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Therapeutic Effect of *Boerhaavia Diffusa* L. on Scanning Electron Microscopy of Rat Exocrine Pancreas Induced by Fluoride

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Abstract

Excessive fluoride ingestion has been identified as a risk factor for fluorosis. Fluoride gets accumulated in hard and soft tissues, and disturbs the metabolic processes. It produces noticeable changes in subsequent morphological pictures of many organs such as liver, kidney, heart and muscle including endocrine glands. The aim of this study was to analyze the ultrastructural changes in exocrine pancreas induced by fluoride. Wistar albino rats were treated with 300 and 600 mg of sodium fluoride/kg b.w./day for 40 days via oral gavage. The fluorotic groups of rats were post treated with 500 mg/kg b.w./day leaf extract of Boerhaavia diffusa L. for 20 days. Control and positive control groups received 1ml of deionized water and 500 mg/kg b.w./day of leaf extract of Boerhaavia diffusa L. for a period of 40 and 20 days respectively. The exocrine pancreas of fluoridated rats exhibited fibrosis and holes through the surface of acini. The apical membrane of superficial cells was broken and collapsed. Acinar cells exhibited collagen fibrils, irregular protrusions and secretory vesicles. Pancreatic duct had many luminal blebs and crystal depositions over the surface. The post-treatment with leaf extract of Boerhaavia diffusa L. had therapeutic effect against fluoride induced toxicity.

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Key words: Albino rats, *Boerhaavia diffusa* L., Fluorosis, Pancreas, Scanning electron microscopy.

INTRODUCTION

Fluorosis is caused by excessive ingestion of fluoride over a prolonged period and endangers the health of humans as well as animals (Ozsvath, 2009). Endemic fluorosis is prevalent in many parts of the world and cause damage not only to teeth and skeleton, but also to soft organs including endocrine glands (Salam *et al.*, 2013). The exocrine pancreas consists of acini and ducts. Enzyme secretions of exocrine pancreas are required for hydrolysis of nutrients present in food (Agha *et al*., 2012). *Boerhaavia diffusa* L. is one of the renowned medicinal plants used to treat large number of human ailments. This plant contains flavonoids, alkaloids, steroids, triterpenoids, lipids, lignins, carbohydrates, proteins and glycoproteins (Mahesh *et al*., 2012). The present study elucidated the potential effects of *Boerhaavia diffusa* L. against sodium fluoride induced toxicity in the exocrine pancreas of albino rats using scanning electron microscopy.

MATERIALS AND METHODS

Experimental animals:

Wistar albino rats weighing 150-200 g were housed in propylene cages with stainless grill tops and fed with standard commercial rat pellet diet (Hindustan lever limited, Mumbai, India) and water was given *ad libitum*. The experiments were performed under the approval of Institutional animal ethical committee of Punjabi University, Patiala (Animal Maintenance and registration No. 107/GO/ReBi/S/99/CPCSEA/2017-20).

Experimental design:

After two weeks of acclimatization, animals were divided into six groups (six rats in each group). Control and positive control groups received 1ml of deionized water and 500 mg/kg b.w./day of leaf extract of *Boerhaavia diffusa* L. for a period of 40 and 20 days respectively. The experimental groups were treated with 300 and 600 mg/kg b.w./day of sodium fluoride for 40 days via oral gavage. The fluoride

treated groups were post-treated with 500 mg/kg b.w./day leaf extract of *Boerhaavia diffusa* L. for 20 days.

Sample preparation for scanning electron microscopic examination :

At the end of experimental period, the controls and experimental rats were sacrificed. Small pieces of pancreatic tissue were washed with 0.1M phosphate buffer (pH 7.4) and fixed in 2.5% glutaraldehyde buffered with 0.1M phosphate buffer at pH 7.4 for 24 hours at 4°C (Karnovsky, 1965). The tissue was washed with phosphate buffer (0.1M), post fixed in 1% osmium tetraoxide for 2 hours at 4°C. Dehydration was done in ascending grades of acetone and critical-point dried. Specimens were glued on to stubs, covered with gold in a sputter coater (Balzer Union SCD 020) and the images were recorded by using scanning electron microscope (JEOL JSM-6510).

RESULTS

The scanning electron microscopic examination of exocrine pancreas of control rat revealed that each pancreatic lobule was composed of numerous acini (Fig. 1).

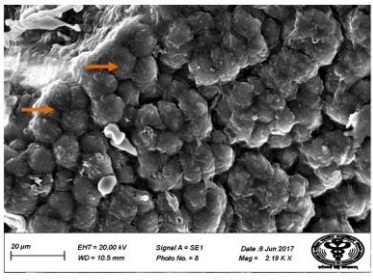

Fig. 1: Scanning electron micrograph of exocrine pancreas of control rat showing groups of acini (↑).

Acini were connected to a long thin duct with a smoother surface structure (Fig. 2).

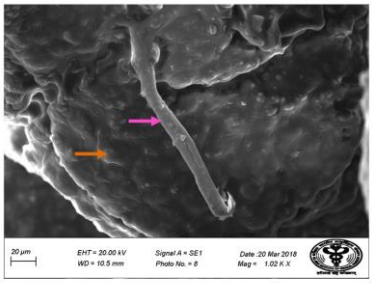

Fig. 2: Scanning electron micrograph of pancreas of control rat showing surface view of acinar cells (↑) and an intercalated duct (↑) with smooth surface.

Pancreatic acini appeared as round or elongated cellular masses. The pancreatic acinar elements were large and irregular ovoid cells. Among these large acinar cells, smaller elements were also noted. Surfaces of the large acinar cells revealed spheroid droplets which represent zymogen droplets (Fig. 3).

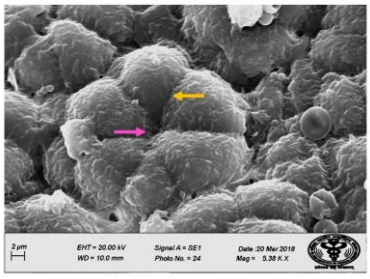

Fig. 3: Scanning electron micrograph of pancreas of control rat showing magnified view of an acini with intercellular canaliculi (↑) arising from the secretory lumen (↑).

Acinar cellular architecture in the exocrine pancreas of rat treated with 500 mg/kg b.w./day of leaf extract of *Boerhaavia diffusa* L. was similar to that of control ones (Fig. 4).

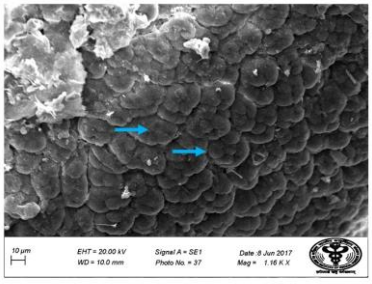

Fig. 4: Scanning electron micrograph of pancreas of rat treated with leaf extract of 500 mg/kg b.w./day of *Boerhaavia diffusa* L. for 20 days showing groups of acini (↑).

The ultrastructure of exocrine pancreas of rat treated with 300 mg/kg b.w./day of NaF for 40 days showed collagen fibrils covering all the surface of the tissue (Fig. 5).

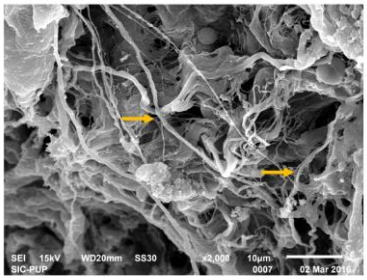

Fig. 5: Scanning electron micrograph of pancreas of rat treated with 300 mg/kg b.w./day of NaF for 40 days showing appearing of collagen fibrils (↑) over the surface.

A small hole was visible on the surface of acinar cells. The acinar cells also showed formation of several irregular protrusions (Fig. 6).

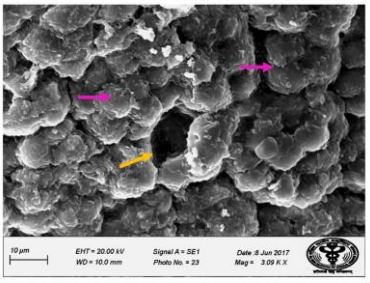

Fig. 6: Scanning electron micrograph of pancreas of rat treated with 300 mg/kg b.w./day of NaF showing surface view of acini. Arrow (↑) indicates small hole through the surface of acinar cell. Some acinar cells exhibit formation of irregular protrusions (↑) over their surface.

The surface of pancreatic duct showed several luminal blebs (Fig. 7).

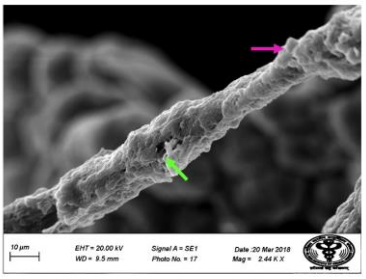

Fig. 7: Scanning electron micrograph of pancreas of rat treated with 300 mg/kg b.w./day of NaF for 40 days showing pancreatic duct with many luminal blebs (↑) and damaged surface (↑).

A part of the acinar cell revealed presence of zymogen granules (Fig. 8).

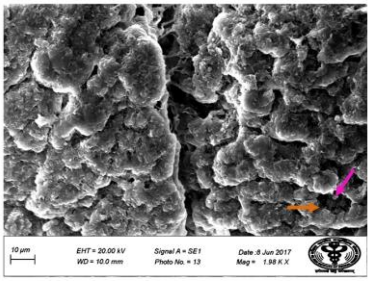

Fig. 8: Scanning electron micrograph of pancreas of rat treated with 300 mg/kg b.w./day of NaF for 40 days showing a broken acinar cell ( ) revealing zymogen granules ( ).

Collagen and reticular fibers covered the surface and obscured the design of acinar cellular assembly (Fig. 9).

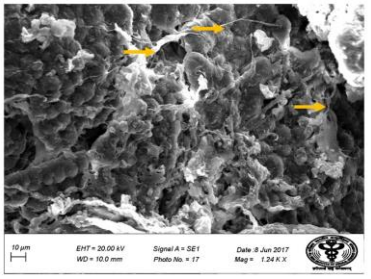

Fig. 9: Scanning electron micrograph of pancreas of rat treated with 300 mg/kg b.w./day of NaF for 40 days showing masses of acinar cells covered with connective tissue fibers ( ).

3D network of the collagen fibers was seen in the decellularized areas of the acini (fig. 10).

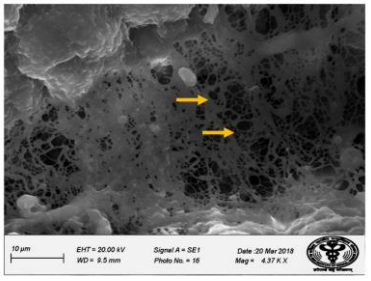

Fig. 10: Scanning electron micrograph of pancreas of rat treated with 300 mg/kg b.w./day of NaF for 40 days showing collagen network ( ) of decellularized areas.

The rats treated with 600 mg/kg b.w./day of NaF for 40 days revealed deposition of crystals and presence of holes over the surface of many acinar cells (Fig. 11).

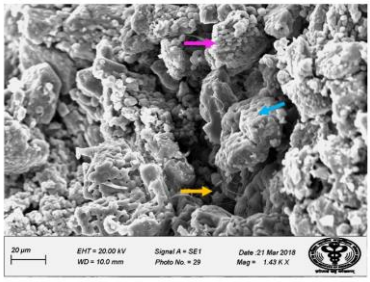

Fig. 11: Scanning electron micrograph of pancreas of rat treated with 600 mg/kg b.w./day of NaF for 40 days showing many entangling fibers ( ) with crystal depositions ( ) over the surface. Small holes were visible over the surface of cells ( ).

Aggregation of large number of prismatic crystals of calcium carbonate all over the surface of the acinar cells was prominent (Fig. 12).

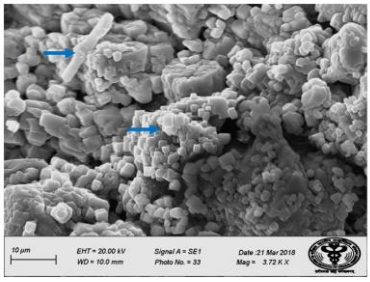

Fig. 12: Scanning electron micrograph of pancreas of rat treated with 600 mg/kg b.w./day of NaF for 40 days showing prismatic calcium carbonate crystals ( ).

The leukocyte had emerged from the underlying connective tissue due to chronic inflammation (Fig. 13).

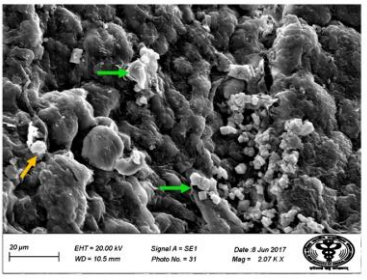

Fig. 13: Scanning electron micrograph of pancreas of rat treated with 600 mg/kg b.w./day of NaF for 40 days showing crystals of variable sizes ( ) and a leukocyte ( ) has emerged from the surface.

The surface of the interlobular duct was covered with many luminal blebs, depressions, and a long cilium (Fig. 14).

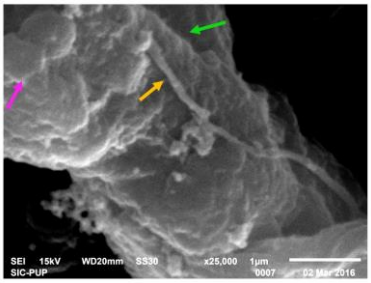

Fig. 14: Scanning electron micrograph of pancreas of rat treated with 600 mg/kg b.w./day of NaF for a period of 40 days showing a pancreatic duct with many luminal blebs ( ), depressions ( ) and a long cilium ( ).

Decellularized pancreas was free of cells leaving only spaces left empty by solubilized cells (Fig. 15).

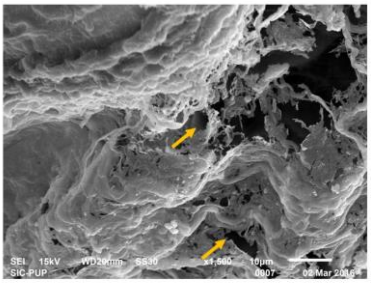

Fig. 15: Scanning electron micrograph of pancreas of rat treated with 600 mg/kg b.w./day of NaF for 40 days showing decellularized tissue with empty cellular spaces ( ).

Crystal deposition and fibrosis was prominent in the acini (Fig. 16).

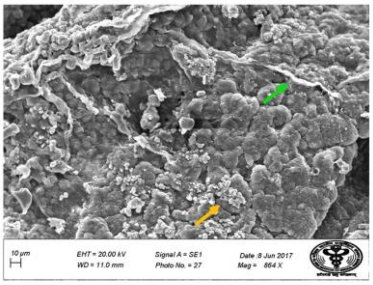

Fig. 16: Scanning electron micrograph of pancreas of rat treated with 600 mg/kg b.w./day of NaF for 40 days showing fibers ( ) and crystal ( ) covering the surface.

Sponge like calcium carbonate spherulites were surrounded by irregular clumps (Fig. 17).

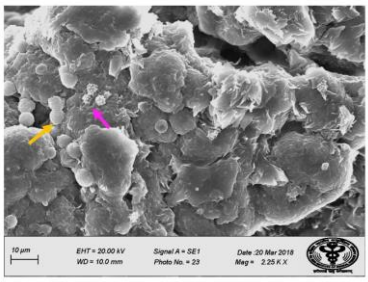

Fig. 17: Scanning electron micrograph of pancreas of rat treated with 600 mg/kg b.w./day of NaF for 40 days showing calcium spherulites ( ) and granules ( ) over the surface of acinar tissue.

The surface of acinar cells was covered by large number of spherical droplets (Fig. 18).

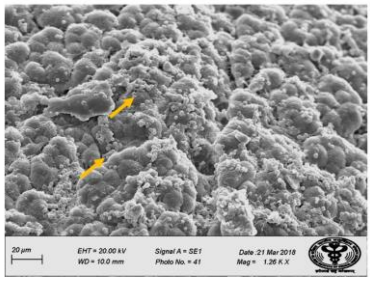

Fig. 18: Scanning electron micrograph of pancreas of rat treated with 600 mg/kg b.w./day of NaF for 40 days showing surface of acinar cells containing large number of spherical droplets ( ).

Scanning electron microscopy of pancreas of rat treated with 300 mg/kg b.w./day of NaF for 40 days post-treated with 500 mg/kg b.w./day of leaf extract of *Boerhaavia diffusa* L. for 20 days showed an improved structure with some crystal depositions over the surface (Fig. 19).

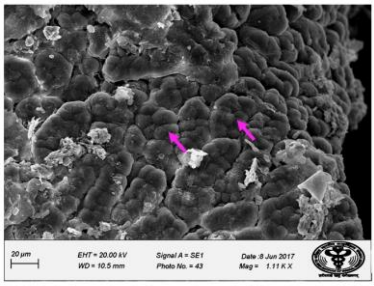

Fig. 19: Scanning electron micrograph of pancreas of rat treated with 300 mg/kg b.w./day of NaF for 40 days post-treated with 500 mg/kg b.w./day of leaf extract of *Boerhaavia diffusa* L. for 20 days showing preserved surface of acinar cells ( ).

The surface showed absence of holes or collagen fibers but few protrusions were still visible (Fig. 20).

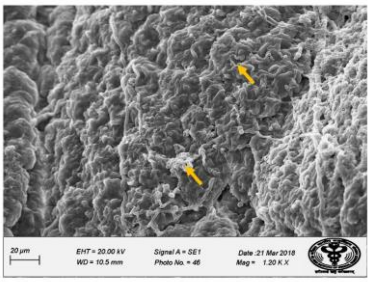

Fig. 20: Scanning electron micrograph of pancreas of rat treated with 300 mg/kg b.w./day of NaF for 40 days post-treated with 500 mg/kg b.w./day of leaf extract of *Boerhaavia diffusa* L. for 20 days showing protrusions over the surface of acinar cells ( )

The pancreas of rat treated with 600 mg/kg b.w./day of NaF for 40 days post-treated with 500 mg/kg b.w./day of leaf extract of *Boerhaavia diffusa* L. for 20 days showed a regained structure with groups of acini and a crystal deposition over the surface (Fig. 21).

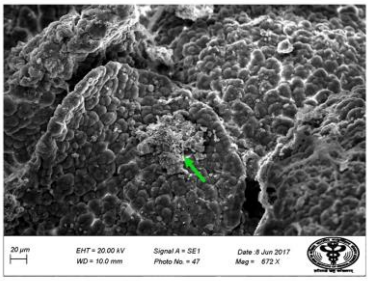

Fig. 21: Scanning electron micrograph of pancreas of rat treated with 600 mg/kg b.w./day of NaF for 40 days post-treated with 500 mg/kg b.w./day of leaf extract of *Boerhaavia diffusa* L. for 20 days showing groups of acini with few crystal depositions ( )

Acinar cells with some protrusions and a duct with smooth surface and few collagen fibrils were seen (Fig. 22).

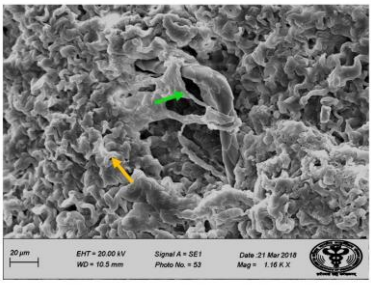

Fig. 22: Scanning electron micrograph of pancreas of rat treated with 600 mg/kg b.w./day of NaF for 40 days post-treated with 500 mg/kg b.w./day of leaf extract of *Boerhaavia diffusa* L. for 20 days showing surface of acini with protrusions ( ) and collagen fibrils ( )

DISCUSSION

The present work has documented a three dimensional architecture study of exocrine pancreas through the use of scanning electron microscopy. The SEM observations revealed that the exocrine

pancreas of control rat was composed of numerous acini which connected to a long thin duct. The findings coincided with the study of Hiromi Takahashi (1984) who demonstrated that exocrine pancreas is a compound acinar gland composed of round or tubular acini.

During the present study, the exocrine pancreas of fluoridated rats showed holes and protrusions through the surface of the acinar cells. There was extensive dense fibrosis and associated diffuse, tiny, crystal depositions over these fibers. These findings are in accordance with the study of Sindhu *et al*. (2015) who performed scanning electron microscopy to study alcoholic chronic pancreatitis. Ducts showed epithelial damage in structures and few fine intraductal calcifications. Pramanik *et al*. (2015) also reported aggregations of crystalline structures in the pancreas of patients suffering from chronic pancreatitis. Calcium carbonate was the major constituent of all the pancreatic calculi studied. Onizuka *et al*. (1994) observed necrosis and degeneration of acinar cells, ductal proliferations, fibrosis and arteriosclerosis in the pancreas of spontaneously hypertensive rats. Campisi *et al*. (2009) reported that pancreatic calcifications were observed in wide spectrum of pancreatic diseases. Certain findings like parenchymal calcifications, diffuse distributions, intraductal calcifications, parenchymal atrophy and cystic lesions were noted more often in chronic pancreatitis than in other pancreatic diseases harboring calcifications. Pancreatic secretions contained bicarbonates, sodium and potassium ions and, water emitted by epithelial cells that line the pancreatic ducts. This secretion had several enzymatic components with high levels of bicarbonates as well as high values of pH which lead to the formation of calcium bicarbonate crystals (Cros *et al*. 2016).

In a recent study, Ishiwata *et al*. (2018) also reported changes like holes in the surface of certain pancreatic cells and irregular protrusions on the surface of others. Esposito *et al*. (2001) observed a heterogenous pattern of fibrosis adjacent to residual acinar parenchayma in chronic pancreatitis. Fibrosis was found mainly in interlobular or perilobular area in the form of nodular pancreatitis. In intralobular fibrosis, alcohol intake had shown effects on initial stage of periacinar collagination through activation of myofibroblast and severe damage to acinar cells (Suda, 2000). Uchida *et al*. (1988) recorded changes like intra or inter lobular fibrosis with lymphocytic infiltrations, degenerative acinar changes, ductal proliferations which

had led to the ischemic changes in the pancreas of rabbit. Bockman *et al.* (1997) explained defects in the basement membrane which provided open pathway from the lumen into extracellular spaces of the duct wall.

The present study demonstrates that the surfaces of the ducts were covered with multiple blebs, and accumulation of crystal depositions. The blood vessels were having notched surfaces. These changes are in consonance with study of Ashizawa *et al*. (1997) who found ductal proliferation, tortuous ductal channels, crater like depressions of ductal inner surfaces and notching of blood vessels surfaces in the stroke-prone spontaneously hypertensive rats. Hamamoto *et al*. (2002) observed increase in the diameter of common bile-pancreatic ducts following the duct ligation. Many pancreatic acini became apoptotic and luminal blebs appeared on the surface of pancreatic duct. Steer *et al.* (1995) had seen dilated ducts, intraductal protein plugs which may be calcified in chronic pancreatitis. Parenchymal necrosis with calcification, intraductal protein plugs, fibrosis and stones were common in alcoholic pancreatitis (Stevens *et al.* 2004, Apte *et al.* 2005).

The pancreas of fluoride intoxicated rats post-treated with 500 mg/kg b.w./day of leaf extract of *Boerhaavia diffusa* L. for 20 days showed improvement in the acinar cells. The surface showed absence of holes, fibrosis and accumulated granules. Few crystal depositions and protrusions still can be seen over the surfaces.

CONCLUSIONS

The scanning electron microscopic analyses of pancreatic acinar cells has given baseline information on the ultrastructural changes caused by sodium fluoride. The administration of leaf extract of *Boerhaavia diffusa* L. showed improvements in the adversity caused.

Conflict of Interests

The authors declare that they have no conflict of interests.

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