A physiological study of the effect of some food additives on the hypothalamic-pituitary-testis axis in male albino rats

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Abstract

This research was conducted to know the effect of food additives Carmoisine, Monosodium Glutamate, and Sodium Benzoate on hypothalamic-pituitary-testis axis by measuring the level of some hormones (Luteinizing Hormone, Follicle Stimulating Hormone, Testosterone Hormone and Gonadotropin-Releasing Hormone) and biochemical parameters (Malondialdehyde, Superoxide dismutase, Catalase and glutathione). The current study included 32 male albino rats distributed in four groups, each group contains 8 animals, and the dose lasted for two months. The first group administered the dose of normal drinking water, the second group carmoisine at a concentration of 250 mg/kg of body weight, the third group-administered Monosodium Glutamate at a concentration of 15 mg/kg of body weight and the fourth group dosed the Sodium Benzoate at a concentration of 15 mg/kg of body weight. The results of the current study showed a significant decrease in the level of concentration of Luteinizing Hormone (LH), Follicle Stimulating Hormone (FSH), Testosterone Hormone (T) and Gonadotropin. Releasing Hormone (GnRH) in animal serum for groups treated with food additives (T₁, T₂, T₃) compared to the control group (C₁). Results as well as was shown a significant increase in the level of Malondialdehyde and a significant decrease in the level of enzymatic and non-enzymatic antioxidants (Superoxide dismutase, Catalase and glutathione) in serum blood for group animals (T₁, T₂, T₃) compared to control group (C₁).

Keywords: Food additives, hypothalamic-pituitary-testis axis, albino rats.
Introduction

Food additives are defined as any substance that has no nutritional value added to food to save it from spoilage and improve its physical characteristics such as color, taste, smell, and texture and make food easy to cook and prepare and increase consumer attractiveness, and the food additives are varied and many, including what adds color to the food or improving its appearance and composition called Food dyes Such as Carmoisine (E122), which prevents food spoilage and chemical and biological corruption called Preservatives such as benzoic acid and its sodium benzoate (E211) or added to give a certain flavor to food called Flavors such as Monosodium Glutamate (E621) Arnold et al., 2012; Pundir and Rawal, 2013; Bawazir, 2016). Because of the many arguments and differences regarding the effect of food additives and their uses, studies related to such dyes have increased to determine the extent of their effect on different body functions (Carochoetal., 2014). Some studies have indicated that the use of Food additives for long periods causes many diseases such as indigestion, anemia, growth retardation, neuropathy in the brain, allergies, asthma, rashes, diseases of the liver, kidneys, and spleen, as well as cancerous diseases (Al-Shinnawy& El-Kattan, 2013; Elbanna et al., 2017 Amin & Al-Shehri, 2018). Also, intestinal irritation and an increase in white blood cells due to an immune system disorder, increased free radicals, oxidative stress, and decreased antioxidants (Soltan et al., 2012).

Hypothalamic-Pituitary-Testicular Axis (HPT) is responsible for secreting hormones of the male reproductive system which produced and excreted by Hypothalamic, Adenohypophysis, and Testes also controlling reproductive functions (Roser, 2008). It also regulates male sexual characteristics and supports the production of sperm (Weinbauer et al., 2010). The hormones released from this axis are responsible for the principle of regulating the activity of the male reproductive system, as the hormone Gonadotropin-Releasing Hormone ( GnRH) is secreted from hypothalamic gland which stimulates the adenohypophysis to secrete stimulating hormones such as Luteinizing Hormone (LH) and Follicle Stimulating Hormone (FSH) with both hormone receptors In reproduction and stimulating editing of Sex Steroids which include Testosterone and Inhibin (Sadate-Nagatchou et al., 2004). So the purpose of the research is to study the effect of some food additives (Carmoisine, Sodium Benzoate, Monosodium Glutamate) on the Hypothalamic-Pituitary-Testicular Axis.

Materials and methods

Experiment design

32 male albino rats were used in this study. This study was conducted in the animal house of the Department of Life Sciences in the College of Education, Al-Qadisiyah University. Weights ranged between (180-200) g and age ranged between (12-14) weeks and distributed in plastic cages with dimensions of 15 x 30 x 50 cm covered with metal mesh covers and spread the floor with sawdust and care was taken to clean the cages and constantly switch the floor and sterilize it with disinfectants as well as care for the cleaning of the irrigation bottles and the quartering room, and all animals underwent similar laboratory conditions in terms of lighting (12 hours lighting -12 hours dark) and temperature 25- 22º C (Ward, 1970). Animals divided into four groups, each group included 8 animals for 60 days, as follows: 1-Control group (C1): Only 1 ml of normal drinking water. 2-First treatment group (T1): 1 ml of Carmoisine at a concentration of 250 mg/kg of body weight. 3-Second treatment group (T2): 1 ml of Monosodium glutamate at a concentration of 15 mg/kg of body weight. 4-Third
treatment group (T3): 1 ml of Sodium benzoate at a concentration of 50 mg/kg of body weight.

**Preparation of Carmoisine**

The Carmoisine dye powder produced by the Indian company RohaDyechem was used at a concentration of 250 mg/kg of body weight and 1 ml orally was administered to each animal (Oyewole & Oladele, 2016).

**Preparation of monosodium glutamate**

MSG was used at a concentration of 15 mg/kg of body weight and the animals were dosed through 1 ml orally per each animal. (El- Imam & Abd El-Salam, 2019).

**Preparation of sodium benzoate**

Sodium benzoate was used at a concentration of 50 mg/kg of body weight and the animals were dosed through 1 ml orally per each animal (Hadi & Mahdi, 2019).

**Blood sampling**

24 hours after end of last dose process, blood samples collected directly from the heart and then the blood was placed in tubes that do not contain anticoagulant and diagonally for 30 minutes, then the tubes were placed inside the centrifuge for 15 speeds (3000 r/min) to obtain a serum Blood drawn with a micropipette and kept in a clean Eppendorf tubes degree at 20 °C until the parameters under study are measured.

**Biochemical parameters**

**Measuring hormone concentrations (FSH, LH, Testosterone)**

The concentrations of these three hormones in blood serum measured by utilizing (Kit) by the French mini-company, Biomerieux, in an immunoassay method.

**Measure the concentration of the GnRH hormone**

GnRH was measured through using the Enzyme-Linked Immunosorbent Assay (ELISA) by using several measurements (Kit) which product by Elabscience company.

**Determination of Catalase**

The level of catalase is measured according to the method (Aebi, 1974).

**Determination of Glutathione**

The level of Glutathione is measured according to method (Sedlak and Lindsay, 1968).

**Determination of Superoxide dismutase (SOD)**

The level of Super Oxide Desmotase was measured depending on the Enzyme-Linked Immunosorbent Assay (ELISA) by using a kit (Kit) produced by the American company Elabscience.
Determination of Lipid Peroxidation in Serum (Malondialdehyde)
The serum MDA was measured depending on the method (Guidet & Shah, 1989).

Statistical analysis
The results of the statistical analysis were subjected to the SPSS software (version 21) to know the differences between the rates of the studied criteria for the different groups. The significant differences were determined at the level of probability of 5% by using a one-way analysis of variance. The moral differences are tested utilizing the Least significant differences (LSD).

Results and Discussion
Effect of some of food additives on some hormones
At present, many Food Additives are added to many foods such as dairy products, fish, juices, jams, ice cream and sweets, in addition to being used in the manufacture of medicines, soaps, shampoo, toothpastes, cosmetics, etc. (Carocho et al., 2014; Masone & Chanforan, 2015). Several studies have indicated that these food additives cause a lot of harmful effects, especially when used for long periods such as diseases of the digestive system, increased cholesterol in the blood, neuropathy in the brain and low body weight and harm the immune system has caused an increase in hypersensitivity (Seetaramaiah et al., 2011; Vojdani, 2015; Hassan& Salman, 2016). Statistical analysis of results of the current study were shown in Table (1) showed an important decrease (P<0.05) in the level of Luteinizing Hormone (LH), Follicle Stimulating Hormone (FSH), Testosterone Hormone (T) and Gonadotropin-Releasing Hormone (GnRH) in animal serum for groups treatment with food additives (T₁, T₂, T₃) compared to control group (C₁). There was also significant differences on the level of probability (P<0.05) with a decrease in the concentration of Testosterone Hormone in the group (T₁) compared with the group (T₂) and no significant differences appeared between-group (T₁), (T₃) as well as between (T₂) and (T₃). As for the group (T₃) of Gonadotropin-Releasing Hormone (GnRH) was showed a significant decrease (P<0.05) compared to group (T₂), and no significant differences appeared between-group (T₁) and group (T₂) as well as group (T₁) and (T₃). Results of our research consistent with (Mahmoud, 2006) who stated that Food dyes cause a decrease in Testosterone Hormone due to a decrease in Acid phosphatase in the serum of rat and consequently causing atrophy of Leydig cells and affects the process of sperm formation or because Glucocorticoids which affect directly or indirectly Hypothalamic and inhibit secretion (GnRH) and this leads to inhibition of secretion of both LH and FSH from Adenohypophysis and thus lower level (Fathollahi et al., 2011) Testosterone Hormone. Also, monosodium glutamate may cause neuron breakdown of the Hypothalamus gland and then decrease in Testosterone Hormone due to work disturbance (Ochiogu et al., 2015) Hypothalamic-Pituitary-Testicular Axis. Exposure to Preservatives such as Sodium benzoate affects the function of the male reproductive system because it affects hormones LH, FSH that is secreted from Adenohypophysis (Kumar et al., 2016).

Table (1): Effect of some of the food additives on some hormones in male albino rats.

| Standards Groups | LH (mlu/ml) | FSH (mlu/ml) | T (ng/ml) | GnRh (pg/ml) |
|------------------|-------------|--------------|-----------|--------------|
| C₁               | 1.71±0.015  | 1.42±0.009   | 0.97±0.039| 217.89±0.616 |

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Effect of some food additives on some biochemical parameters

Malondialdehyde (MDA) is a by-product of the Lipids peroxide process, which has high toxicity and reduces the effectiveness of antioxidants, which consider an important sign of the effectiveness of free radicals (Del Rio et al., 2005; Lobo, 2010). It also one of the most significant indications of an increase in oxidative stress in body tissues (Gopal et al., 2012). Results of the current study were shown in Table (2) that there was a significant increase (P <0.05) in (MDA) blood serum for group animals (T1, T2, T3) compared to the control group (C1). On the other hand, results showed a significant decrease (P <0.05) in the level of Superoxide dismutase (SOD) in groups (T1, T2, T3) compared to the control group (C1). The group (T1) showed a significant decrease compared to the two groups (T2, T3) While there were no significant differences between the groups (T2) and (T3). As for the Catalase level, the results showed a significant decrease (P<0.05) in groups treated with food additives (T1, T2, T3) compared to the control group (C1). A significant decrease was also shown in the group (T1) compared with the group (T2) and group (T3) and a significant decrease between the two groups (T2, T3). Moreover, results were showed a significant decrease (P<0.05) in Glutathione level in groups (T1, T2, T3) compared to the control group (C1). Group (T3) was showed significant differences compared to the two groups (T1, T2) and no significant differences between groups (T1) and (T2).

The reason for the high concentration of MDA may be because when eating foods containing and digesting Azo Dyes it is reduced to aromatic amines and these compounds are oxidized by the presence of oxidation enzymes to N-hydroxy derivatives that interact with Polysaturated fatty acids in membranes of cell and due to interactions of free radicals that form lipid hydroperoxides which break down Other compounds are MDA (Amin et al., 2010; Halliwell & Gutteridge, 2015) MDA also can interact with biomolecules such as DNA and proteins which lead to cell damage and mutations (Del Rio et al., 2005). Infertility in male animals occurs due to the sperm's membranes containing polysaturated fatty acids that are sensitive to lipid peroxidation, resulting in loss of fertility and sperm defects due to damage in their composition (Colagar et al., 2009). Results of our study consistent with studies that have proven that Food dyes are a source of free radical formation and this leads to consumption of antioxidants and reduce their effectiveness and increase the oxidative state due to the imbalance between free radicals and antioxidants (Soltan& Shehata, 2012; Saxena & Sharma, 2015).
The reason for the decrease in Glutathione may also be attributed to the occurrence of oxidative stress resulting from treatment with sodium benzoate. Glutathione is involved in preventing oxidation in cases of oxidative stress either by removing free radicals directly or through enzymes in its composition such as Glutathione peroxidase and this leads to its consumption and its transformation into an ineffective form (Suleyman et al., 2003; Yetuk et al., 2014). Benzoic acid and sodium benzoate are also able to interact with thiol groups of various compounds, including Glutathione, and hence a lack of concentration (Beloborodove et al., 2012) and this is confirmed (Yetuk et al., 2014) that sodium benzoate leads to high MDA and low antioxidant level. Our results are also consistent with his findings (Hamza and AL-Harbi, 2014) in which MSG causes an increase in MDA and decrease in antioxidants SOD and CAT. This effect can be due to increased production of H2O2 and ROS, which in turn stimulates oxidative stress.

Table (2): Illustrated effect of some food additives on some biochemical parameters in male albino rats.

| Standards Groups | MDA (μmol/L) | SOD (U/ml) | Catalase (U/ml) | Glutathione (μmol/L) |
|------------------|--------------|------------|-----------------|----------------------|
| C1               | 1.27±0.004 d | 1.95±0.010 a | 0.86±0.011 a | 2.99±0.083 a |
| T1               | 3.86±0.005 a | 0.88±0.232 c | 0.35±0.006 d | 1.36±0.179 c |
| T2               | 3.64±0.007 b | 1.11±0.020 b | 0.43±0.047 c | 1.19±0.078 c |
| T3               | 3.22±0.120 c | 1.09±0.020 b | 0.65±0.014 b | 1.91±0.069 b |
| L.S.D            | 0.217        | 0.073      | 0.049          | 0.232               |

The numbers represent the mean ± standard error. Different letters show significant differences at the level of significance (P<0.05) between groups.

Conclusions

Treatment with food additives resulted in an inhibition of the hypothalamic-pituitary-testis axis and a decrease in the level of GnRH hormone which leads to inhibition of the secretion of hormones stimulating the genitals LH, FSH from the pituitary gland and thus decrease the concentration of the hormone Testosterone. It also led to a decrease in the level of antioxidant enzymes, the occurrence of oxidative stress, and an increase in the process of Lipids peroxide.
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