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RESEARCH ARTICLE

EFFECT OF FLUORIDE IONS ON THE MICROANATOMY OF PANCREAS IN ALBINO RATS

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ABSTRACT

The present study was conducted to study the effect of graded doses of Sodium fluoride on the microanatomy of Pancreas 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 pancreatic 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 Pancreas.

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INTRODUCTION

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 (Rao, et al.,). 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 (Fluoride in diet). Topical fluorides are found in products containing strong concentrations of fluorides to fight tooth decay (Winston and Bhaskar). 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 systemicfluoride therapy for preventing tooth decay. They are used for water fluoridation and in many products associated with oral hygiene. (Winston and Bhaskar) Originally, sodium fluoride was used to fluoridate water; hexafluorosilicic acid (H2SiF6) and its salt sodium hexafluorosilicate (Na2SiF6) are more commonly used additives, especially in the United States. The fluoridation of water is known to prevent tooth decay (McDonagh *et al*., 2000; Griffin *et al*., 2007) 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". (Community Water Fluoridation, 2016; Ten

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Great Public Health Achievements in the 20th Century, 2016) 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)[24]. 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 (Dietary Reference Intake Tables, 2016). The maximum safe daily consumption of fluoride is 10 mg for an adult.

Many fluoride minerals are known, but of paramount commercial importance is fluorite (CaF2), which is roughly 49% fluoride by mass.16 The 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. (Ambient Water Quality Criteria for Fluoride) For comparison, chloride concentration in seawater is about 19 g/L. The low concentration of fluoride reflects the insolubility of the earth fluorides, e.g., CaF2. Fluoride is present naturally in low concentration in drinking water and foods. Fresh water supplies generally contain between 0.01–0.3 ppm. (Fluoride in Drinking-water, 2016; Liteplo *et al*., 2002) 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. (Liteplo *et al*., 2002; Wong *et al*., 2003) 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 Pancreas of animals fed with graded doses of Sodium Fluoride in drinking water.

Examples of fluoride content

Food/Drink	Fluoride (mg per 100g)	Portion	Fluoride (mg per portion)
Black Tea (brewed)	0.373	1 cup, 240g (8 fl oz)	0.884
Raisins, seedless	0.234	small box, 43g (1.5 oz)	0.033
Table wine	0.153	Bottle, 750ml (26.4 fl oz)	1.150
Municipal tap-water, (Fluoridated)	0.081	Recommended daily intake, 3 litres (0.79 US gal)	2.433
Baked potatoes, Russet	0.045	Medium potato, 140g (0.3 lb)	0.078
Lamb	0.032	Chop, 170g (6 oz)	0.054
Carrots	0.003	1 large carrot, 72g (2.5 oz)	0.002

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 (Murray and Ste-Marie, 1996; Haguenauer *et al*., 2000). 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 (Zajusz, 1996). 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" (Short, 1937). 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.

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.

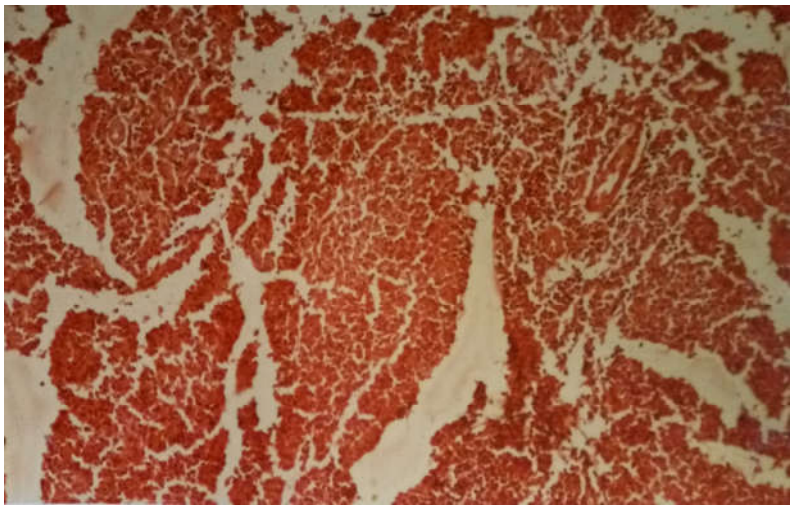

Fig. 1. Microphotograph of Pancreas of Group A after 60 days of therapy showing edema of acinar parenchyma, and occasional haemorrhages and lymphocytic infiltration. (100x)

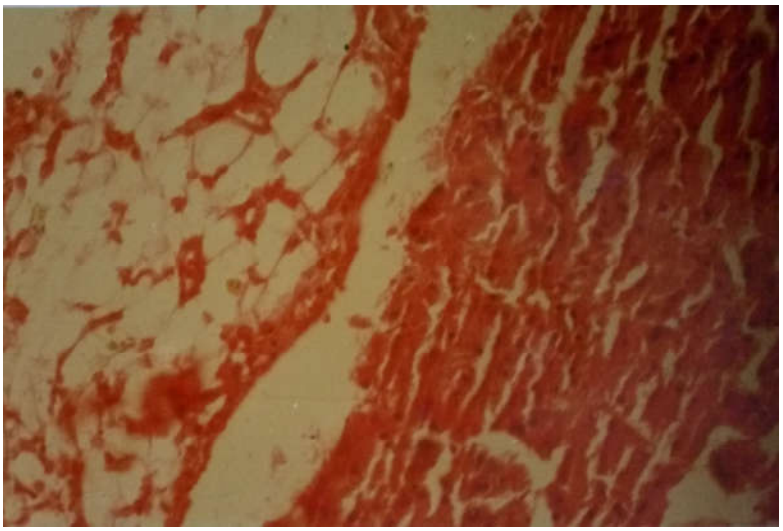

Fig. 2. Microphotograph of Pancreas of Group B after 90 days of therapy showing diffuse fatty infiltration, thickened blood vessels and islet distortion. (200x)

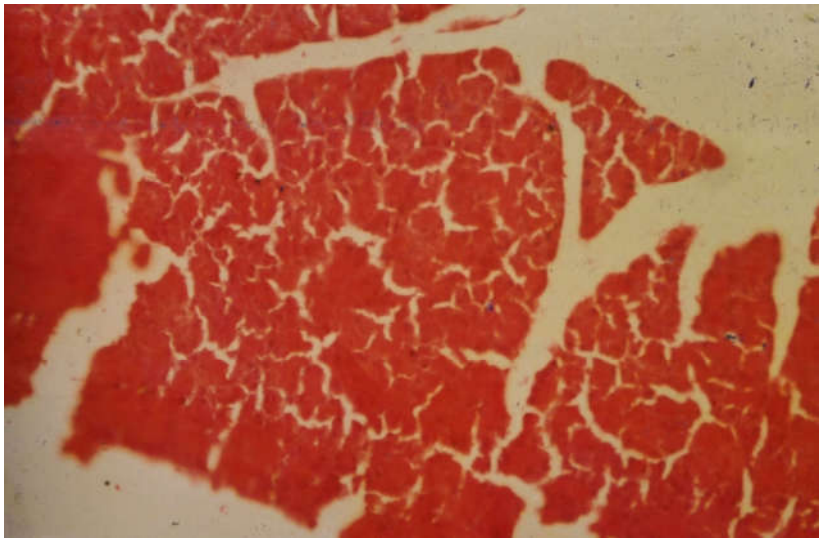

Fig. 3. Microphotograph of Pancreas of Group C after 60 days of therapy showing distortion of islets and necrosis of the pancreatic tissue. (100x)

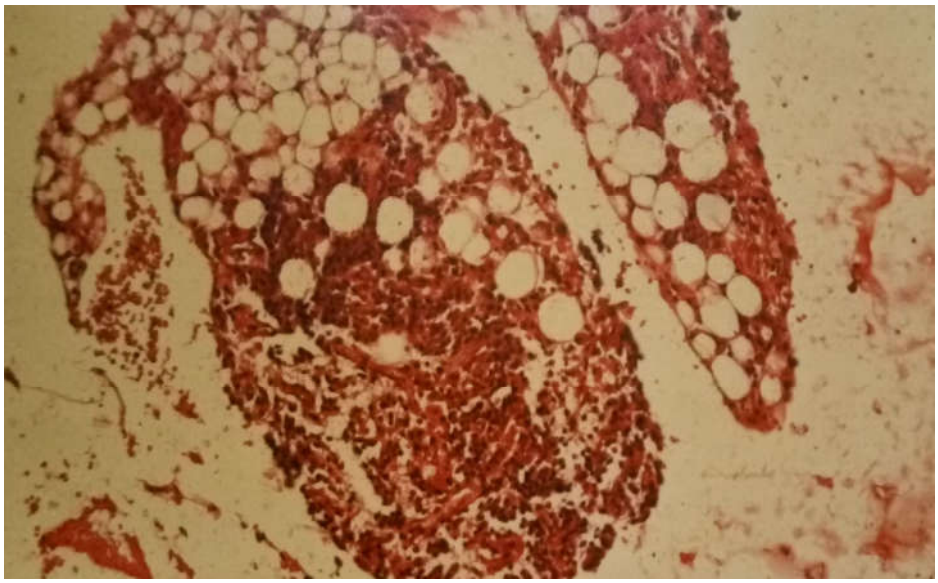

Fig. 4. Microphotograph of Pancreas of Group A after 60 days of therapy showing inflammatory changes, fatty infiltration and fibrotic changes. (100x)

A midline incision was given and pancreas was dissected out and put on a dish containing chloroform. Macroscopic changes if any were observed and compared with the control group. The pancreas was subjected to processing in an automatic tissue processor, sections 0.5 to 0.7micrometer 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 pancreas was slightly lighter and discoloration patches were visible.

Microscopic changes

The pancreas of group A showed minimal microscopic changes after 30 days of treatment. The cellular architecture of the pancreas of this group was maintained even after 60 and 90 days of therapy but mild edema and occasional hemorrhages were seen (Fig. 01). The pancreas of group B after 30 days of treatment showed edema, frequent hemorrhages. However after 60 and 90 days of therapy occasional necrosis was also seen (Fig. 02). The animals of group C were the worst affected. After 30 days of treatment the pancreas of this group showed edema, frequent hemorrhages and occasional necrosis. After 60 days of therapy the Pancreas of this group showed distortion of the pancreatic architecture with frequent hemorrhages and necrosis (Fig. 03). The pancreas of the animals of this group after 90 days of treatment was the worst affected and hardly any normal pancreatic tissue was visible. The histological architecture of the pancreas was distorted and frequent areas of hemorrhages, fatty infiltration and necrotic tissue were found. Fibrosis was also seen in some cases (Fig. 04).

DISCUSSION

The present study was aimed at evaluating the effects of varying strengths of Sodium Fluoride on the pancreas 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 pancreatic parenchyma was maintained but there were occasional haemorrhages and edema of the pancreatic acinar cells. With increased concentration and duration of exposure to fluorides frequent hemorrhages, edema of acini, distortion of cell outline, small areas of parenchymal necrosis and hemorrhages, diffuse areas of parenchymal 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). The present study was aimed at studying the various changes, if any, which take place in various components of the pancreas with varying strengths of sodium fluoride in drinking water. It was observed that the serous acini were disrupted at higher concentrations of 500 to 1000 ppm. Cells lining the acini initially showed edema and latter were degenerated. The islets progressively increased in size with higher concentrations of fluorides but showed signs of degeneration after longer exposure and their outlines were not visible at all. In the present study pancreas showed increased vascularity, thickening of septa and also fatty change. Necrosis, Fatty infiltration and Fibrosis was seen in animals with longer duration of exposure and higher concentration of fluorides in drinking water. It can be concluded that both endocrine and exocrine components of pancreas are adversely affected by fluorides which suggests that damage to the pancreatic tissue will result in indigestion of food and

disturbed glucose metabolism in the experimental animals, causing loss of weight besides other effects.

Effects of fluorides on pancreas have not received much attention in the previous studies. It was Oglivie (1953) who studied the effect of fluorides on pancreas and observed that Fluorosis was associated with greater mitotic division of the alveolar cells and proliferation of connective tissue in interlobular and intralobular septa. Lin *et al*. (1976) reported that fluoride inhibited the oxidation of glucose by islets of Langerhans in the rat pancreas. However it was Mehmood (2002) who conducted a similar study to observe the effects of fluorides on the pancreas in guinea pigs. He reported swelling of cells with increased vascularity and increased connective tissue compactness. Changes were prominent in the higher dose groups which showed disturbance of the acinar pattern and an increase in the number and size of islets. The findings in our study were in conformity with the findings of Mehmood (2002), but it was observed that islet cells were also necrosed in the latter stages. In view of the prevalent clinical impression that acute hemorrhagic pancreatitis involves autodigestion of the pancreas by prematurely activated pancreatic proteolytic enzymes and of reports in the literature that phenylmethylsulfonyl fluoride (PMSF) and related compounds inactivate trypsin and chymotrypsin (from bovine tissues) without affecting acetylcholinesterase (from electriceel), PAOLA TURINI *et al*. (1969) studied the action of PMSF on human enzymes and its toxicity in vivo. PMSF rapidly inactivates purified chymotrypsin from human pancreas, although human trypsin is less susceptible to inhibition by PMSF. In contrast to the insensitivity of acetylcholinesterase from electric eels to PMSF, which was confirmed, the enzyme from human erythrocytes is rapidly inhibited by low concentrations of PMSF. This appears to be the first major difference detected between acetylcholinesterases from mammalian sources and from the electric eel. C_{14}-labeled PMSF penetrates human red cells rapidly and inhibits acetylcholinesterase in situ. The labeled inhibitor also penetrates freely through the blood-brain barrier. Fluoride is also known to be an inhibitor/activator of numerous enzymes. Although the relationship in both human and animal fluorosis between free radical generation, lipid peroxidation, and antioxidant defense systems has been investigated extensively, these various studies have produced conflicting results. Soni *et al*. (1984) 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. With the higher dose, peroxidation continued in the kidneys and intestine, but was inhibited in the liver, lungs, and testes. Interesting results were reported by Jain (1989)[14] from studies on the peroxidation of lipids in human erythrocyte membranes incubated in hyperosmotic solutions of glucose (experimental hyperglycemia). (IPCS 1984) When glucose was used alone, peroxidation was faster, but when erythrocytes were preincubated with fluoride ions, peroxidation was inhibited. The authors suggested a rise in reduced glutathione levels caused by fluoride (as phenylmethylsulphonyl fluoride — PMSF) with subsequent removal of hydrogen peroxide and oxygen free radicals by glutathione peroxidase, and, in effect, inhibition of peroxidation. In a report by Chlubek *et al*. (2003), the effect of increasing concentrations of NaF (2.5, 50, and 500 μM) on lipid peroxidation in the mitochondrial fraction from human placenta was described. Incubation with fluoride induced MDA formation, but higher concentrations of NaF were less potent in raising levels of MDA. The strongest effect (highest MDA levels) was observed with the lowest fluoride concentrations, normally found in plasma of humans unexposed to environmental contamination with fluorine compounds. These data support the view that fluoride at relatively low concentrations stimulates lipid peroxidation, but at high and very high concentrations may act as inhibitor of MDA generation. In a recent study, Chlubek *et al*. (2003) elicited hyperglycemia in rats exposed to 50 or 100 ppm fluoride in drinking water during four months and studied the effect on pancreatic antioxidative systems. Cytoplasmic Cu-Zn SOD ac-tivity was reduced by 50%, with little effect on mitochondrial Mn-dependent SOD. No change was observed in GSH-Px activity and MDA levels in pancreatic homogenates. Even stronger evidence has been presented by Reddy *et al*. (2003). They reported finding no changes in lipid peroxides, GSH, and vitamin C levels, as well as in SOD, GSH-Px, and CAT activities in red blood cells of fluorotic humans and fluoride-intoxicated rabbits. In contrast to the above reports, a number of studies on oxidative stress in fluorotic humans and fluoride-intoxicated animals indicate that generation of ROS and lipid peroxidation (MDA formation) can be directly induced by fluoride. Moreover, there is evidence that both ROS and lipid peroxides play an important role in fluorosis. Shivarajashankara *et al*. (2001) showed that rats receiving 100 ppm fluoride (as NaF) in drinking water for four months have increased levels of MDA and glutathione (GSH) and higher activity of GSHPx in erythrocytes, brain, and liver, but decreased activity of erythrocyte SOD. Another study by Shivarajashankara *et al*. (2001) on fluorotic children revealed the following changes: elevated levels of MDA, decreased GSH levels, increased GSH-Px activity and decreased SOD activity in red blood cells. Chinoy and Patel administered 10 mg of NaF/kg body mass during 30 days to female mice and found that cerebral levels of GSH and ascorbic acid decreased, as well as the activities of SOD, GSH-Px, and CAT. These effects correlated with increased levels of lipid peroxides. Administration of vitamins C, E, and calcium fully reversed these changes. Studies reported by Vani and Reddy carried out on mice treated with NaF (20 mg/kg body mass) for 14 days revealed decreased SOD, CAT, and glutathione transferase (GST) activities in brain and gastrocnemius muscle. (Sidiqui, 1955) 26 The effect of fluoride on muscle enzymes was comparatively larger, evidently owing to a greater accumulation of fluoride in muscle than brain.

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