IARJSET ISSN (O) 2393-8021, ISSN (P) 2394-1588

International Advanced Research Journal in Science, Engineering and Technology

Impact Factor 8.066 ⁜ Peer-reviewed & Refereed journal ⁜ Vol. 11, Issue 5, May 2024

DOI: 10.17148/IARJSET.2024.11582

Histological changes in Sodium Fluoride induced Kidney of Swiss Albino mice and its Amelioration by Ascorbic acid and Calcium

Mahendra Singh Solanki[1], Renu Agarwal[2]

Histological Laboratory, P.G. Department of Zoology, Govt. Dungar College, Bikaner, Rajasthan, India – 334001[1,2]

Abstract: Water pollution has become world-wide phenomenon. Both deficiency and excess of minerals and trace elements in water can have deleterious consequences on biological system. The major ecological problem are the pollutants from industries, pesticides, herbicides, fertilizers and chemicals. The underground water is polluted by many hazardous pollutants like colored dyes, nitrates, metals, pesticides and fluoride. Fluoride is one of major concern among these pollutants. The problem due to high concentration of fluoride in ground water has now become one of the most important health geo-enviornmental issues in india. Fluorosis which was considered to be a problem related to teeth only has now turned up to serious health hazard. However no system of the body can be considered as exempt. 2 3- at At the normal levels of fluoride ingestion (approximately 6 mg/day) almost 87% of the absorbed fluoride is excreted through kidneys (McClure et al.,1945, Hodge 7 and Smith, 1972).Thus among the soft tissues, kidneys supposedly have the highest fluoride content as both excreation and retention of fluoride are affected by kidneys, thus kidney are more prone to fluoride toxicity then other soft organs which generally do not attain high fluoride levels, to which kidneys are subjected S to. bund 2023/06/30 22:06 Experimental studies have shown that the dietary factors such as calcium, amino-acids and vitamin C,D and E can mitigate the toxic effects of individual treatment of fluoride. Thus the present investigation is undertaken to investigate the effects of sodium fluoride (5 and 50 ppm), ascorbic acid (25 ppm) and calcium (calcium phosphate 25 ppm) alone and with calcium and ascorbic acid with sodium fluoride in producing various changes at histological levels in the kidney of mice for understanding the mechanism of action of fluoride. Recovery studies for 10,20 and 30 days were also undertaken with a hope that it is quite possible that recovery will play an important role in the restoration of normal structure once the kidney is effected. T dependent. After withdrawal of treatment, the signs of recovery were evident on day 10.The recovery process continued till day 30 and it was more pronounced with ascorbic acid+ calcium treatment.

Key Words: Histological changes, Fluorosis, Ascorbic acid, Sodium Fluoride.

I. INTRODUCTION

Fluorosis is an important public health problem in 24 countries, including India, which lies in the geographical fluoride belt that extends from Turkey to China and Japan through Iraq, Iran and Afganisthan[1]of the 85 million tons of fluoride deposits on the earth's crusts, 12 million are found in India[2].Hence it is natural that fluoride contamination is widespread intensive and alarming in India. Endemic fluorosis is prevalent in India since 1937[3].It has been estimated that the total population consuming 4 drinking water containing elevated levels of fluoride is over 66 million[4].Choronic fluoresis may cause skeletal florosis.dental florosis,non-skkeleta manifestation and premature aging in human[5,]The acute effects of the investigation of massive doses of fluoride are, first those of an irritant poison, and later become apperent in enzyme system, such as those engaged in metabolism, energetic cellular respiration and in endocrine function.However, no system of the body can be considered as exempt.

Moreever because of its tendency to bind with magnesium and manganese cation, fluoride interfere with the activity of many enzyme system that require these two cations and those involved in carbohydrate,lipid and protein metabolism[6].The biological response and severity of fluoride toxicity depends on concentration of fluoride in drinking drinking water daily intake,continuity and duration of exposure,calcium nutrition, composition of foods (in respect to Ca, Mg,P,Al)age,sex,occupation growth and re:nodeling of bone. The major route for the removal of fluoride from the body is by the kidney. Urinary fluoride is regarded as the best indicator of exposure to fluoride compounds, and usually it correlates well with the level of fluoride in drinking water [7].Various studies[8-9]also revealed that the treatment of vitamin C,D and calcium showed significant improvement in skeletal, clinical and biochemical parameters in children consuming water containing 4.5 ppm of fluoride[10]reported that consumption of food rich in vitamins C and E, which acts as antioxidants, scavenging the free redicals and eliminating them, also reduces the fluoride levels in the body.

IARJSET ISSN (O) 2393-8021, ISSN (P) 2394-1588

International Advanced Research Journal in Science, Engineering and Technology

Impact Factor 8.066 ⁜ Peer-reviewed & Refereed journal ⁜ Vol. 11, Issue 5, May 2024

DOI: 10.17148/IARJSET.2024.11582

Vitamin C in the diet is an important entity to ameliorate the ill effects of fluoride[11-13] Vitamin C, are prescribed in an attempt to reverse toxic effects of fluoride.[14-18].Earlier workers have demonstrated that calcium ingetion by fluoride intoxicated male and female mice and rats brought about a significant regain in the NaF-inhibited enzyme activities[19-23]. Several investigations regarding the protection of mammals against sodium fluoride toxicity have been carried out but there are only few reports with ascorbic acid[24-26].In the view of the above facts and lacunae in the information available on the varied aspects of fluoride toxicity, the present investigation is undertaken to investigate the effects of sodium fluoride, ascorbic acid with sodium fluoride on kidney of mice for understanding the mechanism of action of fluoride. Recovery studies were also observed with ascorbic acid which played an important role for the mitigation of fluoride induced nephrotoxicity by restoring the normal structure once the kidneys is affected with sodium fluoride.

II. MATERIAL AND METHODS

Procurement

Healthy, Swiss strain adult male mice (*Mus musculus*)Weighing between 30 to 40 gram were obtained from CCS University, Hissar (Haryana) under the Animal Maintanance and Ragistration No--/1066/ac/07/CPCSEA from the Ministry and Social justice and Empowerment Govt of India and Committee for the purpose of control and Supervision of Experiments on animals, Chennai, India. The animals we kept in polypropylene cages; saw dust was put on bottom of cages. The cages were cleaned daily. Water bottles and nipples were autoclaved periodically. Mice were fed with standard pellet feed. Water was given ad-libitium.

Design of Experiment

In order to investigate the effects, the animal were divided into following groups

Group I (Normal)

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

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 sub groups on the basis of sodium fluoride dose.

Sub group 1 -5ppm
Sub group II -50ppm

The animals of these sub groups were given sodium fluoride and were sacrified after 10,20,30 days of treatment.

Group III (after withdrawal of sodium fluoride treatment)

The animals were divided into following sub groups
Sub group 1 - 5ppm
Sub group II -50ppm
animals In these sub groups animals were treated with sodium fluoride for 30 days as in group II and were sacrified after 10,20, and 30 days of cessation of treatment.

GroupIV(Ascorbic Acid treated animals)

The animals of this group received Ascorbic acid at the dose rate of 25 ppm (until autopsy) and were sacrified after 10,20,and 30 days of treatment.

Group V: This group comprised of different subgroups: Subgroup I 25ppm

Group VI (A):(Sodium fluoride + Ascorbic Acid)

Subgroup I - 5ppm NaF+25ppm Ascorbic acid
Subgroup II - 50ppm NaF+ 25ppm Ascorbic acid

In these subgroups, animals were treated with sodium Fluoride + Ascorbic acid and were sacrificed after10,20,30 days treatment.

GroupVI (B):(Sodium fluoride+Ascorbic Acid treatment followed by recovery):- Likewise animals were treated with sodium fluoride+ Ascorbic acid for 30 days as in group VI A and were sacrified after 10,20 and 30 days of cassation of treatment.

IARJSET ISSN (O) 2393-8021, ISSN (P) 2394-1588

International Advanced Research Journal in Science, Engineering and Technology

Impact Factor 8.066 ⁜ Peer-reviewed & Refereed journal ⁜ Vol. 11, Issue 5, May 2024

DOI: 10.17148/IARJSET.2024.11582

Group VI (B):(Sodium fluoride+Ascorbic Acid treatment followed by continuation of ascorbic acid in recovery groups):- In this subgroup, animals were treated and sacrificed as in group VII(B) but during recovery period the ascorbic acid alone was given continuously until autopsy.

Autopsy

Animals from each group were autopsied by cervical dislocation at each post treatment interval of 10. 20 and 30 days The weight of the animals was recorded and kidney was removed for various studies (for histological studies. changes in The Kidnay.

HISTOLOGICAL STUDIES

After sacrificing the mice, the pieces of tissue(kidney)from control as well as experimental groups were put in the Bouin's fixative for overnight at room tempreture.The material was washed thoroughly with 50% alcohol and dehydrated by passing through 70%,90% and absolute alcohol.Finalllly the tissue were cleared in xylol and embedded in paraffin wax at 58-60ºC.The blocks were made and sections cut t 5µ(micron) with the help of rotary microtome and stained with Haematoxyline-eosin stains for qualitative studies. Cross section of each kidney from different groups were observed in order to record the histological changes.

Following parameters were taken into consideration:

1.To observe histological changes in kidney
2.changes in overall weight of kidney
3.To observe degeneration of the tubular epithelium
4.to observe vacuolization in the cell lining the convoluted
5. Increased damage of kidney with respect to the increased doses of fluoride

BIOCHEMICAL STUDIES

The biochemical studies were performed on normal as well as on treated mice and in recovery groups after sacrificing the animals, the kidneys were taken out immediately and weighed on electrical balance. These were stored at -20°C.

The following biochemical estimation were carried out in kidney:

1. Total protein (Lowry et al., 1951)
2. Acid phosphatase (Fiske and Subbarow, 1925)
3. Alkaline phosphatase (Fiske and Subbarow, 1925)
4. DNA (Ceriotti, 1952)
5. RNA (Ceriotti, 1952)
6. Ascorbic acid (Natelson, 1963)
7. Glycogen (Montgomery, 1957)
8. Cholesterol (Oscer, 1965)

The value of organo-somatic index decreased on day 10 in all the experimental groups. This decrease continued up to day 30.The difference between control and experimental value was significant at the dose group of 50ppm.in all recovery groups, the value increased gradually from day 10 till day 30 and it was more pronounced with ascorbic acid + calcium than to normal recovery. 2. Histological studies Conclusion

III. OBSERVATION

In the present study, histological changes in the corticular and medullary regions were noticed after administration of sodium fluoride. The general histological changes noticed in the proximal and distal convoluted tubules are intertubular oedema, vacuolization, distortion of tubular epithelium, cytoplasmic degranulation, hyperaemia, pycnosis, necrosis and dead cells and cellular infiltration. Tubular luman filled with fluid or cast. T

he histological changes were mild in nature on day 10 in all experimental groups, which became more servere on days 20 and 30.All the changes were dose and duration dependent. After withdrawal of treatment, the signs of recovery were evident on day 10.The recovery process continued till day 30 and it was more pronounced with ascorbic acid + calcium treatment.

IARJSET ISSN (O) 2393-8021, ISSN (P) 2394-1588

International Advanced Research Journal in Science, Engineering and Technology

Impact Factor 8.066 ⁜ Peer-reviewed & Refereed journal ⁜ Vol. 11, Issue 5, May 2024

DOI: 10.17148/IARJSET.2024.11582

REFERENCES

[1]. Al-Hashem, F., (2009). Camel's milk protects against aluminum chloride-induced toxicity in the liver and kidney of white albino rats. Am. J.Biochem. Biotechnol., 5: 98-101.
[2]. Banks and Gouldwhite Banks R; E. and Gould white; H(1966) Fluorine Chemistry, in ; Smith, FA ed Hand book Experimental Phemacology New York Sprnger vol. 20, 608
[3]. Cooke, Gould MH,(1991) the Health effects of Aluminum. Are view. J Royal Soc Health Oct. ; 163-7
[4]. Chinoy NJ, et al. (1991). Microdose vasal injection of sodium fluoride in the rat. Reproductive Toxicolology 5(6):505-12.
[5]. Chinoy NJ, et al. (1992). Effects of fluoride ingestion on the physiology of reproductive organs of male rats. Journal of Environmental Biology 13: 55-61
[6]. Chinoy NJ, Sequeira E. (1992). Reversible fluoride induced fertility impairment in male mice. *Fluoride 25* (2) 71-76
[7]. Chinoy NJ, Sorathia HP, Jhala DD. (2005) Fluoride + aluminium induced toxicity in mice testis with giant cells and reversal by vitamin C. Fluoride; 38(2):109-14
[8]. Chubb C. (1985). Reproductive toxicity of fluoride. Journal of Andrology 6: 59
[9]. Chinoy NJ, Sequeira E. (1989) Fluoride induced biochemical changes in reproductive organs of male mice. Fluoride;22(2):78-85.
[10]. Chinoy NF, et al. (1995). Amelioration of fluoride toxicity in some accessory reproductive glands and spermatozoa of rat. Fluoride 28: 75-86.
[11]. Chinoy NJ , Sequeira E. (1989). Effects of fluoride on the histo architecture of reproductive organs of the male mouse. Reproductive Toxicolology 3(4):261-7.
[12]. Gupta, SK (1999) Enviormantal Health Perspective of Fluorosis in Children (Ph. D Thesis) , Jaipur, Rajasthan; University of Rajasthan .
[13]. Gupta SK, Gupta, RC, Seth, AK, Gupta, A. (2005) Reversal of Fluorasis in Children, Wei sheng Xanjiv. My ; 34 (3);287-288;
[14]. Jones KC, Bennentt BG.(1985).Exposure commitment assessment of Enviormantal pollutents .monitoring and assessment res centra;4(33)1-35
[15]. Mullenix PJ, Denhesten P.K. Schun or A, Kernan WJ.(1995)Neurotoxicity of Sodium Fluoride in Rates, Neurotoxicol Treatment;17 : 169-17
[16]. Nair SB. , Jhala, D.D. Chinoy. NJ. (2004) : Benfical effects of Certain antidots in mitigation fluoride and arsenic induced Hepatotoxicity in mice Fluoride 2004; 37 (2) 60-70
[17]. Ghosh D, et al. (2002). Testicular toxicity in sodium fluoride treated rats: association with oxidative stress. Reproductive Toxicolology 16(4):385.