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The Effect of Vitamin D3 on Physiological and Biochemical Parameters of Local Adult Male Rabbits

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Abstract

Vitamin D3 is considered as a biological form of vitamin D, it is important for bone and skeletal muscle growth, immune function, and inflammation. In the past decade, several studies demonstrated a potential link between vitamin D3 deficiency and various diseases. To evaluate the effect of vitamin D3 supplementation on certain hematological (blood parameters) and biochemical (liver enzymes) parameters of rabbits, which reflects the effect of this vitamin on the health status and general functions of the body. For this study, vitamin D3 was used in different dosage levels, in sixteen male rabbits (6-12 months old, 2.5±0.5kg body weight) grouped into CG (control), G1 (10000 IU/ml), G2 (20000 IU/ml) and G3 (50000 IU/ml)with four rabbits in each group. Each group was injected with vitamin D3 subcutaneously as 0.1 mL/kgB.W. weekly for 4 weeks. To assess hematological parameters and liver function, blood samples (3-5ml for each sample) were obtained. Results revealed a significant increase (P≤0.05) in the mean value of (RBC, Hb, PCV, HCT, Lymphocytes, and PLT) in all treated groups as compared to controlgroup, and an increase in WBC was noted in G2 and G3 although this increase was not significant while in G1 there was a substantial drop in the overall leukocyte number. Concerning PLT indices (MPV and PDW) values, there was a statistically marked increase (P≤ 0.05) in G3 while in G1and G2 no significant differences were noticed as compared to the control. In addition, we did not notice any significant difference between the treated groups and the control for red blood cell indices (MCV, MCH, and MCHC). Conclusion: It can be concluded that vitamin D3 in the all doses used in the current study particularly the high dose (50000IU/mlas 0.1 mL/kg.B.W) is safe for therapeutic use for the treatment of anemia and immune deficiency problems

Key words: Vitamin D3, Hematological parameters, Liver enzymes, rabbits.

Vitamin D is a fat-soluble vitamin. It binds to distinct receptors referred to as Vitamin D receptors (VDR) presented in the majority of tissue cells and this binding will produce a variety of biological processes associated with the activity of vitamin D (Christakos, *et al.*,2016). In addition to the most prominent role, which is the control of calcium homeostasis as well as osteogenesis, it also influences cell division and proliferation, the immune function, the neurological system, insulin production, and blood pressure regulation and it is crucial for preventing disorders of the respiratory system, like COVID-19 virus infection. In addition to its vital significance, it serves in the reproductive system's functions and fertility (AL-Hadrawy, 2022).

There are two ways that vitamin D can get into the body: either orally or parent rally through intramuscular injection. Additionally, most mammals may produce vitamin D on their own in the skin with the use of UVB sunlight or synthetic UV lights (Sulaiman, *et al.*,2022). Vitamin D is available in two different forms: cholecalciferol and ergocalciferol (Mertens and Muller, 2010).

Vitamin D3 is synthesized endogenously from 7-dehydrocholesterol following skin exposure to UV light (from sun exposure), then the liver and kidneys convert the produced

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product to 1α, 25-dihydroxycholecalciferol (active vitamin D3 form) (Adams, *et al.*, 2013). It can be retained in plasma, muscle cells, lungs, liver, and adipose tissue (Mawer *et al.*, 1972). The purpose of the study is to examine the impact of vitamin D3 treatment on some hematological and biochemical parameters in male rabbits.

Materials and Methods

Chemicals: Vitamin D3 was obtained as Dorev-it-D3 ampoule, 7.5 mg/1 mL (300,000 IU/ mL), supplied by Pharma Dor Ilaç San.ve Tic. Lit. Şti. Istanbul /Turkey. Doses of Vitamin D3 were prepared and diluted using sesame oil extracted by hydrodistillation, taking into consideration the rabbits› body mass, age, and daily feed consumption over the course of the experiment

Experimental animals: Sixteen local male rabbits, (6-12 months old, 2.5±0.5kg weight) were housed in cages maintained at typical humidity and temperature levels of (40%±10°C) and (21±1°C) respectively, with lighting control (12 h (light) –12 h (dark) cycle). The rabbits received free access to water and standard rabbit pellets at all times throughout a two-weeks before initiation of the experimental protocol and the experiment duration. Procedures involving the care of rabbits were governed by international laws and policies.

Experimental design: The rabbits were randomly distributed equally into four groups (n = 4):

GC or Control group: Rabbits set control with normal diet

G1 group: Rabbits received 10000 IU/ml of vitamin D3 injected subcutaneously as 0.1 mL/ kg.B.W. once in week until completion of the trial

G2 group: Rabbits received 20000 IU/ml of vitamin D3 injected subcutaneously as 0.1 mL/ kg. rabbits B.W., once in week until completion of the trial

G3 group: Rabbits received 50000 IU/ml of vitamin D3 injected subcutaneously as 0.1 mL/k g. rabbits B.W., once in week until completion of the trial

At the end of fourth week, all the rabbits were sacrificed after fourteen hours of fasting. Each rabbit's trunk blood was gathered and stored in EDTA and polymer gel-containing tubes, all the samples were centrifuged at 3000×g for 10 min at 8°C, the supernatant was carefully transferred to microtubes and then kept frozen at -80°Cuntil analysis.

Blood analysis: Blood EDTA samples were tested for complete blood count (CBC) including total red blood cells count, haemoglobin, hematocrit, platelets count, and total white blood cells count using a hematology analyzer (Cobas e411). Blood serum samples were analyzed via (a spine 120analyzers) to estimate the ALT, AST, and ALP enzymes using commercial kits (Biolabo, Maizy, France)

Statistical analysis: Results were expressed as mean ± SD (standard deviation of the mean). Data were analyzed by one-way analysis of variance. The DMK's multiple range test was used as a method to compare the significance between groups.

Results and Discussion

Results of the hematological parameters (WBC, lymphocytes and neutrophils) values of rabbit's blood treated with different doses of vitamin D3 as compared to the control group were summarized in Fig (1). The (RBC, Hemoglobin and hematocrit) values of rabbit's blood treated with different doses of vitamin D3 as compared to the control group were summarized in Fig (2). Fig (3) summarized the effects of erythrocytes indices (MCV, MCH, and MCHC) for rabbit's blood treated with different doses of vitamin D3 as compared to the control group, while Fig (4) summarized the results of platelets and platelet indices (MPV and PDW) for rabbit's blood treated with different doses of vitamin D3 as compared to the control group. Fig (5) shows the effects of supplementation with different doses of vitamin D3 on certain liver enzymes (AST, ALT, and ALP) as compared to the control group.

Hematological profile

Total WBC count: The data confirmed that vitamin D3 did not affect the total number of white blood cellsin G1, G2 and G3 groups as compared with CG ($P \leq 0.05$). Although there was a significant increase ($P \leq 0.05$) in the number of WBC in G3 compared to G1, this may explains an effective anti-body production and better disease resistance by the rabbits exposing to high levels of vitamin D3 (Mbanasor *et al.,*2003),

or it could be due to increased WBC migration to blood from tissues as the underlying mechanism as Bella *et al.* (2017) have reported (Fig 1A).

The results obtained in the present study are following the findings of Asebe *et al.* (2020) who reported that rabbit bucks fed vitamin E supplementation in a 60mg/kg diet decreased the WBC count as compared to the control group. Amaravadhi *et al.*(2012) have also reported a non-significant increase in the total leukocyte count in broiler rabbits fed rations supplemented with probiotics. Another study conducted by Petruška *et al.* (2013), they reported that rabbits treated with different concentrations of quercetin have a non-significant differences values of WBC in comparison with the control.

Lymphocytes: In this study, the rabbits on G2 and G3 have shown a significant increase (P≤0.05) in lymphocyte count ascompared to CG. (Fig 1B). This result is in agreement with (Shinde *et al.,* 2007) who confirmed that the rabbits fed on a diet supplemented with vitamin E may improve their immune responses. Since vitamin D was hypothesized to have multiple functions in the immune system (Bikel, 2009). The elevation of lymphocytes may be possibly due to the ability of vitamin D for proliferation of lymphocytes (Payne *et.al.,* 2012) or it may be due to the activation of lymphoid tissue in response to treatment with vitamin D (Yamamoto and Jørgensen,2020)

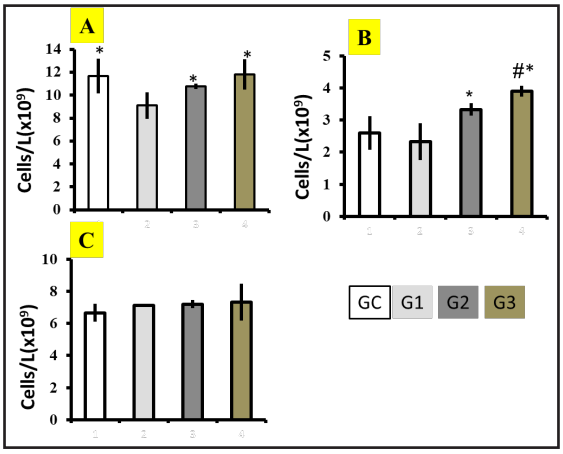

Fig 1. Blood cell counts of the rabbit's blood in different groups (C= control, G1 exposed to 10000IU, G2 exposed to 20000IU, and G3 exposed to 50000IU, single dose, S/C on weekly basis, 4 consecutive weeks). (A) white blood cells, (B) lymphocytes, (C) Neutrophils. Data expressed as mean±SD, *#p>0.05. * as compared to Control and G1. #as compared to Control, G2, and G3.

Neutrophils: Theneutrophils count obtained in this study in all treated groups are slightly higher than that of CG but no significant differences (P≤0.05) were observed between groups. Similar findings of Dawood (2015) who reported non-significant differences in neutrophil in rabbits treated with vitamin C and vitamin B complex. Note that all values were higher than the neutrophils valuesfor healthy rabbits according to Archetti *et al.* (2008), this might imply that the animal might be fighting some infections during the cause of the experiment (Fig 1C). According to a study by (Jankar *et al.*, 2021), vitamin D treatment did not significantly reduce the neutrophil/lymphocyte ratio. This suggests that Vitamin D may have a favorable effect by decreasing inflammation, this suggestion is confirmed by Akbas *et al.*, (2016) who reported that the anti-inflammatory and anti-oxidant role of Vitamin D helps decrease inflammation whereas vitamin D receptors are expressed on the surface of neutrophils and can influence the activity of all types of T cells and modulates the antimicrobial response of these cell types (Payne *et.al.,* 2012).

Total RBC count, hemoglobin, and hematocrit: The results of the present study showed that the average RBC counts, hemoglobin levels, and hematocrit levels increased over the duration of the study in all treatment groups, and also the analysis of the data revealed significant differences (P≤0.05) across groups G1, G2, and G3 as compared with the control group (CG), however no significant differences (P≤0.05) were observed between all treatment groups. This result is in agreement with Asebe *et al.,*(2020) who suggested that the (RBC, Hb, and HCT) values in rabbit bucks fed vitamin E supplementation at different inclusion levels are significantly increased compared with the control group. This is confirmed by other research that rabbits treated with vitamin C and nitrate showed a significant increase in RBC count compared to the group that only consumed nitrate (Alrawi, *et al.,* 2019). Previous research performed by (Al-Kurdy *et al.*,2021) pointed to the treatment of rabbits with ascorbic acid produced a significant increase in RBC However, the same study in disagreement with this finding did not impact any changes in Hb and PCV (Fig 2).

The increase in the (RBC, Hb, and HCT) values might be due to involvement of vitamin D on erythropoiesis as well as the significant effect on these hematological parameters since vitamin D receptors have been detected in the cells of the intestines (Bella, *et.al.,*2017). The impact of vitamin D on hematological parameters may be caused by an increase in gastrointestinal iron absorption and improved iron bioavailability (Lopez *et al.,* 2016). This showed that vitamin D3 stimulated the production of red blood cells from the bone marrow, and improved the oxygen-carrying capacity of the hemoglobin, which could enhance the muscular activities of the rabbits.

Erythrocyte indices: MCV, MCH, and MCHC: The results revealed that The MCV, MCH, and MCHC mean values did not differ significantly (P≤0.05) from one another in all treated groups (G1, G2, and G3) compared to the control (CG). However, in G3 the value of MCH and MCHC were higher than the CG but the increase was not significant. The blood indices values in this study were within the reported physiological range for normal rabbits reported by (Popoiu,*et al*., 2021) (Fig 3).

This result agrees with the findings of (Al-Kurdy *et al*., 2021) who did not impact any change in MCV, MCH, and MCHC values in rabbits treated with vitamin C. Another study was conducted by demonstrating that the mean values of MCV, MCH, and MCHC in rabbits treated with folic acid (vitamin B9) were not significantly different as compared to the control group. (Amaduruonye *et al.,* 2020).

PLT count: The mean values of PLT count were significantly increased (P≤0.05) among the treated groups of rabbits (G1, G2, and G3) compared to those in the control one (CG), G2 recorded significantly the highest values of PLT count followed by G1 and G3 (Fig 4).

This result is coordinated with study of Najim and Mohsen (2022) who observed that the rabbits fed on a diet supplemented with Vitamin B12 showed an increase in PLT count, in a similar study, rabbits fed on dates (fruit enriched with vitamins and minerals) causes significant increase in PLT count (Abdul Ameer and Hassan, 2022).

The PLT values were however lower than the reported physiological range for normal rabbits reported by (Popoiu, *et al*.,2021). The decrease might be due to several factors such as splenic platelet sequestration, immunological processes, reduced levels or activity of thrombopoietin (Peck-Radosavljevic,2000), lung diseases and injuries (Borges, *et al.,* 2017) since the lungs synthesize almost half of all platelets (Lefrançais,2017) or might be due to the hypoproteic diet that rabbits fed (Olayanju,2016).

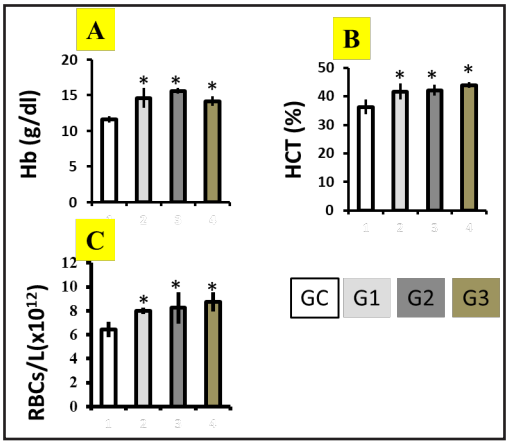

Fig 2. Red blood cell counts and related parameters of the rabbit's blood in different groups (C= control, G1 exposed to 10000IU, G2 exposed to 20000IU, and G3 exposed to 50000IU, single dose, S/C on weekly basis, 4 consecutive weeks). (A) Hemoglobin, (B) Hematocrit, (C) Red blood cells. Data expressed as mean±SD, *p>0.05. * as compared to the control group.

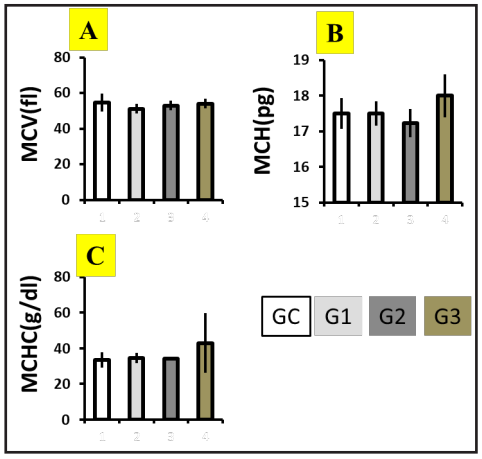

Fig 3. Red blood cell indices and related parameters of the rabbit's blood in different groups (C= control, G1 exposed to 10000IU, G2 exposed to 20000IU, and G3 exposed to 50000IU, single dose, S/C on weekly basis, 4 consecutive weeks). (A) Mean corpuscular volume, (B) Mean corpuscular hemoglobin concentration, (C) Red blood cells. Data expressed as mean±SD.

Platelet indices (MPV and PDW): The G3 group recorded the highest value with a significant increase (P≤0.05) in MPV and PDW as compared to the control (Fig 4). In contrast, the mean values of MPV and PDW in the G1 and G2 groups were virtually identical without significant differences (P≤0.05). The values (including the control) were however higher than the reported physiological range for normal rabbits reported by (Popoiu, *et al*., 2021).

MPV and PDW are common parameters related to the PLT function. MPV is one of the platelet indices and considers a marker of inflammation. The increase in MPV value might be because the platelets that come into the environment in case of inflammation are young, more recently released and larger than average in volume (Çeliker, *et. al.,* 2021); (Engström, *et. al.,* 2014), Nonetheless, Vagdatli *et al.* (2010) stated that platelet activation is typically responsible for the transient elevation of MPV throughout the storage of blood samples because it is related to a reduction in platelet component concentration. Bearing in mind that MPV only refers to the size of platelets, not the actual number of them. PDW serves as a measurement for PLT size variation. The high value of PDW that we observed in the current study might be described as platelet anisocytosis i.e. a large variation in the size of platelets in the blood (Vagdatli, *et al.,* 2010), the inflammatory response and lung damage brought on by oxidative stress is another hypothesis that could apply (Wang,*et al.,* 2022).

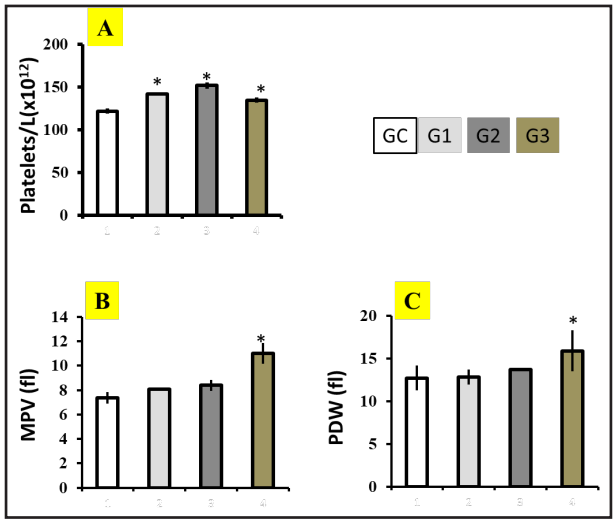

Fig 4. Platelet and related parameters of the rabbits in different groups (control, G1 exposed to 10000IU, G2 exposed to 20000IU, and G3 exposed to 50000IU, single dose, S/C on weekly basis). (A) Platelets, (B) mean platelet volume, (C) Platelet Distribution Width. *p>0.05. * as compared to Control.

Biochemical parameters

The activity of alanine amino transferase (ALT) in blood plasma was significantly lower (P≤0.05) in all treatment groups as compared to CG, the lowest level of ALT was found in G3 followed by G2 than in G1. However, there was no significant difference (P≤0.05) in the activity of a spartate aminotransferase (AST) in G1 and G2 as compared to CG, while in the G3 group the AST enzyme showed a significant difference (P≤0.05) comparing the control one. The results showed a significant decrease (P≤0.05) in alkaline phosphatase (ALK) activity in G2 and G3 as compared to CG, while there was no significant difference (P≤0.05) in G1comparing to CG. These results are similar to the findings of Naseer, *et al* (2020) who point to the protective effect of vitamin E against liver injury in rabbits, they recorded significantly low levels in (AST, ALT, and ALP). Moreover, in a study by Reiter *et al.* (2015), they demonstrated that calcitriol inhibits activation and proliferation of HSCs (source of extracellular matrix proteins deposited in liver-fibrosis) in vitro and Abcb4 (-/-)-mice ameliorate inflammatory liver-damage after 4 weeks of treatment. The values of liver enzymes obtained in the current study were within the physiological range for normal rabbits (Fig 5).

As a general rule, the liver enzymes a spartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) are used as reliable biomarkers for evaluating the health of the liver; they produce more free radicals and experience more oxidative stress as a result of their metabolism, which compromises healthy liver function, increased levels of these enzymes in the blood are a sign of cellular damage (Naik and Vandana, 2008); (Islam *et al*.,2020), on the other hand, when these enzyme levels drop, it shows that the liver functions are stable and in a healthy state (Abdul Ameer and Hassan, 2022). In our study we observed significant decrease in the levels of liver enzymes (ALT, AST, and ALP) this drop of enzymes means that vitamin D3 has a positive effect on liver function through binding with a specific receptors found in the hepatocytes

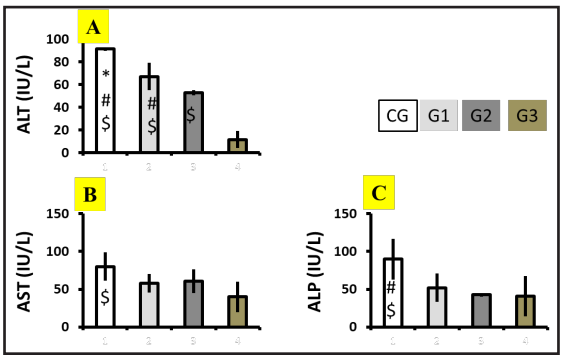

Fig 5. Liver function tests of the rabbit's plasma in different groups (control, G1 exposed to 10000IU, G2 exposed to 20000IU, and G3 exposed to 50000IU, single dose, S/C on weekly basis, 4 consecutive weeks). (A) Alanine Transaminase, (B) Aspartate Transaminase , (C) Alkaline Phosphatase. Data expressed as mean±SD, *#$p>0.05. * as compared to G1, G2, and G3. #as compared to G2, and G3, $ as compared to G3.

(vitamin D receptors VDR), which provides hepatocyte protection and maintaining normal liver function.

Conclusions

The results suggested that supplementation of rabbits with vitamin D3 would be effective in improving the body's general state of health represented by enhancing the hematological parameters such as RBC, HGB, HCT, Lymphocytes, Platelet count, and PLT indices (MPV and PDW) Moreover, Vitamin D3 has a remarkable protective effect on liver function tests represented by low concentrations of liver enzymes (ALT, AST, and ALP). Therefore, the current study concluded that all doses used of vitamin D3 in (particularly 50000IU/ml of vitamin D3 injected as 0.1 ml/kg) are safe for therapeutic use, so we recommend using vitamin D3 as a therapeutic agent for the treatment of various medical disorders, including anemia and immune deficiency problems. However, studies with larger sample sizes and interventions can help to assess the role of vitamin D3 in reducing disease severity or preventing disease.

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