Research Artilcle

Fluoride Induced Histopathological Changes in Liver of Albino Rabbit - An Experimental Study

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ABSTRACT

Fluoride causes serious health problems, as it is a well determined non-biodegradable and moderate pollutant. The present study was designed to investigate the histopathalogical changes in liver of albino rabbits after exposing them to sodium fluoride. 24 adult albino rabbits were divided into 3 groups (8 rabbits per each group). The first group served as controls and received de-ionized water. The second group was treated with 0.5% of sodium fluoride solution and third group was treated with 3% of sodium fluoride solution for 16 weeks. At 2 weeks interval of the treatment period, 1 animal from each group were sacrificed by cervical dislocation and liver was dissected out. The liver tissue was cleared and processed to assess the histopathalogical changes. The histopathological results in the present study indicate that exposure to sodium fluoride from 15 days to 16 weeks in high doses caused necrotic changes in hepatocytes and liver sinusoids.

Keywords: Albino rabbit, Histopathalogical changes, Sodium fluoride, Liver.

INTRODUCTION

Fluoride is largely present in earth and essential trace element for human being and animals[1]. With oral route along with food and water, fluoride is found in small quantities in almost all foods and enters into the human body[2].

Shulman and Wells[3] have demonstrated that fluoride problem occur with releasing of fluoride dust and fumes from different industries using hydrofluoric acid and fluoride salts. All the age groups in several countries have suffered from severe fluorosis due to ingestion of sodium fluoride[4]. Furthermore, in India, fluorosis is an irreversible disease and a major public health hazard. Approximately, 66 million people in 19 states in India are affected with fluorosis.

Though, consumption of fluoride over a long period of time affects the soft tissues like muscle, liver, gastrointestinal tract and several other reproductive and endocrine organs by the property of simple diffusion and caused to impairment of soft tissues[5-7].

Recent study has demonstrated that accumulation of fluoride is due to decreased aerobic metabolism and altered free radical metabolism in liver [8].

In addition, ingestion of fluoride is inhibiting the Kreb's cycle and leads to toxicity in liver[9-11]. However, the effect of fluoride on liver is far from clear.

Earlier studies have shown that fluoride causes degenerative and inflammatory changes, dilatations of sinusoids, hepatic hyperplasia and accumulation of amorphous and crystalline bodies in the hepatocytes in liver[12,13]. Hodge and Smith[14] well recognized liver for its histopathalogical and functional responses to excessive amounts of fluoride.

Many studies have shown that high levels of fluoride could accumulate in the kidney and this organ is the major route for removal of fluoride from the body[15,16]. Studies are also available on fluoride toxicity on kidney that show fluoride induced kidney lesions through apoptosis[17].

However, we made an attempt to study the toxicity in liver induced by exposure to sodium fluoride in high doses.

MATERIALS AND METHODS

Animals

Healthy adult male albino rabbits (60 ± 2) days and weight (1 to 1.5Kg) were maintained at laboratory conditions (26 ± 2 °C) 12 hrs light and 12 hrs dark cycle. They were kept in well cleaned and husk filled sterilized cages.

The animals were provided with standard rabbit-feed and adlibitum tap water. This study was carried out according to guidelines for the care and use of laboratory animals and approved by the Institutional Animal Ethical Committee.

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Chemical and Dosing

Sodium fluoride (99%) is used as a toxicant supplied by BDH Chemical Division, Bombay.

Normal male rabbit were divided into three groups, each group contained six animals. The first group animals served as control and received de-ionized water.

Second group animals treated with 0.5% sodium fluoride solution (5 mg/kg body wt.) through oral route daily up to 16 weeks. The third group animals treated with 3% sodium fluoride solution (30 mg/kg body wt.) through feeding tube orally for 16 weeks.

Necropsy

After 2 weeks interval of treatment period (16 weeks), the body weights of male rabbits were recorded and necropsied by cervical dislocation. Liver was isolated, cleaned from adhering tissue or fluid and their weights were recorded using an electronic balance.

Histopathology

Histopathalogical examination of the tissues was followed as per Humason[18]. Liver tissue was isolated from the control and experimental rabbits. They were gently rinsed with physiological saline solution (0.9% NaCl) to remove blood and debris adhering to the tissues. They were fixed in 5% formalin for 24 hours. The fixative was removed by washing through running tap water overnight.

After dehydration through a graded series of alcohol, the tissues were cleared in methyl benzoate embedded in paraffin wax. Sections were cut at 6 mm thickness and stained with Harris haematoxylin[19] and counter stained with eosin (dissolved in 95% alcohol). After dehydration and clearing the sections were mounted with DPX and observed under microscope. It is believed that histology helps to determine the pathological lesion in tissue caused by the toxicant. The transverse section of liver in control rabbit comprises of continuous mass of hepatic cells, with cord formation. The cells are large in size with more or less centrally placed nucleus and homogenous cytoplasm. A fine network of vascular sinusoids running in between the parenchyma cells observed in the liver (Fig. 1).

The transverse section of liver in Group C rabbit exposed to sodium fluoride for 15 days has shown remarkable changes when compared to control, such as cellular disarray, congestion, cellular degeneration, and cellular vacuoles (Fig. 2).

It has been revealed, that cellular degeneration, severe necrosis in hepatocytes, nuclear fragmentation, nuclear degenerative changes, binucleated condition, pushing of nucleus to periphery of hepatocytes, hemorrhage in central vein and pycnotic nucleus is observed in transverse section of liver in rabbit exposed to sodium fluoride for 8 weeks (Fig. 3).

Under experimental condition the transverse section of liver of 10 weeks fluoride exposed rabbit has shown necrosis in liver cells, degenerative changes in (Fig. 4). Moreover, the transverse section of liver in rabbit treated with fluoride for 16 weeks has exhibited severe necrosis in liver cell, large vacuoles (Fig. 5). Severe fluorosis in humans occurred due to consuming fluoride through drinking water. Therefore, in the present study sodium fluoride was administered in rabbit through the same route (Table 1).

DISCUSSION

Liver is the principal organ responsible for metabolism and involved in the metabolism of toxic compounds produced during systemic processes and exogenous toxins entering into the organisms from the environment[20]. Furthermore, it was assumed that sodium fluoride would induce both pathomorphological and metabolic changes in liver[21]. The results in present study have revealed, cellular disarray, congestion, cellular degeneration, and cellular vacuoles, severe necrosis in hepatocytes, nuclear fragmentation along with nuclear degeneration. Hemorrhage in central vein and pycnotic nucleus is observed in liver of rabbit exposed to sodium fluoride for 15 days to 16 weeks. Several studies are consonance with our results[22]. Previous reports determined hyalinized hepatic tubules with loss of cells and the vocalized cytoplasm and zonal necrosis in the liver cells of sodium fluoride treated rabbits[12]. In addition, Shashi and Thapar[23], have reported albino rabbits exposed to sodium fluoride show hepatocellular necrosis, hepatic hyperplasia, extensive vacuolization in hepatocytes, dilation of central vein and sinusoids in liver. Fluoride induces hepatotoxicity in rabbit evidenced by oxidative stress[24]. Besides, Trivedi[25] also reported that cellular necrosis and degeneration in liver due to significant increase in serum glutamate oxalate transaminase (SGOT) and serum glutamate pyruvate transaminase (SGPT) levels in rabbit after oral administration of sodium fluoride for 30 days. It is believed that increased levels of SGOT and SGPT caused to liver damage[24].

There are many conflicting reports regarding fluoride-induced toxicity in liver. According to the literature it was shown remarkable changes in the liver from 15 days to 16 weeks of fluoride exposed rabbits which include degenerative change in the liver cells as above. Many reports are similar to our results[26,27]. In rabbits, exposure to high concentration of sodium fluoride for 16 weeks caused necrotic and degenerative changes in liver[6]. In contrast, Bosworth and McCay[28] recorded no histopathalogical effect in liver of rabbits administered of 10 ppm sodium fluoride through drinking water. The blood with extreme levels of fluoride caused to selective damage in the tubular structures of the liver by passage of the internal cells of liver[29]. There are reports on

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hypertrophy and hyperplasia in the renal tubules of 1, 5 and 100 ppm fluoride administered rabbits for 500 days and shrunken liver structure, atrophy of glomeruli, degeneration of tubular cells and dilation of convoluted tubules has observed in treated rabbit[30,31]. In addition, fluoride exposure induce oxidative stress in liver and leads to apoptosis in renal tubules and damage the architectural structure of kidney[16,32-34].

Table 1: Histopathological changes of liver of all the 3 groups after treatment of Sodium Fluoride.

Sl. No.	Duration of NaF exposure	Control rabbits	Group B rabbits	Group C Rabbits
1	After 8 weeks	No changes	No changes	Ballooning degeneration of hepatic cells with congested and dilated sinusoids
2	After 10 weeks	No changes	No changes	Proliferative changes in sinusoids
3	After 14 weeks	No changes	No changes	Centrilobular Necrosis
4	After 16 weeks	No changes	No changes	Honeycombed liver with focal infiltration of inflammatory cells around dilated sinusoids

Note: NaF; Sodium Fluoride

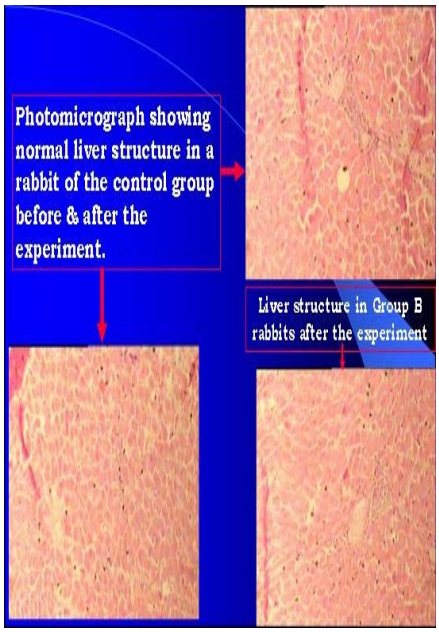

Figure 1: Normal architecture of liver.

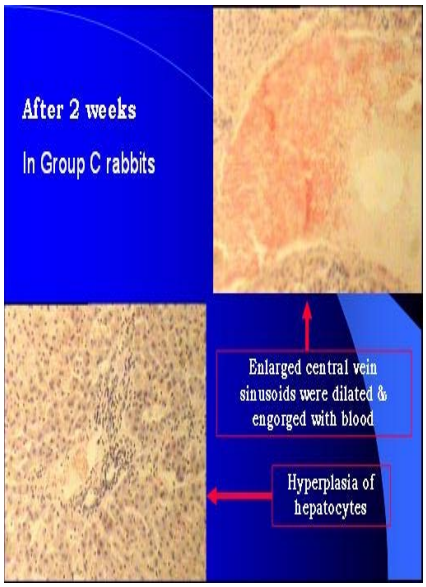

Figure 2: After 2 weeks shows hyperplasia of hepatocytes and enlarged central veins.

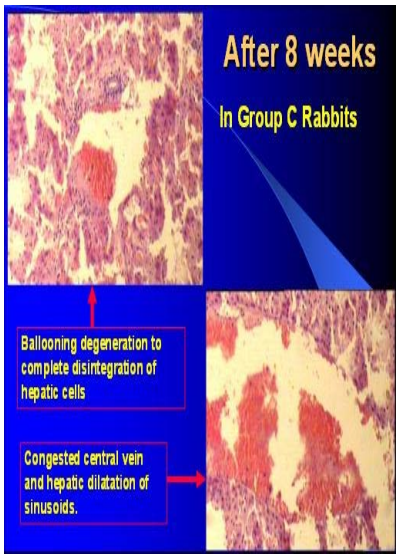

Figure 3: After 8 weeks shows ballooning degeneration of hepatocytes and congested central vein.

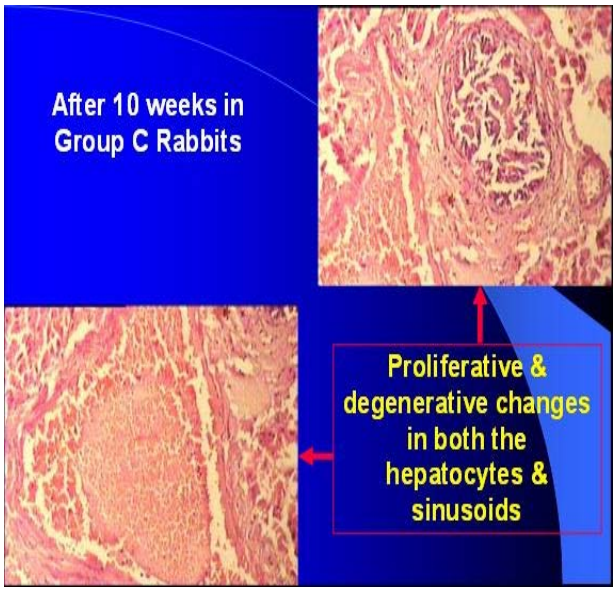

Figure 4: After 10 weeks shows proliferative and degenerative changes in both the hepatocytes and sinusoids.

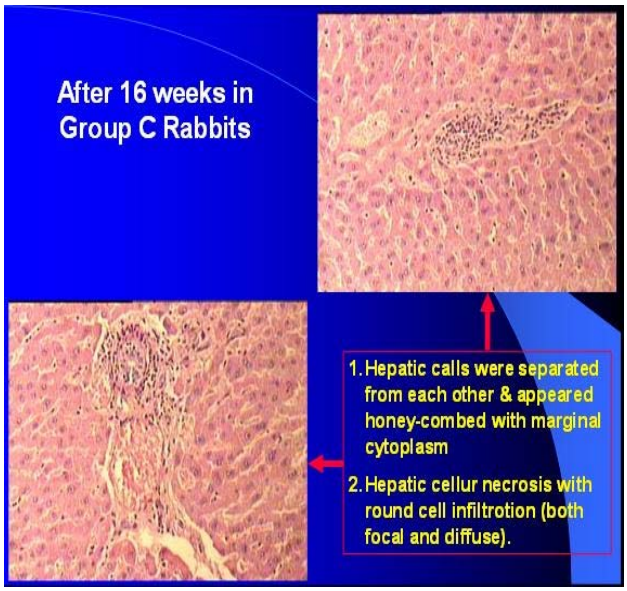

Figure 5: After 16 weeks shows honey combed appearance of hepatic cells and round cell infiltration.

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CONCLUSION

From the results, it is clearly indicated that 16 weeks of sodium fluoride exposure to rabbits exhibits more hepatotoxicity when compared to rabbit exposed to sodium fluoride for low doses. These hepatotoxicity in rabbit exposed to sodium fluoride for 15 and 16 weeks might be due to oxidative stress. Histopathalogical changes in the liver interrupt the normal hepatic architecture.

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