

Effect Of Sodium Fluoride On The Liver Of Male Albino Rats And The Possible Protective Role Of Vitamin C

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Abstract

Background: The liver alters exogenous and endogenous chemicals, foreign molecules and hormones to make them less toxic or less biologically active. The Fluoride causes impairment of liver function, causing decrease in metabolic activities, increased serum indices of liver function tests and inhibits certain antioxidant enzymes and molecules.

Objectives: To evaluate the effect of Sodium Fluoride (NaF) on the liver of male albino rats and the role of Vitamin C.

Animals and methods: This study was carried out on 30 adult male albino rats. The rats were randomly categorized into 3 equal groups of animals, 10 animals for each. Animals were given normal saline in Group (A), sodium Fluoride in Group (B), sodium Fluoride and Vitamin C in Group (C).

Results: Sodium Fluoride caused toxic effects on the liver. In the form of loss of normal pattern, dilated congested thick walled portal vein that contained hemolysed blood cells. Also there was mild dilatation in the thick walled hepatic artery and in the bile duct. After treatment with Vitamin C, there was an improvement in the picture of the liver.

Conclusion: Vitamin C can diminish these toxic changes induced by NaF on the liver tissue.

Key words: Sodium Fluoride; Vitamin C ; Liver.

Introduction

Liver is associated with metabolism and elimination of toxicant from the body and its histologic and biochemical parameters are considered as key points to elucidate toxicity of the chemicals. Evidences of changes in liver due to toxicants has been revealed by abnormal metabolic functions, reduced activity of detoxification reaction & altered structure of sub cellular organelles (Wang et al., 2000). Long-term exposure to a high-fluorine environment may result in

endemic fluorosis among susceptible populations. However, the pathogenesis of this condition remains unclear. Fluorine-induced free radical injury theory has been recognized by a large number of scholars (Lulu, 2016). Fluoride distributes very extensively in the natural environment, and is widely used among industry, agriculture as well as medicine. Fluorine is one of the essential trace elements for human body (Song et al, 2014). Vitamin C is a water soluble antioxidant that can ameliorate the free

radicals. Since ascorbic acid is water soluble, it can work both inside and outside the cells to combat free radical damage (Costa et al, 2016).

Animals and Methods

This study was carried out on 30 adult male albino rats (2 months old and 200-250 g weight) obtained from the Animal House of Sohag Faculty of Science from March 2021 to the end of June 2021. The rats were housed at a constant temperature of 21 ± 2 °C, fed standard diets with water available *ad libitum* and kept under a 12h light/12h dark cycle (lights on at 8:00 am). They were randomly categorized into 3 equal groups, 10 animals for each, treated orally by gastric feeding tube as described below for 30 days.

1- Group (A): Animals were given normal saline orally in a dose of 25mg/kg body weight every day for 30 days.

2- Group (B): Animals were given only a daily oral dose of 30mg/kg body weight sodium Fluoride (powder) dissolved in distilled water for 30 days.

3- Group (C): Animals were given a daily oral dose of 30mg/kg body weight sodium Fluoride (powder) and 500 mg/kg body weight of Vitamin C (powder) dissolved in distilled water for 30 days.

Dose selection of normal saline, NaF and Vitamin C was based on previous established studies (Sewelam, 2017) At the end of the experiment, the rats were anaesthetized by ether then perfused with saline then with the appropriate fixator (formalin 10%). Their

abdomens were opened and the livers were rapidly extracted of, dissected out carefully and processed for light and transmission electron microscopic studies.

I-Preparation for light microscopy

For the light microscopic study: The specimens were fixed in 10% neutral buffered formalin for 48-72 hours. The tissues were trimmed and processed for routine histological examination. Then they were put in paraffin wax and sections of 6µm thickness were cut. Hematoxylin and Eosin staining were used for all tissue sections. Tissue slides were examined under a light microscope (Leitz Orthoplan light microscope) in the Department of Histology, Faculty of Medicine, Sohag University.

II-Preparation for Transmission electron microscopy (TEM)

Samples from fresh liver specimens obtained for transmission electric microscope (TEM) from animals of each experimental group were immediately cut in small pieces and fixed in 2.5 % glutar aldehyde for 24 hours and dehydrated in graded alcohol series, semi-thin sections (one micron) were stained with toluidine blue. Ultrastructural sections were examined by transmission electron microscope (Jeol-1010) in the Department of Histology, Faculty of Medicine, Sohag University.

Ethical consideration

This study were carried out at Sohag University with the approval of the Ethics Committee.

Statistical analysis

The data were studied using the statistical software for social sciences (SPSS) version 18.0. Quantitative data was shown as mean \pm Standard deviation ($M \pm SD$). The chi-square test was used for comparison of non-parametric data. $P < 0.05$ was considered significant.

Results

Light microscopic results (Hematoxylin & Eosin stains)

Group A (control liver)

Light microscopic examination of tissue sections showed normal architecture of liver. The hepatic lobule consisted of the central vein in the center of the lobule and plates hepatocytes radiating from it towards the periphery. The portal triad appeared at the periphery of the hepatic lobule having a branch of the portal vein, hepatic artery and bile duct (**Figure 1A**). The hepatocyte appeared polyhedral in shape with centrally located rounded vesicular nuclei. Some hepatocytes were binucleated. The cytoplasm of hepatocytes appeared acidophilic with basophilic granules. The blood sinusoids appeared in between hepatocytes and lined by flat endothelial cell. Blood sinusoids also contained Von Kupffer cells (**Figure 1B**).

Group B (NaF treated Group)

Light microscopic study of sections of liver after treatment with NaF showed loss of normal pattern of the liver. In the portal area, there was dilated congested thick walled portal vein that contained hemolysed blood cells. Also there was mild dilatation in the thick walled hepatic artery and in the bile duct in comparison with the control group. There was extensive periportal infiltration with inflammatory cells (**Figure 1C**). Some specimens showed cellular infiltration around the central vein. Marked dilatation in the central veins those containing hemolysed red blood cells with necrosis of its endothelial lining. Blood sinusoids showed dilatation; congestion and hemorrhage inside them (**Figure 1D**).

Group C (NaF and Vitamin C treated Group)

There was an improvement in the picture of the liver in the form of regular cords of hepatocytes. In the portal area, there were marked dilated congested portal vein with normal hepatic artery and bile duct. (**Figure 1E**) No dilatation in the central vein. Blood sinusoids were improved but mild hemorrhage in some of them was seen. Some hepatocytes had pale stained nuclei, while other hepatocytes showed absent nuclei, some appeared with shrunken nuclei but some hepatocytes appeared with normal large nuclei and normal cytoplasm. Vonkupffer cells were enlarged (**Figure 1F**).

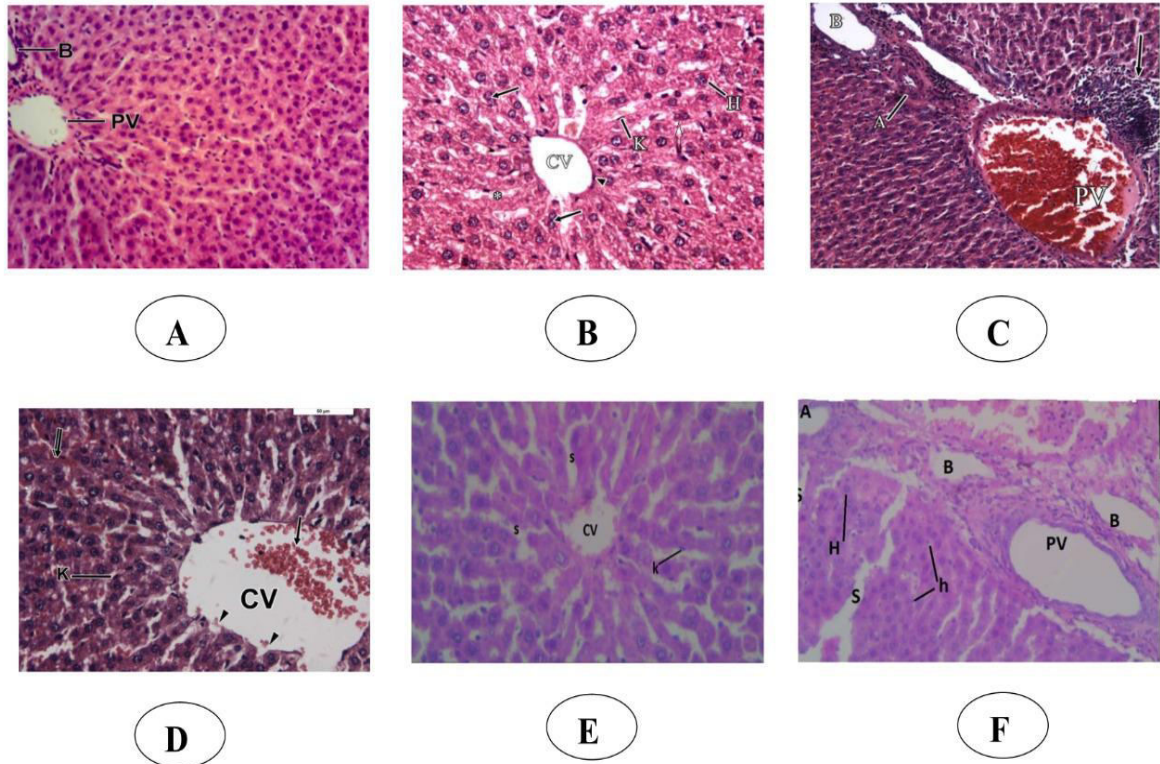


Fig. 1. A photomicrograph of **A:** Liver section of a control rat (group A) showing the portal area containing the portal vein (PV) and the bile duct (B) (H&Ex200). **B:** Liver section of the control group (Group A) showing group of hepatocytes (H) radiating from a central vein (CV) which is lined by thin endothelial cells (head of arrow). These cords are separated sinusoids (Blood sinusoids also contained Von Kupffer cells (k). Some binucleated hepatocytes are noticed (arrow). The cytoplasm of hepatocytes appeared acidophilic with basophilic granules (H & E x400). **C:** Section of NaF treated rat liver (Group B) showing markedly dilated, congested thick walled portal vein containing hemolysed blood cells (PV), extensive periportal infiltration with inflammatory cells (arrow), dilated bile ducts (B) and thick walled hepatic artery (A) (H&E x200). **D:** Section of NaF rat liver (Group B) showing markedly dilated central vein (CV) containing hemolysed red blood cells (arrow) with necrosis of its endothelial lining (arrow heads). Congested dilated blood sinusoids (double arrows) with increased Kupffer cells (k) are recognized (H&Ex400). **E:** Section of Vitamin C treated rat liver (Group C) showing regular cords of hepatocytes. No dilatation in the central vein (CV). Blood sinusoids (S) were improved but mild hemorrhage in some of them was seen. Von Kupffer cells (K) appeared enlarged (H & E x400). **F:** Section of Vitamin C treated rat liver (Group C) showing marked dilated congested portal vein (PV) with normal hepatic artery (A) and bile duct (B). Some hepatocytes appeared with shrunken nuclei (h). But other hepatocytes appeared with normal large nuclei (H) and normal cytoplasm (H & E x200).

Electron microscopic Results

Group A (Control Group)

The hepatocyte appeared with a well defined border and large rounded centrally located nucleus with regular nuclear envelopes. The cytoplasm showed abundant polymorphic mitochondria with well developed transverse cristae and electron dense matrix, well developed endoplasmic reticulum profiles exhibiting regular orientation around the nucleus and clusters of ribosome.(**Fig. 2A**). Kupffer cells were seen lining sinusoids and exhibited thin filipodia ,cytoplasmic phagolysosomes with clear Dissepaces between them and hepatocytic microvilli.(**Fig.2B**).

Group B (Sodium Fluoride treated group):

In Sodium Fluoride treated group (**GroupB**), the hepatocytes showed shrinkage, cytoplasmic vacuolization, hazy organelles and deformed nuclei. Most hepatocyte vacuoles were large compressing cytoplasmic organelles and closely opposed to mitochondrion

with unclear ridges. Fragmented and decreased endoplasmic reticulum, dispersed ribosome. Kupffer cells characterized by numerous phagolysosomes were seen lining dilated congested hepatic sinusoids (**Fig. 2C**). Disrupted hepatocyte cell membrane and disintegrated nuclear membrane were also noticed(**Fig.2D**).Disrupted hepatocyte cell membrane and disintegrated nuclear membrane were also noticed(**Figure 2E**).

Group C (Sodium Fluoride and Vitamin C treated group):

The rats treated with Vitamin C and NaF showed an improvement in the ultrastructural changes in the form of diminution of cytoplasmic dissolution and a well defined large nucleus and nuclear envelope. There were normal shaped rough endoplasmic reticulum and abundant polymorphic mitochondria. Normal shaped endothelial cell was noticed(**Fig. 2F**). There was moderate dilatation of blood sinusoids and a well defined border of Kupffer cell was also noticed(**Fig.2G**).

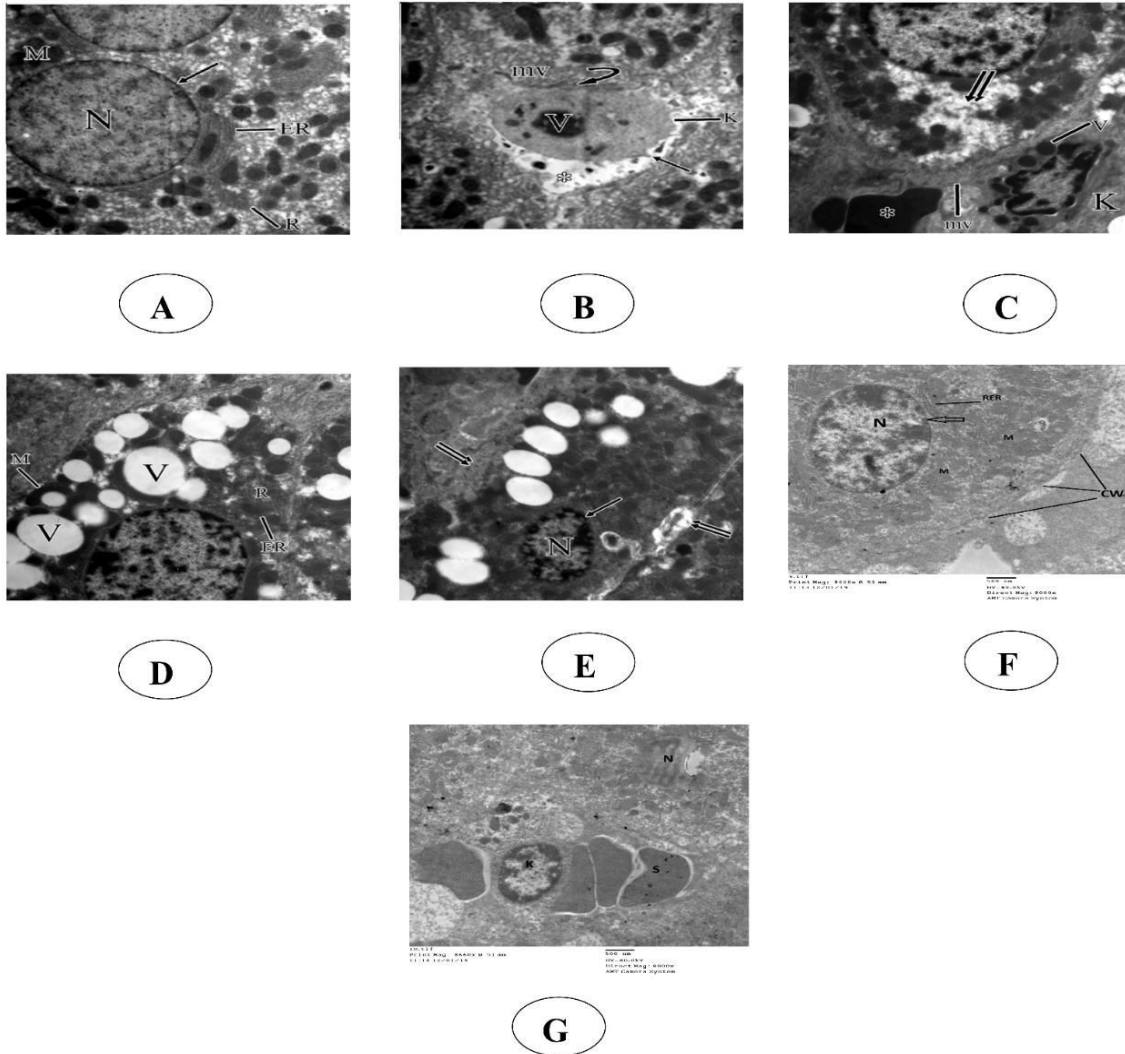


Fig. 2: An electron micrograph of **A:** Liver section of control rat (Group A) showing the hepatocyte exhibiting rounded euchromatic nuclei (N) with regular nuclear envelopes (arrow), polymorphic mitochondria (M), endoplasmic reticulum (ER) and clusters of ribosomes (R) (X8000). **B:** Liver section of control rat (Group A) showing the Kupffer cell (K) lining hepatic sinusoid and exhibiting thin filipodia (arrow), cytoplasmic phagolysosomes (V) and clear Disse space (curved arrow) between it and hepatocytic microvilli (mv) (X8000). **C:** Section of NaF treated rat liver (Group B) showing decreased cytoplasmic density of hepatocytes (double arrows) with fragmented microvilli (mv). Active Kupffer cell (K) containing numerous phagolysosomes (V) was seen, dilated congested sinusoid (*) (X 8000). **D:** Section of NaF treated rat liver (Group B) showing large hepatocytic vacuoles (V) closely opposed to hazy mitochondrion (M), fragmented and decreased endoplasmic reticulum (ER) and dispersed ribosomes (R) (X 8000). **E:** Liver section of NaF treated rat (Group B) showing pyknotic nucleus (N) with disintegrated nuclear membrane (arrow) and disrupted hepatocyte cell membrane (double arrows) (X 8000). **F:** Section of NaF and Vitamin C treated rat liver (Group C) showing the hepatocyte appearance with a well defined border (CW) and large rounded centrally located nucleus (N) with regular nuclear envelopes (arrow). The cytoplasm showed abundant polymorphic mitochondria (M) and normal shaped rough endoplasmic reticulum (RER) (X 8000). **G:** Section of NaF and Vitamin C treated rat liver (Group C) showing well defined border of Kupffer cell (K) and dilatation in blood sinusoids (S). The hepatocyte appeared with a well defined border and rounded centrally located nucleus (N) (X 8000).

Morphometric Analysis

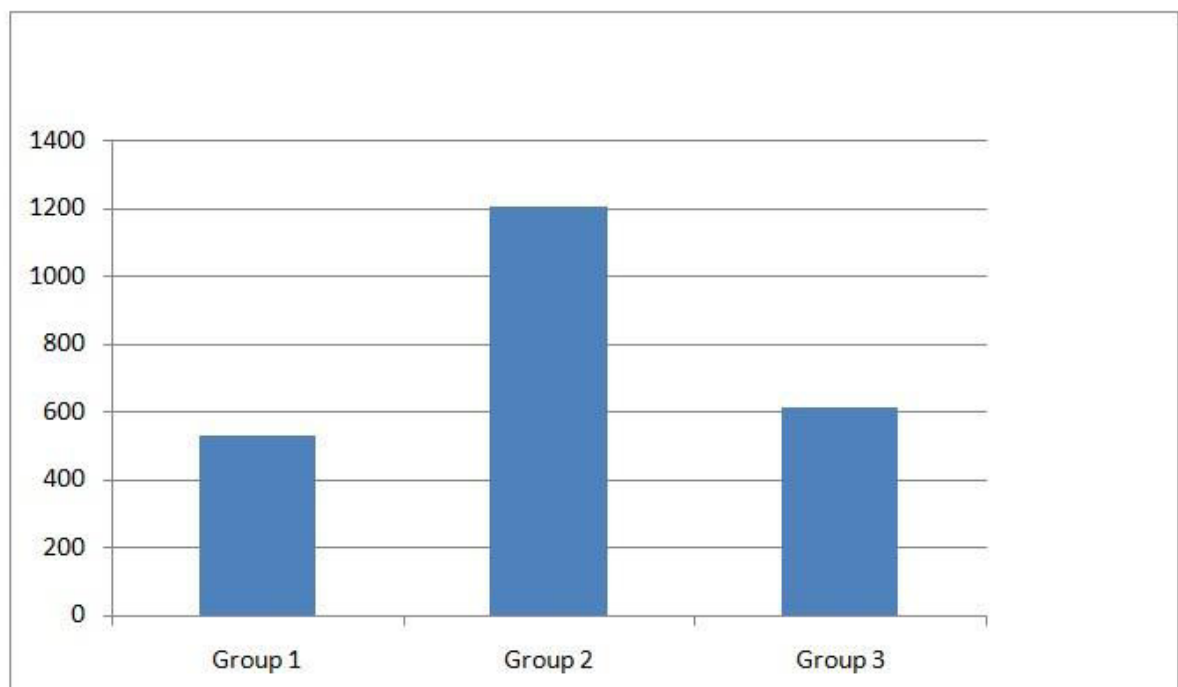
-Diameter of central vein: (Table 1, Histogram1)

Table 1. Mean diameter (in pixels) of Cental vein in the control and the experimental groups

Parameters	Group 1 (control)	Group 2 (NaF treated)	Group3 (Vit C treated)	P1 value	P2 value	P3 value
Diameter of cental vein(Pixels)	529.20	1203.25	610.86	0.000***	0.401	0.000***

P1 value: comparison between Group 1 and Group 2, P2 value: comparison between Group 1 and Group 3, P value: comparison between Group 1,2 and Group 3.

P, P1<0.000(***) high significant difference, P2>0.05non significant.



Histogram (1). Showing the mean diameter (in pixels) of Cental vein in the control and the experimental groups.

Mean central vein diameter in group 2 (1203.25 pixels) showed a highly significant ($P < 0.000$) increased when compared with group 1 (529.20 pixels) and group 3 (610.86 pixels).

Discussion

In the current study the structural alteration, vacuolation, degeneration, necrosis of hepatic cell nucleus and dilatation of central vein in the liver were seen after Fluoride treatment. Necrosis of hepatic cells were also observed by **Shweta et. al (2013)**. In the present study the histoarchitecture of liver showed radially arranged hepatic cords around the central vein in the control group. Fluoride can produce distortion of the liver architecture including degenerative and inflammatory changes. Similar results were also reported by **Chinoy et. al, (1993)**.

Compared to the control group, NaF group in the present study showed marked including histopathological changes disrupted hepatic cords, vascular dilatation and congestion, Kupffer cell enlargement and inflammatory cell infiltration. In addition, the hepatocytes exhibited vacuolation, pyknosis, necrosis and complete lysis after treatment of rats with NaF. Similar degenerative changes have been detected by **Sewelam (2017)** in liver tissue exposed to toxic dose of Fluoride in rats. It has been suggested that Fluoride induced vascular dilatation

might be due to the direct Fluoride toxicity (**Klatskin et al, 1993**).

Fluoride induced inflammatory cell infiltration in the hepatic tissue was noticed by (**Johar, 2004**). The cytoplasmic vacuolation might be a consequence to disturbed lipid and fat metabolism or due to an imbalance between rates of synthesis and release of substances in hepatocytes (**Blankenship et al., 1994**).

As noted in the present results, the ultrastructural changes of hepatic cells in NaF treated group confirmed the light microscopic findings of Fluoride cytotoxicity. Furthermore, important morphological features were recognized especially structural membrane damage of mitochondria, endoplasmic reticulum, nuclear and plasma membranes. Data in this study confirmed the findings depicted by other researchers (**Cao et al.,2013**).

Kupffer cell hyperplasia depicted in this work might be attributed to increased phagocytic activity of sinusoidal cells as a defense mechanism for detoxification or might be a hepatic response to oxidative stress (**Neyrinck, 2004**).

Several studies have concluded that Fluoride induced apoptosis might be

due to increased lipid peroxidation, oxidative stress, mitochondrial functional disturbances, downstream pathways activation and signals imbalance (Flora et al, 2009). According to the present study, in the NaF treated group the bile ducts showed manifest luminal dilatation and disruption of hepatocytes microvilli. These changes were very similar to the hepatocyte damage found by Alemmari et al, (2011). Abou-Zeid (2014) attributed these changes to direct excretion of toxin or drug in bile causing bile duct damage. The present study stated that the exogenous feeding of Vitamin C to fluoride treated rats can play prophylactic role to maintain normal liver histology. After giving the food supplements the structural abnormalities could not be observed peculiarly as compared to the treated groups. The hepatic cells were initialized to recover after the administration of Vitamin C for 30 days. The structural abnormalities could not be observed peculiarly as compared to the treated groups.

Conclusion: Chronic use of NaF causes toxic effect on the liver tissue in the form of dilatation of central veins and sinusoids, vacuolation of the cytoplasm and degeneration of the

hepatocytes and Vitamin C can diminish these toxic changes in the liver tissue.

Abbreviation: Sodium Fluoride (NaF), Hematoxylin and Eosin (H&E) and Transmission electron microscopy (TEM).

Study's limitations

The main limitation of this study was the small sample size, further studies with a large sample sizes were recommended in this field.

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