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RESEARCH ARTICLE

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Evaluation of the impact of vitamin D supplementation on the coagulation profile in mice

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ABSTRACT

Background: Vitamin D is an essential molecule involved in several physiological processes and has demonstrated considerable benefit as a medicinal agent. Thus, we aimed to examine the effects of vitamin D supplementation on hematological, coagulation parameters, and liver markers.

Methods: Thirty mice were evenly allocated into three groups: the control group, the vitamin D-treated group, and the heparin-treated group. Following a four-month period, body weight, hematological and coagulation parameters, along with liver enzymes, were assessed and examined utilizing one-way ANOVA.

Results: Data showed that the mice treated with vitamin D had higher body weights than those in the control group. The data also revealed no significant differences in complete blood count parameters between the mice administered with vitamin D, the controls and the heparin group. However, the levels of prothrombin time (PT) and activated partial thromboplastin time (aPTT) were markedly increased in the vitamin D–treated group relative to the controls and the heparin group. In addition, no significant differences were observed in fibrinogen levels between the three groups. The data also showed that vitamin D supplementation had no major impact on alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) levels with a normal liver architecture.

Conclusions: Vitamin D supplementation resulted in prolonged PT and aPTT levels. These findings indicate that vitamin D potentially possesses antithrombotic properties and may be used in thrombotic episodes. A comprehensive assessment of vitamin D impact on the hemostatic system using supplementary indicators for coagulation and thrombosis is necessary.

Keywords: coagulation, liver, thrombosis, vitamin D

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INTRODUCTION

Thrombosis may occur in either veins or arteries and is classified into two types: venous and arterial thrombosis. In veins, it results in deep vein thrombosis and pulmonary embolism, together referred to as venous thromboembolism (VTE) [1]. It frequently results in acute limb ischemia, ischemic stroke, and myocardial infarction in the arteries [1]. VTE is among the most prevalent cardiovascular disorders, manifesting in around 1 in 1000 individuals. The incidence of VTE rises with advancing age and is linked to considerable morbidity and death [2]. Approximately 50% of all stroke-related deaths are caused by ischemic stroke, which is induced by thrombosis [3]. In addition, stroke accounts for the greatest mortality percentage (87%) of cardiovascular disease (CVD) in Asia [4].

Over the past few decades, numerous advancements in diagnostic and therapeutic interventions have resulted in a significant decline in the incidence and mortality associated with VTE, declining from 12.8 to 6.5 per 100,000 individuals [5]. Anticoagulants, such as heparin and warfarin, serve as first-line therapies to treat and diminish the cases of VTE, as well as to mitigate the risk of stroke [6]. They inhibit the production of detrimental thrombi by addressing many stages of the coagulation cascade [7]. However, prolonged administration of anticoagulants is linked to hemorrhagic consequences [1]. They are also linked to a restricted therapeutic window and require regular monitoring [7]. Therefore, it is essential to find an alternative approach to treat and manage thrombosis safely.

Vitamin D is a lipophilic hormone essential for a wide range of physiological activities. It is crucial for maintaining homeostasis of calcium and phosphorus. However, vitamin D insufficiency is also a global health concern, with numerous studies indicating associations between vitamin D insufficiency with various health conditions,

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such as metabolic syndrome, autoimmune disorders, and CVD [8]. In addition, higher amounts of vitamin D have been related to a lower risk of CVD, as individuals with CVD often show low levels of vitamin D [9]. Vitamin D insufficiency is hypothesized to disrupt hemostatic equilibrium, resulting in a prothrombotic profile [10]. Furthermore, vitamin D insufficiency is common in persons with chronic liver illnesses, irrespective of the underlying cause, and the extent of deficiency correlates with the severity of liver dysfunction [11]. This study was conducted to explore the effects of vitamin D supplementation on the coagulation profile and the safety of vitamin D dosage on the liver enzymes in a mouse model.

METHODS

Experiment design

The study has received approval from the National Committee for Bioethics at Taif University (Approval No. 45-238). The experimental research was executed in accordance with Directive 2010/63/EU of the European Parliament and Council. Thirty male BALB/c mice, two months old and weighing between 20 g and 25 g, were obtained from the animal facility at Umm Al-Qura University. The mice were kept in typical rodent cages with woodchip bedding in a spacious, well-ventilated area on a 12 h light cycle and kept at 25°C. During the experiment, all mice were given standard rodent food and tap water. Once they acclimated to their new surroundings, the mice were assigned randomly to three groups, with each group consisting of 10 mice. The first group was the negative control; the mice in this group were not subjected to any interventions. The second group was the vitamin D–treated group. The mice in this cohort received 300 international units (IU) of vitamin D3 daily for two months by intragastric gavage for the duration of the experiment [12]. The mice in the third group were administered a single subcutaneous dosage of heparin at 40 U/kg of body weight, and blood samples were obtained four hours later [13]. The heparin group was used as a measure of induced hypocoagulability in order to assess vitamin D's antithrombotic properties compared to heparin.

Body weight measurement

The body weight of each mouse was recorded at four-week intervals for a period of 4 months using a digital balance (OHAUS, Model: Scout Pro SPU601, Shanghai, China).

Blood collection

Blood samples were collected from each mouse via cardiac puncture (1mL) using a 23- to 25-gauge needle while the mice were anesthetized. Anesthesia was administered intraperitoneally with 2% sodium pentobarbital solution at a dose of 50 mg/kg. Blood was collected in ethylenediaminetetraacetic acid (EDTA) tubes for the measurement of a complete blood count (CBC). Sodium citrate tubes were used to assess the coagulation profile characteristics, while blood was taken in plain tubes to measure liver enzymes.

Estimation of complete blood count (CBC)

Blood samples were obtained in EDTA tubes from each mouse in both groups to evaluate various CBC parameters (red blood cell count, white blood cell count, platelets, hemoglobin, hematocrit) using Beckman Coulter UniCel® D×H 500.

Estimation of coagulation profile

Several parameters of the coagulation profile were estimated, including prothrombin time (PT), activated partial thromboplastin time (aPTT), fibrinogen, and D-dimer. Their levels were quantified in each animal using an automatic coagulation analyzer (a Sysmex CS5100).

Biochemical and histopathological assessments of the liver

Liver function enzymes involving serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) were evaluated using a colorimetric assay (Dimension EXL 200, SIEMENS). The liver was subsequently dissected and preserved in 10% formaldehyde. The section was stained with hematoxylin and eosin.

Statistical analysis

Statistical analysis was conducted using GraphPad Prism software. The one-way ANOVA, followed by Tukey's post hoc test, was employed for all comparisons between the study groups. The data is presented as means ± Standard Error of the Mean (SEM), and the significance level was established at $p<0.05$.

RESULTS

The findings indicated that the mice administered vitamin D exhibited greater body weights (30.58 ± 1.75) than those in the control group (24.98 ± 0.44, Figure 1). The impact of vitamin D supplementation on several hematological parameters was assessed using CBC analysis. The data revealed no substantial variations in red blood cell counts (10.14 ± 0.30, 9.51 ± 0.28, 9.64 ± ± 0.15), hemoglobin (13.38 ± 0.19, 14.06 ± 0.33, 13.20 ± 0.25), or hematocrit levels (45.80 ± 1.59, 46.98 ± 1.59, 46.50 ± 1.59) between the mice administered vitamin D and those in the control and heparin group, respectively. Moreover, vitamin D exerted no substantial effect on white blood cell counts (9.22 ± 0.33), compared with the control (8.96 ± 0.24) and the heparin group (9.35 ± 0.32).

Platelet counts were lower in the mice administered vitamin D (943.00 ± 30.83) and the heparin group (893.20 ± 30.25) relative to the controls (1073.00 ± 28.62), but no significant difference was noted (Figure 2A).

We also evaluated the coagulation profile in both the vitamin D–treated group, the control, and the heparin group (Figure 2B). The findings indicated that PT levels were markedly increased in the vitamin D–treated group (35.24 ± 1.69) relative to the controls (9.66 ± 0.35, $p<0.0001$) and the heparin group (29.66 ± 0.52, $p<0.01$). In addition, the data found that aPTT levels were significantly increased in the vitamin D–treated group (86.58 ± ± 1.67) relative to the control (36.40 ± 0.91) and heparin group (68.94 ± 2.11, $p<0.0001$).

Results revealed significant differences in the levels of D-dimer (1.50 ± 0.08, 1.00 ± 0.07) between the vitamin D group and the heparin group ($p<0.001$, Figure 2B). However, no notable variations were observed in the levels of fibrinogen (148.60 ± 3.58, 166.80 ± 4.70, 138.80 ± 3.52) between the vitamin D group, the control, and the heparin group, respectively (Figure 2B).

Finally, the safety of the vitamin D dose was evaluated on various liver markers. The data showed that there were no significant changes in ALT (66.80 ± 2.29, 75.00 ± 2.24, 66.62 ± 2.65), AST (98.40 ± 2.16, 105.20 ± 1.86, 98.78 ± 1.53), and ALP levels (140.20 ± 5.07, 151.60 ± 13.07, 143.20 ± 5.33) in the vitamin D–treated group, the control, and heparin group, respectively (Figure 3A). A normal liver architecture was also observed in the mice supplemented with vitamin D, with no signs of inflammation or hepatotoxicity (Figure 3B).

DISCUSSION

Thrombosis is a significant health issue associated with a considerable rate of morbidity and mortality. Vitamin D has emerged as a crucial regulatory element in various physiological functions and has been demonstrated to have significant potential as a prognostic and therapeutic agent. A meta-analysis indicates that patients randomized to have vitamin D supplementation, at an average daily dosage of 528 IU, have a statistically substantial decrease in all-cause death during an average follow-up period [14]. Furthermore, a previous investigation indicates that vitamin D insufficiency is correlated with a high risk of thrombosis [15]. Thus, we aimed to investigate the efficacy of vitamin D supplementation on the coagulation and liver markers in a murine model.

The total RBC counts in mice vary from 7 to 10 million cells per microliter. The white blood cell count in mice ranges from 2,000 to 10,000. Mice possess elevated

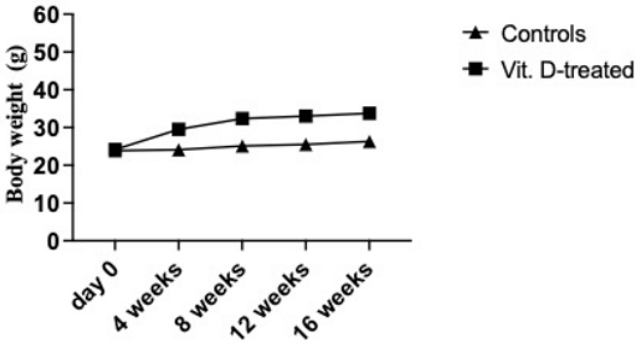

Figure 1. Assessment of body weight in mice groups

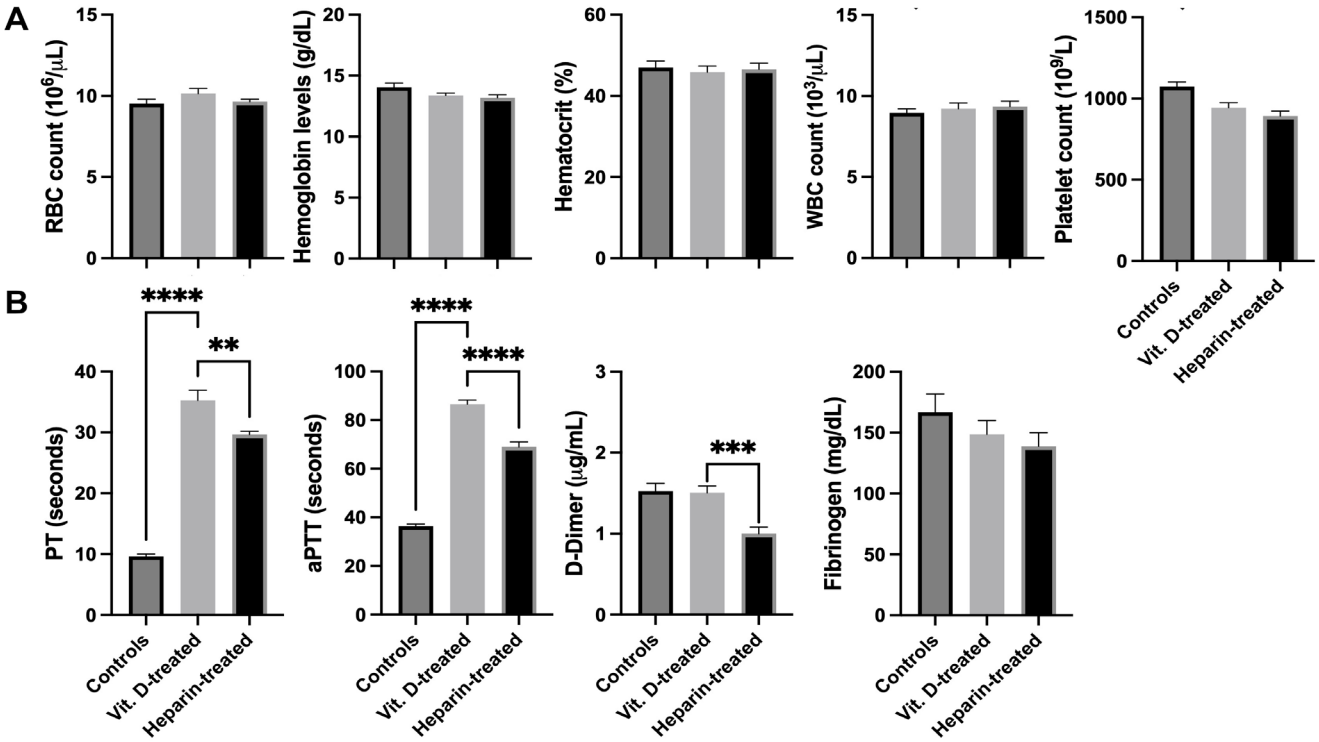

Figure 2. CBC (A) and coagulation (B) parameters in mice groups. Significance: $^{**}p\leq0.01$, $^{***}p\leq0.001$, $^{****}p\leq0.0001$

platelet counts, ranging from 900,000 to 1,600,000. We have demonstrated that vitamin D had no significant impact on red blood cell count, white blood cell count, or platelet count. However, our findings have demonstrated that vitamin D supplementation led to prolongation of PT and aPTT, compared with the controls. These findings suggest that vitamin D induced hypocoagulability, so it has antithrombotic properties compared to the heparin group. In BALB/c mice, it should be noted that the median value for PT and aPTT is 8.30 and 38.35 seconds. The fibrinogen level in mice is 154 mg/dL. It was previously reported that vitamin D receptor (VDR) knockout mice exhibited prothrombotic conditions [15]. Another study suggested that high doses of vitamin D supplementation were associated with diminished thrombin generation and reduced clot density, suggesting that vitamin D supplementation mitigates the prothrombotic profile [16]. The anti-thrombotic properties of vitamin D within the coagulation system were previously characterized [17]. It is well documented that thrombosis is induced by factors such as fibrinogen, tissue factor (TF), prothrombin, and platelets, whereas antithrombin, heparin, thrombomodulin, and proteins C and S inhibit clot formation [9]. The principal hypothesized mechanisms for the antithrombotic impact of vitamin D encompass the upregulation of thrombomodulin and the downregulation of TF [9]. A study has shown a correlation between vitamin D levels exceeding 20 ng/mL and prolonged PT, which signifies the antithrombotic function of vitamin D [17]. By contrast, another study reported that vitamin D administration elevated blood 25-(OH)D levels without affecting coagulation markers [17]. Meanwhile, another study suggested that vitamin D supplementation produced prothrombotic activity [15]. It should have been suggested that the features of the animal (strain, sex, age), along with the kind of anticoagulant and post-collection methods (centrifugation speed, temperature, bench time), significantly influence hematology and blood chemistry. The sample location can significantly affect blood chemistry and cell count [18]. Given the promising antithrombotic role of vitamin D, it should be noted that a more comprehensive evaluation of the impact of vitamin D on the coagulation system is recommended, including additional platelet function tests, biomarkers of thrombosis and inflammation to directly assess thrombus formation.

The liver is a pivotal organ in homeostasis, and it produces most plasma proteins, including pro- and anticoagulant factors, and the thrombopoietin hormone [19]. Therefore, validating the safety of vitamin D on the liver is essential. In this study, we showed that vitamin D administration had no notable cytotoxicity in the liver. Histologically, the liver appears normal in mice supplemented with vitamin D, with no sign of inflammation or cytotoxicity. In addition, vitamin D had slightly reduced the levels

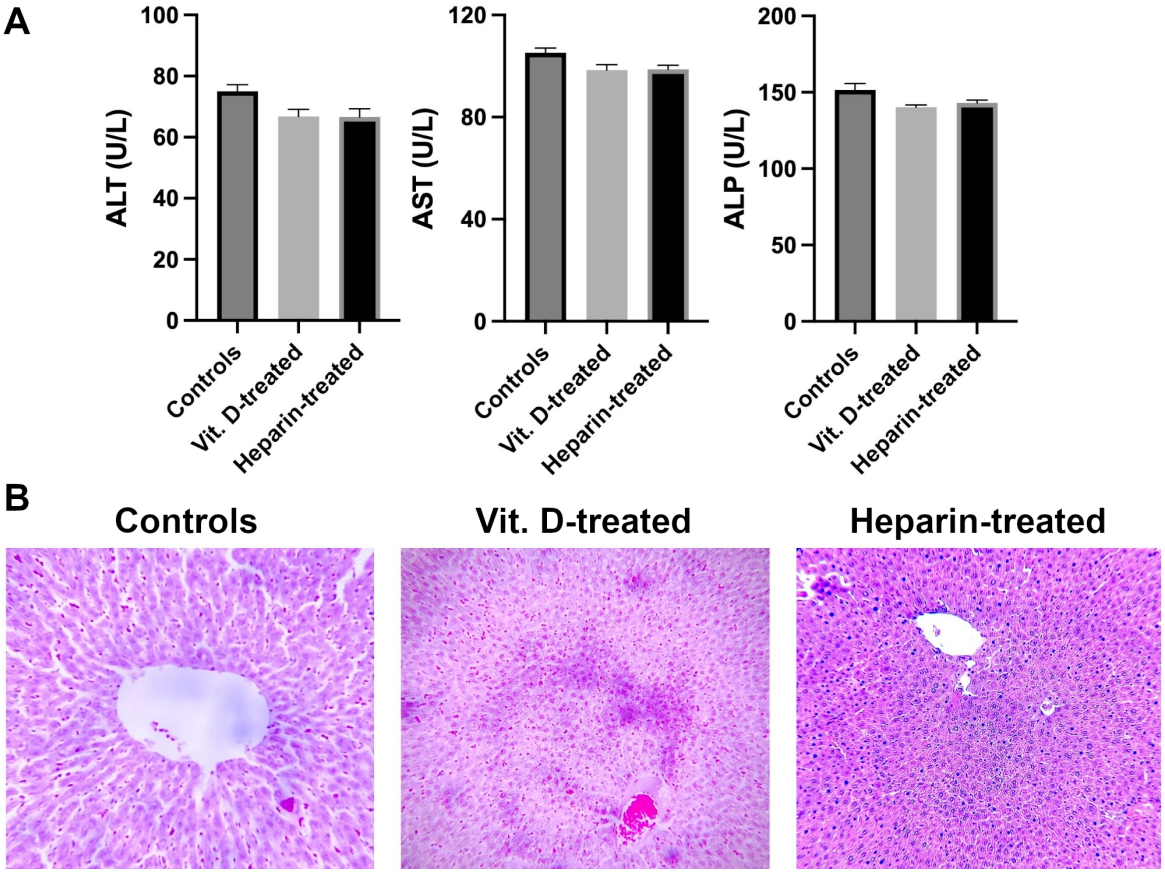

Figure 3. Biochemical (A) and histopathological (B) evaluation of the liver in mice groups

of ALT, AST, as well as ALP but this was not significant. This is in agreement with a previous report indicating that vitamin D administration (2000 IU for 6 weeks) reduces liver enzyme levels in patients with non-alcoholic fatty liver disease (NAFLD) [20]. Recently, a study found that vitamin D improves liver disorders and indicates that the vitamin D/ thioredoxin-interacting protein (TXNIP) axis is a potential therapeutic way for managing liver diseases [21]. In monocytes/macrophages, vitamin D suppressed the synthesis of pro-inflammatory cytokines, including IL-6 and TNF-α [17]. In the liver, vitamin D has anti-fibrotic properties by suppressing the expansion of hepatic stellate cells and downregulating pro-fibrotic mediators, including platelet-derived growth factor (PDGF) and TGF-β [22]. Vitamin D administration enhanced liver enzyme levels, likely via modulating the production of enzymes associated with glucose metabolism [23]. Consequently, mitigating liver inflammation and enhancing mitochondrial activity may aid in the prevention of oxidative stress and damage to hepatic cells [23].

This study has some limitations. A mouse model was used to evaluate the potential effects of vitamin D, so future studies should aim to test vitamin D in human interventions. In addition, the study lacked an experimentally induced thrombosis model, which would directly assess the potential antithrombotic activity of vitamin D. Furthermore, using more coagulation and liver markers would also provide more comprehensive insights.

CONCLUSIONS

Vitamin D supplementation prolonged PT and aPTT in mice as compared with the control and heparin groups. These data revealed that vitamin D has antithrombotic activity and may be used to treat thrombotic events. However, a more thorough evaluation of the impact of vitamin D on the hemostatic system using additional markers for coagulation and thrombosis is required.

ABBREVIATIONS

ALP – alkaline phosphatase

ALT – alanine aminotransferase

aPTT – activated partial thromboplastin time

AST – aspartate aminotransferase

CVD – cardiovascular disease

NAFLD – non-alcoholic fatty liver disease

PDGF – platelet-derived growth factor

PT – prothrombin time

TF – tissue factor

TXNIP – thioredoxin-interacting protein

VDR – vitamin D receptor

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AUTHORS' CONTRIBUTION

MA – conceptualization, methodology, analysis, data curation, writing.

CONFLICT OF INTEREST

None to declare.

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