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ULTRASTRUCTURAL AND FUNCTIONAL CHANGES IN THE RAT COLON EXPOSED TO FLUORIDE AND THE POSSIBLE AMELIORATIVE ROLE OF *BOERHAAVIA DIFFUSA* L.

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ABSTRACT
This experimental study investigates the potential therapeutic effects of *Boerhaavia diffusa* L. leaf extract on the colon of rats exposed to sodium fluoride. Wistar albino rats were divided into six groups. Control group (1) received 1ml of deionized water for a period of 40 days while the control groups (2 and 3) received 250 and 500 mg/kg b.w./day of leaf extract of *Boerhaavia diffusa* L. for 20 days. The experimental group was treated with 600 mg/kg bw/day of sodium fluoride by oral gavage. The fluoride treated groups were post-treated with 250 and 500 mg/ kg b.w./ day of *Boerhaavia diffusa* L. for 20 days respectively. The animals were sacrificed at the end of experimentation period and the colon tissue was taken out and processed for biochemical, histopathological and electron microscopic examination. The results revealed that the rats treated with 600 mg of sodium fluoride showed scattered and complete loss of villi. Hypertrophy of cells, inflammation, damaged crypts, irregular nucleus and decreased villi height were also observed. After post-treatment with leaf extract of *Boerhaavia diffusa* L., the colon of fluoridated rats showed almost normal structures with reappearance of villi, improved structure of villus tips and increase in number of cells and indicated that the administration of leaf extract was proven to lessen the detrimental effects of fluoride. The findings indicate that the leaf extract of *Boerhaavia diffusa* L. reduces the disruptions in the colon of rat caused by fluoride.

KEYWORDS: Albino rats, Antioxidant enzymes, *Boerhaavia diffusa* L., Colon, Electron microscopy, Light microscopy, Sodium fluoride.

ABBREVIATIONS USED: Catalase (CAT), Glutathione peroxidase (GPx), Glutathione reductase (GR), Glutathione-S-transferase (GST), Malondialdehyde (MDA), Mitochondrial Superoxide dismutase (SOD) and Reduced glutathione (GSH)

INTRODUCTION
Excessive fluoride exposure has been linked to various systemic toxic effects, including skeletal fluorosis, nephrotoxicity, and neurotoxicity. The gastrointestinal tract, particularly the colon, is susceptible to fluoride-induced damage due to its exposure to ingested fluoride. Studies have shown that high fluoride levels can lead to oxidative stress, inflammation, and structural changes in colonic tissues.[1]

The colon, as a critical component of the digestive system, is particularly susceptible to fluoride-induced damage, which can manifest through various pathological and functional impairments. The impact of fluoride on the gastrointestinal tract, particularly the colon, has been the subject of several studies. The colon is an essential organ responsible for water absorption, electrolyte balance, and the formation of fecal matter. Fluoride can induce oxidative stress, inflammation, and cellular damage in the colon, which may contribute to gastrointestinal disorders.[2]

Natural remedies such as *Boerhaavia diffusa* L., a plant known for its anti-inflammatory and antioxidant properties, have shown promise in counteracting fluoride-induced damage. Research suggests that *Boerhaavia diffusa* L. may help restore colonic structure and function by reducing oxidative stress and inflammation.[3]

MATERIALS AND METHODS
Wistar albino rats weighing 150-200 g were housed in polypropylene cages with stainless steel grill tops, fed with standard commercial rat pellet diet (Hindustan Lever Limited, Mumbai, India) and water was given *ad*

libitum. The leaf extract of *Boerhaavia diffusa* L. was prepared by the method of Narendhirakannan *et al*.[4]

Experimental design

Wistar albino rats were divided into six groups. Control group (1) received 1ml of deionized water for a period of 40 days while the control groups (2 and 3) received 250 and 500 mg/kg b.w./day of leaf extract of *Boerhaavia diffusa* L. for 20 days. The experimental group was treated with 600 mg/kg b.w./day of sodium fluoride by oral gavage. The fluoride treated groups were post-treated with 250 and 500 mg/kg b.w./day of *Boerhaavia diffusa* L. for 20 days respectively. The animals were sacrificed at the end of experimentation period and the colon tissue was taken out and processed for biochemical, histopathological, transmission and scanning electron microscopy.

Biochemical analysis

The colon tissue from experimental group was homogenized in a 0.1 M phosphate buffer with a pH of 7.4. The homogenate was then centrifuged at 10,000 rpm for 10 minutes. The resulting supernatant was collected and used for the biochemical assays.

Fluoride analysis

The concentration of fluoride in the colon tissue was estimated by the method of Harwood.[5]

Detection of oxidative stress parameters

The level of MDA[6] and GSH[7] in colon tissue was assessed.

Assay of antioxidant enzymes

The activity of catalase[8], glutathione peroxidase[9], mitochondrial superoxide dismutase[10], glutathione-S-transferase[11] and glutathione reductase[12] was determined in the colon tissue of control and treated rats.

Histopathological examination

Colonic tissue from the control group, fluoridated rats, and fluorotic rats treated with leaf extract were collected and fixed in Bouin's fluid for 24 hours. After washing in 70% alcohol, the tissues were dehydrated using 80% and 90% alcohol and tertiary butyl alcohol for 6 hours. They were then cleared in amyl acetate and embedded in paraffin wax. Serial sections of 7 µm thickness were cut and stained with haematoxylin and eosin.[13] Histopathological changes were examined under a research binocular microscope and microphotographed.

Scanning electron microscopic examination

Small samples of colon tissue from the control and experimental rats were collected, washed with 0.1M phosphate buffer at pH 7.4, and then fixed in a 2.5% glutaraldehyde solution buffered with 0.1M phosphate buffer at pH 7.4 for 24 hours at 4°C, following the method of Karnovsky.[14] The tissue samples were then washed with the same phosphate buffer and post-fixed in 1% osmium tetroxide for 2 hours at 4°C. The tissues underwent dehydration through a series of increasing concentrations of acetone and were then dried using a critical point dryer. The specimens were mounted onto stubs, coated with gold using a sputter coater (Balzer Union SCD 020), and the images were captured using a scanning electron microscope (JEOL JSM-6510).

Transmission electron microscopic examination

Small pieces of colonic tissue were fixed in 2.5% phosphate buffered glutaraldehyde (pH 7.4) at 4°C for 24 hours and washed 3-4 times in 0.1M phosphate buffer. Tissues were post-fixed in 1% osmium tetraoxide, 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 were examined under transmission electron microscope (Tecnai 2 Fei Company, The Netherlands) and photomicrographed.

Statistical analysis

Results were expressed as Mean ± SD. All analysis was performed using SPSS 20.0 statistical software (IBM). Statistical significance of difference between experimental groups was evaluated by one way ANOVA followed by Post hoc Tukey's HSD multiple comparison test. The results were considered significant at $P< 0.05$. The relationship between level of colon tissue fluoride and activities of enzymes were determined by Pearson's bivariate correlation and simple linear regression analysis.

RESULTS

Fluoride: A significant ($P<0.001$) increase in the level of fluoride was observed in fluoridated rats as compared to control rats. The fluorotic rats post-treated with 250 and 500 mg of leaf extract of *Boerhaavia diffusa* L. showed significant ($P<0.001$) decline in the level of fluoride (Fig.1).

Malondialdehyde: The mean level of MDA after administration of sodium fluoride showed a significant ($P<0.001$) increase in colon ($F=177.673$; Fig.2) as compared to control.

Post-hoc Bonferroni multiple comparison tests indicated a significant ($P<0.001$) rise in the levels of MDA between groups (95% CI= -4.536 to -3.400) in colon tissue of fluoride-treated rats.

Reduced glutathione: The mean level of glutathione peroxidase (GSH) in the colon tissue of test rats significantly ($F=420.952$, $P<0.001$; Fig.3) declined as compared to control after 40 days of fluoride exposure.

Post-hoc Bonferroni multiple comparison tests after ANOVA exhibited a significant ($P<0.001$) decline in the levels of GSH in colonic tissue between groups (95% CI= 3.942 to 4.822) treated with fluoride for 40 days.

Antioxidant enzymes

Catalase: The activity of catalase (CAT) decreased significantly in colon (F=232.353; Fig.4) of rats treated with 600 mg of NaF in comparison to control.

Post-hoc Bonferroni multiple comparison test after ANOVA indicated significant (P< 0.001) decrease in the activity of CAT in colon tissue between groups (95% CI= 1.740 to 2.295) treated with fluoride for 40 days.

Mitochondrial Superoxide Dismutase: A significant (P<0.001) decrement was observed in the activity of superoxide dismutase in colon (F= 465.672; Fig.5) of fluoride treated group as compared to control.

Post-hoc Bonferroni multiple comparison test after ANOVA indicated significant (P< 0.001) decrease in the activity of SOD in colon tissue between groups (95% CI= 3.621 to 4.536) treated with fluoride for 40 days.

Glutathione Peroxidase: The activity of glutathione peroxidase decreased significantly (P<0.001) in the colon (F=228.133; Fig.6) of fluoridated rats as compared to control.

Post-hoc Bonferroni multiple comparison test after ANOVA showed significant (P< 0.001) decrease in the activity of GPx in colon tissue between groups (95% CI= 3.078 to 3.932) treated with fluoride for 40 days.

Glutathione-S-transferase

The activity of glutathione-S-transferase decreased significantly (P<0.001) in colon (F=115.277; Fig.7) of fluorotic rats as compared to control.

Post-hoc Bonferroni multiple comparison test after ANOVA indicated significant (P< 0.001) decrease in the activity of GST in colon tissue between groups (95% CI= 2.132 to 3.030) treated with fluoride for 40 days.

Glutathione reductase

The activity of glutathione reductase decreased significantly (P<0.001) in colon (F=617.894; Fig.8) of fluoridated rats in comparison to control.

Post-hoc Bonferroni multiple comparison test after ANOVA indicated significant (P< 0.001) decrease in the activity of glutathione reductase in colon tissue between groups (95% CI= 3.981 to 4.733) treated with fluoride for 40 days.

Post-treatment analysis

After mitigation with *Boerhaavia diffusa* L., the level of malondialdehyde decreased (95% CI = 4.608 to 0.447) and reduced glutathione increased (95% CI = -3.966 to -1.086) in post-treated groups as compared to respective fluoride group. The activity of antioxidant enzymes viz., catalase (95% CI = -1.756 to -0.726), superoxide dismutase (95% CI= -3.563 to -0.549), glutathione peroxidase (95% CI= -3.057 to -1.363), glutathione-S-transferase (95% CI= -2.078 to -1.318) and glutathione reductase (95% CI= -4.028 to -1.267) elevated in post-treated groups.

Correlation Analysis

Pearson's bivariate correlation and simple linear regression analysis revealed a significant (P<0.001) positive relationship between levels of colonic tissue fluoride and MDA (R^2= 0.964; Pearson r= 0.982; Fig.9) and negative relationship with GSH in test rats (R^2= 0.965; Pearson r = -0.982; Fig.10). However, there was significant (P<0.001) negative relationship between levels of colon tissue fluoride and activities of CAT (R^2= 0.963; Pearson r = -0.982; Fig.11), MnSOD (R^2= 0.913; Pearson r = -0.956; Fig.12), GPx (R^2= 0.957; Pearson r = -0.978; Fig.13), GST (R^2= 0.954; Pearson r = -0.977; Fig.14) and GR (R^2= 0.953; Pearson r = -0.976; Fig.15) in rats after 40 days of fluoride exposure.

Histopathological examination

The colonic mucosa of control rat consisted of different layers, i.e; mucosa, submucosa, muscularis and serosa. (Fig.16). The colonic tissue from the control rat revealed goblet cells and presence of closely packed microvilli. (Fig.17). The colon of fluorotic rats showed disrupted microvilli. Some microvilli were broken and scattered while in some there was partial loss of villi. (Fig.18,19). The colon of fluoridated rats exhibited depletion of goblet cells. (Fig.20) alongwith inflammation with widened spaces. (Fig.21). Complete villous atrophy and disrupted layers was seen in fluoridated rats. (Fig.22). Dilation of crypts revealing abnormal structure and inflammation of the mucus layer was present.(Fig.23).

The colon of fluoridated rats concomitantly post-treated with 250 mg of leaf extract for 20 days showed increase in the villus height. (Fig.24). The colonic mucosa of fluoridated rats post-treated with 500 mg/kg b.w./day of leaf extract had regular closely packed villi, muscular layers and goblet cells. (Fig.25).

Scanning electron microscopy

SEM examination of colon of control rat revealed uniform arrangement of villi and an intact mucus layer. (Fig.26). The colon exhibited leaf-like and doughnut-shaped villi, some crypt openings were also visible. (Fig.27).

In the colon of rat treated with 600 mg of sodium fluoride, the villi appeared damaged with fissures at their tips. (Fig.28). The colon showed broken and distorted villi, along with mucus discharge from the crypt orifices. (Fig.29). The colon displayed cryptal units and enlarged areas with narrow openings. (Fig.30). Large necrotic regions and mucus clumping were also observed in the colon of fluorotic rats. (Fig.31). Some roughly circular gaps of varying sizes were observed in the surface layer of the basal region. At certain sites, there were extravasations of red blood cells. (Fig.32). In the colon of fluorotic rats, damaged villi, desquamation of

epithelial cells, and disruptions in the surface layer characterized by uneven alterations were noted. (Fig.33).

In the colon of post-treated rats, epithelial cells with long and short microvilli (Fig.34), and mucus secretion in the surface layer were noted. (Fig.35).

Transmission electron microscopy

The colon of the control rat, highlighted the presence of large cells, further identified as macrophages, stretched with the long and thin processes in the intestinal tissue. (Fig.36). Mast cells were present. (Fig.37).

The colon of the fluorotic rat showed significant alterations in cellular architecture. There were visible signs of injury, including aberrant endoplasmic reticulum. Furthermore, irregularly shaped heterochromatic nuclei and the intracellular spaces that appeared narrower than usual were also visible. (Fig.38). Microvilli appeared fragmented and reduced in number with somewhere complete loss. Cytoplasmic vacuolations were notable signs of cell degeneration. Moreover, the nuclei had clumping of chromatin. (Fig.39). The muscles showed disruptions of surface layer. (Fig.40). The muscle cells were almost devoid of mucus. (Fig.41).

The colon of the post treated rats highlighted the presence of basal oval nuclei, and a continuous network of the rough endoplasmic reticulum. The cytoplasm was dispersed with secretory granules, indicating increased secretory activity. (Fig.42). Moreover, lipid droplets and golgi bodies were present. (Fig.43).

RESULTS

FLUORIDE

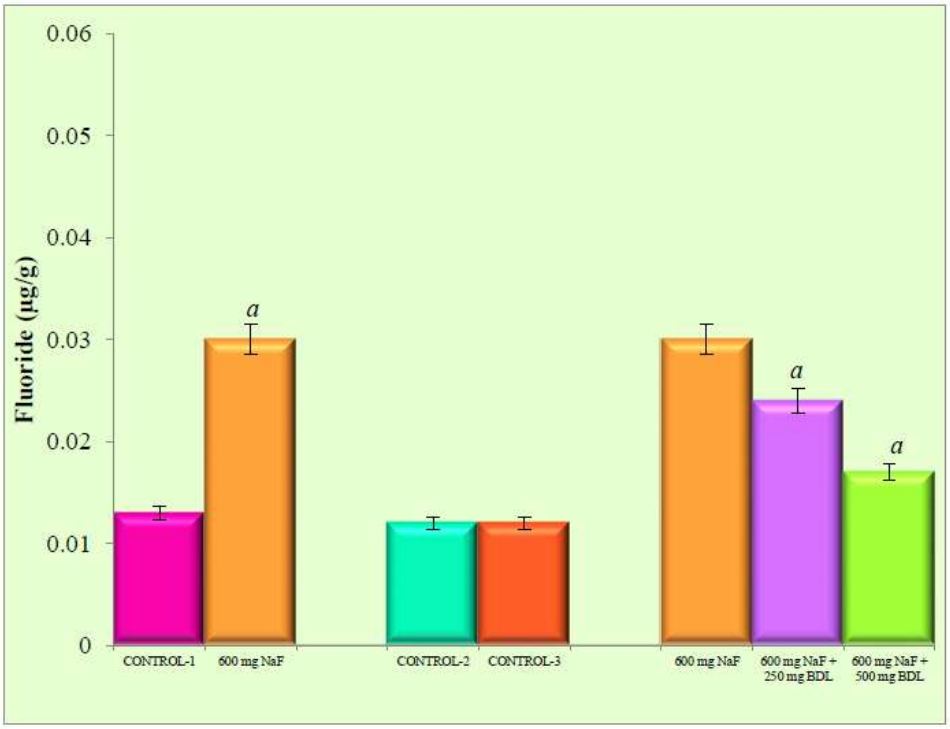

Fig. 1: Mean level of fluoride (µg/g) in colon of control and fluoride treated rats and post-treated with leaf extract of *Boerhaavia diffusa* L.; *aP< 0.001* value is significantly different as compared to NaF treated group and Group 600 mg NaF compared with post-treated groups. One way ANOVA followed by Post-hoc Bonferroni multiple comparison test.

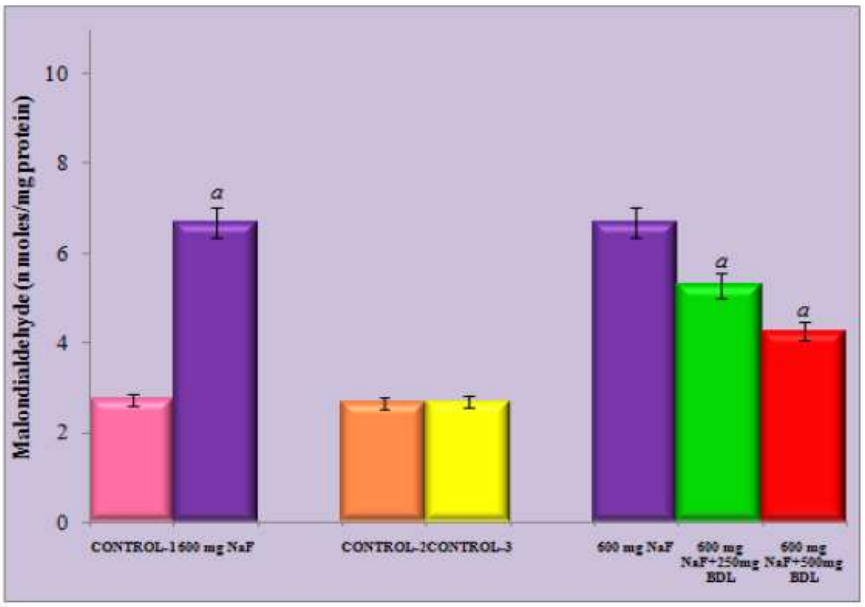

Fig. 2: Mean level of malondialdehyde (MDA) (n moles/mg protein) in colon of control and fluoride treated rats and post-treated with leaf extract of *Boerhaavia diffusa* L.; *aP< 0.001* value is significantly different as compared to NaF treated group and Group 600 mg NaF compared with post-treated groups. One way ANOVA followed by Post-hoc Bonferroni multiple comparison test.

REDUCED GLUTATHIONE

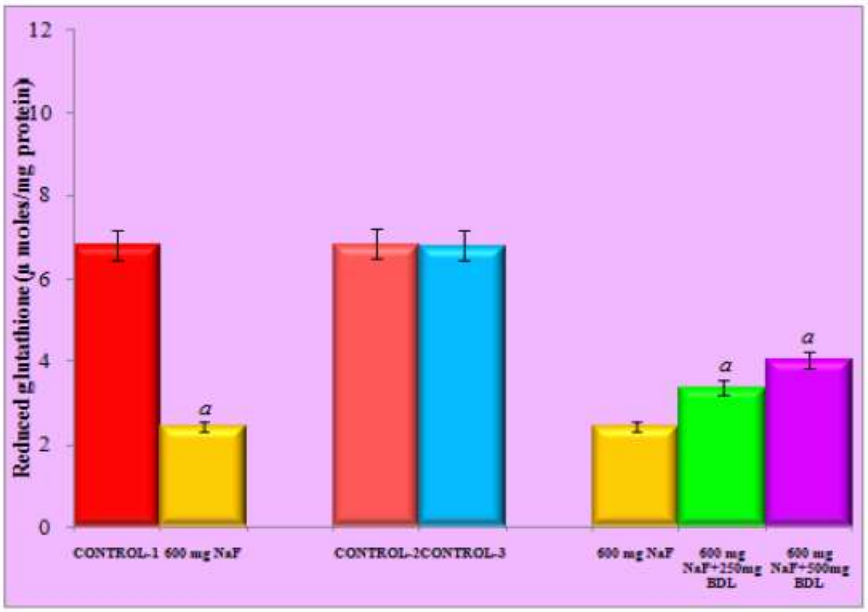

Fig. 3: Mean level of reduced glutathione (µ moles/mg protein) in colon of control and fluoride treated rats and post-treated with leaf extract of *Boerhaavia diffusa* L.; *aP< 0.001* value is significantly different as compared to NaF treated group and Group 600 mg NaF compared with post-treated groups. One way ANOVA followed by Post-hoc Bonferroni multiple comparison test.

ANTIOXIDANT ENZYMES
CATALASE

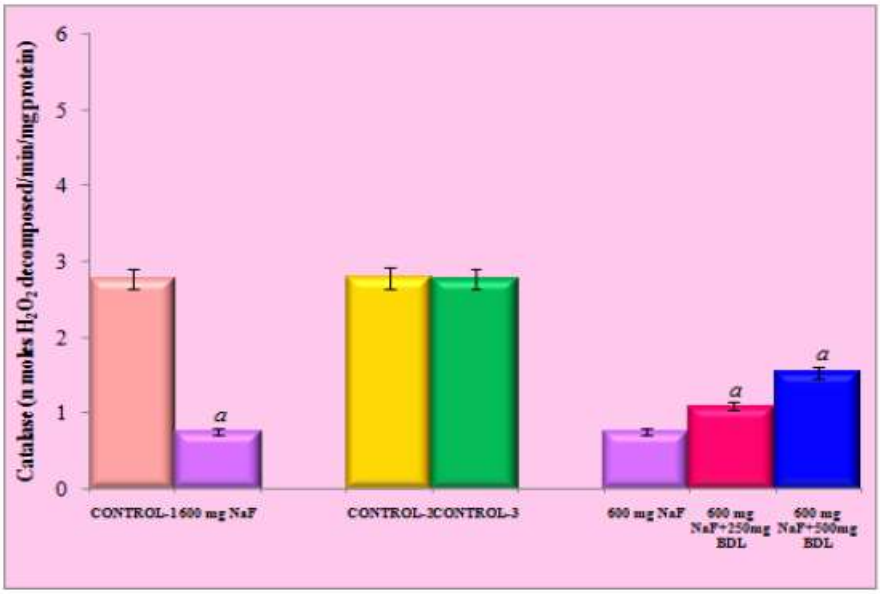

Fig. 4: Mean activity of catalase (CAT) (n moles H2O2 decomposed/min/mg protein) in colon of control and fluoride treated rats and post-treated with leaf extract of *Boerhaavia diffusa* L.; *aP< 0.001* value is significantly different as compared to NaF treated group and Group 600 mg NaF compared with post-treated groups. One way ANOVA followed by Post-hoc Bonferroni multiple comparison test.

SUPEROXIDE DISMUTASE

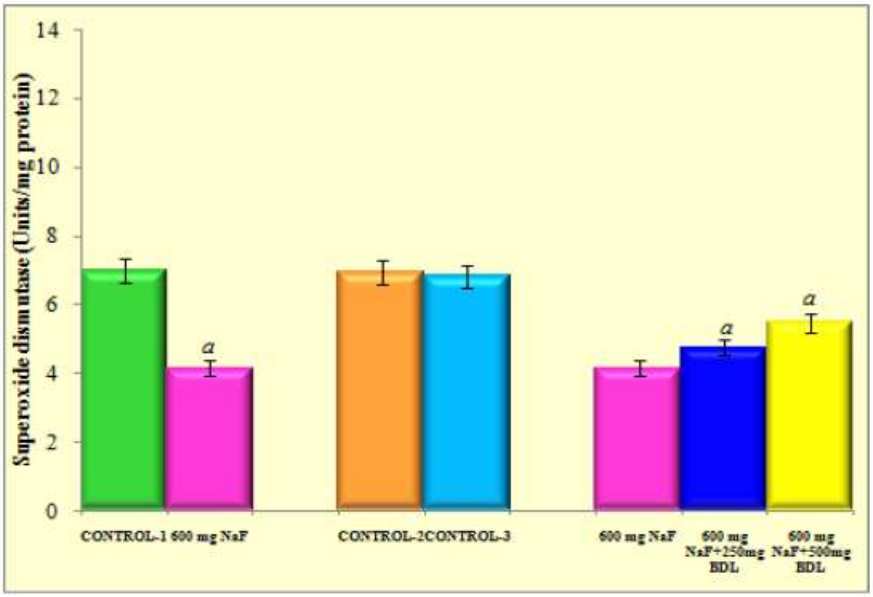

Fig. 5: Mean activity of superoxide dismutase (SOD) (Units/mg protein) in colon of control and fluoride treated rats and post-treated with leaf extract of *Boerhaavia diffusa* L.; *aP< 0.001* value is significantly different as compared to NaF treated group and Group 600 mg NaF compared with post-treated groups. One way ANOVA followed by Post-hoc Bonferroni multiple comparison test.

Glutathione peroxidase

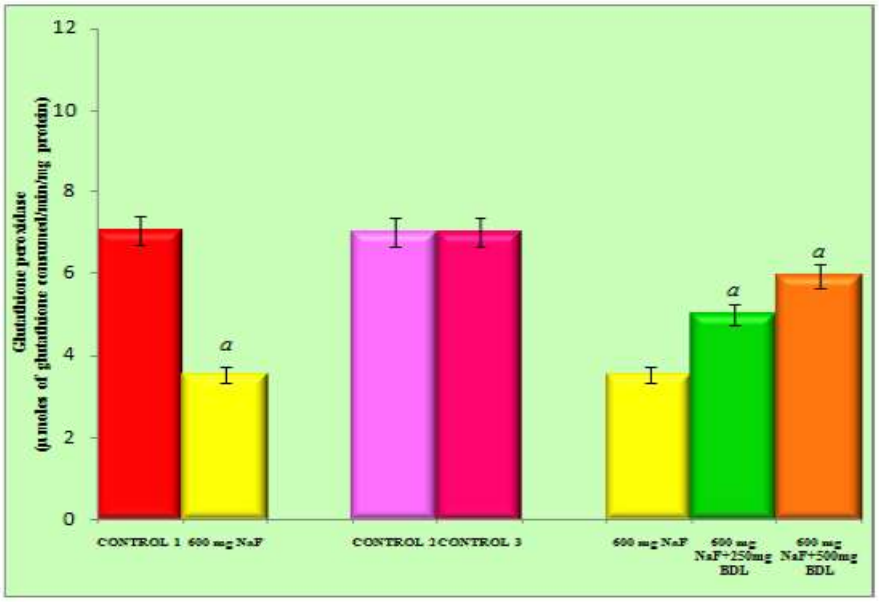

Fig. 6: Mean activity of glutathione peroxidase (GPx) (µ moles of glutathione consumed/min/mg protein) in colon of control and fluoride treated rats and post-treated with leaf extract of *Boerhaavia diffusa* L.; *aP< 0.001* value is significantly different as compared to NaF treated group and Group 600 mg NaF compared with post-treated groups. One way ANOVA followed by Post-hoc Bonferroni multiple comparison test.

Glutathione-S-transferase

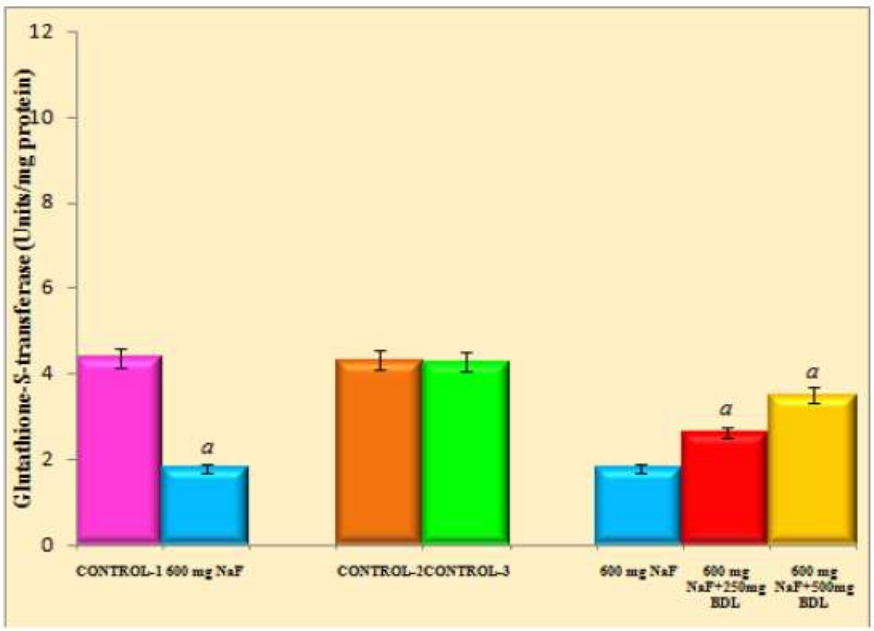

Fig. 7: Mean activity of glutathione-S-transferase (GST) (Units/mg protein) in colon of control and fluoride treated rats and post-treated with leaf extract of *Boerhaavia diffusa* L.; *aP< 0.001* value is significantly different as compared to NaF treated group and Group 600 mg NaF compared with post-treated groups. One way ANOVA followed by Post-hoc Bonferroni multiple comparison test.

Glutathione reductase

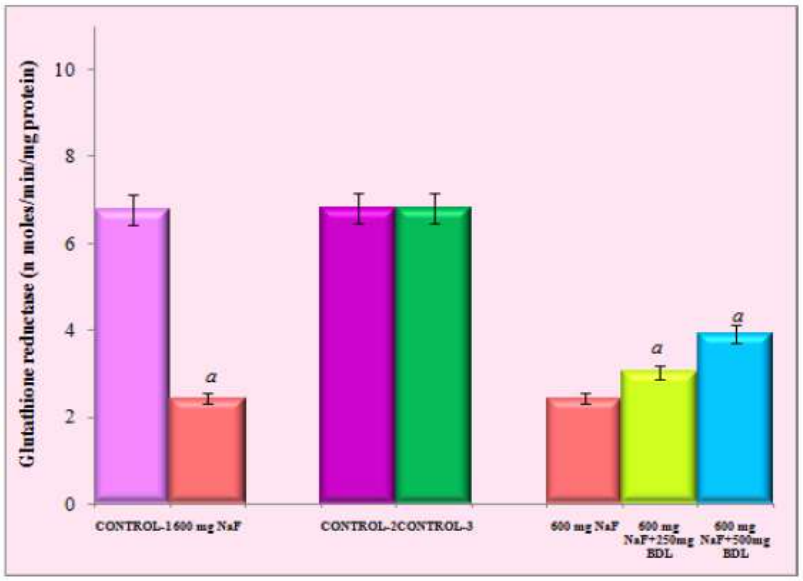

Fig. 8: Mean activity of glutathione reductase (n moles/min/mg protein) in colon of control and fluoride treated rats and post-treated with leaf extract of *Boerhaavia diffusa* L.; *aP< 0.001* value is significantly different as compared to NaF treated group and Group 600 mg NaF compared with post-treated groups. One way ANOVA followed by Post-hoc Bonferroni multiple comparison test.

Correlation analysis

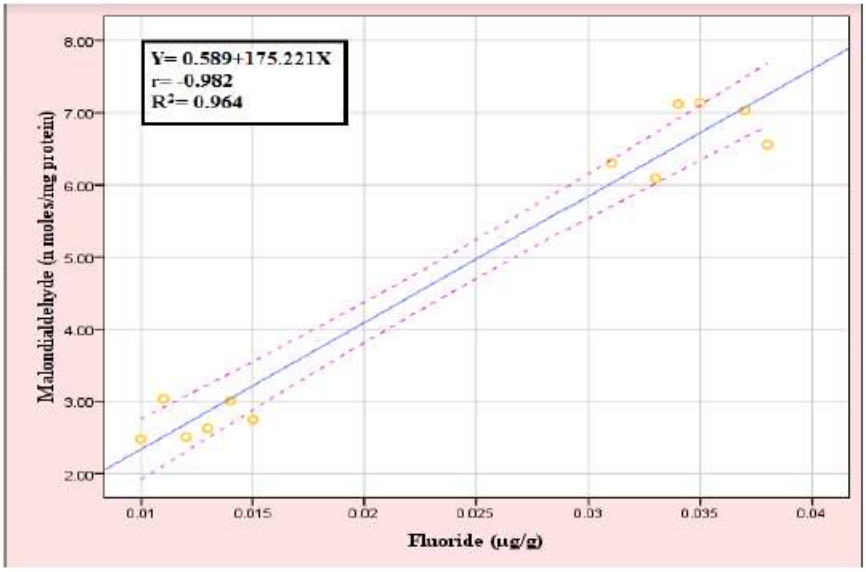

Fig. 9: Scatterplot showing Pearson's bivariate correlation and simple linear regression between levels of colon fluoride (µg/g) and malondialdehyde (MDA) (n moles/mg protein) in test rats after 40 days of fluoride treatment.

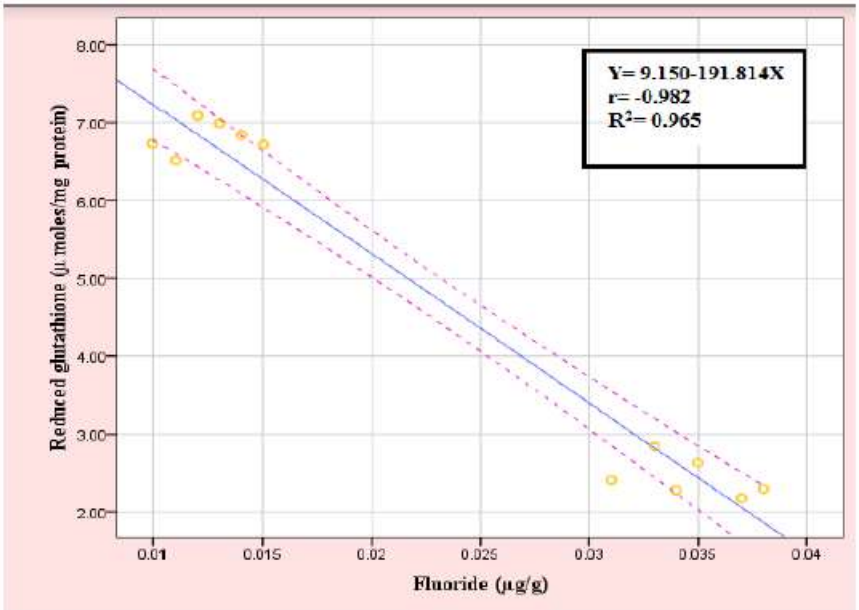

Fig. 10: Scatterplot showing Pearson's bivariate correlation and simple linear regression between levels of colon fluoride (µg/g) and reduced glutathione (µ moles/mg protein) in test rats after 40 days of fluoride treatment.

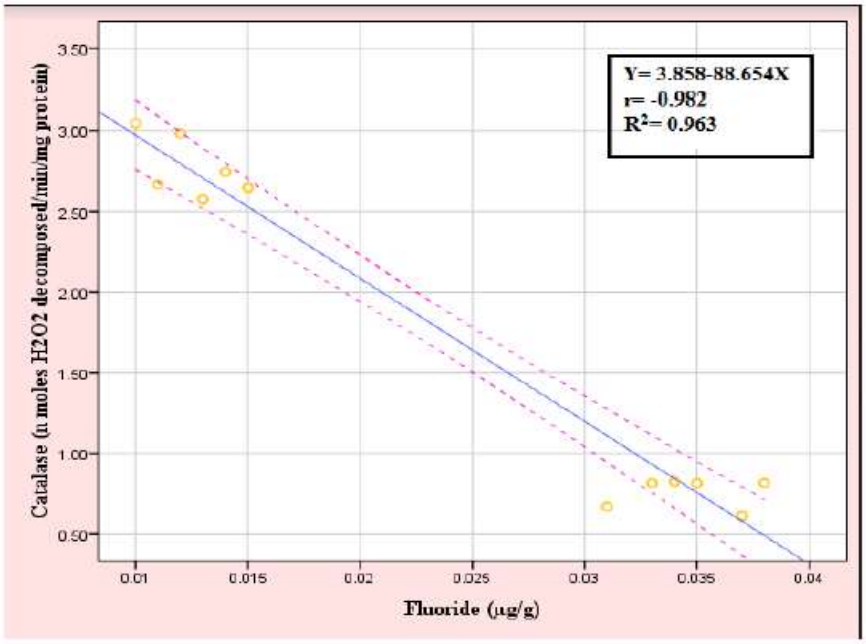

Fig. 11: Scatterplot showing Pearson's bivariate correlation and simple linear regression between levels of colon fluoride (µg/g) and activity of catalase (CAT) (n moles H2O2 decomposed/min/mg protein) in test rats after 40 days of fluoride treatment.

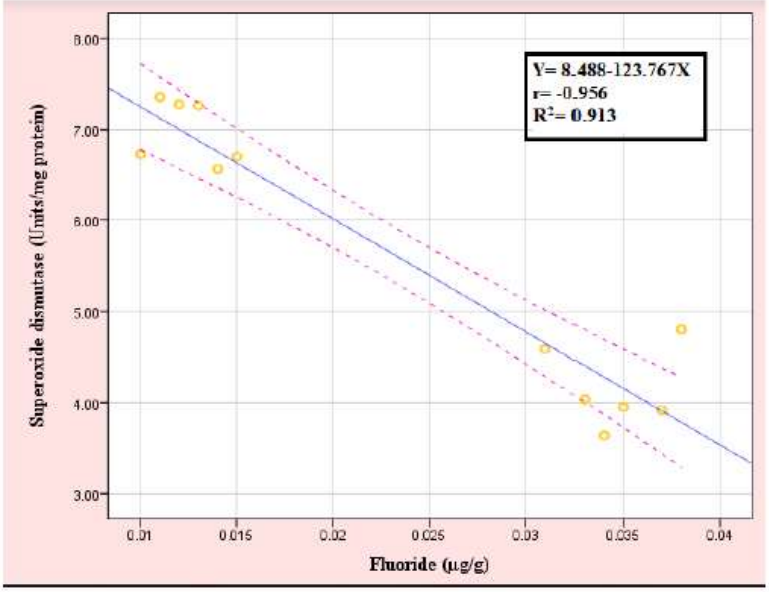

Fig. 12: Scatterplot showing Pearson's bivariate correlation and simple linear regression between levels of colon fluoride (µg/g) and activity of superoxide dismutase (SOD) (Units/mg protein) in test rats after 40 days of fluoride treatment.

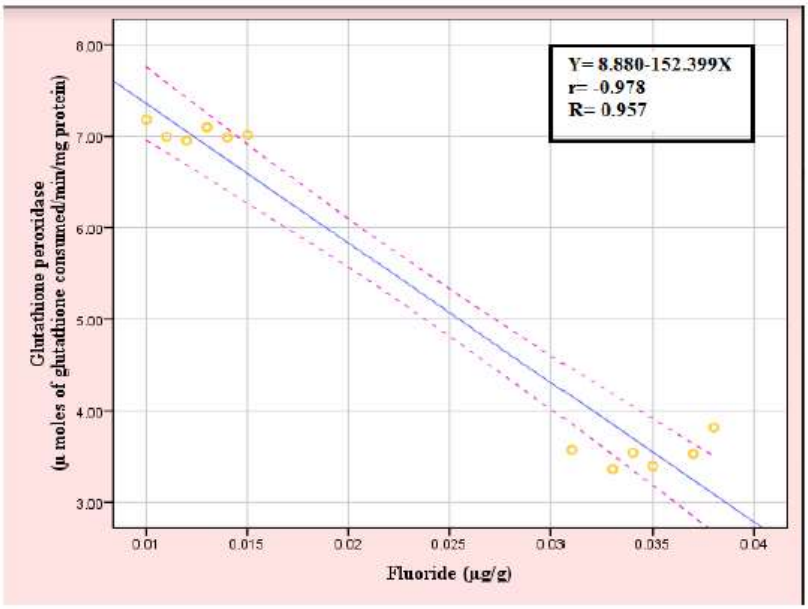

Fig. 13: Scatterplot showing Pearson's bivariate correlation and simple linear regression between levels of colon fluoride (µg/g) and activity of glutathione peroxidase (GPx) (µ moles of glutathione consumed/min/mg protein) in test rats after 40 days of fluoride treatment.

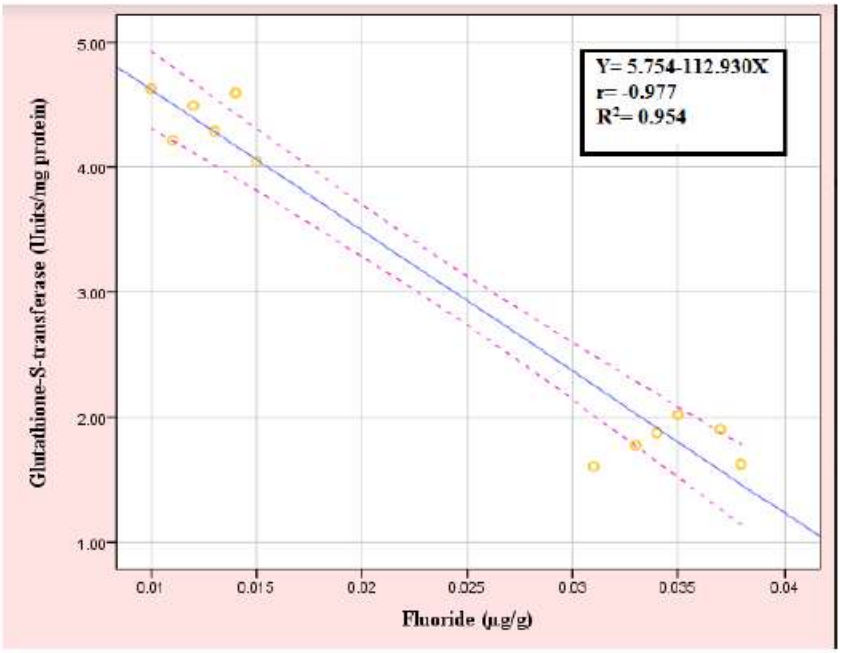

Fig. 14: Scatterplot showing Pearson's bivariate correlation and simple linear regression between levels of colon fluoride (µg/g) and activity of glutathione-S-transferase (GST) (Units/mg protein) in test rats after 40 days of fluoride treatment.

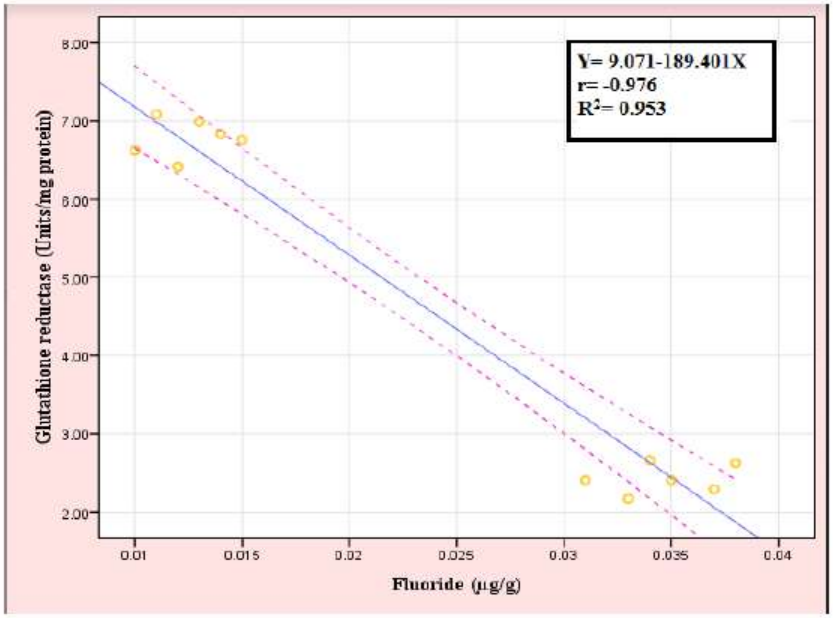

Fig. 15: Scatterplot showing Pearson's bivariate correlation and simple linear regression between levels of colon fluoride (µg/g) and activity of glutathione reductase (n moles/min/mg protein) in test rats after 40 days of fluoride treatment.

HISTOPATHOLOGICAL EXAMINATION

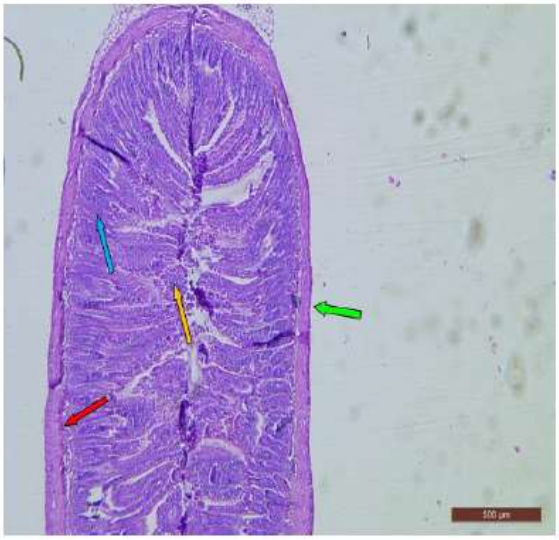

Fig. 16: T.S. of colon of control rat showing the distinctive layers i.e; mucosa (⇧), submucosa (⇧), muscularis mucosa (⇧) and serosa (⇧). H&E× 40

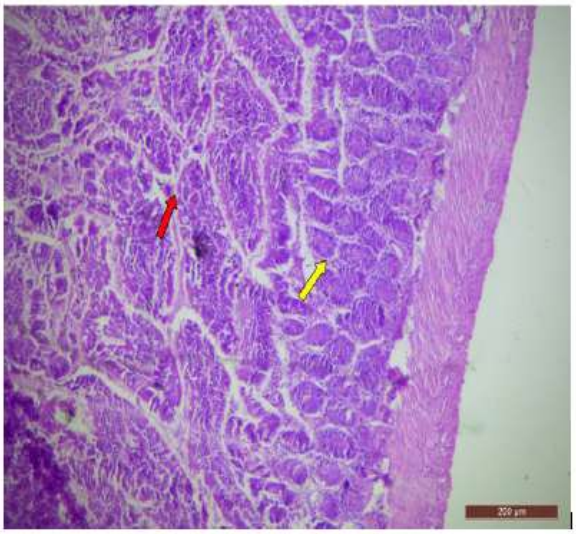

Fig. 17: T.S. of colon of control rat showing closely packed microvilli (⇧) and well developed goblet cells (⇧). H&E× 100

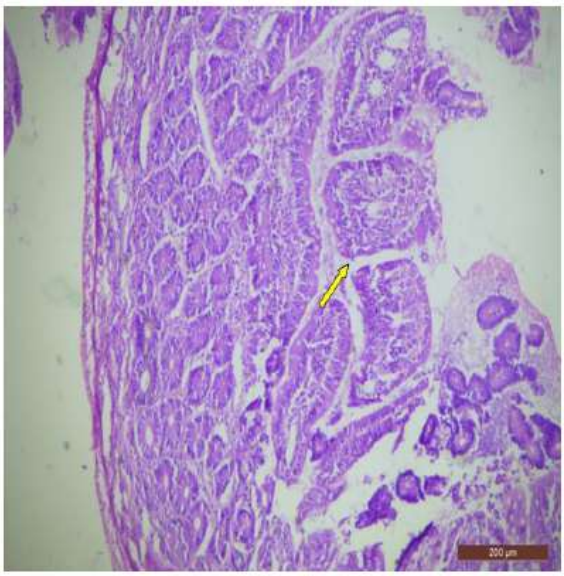

Fig. 18: T.S. of colon of rat treated with 600 mg NaF/kg b.w./day for 40 days showing scattered and broken villi (⇧) with loss of muscular layers. H&E × 100

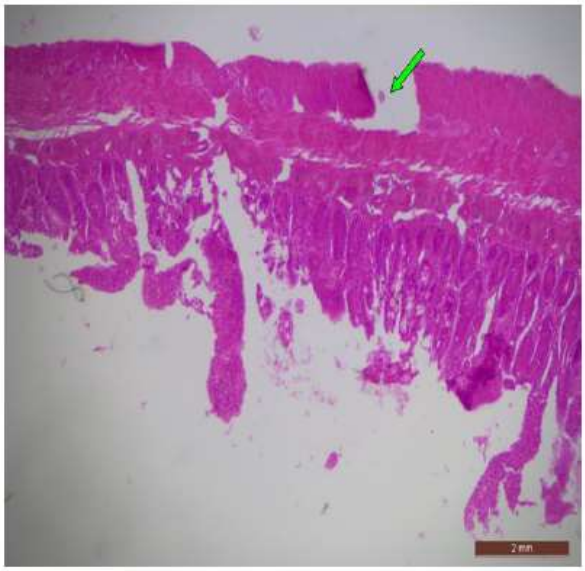

Fig. 19: T.S. of colon of rat treated with 600 mg NaF/kg b.w./day for 40 days showing complete loss of microvilli and disrupted muscular layers (⇧). H & E ×

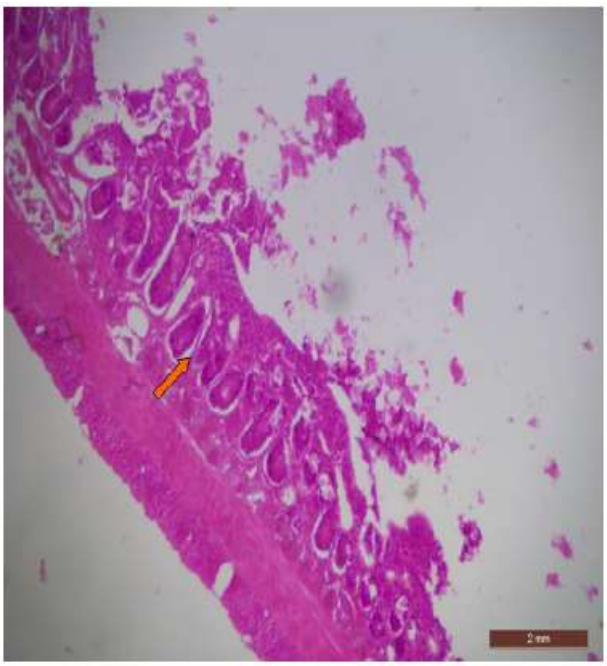

Fig. 20: T.S. of colon of rat treated with 600 mg NaF/kg b.w./day for 40 days showing a decrease in the number of goblet cells (⇧).H&E× 2000

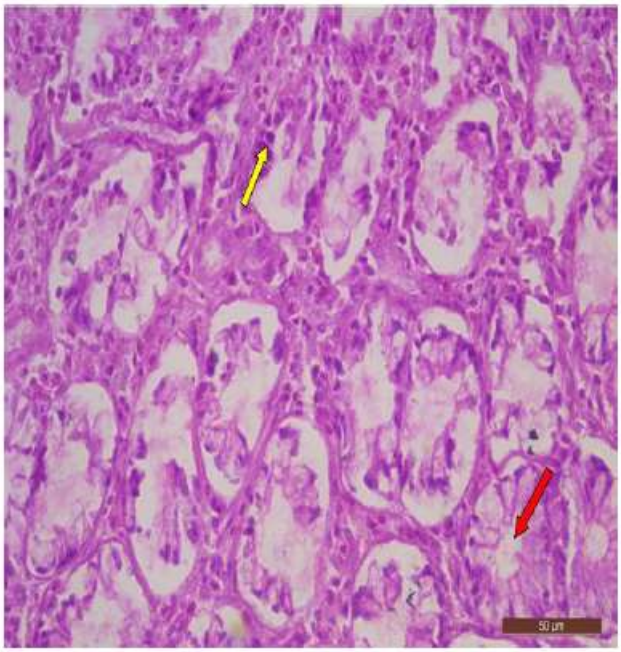

Fig. 21: T.S. of colon of rat treated with 600 mg NaF/kg b.w./day for 40 days showing inflammation of goblet cells (⇧) and dilation of spaces (⇧). H&E × 400

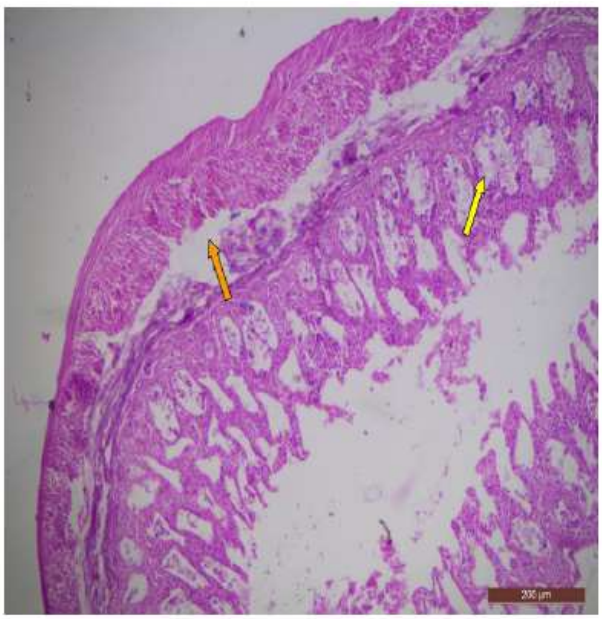

Fig. 22: T.S. of colon of rat treated with 600 mg NaF/kg b.w./day for 40 days showing disruption of layers (⇧) with total villous atrophy along with swollen and reduced number of goblet cells (⇧). H&E×100

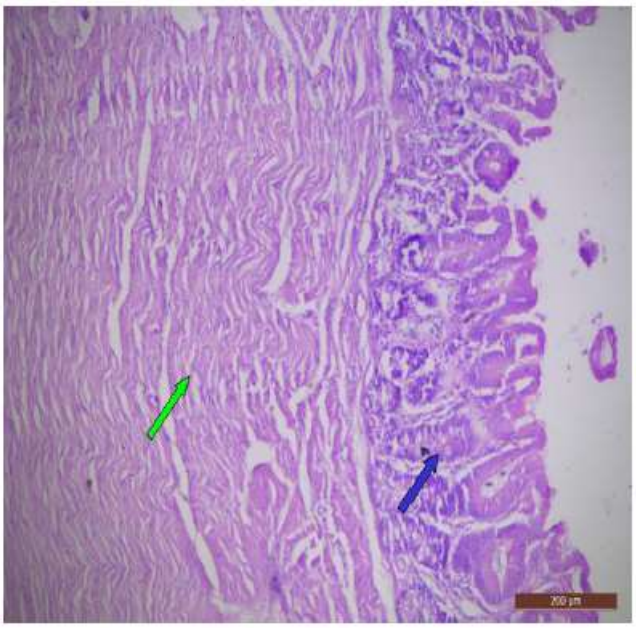

Fig. 23: T.S. of colon of rat treated with 600 mg NaF/kg b.w./day for 40 days showing inflammation of mucus layer (⇧) and abnormal crypts (⇧). H&E×100

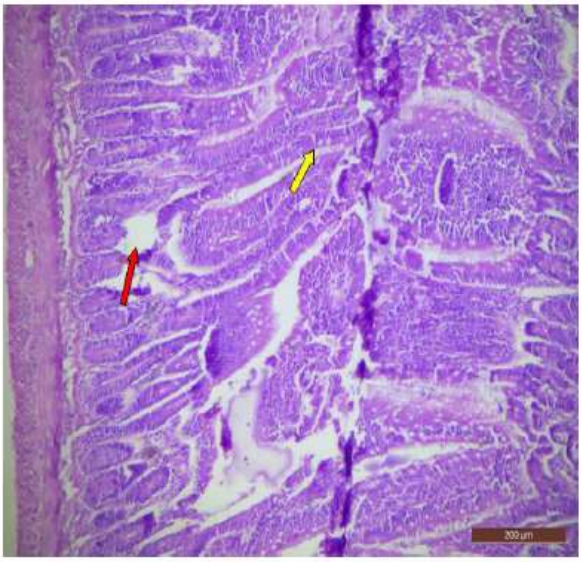

Fig. 24: T.S. of colon of rat treated with 600 mg NaF/ kg bw/day for 40 days and post-treated with 250 mg/kg b.w./day of leaf extract of *Boerhaavia diffusa* L. for 20 days showing an increase in height of villi (⇑) in which nuclei are slightly staggered. Lymphatic dilation (⇑) was also seen. H&E × 100

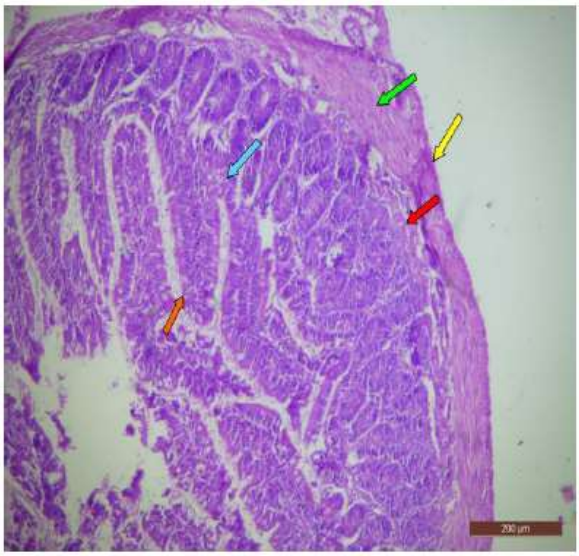

Fig. 25: T.S. of colon of rat treated with 600 mg NaF/kg bw/day for 40 days and post-treated with 500 mg/kg b.w./day of leaf extract of *Boerhaavia diffusa* L. for 20 days showing mucosa (⇑), submucosa (⇑), muscularis (⇑) and serosa (⇑). Long finger-like villi (⇑) were also observed. H&E × 100

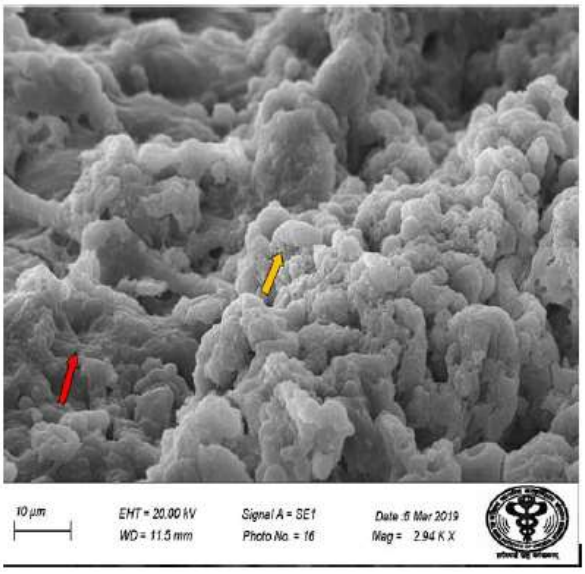

Fig. 26: Scanning electron micrograph of colon of control rat showing the normal appearance of villi (⇑) and mucus layer (⇑). X 2940

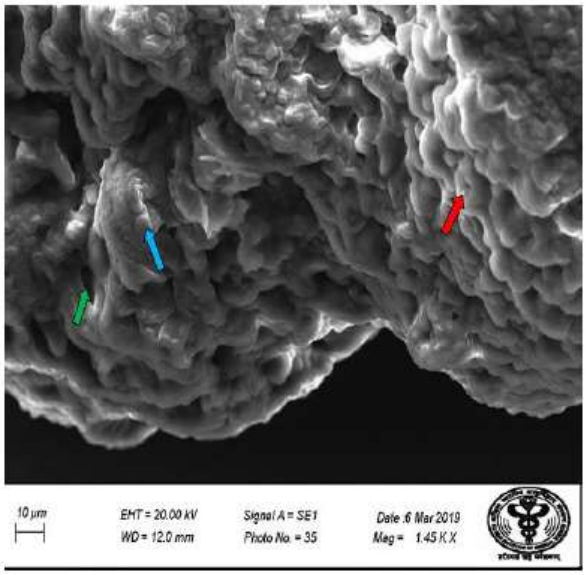

Fig. 27: Scanning electron micrograph of colon of control rat showing leaf-like (⇑) and doughnut shaped villi (⇑). Some crypt mouths (⇑) were also visible. X 1450

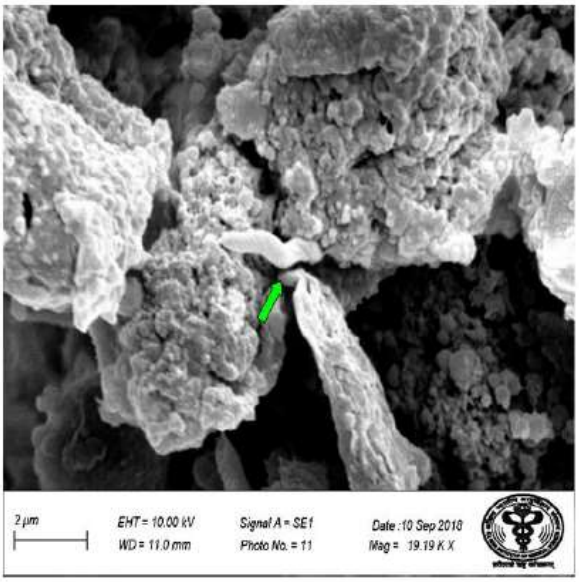

Fig. 28: Scanning electron micrograph of colon of rat treated with 600 mg/ kg b.w./day of NaF for 40 days showing damaged villi (⇧). X 19190

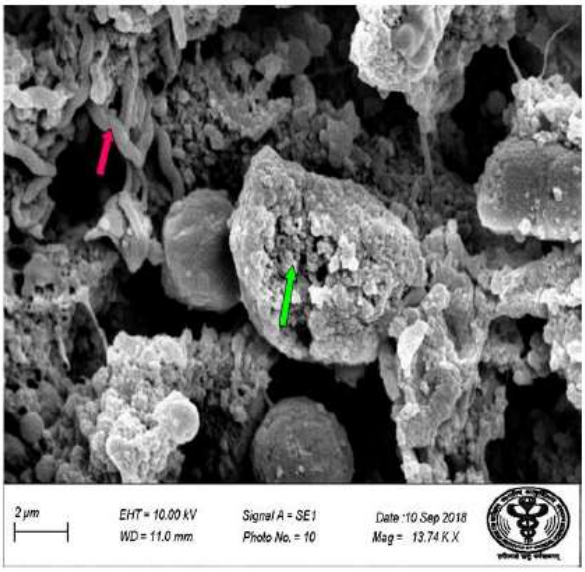

Fig. 29: Scanning electron micrograph of colon of rat treated with 600 mg/kg b.w./day of NaF for 40 days showing broken and distorted villi (⇧) and mucus discharge from crypts (⇧). X 13740

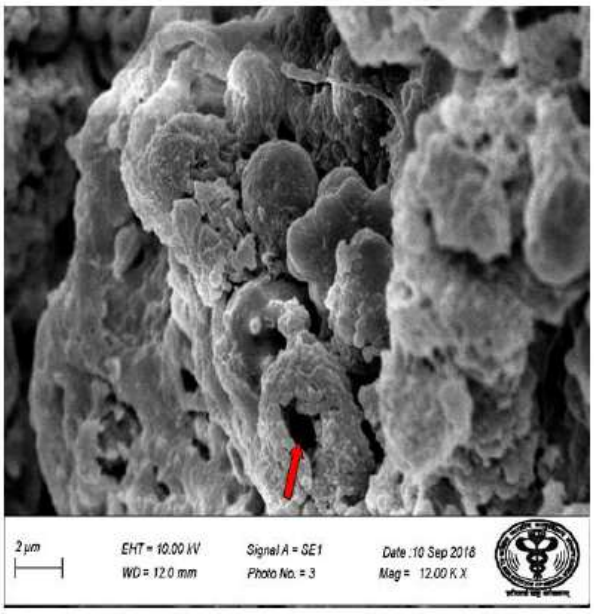

Fig. 30: Scanning electron micrograph of colon of rat treated with 600 mg/kg b.w./day of NaF for 40 days showing cryptal units with narrow openings (⇧). X 12000

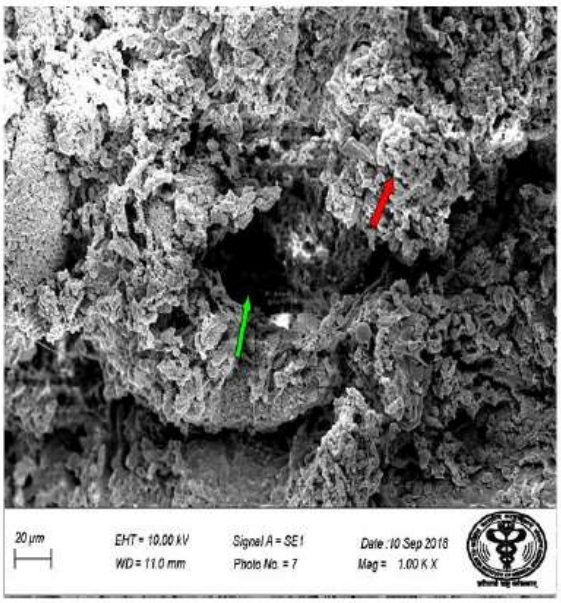

Fig. 31: Scanning electron micrograph of colon of rat treated with 600 mg/kg b.w./day of NaF for 40 days showing a large necrotic area (⇧) and clumping of mucus (⇧). X 1000

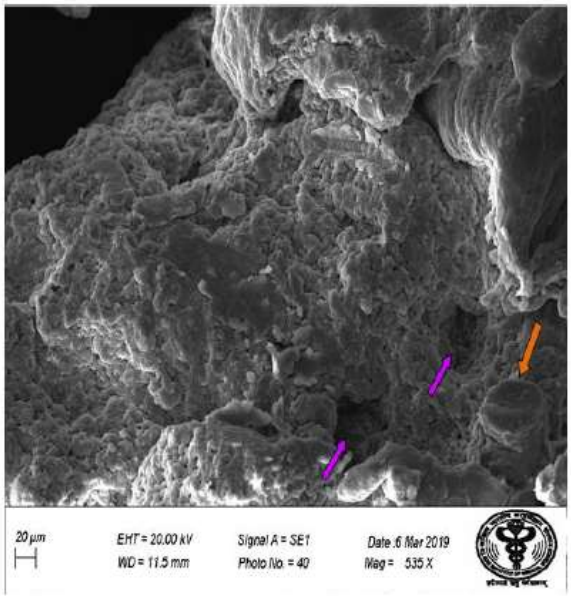

Fig. 32: Scanning electron micrograph of colon of rat treated with 600 mg/kg b.w./day of NaF for 40 days showing two circular gaps (⇧) in the surface layer. Red blood cells (⇧) were also observed at certain sites. X 535

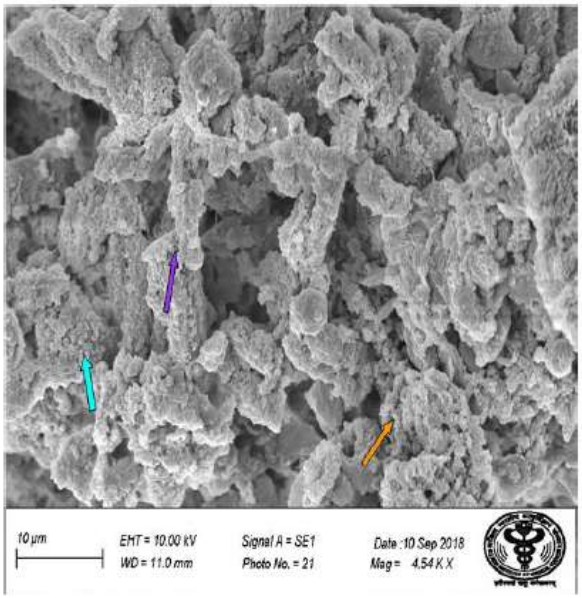

Fig. 33: Scanning electron micrograph of colon of rat treated with 600 mg/kg b.w./day of NaF for 40 days showing damaged villi (⇧), desquamation of epithelial cells (⇧) and disrupted surface layer (⇧). X 4540.

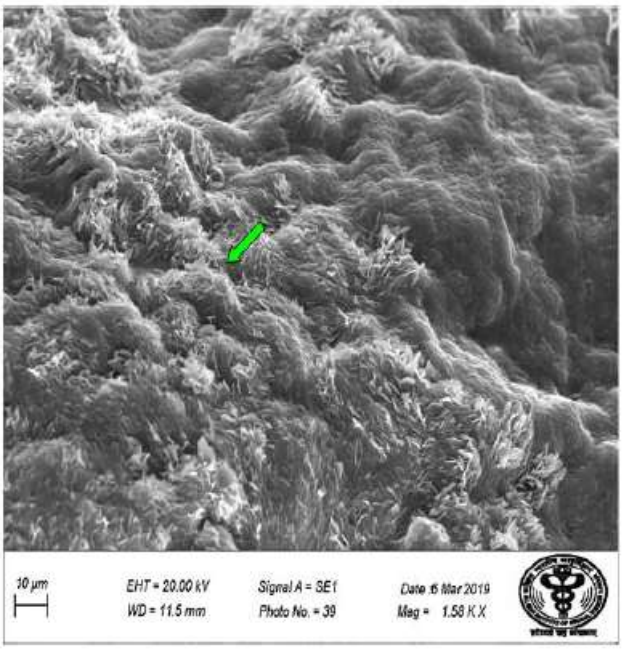

Fig. 34: Scanning electron micrograph of colon of rat treated with 600 mg/kg b.w./day of NaF for 40 days and post-treated with 250 mg/kg b.w./day of leaf extract of *Boerhaavia diffusa* L. for 20 days showing epithelial cells with microvilli (⇧). X 1580

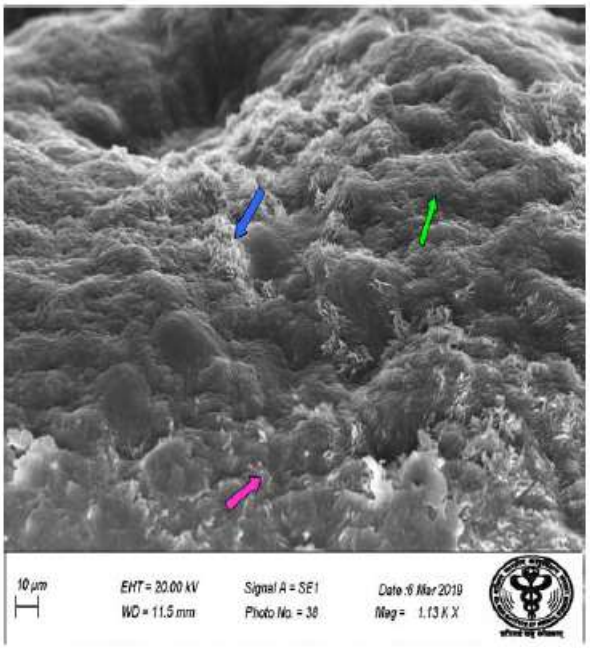

Fig. 35: Scanning electron micrograph of colon of rat treated with 600 mg/kg b.w./day of NaF for 40 days and post-treated with 500 mg/kg b.w./day of leaf extract of *Boerhaavia diffusa* L. for 20 days showing mucus secretion (⇧), epithelial cells (⇧) and numerous short microvilli (⇧). X 1130

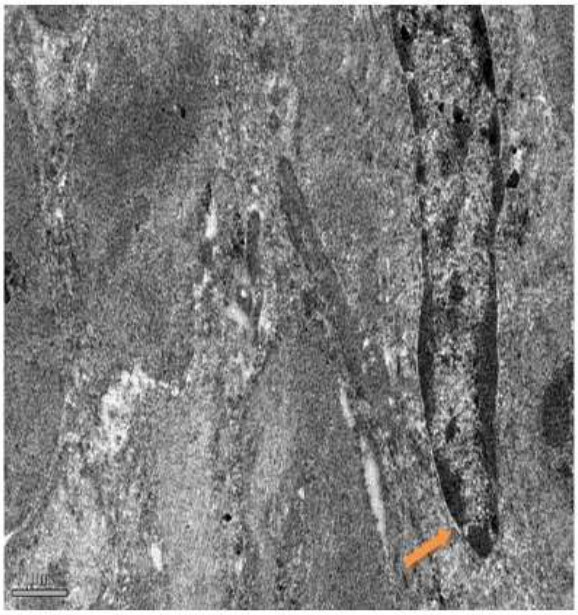

Fig.36: Transmission electron micrograph of colon of control rat showing large cell (macrophage) (⇧). X 2550

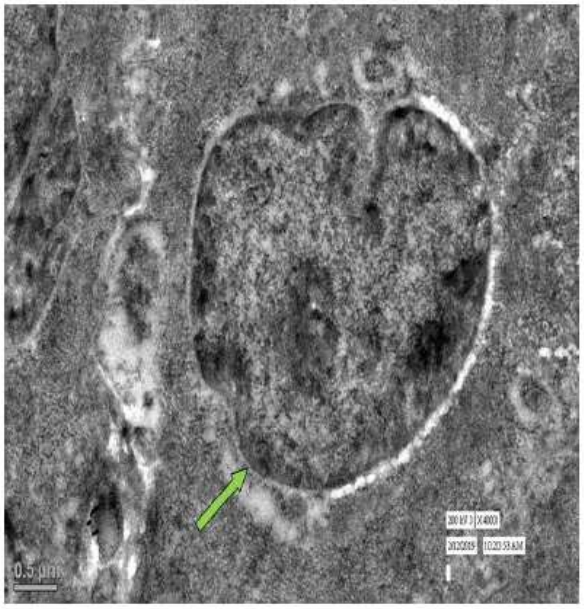

Fig.37:Transmission electron micrograph of colon of control rat showing mast cell (⇧). X 5000

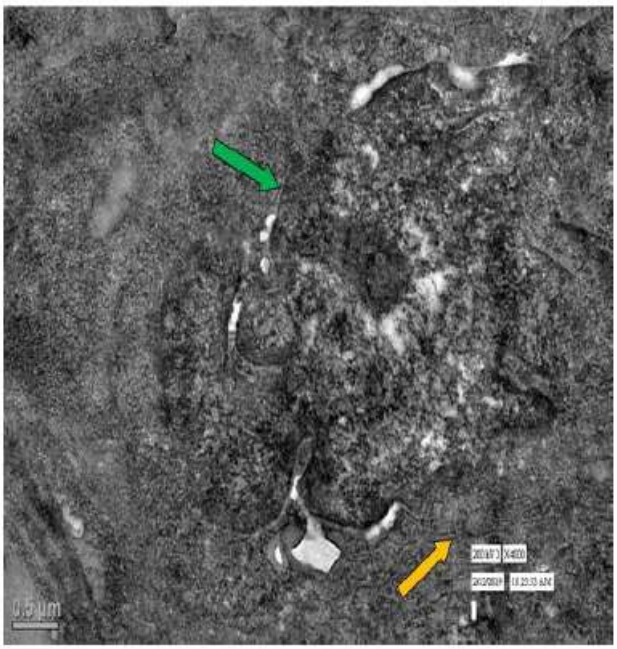

Fig.38:Transmission electron micrograph of colon of rat treated with 600 mg sodium fluoride for 40 days showing irregular heterochromatic nuclei (⇧) with narrow intercellular spaces and dilated reR (⇧). X 5000

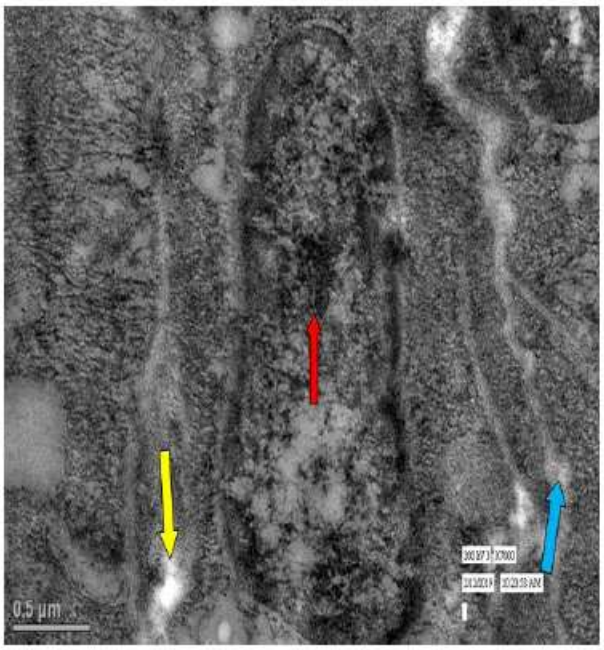

Fig.39:Transmission electron micrograph of colon of rat treated with 600 mg sodium fluoride showing broken and reduced microvilli (⇧), vacoulations (⇧) and clumping of chromatin in the nuclei (⇧). X 5000

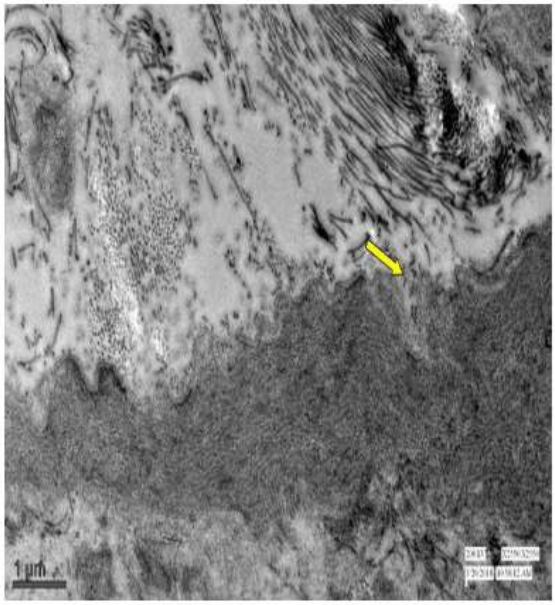

Fig.40:Transmission electron micrograph of colon of rat treated with 600 mg sodium fluoride showing disintegration and breaking of muscle layer (⇧). X 2550

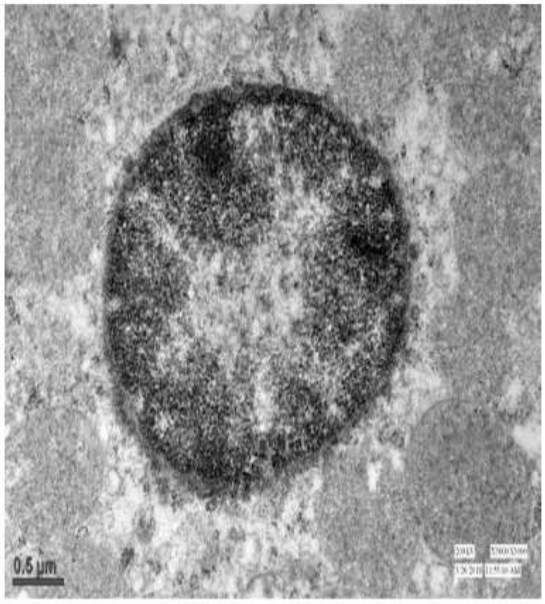

Fig.41: Transmission electron micrograph of colon of rat treated with 600 mg sodium fluoride showing cell almost devoid of mucus and disruptions in the surface layer. X 5000

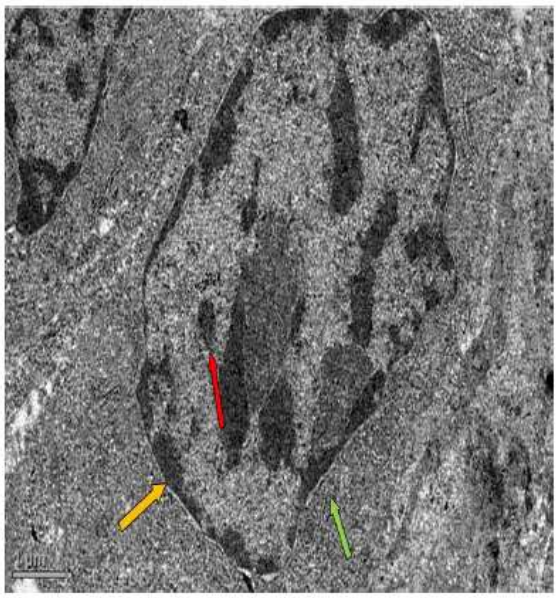

Fig.42:Transmission electron micrograph of colon of rat treated with leaf extract of 250 mg/kg b.w./day of *Boerhaavia diffusa* L. for 20 days showing oval nuclei (⇧) rER (⇧) and mitochondria were also seen. The cytoplasm has secretory granules (⇧). X 2550

Fig.43:Transmission electron micrograph of colon of rat treated with leaf extract of 250 mg/kg b.w./day of *Boerhaavia diffusa* L. for 20 days showing well defined nucleus surrounded by nuclear membrane (⇧). Few fat droplets (⇧) and golgi bodies (⇧) were also observed. X 2550

DISCUSSION

Sodium fluoride is known for its various biological effects, including its role in the induction of oxidative stress. Oxidative stress occurs when there is an imbalance between the production of free radicals and the body's antioxidant defense mechanisms. In the colon,

this imbalance can lead to cellular and tissue damage and inflammation. It is well known that fluoride causes oxidative stress in soft tissues and inhibits the activity of antioxidant enzymes. The present study revealed a close relationship between fluoride treated colon toxicity and oxidative stress.

Malondialdehyde is a byproduct of lipid peroxidation, serves as a marker for oxidative stress. Studies have shown that NaF exposure increases malondialdehyde levels in the colon, indicating enhanced lipid peroxidation and oxidative damage. Increased levels of MDA in the colon upon NaF exposure were reported, suggesting significant lipid peroxidation and oxidative stress in the tissue.

Reduced glutathione is a major intracellular antioxidant that plays a vital role in detoxifying ROS. Glutathione also play a vital role in mitigating oxidative stress.[15] NaF exposure has been shown to deplete reduced glutathione levels in the colon, exacerbating oxidative stress. Significant depletion of reduced glutathione levels in the colonic tissues of rats exposed to NaF was noted.[16]

NaF exposure impacts the activity of several key antioxidant enzymes, such as superoxide dismutase, catalase, glutathione peroxidase, glutathione-S-transferase and glutathione reductase. The activities of these enzymes were reduced in response to NaF, leading to diminished detoxification of ROS and exacerbation of oxidative stress.[17] Catalase and superoxide dismutase are crucial components of defense against oxidative damage. Catalase is another antioxidant enzyme that converts hydrogen peroxide into water and oxygen. Reduced catalase activity has been observed in the colon following NaF exposure, leading to the accumulation of hydrogen peroxide and further oxidative stress. NaF exposure has been associated with decreased SOD activity in the colon, which impairs the colon's ability to neutralize superoxide radicals. This reduction in enzymatic activity implies a compromised antioxidant defense mechanism, making the colonic tissues more susceptible to oxidative damage.[18]

Glutathione peroxidase works in tandem with GSH to reduce hydrogen peroxide and lipid peroxides to non-toxic molecules. NaF exposure has been reported to decrease glutathione peroxidase activity in the colon, further impairing the antioxidant defense mechanism. The reduction in glutathione peroxidase activity indicates a weakened capacity to neutralize peroxides, contributing to oxidative stress and potential damage to cellular components. The decrease in these enzyme activities suggests a compromised antioxidant defense system, leading to an accumulation of ROS and subsequent oxidative damage.[19]

Glutathione-S-transferase is an enzyme involved in the detoxification of electrophilic compounds by conjugating them with glutathione. Studies have demonstrated that NaF exposure can significantly decrease glutathione-S-transferase activity in the colon of rats.[17]

NaF exposure has been shown to impact glutathione reductase activity, affecting the redox balance within colonic cells. Research indicates that NaF exposure can lead to a significant decrease in glutathione reductase activity in the colon. The reduction in activity of glutathione reducatse results in lower levels of reduced glutathione, which impairs the cellular defense against oxidative stress and increases vulnerability to oxidative damage.

The study indicated that treatment with *Boerhaavia diffusa* L. significantly reversed the levels of MDA and increased the levels of antioxidant enzymes.

Histopathological studies are essential to understand the structural alterations in tissues induced by NaF exposure. One of the prominent pathological effects of NaF on the colon is mucosal erosion and ulceration. NaF exposure can lead to the degradation of the colonic mucosal layer, resulting in erosion and ulcer formation. Fluoride treatment has revealed significant mucosal erosion and ulceration in the colon of rats. These lesions are indicative of severe tissue damage and inflammation.[20]

The fluoride induced damage to the colonic crypts and a significant decrease in the number of goblet cells in the colons of rats. This damage affects the regenerative capacity of the colonic epithelium and the production of protective mucus.[21] The colon from NaF-treated rats often show evidence of submucosal edema, contributing to the overall swelling and structural changes in the colon.[22]

Damage to the colonic crypts and atrophy of intestinal villi are severe consequences of NaF toxicity. The extensive crypt damage and villous atrophy in the colonic tissues of NaF-treated rats, reflecting the profound impact of fluoride on colonic architecture has been reported.

NaF exposure leads to significant damage to the colonic mucosa, including epithelial cell degeneration and necrosis. Wang *et al*.[23] documented extensive mucosal damage in the colon of rats exposed to NaF, including epithelial cell degeneration and ulceration, emphasizing the severity of fluoride-induced colonic injury.

Scanning electron microscopy provides detailed imaging of the surface morphology of tissues at a microscopic level, revealing cellular and structural changes that are not visible with light microscopy. Sodium fluoride exposure induces several notable changes in the colon of rats when observed under scanning electron microscope. These changes include loss of microvilli, epithelial cell degeneration, disruption of crypt architecture, and increased inflammatory cell infiltration.

SEM studies have demonstrated that NaF exposure leads to significant alterations in the epithelial surface structure of the colon. These alterations include the loss of microvilli. The microvilli appear shorter and more irregular, reflecting disruption in their normal structure and function.[24]

The colon from NaF-treated rats show significant degeneration of epithelial cells, including cell shrinkage and blebbing. NaF-exposed rats showed extensive epithelial cell degeneration, with noticeable blebbing and detachment, highlighting the cellular impact of fluoride exposure.[25] A significant disruption in the crypt architecture of NaF-treated rats, indicating fluoride's detrimental effects on epithelial cell regeneration.[26]

Inflammatory responses can be visualized using SEM, showing the infiltration of inflammatory cells into the colonic mucosa. NaF exposure can lead to an increase in such inflammatory cells, contributing to tissue damage. It has been demonstrated that increased inflammatory cell infiltration in the colonic tissues of rats exposed to NaF, reflecting the inflammatory response induced by fluoride.[27]

NaF exposure has been shown to induce various ultrastructural changes in the colon, which can be observed using TEM. NaF-exposed rats often show mitochondrial swelling, loss of cristae structure, and increased density of matrix granules.[28] These changes are indicative of mitochondrial dysfunction and oxidative stress.

Significant alterations in tight junctions, including widening of intercellular spaces and loss of tight junctions were also reported. The tight junctions appear less organized, with widening of intercellular spaces and loss of structural integrity. NaF treatment caused considerable mitochondrial swelling and endoplasmic reticulum dilation in the colonic epithelial cells of rats, indicating severe cellular stress.[29] Mitochondrial swelling, distortion of cristae, and increased density of matrix granules was observed in the colon of NaF-exposed rats.[30]

Boerhaavia diffusa L. demonstrates significant therapeutic potential in mitigating the adverse effects of sodium fluoride (NaF) on the colon. Through light microscopy, *Boerhaavia diffusa* L. has been shown to reduce mucosal damage and inflammatory changes. Studies reveal that *Boerhaavia diffusa* L. treatment in fluoride-exposed rats helps restore the number and integrity of microvilli on the epithelial surface and normal architecture of colonic crypts. Electron microscopic analysis further reveals its efficacy in ameliorating subcellular damage, including mitochondrial dysfunction, tight junction disruption, and lysosomal alterations. These findings underscore the potential of *Boerhaavia diffusa* L. as a therapeutic agent against fluoride-induced colonic toxicity.

CONCLUSION

The study concluded that the biochemical, histopathological, scanning, and transmission electron microscopy changes observed in the colon highlight the toxic effects of fluoride as evidenced by morphological abnormalities and disruptions which affect both structure and function of the tissue. These effects result in alterations to key enzymatic activities and increased oxidative stress. Furthermore, *Boerhaavia diffusa* L. has a therapeutic effect and mitigates the negative effects of fluoride treatment.

ETHICAL STATEMENT: The experimental protocols were conducted in accordance to the guidelines approved by Institutional Animal Ethical Committee of Punjabi University, Patiala (Animal maintenance and Registration No. 107/GO/ReBi/S/99/CPCSEA/2017-41).

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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