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Pathological evaluation of pancreatic exocrine glands in experimental fluorosis

Shashi A*, Sharma N, Bhardwaj M

Department of Zoology, Punjabi University, Patiala- 147 002, Punjab, India

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ABSTRACT

Objective: To monitor the pathological alterations in pancreas of rat during experimental fluorosis. **Methods:** Sixty Sprague Dawley albino rats of both sexes were divided into 12 experimental groups and one control group. The rats of control group were administered subcutaneously double distilled water 1 mL/kg bw daily. The experimental groups were injected with 30, 45, and 75 mg NaF/kg bw/day. The experimental period was divided into 4 phases at interval of 15, 22, 30, and 36 days. Animals were sacrificed from each group at the end of 16, 23, 31, and 37 days. **Results:** The following changes were observed in this study: (1) Pathological examination of pancreas after 15 days of fluoride treatment revealed: hypertrophy of acini, leucocytes infilteration and pycnotic nuclei due to necrosis of acini in group 1; uremic alterations, invulsion and infoldings of reticular layer of islets of Langerhans in group 2; and a decrease in number of acini and interlobular connective tissues resulted in an increase in intercellular spaces in pancreas of rats in group 3. (2)Hyalinization and hypertrophy in the lobules of acini and hyperplasia and hypertrophy in intercalated duct with mucinous secretion in pancreas of rat of group 4; squamous metaplasia of pancreatic duct, adenoma of pancreas, hemorrhagic necrosis in group 5; and hyperplasia of acini and reduction in number of pancreatic islets in group 6. (3) Disorganization and atrophy of pancreatic lobules and presence of vacuoles in a group of six were visible in pancreas of rats in group 7; acute pancreatic and lamellated inflammatory cells in test rats of group 8; and islet adenoma and decrease in number of islets cells, and exudation in acini were noticed in experimental rats of group 9. (4) In the last phase of experimentation, atrophic alterations in pancreatic acini, invulsions, and necrosis was prominent in group 10, deep inflammation and proliferation of connective tissue of pancreas in experimental group 11, and periodical fibrosis, hyperplasia of acini, degenerative changes in pancreas of rats in group 12. **Conclusion:** The histopathological examination of pancreas of fluoridated rats exhibited structural alterations in the exocrine glands. The acini revealed hypertrophy, pyknotic nucleus, necrosis and uremic alterations. Acini became lobulated and reveal increased pigmentation.

1. Introduction

The pathological changes in structure and function of some endocrine glands such as parathyroid gland [1], adrenal gland [2], hypothalamus [3], and thyroid gland [4] have been described adequately; however, little information is available on the extent and mode of involvement of the pancreas in fluorosis. As a very active site of regulation of metabolism and due to its role in maintaining sugar and insulin secretion, pancreas is especially susceptible to fluoride intoxication. The present study was designed to monitor the pathological alterations in pancreas of rat during experimental fluorosis.

2. Materials and methods

Sixty Sprague Dawley albino rats of both sexes were given a standard pellet diet obtained from Hindustan Lever Limited Mumbai, India. Water was supplied ad libitum. All the animals were acclimatized for one week and were weighed prior to the start of experiment.

After the treatment period, the overnight fasted rats were sacrificed. The pancreas from all the 13 groups, were taken out in chilled normal saline, washed and cut into small pieces using sharp blade followed by fixation in various fixatives viz. Bouin´s fluid and Carnoy´s fixative. The tissues were dehydrated in 95% alcohol for 45 minutes,

*Corresponding author: Dr. Shashi Aggarwal, Department of Zoology, Punjabi University, Patiala -147002, India.
E-mail: shashiuniindia@yahoo.co.in

tertiary-butyl alcohol for 6 hours, cleared in amyl acetate for overnight and embedded in paraffin wax (temperature 60- 62 ℃). Wax blocks were prepared and 7 μm thin serial sections were cut with microtome. The histopathology of pancreas was studied using various stains viz: Iron haemotoxylin and eosin, Mallory's Triple stain, Feulgen nucleal stain [5] Periodic acid schiff's stain [6], Gallocyanin-Chrome alum [7] and Ninhydrin schiff's stain [8].

The stained sections for pathological lesions were studied under the research microscope. The microphotography was done in the Bimolecular electronics and Nanotechnology laboratory of Central Scientific Instrument Organization (CSIO), Chandigarh, India. The microphotographs were taken with a digital camera attached on inverted research fluorescent microscope (Axivort-200) interphase with computer.

3. Results

All the animals were injected with NaF dissolved in deionized double distilled water/kg bw/day as depicted in Table 1. Results of experiment are documented in Figures1-15. Figure 1 records photomicrographs of transverse section of pancreas of rats in the control group.

3.1. Phase 1

In the first phase of experimentation, the rats were treated with 30, 45 and 75 mg NaF/kg bw/day for 15 days respectively.

3.1.1. Group 1- 30 mg NaF/kg bw/day for 15 days

The pathological examination of pancreas of test rat in this group revealed structural alterations in the exocrine glands. There was thickening and invulsion of capsular membrane and increase in the intercellular connective tissue. Acini showed lot of pigmentation. The hypertrophy of acini and leucocyte infiltration was most marked. Some zymogenic cells stained darkly. Mild acinus cells dissociation accompanied by cytoplasmic vacuolization, pycnotic and deeply stained nuclei occurred. Due to the necrosis of acini, lumens become wide and fragmentation of nuclei was also evident (Figure 2).

3.1.2. Group 2- 45 mg NaF/kg bw/day for 15 days

The pathological alterations in the pancreas of rat of this experimental group showed increase in interlobular connective tissue. Acini became lobulated and uremic alterations occurred. The acinar glands were dilated, hypertrophied, and filled with a mucinous secretion. Some of exocrine glands were present in a state of disintegration. The number of nuclei and zymogenic granules were decreased. Acinar glands were replaced by fibrous tissue. The pancreatic atrophy due to ductal obstruction was most marked (Figure 3).

Table 1
Experimental protocol.

Phase	Group	Dose mg/kg bw/ day	Duration of treatment (Days)	Day of autopsy
	Control	1 mL double distilled water	-	Sacrificed along with treated
Phase -I	1	30 mg NaF	15	16
	2	45 mg NaF	15	16
	3	75 mg NaF	15	16
Phase-II	4	30 mg NaF	22	23
	5	45 mg NaF	22	23
	6	75 mg NaF	22	23
Phase - III	7	30 mg NaF	30	31
	8	45 mg NaF	30	31
	9	75 mg NaF	30	31
Phase -IV	10	30 mg NaF	36	37
	11	45 mg NaF	36	37
	12	75 mg NaF	36	37

3.1.3. Group 3 - 75 mg NaF/kg bw/day for 15 days

The histopathological changes in pancreas of rats of this highest dose group showed decrease in number of acini. A decrease in the interlobular connective tissues occurred resulted in an increase in intercellular spaces. (Figure 4).

3.2. Phase -II

In the second phase of experimentation, the rats were treated with 30, 45, and 75 mg NaF/kg bw/day for 22 days respectively.

3.2.1. Group 4- 30 mg NaF/kg bw/day for 22 days

The microscopic changes in pancreas of this fluoride -treated

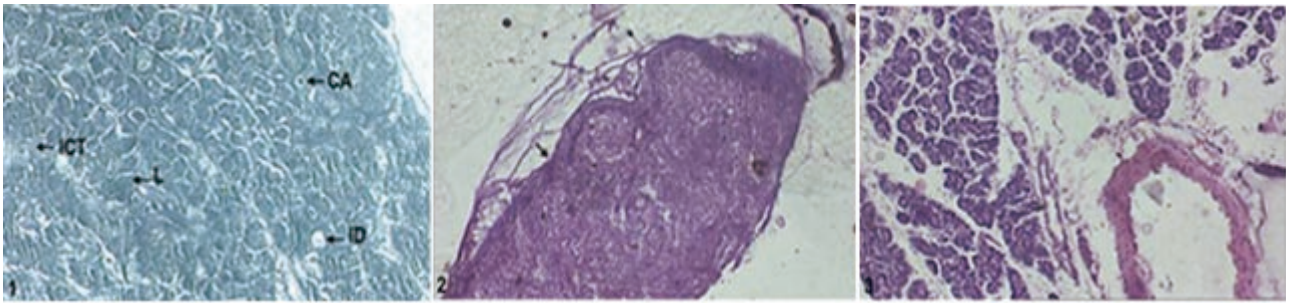

Figure 1. T.S. of pancreas of rat of control group showing pancreatic Lobule, Interlobular connective tissue, Centroacinar cells Intercaleted duct and Islet's of Langerhans (Mallory's Triple stain, × 100).
Figure 2. T.S. of pancreas of rat treated with 30 mg NaF/kg bw/day for 15 days showing invulsion of the capsular membrane (Feulgen nucleal stain, × 100).
Figure 3. T.S. of pancreas of rat treated with 45 mg NaF/kg bw/day for 15 days showing pancreatic atrophy due to ductal obstruction (PAS, × 100).

group of rats showed extensive proliferation of interlobular connective tissues and hypertrophy of intercalated duct. Focal areas of necrosis were observed in exocrine glands (Figure 5). There was hyalinization, uremic alterations and hypertrophy and hyperplasia in the acinar lobules compared to the control group. There was increase in number and size of zymogen granules indicating increased synthetic activity. There was formation of globose tangle, increase in the intercellular spaces between acini and extensive necrosis of exocrine glands (Figure 6).

3.2.2. Group 5– 45 mg NaF/kg bw/day for 22 days

The pathological changes in the pancreas of rat that were given 45 mg NaF for 20 days exhibited squamous metaplasia of pancreatic ducts, virtually obliterating its lumen. Pancreatic hypersecretion and obstruction was observed. The dilated ducts were filled with inspissated secretions. The nuclei moved towards the periphery. The size of nuclei was increased. The centroacinar cells disappeared. The size of intercalated duct was also increased. Infiltrations of leucocytes were prominent. Nodule lesions were formed. There was shrinkage of acini. There was migration of the zymogen granules towards the basal position. The adenomatous alterations and acute pancreatis were most prominent. The cytoplasm decreased and acinar nuclei were hypertrophied. Sodium fluoride induced hypertrophy of intercalated ducts. An increase in connective tissue, hemorrhagic necrosis, fibrosis of the parenchyma, and inflammatory infiltration of the leucocytes occurred. The hypertrophy of the acinar cells and nuclei was noted. The acinar cell membrane and nuclei revealed degenerative changes (Figure 7).

3.2.3. Group 6– 75 mg NaF/kg bw/day for 22 days

The pathological alteration in the pancreas of highly fluoridated rats exhibited hyperplasia of acini. Acinus cell dissociation was intense. The inflammatory infiltrate of acini along with increased number of nuclei were observed. Cytoplasm showed vaculation (Figure 8).

3.3. Phase–III

In the third phase of experimentation, rats were treated with 30, 45, and 75 mg NaF/kg bw/day for 30 days respectively.

3.3.1. Group 7– 30 mg NaF/kg bw/day for 30 days

A decrease in intercellular connective tissue, acinar disintegration was prominent (Figure 9).

3.3.2. Group 8– 45 mg NaF/kg bw/day for 30 days

The pathological alterations in the pancreas of rats of fluorotic group exhibited acute pancritis, fibrocalculous pancreatic regions, and dilated and thinned out pancreatic duct. In the lumen of acinar glands, lamellated inflammatory cells were present (Figure 10).

3.3.3. Group 9– 75 mg NaF/kg bw/day for 30 days

The histopathological changes revealed atrophied acini, exudation in acini, increase in intercellular and intracellular connective tissue spaces and ductal disintegration (Figure 11).

3.4. Phase–IV

In the final phase of experimentation, the rats were treated with 30, 45 and 75 mg NaF/kg bw/day for 36 days respectively

3.4.1. Group 10– 30 mg NaF/kg bw/day for 36 days

There was decrease in the number of acini, atrophy of acinar exocrine glands, and intensive vacuole formation occurred in pancreas. Necrosis was more pronounced (Figure 12). The capsular membrane revealed invulsions and infoldings. The acini showed inflammatory changes (Figure 13).

3.4.2. Group 11– 45 mg NaF/kg bw/day for 36 days

The pathological changes in the pancreas of rats intoxicated with 45 mg NaF, exhibited deep infoldings and invulsions, inflammation, spongy appearance, leucocytic infiltration and acute pancritis as compared to control group and previous fluoridated rats (Figure 14).

3.4.3. Group 12– 75 mg NaF/ kg bw/ day for 36 days

The pathological alterations in the pancreas of this group fluoridated for 36 days revealed that pancreatic acinar lobules underwent degenerative changes. The number of acini was markedly decreased. There was increase in the intercellular spaces between acini, periductal fibrosis, and inflammation of acini. Perilobular and periductular inflammation were most advanced. Acini underwent disintegration and consequently their number decreased. There was extensive proliferation of connective tissue. The size of intercalated ducts was decreased. The numbers of acini declined in each lobule (Figure 15).

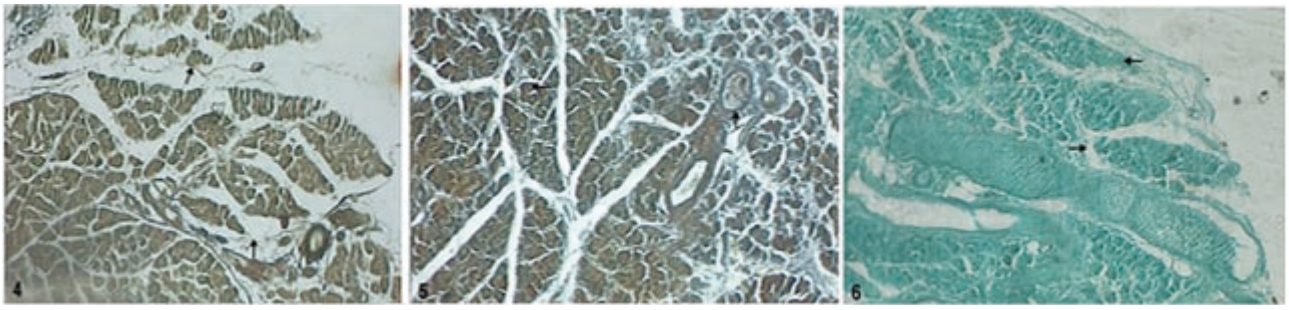

Figure 4. T.S. of pancreas of rat treated with 75 mg NaF/kg bw/day for 15 days showing increase in intercellular spaces due to decrease in interlobular connective tissue (Mallory´s Triple stain, × 100).
Figure 5. T.S. of pancreas of rat treated with 30 mg NaF/kg bw/day for 22 days showing hypertrophy of acini (Mallory´s Triple stain, × 100).
Figure 6. T.S. of pancreas of rat treated with 30 mg NaF/kg bw/day for 22 days showing increase in the intercalated ducts filled with mucinous secretion and extensive necrosis of exocrine gland (Fuelgen nucleal stain, × 100).

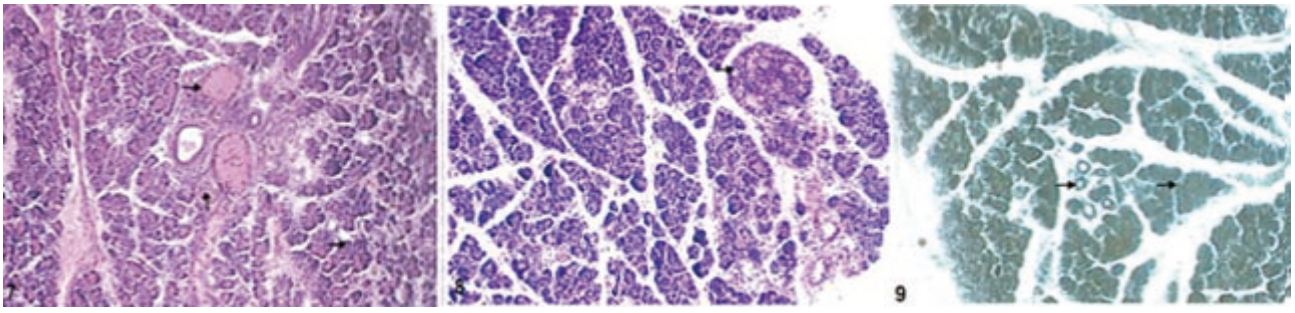

Figure 7. T.S. of pancreas of rat treated with 45 mg NaF/kg bw/day for 22 days showing squmous metaplasia of pancreatic ducts (Hematoxylin–eosin, × 100).
Figure 8. T.S. of pancreas of rat treated with 75 mg NaF/kg bw/day for 22 days showing hyperplasia of acini. (PAS, × 100).
Figure 9. T.S. of pancreas of rat treated with 30 mg NaF/kg bw/day for 30 days. There was decrease in intercellular connective tissue acinar disintegration was prominent. (Mallory´s Triple stain, × 100).

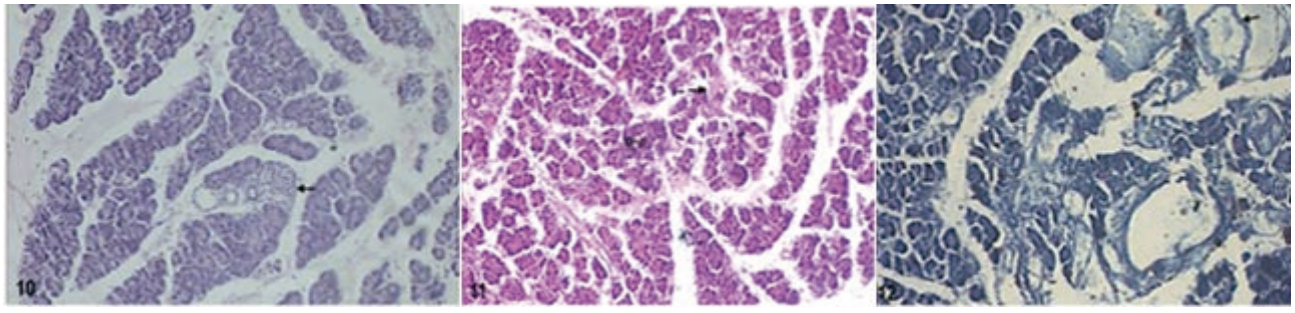

Figure 10. T.S. of pancreas of rat treated with 45 mg NaF/kg bw/day for 30 days showing acute pancritis, and fibrocalcuous pancreatic region (PAS, × 100).
Figure 11. T.S. of pancreas of rat treated with 75 mg NaF/kg bw/day for 30 days showing atrophied acini (Ninhydrin – Schiff´s stain, × 100).
Figure 12. T.S. of pancreas of rat treated with 30 mg NaF/kg bw/day for 36 days showing atrophy of acinar cells and intensive vacuole formation (Gallocyanin– Chrome alum stain, × 100).

Figure 13. T.S. of pancreas of rat treated with 30 mg NaF/kg bw/day for 36 days showing infolding of capsular membrane. (Mallory㈜s Triple stain, × 100).
Figure 14. T.S. of pancreas of rat treated with 45mg NaF/kg bw./day for 36 days exhibiting deep infolding and invulsion.(Mallory㈜s Triple stain, × 100).
Figure 15. T.S. of pancreas of rat treated with 75 mg NaF/kg bw/day for 36 days showing degenerative changes in the pancreatic lobules (Gallocyanin–Chrome alum stain, × 100).

4. Discussion

The histopathological examination of pancreas of fluoridated rats exhibited structural alterations in the exocrine glands. The acini revealed hypertrophy, pyknotic nucleus, necrosis and uremic alterations. Acini became lobulated and revealed increased pigmentation.

In the present study, proliferation in the interlobular and intralobular connective tissue as well as infolding and deep invulsion in capsular membrane were observed. Similar finding have also been reported earlier in pancreas of young rats supplied with 820 ppm fluoride in drinking water for 100 days [9]. The number of zymogen granules was reduced in rats exposed to fluoride treatment. Fluoride disrupts the export of zymogen granules from rough endoplasmic reticulum, resulting in formation of intracisternal granules and autophagosomes [10].

Dobrowska *et al*. [11] reported in the rats, effect of 10.6 to 31.0 mg NaF/dm^3 for 30 days on ultrastructural alterations of

the mitochondria in the submandibular gland, the pancreas and the liver. The mitochondria were mostly damaged in higher dose groups. The mitochondria were polymorphic, with condensed matrix and blurred internal structure. The acinar cells of submandibular glands revealed presence of swollen mitochondria and destruction of mitochondrial crest. In the ultrastructural examination of the pancreas, which structurally resembles the submandibular gland, intensification of changes also increased with the administered dose and duration of fluoride.

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