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Effect Of Vitamin D_3 Against Sodium Fluoride Induced Nephrotoxicity In Male Wister Albino Rats

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ABSTRACT:

Background: In the present study, the protective effect of vitamin D_3 against sodium fluoride induced nephrotoxicity was evaluated in rats. A vital role in kidney protection and ensuring the longevity of healthy kidneys is played by vitamin D3. The risk of autoimmune disorders, internal diseases, cancer, and infections is increased when vitamin D_3 deficiency is present.

Objective: a consequence of prolonged exposure to numerous pharmaceutical products, including industrial material, fluoride-containing herbicides, medications, and fire extinguishers. Concerns concerning fluoride toxicity have grown recently. As a result, scientists are constantly hunting for medications that could reduce toxicity in different human organs. This study will evaluate the nephroprotective effects of vitamin D_3 against renal damage caused by sodium fluoride.

Methods: In this study, 30 rats were divided into 5 groups of 6 rats each. Rats were fed water containing 100 ppm of sodium fluoride, and vitamin D3 was administered orally for 30 days in three different doses: 250 IU per kg, 500 IU per kg, and 1000 IU per kg. The Nephrotoxicity indicators were assessed**.**

Results: Sodium fluoride considerably decreased total protein while dramatically increasing urea, uric acid, and creatinine. It also helps people lose weight. On the other hand, in the test group, vitamin D_3 significantly decreased levels of urea, uric acid, and creatinine while also raising levels of total protein. Furthermore, it enhanced the rats' body weight in test groups.

Results: The test animals that drank 100 parts per million water for 30 days had high levels of urea, uric acid, and creatinine in their blood, but lower levels of total protein. The test group received vitamin D_3 doses of 250, 500, and 1000 IU/kg, leading to weight gain, decreased urea, uric acid, and creatinine levels, and increased levels of total protein. The study group that received 1000 IU/kg performed well. Vitamin D_3 considerably reduced the nephrotoxicity brought on by sodium fluoride in a dose-dependent manner.

Key words: Vit D_3 , NAF, Nephrotoxicity, urea, uric acid.

1. INTRODUCTION

The kidneys, a pair of organs that are positioned in the retroperitoneal area and have the medulla as their innermost layer, serve a crucial role as an excretory organ. Urine is a metabolic waste product made up of nitrogenous chemicals like urea and uric acid that is produced by the kidney. Each weighs approximately 135 g for adult females and 150 g for adult males. The arteries, veins, lymphatics, and ureters are positioned inside the renal hilum, which is centrally situated. A thin fibrous capsule that connects to the hilum surrounds the kidney [1]. Each kidney contains 1,000,000 to 1,250,000 nephrons and filters all 5 literss of water in your blood every 45 minutes. The main routes of fluoride removal are: via the kidney. 50% - 80% of the absorption is fluorine is excreted via the kidney; the kidney is sensitive to fluoride. [2] The kidneys are critical for overall health, and maintaining a stable internal environment depends on the kidneys' healthy functioning. Without healthy kidneys, the body would build up waste products and excess chemicals, which could result in a number of health issues and even life-threatening disorders. These processes can be hampered by kidney illnesses, which can result in conditions including kidney stones, UTIs, and chronic renal disease. It is defined as the rapid degeneration of kidney function due to the noxious effects of medications and chemicals [3]. Kidney problems happen when our body exhibits drugs or any toxic and hazardous chemicals that cause damage to the kidneys. Several therapeutic agents can cause toxic effects on the kidneys and lead to renal failure [4]. Nephrotoxins are the substances that cause nephrotoxicity. Nearly 20% of nephrotoxicity is induced by drugs. There are different types of nephrotoxicity, such as Fluoride induced nephrotoxicity, aminoglycoside-nephrotoxicity, amphotericin-induced nephrotoxicity, crystal-forming drug nephrotoxicity, and drug-induced nephrotoxicity. Fluoride is present in every environment**.** The population is now exposed to considerable amounts of fluoride through non-food and fluoridated water sources. [5]

The mostly people are exposed to fluoride via drinking water with this mineral's inorganic form, which often comes from geological sources and may reach quantities of 30 to 50 mg/l. [6]**.** Fluorosis can develop as a result of excessive fluoride ingestion. One of the pathophysiology of chronic sodium fluoride (NaF) toxicity's most significant impacts is more is being produced (ROS). On several biochemical parameters, fluoride is known to have harmful effects. It is suggested that the harmful effects of fluorine on soft tissues can be reduced by increasing levels of lipid peroxidation (LPO) and free radicle production [6] Fluoride exposure above 1.5 mg/L may result in major health issues.

Excessive concentrations of sodium fluoride can have dangerous effects. It significantly increases urea, uric acid and creatinine, and inhibits the activation of antioxidant enzymes such as superoxide dismutase and glutathione peroxidase, which cause oxidative stress. Sodium fluoride prevents protein synthesis and calcium imbalance prevents the formation of free radicals. This leads to oxidative stress, which affects proteins, lipids, and DNA in kidney cells, causing structural and functional abnormalities in the liver, and leading to metabolic, proliferative, and inflammatory diseases [7]. Vitamin D is a fat-soluble secosterols includes vitamin D, commonly known as calciferol. Vitamin D2 and vitamin D3 are the two primary types. Vitamin D_3 is also obtained through the diet from foods. The activation steps for dietary vitamin D conversion and cutaneous synthesis are shown below. Until two enzymes hydroxylate vitamin D, it is regarded as

physiologically inactive in either the D2 or D3 form. The first phase, which results in 25-hydroxyvitamin D (also known as 25OHD), is carried out in the liver and is mediated by a 25-hydroxylase (perhaps a cytochrome P450 2R1 [CYP2R1]). The second reaction transforms 25OHD into the physiologically active hormone calcitriol (1, 25-dihydroxyvitamin D) in the kidney and is mediated by 1-hydroxylase (CYP27B1). Although the 1-hydroxylase gene is expressed in various extra-renal organs, it is unknown if these tissues conduce to the synthesis of calcitriol. The main form of vitamin D found in circulation is 25OHD that is a precursor to calcitriol D Vitamin metabolism plays an essential role in chronic renal disease. kidney disease, caused due to the gradual decrease of vitamin D As evidenced by lower 25-hydroxyvitamin D levels, recent data imply that renal illness is linked to a high incidence of vitamin D deficiency or insufficiency.[14] Transporters and channels controlled by 1,25-dihydroxyvitamin (1,25(OH) 2D) and parathyroid hormone (PTH) enable glomerular filtration and reabsorption of calcium and inorganic phosphorus from the tubular segment. [8]

2. Materials and Methods

2.1Materials

2.1.1 Chemicals

Vitamin D_3 (Pulse Pharmaceutical Pvt Ltd) and sodium fluoride (SD fine powder), kits were purchased from Excel Diagnostic Pvt Ltd

2.1.2 Experimental animals

Animals are obtained from Jeeva Life Sciences, Uppal, Hyderabad, and Telangana, and are approved by IAEC. The Wister rats utilized in this investigation were adult healthy rats weighing 150–250 gms. In Hyderabad and Telangana state, India's Sai Tirumala Enterprises Pvt Ltd, was where the animals were purchased. Paddy husk was used as bedding in polypropylene cages where the animals were randomly assigned to the treatment group. The temperature of the animal's enclosure was 24 to 26 C, and a 12:12 light-to-dark cycle was employed to keep the animals alive. Throughout the test, they had a free supply of water and regular commercial pellet rat chaw. The Institutional Animal Ethics Committee (IAEC) examined all the experimental techniques and protocols utilized in this work and determined that they all adhered to the IAEC's rules

2.1.3 Preparation of 100ppm fluoride water

100 ppm fluoride was prepared by dissolving 0.22 g of sodium fluoride in 500 ml of distilled water.

2.1.4 Treatment Protocol

The Wister male rats were divided into 5 groups each group consists of 6 rats.

Group-1 severs as normal control animals were given distilled water for 30 days.

Group-2 severs as toxic control animals were given 100ppm sodium fluoride through drinking water.

Group 3 serves as treatment control 250 IU/kg b. wt. (Low Dose).

Group 4 serves as treatment control at 500 IU/kg b. wt. (Medium Dose).

Group-5 serves as treatment control 1000 IU/kg b. wt. (High Dose).

2.1.5 Measurement of biochemical parameters:

The activities of urea, uric acid, and creatinine were determined using commercial kits from Excel diagnostic Pvt Ltd, and Total protein concentration were also measured using commercial kits from TRANSASIA BIO-MEDICAL Ltd. The procedure given in the kits were followed.

2.1.6 Estimation of Complete Blood Profile (CBP):

The blood samples are collected from the experimental animals to determine the complete blood profile with the help of cell analyzer (AD-3200)

2.1.7 Statistical Analysis:

The values will be presented as Mean ± SEM with n=6 in each group. One-way ANOVA has been used for statistical analysis. At *p<0.005, **p< 0.01 and ***p<0.001 the values will be significant and Vs Toxic control.

3.0 Results and Discussion

3.1 Effect of Vitamin D3 treatment on Changes in Body Weight

The body weight of each group animal was recorded. The obtained results were specified as mean ±Se. Sodium fluoride control group animals were treated with 100ppm of sodium fluoride, which showed a drop in body weight compared to the normal group. Treatment of vitamins at a dose of 500IU and 1000IU/kg body weight showed increased body weight in comparison to a NaF control group. A remarkable rise in the body Wight was observed in the vitamin D3 control group in contrast to the normal group

Table 1: Effect of vitamin D_3 treatment on changes in body weight

Name of the group	**1st Day**	**15th Day**	**30th Day**
Normal Control	205±4.17	219±2.11***	236±5.43***
Toxic Control (100ppm of NaF)	175.833±4.5	168.3333±4.5***	160±3.651484***
Low Dose (250IU/kg of Vitamin D_3	210±10.6	231.6667±7.9***	253.333±7.49***
Medium Dose 500IU/kg of Vitamin D_3	181.6667±10.1	215±9.5***	254.1667±10.3***
High Dose 1000IU/kg of Vitamin D_3	165±4.2	205±7.63***	260±4.9***

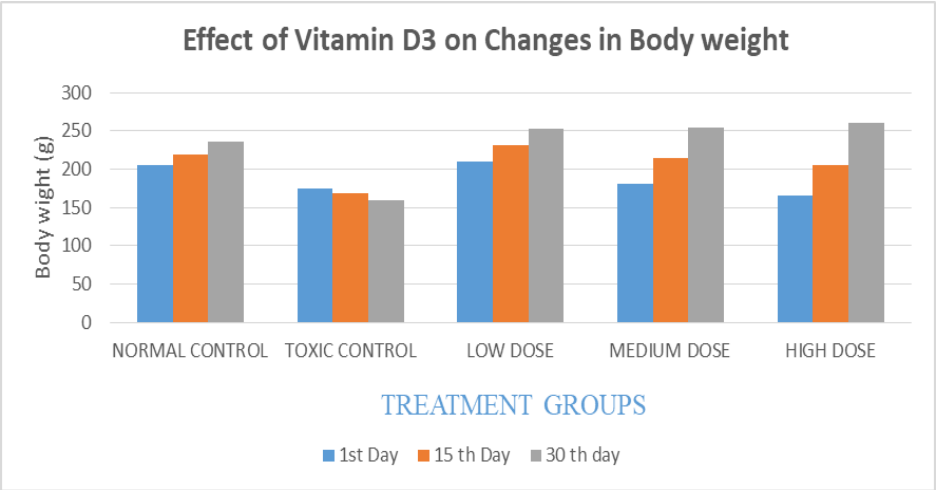

Figure 1: Effect of vitamin D3 changes in body weight

3.2 Estimation of biochemical parameters

The impact of vitamin **D_3** on blood serum levels of urea, uric acid, and creatinine was calculated and shown as Mean± SEM. When contrast to the normal group, urea, uric acid, and creatinine were suggestively rise in the toxic group. Total protein levels were declining. Following vitamin **D_3** therapy, urea, uric acid, creatinine, and total protein were significantly decreased.

Table 2: Effect of vitamin D_3 therapy on NaF induced sodium fluoride

S.NO	Name of the group	Urea Mg/dl	Uric acid Mg/dl	Creatinine mg/dl	Total protein Mg/dl
1	Normal	9±4.1	1.25±0.19	0.68±0.22	8.2±0.15
2	Toxic control	29±0.2	1.8±0.07	2.4±0.12	5.08±0.16
3	Vitamin D_3 250IU Low Dose	26.7±1.51***	1.66±0.49***	1.98±0.704***	6.5±0.94***
4	Vitamin D_3 500IU Medium Dose	12.5±1.60***	1.58±0.52***	0.58±0.459***	7.76±1.20***
5	Vitamin D_3 1000IU High Dose	8.26±1.10***	1.26±0.404***	0.66±0.46***	8.01±0.95***

Table 3: Vitamin D_3 effect on CBP

S. No	Complete blood profile	Normal control	Toxic control	Test Group-1	Test Group-2	Test Group-3
1	WBC	7.55±0.035	11.098±0.30	9.863±0.1258***	8.618±0.489***	7.9466±0.615***
2	Lymphocytes	5.36±0.091	7.296±0.234	6.831±0.082***	6.38±0.4210***	5.811±0.155***
3	Granulocytes	1.18±0.07	2.4083±0.180	2.161±0.0086***	1.71±0.2179***	1.416±0.0984***
4	RBC	6.59±0.049	4.535±0.1092	5.163±0.0140***	5.956±0.406***	6.341±0.098***
5	Hgb	13.65±0.106	10.7883±0.194	11.416±0145***	12.29±0.584***	13.066±0.177***
6	HCT	43.4±0.282	38.05±0.228	40.08±0.349***	41.148±1.069***	42.2±0.196***
7	MCV	67±0.707	55.13±0.288	57.806±0.220	60.172±1.29***	62.589±0.260***
8	MCH	23.7±0.212	18.011±0.179	19.0266±0.144***	20.085±0.765***	22.528±0.152***
	MCHC	33.85±0.813	29.356±0.185	30.83±0.190***	31.11±0.929***	32.141±0.129***
	Platelets	544±0.707	365.6±1.455	427.3±0.819***	490.5±3.691***	321±1.320***

DISCUSSION

Numerous studies have shown that fluoride can raise MDA levels and levels of LPO in the blood and tissues of experimental animals, suggesting that fluoride increases oxidative stress in rats. Additionally, a number of studies revealed that foods high in antioxidants, including blackberry juice, and antioxidants, like methionine, vitamins, N-acetyl-cysteine, and polyphenolic flavonoids could be used to treat fluorosis. In numerous experimental animal models, the connection between level of oxidative stress and fluoride-induced nephrotoxicity has been extensively established. action with fewer side effects and readily available in nature, herbal medication treatments have gained popularity over the past 10 years According to the current study, administering sodium fluoride to rats for 30 days caused a considerable rise in blood levels of urea, uric acid, and creatinine, which indicated that the rats had been exposed to induced nephrotoxicity contrast to normal controls. Therefore, urea, uric acid, and creatinine levels in the treatment control groups as contrast to the toxic control group have significantly changed after receiving vitamin D_3 treatment for 30 days. The delivery of sodium fluoride in the current investigation may have impaired glomerular function as seen by an increase in blood levels of urea, uric acid, and creatinine. These increased serum levels show decreased clearance and filtration rates, which impair the kidney's ability to get rid of the poisonous metabolic material. The kidneys

are crucial for removing fluoride from the body. It illustrates how fluoride retention has negative consequences and compromises kidney function. Additionally, the NaF-treated group displayed a marked increase in lipid peroxidation due to an increase in ROS, and free radical fluoride ion decreased the activity of reduced glutathione and catalase, indicating the low level of oxidative stress and significant damage to biological membrane structure and cell functions. Treatment with 1000 IU/kg body weight of vitamin D_3 administered daily for 30 days reduced lipid peroxidation and increased reduced glutathione and catalase activities by producing dose dependent effects on the oxidative stress parameters of lipid peroxidation, reduced glutathione.

SUMMARY AND CONCLUSION

Multifarious animal diseases and toxicity are often associated with oxidative stress in vital organs such as kidney. This pathophysiological emphasis multiple effects but is specially characterized by reduction in enzymatic activity such as catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx) and glutathione reductase (GR). In many diseases increased in generation of reactive oxygen species (ROS) implicates in the pathogenesis and also in the toxicity of a wide range of compounds including fluoride. Fluoride exerts different effects on number of cellular functions and physiology systems, including inhibition of various enzymes. High exposure to fluoride increases oxidative stress and reactive oxygen species so free radicals are generated which shows effects on soft tissues on various organs. Damage to kidney cells results in nephrotoxicity. It impairs the immune system and damages all the parts of the body. Fluoride toxicity is becoming a matter of grave concern as many countries have been declared endemic for fluoride. The experimental animals were treated with sodium fluoride (100ppm) drinking water for one month. This study showed that there was a significant increase in serum urea, uric acid and creatinine levels in the serum. In in vivo antioxidant parameters showed an increase in the kidney lipid peroxidation levels, and decreased in reduced glutathione and catalase levels. The treatment groups that were treated with 500 IU/Kg and 1000 IU/kg dose of the Vitamin D_3 have shown good Results by increase in body weights, decrease in serum urea, uric acid and creatinine and increase level of reduced glutathione and catalase levels.

Results have revealed that the ingestion of fluoride significantly showed decrease in the growth of the animal and production performances due to decreased consumption of feed and decrease apatite. The possible mechanism behind was the fluoride induced nephrotoxicity by increased production of free radicals and decreased levels of endogenous antioxidants (enzymatic and non-enzymatic). Treatment with the vitamin D_3 sodium fluoride induced nephrotoxicity in a dose dependent manner. Thus, we concluded that the present results strongly support for further drug development for the treatment toxicity.

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