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**Original article**

# Effects of Vitamin D3 against Sodium Fluoride Induced Hepatotoxicity in Male Wistar Albino Rats

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**ABSTRACT:**

It has been demonstrated that vitamin D3 plays a critical function protecting hepatocytes and guaranteeing the life of healthy liver. Lack of vitamin D3 has been linked to an elevated risk of autoimmune disorder, liver diseases, cancer and infections, a consequence of prolonged exposure to numerous man-made sources, including industrial settings, fluoride-containing herbicides, medications, and fire extinguishers. Recently worries about fluoride toxicity have increased. As a result, researchers are always looking for drugs that might lessen toxicity in various human organs. In this study, the hepatoprotective effects of vitamin D3 against liver damage caused by sodium fluoride will be evaluated. In this experiment, 30 rats were allocated into 5 groups, each with 6 rats. Rats were exposed to 100ppm of NaF through drinking water and vitamin D3 is given in three different doses i.e., 250 IU per kg, 500 IU per kg, 1000 IU per kg orally for 30 days. The indicators of hepatotoxicity were evaluated. AST, ALP and ALT were all significantly increased by sodium fluoride whereas albumin and total protein were significantly decreased. Additionally, it reduces body weight. On the other hand, the test group's vitamin D3 led to a considerable reduction in ALP, AST and ALT levels as well as an increase in albumin and total protein levels. Additionally, it increased the body weight of rats in test groups. The experimental animals which were given 100 ppm water to drink for 30 days had high blood concentration of AST, ALP and ALT but lower levels of total protein and albumin. Vitamin D3 doses of 250, 500 and 1000 IU/kg in the test group caused weight growth, lowered AST, ALT and ALP levels and increased total protein and albumin levels. The test group receiving 1000 IU/kg did well. In a dose-dependent way vitamin $D_3$ significantly mitigated the hepatotoxicity caused due to sodium fluoride.

**Keywords:** vitamin $D_3$, hepatotoxicity, sodium fluoride, albumin and total protein.

## 1. INTRODUCTION

The liver is a multifunctional biological organ that ranges in weight from 1 to 2.3. It is principally involved in the cellular breakdown of lipids and carbohydrates [1]. It plays an important role in maintaining the body's metabolic equilibrium. It comprises the conversion of ingested amino acids, carbohydrates, vitamins and lipids, the synthesis of serum proteins, the purification of endogenous waste product, and the excretion of xenobiotics via bile. Except in severe hepatic condition, it regenerates and has a large functional reserve. When 60% of the liver is surgically removed, it produces little hepatic damage and recovers within 4 to 6 weeks. The clinical effects of liver damage, on the other hand, dictate the liver's reserve and regeneration capabilities. Because other organs rely on the metabolic processes of the liver, hepatic inactivity has long-term consequences. The liver is an exceptionally active organ. It manages most of the blood's chemical level and excretes bile. Bile assists in the removal of waste from the liver. The liver breaks downs regulate and produce nutrients in the blood. It also metabolises medication, breaking them down into simpler form that are easier for the body to absorb. More than 500 biological functions have been connected to the liver. It also control's blood clotting.

Hepatotoxicity is described as liver damage or inflammation. The level of ALT in blood shows hepatocyte injury and is a very sensitive and specific clinical and preclinical indicator of hepatotoxicity [2]. Hepatotoxicity is caused by hepatotoxins. Hepatotoxins, which can be found in dietary additives, chemicals compounds, herbal product and pharmaceuticals, can cause hepatotoxicity or liver damage [3]. Fluoride is fluoride is fund in all environments. It is a

major toxin generated both artificially and naturally. Fluoride prolonged exposure affects individuals as well as livestock on a daily basis, whether directly or indirectly [4]. Fluoride exposure at in excess of 1.5 mg/L may cause serious health problems. Excess fluoride intake has been shown in studies to cause cellular death via an oxidative injury- dependent path, which increases lipid peroxidation levels on cells, which results in mitochondrial malfunction and activation of downstream pathways [5, 6, 7]. When sodium fluoride is administered in excessive concentration, it has dangerous repercussions. It greatly raises blood transaminases as ALT, AST and ALP while supressing the activation of antioxidants enzymes such as superoxide dismutase and glutathione peroxidase which produces oxidative stress. Sodium fluoride prevents protein synthesis and calcium ion imbalance both of which have an effect on the generation of free radical. This lead in oxidative stress, which affects the proteins, lipids and DNA in hepatocytic cells, causing structural and functional abnormalities in the liver that cause metabolic, proliferative and inflammatory disease [8]. Vitamin D3 has a pleiotropic effect on human health and a number of chronic diseases. It is a secosteroid hormone that is soluble in fat [9].

The biological forms of vitamin D are called ergocalciferol and cholecalciferol, respectively [10]. Bone deformities have been linked to vitamin D deficiency. Vitamin D insufficiency is indicated by blood levels of 1, 25-dihydroxycholecalciferol that are lower than 25n mol/L [11]. The pro hormone vitamin D is also physiologically inactive. Calcitriol, which is the active form, carries out physiological functions [12]. Numerous biological processes linked to vitamin D3 activity are triggered by the interaction of vitamin D3 with receptor. The conventional role of vitamin D and its receptor is to adjust renal calcium reabsorption and duodenal calcium absorption in order to control calcium absorption from stomach and maintain a healthy balance of bone and calcium. Indirectly, vitamin D3 works by maintains normal blood calcium levels. It's been proven to have a big effect on getting the liver back to working normally. A vitamin D-rich diet lowers fibrogenesis and collagen buildup which lessens serious liver damage [13]. According to a different study, those with hepatic steatosis and low vitamin D levels significantly improved after getting vitamin D replacement therapy. It is capable of reducing inflammation via a number of method. By interacting with the peroxisome proliferator activated receptor and the VDR in hepatocytes, Vitamin S can reduce oxidative stress [14].

## 2. MATERIALS AND METHODS

**Chemicals:**

Sodium fluoride (SD Fine-Chem), Vitamin D3 (Pulse Pharmaceutical Pvt Ltd), Biochemical kits were purchased AEKRAY Healthcare Pvt Ltd and TRANSASIA BIO-MEDICAL Ltd

**Experimental Animals:**

Adult male Wistar albino rats weighing between 150 to 225gm were utilised in this investigation. The animals were brought from Vab bioscience at Bapuji Nagar, Musheerabad in Hyderabad, Telangana state. The animals were kept in polypropylene cages with rice husk as bedding. Animals were housed in 12:12 light: dark cycle with an ambient temperature of 23±2oC and humidity of 50±5%. The rats were fed a standard rat pellet diet and had unrestricted access to water. The institutional animal ethics committee of CMR collage of pharmacy kandlakoya village, Medchal Road, Hyderabad, Telangana reviewed all the experimental procedures and protocol used in this study.

**Preparation of 100ppm fluoride water**

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

**Treatment Protocol**

The Wistar albino 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 500 IU/kg b. wt (Medium Dose).

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

**Measurement of biochemical parameters:**

Different biochemical parameters like SGOT, SGPT and ALP were determined using commercial kits from AEKRAY Healthcare Pvt Ltd and Total protein concentration was measured using commercial kits TRANSASIA BIO-MEDICAL Ltd. The procedure given in the kits were followed.

**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)

**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. RESULTS AND DISCUSSION

**Result:**

Effects of Vitamin D3 treatment on change in body weight:

Each group of animal's body weight was measured for every 15 days. In Table 1, the obtained findings were specified as Mean ± SEM. Toxic control animals were treated with 100 ppm sodium fluoride showed a reduction compared to the normal control group. The Low dose group animals were treated with 250 IU/kg showed raise in the body weight.

Medium dose group animals were treated with 500 IU/kg showed increased body weight more than low dose. A significant increase in body weight is seen in the high dose group where the experimental animals were treated with 1000 IU/kg

**Table 1: Effects of Vitamin D3 treatment on change in body weight (g)**

| 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) | 175.8333±4.5 | 168.3333±0.55*** | 160±0.498*** |
| Test Group -1 Low dose (250 IU/ kg of Vitamin D3) | 210±10.6 | 231.6667±0.734*** | 253.333±0.713*** |
| Test Group-2 Medium dose (500 IU/ kg of Vitamin D3) | 181.6667±10.1 | 215±0.8077*** | 254.1667±0.83*** |
| Test Group-3 High Dose (1000 IU/kg of Vitamin D3) | 165±4.2 | 205±0.720*** | 260±0.580*** |

## Estimation of biochemical parameters:

Effect of vitamin D3 on ALP, AST, ALT, Albumin and Total protein were estimated and presented as MEAN±SEM. When compared to normal route there was a substantial rise in AST, ALP, ALT and decrease in albumin and total protein levels (Figure 2, 3 and 4). After treatment with vitamin D3 the levels of AST, ALP and ALT were decreased and albumin and total protein levels were increased (Figure 5, 6, Table 2).

**Table 2: Vitamin D3's effect on Biochemical parameters of liver**

| S. No | Name of the group | AST | ALT | ALP | Albumin | Total Protein |
|---|---|---|---|---|---|---|
| 1 | Normal Control | 85.71±0.57 | 85.53±0.73 | 46.33±0.49 | 5.37±0.07 | 6.528±0.13 |
| 2 | Toxic Control (100ppm of NaF) | 241.66±1.18 | 286.37±1.18 | 210.83±1.26 | 3.03±0.094 | 4.7±0.157 |
| 3 | Test Group -1 Low dose (250 IU/ kg of Vitamin D3) | 225.26±1.341*** | 252.66±0.68*** | 201.5±0.84*** | 3.5±0.225*** | 4.95±0.15*** |
| 4 | Test Group-2 Medium dose (500 IU/ kg of Vitamin D3) | 217.76±1.155*** | 201.01±0.953*** | 171.5±0.733*** | 4.13±0.14*** | 5.11±0.154*** |
| 5 | Test Group-3 High Dose (1000 IU/kg of Vitamin D3) | 155.80±0.628*** | 161.83±0.87*** | 154.16±0.651*** | 5.23±0.079*** | 6.31±0.111*** |

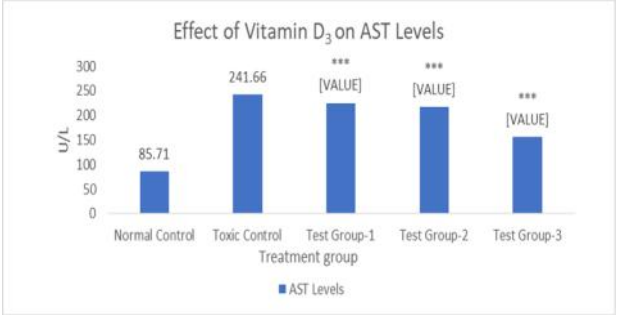


**Fig 2: Effects of Vitamin D3 on Biochemical parameters (AST)**

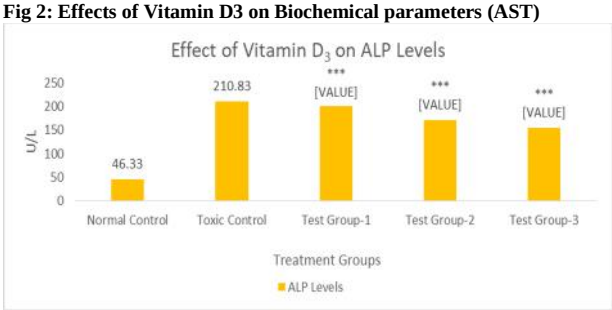


**Fig 3: Effect of Vitamin D3 on ALP**

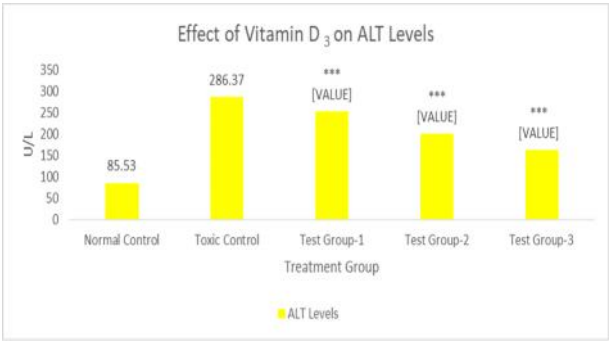


**Fig 4: Effect of Vitamin D3 on ALT**

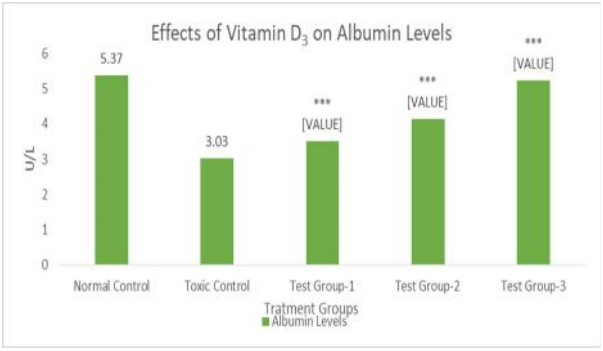


**Fig 5: Effect of Vitamin D3 on Albumin levels**

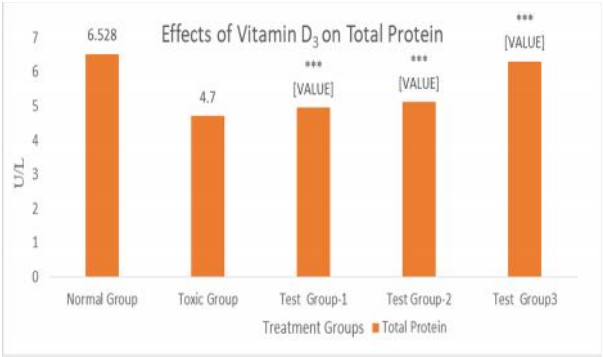


**Fig 6: Effects of Vitamin D3 on Total protein levels**

**Table 3: Complete Blood Profile of experimental animals**

| S. NO | Complete blood profile | Normal Control | Toxic Control | Test Group -1 | Test Group-2 | Test Group-3 |
|---|---|---|---|---|---|---|
| 1 | WBC | 7.5 5 ± 0.035 | 11.098 ± 0.30 | 9.863 ± 0.125 *** | 8.618 ± 0.489*** | 7.9466 ± 0.1615*** |
| 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 ± 0.086*** | 1.71 ± 0.2179*** | 1.416 ± 0.0984*** |
| 4 | RBC | 6.59 ± 0.049 | 4.535 ± 0.1092 | 5.163 ± 0.140*** | 5.956 ± 0.406*** | 6.341 ± 0.098*** |
| 5 | Hgb | 13.65 ± 0.106 | 10.7883 ± 0.194 | 11.416 ± 0.145*** | 12.29 ± 0.584*** | 13.066 ± 0.077*** |
| 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*** | 21.085 ± 0.765*** | 22.528 ± 0.152*** |
| 9 | MCHC | 33. 85 ± 0.813 | 29.356 ± 0.185 | 30.83 ± 0.190*** | 31.11 ± 0.929*** | 32.141 ± 0.129*** |
| 10 | Platelets | 544 ± 0.707 | 365.6 ± 1.455 | 427.3 ± 0.819*** | 490.5 ± 3.691*** | 521 ± 1.320*** |

In the above table-3, normal control is referred as NC. Toxic control is referred as TC and test group is referred as TG.

The toxic group has showed decrease in RBC, haemoglobin level and platelet count and decrease in WBC, lymphocytes and granulocytes due to exposure to NaF. Whereas, the groups which treated with vitamin D3 significant raise in the RBC, hemoglobin level and platelet count and reduced WBC, lymphocytes and granulocytes.

**Discussion:**

The most prevalent negatively charged halogen element is fluoride and occurs in nature as fluoride ion. D. Fluoride is available in nature in conjugation with other elements these chemicals are mineral components present in rock and soil which are the natural fluoride doners [15]. Fluorspar, commonly known as fluorite is the most prevalent fluoride containing minerals and soil often included calcium fluoride. Although increasingly unusually NaF and Na2 [SiF6] were used as insecticide and antiparasitic drugs. It is substantially more physiologically accessible than fluoride derivatives from feed or ambient sources and is easily absorbed from the stomach. In all regions of the body the fluoride is distributed, with kidney excreting around 50% of the fluoride that was absorbed. Fluoride levels in the blood, urine and soft tissues can suggest freshly ingested fluoride but the can gradually rise over time with on-going fluoride exposure.

Fluoride has the power to alter intracellular redox equilibrium and to generate oxidative stress. Fluoride levels in protein carbonyl and lipid peroxidation. It is believed that fluoride inhibits the activity of antioxidant enzymes. Increased ROS production at the mitochondrial level is brought on by glutathione depletion, harming cellular components. The liver and kidney experienced histological and functional changes as a result of increased fluoride exposure. Fluoride has been associated with oxidative stress due to its ability to increase free radical production and impair the activity of antioxidant enzymes. Fluoride causes neutrophils to produce free radicals and have respiratory outbursts. Enzymes, particularly those necessary for mitochondrial respiration, are inhibited by it. When cellular respiration is interrupted the concentration of ATP decreases, which causes the generation of ROS and Hydrogen peroxide. Fluoride ingestion has been liked to oxidative damage in RBC and liver. As a result, oxidative stress and injury/ damage caused by free radical may play a key role in inducing the fluoride toxicity [15]. The liver is in charge of maintaining the body's metabolic equilibrium and is vulnerable to fluoride toxicity. It disrupts the equilibrium of pro-oxidants and oxidants in the liver resulting in functional, biochemical and morphological problems. Fluoride in excess has an effect on hepatic proteins involved in energy metabolism, as well as altering mitochondrial process. Fluoride has been linked to morphological alterations in hepatic tissue such as necrosis, hyperplasia, fatty changes, dilated central veins and vacuolization [16]. AST and ALT catalyse aminotransferase reaction and are thought to be indictors for medical diagnosis damage indicator is ALP, an enzyme that hydrolyzes protein and nucleotide to remove the phosphate group, serves as a damage marker. The marker of fluoride toxicity and pathology f bones is ALP. Fluoride- induced cell damage to osteoblast and osteocytes, which promote increased osteoblast division, matrix formation and ALP production, may be the reason for elevated ALP activity [17].

The functional form of D vitamin of Vitamin D3 serves a variety of vital biological role. Antioxidative activity is one of these biochemical properties. Vitamin D3 administered systemically reduced the increase lipid peroxidation seen in vitamin D3 deficient mice. Furthermore, vitamin D3 decreases the oxidative stress by increasing the antioxidative defensive system in astrocytes and the liver including the increasing the action of glutathione content, SOD and GPx. Diet and sun exposure are the two main sources of vitamin. As a result of skin exposure to the sun's UV radiation, vitamin D3 is produced in the skin [24]. Cholecalciferol is a type of vitamin D which is obtained from animal resources. Yolks of egg, oily fish, shitake mushroom, milk coca and coca-based products are the other foods that contain a modest amount of vitamin D that can be obtained through diet [18, 19, 20].

Vitamin D3 and its dose were chosen for this study after a thorough review of the literature on its effects on liver and antioxidant activity. In the current investigational study, fluoride (100ppm) was induced to rats through drinking water because it is the primary route of fluoride to induce chronic toxicity. Throughout the study, no significant difference in animal behaviour or appearance was observed. In both vitamin D3 and sodium fluoride induced groups, no deaths were detected during the period of 30-day research. Vitamin D3 may regulate body weight by controlling fat cell formation and influencing the expression of specific genes as well as the level of hunger related hormones such as leptin [21]. Vitamin D3 treatment groups showed significant

increase in the animal body. The body weight of the mice that were exposed to sodium fluoride, however, significantly decreased.

Previous investigational studies stated that exposure to NaF caused decrease in RBC, haemoglobin, MCV, MCHC, MCH and decrease in WBC [22]. The data acquired reported considerable reduction in RCB, Hgb, MCV, MCHC, MCH and platelets and decrease in white blood cells. In contrast to toxic group, treatment groups showed increase RCB, Hgb, MCV, MCH, MCHC and platelets. An earlier study revealed that vitamin D3 decreased white blood cells count while increasing levels of red blood cells, hematocrite, hemoglobin and platelets [23, 24]. However, further study should be carried out to determine the role of vitamin D3 on blood cells.

Previous study stated that sodium fluoride causes increased serum concentration of AST, ALP and ALT and decrease in total protein and albumin levels [25]. Data obtained in this research also demonstrated that the toxic control had higher concentration of AST, ALP and ALT and reduced albumin and total proteins level. Earlier researches showed administration of vitamin D3 decreases the level of AST, ALT and ALP and increases in total protein and albumin. Data acquired in this study also revealed that the Vitamin D3 treatment at dose of 250 IU, 500IU and 1000 IU/kg decreased the level of AST, ALP and ALT and increase the level of the albumin and total protein which confirms the hepatoprotective activity of Vitamin D3 against sodium fluoride.

## 4. CONCLUSION

The Experimental animals were given drinking water containing 100ppm for 30 days showed higher serum concentration of AST, ALP and ALT and decreased in total protein and albumin level. The treatment group that was treated with 250 IU, 500IU and 1000 IU/ kg of Vitamin D3 showed good result by increasing body weight, total protein and albumin levels increased whereas ALT, AST and ALP levels decreased. Test Group which is given 1000 IU/ kg showed good results. Results also demonstrated that exposure of fluoride substantially lowered

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