The Effect Study of Using Different Concentrations of Chocolate Brown Dye (Chocolate Brown HT E155) on Some Physiological Parameters and Histological Structure of Stomach and Intestine on Albino Rats

Receved :23/1/2016 Accepted :30/3/2016

Ahmed Jassim Hassan **Hussein Abbas Salman**
Department of Biology/ College of Education/ Al-Qadisiya University

Abstract

The present study was conducted to check the effect of different doses of chocolate brown dye on some physiological parameters(SOD, GSTs and MDA), and the concentration of amine transfer enzymes (AST and ALT) and histological structure of stomach and intestines.

The current study experience forming of 20 rats, divided into four major groups; consisted of each group from five animals which given each group a different concentration from chocolate brown dye concentration for six weeks, as follows: The first group (control group C) animals doses by normal drinking water, the second group (T_1) animals doses by chocolate brown dye at the concentration of 200 mg/ kg of body weight, the third group (T_2) animals doses by chocolate brown dye at the concentration of 400 mg/ kg of body weight and the fourth group (T_3) animals doses by chocolate brown dye at the concentration of 600 mg/ kg of body weight.

Results showed that a significant decreased ($P<0.05$) in body weight and the concentrations of antioxidant enzymes (SOD and GSTs) in exchange for a significant increase in MDA and amine transfer enzymes (AST and ALT) concentrations in animals groups (T_1, T_2 and T_3) by increased the concentration of chocolate brown dye compared with control group animals, as well as, occur pathogenic textile changes in stomach and intestines of groups animals (T_2 and T_3) represented with necrosis, cell death in intestines and stomach an cases of hemorrhage in stomach endothelial layer induced by free radicals.

It was concluded from this study that the high dose of chocolate brown dye have some negative effects on liver functions, some physiological parameters and histological structure of stomach and intestines.

Biology Classification QR1 −74.5

Keywords: Chocolate brown dye, Stomach, Intestines, Albino rat.

Introduction

In recent years, after the great advances made in the field of food and marketing industry and the increasing consumption it has become necessary to add a lot of material it like preservatives, dyes and flavors that increase the nutritional value and taste, as well as, consumer attraction to it, is the dyes of additives important for food and medicines, which are wide used at the moment. There are more than 2,500 type of which produces more than 80 million tons per annum (1, 2,3). These dyes may be natural or artificial, but 95% of which are currently used it food dyes industrial and because of the ease of production and cheap price (4).

Food dyes are added to a lot of basic foods such as cheese, other dairy products, fried fish, meat products, ice cream, juices, pastries and jams (5,6). Food dyes industrial became a lot of controversial because of the toxic effects or carcinogens, hence preventing the use of many of them, and there are many researchers have studied the metabolic disorders and toxicity that occur because of these industrial dyes on rats and some other mammals (7,8, 9), as well as so many of the azo compounds had toxic effect or carcinogen on laboratory animals (10,11). Chocolate brown dye (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))] (European Food Safety Authority, 2010). The complex structureof chocolate brown dye is show in figure (1) According to (12Leo, 2012).

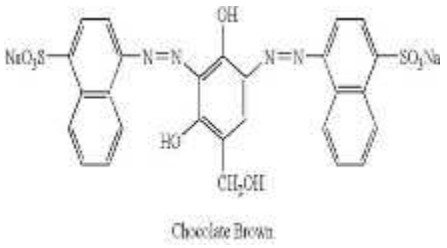


Figure 1: Structure complex of chocolate brown dye (12Leo, 2012)

European Food Safety Authority (13) showed that the limit taken daily from the chocolate brown dye in food between (0 - 1.5 g/ kg body weight); they are widely used in food and gaseous, as well as with sweets (14). Many studies have refer to the harmful effects of this dye on DNA liver and kidney cells, as well as its role in reducing body weight and cholesterol (HDL) and increased liver enzymes in blood (15,16,17). The lack of studies on the potential negative effects of chocolate brown dye on stomach and intestines, the current study was to determine the effect of different concentrations of this dye on some physiological parameters (Oxidation parameters) and histological structure of stomach and intestine in albino rats.

Materials and Methods

- ✓ **Experience design:** The study of experience was conducted in animal house of Biology department/ College of Education/ Al-Qadisiya University, and used albino rats that have been purchased from the animal house of College of Veterinary Medicine/ Al-Qadisiya University, a total of 20 albino 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 five animals per cage.

All animals were offered for the same conditions of temperature (20-25) ºC, which was organized by the air conditioner, and the rate of lighting (12 hour light: 12 hour dark). Animals were given intensive diet and water by free method, and then random distributed and left for two weeks to acclimatize and then weighed to determine the appropriate dosage; it divided into four groups where each group contained five animals, which are as follows:

1. Control group (C) consisted of five animals were dosage with normal drinking water for a six weeks.
2. The first treatment group (T_1) consisted of five animals were dosage with chocolate brown dye concentration of 200 mg/ kg of body weight for a six weeks.
3. The second treatment group (T_2) consisted of five animals were dosage with chocolate brown dye concentration of 400 mg/ kg of body weight for a six weeks.
4. Third treatment group (T_3) consisted of five animals were dosage with chocolate brown dye concentration of 600 mg/ kg of body weight for a six weeks.

- ✓ **Chocolate brown dye:** purchased from a (Ajanta Chemical Industries - India) company which is one of the types of Azo dye, characterized with a reddish-brown color, quick soluble in water, the molecular weight is 652.56 g/ mol, and chemically called is [disodium 4,4 ' - (2,4-dihydroxy-5-hydroxymethyl-1,3-phenylene bis-azo) di-(naphthalene-1-sulfonate))]. The dose prepared by depending on body weight, 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.
- ✓ **Animals test:** animals weight was token after the end experiment, and then the anesthesia animals by chloroform were taking blood from the heart directly and saved in tubes is a container on the anti-clotting substance (EDTA) for the purpose of obtaining serum to measure some biochemical parameters, then animals anatomic and excise of stomach and intestines that have putted in Petri dishes container on physiological solution and cleaned from waste and then preserved by 10% formalin until the preparation of histological sections.
- ✓ **Determination of some biochemical parameters**

1. Determination of glutathione-s-transferase (GSTs) in blood depending on (18) method.

2. Determination of Lipid Peroxidation (Malondialdehyde) in blood depending on the method used and modified by (19)
3. Determination of amino carrier enzymes activity (AST and ALT)by followingmethod of color (20) and several tests were used (Kit) is providedfrom Italian Giesse company.
4. Determination of superoxide dismutase (SOD) by following method described from (21).

- ✓ **Histological study:** Histological sections of stomach and intestine were prepared according to the method (22)
- ✓ **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 (23).

Results and Discussion

1. Effect of chocolate brown dye on body and liver weights (g) of albino rats

Results shown in Table (1) significantly decreased ($P<0.05$) in the rate of body weight in all groups compared with control group; the observed increase this decrease by increasing the dose of dye, where there was a significant difference between the first treatment group compared with second and third groups, which did not show a significant difference between them, on the other hand showed the results of the current study, a significant increase in liver weight for a first, second and third treatments compared with control group, while did not difference shown between second and third groups when compared with each other. Results of this study agreed with a combination of the studies result which refers to the dosage of food dyes for animals adversely affected on weights (24,25,15,9) and(26) refers to that food additives such as tartrazine and amaranth lead to a significant decreased in body weight was proportionally increases with dose increasing. While these results differed with the results of (27,4) which refer that some azo dyeastartrazine and chocolate brown lead to increased body weight in laboratory animals, as well as 28Sahar *et al*., (2012) observed that there was no significant effect of food dyes as chocolate dye on body weight, and possibly was attributable body weight to the negative impact of azo dyes in loss of appetite leading to decreasing of major and necessary nutrients for body growth, as well as dyes were lead to reduce the percentage of fat in blood, such as cholesterol is important to cell membranes (24,25 15).

The obtained increased in liver weight was responded to a case of poisoning; as a center to metabolize poisons and drugs, as well as free radicals, which consists by azo dyes, which may cause to damage in fabric of the liver and thus amplified (10,11, 29)

Table 1: Effect of chocolate brown dye dosage on body and liver weights (g) on albino rats

Parameter	Groups (Mean ± StandardError)			
	C	T_1	T_2	T_3
Body weight (g)	211.09 ± 2.43 A	189.12 ± 1.55 B	181.47 ± 1.22 C	177.23 ± 1.76 C
Liver weight (g)	5.89 ± 0.53 C	6.50 ± 0.22 B	7.71 ± 0.14 A	7.91 ± 0.11 A

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

2. The effect of chocolate brown dye on some parameters of blood biochemical to albino rats

Researchers were depended on activity measure of amino transfer liver enzymes (AST and ALT)as important evidence of the clinical to knowledge of early inflammatory pathological changes that occur in the liver due to chemicals (30,31). So it has been measuring these parameters to determine the effect of chocolate brown dye on body biological systems; as these enzymes act as liaison between carbohydrates and amino acids. Results of this study (Table 2) a significant increase given ($P<0.05$) in amino transfer liver enzymes (AST and ALT) in (T_1, T_2 and T_3) groups compared with control group; because of this increase suited proportional directly with increasing of chocolate dye dose, and these results were reinforce the findings of (32) as a result of which he referred to chocolate brown dye caused a rise in enzymes concentration (AST and ALT) in blood. (14)) observed to increase the enzymes concentration (AST and ALT) in blood by effect of chocolate brown dye due to type, damage degree and poisoning that occurred in liver, muscles and intestinal tissues. On the other hand (26) explained that the azo dyes such as tartrazinecause an increase in amino transfer liver enzymes. Perhaps the reason for that is due to the azo dyes were generate many free radicals that affect on effectiveness and vitality of cells it so attacking their membranes and lose itof permeability optional feature and increases the enzymes leakage, and the oxidative stress leads to liver cell degeneration and its enzymes exudes into blood (29).

The measuring of antioxidant enzymes activity such as SOD, GSTs and MDA measurement is final products of lipid peroxidation there are an important parameters of the oxidative stress state only, but is a clear sign of the lack of antioxidant regulation (33) Results of the present study (Table 2) a significantly decreased ($P<0.05$) in antioxidant enzymes concentrations (SOD and GSTs) on groups (T_1, T_2 and T_3) compared with control group, also found there is a significant difference in the concentration of SOD between the two groups (T_2 and T_3) with a (T_1) group, which did not show differences between them, while there was a significant difference between the groups (T_3) and (T_1) in the concentration of GSTs. Results also showed a significant increase ($P<0.05$)in MAD with proportional directly to increased dose in all groups treated chocolate dye (T_1, T_2 and T_3) compared with control group, and the reason of

changes in the oxidative parameters perhaps is due to the role of main to azo dyes including chocolate brown dye in bringing oxidative stress through are many types of free radicals that cause many health problems and diseases such as anemia, congestion and some tumors as well as the occurrence of an imbalance between oxidants and antioxidants (14, 34). Some researchers were refereed to the free radicals produced by fatty acids oxidation in cell membranes due to food dyes, including azo dyes, lead to the consumption of large amounts from antioxidants that cells protect from the effect of free radicals and thereby decrease the concentration of antibodies in blood (35,28). On the other hand, the concentration of MAD increasing may be due to free radicals targeting free radicals increased of fatty acids in cell membranes (28)

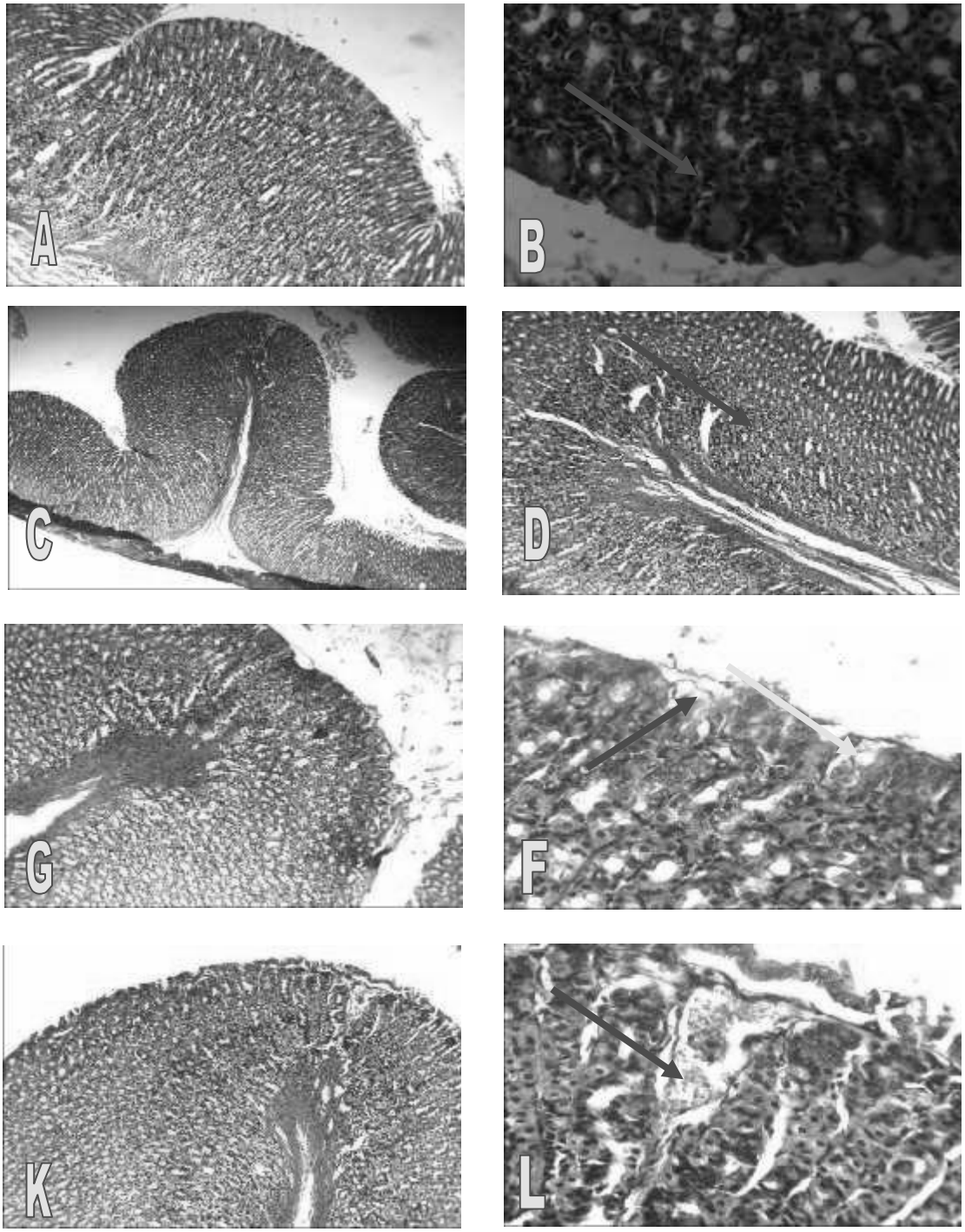
Table 2: Effect of different concentration of chocolate brown dye on some biochemical parameters on albino rats

Groups (Mean ± Standard Error)				Parameter
T_3	T_2	T_1	C	
1.03 ± 0.11 C	1.21 ± 0.04 C	1.70 ± 0.12 B	2.30 ± 0.13 A	SOD (U/L)
6.20 ± 0.11 C	7.30 ± 0.97 BC	8.80 ± 0.92 B	12.00 ± 0.87 A	GSTs (U/L)
70.00 ± 2.17 A	62.11 ± 2.14 B	56.20 ± 1.12 C	44.00 ± 2.23 D	AST (U/L)
78.11 ± 1.31 A	65.61 ± 1.54 B	62.30 ± 2.22 B	49.00 ± 2.00 C	ALT (U/L)
12.80 ± 0.45 A	9.50 ± 0.12 B	7.20 ± 0.44 C	4.50 ± 0.58 D	MDA (µmol/L)

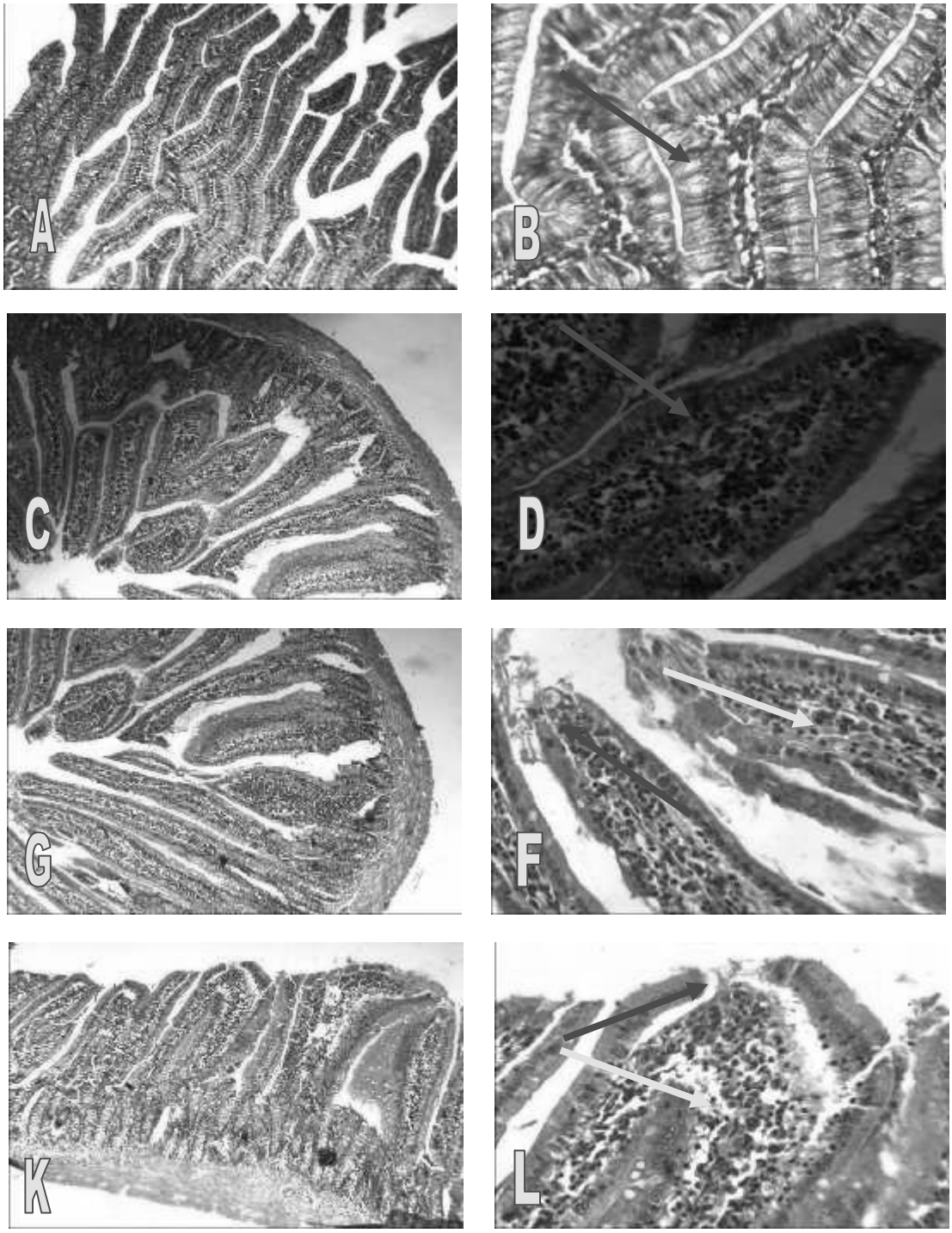
Different English letters (A, B, C) refers to the significant superiority of means between the groups using F-test at ($P \leq 0.05$)a level of probability

3. Histological study

Results microscopic test of taken histological slides from stomach and intestines showed that presence of pathogenic tissues changes in stomach and intestinal tissue for two groups (T_2 and T_3) compared with control group (C), and these changes included damage and necrosis in the cells as well as soreness and change in the intestinal glands of the stomach and damage to the stomach layers, has been reason for this to the state of oxidative stress caused by free radicals generated from the chocolate brown dye and caused by oxidizing unsaturated fat component of the cell wall and thus cell death and tissue damage (29). These results agreed with some studies results that have shown azo dyes as tartrazine and chocolate brown caused many of the pathological changes, cell death, decomposition in the liver tissue and the kidney through of free radicals generation (15,28). (11) indicate through his study on many chemicals, including food dyes it cause damage in stomach glands, colon and the bladder lining, as well as (36) refereed that tartrazine dye that is one of the azo dyes types cause a lot of inflammatory conditions in the stomach and intestines, increasing the number of lymphocytes. Histological slides test shown absence of abnormal changes in (T_1) group compared with control group of the reason may be due to the low-lying doses of the dye not cause pathological changes..



Fig(1) Photomicrographs of paraffin embedded rat stomach tissue: Section (A) and B) is the control showing normal cells stomach. Section (C and D) T1 group Showing normal cells of stomach. Section (G and F) T2 group showing necrosis in stomach cells (red and yellow arrow) and Section (K and L) T3 group showing blooding in mucosa layer of stomach (red arrow) (H& E, 10x and 40x).



Fig(2) Photomicrographs of paraffin embedded rat intestine tissue: Section (A and B) is the control showing normal cells intestine. Section (C and D) T1 group Showing normal cells of intestine. Section (G and F) T2 group showing necrosis and lymphoid infiltration in intestine tissue (red and yellow arrow respectively) and Section (K and L) T3 group showing necrosis and lymphoid infiltration in intestine tissue (red and yellow arrow respectively) .

References

1-Hirschbruch, M.D. and Torres, E.A.F.S. (1998). Toxicological de Alimentos: UmaDiscussao. Hig-Alim., 12(53): 21-25.

2-Toledo, M.C.F. (1999). Regulamentacao de uso de corantesnaturis. Arch. LantinoamNutr., 49(1): 67-70.

3-AL-Shinnawy, M.S. (2009). Physiological effect of a food additive on some haematological and biochemical parameters of male albino rats. Egypt Acad. J. Biol. Sci., 2(1): 143-151.

4- Gautam, D.; Goyal, R. P. and Sharma, G. (2010).Evaluation of toxic impact of tartrazine on male Swiss albino mice.Pharmacol. Online, 1: 133-140.

5-Madsen, C. (1997). Chemicals in food and allergy: food fiction. Environ. Toxicol.Pharmacol., 4(1-2): 115-120.

6-Tripathi, M.; Khanna, S.K. and Das, M. (2007). Surveillance on use of synthetic colors in eatables vis prevention of food adulteration act of India. Food Control, 18(3): 211-219.

7-Reyes, F.G.; Valim, M.F. and Vercesi, A.E. (1996).Effect of organic synthetic food colours on mitochondrial respiration. Food Additives and Contaminants, 13(1): 5.

8-Tanaka, T. (2005).Reproductive and neurobehavioral toxicity study of tartrazine administration to mice in the diet. Food and Chemical Toxicol., 5: 16-25.

9-Zraly, Z.; Pisarikova, B.; Trckova, M.; Herzig, I.; Juzl, M. and Simeonovova, J. (2006).Effect of lupine and amaranth on growth efficiency, health and carcass characteristics and meat equality of market pigs.Acta. Veterinarian Brunenssis, 75(3): 363-372.

10-Combes, R.B. and Haveland-Smith, A. (1982). A review of the genotoxicity of food, drug and cosmetic colours and other azo, triphenylmethane and xanthenes dyes. Mut. Res., 98: 101-148.

11-Sasaki, Y.F.; Kawaguchi, S.; Kamaya, A.; Ohshima, M.; Kabasawa, K.; Iwama, K.; Taniguchi, K. and Tsuda, S. (2002). The comet assay with 8 mouse organs: Results with 39 currently used food additives. Mut. Res., 519: 103-119.

12-Leo, M.L.; Nollet, A. and Fidel, T (2012). Food analysis by HPLC (3ed Ed), CRC Press, P: 513-525.

13-European Food Safety Authority (EFSA) (2010).Scientific opinion on the re-evaluation of brown HT (E 155) as a food additive. EFSA J., 8(3): 1536.

14-Bawazir, A.E. (2012). Effect of chocolate brown HT with olive oil on some neurotransmitters in different brain regions, physiological and histological structure of liver and kidney of male albino rats. J. Evolutionary Biol. Res., 4(1): 13-23.

15-Abou El-Zahab, H. and El-Khyat, Z.G. (1997).Physiological effect of some synthetic food coloring additives rats. Pro-Boll Chemical Farm, 136(10): 615-627.

16-Helal, G.E.; Zaahkouk, A.M. and Mekkaway, A.H. (2000).Effect of some food colorants (synthetic and natural products) of young albino rats. Egyptian J. Hospital Med., 1(2): 103-113.

17-Hassan, G.M. (2009). Effect of some synthetic coloring Addi-tives on DNA damage and chromosomal aberration of rats. Arab J. Biotech., 13(1): 13-24.

18-Habing, W.H.; Pasbst, M.J. and Jacoy, W.B. (1974).The first enzymatic step in mercapturic acid formation. J. Biol. Chem., 249: 7130-7139.

19-Guidet, B. and shah, S.V. (1989). Am J. Physiol 257 (26). F440 cited by Muslih, R. K., Al- Nimer, M.S; Al-Zamely, O.Y. (2002). The level of Malondialdehyde after activation with H_2O_2 and $CuSO_4$ and inhibition by deferoxamine and Molsidomine in the serum of patient with acute Myocardial infraction. National. J. of chemistry. 5: 139-148

20- Reitman, S. and Frankel, S. (1957). Acolorimetric method for the determination of serum glutamic oxaloacetic and glutamic pyruvic transaminase. Am. J. Clin. Path., 28: 56-63.

21-Misra, H.P. and Fridovich, I. (1972).The role of superoxide anion in the out oxidation of epinephrine and a simple assay for superoxide dismutase. J. Biol. Chem., 247: 3170-3175.

22-Al-Mukhtar, K.A.; Allaf, S.M. and Al-Ataar, A.A. (1982).Microscopic Preparations.University of Baghdad, Ministry of Higher Education and Scientific Research, Iraq.

23-Al-Rawi, K.M. and KhalafAllah, A.M. (2000).Design and Analysis of Experiments.National Library for Publication, University of Mosul, Ministry of Higher Education and Scientific Research, Iraq.

24-Shaker, A. M. H.; Ismail, I. A. and El-Nemr, S. E. (1989). Effect of different foodstuff colourants added to casein diet for biological evaluation. Bull.Nutr. Inst .Cairo, Egypt. 9(1): 77.

25-Takeda, H.; Nakajima, A. and Kiriyama, S. (1992). Beneficial effects of dietary fiber on the upper gastrointestinal transit time in rats suffering from toxic dose of amaranth . Biosci . Biotech. Biochem. 56: 551-555.

26-Al-Shinnawy, S.M. and Elkattan, A.N. (2013). Assessment of the changes in some diagnostic parameters in male albino rats fed on an Azo dye. Int. J. Environ. Sci. Eng., 4: 85-92.

27- Sharma, A.; Goyal, R.P.; Chakravarty, G. and Sharma, S. (2005). Hemotoxic

effects of chocolate brown. A commonly used blend of permitted food colour on Swiss albino mice. Asian J. Environ. Sci., 19: 93-103.

28-Soltan, S.A and Shehata, M.E. (2012). The Effects of using color foods of children on immunity properties and liver, kidney on rats. Food and Nutr. Sci., 3: 897-904.

29-Demirkol, O.; Zhang, X. and Ercal, N. (2012).Oxidative effects of tartrazine (CAS No. 1934-21-0) and new Coccin (CAS No. 2611-82-7) azo dyes on CHO cells. J. Verbr. Lebensm, 7: 229-236.

30-Govindwar, S. P. and Dalvi, R. R. (1990). Age dependent Toxicity of a corn extract in young and old male rats.Vet. Hum. Toxicol; 32:23-6.

31-Kowalczyk, E.; Kopff, A.; Fijalkowsk, P.; Kopff, M.; Niedworok, J.; Blaszczyk, J.; kedziora, J. & Tyslerowicz, P. (2003). Effects of anthocyanins on selected biochemical parameter in rats exposed to cadmium. J. Acta. Bio. Chichimec polonica., 50(2): 543-548.

32-Abdel-Rahim, E. A.; Ashoush, Y. A.; Afify, A. S. and Hewedi, F. (1989). "Effect of some synthetic food additives on blood haemoglobin and liver function of rats."Minufiya J.Agric. Res. 14(1): 557.

33- Carocho ,M. and Ferreira,I.C.(2013). A review on antioxidants, peroxidant s and related controversy :Natural and synthetic compounds, screening and analysis methodologies and future perspectives .Food chem. Toxicol.51:15-25

34- 34- Sayed, H.M., Fouad, D., Ataya, F.S., Hassan, N.H., Fahmy, M.A., 2012. The modifying effect of selenium and vitamins A, C, and E on the genotoxicity induced by sunset yellow in male mice. Mutation Research. 744, 145-153.

35-Amin, K.A.; Hameid, H.A. and AbdElsttar, A.H. (2010). Effect of food azo dyes tartrazine and carmoisine on Bio-chemical parameters related to renal, hepatic function and oxidative stress biomarkers in young male rats. Food and Chemical Toxicol., 48(10): 2994-2999.

36-Moutinho, I.I.D.; Bertges, L.C. and Assis, R.V.C. (2007).Prolonged use of the food dye tartrazine (FD & C Yellow n degrees 5) and its effects on the gastric mucosa of Wistar rats. Braz. J. Biol., 67: 141-145.

دراسة تأثير استخدام تراكيز مختلفة من صبغة الشوكولا البنية (Chocolate Brown HT E155) على بعض المعايير الفسلجية والتركيب النُسجي للمعدة والأمعاء في الجرذان البيض

تاريخ الاستلام 2016/1/23 تاريخ القبول 2016/3/30

أحمد جاسم حسن حسين عباس سلمان
قسم علوم الحياة/ كلية التربية/ جامعة القادسية

الخلاصة

أجريت الدراسة الحالية للتحقق من تأثير جرع مختلفة من صبغة الشوكولا البنية على بعض المعايير الفسلجية (SOD و GSTs و MDA), وتركيز الإنزيمات الناقلة للأمين (AST و ALT) والتركيب النُسجي للمعدة والأمعاء.

تكونت تجربة الدراسة الحالية من 20 جرذاً موزعاً على أربعة مجاميع رئيسة؛ تألفت كل مجموعة من خمسة حيوانات جُرّعت فيها كل مجموعة بتركيز مختلف من تراكيز صبغة الشوكولا البنية لمدة ستة أسابيع, وهي كالآتي: المجموعة الأولى (مجموعة السيطرة C) جُرّعت حيواناتها بماء الشرب العادي, المجموعة الثانية (T_1)جُرّعت حيواناتها بصبغة الشوكولا البنية بتركيز 200 ملغم/ كغم من وزن الجسم, المجموعة الثالثة (T_2)جُرّعت حيواناتها بصبغة الشوكولا البنية بتركيز 400 ملغم/ كغم من وزن الجسم, والمجموعة الرابعة (T_3) جُرّعت حيواناتها بصبغة الشوكولا البنية بتركيز 600 ملغم/ كغم من وزن الجسم.

أوضحت النتائج حدوث إنخفاض معنوي ($P<0.05$) في وزن الجسم وتراكيز الإنزيمات المضادة للتأكسد (SOD و GSTs) مقابل حصول زيادة معنوية في تراكيز MDA والإنزيمات الناقلة للأمين AST و ALT في حيوانات المجاميع (T_1 و T_2 و T_3) بزيادة تركيز صبغة الشوكولا البنية مقارنة بحيوانات مجموعة السيطرة, فضلاً عن حدوث تغيرات نُسجيَّة مرضية في المعدة والأمعاء لحيوانات المجموعتين (T_2 و T_3) تمثّلت بتنخر وموت الخلايا في المعدة والامعاء وحصول بعض حالات نزف دموي في بطانة المعدة بسبب الجذور الحرة.

واُستُنتِجَ من هذه الدراسة أنَّ الجرعة العالية من صبغة الشوكولا البنية لها بعض التأثيرات السلبية على وظائف الكبد وبعض المعايير الفسلجية والتركيب النُسجي للمعدة والأمعاء.

كلمات مفتاحية: صبغة الشوكولا البنية, معدة, أمعاء, جرذ أبيض.