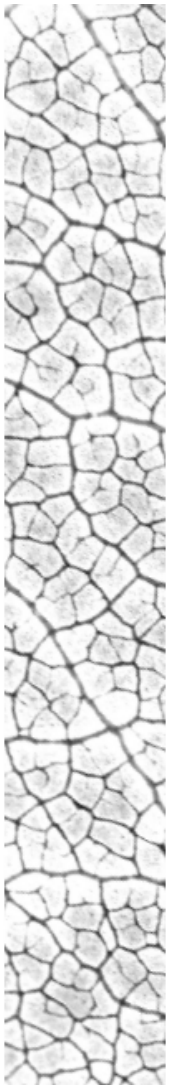

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Effect of chocolate brown HT E155 on some hormones in male albino rats

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Abstract
Today, food colorants additives are randomly used in food products, drugs and cosmetics. It causes health problems to human therefore this study was carried to evaluate the possible effects of Chocolate Brown HT E155 on body weight and some sex hormones in male albino rats. The present study including eighteen of healthy male rats, divided into three groups as follows: The first group (control group C) animals were treated with normal drinking water, the second group (T1) animals were treated with chocolate brown dye at the concentration of 200 mg/ kg of body weight and the third group (T2) animals were treated with chocolate brown dye at the concentration of 400 mg/ kg of body weight. Chocolate brown dye was given for eight weeks to all the experimental animals at experiment end blood serum was collected to determine concentration of some sex hormones included GnRH, testosterone, follicular stimulating hormone (FSH) and luteinizing hormone (LH) in the serum of rats. Our results showed that significant decreased ($P<0.05$) is found in body weight and level of FSH, LH, GnRH and testosterone hormone in T1 and T2 group when compared with control group, as well as, occur significant decreased ($P<0.05$) in level of GnRH and testosterone hormone in T2 group compared with T1 group. From this study, we concluded using of Chocolate brown dye in various foods led to reduce body weight and some negative effects on hypothalamic-pituitary-testis axis function.

Keywords: chocolate brown dye, sex hormones, testis, rats

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INTRODUCTION

Food additives are used for various purposes, including preservation, colouring and sweetening (AL-Shinnawy 2009). Food dyes represent a large part food additives and they were used extensively to color foods, drugs and cosmetics (Himri *et al*. 2011, Soltan and Shehata. 2012). Although these Food dyes may come from both natural and synthetic origin, however 95% of those used now-a-days are synthetic dyes because they are produced easily, cheaper and provide better coloration (Saxena and Sharma 2014).

Azo dyes have a wide range of applications in the textile, leather, paper, food, pharmaceutical and cosmetic industries (Mansour *et al*. 2007). Azo dyes are one of these food additives which widely used as colorants in foods (Al-Shinnawy and Elkattan 2013). Azo colors are characterized by azo groups (–N=N) bound to aromatic rings in their molecular structures (Demirkol *et al.* 2012). But the substances used as food additives may be toxic, causing harmful effects on human beings. It has been reported that some food additives are carcinogenic (Davooabadi and Shahsavari 2013, Shubik 1975).

Among the food dyes which are widely used is chocolate brown dye. Chocolate brown dye (Chocolate Brown HT) is one types of azo (European Food Safety Authority 2010). It is a brown-colored, water soluble dye which is used to enhance the color in several food products such soft drinks, candies, ice-creams and beverages (Sharma *et al.* 2005). The complex structure of chocolate brown dye is shown in **Fig. 1** according to Leo (2012).

Some studies reported toxic effects of Chocolate Brown HT on some organs in experimental animals such as ovary, stomach and intestine, liver and kidney cells, as well as its role in reducing body weight and cholesterol (HDL) and increased liver enzymes in blood (Abou El-Zabhab and El-Khyat 1997, Battal et al. 2015, Hassan 2010, Hassan and Selman 2016, Helal *et al*. 2000, Khatun *et al*. 2017). Therefore, this study was designed to determine the possible effect of Chocolate Brown HT on some sex hormones in male albino rats.

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Fig. 1. Structure complex of chocolate brown dye (Leo 2012)

MATERIALS AND METHODS

The current study was carried out in animal house of Biology department/ College of Education/ University of Al-Qadisiyah, and The experimental animals used in this study were 18 albino male rat healthy and sexually mature (Six weeks aged) and average of weight ranged between (180-200) g/ rat. Animals putted at room (12 m^2) inside plastic cages (length 15 width × 35 × height 15) cm, at a rate of three animals per cage.

All animals were subjected to one week acclimatization before the start of experimental procedures and They were maintained at standard laboratory conditions in cages (12-h light/dark cycle; 25±3°C temperature; 35–60 relative humidity) after animals divided into three groups where each group consist of six animals, which are as follows:

1. Control group (C) consisted of six animals were dosage with normal drinking water for a eight weeks.
2. The first treatment group (T_1) consisted of six animals were dosage with chocolate brown dye concentration of 200 mg/ kg of body weight for a eight weeks.
3. The second treatment group (T_2) consisted of six animals were dosage with chocolate brown dye concentration of 400 mg/ kg of body weight for a eight weeks.

Dye Used: dye Chocolate brown (Chocolate Brown HT) is one types of azo dyes is a reddish-brown color and a molecular weight at 652.56 g/mol, chemically called is [disodium 4,4 '- (2,4-dihydroxy-5-hydroxymethyl-1, 3-phenylene bis-azo) di-(naphthalene-1-sulfonate). **The** dye Chocolate brown used in present study was purchased from a (Ajanta Chemical Industries - India) company.

Selection of doses: The dye dose determinate by depending on report of Neshe *et al.* (2016) after the dye was weighed according to body weight and then dissolved in water to be directly doses to animals by using stomach tube and the rate of 1 ml/ animal.

Parameters studied: animal's weight was token after the end experiment, and then the anesthesia animals by chloroform were taking blood from the heart directly and saved in dry centrifuge tubes isn't a container on the anti-clotting substance (EDTA) and left to clot, then centrifuged at 3000 r.p.m. for 15minutes. The clear supernatant serum was used for determination the concentration of hormones.

Assay of hormones: measurement of testosterone, follicle stimulating hormone (FSH) and luteinizing hormone (LH) were carried out adopting Mini Vidas system, Biomerieux Company France, using kits specific for rats, according to the protocol provided with each kit.

Estimation of serum GnRH hormone: The levels of GnRH hormone were carried out adopting ELISA technique using kits specific for rats purchased from (Elabscience, Made in USA) according to the protocol provided with each kit.

Statistical Analysis: All results under study were subjected for statistical analysis in order to know the significant differences between the control group and other groups by using F-test at 0.05 probability level (Al-Rawi and Khalaf Allah 2000).

RESULTS AND DISCUSSION

Change of Body Weight

The dyes are the most important food colorants additives which are widely used in many drugs and foods such as cheese, fried fish, meat products, ice cream, juices, desserts and jams (Madsen 1997, Tripathi et al. 2007). Many researches has indicated that dietary dyes generate free radicals that cause a lot of problems in the body such as harmful effects on DNA , decreasein body weight, HDL cholesterol and increased of liver enzymes in the blood (Abou El-Zabhab and El-Khyat 1997, Hassan 2010, Helal et al. 2000).

The results of this study in **Table 1** showed a decrease in the rate of overweight with a significant difference ($P \leq 0.05$) between T_1 (at a concentration 200 mg / kg bw) and of T_2(at 400 mg / kg bw) compared with the rate of overweight of C group. Our results were agreed with some studies that recorded decrease in the rate of overweight for experimental animals which treated with brownish chocolate dye (Hashem et al. 2010, Neshe et al. 2016), also other food Azo dyes have a similar effect (Al-Shinnawy 2009, Mashhady 2012), while this study differed with other studied which mentioned to Azo dyes cause increasing in body weight and thus lead to obesity (Chatterjea and Shinde 2002,

Table 1. Effect of chocolate brown dye on mean body weight and concentration of testosterone, GnRH , LH and FSH hormone in the serum of male albino rats

Parameter / Groups	Change of body weight (gm)	GnRH (pg/ ml)	LH (mlu/ml)	FSH (mlu/ml)	Testostrone (ng/ml)
C Group	106.00±2.91 A	219.64±6.15 A	0.388±0.058 A	0.184±0.0147 A	10.40±0.27 A
T1 Group	72.00±7.64 B	183.50±15.65 B	0.346±0.0051 B	0.146±0.0051 B	8.72±0.67 B
T2 Group	62.40±12.38 B	161.50±13.08 C	0.336±0.0116 B	0.124±0.0051 B	3.62±0.096 C
LSD	21.6	3.10	0.0196	0.0229	1.125

Different English letters (A, B, C) refers to the significant superiority of means between the groups using F-test at ($P \leq 0.05$) a level of probability and number refers to (Mean ± Standard Error)

Gautam et al. 2010, Orman et al. 2016, Sharma et al. 2005).

Decrease in body weight may be due to the hypocholesterolemic in the blood. Many studies mentions to the effect of a number of Azo dyes on rats with a decrease in a total cholesterol, especially with high concentration doses (Al-Shinnawy 2009). The reducing in body weight is likely due to the degradation of proteins, fats and dysfunction of organs which caused by oxidative stress and fat oxidation (Vanaja and Palanimuthu 2014). Ajibye (2012)was mentioned to the role of free radicals which resulting from dye have an effect on cells , tissues as well as liver, disorder in metabolism, all of these may cause reducing weight.

On the other hand, Hassan and Selman (2016) were explicate the reason of weight loss, they return it to gastric and gastrointestinal changes in the gastrointestinal tract which due to increased in concentration of brown chocolate dye which lead to negatively affects on digestion, food absorption and loss of appetite.

Hormonal Changes

The statistical analysis of the results in current study (**Table 1**) showed a significant decrease (P≤0.05) in the levels of some sex hormones in the serum of experimental animals of T_1 and T_2 when compared with control group (C), these hormones are: testosterone concentration (T), gonadotropin-releasing hormone (GnRH), follicle stimulating hormone (FSH) and Luteinizing Hormone (LH). There was also a significant decrease in concentration of testosterone (T) and GnRH in T_2 group compared with T_1 group, while the results showed no significant difference between $T_1$and T_2 for concentration of FSH and LH.

These results were consistent with study of Khatun *et al*. (2017) which noted the low concentration of LH, FSH and estradiol with increased dose of brown chocolate dye and this will effect on fertility, which may be the cause of this reduction of hormones return to the role of chocolate dye in inhibition of hypothalamic-pituitary-testis axis function by producing many of free radical.

Also Al-anbaki (2018) demonstrated low concentration of the hormones T, LH, and FSH due to oxidative stress resulting from the free radicals generated by the drug. The low concentration of testosterone may be due to the role of free radicals in inhibiting the activity of acute regulatory steroidogenic (STAR) genes and CYP11A1, which is responsible for the transport of cholesterol in Leydig cells and thereby inhibiting the synthesis of steroids (AL-Awady 2015, Diemer *et al*. 2003, Tsai *et al*. 2003)

Furthermore, Mahmoud (2006) reported that the treatment of blue-dye on rats caused reduction of acid phosphatase in the serum and this leads to atrophy of Leydig cell and reduces testosterone and negatively affects on process of semen formation. Also reasons maybe due to the increase of glycoproteins because the ratio between the level of cortisol and testosterone is an indicator of stress (Uchida *et al*. 2004). In addition, Gore et al. (2006) suggests that glycoproteins directly or indirectly affect on hypothalamus and inhibit the secretion of GnRH and its low level in the portal system leads to inhibition of the process of building both LH and FSH of the anterior pituitary gland.

On the other hand, Scheuer (2010) and Santosh *et al*. (2011) noted that stimulation of the HPA axis (hypothalamic-pituitary-adrenal) due to free-radical stress which leads to inhibition of the HPG axis (hypothalamic pituitary- Gonad) through increased CRH excretion (corticotropin releasing hormone), which stimulates the adrenal cortex to release cortisol. The CRH hormone reduces the secretion of GnRH, LH and FSH hormones and this lead to reduction of function of the testis and thus decreases the concentration of testosterone (Whireledge and Cidlowski 2013). The increasing levels of mRNA for CRH will inhibit the secretion of LH hormone and decrease the level of testosterone and this condition occurs in response to various types of stress (Li et al. 2003).

Also CRH contributes to release of opioid peptides, which inhibit secretion of GnRH. These peptides also affect on secretion of Gonadotropins from pituitary. Many of the axons and nerve endings which formed the opioid peptides, as well as formed of GnRH, are located in the median eminence near the portal vesicular of pituitary gland which is connected to the glandular portion of pituitary gland where the central and frontal portion of the pituitary builds peptides and this confirming their role in regulating the secretion FSH and

LH hormone (Ciechanowska et al. 2010, Dhandapani and Brann 2002).

In addition, oxidative stress causes multiple damages to the hypothalamus cells, reducing their binding to the GPR54 receptor of kisspeptin, which is responsible for GnRH secretion which produced by KISS1 gene on chromosome 1q32.1. GnRH is transported in vessels Under the portal system to affect on the cells of the frontal part of pituitary that respond to the secretion of both LH and FSH hormones (Popa et al. 2008).

The low concentration of GnRH has a direct effect on the frontal lobe of the pituitary gland, which inhibits its secretion of LH and FSH which both of them controlling on Cholestrol synthesis by binding to G protein and cholesterol is the essential molecule in the Steroidogenesis (Rosati et al. 2011). Therefore, the low production of LH and FSH inhibits the process of building cholesterol and the transfer of cholesterol esters to the tissues, leading to lower cholesterol in the formed cells of the steroids and this causes a decrease in testosterone concentration (Aseel 2015).

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