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Possible Effects of Vitamin D3 and Levofloxacin on Selected Hematology Parameter of Rats

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Abstract

Vitamin D is a fat-soluble vitamin with antioxidant and DNA protecting properties, Levofloxacin is a member of the fluoroquinolone drug class, its broad-spectrum bactericidal effect affects both Gram-positive and Gram-negative bacteria.

The goal of the study is to analyze the hematology analysis in rats that received levofloxacin and show the preventive impact of vitamin D3 by analyzing the hematology parameters: packed cell volume (PCV), mean corpuscular hemoglobin concentration (MCHC), hemoglobin (HB), red blood cell (RBC), mean corpuscular volume (MCV), meancorpuscular hemoglobin (MCH), WBC, differential WBC, and Platelets.

The study included 42 rats divided into 6 groups each group 7 rats. group I negative control : healthy animals received normal saline 0.9%, group II: received levofloxacin LFX dose of 50mg/kg/day (IP) for fourteen days, group III : received LFX 100mg/kg/day (IP) for fourteen days, group IV : received vitamin D3 500 IU/day orally by oral gavage for twenty one days, group V received vitamin D3 500 IU/day orally for twenty one days and levofloxacin 50 mg/kg/day IP injected at day 8 for fourteen days, group VI received vitaminD3 500IU/day for twenty one days orally and levofloxacin 100 mg/kg/day IP injected at day 8 for fourteen days.

Blood samples taken from rats treated with levofloxacin, showed a decrease in the values of RBC, HB, PCV, MCH, MCHC, as well as a decrease in the total white blood cells, then returned approximately to normal levels in the group V and group VI.

From the results, the study concludes that some hematological changes caused by levofloxacin ameliorated by vitamin D3 may be due to indirect effect of vitamin D3 on hematology parameters.

Keywords: Levofloxacin, vitaminD3, Hematology, Anemia, Rats.

التاثيرات المحتملة لفيتامين د٣ والليفوفلوكساسين على عينة دم مختارة للجرذان#

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الخلاصة

فيتامين د٣ هو فيتامين قابل للذوبان في الدهون وله تاثيرات واقيه من الحامض النووي ومضادات الاكسدة مع فئة من الادويه المعروفة باسم الفلوروكوينولونات الليفوفلوكساسين واسع الطيف للجراثيم الذي يعمل على البكتريا موجبة الجرام وسالبة الجرام

الهدف من الدراسة المقدمة هو تحليل معايير امراض الدم المختارة (حجم الخلية المعبئه,معدل تركيز الهيموكلوبين الجسيميه,هيموكلوبين الدم, كريات الدم الحمراء,متوسط حجم الكريات,هيموكلوبين الكريه الوسطي و وكريات الدم البيضاء وكريات الدم البيضاء التفضلي والصفائح الدمويه) لتقييم تحليل امراض الدم لدى الجرذان الذين تم حقنهم بعلاج الليفو فلوكساسين واظهار التاثير الوقائي لفيتامين د٣ على معايير الدم المختارة .

اشتملت الدراسة على ٤٢ جرذا مقسمة الى ستة مجاميع المجموعة الاولى تم حقنها داخل الصفاق ب٠,٥مل من محلول ملحي عادي من كلوريد الصوديوم مرة واحدة يوميا لمدة ٢١ يوما تم تقديم هذه المجموعة كمجموعة تحكم وعولجت المجموعة الثانية بجرعة واحدة من الليفوفلوكساسين ٥٠ملغ لكل كيلوغرام في اليوم الواحد داخل الصفاق لمدة ١٤ يوما, المجموعة الثالثة تم علاج الجرذان بجرعة وحيدة من الليفوفلوكساسين ١٠٠ملغ لكل كيلوغرام داخل الصفاق لمدة ١٤ يوما والمجموعة الرابعة كانت الجرذان تعالج بجرعة وحيدة من فيتامين د٣ (٥٠٠ وحدة دوليه لكل جرذ يوميا) عن طريق الفم لمدة ٢١ يوما , المجموعة الخامسة عولجت الجرذان بفيتامين د٣ ٥٠٠ وحدة دولية يوميا عن طريق الفم لمدة ٢١يوما مع الليفوفلوكساسين ٥٠ملغ لكل كيلوغرام عن طريق حقنها داخل الصفاق في اليوم الثامن لمدة ١٤ يوما , المجموعة السادسه عولجت الجرذان بفيتامين د٣ ٥٠٠ وحدة دوليه يوميا لمدة ٢١ يوما مع الليفوفلوكساسين ١٠٠ملغ لكل كلغ عن طريق الحقن داخل الصفاق في اليوم الثامن لمدة ١٤ يوما.

تم اخذ عينات الدم من الجرذان المعالجة ب الليفوفلوكساسين اظهرت انخفاضا في معدل كريات الدم الحمراء ,الهيموكلوبين الدم ,حجم الخلية المعباة ,هيموكلوبين الخليه , تعداد الهيموكلوبين الخلوي وكذالك انخفاضا في كريات الدم البيضاء والصفائح الدمويه ثم عادت بصورة تقريبيه الى مستوياتها الطبيعيه في المجموعات الخامسة والسادسة .

لخصت نتائج الدراسة إلى أن بعض التغيرات الدموية التي يسببها الليفوفلوكساسين تعالج بفيتامين D3 قد تكون بسبب التأثير غير المباشر لفيتامين D3 على معايير أمراض الدم.

الكلمات المفتاحية: الليفوفلوكساسين , فيتامين د٣ ،علم الدم ، فقر الدم ، جرذان

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Introduction

Fluoroquinolones are a class of antibiotics derived from the basic structure of nalidixic acid، which was discovered in the early 1960s [1]. Fluoroquinolone generations have been created as a result of additional quinolone molecular replacement [2]. This class of medications is required for the treatment of severe gram-negative infections [3]. levofloxacin، a third-generation fluoroquinolone [4]، fluoroquinolones are one of the antibiotic types that are most frequently prescribed to both humans and animals. For a number of infections، such as genitourinary infections and lung infections، this class of antibiotics is the first line treatment; Levofloxacin is broad spectrum antibiotics that are delivered or re-distributed to these places by the blood and then reach therapeutic levels in many bodily secretions، including articular fluids. These medications are therefore highly pertinent to clinical practice today [5] compared to other antibiotic medication classes; fluoroquinolones are more likely to cause serious side effects. The majority of side effects are mild to severe[6]. levofloxacin has been documented to aggravate myasthenia gravis، rhabdomyolysis، and spontaneous tendon ruptures[7][8] . Due to the serious side effects associated with fluroquinolones، such antibiotics are unsuitable in elderly، pediatric، pregnant، or lactation individuals unless for very severe bacterial infections[9][10]. To evaluate potential hematological alterations that might be seen after giving rats levofloxacin antibiotics this study was conducted.

Vitamin D3 fat soluble vitamin that has antioxidant and DNA-protective properties، and it can control the maturation of erythrocytes، which controls the production of red blood cells (RBCs)، as well as iron metabolism and hemoglobin synthesis [11] [12]. In addition، vitamin D has a number of physiological processes [13]. Clinical evidence suggests that vitamin D may play a role in erythropoiesis. There are many clinical studies that look at how 1-25(OH)D deficiency affects blood hemoglobin (Hb) levels [14]. Anemia and decreased hemoglobin levels، according to Patel et al.، are independently linked to 25 hydroxy vitaminD and 1،25 dihydroxy vitaminD deficiency in people with chronic kidney disease [15]. Despite the fact that anemia and vitamin D appear to be related، the exact mechanism is unknown. However، the majority of studies examining the relationship between renal anemia level، vitamin D supplementation، and deficiency emphasize the pervasive importance of inflammation in the underlying mechanisms [16] [17].

The vitamin D receptor (VDR)، which is expressed by immune cells، is involved in the control of innate and adaptive immunity، study conducted *in vitro* and *in vivo* have shown that calcitriol decreases cytokine production Interleukin-10 (IL-10) is released lymphocytically when VDR is activated، and this increases the production of inflammatory cytokines in stromal and accessory cells [18]، while also having anti-inflammatory and proliferative effects on erythroid progenitors. Another theory is that calcitriol directly activates erythroid progenitors because vitamin D has been shown to have an impact on bone marrow function [19]. Additionally، the active form of vitamin D، 1،25 hydroxyvitamin D (1-25(OH)2D)، is present in bone marrow at levels that are hundreds of times higher than in plasma. According to research by Aucella et al.، giving patients with end stage renal disease (ESRD) 1،25(OH)2D boosted burst-forming unit erythroid proliferation. Both in vitro and in vivo studies have shown that calcitriol has a direct impact on the proliferation of erythroid precursors and that it has a synergistic effect with epoetin alfa [20].

Vitamin D receptors have been discovered in a variety of non-renal target tissues، including bone marrow. Restoring normal tissue 25(OH)D levels may provide a sufficient substrate for local 1،25(OH)2D synthesis in hematopoietic tissues via extra-renal tissue activity of the enzyme 1-alpha hydroxylase. It has been demonstrated that hematopoietic cells، which include erythroid precursors، fibroblasts، endothelial cells، lipid-laden cells، and macrophages، have significantly higher levels of 25(OH)D and 1،25(OH)2D than bone marrow plasma [21] High local concentrations of 1،25(OH)2D in hematopoietic regions may then result in direct paracrine activation of erythroid precursor cells.

Objective

This study is designed to study the effects of levofloxacin and vitamin D3 on selected hematology parameters in rats.

Materials and Methods

Chemicals and Drugs

Levofloxacin 750mg/30ml from (Bader pharma/ Egypt)، vitamin D3(10000IU/ml) oral drop from (ABIOGEN Pharm a، Italy); 0.9% Normal Saline 100ml I.V Infusion (Pioneer، Iraq).

Experimental animals

42 white Albino rats of both sexes، weighing 150-250 grams، in a climate-controlled environment. The rats were housed in polycarbonate cages and kept in a room with a humidity of 60±5 percent and a set temperature of 22 c◦ commercial pellets were given to the rats، and a 12/12-hour light-dark cycle was maintained throughout the experiment، the study was approved by the Scientific- and the Ethical Committees of the College of Pharmacy/ University of Baghdad.

Experimental protocol

Following two weeks of adaption، rats were randomly placed into six groups (seven rats/each group). group I negative control : healthy

animals received 0.5ml normal saline ، group II positive control: received levofloxacin (LFX) dose of 50mg/kg/day intraperitoneally (IP) for fourteen days[22]، group III positive control : received LFX 100mg/kg/day intraperitonially for fourteen days[23]، group IV negative control : received vitamin D3 500 IU/day[24] orally by oral gavage for twenty one days، group V received vitamin D3 500 IU/day orally for twenty one days and levofloxacin 50 mg/kg/day IP injected at day 8 for fourteen days، group VI received vitaminD3 500IU/day for twenty one days orally and levofloxacin 100 mg/kg/day IP injected at day 8 for fourteen days.

Laboratory Analysis

After the course of treatment was complete، blood was drawn from the rats by slitting their carotid arteries، two milliliters of blood were then drawn and placed in an EDTA tube with rolling to prevent blood clots. the blood was then sent for evaluation of analysis [red blood cell (RBC) counts، hemoglobin، hematocrit، mean corpuscular volume (MCV)، mean corpuscular hemoglobin (MCH)، white blood cell (WBC) counts، differential white blood cells and platelet numbers] by using Auto Hematology-Analyzer(BC-30-Mindray،china)[25] .

Statistical analysis

Data were presented as mean and standard deviation values (mean ±SD). The computerized IBM SPSS statistics 25.0 application was used to examine the data. One-way analysis of variance (ANOVA) and kruskal wallis. is used to assess whether the differences between different groups are statistically significant When P value was less than 0.05 (P<0.05)، statistically significant differences were taken into consideration. Graph Pad Prism 7.04 was used to develop the Figures.

Results

The results of hematological changes can be summarized in Figure 1 and Table 1، Regarding the levels of (PCV، HB، MCHC) declare non-significant difference changes (P>0.05) in groups of rats that were injected with levofloxacin (50mg/kg/day) (Group II) compared to the levels in the negative control (Group I) rats

Similarly، non-significant (*P*>0.05) was observed in (PCV ، HB، MCHC) level in group of rats IP injected with levofloxacin (100mg/kg/day) (Group III) compared to the corresponding level in the negative control (Group I) rats ، in addition there were no statistical significance difference was observed (P>0.05) in rats of (Group IV) compared to negative control (Group I) rats ، moreover (Group V) showed statistically non-significant elevation in(PCV،HB،MCHC) levels (P>0.05) compared to the corresponding level in rats treated with levofloxacin(50mg/kg/day) alone (Group II) ; Furthermore there is a non-significant elevation in (Group VI) showed non-significant difference compared to the corresponding level in levofloxacin100mg-treated alone(Group III) ، and Group V showed non-significant elevation compared to Group VI.

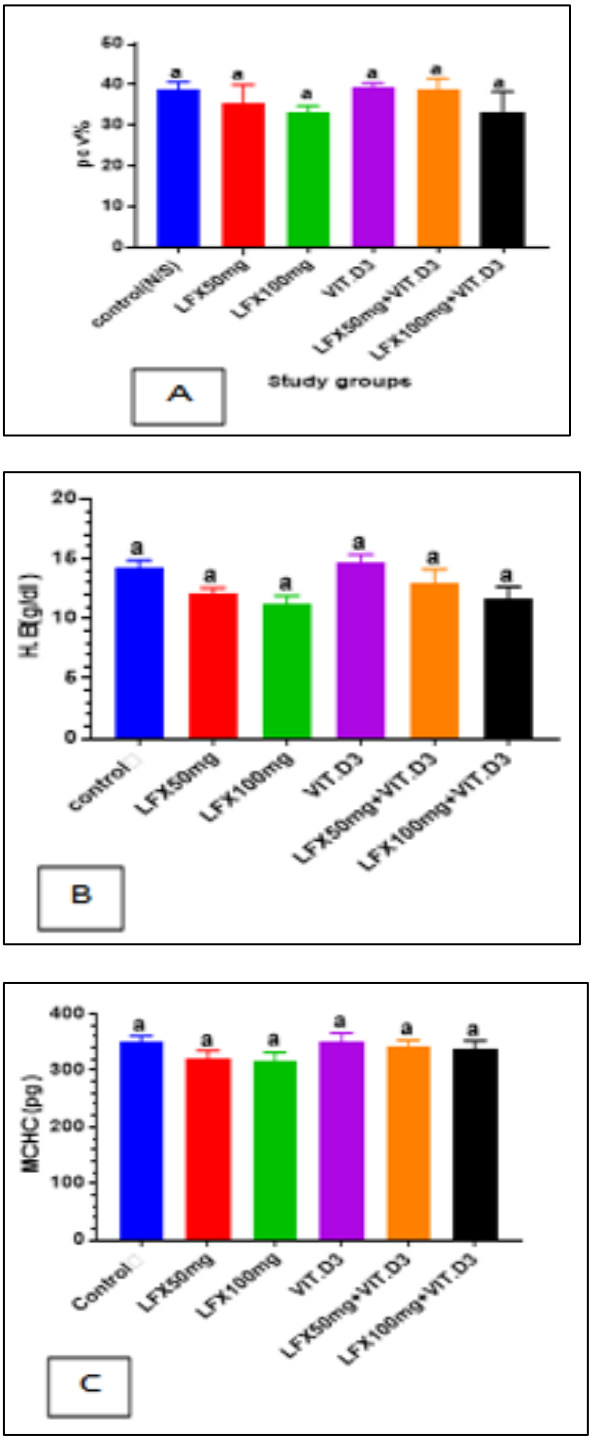
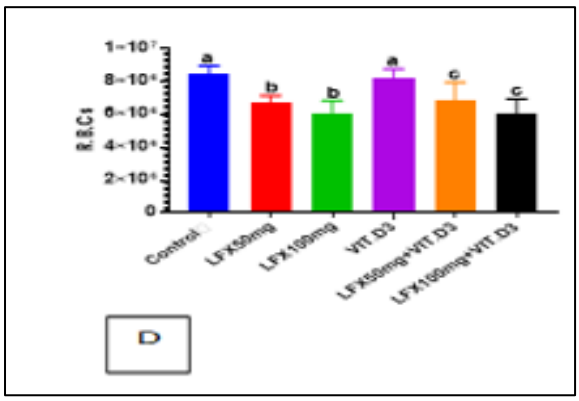
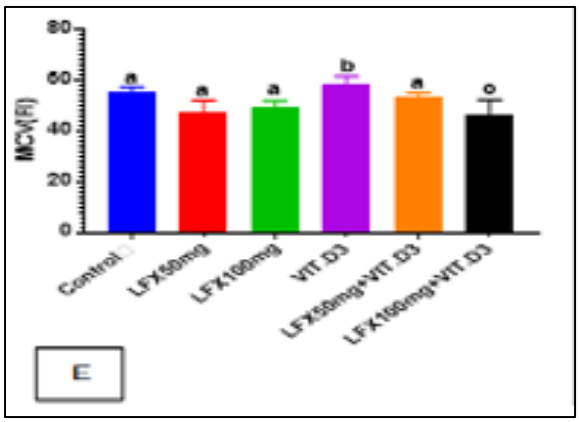


Figure 1. The difference in PCV، HB، MCHC level across the study groups showed non-significant (p>0.05) difference among the groups according to ANOVA، A: show difference in PCV، B: show difference in HB، C: show difference in MCHC.

-Value expressed in identical small letters (a) are non-significantly different (P>0.05).

In addition، the levels of RBC، MCV، MCH show a significant difference (*P*<0.05) of rats that were treated with a dose of levofloxacin (50mg/kg/day) once daily (Group II) for R.B.C but non-significant difference for MCV، MCH compared to the corresponding level in control Group I rats. Similarly significant reduction (P<0.05) was observed in RBC، MCH except for MCV level in group of rats I.P injected with a dose of levofloxacin (100mg/kg/day) once daily Group III compared to the corresponding level in negative control group Group I rats as shown in table 1، Figure 2. Group IV show that there were statistically non significance differences observed (P<0.05) in (R.B.C) and significant difference in(MCV)،MCH) levels in rats of (500IU/rat/day) of vitamin D3 compared to negative control (Group I) rats.Animals treated with (500IU) vitamin D3 and levofloxacin (50mg/kg) once a day Group V showed statistically non- significant elevation in RBC ،MCV and MCH levels (P>0.05) compared to the corresponding level in rats treated with levofloxacin(50mg/kg/day) alone Group II، Table 1،Figure2 Group VI demonstrate a significant difference in MCV ، ،MCH and non-significant in RBCof rats receiving (500IU) of vitamin D3 and levofloxacin (100mg/kg/day) once daily compared to the corresponding level in levofloxacin100mg-treated alone (Group III)،. Furthermore، group V showed non-significant elevation compared with group VI.





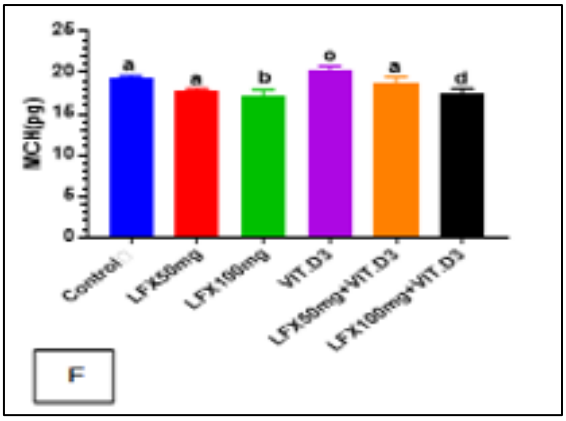


Figure2. The difference in R.B.C، MCV، MCH level across the study groups، D: show difference in R.B.C E: show difference in MCV، F: show difference in MCH.

-Value expressed in identical small letter (a) non-significant difference.

-Values expressed in non- identical small letters (a،b،c and d) are significantly different (P<0.05).

Moreover the animals declare non - significant elevated difference (*P*>0.05) of rats (neutrophil، lymphocyte، monocyte، eosinophil) level that were treated with levofloxacin once daily (Group II) compared to negative control (Group I) rats Similarly no significant elevation (P>0.05) was observed in group of rats treated with a levofloxacin (100mg/kg/day) once daily (Group III) compared to negative control (Group I) rats،table 1 and figure 3. According to Table 1 resulted in statistically significant differences (p<0.05) in (Neutrophil، Lymphocyte، Monocyte، and Eosinophil) levels in rats of Group IV compared to (Group I) rats negative controls group ، Animals treated with Group V showed statistically significant elevation in (monocyte ، Eosinophil) levels (P<0.05) and non-significant reduction in (neutrophil and lymphocyte) compared to Group II ;show Table 1 According to Table 1 revealed a significant reduction in (neutrophil ، monocyte) and non-significant with (lymphocyte، eosinophil). (P<0.05) of rats treated with Group VI compared to Group III، furthermore Group V showed significant reduction in neutrophil، lymphocyte and elevation of monocyte and eosinophil compared to group VI.

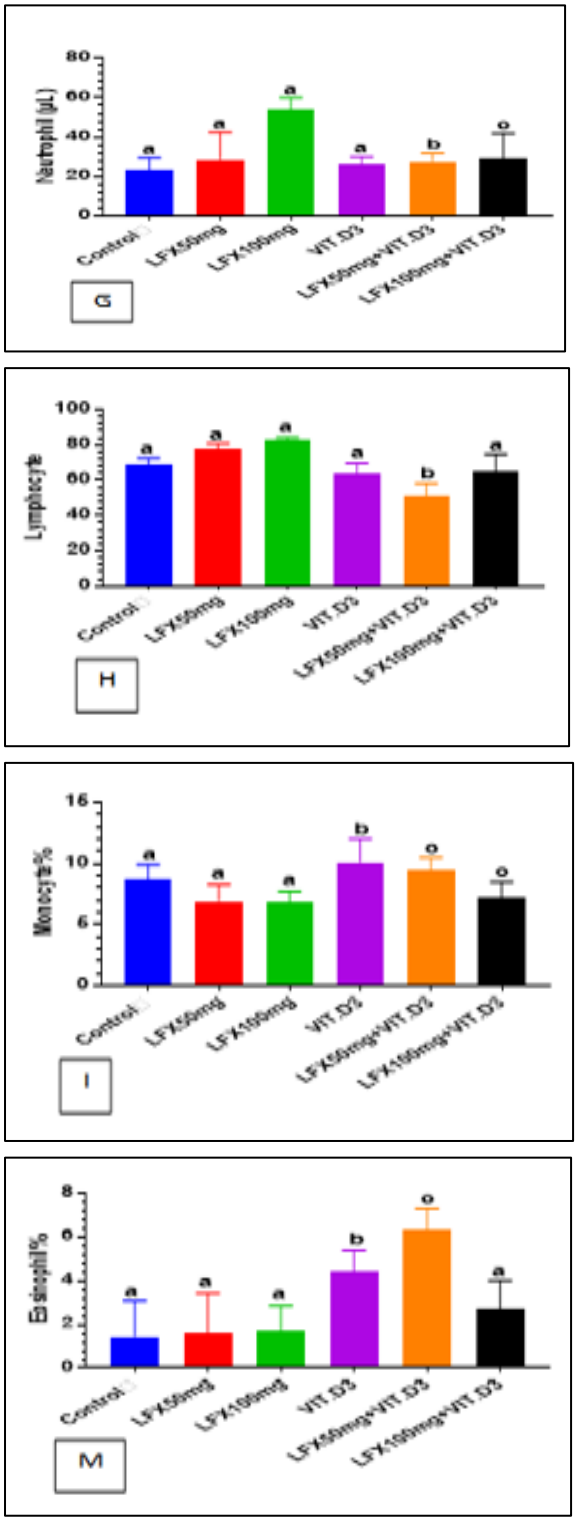


Figure 3. The difference in the Neutrophil، Lymphocyte، Monocyte، Eosinophil level across the study groups، G: show difference in neutrophil، H: show difference in lymphocyte، I: show difference in monocyte، M: show difference in eosinophil.

-Value expressed in identical small letter (a) non-significant difference.

-Values expressed in non- identical small letters (a، b، c) are significantly different (P< 0.05).

The levels of Platelets and W.B.C showed non-significant difference (*P*> 0.05) in Group II (reduction platelets and elevation WBC) comparing with the corresponding level in the negative control Group I rats in addition non-significant difference (P>0.05) was observed in platelets، WBC in Group III compared to the corresponding level in the negative control (Group I) rats as shown in table 1 and Figure 4. In (Group IV) there were no statistically significance differences observed (P>0.05) in levels of platelets and WBC in comparison with the negative control (Group I) rats، Table 1، Animals treated in (Group V) showed statistically non-significant reduction in W B C and elevation in platelets levels (P>0.05) compared to the corresponding level in the (Group II) as show in Table 1According to Table 1 showed non-significant elevation in platelets and reduction WBC. (P>0.05) of rats with the (Group VI) compared to (Group III) rats.

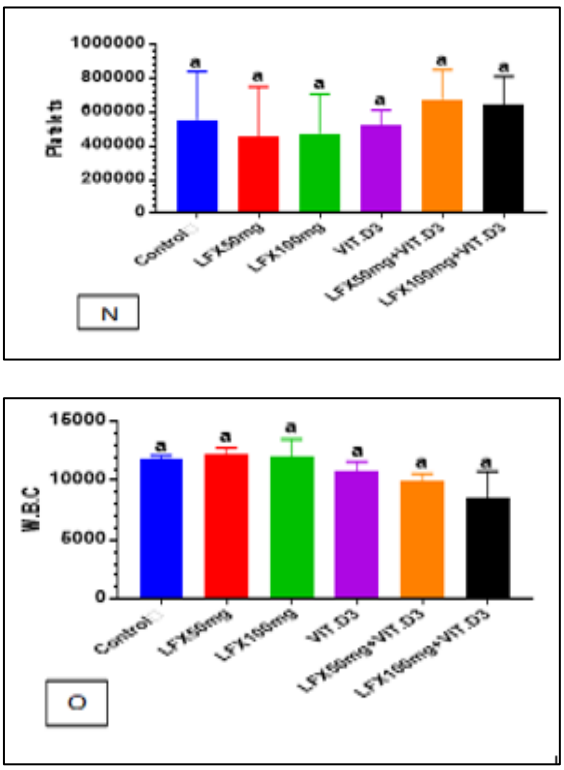


Figure 4. The difference in the platelets، W.B.C level across the study groups (non-significant difference p-value > 0.05 among the study group)، N: show difference in platelets، O: show difference in W.B.C.

-Values expressed in identical small letter (a) non- significant difference.

Table 1. The hematology analysis in different groups of rats

	Group I N=7	Group II N=7	Group III N=7	Group IV N=7	Group V N=7	Group VI N=7
P.C.V%	39.99±3.1 8 [a]	35.27±4.86 a	33.96±3.46 [a]	39.414±3.14 [a]	38.94±5.56 [a]	33.52 ±4.784 [a]
HB g/dl	14.27± 1.19 [a]	12.05±1.59 [a]	11.25±1.15 [a]	14.63±0.71 [a]	13.01±1.64 [a]	11.643±1.0245 [a]
MCHC pg	353.29±7.95 [a]	322.84±9.97 [a]	317.71±11.64 [a]	351.00±15.78 [a]	344.73±13.93 [a]	338.29±14.874 [a]
R.B.C µL	7095000±1070000 [a]	6465000±28327 [b]	6160000 ±3045000 [b]	68100±1420000 [a]	6755000±12975500 [c]	6545000 ±1160000 [c]
MCV FI	53.700±4.8 [a]	51.700±5.6 [a]	52.900±3.3 [a]	53.750±4.3 [b]	53.700±3.8 [a]	52.950±3.8 [c]
MCH pg	19.35±1.9 [a]	±1.8 17.65 [a]	17.18±2.3 [b]	20.30±1.9 [c]	18.82±4.0 [a]	17.60±2.1 [d]
Neutrophil µL	23.52±15.8 [a]	28.71±16.2 [a]	53.61±13.8 [a]	25.84±10.9 [a]	27.55±18.3 [b]	29.17±15.8 [c]
Lymphocyte µL	69.00±23.1[a]	77.64±14.9 [a]	82.52±35.7 [a]	64.04±8.4 [a]	46.58±10.5 [b]	67.84±10.8 [a]
Monocyte µL	8.74±3.6 [a]	6.94±2.9 [a]	6.90±2.4 [a]	10.11±1.9 [b]	9.42±2.1 [c]	7.27±2.5 [c]
Eosinophil µL	1.44±4.6 [a]	1.61±3.9 [a]	1.70±4.3 [a]	4.44±5.1 [b]	6.35±6.0 [c]	2.71±1.6 [a]
Platelets µL	556429±242500 [a]	457286±305250 [a]	475714±435250 [a]	524571±283000 [a]	679286±787250 [a]	641286±581000 [a]
W. B. C µL	11571.4±3175 [a]	12028.6±3750 [a]	11714.3±4150 [a]	11200±3925 [a]	9514.29±3800 [a]	9542.86±4050 [a]

N= Number of animals in each group.
- Each value represent as Mean ±standard deviation (M±SD) for normally distributed and median ±interquartile range for not normally distributed .
-Values expressed in identical small letter (a) non- significant difference
-Values expressed in non- identical small letters (a، b، c and d) are significantly different (*P*<0.05).

Discussion

Different drugs effects on hematology parameters by different mechanisms and this study was designed to highlight the effects of vitamin D3 on hematology parameters. as shown in table 1، In this study، the hematology of rats treated with levofloxacin revealed significant ($p<0.05$) decline of the measured hematological parameters the (PCV، HB and MCHC) level declare non- significant difference ($P>0.05$) level of rats in Group II compared with the negative control (Group I) rats، Similarly، non-significant ($P>0.05$) decline was observed in (PCV، HB and MCHC) level in Group III compared to corresponding level in negative control Group I rats . in addition، there were no statistical significance differences observed ($P>0.05$) in (PCV، HB، MCHC) levels in rats of Group IV compared to control (Group I) rats. Animals treated with Group V showed statistically non-significant elevation in (PCV، HB and MCHC) levels ($P>0.05$) compared to the rats treated with Group II. Furthermore، there are a non-significant elevation in (PCV، HB and MCHC). ($P>0.05$) of rats in Group VI showed non-significant difference compared to the Group III and group V showed non -significant elevation compared to group VI. These results consistent with that observed in previous studies [26][27] Although levofloxacin has been proven to have certain negative effects on blood parameters in rats، there is no obvious mechanism by which it reduces levels of packed cell volume (PCV)، hemoglobin (HB)، and mean corpuscular hemoglobin concentration (MCHC). Some studies show that the decrease in PCV، HB، and MCHC may be attributable to levofloxacin's ability to inhibit bone marrow [28]. Bone marrow suppression is defined as a decrease in the synthesis of blood cells in the bone marrow، which can lead to a drop in the amount of red blood cells، resulting in a fall in PCV، HB، and MCHC [29] Other research suggests that levofloxacin may cause oxidative stress in the body، resulting in the loss of red blood cells and a drop in PCV، HB، and MCHC levels. This technique involves the production of reactive oxygen species (ROS)، which can damage and destroy red blood cells' cell membranes [30]. While several studies have demonstrated levofloxacin's effects on blood parameters in rats، more research is needed to validate the mechanism of action and comprehend the therapeutic implications of these findings [31] According to previous studies different drugs such as cephalosporins، penicilins، and certain fluroquinolones may be linked with anemia [32]،[33]. the (RBC ، MCV، MCH) declare a significant reduction difference ($P<0.05$) of rats that were treated with (Group II) for (R.B.C) but non-significant difference for (MCV، MCH) compared to negative control (Group I) rats.

In addition، significant reduction ($P<0.05$) was observed in (R.B.C، MCH) except MCV level in Group III compared to the negative control group Group I rats and these results consistent with previous studies [34][35]. Group IV declare that there were statistical non significance difference was observed ($P<0.05$) in (RBC) and significant difference in (MCV)، MCH) levels in Group IV compared to negative control Group I rats. animals treated with (Group V) showed statistically non-significant elevation in (RBC، MCV and MCH levels ($P>0.05$) compared to rats treated with Group II. Group VI demonstrate a significant difference in (MCV and MCH) and non- significant in (RBC) compared to the Group III Furthermore، group V showed non-significant elevation compared with group VI. (HB، MCV، MCHC، and MCH) even though there were attempts at recovery between treatment groups V and Group VI after 21 days of treatment except (eosinophil and monocyte was increased may be because allergic reaction for eosinophil and because blood disorder(anemia or bleeding) for monocyte).drug-mediated anemia has been theorized to have lower RBC، HB، and PCV together with a comparable fall in PLT count (thrombocytopenia)، This change in the erythrocytic parameters may be caused by levofloxacin's inhibitory effect on erythropoiesis in the bone marrow [36]. this study shows that the levofloxacin-induced anemia may take longer to go away.

Therefore، it is advisable to use with caution when administering these antibiotics to individuals who already have anemia. Furthermore، the animals declare non -significant elevated difference ($P>0.05$) of rats (neutrophil ،lymphocyte ،monocyte and eosinophil) level in Group II comparing negative control Group I rats ، in addition no significant ($P>0.05$) was observed in (neutrophil ،lymphocyte ،monocyte ، eosinophil) level in Group III compared to negative control Group I rats ، moreover Group IV resulted in statistically significant differences ($p<0.05$) in (Neutrophil، Lymphocyte، Monocyte، and Eosinophil) levels compared to Group I rats negative control group ، Animals treated in Group V showed statistically significant reduction in (Monocyte and Eosinophil) levels ($P<0.05$) and non- significant reduction in (neutrophil and lymphocyte) compared to Group II. Group VI revealed a significant reduction in (neutrophil and monocyte) and non-significant with (lymphocyte and eosinophil) ($P<0.05$) of rats compared to Group III ،furthermore group V showed significant reduction in neutrophil ، lymphocyte and elevation of monocyte and eosinophil compared with group VI ، these results consistent with previous studies [37] but inconsistent with some studies [38] authors mentioned all blood parameters decline except lymphocyte and eosinophil after levofloxacin treatment (25mg) ، in these study all blood parameters decline when dose of levofloxacin increased to (100mg/kg/day).the

level of (platelets and W.B.C) showed non-significant difference (P>0.05) of rats that were-treated in Group II (reduction platelets and elevation WBC) compared with corresponding level in negative control Group I rats maybe due to inflammation . Similarly، non -significant difference (P>0.05) was observed in (platelets and WBC) level in Group III compared to negative control Group I rats. In Group IV with (500IU/rat/day) of vitamin D3، there were no statistically significant difference was observed (P>0.05) in levels of (platelets and WBC) in comparison to group control (Group I) rats.

Animals treated with Group V showed statistically non-significant reduction in (WBC) and (elevation in platelets) levels (P>0.05) compared to level in rats with levofloxacin(50mg/kg/day) alone Group II while animals showed non- significant elevation in platelets and reduction WBC (P>0.05) of rats with Group VI compared to Group III and group V had higher platelets and lowest WBC in contrast group VI these results consistent with previous studies [39][40].Additionally، levofloxacin، which decreased the rats platelet counts during treatment، may cause mild blood coagulation abnormalities [41]. The thrombocytopenia seen in this study could be caused by either enhanced drug-mediated destruction or decreased platelet synthesis as seen in bone marrow suppression [42]، Given that the rats utilized in this investigation were healthy and had normal blood parameters، this study concluded that extended treatment of levofloxacin may result in anemia as well as thrombocytopenia [43][44]. so، because the effect of levofloxacin on hematology parameters a complete hemogram is preferred before and during the treatment this will allow the practitioner to make an informed decision about whether to discontinue treatment or closely monitor the patient during the course of treatments to ensure safety. Levofloxacin side effects were mitigated by oral vitamin D treatment prior to the drug for 21 consecutive days (groups V and VI) as seen by the noticeably higher erythrocyte count، Hb content، PCV%، MCV، MCH، and PLT count compared to rats subjected to levofloxacin (Group I) ، enhancements in erythrogram and leukogram following treatment of vitamin D to rats receiving levofloxacin may be attributable to the antioxidant properties of vitamin D3 [45].

In addition، vitamin D has improved erythropoiesis، inhibited premature erythrocyte lysis، prevented polyunsaturated fatty acid oxidation in the erythrocyte membrane، and increased the amount of erythroid precursor units forming colonies [46][47]. Levofloxacin has been linked to changes in a variety of blood parameters in rats. Here are some possible mechanisms that could explain these effects: Levofloxacin can suppress the generation of blood cells in the bone marrow، resulting in a decrease in the number of red blood cells (RBC)، which can result in a drop in mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH). Similarly، it might induce a drop in platelet count، which can lead to thrombocytopenia[48] and may be by Oxidative stress: Levofloxacin has been demonstrated to cause oxidative stress in the body، which can damage and destroy the cell membranes of red blood cells، resulting in a drop in RBC count and hemoglobin levels[49].or may be effects on Immune system stimulation: Levofloxacin has the ability to stimulate the immune system، resulting in an increase in the production of white blood cells (WBC)، such as neutrophils، lymphocytes، monocytes، and eosinophils. The drug's capacity to stimulate immune cells such as macrophages and natural killer cells is suggested to be responsible for this impact [50]. Inflammation: Levofloxacin has been reported to raise levels of pro-inflammatory cytokines such as interleukin-1 beta (IL-1) and tumor necrosis factor-alpha (TNF-)، which can lead to an increase in WBC count and contribute to anemia development [51].

Vitamin D has a variety of effects on the body، including blood parameters in rats. Here are some potential pathways for how vitamin D may increase blood parameters while decreasing particular cell types may be by:

Erythropoiesis stimulation: It has been demonstrated that vitamin D stimulates the production of erythropoietin، a hormone that regulates the creation of red blood cells (RBC) in the bone marrow. This can raise RBC count، hematocrit (PCV)، and hemoglobin (Hb) levels، as well as mean corpuscular volume (MCV) and mean corpuscular hemoglobin concentration (MCHC) [52].

Inflammation inhibition: Vitamin D has anti-inflammatory characteristics and has been proven to decrease the production of pro-inflammatory cytokines such as interleukin-1 beta (IL-1) and tumor necrosis factor-alpha (TNF-). This can result in a reduction in the number of white blood cells (WBC)، which include neutrophils، lymphocytes، monocytes، and eosinophil [53].

Immune system modulation: Vitamin D can modulate the immune system by modulating immune cell synthesis and function. It might increase the production of anti-inflammatory cytokines like interleukin-10 (IL-10) while suppressing the production of pro-inflammatory cytokines like TNF. This can result in a decrease in the quantity of WBC and the aforementioned subtypes [54].

Regulation of calcium homeostasis: Vitamin D is needed for calcium homeostasis، which is required for RBC synthesis and maturation. It can improve calcium absorption from food and induce calcium release from bone tissue. This may result in increased RBC synthesis and maturation، which may enhance blood parameters [55].

Conclusions

The findings of this study demonstrated that the some hematological changes caused by levofloxacin treatment ameliorate by vitamin D3.

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Ethics Statements

This study was approved by the Ethical and the Scientific Committee of the Department of Pharmacology and Toxicology of the College of the Pharmacy/University of Baghdad at march 2022.

Conflict of interest

The author(s) declare that there is no conflict of interest

Author contributions

Pharmacist Abbas Muslim Mhaibes: contributed to data gathering, analysis, practical (follow the procedure) and written parts of the study. abbasmmph@Gmail.com

Dr. Farah Kais Abdul- Wahab: gave final approval and agreement for all aspects of the study, supervision, revision and rearrangement. farah77kais@yahoo.com.

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