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ORIGINAL ARTICLE

EFFECT OF FLUORIDE ON KIDNEY OF ALBINO RABBIT-AN EXPERIMENTAL STUDY

Santosh K. Sahu[1], Sitansu K. Panda[2], Sujita Pradhan[3], Jami Sagar Prusti[4], Dharma N. Mishra[5], Geetanjali Arora[6], Mahesh C. Sahu[7], Prafulla K. Chinara[8]

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ABSTRACT: **BACKGROUND:** Fluoride is present in environment in various forms and ingested by man from solid foods, drinks, drinking water and inhaled from the air. Out of these, fluoride is present in large quantities in dissolved state in many sources of drinking water producing toxicity in man. Fluoride, being excreted mainly through the kidneys, seems to damage it causing renal dysfunction. Kidneys are among the most sensitive body organs in their histopathological and functional responses to excessive amounts of fluoride. Trace elements are essential and beneficial to human being in minute concentrations. However, intake in large quantities produces adverse and toxic effects on our body. **OBJECTIVE:** The present study was designed to investigate the toxic effects (evaluated as histopathological changes) of sodium fluoride on the kidney in albino rabbit. **MATERIALS AND METHODS:** Total 40 albino rabbits were used for this study, among them 8 rabbits were taken in the control group (Group A) and16 rabbits each were taken in both group B and group C who were administered low and high dose of fluoride respectively. After 2 weeks interval, up to 16 weeks the histology of the kidney of each group of the rabbit was studied for histological analysis. **RESULT:** Histological changes in the kidneys of both Group B and Group C rabbits, following continuous daily exposure to sodium fluoride solutions in two different doses (0.5% solution for Group B and 3% solution for Group C) for different durations of time were studied in detail and compared with those of the controls (Group A). **CONCLUSION:** It is concluded that Sodium Fluoride solution in high doses for prolonged period has a definite adverse effect on the renal parenchyma.
KEYWORDS: Albino rabbits, Fluorosis, Kidney histology, Renal damage, Sodium fluoride.

INTRODUCTION: Fluoride, being excreted mainly through the kidneys, seems to damage it causing renal dysfunction. Kidneys are among the most sensitive body organs in their histopathological and functional responses to excessive amounts of fluoride.[1] Trace elements are essential and beneficial to human being in minute concentrations. However, intake in large quantities produces adverse and toxic effects on our body.

Fluoride is one such element present in environment in various forms and ingested by man from solid foods (Vegetables and fruits, cereals, barley, rice, meat, fish), drinks (drinking water, human breast milk, tea leaves, fresh fruit juices) and inhaled from the air. Out of these, fluoride is present in large quantities in dissolved state in many sources of drinking water producing toxicity in man.[2]

Low amount of fluoride (0.3 to 1.0 mg/litre) in drinking water prevents dental carries and osteoporosis.[3] and the dosage is not important when the administration is permanent in the drinking water.[4, 5] Also, the researchers found the correlations between plasma level of fluoride and urinary

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excretion of the substance.[6]However, fluoride intake in large quantities (>1.5mg/litre) for a prolonged period is known to cause toxicity of soft tissues, skeletal elements and dental enamel.[7]

The exposure to high acute doses of fluoride may occur following administration of certain halothane anesthetics, which are defluorinated by the liver; this can result in serum fluoride concentrations that are 50-fold higher than normal, and these serum concentrations of fluoride have been associated with nephrotoxicity. Several halogenated anesthetics such as Methoxyflurane, Enflurane, induce a urinary concentrating defect, partly related to inorganic fluoride toxicity in collecting duct cells.[8]

Studies suggest that the mitochondrion is a target of fluoride toxicity and its alteration is partly responsible for the sodium and water disturbances observed in patients. Animal studies have been conducted to evaluate the effects of fluoride on kidney tissue and function. Chronic excessive fluoride exposure of young pigs causes various histological structure changes of the kidney, including extensive induction of cell apoptosis, resulting in impairment of renal function and metabolism.[9]

It has been shown that high concentrations of fluoride (5 mmol) affect the ATPase pump in cultured rabbit ascending loop cells.[10]

High levels of lipid peroxidation were detected in rat kidney using an animal model of chronic fluorosis produced with high doses of fluoride in drinking water for a prolonged period. The results of the study suggest that the oxidative stress and modification of cellular membrane lipids may be involved in the pathogenesis of chronic fluorosis and provide a possible explanation for the gross system damage observed in the body, especially in soft tissues and organs.[11]

In Wistar rats, the administration of sodium fluoride in doses of 10 mg/kg for 35 days, result in the impairment of the antioxidative system in the kidney (increase in the concentration of MDA, decrease activity of all antioxidative enzymes – SOD, total and both its isoenzymes, GPX, GST, GR, and CAT).[12]

Our study aimed to investigate the possible pathological changes in rabbit kidney. In this study, a histological analysis was done of renal tissue of albino rabbits and these were exposed to sodium fluoride solution for a period of 4 months in 2 different doses. And data obtained from 2 week interval up to 16 week were documented.

MATERIALS AND METHODS: The present study had been undertaken in the department of Anatomy, S.C.B. Medical College, Cuttack, in collaboration with Department of Pharmacology and Department of Pathology, S.C.B. Medical College, Cuttack and IMS and SUM Hospital, Bhubaneswar. Ethical clearance from the Institute Ethical committee was obtained for the study.

Forty (40) healthy, mature, male albino rabbits (Class-Mammalia, Order-Logomorpha, Family-Leporidae, Genus-Oryctolagus, Species-Cuniculus) were selected for the study which weighed between 1.5 and 2.0 Kg. and divided into 3 groups A, B, and C (Fig. 1a-f). Group A of 8 rabbits was the control group whereas Groups B and C were test groups of 16 rabbits in each.

All 3 groups were housed separately where in addition to normal diet; Groups B & C were supplemented with 0.5% and 3% of Sodium fluoride solution orally through feeding tube which provided 5 mg and 30 mg of Sodium fluoride per Kg. body weight respectively.[13]

One animal from group A and 2 each from group B & C were sacrificed at 2 weeks intervals upto 16 weeks and kidneys were removed and processed for histological study. The tissue slides were stained with Haematoxylin and Eosin and examined under low power microscope.

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RESULT: Gross changes in the body and histological changes in the kidneys of both Group B and Group C rabbits, following continuous daily exposure to sodium fluoride solutions in two different doses (0.5% solution for Group B and 3% solution for Group C) for different durations of time were studied in detail and compared with those of the controls (Group A). Therefore, in the present study, the observations are grouped as: (1) effect on control animals and (2) effect of fluoride on test animals.

Sixteen (16) animals of Group-B and 16 animals of Group-C were sacrificed as test animals in 8 different periods of the experiment, i.e. 2 each from Group-B and Group C, at 2 weeks intervals upto 16 weeks. In all the Group-B animals the histological findings remained unchanged throughout the course of the experiment as was observed in case of the controls. However, in Group-C animals both the histological findings changed from time to time, which have been correspondingly grouped and tabulated below (Table 1).

In histological study, Edematous, atrophied, highly lobulated and hyperemic glomeruli were found throughout the cortex with slight thickening of Bowman's capsule. Inflammatory changes in the glomeruli with increased cellularity in the cortical interstitium were evident. There were round cell infiltrations into the cortical interstitial tissue (Fig. 2a-c, Fig. 3a-c, Fig. 4a-c).

Cell necroses along with interstitial edema were present. Edematous convoluted tubules were prominently dilated along with cloudy swellings and sloughing out of lining epithelia. There were very largely dilated collecting tubules and ducts with granular degeneration & sloughing out of lining epithelia. Both focal and diffuse inflammatory cell infiltrations were seen throughout the medulla. Vascular degeneration and blood in some tubular lumens were also seen.

DISCUSSION: In the present study, the Group-B rabbits treated with 5mg NaF/kg bw/day showed no abnormalities in the gross features of the body as well as in the cytoarchitecture of the kidneys. The histology of the glomeruli, convoluted tubules, collecting ducts and tubules and the interstitial tissue were the same as those of control animals.

Moreover, the malphigian corpuscles did not show any exudation of plasma into subcellular spaces. No significant clinical signs of toxicity were also found in young albino rabbits who were injected with 5mg NaF/kg bw/day for 15 weeks.[14] Excessive intake of fluoride in the dose of 30mg NaF/kg body weight/day for 16 weeks, affected both the gross features of the body and the histology of the kidneys, as were observed in Group-C rabbits.

The urinary elimination is determined for two consecutive generations with an ion selective electrode for fluoride. The method is sensitive for F-, but more significant data are obtained when also the plasma studies areinvolved.[15] The idea of studying the administration of fluoride is that the excess quantities of dietary fluoride can be harmful.[16]

Some researchers found insignificant differences of urinary elimination of fluoride in children between the non-fluoridated areas and water fluoridated areas.[17] Other researchers found that the higher fluoride levels in the urine of participants may be associated to higher fluoride in drinking water.[18] It has been shown that in humans, even the toothpastes could be involved in the increase of the urinary elimination of fluoride.[19]

CONCLUSION: In the histological study, the early changes in the kidneys of rabbits were minimal dilatation of convoluted and collecting tubules, hemorrhages inside the glomeruli, interstitial cellular

crowding, cloudy swellings, hypertrophy and/or atrophy of glomeruli and increase in Bowman's space. The intensity of cloudy swellings was observed to increase with increased duration of exposure to fluoride. There were extensive cellular necroses leading to degeneration of convoluted and collecting tubules.

The remaining cells showed vacuolization of cytoplasm owing to which the cell nuclei were pushed to the basement membranes. Tubular lumens were widened, nuclei showed disintegration and there were serious exudations or blood in the tubules. Increased cellularity with proliferation of interstitial connective tissue was also observed. Vascular degenerations resulting in dilatation and congestion of blood vessels were marked either in cortex or in medulla or inside the glomeruli as hyperemia or hemorrhages. Interstitial nephritis, interstitial edema and necrosis of cells in the interstitium were most pronounced towards the later part of the experiment.

In renal fluoride studies, there were marked discrepancies in the histological findings of the kidneys which could be attributed in part to differences in the fluoride compounds used, the methods of their administration and the various species of the animals used. The histological changes, in the present study, were common to all the rabbits exposed to high dose of sodium fluoride. Thus, it was concluded that sodium fluoride in high dose has a definite adverse effect on the renal parenchyma.

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Sl. No.	Time of sacrifice	Status of Renal corpuscles	Lumens of convoluted tubules (PCT & DCT)	Lumens of collecting tubules and ducts	Interstitium	Blood vessels	Other changes
1	12 weeks	Edematous hypertrophic and atrophic glomeruli associated with hyperemia. Hyaline degeneration was seen in some atrophied glomeruli.	Markedly dilated with cloudy swellings, vacuolization and cell necrosis.	Markedly dilated with nuclear disintegration, exudation and inflammation.	Focal infiltrations of intertubular mononuclear cells in the medullary region.	Highly dilated and congested with extravasation of RBC.	Inflammatory changes mostly marked in the medullary interstitium.

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2	14 weeks	Atrophied & lobulated glomeruli with increase of urinary spaces to a greater extent.	Widened convoluted tubules with cloudy swellings and dropping out of lining epithelia.	Edematous and prominently dilated tubules with cloudy swellings and dropping out of lining epithelia containing secretions or blood inside.	Focal and diffuse infiltrations of round cells in the medullary interstitium.	Dilated and congested.	Inflammatory changes mostly marked in the medullary region.
3	16 weeks	Edematous, atrophied, highly lobulated and hyperemic glomeruli with slight thickening of Bowman's capsule along with inflammatory cell infiltrations into the glomeruli.	Prominently dilated & edematous convoluted tubules with cloudy swellings and sloughing out of lining epithelia with inflammatory cell infiltrations.	Very largely dilated collecting tubules with granular degeneration and sloughing out of lining epithelia with intertubular inflammatory cell infiltrations & blood/secretions in some lumens.	Round cell infiltration with interstitial cell necrosis and edema.	Marked vascular congestion in both cortex and medulla.	Inflammatory changes were equally marked in both the cortical and medullary regions.

Table 1: Histopathological findings of kidney of Group C animal after treating with sodium fluoride solution

Fig. 1a-f: Different stage of observation after fluoride treatments

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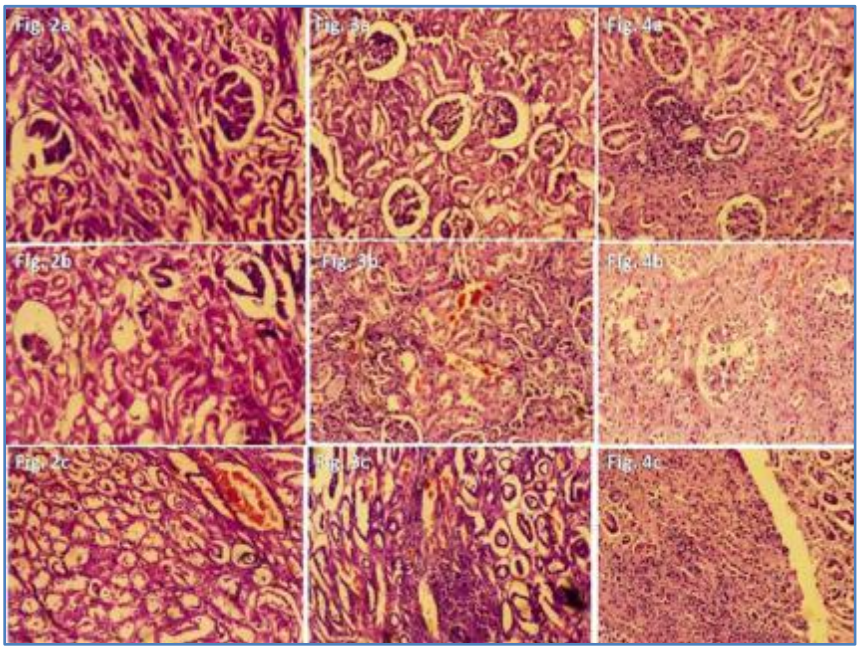

Fig. 2: Histophathological sections of the albino rabbit's kidney

AUTHORS:

1. Santosh K. Sahu
2. Sitansu K. Panda
3. Sujita Pradhan
4. Jami Sagar Prusti
5. Dharma N. Mishra
6. Geetanjali Arora
7. Mahesh C. Sahu
8. Prafulla K. Chinara

PARTICULARS OF CONTRIBUTORS:

1. Assistant Professor, Department of Anatomy, SCB Medical College, Cuttack, India.
2. Associated Professor, Department of Anatomy, IMS and SUM Hospital, Siksha 'O' Anusandhan University, Kalinga Nagar, Bhubaneswar, India.
3. Tutor, Department of Anatomy, IMS and SUM Hospital, Siksha O Anusandhan University, Kalinga Nagar, Bhubaneswar, India.
4. Associate Professor, Department of Anatomy, MKCG Medical College, Berhampur, India.
5. Assistant Professor, Department of Anatomy, VSS Medical College, Burla. India.
6. Associate Professor, Department of Anatomy, Hitech Medical College, Bhubaneswar, India.
7. Research Associate, Central Research Laboraotory, IMS and SUM Hospital, Siksha 'O' Anusandhan University, K8, Kalinga Nagar, Bhubaneswar-751003, India.
8. Professor, Department of Anatomy, IMS and SUM Hospital, Siksha 'O' Anusandhan University, Kalinga Nagar, Bhubaneswar, India.

NAME ADDRESS EMAIL ID OF THE CORRESPONDING AUTHOR:

Sitansu K. Panda,
Department of Anatomy,
IMS and SUM Hospital,
Siksha O Anusandhan University,
K8, Kalinga Nagar.
Email: sitansupanda2011@gmail.com

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