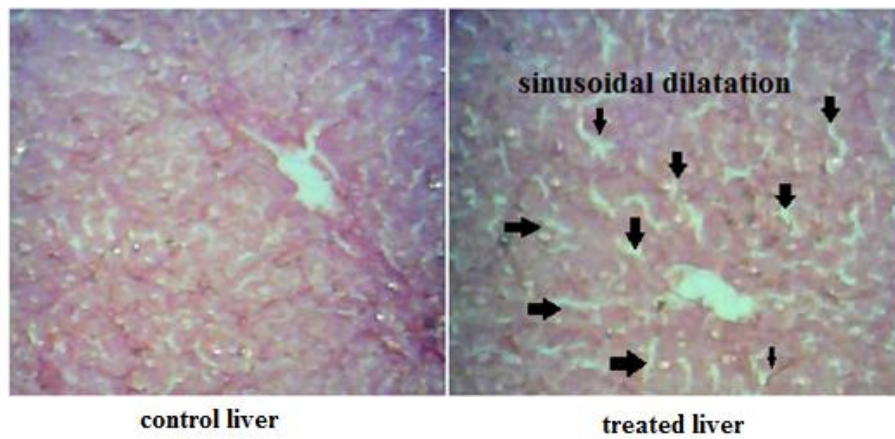
(Fig 3A, B)

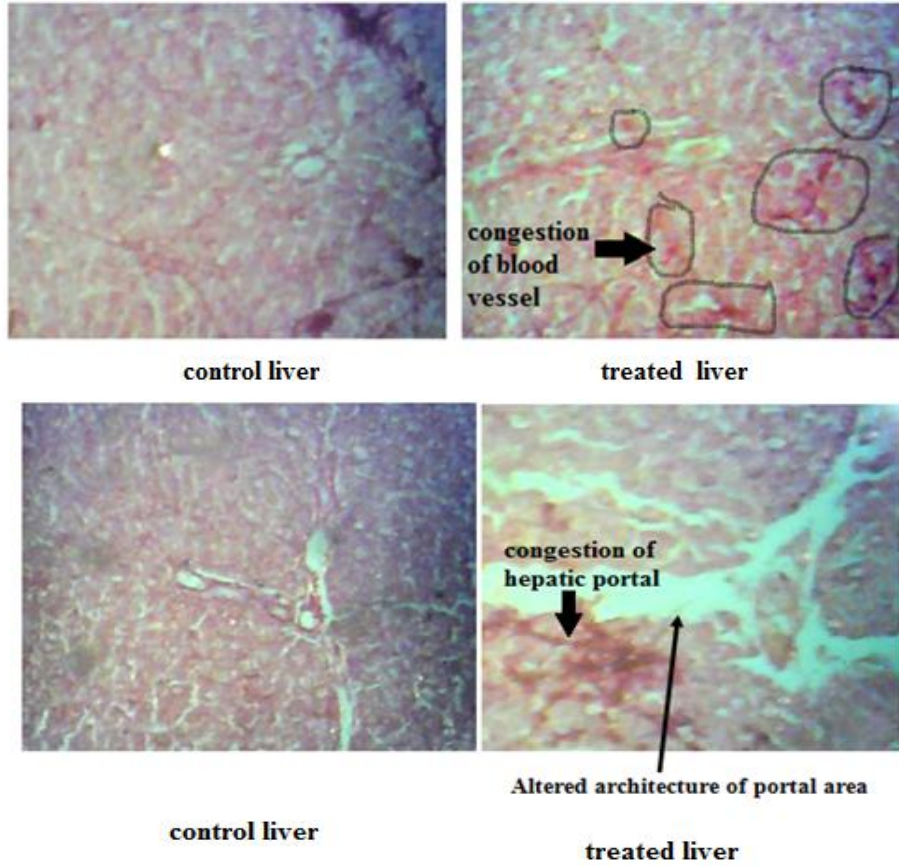
Figure 3 (A, B): (A) H-E stained section of normal mice liver (x 400), (B) H-E stained section of treated mice liver (x 400). Notice the necrotic cells and PFI



(Fig 3C)

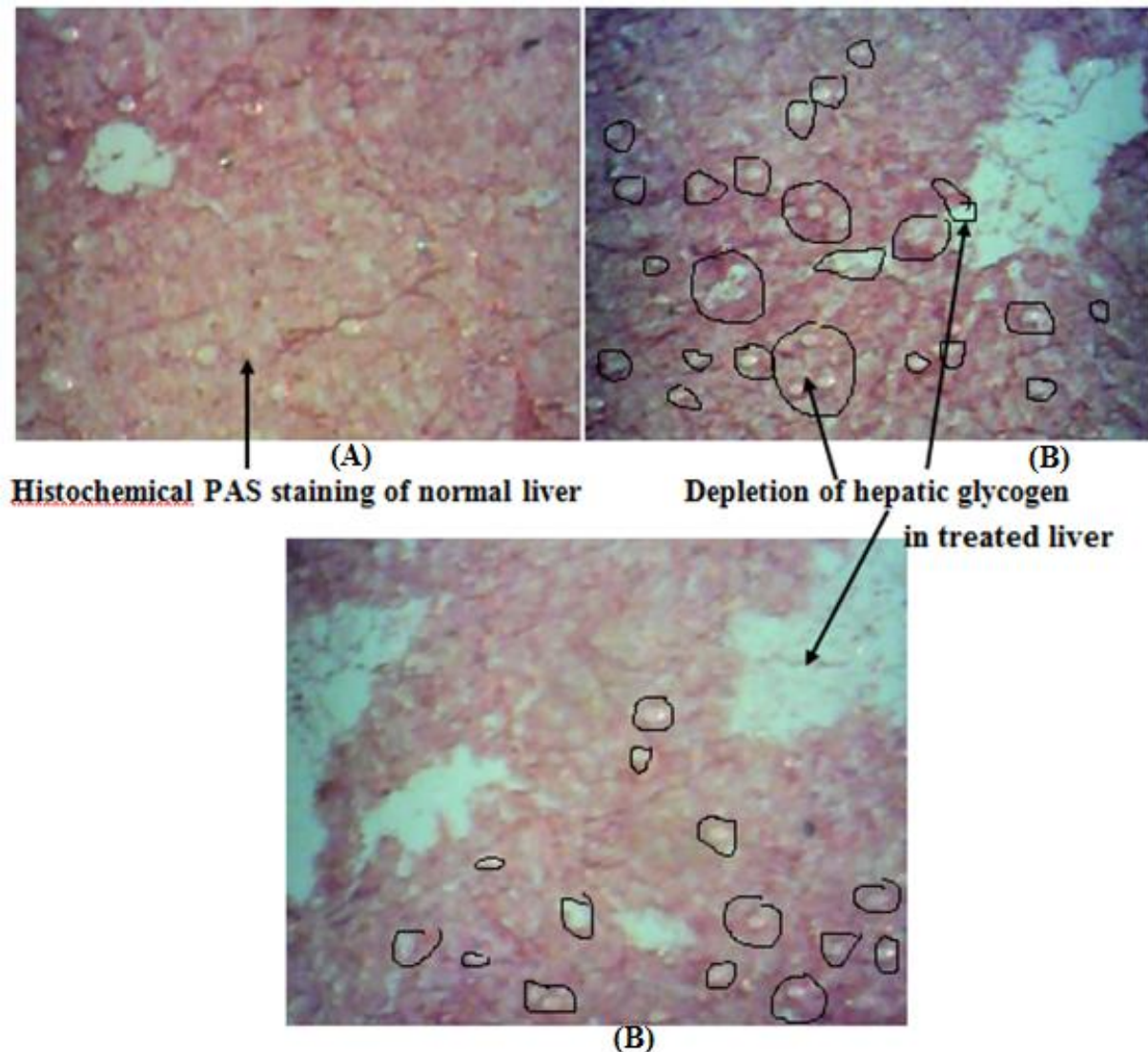
Figure 3(C): H-E stained section of treated mice liver (x 400)





(Fig 4)

Figure 4: PAS stained section of control and treated mice liver (x 400). Notice the dilatation of sinusoidal spaces, congestion of blood vessel and increasing amounts of connective tissue in the portal area.



(Fig 5)

Figure 5(A, B): (A) PAS stained normal mice liver section (x 400), (B) PAS stained treated mice liver section (x 400).

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