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The protective effects of L-Carnitine against oxidative toxicity in adult male New Zealand Rabbits

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ABSTRACT

To determine the effectiveness of L-carnitine in reducing oxidative stress in male New Zealand rabbits, twenty adult rabbits were used in this study and randomly divided into four groups: G I (Control): Standard diet and water, G II (OX): 0.5% H2O2 in drinking water with a standard diet, G III (LCA): 150 mg L-carnitine/kg feed with regular water, and G IV (PCO): 150 mg L-carnitine/kg feed with 0.5% H2O2 in drinking water. There was no significant effect of adding L-carnitine on most blood parameters in normal or oxidative stress-exposed rabbits. However, LCA treatment significantly increased total protein and albumin levels and decreased glucose levels. OX group showed higher levels of GOT, GPT, and MDA, while these parameters improved in the LCA group, which also showed superior GSH levels. L-carnitine treatment significantly improved cortisol and testosterone levels, suggesting it protects the male reproductive system from free radical damage by improving blood parameters and reducing oxidative stress.

التأثيرات الوقائية للـ L-كارنيتين ضد السمية التأكسدية في ذكور الأرانب النيوزيلندية البالغة

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الخلاصة

هدفت هذه الدراسة الى تقييم الدور الوقائي المحتمل لـ L-carnitine في الحد من السمية الناجمة عن الإجهاد التأكسدي في ذكور الأرانب. صممت هذه الدراسة باستخدام 20 ارنب ذكرا من النوع النيوزلندي قسمت عشوائيا الى أربع مجموعات **G I**: تم استخدامها كمجموعة سيطرة سالبة وتم إعطاؤها طعامًا وماءً قياسي. **G II**: السيطرة الموجبة مع إضافة 0.5% H2O2/L. اما المجموعة الثالثة **G III** فقد اضيف الى عليقتها 150 ملغم من L-carnitine / كغم علف. المجموعة الرابعة **G IV**: تتم معاملتها بـ 150 ملغم من L-carnitine /كغم علف و0.5% H2O2/لتر من ماء الشرب. وفي نهاية الفترة التجريبية تم سحب عينات الدم لإجراء الفحوصات الدمية وفحوصات هرمون الكورتيزول والتستوستيرون ومؤشرات مضادات الأكسدة. ومن خلال ما اظهرته النتائج لم يكن لإضافة ل-كارنيتين الى العلف (150 ملغم/كغم علف) تأثير معنوي على معظم المؤشرات الدموية، كما انخفض مستوى هرمون الكورتيزول ونشاط أنزيمات ALT وAST وMDA وتركيز الكلوكوز في الأرانب الطبيعية والمجهدة تأكسدياً. وكذلك كان للمعاملة بـ L-carnitine تأثير إيجابي على تركيز هرمون التستوستيرون. تشير نتائج الدراسة الحالية إلى أن L-carnitine، من الممكن ان يحد من الآثار السلبية للإجهاد التأكسدي في الجهاز التناسلي الذكري للأرانب، ويزيد من نشاط الجهاز التناسلي للأرانب السليمة. وتشير نتائج الدراسة الحالية إلى المعاملة ب L-carnitine يزيد بشكل ملحوظ من مستويات هرمون التستوستيرون مع تحسن في مؤشرات الاجهاد التأكسدي.

الكلمات الافتتاحية: ل- كارنتين ، الاكسدة ، الدم ، الهرمونات الجنسية ، الارانب

INTRODUCTION

One of the most significant variables influencing reproduction is oxidative stress (Hameed *et al.*,2023) which is characterized as an imbalance between the body's defense mechanisms against oxidation and its generation of free radicals. This results in lipid peroxidation, which weakens the body's defenses against antioxidants and damages the body's tissues. Illnesses and the ability of stressed animals to reproduce (Du *et al.*,2024). To lessen the damaging effects that free radicals and their byproducts can have on the bodies of organisms, antioxidants act as a line of defense (Marín *et al.*,2023). The number of free radicals produced and the body's capacity to withstand these stressors are determined by the vital activity and composition of the body's tissues and organs. The higher the cells' content of long-chain unsaturated fatty acids, the greater their chances of being exposed to oxidative damage (Reddy,2023; Majeed& Mustafa ,2023). The male reproductive system is considered one of the most active systems in the body due to its high production of sperm (Sengupta *et al.*,2024). It is also characterized by its high-fat content, which is one of the basic requirements for the production of sperm and the production of male sex hormones (Tsametis *et al.*,2023). Among the most important cells that produce sex hormones

responsible for regulating sperm production and the manifestation of secondary sexual characteristics are Leydig cells (Gurung *et al.*,2022). After being stimulated by the hormone LH secreted from the anterior lobe of the pituitary gland, which is in turn stimulated by the hormone GnRH secreted from the hypothalamus, these cells begin to release the hormone testosterone. They are considered cells. Leydig -cell is one of the cells that are candidates for oxidative damage due to the nature of its production of steroid hormones and its content of unsaturated fatty acids (Wistuba *et al.*,2023). An essential cofactor of the metabolism of fatty acids, L-carnitine is produced either endogenously or through diet. One of the antioxidants that can be acquired from a diet rich in meat and dairy products is L-carnitine, which has the chemical formula 3-hydroxy4-N-trimethylaminobutyric acid (Savic *et al,* 2020) .

The L-isomer of L-carnitine was found to be the only bioactive form when it was initially isolated from cow muscle in 1905. Passive diffusion and active transport are both used by mammals' small intestines to absorb ingested L-carnitine (Alhasaniah, 2023). L-carnitine is a chemical that is structurally similar to a vitamin and is water soluble. 25% of L-carnitine is generated endogenously in the body from the two essential amino acids lysine and methionine, while the remaining 75% is absorbed through diet. These nutrients are stored in the testes, heart, brain, and skeletal muscles. The concentration of L-carnitine is approximately 2000 times higher in sperm and the epididymis than it is in plasma (Mateus *et al.*,2023). 10 Additionally, Energy production requires both the Beta-oxidation of long-chain fatty acids and their transfer from the cytosol to the mitochondria, which is enhanced by L-carnitine (Maldonado et al,2024). As a potent nonenzymatic antioxidant, L-carnitine protects cells, DNA, and the mitochondrial membrane from oxidative damage caused by free radicals in the body cells. Numerous studies have been conducted on L-carnitine to ascertain its antioxidant properties. The findings point to improved antioxidant enzymes and a decrease in oxidative stress in diverse tissues. Superoxide dismutase (SOD), glutathione (GSH), and catalase were all considerably boosted when L-carnitine was administered after radiotherapy, according to research by (Kanter et al, 2010) and (Virmani et al,2015) (CAT).

According to these data, the idea of this study came to demonstrate the effect of L-carnitine in reducing the damaging effects of oxidative stress induced by the use of hydrogen peroxide in male New Zealand rabbits, expressed in some hematological, chemical and hormonal indicators.

MATERIALS AND METHODS:

Animals

From the nearby laboratory animal center in the city of Samarra, twenty adult male New Zealand rabbits (8-10 weeks, 1505-1720 g) were acquired. For the duration of the experiment, the rabbits were housed in standard housing with a temperature range of 24 to 27 degrees Celsius and 12-HD/12-HL. They were fed a standard rabbit diet. The animals were given care for a week before the experiment's start to help them adjust to their new environment.

Study Design

The rabbits were split into four experimental groups, each consisting of five replicates, at random:

Group I, control (Con): Consume a standard diet and regular water

Group II oxidative stress (OX): received 0.5% H2O2/L of drinking water with a standard diet

Group III L-carnitine (LCA): rabbits were consuming (150 mg L-carnitine) /kg diet and regular water

Group IV Preventive group (PCO): The rabbit received (150 mg L-carnitine) /kg diet and 0.5% H2O2/L of drinking water

Four weeks after the experiment started, blood was drawn from the limbic vein, and serum was separated using a centrifuge set to run for fifteen minutes at 3000 rpm. The prepared serum was stored at -20 degrees Celsius.

Determination of complete blood count (CBC):

Fresh blood was tested shortly after collection for estimating complete blood pictures. The total number of red and white blood cells (RBC and WBC), Hemoglobin concentration (HB), Hematocrit percentage (PCV), Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), and Mean Corpuscular Hemoglobin Concentration (MCHC) All these traits were estimated using the Hematology analyzer (COUNT 60). Blood samples were collected by method of (Mustafa & Hussein, 2023).

Biochemical Analysis:

Among the blood tests for biochemistry were: Armstrong and Corri's (1960) method of measuring total protein concentration was followed. The albumin level was calculated using Doumas et al.'s methodology (1971). The values of albumin were subtracted from the corresponding values of total protein to obtain the globulin level values. Spectrophotometric measurements of serum total glucose, cholesterol, urea, and creatinine were made using Spanish Linear Chemicals kits.

Determination of oxidative stress indicators:

Some indicators that indicate the state of stress to which the animal is exposed were estimated and included all of: Using the pre-made analysis kit made by the British RANDOX, the concentration of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) was measured. The levels of glutathione and malondialdehyde were also estimated, glutathione (GSH) in blood serum was estimated according to the method using Elman's reagent containing D.T.N.B (5,5-Dithio bis-2-Nitrobenzoic acid), which reacts strongly with glutathione and reduces the sulfhydryl group of glutathione to produce a yellow complex (Taha,2008). The method to determining MDA is based on the interaction between lipid peroxides, Especially the MDA and

Thiobarbituric acid – TBA. And the reaction takes place in an acidic medium to form a colored product (Bazzaz *et al.*, 2023).

Measurements for Steroid Hormones

Using commercially available ELISA kits, the serum levels of cortisol and testosterone were determined by the manufacturer's instructions. (The American company Beckman Coulter).

Statistical Analysis:

The data was analyzed using SAS software (2009). The results were shown as mean + SD. A one-way analysis of variance and the Duncan test (1955) were used for multiple comparisons. Significant differences were defined as $P \leq 05$.

RESULTS AND DISCUSSION:

The effects of L-carnitine on the blood profile of male New Zealand rabbits exposed to oxidative stress are seen in Table 1, where it is noted that while there are no significant differences between the study treatments in any of the blood characteristics (RBC, HB, PCV, MCV, MCH, and WBC), there is a significant increase in the MCHC rate in the animals of the second group (OX) when compared to the other treatments. In comparison to the fourth and first groups, the animals receiving the third treatment (LCA) showed a noteworthy rise in the MCHC rate.The effects of adding L-carnitine on a few biochemical parameters of blood serum in both normal and oxidatively stressed rabbits are shown in Table 2, where we can see that the H2O2 treatment significantly reduced the concentration of albumin and total protein. However, there were no appreciable variations in the levels of urea, creatinine, cholesterol, or globulin among the study groups. All of these characteristics, on the other hand, significantly improved as a result of L-carnitine treatment, and no discernible variations were seen between the PCO and Con groups—except the PCO group's significantly lower albumin concentration.

Table 1 Effect of L-carnitine on the complete blood picture (CBC) in rabbit mails exposed to oxidative stress

Treatment / Treats	Con	OX	LCA	PCO	P.V
RBC (*10^6/µ l)	6.0 ± 0.15	6.03 ± 0.17	6.50 ± 0.15	6.16 ± 0.18	N.S
PCV %	40.30 ± 1.45	41.33 ± 2.33	44.33 ± 2.02	42.0 ± 2.08	N.S
H b (g/100 ml)	12.22 ± 0.43	12.91 ± 0.72	13.68 ± 0.62	12.72. ± 0.63	N.S
MCV (fl)	62.13 ± 2.71	68.84 ± 5.83	68.40 ± 4.62	68.10 ± 2.62	N.S
MCH(Pg)	18.82 ± 0.82	21.51 ± 182	21.11 ± 1.42	20.64 ± 0.79	N.S
MCHC %	30.30 ± 0.003 c	31.25 ± 0.001 a	30.86 ± 0.002 b	30.30 ± 0.001 c	*
WBC (*10^3/ µ l)	9.13 ± 0.42	8.73 ± 0.52	8.33 ± 0.34	9.10 ± 0.20	N.S

(**G I, Con, G II (OX)**: received 0.5% H2O2/L of drinking water with a standard diet, **G III (LCA):** 150 mg L-carnitine /kg diet and regular water, **G IV (PCO)**: received 150 mg L-carnitine /kg diet and 0.5% H2O2/L of drinking water). The values are presented as mean + SD. The different English letters with in row indicate the presence of significant differences at the probability level P <0 .05.

Table 2 Effect of L-carnitine on the blood biochemical measurements in rabbit mails exposed to oxidative stress

Treatment / Treats	CON	OX	LCA	PCO	P.V
Protein (g/100 ml)	6.86 ± 0.12 a	5.76 ± 0.32 b	7.13 ± 0.23 a	5.83 ± 0.37 b	*
Albumin (g/100 ml)	3.5 ± 1.11 a	2.63 ± 0.26 b	3.70 ± 0.11 a	3.10 ± 0.20 ab	*
Globulin (g/100 ml)	3.30 ± 0.05 a	3.13 ± 0.21 a	3.43 ± 0.12 a	2.73 ± 0.36 a	N.S
Glucose (mg/100 ml)	129.0 ± 3.78 b	150.0 ± 3.21 a	117.6 ± 1.45 c	134.0 ± 3.05 b	*
Cholesterol (mg/100 ml)	41.0 ± 1.7 a	44.0 ± 2.3 a	42.0 ± 2.08 a	41.6 ± 2.33 a	N.S
Urea (mg/100 ml)	41.0 ± 1.15 a	42.0 ± 1.15 a	40.6 ± 1.45 a	41.0 ± 1.52 a	N.S
Creatinine	0.87 ± 0.05 a	0.93 ± 0.13 a	0.83± 0.08 a	0.97 ± 0.11 a	N.S

(**G I, Con, G II (OX)**: received 0.5% H2O2/L of drinking water with a standard diet, **G III (LCA):** 150 mg L-carnitine /kg diet and regular water, **G IV (PCO)**: received 150 mg L-carnitine /kg diet and 0.5% H2O2/L of drinking water). The values are presented as mean + SD. The different English letters with in row indicate the presence of significant differences at the probability level P <0 .05

Regarding indicators of oxidative stress, we note in Figure 1 (A, B, C, D) that male rabbits treated with hydrogen peroxide (OX) recorded a significant increase in the levels of GOT, GPT, and MDA, with a significant decrease in the levels of GSH, while the treatment natural rabbits with carnitine (LCA) showed a significant improvement in these traits compared with CO N and OX treatment. In addition, adding carnitine to the diets of rabbits exposed to oxidative stress using hydrogen peroxide (PCO) led to an improvement in some indicators of oxidative stress (GOT, GPT, and MDA) compared with OX Group. In reference to the hormone testosterone, Figure 2B shows that the third group, LCA, significantly increased the hormone's measure in comparison to the other groups, while no significant differences were observed between PCO, OX, and CON. Because L-carnitine increases the production of IGF-I from liver (insulin-like growth factor-I), which lowers blood glucose levels by increasing glucose entry into cells and oxidizes it through the Krebs cycle to produce energy, there may be a significant decrease in blood glucose concentration when treated with L-carnitine (Zhang *et al.*, 2020).

An observation drawn from our current study's Figure 2 results. According to Yedgar et al. (1983), the liver is thought to be the primary location for the body's synthesis of proteins, especially albumin. Total protein and albumin concentrations in the OX group may have decreased as a result of oxidative stress brought on by hydrogen peroxide use, which increased the production of free radicals and active oxygen species in the liver tissue's cells, causing oxidative damage to the liver tissue (Balkan *et al.*, 2002). In a way that surpasses its capacity to eliminate it or fix its harm. This assumption might be supported by Figure 1's findings, which show that there is a marked rise in markers of liver damage brought on by hydrogen peroxide.

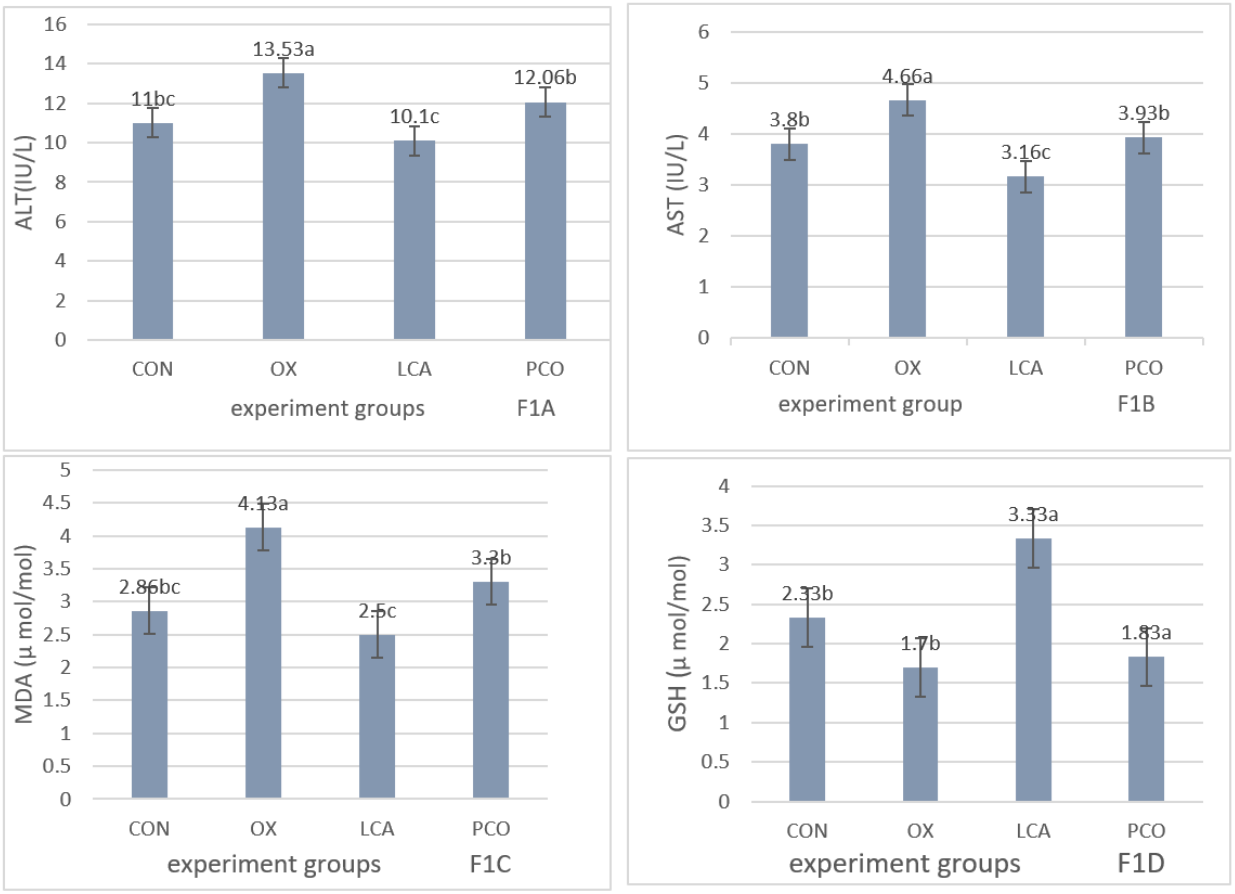

Figure (1): Effect of L-carnitine on the levels of some oxidative stress measurements (GOT enzyme (F1A), GPT enzyme (F1B), MDA, malondialdehyde (F1C), GSH, glutathione (F1D),) in adult male rabbit exposed to oxidative stress

(**G I, Con, G II (OX)**: received 0.5% H2O2/L of drinking water with a standard diet, **G III (LCA):** 150 mg L-carnitine /kg diet and regular water, **G IV (PCO)**: received 150 mg L-carnitine /kg diet and 0.5% H2O2/L of drinking water).. The values are presented as mean + SD. The different English letters above the columns indicate the presence of significant differences at the probability level P <0 .05

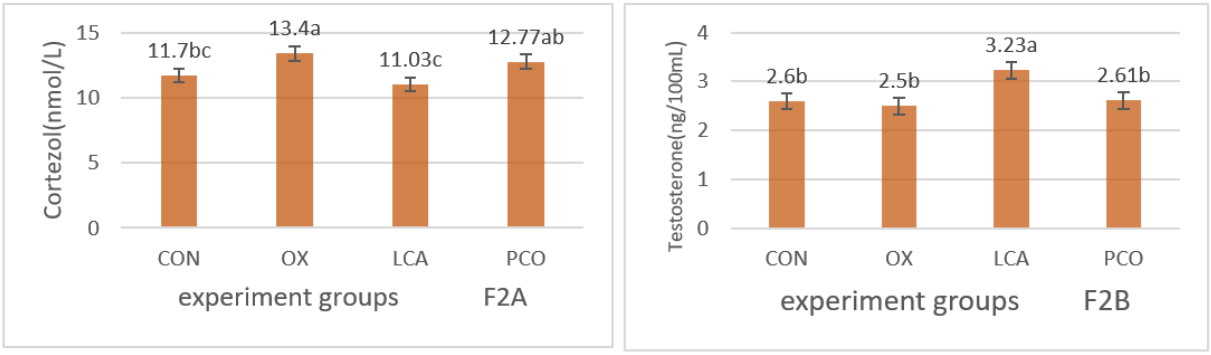

Figure (2): Effect of L-carnitine on the levels of steroid hormones (cortisolF2A, and testosteroneF2B) in adult male rabbit exposed to oxidative stress

(**G I, Con, G II (OX)**: received 0.5% H2O2/L of drinking water with a standard diet, **G III (LCA):** 150 mg L-carnitine /kg diet and regular water, **G IV (PCO)**: received 150 mg L-carnitine /kg diet and 0.5% H2O2/L of drinking water). The values are presented as mean + SD. The different English letters above the columns indicate the presence of significant differences at the probability level P <0 .05.

L-carnitine-containing diets may be the cause of the male rabbit figure (F 2B)'s elevated blood serum concentration of testosterone, as evidenced by the compound's direct impact on the hypothalamic-pituitary axis. By inducing the release of GnRH from the hypothalamus, which in turn stimulates the release of LH and FSH from the anterior lobe of the pituitary, the testicles can produce testosterone (Hadi *et al.*,2020). This could be taken into consideration as a potential explanation for the rise in testosterone activity and concentration in the blood plasma of male rabbits receiving carnitine treatment. Because carnitine suppresses free radicals and inhibits their formation, it is an effective antioxidant (Kooshesh et al.2023). By shielding Leydig cells from free radical damage, it raises the amount of testosterone that these cells produce. Leydig cells collapse as a result of free radical accumulation, which also lowers their quantity and efficiency in producing testosterone (Monageng *et al.*,2023). Free radicals also inhibit the biosynthesis of steroid hormones by destroying some important compounds involved in the formation of these hormones (Chainy and Sahoo,2020). Hydrogen peroxide works to destruction of the protein that regulates the transfer of cholesterol from the smooth endoplasmic reticulum to the cell membrane of the mitochondria to form pregnenolone, which is the first stage of testosterone production. Peroxides also work to obstruct the process of converting cholesterol to pregnenolone, which cleaves p 450 said - Chain Cleavage enzyme by inhibiting the effectiveness of the Pregnenolone side chain enzyme. for cholesterol (Miller, 2008). Free radicals also reduce the number of LH receptors on Leydig cells, leading to a decrease in their production of testosterone (Beattie *et al.*, 2013).

When we observe the results of the experiment regarding the concentration of glucose, ALT, AST, GSH, MDA, and hormone cortisol, we will notice that treatment with hydrogen peroxide caused oxidative stress in the rabbits, as we notice a significant increase in the glucose concentration, coinciding with an increase in the concentration of the AST enzyme and a reducing of GSH percentage. With an increase in the MDA percentage cortisol hormone in the rabbits of group OX, the significant increase in the concentration of glucose in blood serum may be due to the body's tendency to use some non-carbohydrate sources for the purpose of producing energy (Al-Samarai,2021) and this Which led to an increase in blood glucose concentration, and the increase in the activity of AST enzymes may be closely related to this increase, as the levels of this enzyme increased in rabbits treated with H2O2. The breakdown of the majority of the hepatic cells' cellular membranes as a result of oxidative stress could be the cause of this increase. Because of the detrimental effects of increased reactive oxygen species and lipid peroxidation in cell membranes, it eventually causes a defect in cell membrane permeability, which allows liver enzymes to leak into blood serum Malik *et al.*, (2013). We will observe a decline in antioxidant capacity when examining the levels of GSH and MDA. According to Abdel Daim et al. (2019), oxidation (decreased GSH with an increase in free radical production; high MDA) in liver cells, in particular, leads to hepatocellular toxicity. Alternatively, hepatotoxicity may originate from the hepatic portal vein area, as these enzymes have an impact on the cells in this area. Reactive oxygen species are produced more when blood from the hepatic vein is supplied to the liver because this area has the highest concentration of oxygen. Therefore, hepatotoxicity and damage to numerous

liver cells may be the cause of an increase in the concentration of the enzymes AST and ALT in the serum. Free radicals harm the cell membranes of hepatocytes (Fakoya and Olusola, 2019).

Stressful hydrogen peroxide may have had an impact on the drop in testosterone concentration (Taha., 2008). Stress frequently causes the hypothalamus to release the hormone corticotropin-releasing hormone (CRH), which in turn causes the anterior pituitary gland to release the hormone adrenocorticotropic hormone (ACTH). In turn, the increase in ACTH secretion inhibits the secretion of gonadotropin-releasing hormone (GnRH), which hurts the secretion of the hormones FSH and LH (Tsutsui *et al.*, 2000). This process occurs by stimulating the adrenal cortex to secrete the hormone cortisol (Chainy and Sahoo , 2020). This The results of Figure 2 support the explanation, as does the observed significant increase in the hormone cortisol concentration in the OX rabbits in this study. Given that the LH hormone is concentrated in this group of OX rabbits, Bergmann (2006) suggested that this decrease could be the primary cause of the drop in the testosterone concentration in that group. The hormone testosterone is released as a result of its action on Leydig cells, which are found on its receptors in the crevices of the testicular tissue.

CONCLUSION

When male New Zealand rabbits underwent hydrogen peroxide treatment to induce oxidative stress, it led to the degradation of several biochemical markers such as total protein, albumin, and glucose levels, alongside increased levels of MDA, amino acid transporter enzymes, and cortisol hormone. This oxidative stress was accompanied by a significant decrease in testosterone levels. In contrast, administration of L-Carnitine improved the biochemical blood profiles of rabbits experiencing oxidative stress. This improvement was associated with notable enhancements in stress indicators, including reduced levels of cortisol hormone, amino acid transporter enzymes, and MDA. These findings highlight the beneficial impact of L-Carnitine on male reproductive system function, as evidenced by a meaningful increase in testosterone levels. Importantly, overall blood composition remained unaffected, indicating that carnitine effectively restored oxidatively stressed animals to a state resembling normal physiological conditions.

CONFLICT OF INTEREST

The authors declare no conflicts of interest associated with this manuscript.

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