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EFFECTS OF CASTRATION AND SEX HORMONES ON ANTIOXIDANT STATUS AND SOME BIOCHEMICAL PARAMETERS OF MALE RABBITS EXPOSED TO OXIDATIVE STRESS

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ABSTRACT

The present study was aimed to investigate the effect of estrogen and testosterone as antioxidants and their ability to prevent the effect of H2O2-induced oxidative stress and their reflections on blood parameters and antioxidant status of male rabbits. 72 adult male rabbits were divided randomly into 12 groups (6/group), 1st group: Intact, 2nd group: Intact-H2O2, 3rd group: Castrated, 4th group: Castrated-H2O2, 5th group: Intact-H2O2-Testosterone, 6th group: Castrated-H2O2-Testoterone, 7th group: Intact-H2O2-Estrogen, 8th group: Castrated-H2O2-Estrogen, 9th group: Intact-Testosterone, 10th group: Castrated-Testosterone, 11th group: Intact-Estrogen, 12th group: Castrated-Estrogen, treatments continued for 4 weeks. Results showed that castration increased significantly GSH, TAC, TG, HDL-C levels, and significantly decreased MDA, AST, ALT levels. Treatment with H2O2 caused a significant decrease in the levels of GSH, TAC, HDL-C, total proteins, and a significant increase in the levels of MDA, cholesterol, TG, LDL-C, ALT, AST compared to untreated group. On the other hand, estrogen treatment improves TAC, GSH and lipid profile and reduce MDA significantly as compared with testosterone treatment and control group. In regard to interaction effects, castration without H2O2- estrogen treatment reduces the stress as represented by the reduction of MDA, risk index, ALT and elevate the GSH and TAC levels. These results indicate that castration and estrogen treatment of castrated and intact male rabbits reduce stress effects and improve the lipid profile and some immunological measures.

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INTRODUCTION

According to Garcia-Sánchez et al, (2020), oxidative stress is a syndrome brought on by an imbalance between elevated amounts of reactive oxygen species (ROS) and low antioxidant mechanism activity. Increased oxidative stress has the ability to harm cellular structure and even destroy tissues (Preiser, 2012). Under normal circumstances, the cell can maintain a proper balance between the production of ROS and its elimination via enzymatic or non-enzymatic antioxidant mechanisms (Abdel Moneim, 2014).

Testosterone is principally important for the formation of male-specific phenotypes throughout embryogenesis, the establishment of sexual maturity at

puberty, and the maintenance of male reproductive function, spermatogenesis, and sexual behaviour during adulthood (Kalfa et al., 2019). Testosterone is regarded as an anabolic hormone; it typically increases the metabolic rate, an increased metabolic rate results in a higher O2 consumption, which may increase ROS generation. Consequently, elevated testosterone levels would likely increase OS (Chainy and Sahoo, 2020). However, this involvement of testosterone in causing OS is contested. Several researches have demonstrated that testosterone induces oxidative stress in testis, muscle, and human placenta (Chainy et al., 2009 and Aydilek et al., 2004); others imply that testosterone has antioxidant capabilities in prostate and neurological tissue (Ahlbom et al., 2001and Tam et al., 2003).

Estrogen is a well-known female steroid hormone produced by the ovary that regulates the estrous or menstrual cycle in females; hence, estrogen is essential for female reproduction. Estrogen is essential also for male reproduction and countless other systems, including the neuroendocrine, skeletal, and immunological systems of both sexes. A decrease in ovarian hormones promotes oxidative stress (Ha et al., 2006), but the molecular mechanisms by which these steroids influence the oxidant-antioxidant balance in various tissues are still not fully understood. Free phenolic hydroxyl groups on the A-ring of all estrogens confer antioxidant capabilities and are the key structural factor of free radical scavenging (Badeau et al., 2005). Due to its lipophilicity, estrogens concentrate in lipid-rich regions of cell membranes, where it functions as a local antioxidant in vivo (Prokai et al., 2005).

Experimentally, H2O2 was used orally to induce oxidative stress in rabbits by (Al–Kattan et al., 2007), and also H2O2 was used to induce oxidative stress in birds by Taha and Abdul-Rahman (2013) in broiler breeder males, and by Abdulmajeed et al, (2013) in quail as well as by Al-Nuaimmi and Abdul-Rahman (2018) in quail.

The aim of this study was to investigate the effects of castration, hydrogen peroxide, and sex hormones treatment on the antioxidant status as well as some other biochemical indicators of male rabbits.

MATERIALS AND METHODS

Experimental animals

The current study was carried out on (72) adult male local rabbits, their weights between (1.5-2) kg. They were collected from local fields and numbered with plastic numbers for easy identification and handling. They were housed in a well-ventilated room inside wooden cages (6 Rabbits/cages) and kept on standard ration (16.5 % protein and 2213 kcal/kg ration metabolized energy) and tap water throughout the experiment. Along the experiment period, the room temperature was kept at 20-25ºC and 12hours light/ dark cycle with the light on from 6 pm to 6 am. Each group was housed in a separate cage (120 x 100 x 100) cm for length, width and height respectively (6 rabbits/ group) during the experimental period. Animals were allowed to adapt to the experimental conditions and fed for 14 days prior to the commencement of the study.

Table (1). Percentages of feed ingredients used in experiment

Ingredient	%	Crude Protein%*
Wheat bran	47	7.5
Barley	38	3.6
Soybean meal (44%)	10	4.4
Protein (50% concentrated).	2	1.0
Calcium	1	…
Salt	1.5	…
Vitamin and minerals mixture	0.5	…
	100%	16.5
Metabolizable Energy*	2213 kcal. /Kg ration.	

*Al-Khawaje *et al*, (1978).

Rabbits Castration

After the end of acclimation period (14 days), (36) rabbits were castrated surgically as follows:

- Rabbits starved for at least 12 hours (overnight).
- Rabbits anesthesized by using of ketamine*and xylazine*.
- Rabbits castrated bilaterally.
- Castrated rabbits left for 4 weeks after castration then introduced into the experiment.

*Manufactured by Interchemie De Adelaar. Netherlands (Holland).

Materials used in treatments

Estrogen: O.S.T *fort* (10 ml injection solution), composition: Oestradiol cypionate… 5 mg. Exp.qs…1 ml. Vemedim Coporation, Cantho City, Vietnam.
Testosterone: Testosteron Depo (250 mg/ml testosterone. Solution for Injection). 1 ml of solution for injection contains: testosterone enanthate 250 mg. Galenika a.d. Beograd, Belgrad, Republic of Serbia.
Hydrogen Peroxide: (H_2O_2) 0.05%. Made in Turkey.

Experimental Design:

Seventy two (72) adult male rabbits were divided into 12 equal groups (6 rabbits/group). Rabbits of all groups treated for 4 weeks as follows:
T_1: Intact rabbits reared on standard ration and tap water.
T_2: Intact rabbits reared on standard ration and drink 0.5% H_2O_2 supplemented tap water.
T_3: Castrated rabbits reared on standard ration and tap water.

T_4: Castrated rabbits reared on standard ration and drink 0.5% H_2O_2 suplemented tap water.
T_5: Intact rabbits reared on standard ration and drink 0.5% H_2O_2 suplemented tap water and injected i.m with testosterone 10 mg/kg B.wt. (every other day: i:e: 3 injections/ week).
T_6: Castrated rabbits reared on standard ration and drink 0.5% H_2O_2 suplemented tap water and injected i.m with testosterone 10 mg/kg B.wt. (every other day: i:e: 3 injections/ week).
T_7: Intact rabbits reared on standard ration and drink 0.5% H_2O_2 suplemented tap water and injected i.m with estrogen 0.5 mg/kg B.wt. (every other day: i:e: 3 injections/ week).
T_8: Castrated rabbits reared on standard ration and drink 0.5% H_2O_2 suplemented tap water and injected i.m with estrogen 0.5 mg/kg B.wt. (every other day: i:e: 3 injections/ week).
T_9: (Intact) male rabbits, reared on standard ration, and injected i.m with testosterone 10 mg/kg B.wt. (every other day: i:e: 3 injections/ week) for 4 weeks.
T_{10}: (Castrated) male rabbits, reared on standard ration, and injected with testosterone as in T_9.
T_{11}: (Intact) male rabbits, reared on standard ration, and injected with i.m estrogen 0.5 mg/kg B.wt. (every other day: i:e: 3 injections/ week) for 4 weeks.
T_{12}: (Castrated) male rabbits, reared on standard ration, and injected with estrogen as in T_{11}.

Blood collection

At the end of the experimental period for (4 weeks), (5 ml) of the blood of each rabbit was collected from ear vein. The sample was emptied into plain tube and left for 2 hrs at room temperature, then centrifuged (3000 RPM) for 15 minutes and the serum was separated by micropipette and emptied into tubes then stored at -20C° until biochemical analysis was carried out.

Determination of biochemical parameters

Biochemical parameters were determined using kits from Roche Diagnostics Company (Germany), for analysis of Total protein, Albumin, Globulin, Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT), Glucose, Cholesterol, Triglyceride (TG), High-density lipoproteins- cholesterol (HDL-C), Low-density lipoproteins-cholesterol (LDL-C) by automated method using Biochemical auto analyzer Cobas C 501. Globulin was calculated mathematically as the difference between total protein and albumin, also VLDL-C as: VLDL-C = TG/5 and Risk index as: RI = Cholesterol/HDL-C.

Antioxidant status: Total antioxidant capacity (TAC), Glutathione (GSH), Malondialdehyde (MDA) were determined using Rabbit Elisa kit from Sunlong Biotech Co., Ltd (China), by using an automated analyzer (BioTek), BioTek Instruments, Inc., USA.

Statistical analysis

The data were analyzed as factorial experiment by three-way analysis of

variance using General linear model (SAS, 2013) to study the effect of castration, H_2O_2, hormonal treatment and interaction between them on different traits. Duncan multiple tests (1955) within SAS (2013) were used to detect differences among treatments.

RESULTS & DISCUSSION:

Anti-oxidative/oxidative parameters

The antioxidant status measured in the current study is shown in Table (2). The results revealed that there was a significant difference between castrated and intact rabbits in MDA, GSH and TAC levels. Castrated rabbits showed a significantly lowest value of MDA and significantly highest value of GSH and TAC than intact rabbits at $P \leq 0.05$. Regarding to the effect of experimentally H_2O_2 - induced oxidative stress, the results showed that animals treated with H_2O_2 had a significant increase in MDA level with a significant decrease in GSH and TAC levels as compared with untreated animals.

Results of hormonal treatments showed that estrogen treated group rabbits recorded a significantly lower value of MDA with significantly higher value of GSH and TAC compared to testosterone treated group and control. Also, the testosterone treated rabbits showed a significant increase in MDA and a significant decrease in GSH and TAC as compared with control group rabbits at $P \leq 0.05$.

As related to interaction effect as shown in Table (2), the results revealed that castration-without H_2O_2-estrogen group recorded the lowest significantly value in MDA level with the highest significantly values in GSH and TAC levels as compared with other groups. On the other hand, the intact-H_2O_2-testosterone treated rabbits showed the highest significant value in MDA and the lowest significant values in GSH and TAC among the interaction groups at $P \leq 0.05$.

Table (2). Effect of Castration, H_2O_2, Sex hormones and interaction on MDA, GSH and TAC levels of male rabbits (Mean ± S.E.)

Treatments	MDA ng/ml	GSH ng/L	TAC U/ml
Effect of castration			
Intact	32.71±1.08 a	32.82±0.95 b	0.55±0.01 b
Castration	29.78±0.98 b	35.98±1.01 a	0.62±0.02 a
Effect of H_2O_2			
Without H_2O_2	27.83±0.81 b	35.47±1.10 a	0.62±0.02 a
With H_2O_2	34.66±0.96 a	33.33±0.90 b	0.55±0.01 b
Effect of sex hormones treatment			
Control	33.25±0.94 b	31.70±0.64 b	0.57±0.00 b
Testosterone	35.25±1.03 a	30.03±0.71 c	0.48±0.01 c
Estrogen	25.24±0.87 c	41.48±0.73 a	0.71±0.01 a
Interaction			
Intact-without H_2O_2-Control	29.72±0.87 e	34.06±0.81 d	0.58±0.00 de
Intact-without H_2O_2-Testosterone	33.54±1.76 cd	27.06±0.48 g	0.42±0.01 g

Intact-without H_2O_2-Estrogen	24.32±0.71 f	41.94±0.77 b	0.73±0.01 b
Intact-H_2O_2-Control	38.11±0.74 ab	29.65±1.06 fg	0.54±0.00 e
Intact-H_2O_2-Testosterone	41.07±1.97 a	27.49±1.04 g	0.45±0.01 g
Intact-H_2O_2-Estrogen	29.50±1.52 e	36.75±0.55 c	0.61±0.01 d
Castration-without H_2O_2-Control	28.74±0.77 e	32.66±1.33 de	0.61±0.01 d
Castration-without H_2O_2-Testosterone	30.38±0.48 de	31.87±1.02 def	0.55±0.01 e
Castration-without H_2O_2-Estrogen	20.30±0.53 g	45.26±0.78 a	0.84±0.01 a
Castration-H_2O_2-Control	36.42±1.09 bc	30.42±1.23 fe	0.54±0.01 ef
Castration-H_2O_2-Testosterone	36.00±0.58 bc	33.72±0.72 d	0.50±0.01 f
Castration-H_2O_2-Estrogen	26.84±1.30 ef	41.96±0.99 b	0.67±0.01 c

-Means with different letters within each column differ significantly (P≤0.05).

Biochemical parameters

The data regarding lipid profile was illustrated in Table (3), which showed non-significant changes in cholesterol, LDL-C and risk index levels with significant increase in TG, HDL-C and VLDL-C were observed in castrated group compared to intact group at P≤0.05. As related to the effect of H_2O_2, the results showed that animals treated with H_2O_2 showed a significant increase in cholesterol, TG, LDL-C, VLDL-C and risk index with significant decrease in HDL-C levels as compared with untreated animals at P≤0.05. Estrogen treated rabbits showed a significant decrease in serum cholesterol, TG, LDL-C, VLDL-C and risk index with significant increase in HDL-C as compared with testosterone treated rabbits and with control rabbits. Also, testosterone treatment reduced cholesterol, TG, VLDL-C and risk index significantly and increased HDL-C significantly as compared with control values at P≤0.05.

In regard to interaction effects, Table (3) showed that the lowest significant values in cholesterol was recorded in intact-H_2O_2-estrogen and castration without-H_2O_2 estrogen treated rabbits, meanwhile the lowest significant value in TG was recorded in castration without-H_2O_2-estrogen treated rabbits, for LDL-C and risk index the highest significant value were recorded in the intact-H_2O_2-control treated rabbits, for VLDL-C the highest significant values was recorded in castrated-H_2O_2-testosterone and intact-H_2O_2-control treated rabbits. On another hand, castration without-H_2O_2- estrogen treated animals showed the highest significant value in HDL-C and the lowest significant value in risk index at P≤0.05.

The findings related total proteins, glucose, ALT and AST are summarized in Table (4). The results showed that there were no significant changes in total protein, albumin, globulin and globulin/albumin levels between castrated and intact rabbits. While, castrated rabbits recorded a significant decrease in glucose, ALT and AST levels compared to intact rabbits at P≤0.05. Animals treated with H_2O_2 showed a significant decline in total protein, albumin, globulin and globulin/albumin levels with significant increase in glucose, ALT and AST levels as compared with untreated animals at P≤0.05.

Table (3). Effect of castration, H_2O_2, sex hormones and interaction on Cholesterol, TG, HDL-C, LDL-C, VLDL-C and Risk index of male rabbits (Mean ±SE).

Parameters	Cholesterol mg/dl	Triglyceride mg/dl	HDL-C mg/dl	LDL-C mg/dl	VLDL-C mg/dl	Risk Index (HDL/LDL)
Effect of castration						
Intact	58.58±1.58 a	50.00±1.47 b	22.77±0.93 b	22.86±0.66 a	10.00±0.29 b	2.83±0.20 a
Castration	59.25±1.29 a	53.33±1.99 a	24.58±1.32 a	20.83±0.67 a	10.66±0.39 a	2.68±0.15 a
Effect of H_2O_2						
Without H_2O_2	56.50±1.18 b	45.88 ±1.73 b	27.16±1.08 a	20.19±0.56 b	9.17±0.34 b	2.20±0.09 b
With H_2O_2	61.33±1.56 a	57.44±1.18 a	20.19±0.89 b	23.50±0.69 a	11.48±0.23 a	3.31±0.19 a
Effect of sex hormones treatment						
Control	64.50±1.76 a	59.12±1.17 a	18.87±0.88 c	22.50±0.83 a	11.82±0.23 a	3.61±0.21 a
Testosterone	59.95±1.14 b	50.66±2.35 b	22.41±0.87 b	23.95±0.79 a	10.13±0.47 b	2.79±0.14 b
Estrogen	52.29±1.34 c	45.20±1.79 c	29.75±1.38 a	19.08±0.54 b	9.04±0.35 c	1.87±0.11 c
Interaction						
Intact-without H_2O_2-Control	57.50±1.99 cd	53.16±1.53 b	20.83±1.01 ef	22.16±1.13 cde	10.63±0.30 b	2.78±0.11 de
Intact-without H_2O_2-Testosterone	57.66±1.22 cd	41.50±1.17 d	25.16±1.24 cd	24.00±1.15 abc	8.30±0.23 d	2.32±0.12 ef
Intact-without H_2O_2-Estrogen	54.00±1.43 de	39.66±1.05 d	27.33±1.45 bc	20.00±1.29 def	7.93±0.21 d	2.00±0.09 fg
Intact-H_2O_2-Control	74.50±1.85 a	62.83±0.94 a	14.66±0.88 h	27.00±0.96 a	12.56±0.18 a	5.14±0.23 a
Intact-H_2O_2-Testosterone	61.16±2.18 bc	55.16±2.34 b	19.66±0.95 f	25.66±1.28 ab	11.03±0.46 b	3.15±0.22 cd
Intact-H_2O_2-Estrogen	46.66±2.06 f	47.66±1.81 c	29.00±1.18 b	18.33±0.66 f	9.53±0.36 c	1.62±0.09 gh
Castration-without H_2O_2-Control	65.66±3.80 b	64.16±2.12 a	23.83±1.13 de	17.50±0.76 f	12.83±0.42 a	2.76±0.12 de
Castration-without H_2O_2- Testosterone	55.66±2.20 cd	40.16±1.24 d	26.33±1.22 bcd	19.66±1.05 ef	8.03±0.24 d	2.15±0.16 f

Castration-without H_2O_2-Estrogen	48.50±0.99 ef	36.66±1.58 d	39.50±0.76 a	17.83±0.94 f	7.33±0.94 d	1.23±0.03 h
Castration-H_2O_2-Control	60.33±1.45 bcd	56.33±1.11 b	16.16±0.87 gh	23.33±0.91 bcd	11.26±0.22 b	3.77±0.18 b
Castration-H_2O_2-Testosterone	65.33±1.54 b	65.83±2.12 a	18.50±0.88 fg	26.50±1.43 ab	13.16±0.42 a	3.56±0.17 bc
Castration-H_2O_2-Estrogen	60.00±2.08 bcd	56.83±1.83 b	23.16. ±1.44 de	20.16±1.30 def	11.36±0.37 b	2.62±0.14 e

Means with different letters within each column differ significantly (P≤0.05).

Estrogen treated and testosterone treated groups recorded significantly lower values of total protein, and albumin with significantly higher value of globulin and globulin/albumin as compared with control at P≤0.05. Also, animals treated with testosterone had significant decrease in albumin level as compared with animals treated with estrogen. Testosterone treated group recorded significantly decrease in glucose concentration compared to control with significantly increase in ALT and AST level as compared to estrogen treated and control group at P≤0.05, meanwhile, estrogen treated group recorded significantly lower value in ALT and AST than testosterone treated and control group at P≤0.05.

As related to interaction effect as shown in Table (4), total protein recorded lowest value in castration-H_2O_2-testosterone treated rabbits compared to other groups. As well, intact-without H_2O_2-control rabbits and castrated-without H_2O_2-control rabbits groups showed significantly higher value of albumin than other groups. Globulin and globulin/albumin levels were significantly higher in castration-without H_2O_2-estrogen treated rabbits group as compared with other groups. In addition, glucose concentration showed lowest value in castration-H_2O_2-testosterone treated rabbits groups than other groups. ALT and AST levels showed significantly highest value in intact-with H_2O_2-testosterone treated rabbits group compared to other groups at P≤0.05. Also, ALT showed significantly lowest value in castrated-without H_2O_2 estrogen treated group compared to other groups. While, AST showed significantly lowest value in castrated-with H_2O_2-estrogen treated rabbits group as compared with other groups.

Table (4). Effect of castration, H_2O_2, sex hormones and interaction on total protien, albumin, globulin, globulin/albumin, glucose, ALT and AST of male rabbits (Mean ± S.E.)

Treatments	TP gm/dl	ALB gm/dl	GLOB gm/dl	GLOB/ALB	Glucose mg/dl	ALT U/L	AST U/L
Effect of castration							
Intact	6.04±0.06 a	3.49±0.06 a	2.58±0.04 a	0.74±0.02 a	109.25±1.48 a	68.27±1.65 a	43.30±1.15 a
Castration	6.11±0.06 a	3.53±0.06 a	2.60±0.04 a	0.74±0.02 a	105.44±1.45 b	58.58±1.91 b	39.47±1.11 b
Effect of H_2O_2							
Without H_2O_2	6.31±0.06 a	3.57±0.08 a	2.75±0.04 a	0.78±0.02 a	103.58±1.13 b	57.38±2.07 b	40.66±0.83 b
With H_2O_2	5.85±0.03 b	3.44±0.05 b	2.43±0.02 b	0.70±0.01 b	111.11±1.55 a	69.47±1.16 a	42.11±1.43a
Effect of sex hormones treatment							
Control	6.26±0.08 a	3.83±0.09 a	2.50±0.03 b	0.65±0.01 b	109.62±1.90 a	63.25±2.20 b	41.75±1.12 b
Testosterone	5.95±0.07 b	3.27±0.04 c	2.67±0.04 a	0.80±0.01 a	105.12±1.75 b	72.00±1.62 a	47.54±0.87 a
Estrogen	6.03±0.05 b	3.42±0.06 b	2.60±0.07 a	0.76±0.03 a	107.29±1.77 ab	55.04±1.97 c	34.87±0.92 c
Interaction							
Intact-without H_2O_2-Control	6.62±0.09 a	4.18±0.14 a	2.60±0.11 cde	0.61±0.02 de	104.00±3.17 cde	67.83±2.67 cd	40.83±1.16 cd
Intact-without H_2O_2-Testosterone	6.31±0.16 bc	3.40±0.11 cd	2.90±0.06 b	0.83±0.06 bc	104.83±2.66 cde	75.33±1.45 ab	45.83±1.01 b
Intact-without H_2O_2-Estrogen	5.80±0.08 fg	3.10±0.06 e	2.68±0.05 cd	0.86±0.02 b	100.33±3.07 de	51.33±1.45 f	39.00±1.15 de
Intact-H_2O_2-Control	5.86±0.05 def	3.33±0.05 de	2.53±0.05 def	0.75±0.02 c	117.00±2.50 a	70.33±1.40 bcd	47.33±1.70 b
Intact-H_2O_2-Testosterone	5.82±0.07 efg	3.19±0.03 de	2.62±0.03 cde	0.81±0.00 bc	113.50±2.43 ab	79.66±1.70 a	53.16±0.94 a
Intact-H_2O_2-Estrogen	5.86±0.05 def	3.71±0.04 b	2.15±0.02 g	0.57±0.01 e	115.83±2.05 a	65.16±1.44 de	33.66±1.14 f

Castration-without H_2O_2-Control	6.66±0.08 a	4.19±0.05 a	2.47±0.06 ef	0.58±0.01 de	103.16±2.99 cde	47.00±1.46 f	35.00±0.57 f
Castration-without H_2O_2-Testosterone	6.08±0.07 cde	3.35±0.08 de	2.73±0.03 c	0.81±0.02 bc	106.16±2.90 cde	60.33±1.01 e	46.16±1.40 b
Castration-without H_2O_2-Estrogen	6.36±0.08 b	3.23±0.03 de	3.13±0.07 a	0.96±0.02 a	103.00±2.58 cde	42.00±1.29 g	37.16±1.53 ef
Castration-H_2O_2-Control	5.88±0.04 def	3.63±0.15 bc	2.41±0.04 f	0.66±0.02 d	114.33±3.43 ab	67.83±2.67 cd	43.83±1.49 bc
Castration-H_2O_2-Testosterone	5.58±0.05 g	3.14±0.04 de	2.43±0.05 ef	0.77±0.02 bc	96.00±2.14 e	72.16±1.86 cb	45.00±1.21 b
Castration-H_2O_2-Estrogen	6.09±0.08 cd	3.65±0.10 bc	2.44±0.03 ef	0.66±0.02 d	110.00±2.85 abc	61.66±0.55 e	29.66±0.66 g

Means with different letters within each column differ significantly ($P \leq 0.05$).

Anti-oxidative/oxidative parameters

In the current study, as shown in Table (2), there was a significant decrease in the level of MDA, conversely a significant increase in the levels of GSH and TAC in the castrated rabbits as compared with intact rabbits. Castration which leads to testosterone deficiency is reflected as well in variation of anti-oxidative/oxidative parameters. This deficiency of testosterone may be the cause of the low MDA level and rise in GSH and TAC levels. Chainy and Sahoo (2020) have reported that testosterone is an anabolic hormone, it elevates the metabolic rate which in turn increased O_2 consumption which might augment ROS production. According to Al-Hiti (2016), castration resulted in a significant increase in GSH in the pancreas, which is supported by the results of the current study in serum GSH. On the other hand, testosterone replacement therapy in gonadectomized male rats resulted in a decrease of GSH in pancreatic and heart homogenates to virtually normal levels and a considerable loss of GSH in kidney and liver homogenates. In contrast, castration decreased MDA level in the pancreas, heart, kidney, and liver. MDA concentrations in the pancreas, heart, kidneys, and liver are all significantly increased by testosterone replacement therapy. Aydilek and Aksakal, (2005) also reported that rabbits injected with testosterone had significantly higher levels of MDA when compared to the castration group. Furthermore, TAC levels were found to be higher in sham mice and groups that did not receive testosterone (Choobineh *et al.*, 2016). In contrast to above findings, Verma and Rana (2008) found that bilateral castration reduced glutathione in both the liver and the kidney, testosterone administration reduced lipid peroxidation in the livers of castrated and benzene-treated rats, but it did not restore glutathione status. However, testosterone treatment of castrated and benzene-treated rats resulted in a moderate increase in lipid peroxidation when compared to castrated and benzene-treated rats, testosterone may influence free radical generation by influencing membranous polyunsaturated fatty acids, testosterone administration to rats and rabbits is known to increase lipid peroxidation in the liver (Aydilek and Aksakal, 2005).

Regarding to the effect of H_2O_2 as shown in Table (2), the results showed that animals treated with H_2O_2 had a significant increase in MDA level with a significant decrease in GSH and TAC levels as compared with untreated animals at $P \leq 0.05$. The current findings are in agreement with the study of Abdul-Majeed and Abdul-Rahman (2022), who stated that rabbits treated with H_2O_2 showed significant increase in MDA level with significant decrease in GSH level compared to untreated rabbits. In addition, Al-Kattan *et al*, (2007) found that rabbits received H_2O_2 recorded significant decrease in GSH level with significant increase in MDA level in the liver. Furthermore, Taha *et al*, (2020) noticed that the addition of hydrogen peroxide to drinking water at 1% of adult quail males led to a significant increase in MDA level, and a significant decrease in the GSH level compared to control group. Pravda (2020) and Ayed *et al*, (2021) stated that hydrogen peroxide depletes the glutathione in the blood and tissues and causes a decrease in its level in the body and that its decrease is associated with an increase in the level of MDA, which is considered one of the main indicators of the occurrence of the lipid peroxidation. The reason for this decrease in the concentration of GSH is due to an increase in its depletion or a decrease in its synthesis (Loven *et al.*, 1986), and this decrease may also be due to the occurrence of oxidative stress resulting from the ingestion of one of the effective

types of oxygen represented by H_2O_2, the decrease in the concentration of GSH in the cell is an indication of an increase in oxidative stress (Mclennan *et al.*, 1991). As for the high concentration of MDA in the blood serum of treated rabbits with H_2O_2, it could be attributed to the stimulation of the enzyme Fatty Acyl-CoA Oxidase and the start of fatty acid oxidation, which leads to the production of endogenous H_2O_2, which contributes to the production of lipid peroxidation, as MDA is one of the final products of it (Osumi and Hashimoto, 1978).

Data presented in Table (2) show a significant decrease ($P\leq0.05$) in MDA level with a significant increase ($P\leq0.05$) in GSH and TAC levels in the estrogen group compared to the testosterone and control groups. In terms of group interaction, the results revealed that castration-without H_2O_2-estrogen group recorded the lowest significantly value in MDA level with the highest significantly values in GSH and TAC levels as compared with other groups at $P\leq0.05$. On the other hand, the intact-H_2O_2-testosterone treated rabbits showed the highest significant value in MDA and the lowest significant values in GSH and TAC among the interaction groups at $P\leq0.05$. This result was similar to the findings of Al-Rahbi *et al*, (2014) who reported that oestrogen increased the mean level of TAC and GSH while decreasing the mean level of MDA comparable to that of sham-opreated control rats. Also, Mortensen *et al,* (2001) found that chronic oestrogen treatment causes a significant increase in GSH in the aorta of OVX rabbits when compared to the untreated group. MDA was also found to be lower in ovariectomized (OVX) rats given chronic oestrogen (Zhou *et al.,* 2012). Furthermore, compared to the untreated group, chronic E2 treatment causes a significant increase in glutathione transferase (GST) and glutathione reductase (GR) activities in the OVX rabbit aorta (Mortensen et al. 2001). In OVX rats receiving chronic estrogen treatment, increased SOD and GPX activity and decreased MDA were also observed (Zhou *et al.,* 2012). As well, Ceravolo *et al*, (2013) found that chronic conjugate equine E2 therapy significantly increases catalase (CAT) expression in OVX rats when compared to untreated rats. This result may be due to antioxidative properties of estrogen. The antioxidative activities of E2 possibly act via two different mechanisms, the first mechanism is by using the hydroxyphenolic structure of E2 as an important free radical scavenger (Escalante and Quesada, 2012) independent of the ER activation (Petrovska *et al.,* 2012). Modification or removal of the phenolic group blocks the antioxidant activities of E2 (Behl and Manthey, 2000). The second mechanism is related to the influence of E2 on endogenous antioxidative enzyme systems (Unfer *et al.,* 2006). E2 is highly lipid soluble and largely resides in the membrane component of cells (Cegelski *et al.,* 2005), where they are ideally suited to affect the oxidation of unsaturated bonds in phospholipids. This membrane localisation allows E2 to interact synergistically with abundant antioxidants, such as glutathione (Gridley *et al.,* 1997 and Green *et al.,* 1998). Another study revealed that E2 can prevent lipid peroxidation by sacrificing itself, resulting in a quinol product (Simpkins *et al.,* 2005). However, not all E2 results are consistent with a protective function. Several investigations have demonstrated, for instance, that E2 may actually aggravate oxidative stress (Gomez-Zubeldia *et al.,* 2001and Sverko *et al.,* 2004).

Biochemical parameters

Data related to the effect of castration on lipid profile in current study as shown in Table (3), the results showed that non-significant changes in cholesterol, LDL-C and risk index levels with significant increase in TG, HDL-C and VLDL-C were observed in castrated group compared to intact group at P≤0.05. In a study of (Zhao *et al.*, 2013), they noticed that castration significantly increased the levels of HDL-C, total cholesterol and triglyceride in rabbit's serum. Hassan (2010) demonstrated a significant increased (P≤0.05) in the level of triglyceride, cholesterol and LDL-C with no difference in HDL-C in castrated rats group compared with control value. A significantly increased of HDL-C, triglyceride and VLDL-C levels in castrated group may be attributed to the decrease of hepatic lipase (HL) and lipoprotein lipase (LPL) activities due to the absence of gonadal hormones.

In regard to the effect of H_2O_2, the results showed that animals treated with H_2O_2 showed a significant increase in cholesterol, TG, LDL-C, VLDL-C and risk index with significant decrease in HDL-C levels at P≤0.05. These findings are in agreement with the study of Abdul-Majeed and Abdul-Rahman (2022) who stated that male quail treated with H_2O_2 showed significant increase in cholesterol and TG level compared to control group. As well, Al-Samarai and Al-Janabi (2021) reported that there was a significant increase in the level of triglycerides and cholesterol of rabbits treated with H_2O_2 compared to untreated rabbits. Also, Abdul-Rahman and AL–Kattan (2007) noticed a significantly increased level of cholerstrol and triglyceride in the serum of laying hens treated with H_2O_2 compared to control group.

The high level of cholesterol in H_2O_2-stressed rabbits may be due to a decrease in the activity of the thyroid gland, as the decrease of the thyroxine in stressed rabbits has a negative effect on the excretion of cholesterol with the bile, thus increasing its level in the blood (Duntas and Brenta, 2018). On the other hand, the significant increase in triglycerides was in parallel with the cholesterol increase due to the positive correlation between both parameters (Abdul-Majeed *et al.*, 2013). Furthermore, such changes in serum lipid may reflect the suppression of lipid metabolism due to H_2O_2 induced oxidative stress. Partial deficiency of lipoprotein lipase (the key enzyme determining the removal rate of TG from plasma), associated with increased output of lipoprotein from the liver may contribute to the elevation of serum TG level in H_2O_2 treated group (Erikson *et al.*, 2016). Estrogen treated rabbits showed a significant decrease in serum cholesterol, TG, LDL-C, VLDL-C and risk index with significant increase in HDL-C as compared with testosterone treated rabbits and with control rabbits.

In regard to interaction effects, Table(3) showed that the lowest significant values in cholesterol was recorded in intact-H_2O_2-estrogen and castration without-H_2O_2 estrogen treated rabbits, meanwhile the lowest significant value in TG was recorded in castration without-H_2O_2-estrogen treated rabbits, for LDL-C and risk index the highest significant value were recorded in the intact-H_2O_2-control treated rabbits, for VLDL-C the highest significant values was recorded in castrated-H_2O_2-testosterone and intact-H_2O_2-control treated rabbits. On the other hand, castration without-H_2O_2- estrogen treated animals showed the highest significant value in HDL-C and the lowest significant value in risk index at P≤0.05. Stevenson *et al*, (2005) reported similar results when they stated that 17 estradiol increased HDL-C levels and decreased LDL-C levels. In addition, the results of the present study were

supported by Skouby *et al,* (2005), who reported that contraceptives containing estradiol reduced LDL-C. Al–Mahmod (2009) found that treatment with 17 estradiol decreased cholesterol, TG, and HDL-C, while LDL-C significantly increased. Also, Hassan (2010) reported that when testosterone was administered to castrated rats, total cholesterol, TG, and LDL-C levels decreased significantly while HDL-C levels remained unchanged. Moreover, Aydilek and Aksakal, (2005) noticed that the concentration of HDL-C and HDL-C:LDL-C ratio decreased in the testosterone group, ratio of HDL-C/LDL-C and the levels of HDL-C, TC, TG increased in the castration group when compared with testosterone group.

Exogenous testosterone has been reported to increase the activity of hepatic lipoprotein lipase (LPL), an enzyme involved in HDL-C catabolism, therefore suggesting that testosterone treatment should reduce HDL-C levels (Zmuda *et al.*, 1993). Dihydrotestosterone (DHT) inhibited the differentiation of human mesenchymal stem cells (hMSCs) into adipocytes, as well as lipid accumulation in existing adipocytes. DHT also inhibited the maturation of pre-adipocytes into mature adipocytes (Gupta *et al.,* 2008).

The findings related to protiens, glucose, ALT and AST as shown in Table (4). A similar result was obtained by Hassan (2010) who stated that there were no significant changes between castrated and control groups in levels of total protein, albumin and globulin. As well, Al-Hiti (2016) stated that efficacy of castration had lowered glucose level but not significantly. In the current study, testosterone significantly reduced the glucose and albumin levels in the serum of treated rabbits. It is well known that testosterone deficiency is linked to decreased insulin sensitivity and glucose tolerance (Wang *et al.,* 2011).

Albumin is the major plasma protein that circulates in the bloodstream, which is only produced in the liver. Low serum albumin levels indicate impaired liver function. Other than liver disease, albumin levels can be low in conditions such as severe malnutrition and some kidney diseases that cause extensive protein wasting, because the rabbits were well fed and gained weight, severe malnutrition should be ruled out. Furthermore, it is believed that testosterone reduced kidney filtration, so low serum albumin cannot be due to wasting or leakage through the kidney. As a result, this low serum albumin level could be due to impaired liver function. This is a possibility because liver enzymes were found to be elevated, indicating poor liver function (Table 4). Young *et al,* (1993) also found that testosterone reduced serum albumin levels.

Al–Mahmod (2009) reported that 17 estradiol treatment increased total protein concentration. Verma *et al,* (2005) stated that 17B estradiol lowers glucose levels by stimulating insulin. Another study demonstrated that the glucose level decreased following oestrogen therapy (Herrmann *et al.,* 2005). In contrast, neither gonadectomy nor estradiol affected blood glucose levels in male mice (Iakovleva *et al.,* 2020).

In agreement with results of current study, Rebaz *et al,* (2019) found that rabbits injected with a high level of testosterone had higher ALT levels than rabbits injected with a low level of testosterone and the control group.

Consistent with the findings of the current study, Al-Samarai and Al-Janabi, (2021) noticed that treatment with H_2O_2 in rabbits led to significant decrease in total protein and globulin. As well, the result of the current study is in agreement with

Abdul-Majeed and Abdul-Rahman, (2022) who noticed that the addition of hydrogen peroxide led to a significant increase in the level of both AST and ALT enzymes in the blood of male quail compared to the control group at ($P \leq 0.05$), and also agreed with the results of (Al-Kattan *et al.,* 2007) who reported that rabbits treated with H_2O_2 showed significant increase in the level of ALT and AST. Moreover, Abdul-Rahman and Al- Kattan (2007) investigated that treatment H_2O_2 led to significant increase in the level of ALT and AST in the serum of laying hens. This indicates the ability of hydrogen peroxide to cause oxidative stress. The high level of AST and ALT in the blood may be attributed to the oxidative stress induced by hydrogen peroxide, which leads to increased cellular oxidative stress, and produce many ROS which oxidizes the polyunsaturated fatty acids in cellular membranes and damage the channels pumps (Walia *et al.*, 2003), as a result, the membrane loses its selective permeability, due to the lipid peroxidation of the cell membrane and the leaching of these enzymes to the outside of cells (Catala and Díaz, 2016). The validity of this assumption is further enhanced by an increase of MDA level and a decrease in GSH level in the blood of rabbits that were given hydrogen peroxide (Table 1).

In the current study, the ability of estrogen to lower the level of ALT and AST is due to the enhancement of the antioxidant status, the reduction of oxidative stress, i.e: the enhancement of the level of GSH and TAC and the reduction of the level of MDA Table (2).

Treatment with H_2O_2 led to a significant decrease in total protein, albumin and globulin with significant increase of glucose level compared to control. 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 hydrogen peroxide also 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, a raise of blood sugar level. Similarly, (Abdul-Majeed and Abdul-Rahman, 2022), observed that rabbits treated with H_2O_2 showed significant increase in glucose level compared to those of untreated group.

CONCLUSIONS

It could be concluded from the current study that hydrogen peroxide had a significant effect on all of the studied parameters, and testosterone also played a role in increasing oxidative stress, but estrogen reversed the negative effects of oxidative stress caused by hydrogen peroxide and testosterone, indicating that estrogen has the ability or role in improving antioxidant status.

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CONFLICT OF INTEREST

The authors state that there is no conflict of interest in the publishing of this paper.

تأثير الاخصاء والهرمونات الجنسية على حالة مضادات الأكسدة وبعض المعايير الكيموحيوية لذكور الارانب المعرضة للإجهاد التأكسدي

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الخلاصة

أجريت الدراسة الحالية لمعرفة تأثير الإستروجين والتستوستيرون كمضادات للأكسدة وقدرتهما على منع تأثير الإجهاد التأكسدي الناجم عن H2O2 وانعكاساتهما على معاملات الدم وحالة مضادات الأكسدة لدى ذكور الأرانب. تم تقسيم 72 أرنب ذكر بالغ بشكل عشوائي إلى 12 مجموعة (6/مجموعة)، المجموعة الأولى: سليمة، المجموعة الثانية: سليمة– H2O2, المجموعة الثالثة: مخصي، المجموعة الرابعة: مخصي– H2O2، المجموعة الخامسة: سليمة– H2O2– التيستوستيرون، المجموعة السادسة: مخصي– H2O2– تستوستيرون، المجموعة السابعة: سليمة– H2O2– استروجين، المجموعة الثامنة: مخصي– H2O2– استروجين، المجموعة التاسعة : التستوستيرون– السليم، المجموعة العاشرة: التستوستيرون– المخصي، المجموعة الحادية عشر: الاستروجين– السليم، المجموعة الثانية عشر: الاستروجين– المخصي، استمرت المعاملات لمدة 4 أسابيع. أظهرت النتائج أن الإخصاء زادت بشكل معنوي من مستويات GSH و TAC و TG و HDL–C وانخفضت بشكل معنوي من مستويات MDA و AST و ALT. كما تسببت المعاملة باستخدام H2O2 في انخفاض معنوي في مستويات GSH و TAC و HDL–C والبروتينات الكلية وزيادة معنوية ملحوظة في مستويات MDA والكوليسترول و TG و LDL–C و ALT و AST مقارنة بالمجموعة غير المعاملة. من ناحية أخرى، يحسن المعاملة بالإستروجين TAC و GSH ومستوى الدهون ويقلل MDA بشكل معنوي مقارنة بمعاملة التستوستيرون. فيما يتعلق بتأثيرات التداخل، فإن الإخصاء بدون معاملة H2O2– الإستروجين تقلل من الإجهاد. تشير هذه النتائج إلى أن الإخصاء ومعاملة الأستروجين للذكور المخصي والسليم يقلل من آثار الإجهاد ويحسن مستوى الدهون وبعض المعايير المناعية.

الكلمات المفتاحية: الإخصاء، الهرمونات الجنسية، حالة مضادات الأكسدة، H_2O_2.

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