المجلة العلمية للتربية النوعية والعلوم التطبيقية
The Scientific Journal of Specific Education and Applied Sciences

# Effect Of Cholecalciferol As An Antioxidant On Hypercholesterolemic Rats.

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## Abstract :

In recent years, much evidence showed that vitamin $D_3$ deficiency could be related to several chronic diseases such as diabetes and cardiovascular disease. So, the goal of the study is to assess the effect of cholecalciferol on hypercholesterolemic rats at different levels of vitamin $D_3$**. Methods**: Fifty six albino rats were in corporate as follows: G (1) (eight rats) was fed a basal diet, like a negative group. The second main group (48 rats) will be received for 8 weeks (a diet containing 10% sucrose, 0.25%bile salts and 1% cholesterol) to induce hypercholesterolemia in rats. For 8 weeks and divided into six subgroups (8rats each): the first group administrated hypercholesterolemic diet as a positive control group from,( $2^{nd}$ : $7^{th}$) groups administrated on hypercholesterolemic diet and vitamin $D_3$ with different levels (0.5, 0.1, 1.5, 2, 2.5 mg /kg diet) . Results: The group of hypercholesterolemic recorded a highly remarkable increase in serum CHO,TG,LDL,VLDL,HBA1c , tissue malondialdehyde ( MDA ) and a decrease in serum HDL,Vit $D_3$ and in tissue SOD and GSH when compared with (-ve)group.But administered with vitamin $D_3$ at different levels ,there were improvements in all lipid parameters , antioxidant enzymes and the best improvement was found in vit $D_3$ at level 2 mg when compared with the (+ve )group. The pathological examination of the liver confirmed these results.**Conclusion:** concluded that Vit$D_3$ at different levels showed an excellent effect on lipid profile but not exceeded that level and need more studies to discover therapeutic effects of vitamin at different levels.

**Key words:** cholecalciferol - hypercholesterolemia - oxidative stress, HDL - MDA- glycosylated hemoglobin.

## Introduction

The phrase "vit D3" is incorrect, is not a genuine vit since, barring extremely rare circumstances where ultraviolet radiation is completely absent, the human body can synthesis cholecalciferol (D3) on its own. Consider it more accurately as a hormone called. The term "vitamin D3" refers to six separate steroid hormones; the endogenous precursors are active at various levels. Cholecalciferol (D3), which is derived from cholesterol; the hydroxylated variant, calcifediol (25(OH) D3), has partial activity; and the active hydroxylated derivative (**Veloudi et al., 2017 and Neale et al., 2022).**

Essential fat-soluble vitamin D3 serves a variety of purposes. Humans get their vitamin D3 mostly from sun exposure on their skin. 7-dehydrocholesterol may become pro vitamin D3 in the body after being exposed to solar ultraviolet B rays, which is then successively hydroxylated by liver and kidney enzymes to generate 1,25-dihydroxy vitamin D3 (an active form of vitamin D3)(**Schnatz et al .,2015).**

Numerous illnesses, including cardiovascular disease, have been linked to vitamin D3 insufficiency (**Nsengiyumva et al., 2015 and Heidari et al., 2015)** Hypercholesterolemia, which is characterized by raised LDL, reduced HDL-C, and raised TG, is recognized as CVD potential risk. (**Dokic etal., 2015 and Mazidi et al., 2015)**.Through altering blood lipids, vitamin D may have an impact on cardiovascular health.

**Bhat et al., 2022** revealed that with prolonged use of about 40,000 IU/day of vitamin D3, toxicity begins to develop (100 of the 400 IU capsules).About 20% of vitamin D3 is thought to be obtained by diet, and the remaining 80% is produced by the skin after UVB exposure from 7-dihydrocholesterol. Cytochrome P450 2R1 (CYP2R1) and Cytochrome P450 27 (CYP27A1) hydroxylated the compound inside the liver to produce 25(OH) D3.

Vitamin D3 becomes physiologically active (**Borel et al.,2015**). (١،٢٥OH)2D has a variety of effects, including controlling the parathyroid gland's release of parathyroid hormone, the pancreas'

release of insulin, the creation of cytokines by macrophages and T cells, encompasses a diverse range of cell proliferation and differentiation, including tumor cells ( **Hossein , 2013**).

Lack of vitamin D3 decreases pancreatic-cells' ability to secrete insulin and increases insulin resistance in target tissues, which are both necessary for the emergence of diabetes type II **(Alan et al., 2019).**

Following weight loss, Serum 25(OH) D levels have been seen to increase **(Rock et al.,٢٠١٢ ).**Similar to this, other research claim that taking vitamin D supplements improves glucose metabolism). Regarding a clinically significant weight reduction, just one study has demonstrated a connection between decreased insulin resistance and elevated blood 25(OH) D concentrations (**Mitri et al., 2011 and von et al., 2010).**

According to several epidemiological studies, a lack of vitamin D3 is associated with lower levels of HDL and higher levels of triglycerides, and higher levels of apolipoprotein E (**Davidson et al., 2013 and Jorde et al., 2011)**. In support of this, a substantial correlation between lower vitamin D3 levels and hypercholesterinemia was found in a significant prospective assessment of vitamin D3 levels and blood lipids. The results regarding vitamin D3 and blood lipids are inconsistent, and they may be confounded by mentioned link between vitamin D3 and being overweight (**Vimaleswaran et al., 2013**).

Strong cross-sectional associations between higher 25-OH vitamin D levels and lower cholesterol, LDL cholesterol, higher high-density lipoprotein (HDL) cholesterol, and lower triglycerides have been reported using a massive community database with a meta- analysis that discovered increasing 25-OH D levels impacted total and HDL cholesterol levels (**Ponda et al ., 2012**).

When vitamin D levels are sufficient, several cellular induced oxidative activities are controlled. By preserving typical mitochondrial processes, vitamin D3 aids in the regulation of cellular oxidation and reduction (redox). Low serum 25(OH) D concentrations worsen the effects of oxidative stress, speed up an intracellular oxidative injury, and accelerate the rate of apoptotic **(Ryan et al., 2016**).

Thus, this study's objective is to look at the impact of vitamin D3 at various dosages, on rats with hypercholesterolemia and explain the role of the vitamin as an antioxidant.

**Material and Methods**:

**Material:**

- Vitamin D3 was purchased from a pharmacy.
- Natural casein was obtained from Morgan Chemical Company, Cairo Egypt
- All vitamins and minerals, casein and cholesterol were purchased from the Elgomhorya Company in Cairo, Egypt.
- Sprague Dawley Strain rats of the normal albino male variety weighing between ١٣٠and 140 grames were purchased from the Ministry of Health and Population's Institute of Animals in Helwan, Cairo, Egypt.
- kits were purchased from Bio-diagnostic laboratory reagents and products.

## Methods:

**Biological investigation**:

**Diet:** Standard diet was prepared to give the efficiency for normal growth and maintenance of experimental animals. It was created with high-quality components for a 100 g diet. As stated by (**Reeves et al., 1993)**. The diet's components were as follows: maize starch 70.5%, corn oil 10%, and salt combination 4%. (**Hegested et al., 1941)** and vit mix 1% As stated by (**Campbell 1961)**.DL-methionine 0.3gm and choline chloride 0.2%. The protein was added at 14% level at the expense of starch and corn starch up to 100 gm.

**Animals:** Adult albino rats Weight of the Sprague Dawely strain is (130 ±5 g). The creatures were housed in metal cages with copper floors. Water was given to the rat through a glass tube that protruded through its wire cage after the diet was delivered to it in a customized meal bowl.

**Experimental Animal Design:**

Fifty-six albino rats weighing (130±5)g were housed in cages under hygienic conditions and were fed on a basal control diet for

one week for adaptation, the basal diet prepared according to **(Reeves et al., 1993**). After then, all rats were split into two major groupings as follows: Eight rats from the first major group were fed a baseline diet as a check group. To cause hypercholesterolemia, the second major group of 48 rats will consume (10% sucrose, 0.25% bile salts, and 1% cholesterol) for 8 weeks (**skipski and Barclay 1976)**. The second main group was split into six groupings, each with eight rats: the first group served as a positive control group and received a hyperlipidemic diet; (2nd:7th) groups received a hypercholesterolemic diet and vitamin D3 at varying doses (0.5 mg/kg, 0.1 mg/kg, 1.5 mg/kg, 2 mg/kg, and 2.5 mg/kg diet).

Throughout the eight-week trial, animals were kept in a controlled setting with unrestricted access to food and water. Dietary intake and body weight were monitored twice weekly. According to **Chapman et al. (1959**), the weight increase and food consumption of the rat groups will be monitored and used to estimate the Feed Efficiency Ratio (FER). The following formula was used to determine the body weight gain:

(FER) = Weight gain (g) / food consumption (g).

The rats will be anaesthetized and slaughtered at the conclusion of the research after being starved for the previous night, and blood samples will be taken. To get the serum, blood samples will be 3000 rpm centrifuged for 20 min. They will then be maintained at 20 °C until analysis (**Hassan et al., 2013).** The liver and kidney organs will be taken out for histopathological analysis.

**Biochemical analysis:**

According to **Trinder, 1969** serum glucose was measured.Hemoglobin A1C (HbA1C was determined according to (**Satoh 1978**) Vitamin D3 was determined according to (**wacker and Holick 2013**).

**Lipid profile were estimated as:**

Triglycerides in serum was determined according **to Scheletter and Nussel, (1975**), Serum total cholesterol was measured using the **Stein-reported technique (1986**). **Wieland and Seidel's (1981)** methodology was used to determine HDL-C, or high density lipoprotein cholesterol. According to **frunchart (1982**), Low Density Lipoprotein Cholesterol (LDL- C) was measured.

Serum LDL-c and VLDL-c was calculated according to (**Friedewald et al., 1972)**.

LDLc = Total cholesterol - (HDLC + VLDLC)

VLDLc = TG / 5

**Antioxidant activity:**

-Using the technique outlined by **Kakkar et al. (1984**). The activity of the tissue antioxidant enzyme superoxide dismutase (SOD) was evaluated

- Malondialdehyde (MDA) is a lipid peroxidation product, that has been determined using the procedure described **by (Draper et al., 1993).**
- Glutathione activity (GSH) was determined spectrophotometrically as stated by (**Weinhold et al., 1990).**

**Histopathological examination**:

Liver and kidney are preserved in 10% buffered formalin during necropsy till analysis. Hematoxylin and eosin will be used to stain sections of liver and kidney tissue after they have been treated for paraffin embedding (using light microscopy). The liver and kidney organs from the control and treatment groups will be examined histopathologically (**Bancroft and Gamble 2002).**

**Statistical analysis:**

The results obtained were analyzed using SPSS program by the one-way analysis of variance (ANOVA), followed by least significant difference (L.S.D) test to compare between groups. Results were expressed as mean ± standard deviation (SD) and values of $P>0.05$ were considered non-significantly different, while those of $P<0.05$, $P<0.01$ and $P<0.001$ were considered significant, highly and very highly significant, respectively **(McCormick and Salcedo 2017**).

**Results and Discussion:**

Data in Table (1) showed the effect of different levels of Vit.D3 on biological evaluation, hypercholesterolemic rats (+ve) positive group compared to the (-ve) negative control group, exhibited a substantial increase in feed intake, body weight gain percentage, and feed efficiency ratio ($P<0.05$).

The results revealed that all hypercholesterolemic rat groups fed on different levels of VitD3 compared to (+ve) positive control group, exhibited a substantial decrease of feed consumption, Body weight gain percentage, and feed efficiency ratio ($P<0.05$). These results were in a harmony with Qingming and Igor, (2012) who reported that intake and increased vitamin D3 status are inversely associated with body weight and fat in humans.

**Table (1) Effect of various levels of vitamin D3 concentrations on biological evaluations in hypercholesterolemic rat groups**

| variables / Groups | Food Intake (gm / day/each rat) | Body Weight gain % | Feed efficient ratio (FER)% |
|---|---|---|---|
| **Negative Control (G1)** | **25.3 ± 0.0 [b]** | **23.3 ± 3.3 [b]** | **0.61 ± 0.00 [b]** |
| **Positive Control (G2)** | **31.9 ± 2.3 [a]** | **32.9 ± 1.4 [a]** | **0.77 ± 0.01 [a]** |
| **HCD and 0.5 mg of $VitD_3$ (G3)** | **28.6 ± 1.8[b]** | **25.7 ± 3.8 [b]** | **0.58 ± 0.03 [b]** |
| **HCD and 1 mg of $VitD_3$ (G4)** | **28 .1 ± 1.6 [b]** | **25.1 ± 2.3 [b]** | **0.50 ± 0.00 [b]** |
| **HCD and 1.5 mg of $VitD_3$ (G5)** | **27.4 ± 1.5 [b]** | **24.7 ± 2.7 [b]** | **0.57 ± 0.05 [b]** |
| **HCD and 2 mg of $VitD_3$ (G6)** | **27.0 ± 1.8 [b]** | **24.2 ± 1.7 [b]** | **0.59 ± 0.00 [b]** |
| **HCD and 2.5 mg of $VitD_3$ (G7)** | **27.3 ± 1.9 [b]** | **24.1 ± 0.8 [b]** | **0.58± 0.02 [b]** |

The mean + SD of each value.
Significant differences exist between the values in each column with a different superscript. At ($p< 0.05$).

Table (2) showed that the Hypercholesterolemic rats (+) positive group had a significant increase in TC, TG, VLDL-C and LDL-C when compared to the (-ve) negative control group ($P<0.05$).

The results revealed that all hypercholesterolemic rats groups administered varied levels of VitD3 reported a significantly decreased in these variables in comparison to the (+ve) positive group ($P<0.05$).The best result recorded by the group treated with 2 mg/kg diet which it nearest value to (-ve) control group.

On the contrary, the level of serum HDL significantly declined in the positive group in comparison to the negative group. While, all treated groups fed on high cholesterol diet (HCD) and different levels of vit D3 showed a significant decrease in the level of HDL-C

According to (**Farhangi et al., 2013 and McGill et al., 2008**), vitamin D3 reduced lipid abnormalities and lowered blood levels of LDL, TG, and TC. However, this result was incongruent with their claims. Dyslipidemia is a common complication of obesity, and it might be useful to create a dyslipidemia form in humans or animals in order to study how vitamin D3 affects these models.

The responsibilities of vitamin D3 in modifying lipid abnormalities, which have previously been demonstrated by several researches, further clarify the great cardio-protective potential of this steroid vitamin in the management of many obesity-related disorders (**Asemi et al., 2013 and Nagpal et al., 2009**).

**Ponda et al. (2012)** found minor but favorable differences among 25(OH) D levels and TC, LDL, HDL, and TG in a large medical laboratory database of vitamin D levels and blood lipids. **Jorde et al. (2010**) found that HDL and TG had significant and positively distinct correlations for people in the highest quartile of vitamin D levels. According to the same group's assessment, the association between HDL and vitamin D was the clearest; studies included in the analysis demonstrated a positive link between vitamin D levels and HDL, with 50% of these correlations being statistically significant (**Jorde and Grimnes 2011).** The limited sample size prohibited us from detecting any possible statistically significant changes in this parameter, even though we saw a tendency toward higher HDL values in people with better vitamin D status.

According to **Jorde et al. (2010)** findings, vitamin D level is negatively and favorably correlated with atherogenic LDL cholesterol. High-density lipoprotein (HDL) inhibits macrophages-derived from cell cycle progression by promoting cholesterol release and transmitting it to the liver for tubular secretion, a process known as reverse transport of cholesterol. It is well established that an atherogenic lipid profile, including rising serum low-density lipoprotein (LDL), helps promote the huge formation of cholesterol in phagocytosis. **( Das et al., 2013)** , The study discovered that the LXRs/ATP-binding membrane cassette transporter A1 (ABCA1) pathway, a critical regulator in the production and function of HDL, was disrupted in vitamin D deprivation, which lowered plasma HDL levels and accelerated

atherosclerosis in hypercholesterolemic pigs. Through ABCA1-mediated cholesterol export in human monocytic cells, our investigations showed that 1, 25(OH) 2D3 stimulates emerging HDL synthesis in human hepatocellular carcinoma cell line (HepG2) cells.

Table (2) Effect of various levels of vitamin D3 concentrations on the lipid profile of blood in hypercholesterolemic rat groups

| parameter / Groups | Total Cholesterol (mg/dl) Mean ± SD | TG (mg/dl) Mean ± SD | HDL-C (mg/dl) Mean ± SD | VLDL-C (mg/dl) Mean ± SD | LDL-C (mg/dl) Mean ± SD |
|---|---|---|---|---|---|
| (G1)Negative Control | 83.5± 4.4 $^{c}$ | 54.5 ± 2.7 $^{c}$ | 45.6 ± 2.4 $^{a}$ | 10.90±1.1$^{c}$ | 27.0 ± 2.2 $^{f}$ |
| (G2)Positive Control | 135.8 ±8.3 $^{a}$ | 95.5 ± 4.0 $^{a}$ | 26.6 ±2.4 $^{c}$ | 19.11±1.7$^{a}$ | 90.1 ± 7.9 $^{a}$ |
| (G3)HCD and 0.5 mg of vitD3 | 107.8 ±6.8 $^{b}$ | 63.2 ±2.6 $^{b}$ | 31.0 ±2.0 $^{b}$ | 12.64±1.2$^{b}$ | 64.16 ± 5.4 $^{b}$ |
| (G4)HCD and 1 mg of vitD3 | 102.3 ±5.9 $^{b}$ | 64.7 ±3.9 $^{b}$ | 35.2 ±2.1 $^{b}$ | 12.94±1.3 $^{b}$ | 54.2 ±3.0 $^{c}$ |
| (G5)HCD and 1.5 mg of vitD3 | 95.0 ±3.0 $^{b}$ | 63.3 ±3.1 $^{b}$ | 37.8 ±2.3 $^{b}$ | 12.66±1.5 $^{b}$ | 44.54 ±3.5$^{d}$ |
| (G6)HCD and 2 mg of vitD3 | 86.0 ±2.0 $^{c}$ | 59.0 ±2.0 $^{bc}$ | 39.9 ±2.7 $^{b}$ | 11.80±1.4 $^{b}$ | 34.3 ± 3.3 $^{ef}$ |
| (G7)HCD and 2.5 mg of vitD3 | 99.3 ±4.5 $^{b}$ | 60.7 ±2.9 $^{b}$ | 36.3 ±2.8 $^{b}$ | 12.14±1.6 $^{b}$ | 50.56 ±3.5 $^{c}$ |

Each value is the mean ± SD
At ($p< 0.05$), the values in each column with a distinct superscript are statistically different.

Results presented in table 3 revealed that, in comparison to the (-ve) negative control group, hyperlipidemic rats (+ve) positive group had significantly higher blood HBA1c and glucose levels. ($P<0.05$).While, the results revealed that all hypercholesterolemic rat groups fed on different levels of Vit.D3 As compared to the positive group, these metrics revealed a substantial reduction ($P< 0.05$). The group treated with a 2 mg/kg diet had the best results, and the chart shows that this group's value was closest to that of the negative group( 3).

As opposed to the negative control group, the level of blood vitamin D3 was considerably lower in the positive control group.

While, all HCD-treated groups that consumed various amounts of vitamin D3 showed improvement, which recorded a significant increase in the level of VitD3 by increasing the dose of VitD3 added to food from (0.5 gm to 2.5 gm/kg diet).

Vitamin D3 therapy caused a notable rise in insulin concentration, which might less hyperglycemia in diabetic rats. In rats given vitamin D3 treatment, serum HbA1c considerably decreased, which is in line with the research of (**Derakhshanian et al., 2019).**

After observing a reduction in fasting glucose levels in adults who had received vitamin D3 supplements**, Liu et al. (2009**) came to the conclusion that there is an antagonistic relationship between blood vitamin D3 concentration, fasting glucose, and insulin resistance according to **(Von et al., 2010**).Hyperglycemia and metabolic syndrome have been linked to vitamin D3 insufficiency **(Forouhi et al., 2008 and Liu et al., 2009).** Similar to how low HDL-C and high triglyceride levels have been connected to insulin resistance, dyslipidemia has not been linked to insulin sensitivity; it has been reported by **(Chonchol and Scragg 2007**).

**Chagas et al. 2012** found that, vitamin D3 may affect glucose metabolism through a variety of methods, including a quick non-genomic effect and a slower genomic one. One such process involves increasing insulin release through enhanced Vitamin D receptor expression (**Szymczak et al., 2019).** The suppression of cytokines thought to be responsible for mediating insulin resistance is another potential mechanism. Studies demonstrating a connection between reduced blood levels of 25(OH) D and high rates of C-reactive proteins lend weight to the latter idea. In addition, calcium control could well be indirectly impacted by vitamin D3, which is essential for modulating glucose uptake in target tissues.In an effort to comprehend the function of vitamin D3 in - cells, In STZ-induced diabetic mice, These flaws were connected to altered vitamin D metabolism as a result of insulin deficiency's inhibitory influence on 25(OH) D3 $^{-1}$ hydroxylase action in the kidneys. The concentration of HbA1c decreased somewhat but not significantly in the diabetic control group receiving vitamin D, and it dramatically decreased in the diabetic groups receiving standard treatment (glimepiride tablets) and standard treatment plus vitamin D3. Additionally, a noticeable improvement in the insulin level was

seen. Previous research offers several possible explanations for how vitamin D3 helps to normalize blood sugar levels (**Gurudatta et al., 2020).**

Table (3) the impact of various vitamin D3 concentrations on serum (HBA1c,glucose, Vit D3) in hypercholesterolemic rat groups

| parameterS / Groups | HBA1c (mg/dL) Mean ± SD | Glucose (mg/dL) Mean ± SD | Vit D3(ng/ml) Mean ± SD |
|---|---|---|---|
| (G1)Negative Control | 4.1 ± 0.5 [d] | 77.3 ± 5.7 [d] | 7.4 ± 0.7 [a] |
| (G2)Positive Control | 8.1 ± 0.4 [a] | 140.3 ± 10.4 [a] | 2.2 ± 0.1 [d] |
| (G3)HCD and 0.5 mgof VitD3 | 6.9 ± 0.6 [b] | 119.2 ± 9.3 [b] | 3.7 ± 0.3 [c] |
| (G4)HCD and 1 mg of VitD3 | 6.5 ±0.7 [b] | 111.0 ± 9.8[b] | 4.2 ± 0.9 [bc] |
| (G5)HCD and 1.5 mgof Vit D3 | 6.8 ± 0.8[b] | 97.3 ± 4.5 [c] | 4.3 ±0.1 [bc] |
| (G6)HCD and 2 mg of VitD3 | 5.8 ± 0.6 [c] | 98.3 ± 8.0 [c] | 5.2 ± 0.5 [b] |
| (G7)HCD and 2.5 mgof Vit D3 | 6.7 ±0.8[b] | 106.8 ± 8.7 [b] | 5.3 ± 0.6 [b] |

The mean SD of each value.
There are considerable differences between the values in each column's superscript at ($p < 0.05$).

According to data in the table (4), hyperlipidemic rats in the (+ve) positive group had significantly lower SOD and GSH activity than the (-ve) negative control group ($P<0.05$). While,the results revealed that all hypercholesterolemic rat groups fed on different levels of VitD3 compared to the (+ve) positive control group,there was a substantial rise in these variables at ($P<0.05$). The best result was recorded by the group treated with 2mg/kg diet which is the nearest value to (-ve) control group.

Concerning, when compared to the negative control group, the mean MDA level in the positive control group showed a substantial rise. Improvement was shown in all HCD-treated animals fed on various vitamin D3 doses, which documented a substantial drop in MDA levels when compared to the positive comparison group.

The current investigation discovered that the livers of diabetic rats had greater levels of lipid peroxidation and lower levels of hepatic antioxidant enzymes than those of the control group. By lowering the discrepancy between the rate of reactive oxygen species generation and the activity of antioxidant enzymes, vitamin D3 and insulin therapies decreased hepatic oxidative stress. Increased GPx and SOD expression prevent free radicals from inactivating cellular proteins (**Maria et al., 2021**). Consequently, vitamin D3 supplement may help manage hepatic issues brought on by a free electrons by reducing the generation of free radicals, increasing the activity of antioxidant enzymes.

Superoxide anion generation in the mitochondria may rise as SOD activity. Since un neutralized superoxide radicals might result in the generation of hydroxyl radicals, converting superoxide radicals to hydrogen peroxide (H2O2) is a crucial function of SOD (**Hauck et al., 2016).**

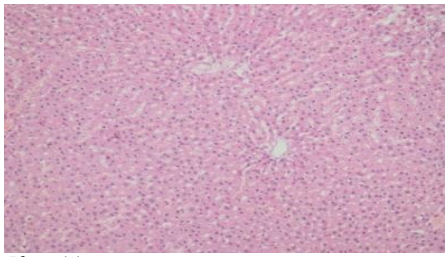
Table (4) Effect of different levels of vit D3 on activity of antioxidant enzymes Superoxide dismutase (SOD), and Glutathione Reductase (GSH) and lipid peroxidation indicators Malondialdehyde (MDA) in hypercholesterolemic rat groups

| Groups \ parameters | Superoxide dismutase(SOD) (n.mol/g.tissue) | Malondialdehyde MDA (n.mol/g.tissue) | Glutathione Reductase (GSH)(U/g.tissue) |
|---|---|---|---|
| (G1)Negative Control | 91.8 ± 0.7 [a] | 49.9 ± 1.2 [b] | 73.1 ± 2.1 [a] |
| (G2)Positive Control | 39.1 ± 0.6 [c] | 89.7 ± 1.8 [a] | 46.8 ± 0.5 [d] |
| (G3)HCD and 0.5 mg of vit D3 | 85.8 ± 0.7 [b] | 59.7 ± 0.6 [b] | 59.1 ± 2.9 [cb] |
| (G4)HCD and 1 mg of vitD3 | 88.0 ± 1.1 [b] | 52.3 ± 0.8 [b] | 61.4 ± 1.5 [b] |
| (G5)HCD and 1.5 mg of vitD3 | 89.8 ± 2.1 [b] | 50.0 ± 2.6 [b] | 64.9 ±0.6 [b] |
| (G6)HCD and 2 mg of vitD3 | 90.7 ± 6.4 [ab] | 48.8 ± 0.4 [b] | 70.1 ± 1.2 [ab] |
| (G7)HCD and 2.5 mg of vit D3 | 50.5 ± 5.5 [b] | 58.5 ± 1.4 [b] | 64.9 ± 2.1[b] |

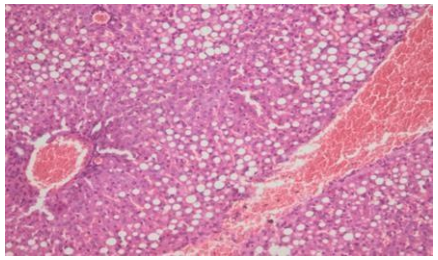
The mean SD of each value.
There are considerable differences between the values in each column's superscript at ($p < 0.05$).

**Histopathological examination for liver and kidney tissue:**

**Liver:**



Photo(1):
Microphotograph demonstrates a normal histological pattern of the liver in the negative control group segment. of hepatocytes, moderate congestion of central veins (HE, 200x)



Photo(2
Microphotograph of liver in 2mg vit D3 group sectionshowing severe macrovesicular steatosis in hepatocytesand congestion of hepatic veins (HE, x200).

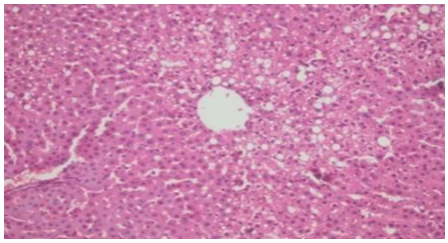


Photo (3):
Microphotograph of liver in 0.5 mg vit D3 group section showing shows moderate multifocal macrovesicular and microvesicular steatosis in hepatocytes surrounding the central vein (HE, 200x)

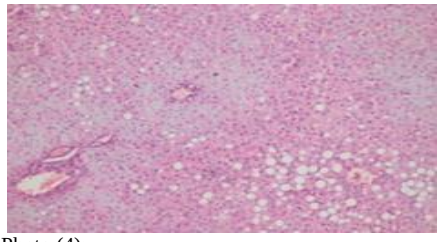


Photo (4):
Microphotograph of liver in 1 mg vit D3 group section shows focal macrovesicular steatosis in hepatocytes around central vein. Mild congestion of central and portal veins (HE, 200x)

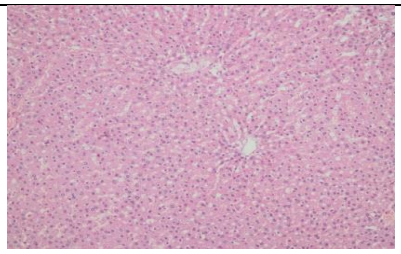


Photo (5):
Microphotograph of liver in 1.5 mg vit D3 groupsection showing moderate macrovesicular steatosis in hepatocytes and congestion of central vein (HE, x200).

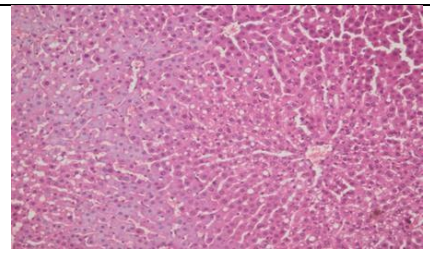


Photo (6):
Microphotograph of liver in positive control group section showing shows normal histological pattern moderate macrovesicular and microvesicular steatosis inhepatocytes (HE, x200)

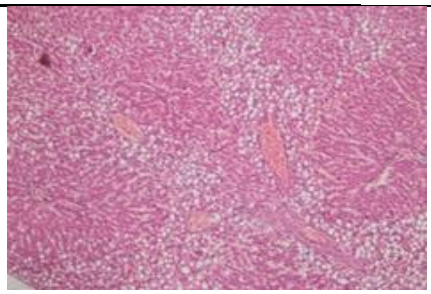


Photo (7):
Microphotograph of liver in 2.5mg vit D3 group section shows multifocal macrosteatosis in hepatocytes. Moderate congestion of central and portal veins (HE, 200x).

The methods that follow cause more intrahepatic fat to accumulate. In our study, Extra vitamin D intake improves blood levels of glucose, insulin, lipids, and the Insulin resistance index while decreasing hepatic steatosis (HOMA-IR). This means that vitamin D lowers the biochemical and histological indicators of the metabolic syndrome, most likely by inhibiting specific metabolic pathways connected to the processes governing the production and accumulation of lipids in the liver **(Wang et al. 2015).**

**Kidney:**

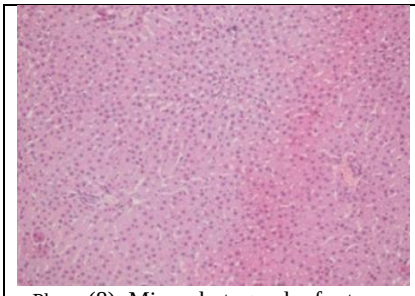


Photo (8): Microphotograph of rat kidney in negative control group section shows normal histological patternof renal tubules (HE, 200x)

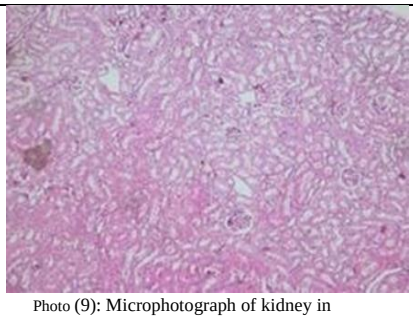


Photo (9): Microphotograph of kidney in positive controlgroup show section renal glomeruli. Some renal tubulesexhibit degenerative changes and renal casts are found inside the tubular lumen (HE, 200x)

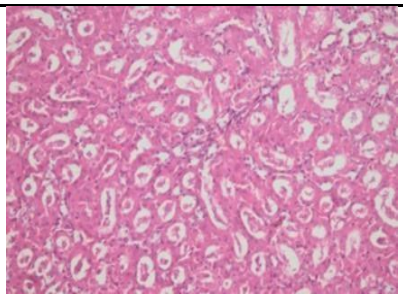


Photo (10) Microphotograph of kidney in 0.5 mg vit D3 group section shows renal casts in the tubular lumen with scattered inflammatory cells in renal interstitial tissue (HE, 200x)

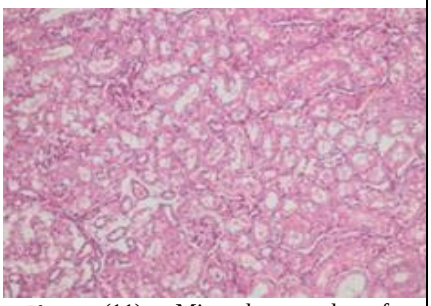


Photo (11): Microphotograph of kidney in 1 mg vit D3 group section shows renal tubular degeneration with congestion of renalblood vessels surrounded with perivascular edema (HE, 200x)

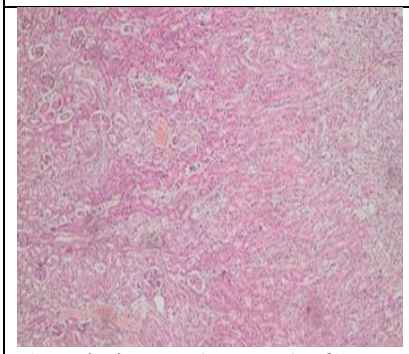


Photo (12):Microphotograph of liver in 1.5 mg vit D3 group section showing moderate congestion of renal blood vessels (HE, x200)

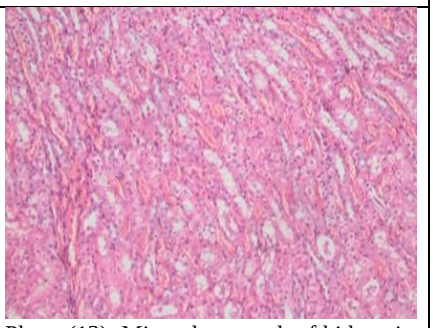


Photo (13): Microphotograph of kidney in 2mg vit D3 group section severe congestion of renal blood vessels (HE, x200

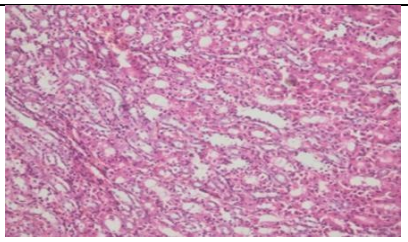


Photo (14): Microphotograph of kidney in 2.5mg vit D3 group section shows mild degenerative change in renal tubules. Mild congestion of renal biood vessels is (HE, 200x)

**Conclusion**: In conclusion, taking vitamin D improved blood lipid parameters,antioxidant enzymes, and glycemic status. The same beneficial effects was noticed in liver tissues.More investigation must be undertaken.

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المجلة العلمية للتربية النوعية والعلوم التطبيقية
The Scientific Journal of Specific Education and Applied Sciences

# تأثير الكولى كالسيفيرول المضاد للاكسدة على الجرذان المصابة بارتفاع كوليسترول الدم

**المستخلص العربى**

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أظهرت الكثير من الأدلة أن نقص فيتامين د يمكن أن يكون مرتبطًا بالعديد من الأمراض المزمنة مثل مرض السكري وأمراض القلب والأوعية الدموية. لذا ، فإن الهدف من الدراسة هو تقييم تأثير الكولي كالسيفيرول على الجرذان المصابة بفرط كوليسترول الدم عند مستويات مختلفة من فيتامين د ٣. الطريقة: ستة وخمسون فأر ألبينو تم وضعهم على النحو التالي: تم تغذية الجرذان بالنظام الغذائي الأساسي( المجموعة السلبية). المجموعة الرئيسية الثانية (ثمانية واربعون جرذاً) تلقت (١٠٪ سكروز ، ٠,٢٥٪ أملاح صفراوية ، ١٪ كوليسترول) لمدة ٨ أسابيع ، وتم تقسيمهم إلى ست مجموعات فرعية (ثمانية جرذان لكل منهما): المجموعة الأولى التي خضعت لحمية فرط كوليسترول الدم كمجموعة ضابطة إيجابية ومن ، (٢: ٧) بقية المجموعات تغذت على نظام غذائي عالي الكوليسترول وفيتامين د بمستويات مختلفة (٠,٥ ، ٠,١ ، ١,٥ ، ٢ ، ٢,٥ مجم / كجم من النظام الغذائي). النتائج: سجلت مجموعة فرط الكوليسترول الدم زيادة ملحوظة في السيرم فى كل المعاملات كالكوليسترول وبقية دهون الدم بالمقارنة مع مجموعة الضابطة لكن عند تناول فيتامين د ٣ بمستويات مختلفة ، كان هناك تحسن في جميع معاملات الدهون ، والإنزيمات المضادة للأكسدة وأفضل تحسن قد لوحظ فى فيتامين د ٣ عند المستوى ٢ ملغ/كجم من الغذاء الاساسى بالمقارنة مع باقى المجموعات وأكد الفحص المرضي للكبد هذه النتائج. الخلاصة تهدف إلى أن فيتامين بمستويات مختلفة أظهر تأثيرًا ممتازًا لملف الدهون ولكن بشرط عدم تجاوز هذة النسبة ويحتاج إلى مزيد من الدراسات لاكتشاف التأثيرات العلاجية للفيتامين بمستويات مختلفة.

**الكلمات المفتاحية : كولي كالسيفيرول - فرط كوليسترول الدم - الإجهاد التأكسدي- مالون داى الدهيد – جلايكولاتيد هيموجلوبين**