THE IN-VIVO EFFECT OF SODIUM FLUORIDE ON THE ACTIVITIES OF RENAL ENZYMES IN THE BRUSH BORDER MEMBRANE OF RAT KIDNEY IS MITIGATED BY SUPPLEMENTING WITH LEAF EXTRACT OF *BOERHAAVIA DIFFUSA* L.

Jawant Kaur[1]*

Article History: - **Received: 18.04.2022** **Revised: 17.05.2022** **Accepted: 09.06.2022**

ABSTRACT

The present study was carried out to investigate the toxic effects of fluoride on the brush border membrane enzymes in kidney of rats and also to evaluate the ameliorative effect of *Boerhaavia diffusa* L.Eight groups of six rats each were randomly selected from a total of forty-eight animals. Rats in Control I were given deionized water orally. For 40 days, rats in the first and second groups received daily doses of 300 and 600 mg NaF/kg bw, respectively. For 20 days, 500 mg/kg b.w./day of *Boerhaavia diffusa* L. leaf extract was given orally to Control II rats. After receiving a 20-day pretreatment of 500 mg/kg bw/day of *Boerhaavia diffusa* L. leaf extract, groups third and fourth were subjected to 40 days of exposure to 300 and 600 mg NaF/kg bw/ /day. Groups fifth and sixth were exposed firstly to 300 and 600 mg NaF/ kg bw /day and then post-treated with leaf extract of *Boerhaavia diffusa* L. for 20 days. The renal cortex brush border membrane enzymes viz; acid (ACP) and alkaline phosphatase (ALP) , leucine aminopeptidase (LAP), γ-glutamyl transferase (γ-GGT) were analyzed .The activities of ALP,GGT, and LAP were significantly ($P<0.001$) declined , whereas activity of ACP was elevated in animals treated with 300 and 600mg NaF for 40 days. Pre and post treatment with leaf extract of *Boerhaavia diffusa* L. significantly restored ($P<0.001$) activities of these enzymes .Maximum decrease of (-48.14) ALP , (-58.97) γ-GGT , (-72.70) LAP , and maximum increase of (+482.29) ACP was recorded in rats exposed to 600 mg NaF in drinking water . The results of the present study show that the enzymatic alterations in the brush border membrane in the kidney of rats treated with sodium fluoride were considerably reduced by leaf extract of *Boerhaavia diffusa* L. both before and after the treatment.

KEYWORDS: Albino rats, *Boerhaavia diffusa* L., Brush border membrane enzymes, Sodium fluoride, Serum biochemistry.

[1*]Assistant Professor, Department of Zoology, Guru Teg Bahadur Khalsa College for Women, Dasuya, Distt. Hoshiarpur Pin code-144205,Punjab, India.
Department of Zoology and Environmental Sciences, Punjabi University Patiala-147002, Punjab, India

***Correspondence author: -** Jawant Kaur
*Assistant Professor, Department of Zoology, Guru Teg Bahadur Khalsa College for Women, Dasuya, Distt. Hoshiarpur Pin code-144205,Punjab, India. Department of Zoology and Environmental Sciences, Punjabi University Patiala-147002, Punjab, India, Email: drjawantkaur@gmail.com Contact no. 09464746670

DOI: 10.53555/ecb/2022.11.6.122

Introduction

Fluoride enhances the generation of reactive oxygen species and decreases antioxidant enzyme capacity that plays critical roles in fluoride-induced organ toxicity (Thanganpandiyan and Miltonprabu, 2014). Kidney is the favourite target organ to study stress proteins due to its physiological reaction against chemical and physical stress (Borkan and Gullans, 2002). The deposition of fluoride is characterized by extensive gastrointestinal absorption which is followed by distribution in calcified tissue (Cardenas *et al*., 2013). Both *in vivo* and *in vitro* studies have revealed that the mechanism of induction of lipid peroxidation by oxalate may be involved through the inhibition of catalase activity (Selvan and Kurien, 1987). Damage to the proximal tubular epithelium has been associated with the shedding of brush border membrane and calcium oxalate retention (Khan and Hackett, 1993).

Brush border membrane lines the epithelial cells and is the first barrier for various solutes during absorption in the kidney (Ahmad *et al*., 2013). The plasma membrane of the epithelial cells lining the proximal tubule is composed of two morphological and functionally distinct regions: the luminal brush border and the basolateral membranes. The brush border membrane faces the lumen and is the first barrier for various solutes during absorption in the kidney. It contains a number of hydrolytic enzymes and transported systems (Murer and Biber, 1993). Many morphological studies have shown that the renal proximal tubule and brush border membrane are major targets for ischemic and toxic injury. Impairment in the activity of brush border membrane enzymes and transport processes can affect the reabsorptive properties of the kidney and lead to renal failure (Scherberich *et al*., 1993; Fatima *et al*., 2005). When brush border membrane is damaged by the toxic insult, these enzymes are dissociated from damaged brush border membrane, lost in the lumen and sometimes are excreted into the urine (Farooq *et al*., 2006). The transport processes depend upon the structural integrity of brush border membrane and available energy as ATP which is supplied by various metabolic pathways (Ahmed *et al*., 2013).

Alkaline phosphatase is a family of cell surface glycoprotein that catalyzes the hydrolysis of phosphomonoesters with release of inorganic phosphate. Four major alkaline phosphatase isoenzymes have been characterized from human, each encoded by a separate gene, three of them are expressed in intestine, placenta and germ cells whereas the fourth isoenzyme is highly expressed in liver, bone and kidney and low in other tissues (Sasikala *et al*., 2019).

Gamma glutamyltransferase is a brush border membrane enzyme which reflects damage to proximal tubules. Gamma glutamyltransferase catalyzes the transfer of gamma glutamyl groups with peptides (as glutathione) to other peptides or amino acids. Gamma glutamyltransferase plays an important role in the glutathione metabolism. High enzyme concentration are found in kidney (proximal tubules), pancreas (acinar cells), prostate and liver. Gamma glutamyltransferase is mostly located on the external part of the plasma membrane (Spasovski *et al*., 2013).

All across India, *Boerhaavia diffusa* L. is a common weed. This perennial plant has many upright branches and a strong root system that allows it to creep and spread. Because the top of the plant dies in the sweltering summer months and regrows after rains, the plant is generally known as *Punarnava* in Ayurveda and is said to be a rejuvenator (Chaudhary and Dantu, 2011). For the treatment of inflammatory renal illnesses and frequent clinical issues such nephrotic syndrome, oedema, and ascites, *Boerhaavia diffusa* L. is considered therapeutically effective because to its combination of diuretic, antioxidants, and anti-inflammatory actions (Padmini and Kumar, 2013).*Boerhaavia diffusa* L. is now required to help reduce NaF toxicity, and it may eventually be added to normal human supplements due to fluoride's widespread presence in drinking water and our surroundings.

MATERIAL AND METHODS

Young Wistar albino rats, weighing between 100-200g were housed in polypropylene cages with stainless grill tops and fed with commercial rat pellet diet (Hindustan Lever Limited, India) and water was provided *ad libitum*. 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).

EXPERIMENTAL STUDY

The animals were randomly divided into eight experimental groups with six animals in each group:

- Rats served as control 1 and were given 1 ml of deionized water per kilogram of body weight every day for 40 days.

- For 40 days, Group I and II received oral gavage treatments of 300 and 600 mg NaF/kg b.w./day.
- For 20 days, rats were fed 500 mg/kg b.w./day of *Boerhaavia diffusa* L. leaf extract (Control-II).
- Rats in Groups III and IV were given a single oral dosage of 500 mg/kg b.w./day of *Boerhaavia diffusa* L. leaf extract for 20 days, and then they were given 300 and 600 mg of NaF/kg b.w./day for 40 days.
- After 40 days of treatment with 300 and 600 mg NaF/kg b.w./day, rats in Groups V and VI received a 20-day post-treatment of 500 mg/kg b.w./day of *Boerhaavia diffusa* L. leaf extract. During the exposure period, animals were placed in metabolic cages, the rats were weighed, fasted overnight, and were excised under ether anesthesia. The kidney tissues were rapidly excised, weighed, and processed for biochemical parameters.

PREPARATION OF *BOERHAAVIA DIFFUSA* L. PLANT EXTRACT

Fresh leaves of *Boerhaavia diffusa* L. were collected from Botanical Garden, Punjabi University, Patiala and got identified in Department of Botany, Punjabi University, Patiala. The plant leaf extract was prepared by the method of Narendhirakannan *et al*. (2006).The collected leaves were shade dried and ground to a coarse powder. The powder was extracted in 95% ethanol in a soxhlet apparatus at 60^{o} C for 35 hours. After cooling and filtration, the filtrate was concentrated at 65^{o} C in rota vapor to obtain dry powder.

PREPARATION OF BRUSH BORDER MEMBRANE:

Brush border membrane was prepared from whole cortex using the $MgCl_2$ precipitation method of Yusufi (1994). Freshly minced cortical slices were homogenized in 50 mM mannitol and 5 mM Tris-HEPES buffer pH 7.0, in a glass Teflon homogenizer. $MgCl_2$ was added to the homogenate to a final concentration of 10mM and the mixture stirred for 20 min on ice. The homogenate was centrifuged at 2000 rpm for 10 min and the supernatant was then recentrifuged at 35,000 rpm for 30 min. The pellet was resuspended in 300 mM mannitol and 5mM Tris-HEPES, pH 7.4, with four strokes by homogenizer and centrifuged at 35,000 g for 20 min in a 15 ml corex tube. Aliquots of homogenates were stored at -20˚C for brush border membrane enzyme analysis (Farooq *et al*., 2004).

ASSESMENT OF BRUSH BORDER MEMBRANE ENZYMES AND LYSOSOMAL MARKER ENZYME (BIOCHEMICAL PARAMETERS)

The activity of acid phosphatase was determined by the method of King and Jagatheesan (1959), alkaline phosphatase by the method of Kin and Chem, (1972),gamma-glutamyl transferase (γ-GT) was determined by the method of Szasz (1976).The activity of leucine aminopeptidase (LAP) was determined by the method of Mitz and Schlueter (1958).

STATISTICAL ANALYSIS

The statistical analysis of the data was performed using statistical package SPSS (Version 20.0 SPSS Inc., Chicago, IL, USA).Significant differences between means were determined by one way analysis of variance (ANOVA) followed by Bonferroni and Dunnett's t (2-sided) multiple comparison test. Results were considered significant at $P < 0.05$.

RESULTS AND DISCUSSION

BRUSH BORDER MEMBRANE ENZYMES

Acid phosphatase

There was significant ($P<0.001$) elevation in the activity of acid phosphatase in kidney tissue of test rats after 40 days ($F= 3745$) of fluoride treatment (Fig. 1). The highest increase (482.29%) was noted in renal tissue of rats treated with 600 mg NaF / kg b.w./day.

Bonferroni multiple comparison test after ANOVA showed significant ($P < 0.001$) increase in the activity of acid phosphatase in kidney tissue between and within groups (95 % CI = -13.64 to -20.53; mean difference = -12.63 to -21.54) after 40 days of fluoride exposure.

Dunnett's t (2-sided) multiple comparison test revealed that the renal activity of acid phosphatase was significantly ($P < 0.001$) decreased in all pre-treated (95% CI = 6.560 to -10.191; Fig. 2) as well as in post- treated groups (95% CI = 2.680 to -14.270) with 500 mg / kg b.w. /day of leaf extract of *Boerhaavia diffusa* L. for 20 days compared to respective NaF treated groups.

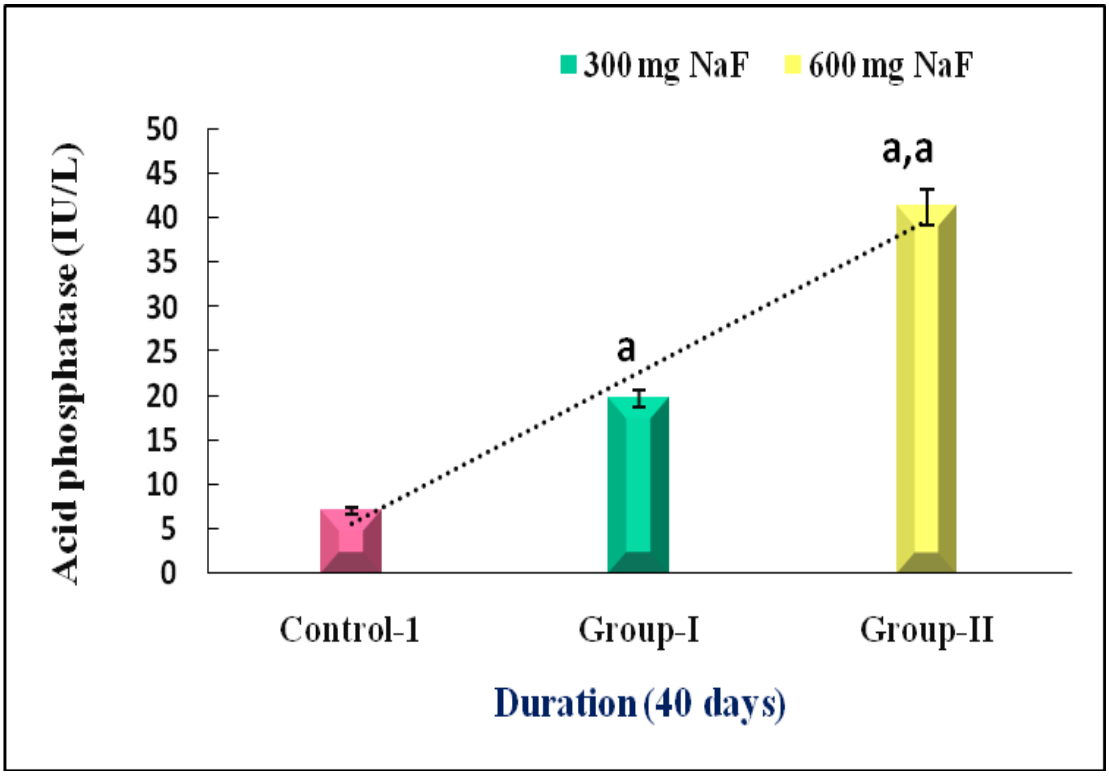

Fig 1. The activity of acid phosphatase (IU/L) in kidney tissue of fluoridated rats
[a] *P < 0.001 Group I - II compared with control-1*
[a,a] *P < 0.001 Group I compared with group - II*
One way ANOVA followed by Bonferroni multiple comparison test

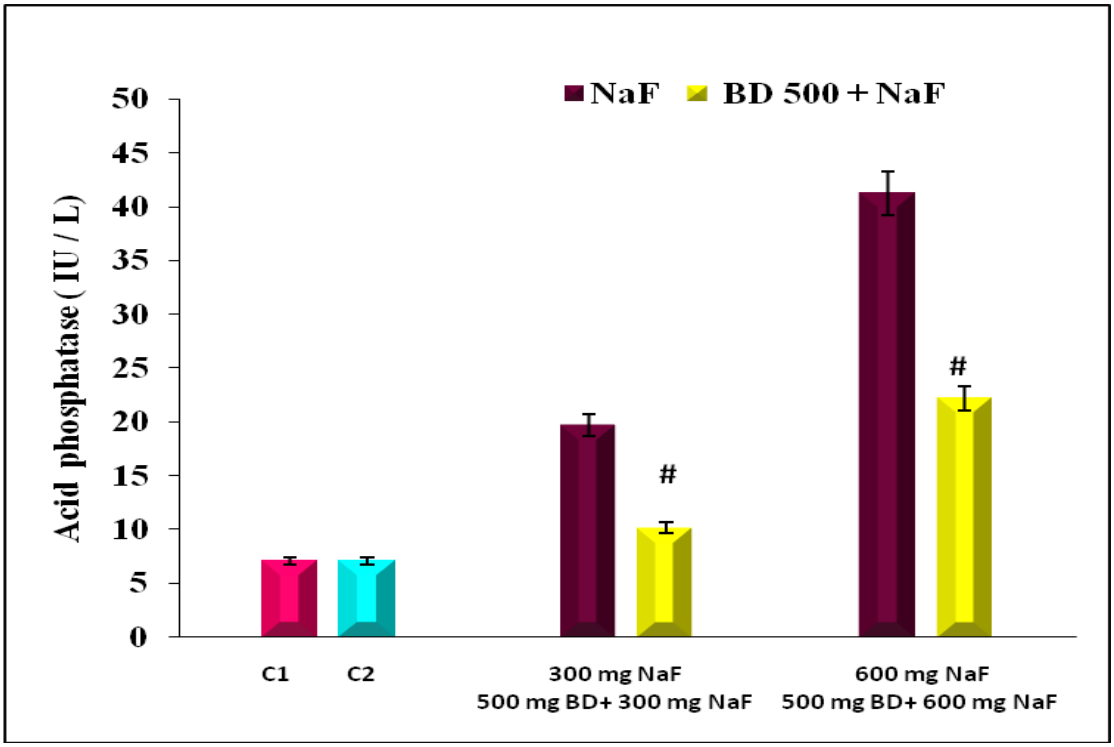

Fig. 2: The activity of acid phosphatase (IU/L) in kidney tissue of fluoride treated rats, pre-treated with leaf extract of *Boerhaavia diffusa* L.
BD = *Boerhaavia diffusa* L.
Control-1= 1 ml deionized water
Control-2 = 500 mg / kg b.w. BD leaf extract
[#] *P < 0.001 values are significantly different as compared to respective NaF treated groups.*

Alkaline phosphatase

The activity of alkaline phosphatase in kidney tissue of test rats revealed significant ($P < 0.001$) decrease after 40 days ($F = 1294$) of fluoride treatment (Fig 3). Bonferroni multiple comparison test after ANOVA showed a significant ($P < 0.001$) fall in the activity of alkaline phosphatase in kidney tissue between and within groups (95 % CI = 52.621 to 26.760; mean difference = 52.54 to 26.64) after 40 days of fluoride exposure.

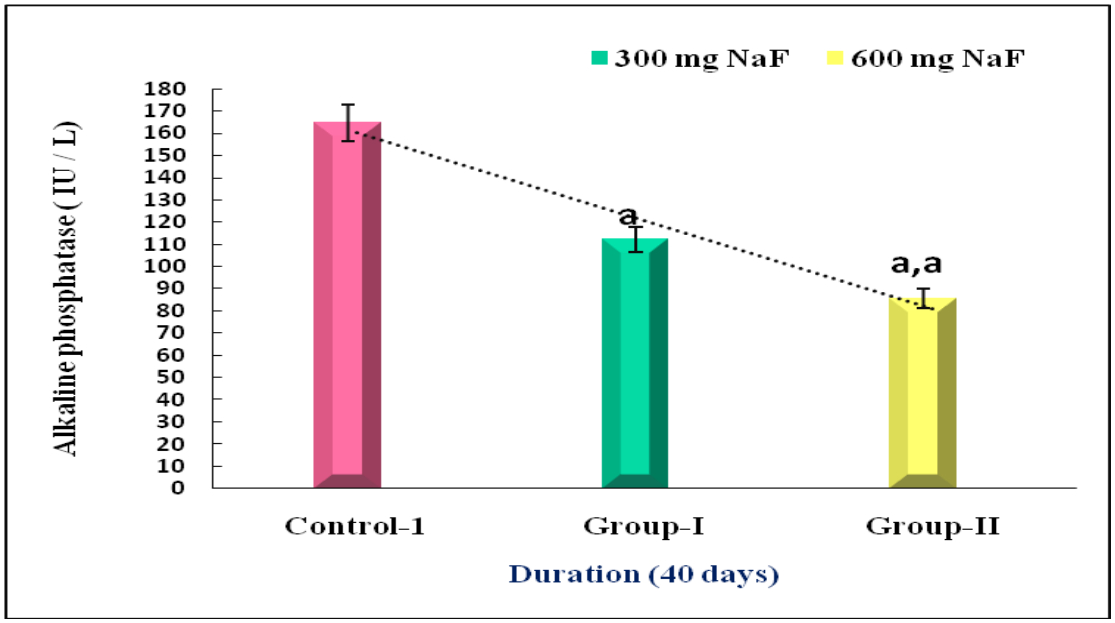

Fig. 3: The activity of alkaline phosphatase (IU/L) in kidney tissue of fluoridated rats
[a]P < 0.001 Group I - II compared with control-1
[a,a]P < 0.001 Group I compared with group- II
One way ANOVA followed by Bonferroni multiple comparison test

Dunnett's t (2-sided) multiple comparison test revealed that the renal activity of alkaline phosphatase was significantly ($P < 0.001$) increased in all pre-treated (95% CI = -21.71 to 10.79; Fig .4) as well as in post- treated groups (95% CI = -15.38 to 15.13) with 500 mg / kg b.w. /day of leaf extract of *Boerhaavia diffusa* L. for 20 days compared to respective NaF treated groups.

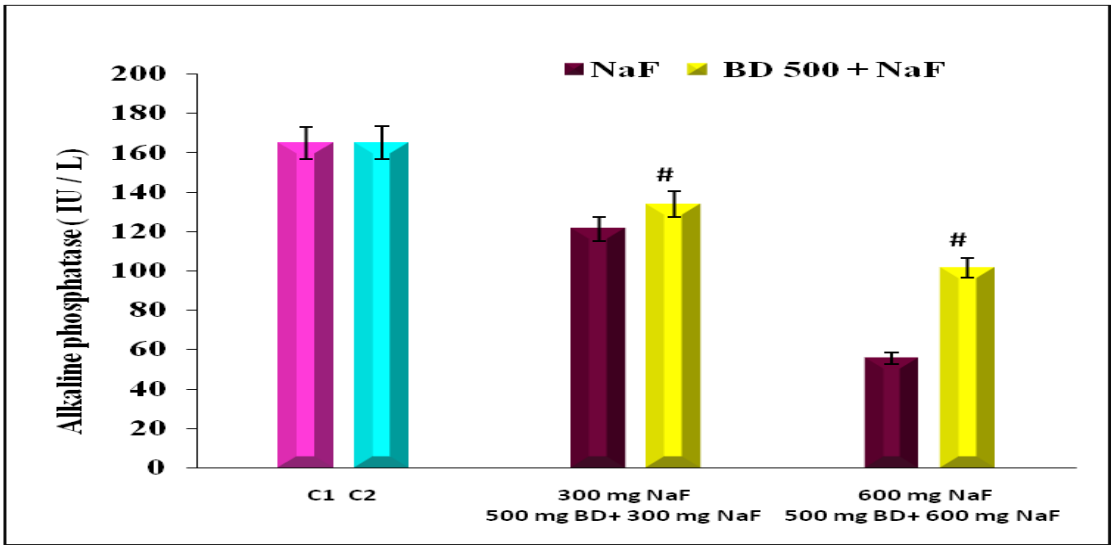

Fig. 4: The activity of alkaline phosphatase (IU/L) in kidney tissue of fluoride treated rats pre-treated with leaf extract of *Boerhaavia diffusa* L.
BD = *Boerhaavia diffusa* L.
Control-1= 1 ml deionized water
Control-2 = 500 mg / kg b.w. BD leaf extract

[#]P < 0.001 values are significantly different as compared to respective NaF treated groups.

Gamma glutamyl transferase

There was significant (P<0.001) decrease in the activity of gamma glutamyl transferase (γ-GT) in kidney tissue of test rats after 40 days (F= 225) of fluoride treatment. More prominent decrease (58.97 %) was reported in rats treated with 600 mg NaF / kg b.w./day (Fig.5).

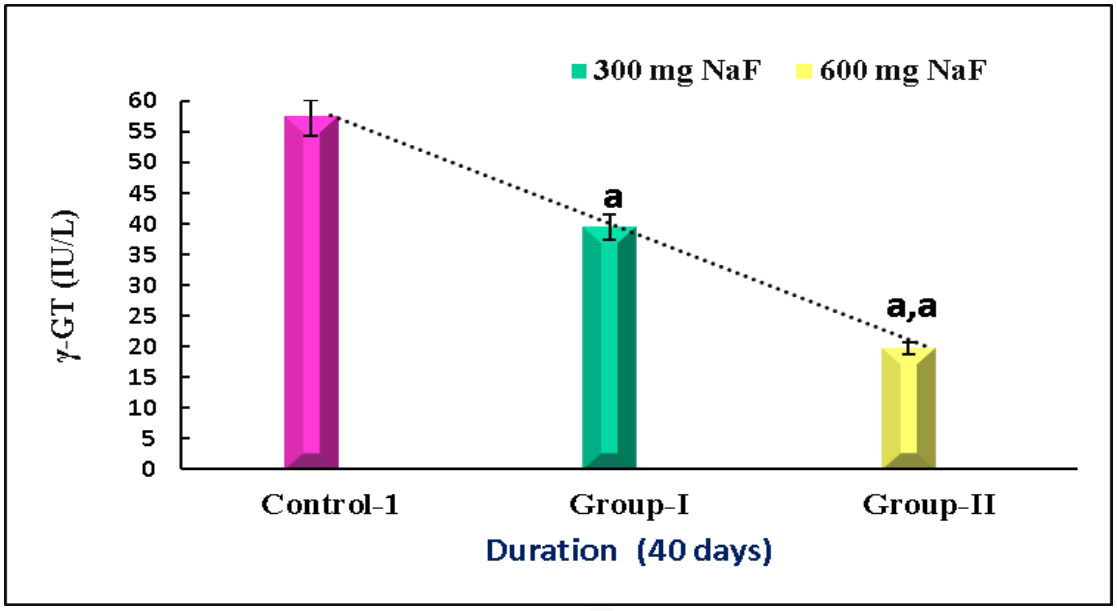

Fig 5: The activity of gamma glutamyl transferase (γ-GT) (IU/L) in kidney tissue of fluoridated rats
[a]P < 0.001 Group I- II compared with control-1
[a,a]P < 0.001 Group I compared with group-II
One way ANOVA followed by Bonferroni multiple comparison test

Bonferroni multiple comparison test after ANOVA showed significant (P < 0.001) decrement in the activity of γ-GT in kidney tissue between and within groups (95 % CI = 12.05 to 20.56; mean difference = 15.85 to 16.76) after 40 days of fluoride exposure.

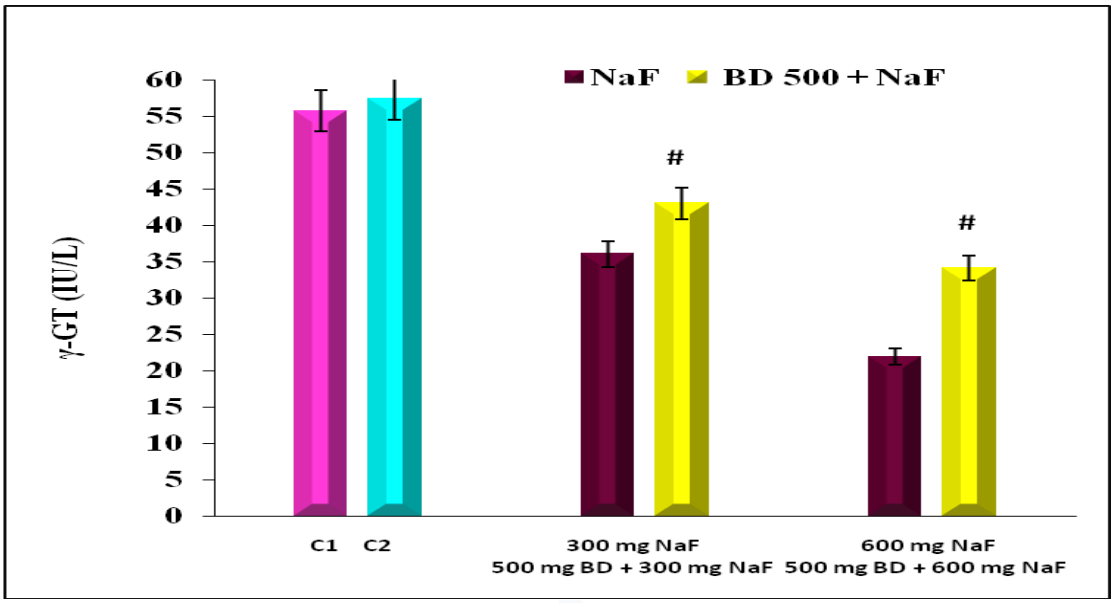

Fig. 6: The activity of gamma glutamyl transferase (γ-GT) (IU/L) in kidney tissue of rats pre-treated with leaf extract of *Boerhaavia diffusa* L.
BD = *Boerhaavia diffusa* L.
Control-1= 1 ml deionized water

Control-2 = 500 mg / kg b.w. BD leaf extract
#P < 0.001 values are significantly different as compared to respective NaF treated groups.

Dunnett's t (2-sided) multiple comparison test revealed that the renal activity of γ-GT was significantly (P < 0.001) increased all pre-treated (95% CI = - 15.46 to 9.099; Fig. 6) as well as in post-treated groups (95% CI = 12.46 to 11.200) with 500 mg / kg b.w. /day of leaf extract of *Boerhaavia diffusa* L. for 20 days compared to respective NaF treated groups.

Leucine amino peptidase

The activity of leucine amino peptidase (LAP) in kidney tissue of test rats revealed significant (P < 0.001) decrease after 40 days (F = 5644) of fluoride treatment. More prominent decrease (-72.70 %) was registered in rats treated with 600 mg NaF / kg b.w./day (Fig. 7).

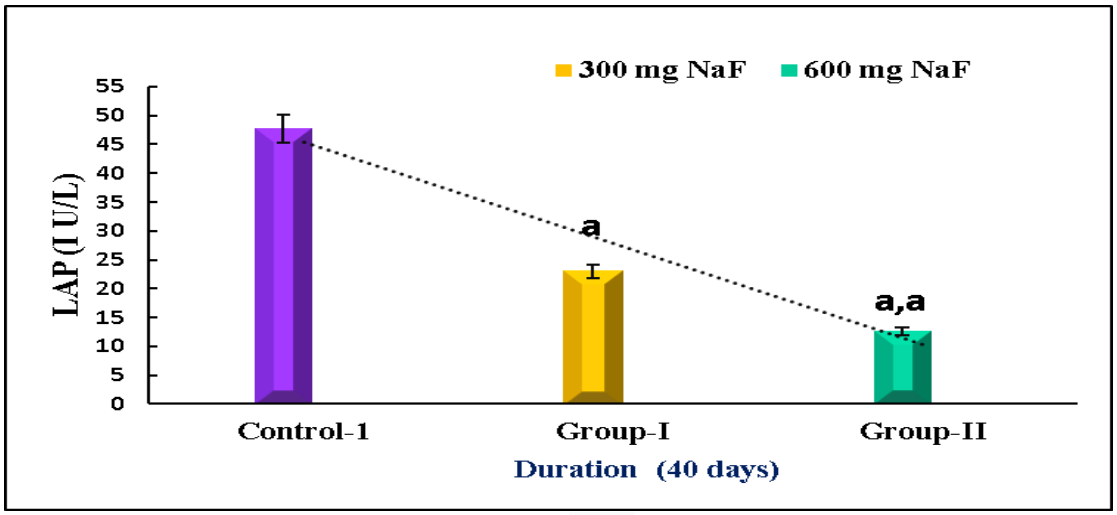

Fig 7: The activity of leucine amino peptidase (LAP) (IU/L) in kidney tissue of fluoridated rats
[a]P < 0.001 Group I - II compared with control-1
[a,a]P < 0.001 Group I compared with group II
One way ANOVA followed by Bonferroni multiple comparison test

Bonferroni multiple comparison test after ANOVA showed significant (P < 0.001) decrease in the activity of LAP in kidney tissue between and within groups (95 % CI = 24.47 to 10.66; mean difference = 24.74 to 10.39) after 40 days of fluoride exposure.

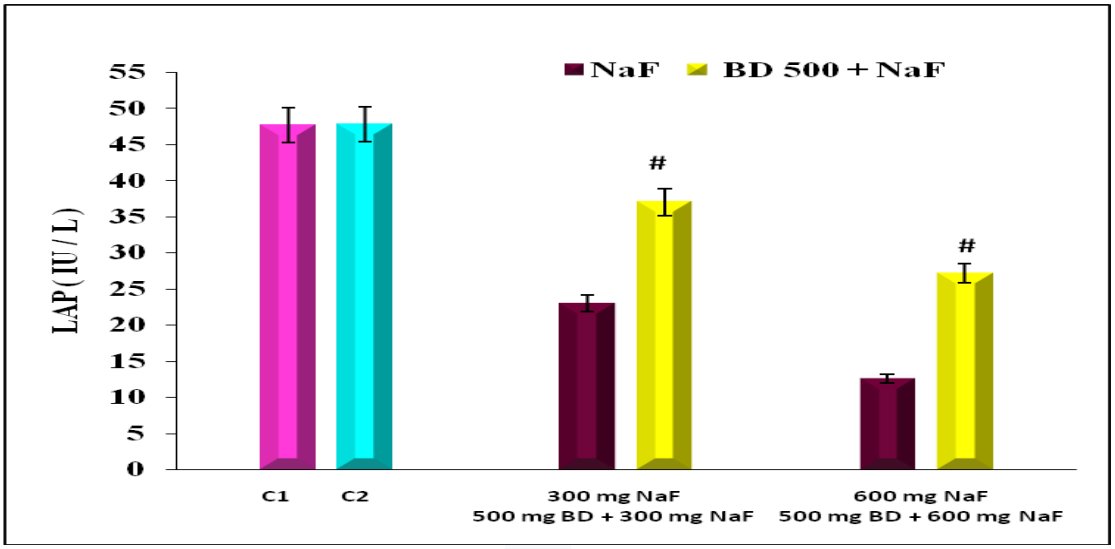

Fig. 8: The activity of leucine amino peptidase (LAP) (IU / L) in kidney tissue of rats pre-treated with leaf extract of *Boerhaavia diffusa* L.
BD = *Boerhaavia diffusa* L.
Control-1= 1 ml deionized water

Control-2 = 500 mg / kg b.w. BD leaf extract

$^{\#}P < 0.001$ values are significantly different as compared to respective NaF treated groups.

Dunnett's t (2-sided) multiple comparison test revealed that the renal activity of LAP was significantly ($P < 0.001$) increased in all pre-treated (95% CI = 0.3473 to - 1.093; Fig. 8) as well as in post-treated groups (95% CI = 3.303 to 6.067) with 500 mg / kg b.w. /day of leaf extract of *Boerhaavia diffusa* L. for 20 days compared to respective NaF treated groups.

The present study revealed significant ($P<0.001$) decrease in the activities of brush border membranes enzymes viz; alkaline phosphatase, gamma glutamyl transferase, leucine aminopeptidase, and significantly ($P<0.001$) increased the activity of lysosomal enzyme acid phosphatase in cortex of rats treated with 300 and 600 mg of NaF /kg b.w./ day for 40 days.

The decrease in brush border membranes enzyme activities could be due to the oxidative modification and consequent inactivation of enzymes by sodium fluoride generated free radicals and reactive oxygen species. Increased lipid peroxidation, which affects membrane structure and function, could also have resulted in a decrease in the activities of these enzymes. There could have been leakage or loss of brush border membranes into the tubular lumen following reactive oxygen species induced damage to the epithelial cells, especially to the membrane and these enzymes later appear in the urine as demonstrated previously for other toxicants (Banday *et al*., 2008; Rizwan *et al*., 2014) and reflected by observed proteinuria. The present clearly indicate that lysosomes alongwith plasma membrane were selectively damaged by sodium fluoride administration. Similar findings were consistent with kidneys of experimental animals upon exposure to other toxicants such as potassium dichromate (Fatima *et al*., 2005), gentamicin (Banday *et al*., 2008), cisplatin (Khan *et al*., 2009, Naqshabandi *et al*., 2012, Naqshabandi *et al*., 2017); benzene (Khan and Yusufi, 2009) and hexachlorobenzene (Khan *et al*., 2017). *Boerhaavia diffusa* L. leaf extract as a supplement significantly ($P < 0.001$) restores the activities of brush border membranes enzymes viz; alkaline phosphatase, gamma glutamyl transferase, leucine amino peptidase, and lysosomal acid phosphatase in the fluoride exposed animals in the present investigation.

Several nephrotoxins such as mercuric chloride, ethanol and cefotiam result in loss of microvilli from proximal tubular cells. However, if the damage is not severe, the brush border membrane can be reformed and the integrity of proximal tubule restored. The effect of potassium dichromate administration on renal brush border membrane marker enzymes and inorganic phosphate transport has been reported by Fatima *et al*. (2005). They observed that the activities of brush border membrane enzymes were significantly decreased in cortical and medullary homogenates after potassium dichromate administration, but the extent of the decrease was greater in cortex and medulla.

Banday *et al*. (2008) explored the effect of gentamicin that elicited deleterious nephrotoxic effects by causing major damage to mitochondria, lysosomes, and basolateral and brush border membranes which were reflected by significant decrease in the activities of specific biomarkers of these intracellular organelles. Gentamicin caused greater damage to cortex compared to medulla.

Khan *et al*. (2009) demonstrated protective effect of green tea against cisplatin induced nephrotoxicity. Green tea consumption increased the activities of the enzymes of carbohydrate metabolism, brush border membrane, oxidative stress and inorganic phosphate transport.

The NaF-induced decrease in brush border membrane enzymes suggested severe damage to the structural architecture of the brush border membranes affecting its transport functions. A variable increase in the activity of alkaline phosphatase, gamma glutamyl transferase, and leucine amino peptidase in the renal cortex may be due to their localization in the thickness of brush border membranes (Yusufi *et al*., 1994) or due to differential accumulation of leaf extract of *Boerhaavia diffusa* L. in these tissues.

CONCLUSION

Our study describes fluoride induced disturbances in the filtrating function of kidneys in rats, hypertension and kidney dysfunction such as reduced ability to excrete urea, The leaf extract of plant *Boerhaavia diffusa* L. consumption, however, significant increase in the activities of brush border membranes enzymes in the cortex homogenate and brush border membranes, indicating an overall improvement in renal brush border membranes integrity.

ACKNOWLEDGMENT

The financial assistance from Rajiv Gandhi National Fellowship (RGNF) program, University Grants Commission, Govt. of India is gratefully acknowledged.

REFERENCES

1. Ahmad, M.K., Khan, A.A. and Mahmood, R., 2013. Taurine ameliorates potassium bromate-induced kidney damage in rats. *Amino Acids*, **45** (5):1109-1121.
2. Banday, A.A., Farooq, N., Priyamvada , S., Yusufi, A.N.K. and Khan, F. 2008. Time dependent effects of gentamicin on the enzymes of carbohydrate metabolism, brush border membrane and oxidative stress in rat kidney tissues. *Life Sciences*, **82**: 450-459.
3. Borkan, S. and Gullans, S. 2002. Molecular chaperones in the kidney. *Annual Review of Physiology*, **64**: 503-527.
4. Cardenas, G.M.C., Del Razo, L.M., Barrera-Chinol, J., Estrada, T.J., Bayghen, E.L., Bobadilla., N. A. and Barbier, O. 2013. Proximal renal tubular injury in rats, sub chronically exposed to low fluoride concentrations. *Toxicology and Applied Pharmacology*, **272** (3): 888 – 894.
5. Chaudhary, G. and Dantu P.K. 2011. Morphological, phytochemical and pharmacological studies on *Boerhaavia diffusa* L. *Journal of Medicinal Plant Research*, **5** (11): 2125-2130.
6. Farooq, N., Priyamvada, S., Arivarasu, N. A., Salim, S., Khan, F. and Yusufi, A.N.K. 2006. Influence of Ramadan-type fasting on enzymes of carbohydrate metabolism and brush border membrane in small intestine and liver of rat used as a model. *British Journal of Nutrition*, **96** (6): 1087–1094.
7. Farooq, N., Yusufi, A. and Mahmoo, R. 2004. Effect of fasting on enzymes of carbohydrate metabolism and brush border membrane in rat intestine. *Nutrition Research*, **24** (6) : 407-416. : 10.1016/j.nutres.2004.01.004
8. Fatima, S., Arivarasu, N.A., Banday, A.A., Yusufi, A.N.K. and Mahmood, R. 2005. Effect of potassium dichromate on renal brush border membrane enzymes and phosphate transport in rats. *Human and Experimental Toxicology*, **24**: 631-638.
9. Khan, A.N.K. and Yusufi. 2009. Effect of benzene on the enzymes of carbohydrate metabolism, brush border membrane and oxidative stress in kidney and other rat tissues. *Biology and Medicine*, **1** (2): 28-41.
10. Khan, S., Priyamvada, S., Khan, S.A., Khan, W. and Yusufi, A.N.K. 2017. Studies on hexachlorobenzene induced toxicity and oxidative damage in the kidney and other rat tissues. *International Journal of Drug Metabolism and Toxicology*, **1**(1): 1-9.
11. Mitz, M.A. and Schleuter, R.J. 1958. Direct spectrophotometric measurement of the peptide bond: Application to the determination of acylase I. *Biochimica Biophysica Acta*, **27**:168-172.
12. Murer, H. and Biber, J. 1993. Structural identification of brush border membrane transport systems-towards an understanding of regulatory mechanisms. *The Clinical Investigator*, **71**: 852-854.
13. Naqshbandi, A., Farooqui, Z., Khan, M.W., Rizwan, S., Khan, S.A., Khan, F. and Yusufi, A.N.K. 2017. Fish oil supplementation ameliorates long term cisplatin treatment induced toxicity and oxidative damage in rat kidney. *Journal of Nephrology and Renal Diseases*, **1**(2): 1-8.
14. Naqshbandi, A., Khan, M.W., Rizwan, S., Rehman, S. and Khan, F. 2012. Studies on the protective effect of dietary fish oil on cisplatin induced nephrotoxicity in rats. *Food and Chemical Toxicology*, **50:** 265-73.
15. Narendhirakannan, R.T., Subramanian, S. and Kandaswamy, M. 2006.Bio-chemical evaluation of antidiabetogenic properties of some commonly used Indian plants on streptozotocin induced diabetes in experimental rats. *Clinical Experimental Pharmacology and Physiology*, **33**:1150-1157.
16. Padmini, M.P. and Kumar, J.V. 2013. An experimental study of biochemical and histopathological study on gentamycin induced renal failure in albino rat and the effectiveness of punarnava *(Boerhaavia diffusa)* on reversal of renal damage. *Journal of Medical and Dental Sciences*, **9** (6): 17-21.
17. Rizwan, S., Naqshbandi, A., Farooqui, Z., Khan, A.A. and Khan, F. 2014.Protective effects of dietary flaxseed oil on arsenic induced nephrotoxicity and oxidative damage in rat kidney. *Food and Chemical Toxicology*, **68***:*99-107.
18. Sasikala, T. Bitla, A. R. and Sachan, A. 2019. Urinary brush border enzymes for early diagnosis of tubular dysfunction in patients with type 2 diabetes mellitus. *International Journal of Research in Medical Sciences*, **7** (6): 2015-2021.
19. Scherberich, J.E, Wolf, G. and Schoeppe W.1993. Shedding and repair of renal cell membranes following drug-induced

nephrotoxicity in humans. *European Journal of Clinical Pharmacology*, **44**: S33-S38.

20. Selvam R. and Kurien, T.B. 1987. Induction of lipid peroxidation by oxalate in experimental rat Urolithiasis. *Journal of Bio Sciences*, **12**: 367-373.
21. Spasovski, D. 2013. Renal markers for assessment of renal tubular and glomerular dysfunction. *Journal of Nephropharmacology*, **2** (2): 23–25.
22. Szasz, G.1976. Reaction rate method for gamma-glutamyl transferase activity in serum. *Clinical Chemistry*, **22**: 2051-2055.
23. Thanganpandiyan, S. and Miltonprabu, S.2013.Protective effect of epigallocatechin gallate on sodium fluoride induced changes in histology of selected organs in rats. *A Journal of Toxicology*, **3** (1): 2231-3834.
24. Yusufi, A.N., Murayama, N., Gapstur, S.M., Szczepanska-Konkel, M. and Dousa, T.P. 1994. Differential properties of brush border membrane vesicles from early and late proximal tubules of rat kidney. *Journal of Nephrolology and Renal Disease*, **1** (2): 1-8.