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Effect of food azo dyes tartrazine and carmoisine on biochemical parameters related to renal, hepatic function and oxidative stress biomarkers in young male rats

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ABSTRACT

Tartrazine and carmoisine are an organic azo dyes widely used in food products, drugs and cosmetics. The present study conducted to evaluate the toxic effect of these coloring food additives; on renal, hepatic function, lipid profile, blood glucose, body-weight gain and biomarkers of oxidative stress in tissue.

Tartrazine and carmoisine were administered orally in two doses, one low and the other high dose for 30 days followed by serum and tissue sample collection for determination of ALT, AST, ALP, urea, creatinine, total protein, albumin, lipid profile, fasting blood glucose in serum and estimation of GSH, catalase, SOD and MDA in liver tissue in male albino rat.

Our data showed a significant increase in ALT, AST, ALP, urea, creatinine total protein and albumin in serum of rats dosed with tartrazine and carmoisine compared to control rats and these significant change were more apparent in high doses than low, GSH, SOD and Catalase were decreased and MDA increased in tissue homogenate in rats consumed high tartrazine and both doses of carmoisine.

We concluded that tartrazine and carmoisine affect adversely and alter biochemical markers in vital organs e.g. liver and kidney not only at higher doses but also at low doses.

1. Introduction

Food additives play a vital role in today's bountiful and nutritious food supply, they allow our growing population to enjoy a variety of safe, wholesome and tasty foods year round, and they make possible an array of convenience foods without the inconvenience of daily shopping.

The Egyptian famous food additives which are used as coloring substances are tartrazine and carmisione.

Food colors are materials of natural origin have been used to provide color in foods; drugs and cosmetics for thousands of years, Ash from fires mineral compounds and plants were probably among the first materials used for cosmetic purposes (Gaunt et al., 1972).

By the early 1995, natural and synthetic color additives were used extensively to color foods, drugs and cosmetics (Hallagan et al., 1995). Color is an important characteristic and selection criterion for food choice, recent studies have high lighted this importance and have shown how selection may change among certain, populations, and over time (Clydesdale, 1993). Colorant plays a significant role in enhancing the artistic appeals of foods that are aesthetically pleasing more likely to be consumed and to contribute to a varied diet (Hallagan et al., 1995).

Tartrazine known as E102 or FD&C Yellow 5 or C.I. 19140 is a synthetic lemon yellow azo dye used as a food coloring. It is derived from coal tar. It is water soluble and principally is the trisodium 5-hydroxy-1-(4-sulfonatophenyl)-4-(4-sulfonatophenylazo)-H-pyrazol-3-carboxylate.

Many products contain tartrazine like foods cotton candy, soft drinks, flavored chips (Doritos, Nachos, etc.), cereals (corn flakes, muesli, etc.), cake mixes, soups, sauces, some rice, ice cream, candy, chewing gum, marzipan, jam and jelly, some of non food products include tartrazine such as soaps, cosmetics, shampoos and other hair products, also some medical preparations contain tartrazine such as vitamins, antiacids, medicinal capsules and certain prescription drugs. The ADI for tartrazine is 7.5 mg/kg/day (Walton et al., 1999).

Carmoisine and its different names as Azorubine, Food Red 3, Azorubin S, Brillant carmoisine O, Acid Red 14, or C.I. 14720 is a synthetic red food dye from the azo dye group. It usually comes as a disodium salt. It is a red to maroon powder; it has been used for the purposes where the food is heat-treated after fermentation. It has E number E122 carmoisine present in food like blancmange, marzipan, Swiss roll, jams, preserves, yoghurts, jellies, breadcrumbs, and cheesecake mixes. It is also present in oraldene mouthwash.

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Because tartrazine and carmoisine are a nitrous derivatives (azo class) they can be reduced in the organism to an aromatic amine which is highly sensitizing. The main metabolite identified to date is sulfanylic acid (Maekawa et al., 1987; Chung et al., 1992).

Two doses of synthetic dyes (low or high doses) mostly attributable to hepatocellular damage when the toxic effects of synthetic dyes that tartrazine and carmoisine were among of them (ponceau, carmoisine, erythrosine, sunset yellow, tartrazine, fast green, indigotine, brilliant blue and brilliant black) were tested in rats by the biochemical and histopathological examinations. Dyes exert histopathological effects on the hepatic and renal tissues of the rats, indicated by vacuolation, swelling, necrosis and pyknosis of their cells (Mekkawy et al., 1998).

Histopathological studies showed brown pigment deposition in the portal tracts and Van Küpffer cells of the liver and renal mainly induced by colorant that include carmoisine (Aboel-Zahab et al., 1997).

The individual response varies not only according to dose, age, gender, nutritional status and genetic factors, but also according to long term exposure to low doses (Sasaki et al., 2002).

Therefore the aim of this work is to study the effects of tartrazine and carmoisine as coloring agent widely used in food products, drugs and cosmetics on on some biochemical parameters in serum of young male albino rats (as a model of children) associated with liver and kidney functions, blood glucose and serum lipids, also our study was extended to evaluate the effect of these additives on the biomarkers of the oxidative stress in tissue homogenates.

2. Materials and methods

2.1. Chemicals utilized in the experiment

Tartrazine is a yellow color substance known as E102 or FD&C Yellow 5, dye content in the substance is 87%, loss in drying and sodium chloride/sulphate is less than 13%, water insoluble matter less than 0.2% lead less than 0.01 ppm Arsenic less than 1 ppm, heavy metals less than 40 ppm, tartrazine was labeled as food colorant.

Carmoisine is a red color substance known as E122 or Food Red 3 Azorubin S, Brillant carmoisine O, Acid Red 14, or C.I. 14720, dye content in the substance is 87%, loss in drying and sodium chloride/sulphate is less than 13%, water insoluble matter less than 0.2% lead less than 0.01 ppm Arsenic less than 1 ppm, heavy metals less than 40 ppm, carmoisine was labeled as food colorant. Tartrazine and carmoisine were obtained from Star Aromatic Company for flavors and fragrance food colors (Egypt), which obtain these chemicals from Ajanta Chemical Industries (India).

2.2. Animals and treatments

A total of 36 young male (Rattus Norvegicus) albino rats weighting about 60–80 g were used in the present study. They were obtained from (National Research Center, Cairo, Egypt). Animals were kept under observation for about 7 days before the onset of the experiment to exclude any intercurrent infection. They were maintained in stainless steel cages at normal atmospheric temperature of 27 ± 5 °C as well as under good ventilation. Our work was carried out in accordance with the guidelines of Beni Suef University for animal use.

Tartrazine, and carmoisine were in a solid state so we prepared two solutions of each substance (one low and the other high) by dissolving the solid in distilled water, low doses of tartrazine and carmoisine were 15 and 8 mg/kg bw respectively while high doses were were 500 and 100 mg/kg bw respectively.

Animals were fed on the standard basal diet and provided with tap water a composition of experimental diets according to Kim et al. (2005) as follows: (fat 5%, carbohydrates 65%, protein 20.3%, fiber 5%, salt mixture 3.7%, vitamins mixture 1%).

2.3. Sampling and tissue preparation

By the end of the experimental periods, venous blood samples were collected from the orbital sinus of control, and food dyes treated rats via glass capillaries at fasting state. The blood samples were collected in dry glass centrifuge tubes and allowed to coagulate at room temperature and centrifuged at 3500 rpm for 15 min at room temperature for separation of serum.

Homogenates of liver tissues were prepared by dissolving 0.25 g of liver tissue in 5 ml of NaOH 0.9% in test tube then homogenized by the homogenizer for 15 min then centrifuged by centrifuge for 10 min at 3000 rpm then the supernatants were collected for determination of biomarkers of oxidative stress.

Activity of serum ALT and AST were determined by the method of Reitman and Frankel (1957). Total protein was determined according to the method of Henry (1964). Urea was determined in serum by the method of Patton and Crouch (1977). Total cholesterol was estimated in serum by enzymatic colorimetric method according the method of Allain et al. (1974) and Tamaoku et al. (1982). Triglycerides in serum were estimated by enzymatic colorimetric method of Buccolo (1973). HDL-cholesterol in serum was determined by enzymatic colorimetric method of Fruchart et al. (1982). Glucose concentration in serum was estimated by enzymatic colorimetric method according to Trinder (1969). Catalase activity was estimated in tissue homogenate by method of Cohen et al. (1970). Liver reduced glutathione content (GSH) was determined according to the procedure of Beuther et al. (1963). Determination of lipid peroxidation in tissue homogenate was determined according to the method of Presuss (1998).

2.4. Statistical analysis

The statistical analysis was carried out using GraphPad Instat software (version 3, ISS-Rome, Italy). Unless differently specified, groups of data were compared with un-paired *t*-test and one-way analysis of variance (ANOVA) followed by Tukey–kramer (TK) multiple comparisons post-test. Values of $P < 0.05$ were regarded as significant. The data, as clearly indicated are reported in tables and figures as mean ± standard error (SE).

3. Results

The present study revealed that high dose of tartrazine and low and high doses of carmoisine that were 15.4 ± 0.80, 14.2 ± 1.19, and 17.2 ± 0.94 u/l respectively induced a significant increase in serum ALT activity in comparison to control group where being 8.91 ± 0.27 u/l. Also high doses of tartrazine and carmoisine and low and high doses of saccharin that their values are 55.38 ± 1.00 and 52.91 ± 2.54 u/l showed a significant increase in serum AST activity when compared to control rats where being 47.85 ± 2.70 u/l (Table 1).

Both low and high doses of tartrazine, carmoisine that their values are 117.16 ± 2.3, 119 ± 2.93, 114.16 ± 3.85, and 115.45 ± 2.89 iu/l exhibited a significant increase in serum ALP activity when compared to control group where being 87.56 ± 2.79 iu/l (Table 1).

Table 1 showed that high dose of carmoisine and both low and high doses of tartrazine that their values were 6.16 ± 0.10, 6.66 ± 0.18, and 6.70 ± 0.18 g/dl respectively induced a significant increase in serum total protein concentration when compared to control group where being 5.59 ± 0.12 g/dl, also serum albumin le-

Table 1
Effect of food colorants both low and high doses on serum ALT, AST and ALP activities also serum level of T proteins, albumin and globulin in rats.

Groups	Control	Low tartrazine	High tartrazine	Low carmoisine	High carmoisine
ALT (u/l)	8.91 ± 0.27^{a}	10.78 ± 0.65^{a}	15.4 ± 0.80^{b}	14.2 ± 1.19^{b}	17.2 ± 0.94^{c}
AST (u/l)	47.85 ± 2.70^{a}	48.96 ± 0.96^{a}	55.38 ± 1.0^{b}	47.68 ± 2.41^{a}	52.91 ± 2.54^{b}
ALP (iu/l)	87.56 ± 2.79^{a}	117.16 ± 2.3^{b}	119 ± 2.93^{b}	114.16 ± 3.85^{b}	115.45 ± 2.89^{b}
T protein (g/dl)	5.59 ± 0.12^{a}	6.66 ± 0.18 ^{c}	6.70 ± 0.18^{c}	5.71 ± 0.26^{a}	6.16 ± 0.10 ^{b}
Albumin (g/dl)	3.81 ± 0.14^{a}	4.57 ± 0.08^{c}	4.76 ± 0.13^{c}	3.94 ± 0.03^{a}	4.27 ± 0.27^{b}
Globulin (g/dl)	1.77 ± 0.05^{a}	1.88 ± 0.08^{a}	2.13 ± 0.04^{b}	1.8 ± 0.07^{a}	1.89 ± 0.05^{a}

Data expressed as means ± SE. Number of animals is eight for low dose groups and ten for high dose groups. Means which share the same letter are not significantly different. Means which have different letters are significantly different ($P < 0.05$).

vel increased in these groups where their values are 4.27 ± 0.27, 4.57 ± 0.08, and 4.76 ± 0.13 g/dl, respectively when compared to control value where being 3.81 ± 0.14 g/dl. High tartrazine dosed group showed a significant increase in serum globulin concentration when compared to control group.

Low and high doses of tartrazine, and carmoisine where their values were 1.65 ± 0.05, 1.80 ± 0.05, 1.55 ± 0.05, and 1.56 ± 0.09 mg/dl induced a significant increase in serum creatinine level when compared to control value that was 1.32 ± 0.06 mg/dl (Table 2).

Low and high doses of tartrazine, and carmoisine where their values were 37.41 ± 1.59, 37.46 ± 0.94, 37.76 ± 2.05, and 42.15 ± 1.03 mg/dl respectively, induced a significant increase in serum urea level when in comparison to control value where being 17.54 ± 0.68 mg/dl.

Table 2 showed that serum total cholesterol levels were significantly reduced in groups of rats dosed with tartrazine or carmoisine in both low and high doses and their values were 115.86 ± 4.03, 104.1 ± 2.009, 121.71 ± 4.68, and 118.2 ± 3.31 mg/dl when compared to control value where being 143.41 ± 4.32 mg/dl, as a result serum HDL-cholesterol and LDL-cholesterol were significantly reduced in azo dyes dosed rats.

Rats consumed low and high doses of carmoisine and that consumed high dose of tartrazine where their values were 36.15 ± 1.44, 27.25 ± 1.86, and 34.2 ± 1.16$k \times 10^{-2}$ showed a significant decrease in liver catalase activity when compared to control value where being 47.03 ± 2.01$k \times 10^{-2}$ (Table 3)

Low and high doses of tartrazine and high dose of carmoisine where values were 64.8 ± 0.68, 53.0 ± 1.27, and 50.18 ± 2.36 u/g showed a significant decrease in liver SOD activity in comparison to control value that being 70.68 ± 1.50 u/g (Table 3).

Rats consumed low and high doses of carmoisine and that consumed high dose of tartrazine where their values were 66.25 ± 1.83, 61.12 ± 1.07, and 67.37 ± 2.73 nmol/100 mg showed a significant decrease in liver GSH content in comparison to control value where being 72.9 ± 1.41 nmol/100 mg. Low and high doses of carmoisine and high dose of tartrazine where their values were 6.27 ± 0.44, 8.32 ± 0.36, 6.63 ± 0.16, and 7.64 ± 0.62 nmol MDA/g/h showed a significant increase in liver MDA in comparison to control value where being 4.82 ± 0.41 nmol MDA/g/h (Table 3).

In Table 3 all groups of rats dosed with food azo dyes showed significant reduction in body-weight gain when compared to control group.

4. Discussion

In this study some trials were adopted to throw a light on the side toxic effects and biochemical changes in some constituents in serum of experimental rats treated with 2 compounds (each of low and high doses) that are commonly used in Egyptian field of food additives.

We considered low dose (double of ADI) because our young children in Egypt can consume a double of ADI (or more) daily in several products without control, in addition we used the high dose (a much higher than ADI) to evaluate the toxicity and health hazards of these additives on biochemical assay and oxidative stress.

High dose of carmoisine showed a significant decrease more than low carmoisine dose in body-weight gain; also low dose of tartrazine produced a significant decrease more than low carmoisine dose in body-weight gain (Fig. 1).

These data are in agreement with Ford et al. (1987) who reported that high carmoisine dose groups had reduced body-weight gain compared with that of the controls.

Body weight loss is considered by some authors to be a good reliable sensitive toxicity indicator (Ezeuko et al., 2007). Thus the body weight loss in the present study may represent the primary marker of dye bad effect.

4.1. Effect of food azo dyes on liver enzymes

The present study revealed that rats consumed high dose of tartrazine (500 mg/kg bw) or high dose of carmoisine (100 mg/kg bw) exhibited a significant increase in serum ALT, AST and alkaline phosphatase activities when compared to control rats, while low dose of carmoisine (8 mg/kg bw) showed a significant increase in serum ALT and alkaline phosphatase activities when compared to control rats in addition, low dose of tartrazine (15 mg/kg bw) showed a significant increase in serum alkaline phosphatase activity when compared to control rats (Table 1).

The present findings are in a agreement with Mekkawy et al. (1998) who indicated that two doses of synthetic dyes (low or high doses) where tartrazine and carmoisine were among of them (ponceau, carmoisine, erythrosine, sunset yellow, tartrazine, fast green, indigotine, brilliant blue and brilliant black) showed a significant increase in serum AST, ALT, and alkaline phosphates activities.

Table 2
Effect of food colorants both low and high doses on serum level of creatinine and urea, TC, TG, HDL, LDL in rats.

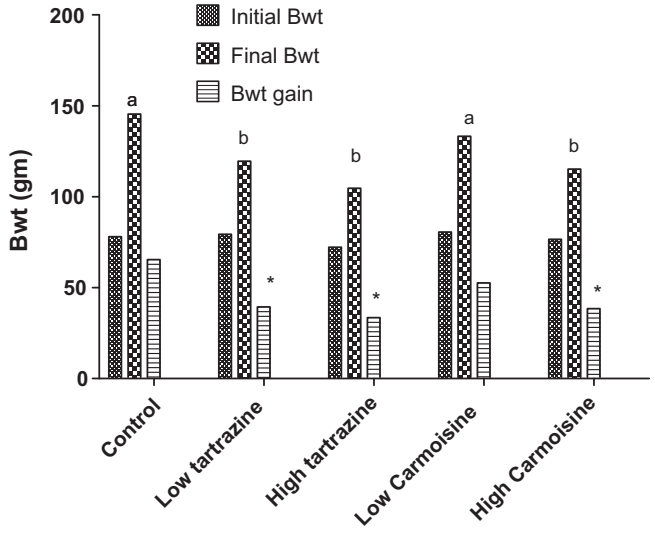
Groups	Control	Low tartrazine	High tartrazine	Low carmoisine	High carmoisine
Urea (mg/dl)	17.54 ± 0.68^{a}	37.41 ± 1.59^{b}	37.46 ± 0.94^{b}	37.76 ± 2.05^{b}	42.15 ± 1.03^{c}
Creatinine (mg/dl)	1.32 ± 0.06^{a}	1.65 ± 0.05^{b}	1.80 ± 0.05^{c}	1.55 ± 0.05^{b}	1.56 ± 0.09^{b}
TC (mg/dl)	143.41 ± 4.32^{a}	115.86 ± 4.03^{b}	104.1 ± 2.009^{b}	121.71 ± 4.68^{b}	118.2 ± 3.31^{b}
TG (mg/dl)	130.6 ± 3.98^{a}	143.5 ± 1.50^{a}	144 ± 3.24^{a}	132.95 ± 2.8^{a}	144.6 ± 3.01^{a}
HDL (mg/dl)	87.11 ± 1.47^{a}	65.63 ± 1.68^{b}	58.11 ± 1.14^{c}	71.33 ± 2.89^{b}	59.93 ± 1.01^{c}
LDL (mg/dl)	34.18 ± 1.52^{a}	17.45 ± 0.91^{b}	15.78 ± 1.43^{b}	28.18 ± 1.91^{a}	17.83 ± 1.30^{b}

Data expressed as means ± SE. Number of animals is eight for low dose groups and ten for high dose groups. Means which share the same letter are not significantly different. Means which have different letters are significantly different ($P < 0.05$).

Table 3
Effect of food colorants both low and high doses on biomarkers of Oxidative stress {catalase, SOD, GSH, and malondialdehyde (MDA)} in liver homogenate.

Groups	Control	Low tartrazine	High tartrazine	Low carmoisine	High carmoisine
SOD (u/g)	70.68 ± 1.50^{a}	64.8 ± 0.68^{b}	53.0 ± 1.27^{b}	69.3 ± 1.91^{a}	50.18 ± 2.36^{b}
Catalase ($k \times 10^{-2}$)	47.03 ± 2.01^{a}	46.01 ± 1.83^{a}	34.2 ± 1.16^{b}	36.15 ± 1.44^{b}	27.25 ± 1.86^{c}
GSH (nmol/100 mg)	72.9 ± 1.41^{a}	70.21 ± 3.85^{a,b}	67.37 ± 2.73^{b}	66.25 ± 1.83^{b,d}	61.12 ± 1.07^{c,d}
MDA (nmol/g/h)	4.82 ± 0.41^{a}	4.93 ± 0.23^{a}	6.63 ± 0.16^{b}	6.27 ± 0.44^{b}	8.32 ± 0.36^{c}

Data expressed as means ± SE. Number of animals is eight for low dose groups and ten for high dose groups. Means which share the same letter are not significantly different. Means which have different letters are significantly different ($P < 0.05$).



* *Indicated significant changes (P <0.05) between control and both tartrazine and carmoisine either low or high dose.*

Fig. 1. Effect of food colorants both low and high doses on initial, final body weight and body-weight gain in rats.

Mekkawy et al. (1998) attributed these results to hepatocellular damage caused by the toxic effects of these synthetic dyes which indicated by vacuolation, swelling, necrosis and pyknosis of the liver cells.

Also these results are deal with Aboel-Zahab et al. (1997) who found that liver enzymes ALT, AST, and Alkaline phosphatase were elevated in rats whose diets were supplemented with chocolate colors A and B (sunset yellow, tartrazine, carmoisine and brilliant blue in varying concentrations), the histopathological studies showed brown pigment deposition in the portal tracts and Van Küpffer cells of the liver, in addition congested blood vessels and areas of haemorrhage in both liver and renal sections were revealed in those rats given colorants B and C (sunset yellow, tartrazine, carmoisine and brilliant blue in varying concentrations).

Furthermore, these results are in accordance with Sharma et al. (2006) who found that the two doses of Tomato Red (blend of carmoisine and ponceau 4R) showed a significant increase in alkaline phosphatase activity when swiss albino mice consumed these colorants for 21 days as short term or 42 days as long term.

Also, Sharma et al. (2005) observed a significant increase in serum transaminases in rats whose diets were supplemented with chocolate colors A and B (sunset yellow, tartrazine, carmoisine and brilliant blue in varying concentrations).

The present findings are in agreement with Helal et al. (2000) who found that oral administration of synthetic or natural colorants induced a marked increase in the serum AST and ALT level of all treated groups after 30 days of treatment.

Abdel-Rahim et al. (1987) found a significant increase in both serum AST and ALT of rats fed on brown food dye for three months, he attributed these changes in liver function to hepatocellular impairment which subsequently caused the release of greater than normal levels of intracellular enzymes into the blood.

The elevation of aminotransferases activities in serum may be due to tissue damage particularly in liver, kidney and heart (Varely et al., 1988). And increased permeability of cell membrane or increased synthesis or decreased catabolism of transaminases may be involved (Malik et al., 1980), also Westlake et al. (1981) mentioned that the release of abnormally high levels of specific tissue enzymes into blood stream is dependent on both the degree and the type of damage exerted by the toxic compound administration.

In the same concern, Webner (2003) reported that the damaged or diseased tissues release enzymes into the blood, so serum alkaline phosphatase measurements can be abnormal in many conditions including bone diseases and liver diseases.

The significant elevation of serum aminotransferases may be attributed to what mentioned by Shakoori et al. (1987) who revealed that under pathological conditions the parenchymal cells of hepatic lobules fail to carry out vital functions, which usually results in disturbed or imbalanced intermediatory metabolism, as a result of cellular damage, several enzymes like ALT, AST, LDH and ALP beach out into the serum and hence their level indicate the type and extent of damage inflicted.

4.2. Effect of food azo dyes on serum proteins

Our work revealed that rats consumed high dose of tartrazine (500 mg/kg bw) or low dose of tartrazine (15 mg/kg bw) and that consumed high dose of carmoisine (100 mg/kg bw) exhibited a significant increase in serum total protein and serum albumin concentration when compared to control rats (Table 1).

These results are in accordance with Mekkawy et al. (1998) who found a significant increase in serum total protein also this is in agreement with Aboel-Zahab et al. (1997) who found the same effect on serum total protein with rats whose diets were supplemented with chocolate colors A and B. Also Sharma et al. (2006) found that total protein was significantly elevated when Tomato Red (blend of carmoisine and ponceau 4R) was consumed by swiss albino mice.

Sharma et al. (2005) observed significant increase in serum total protein and globulin in rats whose diets were supplemented with chocolate colors A and B.

Moreover, these results are in agreement with Ali et al. (1998) who found that the nucleic acids and the total protein had marked increase during the various periods of treatment when the rats were treated with two doses of carmoisine limdose (0.011 mg/100 g/day) and high dose (0.022 mg/100 g/day) during different periods of treatment 30, 60, and 90 days.

Furthermore, our study demonstrated that high dose of tartrazine caused a significant increase in serum globulin, which were in accordance with Mekkawy et al. (1998) and Aboel-Zahab et al. (1997).

Proteins are the fundamental components of all living cells and include many substances such as enzymes, hormones and antibodies that are necessary for the proper functioning of an organism, increased release of enzymes by the damaged tissues and the antibodies to counter act the dye might be the cause of increase in serum protein (Sharma et al., 2005), so increase of release of liver enzymes caused by toxic effect of synthetic food dyes tartrazine and carmoisine can result in increasing of serum protein concentration.

Furthermore, Al-Shinnawy (2009) attributed the elevation in serum total protein of rats whose supplemented with synthetic food dye to the stimulation of protein biosynthesis to produce the specific enzymes required for all processes.

In our opinion these hypersensitivity reactions caused by yellow food dye tartrazine can increase production of immunoglobulin which lead to elevation of serum globulin concentration and this phenomenon is more appreciable in the group of rats consuming high dose of tartrazine due to increasing of immune hyperactivity caused by this food colorant.

Our opinion is agreed with that reported by Al-Shinnawy (2009) who mentioned that the specific elevation in globulin fraction points towards increased immunoglobin synthesis, the defense mechanism which aims to protect the body from the toxic effects of this synthetic food dye.

4.3. Effect of food azo dyes on kidney function

Our study demonstrated that the daily intake for 30 day of tartrazine or carmoisine either low or high doses exhibited a significant increase in serum creatinine and urea concentration when compared with control rats, while high dose of tartrazine exhibited a significant increase more than low dose in serum creatinine level (Table 2).

Our results were parallel to our findings those recorded by Helal et al. (2000) who found a significant elevation in serum creatinine and urea in rats consumed a synthetic or natural food colorants after 30 days of treatment.

Furthermore, the present findings are in accordance with data reported by Ashour and Abdelaziz (2009) who observed a significant elevation in serum creatinine and urea level of rats dosed with organic azo dye (fast green) orally for 35 days.

We believe that the significant elevation in urea and creatinine levels is closely related to the impairment of renal function. These results are in agreement with Varely (1987) who determined that the blood urea can be increased in all forms of kidney diseases such as hydronephrosis congenital cystic, kidney renal tuberculosis, condition in which deposition of calcium occurs as hypervitaminosis D. Also plasma creatinine increases in renal diseases gave prognostic significance than those of other nitrogenous substances.

4.4. Effect of food azo dyes on lipid profile

The reduction in serum cholesterol levels obtained in this study are in accordance with results recorded by Sharma et al. (2006) who reported that two doses of Tomato Red (blend of carmoisine and ponceau 4R) showed a significant decrease in serum total cholesterol and triglycerides when swiss albino mice consumed these colorants for 21 days as short term or 42 days as long term.

Also, these results are correlate well with that reported by Ashour and Abdelaziz (2009) who obtained a significant reduction in serum total cholesterol and triglycerides level when food color azo dye (fast green) was consumed orally to male albino rats for 35 days. While our results are in a contrary with Aboel-Zahab et al. (1997) who observed a significant increases in serum total lipids, cholesterol and triglycerides in rats whose diets were supplemented with chocolate colors A and B that tartrazine and carmoisine were among of them (sunset yellow, tartrazine, carmoisine and brilliant blue) in varying concentrations. Cholesterol is a soft waxy substance found among the lipids in the blood stream and in the body's cells. It is an important part of healthy body because it is used to form cell membranes and to produce certain hormones. The total body content of cholesterol depends on the balance between the amount of cholesterol formed in the body plus that absorbed from diet. Intestinal cholesterol absorption represents another major route for the entry of cholesterol into the body, and, thus, this source can influence the plasma LDL-cholesterol concentration (Turley, 2004). The cholesterol pool in the intestine comes from dietary cholesterol and the majority from biliary excretion. Approximately 50% of the intestinal cholesterol pool is reabsorbed by the intestines (Turley and Dietschy, 2003) and recirculated through the body (via the enterohepatic circulation), with the remainder excreted in feces (Grigore et al., 2007). The deviation from normal values of cholesterol, in the blood serum is considered as symptoms of liver diseases (Singh et al., 1988). In the present study the decreased cholesterol level implies liver damage which is in accordance with increased alkaline phosphatase level discussed earlier.

4.5. Effect of food colorants (azo dyes) on oxidative stress biomarkers

The present study revealed that rats consumed high and low doses of carmoisine and that consumed high dose of tartrazine showed a significant decrease in liver GSH content and catalase activity. Also high and low doses of tartrazine and high dose of carmoisine showed a significant decrease in liver SOD activity when compared to control group, while high and low doses of carmoisine and high dose of tartrazine showed a significant increase in liver MDA (Table 3).

There is a shortage in the literatures dealing with this subject so we have not been able to find a studies targeting oxidative stress with a given food additives.

While our results may be in accordance with Sweeney et al. (1994) who suggested that various azo dye products are genotoxic, not through N-hydroxylation and esterification, which is characteristic of many aromatic amines but rather through a mechanism involving oxygen radicals and the superoxide free radical was produced by the azo dyes only after reduction by the intestinal bacteria *Enterococcus faecalis*.

Moreover, Siraki et al. (2002) found that incubation of hepatocytes with aromatic amines caused a decrease in the mitochondrial membrane potential before cytotoxicity ensued. Hepatocyte GSH was also depleted by all arylamines tested and extensive GSH oxidation occurred with *o*-anisidine and aminofluorene.

Azo compounds contain an aromatic ring linked by an azo bond to second naphthalene or benzene ring.

Many intestinal bacteria are able to reduce the azo bond (termed azofission), which liberates the substituted naphthol compounds. Coloring matter entering the intestinal tract is subjected to the action of acid, digestive enzymes, and microflora. Azo compounds may reach the intestine directly after oral ingestion or through the bile after parenteral administration. They are reduced by azo reductases from intestinal bacteria and, to a lesser extent, by enzymes of the cytosolic and microsomal fractions of the liver. The first catabolic step in the reduction of azo dyes, which is accompanied by a decrease in the visible light absorbance and then decoloration of the dye, is the reduction of the azo bond to produce aromatic amines. Aromatic amines, some of which are known carcinogens, have been found in the urine of dyestuff workers and test animals following administration of azo dyes (Cerniglia et al., 1986).

Tartrazine is transformed into aromatic amine sulfanilic acid after being metabolized by the gastrointestinal microflora (Moutinho et al., 2007).

Because the food dyes (tartrazine and carmoisine) are from the group of azo dye food colorants, they are metabolized into aromatic amine by intestinal flora and the formed aromatic amines can generate reactive oxygen species as part of their metabolism (NOS) by interaction of these amino groups with nitrite or nitrate containing foods or in the stomach., The reactive oxygen species (ROS) such as superoxide anion, hydroxyl radical and H_2O_2 could be produced in the metabolism of nitrosamines and increase oxidative stress (Bansal, 2005). As a result of the ROS formation of the antioxidant defense mechanism of the cells including catalase, SOD, and GSH began to consumed to prevent the cell death by these toxic radicals so their levels in the tissue homogenate were decreased specially at higher doses when the need for them was increased, on the other hand MDA level was increased as a product of lipid peroxidation occurred by the ROS action on lipids of cellular membrane.

Reactive oxygen species play an important role in pathological changes in the liver (Poli and Parola, 1997). Biological membranes are particularly prone to the ROS effect, the peroxidation of unsaturated fatty acids in biological membranes leads to a decrease of membrane fluidity and disruption of membrane integrity and function, which is implicated in serious pathological changes (Halliwell, 1987).

Increased generation of ROS or free radicals is able to cause auto-oxidation of the hepatic cells, resulting in marked hepatic lesions (Suzuki et al., 1998). In the present study, the increased activities of serum enzymes (AST and ALT) have been detected in food

colorants administrated in rats specially at higher doses (Table 2), implying the increased permeability, damage and injuries of hepatocytes. Because the enzyme ALT is located in the cytoplasm and the soluble enzyme AST is located mainly in organelles such as mitochondria (Senthil et al., 2003). Increased levels of AST and ALT suggested damage of both hepatic cellular and mitochondrial membranes in food azo dyes administered rats.

This study suggests a potential role for commonly consumed beverages in elevating the risk of pathophysiologies associated with peroxyl radical-mediated events.

5. Conclusion

Food azo dyes like tartrazine and carmoisine can affect adversely and alter biochemical markers in vital organs e.g. liver and kidney not only at higher doses but also at low doses. Tartazine and carmoisine not only cause changes in hepatic and renal parameters but also their effect become more risky at higher doses because they can induce oxidative stress by formation of free radicals. Therefore, it is necessary to create consumer awareness regarding the ill effects of these food azo dyes and mention the type and concentration of each material added to food.

Based on our results, we believe that more extensive assessment of food additives in current use is warranted.

Conflict of Interest

The authors declare that there are no conflicts of interest.

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