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RESEARCH ARTICLE

A STUDY OF FLUORIDE INDUCED TOXIC EFFECTS ON THE MICROANATOMY OF LIVER IN ALBINO RATS

*Bashir Ahmad Shah, Gousia Nisa, Fowzia Farzana, Gh. Mohd. Bhat, Mohd. Saleem Itoo and Showkat Ahmad

Department of Anatomy Government Medical College, Srinagar, India

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ABSTRACT

The present study was conducted to study the effect of graded doses of Sodium fluoride on the microanatomy of Liver in Albino Rats over different periods of time. Sixty male adult albino rats taken from the animal house of Govt. medical College Srinagar were divided randomly into four groups of 15 animals each. Animals of the first three groups were given fluorinated water in various concentrations to drink and fourth group served as the control group getting plain tap water to drink. Animals from each group were sacrificed and examined after 30, 60, and 90 days of therapy and gross and microscopic changes recorded. It was observed that fluorides induce dose and duration dependent microscopic changes in Liver tissue ranging from mild edema to gross necrosis.Fluoride ions are known to affect the bones and teeth of human beings for a pretty long time now. On the one hand presence of fluoride ions in drinking water is essential for the normal development of the various organ systems of the body, particularly skeletal system and teeth. But presence of Fluoride ions in food and drinking water has increased worldwide resulting in toxic effects on various tissues particularly the skeletal system and teeth. However it is now a well known fact that the toxic effects of a substance cannot be limited to a particular system only and the present study was focused on dose and duration related effects of fluorides on Liver.

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INTRODUCTION

Fluoride is a naturally occurring element, abundant in the Earth's crust. It is not essential for growth and development of humans or other organisms. Most fluorine occurs as insoluble fluorides, but there is some ionised fluoride in soil and groundwater .People's exposure to fluoride varies a lot, depending on levels in water, what toothpaste they use, and other factors like use of mineral water and some kinds of tea. The existing collection of risk assessments suggests that there is quite a narrow gap between the fluoride intake recommended to safeguard teeth and the maximum recommended exposure. Water fluoridation has been described by the Centre for Disease Control as one of the ten most important public health advances of the 20th Century (Baselt, 2008). Consumption of large amounts of fluoride can lead to fluoride poisoning and death; the lethal dose for most adult humans is estimated at 5 to 10 g (which is equivalent to 32 to 64 mg/kg elemental fluoride/kg body weight.

***Corresponding author: Dr. Bashir Ahmad Shah,**
Associate Professor, Department of Anatomy Government Medical College, Srinagar, India.

Ingestion of fluoride can produce gastrointestinal discomfort at doses at least 15 to 20 times lower (0.2–0.3 mg/kg) than lethal doses. Chronic intake and topical exposure may cause dental fluorosis, and excess systematic exposure can lead to systemic effects such as skeletal fluorosis. Young children are at risk for receiving excess fluoride, and the ADA has recently issued an interim guidance on their fluoride consumption. In 1974 a 3-year old child swallowed 45 milliliters of 2% fluoride solution, estimated to be triple the fatal amount, and then died. The fluoride was administered during his first visit to the dentist, and the dental office was later found liable for the death. Fluorine exists abundantly in living tissue as an ion and is absorbed easily into the enamel of teeth especially in children's growing teeth. Halfway through the twentieth century, fluoride piqued the interest of toxicologists due to its deleterious effects at high concentrations in human populations suffering from Fluorosis and in in vivo experimental models. Until the 1990s, the toxicity of fluoride was largely ignored due to its "good reputation" for preventing caries via topical application and in dental toothpastes. However, in the last decade, interest in its undesirable effects has resurfaced due to the awareness that this element interacts with cellular systems even at low doses.

In recent years, several investigations demonstrated that fluoride can induce oxidative stress and modulate intracellular redox homeostasis, lipid peroxidation and protein carbonyl content, as well as alter gene expression and cause apoptosis. Genes modulated by fluoride include those related to the stress response, metabolic enzymes, the cell cycle, cell–cell communications and signal transduction. The chief sources of fluorides to human beings in addition to drinking water are vegetables, edible marine oils, animals, drugs and certain other varieties like tea, coffee, tobacco, detergents, cleansing materials, pollen grains etc. Topical fluorides are found in products containing strong concentrations of fluorides to fight tooth decay. Fluoridated water is believed to protect against dental cavities and root caries. Hence fluoridation of water is considered to be the most efficient and cost effective dental caries prevention measure available. Fluoride-containing compounds, such as sodium fluoride or sodium monofluorophosphate are used in topical and systemic fluoride therapy for preventing tooth decay. They are used for water fluoridation and in many products associated with oral hygiene. Originally, sodium fluoride was used to fluoridate water; hexafluorosilicic acid (H_2SiF_6) and its salt sodium hexafluorosilicate (Na_2SiF_6) are more commonly used additives, especially in the United States. The fluoridation of water is known to prevent tooth decay and is considered by the U.S. Centers for Disease Control and Prevention as "one of 10 great public health achievements of the 20th century". In some countries where large, centralized water systems are uncommon,fluoride is delivered to the populace by fluoridating table salt.

It has been recognized for over five decades that fluoride may have both beneficial and potentially harmful effects on dental health. While the prevalence of dental caries is inversely related to a range of concentrations of fluoride in drinking-water consumed, the prevalence of dental fluorosis has been shown to be positively related to fluoride intakefrom many sources (Fejerskov *et al.*, 1981). Public health programmes seeking to maximize the beneficial effects of fluoride on dental health through the introduction of fluoridated drinking-water have, at the same time, strived to minimize its adverse fluorotic effects on teeth. Based upon the studies conducted by Dean and colleagues five decades ago, the "optimum" level of fluoride in drinking-water, associated with the maximum level of dental caries protection and minimum level of dental fluorosis, was considered to be approximately 1 mg/litre. The effects of fluoride on dental health were examined by a WHO Expert Committee (WHO, 1994). Daily intakes of fluoride can vary significantly according to the various sources of exposure. Values ranging from 0.46 to 3.6–5.4 mg/day have been reported in several studies (IPCS, 1984). In areas where water is fluoridated this can be expected to be a significant source of fluoride, however fluoride is also naturally present in huge range of foods, in a wide range of concentrations. The maximum safe daily consumption of fluoride is 10 mg for an adult. Fluorides have also found to be one of the essential constituents of certain tranquilizers, diuretics, and anticancer drugs. Fluoridated corticosteroids are widely used for various dermatological conditions. Anaesthetics like Fluroxane contain fluorides and are widely used. Fluorides are either used or produced in various industries producing fertilizers, insecticides etc. Fluorides have been used for treating diseases like Pagets disease of bone, Osteogenesis imperfecta and Ostosclerosis. Radioactive Fluoride 18F has been used for bone imaging. Fluorides are absorbed through GIT, Lungs and Skin. About 75-90% of ingested fluoride is absorbed. Fluoride is distributed to whole of the body through blood stream. Twentieth century was considered to be the age of industrialization but unfortunately rapid industrial growth has resulted in complex range of health problems due to environmental pollution and one of the most important health hazards of environmental pollution is “Fluorosis”. The main clinical signs of Fluorosis are manifest in the skeletal system but there are direct and indirect toxic effects on other systems of the body including Nervous, Urinary, Respiratory, Reproductive, and Digestive System including Pancreas. Many fluoride minerals are known, but of paramount commercial importance is fluorite (CaF_2), which is roughly 49% fluoride by mass.16The soft, colorful mineral is found worldwide. Seawater fluoride levels are usually in the range of 0.86 to 1.4 mg/L, and average 1.1 mg/L. For comparison, chloride concentration in seawater is about 19 g/L. The low concentration of fluoride reflects the insolubility of the alkaline earth fluorides, e.g., CaF_2. Fluoride is present naturally in low concentration in drinking water and foods. Fresh water supplies generally contain between 0.01–0.3 ppm. In some locations, the fresh water contains dangerously high levels of fluoride, leading to serious health problems. All tea leaves contain fluoride; however, mature leaves contain as much as 10 to 20 times the fluoride levels of young leaves from the same plant.

Aims of the study

Fluoride levels vary in the drinking water throughout the world and fluoridation of drinking water to prevent tooth decay is done in many parts of the world. Besides this fluorides are ingested in various other forms like food products and medicines. Growing industrialization has enormously increased fluoride pollutants in the environment. The present study was therefore aimed at studying dose related microscopic changes in the Liver of animals fed with graded doses of Sodium Fluoride in drinking water.

MATERIALS AND METHODS

A total of 60 male adult albino rats were randomly selected and divided into four groups of 15 animals each. Group A: The animals of this group were given drinking water with 10 ppm concentration of fluoride besides standard diet. Group B: The animals of this group were given drinking water with 500 ppm concentration of fluorides besides standard diet. Group C: The animals of this group were given drinking water with 1000 ppm concentration of fluoride besides standard diet. Group D: The animals of this group were given plain tap water to drink besides standard diet. This served as the control group. Fluorinated water was prepared by dissolving sodium fluoride in tap water. Addition of one mg of sodium fluoride to one liter of water makes a concentration of one part per million (ppm). The animals were observed daily for changes in appearance and body weight. Animals of different groups were studied after 30, 60, and 90 days of therapy when 15 animals from each of the four groups were sacrificed and examined. At the time of each examination the animals were weighed and anaesthetized by chloroform. A midline incision was given and Liver was dissected out and put on a dish containing chloroform. Macroscopic changes if any were observed and compared with the control group. The liver was subjected to processing in an automatic tissue processor, sections 0.5 to 0.7 micrometer thick were cut with a rotatory microtome, stained with Haematoxylin & Eosin stain and observed under compound light microscope.

Observations

On gross examination the pancreas of both control and experimental group of animals was normal in appearance. However in the animals of group B and C after 60 and 90 days of therapy the color of the liver was slightly lighter and discoloration patches were visible.

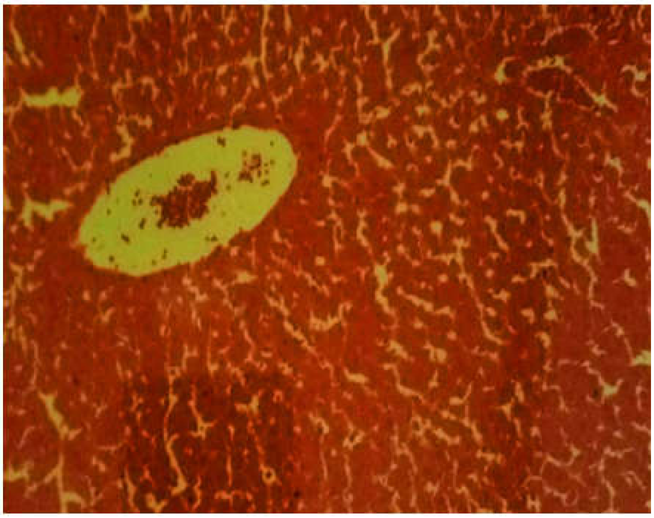

Fig. 1. Microphotograph of Liver of Group A after 60 days of therapy showing edema of hepatic parenchyma, and occasional haemorrhages and lymphocytic infiltration. (200x)

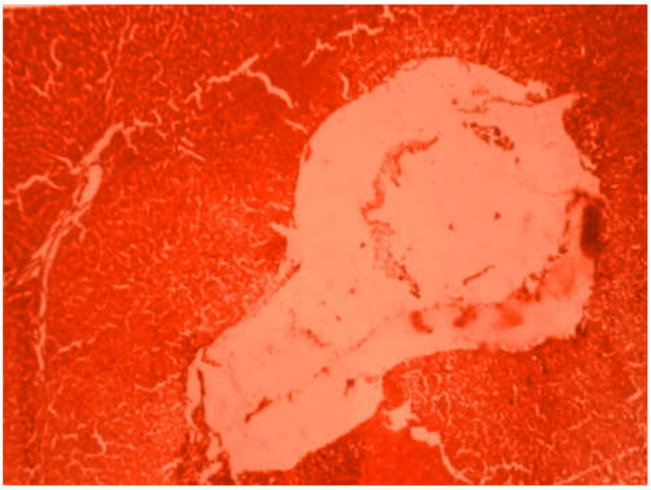

Fig. 2. Microphotograph of Liver of Group B after 90 days of therapy showing diffuse edema, lymphocytic infiltration, central vein dilatation and cellular distortion. (100x)

Microscopic changes: The liver of group A showed minimal microscopic changes after 30 days of treatment. The cellular architecture of the liver of this group was maintained even after 60 and 90 days of therapy but mild edema and occasional hemorrhages were seen (Fig No 01). The liver of group B after 30 days of treatment showed mild edema, and occasional hemorrhages. However after 60, and 90 days of therapy, in this group of animals, sinusoidal hemorrhages, central vein dilatation, and occasional necrosis was seen (Fig No 02).

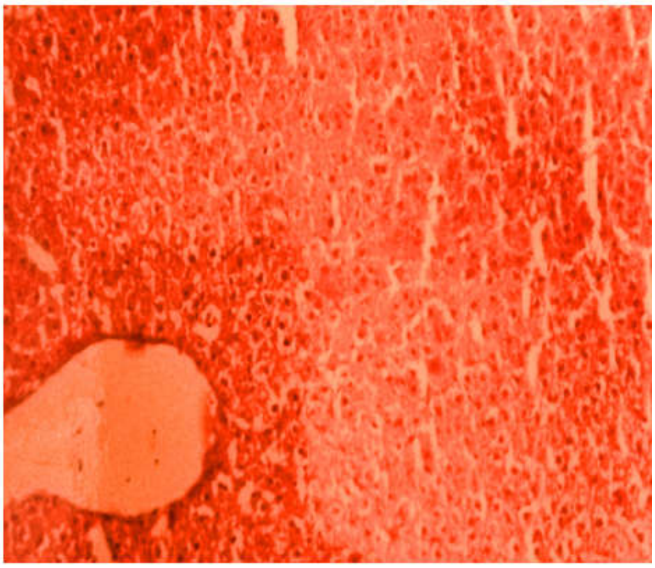

Fig. 3. Microphotograph of Liver of Group C after 60 days of therapy showing distortion of islets and necrosis of the pancreatic tissue. (200x)

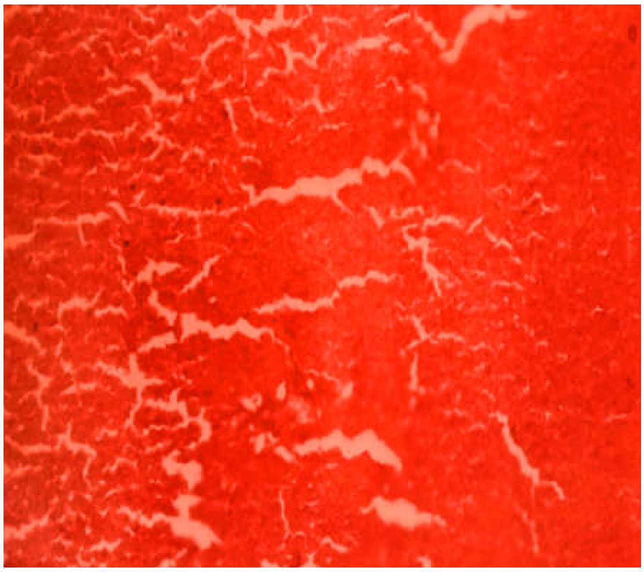

Fig. 4. Microphotograph of Liver of Group A after 90 days of therapy showing inflammatory changes, lymphocytic infiltration, necrosis of cells and central vein dilation and some fibrotic changes. (100X)

The animals of group C were the worst affected. After 30 days of treatment the liver of this group showed edema, frequent hemorrhages and occasional necrosis. After 60 days of therapy the Liver of this group showed distortion of the liver architecture with frequent hemorrhages and necrosis (Fig No. 03). The liver of the animals of this group after 90 days of treatment was the worst affected and hardly any normal hepatic tissue was visible. The histological architecture of the liver was distorted and frequent areas of hemorrhages, fatty infiltration, central vein dilatation and necrotic tissue were found. Fibrosis was also seen in some cases (Fig No. 04).

DISCUSSION

The present study was aimed at evaluating the effects of varying strengths of Sodium Fluoride on the liver of Albino Rats. A notable reduction in the body weight of experimental animals was seen after Fluoride administration which was more obvious in the animals of higher dose group. It is obvious that fluoride toxicity causes metabolic and structural changes which in turn cause wasting of muscle mass and loss of body weight. With low concentrations of fluorides the histological architecture of liver tissue was maintained but there were occasional hemorrhages and edema of the liver cells. With increased concentration and duration of exposure to fluorides frequent hemorrhages, edema, distortion of cell outline, focal areas of parenchymal necrosis and hemorrhages, diffuse areas of hepatic cell necrosis and finally fatty infiltration and fibrosis was observed. Taylor *et al*. (1961) and Simon *et al*. (1968) have also reported weight loss in the animals fed on fluorides. Weight loss in fluorotic humans was reported by Short *et al* (1937) and Sidiqui (1955). Koul *et al.* (1976) also reported weight loss in fluorotic humans and rabbits and concluded that it was due to altered biochemical reactions.

The present study was aimed at studying the various changes, if any, which take place in various components of the liver, an organ of vital importance with varying strengths of sodium fluoride in drinking water. It was observed that extensive degenerative changes occur in liver tissue and cellular architecture is disrupted at higher concentrations of 500 to 1000 ppm. Cells lining the hepatic cords initially showed edema and latter were degenerated. In the present study liver changes ranging from focal areas of necrosis, increased vascularity, central vein dilatation and fatty change were seen. Necrosis, Fatty infiltration and Fibrosis were seen in animals with longer duration of exposure and higher concentration of fluorides in drinking water (Group B and C after 60 and 90 days of therapy). A similar picture of liver of experimental rabbits fed with fluorides was earlier reported by Muchelberger in 1930 and Philips in 1934.From the present study it can be concluded that fluorides do not spare liver tissue in exerting their toxic effects and various components of liver are adversely affected by fluorides which suggests that damage to the liver tissue will result in indigestion of food and disturbed metabolism in the experimental animals, causing loss of weight besides other effects. Effects of fluorides on liver have not received much attention in the previous studies. It was Lidbeck *et al* in 1943, who studied the effect of fluorides on liver and observed that Fluorosis was associated with acute congestion of abdominal organs including liver. The Feb 10 1951 issue of JAMA reported liver damage in areas of endemic fluorosis. Wallace Durban 1954 reported that liver an organ with high vascularity took more fluorides than less vascular tissues. Geal *et al* 1964 reported that fluoride taken orally causes hepatitis. It was Kulbir Kour *et al* in 1978, who conducted a similar study and reported similar results, which included degenerative changes ranging from focal areas of liver cell necrosis to complete fatty degeneration of liver in Albino Rats fed fluorides in drinking water. Our present study observed that hepatic architecture was maintained at lower doses of fluorides but with increasing doses and longer exposure changes in liver varied from dilated and congested central vein, dilated and congested sinusoids, increased vascularity, and focal areas of liver cell necrosis, diffuse areas of necrosis, fatty infiltration and occasional fibrosis. Plummer J L 1985 reported that methoxyfluorane caused liver cell degeneration and necrosis. Fatty infiltration was also seen. Battelle 1989 reported that fluorides lead to the formation of an extremely rare form of liver cancer known as hepatocholangiocarcinoma. M Humcizska *et al* 1994 observed that fluorides caused the liver cells to accumulate excess glycogen. He also reported extension of blood vessels and occasional fibrosis. Soni *et al* studied the influence of sodium fluoride (NaF) intoxication at 5 and 20 mg/kg body mass on some tissues of the rat. The lower dose was accompanied by increased peroxidation of lipids in all examined tissues, i.e., liver, kidneys, lungs, intestine, and testes.

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