ATTAHADI MEDICAL JOURNAL
Journal homepage:
http://attahadi.edu.ly/journal/index.php/amj/index

Effect of Folic Acid and Ascorbic Acid Supplementation on Hematological Parameters of Male Rabbits

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Keywords:
Ascorbic Acid, Folic Acid, Rabbits, Hematological Parameters.

Received *21 Nov 2024*
Accepted *29 Jan 2025*
Published *11 Feb 2025*

ABSTRACT

Ascorbic acid (Vitamin C) and folic acid (Vitamin B9) are essential micronutrients involved in hematopoiesis, immune function, and antioxidant defense. Ascorbic acid enhances iron absorption and red blood cell (RBC) production, while folic acid is crucial for DNA synthesis and erythropoiesis. Deficiencies in these vitamins are associated with anemia and impaired immune responses. This study investigates the effects of ascorbic acid, folic acid, and their combination on hematological parameters in male rabbits, focusing on hemoglobin (Hb), RBC count, white blood cell (WBC) count, platelets (PLAT), and hematocrit (HCT). Male rabbits were divided into four experimental groups: control, ascorbic acid, folic acid, and a combination of both. Hematological parameters were assessed after treatment, and statistical significance was evaluated. The findings demonstrated that supplementation with ascorbic acid and folic acid significantly improved Hb and RBC counts compared to the control, suggesting enhanced erythropoiesis. The combination group exhibited the highest RBC count (8.60 ± 0.417 $\times 10^6/\mu l$), indicating a potential synergistic effect. The WBC count decreased in the folic acid group but remained stable in the ascorbic acid and combination groups, suggesting an immunomodulatory role. Platelet counts increased significantly in the combination group (489.12 ± 209.7 $\times 10^3/\mu l$), highlighting a possible stimulatory effect on megakaryocyte maturation. HCT levels were highest in the combination group (39.66 ± 1.016 $\times 10^3/\mu l$), indicating improved oxygen-carrying capacity.

Citation info. Khaled F, Ali R, Asbaaq M. Effect of Folic Acid and Ascorbic Acid Supplementation on Hematological Parameters of Male Rabbits. Attahadi Med J. 2025;2(1):35-38. https://doi.org/10.69667/amj.25109

INTRODUCTION

Ascorbic acid, also referred to as vitamin C, is an essential water-soluble nutrient that is involved in many physiological processes, including antioxidant defense, collagen production, and immunological support [1]. Because the human body is unable to produce it on its own, a steady supply of it must be obtained from diet in order to avoid illnesses caused by deficiencies [2].

Ascorbic acid's antioxidant activity, which counteracts free radicals and lowers oxidative stress a recognized cause of a number of degenerative diseasesis one of its main functions [3]. By improving iron absorption, which promotes hemoglobin synthesis and helps avoid anemia, ascorbic acid contributes significantly to hematological health in terms of blood profile[4]. According to studies, ascorbic acid also supports immunological defense and coagulation processes by preserving platelet function and white blood cell count[5]**.** For a balanced and healthy blood profile, it is therefore essential to maintain appropriate amounts of ascorbic acid, particularly for those with oxidative stress conditions or elevated metabolic demands. Folic acid is essential for erythropoiesis, the process that produces red blood cells, and for blood health [6].

Megaloblastic anemia, which is defined by the presence of unusually large and immature red blood cells, develops when folic acid shortage interferes with the process of "DNA" synthesis, which is essential for the formation of healthy red blood cells. Folic acid treatment has been shown to successfully lower the risk of this anemia and preserve a balanced hematological profile, both of which are critical for immunological response and oxygen transport [7].

Essential vitamins including folic acid (vitamin B9) and ascorbic acid (vitamin C) are vital for a number of physiological functions, such as liver and hematological health [8]. Water-soluble antioxidant ascorbic acid is essential for immune system support, collagen formation, and cell defense against oxidative stress [9]. Because ascorbic acid cannot be synthesized by humans, it must be obtained through food in order to prevent deficiencies and associated illnesses [10].

In contrast, folic acid is an essential component for nucleotide synthesis and amino acid metabolism, which makes it needed for "DNA" repair and cell division, particularly in rapidly proliferating cells like red and white blood cells [11]. Both folic acid and ascorbic acid play a major role in preserving an ideal hematological profile in relation to blood health. According to study [12], ascorbic acid improves intestinal absorption of iron, which is necessary for hemoglobin production and the avoidance of iron-deficiency anemia. It also promotes leukocyte function, which keeps the immune response strong [13].

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However, the synthesis of purines and thymidylate, which are essential for the creation of red and white blood cells, depends on folic acid [14]. Megaloblastic anemia, which is defined by the presence of abnormally large red blood cells and affects overall blood health, can result from a folic acid deficiency [15].

MATERIALS AND METHODS

Tested compounds

Folic acid and ascorbic acid were used in this investigation. Folic acid was purchased from a pharmacy in El-Bayda, Libya, whereas ascorbic acid was transported from the chemistry department of Omar Al-Mokhtar University's college of science to Al-Bayda City.

Experimental animals

We purchased twenty healthy, robust male rabbits from reputable local farms. The room in which these rabbits were kept was suitable for the trial period and was equipped in compliance with US-EPA 2004. The principles and standards of the Libyan Ministry of Agriculture as well as the US-EPA2004 for animal care were followed in the care of the rabbits. Every rabbit was housed in an appropriate steel cage that had a 12-hour light cycle, a temperature between 22 and 26°C, and a humidity level between 40 and 70%. A suitable diet comprising clean water and balanced feed has been provided for the duration of the study.

After being divided into four groups of five rabbits each at random, the animals were given the following treatment: Group 1: For six weeks, each rabbit received an oral dose of ascorbic acid (100 mg/kg body weight) on alternate days [16]. Group 2: the rabbits were given folic acid. Folic acid was given by gavage for twelve consecutive weeks at a dosage of 5 mg/kg BW [17]. Group 3: Each rabbit received oral ascorbic acid (100 mg/kg body weight) and folic acid (5 mg/kg body weight) daily. Group 4: As a control, received eight milliliters of distilled water orally for six weeks.

Hematological parameters

Weekly blood samples were drawn from each animal's ear vein for the duration of the six-week study. Blood samples were taken prior to feeding and drinking the cinnamion. figures based on the total amount of blood cells. All "CBC" tests were performed automatically using Sysmex American, Inc.'s XP-300 Automated Hematology Analyzer**.** Anticoagulated "EDTA" samples were subjected to "CBC" assays. The differentiating cells were manually counted using Dif-Quik-stained blood smears. The categories used to record data were hemoglobin (abbreviated "HB"), red blood cells (abbreviated "RBC"), and white blood cells (abbreviated "WBC").

Statistical analysis

When necessary, statistical analysis was performed using GraphPad Prism 8 or Minitab software (version 17). After determining that the data had a normal distribution, a "ANOVA" analysis was conducted using the Tukey multiple comparison test in order to achieve a significance level of $P < 0.05$.

RESULTS

The results in table 3 illustrate the impact of Ascorbic acid, Folic acid, and their combination on hematological parameters in male rabbits, including hemoglobin "Hb", red blood cells "RBCs", white blood cells "WBCs", platelets "PLAT", and hematocrit "HCT".

Hemoglobin "Hb" (g/dl): The control group exhibited an "Hb" level of 12.5 ± 0.12 g/dl, which was lower than all treatment groups. Ascorbic acid supplementation led to the highest Hb level (13.60 ± 0.21 g/dl), followed by Folic acid (13.50 ± 0.81 g/dl) and the combination group (13.38 ± 0.31 g/dl). The statistical analysis indicates that the Hb levels in the Ascorbic acid and Folic acid groups were significantly higher compared to the control, suggesting enhanced erythropoiesis. The increase in Hb levels with supplementation suggests that both Ascorbic acid and Folic acid contribute to improved hemoglobin synthesis, likely due to their role in iron absorption and erythropoiesis. The slight decline in the combination group compared to individual treatments may indicate a non-additive effect.

Red Blood Cells "RBCs" ($\times 10^6/\mu l$): "RBC" count was significantly higher in the combination group (8.60 ± 0.417 $\times 10^6/\mu l$) compared to the control (6.02 ± 0.288 $\times 10^6/\mu l$). Ascorbic acid (8.25 ± 0.176 $\times 10^6/\mu l$) and Folic acid (7.58 ± 0.79 $\times 10^6/\mu l$) also increased "RBC" counts compared to the control. The increase in "RBC" count suggests that both Ascorbic acid and Folic acid play a role in red blood cell production, possibly by enhancing iron metabolism and "DNA" synthesis in erythropoiesis. The combination group exhibited the highest "RBC" count, indicating a synergistic effect in supporting red blood cell formation.

White Blood Cells "WBCs" ($\times 10^3/\mu l$): The "WBC" count was highest in the control (8.10 ± 0.30 $\times 10^3/\mu l$) and Ascorbic acid (7.9 ± 0.26 $\times 10^3/\mu l$) groups. The Folic acid group showed the lowest "WBC" count (6.4 ± 0.34 $\times 10^3/\mu l$), while the combination group exhibited a moderate increase (7.2 ± 0.27 $\times 10^3/\mu l$). The decline in "WBC" count in the Folic acid group may suggest a mild immunomodulatory effect, while the Ascorbic acid and combination groups maintained relatively stable "WBC" levels. Since Ascorbic acid is known for its immune-boosting properties, its presence in the combination group may have counteracted the decline observed with Folic acid alone.

Platelets "PLAT" ($\times 10^3/\mu l$). Platelet counts remained relatively stable across all groups except for the combination treatment, which exhibited a marked increase (489.12 ± 209.7 $\times 10^3/\mu l$) compared to the control (293.02 ± 10.20 $\times 10^3/\mu l$). The Ascorbic acid (300.58 ± 25.702 $\times 10^3/\mu l$) and Folic acid (305.4 ± 22.93 $\times 10^3/\mu l$) groups showed only slight, non-significant increases. The significant increase in platelet count in the combination group suggests a possible stimulatory effect of Ascorbic acid and Folic acid on platelet production. This effect could be related to their roles in "DNA" synthesis and megakaryocyte maturation, although the variability in data suggests the need for further investigation.

Hematocrit "HCT" ($\times 10^3/\mu l$): "HCT" levels were significantly higher in the combination group (39.66 ± 1.016 $\times 10^3/\mu l$) compared to the control (34.73 ± 1.172 $\times 10^3/\mu l$). Both Ascorbic acid (36.81 ± 0.415 $\times 10^3/\mu l$) and Folic acid (36.17 ± 0.413 $\times 10^3/\mu l$) showed moderate increases. The increase in "HCT" levels suggests enhanced oxygen-carrying capacity due to higher "RBC" production. The combination treatment provided the most significant improvement, indicating a potential synergistic effect on erythropoiesis and overall blood volume.

Table 1. Shifts Male rabbits treated with Ascorbic acid, Folic acid, and their combination had complete blood counts that included red blood cells "RBCs", white blood cells "WBCs", hemoglobin "Hb", packed cell volume "PCV", platelets, and hemoglobin "Hb".

Parameter	Experimental groups			
	Control	Ascorbic acid	Folic acid	Ascorbic acid +Folic acid
Hb (g/dl)	12.5 ± 0.12^{bc}	13.60 ± 0.21^{c}	13.50 ± 0.81^{ab}	13.38 ± 0.31^{a}
RBC ×10⁶ (µl)	6.02 ± 0.288^{c}	8.25 ± 0.176^{ab}	7.58 ± 0.79^{b}	8.60 ± 0.417^{a}
WBC ×10³(µl)	8.10 ± 0.30^{a}	7.9 ± 0.26^{a}	6.4 ± 0.34^{b}	7.2 ± 0.27^{a}
PLAT ×10³(µl)	293.02 ± 10.20^{a}	300.58 ± 25.702^{a}	305.4 ± 22.93^{a}	489.12 ± 209.7^{a}
HCT×10³(µl)	34.73 ± 1.172^{b}	36.81 ± 0.415^{b}	36.17 ± 0.413^{b}	39.66 ± 1.016^{a}

Values are expressed as means ± SE; n = 5 for each treatment group. Mean values within a row not sharing a common superscript letter (a, b, c) were significantly different, p<0.05.

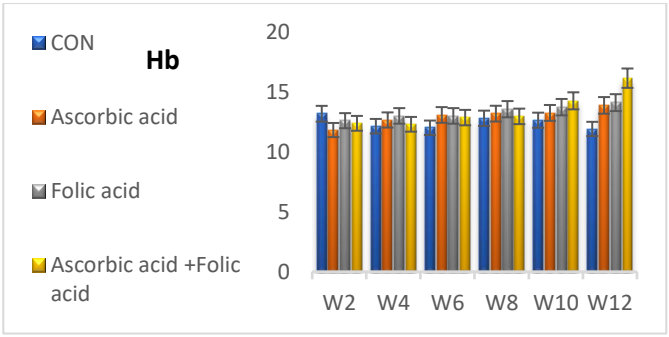

Fig 1. Changes in hemoglobin Hb during treatment of male rabbits with Ascorbic acid, Folic acid and their combination.

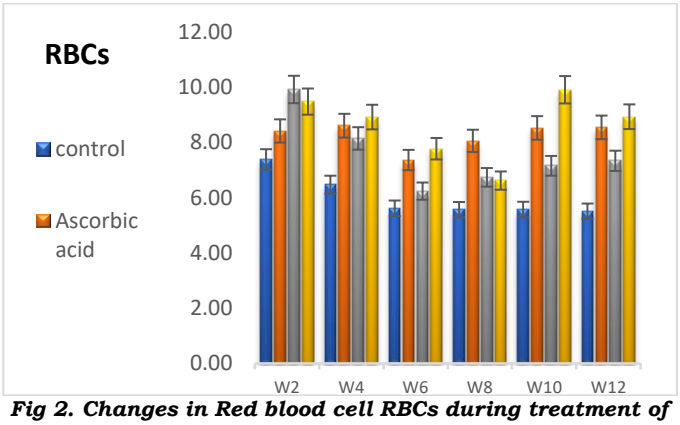

Fig 2. Changes in Red blood cell RBCs during treatment of male rabbits with Ascorbic acid, Folic acid and their combination.

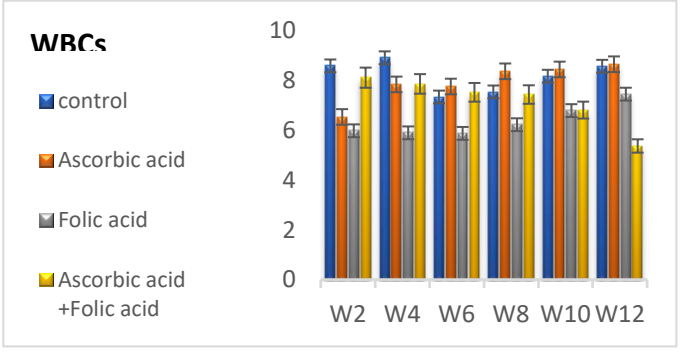

Fig 1. Changes in White blood cell WBCs during treatment of male rabbits with Ascorbic acid, Folic acid and their combination.

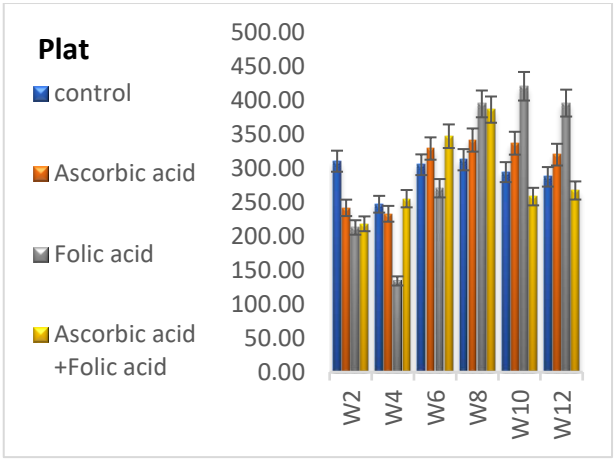

Fig 4. Changes in platelets "plat" when male rabbits are treated with Ascorbic acid, Folic acid, and their combination.

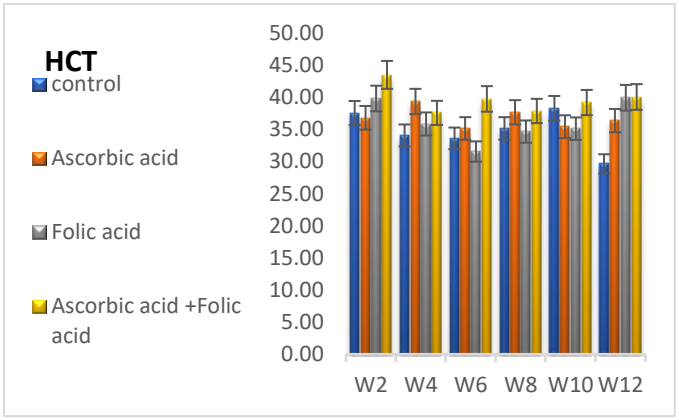

Fig 2. Changes in hematocrit HCT when male rabbits are treated with Ascorbic acid, Folic acid, and their combination.

DISCUSSION

The hematological parameters analyzed in the study provide valuable insights into the effects of ascorbic acid, folic acid, and their combination on male rabbits. The results show a significant increase in hemoglobin (Hb) levels in all treatment groups compared to the control, with the combination of ascorbic acid and folic acid showing the most notable enhancement. This suggests that both ascorbic acid and folic acid play critical roles in improving hemoglobin synthesis [18].

Ascorbic acid enhances the absorption of non-heme iron in the intestine and reduces ferric iron to its ferrous form, which is essential for hemoglobin production. Folic acid, on the other hand, supports erythropoiesis by contributing to DNA synthesis and cell division in red blood cell (RBC) precursors [19]. The combined effect likely results from the synergistic interaction between ascorbic acid's antioxidant and iron-enhancing properties and folic acid's role in cellular proliferation [20]**.** The RBC count significantly increased in the groups treated with ascorbic acid and folic acid, with the highest levels observed in the combination group. This finding highlights the role of these vitamins in promoting erythropoiesis.

Ascorbic acid prevents oxidative damage to RBCs, thereby extending their lifespan, while folic acid ensures the proper synthesis and maturation of erythrocytes in the bone marrow [21]. The combination of these two compounds enhances RBC production by reducing oxidative stress and improving the efficiency of red cell turnover. This observation is consistent with studies demonstrating the importance of antioxidants and

vitamins in supporting hematopoietic function [22]**.** The WBC count showed slight variations among the groups, with a significant reduction in the folic acid group. While ascorbic acid maintained WBC levels similar to the control, the combination group showed a moderate effect. These findings suggest that ascorbic acid may play a protective role in maintaining immune cell integrity due to its ability to reduce oxidative damage and support neutrophil function [18]**.** The slight decrease observed in the folic acid group may be attributed to its primary role in erythropoiesis rather than leukocyte production.

Combined treatment may moderate these effects, balancing antioxidant and metabolic benefits [23]. Platelet levels were elevated in all treatment groups, with a particularly high increase observed in the combination group. This indicates that ascorbic acid and folic acid enhance thrombopoiesis, potentially through their roles in reducing oxidative stress in megakaryocytes and supporting DNA synthesis required for platelet production [24]. Enhanced platelet count could contribute to improved hemostatic balance and vascular health, highlighting the combined efficacy of these vitamins in promoting overall hematological function [25].

The PCV values showed a significant increase in the combination group compared to the control, with moderate increases in the ascorbic acid and folic acid groups individually. This is indicative of improved oxygen-carrying capacity and overall hematological health. Ascorbic acid's role in iron metabolism and folic acid's involvement in RBC maturation likely contribute to this improvement. The synergistic effect of both compounds is particularly evident, as they support different yet complementary aspects of hematopoiesis [26]. The results highlight the biochemical and physiological roles of ascorbic acid and folic acid in enhancing hematological parameters. Their individual and combined effects demonstrate their potential to improve blood health by reducing oxidative stress, enhancing erythropoiesis, and supporting platelet production. Further studies should explore the molecular mechanisms underlying these effects to confirm their therapeutic potential in managing hematological disorders [27,28].

CONCLUSION

The results suggest that ascorbic acid and folic acid enhance hematological parameters, with their combination providing superior benefits in RBC production and HCT levels. These findings highlight their potential therapeutic role in preventing anemia and supporting hematopoiesis in clinical and veterinary applications. Further research is needed to elucidate the underlying mechanisms and long-term effects.

Conflicto of interest. Nil

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