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HISTOPATHOLOGY OF FLUORIDE-INDUCED HEPATOTOXICITY IN RABBITS

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SUMMARY: The effect of chronic and acute exposure to sodium fluoride (5, 10, 20, and 50 mg/kg body weight/day) for fifteen weeks on hepatic damage in young albino rabbits was evaluated. Histopathological examination revealed increasing degrees of hepatocellular necrosis, degenerative changes, hepatic hyperplasia, extensive vacuolization in hepatocytes, and centrilobular necrosis in the liver of the exposed animals. The central vein and sinusoids of the liver were dilated and engorged with blood and were associated with small areas of haemorrhages. These effects were not observed in the control group.

Keywords: Albino rabbits, fluoride hepatotoxicity, Liver histopathology, Sodium fluoride.

INTRODUCTION

As a very active site of metabolism, the liver is especially susceptible to fluoride intoxication. Earlier studies show that fluoride can produce abnormalities in the liver including degenerative and inflammatory changes,[1] dilatations of sinusoids,[2] hepatic hyperplasia,[3] and accumulation of amorphous and crystalline bodies in the hepatocytes around the hepatic vein.[4]

Fluoride-linked occurrence of a rare form of liver cancer (hepatocholangiocarcinoma) in mice has been reported.[5] Gross observations made at necropsy of rats supported histological observations of hepatic hemangiosarcoma, hepatocellular adenoma and carcinoma, metastatic lung tumors, and Zymbal's gland tumors in rats exposed to vinyl fluoride.[6] Hepatic hemangiosarcoma was the sentinel lesion in rats.

This study examines fluoride-induced hepatic damage in albino rabbits.

MATERIALS AND METHODS

Animals and treatments: Experiments were done with 60 young male and female albino rabbits weighing 400 - 650 g. They were kept under standardized conditions, including a standard diet and free access to normal (low-fluoride) tap water. Animals were divided equally into the following five groups.

Control: One mL of double distilled water/kg body weight/day was administered subcutaneously for fifteen weeks.

Experimental: Doses of 5, 10, 20, and 50 mg of sodium fluoride dissolved in 1 mL of double distilled water/kg body weight/day were injected subcutaneously for the same 15-week period.

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Assessment of hepatic damage: The rabbits were decapitated under ether anaesthesia, and the liver was removed, blotted free of blood, fixed in Carnoy's fixative and alcoholic Bouin's fluid. The tissue was washed in 70% alcohol and then 95% alcohol, dehydrated in tert-butyl alcohol, cleared in amyl acetate, and embedded in paraffin. Serial sections were cut at 7 μm and stained with iron haematoxylin and eosin for histopathological examination.

RESULTS

On gross examination the liver of the rabbits in the fluoride-treated groups was pale in color but normal in size, whereas in controls, the liver was reddish brown.

In all animals administered 5 mg of NaF/kg body weight/day, the lobular pattern of the liver was normal compared with that of the control rabbits. Ballooning or hyaline degeneration was absent. There was no evidence of proliferation of Kupffer's cells or abnormal pigment in them. Hepatic cell necrosis of focal type was absent. Round cell infiltration in the periportal area (pp) was absent. There was also no evidence of bile duct (bd) proliferation or periportal fibrosis. Sinusoids did not show any dilatation. The blood vessels in the periportal area were normal in all the rabbits as compared to the controls (Figures 1 and 2).

In group II treated with 10 mg of NaF/kg/day the rabbits showed normal hepatic lobular pattern. Focal areas of necrosis of hepatic cells was present. There was proliferation of hepatic cords (Figure 3). However, in Kupffer's cells, proliferation was absent. No abnormality was detected in the periportal tissue in regard to bile duct proliferation and periportal fibrosis. Sinusoids showed dilatation (Figure 4).

In animals of group III treated with 20 mg of NaF/kg/day, the normal lobular pattern of hepatic cords was distorted. There was ballooning degeneration and cell necrosis (Figure 5). Hepatocellular hyperplasia and hepatic sinusoidal dilatation were more marked. The hepatic cords exhibited distortion and linear contraction. In certain areas the spaces normally occupied by hepatic cells appeared empty. Around the central vein, hepatic cells showed hyperplasia of parenchymatous cells. Hepatic cells exhibited proliferation, and the central vein was enlarged and filled with pitch-black erythrocytes.

An extensive cell necrosis along with ballooning degeneration of hepatic cells together with the nucleus was observed (Figure 6) in rabbits of the highest dose group (50 mg of NaF/kg/day). The cytoplasm in some hepatic cells exhibited extensive vacuolization. Hepatic cellular hyperplasia showing dilatation of sinusoids with a large number of erythrocytes in them was most pronounced (Figure 7). Some hepatic cells were binucleated indicating hepatocellular adenoma and carcinoma. The central vein was enlarged and dilated. The cells lining the central vein exhibited distortion suggestive of centrilobular necrosis (Figure 8).

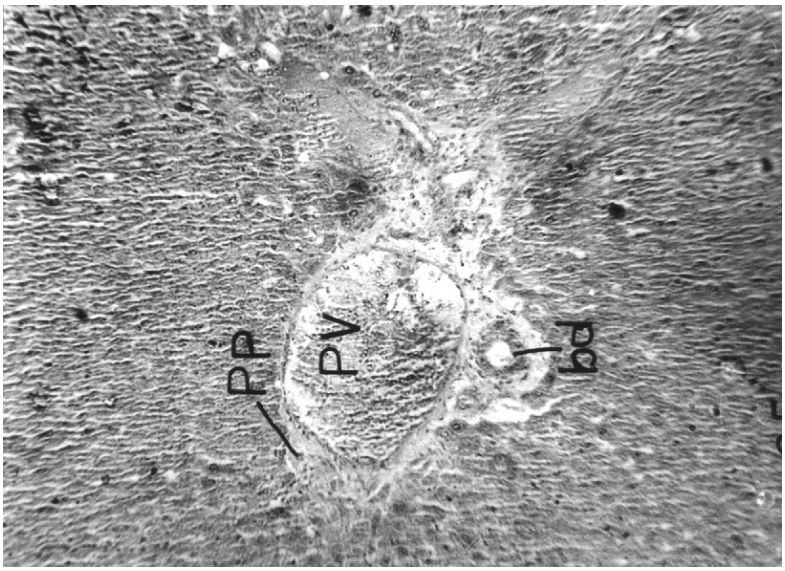

Figure 2. Photomicrograph showing normal liver structure in a rabbit of the control group x 100 (bd - bile duct, PV - portal vein, pp - periportal area)

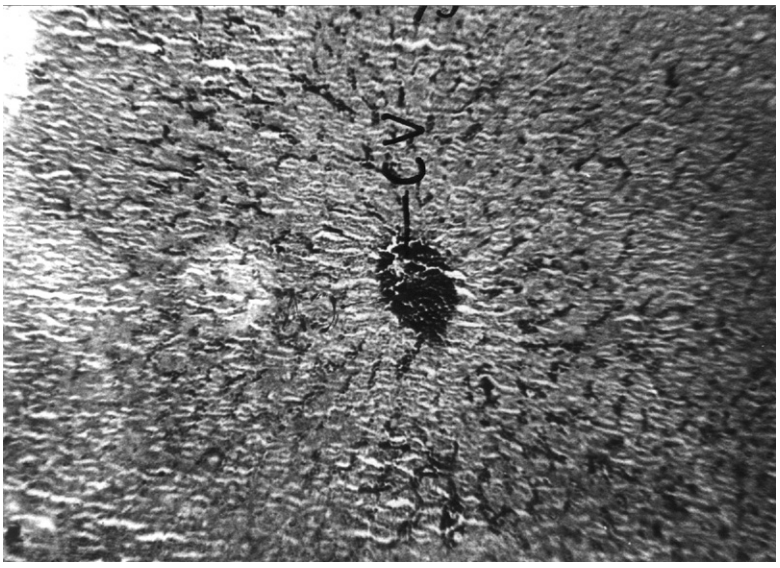

Figure 1. Photomicrograph showing normal liver structure in a rabbit of the control group x 70 CV-(Central vein)

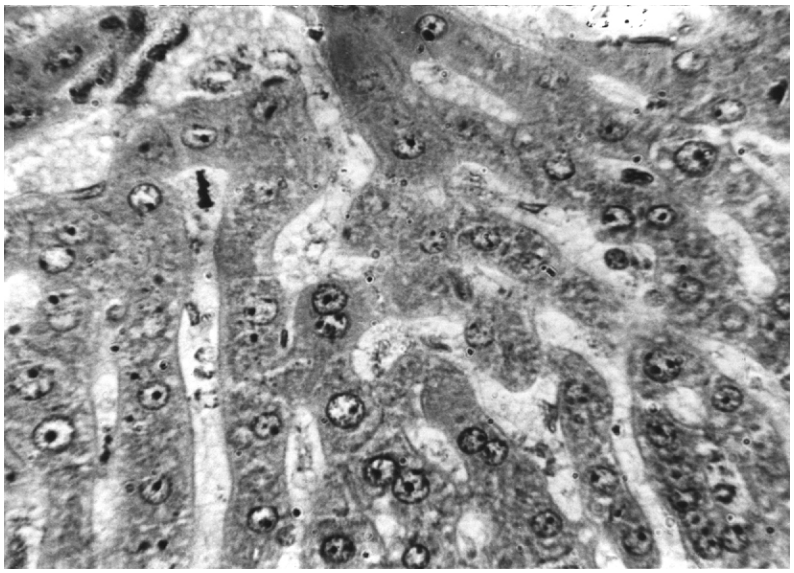

Figure 4. Photomicrograph showing dilatation of sinusoids in the liver of a rabbit treated with 10 mg of NaF/kg body weight x 400

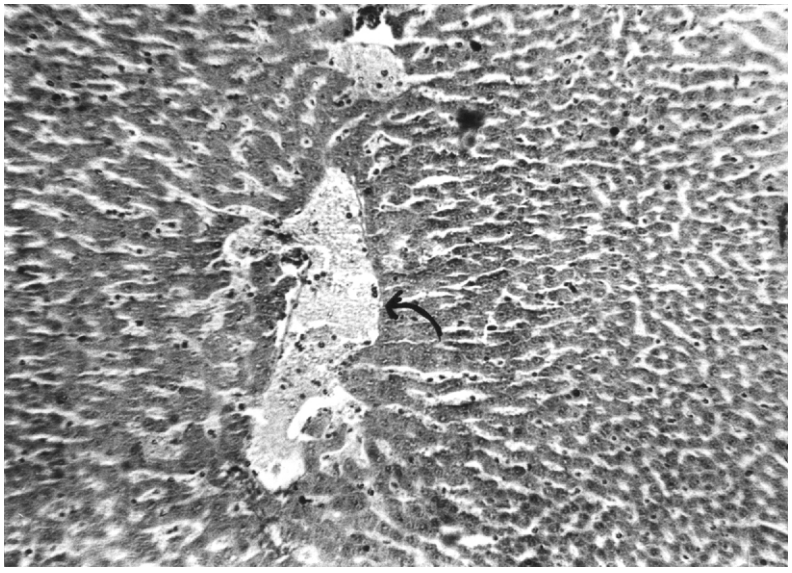

Figure 3. Photomicrograph showing focal areas of liver cell necrosis and proliferation of hepatic cells x 100 in a rabbit treated with 10 mg of NaF/kg body weight

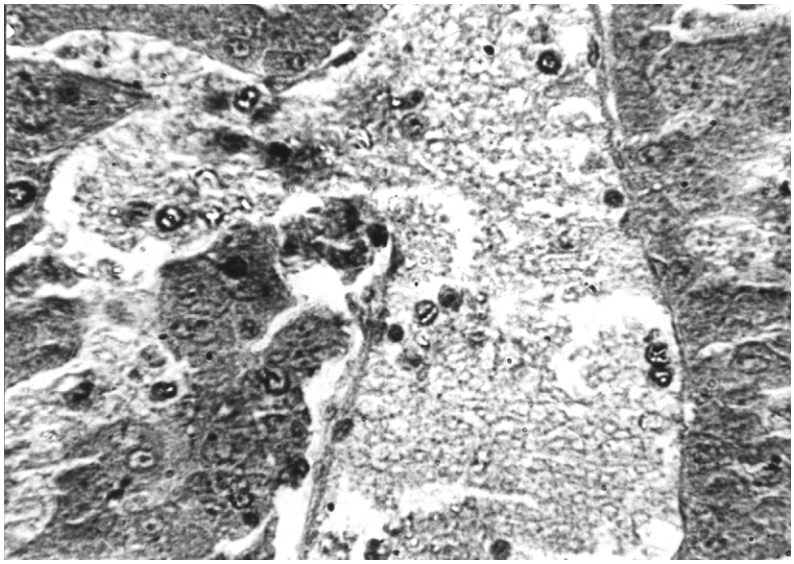

Figure 6. Photomicrograph showing extensive cell necrosis along with ballooning degeneration of liver cells in a rabbit treated with 50 mg of NaF/kg body weight x 400

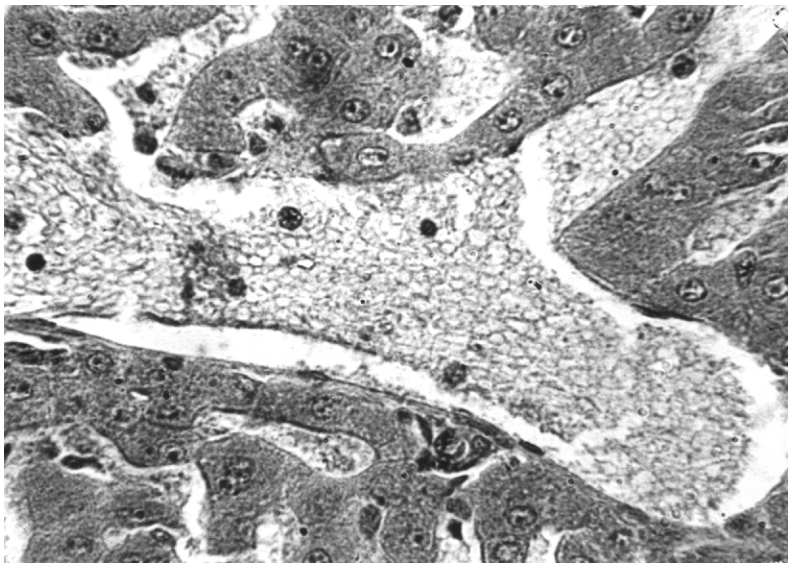

Figure 5. Photomicrograph showing ballooning degeneration and cellular necrosis in the liver of a rabbit treated with 20 mg of NaF/kg body weight x 400

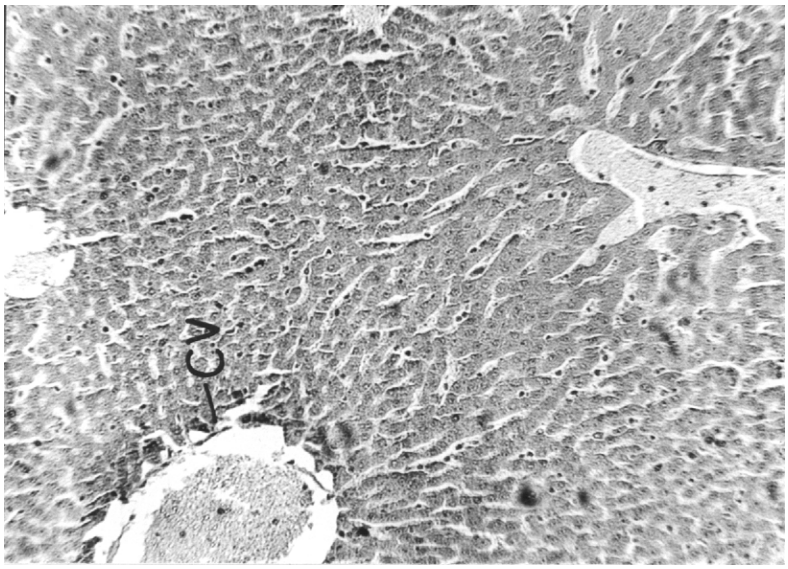

Figure 8. Photomicrograph showing enlarged central vein (CV) and hyperplasia of cells in the liver of a rabbit treated with 50 mg of NaF/kg body weight x 100

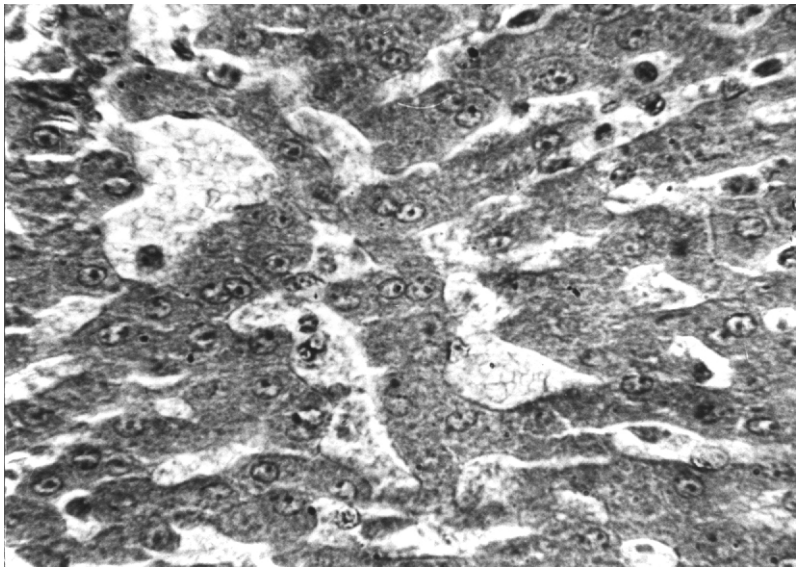

Figure 7. Photomicrograph showing dilatation of sinusoids and necrosis of hepatocytes in liver of a rabbit treated with 50 mg of NaF/kg body weight x 400

DISCUSSION

In the past some workers have failed to observe any change in the liver tissue of fluoridated animals.[7-12] In rats, for example, treatment with 1, 10 and 100 ppm sodium fluoride in drinking water for a period of 180 days, no abnormality in body weight gain and histopathological structure of the liver was recorded.[13]

Liver, an organ of vital importance, was severely damaged by fluoride in the present investigation on rabbits. Excessive fluoride intake resulted in extensive degenerative changes in the liver varying from ballooning degeneration to complete disintegration of hepatic cells. Focal areas of hyperplasia of the hepatic cells, congested central vein, and hepatic dilatation of sinusoids were also observed. Degenerative changes in the liver of animals fed excessive amounts of fluoride have been reported earlier[2,4,14,15] by others. Muehlberger[14] found hydropic degeneration in fatally poisoned rats. Smyth and Smyth[15] recorded degeneration of hepatic cells in rats intoxicated with barium fluorosilicate. Phillips *et al*[4] recorded degeneration of hepatic cells especially around hepatic vein and accumulation of both amorphous and crystalline bodies in the liver of experimental animals. Mello *et al*[16] observed degenerative lesions in the liver of rats, even with only 1 ppm fluoride in their drinking water. Chinoy *et al*[1] recorded zonal necrosis and pycnosis of hepatocyte nuclei and disturbed arrangement of hepatic cords in fluoride-treated rats. Bogdanffy *et al*[6] found that rats and mice exposed up to 2500 ppm vinyl fluoride had hepatic hemangiosarcoma, hepatocellular adenoma and carcinoma, hepatic foci of clear cell and basophilic alteration, and sinusoidal dilation.

In our studies, the liver in the fluoride-treated rabbits appeared pale in color as compared to the red color of the controls. In guinea pigs given sodium fluoride as 10, 500, and 1000 ppm in drinking water for 1-2 months, Kour *et al*[2] recorded hepatic focal areas of reticuloendothelial cell hyperplasia accompanied by areas of liver cell necrosis. These structural changes were even more advanced after three months of fluoride administration when massive hepatic cell necrosis had occurred. Calcified areas surrounded by degenerated liver parenchyma, congested and dilated central veins, and sinusoids were also observed. Similar changes were detected in rabbits during the present study. In rabbits, Wang *et al*[17] also reported necrotic areas in the liver cells associated with vacuolar changes and proliferation of connective tissue fibres.

In fluorotic patients, hepatic cellular necrosis with round cell infiltration has been recorded.[18] The hepatic cells were separated from each other and appeared honey-combed with marginal cytoplasm. Empty spaces were present at places which had been occupied by hepatic cells. Between the plates of hepatic cells, a greasy, protein-rich edematous substance and fatty drop-

lets of different sizes were present in the cytoplasm. Haber[19] even found mid-zonal hepatic cell necrosis caused by fluoride ions in patients receiving fluorinated anesthetics.

ACKNOWLEDGEMENT

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