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Article

Protective Effect Of The Antioxidant Butylated Hydroxytoluene (Bht) On Some Physiological And Biochemical Variables Of Male Rats Exposed To Stress By Starvation.

Wedyan Yousif Al-tikrity[1,] and Thaer Mohamed Al-Mashhadani[2]*
[1] *Department of Biology, College of Science, Mosul University, Iraq*
[2] *Department of Biology, College of Science, Mosul University, Iraq*
***** *Correspondence: wedyan.20scp34@student.uomosul.edu.iq*
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ABSTRACT

This study investigated the effect of Butylated Hydroxytoluene (BHT) on some physiological and biochemical parameters of rats subjected to stress by starvation, and 56 mature males were divided into eight groups.(Control, Olive Oil, Starvation, BHT Concentration (100 mg/kg), BHT Concentration (50 mg/kg), Starvation + BHT concentration (100 mg/kg), starvation + BHT concentration (50 mg/kg), starvation + olive oil. The results showed that the olive oil group and BHT groups at a concentration (50, 100 mg/kg) There was no significant difference in most of the treatments within the studied variables. In contrast, the starvation group showed a significant decrease in the concentration of (Total protein, Albumin, Lipase Enzyme, Lactate Dehydrogenase Enzyme (LDH), and Total Antioxidant Capacity (TAC). The control group, while the starvation + BHT group, did not show a concentration (100,50 mg/kg) Significant difference compared with the control group in all the variables as mentioned earlier, which shows clearly the protective effect of BHT against the negative effect of starvation by maintaining its concentrations within its normal level.

Keywords: BHT; Starvation; Total Protein; Albumin; Lipase Enzyme; LDH; TAC.

INTRODUCTION

Starvation is the absence of essential food components (carbohydrates, proteins, fats) necessary for survival. Starvation affects the body of the organism in terms of its destruction or death, and starvation is divided according to the time it takes into two types: short-term starvation and long-term starvation cause changes in the destruction of nutrients of various kinds[1]. Starvation has severe effects on the body in that it leads to changes in weight and also causes modifications in the action of organs and organs in the body, the most important of which are the liver, small intestine and pancreas, which leads to a defect in the work of these organs and causes a deficiency or increase in the secretion of digestive enzymes and proteins in the blood [2] Several studies have indicated the adverse effects of starvation on total proteins in the blood serum, as the protein content in the body is used to obtain energy through protein decomposition, and this causes the protein to run out by

40% of the total organs of the body and thus life becomes threatened. Serum proteins play an active role in the body through their importance to the immune system, as any decrease or defect in these proteins is an indication of weak immunity and thus indicates injury to one of the organs, the most important of which is the liver, and one of the most critical factors affecting albumin levels is nutritional deficiency and inflammatory injuries Which causes protein loss from the body [3]. Starvation leads to the breaking down of hepatic glycogen to the most straightforward units (glucose) to compensate for the lack of blood sugar concentration. In starvation, fats are decomposed into their primary units by the action of lipase-breaking enzymes [4] Among the harmful effects of starvation is the occurrence of oxidative stress and the formation of free radicals through the imbalance between the free radicals generated as a result of starvation and the susceptibility of antioxidants and defense mechanisms. Oxidative stress does not remove the oxidative effects in the body, and oxidative stress leads to cell death by free radicals that were formed due to starvation, lack of energy intake from food and the occurrence of changes in the membrane. Mitochondrial oxidative stress is one of the leading causes of the emergence of most modern diseases in Humans, such as cardiovascular diseases, cancer, diabetes, hepatitis, arthritis, and diseases of the central nervous system[5]. The body secretes complex substances that ward off the action of free radicals, as these substances act as a defensive and protective system against oxidative stress and are called antioxidants [6] It has biological properties that can prevent or inhibit the oxidation of other molecules, as it works to protect the cells of the body from the harmful 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, and works in maintaining health Especially in the advanced age stages [4]. Butylated Hydroxytoluene (BHT) of industrial antioxidants, a white crystalline, is unsolvable in water but soluble in fat because of its phenolic structure and presence of Hydrocarbon strings; when used within recommended levels, BHT has shown a positive impact through its role as an anti-cancer and anti-mutation and antiviruses such as herpes and HIV. The use of BHT reduces the free radicals in the body, helps prevent fat eating, enhances glutathione levels in cells, enriches associated with and reduces the risk of cancer diseases arteries, and reduces signs of Aging by preventing cells [5]. The permissible limits for the use of the synthetic antioxidant (BHT), according to the World Health Organization (WHO), are 125-100 mg/kg as a maximum in food [7].

MATERIALS AND METHODS

This study was conducted in the animal house of the College of Veterinary Medicine, University of Mosul; 56 male white rats were used, aged (3-4) months, and their average weight ranged between (200-400) grams, and the appropriate conditions were provided in terms of temperature (20-25 Cº) and lighting. The rats were given standard ration and water.

Rats Were Divided into Eight Groups:

- The control group: It included 7 males, not exposed to any treatment, and they were given the standard diet and water daily.
- Olive oil group: It included 7 males who took olive oil orally (0.5 ml) daily for 60 days
- The starvation group: It consisted of 7 males who were subjected to 24 hours of starvation and 24 hours of feeding consecutively for 60 days with water being given.
- BHT group (100 mg/kg): It included 7 males who took BHT orally at a concentration of 100 mg/kg of body weight daily for 60 days.

- The BHT group 50 (mg/kg): It included 7 males who took BHT orally at a concentration of 50 mg/kg of body weight per day for 60 days.
- The starvation group + BHT 100 (mg/kg): It included 7 males subjected to starvation and dosed orally with BHT at a concentration of 100 mg/kg of body weight per day for 60 days.
- The starvation group + BHT 50 (mg/kg): It included 7 males subjected to starvation and dosed orally with BHT at a concentration of 50 mg/kg of body weight per day for 60 days.
- The group of starvation + olive oil: It included 7 males who were subjected to starvation and were administered orally with olive oil daily for 60 days.

Preparation of Butylated Hydroxytoluene (BHT):

The antioxidant Butylated Hydroxytoluene (BHT) was obtained from the Indian company Himedia. A concentration of 100 mg/kg was prepared by dissolving (0.4 mg) in (10 ml) of olive oil. A concentration of 50 mg/kg was prepared by dissolving (0.2mg) in 10 ml) of olive oil.

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 left at room temperature for 30 minutes, and then centrifuged at 3000 rpm. Furthermore, for 15 minutes, the serum was obtained, which was kept at a temperature of -20 degrees Celsius until the required analyses were performed.

Estimation of Biochemical Parameters

The concentration of total protein, albumin, lipase, and lactate dehydrogenase (LDH) in blood serum was determined using a FUJIFILM NX500 chemical analyzer (Japanese origin) and unique strips for each assay.

Determination of Total Antioxidant Capacity (TAC)

The total concentration of antioxidants (TAC) in the blood serum was estimated using a ready-made assay kit from Bioassay Technology Laboratory Company (BT LAB), which is based on competitive linkage analysis using the enzyme-linked immunosorbent adsorption technology.

Statistical Analysis

The results were statistically analyzed using the SPSS V.16 program to show the mean and standard error. Then, the statistical program SAS V.9.0 was used to compare the totals by the Duncan test, and the results were considered significant at the level of $P \leq 0.05$ [8]. The graphs were drawn using the ready-made statistical program Excel (2010).

RESULTS

Figure (1) shows the total protein concentration in the serum of treated rats, which is evident from the results. There was no significant difference in the concentration of total protein at the probability level ($p \leq 0.05$)) between the groups. The control 7.13 g/dl, the olive oil group 7.80 g/dl and the BHT group concentration (100 mg/kg, 50 mg/kg, 7.08 g/dl, 6.95 g/dl), respectively, while the group exposed to stress by starvation 3.98 g/dl showed a significant decrease in the protein concentration. Total in serum, while the group exposed to starvation and treated with BHT at a concentration of 100 mg/kg did not show a significant difference compared with the control group, while the group exposed to starvation and treated with BHT showed a concentration of 5.57 g/dl, 50 mg/kg, as well as the starvation group and olive oil 5.30 g/dl decrease Significant in the concentration of total protein in the blood serum compared with the control group. The results show the harmful effect of starvation on the concentration of total protein in the blood serum, which is consistent with what was indicated by Şenay et al. (2021). that the decrease in the

concentration of total protein in the blood serum is due to poor liver function due to damage to liver tissues as a result of oxidative stress. The result of starvation led to the inhibition of the biosynthesis of protein, so one of the most important functions of the liver is its ability to form the basic structural units of protein (amino acids). The results also show the protective effect of BHT in maintaining the concentration of total protein in the blood serum from the negative effect of starvation. This result is consistent with what was confirmed by Hosseini et al. (2020). The antioxidant (BHT) has a positive effect on maintaining protein in the blood serum, as it helps The cell membrane maintain the total protein in it. It is considered a line of defense for the cell by being able to neutralize or prevent oxidation of stray molecules (free radicals).

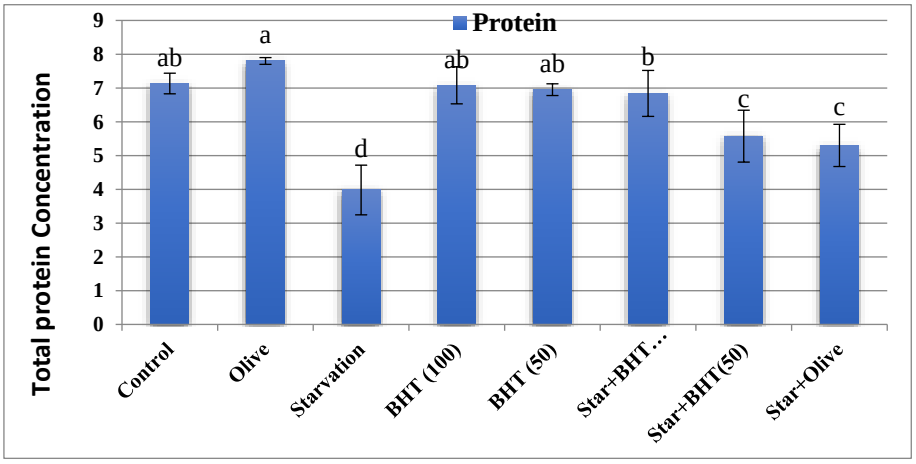

Figure 1. Total protein concentration in serum of treated rats

Figure (2) shows the albumin concentration in the blood serum of group rats. The results showed that there was no significant difference in the albumin concentration at the probability level ($p \leq 0.05$)) between the control group 4.2 g/dl, the olive oil group 4.6 g/dl and the BHT group (100 mg/kg) 4.2 g/dl, and BHT group (50 mg/kg, 3.6 g/dl), while the group exposed to stress by starvation (2.6 g/dl) showed a significant decrease in the concentration of albumin in the blood serum, while the group exposed to starvation + BHT 100 mg/kg did not show a significant difference in comparison. The control group, exposed to starvation + BHT (50 mg/kg), showed 3.3 g/dl. In contrast, the starvation group + olive oil showed 2.7 g/dl, a significant decrease in the albumin concentration in the blood serum compared with the control group. The results showed the harmful effect of starvation on the concentration of albumin in the blood serum. This result is consistent with what [9]. indicated that the decrease in the concentration of albumin in the blood serum upon starvation stress indicates liver damage, as albumin constitutes more than half of the amount The total plasma proteins in the body and formed in the liver, and the synthesis of albumin in the body is related to nutrition, especially protein intake, and damage to liver tissues occurs as a result of starvation, which leads to a decrease in the proportion of protein and causes a decrease in the proportion of albumin and damage to kidney tissues, which leads to a high concentration of albumin in the urine and a decrease in its concentration in serum. The results also show that there is no significant difference between the starvation group + BHT (100 mg/kg) and the control group, and this shows the protective effect of BHT in maintaining the serum albumin concentration, and this result is consistent with what Wang et al (2021). confirmed that BHT maintains The chemical composition of some essential components in the body, such as proteins, and BHT works to resist the harmful effects of free radicals that are formed in the body, as

it works to protect the liver from free radical damage and maintains the integrity of the cell membrane in the liver.

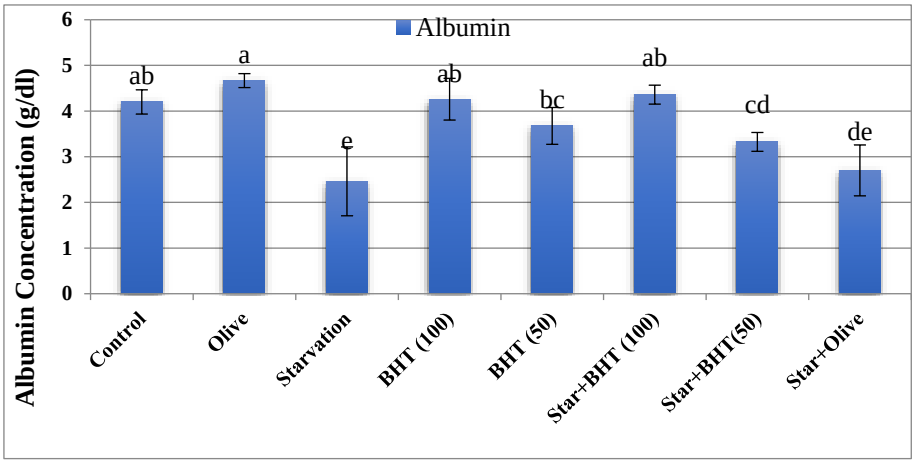

Figure 2. The serum albumin concentration of treated rats

Figure (3) shows the concentration of Lactate dehydrogenase (LDH) enzyme in the blood serum of groups of rats. The results show no significant difference in the concentration of LDH enzyme at the probability level ($p \leq 0.05$)) between the control group 826.33 U/L and the olive oil group 820.3 U/L. The BHT concentration range (100,50 mg/kg) is 711.0 U/L and 714.2 U/L, respectively. In contrast, the starvation-exposed group showed 328.4 U/L significant decrease in the concentration of LDH enzyme in the blood serum, while the group exposed to starvation and treated with BHT concentration, 100 mg/kg,696.2 U/L did not show a significant difference compared to the control group, while the group exposed to starvation and treated with BHT showed concentration, 50 mg/kg,569.7 U/L and the starvation and olive oil group had a significant decrease in the concentration of LDH enzyme in the blood serum compared with the control group. The results show the harmful effect of starvation on the concentration of LDH enzyme in the blood serum. This result with what [10] indicated the decrease in the concentration of LDH enzyme in the blood serum.Refers to the breakdown of glucose due to starvation and the lack of energy intake from food and a change in the functions of the mitochondrial membrane or the destruction of mitochondria in rats exposed to stress by starvation. The results also show the protective effect of BHT in maintaining the concentration of LDH enzyme in the blood serum, which agrees with the assertion of [11] that BHT can reduce harmful effects and maintain physiological functions within cells as well as protect the membrane of hepatocytes by scavenging free radicals and exerting antioxidants The oxidative stress (BHT) defends against the continual production of ROS inside the cell by cleaning up toxic molecules.

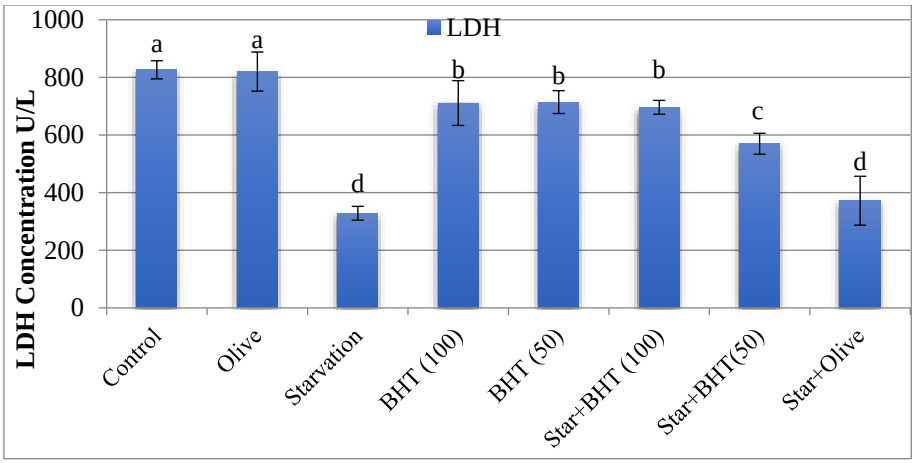

Figure 3. The concentration of lactate dehydrogenase (LDH) enzyme in the serum of treated rats.

Figure (4) shows the concentration of lipase enzyme in the blood serum of groups of rats. The results showed that there was no significant difference in the concentration of lipase enzyme at a probability level ($p \leq 0.05$) between the control group 45.3 U/L, the olive oil group 40.6 U/L and the BHT concentration group (100,50 mg/kg) 40.6 U/L, 41.0 U/L, respectively, while the group exposed to stress by starvation 21.8 U/L showed a significant decrease in the concentration of lipase enzyme in the blood serum, while the group exposed to starvation and treated with BHT concentration of 100 mg/kg, 41.0 U/L did not show a significant difference compared to the control group, while the group exposed to starvation and treated with BHT showed a concentration of 50 mg/kg, 38.2 U/L, as well as the starvation group and olive oil 40.3 U/L a significant decrease in the concentration of lipase enzyme in the blood serum compared to with a control group. The results show the negative effect of starvation on the concentration of the lipase enzyme through an apparent decrease in the concentration of the enzyme in the starvation group compared to the control group. For the production of glucose and energy, lipase is one of the digestive enzymes responsible for the Digestion and breakdown of fats during Digestion. During starvation, body fat is used up by the lipase enzyme [11]. The results also confirm that the groups of (100,50 mg/kg) BHT and the starvation group + BHT (100 mg/kg) did not show a significant decrease in the concentration of the enzyme, and this indicates the protective role of BHT through its role as an antioxidant and radical removal. Free if it makes it unable to destroy cells or damage tissues and plays a defensive role in the body. [12]

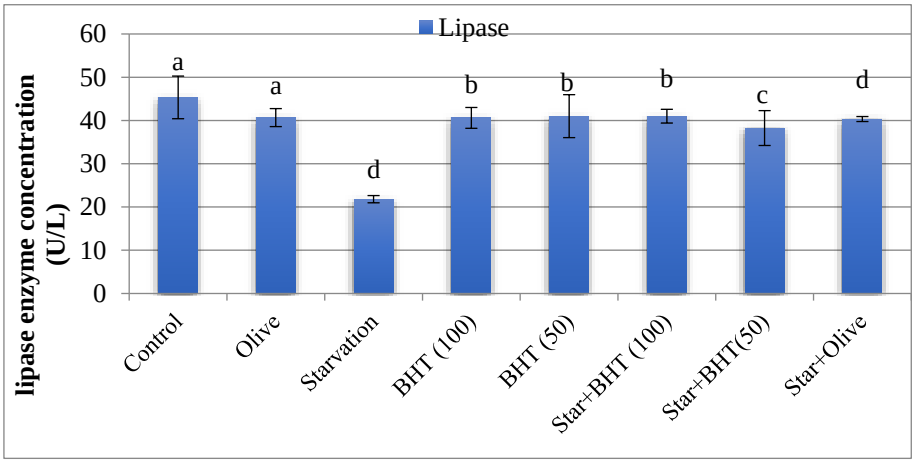

Figure 4. Lipase enzyme concentration in serum of treated rats.

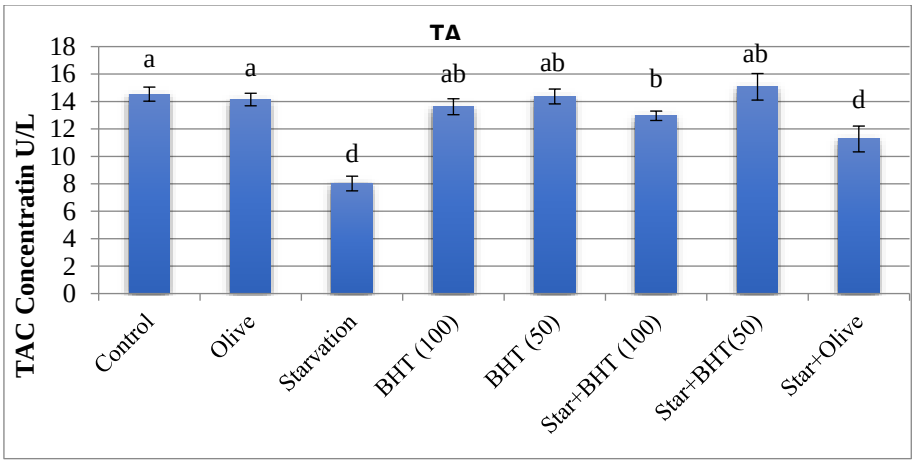

Figure 5. TAC concentration in serum of treated rats.

DISCUSSION

Figure (5) shows the total concentration of TAC antioxidants in the blood serum of groups of rats, and the results showed that there was no significant difference in the concentration of TAC at the probability level ($p \leq 0.05$) between the control group 14.5 U/L, the olive oil group 14.1 U/L and the BHT group. The concentration of (100,50 mg/kg) was 13.6 U/L and 14.3 U/L, respectively, while the group exposed to stress by starvation 8.0 U/L showed a significant decrease in the concentration of TAC in the blood serum, while the group exposed to starvation and treated with BHT showed a concentration of (50 mg/kg) 15.7 U/L, no significant difference compared to the control group, and the starvation group and olive oil 11.26 U/L showed a significant decrease in the concentration of TAC in the blood serum compared with the control group. The results show the negative effect of starvation on the total concentration of TAC antioxidants, and [15] confirmed that the decrease in serum TAC in the case of starvation occurs as a result of oxidative stress when there is an imbalance in the ratio of biological oxidation to the ratio of antioxidants and leads to damage to biomolecules Such as lipids, proteins, carbohydrates and nucleic acids, and in most cases the abnormal production of reactive oxygen species ROS that can lead to significant damage in the cell environment as a signal of oxidative damage. The results also show the protective effect of BHT in maintaining the concentration of TAC in the serum of rats, which may be attributed to the effectiveness of BHT as an anti-oxidative stress, which in turn causes damage to body tissues. Free radicals cause it and thus contribute to limiting the spread of some diseases.

CONCLUSIONS

This study concludes the preventive influence of antioxidant Butylated Hydroxytoluene (BHT) against stress by starvation for a certain period in some physiological and biochemical parameters in male albino rats.

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