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Effect of wheat germ oil and Coenzyme Q10 on physiological performance and testicular oxidative stress markers in rabbit bucks

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Abstract

The aim of this study is to investigate the efficacy of WGO and Coenzyme Q10 low and high doses on physiological performance and testicular oxidative status of male rabbit bucks. In addition tracing the best suitable interval which modulates physiological response under Egyptian summer condition. This experiment was done on a pure strain of Sinai gabali bucks 3 months old. The animals were randomly divided into four groups each one comprise 6 animals. The 1st group served as normal control (C), the 2nd group treated with wheat germ oil (WGO) (300 mg/kg BW), the 3rd group treated with Coenzyme Q10L (10 mg/kg BW), and the 4th group treated with Coenzyme Q10H (20 mg/kg BW). Rabbits of all treatments were given oral administration daily for 60 days, and blood samples were collected monthly along 6 months after the end of the treatment. Moreover, the rabbits were decapitated and the testes tissues were exceed for evaluation Reduced Glutathione (GSH), Malondialdehyde (MDA), Oxidized Glutathione (GSSG) and Nitric oxide (NO) contents. Data revealed that liver function did not show any significant differences due to treatment. Month effect did not show any differences in ALT and AST values. According to the interaction of treatment and month data showed the best value of ALT and AST (26.48 and 32.72 U/L, respectively) in C×6th and Coenzyme Q10L×5th month, respectively. With regard to Lipid profile data showed significant decrease (P<0.05) of total cholesterol, LDL and triglyceride due to Coenzyme Q10L treatment and significant (P<0.05) increase in HDL due to Coenzyme Q10L treatment and the interaction showed the best values for TC, TG, HDL and LDL (89.50 mg/dL Coenzyme Q10L×2nd month, 87.50 mg/dL Coenzyme Q10L×3rd month, 36.00 mg/dL Coenzyme Q10L×3rd month and 35.96 mg/dL Coenzyme Q10L×3rd month, respectively). Results did not show any significant (P> 0.05) differences in blood proteins in the 4 groups due to the effect of treatments on male rabbits. Also month effect data did not show any changes (P> 0.05) in blood proteins. The interaction showed the best values of blood proteins, total protein, albumin, globulin and A/G ratio, 7.63g/dL in Coenzyme Q10H×4th month (hot THI), 4.17g/dL in WGO×2nd month (hot THI), 3.58g/dL in Coenzyme Q10H×4th month (hot THI) and 1.30 in Coenzyme Q10H×6th month (mild THI), respectively). Oxidative markers of testicular MDA and GSH showed decreases in MDA and increase in GSH due to WGO and Coenzyme Q10 treatments, respectively. In conclusion, the present study suggests that Coenzyme Q10 and WGO treatments improve physiological performance, reduced heat stress and testicular oxidative stress markers in Sinai gabali male rabbits under Egyptian summer condition.

Keywords: Coenzyme Q10, Wheat germ oil, Sinai gabali rabbits, Lipid profile, Oxidative markers.

Introduction

Heat stress (HS) and oxidative stress often blamed for suboptimal reproductive efficiency and is a worldwide problem, which inflicts heavy economic losses reflected in limiting the breeding season of rabbits to be normally from September to May in northern hemisphere and subtropical regions.

Oxidative stress is defined as the unbalancing between production of free radicals, molecules characterized by high reactivity due to one or more unpaired electrons in the external orbital, and antioxidant defenses in the biological systems. In addition, it is considered an important pathogenetic mechanism in different diseases (Halliwell and Gutteridge, 1979). An augmented ROS production can be the consequence of an augmented electronic flow in the respiratory chain, when it is activated by an increased energetic demand or contribution of

substrates (Turrens and Boveris, 1980). There are many substances that used as antioxidants and their role to protect the body against the free radicals. These substances can be liposoluble, such as vitamin E and coenzyme Q10, the only liposoluble antioxidant synthesized in living organisms and herbal extract as wheat germ oil (WGO). WGO contains alpha- and gamma- tocotrienols and induces the tocopherol-mediated redox system and inhibits the synthesis of eicosanoid, which activates the lipid peroxidation process (Paranich *et al.*, 2000).

Coenzyme Q10 (CoQ10), ubiquinone-10, is an important lipid-soluble molecule, which exists in the inner membrane of mitochondria. It works as hydrogen carrier in the respiratory chain and plays an important physiological role. It is not only activates enzymes but also enhances the immunity of organisms. It is an antioxidant that has great importance against free radicals, protects the stability

of the cell membrane, DNA from free radicals induced oxidative damage and helps recycling of vitamin E and maintain healthy energy levels. It plays a crucial role in the production of cellular adenosine triphosphate which provides modulating antioxidants defense system. Studies have shown that antioxidants are uniquely different from each other and each have a specific function in the body. They are attracting more and more attention and the range of clinical applications is gradually being expanded (Roffe *et al.*, 2004; Sohmiya *et al.*, 2004; Yalcin *et al.*, 2004).

The aim of this study is to investigate the efficacy of WGO and Coenzyme Q10 low and high doses on physiological performance and testicular oxidative status of male rabbit bucks. In addition tracing the best suitable interval which modulates physiological response under Egyptian summer condition.

Materials and methods

Experimental design:

This experiment was done on 24 mature Sinai gabali bucks 3 months old with average weight 1.9 ±0.1kg. The animals were randomly divided into four groups each one comprise 6 animals. The 1st group served as normal control (C) treated with distilled water (0.5mL / kg BW), 2nd group treated with daily oral dose of wheat germ oil (300 mg/kg BW), 3rd group treated with daily oral low dose of Coenzyme Q10 (10 mg /kg BW), and 4th group treated with daily oral high dose of Coenzyme Q10 (20 mg/kg BW).

Management:

These animals were housed in animal house of rabbitary farm at cages with wire-mesh bases constructed of galvanized steel. Dimensions of cages were (60 length × 40 width × 40 cm height). All animals were housed in a room with controlled lighting (14 h/day) and natural ventilation. Animals were supplied with adequate standard diet pellets purchased from (IBEX) company with ingredient composition designed from Nutrition Requirement Center (NRC, 1977). The pelleted ration was provided in the morning. Fresh clean water was available at all time. After adaptation period (7 days), animals treated

for 60 days under the same conditions of adaptation period.

Blood samples:

Blood samples from individual animals in each group were withdrawn from the marginal air vein of the ears which visualized and dilated by a warm-wash cloth before sampling. The samples were taken using gauge butterfly catheter according to the method of Moore (2000). Blood samples were withdrawn from each animal at zero time (at five months of age, after two months of treatment), and monthly within for next six months. Samples collected from each animal in clear centrifuge tubes and were kept at room temperature for one hour and, half. The tube centrifuged at 3500 r.p.m for 20 min, the supernatant layer for clear serum was carefully withdrawn and kept at -20 °C until subsequent analysis.

Testes tissues: At the end of the experiment, the rabbits were decapitated and dissected testes tissues were excised for evaluation a testicular Reduced Glutathione (GSH), Malondialdehyde (MDA), Oxidized Glutathione (GSSG) and Nitric oxide (NO) contents.

Ambient temperature, relative humidity and temperature humidity index:

The ambient temperature and relative humidity were obtained daily from Meteorological Authority in Qalioubia throughout the whole experimental period. The temperature humidity index (THI) was calculated during mild and hot months according to Marai *et al.* (2000) as:

$$THI = db^{\circ}C - [(0.31 - 0.31 RH/100) \times (db^{\circ}C - 14.4)].$$

Where, THI= temperature humidity index, db^oC= dry bulb temperature in Celsius and RH= relative humidity ÷100.

A value of THI < 22.2 was considered remarkably an absence of heat stress, while the values for 22.2 to 23.3 referred to moderate (mild) heat stress, 23.3 to < 25.6 referred to several heat stress and > 25.6 referred to very severe heat stress. Values of THI during the experimental period are presented in Table 1:

Table 1. The monthly average temperature, humidity and temperature- humidity index (THI) in the area of farm during the experimental period (May- October, 2017).

| Month | Average of air temperature(°C) | Average of humidity (%) | Average of THI | Heat stress |
|-----------|---------------------------------|-------------------------|----------------|-------------|
| May | 27 | 44.86 | 24.84 | Sever |
| June | 29 | 48.73 | 26.67 | |
| July | 31 | 53.32 | 28.59 | Very Sever |
| August | 30.5 | 54.90 | 28.24 | |
| September | 28 | 54.30 | 26.07 | |
| October | 25 | 50.79 | 23.33 | Mild |

Biochemical parameters:

Serum total cholesterol was quantified spectrophotometrically using Stanbio Cholesterol Liquid Color® Kit, (Proc. No. 1010) produced by Stanbio Laboratory Inc., Boerne, Texas, USA according to **Allain et al. (1974)**. Serum triglycerides concentrations were quantified spectrophotometrically according to **Fossati and Prencipe (1982)** by using Stanbio Liquid Color®, Triglycerides Kit (Proc. No. 2100) employed from, Stanbio Laboratory Inc., Boerne, Texas, USA. The serum high-density lipoprotein HDL- cholesterol (mg/dL) was determined by the method of **Lopez (1977)** and the serum low-density lipoprotein LDL- cholesterol (mg/dL) was according to the method of **Fossati and Prencipe (1982)** and the transaminases enzyme (ALT, AST) activities was preformed according to **Reitman and Frankel (1957)**. Concentration of total protein and albumin was estimated using Biuret method in the presence of alkaline cupric sulfate according to the methods of **Dumas et al (1971)**. Globulin value was obtained by subtracting the value of albumin from the corresponding value of total protein. The albumin to globulin ratio (A/G) was calculated by dividing A/G value and determined the Malondialdehyde (MDA) in testes tissue according to **Karatepe (2004)**. Determination of Oxidized Glutathione (GSSG) and Reduced Glutathione (GSH) in tests tissue detected by using the method of **Jayatilleke and Shaw (1993)** and the Determination of testes tissue Nitric Oxide (NO) ($\mu\text{mol/ g tissue}$) detected by using the method of **Papadoyannis et al. (1999)**.

Statistical analysis:

Analysis of variance in factorial was carried out using SAS procedure guide (**SAS, 2004**). According to the following liner model:

$$X_{ijk} = \mu + T_i + M_j + (TM)_{ij} + e_{ijk} \dots \dots \dots \text{(Model)}$$

Whereas:

X_{ij} = the observation of traits for ijk^{th} buck, μ = the overall mean, T_i = the effect of the i^{th} treatments, M_j = the effect of the j^{th} month, $(TM)_{ij}$ = the fixed effect of the interaction between the i^{th} treatments and the j^{th} months and e_{ij} = random error assumed to be independently and randomly distributed.

Significant differences among means were tested using Duncan multiple rang test (**Duncan, 1955**).

Data expressed via factorial design and extracted to show significant between groups epically interaction effect

The following linear model was applied for tissues measurements:

$$Y_{ij} = \mu + \alpha_i + e_{ij} \dots \dots \dots \text{(Model 2)}$$

Whereas:

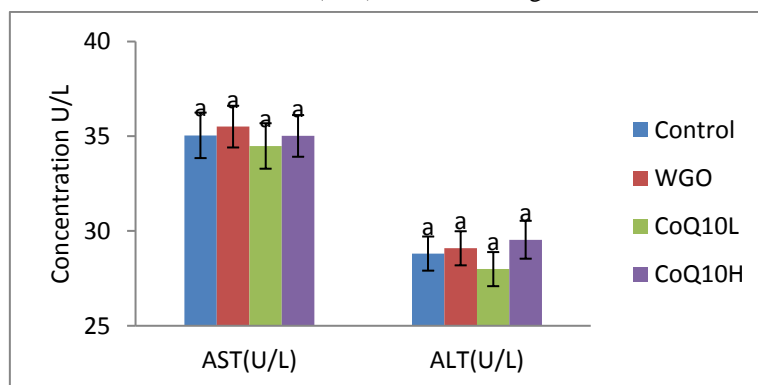
Y_{ij} = observation measured, μ = the overall mean, α_i =effect of the i^{th} treatment and e_{ij} = experimental error assumed to be randomly distributed with $IND \sim (0.6 \ 2e)$.

Results and Discussion.

Liver function:

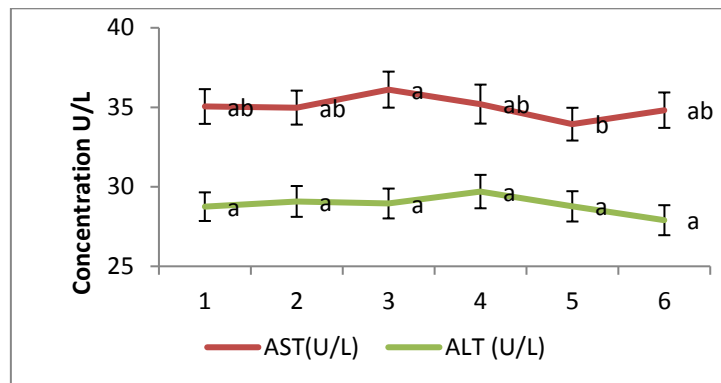
Observed data in Figure 1 showed non-significant differences between treatments and control on liver enzymes. It was found that the group of male rabbit which treated with daily oral dose of Coenzyme Q10 (10 mg / kg B.W) revealed non- significant decreases AST and ALT values (34.49 and 27.99 U/L), respectively compared with other treatments. This means that low dose of Coenzyme Q10 and WGO did not affected liver enzymes.

Fig. 1. Effect of Coenzyme Q10 (CoQ10) and Wheat Germ Oil (WGO) on Aspartate aminotransferase AST (U/L) and Alanine aminotransferase ALT (U/L) in male Sinai gabali rabbits after 60 days of treatment .



WGO = Wheat GermOil (300mg/Kg B.W), CoQ10 L= 10 mg/ Kg B.W. CoQ10H = 20 mg/Kg B.W.

Observed data in Figure2 did not show any significant differences between different 6 months in ALT activity the other hand results showed significant differences ($P < 0.05$) in AST values. These results showed that the least value of AST (33.94 U/L) was in the 5th month of experimental period and the least value of ALT (27.90 U/L) was in the 6th month of experimental period (October).

Fig. 2: Effect of month on Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT) in male Sinai gabali rabbits after 60 days of treatment with Coenzyme Q10 and WGO.

ALT = Alanine aminotransferase, AST = Aspartate aminotransferase.

1st = May, 2nd = June, 3rd = July, 4th = August, 5th = Septembre, 6th = Octobre.

According to the interaction between treatment and month, data in Table 2 showed the best value of ALT and AST (26.48 and 32.72 U/L) in C × 6th and Coenzyme Q10L × 5th month, respectively. And the data showed the lowest measurements of AST in Coenzyme Q10L in the 5th month and the lowest value of ALT was in the control in 6th month (October, mild THI) with a mean value of 26.48 U/L. These results showed non-significant differences between treatments and control on liver enzymes. This means that Coenzyme Q10 and WGO did not show any pathological alteration in liver and indicated the safety

for liver enzymatic- profile after treatment along 2 months.

The decrease of liver enzymes may be due to the role of stability of Coenzyme Q10 membrane which leads to reduction of ALT and AST leakage from liver. These results are in agreement with the finding of *Ali et al. (2010)* who suggested that Coenzyme Q10 protected rats against Carbon Tetrachloride (CCl₄) induce liver injury. Ubiquinone is an internal synthesized lipid soluble benzo-quinone compound that is found in most living cells in the body act as an expansion electron carrier in the mitochondrial respiratory chain (*Forbes et al., 2008*).

Table 2. Effect of wheat germ oil (WGO) and Coenzyme Q10 (CoQ10) on aspartate aminotransferase (AST) and alanine aminotransferase (ALT) in blood serum of male Sinai gabali rabbits after 60 days of treatment.

| Interaction (treatment × month) | AST (U/L) | ALT (U/L) |
|---------------------------------|-----------------|----------------|
| Control × 1 st m. | 34.3 ± 1.049ab | 28.82 ± 0.914a |
| WGO × 1 st m. | 35.57 ± 1.09ab | 29.73 ± 1.059a |
| CoQ10L × 1 st m. | 35.11 ± 1.072ab | 27.01 ± 0.888a |
| CoQ10H × 1 st m. | 35.23 ± 1.114ab | 29.43 ± 0.996a |
| Control × 2 nd m. | 35.13 ± 1.113ab | 28.33 ± 1.007a |
| WGO × 2 nd m. | 35.26 ± 1.102ab | 30.33 ± 0.95a |
| CoQ10L × 2 nd m. | 34.83 ± 1.157ab | 27.3 ± 0.971a |
| CoQ10H × 2 nd m. | 34.68 ± 1.06ab | 30.35 ± 0.921a |
| Control × 3 rd m. | 37.33 ± 1.151a | 30.53 ± 1.089a |
| WGO × 3 rd m. | 36.07 ± 1.176ab | 29.85 ± 1.03a |
| CoQ10L × 3 rd m. | 34.72 ± 1.162ab | 27.88 ± 0.936a |
| CoQ10H × 3 rd m. | 36.33 ± 1.27ab | 27.55 ± 0.861a |
| Control × 4 th m. | 33.9 ± 1.122ab | 30.27 ± 0.917a |
| WGO × 4 th m. | 36.9 ± 1.292a | 30.18 ± 1.015a |
| CoQ10L × 4 th m. | 35.21 ± 1.2ab | 28.8 ± 0.9a |
| CoQ10H × 4 th m. | 34.81 ± 1.184ab | 29.53 ± 0.9a |
| Control × 5 th m. | 34.38 ± 1.11ab | 28.4 ± 1.008a |
| WGO × 5 th m. | 34.38 ± 1.102ab | 27.71 ± 0.906a |
| CoQ10L × 5 th m. | 32.72 ± 1.103b | 28.12 ± 0.903a |
| CoQ10H × 5 th m. | 34.27 ± 1.08ab | 30.85 ± 0.992a |
| Control × 6 th m. | 35.23 ± 1.109ab | 26.48 ± 0.849a |

| | | |
|-----------------------------------|-----------------|----------------|
| WGO × 6thm. | 34.87 ± 1.114ab | 26.76 ± 0.828a |
| CoQ10L × 6th m. | 34.36 ± 1.10ab | 28.82 ± 0.91a |
| CoQ10H × 6th m. | 34.81 ± 1.18ab | 29.56 ± 0.88a |

Data are expressed as Mean ± S.E.M for 6 rabbits /group.

Means having different letters in the same column are significantly different at P<0.05.

WGO = Wheat Germ Oil (300 mg/Kg B.W), CoQ10 L= 10 mg/ Kg B.W, CoQ10H = 20 mg/Kg B.W.

1st m.= May, 2ndm. = June, 3rd m.= July, 4th m.= August, 5th m.= Septembre, 6th m.= Octobre

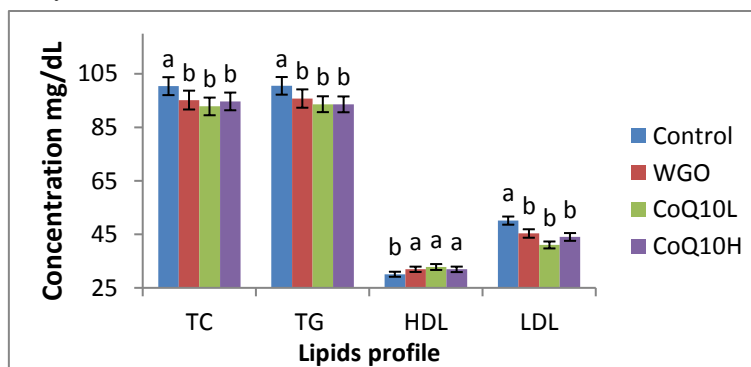
In the same manner obtained data are in agreement with **Pari and Arumugam (2008)** who reported that in rats the WGO decrease the levels of serum AST and ALT which indicate the decrease permeability and protect cell damage or necrosis of hepatocytes. In addition, the activities of antioxidant enzymes may have arisen from the individual and synergistic effects of vitamins, fatty acids, phytosterols and phenolic compounds found in wheat germ oil. It is considered that this compound has antioxidant activity, owing to its composition, and has the prospect motivate positive effects on the antioxidant defense system (**Paranich et al., 2000; Leenhardt et al., 2008**). Liver

is the main detoxifying organ in the body, and as such it possesses a high metabolic rate and it is subjected to many insults potentially causative of oxidative stress. Consequently, a correct status of the hepatic antioxidant defense system is of major importance for the maintenance of health according to **Mario et al. (2003)**.

Lipid profile:

Obtained data in Figure 3 showed a significant decrease in TC, TG and LDL values compared to control and increase in HDL values due to the treatment with Coenzyme Q10 and WGO.

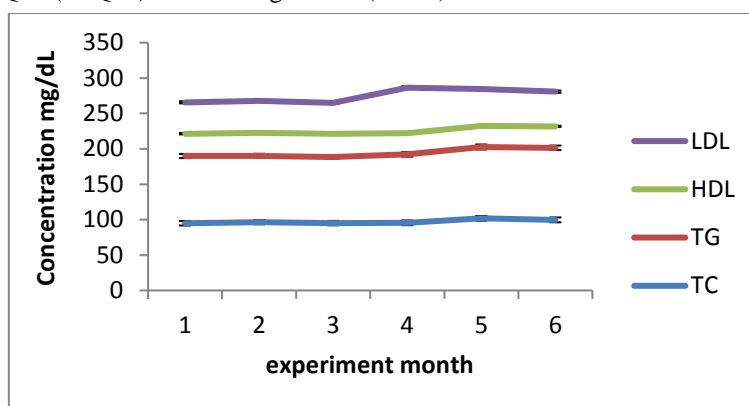
Fig. 3: Effect of Coenzyme Q10 (CoQ10) and Wheat germ oil (WGO) on lipid profile Total cholesterol (TC), Triglyceride (TG), High density lipoprotein (HDL) and Low density lipoprotein (LDL) in male Sinai gabali rabbits after 60 days of treatment.



TC= Total cholesterol, TG= Triglyceride, LDL= Low density lipoprotein, HDL= high density lipoprotein
WGO= Wheat germ oil (300mg/Kg B.W), CoQ10L = (10mg/kg B.W), CoQ10H= (20 mg/kg/B.W)

According to the effect of months observed data in Figure 4 showed significant differences (P< 0.05) between different 6 months in lipid profile. These results found the least value of TC (94.93 mg/ dL) was in the 1st month (May) and showed the least value of TG (93.31 mg/ dL) was in the 3rd month (July) and the least value of HDL (29.78 mg/ dL) was in the 5th month (September) of experimental period, while the highest value of HDL (32.81 mg/ dL) was in the 3rd month (July).

According to the interaction between treatment and month data in Table 3 showed the values of lipid profile, where TC recorded the lowest value (89.50 mg/ dL) for Coenzyme Q10L × 2nd month (June) after two months of treatment, TG least value (87.50 mg/ dL) was in Coenzyme Q10L × 3rd month (July), LDL lowest value was 35.96 mg/dL for Coenzyme Q10L group × 3rd month (July), HDL highest value of HDL was 36.00 mg/ dL in Coenzyme Q10L × 3rd month (July).

Fig. 4: Effect of experimental month on Lipid profile in male Sinai gabali rabbits after 60 days of treatment with Coenzyme Q10 (CoQ10) and wheat germ oil (WGO).

TC= Total cholesterol, TG = Triglyceride, HDL = High density lipoprotein, LDL = Low density lipoprotein 1st = May, 2nd = June, 3rd = July, 4th = August, 5th = September, 6th = October.

The decrease of lipid profile may be due to the monounsaturated fatty acid which reduces serum lipid profile level. These results are in agreement with **Jenkins *et al.* (1999)**; **salmeron *et al.* (1997)** and **Mensink and Katan, (1987)**, who reported that the administration of WGO could reduce the level of lipid profile when compared with the control value. In addition, WGO has a number of other nutritional and health benefits factors like high content of vitamin E and phytosterol (**Jonnala *et al.*, 2005**) which may be the reason of its lowering effect on triglyceride, cholesterol and LDL. Thus the reduction effect of WGO on triglyceride, cholesterol and LDL and increasing HDL level is a positive finding of this study.

The fatty acid composition of wheat germ oil unsaturated and multiple saturated fatty acid saturates of 81% and 64%, respectively (**Zacchi *et al.*, 2006**; **Eisenmenger and Dunford, 2008**). These fatty acids, alfa-linolenic acid, in relation to its anti-inflammatory effect, decreases O₂- production and NADPH oxidase activity, and thereby, has antioxidant activity (**Alessandri *et al.*, 2011**). These compounds are the portent of prostaglandins, which are embroiled in muscle constriction and the speedy recuperation of the inflammatory process. Furthermore, linoleic acid aids in the abstraction of cholesterol and acts as the precursor of cell membrane phospholipids (**Zacchi *et al.*, 2006**; **Piraset *et al.*, 2009**). Wheat germ oil also contains phytosterols, mainly campesterol, beta-cytosterol, and to a less extent, 5-stigmasterol, 7-stigmassterol, isofucosterol, 7-avenasterol and D5-avenasterol. These compounds, in particular D5-avenasterol has strong antioxidant activity (**Malecka, 2002**; **Hassanein and Abdel-Razek, 2009**). Steryl

glycosides constitute another component to the structure of wheat germ oil, with isofuco-SG, campestigma-SG and B-cyto-SG sharing the highest levels (**Hassanein and Abdel-Razek, 2009**). It is known that the phenolic compounds found in this oil also have antioxidant effect (**Niu *et al.*, 2011**). Flavonoids are also thought to enhance the efficiency of liver cells to remove LDL from the blood circulation by increasing LDL receptor densities in the liver and binding to a poli- protein B (**El-Beshbishy *et al.*, 2006**).

In the same manner oral administration with Coenzyme Q10 enhances lipids profile by increasing consumption leading to a lower absorption rate of Coenzyme Q10, as roughly 60%, of oral dosage forms are excreted in the feces (**Potgieter, *et al.*, 2013**). Plasma Coenzyme Q10 concentrations increase with increasing doses of Coenzyme Q10 at 2,400 mg, with a decreased efficiency of absorption at higher dosages. Either during absorption or after absorption, Coenzyme Q10 is reduced to ubiquinol and incorporated into chylomicrons and transported to the liver (**Bhagavan and Chopra, 2006**). Lastly, these are packaged into lipoprotein particles and released into circulation (**Potgieter, *et al.*, 2013**). Plasma Coenzyme Q10 is mainly packaged into very low-density lipoprotein VLDL and LDL particles, with a small amount incorporated into high-density lipoprotein (HDL) particles. Among its many functions, it transports mechanism of Coenzyme Q10 along with α -tocopherol that protects lipoproteins from lipid peroxidation. (**Bhagavan and Chopra, 2006**).

Table 3. Effect of wheat germ oil (WGO) and coenzyme Q10 (CoQ10) on lipid profile, total cholesterol, triglyceride (TG), LDL and HDL in blood serum of male Sinai gabali rabbits after 60 days of treatment.

| Interaction(treatment×month) | Cholesterol (mg/dL) | Triglyceride (mg/dL) | HDL (mg/dL) | LDL (mg/dL) |
|------------------------------|---------------------|----------------------|-------------|-------------|
|------------------------------|---------------------|----------------------|-------------|-------------|

| | | | | |
|------------------------------------|--------------------|------------------|-------------------|----------------------|
| Control × 1st m. | | | | 50.26 ± 1.586 abc |
| WGO × 1st m. | 98.12 ± 3.458 bc | 100.37 ± 3.074 a | 27.75 ± 0.933 e | 50.62 ± 1.56 abc |
| CoQ10L × 1st m. | 100.37 ± 3.505 abc | 98.75 ± 3.233 a | 30 ± 0.986 de | 37.86 ± 1.167 e |
| CoQ10H × 1st m. | 90.37 ± 3.136 d | 89.12 ± 2.821 b | 34.37 ± 1.176 ab | 38.4 ± 1.295 e |
| Control × 2nd m. | 90.87 ± 2.922 d | 91.62 ± 3.188 b | 34 ± 1.211 abc | 51.45 ± 1.59 abc |
| WGO × 2nd m. | 102.5 ± 3.451 ab | 97.87 ± 3.047 a | 31.37 ± 0.953 bcd | 45.42 ± 1.56 ab |
| CoQ10L × 2nd m. | 103.62 ± 3.31 ab | 98 ± 3.185 a | 29.62 ± 0.963 de | 37.53 ± 1.278 e |
| CoQ10H × 2nd m. | 89.5 ± 3.144 d | 91 ± 3.116 b | 34 ± 1.184 abc | 37.01 ± 1.124 e |
| Control × 3rd m. | | | | 50.28 ± 1.762 abc |
| WGO × 3rd m. | 100.12 ± 3.523 abc | 98.75 ± 3.027 a | 30 ± 0.967de | 48.76 ± 1.486 bc |
| CoQ10L × 3rd m. | 99.5 ± 3.269 bc | 98.25 ± 2.981 a | 31.25 ± 0.99 bcde | 35.96 ± 1.282 e |
| CoQ10H × 3rd m. | 89.62 ± 2.975 d | 87.5 ± 2.822 b | 36 ± 1.165 a | 39.62 ± 1.299 e |
| Control × 4th m. | 91.37 ± 3.139 d | 88.75 ± 2.901 b | 34 ± 1.04 abc | 50.12 ± 1.531 abc |
| WGO × 4th m. | 103 ± 3.184 ab | 102.87 ± 3.163 a | 32 ± 1.117bcd | 52.66 ± 1.642 abc |
| CoQ10L × 4th m. | 100.75 ± 3.595 abc | 101.25 ± 3.109 a | 27.75 ± 0.965 e | 39.66 ± 1.267e |
| CoQ10H × 4th m. | 88.75 ± 2.835d | 91.5 ± 3.158 b | 30.87 ± 1.01 cde | 42.03 ± 1.438 de |
| Control × 5th m. | 89.62 ± 3.019 d | 91.25 ± 2.825 b | 29.37 ± 1 de | 52.25 ± 1.614 abc |
| WGO × 5th m. | 100 ± 3.438 abc | 102 ± 3.444 a | 29.25 ± 0.9 de | 49.92 ± 1.705 abc |
| CoQ10L × 5th m. | 100 ± 3.26 abc | 99.5 ± 3.065 a | 30.37 ± 1.054 de | 50.13 ± 1.629 abc |
| CoQ10H × 5th m. | 100.62 ± 3.58 abc | 102.12 ± 3.56 a | 30.25 ± 0.929 de | 55.96 ± 1.833 a |
| Control × 6th m. | 105 ± 3.52 a | 99.37 ± 3.137 a | 29.25 ± 0.929 de | 46.41 ± 1.407 cd |
| WGO × 6th m. | 96.75 ± 3.35 c | 101.12 ± 3.61 a | 30.12 ± 1.04 de | 51.5 ± 1.573 abc |
| CoQ10L × 6th m. | 102.87 ± 3.23 ab | 102.75 ± 3.316 a | 30.62 ± 0.987cde | 46.67 ± 1.421 cd |
| CoQ10H × 6th m. | 98 ± 3.148 bc | 100.62 ± 3.561 a | 31.25 ± 0.961bcde | 51.11 ± 1.817 abc |

Data are expressed as Mean ± S.E.M for 6 rabbits /group.

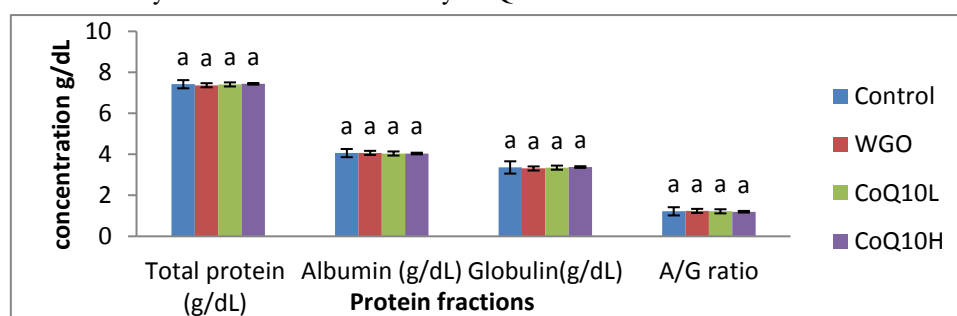
Means having different letters in the same column are significantly different at P<0.05.

WGO = WheatGermOil (300 mg/Kg B.W), CoQ10 L= 10 mg/ Kg B.W, CoQ10H = 20 mg/Kg B.W.

1st m.= May, 2nd m. = June, 3rd m.= July, 4th m.= August, 5th m.= Septembre, 6th m.= Octobre TC = Total cholesterol, TG = Triglyceride, HDL = High density lipoprotein, LDL = Low density lipoprotein

Data in Figure 5 did not show any significant changes in blood proteins due to the treatments with Coenzyme Q10 and WGO. This means that Coenzyme Q10 and WGO did not affect in blood proteins.

Fig. 5. Effect of treatment on Total protein, Albumin, Globulin g/dL and A/G ratio on Sinai gabali rabbits after 60 days of treatment with Coenzyme Q10 and WGO.



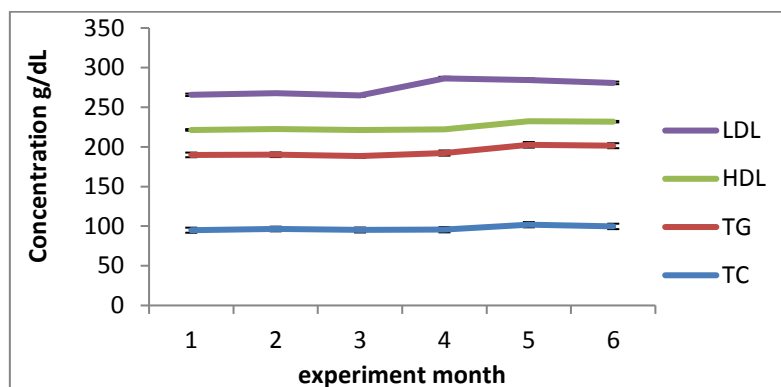
TP= Total protein, Alb = Albumin, Glob = Globulin.

WGO= Wheat germ oil (300mg/Kg B.W), CoQ10L = Q10 (10mg/kg B.W),

CoQ10H= (20mg/kg/B.W).

Data in Figure 6 did not show any significant changes in blood proteins in different 6 months after 60 days of treatment.

Fig. 6: Effect of experiment month on blood serum proteins (Total protein (TP), Albumin (Alb), Globulin (Glob) and A/G ratio) in male Sinai gabali rabbits after 60 days of treatment with Coenzyme Q10 and WGO.



1st = May, 2nd = June, 3rd = July, 4th = August, 5th = September, 6th = October.

Data in Table 4 showed the means of interaction between treatment and months and showed the highest values of total protein, albumin, globulin and A/G ratio were 7.63 g/dL in Coenzyme Q10H × 4th month (August), 4.17 g/dL in WGO × 2nd month (June), 3.58 g/dL in Coenzyme Q10H × 4th month (August) and 1.30 in Coenzyme Q10H × 6th month (October), respectively.

Observed data in Table 4 did not show any satisfactory changes in blood proteins. This means that oral administration with Coenzyme Q10 or WGO did not affect the vital processes in the body and did not affect the proportion of blood proteins created in the body, may be due to the improvement in protein synthesis in the liver as a result of antioxidant effect which act as a free radical and could protect against lipid peroxidation according to **Abd El Dayem and Moawad, (2001)**.

Data in Table 5 showed the effect of WGO and Coenzyme Q10 on oxidative stress markers in testes tissues in rabbit bucks. The results showed decreases in GSSG and NO due to the treatment with WGO and Coenzyme Q10H, while there was a non-significant change in Coenzyme Q10L. Also this study showed a significant decrease in testes tissue oxidative stress

marker MDA and increase GSH that may be due to Coenzyme Q10 and WGO antioxidant activity. **Bentinger et al. (2007)** found that Lipid peroxidation triggers the denaturation of cell membrane, causing increased cell permeability, enzyme inactivation, and structural damage led to DNA mutations, and cell death. It could be involved in the protection of cell membranes from oxidative insult. Coenzyme Q10 shows effectiveness in counteracting free radicals-induced tissue damage. Antiradical effects of Coenzyme Q10 may prevent the initiation and propagation of lipid peroxidation in cellular membranes. Our results are similar to **Mohammad et al. (2015)** who found that Coenzyme Q10 synergistically all eviate Aluminum induced suppression of testicular Steroidogenesis and antioxidant defense.

Table 4. Effect of wheat germ oil (WGO) and coenzyme Q10 (CoQ10) on blood serum proteins, total Protein (TP), Albumin (Alb), globulin (Glob) and A/G ratio in male Sinai gabali rabbits after 60 days of treatment.

| Interaction (month × treatment) | Total protein (g/dL) | Albumin (g/dL) | Globulin (g/dL) | A/G ratio |
|---------------------------------|----------------------|------------------|-----------------|----------------|
| Control × 1 st m. | 7.58 ± 0.255 ab | 4.1 ± 0.14 abc | 3.5 ± 0.11 a | 1.2 ± 0.039 a |
| WGO × 1 st m. | 7.38 ± 0.258 abc | 4.07 ± 0.14 abc | 3.3 ± 0.104 a | 1.26 ± 0.039 a |
| CoQ10L × 1 st m. | 7.45 ± 0.237 abc | 4 ± 0.126 abc | 3.43 ± 0.106 a | 1.17 ± 0.038 a |
| CoQ10H × 1 st m. | 7.51 ± 0.253 abc | 3.96 ± 0.137 cb | 3.53 ± 0.12 a | 1.12 ± 0.035 a |
| Control × 2 nd m. | 7.33 ± 0.245 abc | 4.02 ± 0.132 abc | 3.35 ± 0.102 a | 1.22 ± 0.043 a |
| WGO × 2 nd m. | 7.52 ± 0.239 abc | 4.17 ± 0.141 a | 3.37 ± 0.105 a | 1.23 ± 0.042 a |
| CoQ10L × 2 nd m. | 7.28 ± 0.25 abc | 3.95 ± 0.126 c | 3.32 ± 0.109 a | 1.2 ± 0.041 a |
| CoQ10H × 2 nd m. | 7.48 ± 0.254 abc | 3.98 ± 0.124 cb | 3.45 ± 0.105 a | 1.16 ± 0.041 a |
| Control × 3 rd m. | 7.51 ± 0.231 abc | 4.05 ± 0.142 abc | 3.46 ± 0.111 a | 1.18 ± 0.042 a |
| WGO × 3 rd m. | 7.2 ± 0.254 c | 3.98 ± 0.123bc | 3.2 ± 0.105 a | 1.26 ± 0.041 a |
| CoQ10L × 3 rd m. | 7.26 ± 0.256 bc | 4.06 ± 0.145 abc | 3.21 ± 0.115 a | 1.27 ± 0.041a |
| CoQ10H × 3 rd m. | 7.3 ± 0.223abc | 4.01 ± 0.132 abc | 3.28 ± 0.112 a | 1.21 ± 0.043 a |
| Control × 4 th m. | 7.26 ± 0.233 bc | 4.05 ± 0.128 abc | 3.2 ± 0.114 a | 1.27 ± 0.039 a |
| WGO × 4 th m. | 7.31 ± 0.224 abc | 4.08 ± 0.128 abc | 3.25 ± 0.107 a | 1.27 ± 0.041 a |
| CoQ10L × 4 th m. | 7.57 ± 0.239 ab | 4.1 ± 0.144 abc | 3.47 ± 0.118 a | 1.18 ± 0.042 a |

| | | | | |
|------------------------------------|------------------|------------------|----------------|----------------|
| CoQ10H × 4th m. | 7.63 ± 0.272 a | 4.05 ± 0.128 abc | 3.58 ± 0.113 a | 1.15 ± 0.037 a |
| Control × 5th m. | 7.41 ± 0.25 abc | 4.11 ± 0.136 abc | 3.28 ± 0.106 a | 1.26 ± 0.041 a |
| WGO × 5th m. | 7.52 ± 0.232 abc | 4.01 ± 0.126 abc | 3.52 ± 0.107 a | 1.13 ± 0.037 a |
| CoQ10L × 5th m. | 7.4 ± 0.26 abc | 4.1 ± 0.126 abc | 3.32 ± 0.101 a | 1.25 ± 0.041 a |
| CoQ10H × 5th m. | 7.38 ± 0.235 abc | 4.12 ± 0.144 abc | 3.27 ± 0.107 a | 1.28 ± 0.041 a |
| Control × 6th m. | 7.45 ± 0.265 abc | 4.02 ± 0.139 abc | 3.41 ± 0.108 a | 1.18 ± 0.036 a |
| WGO × 6th m. | 7.32 ± 0.244 abc | 4.13 ± 0.126 ab | 3.21 ± 0.108 a | 1.28 ± 0.041 a |
| CoQ10L × 6th m. | 7.45 ± 0.245 abc | 4.07 ± 0.126 abc | 3.37 ± 0.112 a | 1.23 ± 0.038 a |
| CoQ10H × 6th m. | 7.32 ± 0.226 abc | 4.13 ± 0.143 ab | 3.2 ± 0.111 a | 1.3 ± 0.04a |

Data are expressed as Mean ± S.E.M for 6 rabbits /group.

Means having different letters in the same row are significantly different at P<0.05.

WGO = Wheat Germ Oil (300 mg/Kg B.W), CoQ10L = 10 mg/ Kg B.W, CoQ10H = 20 mg/Kg B.W.

1st m.= May, 2ndm. = June, 3rd m.= July, 4th m.= August, 5th m.= Septembre, 6th m.= Octobre)

TP= Total protein, Alb = Albumin, Glob = Globulin.

Table 5. Effects of Co-enzymeQ10 (Coenzyme Q10) and Wheat germ oil (WGO) on oxidative stress markers Reduced Glutathione (GSH) , Malondialdehyde (MDA), Oxidized Glutathione and NO in testes tissue of rabbits.

| groups | GSH µmol / g tissue | NO µmol / g tissue | GSSG µmol / g tissue | MDA nmol / g tissue |
|----------------|------------------------|-----------------------|-------------------------|------------------------|
| Control | 17.72 ± 1.15c | 0.41± 0.03a | 0.44 ± 0.04a | 25.43± 2.67a |
| WGO | 28.26 ± 2.33a | 0.31 ±0.03b | 0.28 ± 0.02b | 20.22 ± 1.34bc |
| CoQ10L | 17.78± 1.17c | 0.48 ±0.04a | 0.52 ±0.04a | 23.41 ±2.67ab |
| CoQ10H | 24.36 ±1.78b | 0.28± 0.02b | 0.29 ±0.03b | 19.69 ± 1.37c |

Data are expressed as Mean ± S.E.M for 6 rabbits /group.

Means having different letters in the same column are significantly different at P<0.05.

WGO= Wheat germ oil (300mg/Kg B.W), CoQ10L = (10mg/kg B.W), CoQ10H= (20 mg/kg/B.W).

Obtained data in Table 5are consisted with **Lee *et al.* (2011)** who found that, Coenzyme Q10 supplementation reduces oxidative stress and increase up regulation of antioxidant enzymes activity in patients with coronary artery disease. Increase ATP production for Coenzyme Q10 may be due to the role of Coenzyme Q10 in mitochondrial respiratory chain (MRC) to synthesize ATP via oxidative phosphorylation. MRC is consists of 5 enzymes complex I-V. Coenzyme Q10 is the predominant form of ubiquinone and serves as an electron carrier in MRC (**Rahman and Hanna, 2009**).**Goel *et al.* (2005)** reported that highly reactive oxygen metabolites, especially hydroxyl radicals, act on unsaturated fatty acids of phospholipid components of membranes to produce malondialdehyde, a lipid peroxidation product. This is in the same trend with our results which reported to induce oxidative stress, as shown by enhanced MDA production and decrease GSH.

Conclusion

The results of the present study indicate that administration of WGO or Coenzyme Q10presented positive effects of reduced total cholesterol, triglycerides, low density lipoprotein and increased high density lipoprotein, also the data showed that oral administration of WGO and Coenzyme Q10 did not affect Total protein, Albumin, Globulin and A/G ratio, liver functions (AST and ALT) and decrease testicular

oxidative marker MDA, GSSG, NO and increase GSH.in addition air temperature did not affect on physiological performance due to the use of antioxidants.

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تأثير زيت جنين القمح و معامل الانزيم Q10 على الاداء الفسيولوجي ودلائل الإجهاد التأكسدي على خلايا الخصية في ذكور الأرانب.

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الهدف من هذه الدراسة هو التحقق من فعالية جرعات زيت جنين القمح ومعامل الانزيم Q10 بالجرعات المنخفضة والعالية على الأداء الفسيولوجي ودلائل الإجهاد التأكسدي على الخلايا الخصوية في ذكور الأرانب بالإضافة إلى ذلك تتبع أفضل فترة زمنية مناسبة تحت الظروف المصرية صيفا. أجريت تجربته على 24 من ذكور الأرانب الجبلى النقية عمر 3 شهور . حيث تم تقسيم الحيوانات عشوائيا الى أربع مجموعات بواقع ستة أرانب لكل مجموعة . المجموعة الأولى كانت المجموعه الضابطة حيث أعطيت 0.5 مل /كجم وزن الجسم من الماء المقطر , المجموعة الثانية تم معاملتها بزيت جنين حبة القمح (300 مل/كجم وزن الجسم) , المجموعة الثالثة تم معاملتها بمعامل الانزيم Q10 (10 مل/كجم وزن الجسم) والمجموعة الرابعة تم معاملتها بمعامل الانزيم Q10 (20 مل /كجم/وزن الجسم) . جميع المعاملات تم إعطاؤها تجريبا يوميا لمدة 60 يوم . بعد ذلك تم سحب عينات الدم شهريا لمدة 6 شهور تحت نفس الظروف. لم تسجل النتائج أى زيادة معنوية فى نشاط الإنزيمات الداله على وظائف الكبد (ALT و AST) مقارنة بالمجموعة الضابطة. ولم يسجل تأثير الشهر أى زيادة معنوية على نشاط إنزيم ALT ولكن سجل فروق معنوية على انزيم AST فى الشهر الثالث (يوليو) والخامس (سبتمبر) كما أوضح التداخل بين المعاملات والشهور أفضل النتائج لكل من ALT,AST و 26.48 و 32.72 وحده/لتر) على التوالي للمجموعه الضابطة فى الشهر السادس والمجموعة المعاملة بمعامل الإنزيم (CoQ10L) فى الشهر الخامس (سبتمبر) على التوالي.

وأظهرت المعاملات انخفاضا معنويا ($P < 0.05$) على كل من الكوليستيرول الكلي و الجلسريدات الثلاثية والدهون منخفضة الكثافة LDL.نتيجة لتعاطى معامل الانزيم Q10 بجرعه (10 mg/kg BW) بمتوسطات 92.81, 93.64 و 41.03 مجم/ديسيلتر مقارنة بباقي المجموعات . بينما حدث إرتفاع معنوي فى الدهون مرتفعة الكثافة HDL نتيجة المعاملة بمعامل الانزيم (CoQ10 L) (32.79 مجم/ديسيلتر) مقارنة بالمجموعه الضابطة . وأوضحت نتائج التداخل بين المعاملات والشهور أفضل القيم لكل من الكوليستيرول الكلي و الجلسريدات الثلاثية والدهون منخفضة الكثافة LDL وكذلك عالية الكثافة 89.50 مجم/ديسيلتر لمعامل الإنزيم Q10L فى الشهر الثانى (يونية) و 87.50 مجم/ديسيلتر لمعامل النزيم Q10L فى الشهر الثالث (يوليو) و 36.00 مجم/ديسيلتر لمعامل الانزيم Q10L فى الشهر الثالث (يوليو) وأيضا 35.96 مجم/ديسيلتر لمعامل الانزيم Q10L فى الشهر الثالث (يوليو) على التوالي. لم تظهر النتائج أيضا أى زيادة معنوية على كل من البروتين الكلى والألبومين والجلوبولين والنسبة بينهما وفى المجموعات الأربعة المختلفه نتيجة للمعاملات وتأثير الشهور . وأظهرت نتائج التداخلات بين المعاملات والشهور أفضل القيم لبروتينات الدم وكانت للبروتين الكلى والألبومين والجلوبولين والنسبة بينهما 7.63 جم/ديسيلتر لمعامل الإنزيم Q10H فى الشهر الرابع (أغسطس) و 4.17 جم/ديسيلتر فى زيت جنين القمح فى الشهر الثانى (يونيو) و 3.58 جم/ديسيلتر لمعامل الإنزيم Q10H فى الشهر الرابع (أغسطس) و 1.30 جم/ديسيلتر لمعامل النزيم Q10H فى الشهر السادس (أكتوبر) على التوالي . وسجلت النتائج انخفاضا معنويا عند مستوى 5% فى المألون داي ألديهيد وزيادة فى الجلوتاثيون المختزل نتيجة للمعاملة بزيت جنين القمح ومعامل الإنزيم (Q10).

وقد خلصت نتائج الدراسة الحالية الى ان المعامله بزيت جنين القمح ومعامل الإنزيم (Q10) تحسن من القياسات الفسيولوجية وتعمل كمضادات لعمليات الأكسده فى نسيج الخصية لذكور الأرانب الجبلى . ولا تؤثر درجات الحرارة فى الشهور الستة المختلقة على الأداء الفسيولوجى نتيجة لإستخدام مضادات الأكسده.