Effect of Hypervitaminosis D\textsubscript{3} on Liver, Kidney Functions in White Rats

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ABSTRACT
The research was undertaken to evaluate the effects of vitamin D\textsubscript{3} on the liver and kidney functions through some haematological and biochemical parameters. The results illustrate that high doses (30000 and 50000 IU) of vitamin D\textsubscript{3} significantly reduce the rats' body weights in about 46% additionally much of symptoms like weakness, the rigidity of limbs, neurological irritation, difficulty in movement, respiration and deaths occurrence. The results also showed that the high doses vitamin D\textsubscript{3} has a significant decreased in Red Blood Corpuscles (RBCs), Hemoglobin (Hb), Packed Cell Volume (PCV), Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), White Blood Corpuscles (WBCs), Lymphocytes (Lymph), Monocytes (Mono), Granulocytes (GRA) and Platelets (PLT) compared with the control group. In contrast, the Mean Corpuscular Hemoglobin Concentration (MCHC) and Platelet Distribution Width (PDW) significantly increased after 20-30 days of treatment. Besides, the results demonstrated a significant decrease in Alanine aminotransferase (ALT), Total Protein (TP), Creatinine (Cr) and increase in aspartate aminotransferase (AST), Blood Urea Nitrogen (BUN), Urea. While albumin did not give any significant differences. As well the study proved significantly increase in serum vitamin D\textsubscript{3} concentration compared with the control group after 20-30 days of treatment. Since vitamin D\textsubscript{3} can be beneficial for the organisms, but the overdoses of the vitamin can alter some parameters in the body.

INTRODUCTION
Vitamin D is a fat-soluble steroid hormone, may found in two forms: (1) Ergocalciferol (vitamin D\textsubscript{2}) produced by plants and fungi. (2) Cholecalciferol (vitamin D\textsubscript{3}: 1,25-dihydroxyvitamin D\textsubscript{3} (1,25(OH)\textsubscript{2}D\textsubscript{3}) produced by the animal tissue and the cutaneous synthesis under the action of ultraviolet light at 7-dehydrocholesterol present in the skin (Zhang \textit{et al.}, 2015; Buyuker, 2019; de la Puente Yagüe \textit{et al.}, 2020).

Additionally, vitamin D may be obtained from the diet what is particularly important to people who have limited exposure to the sunlight. The primary dietary sources of this vitamin include oily fish, egg yolk and supplemented milk (Detregiachi \textit{et al.}, 2016). Both forms of Vitamin D\textsubscript{3} transported to the liver where they hydroxylated to 25(OH) D (the inactive form of Vitamin D) then to the kidneys where they undergo another hydroxylation by the enzyme 1-\textalpha hydroxylase to 1, 25(OH) D, the active form (Al-
Saady, 2018). According to the Endocrine Society, assumed that deficiency of serum vitamin D₃ below 20 ng/ml; 20-30 ng/ml considered as insufficiency; while levels above 30 ng/ml are sufficient to maintain normal physiological functions (Gupta et al., 2014; Sowah et al., 2017).

Over the past two decades, vitamin D level measurements have become one of the most frequently ordered tests in the laboratory. This increase is due to a growing awareness of vitamin D deficiency and related health problems, it became a popular supplement, and its use has increased markedly. More intake of vitamin D supplements by the general population and a growing number of prescriptions of therapeutic doses (included very high doses) without medical monitoring might result in a greater risk of exogenous hypervitaminosis D, with symptoms of hypercalcemia also known as vitamin D toxicity (Marcinowska-Suchowierska et al., 2018). Physicians also recommend a high daily dose of vitamin D₃ for many weeks as a treatment for chronic bone diseases (osteomalacia and osteoporosis) and autoimmune diseases such as rheumatoid arthritis, systemic lupus erythematosus, and psoriasis because they are associated with low blood levels of vitamin D₃ metabolites; therefore, the incidence rate of hypervitaminosis D₃ may increase (Elshama et al., 2016).

Failure to determine appropriate doses in long-term treatments, age-inappropriate doses, and variable response to contraindication may result in toxicity (Buyuker, 2019). Therefore, in humans, vitamin D hypervitaminosis leads to increased calcium concentration in the blood leading to the calcification of soft tissues and organs, such as the liver, kidney, heart and over calcification of bones. As well in rats, vitamin D toxicity leads to the symptoms that are similar to humans, such as ruffled coat, dullness, anorexia, cachexia, progressive weight loss, the rigidity of limbs, ataxic movement, difficulty in respiration, diarrhea, decreased food and water consumption, epistaxis, shivering, subnormal body temperature, nervous signs, and possibly death (Ali et al., 2018). Finally, because hypervitaminosis D leads to many deleterious effects and increased mortality, the study is aimed to evaluate the effects of vitamin D₃ toxic doses on liver and kidney functions in rats.

MATERIALS AND METHODS

Animals housing

Forty-eight young, healthy male rats aged about eight weeks and weighed between 155–190 g were assigned to groups by a stratified randomization scheme designed to achieve similar group mean body weights. The rats were housed in collective cages under a dark/light cycle of 12 hours, room temperature of 25 ± 2°C. They were allowed free access to food and water during the entire experimental period.

**Figure 1:** The weight (g) of rats after 20 days dosing vitamin D₃ B: before dosing A: after dosing

**Figure 2:** The weight (g) of rats after 30 days dosing vitamin D₃ B: before dosing A: after dosing

**Experimental design**

The rats randomly divided into six groups (8 rats each). The treatment continued for 20 and 30 days period—the first (control) group given distilled water. The second (milk) group was given milk. The third group was given vitamin D₃ (cholecalciferol) dissolved in milk at the dose rate of 10000 IU/Kg daily (JOSWE medical Health Company). The fourth group was given vitamin D₃ at the dose rate of 20000 IU/Kg daily. The fifth group was given vitamin D₃ at the dose rate of 30000 IU/Kg daily. The sixth group was given vitamin D₃ at the dose rate of 50000 IU/Kg daily. All rats administered their doses by gavage tube orally at the same time every day.

**Blood sample collection and assessment**

Blood samples were obtained from the orbital sinus using capillary tubes. Blood transferred by 1 ml into a heparinized tube for assessment of complete blood count (CBC) and about 5 ml into a non-heparinized tube, after clotting; the samples were
centrifuged at 3000 rpm for 15 min to separate the serum for assessment of alanine aminotransferase (ALT), aspartate aminotransferase (AST), total protein, albumin, urea and creatinine. The haematological parameters measured by RT-7600 Auto Hematology Analyzer (Rayto) and the biochemical parameters in serum were estimated by colourimetric methods using chemistry analyzer smart-150 (GenoTEK). Vitamin D₃ assessed by using enzyme-linked immunosorbent assay RT-2100C microplate reader (Rayto).

**Table 1: Effect of different concentrations of vitamin D3 (IU) on hematological parameters after 20 days**

| Groups Parameters | 50.000 | 30.000 | 20.000 | 10.000 | Milk | Control |
|-------------------|-------|-------|-------|-------|------|--------|
| RBC               | 6.77±0.06 | 13.8±0.05 | 13.8±0.03 | 13.8±0.03 | 7.07±0.01 | 7.54±0.05 |
| Hb                | 48.9±0.00 | 37.3±0.11 | 37.3±0.11 | 37.3±0.11 | 49.9±0.18 | 54.2±0.23 |
| PCV               | 18.3±0.16 | 19.2±0.06 | 19.2±0.06 | 19.2±0.06 | 20.2±0.11 | 20.4±0.05 |
| MCV               | 48.9±0.00 | 37.3±0.11 | 37.3±0.11 | 37.3±0.11 | 49.9±0.18 | 54.2±0.23 |
| MCH               | 36.6±0.11 | 36.6±0.11 | 36.6±0.11 | 36.6±0.11 | 36.7±0.63 | 36.7±0.63 |
| MCHC              | 39.4±0.23 | 37.3±0.11 | 37.3±0.11 | 37.3±0.11 | 49.9±0.18 | 54.2±0.23 |
| WBC               | 10.4±0.05 | 11.4±0.05 | 11.4±0.05 | 11.4±0.05 | 13.6±0.05 | 14.6±0.34 |
| Lymph             | 8.5±0.28 | 9.4±0.05 | 9.4±0.05 | 9.4±0.05 | 12.0±0.26 | 12.9±0.26 |
| Mono              | 1.00±0.05 | 1.10±0.05 | 1.10±0.05 | 1.10±0.05 | 2.00±0.05 | 2.00±0.05 |
| PLT               | 34.2±0.23 | 49.5±0.28 | 49.5±0.28 | 49.5±0.28 | 52.5±0.93 | 52.5±0.93 |
| PDW               | 16.7±0.24 | 16.7±0.24 | 16.7±0.24 | 16.7±0.24 | 10.4±0.11 | 7.32±0.06 |

* For each treatment 3 replicates were used.

**Table 2: Effect of different concentrations of vitamin D3 (IU) on hematological parameters after 30 days**

| Groups Parameters | 50.000 | 30.000 | 20.000 | 10.000 | Milk | Control |
|-------------------|-------|-------|-------|-------|------|--------|
| RBC               | 7.57±0.17 | 13.6±0.11 | 13.6±0.11 | 13.6±0.11 | 14.2±0.00 | 14.6±0.34 |
| Hb                | 37.8±0.63 | 40.9±0.92 | 40.9±0.92 | 40.9±0.92 | 41.7±0.23 | 40.0±0.69 |
| PCV               | 47.6±0.34 | 48.1±0.11 | 48.1±0.11 | 48.1±0.11 | 49.5±0.28 | 52.5±0.93 |
| MCV               | 1.00±0.05 | 2.00±0.05 | 2.00±0.05 | 2.00±0.05 | 2.00±0.05 | 2.00±0.05 |
| MCH               | 16.6±0.17 | 17.0±0.12 | 17.0±0.12 | 17.0±0.12 | 18.5±0.08 | 17.9±0.86 |
| MCHC              | 35.7±0.18 | 35.4±0.34 | 35.4±0.34 | 35.4±0.34 | 38.9±0.86 | 38.9±0.86 |
| WBC               | 6.87±0.04 | 7.38±0.16 | 7.38±0.16 | 7.38±0.16 | 8.10±0.05 | 9.40±0.15 |
| Lymph             | 4.19±0.03 | 4.76±0.12 | 4.76±0.12 | 4.76±0.12 | 14.6±0.04 | 11.0±0.05 |
| Mono              | 1.40±0.01 | 1.32±0.03 | 1.32±0.03 | 1.32±0.03 | 1.40±0.00 | 1.75±0.01 |
| PLT               | 222.0±37.0 | 257.0±4.0 | 257.0±4.0 | 257.0±4.0 | 537.0±6.9 | 537.0±6.9 |
| PDW               | 144.0±5.19 | 200.0±2.88 | 200.0±2.88 | 200.0±2.88 | 10.4±0.11 | 7.32±0.06 |

* For each treatment 3 replicates were used.

**Statistical Analysis**

All data are presented as means ± SE. Differences between groups were analyzed by using the Duncan test, one-way ANOVA at the level of statistical significance $P \leq 0.05$ by SPSS version 21 (Indrayan and Sarmukaddam, 2001).

**Ethical considerations**

In the University of Mosul / College of Science / Biology Department, a high standard of care and animal well-being was promoted at all times. Painful pro-
Twenty days of dosage. The results also show no significant international laws and regulations.

Table 3: Effect of different concentrations of vitamin D3(IU) on Liver functions through some parameters after 20 and 30 days

| Groups | Parameter | Period in Day | Control Mean±SE | Milk Mean±SE | 10.000 Mean±SE | 20.000 Mean±SE | 30.000 Mean±SE | 50.000 Mean±SE |
|--------|-----------|---------------|-----------------|-------------|----------------|----------------|----------------|----------------|
| ALT    | 20        | 44.3±2.70a    | 37.0±0.57ab    | 37.6±8.60ab | 32.3±1.30ab    | 28.3±3.70b    | 28.1±3.80b     |
|        | 30        | 42.0±0.00a    | 40.0±2.86a     | 39.6±2.40a  | 37.0±2.88ab    | 35.6±0.88a    | 30.6±0.88a     |
| AST    | 20        | 95.5±8.94a    | 94.5±9.52a     | 121.0±6.11b | 133.0±0.57ab   | 147.0±2.51ab  | 164.5±4.33c    |
|        | 30        | 121.0±8.08ab  | 120.0±0.57ab   | 125.0±0.56a | 127.0±6.92a    | 148.0±4.97b   | 154.0±5.19b    |
| T. P   | 20        | 6.53±0.2a     | 6.56±0.17a     | 6.50±0.40a  | 6.15±0.08a     | 5.95±0.37a    | 5.85±0.25a     |
|        | 30        | 7.20±0.11a    | 7.00±0.05ab    | 7.16±0.23a  | 7.11±0.28a     | 6.55±0.08bc   | 6.35±0.09c     |
| Albumin| 20        | 3.03±0.12a    | 3.00±0.05a     | 3.15±0.14a  | 3.03±0.08a     | 3.10±0.00a    | 3.10±0.05a     |
|        | 30        | 3.36±0.08a    | 3.30±0.05a     | 3.33±0.12a  | 3.30±0.05a     | 3.30±0.11a    | 3.13±0.06a     |

* ALT-Alaninetransaminase (U/L) – AST-Aspartate transaminase (U/L) – T.P -Total Protein (g/dl) – Albumin (g/dl).
** For each treatment 3 replicates were used.
*** Based on Duncan-test, horizontally different letter refers to a significant difference between treatments at P ≤ 0.05.

Table 4: Effect of different concentrations of vitamin D3(IU) on Kidney functions through some parameters after 20 and 30 days

| Groups | Parameter | Period in Day | Control Mean±SE | Milk Mean±SE | 10.000 Mean±SE | 20.000 Mean±SE | 30.000 Mean±SE | 50.000 Mean±SE |
|--------|-----------|---------------|-----------------|-------------|----------------|----------------|----------------|----------------|
| Urea   | 20        | 21.3±3.75a    | 22.0±0.57a     | 23.6±0.66c  | 24.3±0.88a     | 33.0±0.57b    | 41.5±3.17c     |
|        | 30        | 29.0±0.57a    | 30.0±0.57ab    | 34.0±2.64ab | 37.5±1.44abc   | 43.0±1.73bc   | 49.0±9.45c     |
| BUN    | 20        | 9.95±1.75a    | 10.60±6.42a    | 11.05±0.31a | 11.36±0.41a    | 15.40±0.26b   | 19.37±1.48c    |
|        | 30        | 13.54±0.27ab  | 14.00±0.26ab   | 15.87±1.23ab | 17.51±0.67abc  | 20.08±0.08bc  | 22.87±4.41c    |
| Creat. | 20        | 0.61±0.05a    | 0.62±0.06a     | 0.55±0.00ab | 0.52±0.00ab    | 0.48±0.01b    | 0.46±0.01b     |
|        | 30        | 0.65±0.05ab   | 0.64±0.00ab    | 0.66±0.08a  | 0.65±0.03ab    | 0.60±0.06bc   | 0.58±0.05c     |

* Urea (mg/dl) – BUN (mg/dl) – Creat.- Creatinine (mg/dl).
** For each treatment 3 replicates were used.
*** Based on Duncan-test, different letter refers to a significant difference between treatments at P ≤ 0.05.

Table 5: Vitamin D3 levels in white rats after dosing with different concentrations

| Groups | Days       | Control Mean ± SE | Milk Mean ± SE | 10.000 Mean ± SE | 20.000 Mean ± SE | 30.000 Mean ± SE | 50.000 Mean ± SE |
|--------|------------|-------------------|---------------|-----------------|-----------------|-----------------|-----------------|
|        | 20         | 38.0±2.07a        | 43.5±0.51a    | 127.8±5.94b    | 201.4±3.05c    | 215.6±6.00d     | 236.3±1.84c     |
|        | 30         | 127.4±4.27a       | 123.3±0.89a   | 235.8±0.98b    | 241.5±0.54b    | 248.4±1.21c     | 252.7±0.80c     |

* Vitamin D3 (ng/ml).
** For each treatment 3 replicates were used.
*** Based on Duncan-test, horizontally different letter refers to a significant difference between treatments at P ≤ 0.05.

Table 3: Effect of different concentrations of vitamin D3(IU) on Liver functions through some parameters after 20 and 30 days

RESULTS AND DISCUSSION

The results of the Figure 1 indicate that there was a clear significant decrease in rat's weight after twenty days of dosage. The results also show no significant differences between control and milk group. The figure shows that the increase of weight is about 40 - 50 g in the control and milk groups compared with weight losses in different concentrations of vitamin D3, especially at 50,000 IU. Figure 2 demonstrated a significant difference in rat's weight after thirty days of dosage. The results also show no significant differences between control and milk group. The figure shows that there was a slight increase in the group's weights dosage with different concentrations of vitamin D3 except for a decrease in about 36 g at 50,000 IU group.
The results of Table 1 showed that a significant difference might be increase or decrease in RBCs, Hb, PCV, MCV, MCH, MCHC, WBCs, PLT and PDW between control and vitamin D_3_ groups as well among the concentrations of vitamin D_3_ after 20 days of dosing. The results also indicate no significant between the control and milk groups in most of the haematological variables.

Table 2 indicates that there were a significant difference in RBCs, Hb, PCV, MCV, MCH, MCHC, WBCs, PLT and PDW between control and vitamin D_3_ groups especially at concentrations 30,000 and 50,000 IU after 30 days of dosing. In addition, the results showed no significant between the control and milk groups in most of the hematological variables.

The results in Table 1 demonstrated that there were significant differences in the activity of ALT and AST between the control and the vitamin groups after 20 and 30 days of the dosing, as well significant differences in TP after 30 days while the milk group did not show any significant compared with the control.

Table 4 results indicate the presence of significant differences in Urea, BUN and Creatinine between control group and vitamin groups after 20 and 30 days of the dosing, while there were no significant difference between the control and milk group.

Finally, Table 5 illustrates the highly significant differences between the control and the different concentrations of vitamin groups at period 20 and 30 days of the dosing. Additionally, the table shows that there was no significant between the milk and control group.

The research included studying the effect of hyper-toxicity of vitamin D_3_ on body weight, blood components, liver and kidney’s functions in white rats. Figure 1 shows the effect of different concentrations of vitamin D_3_ that dosing the white rats. The results indicate a significant weight loss in these rats, which ranged between 28-46% after 20 days of dosing. The rats also show other symptoms such as weakness, the rigidity of limbs, neurological irritation, difficulty in movement and respiration after the first week of dose. Additionally, to the deaths, especially at concentrations 30,000 and 50,000 IU. Whereas the results of the Figure 2 revealed a slight increase in weight except 50,000 IU concentration show a decrease in weight and the ratio of weight loss ranged between 5-45% after 30 days of dosing in addition to the previous symptoms and deaths.

Increasing Vitamin D_3_ may cause a reduction in the formation of adipose cells and not stored in the body thus lack fat accumulation and weight loss, or the Vitamin cause increasing serotonin, which is a neurotransmitter, plays a role in controlling appetite and can increase the feeling of satiety (Sabir et al., 2018).

The results, which is in agreement with (Ali et al., 2018) they confirmed that during the second week of treatment with a high dose of vitamin D_3_, rats display decreased in activity, nervous signs, like aimless running and rolling. In the third week and after that, rats were less active than usual, and on the 15th and 18th day, two rats from the groups were found dead; also they demonstrated that the therapeutic or low dose did not have significant effects. Besides, the results agreed with (Tischler, 1999) they reported that rats receiving 20,000 IU/Kg/Day exhibited markedly reduced body weight gain after the end of week 1. The results also are in agreement with (Chavhan et al., 2011a) they demonstrated that the clinical signs observed in vitamin D_3_ toxicity at a dose rate 2 mg/kg/body weight were anorexia, progressive weight loss, difficulty in movement and respiration, diarrhoea, epistaxis and nervous signs. Mortality was observed in treated rats at day 10-19 of treatment. As well, they indicated that the total decrease in average body weight was 23.17%.

Likewise, the result agrees with the study of Thompson (2013), she suggested that vitamin D is associated with weight loss success or higher increases in 25OHD levels predicting had better weight loss. Besides, to agree with other investigators (Smith et al., 2000) explained that the mice treated with vitamin D analogues were underweight compared with the control mice, about 35% after 55 weeks.

The authors (Taylor and Davies, 2018) showed that Vitamin D treatment appears to be safe at doses of up to 10,000 IU/day, delivering 25OHD concentrations below levels associated with toxicity. In addition, (Marcinowska-Suchowierska et al., 2018; Pludowski et al., 2019) indicated that adults who ingested 20,000 IU of vitamin D_3_ per day had significantly increase of 25(OH)D concentrations, up to 60 ng/ml without any toxicity evidence and vitamin D_3_ toxicity resulting from excessive use at 150 ng/ml and more. It is worthy of mentioning that (Suchowierska and Pludowski, 2016) demonstrate that the pathological processes of vitamin D3 toxicity were related to dosage, length of time between doses and exposure duration.

Furthermore, the results showed no significant differences between control and milk group. We have used the milk as a way to dissolve vitamin D_3_ so that it can easily be given to the rats. (Iltkonen et al., 2018; Leskauskaite et al., 2016) They confirmed that
when vitamin D₃ fortified milk products are manufactured, it is incorporated into dairy products in the emulsified form does not degrade. In addition, (Itkonen et al., 2018) indicated that the countries with a national vitamin D₃ fortification policy for fluid milk, milk products contribute substantially to vitamin D₃ intake and the excess of vitamin D₃ not caused by milk, but due to the vitamin in it.

Table 1 indicated a significant decrease in RBCs, Hb, PCV, MCV, MCH, WBCs, Lymph, Mono, GRA and PLT between control and vitamin D₃ groups as well showed a significant increase in MCHC and PDW additionally RBCs in concentration 10.000 and 20.000 IU of vitamin D₃ after 20 days of dosing. The results also indicate no significant between the control and milk groups.

The results agree with the publication of (Gaafer et al., 2019) they illustrate that a significant decrease in RBCs, Hb, PCV and PLT in comparing with normal control as a result of treatment of induced arthritis in rats with vitamin D₃ and losartan for two weeks.

Refaat et al. (2014) showed a significant positive correlation between 25-OH vitamin D levels with serum RBC count and haemoglobin concentrations. As regarding the reduction of monocyte number with vitamin D₃ high dose may be associated with the hepatic macrophages are central in the pathogenesis of chronic liver fibrosis (Tacke and Zimmermann, 2014; Krenkel and Tacke, 2017).

The results of Smith et al. (2000) demonstrate that WBC count was a little lower in the groups in addition to no differences in MCV, MCH, MCHC and PLT at low concentrations in mice treated with some vitamin D₃ analogues three times a week for 55 weeks. On the other hands Korzonek-Szlacheta et al. (2018) indicate that PDW values were the highest in groups treatment with 10 and 20 ng/ml of 25(OH)D.

Results of Table 2 revealed that a significant increase in RBCs, Hb, PCV in concentrations 10.000 and 20.000 IU and then decreased at 30.000 and 50.000 IU. As well, a significant increase in MCHC, Mono and PDW between control and vitamin D₃ groups in addition to a significant decrease in MCV, MCH, WBCs, Lymph, GRA and PLT after 30 days of dosing. The results also showed no significant between the control and milk groups in most of the haematological variables.

The results agree with the reported values of Hasan et al. (2016), they illustrated that there was a dramatically significant increase in RBC count, Hb and PCV level in treated groups and then suddenly decrease with excessive supplementation, and there was no relation with vitamin D concentration and WBC counting, the study continued for 120 days. While Smith et al. (2000) state that there was slightly higher levels of red blood cells, haemoglobin, and hematocrits than did in control when mice treated with some vitamin D₃ analogues. Others showed that when a body exposed to overdose vitamin D, hemopoietic organ produce more blood cells and thus, the PCV level growing gradually (Meguro et al., 2011). As well Coşkun and Şahin (2018) observed that there was no difference between 25(OH)D in concentrations less than 20, 20-32, more than 32 ng/ml in PLT, also PDW was higher at female in healthy children aged 0-18 years.

Table 3 has shown a significant decrease in ALT, a significant increase in AST compared with the control after 20, and 30 days of the dosing also TP indicate significant decrease after 30 days only whereas milk and albumin groups did not show any significant differences as compared with the control.

The results are in agreement with Hasan et al. (2016), they showed that AST level is a significant fluctuation of ALT level in mice with increases vitamin and period. Also, the very high dose may cause toxicity, and the excessive amount causes calcification of soft tissues. This toxicity and calcification may affect both liver, muscle, and secrete more AST in blood serum. As well, the ALT level in mice significantly varied with different treatments.

The results, which agrees with Tavakoli et al. (2019), they demonstrated that significant reductions were observed in serum ALT, AST, LDH, total bilirubin and TGT at the end of supplementation with 50.000 IU one week for nine weeks to a total of 988 adolescent girls were recruited. Additionally, the study of Den et al. (2018) showed a significant decrease (P<0.05) in liver biomarkers such as ALT and AST in rats with fatty liver induced by a choline-deficient diet supplement with different concentrations of vitamin D₃ intraperitoneally twice a week for 12 weeks. While serum albumin levels and total bilirubin showed a non-significant (P>0.05) decrease when compared with the control group. Previous results appear to be consistent with those manifested by Taghvaei et al. (2018), they indicate that there were significant decrease in ALT and AST in a total of 40 patients with the nonalcoholic fatty liver disease received 50.000 IU vitamin D₃ weekly for 12 weeks.

It is worth noting that the study of Podgorska et al. (2018) indicate that Liver toxicity characterized by biochemical parameters, there was only a slight increase in the alanine aminotransferase (ALT) after calcitriol treatment, which was statistically insignificant and increased in the aspartate aminotransferase (AST) values as compared with
the untreated control. In addition to the tested doses, 50 nM and 200 nM of calcitriol (vitamin D₃) may lead to acute effects on the liver and kidneys. The hepatotoxic effect of calcitriol in Syrian golden hamster can be painful and seems to be a primary cause of changes in animal behaviour.

Additionally, the results of Zarghani et al. (2018) elucidate that there were no significant differences in levels of serum ALT and AST when rats were treated with calcium and vitamin D₃ at 10,000 IU (cholecalciferol) combination improve fatty liver disease for 60 days. Other studies suggest that serum ALT activity is a reliable marker of liver disease, cardiovascular disease or mortality and muscle injury. Also showed that low ALT levels are associated with increased risk of cardiovascular mortality (Ndrepepa and Kastrati, 2019). In contrast, the study of Liangpunsakul and Chalasani (2011) demonstrate a significant inverse relationship between serum vitamin D levels and unexplained elevation in ALT.

On the other (Chavhan et al., 2011b) they revealed that on day 6 of treatment with Vitamin D₃, the total plasma protein and albumin were found significantly decreased as compared to control groups.

The results of Table 4 illustrate that a significant increase in Urea and BUN while Creatinine showed a considerable decrease compared with the control after 20 and 30 days of the dosing. Additionally, the milk group has not shown a significant difference.

The results are in agreement with the study of Chavhan et al. (2011a), they revealed that treatment with Vitamin D₃, the plasma concentration of blood urea, BUN and other parameters was found significantly increased. Also, the evaluated level of BUN in the study was indicative renal damage because of Vitamin D₃ toxicity in rats.

The results also agree with Elshama et al. (2016), they showed a significant increase in serum urea associated with a substantial decrease in serum creatinine that received vitamin D₃ in a toxic dose (2 mg/kg), in comparison with the control group. As well, they indicated that differences between the toxic dose of vitamin D₃ and the renal function tests of urea and creatinine in rats. As illustrated that the renal histological sections which received 2 mg/kg of vitamin D₃, showed shrinkage of some vascular glomeruli, tightness of the glomerular capsular space, degeneration of the epithelial lining of most renal tubules, and mineralization of the cortex and medulla with calcified tubular epithelium. In addition, to agree with Chavhan et al. (2011b), they elucidate that the clinical-pathological findings of cholecalciferol (Vitamin D₃) toxicity in animals increase plasma levels of blood urea and nitrogen BUN.

On the other hand, the study of Liyanage et al. (2018) demonstrated that Vitamin D₃, 50,000 IU given intravenously monthly for six months reduces urine albumin, serum creatinine, and renin levels in patients with diabetic nephropathy. As for Ralston et al. (2003); Jones (2008) was proved that low levels of serum creatinine are due to high urine calcium creatinine, which is associated with an increase in the 25-hydroxy vitamin D level also hypercalcemia causes accumulation of calcium phosphate crystals in soft tissues leading to high serum urea as a result of renal function impairment. Whereas Dee and Hovda (2012) concluded that soft tissue mineralization and chronic renal failure might result in chronic illness or death, even after the level of serum calcium returns to normal when mice or rats treated with cholecalciferol.

Finally, the results of the study of Table 5 indicate that a highly significant increase in all concentrations used of vitamin D in serum remarkably comparing with the control at the periods 20 and 30 days of the dosing in addition to a non-significant between the milk and control groups.

The results appear to be consistent with those manifested by Šimoliūnas et al. (2019), they illustrate that vitamin D₃ concentration increased in rat’s blood serum significantly when using three types of these vitamins. Similarly, Wylon et al. (2017); Choi et al. (2016) showed that the serum concentration 25(OH)D increased significantly after 8000 IU oral cholecalciferol was administered daily for 84 days or a single intramuscular injection of 100,000 IU compared to the control group. Additionally, to agree with Close et al. (2013), they demonstrated both 20,000 and 40,000 IU vitamin D₃ supplementation over six weeks elevates serum 25(OH)D concentrations above 50 nmol/l.

The study of the researcher (Bella et al., 2017) indicates that diabetic and nondiabetic mice supplemented with 40000 IU/kg body weight/day of vitamin D₃ had higher serum 25(OH)₂D levels than controls.

Others (Holick et al., 2011) demonstrate that the ingestion of 10,000 IU/day of vitamin D₃ raising blood levels of 25-hydroxyvitamin D above 100 ng/mL and was not associated with hypercalcemia. Whereas Koop et al. (2018) elucidate that toxicity is generally caused by inadvertent ingestion of excessive doses. Doses more than 50,000 IU/day could raise levels of 25(OH)D to >150 ng/mL, which is associated with hypercalcemia and hyperphosphatemia.
**CONCLUSION**

Vitamin D in a high dose remains an ongoing issue, and its incidence is likely to rise, owing to both increasing interest and the widespread prescribing. On this basis, the study was conducted to estimate the toxicity of vitamin D₃, especially in high doses on the liver and kidneys functions through some haematological and biochemical parameters. On the light of the results, blood levels should be monitored while taking high doses of vitamin D₃ due to its effects on cells and their contents. In addition, the overdose of a vitamin can make changes in serum ALT, AST, TP, Urea, BUN and Creatinine concentrations. The increase in the period time had the same effects.

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**Conflict of Interest**

The authors declare that they have no conflict of interest for this study.

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**Didriksen et al. (2015)** showed that there were highly significant correlations between baseline and serum 25(OH)D₃ levels at the study end. In addition, they found considerable storage of vitamin D₃ in the adipose tissue when took 20,000 IU/week for 3-5 years in a human. As well *Jain et al. (2019)* explained that the mechanism of vitamin D toxicity involves an increased concentration of vitamin D metabolites reaching the vitamin D receptor in the nucleus of target cells and causing exaggerated gene expressions. Whereas *Dee and Hovda (2012)* suggest that cholecalciferol serves as rodenticides because of the overproduction of 25-hydroxycholecalciferol (a metabolite with limited activity) in the liver and 1,25-dihydroxycholecalciferol (an active metabolite) in the kidneys. Normally, cholecalciferol is rapidly absorbed and converted to 25-hydroxycholecalciferol in the liver. Enzymes in the kidneys further converted 25-hydroxycholecalciferol to 1,25-dihydroxy-cholecalciferol until there is a sufficient amount in the plasma. Moreover, in greater dose situation, not only dose conversion to 1,25-dihydroxycholecalciferol occur; but the 25-hydroxy-cholecalciferol concentration keep going to rise until the 25-hydroxycholecalciferol plasma level is high enough to become metabolically active.
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