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Effects of Vitamin C, E and K Supplementation on Hematological Indices and Serum Iron in Female Wistar Rats

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ABSTRACT

Background: Vitamins are indispensable micronutrients that support the regulation and optimization of blood cell production, function and hematological health. This experimental study investigates the effect of vitamins C, E and K on hematological indices and serum iron levels in female Wistar rats.

Methods: Forty rats were randomized into 8 groups of five rats each. Group 1 as control was given only food and water. Groups 2-4 (100mg/kg, 500mg/kg, and 10mg/kg body weight of vitamin C, E and K). Group 5 (100mg/kg and 500mg/kg body weight of vitamin C and E), group 6 (100mg/kg and 10mg/kg body weight of vitamin C and K), group 7 (500mg/kg and 10mg/kg of body weight of vitamin E and K), group 8 (100mg/kg, 500mg/kg and 10mg/kg body weight of vitamin C, E and K). These rats were orally administered these vitamins and had food and water also for one month. Blood was collected by cardiac puncture for analysis.

Results: there was significant (P<0.05) decrease in total WBC in vitamin C and C+E groups, significant (P<0.05) increase in neutrophil count in vitamin E, C+E and C+E+K groups with a significant (P<0.05) increase in neutrophil/lymphocyte ratio in vitamin C, C+E and C+E+K groups. A significant (P<0.05) decreased in platelet-lymphocyte ratio in control group. Vitamin C+E group had a significantly (P<0.05) increased PLR. Vitamin E+K and C+E+K had a significant (P<0.05) increased serum iron level.

Conclusion: the overall result shows the regulatory effect of these vitamins on optimal blood health.

Keywords: Vitamin C, E and K; Hematological parameters; Serum iron; Wistar Rats.

INTRODUCTION

Vitamins are essential micronutrients for maintaining numerous physiological functions including immune support, blood formation and antioxidant defense. Specifically vitamin C, E and K are recognized for their significant influence on blood health and hematological parameters. Vitamin C (ascorbic acid), a potent antioxidant plays a crucial role in protecting cells from oxidative damage. It also facilitates collagen synthesis, iron absorption and immune system activity (1). Evidence suggests that vitamin C contributes to improved hematological indices by promoting red blood cell production and safeguarding cells from oxidative injury (2).Vitamin E, a fat-soluble antioxidant function to stabilize cell membrane and prevent lipid peroxidation which can otherwise lead to cell membrane damage and subsequent cell destruction (3). Research involving animal model has indicated that vitamin E supports immune health and maintains red blood cell stability reinforcing its role in maintaining hematological balance (4). By mitigating oxidative stress in blood cells vitamin E has been shown to lower the risk of hemolytic anemia due to its strong antioxidant with properties (5). Vitamin K is renowned for its critical role in blood coagulation which is essential for achieving hemostasis (6). This vitamin is necessary for the synthesis of various clotting factors such as prothrombin making it vital for effective blood clotting and wound repair (7). Studies have demonstrated that a deficiency in vitamin K can result in excessive bleeding and impaired clotting ability underscoring its importance for hematological health (8). Vitamin C recycles Vitamin E from tocopheroxyl radical and Vitamin E recycles Vitamin C but few in vivo hematological tests both. Vitamin E and K are both fat-soluble; share the same absorption pathways (9), but hematological impact of Vitamin C+E+K is unknown. Hematological parameters including red blood cell, white blood cells count and platelet count are crucial indicators of an organism's physiological and pathological status. These metrics are instrumental in assessing overall health particularly regarding oxygen transport, immune function and coagulation processes (10).

Serum iron and neutrophil to lymphocyte and platelet to lymphocyte ratios were measured together in this study because iron and inflammation regulate each other and vitamins C, E and K is seen at the interface of both pathways.

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Estimating both concurrently allow for easy differentiation of the cause of the hematological changes whether due to altered iron handling or by immune modulation. (1, 11).

Vitamin C enhances iron absorption directly (11), while vitamin E and K contribute indirectly by supporting red blood cell stability and coagulation mechanisms respectively (12,13).

Although the individual effects of these vitamins have been well-studied, detailed research investigating the combined influence of vitamins C, E, and K on hematological parameters and serum iron creates a knowledge gap.

Given their interconnected roles in redox cycling, membrane protection and hemostasis, potential synergistic or antagonistic interactions among these vitamins cannot be inferred from single-agent studies. Also no systematic data on NLR, PLR or serum iron when all three vitamins are given together.

Hence this study was to evaluate the effect of vitamin C, E and K both individually and in combined forms on hematological parameters and serum iron levels to determine whether the combined forms confers additional hematoprotection, alter iron status or modulate inflammatory indices (NLR and PLR) beyond the effects of individual vitamins.

MATERIALS AND METHOD

Ethical Approval: Ethical approval for the study was sought and obtained from the Edo State Ministry of Health, Health Research Ethics Committee. Protocol number: HA|737|24|D|1119432). Approval date: 19/11/2024 (HREC Edo SMoH), for this research work.

Experimental Animals

Forty female Wistar rats of the Federer strain were used for this study. The rats were house in clean disinfectant cages in the animal house of the Department of Anatomy, University of Benin, Benin City and maintained on a twelve-hour light and dark cycle. The sample size was calculated using Federer's formula with eight treatment groups and five rats per group total N=40. The error degree of freedom E=N-T=40-8=32. This exceeds the minimum E of 10 required for adequate error estimation, ensuring sufficient power while meeting the principle of reduction.

E= N-T

Where: E= Error degrees of freedom

N = Total number of animals (40)

T= Total number of treatment groups

$T = \frac{N}{animals\ per\ group} = \frac{40}{5}$ =8 treatment groups

E = N – T = 40 – 8= 32

An extra animal per group is included to account for potential loss during terminal blood collection. They were fed on standard rat food and water throughout the duration of the experiment. The rats were allowed to acclimatize to the new environment for a period of fourteen days at room temperature.

The experimental animals were anesthetized using ketamine (80mg/kg) and xylazine (10mg/kg) given intra peritoneal before cardiac puncture for terminal blood collection.

Experimental Design

The experimental animals were randomized into eight groups (1-8), five rats per group and were administered the vitamins orally for one month period.

Group1: were given food and water only (control)

Group2: were given vitamin C (100mg/kg body weight)

Group 3: were given vitamin E (500mg/kg body weight)

Group 4: were given vitamin K (10mg/kg body weight)

Group 5: were given vitamins C and E (100mg/kg and 500mg/kg body weight)

Group 6: were given vitamins C and K (100mg/kg and 10mg.kg body weight)

Group 7: were given vitamins E and K (500mg/kg and 10mg/kg body weight)

Group 8: were given vitamins C, E and K (100mg/kg, 500mg/kg and 10mg/kg body weight).

Vitamin C, E and k were gotten from commercial pharmaceutical store in Benin City. The following dosages were selected: Vitamin C- 100mg/kg/day, Vitamin E- 500mg/kg/day, and Vitamin K- 10mg/kg/day.

After the one month period of oral administration of these vitamins, blood samples were collected via cardiac puncture

Selection Criteria

Inclusion Criteria: Female Wistar rats of the Federer strain. Weight: 119-129 grams. Age - 4 weeks old.

Exclusion Criteria: Male rats. Rats with previous vitamins supplementation

Detection Principle of the Hematology Auto analyzer

The full blood count parameters were evaluated immediately after the collection of samples, making use of an automated five-part hematology analyzer called ERMA Hematology Auto analyzer PCE-210N (manufactured by Diamond Diagnostic, Holliston, USA).

The blood sample was diluted in a conductive liquid and passed through an aperture which generates a resistance signal

because blood cells are poor conductor of electricity. As the cell volume increases while passing through the aperture, the resistance also increases. The amplifying circuit magnifies the voltage signal, removes background noise, and processes the signal for analysis. The white blood cells (WBCs) and red blood cells/platelets (RBC/PLT) are analyzed by two different circuits. The microprocessor unit (MPU) analyzes and calculates the cell counts, then generates histograms. The platelet count utilizes an advanced liquid, electronic, and software system, which can resolve the repetitive counting of cells.

Serum iron concentration was determined by atomic absorption spectrophotometry (AAS) ICSH recommendations for serum iron. Briefly serum was de-proteinized with 1MHCI and centrifuged at 1500 rpm for 10 minutes to release transferrin-bound iron. Ascorbic acid was added to the supernatant to reduce fe3+ to fe2+, and samples were aspirated into an air-acetylene flame AAS system. Absorbance was measured at 248.3 nm against iron standards and concentrations were calculated from the standard curve. All procedures used acid-washed trace-metal free materials to prevent contamination.

Collection of Samples

Three (3) ml of the blood samples were collected from each rat in each group by cardiac puncture after the application of anesthesia. Using sterile syringes and under aseptic conditions, 2ml of blood was dispensed into EDTA container and 1ml was put into plain container for hematological and serum iron analysis respectively

Data analysis: The data were analyzed using graph pad 8.0 (California, USA). Descriptive statistics, including mean and standard deviations, were calculated to summarize the data. To determine the effect of the vitamins on the parameters, a one-way ANOVA was employed followed by Turkey's post-hoc test. A significance level of $p < 0.05$ was set for all statistical tests.

RESULTS

Table 1 showed the changes in hematological parameters following the administration of vitamins C, E and K supplementation. There was significant decrease in total white cell count in Vit C and Vit C + E groups when compared to the control group ($P< 0.05$). Monocyte count was significantly lower in Vit C + E group when compared to Vit C and VitE+K groups. ($P<0.05$) Also, there was a significantly higher neutrophil count in Vit E, Vit C + E and Vit C + E + K groups ($P<0.05$) when compared to the control group.

As shown in Table 2, There was non-significant difference in all red cell and platelet evaluated in the Wistar rats ($p>0.05$).

Table 3 summarizes the impact on inflammatory and oxidative stress markers. There was a significant increase ($P<0.05$) in neutrophil/lymphocyte ratio (NLR) in Vit C (0.19±0.02), Vit C+E (0.23±0.02), and Vit C+E+K (0.18±0.02) groups when compared to control group (0.08±0.01), ($P<0.05$). There was a significant lower platelet-lymphocyte ratio (PLR) in control group (33.1±3.03) when compared to Vit C (70.29±4.63) and VitC+E (87.34±4.14) ($P<0.05$) groups. Also VitC+E group had a significantly higher ($p<0.05$) PLR when compared to VitE (50.90±5.12), VitK (45.15±1.71), VitC+K (58.14±4.35), VitE+K (48.86±6.04) and Vit C+E+K (57.39±8.29) groups respectively. Data from lymphocyte/monocyte ratio (LMR) shows that VitC+E group (9.35±0.60) had a significantly higher ($P<0.05$) LMR when compared to VitC (6.28±0.52) and VitE+K (5.37±0.31) groups. In addition VitE+K group had a significantly lower ($p<0.05$) LMR when compared to VitC+K (8.60±0.81) and VitC+E+K (8.90±0.85) groups.

Table 4 presented a statistically significant increase ($p<0.05$) in serum iron levels across the groups. VitE+K (36.55±12.47mg/l) and VitC+E+K (27.28±6.95mg/l) had a significantly higher values compared to control group (16.02±3.64mg/l).

Table 1. Table1: Effect of vitamin C, E and K on some white cell parameters in Wistar rats

Groups	Total WBC ($\times10^9$/l)	Lymphocyte count (%)	Monocyte count (%)	Neutrophil count (%)	Eosinophil count (%)	Basophil count (%)
Control	20.6±0.36	80.7±2.41	11.59±1.43	6.16±0.86	1.33±0.37	0.22±0.07
VitC	12.32±1.89[a]	72.32±1.06	12.22±0.49	14.13±1.20	1.58±0.44	0.26±0.11
VitE	13.83±0.97	73.26±0.79	9.32±0.7	15.83±1.04[a]	1.29±0.28	0.3±0.09
VitK	15.9±1.16	76.74±2.04	10.55±0.44	11.25±1.99	1.35±0.18	0.11±0.03
VitC+E	10.85±1.69[a]	73.78±1.71	8.01±0.46[b]	16.69±1.49[a]	1.35±0.47	0.18±0.05
VitC+K	12.9±2.05	77.7±2.38	9.48±1.11	10.79±2.39	0.68±0.29	0.22±0.03
VitE+K	15.93±1.48	70.73±5.82	12.2±1.36[e]	10.32±2.43	0.86±0.31	0.11±0.02
VitC+E+K	14.14±1.43	75.72±2.23	9.96±1.15	13.47±1.26[a]	0.72±0.20	0.19±0.02
F Value	**3.231**	**1.072**	**2.594**	**4.05**	**1.013**	**1.464**
P Value	**0.0127**	**0.4079**	**0.036**	**0.0043**	**0.4443**	**0.2219**

Data represents Mean±S.E.M. Means with superscripts [a, b, e,] indicate statistical significance ($P<0.05$) compared to control

Table 2. Effect of vitamin C, E and K on red cells and platelet parameters

Groups	RBC Count (×10⁹cells /l)	Hgb (g/dl)	Hct (l/l)	MCV (fL)	MCH (pg)	MCHC (g/l)	RDW (%)	NRBC (%)	PLT (×10⁹/l)	MPV (fL)
Control	6.56±0.29	13.27±0.18	39.07±0.56	59.9±3.49	20.27±1.14	33.9±0.06	14.73±0.89	2.53±0.31	501.0±50.82	6.96±0.14
VitC	6.15±0.38	12.73±0.47	36.88±1.31	60.33±1.99	20.73±0.61	34.48±0.13	14.95±0.20	2.63±0.34	577.8±30.48	6.8±0.34
VitE	6.56±0.17	13.2±0.51	37.94±1.65	57.82±1.3	20.04±0.32	34.78±0.26	14.1±0.44	2.74±0.31	502.4±27.35	6.64±0.23
VitK	6.97±0.13	13.45±0.29	39.38±0.64	56.5±0.89	19.23±0.23	34.08±0.30	14.55±0.47	3.37±0.05	576.0±45.69	6.72±0.07
VitC+E	6.75±0.19	13.64±0.43	39.70±1.04	59.02±1.83	20.18±0.68	34.28±0.52	14.74±0.29	3.07±0.21	523.4±40.10	6.64±0.26
VitC+K	6.12±0.12	13.03±0.31	37.13±0.76	60.70±0.27	21.23±0.13	35.00±0.14	15.75±0.45	3.09±0.38	526.00±16.98	6.62±0.22
VitE+K	6.31±0.21	12.34±0.23	35.86±1.07	56.90±1.01	19.54±0.47	34.4±0.55	15.28±0.39	2.84±0.27	518.8±30.87	6.54±0.17
VitC+E+K	6.37±0.18	13.08±0.25	37.14±0.77	58.50±1.62	20.52±0.49	35.18±0.29	15.32±0.59	3.17±0.23	581.20±38.12	6.60±0.17
F Value	**1.763**	**1.301**	**1.571**	**0.8884**	**1.374**	**1.374**	**1.247**	**0.9914**	**0.9064**	**0.3038**
P Value	**0.1364**	**0.2876**	**0.1866**	**0.529**	**0.2561**	**0.2562**	**0.3127**	**0.4583**	**0.5162**	**0.946**

Table 3. Effect of vitamin C, E and K on some inflammatory marker in Wistar rats

Groups	Neutrophil/ Lymphocyte Ratio	Platelet/ Lymphocyte Ratio	Lymphocyte/ Monocyte Ratio
Control	0.08±0.01	33.01±3.03	7.21±1.01
VitC	0.19±0.02[a]	70.29±4.63[a]	6.28±0.52
VitE	0.22±0.02[a]	50.90±5.12	8.02±0.54
VitK	0.15±0.03	45.15±1.71	7.31±0.35
VitC+E	0.23±0.02[a]	87.34±4.14[acd]	9.35±0.60[b]
VitC+K	0.12±0.03	58.14±4.35[e]	8.60±0.81
VitE+K	0.14±0.04	48.86±6.04[e]	5.37±0.31[ef]
VitC+E+K	0.18±0.02[a]	57.39±8.29[e]	8.9±0.85[g]
F Value	**3.649**	**8.815**	**5.226**
P Value	**0.0071**	**<0.0001**	**0.0008**

Data represents Mean±S.E.M. Means with superscripts [a, b, acd, e, ef, g] indicate statistical significance (P<0.05) compared to control

Table 4. Effect of vitamins C, E, and K on serum iron (Fe) levels in Wistar rats

Groups	Fe (mg/l)
Control	16.02±3.64
Vit C	18.06±3.48
Vit E	17.79±4.04
Vit K	20.86±4.11
Vit C+E	16.83±2.88
Vit C+K	22.41±9.22
VitE+K	36.55±12.47[a]
VitC+E+K	27.28±6.95[a]

F- Value = 4.46 and P-value = 0.002. Data represents Mean±S.E.M. Means with superscripts [a] indicate statistical significance (P<0.05) compared to control.

DISCUSSION

This research evaluates the impact of supplementation with vitamin C, E and K on hematological and serum iron levels in Wistar rats. The study examines how these vitamins affect white blood cell count, red blood cell count, platelet profile, inflammatory biomarkers and serum levels offering insight into their physiological and immune-regulation. A notable finding was the significant reduction in total WBC count observed in the vitamin C and vitamin C+E groups compared to the control group. This outcome aligns with existing literature highlighting the immune-regulatory properties of vitamin C and E. for instance the research by Oyeka et al. (14) demonstrated that vitamin C administration reduced WBC counts in rats potentially due to its antioxidant activity which helps modulate immune responses. Additional evidence from Oyesola et al. (15) indicates that Vitamin C supplementation at dosages of 100-300mg/kg effectively mitigated chronic low-grade inflammation, emphasizing its therapeutic potential. Furthermore, Fuente et al. (16) underscored vitamin dual role as a critical antioxidant and micronutrient in immune regulation, suggesting its application as an adjunct in enhancing conventional therapies. The study also revealed a significant elevation in neutrophil count in the vitamin E, vitamin C+E and vitamin C+E+K groups compared to controls. Neutrophils being pivotal to innate immunity are enhanced in their function by vitamin supplementation. Supporting evidence from Liugan and Carr (17) Shows that vitamin improves neutrophil chemotaxis, phagocytosis and oxidative burst activity in diverse populations including hospitalized individuals and athletes. Similarly, research by Kizhina et al. (10) reported that vitamin supplementation in Americans led to increases in leukocyte counts especially neutrophils and monocytes in conditions of abnormal granulogenesis. Fuente et al. (16) observed that elderly individuals supplemented with vitamin C and E experienced improvement in neutrophil and lymphocyte functionality, nearing levels seen in younger adults. In contrast no statistically significant differences were found in RBC and platelet parameters across all groups in this study which can be due to the absence of a hemorrhagic or oxidative stress so no erythropoietic or thrombopoietic stimulus to be modify by these vitamins and also due to the short duration for this study which may have led to the red blood cell and platelet count unaltered. This observation concurs with the findings by Olatunde et al. (18) who reported no significant changes in RBC or platelets after administering vitamin A, C, D and E suggesting that such micronutrients may have limited direct influence on erythropoiesis or thrombopoiesis. This study highlighted an increase in the neutrophil to lymphocyte ratio

in the vitamin C and vitamin C+E group also the platelet to lymphocyte ratio was significantly lower in the control group relative to the vitamin C and vitamin C+E groups indicating an altered leukocyte and platelet-lymphocyte relationship. Another significant finding was the elevated serum iron levels in the vitamin E+K and vitamin C+E+K groups compared to the control. This aligns with previous studies associating vitamins E and K with antioxidant effect and iron metabolism modulation. Gambaro et al. (19) demonstrated that combining vitamin E with ferrous sulfate conferred cellular protection and mitigated genotoxic damage in vitro. Additionally Nuszkiewicz et al. (20) highlighted vitamin K role in reducing oxidative stress and inflammation while preventing ferroptosis a type of iron dependent cell death. These findings underscore the complex interplay of vitamin E and K in regulating immune function, and iron homeostasis.

Limitations of this study: firstly a dose-response relationship was not assessed and only male Wistar rats were used. Secondly, the rats were healthy and maintained on standard chow with adequate micronutrients which differs substantially from models of iron deficiency, anemia, inflammation or oxidative stress where individual vitamins might demonstrate benefit. Third, mechanistic parameters were not directly measured: hepcidin level, tissue iron concentrations, inflammatory cytokines, oxidative stress markers, histologic changes and immune function assays were not performed. Therefore proposed mechanisms remain inferred rather than proven. Finally, potential confounding factors were not fully controlled: gavage-related stress was not quantified, food intake was not recorded and dosing was not fully blinded.

Conclusion

This study demonstrated the significant influence of vitamins C, E and K on various hematological, inflammatory parameters and serum iron levels in Wistar rats. Supplementation with these vitamins resulted in a significant decrease in total white cell count in Vitamin C and Vitamin C+E groups. There was a significant decrease in monocyte count in Vitamin C+ E and significant increase in neutrophil count in vitamin E, Vitamin C+E and Vitamin C+E+K groups. There was no significant difference in RBC and platelet counts across all groups. There was significant increase in NLR in vitamin C, vitamin C +E and vitamin C + E +K groups and significant increase in PLR in vitamin C + E group. There was a significant increased lymphocyte/monocyte ratio in vitamin C + E group and a significantly decreased lymphocyte/monocyte ratio in vitamin E + K group. The serum iron level was significantly increased in all groups with higher levels in vitamin E +K and vitamin C +E +K groups. Although these vitamins in their combined forms did not provide additional hematoprotection in healthy Wistar rats, it did produce distinct effect on iron status and inflammatory indices that exceed those of the individual vitamins. Specifically the combinations increased serum iron and elevated NLR/PLR to a greater extent than single vitamins, without improving red cell or platelet parameters. These findings have shown that interactions between vitamins C, E and K alter iron metabolism and immune cell balance in ways not predictable from single-agent data, highlighting the need for combination studies when assessing multivitamin safety and efficacy.

Despite these limitations the study provided novel evidence that combined vitamin C, E and K produced effects on iron and inflammatory indices that are not predictable from single-vitamin data warranting further investigation with mechanistic endpoints and clinically relevant doses.

DECLARATIONS

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This study did not receive any specific funding from public, commercial, or non-profit agencies.

Conflict of Interest
The authors declare no conflict of interest.

Author Contributions
O.J.C. formulated the structure of the study, handled data collection and drafted the manuscript. A.O.B assisted with statistical analysis, and performed laboratory analysis.. All authors read and approved the final manuscript.

Data Availability
The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethical Approval
Ethical approval for the study was obtained from the Edo State Ministry of Health, Health Research Ethics Committee. Protocol number: HA|737|24|D|1119432). Approval date: 19/11/2024 (HREC Edo SMoH), for this research work.

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