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# SODIUM FLUORIDE INDUCED TOXICITY IN THE KIDNEY OF SWISS ALBINO MICE AND ITS AMELIORATION BY ASCORBIC ACID

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## ABSTRACT

The widespread distribution of fluoride in nature is a direct source of adverse health effects in human population. Fluoride in drinking water is easily absorbed by the intestine and is quickly distributed throughout the body. Fluoride easily crosses the membrane and enters tissues, thus affecting every phase of metabolism. The kidney accumulates more fluoride than all other soft tissues (with the exception of Pineal gland) and is a site for potential fluoride toxicity, since it can be exposed to relatively high concentration of fluoride and this fluoride in kidney is associated with structural and physiological changes. The present study has been carried out to investigate the effect of fluoride toxicity and its amelioration by Ascorbic Acid on the kidney of Swiss albino mice. Adult male mice were treated with 5ppm, 10ppm and 40ppm sodium fluoride (NaF) in drinking water for 7$^{th}$, 14$^{th}$, 21$^{st}$ and 28$^{th}$ days until autopsy. Kidney revealed hydropic degeneration, vacuolization, cytoplasmic degranulation in renal tubules and pycnosis in cortical and medullary region. The cessation of NaF treatment could not bring about a complete recovery. However, the administration of Ascorbic Acid to NaF treated mice revealed significant recovery from fluoride toxicity. The effects are transient and reversible with the administration of Ascorbic Acid. So Ascorbic Acid is proposed as an antidotal agent for amelioration of fluoride effects on the kidney.

**KEYWORDS: - Albino Mice, Ascorbic Acid, Kidney, Recovery, Swiss.**

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# INTRODUCTION

Fluoride is one of the important life element of human health. It is essential for normal mineralization of bones and formation of dental enamel with presence in small quantity[1]. At the normal levels of fluoride ingestion almost all of the absorbed fluoride is excreted[2] but when it crosses the permissible limit (WHO, 1.5ppm) it becomes toxic and create metabolic disturbances in animals and human being such as dental and skeletal Fluorosis[3,4]. However, following prolonged excessive fluoride intake, fluoride levels in the plasma increases and consequently the soft tissues are loaded. If the fluoride levels in the soft tissues increase beyond a particular limit, the physiological functioning of the affected organs is impaired[5]. Among the soft tissues, kidneys have the highest fluoride content as both excretion and retention[6]. Thus the kidney is more prone to fluoride toxicity than other soft organs[7,8]. Nephropathy has been established as a major manifestation on fluoride toxicity in its early stages[9]. Large doses of fluoride induce necrosis of convoluted tubules and inflammation of glomeruli, which result in impaired kidney function such as polyuria, polydipsia and increased non-protein nitrogen[10]. In rats at low level (1 to 10 ppm NaF) alteration in kidney structure and functions are also reported[11,12]. Ascorbic Acid is well known for its antioxidant activity. Ascorbate acts as reducing agent to reverse oxidation in aqueous solution, increasing the plasma ascorbate level may have therapeutic effects in oxidative stress individual[13,14]. Adequate vitamin C (Ascorbic Acid) in the diet is an important entity to ameliorate the ill effects of fluoride[15,16,17]. Ascorbic Acid treatment to chronically fluoride exposed fishes helped in overcoming the adverse effects and reversal towards normal structure and function of vital organs[18]. The present investigation was undertaken to elucidate the effects of sodium fluoride on histology of mice kidney for understanding the mechanism of fluoride action and its possible reversibility by feeding Ascorbic Acid which have an important role in the restoration of normal structure once the kidney is affected.

# MATERIALS AND METHODS

### *Procurement*

Healthy, Swiss strain adult male mice (*Mus musculus*) Weighing between 30 to 40 gm were obtained from CCS University, Hisar (Haryana) under the Animal Maintenance and Registration No--/1066/ac/07/CPCSEA from the Ministry of Social Justice and Empowerment, Govt. of India and Committee for the purpose of Control and Supervision of Experiments on animals, Chennai, India.

### *Maintenance of Animals*

The animals were kept in polypropylene cages; saw dust was put on the bottom of the cages. The cages were cleaned daily. Water bottles and nipples were autoclaved periodically. Mice were fed with standard pellet feed. Water was given *ad-libitum.*

### *Source of Drug*

Sodium fluoride and Ascorbic Acid were obtained from Sigma Chemicals Co. USA. 5, 10 and 40ppm of sodium fluoride and 40ppm of Ascorbic Acid were prepared in double distilled water.

### *DESIGN OF EXPERIMENT*

The animals were divided into following groups:-

**Group I: (Normal)**

This group comprised the control group. These were provided with standard pellet feed and they received distilled water *ad-libitum.*

***Group II (Sodium Fluoride treated animals)***

The animals of this group received sodium fluoride at the dose rate of different levels in distilled water (*ad-libitum* until autopsy). This group was further divided into different sub groups on the basis of sodium fluoride doses.

Sub Group I - 5ppm
Sub Group II - 10ppm

Sub Group III - 40ppm
The animal of these three sub groups were given sodium fluoride and were sacrificed after 7, 14, 21 and 28 days of treatment.

***Group III (After Withdrawal of Sodium fluoride treatment and recovery with Ascorbic Acid)***
The animals were divided into following sub groups
Sub Group I - 5ppm
Sub Group II - 10ppm
Sub Group III - 40ppm
In these sub groups animals were treated with sodium fluoride for 28 days as in group II. After cessation of treatment animals were treated with Ascorbic Acid for next 28 days and sacrificed on $7^{th}$, $14^{th}$, $21^{st}$ and $28^{th}$ days.

***Group IV: This group comprised of 4 different sub groups***
***Group IV (A) :( Sodium Fluoride + Ascorbic Acid)***
Sub Group I - 5ppm NaF+40ppm Ascorbic Acid
Sub Group II - 10ppm NaF+40ppm Ascorbic Acid
Sub Group III - 40ppm NaF + 40ppm Ascorbic Acid
In these sub groups, animals were treated with sodium fluoride + Ascorbic Acid and were sacrificed after 7, 14, 21 and 28 days of treatment.

***Group IV (B) - (Sodium fluoride + Ascorbic Acid treatment followed by continuation of Ascorbic Acid in recovery groups)***
Likewise, animals were treated with Sodium fluoride + Ascorbic Acid for 28 days as in group IV A but they were given Ascorbic Acid alone for next 28 days and sacrificed after next 7, 14, 21 and 28 days.

***Group IV (D) – Ascorbic Acid alone (40ppm)***
In this subgroup animals were given 40ppm Ascorbic Acid alone and were sacrificed after 7, 14, 21 and 28 days of treatment.

***Histological Studies***
After sacrificing the mice, the pieces of Tissues (kidney) from control as well as experimental groups were put in theBouin'sfixative for overnight at room temperature. The material was washed thoroughly with 50% alcohol and dehydrated by passing through 70%, 90% and absolute alcohol. Finally the tissues were cleared in xylol and embedded in paraffin wax at 58-60$^0$C. The blocks were made and section were cut at 7µ (micron) with the help of rotary microtome and stained with Haematoxyline-Eosin and PAS-Haematoxyline stains for Histological study of kidney tissues.

***Chemicals***
Analytical laboratory-grade sources of sodium fluoride (NaF), ethanol, xylene, picric acid, glacial acetic acid, formaldehyde, embedding wax; Distyrene Plasticizer Xylene (DPX), haematoxylin, eosin etc were used for various experimental preparations in this study.

***Preparation of NaF solutions***
Analytical grade sodium fluoride was used in the required aqueous NaF solution. the A 1000ppm of F stock solution was prepared by dissolving 2.21g of NaF in 1L of Water. Feeding dilutions of 5ppm , 10ppm and 40ppm were prepared by adding 99.5ml of water to 0.5ml stock solution, 99.0ml of water to 1.0ml of stock solution and 96.0ml of water to 4.0ml of stock solution as per requirement.

## RESULTS

The histological sections in the control group show that the kidney is composed of a huge number of nephrons which are the functional filtering units of the kidney. This nephron consists of the large number of glomeruli surrounded by a double walled epithelial capsule called Bowman's capsule, the proximal convoluted tubule lined by simple cuboidal epithelium with an acidophilic cytoplasm and the apex possesses abundant microvilli which form a brush border and the distal convoluted tubule lined by simple cuboidal epithelium; the thin and thick limbs of the Henle's loop etc (Fig.1)

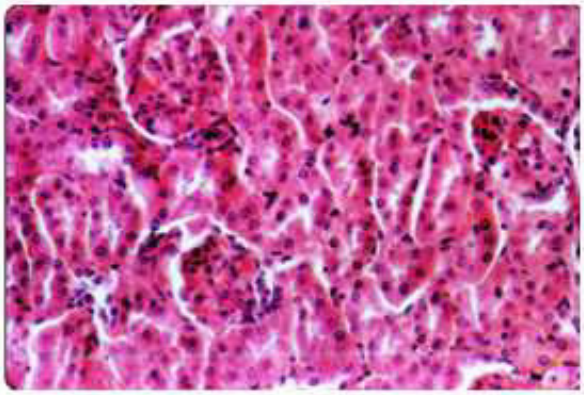

Figure. 1: T.S. kidney of mice from control group showing normal structure of glomerulus with intact Bowman's capsule and tubules in the cortical portion
(H&E X 200)

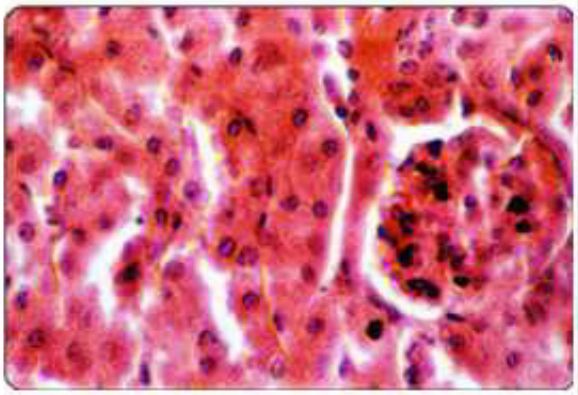

Figure. 2: T.S. kidney showing cytoplasmic degranulation and vacuolization in tubules and glomeruli, tubular cells were devoid of nuclei at certain places 5ppm/28 days NaF treatment
(H&E X 400)

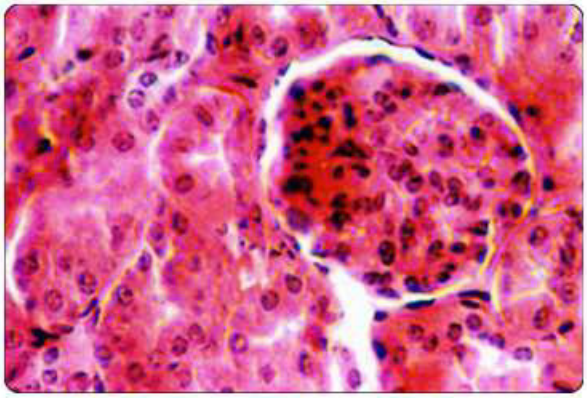

Figure.3: T.S. kidney showing glomeruli with hypercellularity and cell proliferation. No necrosis or tubular damage was noticed after 10ppm/7 days NaF

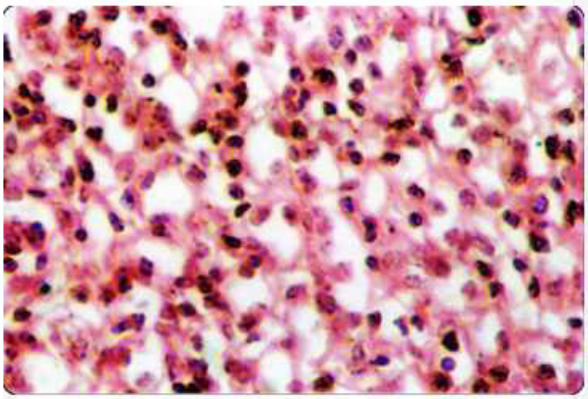

Figure.4: T.S. showing hydropic degeneration in medullar convoluted tubules, number of abnormal cells increased, karyolysis in epithelial lining of urinary tubules after 10ppm NaF for 14 days
(H&E X200)

5ppm treated group showed insignificant changes for an initial two weeks but after 28days treatment animals showed marked renal tubular degeneration with increased number of pyknotic nuclei, cytoplasmic degranulation and vacuolation were observed in very few tubules and glomeruli. Tubular cells were devoid of nuclei at certain places (Fig. 2) In 10ppm sub group after 7 days treatment of sodium fluoride a mild histological changes were observed. Glomeruli with hyper cellularity and mesangial cell proliferation were apparent. Nuclear pyknosis at certain points. No necrosis or tubular damage was noticed (Fig. 3). After 14 days mild hydrophic degeneration in medullary convoluted tubules. number of abnormal cells increased, karyolysis in epithelial lining of urinary tubules at certain points (Fig. 4).

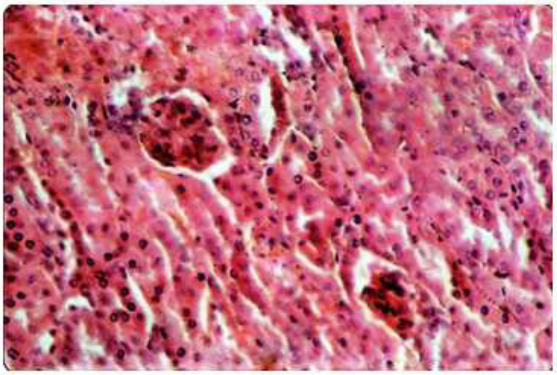

Figure.5: T.S. of kidney showing oedema in the interstitial connective tissue, increased nuclear pycnosis in cortical region and degenerative changes were observed in glomeruli after 10ppm/28 day NaF treatment (H&E X 200)

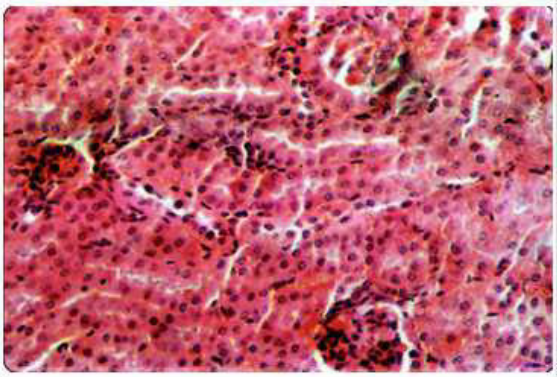

Figure.6: T.S. of kidney showing narrow Bowman's capsule, shrunken glomeruli, nuclear pyknosis both in tubules and glomeruli after 40ppm/7 days NaF (H&E X 200)

After fourth week of treatment cytoplasmic degranulation and vacuolization were observed in cortical region. Increased nuclear pyknosis and more number of dead cells. deep stained mesangial cells as well as degenerative changes were observed in glomeruli (Fig. 5). In 40ppm treated group after 7 days Bowmen's capsule were narrowed, shrunken in glomeruli, nuclear pyknosis in renal tubules and glomeruli (Fig. 6). After the second week of treatment hydropic degeneration with cellular oedema, vacuolization in glomeruli and tubular part become distorted at certain places, epithelium was devoid of nucleus (Fig. 7).

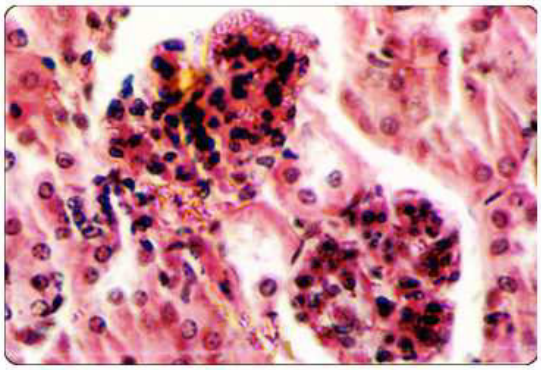

Figure.7: T.S. of kidney showing hydropic degeneration with oedema, vacuolization in glomeruli. Tubular parts distorted at some places after 14 days/40ppm NaF
(H&E X400)

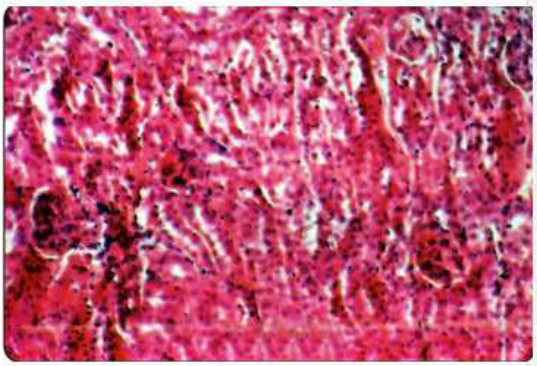

Figure.8: T.S. of kidney showing vacuolization, renal tubules with cytoplasmic degeneration, pyknosis and glomeruli appeared constricted at places after 21days of 40ppm NaF treatment
(H&E X100)

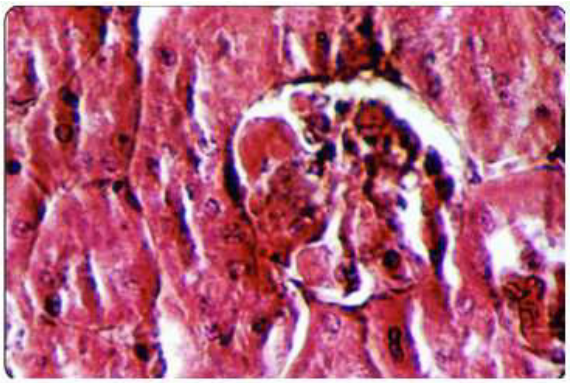

Figure.9: kidney section showing lobulated glomeruli with Hypertrophy. Glomerulus with slightly thickened basement membrane, 21 days/40ppm NaF treatment (H&E X400)

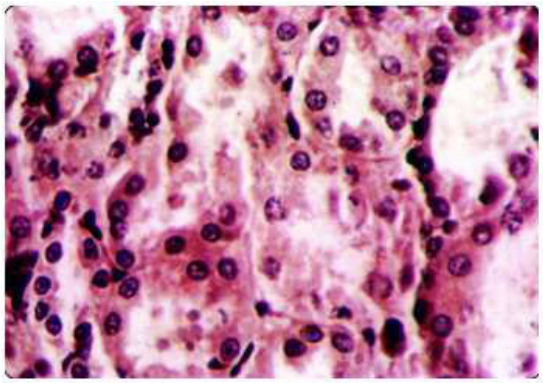

Figure.10: kidney section showing vacuolization in renal tubules. Cytoplasmic degranulation and partial loss of epithelial tissues of proximal convoluted tubules after 40ppm/28 days treatmernt (H&E X400)

After 21 and 28 days treatment vacuolated renal tubules with cytoplasmic degeneration, nuclear fragmentation and pyknosis were observed. Large number of dead cells or tissues necrosis. Glomeruli appeared constricted (Fig. 8, 9) while in the medullary region, vacuolization, cytoplasmic degranulation and pyknosis at certain places was observed (Fig. 10).

***Recovery with Ascorbic Acid***

After cessation of treatment when animals were given 40ppm Ascorbic Acid for next 28 days following histological changes were observed: In 5ppm treated group after 21 days of Ascorbic Acid treatment, glomeruli showed normal architecture without vacuolization encapsulated by normal Bowman's capsule, decreased number of pyknotic nuclei and necrosis in tissue. Medullary cells with proper nuclear arrangement in tubules (Fig. 11)

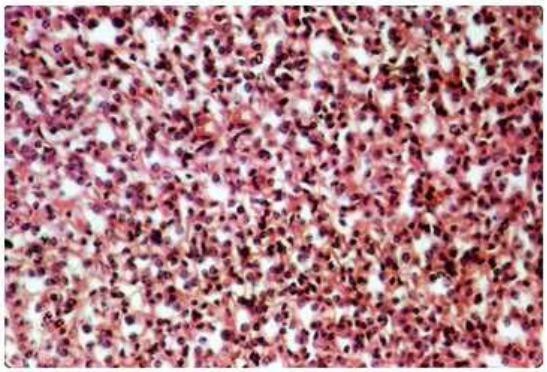

Figure.11: kidney section showing medullary cells with proper nuclear arrangement in tubules after 21 days recovery with 40ppm Ascorbic acid of 5ppm/28 days NaF treatment (H&E X100)

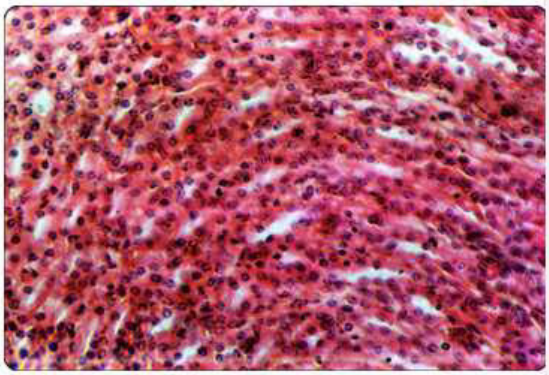

Figure.12: lesser number of pyknotic nuclei, hyperemia at few places. Cortico-medullary region with restored structure 7 days recovery with 40ppm Ascorbic acid of 10ppm/28 days NaF treatment (H&E X100)

In 10ppm After 7 days of recovery with Ascorbic Acid glomeruli were observed with less vacuolization renal tubules, get hydrophic but less degenerated, epithelial cytoplasmic regranulation. Less number of dead cells or pyknotic nuclei, Cortico-medullary region got more restored structure (Fig. 12) After 28 days glomeruli recovered almost completely intact with bowmen's capsule, no monocytic infiltration or mesangial cell proliferation. Proximal and distal tubules with normal structural epithelial cell lining and few

pyknotic nuclei were observed. No nuclear infiltration or hyper cellularity was recorded. Renal architecture returned to normal (Fig. 13) In the 40ppm treated group 21 days treatment with 40ppm Ascorbic Acid showed that the kidney exhibits signs of recovery. The outer surface of capsule appeared normal, no cytoplasmic vacuolization or hemorrhagic inflammation was observed.

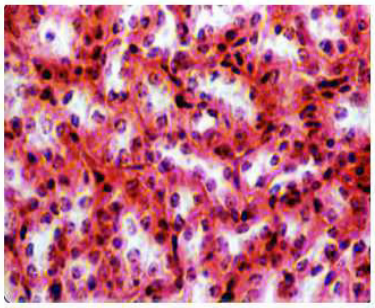

Figure.13: kidney section showing tubular cells were more rearranged than 7 day recovery. Less number Of pyknotic cells after 14 days recovery with 40ppm Ascorbic acid of 40ppm/28 days NaF treatment H&E X200)

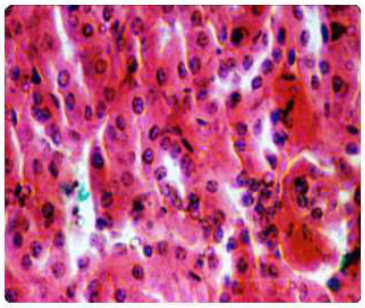

Figure.14: kidney section showing proximal and distal tubules with normal structure epithelial cell lining. A few number of pyknotic nuclei. Renal architecture to normal after 28 days recovery with 40ppm ascorbic acid of 10ppm/28 days NaF treatment (H&E X200)

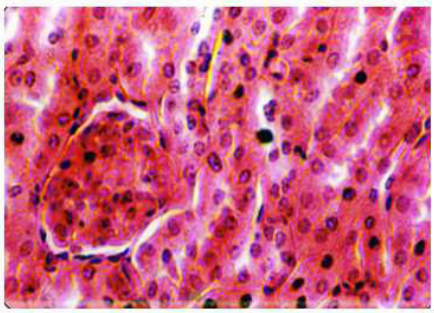

Figure.15: kidney section showing intact glomeruli, tubules with proper lumen, no vacuolization in cytoplasm and very few pyknotic nuclei after 28 days recovery with Ascorbic Acid 40ppm of 28 days 40ppm NaF treatmen (H&E X400)

28 days of recovery with 40ppm Ascorbic Acid showed that the kidney exhibits slightly arranged renal architecture. Decreased vacuolization, cytoplasmic infiltration and few pyknotic nuclei were observed. Proximal and distal renal tubules with normal lumen without obliteration and fluid. No nuclear infiltration or necrotic cells were observed. No hyperemia, atrophy or hemorrhagic condition observed (Fig. 15).

## DISCUSSION

In the kidneys of Swiss albino mice of Group II (sub group- $I^{ST}$, $II^{nd}$ and $III^{rd}$administered with doses 5, 10 and 40ppm NaF) and Group IV (A) (5ppm NaF+40ppm Ascorbic Acid, 10ppm NaF+40ppm Ascorbic Acid and 40ppm NaF+40ppm Ascorbic Acid) for 28 days, nuclear pyknosis, cytoplasmic degranulation, increased number of abnormal cells, vacuolization in tubules, hypertrophy and atrophy of glomeruli, cloudy swellings, cellular oedema and hydropic degeneration have been observed in the present investigation. The appearances of cloudy swellings almost remain constant factor. Their intensity increase with the increase in the degree of the respective doses. Hydropic degeneration of tubular epithelium and congested glomeruli in fluoridated rabbits were observed[19]. Acute congestion and general cloudy swellings are reported for 6 patients ingesting lethal doses of NaF[20]. At the lower doses of NaF 5, 10 and 40ppm for 28 days other changes like vacuolization of the cell lining the convoluted tubules, widening of lumen of tubules, hypertrophic and lobulated glomeruli and varying degree of degeneration of tubular epithelium have been fully detected. [1]. During present endeavor, the vacuolization of cytoplasm in the cells lining the convoluted tubules in treated groups happens to be quite intense[21]. Tubular degeneration, cloudy swelling and protein casts or blood in the lumen are also recorded in our study. As compared to Kawahara's observations, cloudy swelling, tubular degeneration and hypertrophy of glomeruli were recorded in fluoridated mice[22]. The normal glomerular basement membrane, composed of type IV collagen, plays an important function in the process of filtration. Therefore any alteration in the kidney collagen content is also likely to affect the renal function[23]. The ultra structure of the liver and kidney in fluoride treated rats displayed shrinkage of nuclear and cell volume, swollen mitochondria and endoplasmic reticulum and vacuoles formation in the liver and kidney cells[24].

Inflammatory changes in the glomeruli and interstitial oedema, hypertrophy and hyperplasia in the tubules of the rats, which had been given fluoride in the drinking water in the doses of 1, 5 and 10ppm was observed[25,26]. The intensity of kidney histopathology increased with increasing doses of fluoride. Higher doses of fluoride increase lipid peroxidation and inhibits the oxidative enzymes in the kidney[24]. Ascorbic Acid (vitamin C) is a highly effective antioxidant, acting to lessen oxidative stress[27]. Adequate vitamin C (Ascorbic Acid) in the diet is an important entity to ameliorate the ill effects of fluoride[15,16,17]. Hepatic and renal antioxidant status of fluoride exposed animals improved upon feeding *Emblica officinnalis*fruit powder. We, therefore, conclude that *E. officinalis* fruit could be useful in regulating hyperglycemia and enhances antioxidant status of fluoride exposed animals[27]. Ascorbic Acid treatment to chronically exposed fishes helped in overcoming the adverse effects and reversal towards normal structure and function of vital organs[18]. Vitamin C inhibits the peroxidation of membranes phospholipids and acts as a scavenger of free redicals[28]. Tamarind pulp (vitamin C) is able to prevent free radicals induced oxidative stress by F, attributable to its antioxidant property[29]. Our findings are also similar to this report Ascorbic Acid when given with NaF have played a role to ameliorate the oxidative stress induced by NaF. The therapeutic treatment of Tamarindus indica pulp extract manifested the improvement in the recovery and mitigation of devastating toxic effects of fluoride on the concerned parameters in male albino rat[30] The administration of Ascorbic Acid to NaF-treated mice revealed significant recovery from NaF toxicity in our Histological studies. After administration of Ascorbic Acid (vitamin C) manifested significant recovery kidney histology due to its active antioxidant property as well as detoxification properties, is a promising and potent agent in suppressing fluoride toxicity.

## CONCLUSION

This experimental study indicates that fluoride toxicity produces definite effects on kidney which is evident from histological studies. It depends on the dose and duration of NaF exposures. Withdrawal of treatment and administration of Ascorbic Acid caused recovery, suggesting that effects induced by sodium fluoride treatment were transient and reversible by Ascorbic Acid and kidney revealed significant recovery from fluoride toxicity during the histological stages. Ascorbic Acid can be used as therapeutic agents for the mitigation of fluoride induced toxicity.

## REFERENCES


1. Chouhan S and Flora SJS, Arsenic and Fluoride: Two major ground water pollutants. Indian Journal of Experimental Biology, 48; 666-678, (2010).
2. McClure FJ, Mitchell HH, Hamilton TS and Kinser CA, Balances of fluorine ingested from various sources in food and water by five young men. Excretion of fluorine through the skin. J. Ind. Hyg. Toxicol. 27: 159-170, (1945).
3. Arif M, Hussain I, Hussain J and Sharma KC, Potential fluoride contamination in drinking water of Nagaur District, Rajasthan, India. Bulletin of Environmental contamination and Toxicology, 88 (6); 870-875 (2012).
4. Hussain J, Sharma KC and Hussain I, Fluoride in drinking water and its ill effects on human health. A review Journal of Tissue Research, 4(2): 263-273 (2004).
5. Hodge HC and Smith FA, Occupational fluoride exposure. Journal of Occupational Medicine, 19: 12-39 (1997).
6. Inkielewicz I and Krechniak J, Fluoride effects on glutathione peroxidase and lipid peroxidation in rats. Fluoride, 37(1): 7-12 (2004).
7. Whitford G, Fluoride toxicology and health effects. In: O. Fejerskov, J. Ekstrand, and B. Burt. (Eds), *Fluoride in Dentistry*, 2nd Edn, Munksgaard, Denmark, 1996, pp. 167-184.
8. Shashi A, Singh JP and Thapar SP, Toxic effects of fluoride on rabbit kidney. Fluoride, 35: 38-50, (2002).

9. Whitford GM and Taves DR, Fluoride-induced diuresis: plasma concentrations in the rat. Proceedings of the Society for Experimental Biology and Medicine, 137: 458-460, (1971).
10. Jankauskas J, Effects of fluoride on the kidney: A review. Fluoride, 7: 93-105, (1974).
11. De Camarago AM and Merzel J, Histological and histochemical appearance of liver and kidney of rats after long term treatment with different concentration of sodium fluoride in drinking water. Acta, Anaat,. 108: 288-294, (1980).
12. Kessabi M, Hamliri A, Braun JP and Rico AG, Experimental acute sodium fluoride poisoning in sheep: renal, hepatic, and metabolic effects. Fundamentals of Applied Toxicology 5(6 Pt 1): 1025-33, (1985).
13. McGregor GP and Biesalski HK, Rationale and impact of vitamin C in clinical nutrition. Current opinion in clinical nutrition and metabolic care, 9 (6): 697–703, (2006).
14. Padayatty SJ, Katz A, Wang Y, Eck P, Kwon O, Lee J, Chen S, Corpe C, Dutta A, Dutta S and Levine M, Vitamin C as an Antioxidant: evaluation of its role in disease prevention. J Am CollNutr. 22 (1): 18–35 (2003).
15. WHO, Guidelines for Drinking water equality, World Health Organization, Geneva, 13: 249-267 (1984).
16. Boyd CD and Cerklewski NE, Influence of type and level of dietary protein on fluoride bioavailability in the rat. J. Nutr. 117: 2086-2090 (1987).
17. Gupta SK, Environmental Health Perspective of Fluorosis in Children (Ph.D Thesis), Jaipur, Rajasthan: University of Rajasthan (1999).
18. Tripathi M, Gupta R, and Sharma UD, Recovery of adverse effects induced by fluoride after Ascorbic Acid treatment in Channapunctatus (Bloch). Journal of Ecophysiology & Occupational Health, 8(3 & 4): 231-236, (2008).
19. Muehlberger CW, Toxicity studies of fluorine insecticides. J Pharmacol Exptl Therap, 39: 246-248, (1930).
20. Geiger JC, Poisoning Due to the Ingestion of a Mixture of Sodium bicarbonate-Sodium fluoride. Cal West Med, 44: 81-83, (1936).
21. Bond AM and Murrey MM, Kidney function and structure in chronic fluorosis. Brit J Exp Path, 33: 168-176, (1952).
22. Kawahara H, Influence of sodium fluoride on the urine changes and non-protein nitrogen, creatinine and sodium chloride in serum of rabbits. ShikokuActa. Med. 8: 226-272, (1956).
23. Khubchandani SR, Arun RC and Gowrishankar S, Banded collagen in the kidney with special reference to collagenofibrotic glomerulopathy. Ultrastruct Pathol, 34(2): 68-72, (2010).
24. Zhang Z, Zhou B, Wang H, Wang F, Song Y, Liu S and Xi S, Maize Purple Plant Pigment Protects Against Fluoride-Induced Oxidative Damage of Liver and Kidney in Rats. Int. J. Environ. Res. Public Health, 11: 1020-1033 (2014).
25. Ramseyer WF, Smith CAH, and Mccay CM, Effect of sodium fluoride administration on body changes in old rats. J Gerontol 12: 14-19, (1957).
26. Zhan X, Xu Z, Li J and Wang M, Effects of fluorosis on lipid peroxidation and antioxidant system in young pigs. Fluoride, 38(2): 157-161, (2005).
27. Vasant RA and Narasimhacharya A, Amla as an antihyperglycemic and hepato-renal protective agent in fluoride induced toxicity. J Pharm Bioall Sci, 4(3): 250-254, (2012).
28. Frei B, England LS and Ames BN, Ascorbate is an outstanding antioxidant in human blood plasma. Proc Natl Acad Sci USA, 86 (16): 6377-6381, (1989).
29. Ekambaram P, Namitha AT, Bhuvaneswari S, Aruljothi S, Vasanth D, Saravanakumar M, Therapeutic efficacy of Tamarindus indica (l) to protect Against fluoride-induced oxidative stress, In the liver of female rats. Research report Fluoride, 43(2): 134-140, (2010).
30. Singh PK, Feroz AD, Sheeba H, Khalil A and Samir AM, Beneficial Effect of Tamarindus Indica on the Testis of Albino Rat after Fluoride Intoxification, Int J Pharma Bio Sci, 3(3):(B)487-493.