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THE EFFECT OF USING COENZYME Q10 AND WHEAT GERM OIL ON SOME BLOOD CHARACTERISTICS OF STRESSED RABBITS

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ABSTRACT

To determining effect of Coenzyme Q10 (CoQ10)and wheat germ oil(WGO) on hematological , and blood biochemical treats in rabbits buck exposed to oxidative stress, 54 animal aged 4-5 month randomly distributed into six group. Nine animals / group. as following: the first was a productive ration + tap water, the 2nd group consumption normal diet + Water with 0.04%H2O2, G3 and G5: supplemented CoQ10 100 mg/kg fodder without and with H2O2, respectively. Moreover, the G4 and G6: supplied 3 gm. WGO /kg of feed without and with H2O2 in drinking water, respectively. The results showed: No significant difference (P≤0.05) among group in the (RBC), G3 recorded a significant increase in the PCV, corresponding to a significant decrease in the MCV. The sixth group recorded increasing in the hemoglobin concentration, which led to a significant increase in the MCHC. The second treatment recorded decrease total protein, and globulin, Regarding the lipid profile, there was a significant increase in the level of triglycerides and cholesterol with H2O2. There was a significant decrease in ALT and AST levels for the third and fourth treatments compared with the rest of the oxidative stress treatments. With a significant decrease in glucose concentration, creatinine, urea and cortisol levels.

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INTRODUCTION

Oxidative stretch is an imbalance between free radicals and antioxidants in your body. Free radicals are oxygen-containing atoms with an uneven number of electrons. The uneven number permits them to effectively respond with other atoms. Free radicals can cause expansive chain chemical responses in your body since they respond so effectively with other particles. These responses called oxidation. They can be useful or destructive. Natural antioxidants from the basic components of food. A decrease in these compounds in food leads to a decrease in the body's susceptibility to free radicals. and the elimination of their destructive effects on the body and leads to a decrease in its immune susceptibility to diseases and the possibility of increasing the chance of problems associated with the damage caused by free radicals. which are excitable and unstable, have high energy and are very affinity to interact with vital

molecules in the body(Taha & Mawlood,2019) whose distinguishes free radicals is their ability to initiate a series of reactions that lead to an actual amplification of the activity of free radicals, which leads to destruction of essential macromolecules and cell components in biological systems (Matkovics, 2003). Coenzyme Q10 is a critical antioxidant known as ubiquinone, which is found normally in higher and microorganisms and is the as it were fat-soluble antioxidant that can be synthesized within the body (Raouf and Taha, 2020). It has an imperative part in vitality generation by being one of the electron and proton transport compounds within the oxidative phosphorylation forms within the mitochondria of cells (Shukla and Dubey, 2018). It moreover works to secure cell films and lipoproteins in plasma from lipid peroxidation, as well as its part within the recovery of other antioxidants such as vitamin E and C (Ali *et al*., 2019). Wheat germ oil is one of the fat-soluble antioxidants that can be extricated from numerous sorts of plants. It contains alpha- and gamma-tocotrienols that invigorate the arrangement of tocopherolmediated, which incorporates a major part in decreasing the formation of eicosanoid, which is one of the most variables in causing oxidative stress. Moreover, rancidity of fats (Paranich *et al*., 2000). Wheat germ oil decrease the level of total cholesterol, triglycerides, low-density lipoproteins, and exceptionally low-density lipoproteins (Farooq et al., 2021). It moreover has the capacity to make strides in the action of ascorbic corrosive and glutathione and diminish the level of MDA and Diminished Glutathione. (El-Sisy *et al*., 2018). Therefore, this study was designed with the aim of knowing the effect of coenzyme Q10 and wheat germ oil on some blood parameters of rabbits that are normal or exposed to oxidative stress.

MATERIAL AND METHODS

Using fifty-four rabbits, this study was designed, their average weights (1254.82 ± 36.78 g), their ages ranged between 4-5 months. After ensuring the animals' safety and freedom from diseases, they were fed on a typical diet in the form of coarsely crushed fodder, which worked to supply an energy amount of 2800 kilocalories and 16% protein, in addition to providing it with green fodder at 20% of the diet, and the water was given freely to the animals. After that, the animals were randomly distributed into (6) groups, and each included 9 animals, as follows:

G1: control group.
G2: oxidative stress group adding 0.04%H2O2/L drinking water .
G3: adding 100 mg Co Q10/kg fodder.
G4: adding 3gm WGO /kg fodder.
G5: adding 2to drinking water and adding 100 mg Co Q10/kg fodder.
G6: addition of H_2O_2 to drinking water and adding 3gm WGO /kg fodder.

Blood Samples collection

Blood samples were collected from animals by heart thrust and by three animals from each treatment twice during the eight-week study period. After obtaining the blood, samples were placed in two types of tubes, the first containing an anticoagulant substance for the purpose of conducting blood tests, and the second was placed in clean test tubes free of anticoagulants, and then

deposited in a centrifuge, to obtain the blood serum, which was preserved by freezing until biochemical tests were conducted on it.

Hematological tests

All blood parameters were determined using a Chinese-origin Hematology analyzer (COUNT 60). Which included Red Blood Cells (RBC),)Packed Cell Volume (PCV), Hemoglobin (Hb), Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), Mean Corpuscular Hemoglobin Concentration (MCHC), and White Blood Cells (WBC).

Biochemical tests for blood serum

Glucose, total protein, albumin, globulin, urea and creatinine concentration, cholesterol, triglycerides, high-density lipoproteins, and cortisol levels were all estimated using a prepared test kit from BIO LABO. COM.

Statistical analysis

Study samples were analyzed using a one-way Complete Randomize Design (CRD), and to test of significance of differences between groups, Duncan's multiple range test (Duncan, 1955) were tested by using, and the ready-made statistical analysis program SAS (2004) was used.

RESULTS

Hydrogen peroxide or Co Q10 and wheat germ oil (WGO) when added to the diets of rabbits that are normal or exposed to oxidative stress did not have a significant effect on RBC. Moreover, noted that the sixth treatment recorded a significant superiority compared to the second and third treatments. From the results of Table (1), we note that the treatment with Coenzyme Q10 led to a significant increase in the PCV compared to the rest of the study treatments groups, with the exception of the sixth treatment, which did not record significant differences between them. With regard to the effect of treatments on the of hemoglobin concentration, the sixth treatment led to a significant increase in the Hb compared to others.

Table (1): Impact of adding Coenzyme Q10 and wheat germ oil on hematological traits in stressful rabbits buck by H2O2.

Traits / Treatments	RBC* (10^6/μL)	PCV%	HB g/100ml
G1	6.18 ± 0.2 ab	39.73±1.71 b	12.58±0.55 b
G2	6.05±0.11 b	40.70±1.23 b	13.01±0.33 b
G3	5.98±0.11 b	45.55±3.19 a	12.65±0.25 b
G4	6.23±0.17 ab	40.88± b 0.54	13.01±0.24 b
G5	6.30±0.20 ab	40.11±2.24 b	12.63±0.73 b
G6	6.41±0.11 a	43.86±0.85 ab	14.23±0.36 a

[abc] demonstrates that there are significant differences between the means at the probability level ($p \leq 0.05$).

G1: control group. G2: oxidative stress group. G3: adding 100 mg Co Q10/kg fodder. G4: adding 3gm WGO /kg fodder. G5: adding H2O2 to drinking water and adding 100 mg Co Q10/kg fodder. G6: Addition of H2O2 to drinking water and adding 3gm WGO /kg fodder.

The results in Table (2) indicated a significant increase in MCV values when treated with coenzyme Q10 at a concentration of 100 mg/kg feed for local rabbits compared to all treatments. The results of Table (2) a significant increase in the MCH of the sixth treatment compared with the control treatment and the third, fourth and fifth treatment and did not differ significantly from the second group. The third treatment recorded a significant decrease in the MCHC compared to all treatments, as shown by the results of the statistical analysis in Table (2), and the rest of the treatments did not show significant differences between them. The treatment with hydrogen peroxide and coenzyme Q10 did not register a significant difference from the control treatment and the fourth and fifth treatments, while the sixth treatment recorded a significant decrease in the total number of white blood cells compared with the whole study treatments.

Table (2): Impact adding Coenzyme Q10 and wheat germ oil on hematological traits in stressful rabbits buck by H_2O_2

Traits / Treatments	MCV	MCH	MCHC%	WBC x*10^3
G1	64.25±1.08 b	20.33± 0.31 cd	31.95±0.09 a	10.06±0.48 a
G2	67.36±0.88 b	21.53±0.19 ab	31.95±0.20 a	8.96±1.82 ab
G3	76.01±4.96 a	21.15±0.61 bc	28.46±2.02 b	8.80±0.75 ab
G4	66.01±1.70 b	20.98±0.41 bcd	31.80± 0.30 a	7.93±0.49 b
G5	63.53±1.53 b	20.00±0.55 d	31.46±0.19 a	7.91±0.54 b
G6	68.45±1.19 b	22.18±0.42 a	32.41±0.42 a	6.10± 0.23 c

[abc] demonstrates that there are significant differences between the means at the probability level ($p \leq 0.05$).

From table 3 the second group recorded a significant decrease in the total protein concentration compared to all treatments. The treatments added to Co Q10, WGO and the control treatment showed a significant increase in the total protein concentration compared to the second treatment. The treatments had no significant effect on albumin concentration. There was a significant decrease in serum globulin concentration in favor of the second treatment e compared to the G1, G3, G5, and G6.

Table (3): Impact adding Coenzyme Q10 and wheat germ oil on serum proteins in stressful rabbits buck by H_2O_2

Traits / Treatments	Total protein g/100 ml	Total albumin g/100 ml	Total globulin g/100 ml
G1	6.30±0.36 a	4.19±0.15 a	2.10±0.38 a
G2	5.52±0.36 b	4.41± 0.17 a	1.11±0.27 b
G3	6.06±0.15 a	4.34±0.25 a	1.72±0.17 a
G4	6.13±0.19 a	4.54±0.21 a	1.59±0.14 ab
G5	5.94±0.40 a	4.22±0.15 a	1.71±0.32 a
G6	6.05±0.37 a	4.34±0.11 a	1.71±0.29 a

[abc] demonstrates that there are significant differences between the means at the probability level ($p \leq 0.05$).

The results of the statistical analysis in Table (4) indicated a high cholesterol concentration in the G2 compared to the G1, G3, and G5.

Table (4): Impact adding Coenzyme Q10 and wheat germ oil on serum Cholesterol, Triglycerides, and Glucose in stressful rabbits buck by H_2O_2.

Traits / Treatments	Cholesterol mg/100ml	Triglycerides mg/100ml	Glucose mg/100ml
G1	38.25±2.41 ab	121.02±6.21 b	115.00 ±5.64 b
G2	42.00± 2.77 a	137.52±5.17 a	134.87±3.09 a
G3	29.50±2.15 c	98.77±9.15 c	113.25±5.47 b
G4	39.60± 0.86 ab	121.40±5.42 b	115.75±1.13 b
G5	36.00±1.21 b	131.57±3.38 a	117.25±3.68 b
G6	37.85±1.19 ab	119.75± 3.02 b	118.00±3.91 b

[abc] demonstrates that there are significant differences between the means at the probability level ($p \leq 0.05$).

The second and fifth treatment also recorded increase in triglycerides compared to the G1, G4 and G6 treatment. While the glucose concentration increased significantly in G2 compared with the others. The results of the statistical analysis in Table (5) indicated to significant increase in urea concentration at the G2 compared to all treatments except for G1. A significant decrease in creatinine concentration in the blood serum of the G6 compared to the G2 and G1 and it did not differ with others. Notice a significant increase in the second oxidative stress treatment compared to all treatments, the fifth and sixth treatments also recorded a significant decrease compared to the first, third and fourth treatments. On the concentration of GOT enzyme. Significant decrease compared to the first, third and fourth treatments. The third group had a significant decrease in GPT enzyme compared with the G2, G5 and G6.

The results in figure (1) refers to significant increase in cortisol hormone for (G2), compared with the rest of the other experimental treatments. In addition, the rest of the treatments did not register a significant difference from the G1, moreover, there is a significant increase in the animals of the G6 and G4 compared to the G3.

Table (5): Impact adding Coenzyme Q10 and wheat germ oil on liver and kidney function indicators for stressful rabbits buck by H_2O_2

Traits / Treatments	Urea mg/100ml	Creatinine mg/100ml	GOT IU/100ml	GPT IU/100ml
G1	45.57±2.07 ab	0.97±0.10 a	9.17±0.25 c	3.70±0.20 bc
G2	49.68±2.71 a	0.95±0.10 ab	11.60±0.48 a	4.22±0.26 ab
G3	36.90±1.54 d	0.82±0.03 abc	8.65±0.17 c	3.42±0.25 c
G4	42.88± 0.85 bc	0.90±0.06 abc	8.79± 0.15 c	3.76±0.29 abc
G5	40.56±1.70 dc	0.81±0.02 bc	10.06±0.46 b	4.12±0.13 ab
G6	39.24±0.40 dc	0.79±0.02 c	10.51±0.60 b	4.38±0.19 a

[abc] demonstrates that there are significant differences between the means at the probability level ($p \leq 0.05$).

Although the treatments did not differ compared to the control treatment, G2 and G3 showed decrease in RBC. In addition, the reason for the significant decrease in the number of RBC when treated with hydrogen peroxide may be due to causing oxidative stress. Moreover, this decrease number of RBC. (Johns & Heller, 2021). As for the decrease in the product due to the addition of CoQ10. It is associated with PCV values, where these values showed a significant increase in G3 compared to other treatments, and this means that Co Q10 increased MCV (Table 2). This improvement in red blood cell indicators is due to the effect of coenzyme Q10 in raising the concentration of testosterone. Where testosterone considered among the regulating factors for the formation of red blood cells by stimulating the formation of the hormone erythropoietin in the kidney on the one hand and stimulating the formation of red blood cells on the one hand. Red blood in the bone on the other hand (Coles, 1986). Improvement in blood characteristics attributed to the improvement in antioxidants status. Which may give an indication to provide protection for red blood cells from oxidative damage during formation or after production from the bone marrow. We note, through the study of the blood characteristics of the local rabbits treated with WGO, an improvement in some of their indicators, even in the animals treated with hydrogen peroxide. Red blood cells as well as their anti-inflammatory effect (Abdel-Fattah *et al*., 201), and this means that the increase in the values of HB, MCH, and MCV may be due to the effect of WGO, which worked to raise the production of RBC.

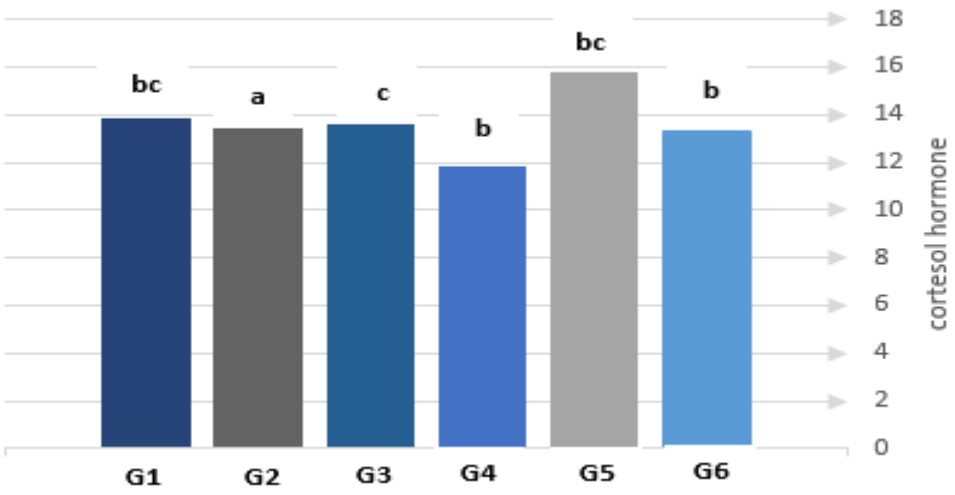

Figure 1: Impact adding Coenzyme Q10 and wheat germ oil on cortisol hormone concentration for stressful rabbits buck by H_2O_2

[abc] demonstrates that there are significant differences between the means at the probability level ($p \leq 0.05$).

Treatment with H_2O_2 led to a significant decrease in total protein compared to other. This decrease may be related to the production of free radicals caused by hydrogen peroxide, which ultimately leads to a defect in the immune system (Lauridsen, 2019), which is achieved through Oxidation processes of protein and the production of nitrous radical or damage to cell membranes and their components, including protein. Treatment with H_2O_2 led to a significant decrease in globulin, while the albumin concentration did not show any significant difference among the six treatments of the experiment. From here, we can note that the addition of Co Q10 and WGO to stressfully rabbits (the fifth and sixth treatments) had a positive effect on total protein and the proportion of each of the albumins and globulins, as their concentrations did not differ significantly from the control treatment (natural group of animals). The absence of significant differences in the concentration of total protein when adding Co Q10 agrees with the results of Raouf and Taha (2019) when adding Co Q10 at a concentration of 100 mg/kg feed for four weeks. It also agrees with the results of researcher Abdou *et al*., (2017), where they did not notice significant differences in the concentration of total protein in mice treated with WGO, even though they were raised under oxidative stress conditions.

By observing the results of Table (3), an improvement in the lipid profile was observed when using CoQ10. During or after the process of absorption of Co Q10, the proportion of CoQ10 often decreases as a result of its conversion to ubiquinol after mixed it with chylomicron molecules to transfer it to the liver, (Bhagavan and Chopra, 2006) Finally, Co Q10 is packaged in lipoprotein molecules to be released into bloodstream. As a result, the bulk of CoQ10 molecules are loaded into LDL and VLDL molecules, as well as small amounts are carried in HDL molecules and through this mode of transport is provided Protection of these molecules from oxidation by a process lipid peroxidation (Potgieter *et al*., 2013). In addition, the treatment of local rabbits under

conditions of oxidative stress with WGO led to an improvement in the lipid profile through the absence of significant differences between the G6 and G1. This result agrees with El-Sayed (2019) and Jankins *et al*., (1999) when they noticed an improvement in the lipid profile of animals treated with WGO. On the other hand, wheat germ oil contains many biological compounds such as vitamin C and phytosterols such as octacosanol, which may be the cause of low levels of cholesterol and triglycerides (Jonnala *et al*., 2005).

Studies have indicated that wheat germ oil contains a mixture of saturated and unsaturated fatty acids. In general, the percentage of unsaturated ones exceeds them, and most importantly, most of these fatty acids are of the alpha type, and the main acid of them is alpha linoleic. Moreover, this provided them with an anti-inflammatory effect, and reduced production Reactive oxygen species and the effect of NADPH oxidase, which may be the reason for its antioxidant action. It was also noted that linoleic acid could predispose the cholesterol involved in the synthesis of biofilm phospholipids after removing it (Zacchi et al., 2006). Wheat germ oil contains many phenolic compounds that actually improve liver function by removing low-density lipoproteins from the bloodstream by increasing their LDL receptors in the liver and binding to polyprotein B (EL-Beshbishy et al., 2006).

Levels of both urea and creatinine are important indicators of kidney function in animals. From the observation of the results of Table (4), we note a significant increase in the concentration of urea and creatinine in the blood serum of rabbits exposed to oxidative stress induced using H_2O_2. The animal consumes proteins as an alternative source of carbohydrates to produce energy, this leads to the formation of large amounts of urea (Yousef et al., 2010). Free radicals lead to the oxidation of proteins and amino acids. This process results in an increase in the concentration of urea in the blood serum as a byproduct (El-Boshy et al., 2015).

Alternatively, researchers mainly to the complications that occur in a number of parts of the body because of oxidative stress, including nephropathy, which characterized by gradual negative changes in kidney function, may explain it. Which results in a high concentration of urea and creatinine, meaning that hydrogen peroxide affects the concentration of filtration of the kidneys. In addition, on the processes of secretion and reabsorption in the renal tubules (Ruiz et al., 2013 and Friederich-Persson et al., 2013). Rabbits exposed to oxidative stress were treated with Co Q10, a significant decrease in urea concentration and creatinine was observed. Which causes an increase in urea production and this was observed in the results of Table (3), where the G5 recorded values that were statistically equal with the normal animals in G1, in the total protein concentration, albumin, and globulin. (Orlando et al., 2018 and Raouf, 2019), Also, the action of Co Q10 may be on rebuilding and restoring the tissues and cells of the renal units, in addition to reducing the processes of programmed cell death and modifying the renal filtration processes, which helps to excrete wastes outside the body (Abdeen et al., 2020). We can note from the results of this study that the treatment with facilities Coenzyme Q10 caused a significant decrease in the concentration of enzymes transporting the amino group table No. (4), which

leads to a decrease in the concentration of urea and creatinine in the blood (H). Agiwara et al., 2012 and Holecek, 2013 and Park et al., 2017), and thus we can say that Co Q10 reduce the damage caused by free radicals, providing glucose as an energy source instead of directing the metabolism processes in stressed animals to producing energy from non-existent sources. Carbohydrates and this may be a reason for lowering the concentration of urea in the blood (Ahmadvand, 2012).

Treatment with hydrogen peroxide led to stimulating the adrenal cortex to secrete the hormone cortisol, which begins to direct the body towards producing energy from non-carbohydrate sources (Charmandari et al., 2005). To cause, in the end, to raise of blood sugar level. In addition, provide the liver needs of glycogen and carbohydrates. accompanied by this action with an increase in the activity of enzymes that transport the amine group and the production of reactive oxygen species and an increase in the products of protein metabolism that appear as a high levels of urea and creatinine (Ranjan et al., 2018).

The reason for the low concentration of GOT and GPT enzymes in the serum of normal and stressed domestic rabbits when treated with Coenzyme Q10 as a result of maintaining the cell membranes from the harmful effects of free radicals and preventing the decomposition of fatty acids in them. This improvement in the levels of enzymes transporting the amino group was accompanied by a significant decrease ($P \leq 0.05$) in the concentration of cortisol, the clear indicator of the state of stress experienced by the stressed animal (Tsigos et al., 2020).

The addition of wheat germ oil also had a positive effect on the concentration of glucose, the enzymes that transport the amino group, and the concentration of cortisol in normal stressed animals. Akool (2015) indicated an improvement in the oxidation and reduction indices of male wisteria rats suffering from oxidative damage in the liver tissue, where he noticed an improvement in the endogenous oxidation indices such as GSH, SOD and CAT, which inhibited the stages of lipid peroxidation and the gene expression of some cofactors. On the other hand, he noticed an improvement in the level of liver enzymes GOT and GPT. This improvement is consistent with what was observed improvement in some indicators of oxidation and reduction. What supports these assumptions is what was found (Mehranjani et al., 2007) of a significant increase in the level of vitamin E in the liver of animals treated with wheat germ oil, which gave them the antioxidant power in the liver tissues.

CONCLUSIONS

We conclude from this study that the treatment of normal and stressed rabbits with Co Q10 and wheat germ oil improved some blood parameters, As lipid profile and indicators of kidney and liver function.

تأثير استعمال المرافق الانزيمي Q10 وزيت جنين القمح في بعض المتغيرات الدموية للأرانب المجهدة

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الخلاصة

اجريت هذه الدراسة لمعرفة تأثير إضافة المرافق الانزيمي Q10 وزيت جنين القمح في عدد من الصفات الفسيولوجية و الكيموحيوية لذكور الأرانب المحلية المجهدة تأكسديا باستعمال H_2O_2 ، تم استعمال 54 من ذكور الارانب المحلية بعمر 4–5 أشهر ، وزعت عشوائياً على ست معاملات بواقع 9 حيوانات / معاملة . وكانت المعاملات كما يأتي المعاملة الأولى (السيطرة) عليقة انتاجية+ ماء اعتيادي و المعاملة الثانية إعطاء عليقة انتاجية + ماء مضاف اليه H_2O_2 بنسبة 0.04 % و المعاملتين الثالثة والخامسة: إضافة المرافق الانزيميQ10 بتركيز 100 ملغم\كغم علف بدون ومع بيروكسيد الهيدروجين على التوالي . والمعاملتين الرابعة والسادسة: إضافة زيت جنين القمح بتركيز 3غم زيت جنين القمح /كغم علف بدون ومع بيروكسيد الهيدروجين في ماء الشرب على التوالي واستمرت الدراسة لمدة شهرين تم اجراء الفحوصات مرتين في منتصف ونهاية التجربة. وتم التوصل الى النتائج الاتية: لم تسجل جميع المعاملات اختلاف معنوي ($P\leq0.05$) عن معاملة السيطرة في العدد الكلي لكريات الدم الحمراء ، وسجلت المعاملة الثالثة ارتفاع معنوي في قيمة مكداس الدم قابلة انخفاض معنوي في متوسط احجام كريات الدم الحمر. وسجلت المعاملة السادسة ارتفاع معنوي في تركيز هيموكلوبين الدم مقارنة مع بقية المعاملات مما أدى ارتفاع معنوي في معدل تركيز هيموكلوبين الكرية. سجلت المعاملة الثانية انخفاض معنويفي تركيز البروتين الكلي والكلوبيولين. في صورة الدهن وجد ارتفاع معنوي في مستوى الدهون الثلاثية والكولسترول عند المعاملة باستعمال بيروكسيد الهيدروجين مقارنةً مع باقي المعاملات. وجود انخفاض معنوي في مستويات الأنزيمات الناقلة لمجموعة الامين للمعاملتين الثالثة والرابعة مقارنةً مع باقي المعاملات. مع انخفاض معنوي في تركيز الكلوكوز والكرياتنين واليوريا وتركيز هرمون الكورتيزول.

الكامات المفتاحية: CoQ10، جنين القمح، الاجهاد، الدم .

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