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Keywords: Albino rats; Antioxidant enzymes; *Boerhaavia diffusa L*; Nephrotoxicity; Oxidative stress; Sodium fluoride

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Review Article

Protective and curative effects of *Boerhaavia diffusa* L. against fluoride induced renal oxidative stress and antioxidant enzymes in rats

Abstract

This work elucidated the protective effect of leaf extract of the *Boerhaavia diffusa* L. (punarnava) on kidney damage following fluoride administration in rats. Forty eight rats were randomly divided into eight group's six rats in each. Group I was administered deionized water orally served as control. Group II and III were administered with 300 and 600 ppm NaF/kg bw/day for 40 days. Group IV were orally administrated with 500mg/kg b.w/day of leaf extract of *Boerhaavia diffusa* L. for 20 days Group V and VI were pre-treated with 500 mg/kg bw/day of leaf extract of the *Boerhaavia diffusa* L. for 20 days and then exposed to 300 and 600 ppm NaF/kg bw/day for 40 days. Group VII and VIII were exposed firstly to 300 and 600 ppm NaF/kg bw/day and then post-treated with leaf extract of the *Boerhaavia diffusa* L. for 20 days. The level of MDA exhibited significantly ($p<0.001$) increase while GSH and activities of SOD, CAT, and GPx revealed significant ($p<0.001$) decline in kidney of rats treated with 300 and 600 ppm of NaF. The results indicate that pre and post-treatment significantly decrements ($p<0.001$) in the activity of antioxidant enzymes superoxide dismutase (SOD), catalase (CAT), reduced glutathione (GSH) and glutathione peroxidise (Gpx) alongwith significant increase ($p<0.001$) in the level of malondialdehyde (MDA).The current work suggests that the leaf extract of *Boerhaavia diffusa* L conferred therapeutic benefits on NaF-induced nephrotoxicity, particularly when administered before more than after the insult.

Introduction

Drug-induced nephrotoxicity is an important cause of renal failure. Kidney is one of the target organs attacked by excessive amounts of fluoride. Fluoride has a role in cellular respiratory process like in free radical reactions. Fluoride reacts with polyunsaturated fatty acids and initiates lipid peroxidation leading to necrosis and apoptosis [1,2]. As the primary organ concerned with excretion and retention of fluoride, kidney is quite sensitive to the toxicity of fluoride [3]. The situation of serious imbalance between oxidant and antioxidant is referred to as oxidative damage. In many diseases, tissue damage is accompanied by an imbalance in the oxidant and antioxidant status. Exposure to fluoride results in generation of anion superoxide, increased oxygen concentration and its downstream consequences such as hydrogen peroxide, hydroxyl radicals, which are important in mediating the toxic effects of fluoride. Intake of high levels of fluoride is known to cause structural changes, altered activities of enzymes, and influence the metabolism of lipid. Acute poisoning can terminate in death due to blocking of cell metabolism since fluoride inhibits enzymatic processes, mainly metalloenzymes responsible for important vital processes [4].

Boerhaavia diffusa L. has many medicinal properties and enjoys an important place among medicinal herbs in India since ancient times [5]. Punarnava leaves are consumed by the people as food supplements with broad spectrum disease defending properties and with no reported side-effects, the results of the present studies may have future therapeutic relevance in the areas where humans are exposed to fluoride either occupationally or environmentally [6]. The aim of the present study is to investigate the oxidative damage caused in renal tissue and the protective effects of leaf extract of *Boerhaavia diffusa* L.

Materials and Methods

Preparation of leaf extract of *Boerhaavia diffusa* L.

Fresh leaves of *Boerhaavia diffusa* L. were washed in running tap water to remove adhering dust and wiped to dryness. The leaves were then dried under shade. The shade dried leaves were finely grind using a mechanical blender. The powder

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obtained was used for ethanol extraction in a soxhlet extractor. The excessive solvent from the extract was recovered with rotary vacuum evaporator and then the concentrated extract was dried to constant weight in a hot air oven at 40°C.The leaf extract of *Boerhaavia diffusa* L. was prepared by the method given by Narendhirakannan [7].

Experimental Protocol

Ethical aspects

Experimental protocols and procedures used in this study were approved by the animal ethical committee of Punjabi University, Patiala (Animal Maintenance and Registration No. 107/99/ CPCSEA /2014-23).

Young Wistar albino rats, weighing between 100-200gm were housed in polypropylene cages with stainless grill tops and fed with standard rat pellet diet (Hindustan lever limited, India) and water was given *ad libitum*. Animals were maintained at a constant room temperature of 20-22°C and 60% humidity. Rats were allowed a 2-week acclimatization period and then they were randomly divided into eight groups. Rats of group I received 1ml deionized water /kg b.w. / day orally daily by a gastric tube for 40 days, and served as control. Rats of group II and III were orally administered with 300 and 600 ppm NaF /kg bw /day for the same duration. Group IV antidote control group was orally administrated with 500mg/kg b.w/day of leaf extract of *Boerhaavia diffusa* L. for 20 days. Animals of Group II and III were pre and post-treated with 500mg/kg bw/day of leaf extract of *Boerhaavia diffusa* L. for 20 days .At the end of the experimental period, rats were fasted overnight and sacrificed under ether anesthesia.

Preparation of tissue homogenate

The renal tissue was washed with ice-cold 0.9% saline and homogenized quickly with ice cold 0.1M phosphate buffer (pH 7.4) using glass teflon homogenizer to give a 10% homogenate. The homogenate was centrifuged at 10, 000 rpm for 20 min and the supernatant were used for estimation of malondialdehyde (MDA), reduced glutathione (GSH), superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx).

Assesment of biochemical parameters

The level of MDA in the kidney tissue of rats was determined by the method of Ohkawa [8]. The GSH content in kidney tissue was measured by the method of Dringen [9]. The activities of SOD [10]. CAT [11], and GPx was determined [12], in kidney tissue of rats.

Statistical analysis

Results were expressed as mean ± standard deviation (SD). Statistical significance of difference between the experimental groups was evaluated by one way ANOVA followed by Bonferroni and Post hoc Dunnetts multiple comparison test. The correlation between two variables was analyzed by STATISTICA 7 software. A two tailed p value < 0.05 was considered statistically significant. All computations were performed using SPSS 17.0 statistical software (IBM).

Results

Malondialdehyde (MDA)

The level of MDA in kidney tissue of test rat showed a significant (F=567.9, p<0.001) increase after 40 days of fluoride treatment. More prominent increase (156.2 %) was registered in highest dose group (600 ppm NaF/kg b.w/day Figure 1).

Bonferroni multiple comparison test after ANOVA showed a significant (p<0.001) increase in the level of MDA in kidney tissue (95%CI=0.0562 to -0.0432; mean difference =0.0231 to -0.0652) as compared between and within all groups after 40 days of fluoride exposure.

Dunnetts (2-sided) multiple comparison test revealed that administration of *Boerhaavia diffusa* L. either pre-treated (95%Cl=-0.0314 to 0.0382, mean difference = -0.0278 to -0.0516) as well as in post-treatment (95% Cl=-0.0328 to -0.0396, mean difference =-0.0312 to 0.0553, p< 0.001) with leaf extract of *Boerhaavia diffusa* L. significantly decreased the levels of MDA (Figure 2).

Reduced glutathione (GSH)

The GSH content in kidney tissue of fluoridated rats showed a significant (F= 48 1.672, p<0.001) decrease after 40 days of fluoride treatment. More prominent decrease (-44.19%) was recorded in animals treated with 600 ppm NaF/kg b.w/day (Figure 3).

Bonferroni multiple comparison test after ANOVA showed a significant (p<0.001) decrease in the level of GSH in kidney tissue (95%CI=0.851 to 1.114, mean difference = 0.765 to 1.739) as compared between and within all groups after 40 days of fluoride exposure.

Dunnetts (2-sided) multiple comparison test revealed that GSH level was significantly (p< 0.001) increased in all pre-treated (pre-treated 95%Cl = 0.851 to 2.123; mean difference = 0.789 to 2.729) and in post- treated groups (95% Cl=0.864 to

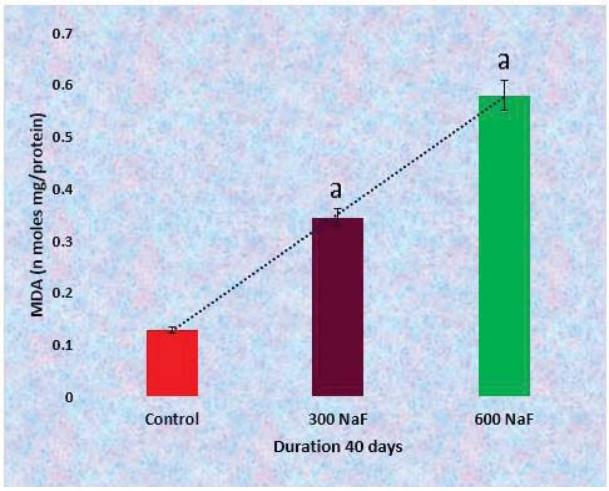

Figure 1: The level of MDA (n moles /mg protein) in kidney tissue of fluoridated rats. Values are given as mean ± SD for 6 rats in each group. ap <0.001 values are significantly different (P<0.001) as compared with control.

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2.119; mean difference 0.851 to 2.744) with 500 mg / kg bw /day of leaf extract of *Boerhaavia diffusa* L. as compared to respective NaF treated groups (Figure 4).

Superoxide dismutase (SOD)

The activity of SOD in kidney tissue of test rat revealed a significant (F=13.0491, p<0.001) decrement after 40 days of fluoride treatment. More prominent decreased (-70.40 %) was reported treated with highest dose group (600 ppm NaF/kg b.w/day) (Figure. 5).

Bonferroni multiple comparison test after ANOVA showed a significant (p<0.001) decrement in the activity of SOD in kidney tissue (95%CI=-0.978 to 0.3927; mean difference =0.0231 to -0.5620) as compared between and within all groups after 40 days of fluoride exposure.

Dunnetts (2-sided) multiple comparison test revealed that administration of *Boerhaavia diffusa* L. either (pre-treated with

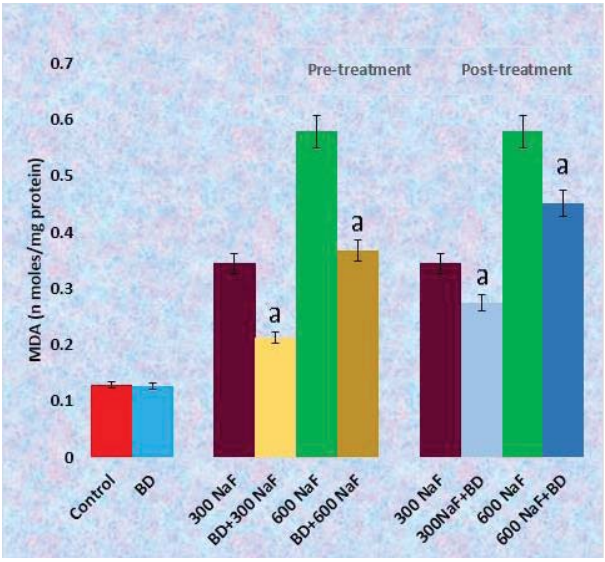

Figure 2: Effect of leaf extract of *Boerhaavia diffusa* L. on renal MDA content in fluoridated rats. Values are given as mean ± SD for 6 rats in each group. aP <0.001 values are significantly different as compared to respective NaF treated groups.

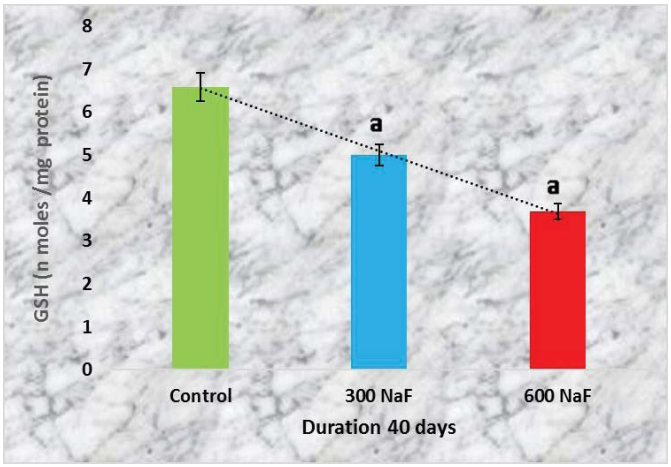

Figure 3: The GSH content (n moles /mg protein) in kidney tissue of fluoridated rats. ap <0.001 values are significantly different (P< 0.001) as compared with control.

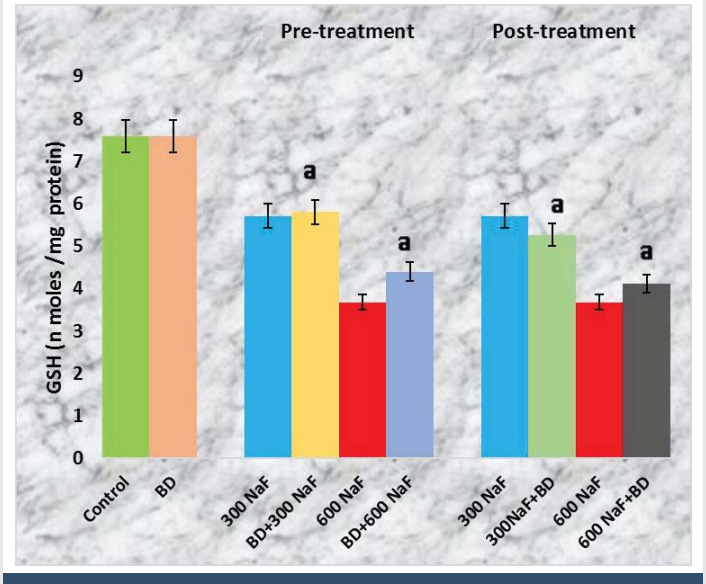

Figure 4: Effect of leaf extract of *Boerhaavia diffusa* L. on renal GSH content in fluoridated rats. Values are given as mean ± SD for 6 rats in each group. aP<0.001 values are significantly different as compared to respective NaF treated groups.

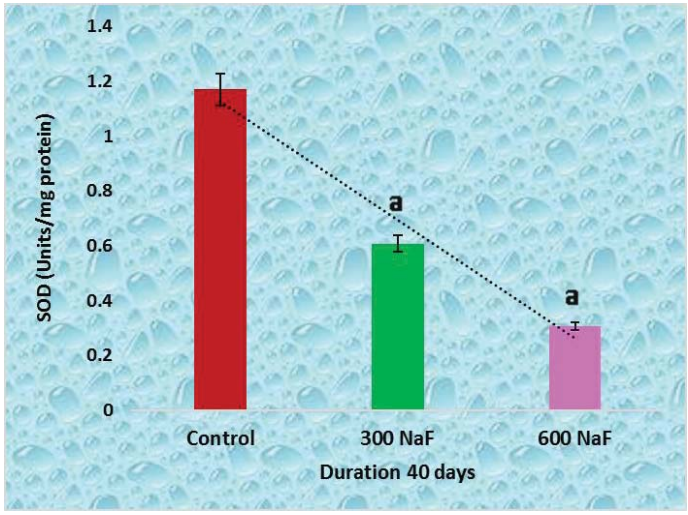

Figure 5: The activity of SOD (Units /mg protein) in kidney tissue of fluoridated rats. Values are given as mean ± SD for 6 rats in each group. ap <0.001 values are significantly different (P<0.001) as compared with control.

95%Cl=-0.0053 to -0.2527; mean difference = -0.0960 to -0.1620) or in (post- treatment 95% CI=-0.0373 to -0.2207; mean difference =-0.1280 to -0.1300) increased (p< 0.001) the activity of SOD. (Figure 6).

Catalase (CAT)

The activity of CAT in kidney tissue of test rat showed a significant (F=2.269, p<0.001) decrease after 40 days of fluoride treatment. More prominent decrease (-62 %) was registered group treated with (600 ppm NaF/kg b.w/day) (Figure 7).

Bonferroni multiple comparison test after ANOVA showed a significant (p<0.001) decrease in the activity of CAT in kidney tissue (mean difference =-0.5440 to 0.3160, 95%CI=-0.6462 to 0.4476) as compared between and within all groups after 40 days of fluoride exposure.

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Dunnetts (2-sided) multiple comparison test revealed that administration of *Boerhaavia diffusa* L. either (pre-treated with 95%Cl=-0.0314 to 0.0382; mean difference = -0.0260 to -0.0202) and in (post- treatment with 95% Cl=-0.0333 to -0.0350; mean difference =-0.0140 to -0.1820) increased (p< 0.001) the activity of CAT (Figure 8).

Glutathione pereoxidase (GPx)

The activity of glutathione peroxidase in kidney tissue of test rat showed a significant (F= 57.278, p<0.001) decrease after 40 days of fluoride treatment. More prominent decreased (-78.73 %) was registered in highest dose group (600 ppm NaF/kg b.w./day) (Figure 9).

Bonferroni multiple comparison test after ANOVA showed a significant (p<0.001) decrease in the activity of glutathione peroxidase in kidney tissue (mean difference =-1.0144 to 2.1940, 95%CI=-1.4875 to 2.548) as compared between and within all groups after 40 days of fluoride exposure.

Dunnetts (2-sided) multiple comparison test revealed that administration of *Boerhaavia diffusa* L. either (pre-treated with 95%Cl=-0.313 to 0.368; mean difference = 0.2042 to 0.3462) and in (post- treatment with 95% Cl=-0.0333 to -0.0350; mean difference =-0.0140 to -0.1820) increased (p< 0.001) the activity of GPx. (Figure 10).

Discussion

This study was undertaken to estimate the prophylactic and curative effect of *Boerhaavia diffusa* L. against sodium fluoride-induced oxidative stress in kidney tissue of rat.

The present study demonstrate an elevation in level of renal MDA in rats treated with 300 and 600 ppm of NaF /kg bw/day. The present study revealed that there was close relationship between fluoride-induced nephrotoxicity and oxidative stress. This finding is consistent with those of previous studies

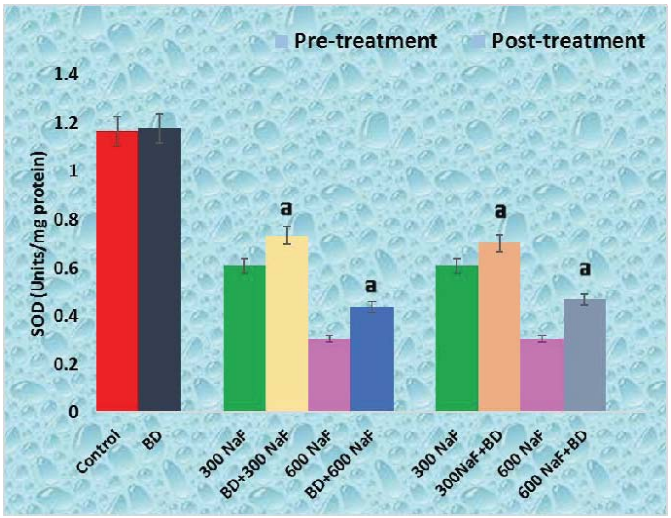

Figure 6: Effect of leaf extract of *Boerhaavia diffusa* L. on renal SOD activity in fluoridated rats. Values are given as mean ± SD for 6 rats in each group. Values with different superscript letters are significantly different (P<0.001). aP <0.001 values are significantly different as compared to respective NaF treated groups.

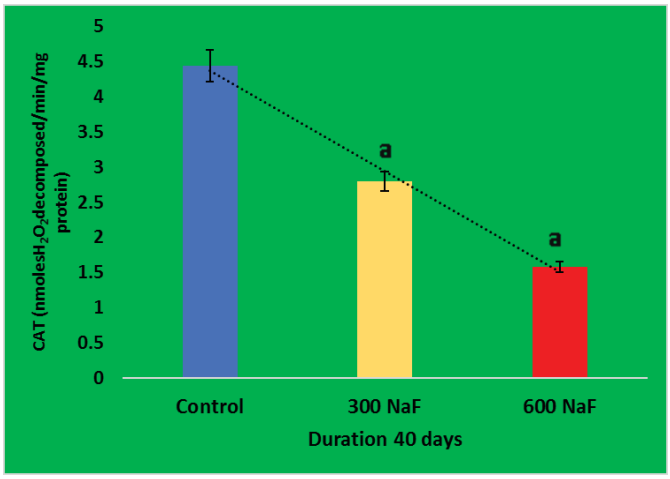

Figure 7: The activity of CAT (n moles H2O2decomposed/min/mg protein) in kidney tissue of fluoridated rats. Values are given as mean ± SD for 6 rats in each group. [a]p<0.001 values are significantly different (P<0.001) as compared with control.

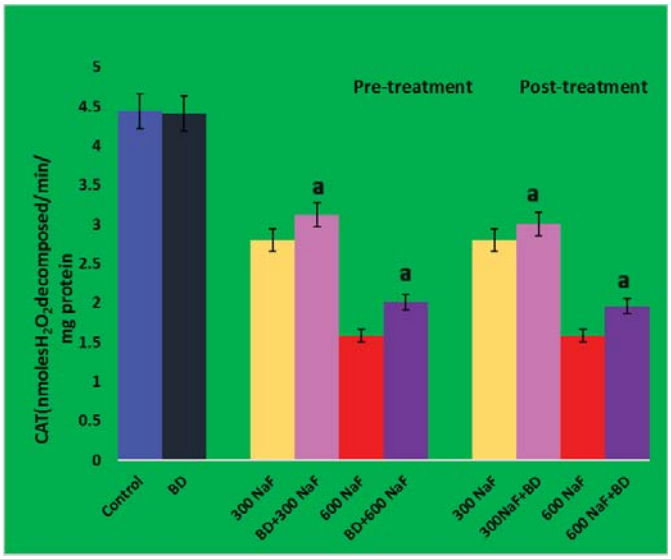

Figure 8: Effect of leaf extract of *Boerhaavia diffusa* L. on renal CAT activity in fluoridated rats. Values are given as mean ± SD for 6 rats in each group. Values with different superscript letters are significantly different (P<0.001). [a]P<0.001 values are significantly different as compared to respective NaF treated groups.

[13-16]. *Boerhaavia diffusa* L. leaf extract as a supplement significantly reversed the fluoride-induced lipid peroxidation in a dose-dependent manner. Active principles of *Boerhaavia diffusa* L. represent a large group of polyphenolic flavonoids that are helpful in preventing lipid peroxidation .MDA is an important reactive metabolite and an indicator of lipid peroxidation. Lipid peroxidation from oxidative stress disturbs the integrity of cellular membranes leading to the leakage of cytoplasmic enzymes [17]. Free radicals and oxidative stress have been implicated in the pathogenesis of several xenobiotic toxicities, compromise in antioxidant defense, and increase in lipid peroxidation products in experimental fluorosis [18].

In our experiments, rats exposed to 300 and 600 ppm of NaF /kg bw/day for 40 days showed decrease in content of reduced glutathione. The present results are in accordance with previous

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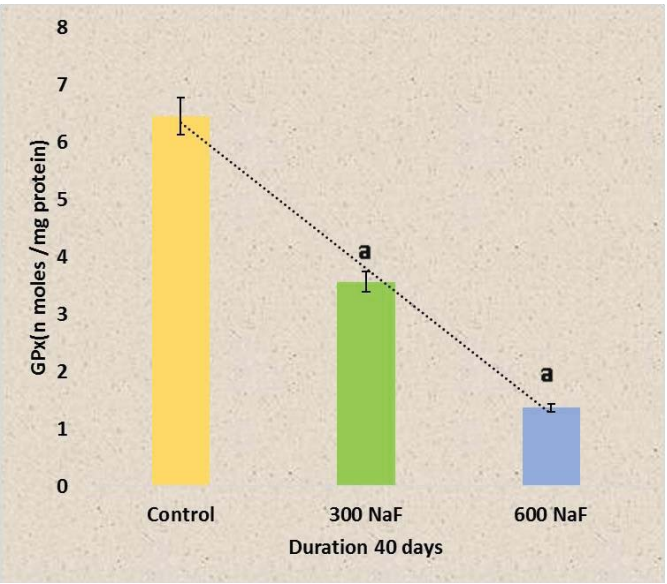

Figure 9: The activity of GPx (nmoles/mg protein) in kidney tissue of fluoridated rats. Values are given as mean ± SD for 6 rats in each group. ap <0.001 valuesare significantly different (P<0.001) as compared with control.

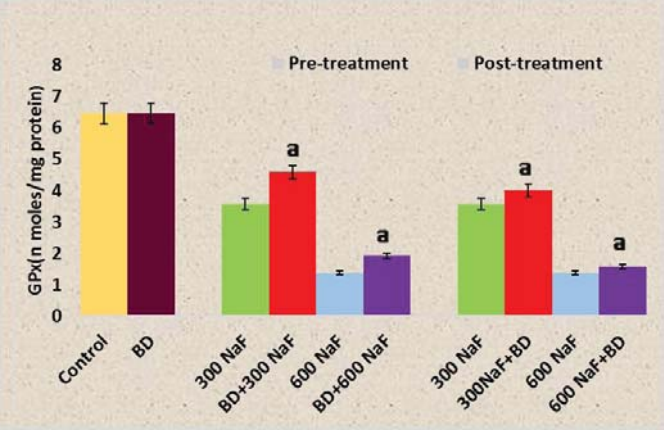

Figure 10: Effect of leaf extract of *Boerhaavia diffusa* L. on renal GPx activity in fluoridated rats. Values are given as mean ± SD for 6 rats in each group. Values with different superscript letters are significantly different (P<0.001). aP <0.001 values are significantly different as compared to respective NaF treated groups.

fluorosis reports [3,13,19]. Decrease in the level of fluoride after mitigation with leaf extract also resulted in significant increase in content of reduced glutathione as compared with 40 days of fluoride treatment. Reduced glutathione neutralizes the hydroxyl radical and plays a major role against inflammatory responses and oxidative stress. Reduced glutathione is an important naturally occurring antioxidant, which prevents free radical damage and helps detoxification by conjugating with chemicals. Under oxidative stress, reduced glutathione is consumed by reduced glutathione related enzymes to detoxify the agents that increase lipid peroxidation [20]. Oxidative stress is induced by increasing production of reactive oxygen species, such as superoxide anion, hydrogen peroxide and hydroxyl radicals. Reactive oxygen species can induce lipid peroxidation, inactive cellular enzymes, depolymerize polysaccharides, and induce deoxyribonucleic acid breaks and chromosome breakage [17]. Experimental evidences have indicated that exposure to fluoride results in oxidative stress in both vitro and in vivo in soft tissues [21].

The investigation indicates the inhibition of oxidative enzymes superoxide dismutase, catalase, and glutathione peroxidase in kidney tissue of rats during 300 and 600 ppm NaF /kg bw/day intoxication. It was observed that sodium fluoride exposure in rats caused a significant ($p<0.001$) decrement in total activity of superoxide dismutase, catalase, and glutathione peroxidase. This findings are in accordance with [13,15,16,22]. *Boerhaavia diffusa* L. leaf extract administration significantly ($p<0.001$) accelerated the renal activities of superoxide dismutase, catalase, and glutathione peroxidase in fluoridated rats kidney tissue. Superoxide dismutase is a naturally occurring intracellular enzyme that catalyzes the breakdown of superoxide radicals. As such, they are an important antioxidant defense in nearly all cells exposed to oxygen. Catalase is a common enzyme found in nearly all living organisms that are exposed to oxygen, where it catalyzes the decomposition of hydrogen peroxide to water and oxygen.

Our studies revealed that leaf extract of *Boerhaavia diffusa* L .has the capability to provide protection against fluoride-induced renal injury mediated, by reactive oxygen species and the other related toxicants. Thus, extract of *Boerhaavia diffusa* L. seems to have the potential to be considered as a beneficial antioxidant. This extract of *Boerhaavia diffusa* L. may function simply by quenching free radicals and the other related toxic intermediates generated during oxidative stress due to fluoride or may improve the antioxidant enzyme status of the tissue in the face of the oxidative stress. Toxicity of superoxide anion free radical and hydrogen peroxide could involve the formation of much more reactive hydroxyl radical (•OH) [23]. The results of the present study may be of future therapeutic relevance particularly in the areas where humans are chronically exposed to fluoride either occupationally or environmentally. *Boerhaavia diffusa* L. can also serve as pharmacological intervention and, the bio-active fractions obtained therefrom may be used also as a future antioxidant supplement to combat oxidative stress-induced renal damage due to fluoride. The present study reflects the antioxidant and free radical scavenging activity of leaf extract of *Boerhaavia diffusa* L. (punarnava).

Conclusion

Our result describes the protective effect of leaf extract *Boerhaavia diffusa* L against fluoride-induced kidney tissue damage in experimental rats. However, the nephroprotective effect of leaf extract of *Boerhaavia diffusa* L. was observed to be significantly higher when it was administered before NaF treatment than after NaF treatment.

Acknowlegment

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