



## Physiological and histological effects of the antioxidant butylated hydroxytoluene (BHT) on white male rats exposed to starvation stress

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### Abstract

This study was done to find out the effect of the antioxidant Hydroxytoluene (BHT) on some physiological and histological variables of rats subjected to stress by starvation. 50 kg starvation + BHT concentration (mg/kg 100) starvation + BHT concentration (50 mg/kg) starvation + olive oil). The data indicate that starvation decreases the sex hormones of rats exposed to stress by starvation Follicle Stimulating Hormone (FSH), Luteinizing Hormone(LH), Testosterone, and damage to testicular tissues, the antioxidant group BHT showed a concentration of (100,50 mg/kg) a protective effect against the negative effect of stress by starvation on sex hormones and testicular tissues by maintaining its concentration is within its normal level, while the starvation group showed a negative effect on the concentration of sex hormones in the rats' blood serum and testes tissue. The antioxidant BHT at concentrations of 50 and 100 mg/kg has an effective role in maintaining most of the physiological, biochemical, and histological variables from the negative effects of stress by starvation.

**Keywords:** BHT, Starvation, FSH, LH, Testosterone Hormone

### Introduction

Hunger is the total or extreme loss of calories, nutrients, vitamins, and minerals needed to sustain life, and it is one of the more severe forms of malnutrition (Hebebrand et al., 2022). Alterations in the work of organs and organs inside the body, the most important of which are the liver, small intestine, and pancreas, lead to a serious defect in the work of these organs and cause a decrease or increase in the secretions of digestive enzymes and proteins in the blood (Al-Gharrawi et al., 2019).

Starvation leads to the release of free radicals in the body, causing damage to cells, and thus the body secretes complex substances that ward off the action of free radicals. These substances act as a defensive and protective system against oxidative stress and are called antioxidants (Parcheta et al., 2021).

Antioxidants are defined as any substance that reduces or reduces the harmful caused by (ROS) that works to protect the

cells of the body from the damage effects of free radicals that are formed continuously in the body and can be produced internally in the body or supplied from an external source (Gulcin et al., 2020).

Oxidative stress represents the state of cells distinguished by the presence of a raise in the concentrations of many types of (ROS) in quantities that exceed the defensive tissues' ability to antioxidants to get rid of these radicals, causing damage and destruction in tissues accompanied by an increase in lipid peroxidation of tissues, which leads to the destruction of multiple fatty acids Unsaturated and results in damage to the various tissues of the body (Janker and Khorshid., 2009).

Description Butylated Hydroxytoluene (BHT) is a synthetic antioxidant. It is white crystalline solid that is insoluble in water but soluble in fats due to its phenolic structures and the presence of hydrocarbon chains. When used within recommended levels, BHT showed a positive effect on our health (Kamemura et al., 2018).

The permissible limits are not the use of synthetic antioxidants (BHT), and according to what is stipulated in the law of the World Health Organization (WHO) at a maximum of 125-100 mg/kg in food, and the Joint Committee of Expert on the Food Additives of the Food and Agriculture Organization and the World Health Organization (WHO) agreed that the intake The acceptable daily allowance for humans is 0.5 mg/kg of body weight and is absorb it quickly from the digestive tract and distributed to the liver and body fat (Basma et al., 2018).

The testicle secretes a number of male sex hormones called androgens, including testosterone which is more abundant and important and is secreted from Leydig cells Located in the connective tissue between the seminiferous tubules, the secretion of testosterone is under the control of Luteinizing Hormone (LH) and Follicle Stimulating Hormone (FSH), and it binds to receptors on Sertoli cells and works on the secretion of Androgen Binding Protein (ABP). The primary role of FSH is to stimulate the testes to produce sperm (Karamichos et al., 2021; Fröhlich & Wahl, 2022).

Studies have indicated that starvation leads to metabolic changes, which are reversed Decreased levels of testosterone in the blood lead to atrophy of the auxiliary sexual structures, which in turn leads to a decrease in the volume of semen and the number of sperm in the testes, and also reduces erection and ejaculation and leads to impotence, infertility and hormonal weakness, and a decrease in the concentration of testosterone is linked By undermining the mitochondrial membrane, increasing ROS production and decreasing antioxidant defenses, starvation leads to a lack of vitamins and ions that protect the testes from oxidative stress and improve sperm quality (Jacobs, 1948; Sakae & Tamaka, 2021).

## Materials and Methods

This study was conducted in the College of Veterinary Medicine, University of Mosul, 56 male white rats, aged (3-4) months, and average weights ranged between (200-400) grams, and provided the appropriate conditions in terms of temperature (20-52) m and lighting, and the rats were given the standard diet and water Rats were divided into eight groups:

1. Control group: It included 7 males who were not exposed to any treatment, and they were given daily feed and water.
2. The Group of animals given olive oil.
3. The starvation group: It included 7 males.
4. The group of animal's doses BHT in concentration of 100 mg/kg animal weight/day.
5. The group of animals doses BHT in concentration of 50 mg/kg animal weight/day.
6. The group of animals doses BHT in concentration of 100 mg/kg animal weight/day + starvation.

7. The group of animals dose BHT in concentration of 50 mg/kg animal weight/day + starvation.

8. The group of animals doses olive oil + starvation.

## Chemicals

The antioxidant Butylated Hydroxytoluene (BHT) was obtained from the Indian company Himedia. The concentration of 100 mg/kg was prepared by dissolving (0.4 g) in (10 ml) of olive oil (Hosseinnia et al., 2021). The concentration of 50 mg/kg was also prepared by dissolving (0.2g) in (10 ml) of olive oil. It was placed on the magnetic stirrer with the heating unit for 5 minutes.

## Blood Sample Collection

Blood samples were drawn from rats from the eye socket vein of each animal using a capillary tube. The blood was allowed to flow through the capillary tube to clean, dry test tubes and left at room temperature for 30 minutes, and then centrifuged at 3000 rpm for 15 minutes to get the serum, which was kept at a temperature of -20°C until the required analyzes were performed.

## Measurement of physiological variables

### Determination of Serum follicle-stimulating hormone (FSH) Concentration

The concentration of FSH hormone in serum of male albino rats was estimated using the ready-made assay kit manufactured by the Chinese company SUNLOG by the Enzyme-Linked Immunosorbent (ELISA) method.

### Determination of Serum Luteinizing Hormone (LH) Concentration

The concentration of LH hormone in the blood serum of male albino rats was estimated using the ready-made assay kit manufactured by the Chinese company SUNLOG by the Enzyme-Linked Immunosorbent (ELISA) method.

### Determination of serum testosterone concentration in male's blood -serum

The concentration of testosterone hormones in the blood serum was assessed using a ready-made analysis kit from Bioassay Technology Lab (BT LAB), which is based on competitive linkage analysis using the enzyme-linked immunosorbent assay technique (ELISA).

## Tissue sample preparation

The testes tissues were left in 10% formalin solution for 72-48 hours, after which they were treated by conventional histological methods, and then the tissues were dyed for the purpose of obtaining well-stained tissue sections (Al-Hajj

2010; Steven & Bancroft 1975). The slides were placed in xylol for half an hour at a temperature of 60-50°C. The slides were passed in a descending series of concentrations of the ethyl solution (70%, 90%, 100%) for five minutes for each stage. The samples were placed in aluminum molds containing molten paraffin wax at 52 degrees. -50 m. Wax molds containing tissue samples were cut with a thickness of 4-6 micrometers. Hematoxylin-Eosin stain was used at this stage.

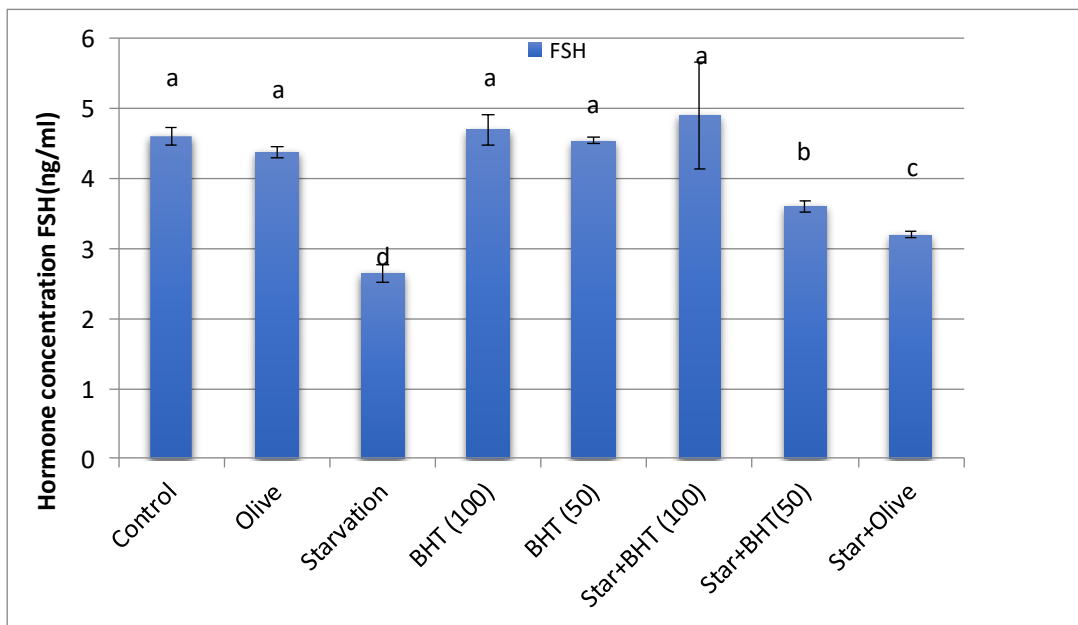
**Statistical analysis**

Statistical analysis was done by using SPSS VERSION 16 program to show the mean and standard error, and the comparison between the treatments was done using the Duncan test, at the level of probability  $P \leq 0.05$  (Steel & Torrie, 1980).

**Results and Discussion**

**Estimation of the concentration of follicle-stimulating hormone (FSH)**

Fig. 1 shows the effect of BHT on the concentration of FSH hormone in the blood serum of male albino rats subjected to stress by starvation. The results showed of starvation a significant decrease in the concentration of FSH at the probability level ( $P \leq 0.05$ ) in the starvation group ( $2.64 \pm 0.11$ ) ng/ml and the group ( Starvation + olive oil) ( $3.19 \pm 0.08$ ) ng/ml and group (starvation + BHT) concentration (50) mg/kg ( $3.60 \pm 0.08$ ) ng/ml compared with the control group ( $4.59 \pm 0.12$ ) ng/ml, notice that no significant difference between the control group and the olive oil group ( $4.37 \pm 0.07$ ) ng/ml, and the BHT with a concentration of (100) mg/kg ( $4.69 \pm 0.22$ ) ng/ml and the BHT group concentration of (50) mg/kg.  $4.53 \pm 0.04$  ng/ml, the protective role of the antioxidant BHT was also shown through the absence of a significant difference between the group (starvation + BHT) concentration (100) mg/kg ( $4.89 \pm 0.76$ ) ng/ml compared with the control group.

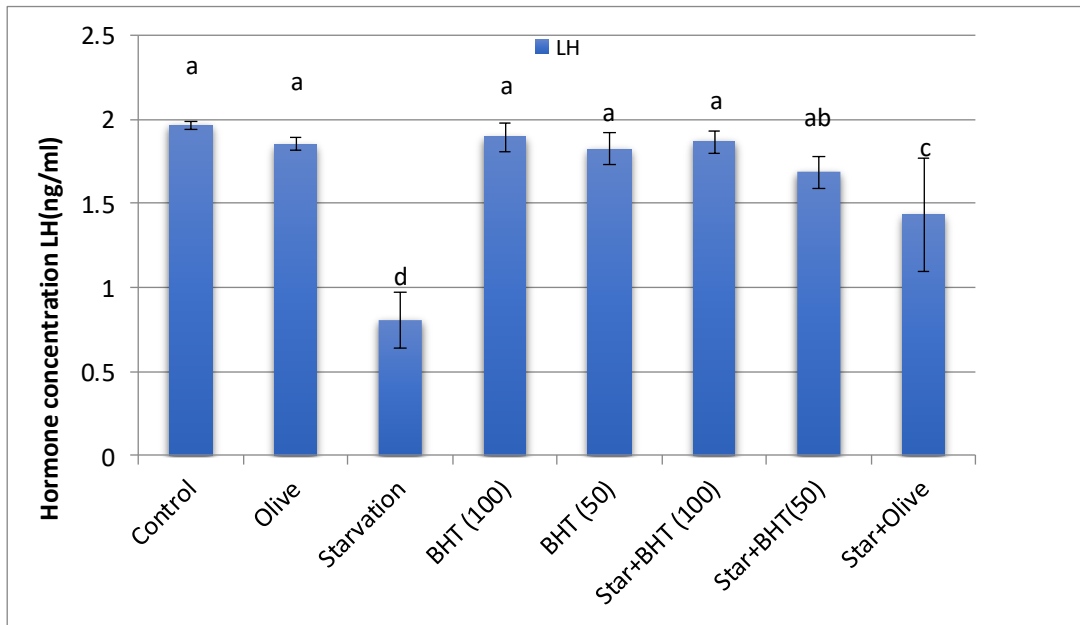


**Fig.1 FSH concentration in serum of treated rats**

**Determination of the concentration of the luteinizing hormone (LH)**

Fig. 2 shows the effect of BHT on the concentration of LH hormone in the blood serum of male albino rats subjected to stress by starvation, the results showed a significant decrease in the concentration of LH at the probability level ( $P \leq 0.05$ ) in the starvation group ( $0.80 \pm 0.16$ ) ng/ml and the group (starvation + olive oil) ( $1.43 \pm 0.33$ ) ng/ml and control group ( $1.96 \pm 0.02$ ) ng/ml, as it is noticed from the results that is no

significant difference between the olive oil group ( $1.85 \pm 0.03$ ) ng/ml, BHT group concentration (100) mg/kg ( $1.89 \pm 0.08$ ) ng/ml and BHT concentration group (50) mg/kg ( $1.82 \pm 0.09$ ) ng/ml compared with the control group, The results showed no significant difference between group (BHT + starvation) concentration (100) mg/kg ( $1.86 \pm 0.06$ ) ng/ml and the group (BHT + starvation) concentration (50) mg/kg ( $1.68 \pm 0.09$ ) ng/ml compared with the control group.

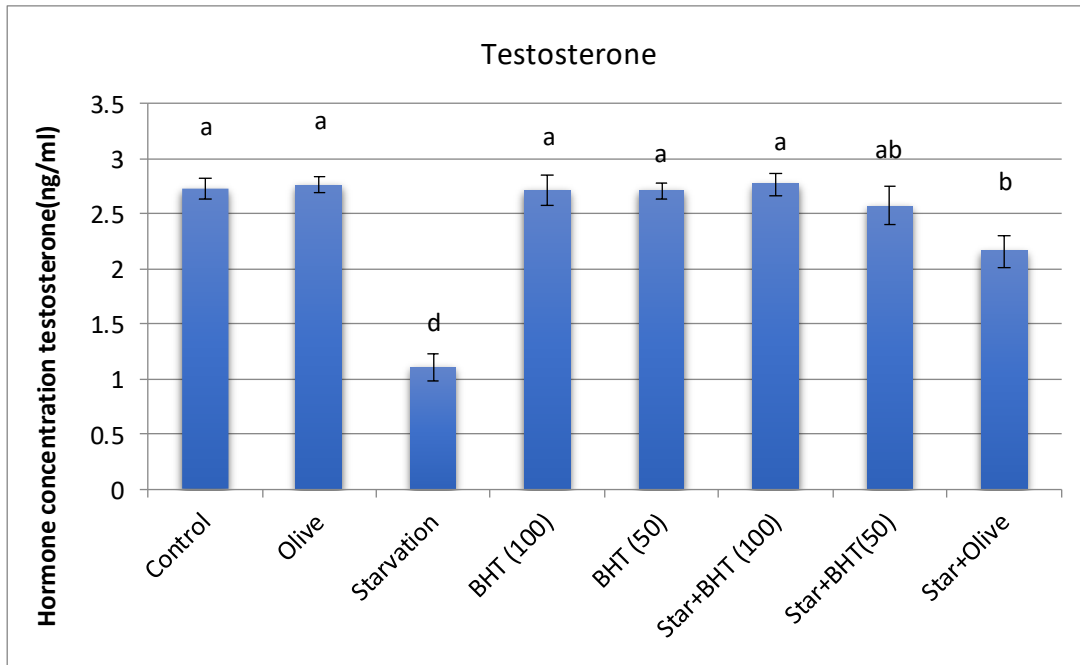


**Fig. 2** The concentration of LH in the serum of treated rats

**Testosterone concentration**

Fig. 3 shows the effect of BHT on the testosterone concentration in the serum of white male rats subjected to stress by starvation. The results showed a significant decrease in testosterone concentration at the probability level ( $P < 0.05$ ) in the starvation group ( $1.10 \pm 0.11$ ) ng/ml and the group (P<0.05). starvation + olive oil ( $2.16 \pm 0.14$ ) ng/ml and the control group ( $2.73 \pm 0.09$ ) ng/ml, notice no significant

difference between the control group and the olive oil group ( $2.76 \pm 0.07$ ) ng/ml, group BHT concentration (100) mg/kg ( $2.71 \pm 0.13$ ) ng/mL and BHT concentration (50) mg/kg ( $2.71 \pm 0.07$ ) ng/mL, It is also evident from the results that the antioxidant effect of BHT, through the absence of a significant difference between the group (BHT + starvation) concentration (100) mg/kg ( $2.76 \pm 0.10$ ) ng/ml and the group (BHT + starvation) concentration (50) mg/kg ( $2.57 \pm 0.17$ ) ng/ml compared with the control group.



**Fig. 3** The concentration of testosterone hormone in the serum of treated rats



The results of our study showed significant decrease in the concentration sex hormones LH, FSH, and testosterone in blood serum of male rats exposed to stress by starvation, which confirms the negative effect of starvation on the concentrations of sex hormones, and this is consistent with what Sales et al. (2020) indicated that starvation negatively affects on sexual ability by inhibiting the secretion of sex hormones from the pituitary gland. Several studies have confirmed that starvation has a negative effect represented in reducing the concentrations of sex hormones, and also leads to a decrease in the number of sperms due to the decrease in the concentrations of sex hormones secreted from the pituitary gland and testis, which in turn affects the process of spermatogenesis. (Bergendahl, 1989; Sakae et al., 2021).

The researcher Liu et al. (2022) indicated that the decrease in the concentration of testosterone hormone in the blood serum as a result of starvation may be due to the effect on the fat content in the body, the weight of the testicles and the diameter of the seminiferous tubes, as nutritional deficiency result to a disturbance in a metabolism process, which leads to decrease in the weights of the testes and epididymis. A decrease in testosterone causes a dysfunction in Sertoli cells and spermatogenesis.

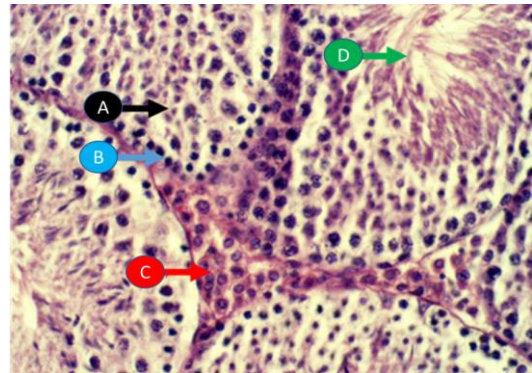
The reason for the decrease in hormone concentrations is due to the effect of oxidative stress on the mechanism of regulating the secretion of hormones stimulating the growth of sperms, which stimulates Sertoli cells to secrete the hormone Inhibin, which works to inhibit the secretion of sperm growth stimulating hormone through its effect on the secreting cells, and since FSH has a previous role to influence The LH hormone works to increase the number of LH receptors on interstitial cells, and decrease in the concentration of FSH is accompanied by a decrease in the concentration of the hormone LH, and this leads to decrease in the manufacture of the male testosterone in Leydig cells because the main role that the interstitial cell-stimulating hormone plays in males is its effect On Leydig cells in the testicles, thus stimulating them to produce and secrete testosterone (Jouloud and Al-Qattan, 2016).

The results also showed the role of the antioxidant BHT in maintaining the concentrations of sex hormones within their normal or close to normal levels through the absence of a significant difference between (starvation + BHT) groups compared with the control group, and many studies have confirmed the antioxidant BHT works to protect Sperm membranes when they are targeted by free radicals, specifically unsaturated fatty acids, membrane proteins, sperm DNA, causing oxidation of membrane lipids and these changes lead to cell damage and death of sperm (Jara et al., 2019; Singh et al., 2017).

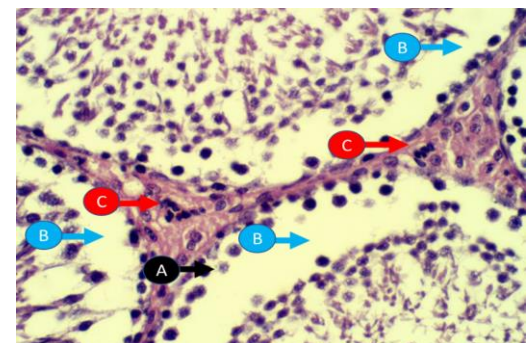
### The Testicular Tissue

Fig. 4 shows a testicular tissue model taken from the control group, which shows the seminiferous tubules in different

shapes, because the seminiferous tubules are highly convoluted. When cutting, they appear with different diameters according to the level of the cut. These tubules are lined with epithelial tissue that clearly represents the cells. Sertoli, Leydig cells, and spermatozoa accumulate in the lumen, where he observes spermatids, prospermids, and mature spermatogonia.



**Fig. 4 A cross-section of the seminiferous tubules in the control group showing the epithelial tissue lining the tubules, which is the sperm in its different stages, namely: A-sperm cells B-Sertoli cells C-Leydig cells D- sperms accumulated in the lumen H. and E.400x.**

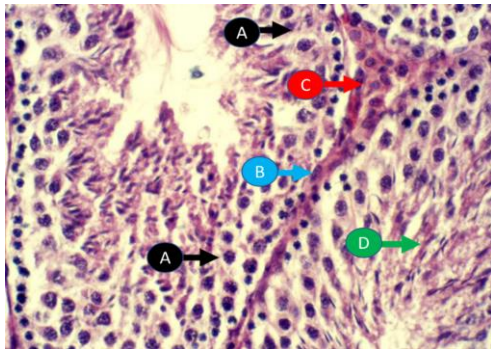


**Fig. 5 A cross section of seminiferous tubules in starvation group A- showing cytolysis in spermatocytes B- edema interstitial C- hyperplasia and hypertrophy of Leydig cells. H.and E.400x**

Fig. 5 shows a sample of testicular tissue taken from the starvation group. The negative effect of starvation on the testicular tissue is noted, as cytolysis and interstitial edema appear in the sperm cells and hyperplasia and hypertrophy in Leydig cells. Necrosis and degeneration of the cells are also noted. The epithelial tissue forms the stages of sperm formation, which led to their reduction with edema in some tubules. It also shows clearly the voids formed inside the seminiferous tubules and the occurrence of shrinkage in their contents.

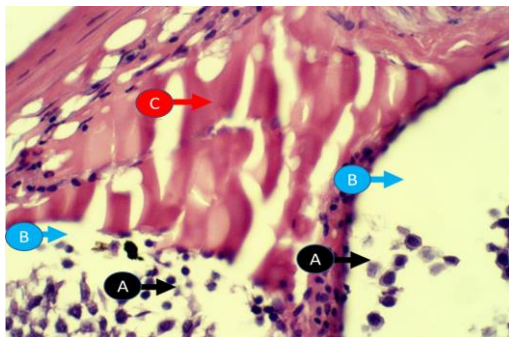
Through the foregoing, we clearly notice the effect of starvation on the testicular tissue, which led to sabotage damage to the lining tissue of the testicle and to the cells that

make up the tissue of its various types. Testosterone concentration, reduction in the number of sperm, and necrosis in testicular tissues. The reason was attributed to the harmful effect on Leydig cells, which affected the cells' ability to secrete testosterone, which has a key role in the formation and maturation of sperm in the testicles.



**Fig. 6** A cross-section of the seminiferous tubules in a group (olive oil) showing the epithelial tissue lining the tubules, which is the sperm in its different stages: A- Sperm cells B-Sertoli cells C-Lydg cells D-Sperm accumulated in the cavity. H.and E .400x.

Fig. 6 shows a testis model taken from the (olive oil) group. It notes sperm cells, Sertoli cells, Leydig cells and sperms in their different stages, accumulating in the cavity.

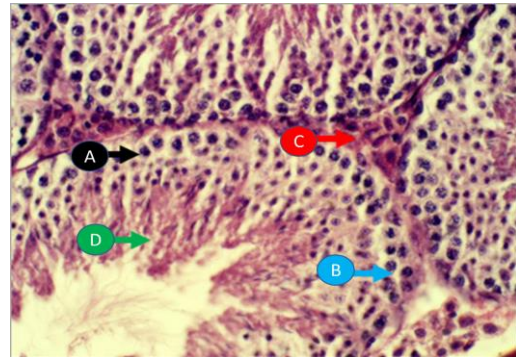


**Fig. 7** A cross-section of the seminiferous tubules in the group (starvation + olive oil) shows the epithelial tissue lining the tubules, which is the sperm in their different stages: A- Cellular decomposition appears in sperm cells B- Interstitial edema C- Deposition of acidic substances. H. and E.400x

Fig. 7 also shows a testicular tissue model taken from the group (starvation + olive oil). The negative effect of starvation on the testicular tissue is noted, as cytolysis appears in sperm cells, interstitial edema and the deposition of acidic substances.

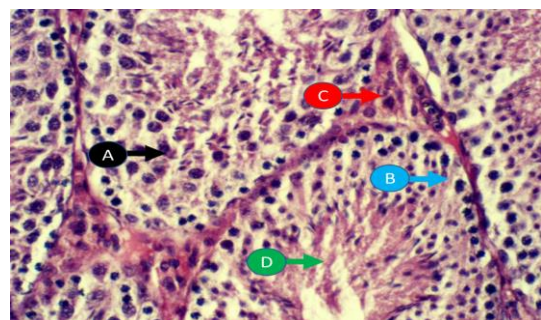
Olive oil has many biological benefits mainly through its function as a natural antioxidant consisting of (Oleic acid, Phenolics, Squalene), as it has been found to reduce oxidative

stress in the body (Abed, 2017). Olive oil has shown some good qualities but it does not compare with the effectiveness of the antioxidant BHT concentration of mg/kg (100, 50) in maintaining testicular tissue better because it has a protective role in protecting sperm and sex hormones from oxidative stress and damage to testicular tissue from stress by starvation.



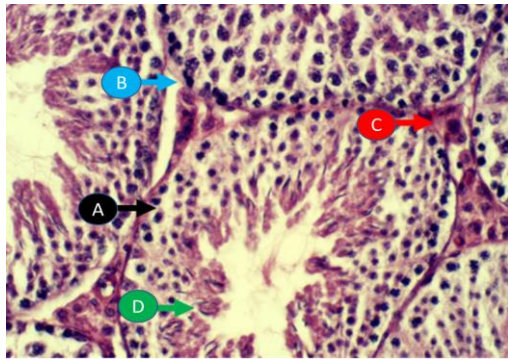
**Fig. 8** A cross-section of the seminiferous tubules in the group that was dosed with the antioxidant BHT at a concentration of (100mg/kg) showing the epithelial tissue lining the tubules, which is the sperm in their different stages: A- Sperm cells B-Sertoli cells C-Leydig cells D-Sperm accumulate in the lumen. H.and E.400x.

Fig. 8 and Fig. 9 show a testicular tissue sample taken from a group of white male rats that were dosed with the antioxidant BHT concentration of (50, 100 mg/kg), which shows the epithelial tissue lining the seminiferous tubules, in which the sperms in their different stages appear in a normal and similar condition to the control group. You also notice the chorion, which are Sertoli cells and Leydig cells in a normal way.

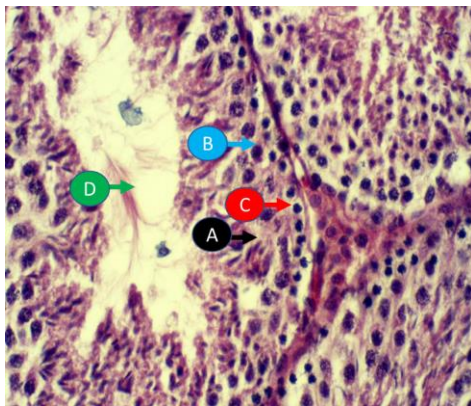


**Fig. 9** A cross-section of the seminiferous tubules in the group that was dosed with the antioxidant BHT at a concentration of (50mg/kg) showing the epithelial tissue lining the tubules, which is the sperm in their different stages: A- Sperm cells B-Sertoli cells C-Leydig cells D-Sperm accumulate in the lumen. H.and E.400x.





**Fig. 10** A cross-section of the seminiferous tubules in the group (starvation + BHT) concentration (100 mg/kg) shows the epithelial tissue lining the tubules, which is the sperm in its different stages: A- spermatocytes B-Sertoli cells C-Leydig cells D-sperm accumulated in the cavity. H. and E.400x.



**Fig. 11** A cross-section of the seminiferous tubules in the group (starvation + BHT) concentration (50mg/kg) showing the epithelial tissue lining the tubules, which is the sperm in their different stages, namely: A- spermatocytes B- Sertoli cells C- Leydig cells D- animals Sperm accumulated in the lumen. H. and E.400.

Fig. 10 and 11 show a testicular tissue model taken from the group (starvation + BHT) concentration (100mg/kg) and concentration (50 mg/kg), respectively in male rats, as it shows a section of the seminiferous tubules that appear in their condition Natural or close to normal in terms of the epithelial tissue lining the seminiferous tubules and sperm cells in their different stages as well as the supporting cells, which gives a conclusive indication of the role of the antioxidant BHT in preserving the components of the testicular tissue from the disruptive effect of starvation on the testicular tissue.

Antioxidants remove reactive oxygen species (ROS) from different body tissues to prevent lipid peroxidation, such as vitamins E and C. They neutralize free radicals and prevent them from harmful cells and tissues, as the antioxidants

produced by the body are not able to neutralize all free radicals, the use of antioxidant supplements It can play an important role in increasing the body's ability to fight free radicals through their positive impact on testicular functions, as the testes rely heavily on antioxidant factors to fight complications resulting from oxidative stress, and protect sperm from reactive oxygen species (Asadi et al., 2017; Soliman et al., 2022).

The normal tissue condition results from the clear protective role of the antioxidant BHT in maintaining the concentrations of the sex hormones FSH, LH, and testosterone within their normal levels and protecting them from the harmful effect of starvation, which directly affects the process of sperm formation and maturation in the testicle. Several studies have confirmed that the antioxidant BHT works to protect the sperm membranes from the damage of oxidative stress caused by starvation stress, which in turn led to changes in the testicular tissue by increasing the thickness of the epithelial tissue lining the seminiferous tubules, resulting of increase in the number of Leydig cells and sperm in tissue. (Singh et al., 2017).

### Conclusions

We conclude from this study that the antioxidant BHT at concentrations 50 and 100 mg/kg has an effective role in maintaining most of the physiological, biochemical, and histological variables from the negative effects of stress by starvation. It maintains the concentrations of hormones in the blood serum FSH, LH, and Testosterone within their normal level and protects them from the effect of stress by starvation.

The absence of harmful histological changes to the tissues of the testes and liver tissues, the preservation of their cellular components and tissue composition from the negative and harmful effect of starvation, and the survival of the tissue in its similar healthy state for the control group.

### Conflict of Interest

The author hereby declares no conflict of interest.

### Consent for publication

The author declares that the work has consent for publication.

### Funding support

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