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MITIGATION OF FLUORIDE INDUCED LIPID PEROXIDATION AND GASTROINTESTINAL BIOCHEMISTRY DYSFUNCTION IN RAT BY BOERHAAVIA DIFFUSA L.

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ABSTRACT
The present study evaluated the toxic effects of oral administration of sodium fluoride on oxidative stress parameters and its possible amelioration upon treatment with leaf extract of *Boerhaavia diffusa* L. in Wistar albino rats divided randomly into six groups with six rats in each. Group 1 served as control (C1) receiving deionized water. Group II was orally administered with 600 mg/kg b.w./day of sodium fluoride for 40 days. Group III (C2) and Group IV (C3) were administered with 250 and 500 mg/kg b.w./day of *Boerhaavia diffusa* L. for 20 days respectively. Group V and Group VI were firstly exposed to 600 mg of NaF and then post-treated with 250 and 500 mg of leaf extract for 20 days. The level of malondialdehyde (MDA) exhibited a significant ($P< 0.001$) increase, while alterations in reduced glutathione (GSH) and activities of superoxide dismutase (MnSOD), catalase (CAT), glutathione peroxidase (GPx), glutathione-S-transferase (GST) and glutathione reductase (GR) revealed a significant ($P<0.001$) decline in all organs viz; stomach, duodenum, jejunum and ileum of fluoridated rats. However, after mitigation with leaf extract in NaF treated rats manifested a significant decrement ($P<0.001$) in the activity of antioxidant enzymes superoxide dismutase, catalase, glutathione peroxidase, glutathione-S-transferase and glutathione reductase. There was significant improvement with leaf extract of *Boerhaavia diffusa* L. on devastating effects of NaF in stomach and small intestine of fluoridated rats.

KEYWORDS: Antioxidant enzymes, *Boerhaavia diffusa* L., Small intestine, Sodium fluoride, Stomach, Oxidative stress, Wistar albino rats.

ABBERVIATIONS: Catalase (CAT), Glutathione peroxidase (GPx), Glutathione reductase (GR), Glutathione-S-transferase (GST), Malondialdehyde (MDA), Mitochondrial superoxide dismutase (MnSOD), Reduced glutathione (GSH).

INTRODUCTION
Fluoride has been linked to acute gastrointestinal symptoms, the most common of which are nausea, vomiting, and stomach aches.[1,2] Fluoride-induced oxidative stress is recognized as a key factor in the toxicity associated with fluorosis.[3] The relationship between the amount of fluoride absorbed and alterations in oxidative stress markers is crucial for understanding the harmful effects of fluoride on cellular functions. Some studies revealed that fluoride can lead to an overproduction of oxygen free radicals, which may reduce the biological activity of certain antioxidant enzymes like catalase, superoxide dismutase, glutathione peroxidase, glutathione-S-transferase and glutathione reductase.

Boerhaavia diffusa L. also known as Punarnava, is a potent medicinal herb that is utilised in many Ayurvedic and Unani treatments worldwide. Due to its numerous therapeutic characteristics, *Boerhaavia diffusa* L. has long held a prominent place among medicinal plants in India.[4] People often consume the leaves of Punarnava as dietary supplements. Phytochemical studies have identified various plant products, and extracts from different parts of these plants are beneficial for treating various diseases without causing side effects.[5]

This study aimed to explore the prophylactic role of *Boerhaavia diffusa* L. on the impact of fluoride exposure on oxidative stress in the gastrointestinal organs of rats.

MATERIALS AND METHODS
Preparation of plant extract
The leaf extract of *Boerhaavia diffusa* L. was prepared by the method of Narendhirakannan *et al*.[6]

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

After acclimatization for two weeks, the animals were divided into six groups with six rats in each. Control group (C1) was administered deionized water for 40 days via oral gavage and the rats in experimental group were treated with 600 mg/kg b.w./day of sodium fluoride for the same period. The control groups (C2) and (C3) were orally administered with 250 and 500 mg/kg b.w/day of leaf extract of *Boerhaavia diffusa* L. for 20 days respectively. The rats treated with 600 mg of sodium fluoride were post-treated with 250 and 500 mg/kg b.w./day of *Boerhaavia diffusa* L. leaf extract for the next 20 days respectively. At the end of experimental period, all rats were fasted overnight, sacrificed and gastrointestinal organs viz; stomach, duodenum, jejunum and ileum were taken out in physiological saline.

Preparation of tissue homogenate
The gastrointestinal organs (viz; stomach, duodenum, jejunum and ileum) were quickly homogenized with ice-cold phosphate buffer (0.1 M, pH = 7.4) and centrifuged at 10,000 rpm for 10 minutes. The supernatant was used for estimation of malondialdehyde, reduced glutathione, catalase, mitochondrial superoxide dismutase, glutathione peroxidase, glutathione-S-transferase and glutathione reductase.

Fluoride analysis
The concentration of fluoride in stomach and small intestine was determined.[7]

Assessment of biochemical parameters
The level of MDA[8] and GSH[9] content was assessed. The activity of antioxidant enzymes such as catalase[10], mitochondrial superoxide dismutase[11], glutathione peroxidase[12], glutathione-S-transferase[13] and glutathione reductase[14] was measured on UV-Vis-spectrophotometer.

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 Bonferroni multiple comparison test. The results were considered significant at $P< 0.05$. The relationship between level of fluoride in gastrointestinal organs and activity of enzymes were determined by Pearson's bivariate correlation and simple linear regression analysis.

RESULTS
Malondialdehyde: The mean level of malondialdehyde in fluoridated rats showed a significant ($P<0.001$) increase in stomach ($F=55.969$, Fig.5), duodenum ($F=62.751$, Fig.6), jejunum ($F=176.608$, Fig. 7) and ileum ($F=142.695$, Fig. 8) as compared to control.

Pearson's bivariate correlation and simple linear regression analysis demonstrated significant ($P<0.001$) positive relationship between the levels of fluoride and malondialdehyde in stomach ($R^2= 0.959$; Pearson $r= 0.979$; $Y= 1.318+101.166X$; Fig.33), duodenum ($R^2= 0.953$; Pearson $r= 0.976$; $Y= 1.436+82.318X$; Fig.34), jejunum ($R^2= 0.944$; Pearson $r= 0.972$; $Y= 0.896+112.164X$; Fig.35) and ileum ($R^2= 0.943$; Pearson $r= 0.971$; $Y= 1.115+113.123X$; Fig.36) after 40 days of fluoride treatment.

Reduced glutathione: The reduced glutathione content exhibited significant ($P<0.001$) reduction in the stomach ($F= 59.384$, Fig.9), duodenum ($F=342.365$, Fig.10), jejunum ($F=756.345$, Fig.11) and ileum ($F=114.946$, Fig.12) of fluoridated rats as compared to control.

Pearson's bivariate correlation and simple linear regression analysis showed a significant ($P<0.001$) negative relationship between the levels of fluoride and GSH in stomach ($R^2= 0.922$; Pearson $r = -0.960$; $Y= 7.119-95.221X$; Fig.37), duodenum ($R^2= 0.912$; Pearson $r = -0.955$; $Y= 7.705-115.422X$; Fig.38), jejunum ($R^2= 0.983$; Pearson $r = -0.9992$; $Y= 8.656-138.869X$; Fig.39) and ileum ($R^2= 0.914$; Pearson $r = -0.956$; $Y= 7.555-115.984X$; Fig.40) after 40 days of fluoride treatment.

ANTIOXIDANT ENZYMES
Catalse
The activity of catalase decreased significantly ($P<0.001$) in stomach ($F=160.195$, Fig.13), duodenum ($F=13.604$, Fig.14), jejunum ($F=4.510$, Fig.15) and ileum ($F=19.479$, Fig.16) of fluoridated rats treated with 600 mg of NaF in comparison to control.

Pearson's bivariate correlation and simple linear regression analysis showed significant ($P<0.001$) negative relationship between the levels of fluoride and activity of CAT in stomach ($R^2= 0.963$; Pearson $r = -0.982$; $Y= 4.690-80.323X$; Fig.41), duodenum ($R^2= 0.959$; Pearson $r = -0.979$; $Y= 1.721-26.860X$; Fig.42), jejunum ($R^2= 0.883$; Pearson $r = -0.940$; $Y= 0.879-12.678X$; Fig.43) and ileum ($R^2= 0.948$; Pearson $r = -0.974$; $Y= 2.205-27.305X$; Fig.44) after 40 days of fluoride exposure.

Mitochondrial superoxide dismutase
The activity of mitochondrial superoxide dismutase in fluorotic rats revealed significant elevation ($P<0.001$) in stomach ($F=113.667$, Fig.17), duodenum ($F=63.406$, Fig.18), jejunum ($F=56.605$, Fig.19), and ileum ($F=44.655$, Fig.20) as compared to control.

Pearson's bivariate correlation and simple linear regression analysis showed significant ($P<0.001$) negative relationship between the levels of fluoride and

activity of MnSOD in stomach (R^2= 0.959; Pearson r = -0.979 Y= 9.063-74.272X; Fig.45), duodenum (R^2= 0.936; Pearson r = -0.968 Y= 8.075-73.469X; Fig.46), jejunum (R^2= 0.856; Pearson r = -0.925 Y= 8.332-70.186X; Fig.47) and ileum (R^2= 0.821; Pearson r = -0.906 Y= 7.252-69.379X; Fig.48) after 40 days of fluoride treatment.

Glutathione peroxidase

The activity of glutathione peroxidase decreased significantly (P<0.001) in the stomach (F=76.367, Fig.21), duodenum (F=135.231, Fig.22), jejunum (F=154.607, Fig.23) and ileum (F=124.189, Fig.24) of fluoridated rats as compared to control.

Pearson's bivariate correlation and simple linear regression analysis showed significant (P<0.001) negative relationship between the levels of fluoride and activity of GPx in stomach (R^2= 0.940; Pearson r = -0.969 Y= 6.876-104.734X; Fig.49), duodenum (R^2= 0.931; Pearson r = -0.965 Y= 7.705-92.360X; Fig.50), jejunum (R^2= 0.971; Pearson r = -0.985 Y= 8.259-101.389X; Fig.51) and ileum (R^2= 0.934; Pearson r = -0.966 Y= 8.238-107.0.32X; Fig.52) after 40 days of fluoride treatment.

Glutathione-S-transferase

The activity of glutathione-S-transferase decreased significantly (P<0.001) in stomach (F=107.198, Fig.25), duodenum (F=97.616, Fig.26), jejunum (F=254.525, Fig.27) and ileum (F=52.986, Fig.28) of fluoridated rats as compared to control.

Pearson's bivariate correlation and simple linear regression analysis showed significant (P<0.001) negative relationship between the concentration of fluoride and activity of GST in stomach (R^2= 0.941; Pearson r = -0.970 Y= 7.961-86.222X; Fig.53), duodenum (R^2= 0.935; Pearson r = -0.967 Y= 5.524-86.413X; Fig.54), jejunum (R^2= 0.970; Pearson r = -0.985 Y= 8.089-94.990X; Fig.55) and ileum (R^2= 0.791; Pearson r = -0.889 Y= 5.850-66.965X; Fig.56) after 40 days of fluoride treatment.

Glutathione reductase

The activity of glutathione reductase decreased significantly (P<0.001) in stomach (F=303.121, Fig.29), duodenum (F=473.048, Fig.30), jejunum (F=265.535, Fig.31) and ileum (F=132.531, Fig.32) of fluorotic rats in comparison to control.

Pearson's bivariate correlation and simple linear regression analysis showed significant (P<0.001) negative relationship between the concentration of fluoride and activity of GR in stomach (R^2= 0.966; Pearson r = -0.983 Y= 5.517-68.038X; Fig.57), duodenum (R^2= 0.942; Pearson r = -0.970 Y= 7.799-125.042X; Fig.58), jejunum (R^2= 0.959; Pearson r = -0.979 Y= 8.288-139.005X; Fig.59) and ileum (R^2= 0.954; Pearson r = -0.977 Y= 8.072-141.784X; Fig.60) after 40 days of fluoride exposure.

Post-treatment analysis

After mitigation with *Boerhaavia diffusa* L., the level of malondialdehyde decreased and reduced glutathione increased in post-treated groups as compared to respective fluoride supplemented group. The activity of antioxidant enzymes viz., catalase, superoxide dismutase, glutathione peroxidase, glutathione-S-transferase and glutathione reductase elevated in post-treated groups.

FLUORIDE

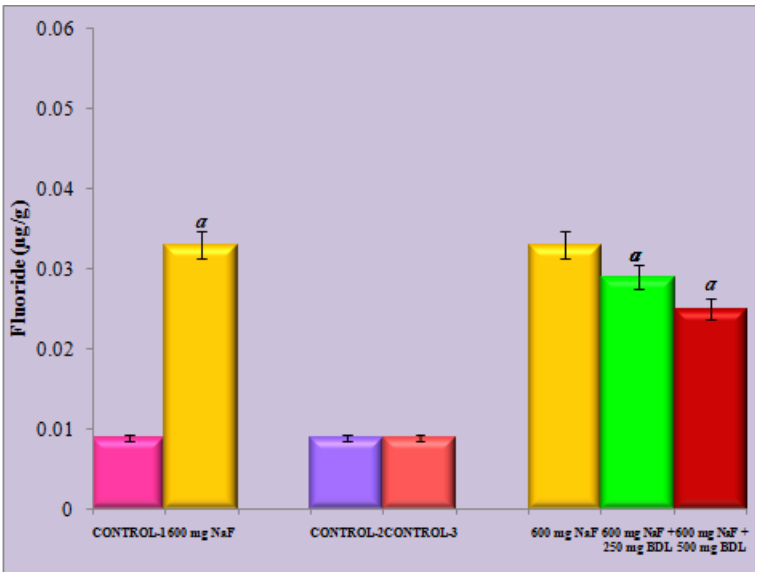

Fig. 1: Mean level of fluoride (µg/g) in stomach of control and fluoride treated rats and post-treated with leaf extract of *Boerhaavia diffusa* L.; *aP< 0.001* value is significantly different as compared to NaF treated group and Group 600 mg NaF compared with post-treated groups. One way ANOVA followed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

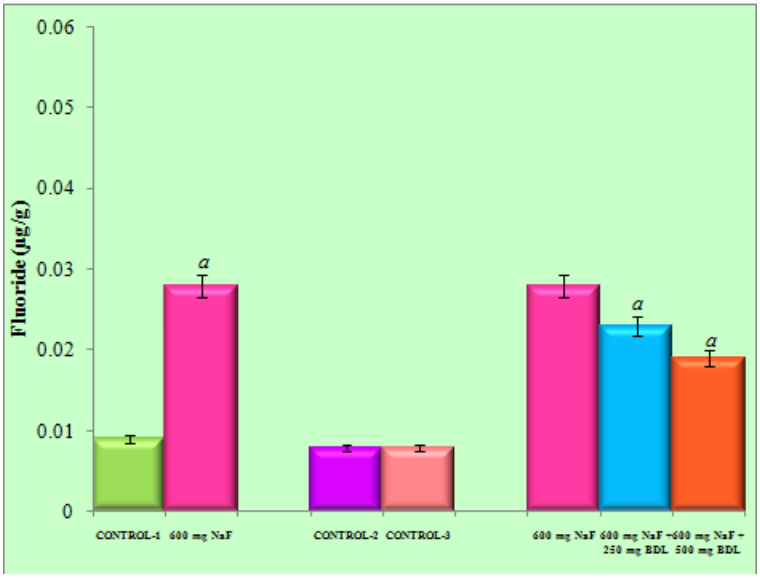

Fig. 2: Mean level of fluoride (µg/g) in duodenum of control and fluoride treated rats and post-treated with leaf extract of *Boerhaavia diffusa* L.; *aP< 0.001* value is significantly different as compared to NaF treated group and Group 600 mg NaF compared with post-treated groups. One way ANOVA followed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

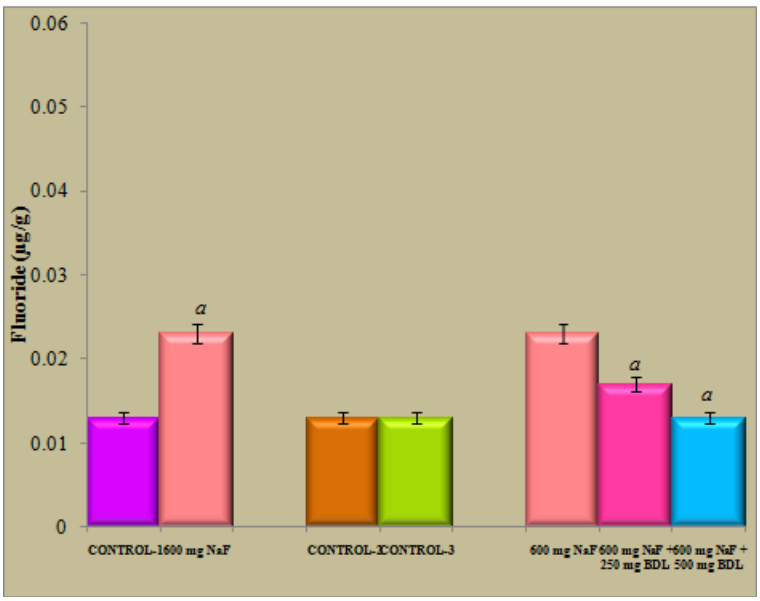

Fig. 3: Mean level of fluoride (µg/g) in jejunum of control and fluoride treated rats and post-treated with leaf extract of *Boerhaavia diffusa* L.; *aP< 0.001* value is significantly different as compared to NaF treated group and Group 600 mg NaF compared with post-treated groups. One way ANOVA followed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

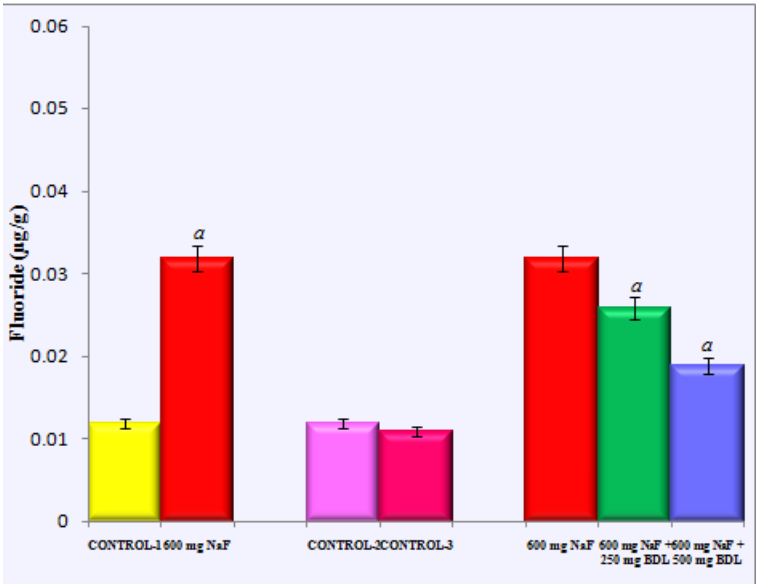

Fig. 4: Mean level of fluoride (µg/g) in ileum of control and fluoride treated rats and post-treated with leaf extract of *Boerhaavia diffusa* L.; *aP< 0.001* value is significantly different as compared to NaF treated group and Group 600 mg NaF compared with post-treated groups. One way ANOVA followed by Post-hoc Bonferroni multiple comparison test.

MALONDIALDEHYDE

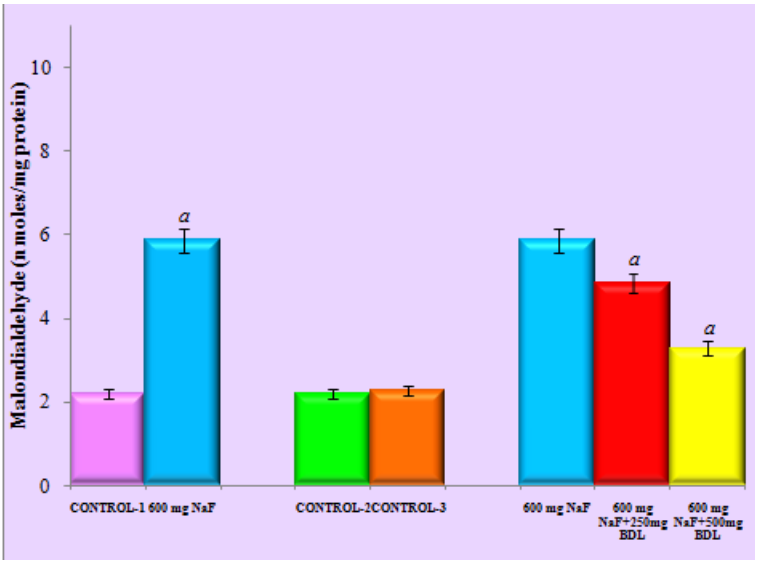

Fig. 5: Mean level of malondialdehyde (MDA) (n moles/mg protein) in stomach of control and fluoride treated rats and post-treated with leaf extract of *Boerhaavia diffusa* L.; *aP< 0.001* value is significantly different as compared to NaF treated group and Group 600 mg NaF compared with post-treated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

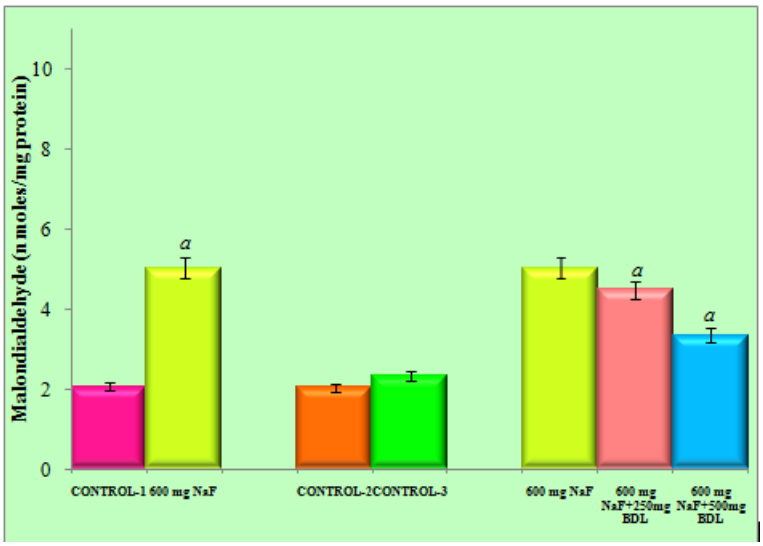

Fig. 6: Mean level of malondialdehyde (MDA) (n moles/mg protein) in duodenum of control and fluoride treated rats and post-treated with leaf extract of *Boerhaavia diffusa* L.; *aP< 0.001* value is significantly different as compared to NaF treated group and Group 600 mg NaF compared with post-treated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

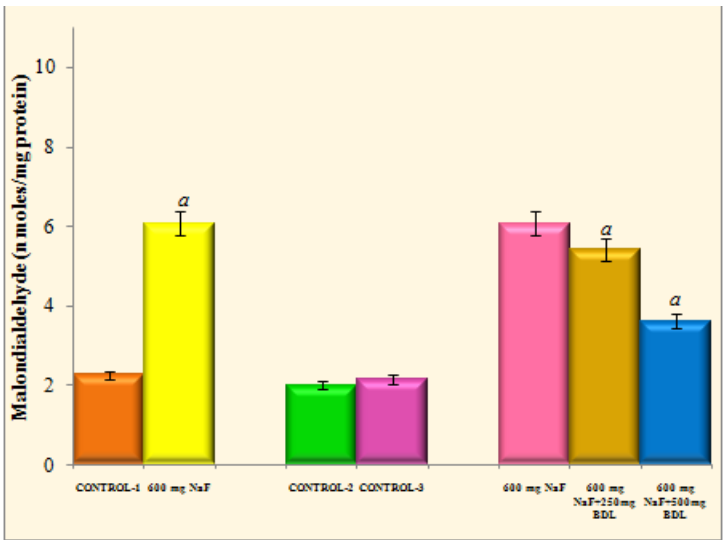

Fig. 7: Mean level of malondialdehyde (MDA) (n moles/mg protein) in jejunum of control and fluoride treated rats and post-treated with leaf extract of *Boerhaavia diffusa* L.; *aP< 0.001* value is significantly different as compared to NaF treated group and Group 600 mg NaF compared with post-treated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

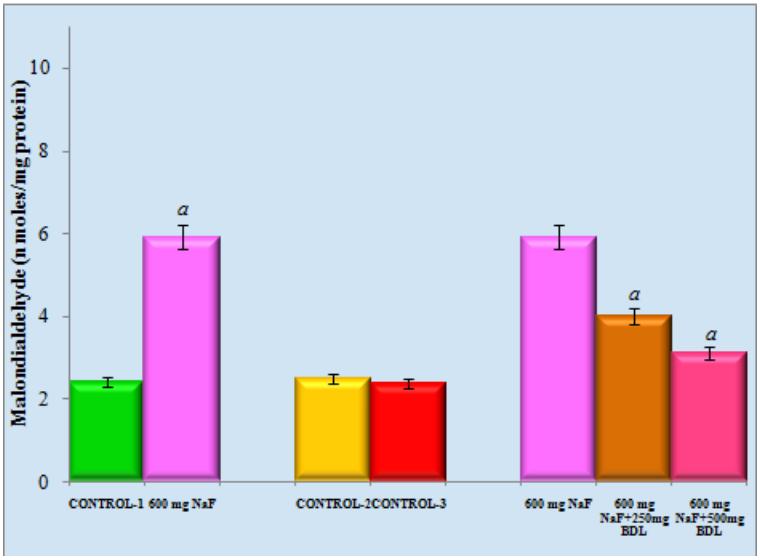

Fig. 8: Mean level of malondialdehyde (MDA) (n moles/mg protein) in ileum of control and fluoride treated rats and post-treated with leaf extract of *Boerhaavia diffusa* L.; *aP< 0.001* value is significantly different as compared to NaF treated group and Group 600 mg NaF compared with post-treated groups.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

REDUCED GLUTATHIONE

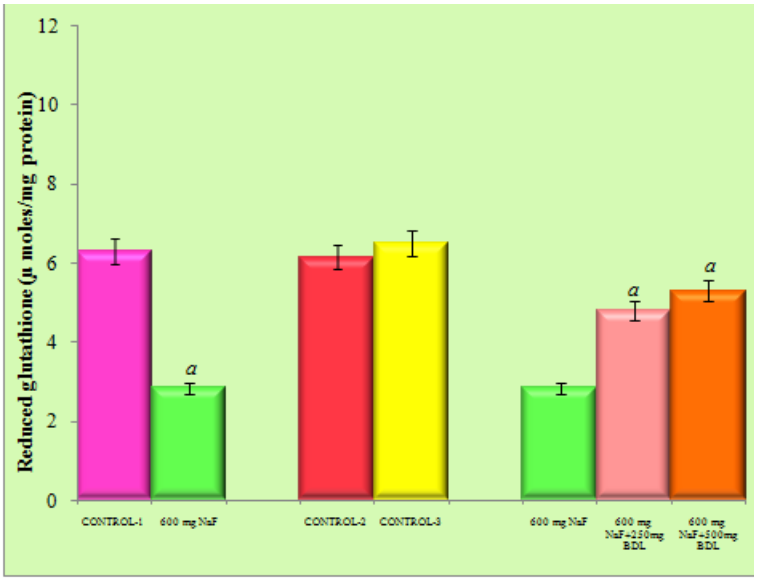

Fig. 9: Mean level of reduced glutathione (µ moles/mg protein) in stomach of control and fluoride treated rats and post-treated with leaf extract of *Boerhaavia diffusa* L.; *aP< 0.001* value is significantly different as compared to NaF treated group and Group 600 mg NaF compared with post-treated groups. One way ANOVA followed by Post-hoc Bonferroni multiple comparison test.

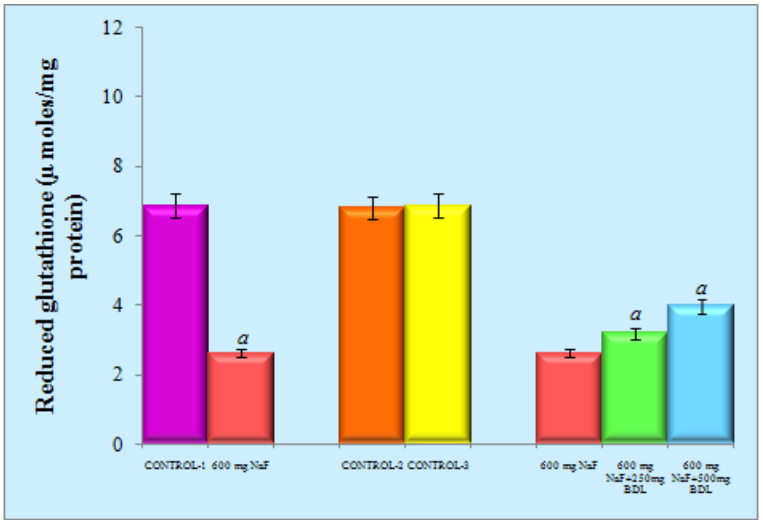

Fig. 10: Mean level of reduced glutathione (µ moles/mg protein) in duodenum of control and fluoride treated rats and post-treated with leaf extract of *Boerhaavia diffusa* L.; *aP< 0.001* value is significantly different as compared to NaF treated group and Group 600 mg NaF compared with post-treated groups. One way ANOVA followed by Post-hoc Bonferroni multiple comparison test.

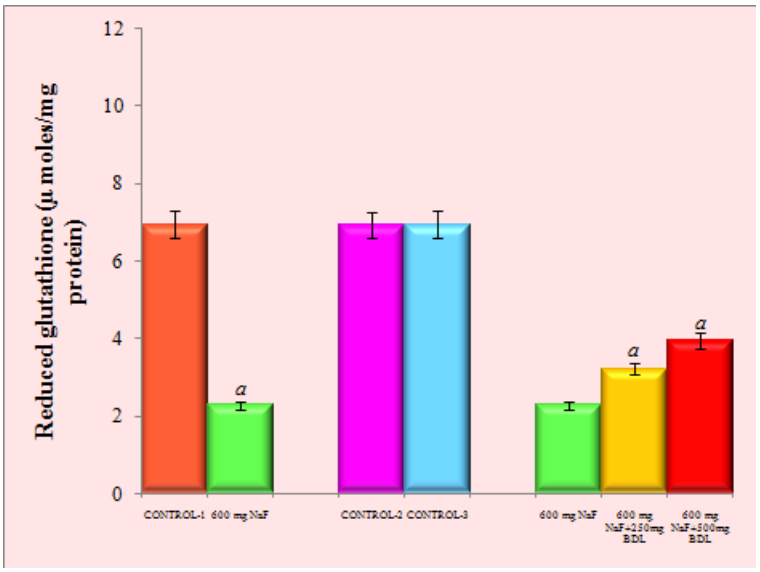

Fig. 11: Mean level of reduced glutathione (µ moles/mg protein) in jejunum of control and fluoride treated rats and post-treated with leaf extract of *Boerhaavia diffusa* L.; *aP< 0.001* value is significantly different as compared to NaF treated group and Group 600 mg NaF compared with post-treated groups. One way ANOVA followed by Post-hoc Bonferroni multiple comparison test.

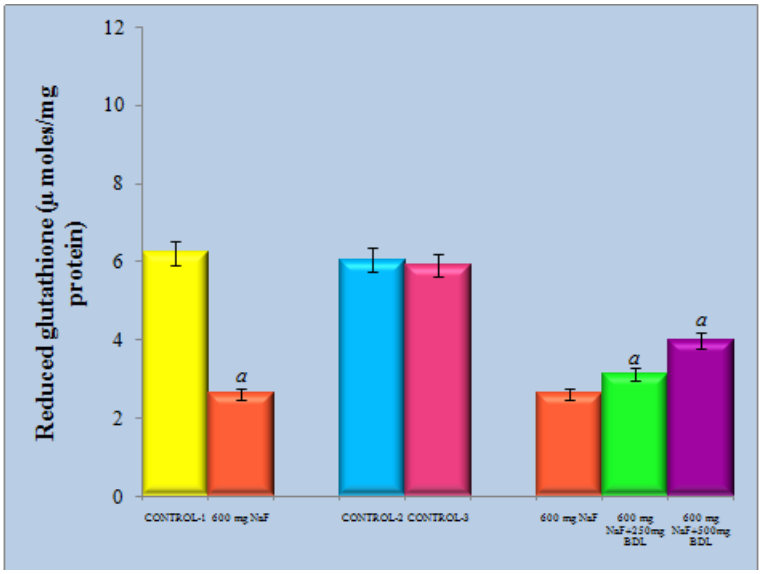

Fig. 12: Mean level of reduced glutathione (µ moles/mg protein) in ileum of control and fluoride treated rats and post-treated with leaf extract of *Boerhaavia diffusa* L.; *aP< 0.001* value is significantly different as compared to NaF treated group and Group 600 mg NaF compared with post-treated groups. One way ANOVA followed by Post-hoc Bonferroni multiple comparison test.

CATALASE

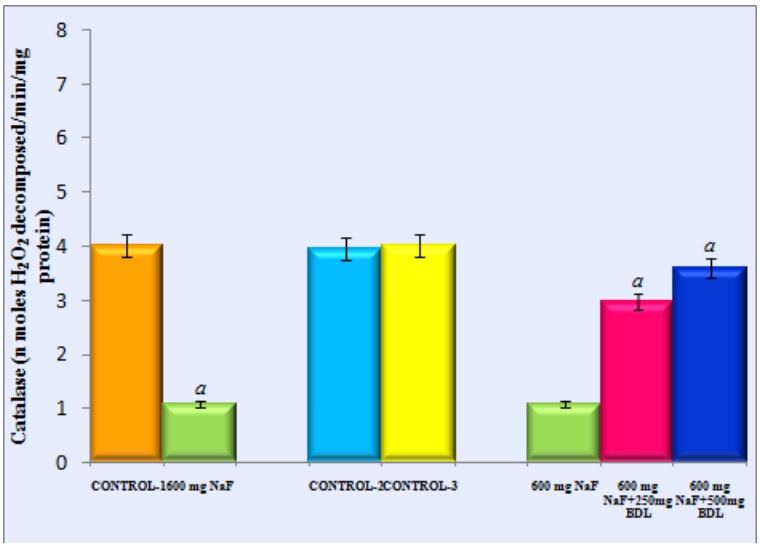

Fig. 13: Mean activity of catalase (CAT) (n moles H2O2 decomposed/min/mg protein) in stomach of control and fluoride treated rats and post-treated with leaf extract of *Boerhaavia diffusa* L.; *aP< 0.001* value is significantly different as compared to NaF treated group and Group 600 mg NaF compared with post-treated groups. One way ANOVA followed by Post-hoc Bonferroni multiple comparison test.

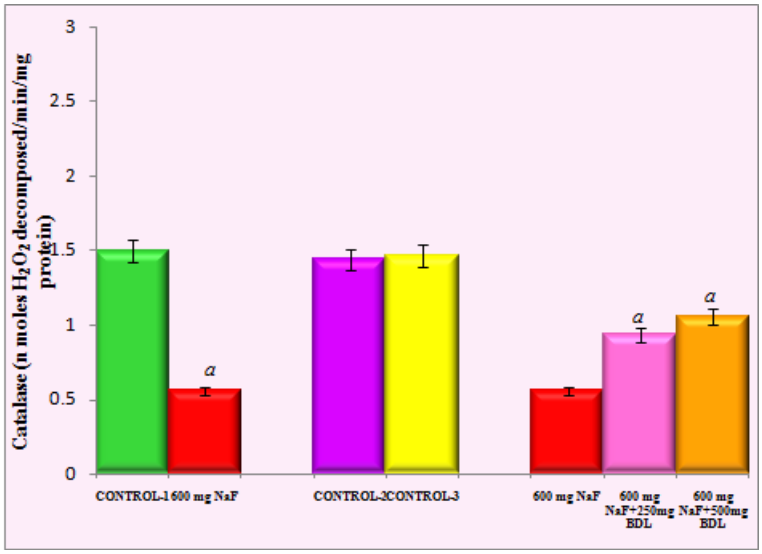

Fig. 14: Mean activity of catalase (CAT) (n moles H2O2 decomposed/min/mg protein) in duodenum of control and fluoride treated rats and post-treated with leaf extract of *Boerhaavia diffusa* L.; *aP< 0.001* value is significantly different as compared to NaF treated group and Group 600 mg NaF compared with post-treated groups. One way ANOVA followed by Post-hoc Bonferroni multiple comparison test.

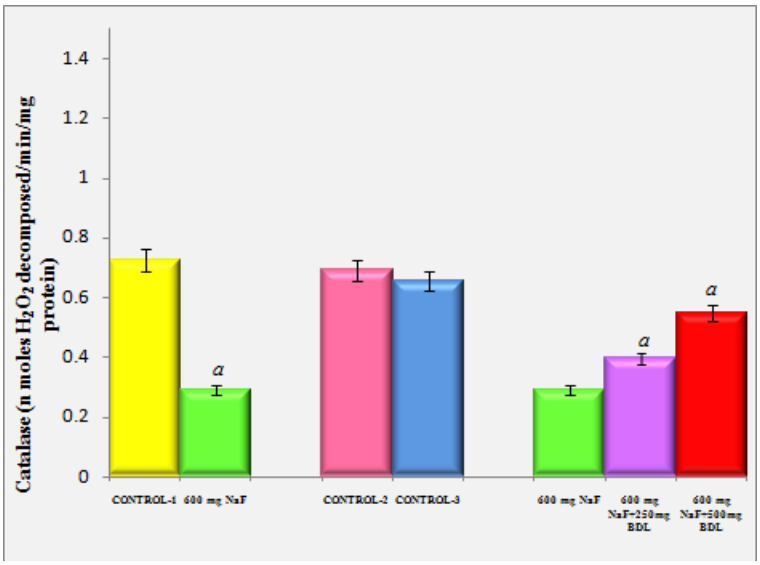

Fig. 15: Mean activity of catalase (CAT) (n moles H2O2 decomposed/min/mg protein) in jejunum of control and fluoride treated rats and post-treated with leaf extract of *Boerhaavia diffusa* L.; *aP< 0.001* value is significantly different as compared to NaF treated group and Group 600 mg NaF compared with post-treated groups. One way ANOVA followed by Post-hoc Bonferroni multiple comparison test.

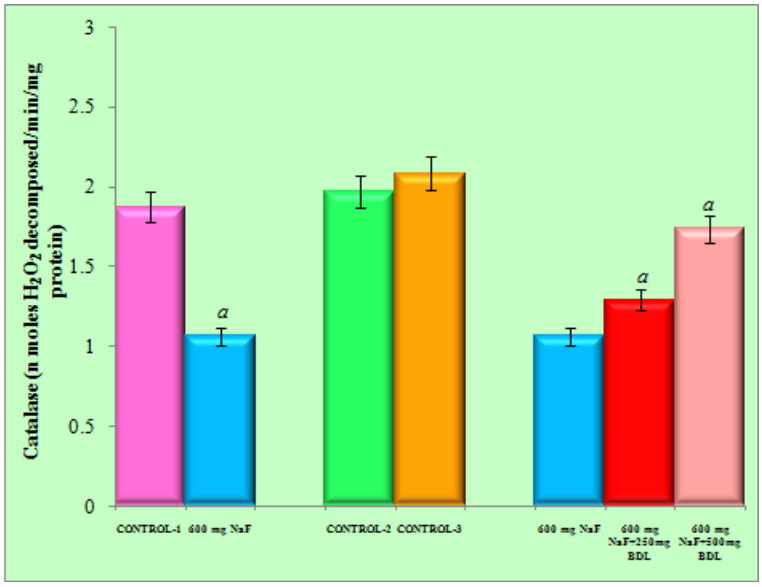

Fig. 16: Mean activity of catalase (CAT) (n moles H2O2 decomposed/min/mg protein) in ileum of control and fluoride treated rats and post-treated with leaf extract of *Boerhaavia diffusa* L.; *aP< 0.001* value is significantly different as compared to NaF treated group and Group 600 mg NaF compared with post-treated groups. One way ANOVA followed by Post-hoc Bonferroni multiple comparison test.

MITOCHONDRIAL SUPEROXIDE DISMUTASE

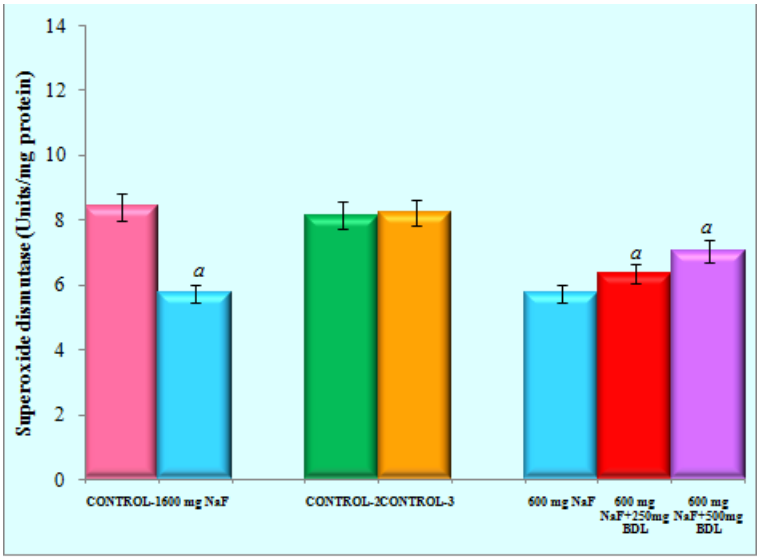

Fig. 17: Mean activity of superoxide dismutase (SOD) (Units/mg protein) in stomach of control and fluoride treated rats and post-treated with leaf extract of *Boerhaavia diffusa* L.; *aP< 0.001* value is significantly different as compared to NaF treated group and Group 600 mg NaF compared with post-treated groups. One way ANOVA followed by Post-hoc Bonferroni multiple comparison test.

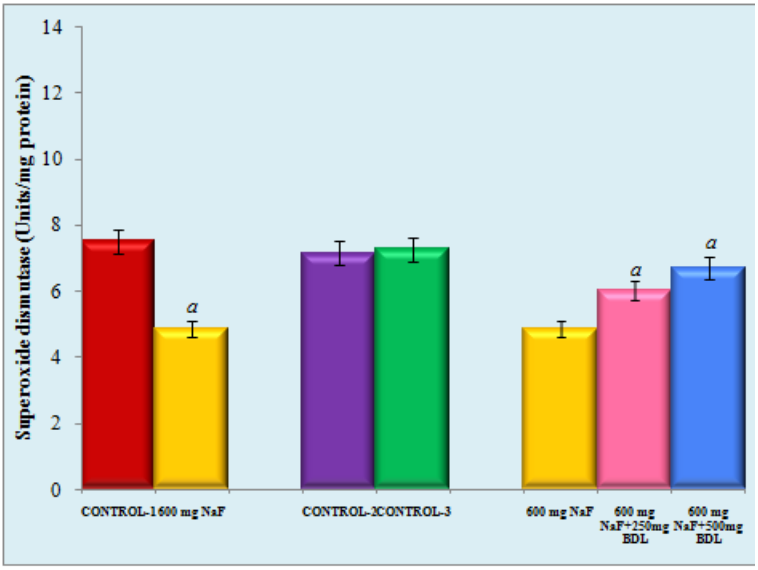

Fig. 18: Mean activity of superoxide dismutase (SOD) (Units/mg protein) in duodenum of control and fluoride treated rats and post-treated with leaf extract of *Boerhaavia diffusa* L.; *aP< 0.001* value is significantly different as compared to NaF treated group and Group 600 mg NaF compared with post-treated groups. One way ANOVA followed by Post-hoc Bonferroni multiple comparison test.

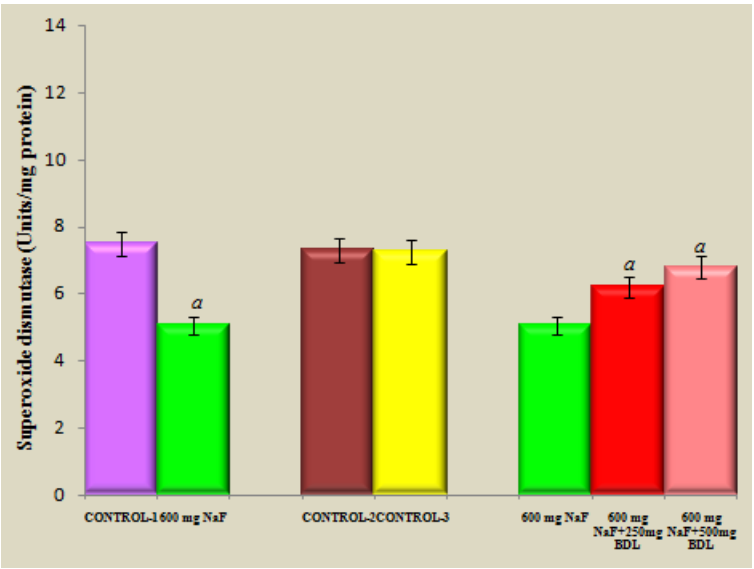

Fig. 19: Mean activity of superoxide dismutase (SOD) (Units/mg protein) in jejunum of control and fluoride treated rats and post-treated with leaf extract of *Boerhaavia diffusa* L.; *aP< 0.001* value is significantly different as compared to NaF treated group and Group 600 mg NaF compared with post-treated groups. One way ANOVA followed by Post-hoc Bonferroni multiple comparison test.

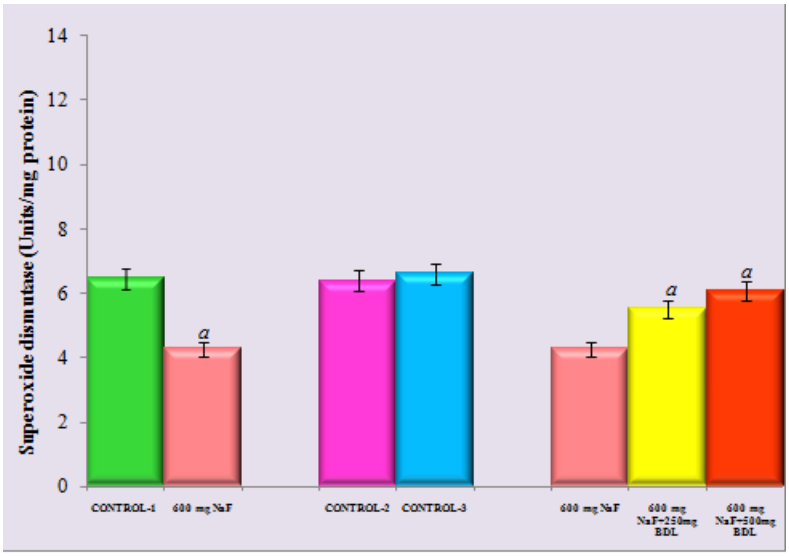

Fig. 20: Mean activity of superoxide dismutase (SOD) (Units/mg protein) in ileum of control and fluoride treated rats and post-treated with leaf extract of *Boerhaavia diffusa* L.; *aP< 0.001* value is significantly different as compared to NaF treated group and Group 600 mg NaF compared with post-treated groups. One way ANOVA followed by Post-hoc Bonferroni multiple comparison test.

GLUTATHIONE PEROXIDASE

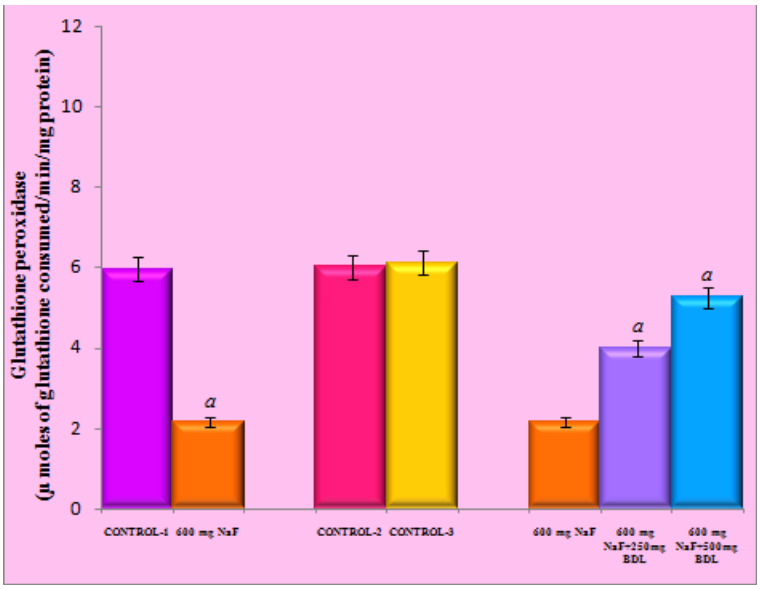

Fig. 21: Mean activity of glutathione peroxidase (GPx) (µ moles of glutathione consumed/min/mg protein) in stomach of control and fluoride treated rats and post-treated with leaf extract of *Boerhaavia diffusa* L.; *aP< 0.001* value is significantly different as compared to NaF treated group and Group 600 mg NaF compared with post-treated groups. One way ANOVA followed by Post-hoc Bonferroni multiple comparison test.

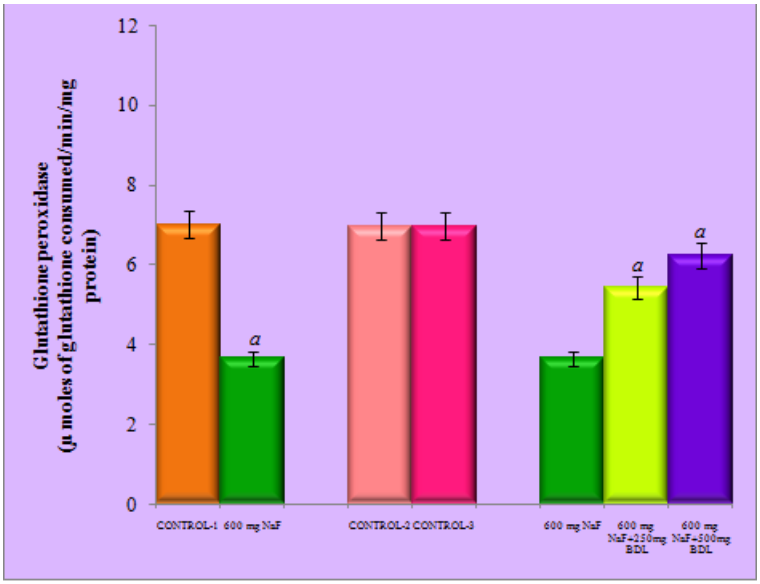

Fig. 22: Mean activity of glutathione peroxidase (GPx) (µ moles of glutathione consumed/min/mg protein) in duodenum of control and fluoride treated rats and post-treated with leaf extract of *Boerhaavia diffusa* L.; *aP< 0.001* value is significantly different as compared to NaF treated group and Group 600 mg NaF compared with post-treated groups. One way ANOVA followed by Post-hoc Bonferroni multiple comparison.

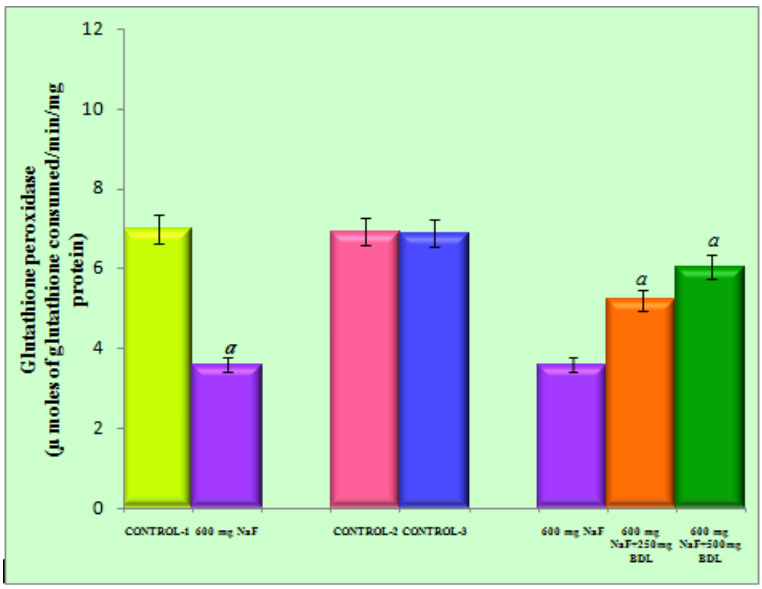

Fig. 23: Mean activity of glutathione peroxidase (GPx) (µ moles of glutathione consumed/min/mg protein) in jejunum of control and fluoride treated rats and post-treated with leaf extract of *Boerhaavia diffusa* L.; *aP< 0.001* value is significantly different as compared to NaF treated group and Group 600 mg NaF compared with post-treated groups. One way ANOVA followed by Post-hoc Bonferroni multiple comparison test.

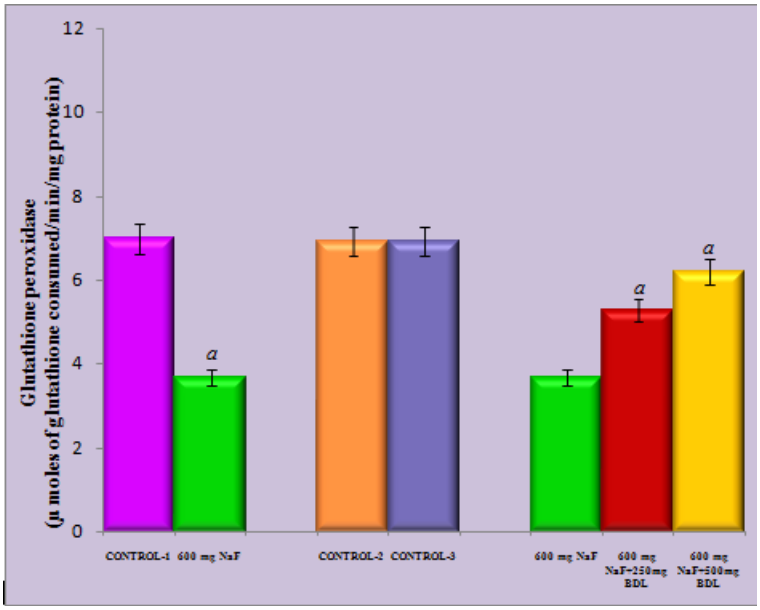

Fig. 24: Mean activity of glutathione peroxidase (GPx) (µ moles of glutathione consumed/min/mg protein) in ileumum of control and fluoride treated rats and post-treated with leaf extract of *Boerhaavia diffusa* L.; *aP< 0.001* value is significantly different as compared to NaF treated group and Group 600 mg NaF compared with post-treated groups. One way ANOVA followed by Post-hoc Bonferroni multiple comparison test.

GLUTATHIONE-S-TRANSFERASE

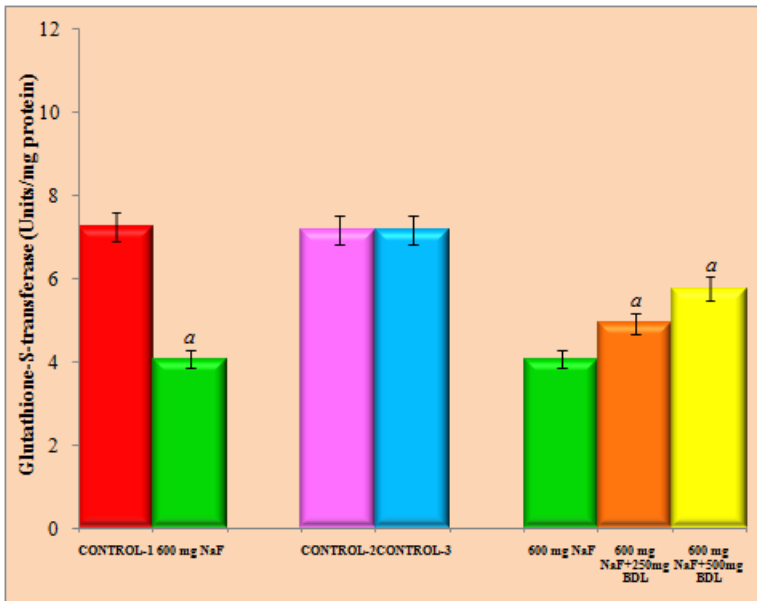

Fig. 25: Mean activity of glutathione-S-transferase (GST) (Units/mg protein) in stomach of control and fluoride treated rats and post-treated with leaf extract of *Boerhaavia diffusa* L.; *aP< 0.001* value is significantly different as compared to NaF treated group and Group 600 mg NaF compared with post-treated groups. One way ANOVA followed by Post-hoc Bonferroni multiple comparison test.

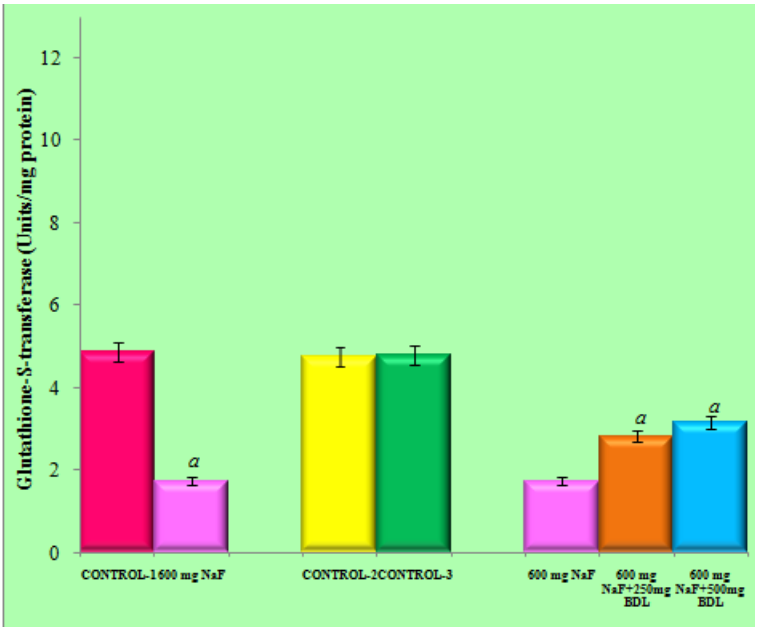

Fig. 26: Mean activity of glutathione-S-transferase (GST) (Units/mg protein) in duodenum of control and fluoride treated rats and post-treated with leaf extract of *Boerhaavia diffusa* L.; *aP< 0.001* value is significantly different as compared to NaF treated group and Group 600 mg NaF compared with post-treated groups. One way ANOVA followed by Post-hoc Bonferroni multiple comparison test.

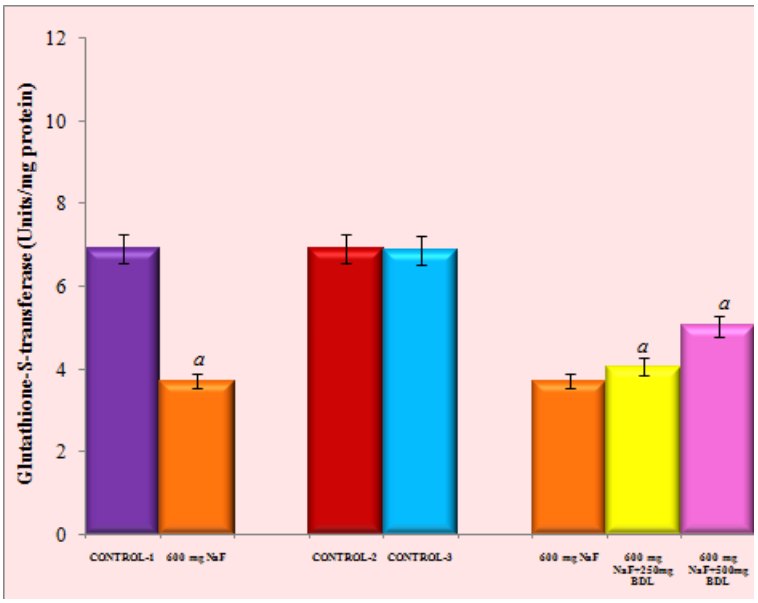

Fig. 27: Mean activity of glutathione-S-transferase (GST) (Units/mg protein) in jejunum of control and fluoride treated rats and post-treated with leaf extract of *Boerhaavia diffusa* L.; *aP< 0.001* value is significantly different as compared to NaF treated group and Group 600 mg NaF compared with post-treated groups. One way ANOVA followed by Post-hoc Bonferroni multiple comparison test.

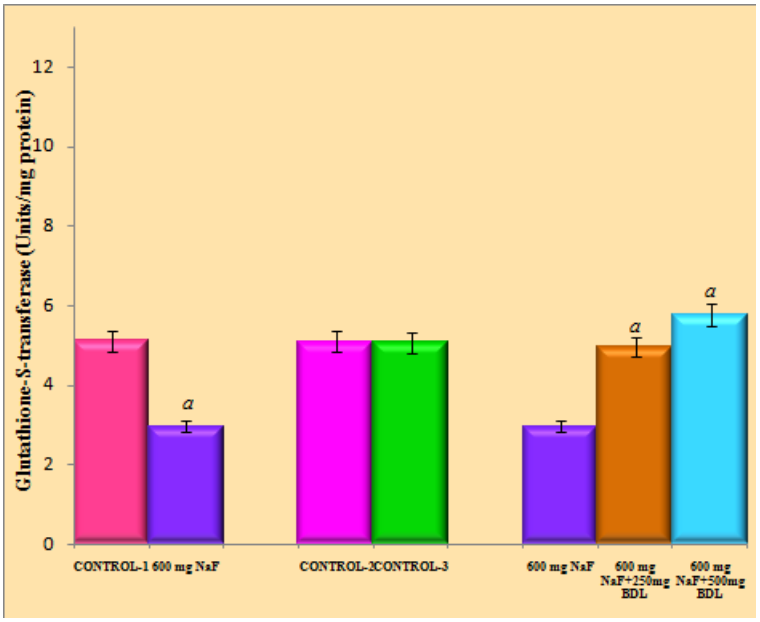

Fig. 28: Mean activity of glutathione-S-transferase (GST) (Units/mg protein) in ileum of control and fluoride treated rats and post-treated with leaf extract of *Boerhaavia diffusa* L.; *aP< 0.001* value is significantly different as compared to NaF treated group and Group 600 mg NaF compared with post-treated groups. One way ANOVA followed by Post-hoc Bonferroni multiple comparison test.

GLUTATHIONE REDUCTASE

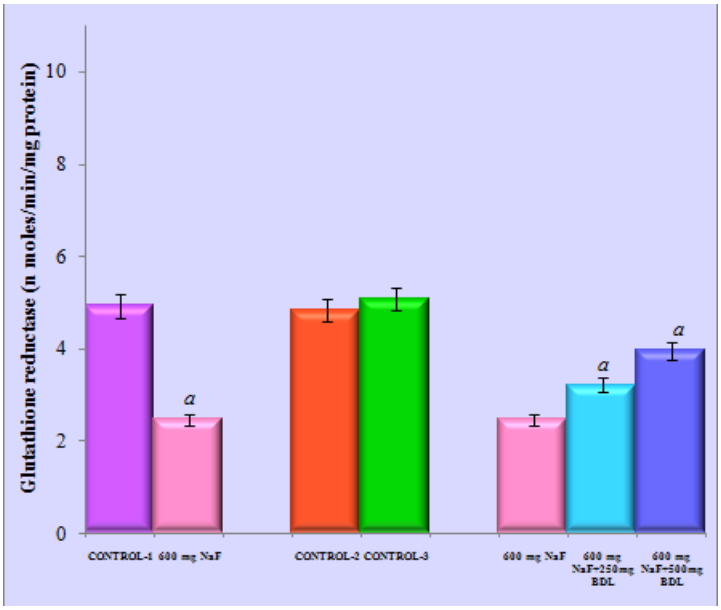

Fig. 29: Mean activity of glutathione reductase (n moles/min/mg protein) in stomach of control and fluoride treated rats and post-treated with leaf extract of *Boerhaavia diffusa* L.; *aP< 0.001* value is significantly different as compared to NaF treated group and Group 600 mg NaF compared with post-treated groups. One way ANOVA followed by Post-hoc Bonferroni multiple comparison test.

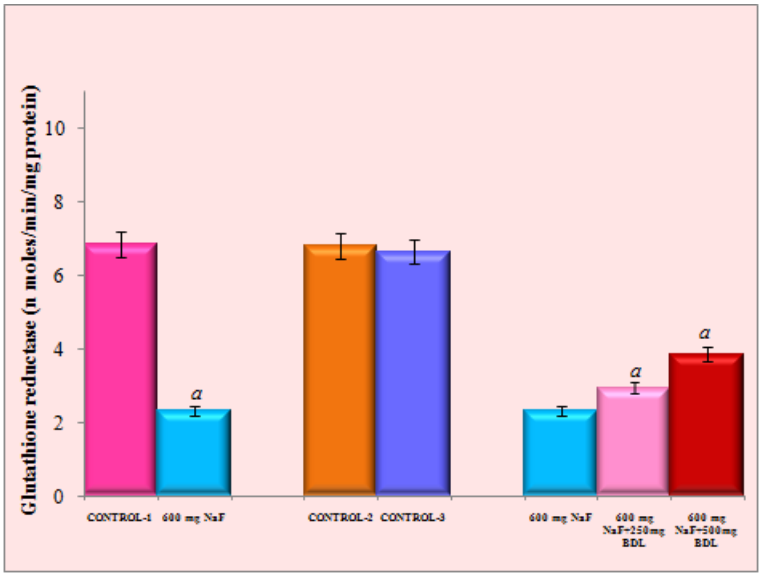

Fig. 30: Mean activity of glutathione reductase (n moles/min/mg protein) in duodenum of control and fluoride treated rats and post-treated with leaf extract of *Boerhaavia diffusa* L.; *aP< 0.001* value is significantly different as compared to NaF treated group and Group 600 mg NaF compared with post-treated groups. One way ANOVA followed by Post-hoc Bonferroni multiple comparison test.

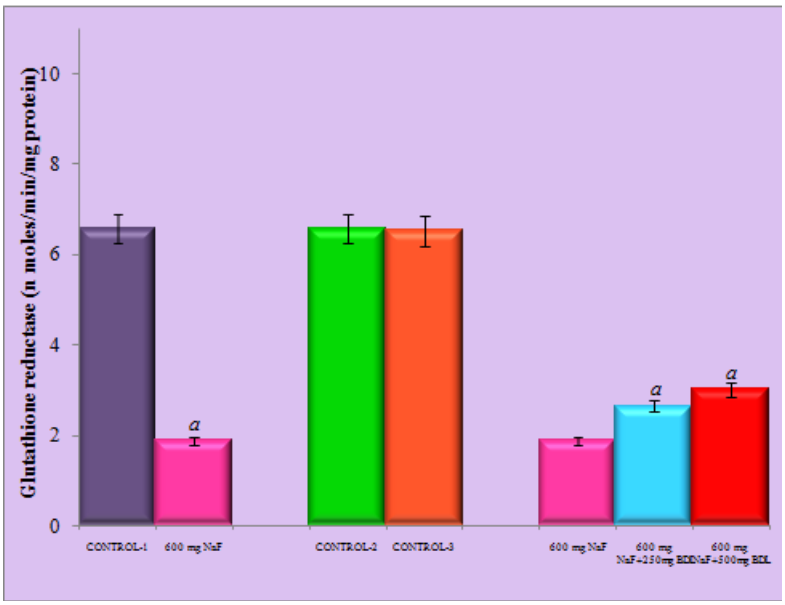

Fig. 31: Mean activity of glutathione reductase (n moles/min/mg protein) in jejunum of control and fluoride treated rats and post-treated with leaf extract of *Boerhaavia diffusa* L.; *aP< 0.001* value is significantly different as compared to NaF treated group and Group 600 mg NaF compared with post-treated groups. One way ANOVA followed by Post-hoc Bonferroni multiple comparison test.

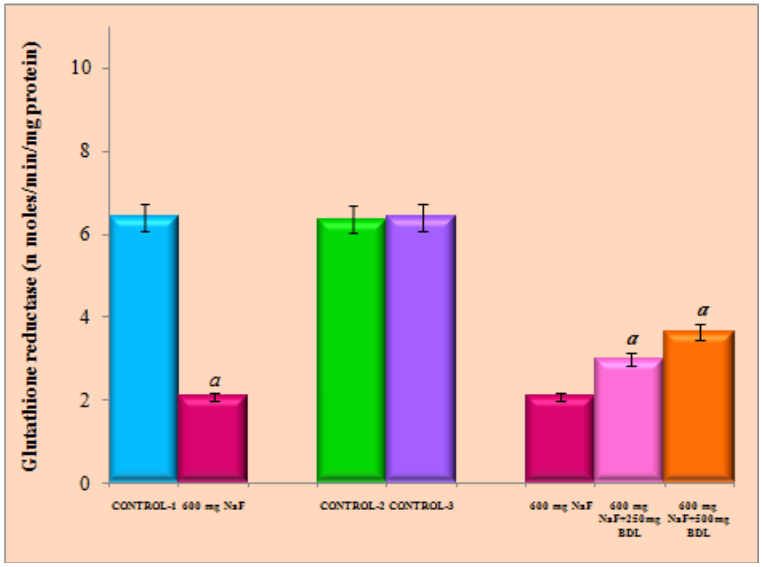

Fig. 32: Mean activity of glutathione reductase (n moles/min/mg protein) in ileum of control and fluoride treated rats and post-treated with leaf extract of *Boerhaavia diffusa* L.; *aP< 0.001* value is significantly different as compared to NaF treated group and Group 600 mg NaF compared with post-treated groups. One way ANOVA followed by Post-hoc Bonferroni multiple comparison test.

MALONDIALDEHYDE

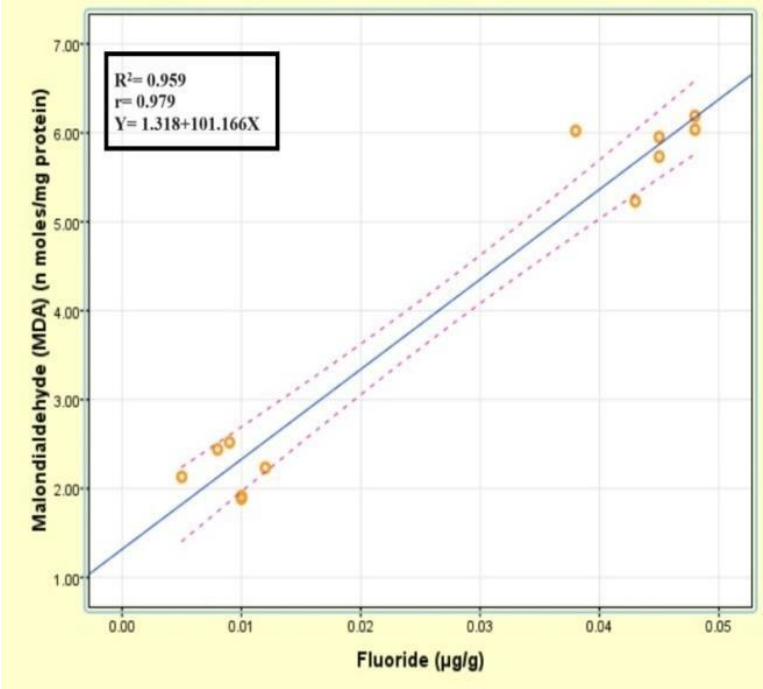

Fig. 33: Scatterplot showing Pearson's bivariate correlation and simple linear regression between levels of stomach fluoride (µg/g) and malondialdehyde (MDA) (n moles/mg protein) in test rats after 40 days of fluoride treatment.

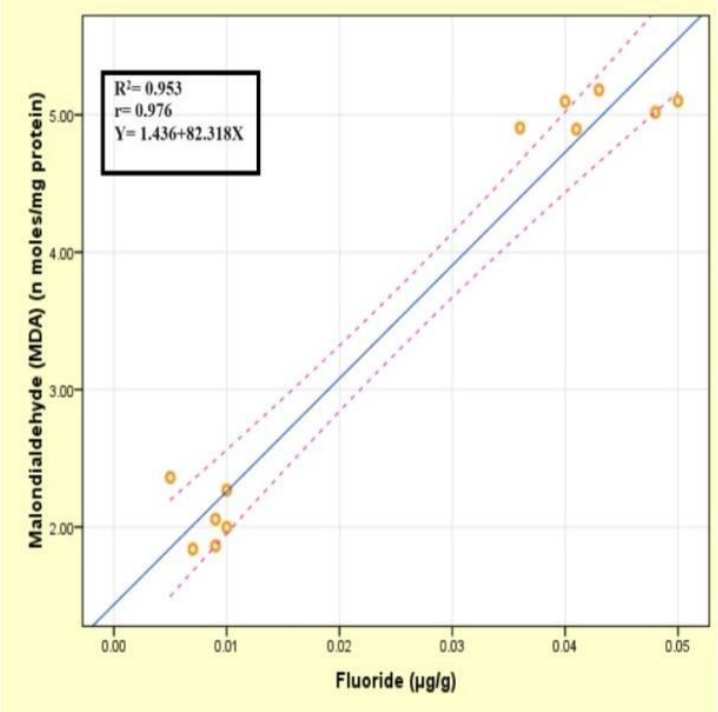

Fig. 34: Scatterplot showing Pearson's bivariate correlation and simple linear regression between levels of duodenum fluoride (µg/g) and malondialdehyde (MDA) (n moles/mg protein) in test rats after 40 days of fluoride treatment.

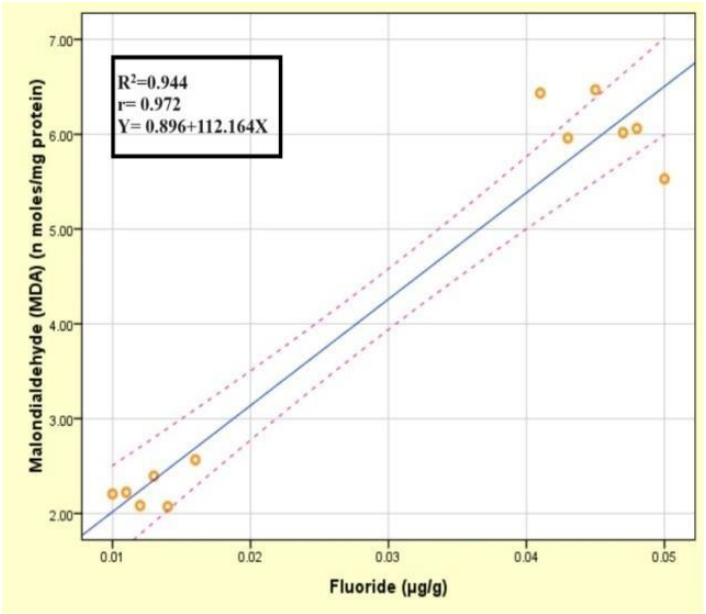

Fig. 35: Scatterplot showing Pearson's bivariate correlation and simple linear regression between levels of jejunum fluoride (µg/g) and malondialdehyde (MDA) (n moles/mg protein) in test rats after 40 days of fluoride treatment.

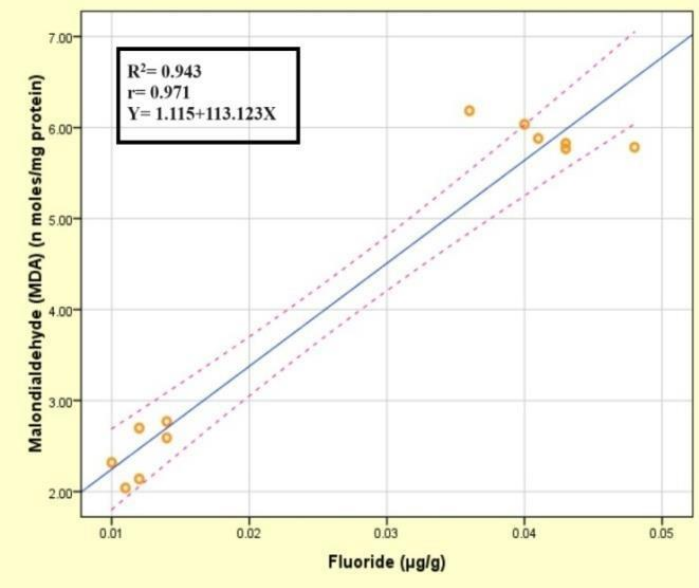

Fig. 36: Scatterplot showing Pearson's bivariate correlation and simple linear regression between levels of ileum fluoride (µg/g) and malondialdehyde (MDA) (n moles/mg protein) in test rats after 40 days of fluoride treatment.

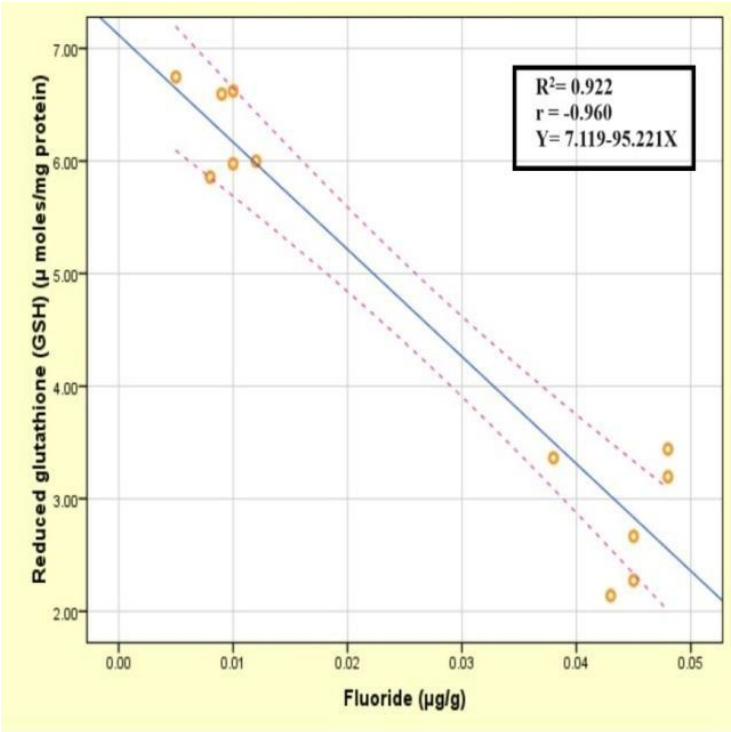

Fig. 37: Scatterplot showing Pearson's bivariate correlation and simple linear regression between levels of stomach fluoride (µg/g) and reduced glutathione (GSH) (µ moles/mg protein) in test rats after 40 days of fluoride treatment.

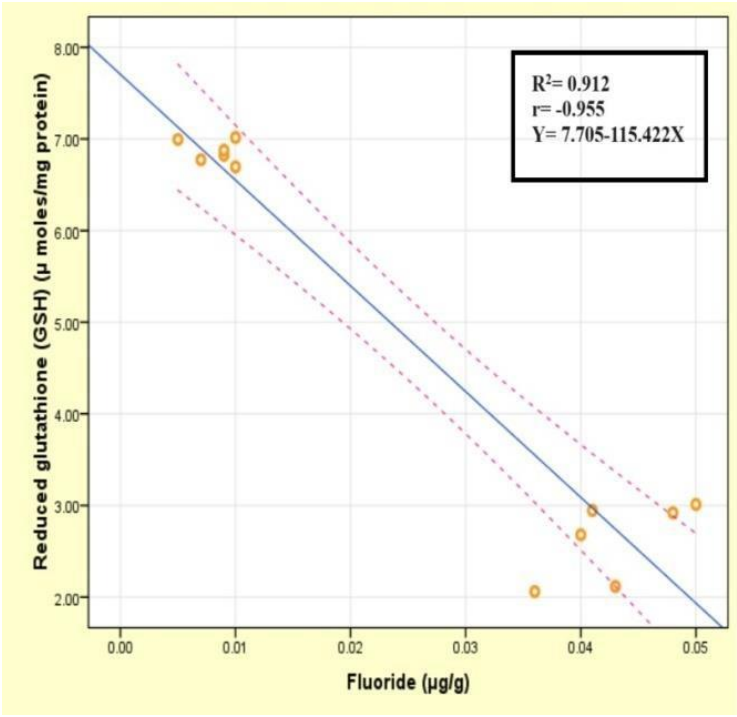

Fig. 38: Scatterplot showing Pearson's bivariate correlation and simple linear regression between levels of duodenum fluoride (µg/g) and reduced glutathione (GSH) (µ moles/mg protein) in test rats after 40 days of fluoride treatment.

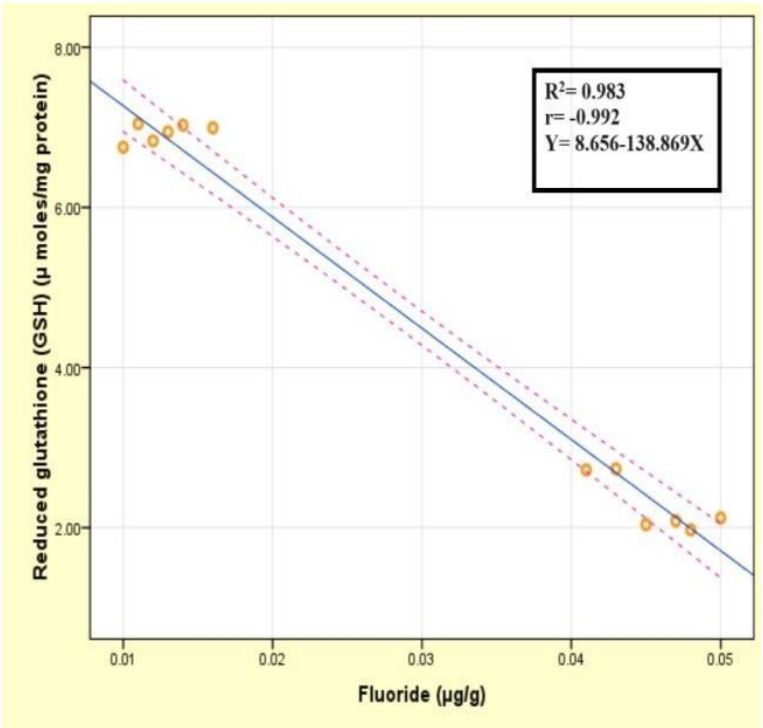

Fig. 39: Scatterplot showing Pearson's bivariate correlation and simple linear regression between levels of jejunum fluoride (µg/g) and reduced glutathione (GSH) (µ moles/mg protein) in test rats after 40 days of fluoride treatment.

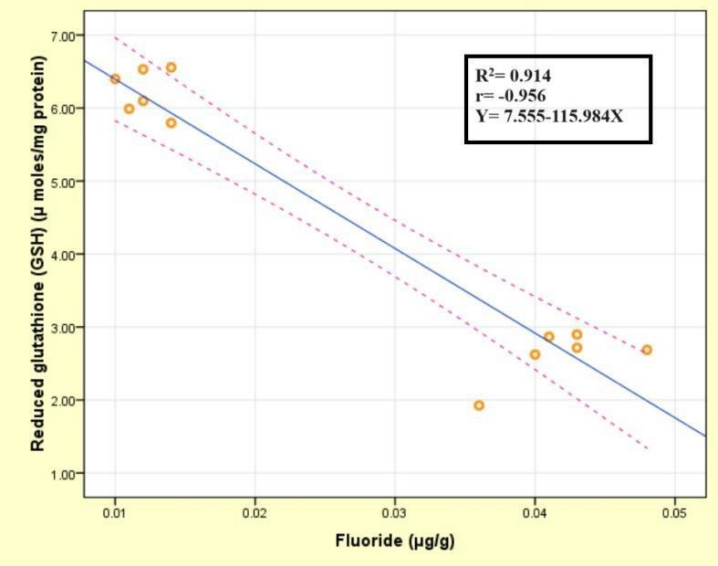

Fig. 40: Scatterplot showing Pearson's bivariate correlation and simple linear regression between levels of ileum fluoride (µg/g) and reduced glutathione (GSH) (µ moles/mg protein) in test rats after 40 days of fluoride treatment.

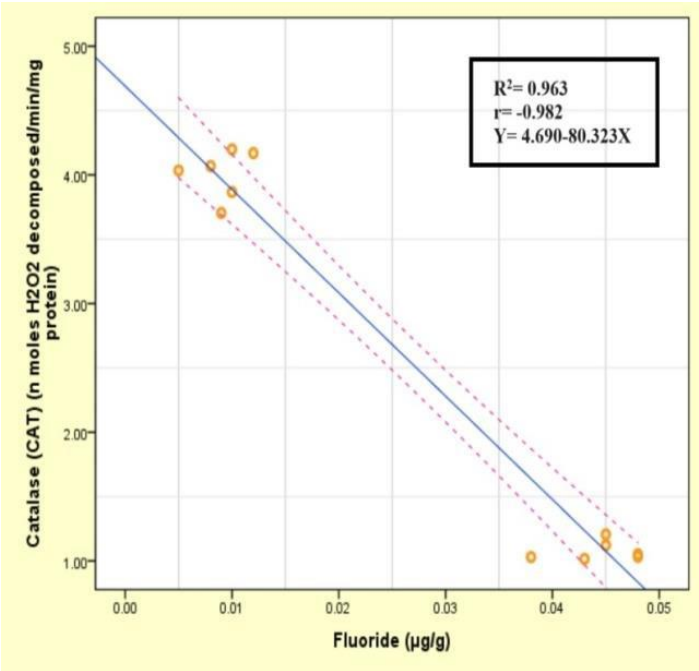

Fig. 41: Scatterplot showing Pearson's bivariate correlation and simple linear regression between levels of stomach fluoride (µg/g) and activity of catalase (CAT) (n moles H2O2 decomposed/min/mg protein) in test rats after 40 days of fluoride treatment.

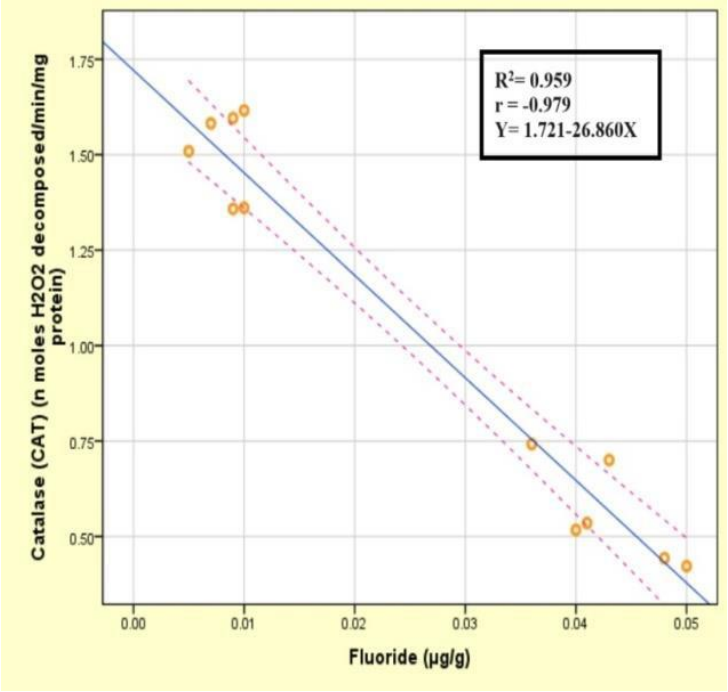

Fig. 42: Scatterplot showing Pearson's bivariate correlation and simple linear regression between levels of duodenum fluoride (µg/g) and activity of catalase (CAT) (n moles H2O2 decomposed/min/mg protein) in test rats after 40 days of fluoride treatment.

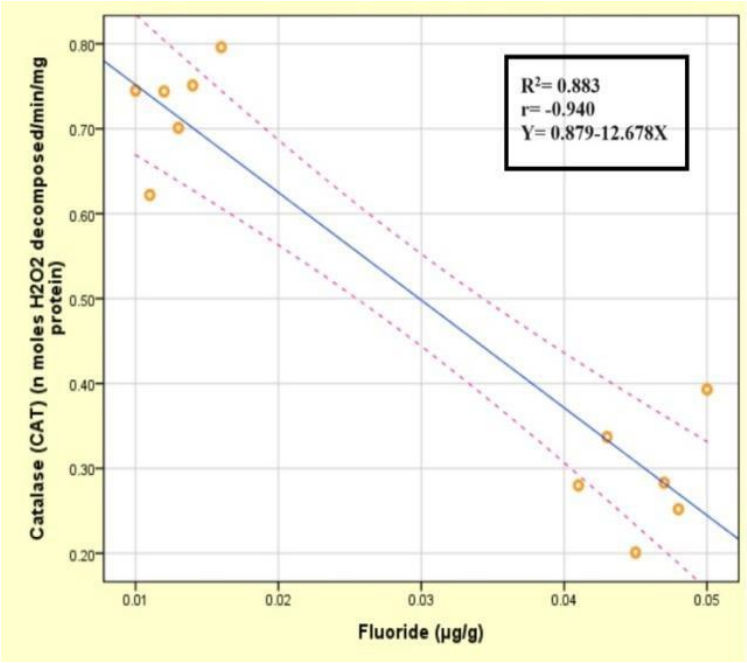

Fig. 43: Scatterplot showing Pearson's bivariate correlation and simple linear regression between levels of jejunum fluoride (µg/g) and activity of catalase (CAT) (n moles H2O2 decomposed/min/mg protein) in test rats after 40 days of fluoride treatment.

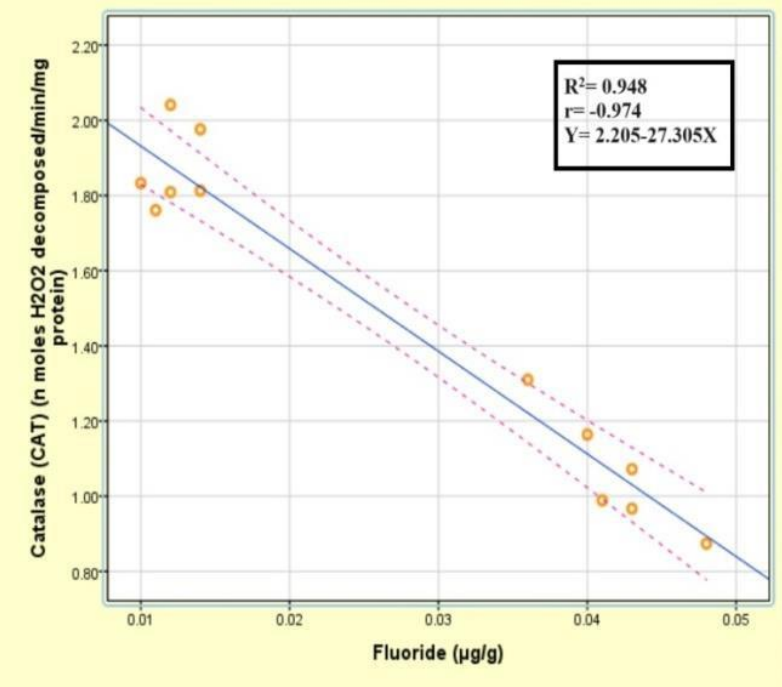

Fig. 44: Scatterplot showing Pearson's bivariate correlation and simple linear regression between levels of ileum fluoride (µg/g) and activity of catalase (CAT) (n moles H2O2 decomposed/min/mg protein) in test rats after 40 days of fluoride treatment.

MITOCHONDRIAL SUPEROXIDE DISMUTASE

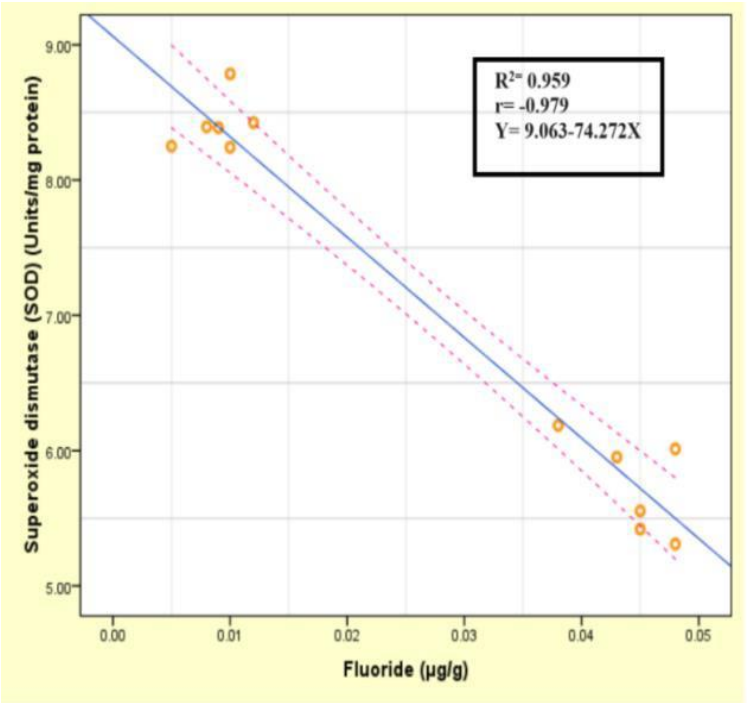

Fig. 45: Scatterplot showing Pearson's bivariate correlation and simple linear regression between levels of stomach fluoride (µg/g) and activity of superoxide dismutase (SOD) (Units/mg protein) in test rats after 40 days of fluoride treatment.

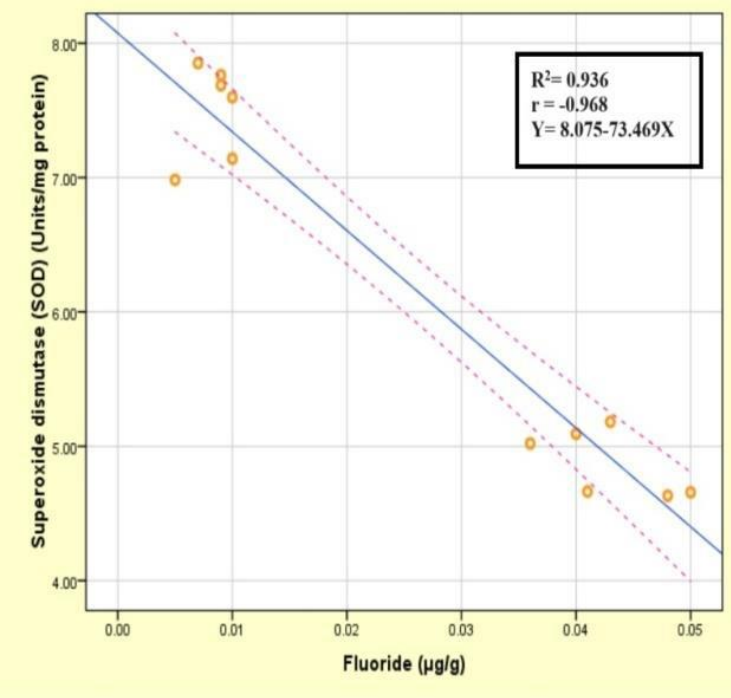

Fig. 46: Scatterplot showing Pearson's bivariate correlation and simple linear regression between levels of duodenum fluoride (µg/g) and activity of superoxide dismutase (SOD) (Units/mg protein) in test rats after 40 days of fluoride treatment.

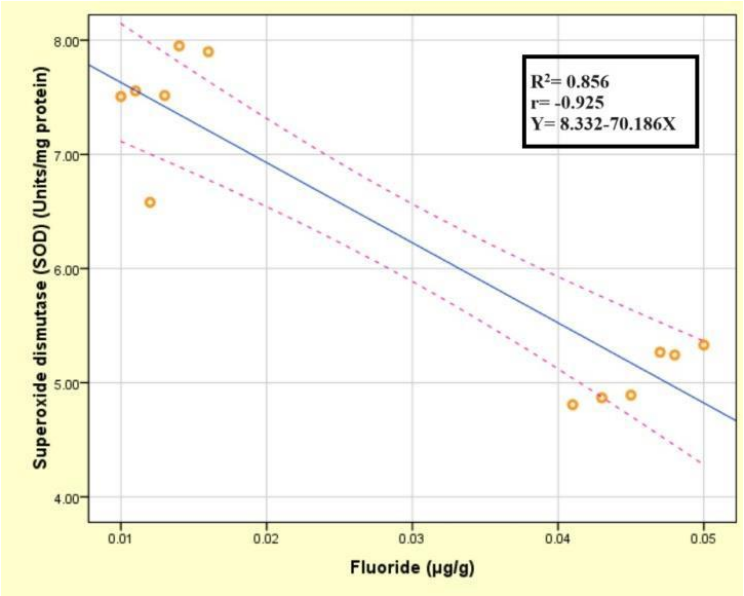

Fig. 47: Scatterplot showing Pearson's bivariate correlation and simple linear regression between levels of jejunum fluoride (µg/g) and activity of superoxide dismutase (SOD) (Units/mg protein) in test rats after 40 days of fluoride treatment.

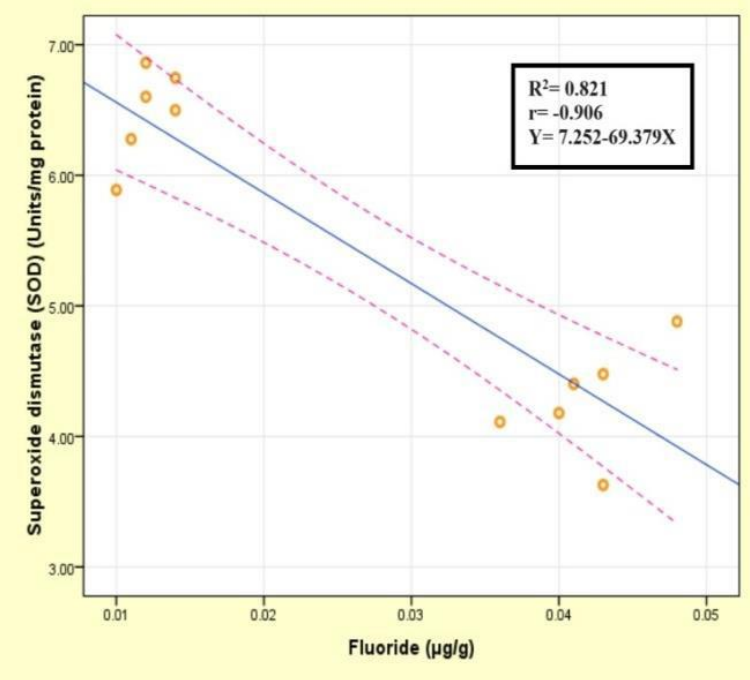

Fig. 48: Scatterplot showing Pearson's bivariate correlation and simple linear regression between levels of ileum fluoride (µg/g) and activity of superoxide dismutase (SOD) (Units/mg protein) in test rats after 40 days of fluoride treatment.

GLUTATHIONE PEROXIDASE

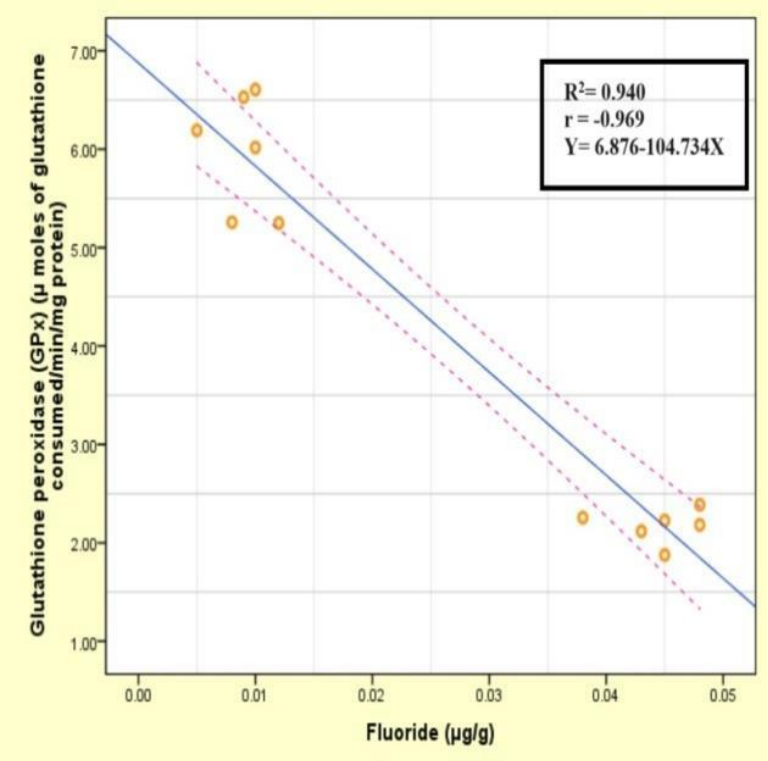

Fig. 49: Scatterplot showing Pearson's bivariate correlation and simple linear regression between levels of stomach fluoride (µg/g) and activity of glutathione peroxidase (GPx) (µ moles of glutathione consumed/min/mg protein) in test rats after 40 days of fluoride treatment.

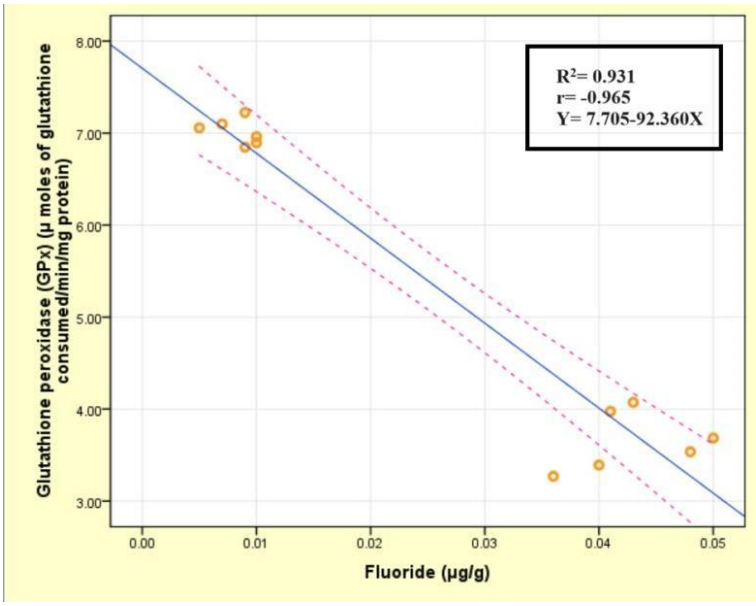

Fig. 50: Scatterplot showing Pearson's bivariate correlation and simple linear regression between levels of duodenum fluoride (µg/g) and activity of glutathione peroxidase (GPx) (µ moles of glutathione consumed/min/mg protein) in test rats after 40 days of fluoride treatment.

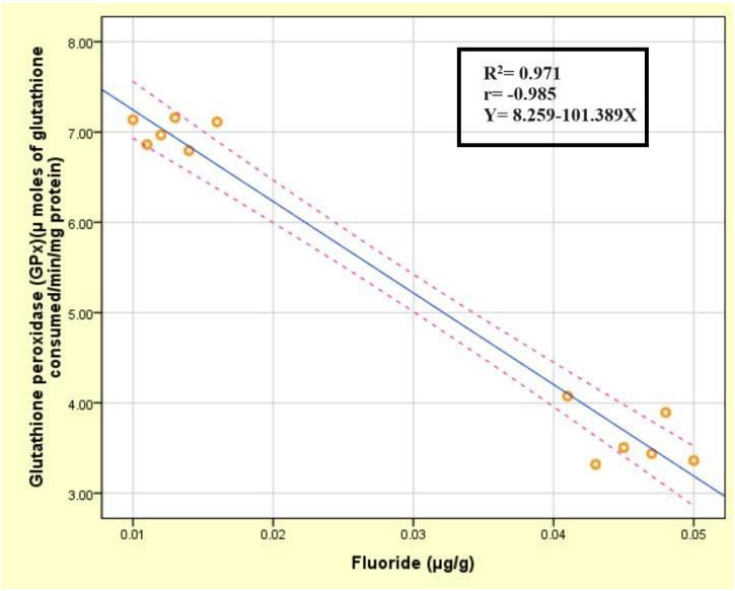

Fig. 51: Scatterplot showing Pearson's bivariate correlation and simple linear regression between levels of jejunum fluoride (µg/g) and activity of glutathione peroxidase (GPx) (µ moles of glutathione consumed/min/mg protein) in test rats after 40 days of fluoride treatment.

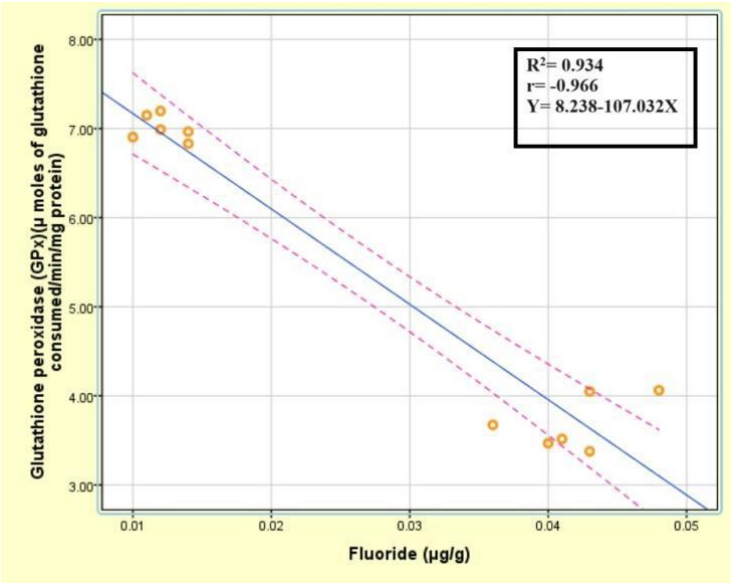

Fig. 52: Scatterplot showing Pearson's bivariate correlation and simple linear regression between levels of ileum fluoride (µg/g) and activity of glutathione peroxidase (GPx) (µ moles of glutathione consumed/min/mg protein) in test rats after 40 days of fluoride treatment.

GLUTATHIONE-S-TRANSFERASE

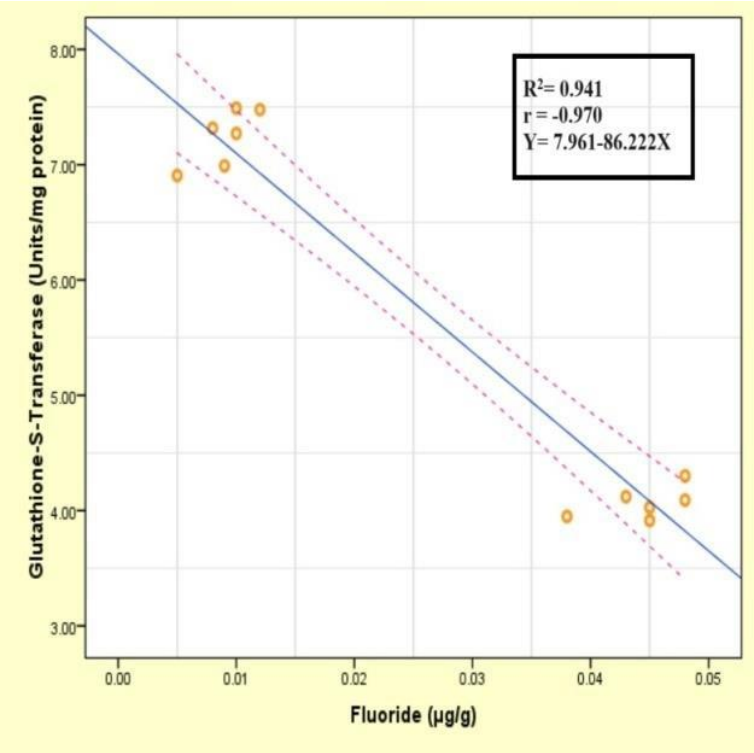

Fig. 53: Scatterplot showing Pearson's bivariate correlation and simple linear regression between levels of stomach fluoride (µg/g) and activity of glutathione-S-transferase (GST) (Units/mg protein) in test rats after 40 days of fluoride treatment.

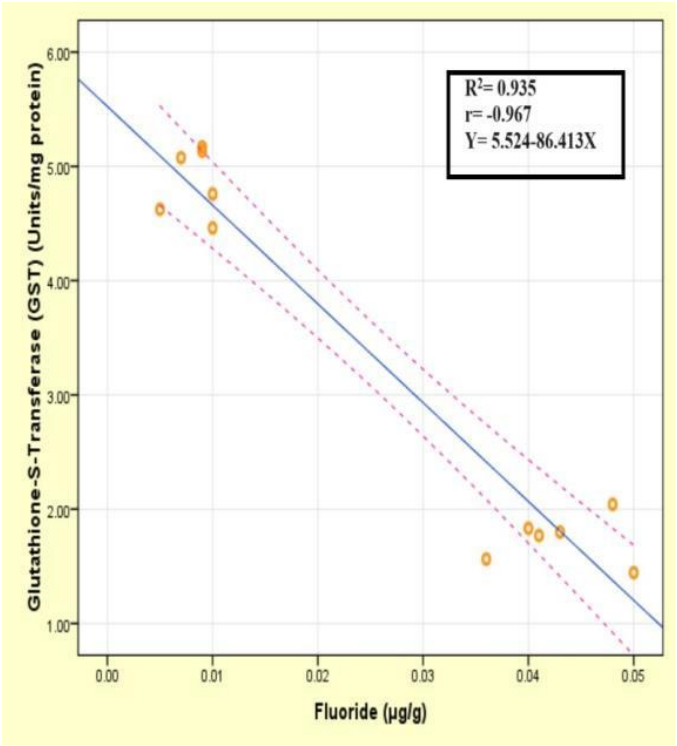

Fig. 54: Scatterplot showing Pearson's bivariate correlation and simple linear regression between levels of duodenum fluoride (µg/g) and activity of glutathione-S-transferase (GST) (Units/mg protein) in test rats after 40 days of fluoride treatment.

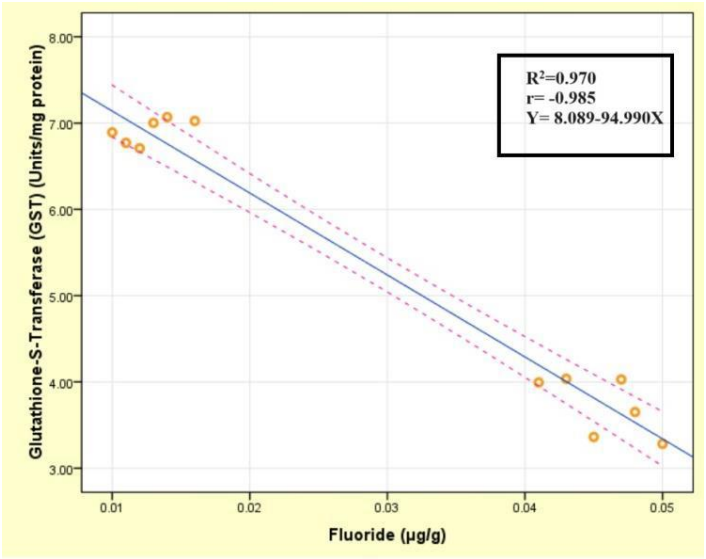

Fig. 55: Scatterplot showing Pearson's bivariate correlation and simple linear regression between levels of jejunum fluoride (µg/g) and activity of glutathione-S-transferase (GST) (Units/mg protein) in test rats after 40 days of fluoride treatment.

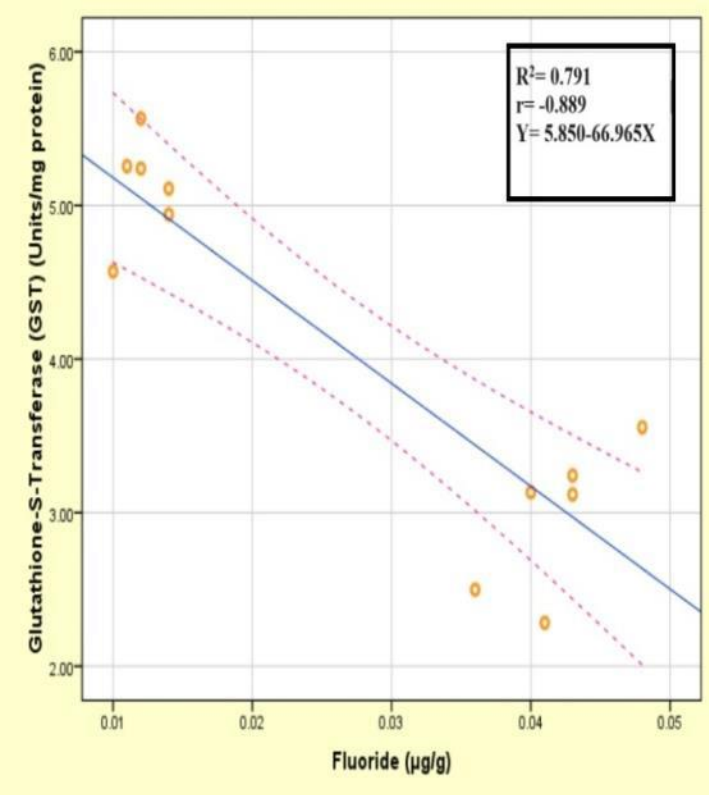

Fig. 56: Scatterplot showing Pearson's bivariate correlation and simple linear regression between levels of ileum fluoride (µg/g) and activity of glutathione-S-transferase (GST) (Units/mg protein) in test rats after 40 days of fluoride treatment.

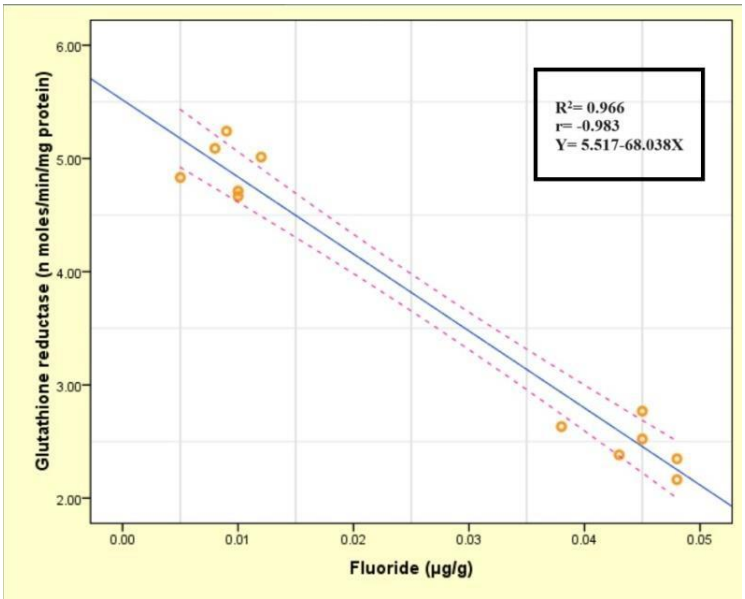

Fig. 57: Scatterplot showing Pearson's bivariate correlation and simple linear regression between levels of stomach fluoride (µg/g) and activity of glutathione reductase (n moles/min/mg protein) in test rats after 40 days of fluoride treatment.

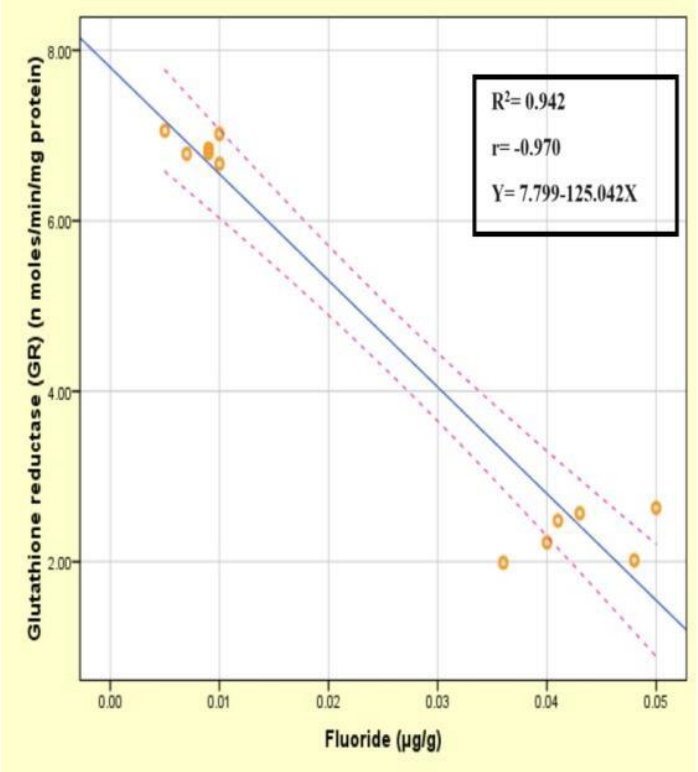

Fig. 58: Scatterplot showing Pearson's bivariate correlation and simple linear regression between levels of duodenum fluoride (µg/g) and activity of glutathione reductase (n moles/min/mg protein) in test rats after 40 days of fluoride treatment.

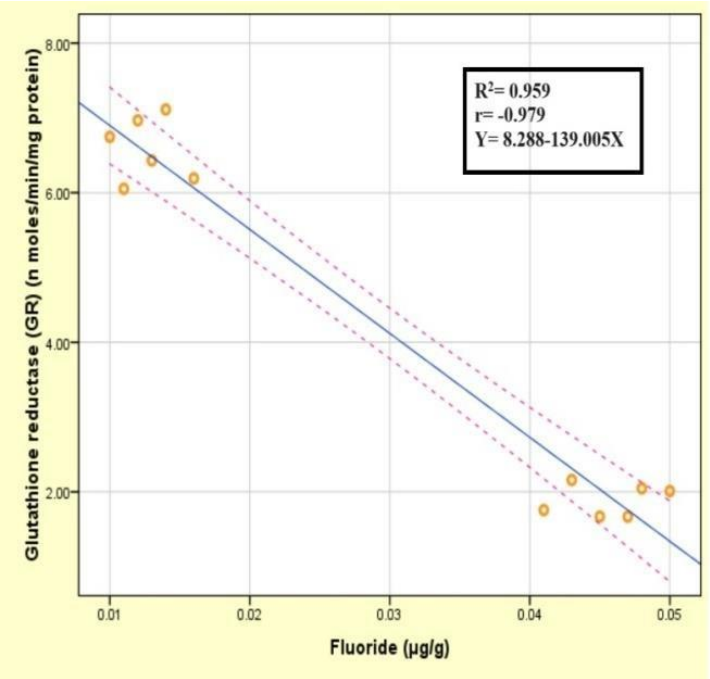

Fig. 59: Scatterplot showing Pearson's bivariate correlation and simple linear regression between levels of jejunum fluoride (µg/g) and activity of glutathione reductase (n moles/min/mg protein) in test rats after 40 days of fluoride treatment.

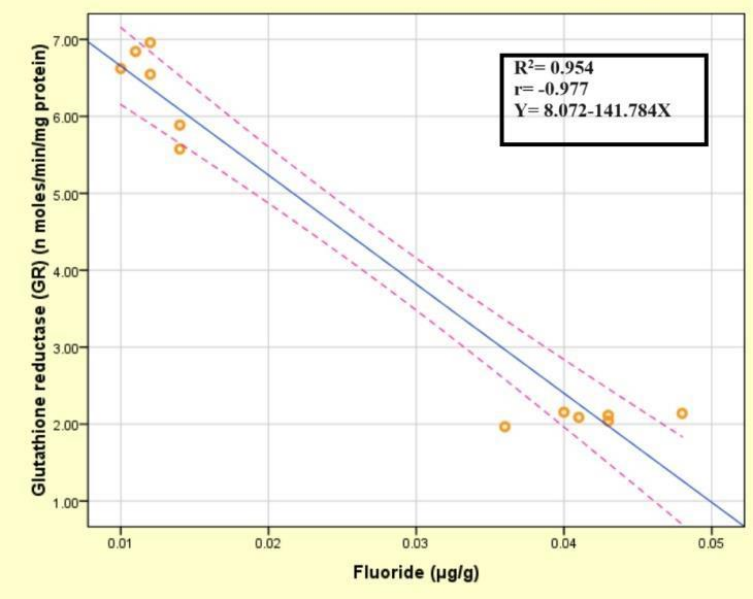

Fig. 60: Scatterplot showing Pearson's bivariate correlation and simple linear regression between levels of ileum fluoride (µg/g) and activity of glutathione reductase (n moles/min/mg protein) in test rats after 40 days of fluoride treatment.

DISCUSSION

Fluoride is toxic to living cells and can cause various biochemical changes, including oxidative stress, when its levels exceed certain limits.[15] Oxidative stress results from the buildup of free radicals in the body, leading to oxidative damage.[16] One of the primary mechanisms through which NaF exerts its toxic effects is by inducing oxidative stress. NaF has been shown to increase the production of reactive oxygen species (ROS) in gastric tissues, leading to lipid peroxidation and oxidative damage to cellular components. Studies have demonstrated elevated levels of malondialdehyde (MDA), a marker of lipid peroxidation, in the stomach tissues of NaF-treated rats.[17] The present study exhibited that rats exposed to fluoride showed an increase in malondialdehyde content and a decrease in the activity of catalse, superoxide dismutase, glutathione peroxidase, glutathione-S-transferase and glutathione reducatse. These findings are consistent with the study of Chauhan *et al*.[18]

Lipid peroxidation is a common reaction caused by free radicals attacking biological structures. MDA content is a reliable biomarker for oxidative stress injury in the body.[19] A significant increase in MDA levels in rats treated with fluoride attributed to increased oxidative stress in cells caused by the fluoride-induced depletion of the antioxidant scavenger system.

GSH serves as a catalyst for disulfide exchange processes and is oxidized to produce a disulfide bond under conditions of oxidative stress.[20] GSH deficiency in living things can result in tissue damage and disorders.[21]

Catalase is a prevalent enzyme that breaks down hydrogen peroxide into water and oxygen in almost all organisms that are exposed to oxygen. Animals treated with fluoride showed a decrease in CAT activity that is consistent with previous findings.[15] This reduction in CAT activity is likely attributed to the oxidative stress induced by fluoride intoxication.

The antioxidant enzymes are crucial components of the initial cellular defense against oxidative damage. Superoxide dismutase is a naturally occurring intracellular enzyme responsible for catalyzing the breakdown of superoxide radicals.

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 gastrointestinal organs, further impairing the antioxidant defense mechanism. GPx functions in conjunction with CAT to effectively scavenge hydrogen peroxide (H_2O_2) and reduce lipid peroxidation during periods of oxidative stress. The activity of glutathione peroxidase relies on maintaining a balance between glutathione and glutathione disulfide levels.[22] A notable reduction in glutathione reductase activity could be linked to a compromised conversion of glutathione disulfide into reduced glutathione, which in turn influences the GSH/GSSG ratio.

Glutathione S-transferase plays a crucial role in removing free radicals and its levels can reflect the antioxidant capacity of tissues. Studies have shown that rats exposed to fluoride exhibit reduced glutathione-S-transferase activity. The gastrointestinal tract reported decline in glutathione reductase and glutathione-S-transferase activity to increased oxidative stress, which increases its susceptibility to oxidative damage brought on by NaF.

The current study demonstrated that the leaf extract of *Boerhaavia diffusa* L. provides therapeutic benefits by protecting against fluoride-induced oxidative stress. The enhancement observed in antioxidant metabolism is attributed to the diverse effects of secondary metabolites present in *Boerhaavia diffusa* leaves. These metabolites play a role in mitigating oxidative stress associated with fluoride intoxication.

CONCLUSION

In conclusion, the present study implies that exposure to sodium fluoride increases oxidative stress in the gastrointestinal tract of rats. However, treatment with *Boerhaavia diffusa* L. leaf extract shows significant ameliorative effects against this fluoride-induced oxidative stress. This is evidenced by the reduced levels of malondialdehyde, a marker of lipid peroxidation, following *Boerhaavia diffusa* L. treatment. Additionally, the treatment restored the levels of glutathione and the activities of glutathione peroxidase, catalase, superoxide dismutase, glutathione-S-transferase and glutathione reductase. Overall, the results revealed that leaf extract of *Boerhaavia diffusa* L. effectively mitigates the cytotoxic effects of NaF on the gastrointestinal tract of rats.

ETHICAL STATEMENT

The animal care and experimental protocols used in this study were approved by Institutional Animal Ethical Committee of Punjabi University, Patiala (Animal maintenance and Registration No. 107/GO/ReBi/S/99/CPCSEA/2017-41).

GRANTS

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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