Acta Chimica & Pharmaceutica Indica

Acta Chim. Pharm. Indica: 4(1), 2014, 58-62
ISSN 2277-288X

SODIUM FLUORIDE INDUCED HISTOPATHALOGICAL CHANGES IN LIVER AND KIDNEY OF ALBINO MICE

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(*Received : 25.12.2013; Revised : 02.01.2014; Accepted : 03.01.2014*)

ABSTRACT

Fluoride is a well determined non-biodegradable and moderate pollutant, which at high levels causes serious health problems. The present study was designed to investigate the histopathalogical changes in liver and kidney of exposure to sodium fluoride in albino mice. Eighteen adult albino mice were divided into 3 groups (6 mice per each group). The first group is served as controls and received de-ionized water. The second group is treated with 5 ppm of sodium fluoride for 15 days and third group is treated with 5 ppm of sodium fluoride for 30 days. At the end of the treatment period, the animals are sacrificed by cervical dislocation and liver, kidney was dissected out. The adhesive tissue is cleared and used for assess the histopathalogical changes. The histopathalogical results in the present study indicate that exposure to sodium fluoride for 15 and 30 days caused to cytoplasmic, hepatocytes, vacuoles cellular and nucleus in liver. Moreover, exposure to sodium fluoride for 15 and 30 days results in necrosis in glomerules, Convolated tubules and bowman's capsule lumen in kidney.

Key words: Albino mice, Sodium fluoride, Histopathalogical changes, Liver, Kidney.

INTRODUCTION

Fluoride is ubiquitously present in earth and essential trace element for human being and animals[1]. Fluoride is found in small quantities in almost all foods and enters into the human body mainly through the oral route along with food and water[2]. Shulman and Wells[3] have demonstrated that fluoride contamination occur through releasing of fluoride dust and fumes from industries using hydrofluoric acid and fluoride salts. All the age groups in several countries are 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, kidney, 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 decreased aerobic metabolism and altered the free radical metabolism in liver and kidney[8]. In addition, ingestion of fluoride is inhibiting the Kreb's cycle and leads to toxicity in liver and kidney[9-11]. However, the effect of fluoride on liver and kidney is far from clear.

Earlier studies have been shown that fluoride is caused to degenerative and inflammatory changes, dilatations of sinusoids, hepatic hyperplasia and accumulation of amorphous and crystalline bodies in the

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hepatocytes in liver[12,13]. Besides, Hodge and Smith[14] is described that kidney is well recognized for its histopathalogical and functional responses to excessive amounts of fluoride. Many studies have shown that high levels of fluoride could be accumulate in the kidney and this organ is the major route for removal of fluoride from the body[15,16]. Few studies are available on fluoride toxicity on kidney that show fluoride induces kidney lesions through apoptosis[17]. However, we made an attempt to study the toxicity in liver and kidney induced by exposure to sodium fluoride due to histopathology.

EXPERIMENTAL

Material and methods

Animals

Healthy adult male albino mice (60 ± 2) days and weight (30 ± 5 g) were maintained at laboratory conditions (26 ± 2^{o}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 rat feed and adlibitum tap water. This study was carried out according to guidelines for the care and use of laboratory animals (National Research Council. 1996) and approved by the Institutional Animal Ethical Committee at Sri Venkateswara University, Tirupati, India (Resolution No. 04a/(i)/a/CPCSCA/IAEC/SVU/KJR-SKB/Dt.18.8.2010).

Chemical and dosing

Sodium fluoride (99%) was used is a toxicant supplied by BDH Chemical Division, Bombay. Normal male mice were divided into three groups, each group contains six animals. The first group animals served as control and received de-ionized water. Second group animals treated with 5 ppm sodium fluoride (5 mg/L) through oral route for 15 days. The third group animals treated with 5 ppm sodium fluoride (5 mg/L) through oral for 30 days.

Necropsy

After completion of treatment period, the body weights of male mice were recorded and necropsied by cervical dislocation. Liver and kidney were 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]. Tissues like liver and kidney were isolated from the control and experimental mice. 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 for 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 μ thick nesses 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.

RESULTS AND DISCUSSION

It is believed that histology helps to determine the pathological lesion in tissue caused by the toxicant. The transverse section of liver in control mouse showed 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 capillaries sinusoids running in between the parenchyma cells observed in the liver (Fig. 1.1.A). The transverse section of liver in mouse exposed to sodium fluoride

for 15 days was shown remarkable changes when compared to control, such as cellular disarray, congestion, cellular degeneration, and cellular vacuoles (Fig. 1.2.B). The Fig. 1.1.C 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 mouse exposed to sodium fluoride for 30 days.

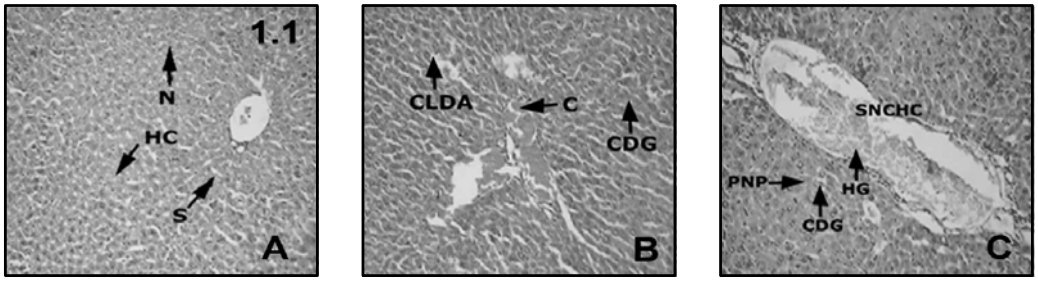

Fig. 1.1

Kidneys are made up of more basic units of nephrons. Each nephron consists of two major parts the glomerulus and tubules. The transverse section of kidney in control mouse showed normal proximal and convoluted tubes with polygonal in shape and distinct nuclei in the center (Fig. 1.2.A). Under experimental condition the transverse section of kidney of 15 days fluoride exposed mouse has shown necrosis in glomerulus, degenerative changes in bowman's capsule and alterations in glomerulus's tubular region (Fig. 1.2.B). Moreover, the transverse section of kidney in mouse treated with fluoride for 30 days has been exhibited severe necrosis in glomeruli and bowman's capsule, large vacuoles and increase in lumen of distal convoluted tubules (Fig. 1.2.C).

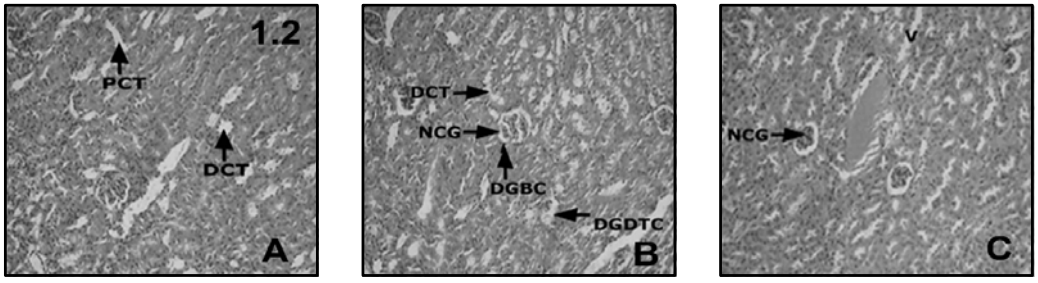

Fig. 1.2

Severe fluorosis in humans is occurred due to consuming fluoride through drinking water. Therefore, in the present study sodium fluoride was administered in mice through the same route.

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 been revealed, that 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 mice exposed to sodium fluoride for 15 and 30 days. Several studies are consonance with our results[22]. Previous reports are determined that hyalinized hepatic tubules with loss of cells and the vocalized cytoplasm and zonal necrosis in the liver of sodium fluoride treated rats[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 mice evidenced by oxidative stress[24]. Besides, Trivedi et al.[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 mice 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 kidney. The present study has been shown remarkable changes in the kidney of 15 and 30 days fluoride exposed mice which include degenerative change in proximal convoluted tubules and distal convoluted tubules, congestion necrosis in glomeruli and bowman's capsule. Many reports are similar to our results[26,27]. In rabbits, exposure to high concentration of sodium fluoride for 15 weeks caused to necrotic and degenerative changes in kidney[5,6]. In contrast, Bosworth and McCay[28] recorded no histopathalogical effect in kidney of rats 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 kidney by passage of the glomerular filtrate and consequent lesions produced in the kidney[29]. There are reports on hypertrophy and hyperplasia in the renal tubules of 1, 5 and 100 ppm fluoride administered rats for 500 days and shrunken kidney structure, atrophy of glomeruli, degeneration of tubular cells and dilation of convoluted tubules has observed in treated mice[30,31]. In addition, fluoride exposure induce oxidative stress in kidney and leads to apoptosis in renal tubules and damage the architectural structure of kidney[16,32-34]

CONCLUSION

From the results, it is clearly indicated that 30 days of sodium fluoride exposed mice exhibits more hepatotoxicity and renal toxicity when compared to mice exposed to sodium fluoride for 15 days. These hepatotoxicity and nephrotoxicity in mice exposed to sodium fluoride for 15 and 30 days might be due to oxidative stress. Histopathalogical changes in the liver and kidney interrupt the normal hepatic and renal function. For overcome to hepatotoxicity and nephrotoxicity by avoiding the intake of fluoride rich food and simultaneous ingestion of adequate protein, vitamin C and calcium diet.

ACKNOWLEDGEMENT

The authors are grateful to the Head, Department of Zoology, S. V. University, Tirupati for providing facilities. One of the authors (KJR) is thankful to Prof. K. V. S. Sarma for the statistical analysis of data and Mr. Anjeneyula Raju for maintaining rat colony. The authors declare that the experiments conducted during these studies comply with the current laws of their country.

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