

ORIGINAL ARTICLE

THE CORRELATION BETWEEN L-CARNITINE UPTAKE AND SOME HEMATOLOGICAL PARAMETERS IN OXIDATIVE STRESSED RATS

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Summary

The objective of this research was to determine the effect of L-carnitine antioxidant ability on some hematological parameters such as the extent of red blood corpuscles (RBCs), "Packed cells volume (PCV)", Hemoglobin (Hb), and Mean cellular volume (MCV) in normal and oxidative stress exposed rats respectively. Using an animal model, "Hydrogen peroxide (H_2O_2 0.5 %)" was mixed with drinking tap water to induce oxidative stress through the experiment period (30 days). Three groups were used in this study (each containing 7 rats) one of them was the negative control group and the other two were the positive control group (oxidative stress group treated with H_2O_2) and the treatment group treated with H_2O_2 + L-carnitine. The oxidative stress group showed a significant decrease ($P < 0.05$) in RBCs count, and no significant differences ($P < 0.05$) were observed in Hb, PCV, and MCV compared with the control group on the other hand the treatment group revealed a significant increase ($P < 0.05$) in RBCs count in cooperation with the oxidative stress group. It can be concluded that L-carnitine has a positive key role toward H_2O_2 induced oxidative stress. This role positively affects the red bone marrow and erythropoiesis through balancing the negative effect of oxidative stress and can be monitored through RBCs count only.

Key words: L-carnitine; Oxidative stress; RBCs count; Haemoglobin; H_2O_2

Introduction

Oxidative stress plays a key role in the pathophysiology of several pathological conditions such as inflammation, carcinogenesis, cardiovascular dysfunctions, reperfusion injury, atherosclerosis, and neurodegenerative diseases (1). It is described as an elevation in the levels of "reactive oxygen species (ROS)" and/or a decrease in the redux system. There are several types of ROS such as "hydroxyl radical (OH)", "superoxide ($O_2^{\cdot-}$)", "peroxyl radical (RO_2^{\cdot})" alongside hydrogen peroxide (H_2O_2) which is a non-radical species (2).

The impacts of oxidative stress on membrane lipid, nucleic acids, and protein appear as a defection of the membrane, function integrity, enzymes inhibition, lipoproteins malfunction, and DNA chemical alteration, giving the diseases the chance to happen such as cardiovascular deceases or even cancer (3, 4).

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L-carnitine or vitamin BT is a cofactor that has been used as a dietary supplement sometimes it was originally found as a growth factor that is a part of the B complex group of vitamins of *Tenebriomolitor* (mealworms) and was labeled as vitamin BT by Fraenkel and Friedmann (5). The molecular formula of L-carnitine is $C_7H_{15}NO_3$ and molecular weight is 161.20 Da. It is an ammonium compound synthesized endogenously from the amino acids lysine and methionine, the carbon backbone is obtained from lysine while the 4-N-methyl groups are originated from methionine (6). The plants represent a poor dietary source of L-carnitine, while the animal dietary sources are considered a good source (5). The carnitine has many benefits such as its ability to improve cardiovascular function and improving exercise performance (7).

Erythropoiesis is the conduit that produces functional RBCs from hematopoietic stem cells. It is controlled by a combination of growth factors and micro-environmental that promote the proliferation, differentiation, and/or subsistence of erythroid precursors (8). The erythrocytes production is the largest output of the hematopoietic system. the estimated rate of production from the bone marrow is 2×10^{11} erythrocytes each day (9). To maintain this constant production of erythrocytes, several components are required including iron for hemoglobin synthesis, vitamin B12, and folic acid for the DNA synthesis (10). the proliferation of early erythroid progenitors is increased by insulin growth factor 1 alongside other cytokines as well as this cooperation may coordinate RBCs production through the development and increase the body mass. Steroid hormones may enhance RBCs production through their nuclear receptors but none of them is involved in the fine erythropoiesis regulation. However, the production of androgen may explain the reason behind the elevation of hematocrit in males more than in females (11).

Erythropoiesis may also be regulated by cytokines which in turn enact a perilous role in its affirmative regulation. They act through all the stages of maturation of the erythroid progenitors and precursors to induce proliferation, prevent apoptosis, and induce or inhibit differentiation (12, 13). Hemoglobin carries the oxygen from the lungs and relinquishes it to other tissues. The circulating erythrocytes number is the tissue oxygenation foremost determinant. The understanding of all those mentioned processes will shed light on the pathophysiology of anemia allied with various erythroid disorders including oxidative stress-induced disorders (14). The current study aimed to reveal the effect of L-carnitine antioxidant action on some hematological parameters.

Materials and Methods

Male albino rats (*Rattus norvegicus*) weighing (275-300) g were used in the current study their ages were ranged (3-4) months. The experimental animals were obtained from the animal house in Mosul University-College of Veterinary Medicine. The lab animals were handled according to the animal research institutional guidelines and housed in clean propylene cages. Animals were kept at standard laboratory conditions (12 h light and 12 h dark, Temperature was maintained at 23 ± 2 °C and 45 ± 1 % relative humidity). The animals were allotted to acclimate to the laboratory conditions for two weeks before starting the experiments. Rats had free access to standard food. They were given water and standard food *ad libitum* in adequate amounts during the experimental period.

The lab animals were randomly divided into 3 groups with the same relative weight, each group included 7 as follows:

The negative control group: receive drinking water and food daily for (30) days.

The positive control group: receive "hydrogen peroxide H_2O_2 (0.5%)" Indian peroxideR with drinking tap water for (30) days.

The treatment group: receive "hydrogen peroxide H_2O_2 (0.5%)" with drinking water and treated with L-carnitine (Sigma aldrich-Germany) 15 mg/kg of body weight using gavage, daily for (30) days.

At the end of the experimental period, the animals were anesthetized and sacrificed through jugular vein severance. 2 ml of blood were collected from each animal using EDTA tubes for assessing the hematological parameters.

Determination of RBCs count: RBCs count were assessed in one cubic millimeter of blood using a hemocytometer (Neubauer Counting Chamber), the blood sample diluted was 1:200 with normal saline.

Determination of packed cells volume (PCV): Measured by using a capillary tube and micro-centrifuge at 12000 rpm for 5 mins. The result was calculated by the special ruler of micro-centrifuge.

Determination of hemoglobin amount (Hb): The Hb amount was determined by dividing the value of PCV on (3.3) as Hb signifying third a size of RBC.

In addition to, these blood tests and MCV were asserted using an automated hematology analyzer (Sysmex model: K-1000, Japan).

Statistical analysis: statistical analysis was performed using a statistical program (SAS, 2001). The comparison between the study groups was made using Duncan's test of multiple ranges to assess significance between groups. The significance level was at ($P<0.05$). The data were expressed as means \pm standard deviation.

The results

Figures 1, 2 and 3 reveal a significant decrease in all the study parameters (RBCs count, PCV, Hb, and MCV) in the oxidative stress group compared with the control and the treatment group ($P<0.05$) while there was no significant change between the control and the treatment-groups ($P<0.05$). However, figure 4 shows a significant increase in the MCV level of the H_2O_2 group compared with the control and the treatment group respectively. The mean level of RBCs count of the control group was $6.185 \pm 0.34 \times 10^6/\text{ml}$ this value was drubbed significantly to $4.655 \pm 0.15 \times 10^6/\text{ml}$ then restored to the normal value after using L-carnitine ($6.315 \pm 0.21 \times 10^6/\text{ml}$).

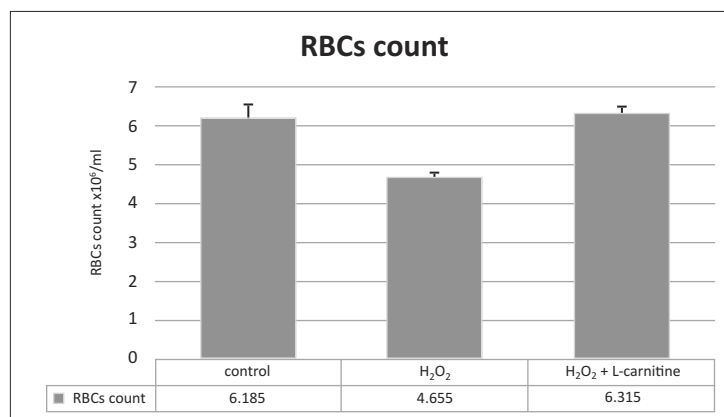


Figure 1. Effect of treatment by L-carnitine (15 mg/kg) on RBCs count in male albino rats that exposure to oxidative stress (H_2O_2) (0.5%) for (30) days.

The mean level of hemoglobin of the control group was 13.1 ± 0.39 g/dl this value was drubbed significantly to 12.7 ± 0.43 g/dl then restored to the normal value after using L-carnitine (13.2 ± 0.35 g/dl).

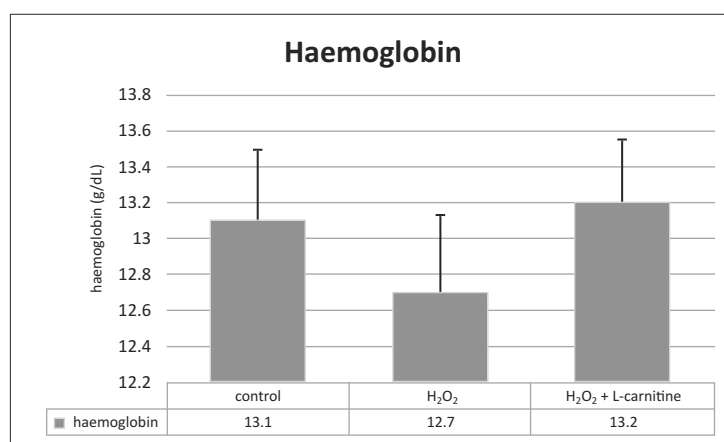


Figure 2. Effect of treatment by L-carnitine (15 mg/kg) on Hb concentration in male albino rats that exposure to oxidative stress (H_2O_2) (0.5%) for (30) days.

The mean percentage level of packed cells volume of the control group was 44.15 ± 2.88 % this value was drubbed significantly to 35.6 ± 3.49 % then restored to the normal value after using L-carnitine (44 ± 1.28 %).

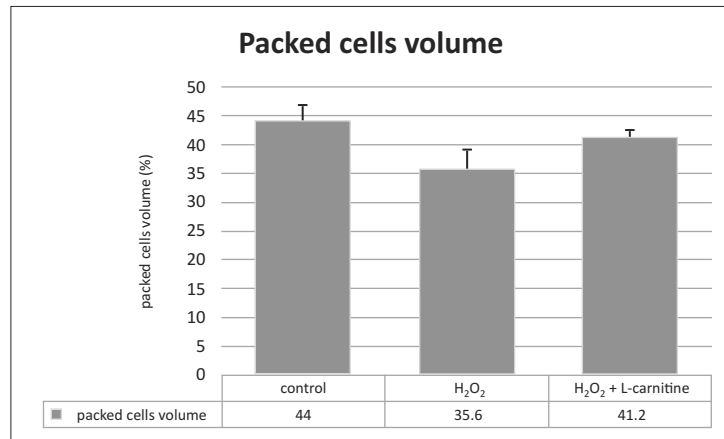


Figure 3. Effect of treatment by L-carnitine (15 mg/kg) on PCV in male albino rats that exposure to oxidative stress (H₂O₂) (0.5%) for (30) days.

The mean value level of MCV of the control group was 64.15 ± 1.83 fL this value was increased significantly to 73.67 ± 2.89 fL then restored to the normal value after using L-carnitine (65.37 ± 1.49 fL) compared to the control group.

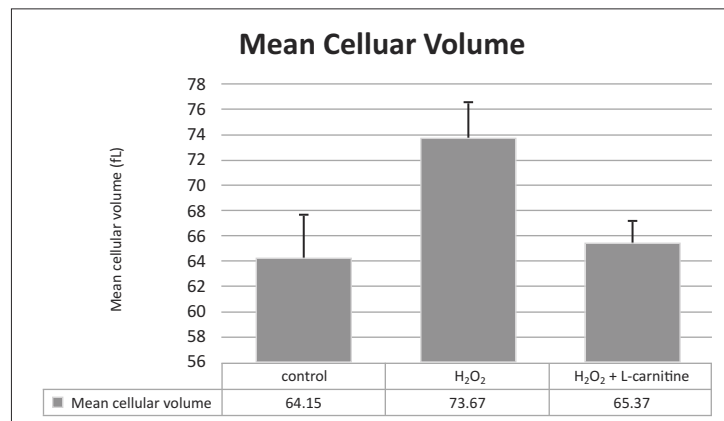


Figure 4. Effect of treatment by L-carnitine (15 mg/kg) on MCV in male albino rats that exposure to oxidative stress (H₂O₂) (0.5%) for (30) days.

Discussion

The present findings may be accredited to the massive oxidative stress provoked by H₂O₂ that will invoke protein oxidation, "lipid peroxidation", ATP exhaustion, and "DNA fragmentation" these influences will lead to rigorous cell injuries and/ or necrosis, which is eminent by cellular organelles and cell membrane interruption (15) thereby subjected to H₂O₂ may lead to "bone marrow suppression", as a result, diminishing in RBCs counts as the RBCs is more susceptible to alterations in the redox/oxidative system quasi-equilibrium. However, a reduction of GSH and increased lipid peroxidation as a result of "oxidative stress" may result in cellular lysis. And that may be the key reason for PCV and Hb decrement(16).

Oxidative stress effect on the blood is one of the well-documented processes of damage (17). Deformation of RBC is plumped by RBC cytoplasmic viscosity (depending on the concentration of the cytosolic hemoglobin),

the RBC membrane viscoelastic features, and cellular architecture (18). Membrane viscoelasticity is ascertained by the RBC skeleton, which is mainly a fine mesh conferred to the integrins (19).

The lipid bilayer backing to the total viscoelasticity is so little, nevertheless, the lunched oxidative reactions in the lipid constituents such as lipid peroxidation lead to cross-linkages configuration within the membrane geometry components or between hemoglobin and the proteins of the membrane skeletal as a result, elevate the viscosity of the membrane (19). Additionally, oxidative stress may affect the processes of transportation across the RBC membrane, affecting the cytosolic viscosity and cell geometry, through the alterations in the cations cytosolic concentration (20).

The results demonstrated an appositive effect for L-carnitine on the hematological features of the study animals. is one of the fundamental key components of energy metabolism, that occur during the fatty acid oxidation in the mitochondrial (21). It has clear effects on the stability and functions of the membrane that are not related to mitochondrial function (22), Arduini *et al.* stated that L-carnitine increased the stability of the RBC membrane, probably through interacting with the skeleton proteins of the membrane (23).

The changes in the treatment group may be due to the acetyl-L-carnitine compound, acetyl part which involves in the preservation of acetyl-CoA levels and can promote glutathione antioxidant production (24), this effect may lead to preservation of RBCs. On the other hand, Palmieri *et al.* reported that L-propionyl carnitine equalized the "oxidative stress" in "β-thalassemic patients" RBC *in vitro*, dose-dependently (25), the mode of action is yet to be discovered. L-carnitine can free radical rummaging potential and to reduce lipid peroxidation, both *in vivo* and *in vitro* (26, 27). Moreover, L-carnitine impact many blood cells features which determine the viability of the erythrocyte such as stability under stress, osmotic resistance, and Na⁺-K⁺ ATPase (28).

Conclusion

This study illustrated extra corroborate for the importance of the significant part of L-carnitine in upholding normal RBC physiology and properties.

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Conflict of Interest

The authors have no conflicts of interest regarding the publication of this article.

Adherence to Ethical Standards

The study was approved by the ethical committee at the University of Mosul. The study is registered by the scientific committee in the College of Pharmacy at the University of Mosul, the study approval number and date UOM/COM/MREC/2021 (5) on 07/08/2021.

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