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THE AMELIORATIVE EFFECTS OF VIRGIN OLIVE OIL ON HEAT STRESS IN MALE RABBITS

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Heat stress (HS) due to climate change is a problem of great concern to rabbits as they very susceptible to heat which negatively affecting their physiological status. Recently usage of natural safe dietary additives was used to alleviate the effects of HS during summer seasons. The current study aimed to investigate the impacts of chronic cyclic HS on the hematological parameter, hormonal levels and testicular histopathological changes of rabbits and the possible ameliorative effects of virgin olive oil (VOO) supplementation. 25 mature male NZW were divided into three groups: control group, HS-group that was exposed to chronic HS for 2 hrs daily, and HS+VOO that was also subjected to HS and supplemented with VOO. The THI values were 31, 32 and 34 for the 10, 20 and 30-day periods of HS application. WBCs, lymphocytes, monocytes, neutrophils and eosinophils counts were significantly lowest in the HS-bucks compared with control. However, VOO-supplemented group showed significant enhancement in lymphocytes and monocytes compared with the HS-group. No significant differences were observed in erythrograms and platelets count among the three studied groups. The HS-bucks recorded the least mean values for both serum LH and T. whereas, VOO-supplemented bucks showed the highest mean value for both parameters, even higher than control. Applied HS has elicited impairment of spermatogenic cycle, however, testicular tissues from VOO-supplemented were still keeping their tissue architecture and seminiferous tubules were well-populated with few damaged cells. In conclusion, HS induced hematological, hormonal and histopathological

changes in tests of male rabbit and supplementation with VOO may ameliorate these changes.

Introduction:

In Egypt, the rabbit industry is regarded as one of the fast-paced and moderately developed rabbit industries **(Oseni & Lukefahr, 2014)**. Recently, the strong correlation between the progress of this industry and the global rise in surface temperature has great interest **(Al-Sagheer *et al.*, 2017)**. In Egypt, the high ambient temperatures in the summer season constitute a clear limitation to rabbit production **(Ondruska *et al.*, 2011)**. Heat stress (HS) is a worldwide problem that occurs when the amount of heat produced by an animal surpasses the animal's capacity to dissipate heat to its surrounding environment. A various combination of environmental factors such as air temperature and humidity in parallel with the animal's characteristics such as species, age, thermal comfort zone, and its physiological status may cause the imbalance between heat input and output by the animal **(Akbarian *et al.*, 2016).** Temperature-humidity index (THI) is widely used to estimate the degree of HS to which the animal is exposed **(De Rensis *et al.*, 2015).** For rabbits, the reproductive performance is impaired with high THI values above 27.8, which implies the beginning of HS **(Maya Soriano, 2012)**. Rabbits, in general, are highly susceptible to HS because their thermal comfort zone ranges between 15-21°C. Accordingly, HS causes heavy economic losses reflected in limiting the rabbits' breeding season to be normally from September to May **(El-Tohamy *et al.*, 2012).** Since HS has been documented to be involved in cellular oxidative stress, several authors sought to use antioxidants supplementation to alleviate the HS drastic impacts.

Olive oil, for example, is regarded as natural functional food because of having a high content of mono and polyunsaturated fatty acids and being rich in several potent bioactive components with possible antioxidant properties **(WHO, 2018)**.

Materials and Methods

Chemicals:

- LH (Human) ELISA Kit (NO. KA0214), Abnova Corporation.
- Testosterone ELISA Kit (NO. 582701), Cayman Chemical.
- A commercial type of natural virgin olive oil (VOO) with max acidity 1.5% produced by KAHRAMAN Oil & Food Ltd Co, Turkey.
- All other chemicals from Sigma-Aldrich- USA

Animals:

A total of 25 sexually mature apparently healthy male NZW rabbits at age 8:12 months with average initial body weight 2653±62 g were purchased from a commercial farm. The study was carried out in the Animal House of the Faculty of Medicine, and Zoology Department, Faculty of Science, Assiut University, Assiut, Egypt. Throughout the experimental period, bucks were individually housed in metal wire mesh cages provided with pottery and automatic drinker to provide free access to feed and freshwater, respectively. The ration and water were offered between (8:30 & 9:00 a.m.) and refilled at between (15:00 & 15.30 p.m.) daily. Bucks were also subjected to a photoperiod of 12 hrs light/day. Animals were fed *ad libitum* a commercial pelleted ration containing 18% crude protein, 2.69% crude fat, 12.39% crude fiber, 2738 Kcal/kg diet digestible energy that met all nutritional requirements of rabbits. All experimental protocols that held on animals were done according to

regulations set by the Institutional Animal Care and approved by Assiut University.

Temperature-humidity index (THI) calculation:

Climatic data were continuously recorded during the experimental period by using a thermometer and a calibrated hygrometer. Then, the average values of ambient temperature in Celsius and relative humidity in percentage at midday inside the rabbit building were estimated for each 10-day period along the experimental period. The THI was computed using the formula established by **Marai *et al.* (2001)** for rabbits as follow: **THI = dbºC – [(0.31 – 0.31(RH)) (dbºC – 14.4)]**, where: dbºC = the dry bulb temperature in Celsius, RH = relative humidity percentage/100. Then, the values obtained are categorized as follows: THI<27.8: absence of HS, 27.8<THI<28.9: moderate HS, 28.9 < THI < 30.0: severe HS, and THI > 30.0: very severe HS.

Experimental design:

After one week of acclimation. The rabbits were randomly allocated into three groups, as follows:

a- Control group: (n=6) the rabbits were maintained at comfort conditions and fed the basal diet without any supplementation.

b- HS group: (n=10) the rabbits were exposed to a high temperature using heaters for about 2hrs daily and fed the basal diet. The temperature was designed to start to be elevated from 9:00 am to about 12:00 pm until it reached the degree wanted for the application of HS (37°C and above), and then being settled at that temperature for continuous 2hrs. Then, heaters were turned off and the animals started a recovery period during which rabbits regain the normal air temperature as in the control group (**Sabés-Alsina, 2016).**

c- HS+EVOO group: (n=9) the rabbits were exposed to a high temperature just like those in the HS group and fed the basal diet. In addition, they were supplemented with VOO at 3 ml/day via oral gavage by a syringe. VOO was given for each buck after the end of HS exposure, at about 3:00 p.m.

Collection of samples:

Animals slaughter was designated to be at the 10^{th}, 20^{th}, and 30^{th} days of the experiment. On the day scheduled, the specified bucks were slaughtered and the blood samples were collected during slaughter. Then, the tests were removed and collected post-slaughter. The blood samples were collected once during the slaughter of the animal. About 1 ml of whole blood was taken directly from the animal in a sterilized test tube with EDTA for complete blood count (CBC). About 5 ml of whole blood was taken directly from the animal in a sterilized test tube without anti-coagulant, blood samples allowed to stand for clotting from 1-2 hrs at room temperature. The clot was detached from the wall of the centrifuge tube and then samples were centrifuged at 3000 rpm for 10 mins in order to separate the serum. Serum samples were then divided into aliquots in eppendorf tubes, and stored at -20˚C until further analysis. Testes from each buck were quickly removed, washed in a saline solution (0.9% NaCl). One testis was fixed immediately in 10 % neutral buffered formalin, dehydrated, cleared, embedded in paraffin wax blocks for histopathological study according to **Drury & Wallington (1980)**. The other was immersed in liquid nitrogen and then kept at -20^0C for biochemical study.

Methods:

CBC was performed in the Central Lab of the Pathology and Clinical Pathology Department, Faculty of Veterinary Medicine,

Assiut University, Assiut using hematological analyzer (Exigo Veterinary Hematology Analyzer).

The quantitative determination of LH and testosterone concentration in rabbits' sera were performed by Kit based on the principle of a solid phase enzyme-linked immunosorbent assay (ELISA) according to **Tietz (1995)** and **Kicman (2010),** respectively.

The morphological analysis was done by Research Microscope type Axiostar Plus made by Zeiss transmitted light bright field examinations upgradeable to professional digital image analysis system (Carl Zeiss Axiovision Product Suit DVD 30).

Statistical Analysis:

The normal distribution of sample data and the homogeneity of the variances were firstly assessed by Shapiro-Wilk's test and Leven's test, respectively. One-way ANOVA was conducted followed by a post-hoc test (Duncan's test) for multiple comparisons among experimental conditions. A student t-test was carried out to compare each period of time in HS group with its corresponding in HS+VOO group. The results were expressed as the mean±standard error (SE). Statistical analysis was performed with IBM® SPSS® for Windows (IBM Corp., Armonk, NY, USA) statistics version 21 for Windows.

Results:

Temperature-humidity index (THI): The THI computed values were 30.75, 32.08 and 33.45 for the 10-day, 20-day and 30-day periods of HS application respectively during the experiment,

which indicates a condition of gradual upwards increase of very severe HS **Table (1).**

Table (1): Average of climate data for each 10 days period during the experiment.

Days	Temperature (°C)	RH (%)	THI
10	**35.00±0.61**	**33.50±2.20**	**30.75**
20	**37.40±0.54**	**25.40±1.19**	**32.08**
30	**39.00±0.42**	**27.20±0.80**	**33.45**

RH: Relative humidity, THI: Temperature humidity index

Table (2) showed that the mean WBCs count decreased significantly in both of HS-group and the VOO-supplemented group in comparison with the control. However, the VOO-supplemented group exhibited a little bit better value when compared with that of the HS- group. With regard to the effect of exposure time, both the HS and VOO groups reflected a general pattern of decrement in the WBCs count by the time. However, this pattern was at different rates in these groups, as the HS group showed a greater rate of loss in comparison with that of VOO group followed by a slight increase in the last ten days. Moreover, when each time period in the HS was compared with its corresponding in the VOO group, the only significant difference was found at 20-days of exposure. In comparison with the control group, HS-group showed a significant decrement in the lymphocytes count, while in VOO-supplemented group was insignificantly different from both HS and control groups. There was a minor enhancement in lymphocytes count of VOO-supplemented group if compared with HS one. With regard to the effect of exposure time, there was a general pattern toward a decrease in the lymphocytes count by time and elevated THI. As for monocytes count, **Table (2)** revealed that HS-group achieved again

the significant least mean value**,** while there was a non-significant difference between the VOO-supplemented and the control groups in monocytes count. Moreover, when each time period in the HS was compared with its corresponding in the VOO group, the only significant difference was found at 10-days of exposure where HS values were lower than VOO group. The neutrophils count was significantly different among the three studied groups as shown in **Table (2).** A significant decrease in the overall neutrophils counts happened in both the HS group and the VOO-supplemented group when compared with the control**.** There was a slight improvement in the number of neutrophils of VOO-supplemented group compared with the HS group. In addition, regarding the effect of the exposure time, the only statistically significant difference between HS and VOO groups was detected at 20-days of exposure to HS. **Table (2)** indicated a highly significant difference in the number of eosinophils among the three studied groups. Both HS and VOO-supplemented rabbits exhibited a decrease in the eosinophils count when compared with control rabbits**.** Both HS and VOO groups showed a growing shortage in the numbers of eosinophils by the passage of time under HS conditions. Regarding the effect of HS and VOO-supplementation to HS adult NZW bucks on their RBC indices, none of them showed any significant change relative to the control group. However, some of the RBCs indices differed by the time between HS and VOO-supplemented groups, namely, the RBCs, HB, Hct and MCHC as shown in **Table (2)**. **Table (2)** indicated that there was not any statistically significant difference **(P>0.05)** among the studied groups regarding the platelets count. Similarly, no statistical differences were found when the HS group was compared with the VOO group at the different periods of the HS application.

The present results showed a significant difference in the levels of LH and T hormone among the three studied groups as in **Table (3)**. The HS-bucks recorded the least mean values for serum LH. While the VOO-supplemented group and the control one recorded the higher values with the highest value was for VOO group. Once again, the HS bucks had the lowest mean value for serum T hormone followed by the control group**.** However, the serum T was insignificantly lower in HS group relative to control. Whilst, compared with these two groups, VOO-supplemented group had a significantly higher serum T hormone.

Table (2): Effects of VOO on the hematological parameters of HS-adult male NZW rabbits at different exposure time.

Parameters	Group	Exposure time			Total
		10 days	20 days	30 days	
WBCs ($x10^9$/L)	Control				12.44±0.43^{b}
	HS	9.73±0.41	6.90±0.59^{C}	7.85±0.58	8.13±0.47^{a}
	HS+VOO	11.27±0.43	9.07±0.38^{D}	6.80±0.32	9.04±0.67^{a}
	P-value	0.061	0.037*	0.212	< 0.0001***
Lymphocytes ($x10^9$/L)	Control				3.34±0.19^{b}
	HS	2.37±0.18^{A}	2.63±0.15	2.53±0.13	2.51±0.08^{a}
	HS+VOO	3.53±0.23^{B}	2.87±0.12	2.27±0.20	2.89±0.21^{ab}
	P-value	0.016**	0.284	0.313	0.012**
Monocytes ($x10^9$/L)	Control				0.90±0.07^{b}
	HS	0.53±0.07^{A}	0.70±0.10	0.65±0.06	0.63±0.04^{a}
	HS+VOO	0.90±0.10^{B}	0.93±0.09	0.60±0.06	0.81±0.07^{b}
	P-value	0.038*	0.155	0.604	0.017**

Table (2) continued,

Parameters	Group	Exposure time			Total
		10 days	20 days	30 days	

Neutrophils ($x10^9$/L)	Control				5.06±0.34[b]
	HS	4.70±0.17	2.37±0.12[C]	3.53±0.17	3.53±0.31[a]
	HS+VOO	4.73±0.20	3.50±0.12[D]	2.97±0.15	3.73±0.27[a]
	P-value	0.907	0.002[***]	0.060	0.013[**]
Eosinophils ($x10^9$/L)	Control				3.14±0.20[b]
	HS	2.17±0.15	1.20±0.12	1.03±0.10	1.42±0.18[a]
	HS+VOO	2.10±0.15	1.77±0.18	0.97±0.12	1.61±0.18[a]
	P-value	0.768	0.055	0.727	< 0.0001[***]
RBCs ($x10^{12}$/1)	Control				5.00±0.13
	HS	5.30±0.14	4.30±0.16	5.27±0.13[F]	4.99±0.17
	HS+VOO	5.75±0.09	4.34±0.11	4.65±0.11[E]	4.91±0.22
	P-value	0.051	0.860	0.018[**]	0.939
HB (g/dl)	Control				10.84±0.43
	HS	12.03±0.15	10.03±0.30	11.68±0.28[F]	11.29±0.31
	VOO	12.67±0.27	9.57±0.54	10.03±0.43[E]	10.76±0.53
	P-value	0.110	0.489	0.020[*]	0.619
Hct/ PCV (%)	Control				31.36±1.12
	HS	34.47±1.07	28.47±1.05	33.90±1.11[F]	32.44±1.04
	VOO	36.57±1.01	27.13±1.04	28.63±0.99[E]	30.78±1.55
	P-value	0.226	0.417	0.020[*]	0.626
MCV (fl/cell)	Control				63.06±2.16
	HS	65.20±2.00	66.53±1.16	64.40±1.63	65.28±0.89
	HS+VOO	63.60±2.80	61.80±2.44	61.60±0.21	62.33±1.12
	P-value	0.667	0.154	0.208	0.190
MCH (pg/cell)	Control				21.88±0.79
	HS	22.77±0.55	23.50±0.67	22.23±0.52	22.77±0.34
	HS+VOO	22.07±0.90	21.93±0.52	21.67±0.18	21.89±0.31
	P-value	0.545	0.138	0.414	0.226
MCHC (g/dl)	Control				34.72±0.66
	HS	34.93±1.39	34.73±0.81	32.05±0.94[E]	33.72±0.71
	HS+VOO	34.73±0.27	35.57±0.61	35.20±0.55[F]	35.17±0.28
	P-value	0.894	0.457	0.042[*]	0.185
Platelets ($x10^9$/1)	Control				500.20±33.48
	HS	418.67±32.20	351.67±34.60	512.25±37.45	436.00±29.01
	HS+VOO	580.33±36.80	335.67±34.91	398.67±27.70	438.22±40.29
	P-value	0.030	0.761	0.072	0.475

- n= 5 for control, 10 for heat stress, and 9 for heat stress-treated with VOO.

- Means in the same column followed by the same letter are not significantly different based on Duncun test at 0.05 significance level.
- Small letters a, b and c are used to denote the significant difference for the overall mean (total). Capital letters (A, B), (C, D), and (E, F) are used to denote the significant difference for 10 days, 20 days and 30 days respectively.
- The used symbols *, **, and *** to represent significance at $P \leq 0.05$, $P \leq 0.01$ and $P \leq 0.001$, respectively.

Table (3): Effects of VOO on the LH and T hormones of HS NZW bucks.

Group	n	LH (m.IU/ml)	T (ng/ml)
Control	**4**	**0.053±0.008** [b]	**1.578±0.265** [a]
HS	**6**	**0.020±0.004** [a]	**1.467±0.156** [a]
VOO	**6**	**0.063±0.008** [b]	**3.853±0.410** [b]
p-value		**0.002**[***]	**<0.0001**[***]

2. **Histopathological investigation:** The effects of 30 days VOO-supplementation to HS adult NZW bucks on the histology of seminiferous tubules of rabbits' testes compared to control are shown in **Fig. (2& 3).**

Fig.2. Photomicrographs for testicular tissues stained with H&E for the control rabbits Figs. (A, B&C) in comparison with the HS-rabbits Figs. (D, E &F) and the HS+VOO Figs. (G, H&I) showing the effect of EVOO supplementation on the HS-rabbits. **Fig. A, B&C:** photomicrographs of testicular tissue of control rabbits stained with H&E showing the normal architecture of the seminiferous tubule. (A) a number of well-populated seminiferous tubules. (B) well-organized tubular germ cells. (C) different stages of spermatogenesis are represented and easily recognized. **Fig. D, E&F:** photomicrographs of testicular tissue of HS rabbits stained with H&E showing the

deleterious effect of HS on the seminiferous tubule architecture. (D) increased number of extensively damaged seminiferous tubules. (E) cells within seminiferous tubules lose their hierarchical organization. (F) collapsed seminiferous epithelium, largely grown seminiferous lumen "red line", tubules were completely void of rounded spermatids and elongated sperms, the leftover cells were deformed, and shrunken. Empty areas are easily noticed of the seminiferous epithelium represented degenerated cells (red stars) and interstitial fibrosis (black asterisk). **Fig. G, H&I:** photomicrographs of testicular tissue of HS+VOO rabbits stained with H&E showing the VOO ameliorative effect on testicular tissue after HS exposure. (G) a number of well-populated seminiferous tubules as well as few damaged ones. (H) a seminiferous tubule maintained its organized architecture. (I) different stages of spermatogenesis are represented and easily recognized, however, the fewer number of cells areas are lost (red stars). Scale bars: A, D&G=5µm, B, E&H=10µm, C, F &I=40µm.

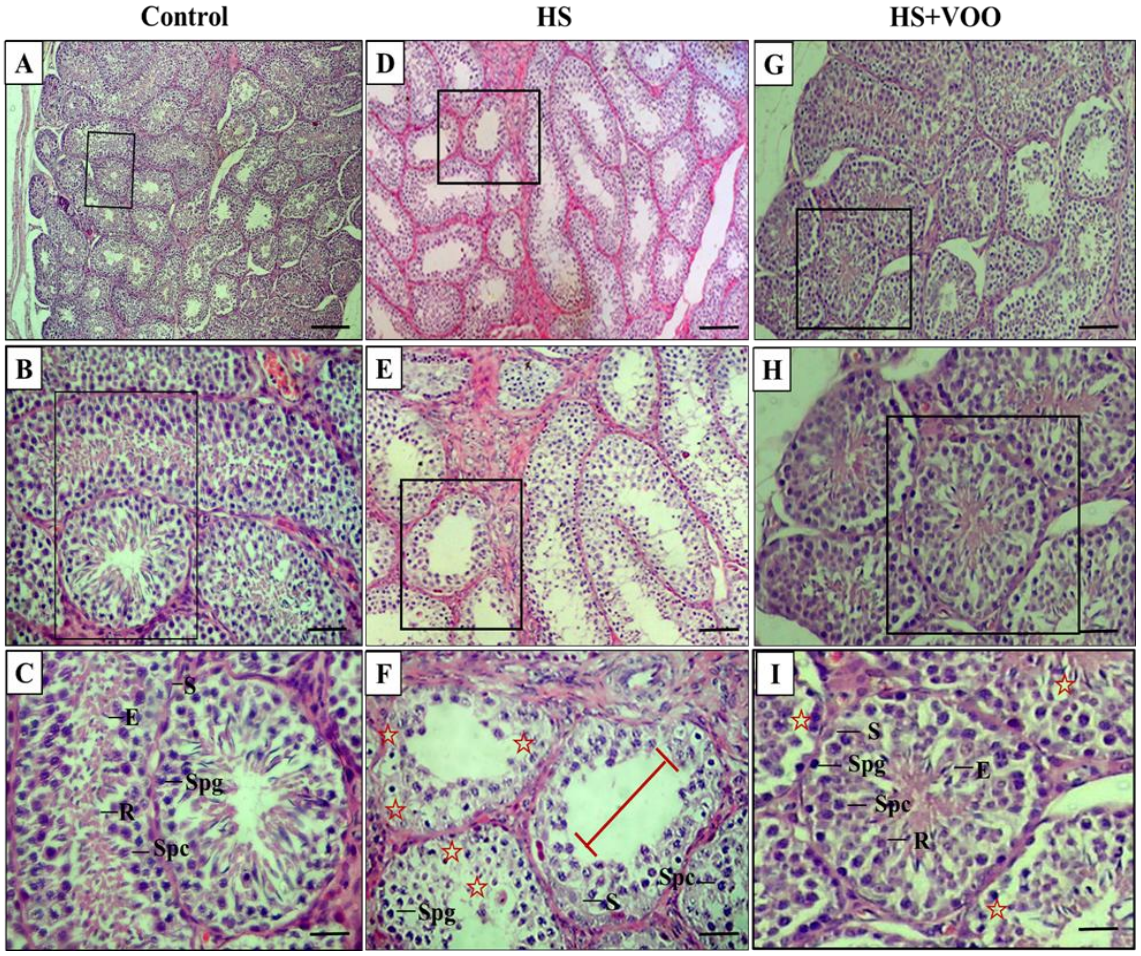
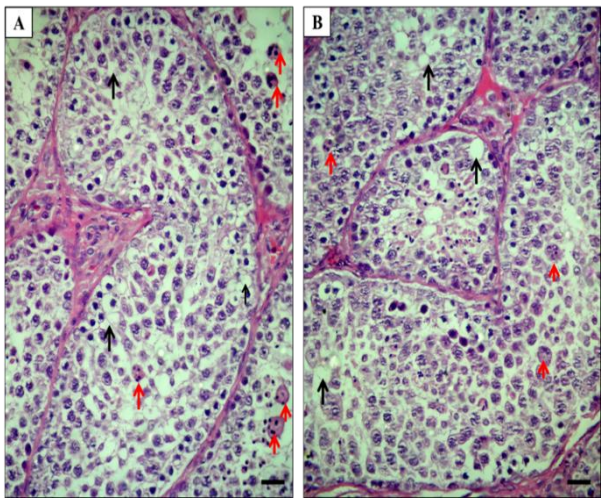


Fig.3. Photomicrographs show the other effect of HS on testicular tissues showing the swelling of degenerating spermatocyte, the formation of intratubular multinucleate giant cells "red arrows", thickened basement membranes, and the vacuolation or disappearance of seminiferous tubules epithelium lining "black arrows". Scale bars: A&B=40µm.



Discussion:

Throughout the experimental period, adult NZW bucks suffered from very severe HS as reflected by the high values of THI, which were 30.75, 32.08 and 33.45 for the 10-day, 20-day and 30-day periods of HS application, respectively that mean very severe HS **(Marai *et al.*, 2001)**. Hematological changes seem to play an important role in adjusting the rabbit's physiology during elevated ambient temperature **(Ondruska *et al.*, 2011)**. HS significantly decreased the overall mean values of each of WBCs, lymphocytes, monocytes, neutrophils and eosinophils counts of HS bucks relative to control. Similarly, **Dyavolova *et al.* (2014)** and **Khalil *et al.* (2015)** found that HS diminishes WBCs and eosinophils, lymphocytes and neutrophils in rabbits. On the contrary, **Okab *et al.* (2008)** and **Mahmoud *et al.* (2013)** found that the count of WBC and differential count were markedly increased in male rabbit in response to chronic HS. Opposing to the preceding views, **Ondruska *et al.* (2011), Dyavolova *et al.* (2014)** and **Khalil *et al.* (2015)** stated that WBCs and eosinophils counts in the male rabbits remained unchanged after exposure to heat. Regarding the effect of increased THI with the increase of the exposure time, for HS group, current results showed that measured CBC parameters except eosinophils exhibited a fluctuation between decrease and increase and general pattern could not be detected accurately. On the contrary, in VOO group, the decreasing pattern was obviously noted for all CPC parameters except for monocytes that fluctuated. Different explanations were reported regarding these fluctuations in WBCs count under HS. Generally, WBCs responses or lymphoid organ regressions are considered as better indicators of chronic stress **(Siegel, 1995).** On one hand, the decrement in WBCs because of HS

was previously considered as a consequence of the physiological response to stress, since the stress hormones (catecholamines and the glucocorticoids) are released and induce significant alternations in the absolute numbers and relative proportions of WBCs in the blood **(Redwine *et al*., 2003).** These glucocorticoids seem to play a certain role in the maintenance of leukocyte counts **(Deutsch *et al.*, 2007).** Additionally, **Mahmoud *et al.* (2013)** indicated that glucocorticoids released under HS caused the dissolution of lymphocytes in lymphoid tissues leading to lymphopenia. Induced corticosteroids and catecholamine increased the accumulation of lymphocytes in the spleen, lymph nodes and in turn decreases the lymphocytes circulating in the blood **(Viswanathan & Dhabhar, 2005). Khalil *et al.* (2015)** concluded that the reduction of WBCs may reflect the large-scale destruction of circulating WBCs **(Cohen, 1992)** and redistribution of leukocytes from the blood to other organs to enhance immune function **(Toft *et al*., 1993)**. While, **Mahmoud *et al.* (2013)** returned this reduction to the atrophy of lymphoid organs which result in reduction in feed intake and fewer nutrients for the proper development of these organs **(Bartlett & Smith, 2003).**

In the present study when each time period in the HS was compared with its corresponding in the VOO group, the HS rabbits in the majority of traits exhibited higher levels of shortage compared with VOO group. However, the significant changes were detected at 10-days and 20-days of HS application. Both WBCs and neutrophils differed significantly at 20-days of exposure between the two groups. However, lymphocytes and monocytes showed a significant difference at the 10-days of HS exposure. Additionally, in these four significantly changed parameters, the higher rates were estimated in VOO group. These results elucidated the ameliorative effects of VOO

on HS male NZW rabbits. In this context, there are supporting results by **AL-Sagheer *et al.* (2017)** who reported that the numbers of WBCs and lymphocytes were significantly increased in VOO-supplemented HS growing NZW rabbits even relative to the levels of control rabbits. **Khalil *et al.* (2013)** attributed **s**uch elevations to the activation of gut-associated lymphoid tissue in response to the diet supplemented with VOO or its cytoprotective activity against free radical-induced injury during HS. On the other hand, with regard to the effect of HS and VOO-supplementation on the overall means of erythrograms, none of the measured parameters was significantly different among the studied groups relative to control. Hence, it may be speculated that neither HS application nor VOO-supplementation affected on RBCs and their indices. These observations were in line with those of **Waltz *et al.* (2014)** who showed that RBCs, Hb, Hct, MCV, MCH, and MCHC did not change significantly during HS application on sheep. Moreover, **Al-Sagheer *et al.* (2017)** reported that the mean values of RBCs, Hb, MCV, MCHC were not significantly different in VOO-supplemented HS growing NZW rabbits relative to those in the control one. Nevertheless, when each time period in the HS was compared with its corresponding in the VOO group, the significant changes were detected at 30-days of HS application. Based on the obtained results, HS application after 30 days was accompanied by elevated RBCs, HB, and Hct, while it decreased MCHC value. According to **Waltz *et al.* (2014),** the increase in RBCs counts may be explained by the fact that HS induces skin blood flow circulation to promote heat loss **(Collin *et al.*, 2001).** This blood redistribution to the skin causes a reduction of blood flow to the other tissues, such as the intestinal epithelium, which may, in turn, cause tissue hypoxia **(Pearce *et al.*, 2013).**

Consequently, the bone marrow is triggered to release reticulocytes in order to increase HB mass and to protect several tissues from hypoxia and in turn raise the Hct mean value. **Chaudhary *et al*. (2015)** reported that HS caused an increase in PCV in buffaloes and attributed it to the loss of water from the body due to dehydration. MCHC decreases by the elevation of ambient temperature due to the decrease of salt content in blood plasma during summer **(Okab *et al*., 2008).** No significant alternations were detected in the platelets count whether among the three studied groups or between HS and VOO-supplemented ones

The present result showed that HS exposure significantly reduced the serum LH, while it insignificantly decreased the serum T level of HS-adult NZW bucks relative to the control group. Meanwhile, supplementation with VOO insignificantly increased serum LH concentration relative to the control rabbits; however, it significantly increased the serum T concentration after HS exposure. While, compared with HS-group, both T and LH levels were significantly increased in VOO. These findings indicated that one of the deleterious effects of HS on male rabbits was disturbing the reproductive hormones balance; namely, LH and T, which would definitely harm the animals' reproductive tissue and compromise their reproductive functions. Unlike, boosting these hormonal secretions is a possible mechanism through which VOO may partly help preserving the reproductive functions of bucks have suffered from extending periods of cyclic HS. The observed declines in both LH and T levels under HS in adult male NZW rabbits were in agreement with **Hansen (2009)** who stated that hyperthermia can alter LH secretion in females and males as well. Also, **Rodrigues-Alves *et al.* (2016)** showed that serum T concentration was significantly lesser in

rams exposed to scrotal insulation at 32°C for 72 hrs. Additionally, **Perumal *et al.* (2017)** found that LH and T secretions were significantly lower in adult *Mithun* bulls in the summer season in comparison with the other seasons of the year. Moreover, **Mohammadghasemi *et al.* (2014)** indicated that both swimming and standing in warm water at 36°C for 5 mins for 5 consecutive days per week through an experimental period of 5 weeks significantly reduced serum LH and T levels in male mice. On the other hand, **Rao *et al.* (2015)** illustrated that none of the plasma LH and T hormones changed significantly by transient scrotal hyperthermia. While **Rasooli *et al.* (2010)** found that there was no significant difference in serum T concentration between two groups of fall-born ram lambs subjected to high and moderate summer ambient temperatures. Similarly, **Li *et al.* (2015)** showed that serum and seminal plasma T concentration of boars were not affected by HS at mean maximum ambient temperature estimated by 35°C. However, a different result was reported by **Li *et al.* (2017)** elucidated that serum T levels of boars increased significantly after HS and then returned to normal levels. High temperature may reduce the ability of the animal's brain to dissipate heat to the external environment causing abnormal secretion of anterior pituitary FSH and LH **(Chen *et al.*, 2015).** Whereas, **Kirby *et al.* (2009)** reported that stressors generally lead to activation of the hypothalamus-pituitary-adrenal (HPA) axis, which in turn leads to suppression of HPT activity via the inhibition of GnRH secretion resulting in decreases of plasma LH and T levels **(Narayan & Parisella, 2017)**. Moreover, HS decreased T biosynthesis by down-regulation of two enzymes necessary for T biosynthesis in Leydig cells within testes **(Li *et al.*, 2015)**.

The current results also showed that serum LH and T levels were elevated in HS-bucks supplemented with VOO. This result is in conformity with that of **Derouiche *et al.* (2013)** who found that consumption of EVOO increased the levels of T and LH. **Oi-Kano *et al.* (2012)** reported that rats fed a high-protein diet supplemented with oleuropein had higher levels of LH and testicular T, which positively affects spermatogenesis and hence semen quality. Conversely, adult male rats orally administered olive oil had an insignificant change in T and LH compared to the control group **(Mansour *et al.*, 2013).** The effect of VOO on LH secretion might be the result of activation of the HPA **Derouiche *et al.* (2013)** or the stimulation of T biosynthesis (**Payne & Hales, 2004)** or by activating the pituitary-testicular axis **(Derouiche *et al.*, 2013)**. **Derouiche *et al.* (2013)** suggested that the rich composition of VOO in tocopherols could be, at least in part, the cause of the increase in the T hormonal profile due to stimulation of T biosynthesis by a mechanism that could involve activation of the hypothalamic-pituitary-testicular axis and/or induction of steroidogenic proteins.

Shen *et al.* (2019) found that the expression levels of Hsp70 was significantly increased after heat treatments and changes Sertoli cells which may induce germ cell apoptosis (**Chen *et al.*, 2008)**. In the present study, H&E stained testicular tissues from HS-rabbits exhibited conclusively the damaging effect of heat treatment on the seminiferous tubule architecture. These observations regarding the impact of HS on testicles were in conformity with **Pei *et al.* (2012), Yadav *et al.* (2017)** and **Kokubu *et al.* (2019).** However, testicular tissues recovered from VOO-supplemented bucks showed much betterment reflected in well-populated seminiferous tubules as well as few damaged ones in comparison with the HS-group. In addition,

different stages of spermatogenesis are represented. On contrary to HS-group, rounded spermatids and elongated spermatids were recognized, however, there was still, even fewer, number of gaps of vacuolations. These results suggested that VOO administration was remarkably successful in protecting the testicular tissue from damage after heat treatment. The ameliorative effect of VOO on testicular tissue was in agreement with **El-Kholy *et al.* (2015)** who reported that VOO restores the reverse effects of GMSB diet on testes of mice. Also, **Simón *et al*. (2018)** found that the diet enriched with olive oil induced an improvement in the progress of spermatogenesis represented in restoring the testicular efficacy demonstrated by the increased number of spermatids and sperm cells in hypercholesterolemic NZW rabbits. The reduced quantity of specific germ cells after heat exposure could be due to apoptosis and/or dysregulation of Sertoli cell junctional complexes **(Durairajanayagam *et al.*, 2015).** While, the enhancement in the testicular tissue from VOO-supplemented rabbits may be attributed to the antioxidant properties of bioactive components of VOO, especially polyphenols. This is in accordance with **Kholy *et al.* (2015)** who mentioned that diet rich in olive oil reduces tissue oxidative stress. Over and above, the previously discussed effects on LH and T that stress response causes significant reduction in these hormones, whereas VOO boosts their secretion or synthesis, and consequently influence on the progression of spermatogenic cycle.

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التَّأثيرات المُحسِّنة لزيت الزيتون البِكر على الإجهاد الحراري في ذكور الأرانب

يمثل الإجهاد الحراري (HS) الناتج عن تغير المناخ مشكلة ذات أهمية كبيرة للأرانب حيث أنها شديدة الحساسية للحرارة التي تؤثر سلبًا على وظائفها الفسيولوجية. في الآونة الأخيرة تستخدم المضافات الغذائية الطبيعية الآمنة للتخفيف من هذه الأثار السلبية خلال مواسم الصيف. تهدف الدراسة الحالية إلى دراسة آثار الإجهاد الحراري المزمن على قياسات الدم، ومستويات هرمونات التكاثر والتغيرات الهستولوجية في الخصية، بالإضافة إلى دراسة الآثار المحسنة المحتملة لزيت الزيتون البكر. وقد أجريت الدراسة على ٢٦ من ذكور الأرانب البالغة، حيث تم تقسيمهم إلى ثلاث مجموعات: المجموعة الضابطة، ومجموعة الإجهاد الحراري المزمن لمدة ساعتين يوميًا ومجموعة الإجهاد الحراري المعاملة بزيت الزيتون. كانت قيم مقياس درجة الحرارة والرطوبة (THI) هي ٣١ و٣٢ و٣٤ للفترات ١٠ و٢٠ و٣٠ يومًا مما يدل على وجود إجهاد حراري شديد. كانت أعداد كرات الدم البيضاء، الخلايا الليمفاوية، الخلايا وحيدة النواه وعديدات النواه والحمضية أقل بشكل ملحوظ في ذكور الأرانب التي تعرضت للإجهاد الحراري مقارنة مع

المجموعة الضابطة. كما أظهرت المجموعة التي عولجت بزيت الزيتون البكر تحسناً كبيراً في اعداد الخلايا اللمفاوية والخلايا وحيدة النواه مقارنة مع مجموعة الإجهاد الحراري. بينما لم يلاحظ وجود فروق ذات دلالة إحصائية في كريات الدم الحمراء وعدد الصفائح الدموية بين مجموعات الدراسة الثلاث. سجلت مجموعة الأرانب التي تعرضت للإجهاد الحراري أقل قيم في مستوي هرموني التستوستيرون والهرمون المحفز للخلايا البينية بالخصية في الدم، في حين تحسنت هذه المستويات في الأرانب التي عولجت بزيت الزيتون البكر. إضافة إلى ما سبق تسبب تعرض ذكور الأرانب إلى الإجهاد الحراري إلى خلل في دورة تكوين الحيوانات المنوية ولكن تحسنت هذه التغيرات الباثولوجية عند معالجة ذكور الأرانب بزيت الزيتون البكر حيث احتفظ نسيج الخصية بالبنية الخاصة به مع وجود عدد قليل من الخلايا التالفة. وخلصت الدراسة إلى قدرة زيت الزيتون البكر في تخفيف التغييرات الضارة التي يحدثها الإجهاد الحراري في القياسات الدموية ونسيج الخصية لدى ذكور الأرانب.