# Prolonged Impact OfVitamin-D Deficiency And 1, 25-$(OH)_2$ D3 (Vitamin-D3 Supplement) Of An Essential Endocrine Gland And Their Associated Hormones On Male Mice Mus Musculus(P).

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**Abstract**
Many people nowadays, avoid getting enough sunlight, which directly leads to less production of vitamin-D, resulting in a weak immune system and a variety of disorders. Our primary goal is to investigate the impact on the Endocrine gland which is caused by Vitamin- D (Sunshine Hormone) deficiency in male adults, particularly the declining level of the Reproductive Hormone and its various metabolic disorders. Vitamin-D deficiency is now a days becoming a worldwide issue. Glands are very important in organism which contains various secretions and properly performs the all the metabolic functions of the body. Sometimes due to insufficient intake of diet (food intake) and many others, this leads to the decline in the level of nutrients such as Carbohydrates, Proteins, fats and Vitamins. Out of these, we are here mainly concern with vitamin which is a fat soluble vitamin (FSV) i.e. Vitamin-D commonly called as sunshine vitamin. Vitamin-D plays very pivot role in the metabolism of the organism, we use the male mice for our experimental work for the duration of the 90 days. We use the various methods to check the deficiency symptom which was caused by the Sunshine Vitamin-D. Reproductive Hormones was estimated statistically and histo-morphologically. We got statistical significant results in terms of certain parameters such as body weight while the prolonged effect of Vit.-D deficiency, reproductive fitness of the organism's body through its important endocrine glands such as (lowering conc. of PTH and Testosterone level). We came to conclusion that due to the prolonged deficiency of Vitamin- D (Cholecalciferol) deficiency weight will be increased and also the level of the testosterone was also decreased which leads to the sexual disorders in males, which is now a day's becomes a major global problem issues among the males and also hampers the reproductive fitness.

**Keywords**: Vitamin-D Deficiency, Supplementation, Histological Remodeling, Reproductive Hormones, and Endocrine Glands.

## Introduction

Glands are very important in organism which contains various secretions and properly secreted and perform the important metabolic functions of the body. Sometimes due to the various conditions like insufficient intake of diet (food intake) and many others, this leads to the decline in the level of nutrients such as Carbohydrates, Proteins, fats and Vitamins. Out of these, we are here mainly concern with vitamin which is fat soluble vitamin (FSV) i.e. Vitamin-D3 commonly called as sunshine vitamin. Vitamin plays very pivot role in the metabolism of the organism, its concentration variations like sufficient, inefficient and deficient. More than its inefficiency and deficiency leads to the various disorders in an organism. Our main objective is to learn more about how Vitamin-D3 (cholealciferol) insufficiency in male adults affects the endocrine system, specifically how it affects the level of the reproductive hormone and its numerous metabolic diseases. Our primary goal is to investigate the impact on the Endocrine gland which is caused by Vitamin-D (Sunshine Hormone) deficiency in male adults, particularly the declining level of the Reproductive Hormone and its various metabolic disorders. Vitamin-D deficiency is now a days becoming a worldwide issue. Many human populationis getting suffered a lot form it below the sufficientconcentration.

## Material and methods:

### Experimental Design

For this experimental study, we take adult Swiss albino male mice Mus musculus having 25±5gms per animal kept in a research center animal house by giving proper 12 hrs Light: Dark condition and having the proper standard pellet diet and water *ab libitium* for the whole experimental duration.

### Chemical and Dose Preparation

Current study contained the chemical name Cholecalciferol 1,000 IU (each dose contains 0.025mg/Kg) which was brought from the market (Ready to use) and a dose was prepared and given orally only once per week with the help of gavage were firstly finalized after
reviewing various previous studies and their successive investigations (Mallya et al., 2016; Shahreza et al., 2020).

**Preparation of Diet**

We feed our animals the standard diet, and we supplement one group with a vitamin-D3 deficient diet (V-DD) that we bought from the National Institute of Nutrition, ICMR Hyderabad, under the terms of Reference Letter No. NIN/ HOD/AF/09/2021/214, dated September 21, 2021.

**Experimental Design**

*Mus musculus* mature male mice total 30 in number were split into three groups of 15 each. The first group (GP-1) Controlled group was retained for 90 days. They received a balanced standard diet, drink water *ab libiitum*, and proper photoperiodic intervals of 12 hours L: D (Light: Dark) for the course of 60 days, the second group (GP-2) was fed a specially developed diet called a Vitamin-D3 deficient diet (V-DD). However, the third group (GP-3) supplemented with 10 µl of Cholecalciferol (V-D3) once a week for 90 days. The following various parameters were performed after the trials, which lasted for 90 days, were completed. All the animals were sacrificed by cervical dislocation on consecutive days of 91.

**Parameters estimated: Body Weight:**

All values for the body weight of the experimental animals were expressed in grams andwere taken at the beginning of each interval, i.e., at day 0 and at91day.

The body weight gain was calculated by the formulae: Bodyweightgain=Final bodyweight–Initialbodyweight

Percentage Weightgain=weight

$$\frac{\text{Final body weight} - \text{Initial body}}{\text{Initial Body weight}} \times 100$$

**Hormonal Analysis:**

Using the Automated Micro Plate Reader for Immunology and Microbiology Lisa Scan ® EM, hormonal estimations were performed in the blood serum of control and experimental groups at the time interval, i.e. 90 days following treatment. Before the animal was euthanized by cervical dislocation, blood was drawn into a separate serum tube (SST) by an ocular puncture. After the blood was drawn, it was allowed to sit undisturbed for 15 to 30 minutes. Then, it was centrifuged at 10,000 to 20,000 g for 10 minutes in a cooled centrifuge to separate the serum, which was then aspirated out for hormonal estimations or stored at -20 to -80 C for hormonal estimations. As repeated freezing and thawing will destroy numerous serum components, it will be avoided. In the endocrinology unit, hormone estimation for T4, and Testosterone was carried out utilizing the relevant assay kits (manufactured by ELK Biotechnology, Wuhan, China) acc. to the manufacture'sprotocol.

Statistical Analysis of Data (Tukey, 1949):

The means and standard errors of the means for the various groups were used to express the results. A one-way analysis of variance (ANOVA) and Tukey's test were used to determine the intergroup variation (Tukey, 1949). Using appropriate statistical analysis, $p<0.05$ was regarded as statistically significant. Additionally, some statistical tests were utilized throughout the entire study project. For independent variables the ($p<0.001$) was regarded as a highly significant in dependent variables. For analysis the Statistical tool (Sigma Stat Ver.4.0) wasused.

**Results:**

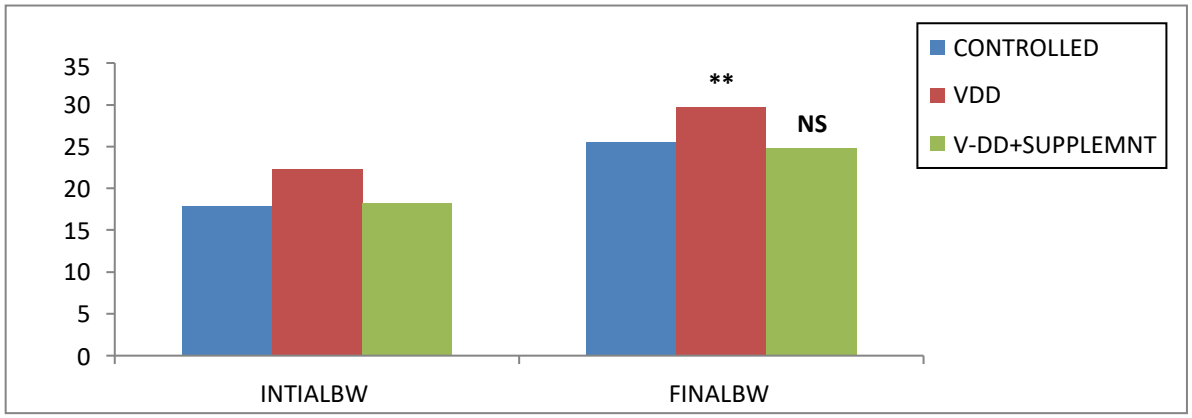
Body weight was an essential consideration in our entire experimental phases. The impact of the Vit-D deficient group showed variations in different time intervals i.e. 90 days as compared with the control group. In the prolonged phase of 90 days, the V-DD group showed a decrease in their body weight as compared to the control, the VDD-treated animals showed significant ($p\leq0.01$) elevations in their body weight as compared with the control group. While, the animals weekly supplemented orally with cholecalciferol to V-DD group for 90 days lowered in their body weights towards normalcy, exhibited recoveries in their body weight when compared with the VDD group **(Table: - 01, Hist. 01).**

**Table: - 01 Bodyweight gain of animals taken initial in the duration of 90 days prior to the animal sacrifice gives the significant body weight gain**

| **DURATION 90$^{th}$Days** | **GROUP-01 CONTROLLED** | **GROUP-02VDD** | **GROUP-03 S-VD3** |
|---|---|---|---|
| **Initial BW** | **17.89±1.21** | **22.31±1.09** | **18.29±1.13** |

| | | | |
|---|---|---|---|
| **Final BW** | **25.50±1.38** | **29.66±1.41**** | **24.83±1.01**$^{NS}$ |

**FIG: - 01 Body Weight gain observed prior to the animal sacrifice duration of 90 days of male mice Mus musculus.**




**±= SEM of six animals.**
***=Significant differences (p<0.05) from the Control v/s V-DD and S-VD3 supplemented.**
*****=Highly Significant differences (p<0.001) from the Control v/s V-DD and S-VD3 supplemented.**
**NS= Non- significant differences from the Control v/s V-DD and S-VD3 supplemented. Hormonal Analysis**:
All the hormones were analyzed by the ELISA kit through the Kit method. In the thyroid gland the posterior surface the four minuterice like tissues are present on the bottom of the neck and it keep the calcium level of the organisms. But here in our experimental phases we also notice the cholecalciferol deficiency in this gland but we find the significant difference not in the initial phase as well as in second phase but we found the prolong effect i.e. in our 90 days means the third phase it shows the significant difference in male mice Musmusculus.

**Table: - 02 Estimation of Hormone parathyroid (PTH) and Vitamin-D3 hormone level was analysed during the duration of 90 days in male mice Mus musculus.**

| **DURATION 90$^{th}$Days** | **GROUP-01 CONTROLLED** | **OUP-02 V-DD** | **OUP-03 S-VD3** |
|---|---|---|---|
| **PTH** | **2.61±0.13** | **2.96±0.18**** | **2.56±1.50**$^{NS}$ |
| **Vit.D3** | **2.84±0.05** | **1.35±0.06**** | **2.79±0.03**$^{NS}$ |

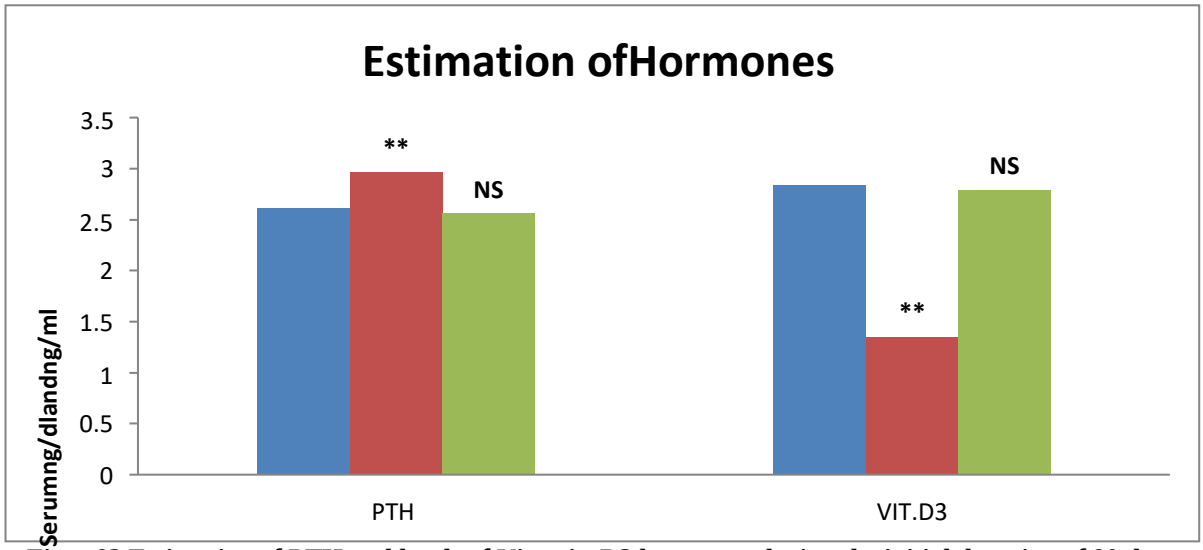
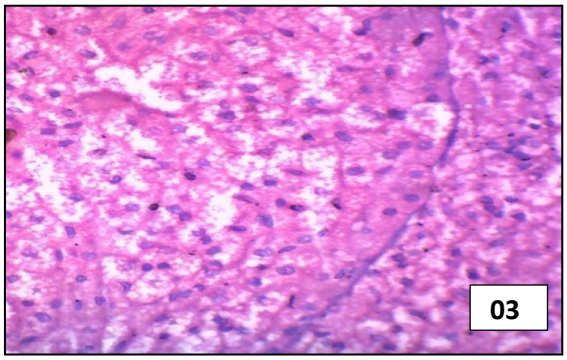



**Fig: - 02 Estimation of PTH and levels of Vitamin-D3 hormones during the initial duration of 90 days experimental study in male mice Mus musculus.**

**±= SEM of six animals.**
***=Significant differences (p<0.05) from the Control v/s V-DD and S-VD3 supplemented.**
*****=Highly Significant differences (p<0.001) from the Control v/s V-DD and S-VD3 supplemented.**
**NS= Non- significant differences from the Control v/s V-DD and S-VD3 supplemented.**

**Histopathological changes in Adrenal gland:**
Over the duration of 90 days showed the appearance of vacuolization around the nucleus in the cortical cells with more degenerative changes **(fig. 03).** While, the cortex region of V-DD
+ cholecalciferol treated of adrenal gland up to 30 days is similar to the control with no degenerative changes **(fig. 04)**, for 90 days, the cell in the cortical regions shown more recoveries in the degenerative cortical cells caused by V-DD **(figs. 05 & 06).**
Fig: 03 Transverse section of VDD cortex region of adrenal gland male Mus musculus up to 90 days showing appearance of vacuolization around the nucleus in the cortical cells along with more degenerative changes (H & E 400X).



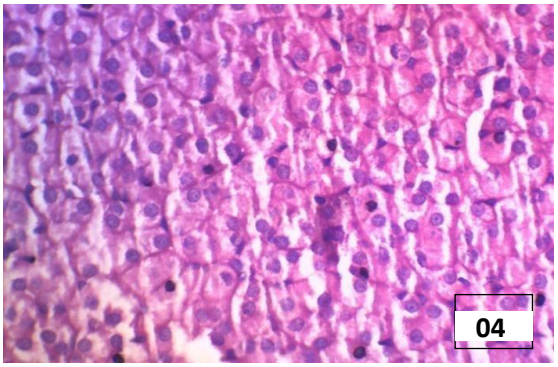
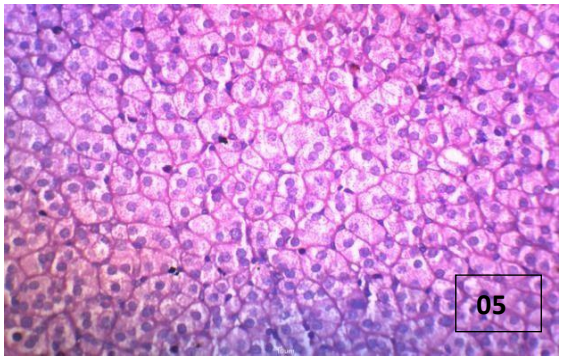
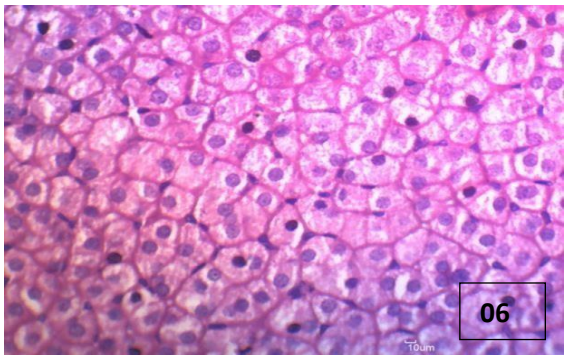




Fig: 04 & 05 Transverse section of cortex region of VDD + Cholecalciferol adrenal gland male Mus musculus for 90 days, the cell in the cortex region showing more recovery for the degeneration caused by VDD. Vacuolization appear to be reduced (H & E 400X)




**Histopathological changes in Testis:**

In 90 days duration of the V-DD exposed animal showed normal germinal epithelial cells with different stages of spermatogenesis were not seen in the seminiferous tubules, but a cluster of sperms seen in middle of the lumen **(fig. 07)**. In 90 days exposed animals with V- DD +cholecalciferol showed prominent recovery in the normal testicular histo-architecture with well-defined seminiferous tubule in different stages of spermatogonial cells, however, at the centre spermatozoa were not clearly visible but interstitial cells were normal in conditions **(fig.08)**.

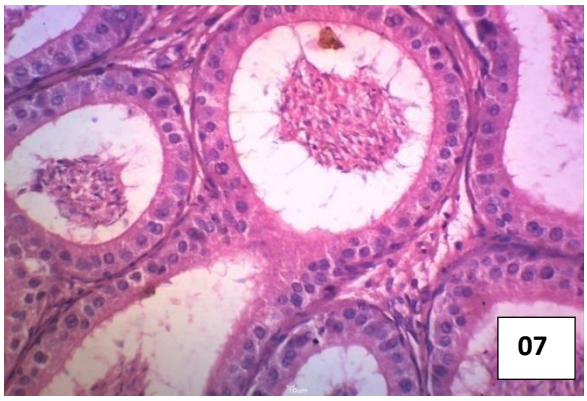



**Fig: 07, Transverse section of VDD testis of male Mus musculus showing for 90 days normal germinal epithelial cell but the different stages of spermatogenesis are not seen in the seminiferous tubules however a cluster of sperm seen in middle of the lumen (H&E 400X).**

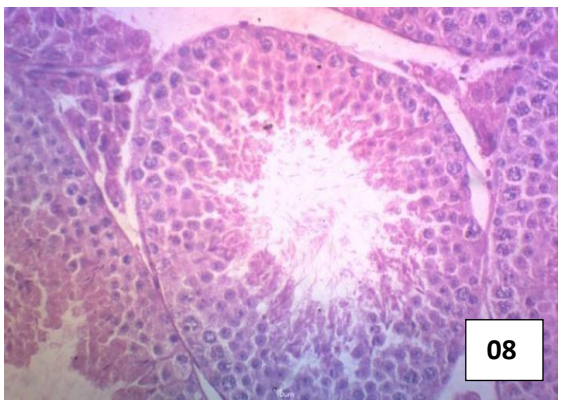



**Fig: 08, Transverse section of VDD+Sup. of testis of male Mus musculus for 90 days showing prominent recovery in the normal testicular histo-architecture with well-defined seminiferous tubule different stages of spermatogonial cells, however at the centre spermatozoa are not clearly visible . Interstitial cells are normal (H&E100X).**

**Discussion:**

Vitamin-D deficiency has turned into a very frequent problem, and it is a global public health issue. Around 1 billion people worldwide are vitamin-D deficient, and 50% are vitamin-D insufficient (Sizar et al, 2023). However, subsequent randomized controlled trials in the general population have failed to establish any cardiovascular benefit from supplementation (Michos et al, 2021). . For decades, vitamin-D treatment in rodents has been utilized as a valid experimental model of vascular calcification and Vitamin-D is a misnomer, technically (Demer et al, 2018). Cholecalciferol (Vit-D3), a member of the secosteroids family, governs both phosphorus and calcium metabolism and

thus stimulates bone mineralization as its primary function. (Holick et al., 2007). Vitamin-D is currently considered a major area of research. Poor health is also linked to low Vitamin-D levels in the body. Although a vast range of ailments, including cancer, metabolic syndrome, autoimmune, psychiatric, and neurodegenerative diseases have recently been linked to its deficiency, it is still unknown how many of these conditions are caused by Vitamin D insufficiency (Autier et al., 2014). The skeletal system, intestine, parathyroid, and kidneys, are the main targets of Vit-D3 As a result, each of these organs is affected biologically by the concentration of vit.-D in different ways but recent research has broadened the range of organs that Vit.- D targets, such as the immune system, adipose tissue, pancreas, thyroid, and reproductive organs ( Altieri et al., 2017; Savastano et al.,2017). The vitamin-D receptor (VDR), a sort of soluble protein, mediates vitamin-D's physiological impacts. The active form of vitamin-D (1, 25(OH)2 D3) acts on the genome through a transcription factor VDR that is found in the target cells' nucleus (Shahrokhi, et al., 2016). PTH level was decreased significantly in VDD ascompared to control in 30 and 60 days however for 90 days significant elevation was observedin the VDD group. Supplementation of cholecalciferol in VDD has brought back the level of PTH to normalcy. According to ( Lofti-Dizaji et al., 2019) obese condition is related to a deficiency of Vit-D3 which positively impacts the serum level of PTH in the long term. The administration of Vit–D3 elevates the level of PTH in the obese group. Petramala and associates reported that patients with primary aldosteronism showed greater plasma PTH levels, decreased serum 25(OH) Vit-D levels, a greater incidence of vitamin-D insufficiency along with osteoporosis/ osteopenia ( Petramala et al., 2014). Vitamin-D insufficiency is related to low levels of serum 25OH (D). In the present study, the serum level of PTH level was low in 90 days in VDD-treated male mice as compared to control animals. Oral administration of cholecalciferol in VDD animals showed significant improvedin their PTH level. The result of the present investigation corroborates the finding of (Lotfi-Dizaji et al., 2019) who observed elevation in serum 25OH (D) in deficient group supplementation of cholecalciferol in a randomized clinical trial (Pliz et al., 2010), observed that vitamin –D supplementation is directly correlated with increased 25( OH) D which otherwise remains below the baseline line level in deficientpatients.

**Conclusion:**

From the above discussions, we concluded that the due to the prolonged deficiency of Vitamin D body weight will be increased and also the level of the parathyroid were also decreased which will leads to the less bone formation and the probability of getting the more frequent fractures in males which is now a becoming a major problem in males which directly leads to the physical as well as the overall fitness and growth of an individual. In future aspects, it's mandatory to eradicate the population from this deficiency by taking the supplement with properconsultation.

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**Ethical Approval:**

For the current piece of work we use the 15 number of animals, male mice Mus musculus (p) for that we have an ethical approval form the Institutional Animal Ethics Committee form the CPCSEA, New Delhi, India under the ethical certificate approval No.1885/GO/Re/16/CPCSEA/IAEC/BU/22.

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