Ameliorative Effect Of Boerhaavia Diffusa L. On Transmission Electron Microscopic Changes In Pancreatic Acinar Cells Of Fluoridated Rats

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Abstract

Background: Prevalent in many parts of the world, fluorosis is caused by excessive ingestion of fluoride over a prolonged period and endangers the health of humans as well as animals. In addition to its well known effects on the skeleton and teeth, fluoride can exert toxic effects on many other tissues and organs, giving rise to a broad array of symptoms and pathological changes.

Objective: The aim of the present study is to evaluate the effect of sodium fluoride followed by post-treatment of leaf extract of *Boerhaavia diffusa* L. on the pancreatic acinar cell of albino rat. The ultrastructure of pancreas is studied through transmission electron microscopy.

Material and Methods: Wistar albino rats were divided into six groups. 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 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. At the end of experimental period, the animals of all groups were sacrificed and pancreatic tissue specimens were immediately fixed in karnowsky fixative.

Results: In control rat, the pancreatic acinar cells had well defined plasma membrane, basal, large, euchromatic nucleus with a prominent nucleolus and large number of zymogen granules present in the apical regions of cells. The mitochondrias were scattered in the cytoplasm surrounded by rough endoplasmic reticulum. In rats, treated with 300 mg sodium fluoride/kg b.w./day damaged acinar cell membranes, irregular shaped, pyknotic and heterochromatic nuclei, condensation and margination of nuclear chromatin were seen. Centroacinar cells with elongated nucleus, large vesicles with membranous amorphous materials, intercellular spaces filled with cellular infiltrations and debris were also present. The animals treated with 600 mg sodium fluoride/kg b.w./day revealed damaged exocrine cell membrane and few or even absence of zymogen granules. Dilated and globular shaped rough endoplasmic reticulum, swelling of mitochondria, intracisternal granule accumulation, excessive autophagy and ribosomal degranulation with necrotic changes were also observed in fluoride exposed acinar cells. Increased intercellular spaces, cytoplasmic vacuolizations, constricted capillary with entrapped degenerating red blood cells and early endothelial degeneration, and collagen deposition were seen. These findings indicate that fluoride induced rough endoplasmic reticulum stress by intracisternal granule accumulation followed by excessive autophagosome formation and degranulation/aggregation of ribosomes. The concurrent administration of sodium fluoride in combination with leaf extract of *Boerhaavia diffusa* L. for 20 days alleviated the adverse effects of fluoride.

Keywords: Albino rats, *Boerhaavia diffusa* L., Exocrine pancreas, Sodium fluoride, Transmission electron microscopy.

Introduction

Excessive fluoride ingestion has been identified as a risk factor for fluorosis. Fluoride is widely distributed in nature in many forms and its compounds are being used extensively. Fluorosis is characterized by dental mottling and skeletal manifestation in the form of crippling deformities, osteoporosis, and osteosclerosis [1]. Deterimental effects of high-fluoride intake are also observed in soft tissues. The pathological changes in structure and function of endocrine glands has been described adequately, however little information is available on the extent and mode of involvement of pancreas in fluorosis. Pancreas is a compound gland that is mixed endocrine and exocrine. The exocrine portion consists of numerous acini composed of tubular and spherical masses of cells. An acinar cell basically has a great amount of zymogen granules in its apical part. The zymogen granules represent the digestive enzymes of pancreas. Enzyme secretions of exocrine pancreas

are required for hydrolysis of nutrients present in food. Fluoride causes degeneration of exocrine pancreatic cells, inflammatory stromal infiltrations as well as congestion and hemorrhage of pancreatic blood vessels [2,3]. As a very active site of regulation of metabolism and due to its role in maintaining sugar and insulin secretions, pancreas is especially susceptible to fluoride intoxication [4]. *Boerhaavia diffusa* L. commonly known as (Punarnava) in Indian system of medicine, is a perennial creeping herb and has got lots of importance because of its various pharmacological, biological, antioxidant, anti-hyperglycemic and antidiabetic activities [5]. The present study evaluated the ultrastructural effect of sodium fluoride on pancreas of albino rats and the impact of synchronous administration of *Boerhaavia diffusa* L.

Materials and Methods

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

Preparation of plant extract

Fresh leaves of *Boerhaavia diffusa* L. were collected and got identified in Department of Botany, Punjabi University, Patiala, India. The plant extract was prepared by the method of Narendhirakannan *et al*. [6]. The collected leaves were shade dried and ground to a coarse powder. The powder was extracted in 95% ethanol in a Soxhlet apparatus at 60^{o}C for 35 hours. After cooling and filtration, the filtrate was concentrated at 65^{o}C in a rotavapour to obtain a dry powder.

After two weeks of acclimatization, animals were divided randomly into six groups having 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 transmission electron microscopic examination

At the end of experimental period, the animals of all groups were sacrificed. Pancreatic tissue specimens were immediately fixed in 2.5% phosphate buffered gluteraldehyde (pH 7.4) at 4^{0}C for 24 hours and washed with 0.1M phosphate buffer 3-4 times [7]. Tissues were post fixed in 1% osmium tetraoxide for one hour, and then dehydrated in ascending grades of acetone. Ultrathin sections were cut with an ultramicrotome (Leica Ultracut UC7, Austria), stained with uranyl acetate and lead citrate and subsequently were examined and photomicrographed under Tecnai, G2 20 high resolution transmission electron microscope (FEI Company, The Netherlands).

Results

In control rat, electron microscopic examination of ultrathin exocrine cells revealed a well defined plasma membrane along with basal, large, euchromatic nucleus with a prominent nucleolus. A large number of zymogen granules were present towards the apical portion of the exocrine cell, mitochondrias were scattered in the cytoplasm which were surrounded by rough endoplasmic reticulum. A centroacinar cell with heterochromatic nucleus was located near the acinar cell (Figures 1 and 2). At higher magnification, each acinar cell had an euchromatic spherical nucleus containing a prominent nucleolus, closely packed cisternae of rough endoplasmic reticulum with ribosomes attached to their surface. The intact, rounded and elongated mitochondria with cristae were present in the cytoplasm (Figure 3). The cytoplasm of exocrine cells of *Boerhaavia diffusa* L. treated animals showed basal nucleus, surrounded by rough endoplasmic reticulum. Mitochondrias and zymogen granules were present in the cytoplasm (Figure 4).

In the rats treated with 300 mg NaF/kg b.w./day for 40 days, most exocrine cells had irregular shaped, pyknotic and heterochromatic nuclei. The rough endoplasmic reticulum saccules showed dilations and exhibited globular shape in some parts (Figure 5). Damaged exocrine cell membrane and cellular infiltrations in inter cellular spaces were visible. In severely affected exocrine cells, cytoplasm and mitochondrias showed vacuolizations (Figure 6). Extensive ribosomal degranulation from rough endoplasmic reticulum and aggregates were present in cytoplasm (Figure 7). There were present multiple vesicles of variable sizes having membranous structures and amorphous materials (Figure 8). Less number of zymogen granules were present in the apical part of exocrine cell. Autophagosomes and cytoplasmic vacuolizations were prominent. The centroacinar cell had elongated and heterochromatic nucleus (Figure 9). Some exocrine cells were binucleated and exhibited complete loss of zymogen granules. Intercellular spaces were filled with cellular infiltrations and debris. Constricted capillaries with degenerating endothelial cells and collagen depositions were observed (Figure 10).

In the pancreas of rats treated with 600 mg sodium fluoride/kg b.w./day for 40 days, exocrine cells had irregular cell membranes, binucleated nucleus and the zymogen granules in some groups of acinar cells were completely lost (Figure 11). Mitochondria had irregular shapes, clumped and became swollen. In some exocrine cells, the ribosomes fell away from the surface of rough endoplasmic reticulum membrane and aggregated throughout the cytoplasm (Figure 12).

Groups of acinar cells with damaged cell membranes and cytoplasmic vacuolizations were seen. Centroacinar cell with heterochromatic, pyknotic, condensed nucleus alongwith margination of chromatin materials were visible (Figure 13). Cytoplasmic vacuolizations, mitochondrial swellings and autophagosomes were seen in many acinar cells (Figure 14). Increased intercellular spaces and damaged cell membranes were prominent in some areas. Zymogen granules were few or even absent in some cells. Constricted capillary with entrapped degenerating red blood cells and collagen depositions were visible (Figure 15). Acinar cells with large intercellular spaces, damaged cellular membranes, and autophagosomes were prominent in the most damaged areas (Figure 16).

In pancreas of fluoridated rats post-treated with 500 mg/kg b.w./ day of leaf extract of *Boerhaavia diffusa* L. for 20 days in 300 mg sodium fluoride group for showed intact acinar cell membranes and the zymogen granules were normal in number. Rough endoplasmic reticulum was not dilated, few mitochondrias were damaged and many had regained their shape The size of vesicles containing amorphous materials was markedly decreased (Figure 17). The exocrine cell had basal, rounded and euchromatic nucleus. Rough endoplasmic reticulum and mitochondrias were normal in their form. A single vesicle with amorphous material was present (Figure 18).

In Pancreas of fluoridated rat post-treated with 500 mg/kg b.w./ day of leaf extract of *Boerhaavia diffusa* L. for 20 days in 600 mg sodium fluoride group, there was improvement in the ultrastructure features of acinar cells where the plasma membrane was least damaged. Basal, heterchromatic nucleus also had intact nuclear membrane. Dilations of rough endoplasmic reticulum were completely absent (Figure 19). In some cells normal, rounded, euchromatic nucleus with damaged nuclear membrane was visible. Round and elongated Mitochondrias were present in the cells (Figure 20).

Discussion

The electron microscopic examination of exocrine cells of fluoridated animals revealed marked ultrastructural changes. The basal, pyknotic, heterochromatic nucleus with somewhat damaged nuclear membrane was seen alongwith condensation and margination of chromatins. There was dilated and globular shaped rough endoplasmic reticulum with degranulation of ribosomes from the surface and extensive autophagosome formations. These findings were in accordance with the study of Matsua *et al.* [8] and Ito *et al*. [9] who stated that fluoride treatment induced condensation of nuclear chromatin in some exocrine cells. Accumulation of intracisternal granules in the rough endoplasmic reticulum of exocrine pancreatic cells, and degranulation/aggregation of ribosomes resulted in the formation of autophagosomes containing cytoplasmic organelles and intracisternal granules. Jinsong *et al*. [10] observed that after vagotomy, the nucleus was found to be condensed, zymogen granules were decreased, the endoplasmic reticulum had reduced in number, and mitochondrias became swollen.

In the present study, swelling, rupturing, irregular shapes of mitochondrias were seen in many exocrine cells of fluorotic rats. The damaged acinar cell membranes, cytoplasmic vacuolization, increase in intercellular spaces in the acinar cells and vesicles of variable sizes containing amorphous materials were visible. Similar findings have also been reported earlier by Zhan *et al*. [11] who investigate the effects of sodium fluoride on ultrastructure of pancreas in young pigs.

The present study demonstrated decreased zymogen content in the acinar cells of fluoridated rats. Constricted capillary with entrapped degenerating red blood cell, early endothelial degenerations and collagen depositions were prominent. Excessive protein accumulations in the rough endoplasmic reticulum resulted in intra-cistrenal granule accumulation and decrease in number of zymogen granules [12].

Salam *et al.* [13] observed that the acinar cell had heterochromatic nucleus, small number of zymogens, vacuolated mitochondria and cisternae of endoplamic reticulum were circular. The nucleus of centroacinar cell was elongated. Vesicles and amorphous materials were seen in rats treated with fluoride.

In the present study, we evaluated ameliorative effect of *Boerhaavia diffusa* L. against pancreatic exocrine cell toxicity induced by fluoride in rats. Improvement in cytoplasmic contents of affected cells, rounded nucleus with prominent nucleolus and marked increase in the number of zymogen granules in the apical region of acinar cells were observed. Dilations or globular shapes of rough endoplasmic reticulum were completely absent and mitochondrias had regained their structure. Cytoplasmic vacuolizations, intercellular spaces or cellular infiltrations and collagen depositions in capillaries and ducts were almost absent. These types of changes have also been reported by Agha *et al*. [14] who observed few or even absence of zymogen granules, rough endoplasmic reticulum saccules dilations and globular-shape in some parts of acinar cells in pancreas of albino rats under sodium fluoride treatment. The cytoplasm of acinar cells of vitamin E, methione and L-carnosine prior to sodium fluoride treatment also regained the normal number of zymogen granules and no or mild dilations in rough endoplasmic reticulum were seen.

Pieta *et al*. [15] reported that administration of vitamin A, E and coenzyme Q had a counteracting influence upon the degenerating changes seen in the acinar cells of pancreas and the cells showed normal pattern.

Suzuki *et al*. [16] observed that L-asparaginase at higher dose had reduced the number of zymogen granules and some vacuoles were formed in the cytoplasm of pancreatic acinar cells of rats. The vacuoles contained recognizable membrane bound-organellles such as endoplasmic reticulum cisternae and zymogen granules. Prior treatment with ostrotide prevented the development of L-asparaginase induced injury in the pancreas.

Conclusion

Fluoride exposure had deleterious effects on the ultrastuctural feature of exocrine cells which could be attributed to redox state disturbances. The leaf extract of *Boerhaavia diffusa* L. showed therapeutic effects on sodium fluoride induced alterations.

Conflict of Interest statement

The authors declare that they have no conflict of interest.

References

1. A Shashi, M Kumar and M Bhardwaj. Incidence of skeletal deformities in endemic fluorosis. Tropical Doc. 2008; 38: 231-233.

2. D Ahmed, V Kumar, A Verma, PS Gupta, H Kumar, V Dhindra, V Mishra and M Sharma. Antidiabetic, renal/hepatic/pancreas/cardiac protective and antioxidant potential of methanol/dichloromethane extract of Albizzia Lebbeck Benth. Stem bark (ALEx) on streptozotocin induced diabetic rats. Complementary Alternative Med. 2014; 14:243.

3. DAM Zaghloul, WM Gad-El-Rab, RR Bushra and AAA Farahat. The possible protective role of methione against sodium fluoride induced pancreatic changes in adult male albino rat: a histological immunohistochemical and morphometric study. Eropean J Haematol. 2019; 42(2): 285-296.

4. A Shashi, N Sharma and M Bhardwaj. Pathological evaluation of pancreatic exocrine glands in experimental fluorosis. *Asian Pacific J. Trop. Med.* 2010; 3: 36-40.

5. AR Mahesh , H Kumar , MK Ranganath and RA Devkar. Detail study on *Boerhaavia diffusa* plant for its medicinal importance- A review. Res J Pharmaceutical Sci. 2012; 1(1): 28-36.

6. RT Narendhirakannan, S Subramanian and M Kandaswamy. Biochemical evaluation of antidiabetogenic properties of some commonly used Indian plants on streptozotocin induced diabetes in experimental rats. Clin. Exp. Pharmacol. Physiol. 2006; 33: 1150-1157.

7. MJ Karnovsky. A formaldehyde-glutarldehyde fixative of high osmolarity for use in electron microscopy. J Cell Biol. 1965; 27: 137-138.

8. S Matsuo, H Nakagawa, K Kiyomiya and M Kurebe. Fluoride induced ultrastructural changes in exocrine pancreas cells of rats: fluoride disrupts the export of zymogens from the rough endoplasmic reticulum. *Arch. Toxicol.* 2000; 73(12): 611-617.

9. M Ito, H Nakagawa, T Okada, S Miyazaki and S Matsuo. ER-stress caused by accumulated intracistanal granules activates autophagy through a different signal pathway from unfolded proteins response in exocrine pancreas cells of rats exposed to fluoride. *Arch. Toxicol.* 2008; 83: 151-159.

10. L Jinsong, C Daoda and T Yuan. DNA content in pancreatic exocrinal cells after vagotomy and electron microscopy in rats. J. Tongji Med Uni. 1999; 19(3): 226-229.

11. X Zhan, J Li, Z Xu and M Wang. Effects of fluoride on pancreatic digestive enzymes activities and ultrastructure in young pigs. *Fluoride.* 2005; 38(3): 215-219.

12. KA Brenneman, SK Romanian, CM Rohde, DM Messing, SP Neil, LM Ganthier, ZS Stewart, SR Mantena, KM Shevlin, CG Leonard, SA Sokohomski, H Lin, DC Carraner, MI Jerson, L Tomlinson, Y Zhan, WF Bobroneski, SA Bailey, WM Vogue, DL Morris, LO Whiteley and JW Davis. Mechanistic investigations of test article-indued pancreatic toxicity at the endocrine-exocrine interface in rat. Toxicol Pathol. 2013; 42: 229-242.

13. GA Salam, EM Mehlab, M Shishtawy and I Zahrani. Ameliorative effects of selenium and vitamins C and E on chronic fluoride pancreatic toxicity: structural and ultrastructural changes in albino rats. J Am Sci. 2013; 9(3): 274-283.

14. FE Agha, MO El-Badry, DAA Hassan and AA Elraouf. Role of vitamin E in combination with methionine and L- carnosine against sodium fluoride-induced hematological, biochemical, DNA damage, histological and immunohistochemical changes in pancreas of albino rats. Life Sci J. 2012; 9(2): 1260-1275.

15. BS Pieta, A Paszczela, E Grucka-Mamczar, E Szaflarska-Stojko and E Birkner. The effect of antioxidative vitamins A and E and coenzyme Q on the morphological picture of lungs and pancreata of rats intoxicated with sodium fluoride. *Food Chem. Toxicol.* 2009; 47: 2544-2550.

16. M Suzuki, T Shimizes, T Kudo, H Shoji, Y Ohtshka and Y Yamashino. Octreotide prevents L-Asparaginase-induced pancreatic injury in rats. Exp Hematol. 2008; 36: 172-180.

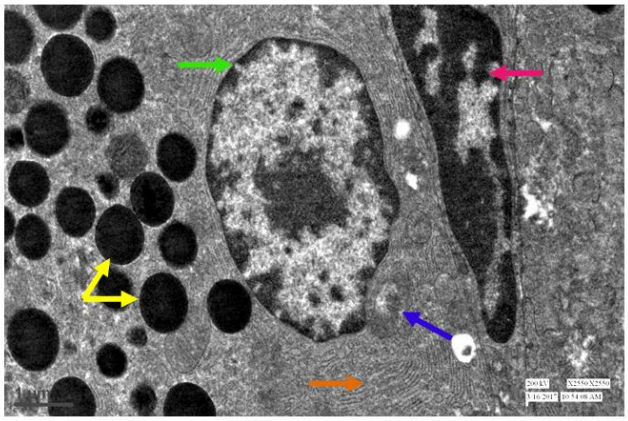

Figure 1: Transmission electron micrograph of pancreas of control rat showing euchromatic nucleus (↑) surrounded by rough endoplasmic reticulum (↑), mitochondria (↑), zymogen granules (↑) and a centroacinar cell (↑). X2550

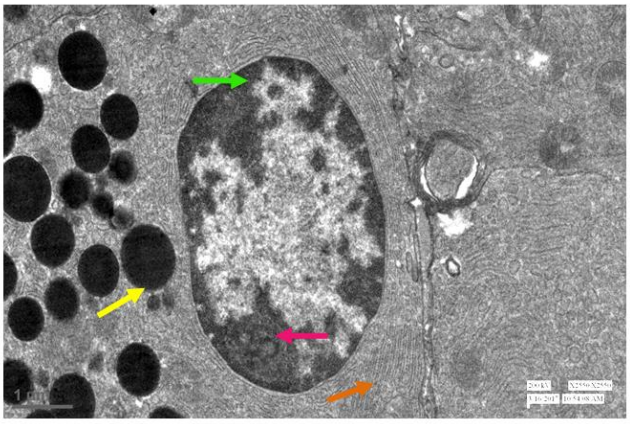

Figure 2: Transmission electron micrograph of pancreas of control rat showing nucleus (↑), nucleolus (↑), rough endoplasmic reticulum (↑) and zymogen granules (↑). X2550

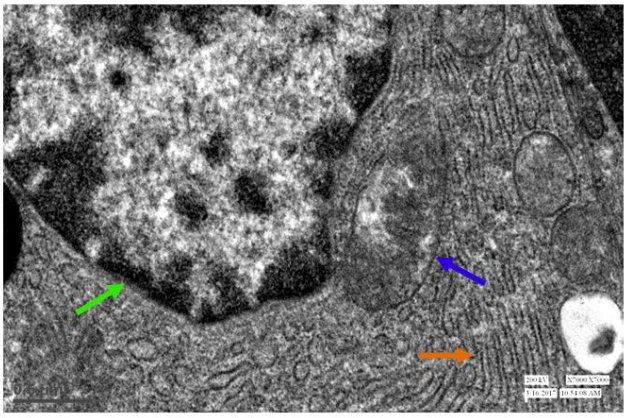

Figure 3: Transmission electron micrograph of pancreas of control rat showing magnified view of acinar cell having euchromatic nucleus (↑), cisternae of rough endoplasmic reticulum (↑) and mitochondria (↑) with visible cristae. X7000

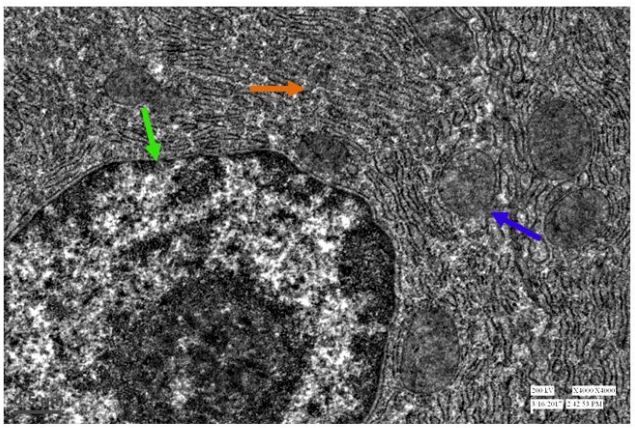

Figure 4: Transmission electron micrograph of pancreas of rat treated with 500 mg/kg b.w./day of leaf extract of *Boerhaavia diffusa* L. for 20 days showing an acinar cell with a nucleus (↑), rough endoplasmic reticulum (↑) and mitochondria (↑). X4000

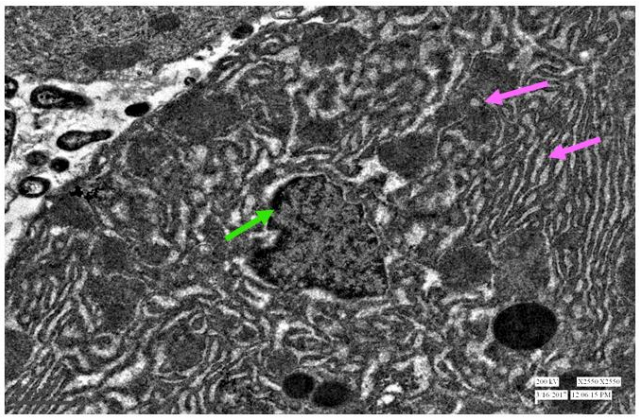

Figure 5: Transmission electron micrograph of pancreas of rat treated with 300 mg NaF/kg b.w./day for 40 days showing pyknotic nucleus (↑) with the rough endoplasmic reticulum exhibited dilations and globular shaped saccules (↑) in some parts. X2550

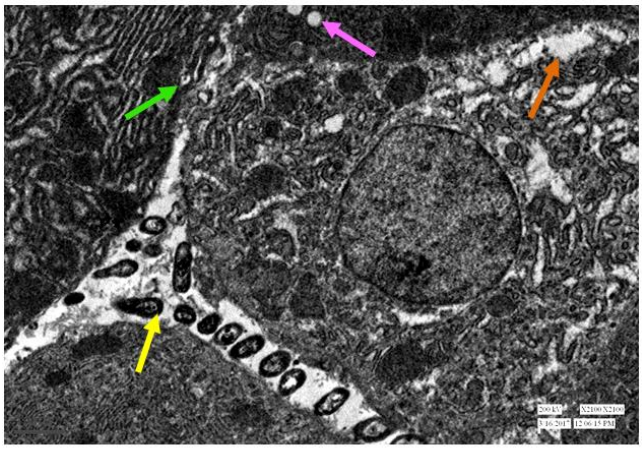

Figure 6: Transmission electron micrograph of pancreas of rat treated with 300 mg NaF/kg b.w./day for 40 days showing damaged acinar cell membranes ( ) and cellular infiltrations ( ) in the spaces. Cytoplasmic vacuolizations ( ) and vacuolated mitochondria ( ) are also visible. X2100

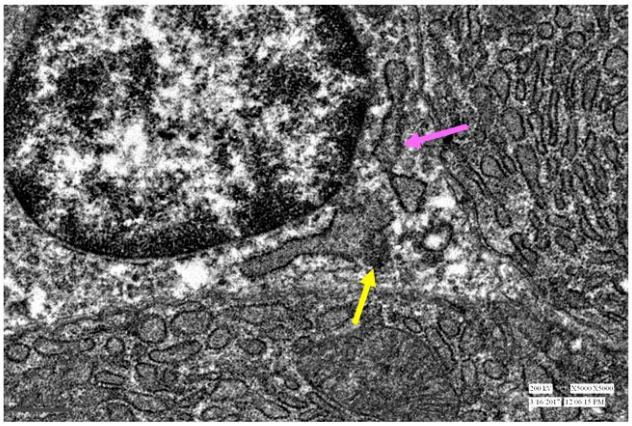

Figure 7: Transmission electron micrograph of pancreas of rat treated with 300 mg NaF/kg b.w./day for 40 days showing damaged endoplasmic reticulum ( ) and ribosomal aggregations ( ) in the acinar cell. X5000

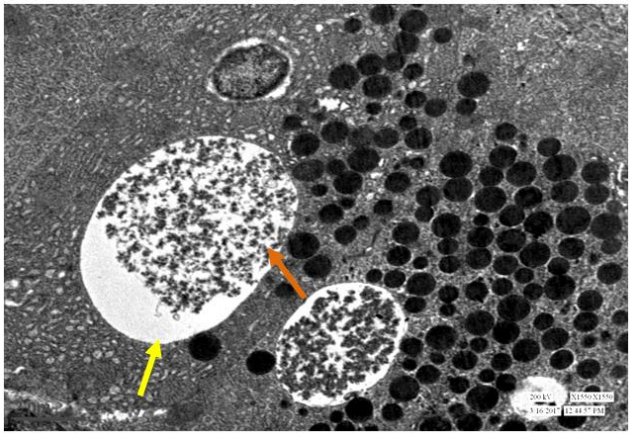

Figure 8: Transmission electron micrograph of pancreas of rat treated with 300 mg NaF/kg b.w./day for 40 days showing vesicles ( ) of variable sizes having amorphous materials ( ). X1550

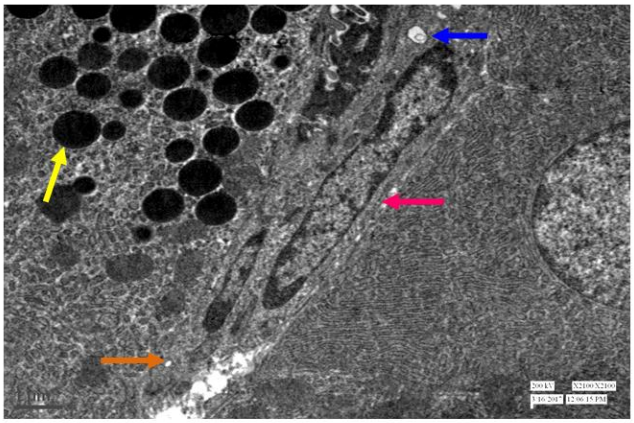

Figure 9: Transmission electron micrograph of pancreas of rat treated with 300 mg NaF/kg b.w./day for 40 days showing acinar cells with few zymogens ( ), autophagosomes( ) and cytoplasmic vacuolizations ( ). A centroacinar cell ( ) with elongated, heterochromatic nucleus is visible. X2100

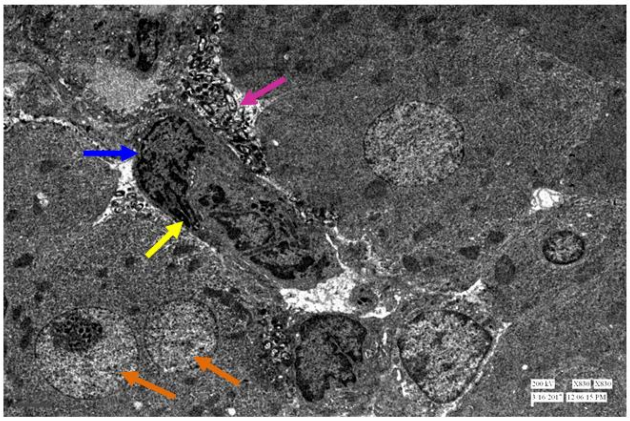

Figure 10: Transmission electron micrograph of pancreas of rat treated with 300 mg NaF/kg b.w./day for 40 days showing acinar cell with two nuclei ( ) and complete loss of zymogen granules. Spaces filled with cellular infiltrations and debris ( ), constricted capillary ( ) with degenerated endothelial cell ( ) and collagen deposition. X830

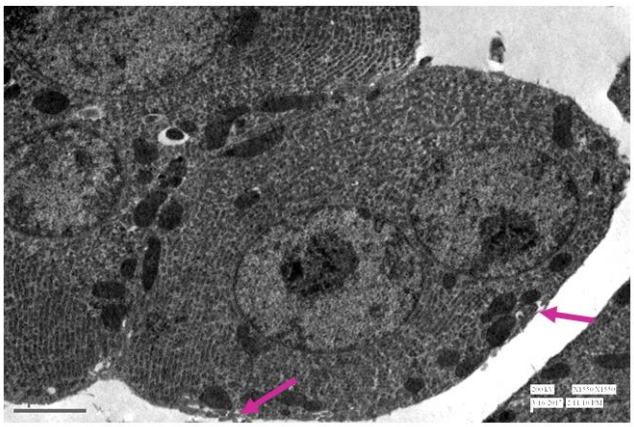

Figure 11: Transmission electron micrograph of pancreas of rat treated with 600 mg NaF/kg b.w./day for 40 days showing an acinar cell with irregular cell membrane ( ), two basal nuclei and complete loss of zymogen granules. X1550

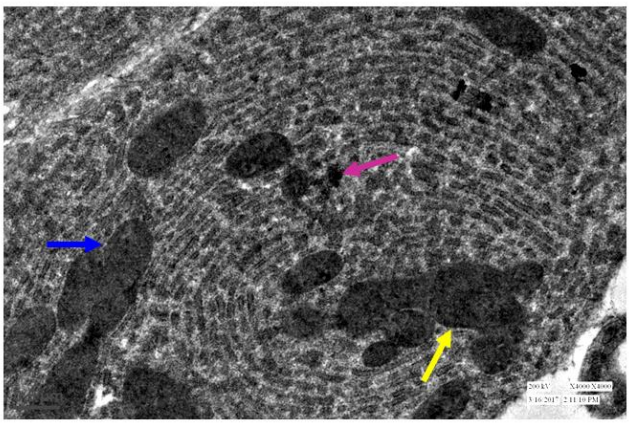

Figure 12: Transmission electron micrograph of pancreas of rat treated with 600 mg NaF/kg b.w./day for 40 days showing clumping ( ) , swelling and irregularly shaped mitochondria ( ). Ribosomal aggregations ( ) throughout the cytoplasm was also seen. X4000

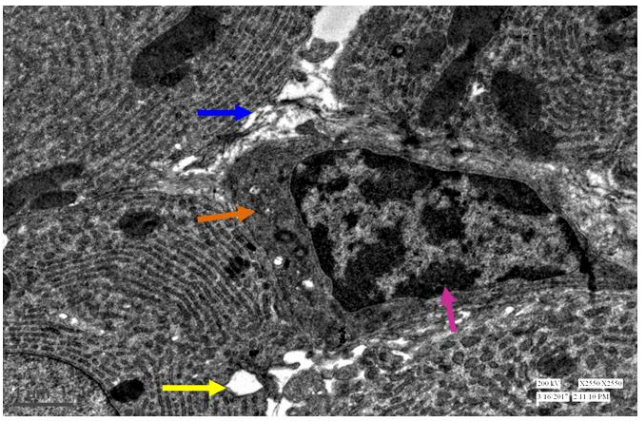

Figure 13: Transmission electron micrograph of pancreas of rat treated with 600 mg NaF/kg b.w./day for 40 days showing acinar cells with damaged cell membranes ( ) and cytoplasmic vacuolizations ( ). Centroacinar cell ( ) with heterochromatic, pyknotic nucleus with condensation and margination of chromatin materials ( ) was visible. X 2550

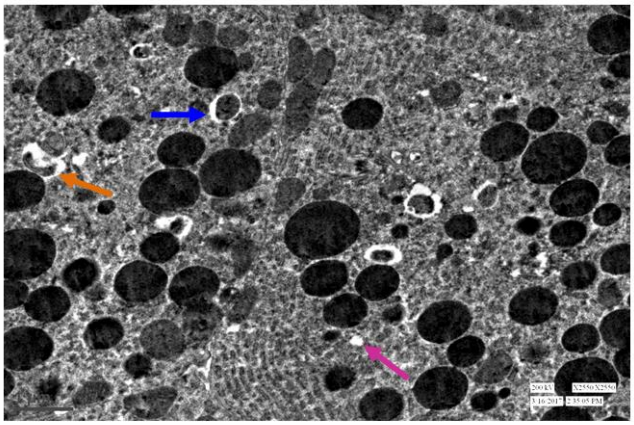

Figure 14: Transmission electron micrograph of pancreas of rat treated with 600 mg NaF/kg b.w./day for 40 days showing cytoplasmic vacuolizations ( ), mitochondrial swellings ( ) and formation of autophagosomes ( ) X 2550

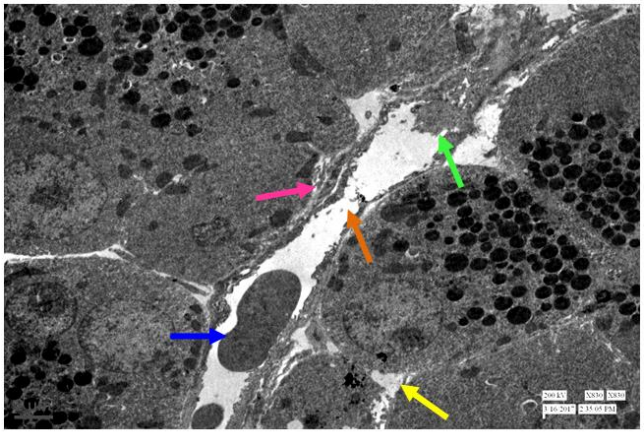

Figure 15: Transmission electron micrograph of pancreas of rat treated with 600 mg NaF/kg b.w./day for 40 days showing increased intercellular spaces ( ), damaged acinar membranes ( ), constricted capillary ( ) with degenerated erythrocytes ( ) and collagen deposition ( ). X 830

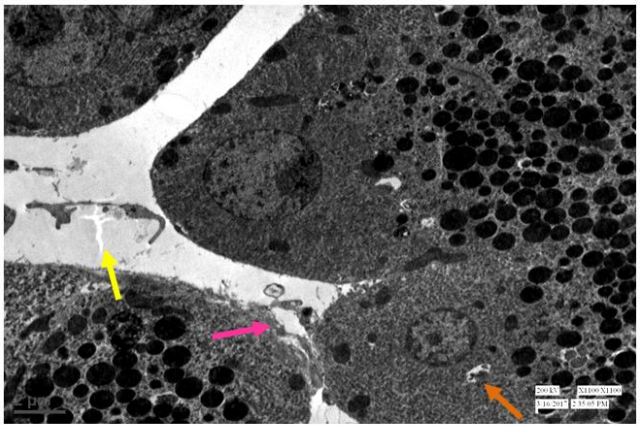

Figure 16: Transmission electron micrograph of pancreas of rat treated with 600 mg NaF/kg b.w./day for 40 days showing acinar cells with large intercellular spaces ( ), damaged cellular membranes ( ) and autophagosmes ( ) which were prominent in most damaged areas. X 1100

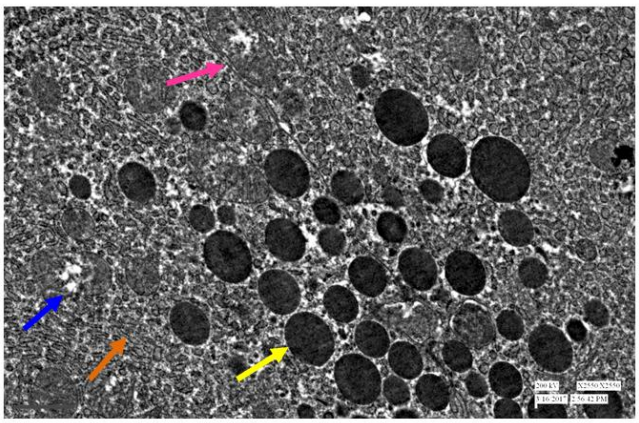

Figure 17: Transmission electron micrograph of pancreas of rat treated with 300 mg NaF/kg b.w./day for 40 days post-treated with 500 mg/kg b.w./day of leaf extract of *Boerhaavia diffusa* L. for 20 days showing less damaged membrane ( ), normal rough endoplasmic reticulum ( ), few damaged mitochondria ( ) but many has regained their shape and zymogen granules ( ) with normal number. X 2550

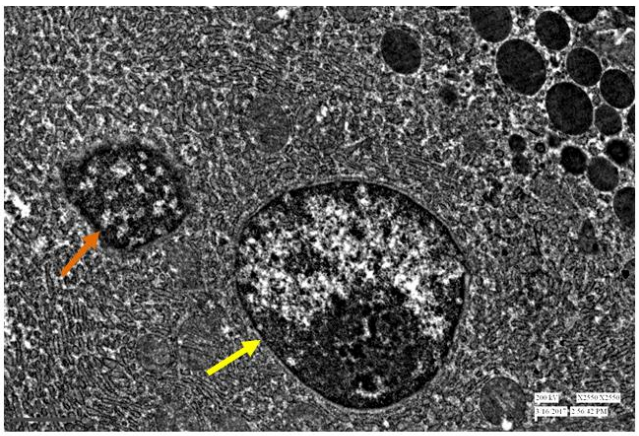

Figure 18: Transmission electron micrograph of pancreas of rat treated with 300 mg NaF/kg b.w./day for 40 days post-treated with 500 mg/kg b.w./day of leaf extract of *Boerhaavia diffusa* L. for 20 days showing round, euchromatic nucleus (↑) and a vesicle (↑) containing amorphous material which is reduced in size. X2550

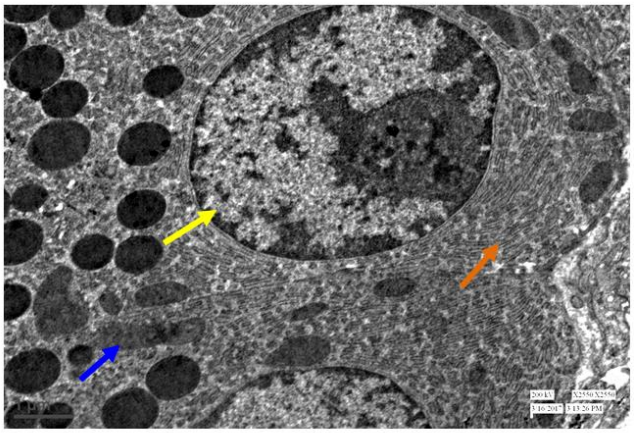

Figure 19: Transmission electron micrograph of pancreas of rat treated with 600 mg NaF/kg b.w./day for 40 days post-treated with 500 mg/kg b.w./day of leaf extract of *Boerhaavia diffusa* L. for 20 days showing basal nucleus (↑) with intact nuclear membrane, rough endoplasmic reticulum (↑), and normal mitochondria (↑). X 2550

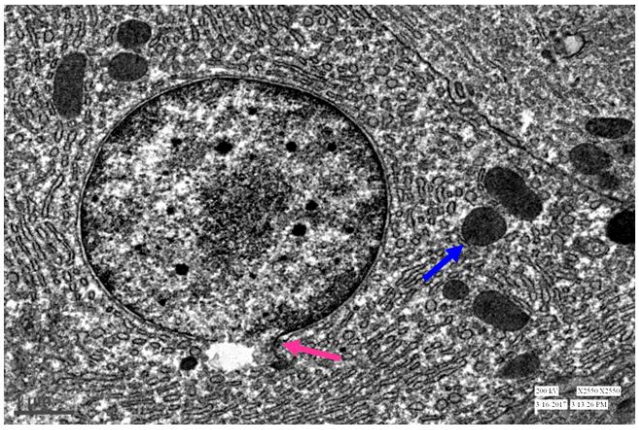

Figure 20: Transmission electron micrograph of pancreas of rat treated with 600 mg NaF/kg b.w./day for 40 days post-treated with 500 mg/kg b.w./day of leaf extract of *Boerhaavia diffusa* L. for 20 days showing slightly damaged nuclear membrane (↑). The mitochondrias (↑) were elongated , rounded and without clumping. X 2550