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Oxidative stress, biochemical and histopathological alterations induced by some synthetic food colorants on Albino rats

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Abstract

This study was performed to evaluate the detrimental effects of some synthetic colorants widely used in manufacturing of soft drinks such as caramel IV, sunset yellow and (tartrazine + brilliant blue) at low dose equal to acceptable daily intake (ADI) and high dose equal to 5 times ADI on oxidative stress markers, biochemical parameters, and histopathological alterations of male rats. Results indicated that treated rats with caramel at low and high doses and sunset yellow at low dose did not cause any toxic effects on tested rats. While, treated animals with high dose of sunset yellow and both low and high doses of (tartrazine + brilliant blue) showed significant decrease in body weights and a significant increase in the relative liver and kidneys weights as compared to control. Results also revealed that a significant decrease in some biomarkers; superoxide dismutase (SOD), glutathione peroxidase (GPX), reduced glutathione (GSH) and catalase (CAT) activities and significant increase in malondialdehyde (MDA), aminotransferases enzymes (AST and ALT), alkaline phosphatase (ALP), urea, and creatinine levels in treated rats with high dose of sunset yellow and both low and high doses of (tartrazine + brilliant blue) compared to control. Some histopathological alterations were observed in liver tissues including congested blood vessels, thickened wall bile duct, pyknosis and cellular infiltration. Also, kidney tissues showed blood congestion, hypercellularity of the glomeruli and narrowing of the urinary space. These alterations were found only at a high dose of sunset yellow and both low and high doses of (tartrazine + brilliant blue) compared to control rats.

Keywords: synthetic colorants, oxidative stress, pathological alterations, rats.

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1. Introduction

Carbonated soft drinks are very popular all over the world including Egypt. A common ingredient in carbonated soft drinks is color (USFDA, 2012). Synthetic colorants such as caramel, sunset yellow, tartrazine, and brilliant blue are widely used in many soft drinks' products such as Coca-cola, Pepsi, Mirinda, Fanta, Schweppes and other beverages because of their coloring characteristics, high stability and inexpensively (Amin and Al-Shehri, 2018; JECFA, 1992). However, many of these colorants become toxic and induced serious adverse effects on human health (Abdel-Wahab and Moram, 2012; Al-Shinnawy, 2009; Sadar *et al.*, 2017; Saxena and Sharma, 2015). Caramel is one of the oldest worldwide consumed colorants of all foods and drinks colorants (JECFA, 1992) and used in cola beverages for over 150 years and still used up today (Delgado-Vargas and Paredes-Lo´pez, 2003). Many chemical reactions take place during the production process of caramel color, and lead to the formation of some compounds such as furan, 5-hydroxy methyl furfural (EFSA, 2011), acrylamide (Ghen and Gu, 2014), 2-acetyl-4-tetrahydroxy-butyl imidazole (Wang *et al.*, 2015), and 4- methylimidazole (Xu *et al.*, 2015). These chemicals induced cytotoxic, genotoxic and mutagenic effects and they are suspected from carcinogenicity (Khalil *et al.*, 2010; Kahoun *et al.*, 2017; Pereira *et al.*, 2011). The international agency for research on cancer classified 4-methylimidazol as (Category 2B), that possibly carcinogenic to humans (IARC, 2013). Administration of caramel IV at dose 3 g/kg caused an increase in renal and hepatic enzymes activities of GPX, CAT, SOD, AChE and several histopathological alterations such as diffuse mild paracentral hydropic degeneration in the liver (Jacobson, 2013; Marins *et al.*, 2020; Yadav *et al.*, 2017). Sunset yellow and tartrazine are the most azo colorants soluble in water to give yellow and orange color (Amin *et al.*, 2010) and used in many food products such as soft drinks, fruit juice, candy, sweet and ice cream (Bawazir, 2016). Hashem *et al.* (2014) found that rat's administrated sunset yellow orally at high dose equaling 10 times ADI decreased hepatic lipid peroxide concentration and increased AST and ALT activities of rats. Demirkol *et al.* (2012) found that tartrazine dye induced oxidative stress in CHO cell because decrease GSH levels and increase MDA levels. Ali *et al.* (2016) reveled that tartrazine caused a significant elevation in serum AST, ALT, total protein, urea and MDA, while a significant decrease was found in liver SOD and GSH of rats compared to control. Also, some histopathological changes were noted in liver of rats such as congestion of the hepatic blood vessel and with hepatic vacuolation. Brilliant blue is one of the most common dyes used in foods, drinks and pharmaceuticals (EFSA, 2010). It is also found in green food products and green soft drinks as a mixture with yellow colorants (Ferreira *et al.*, 2017). Mahmoud (2006) who revealed that higher doses of synthetic brilliant blue dye caused an increase of AST, ALT, ALP and total bilirubin, also, some alterations in kidney tissue include blood congestion, thick-walled blood vessels and hemorrhage with infiltration. Mohamed *et al.* (2019) found

that oral administration of brilliant blue induced a significant increase in body weight, organs weights, serum urea, uric acid, FSH, LH and creatinine levels. Also, oxidative damage were observed due to significant elevation in lipid peroxidation with a defect in the activity of glutathione peroxidase. In addition, some histological alterations include inflammatory infiltration and vacuolation in kidney. Also, some testicular damages such as degeneration and necrosis of spermatogoneal cells lining seminiferous tubules of rats. Therefore, the present study planned to evaluate the possible toxic impact of caramel IV, sunset yellow and a mixture of (tartrazine + brilliant blue) colorants widely used in the manufacturing of soft drinks on body weights, organs weights, antioxidant enzymes activity and some biochemical parameters as well as the histopathological alteration of adult male albino rats.

2. Materials and methods

2.1 Materials

2.1.1 Synthetic colorants

Caramel class IV (sulfite ammonia caramel); CAS number 8028-89-5); FD&C Black No. 4; INS/E number E 150d; black liquid color. Sunset yellow; chemical formula ($C_{16}H_{10}N_2Na_2O_7S_2$); CAS number 2783-94-0; FD&C Yellow No. 6; INS/E number E 110; orange powder, soluble in water. Tartrazine; chemical formula ($C_{16}H_9N_4Na_3O_9S_2$); CAS number 1934-21-0; FD&C Yellow No. 5 (lemon yellow azo); INS/E number E 102; powder, soluble in water. Brilliant blue; chemical formula ($C_{37}H_{34}N_2Na_2O_9S_3$); CAS number 3844-45-9; FD&C Blue No. 1; INS/E number E 133, powder, soluble in water. All chemicals and reagents were of analytical pure grades and purchased from Delta Aromatic International Company, Giza, Egypt.

2.2 Methods

2.2.1 Experimental animals and feeding

Adult male albino rats, weighing about 140-150 g were obtained from Animal house, National Research Center, Giza, Egypt, and used in this study. The animals were housed in polyethylene plastic cages at 25±2^{o}C with a 12-hr light/dark cycle and allowed to acclimate for 15 days prior to experimental use. The animals were fed on a standard basal diet, composed of 70% starch (corn starch), 8% fat (corn oil), 12% casein, 4% salt mixture, 1% vitamins mixture, and 5% cellulose as described by AOAC (2008), and water were available *ad libitum*.

2.2.2 Experimental design

Forty-nine male albino rats were used and randomly divided into 7 groups, each group had seven rats. Based on the acceptable daily intake (ADI) for caramel (IV), sunset yellow, tartrazine and brilliant blue, established by JECFA (1992) and U.S.FDA (2012) we administrated oral doses as: Group (1):

Rats provided with tap water and fed with basal diet only and served as control; group (2): Rats given an oral low dose equal to ADI, 200 mg/kg.bw of caramel color; group (3): Rats given an oral high dose equal to 5 times ADI, 1000 mg/kg.bw of caramel color; group (4): Rats given an oral low dose equal to ADI, 31.5 mg/kg.bw of sunset yellow color; group (5): Rats given an oral high dose equal to 5 times ADI, 157.50 mg/kg.bw of sunset yellow color; group (6): Rats given an oral low dose equal to ADI of (tartrazine 7.5 mg/kg.bw + brilliant blue 12.5 mg/kg); group (7): Rats given an oral high dose equal to 5 times ADI of (tartrazine 37.5 mg/kg.bw + brilliant blue 62.5 mg/kg.bw). Sunset yellow, tartrazine and brilliant blue powders were dissolved in distilled water, while caramel color was in a liquid type. All treated animals under study were administered orally by stomach tube daily for 28 days. The animals were carefully observed daily for clinical sings and mortality. The body weights (g) of the animals in all groups were recorded before treatment, after 7, 14, 21 days and at the end of treatment (28 days).

2.2.3 Blood sampling

At the end of the experiment, blood samples were collected from retro-orbital plexus of all rats. Blood was collected into serum separator tubes, and then the tubes were stood for two hours to clot before centrifugation at 5000 rpm at 4^{o}C for 10 minutes. Serum samples were stored at -18^{o}C until analysis. Rats were sacrificed, and then liver and kidneys were excised, rinsed well in a saline solution, then put on filter paper, and weighed to calculate the relative organ's weight as g/100 g b.w. rats according to Ping *et al*. (2006).

2.2.4 Antioxidant enzymes activities

Serum superoxide dismutase (SOD) was determined according to the method described by (Paglia and Valentine 1967), serum reduced glutathione (GSH) was determined according to the methods described by Ridnour *et al.* (1999), serum glutathione peroxidase (GPX) activities were determined according to the method described by Paglia and Valentine (1967), serum catalase (CAT) activity was determined according to the method described by Aebi (1984) and serum malondialdehyde (MDA) was measured using commercial spectrophotometric analysis according to the method of Ohkawa *et al.* (1979).

2.2.5 Biochemical parameters

Serum aspartate transaminase (AST) and alanine transaminase (ALT) activity were determined spectrophotometrically by using commercial kits, according to the method of Schmidt and Schmidt (1963), while, serum alkaline phosphatase (ALP) activity was determined according to calorimetric method of Belfield and Goldberg (1971). Blood urea was estimated by the enzymatic method of Patten and Crouch (1977) and serum creatinine was determined according to

the method described by Faulkner and King (1976).

2.2.6 Histopathological examination

Livers and kidneys were excised out and put in 10% formal saline for 24 hours. Then, the specimens were dehydrated in ascending grades of ethyl alcohol, cleared in xylene, and embedded in paraffin wax. After that, paraffin sections were cut at (5-6µm thick); the sections were stained with haematoxylin and eosin stain for microscopic examination (Drury and Wallington, 1980).

2.2.7 Statistical analysis

All obtained values are expressed as mean ± SD. Compare means between different groups were obtained by using a one-way analysis of variance (ANOVA) followed by Duncan's tested according to the procedure of Armitage (1971) using SPSS version 20 computer program.

3. Results and Discussion

3.1 Clinical signs of toxicity and body weights of rats after oral administration of tested colorants

There were no clinical symptoms of toxicity in all treated groups except animals treated with high dose of sun set yellow and high dose of (tartrazine + brilliant blue) which showed some signs included diarrhea, anorexia and hyperactivity compared to control animals. These symptoms appeared in the second week and continued to the end of the experiment. The effects of the oral administration of either the low dose (equal ADI) and high dose (equal 5 times ADI) of caramel (IV), sunset yellow and mixture of (tartrazine + brilliant blue) on body weight of male albino rats are presented in Table (1). The present results evident that rats in control and treated groups showed an increase in body weight throughout the experimental period which could be attributed to the normal growth phase. But animals that received sunset yellow in high dose (5×ADI) and mixture of (tartrazine + brilliant blue) in doses equal (ADI) and (5×ADI) showed that significant decrease $P \leq 0.05$ in body weights compared to the control group and other treated groups. This decrease in body weights started in the third week and continued to the end of the experiment. Obtained results are in agreement with studies reported by Mackenzie *et al.* (1992), EFSA (2009), EFSA (2010), and Mohamed *et al.* (2019). Based on these studies it could be concluded that a large amount of sunset yellow, tartrazine and brilliant blue colorants caused significant changes in body weight of male rats.

Table (1): Impact of oral administration of caramel IV, sunset yellow and (tartrazine + brilliant blue) at doses equal to ADI and 5 times ADI on body weight of male rats.

Groups	Dose•	Body Weight (g)				
		Before treatment	After 7 days	After 14 days	After 21 days	After 28 days
Control	---	146.75±1.90^{a}	158.02±2.05^{a}	171.04±2.60^{a}	183.70±1.50^{a}	202.08±1.25^{a}
Caramel	Low	145.96±2.14^{a}	158.59±2.23^{a}	169.95±2.09^{a}	182.92±0.60^{a}	199.29±2.60^{a}
	High	146.80±2.07^{a}	156.74±1.19^{a}	170.22±2.05^{a}	183.15±2.39^{a}	201.75±2.10^{a}
Sunset yellow	Low	148.10±1.65^{a}	159.18±3.82^{a}	170.88±1.95^{a}	182.00±3.84^{a}	200.55±2.44^{a}
	High	146.95±2.86^{a}	157.99±3.86^{a}	164.23±3.10^{b}	171.33±3.15^{b}	180.25±2.77^{b}
Tartrazine + Brilliant blue	Low	145.37±3.90^{a}	157.10±2.76^{a}	171.55±1.94^{a}	171.15±1.89^{b}	181.90±1.65^{b}
	High	147.25±2.18^{a}	156.98±3.05^{a}	163.19±2.75^{b}	169.92±2.70^{b}	174.12±3.08^{c}

Mean ± Standard deviation for body weight; the means having significant different between control and treated groups (*P≤0.05*); Dose: (mg/kg b.w. /day). Low dose: equal ADI; High dose: equal 5 times ADI.

3.2 Impact of the oral administration of tested colorants on relative organs weight of tested male albino rats

From the obtained data (Table 2), it could be observed that the oral administration of caramel IV in doses equal (ADI and 5×ADI) and sunset yellow in dose equal ADI for 28 days did not effect on liver and kidney weights. While animals received sunset yellow in high dose equal (5xADI) and mixture of (tartrazine + brilliant blue) in both of low and high dose showed significant increase in liver and kidney weights compared with control group animals.

Table (2): Impact of oral administration of caramel IV, sunset yellow and (tartrazine + brilliant blue) in doses equal to ADI and 5 times ADI on liver and kidneys weights of male albino rats.

Groups	Dose•	Final body weight (g)	Parameter (Means ± SD)			
			Liver weight		Kidneys weight	
			Absolute (g)	Relative••	Absolute (g)	Relative••
Control	---	202.08±1.25^{a}	6.86±0.08^{b}	3.39±0.05^{b}	1.38±0.02^{b}	0.68±0.009^{b}
Caramel	Low	199.29±2.60^{a}	6.87±0.08^{b}	3.44±0.04^{b}	1.38±0.05^{b}	0.69±0.05^{b}
	High	201.75±2.10^{a}	6.86±0.10^{b}	3.40±0.08^{b}	1.39±0.02^{b}	0.69±0.02^{b}
Sunset yellow	Low	200.55±2.44^{a}	6.96±0.06^{b}	3.47±0.07^{b}	1.38±0.06^{b}	0.68±0.06^{b}
	High	180.25±2.77^{b}	8.05±0.05^{a}	4.46±0.03^{a}	1.50±0.03^{a}	0.83±0.01^{a}
Tartrazine + Brilliant blue	Low	181.90±1.65^{b}	8.02±0.09^{a}	4.40±0.009^{a}	1.49±0.01^{a}	0.81±0.04^{a}
	High	174.12±3.08^{c}	8.11±0.04^{a}	4.65±0.03^{a}	1.52±0.03^{a}	0.87±0.02^{a}

Mean ± Standard deviation for absolute and relative organs weights; the means having significant difference between control and treated groups *(P≤0.05)*; Dose: (mg/kg b.w. /day); Low dose: equal ADI; High dose equal 5 times ADI; •• Relative weight (g /100 g b.w.).

These results are coincidence with Mackenzie *et al.* (1992) who indicated that the relative weights of liver, kidneys, brain and testes were increased in rats treated with 2.5, 5.0, 7.5 and 10 g/kg b.w. of caramel IV for 13 weeks. Himri *et al.* (2011) who observed that relative weight of liver was significantly changes in rats treated with 10 mg/kg b.w. of tartrazine. Balta *et al.* (2019) who found that relative weight of the liver and kidneys of rats which received a high dose 75 mg/kg of tartrazine were significantly increase compared with control.

3.3 Effect of the oral administration of tested colorants on antioxidant enzymes activity of tested male albino rats

The activity of glutathione peroxidase (GPX), superoxide dismutase (SOD) and catalase (CAT) enzyme constitutes a first-line defense against oxidative stress in biological systems (Ighodaro and Akinloye, 2017). Oral administration of tested colorants in doses equal ADI (low dose) and 5 times ADI (high dose) on malondialdehyde (MDA) and some antioxidant enzymes levels of rats are presented in Table (3). The obtained data showed a significant ($P \leq 0.05$) decrease in the activity of SOD, GSH, CAT, and GPX accompanied with significant ($P \leq 0.05$) increase in serum MDA in rats treated with high dose of sunset yellow and both low and high dose of (tartrazine + brilliant blue) when compared to control and other treated groups. This increase in malondialdehyde level in this study may be attributed to its direct effect of induced generation of reactive oxygen species resulted from tested colorants administration. It was explained that malondialdehyde level is increased as a product of lipid peroxidation occurred by reactive oxygen species action on lipids of cellular membrane (Amin *et al.*, 2010) led to increasing free radicals that can cause auto-oxidation of cells (Bu *et al.*, 2015). These results are approximately similar with Amin *et al.* (2010) who reported that treated rats with synthetic colorants (carmoisine and tartarzine) led to decrease in reduce glutathione and catalase activity. Saxena and Sharma (2015) who indicated that food colorant; Tartrazine, metanil yellow and sunset yellow induced a significant increase in serum malondialdehyde and significantly lowered levels of Super oxide dismutase, reduce glutathione and catalase enzymes in albino rats. Tawfek *et al.* (2015) who found that highly significant increase in malondialdehyde, Glutathione Peroxidase and catalase activities in treated rats with sunset yellow and tartrazine. Marins *et al.* (2020) found that administration of caramel IV dye in dose 3 g/kg b.w. induced oxidation damage in liver and kidney by increase of malondialdehyde and reduction of free SH group.

Table (3): Impact of oral administration of caramel IV, sunset yellow and (tartrazine + brilliant blue) on some oxidative stress markers of tested rats.

Groups	Dose•	Oxidative stress markers (Mean±SD)				
		MDA (Mmol/L)	SOD (U/L)	GSH (µg/mL)	CAT (Mmol/L)	GPX (µg /mL)
Control	---	34.83±0.87^{c}	36.44±1.53^{a}	12.40±0.49^{a}	64.33±1.05^{a}	32.40±1.22^{a}
Caramel	Low	33.70±1.19^{c}	35.29±1.70^{a}	12.25±1.20^{a}	65.86±2.66^{a}	30.12±1.97^{a}
	High	35.43±1.50^{c}	37.01±1.51^{a}	11.72±0.90^{a}	65.16±1.91^{a}	32.69±2.55^{a}
Sunset yellow	Low	36.18±0.95^{c}	36.95 ±1.12^{a}	12.26±0.60^{a}	62.24±3.20^{a}	31.25±3.16^{a}
	High	51.14±1.26^{b}	21.88±2.15^{b}	7.14±0.32^{b}	44.35±2.17^{b}	20.16±1.82^{b}
Tartrazine + Brilliant blue	Low	49.55±1.17^{b}	20.75±1.89^{b}	6.98±0.50^{b}	46.80±1.98^{b}	21.87±0.95^{b}
	High	85.07±2.10^{a}	14.66±2.15^{c}	3.24±0.06^{c}	21.05±2.74^{c}	14.74±1.40^{c}

Mean ± Standard deviation; the means having significant different between control and treated groups ($P \leq 0.05$). MDA: Malondialdehyde; SOD: Super oxide dismutase; GSH: reduce glutathione; CAT: Catalase; GPX: Glutathione Peroxidase; Low dose: equal ADI and High dose: equal 5 times ADI.

3.4 Biochemical alterations induced by tested synthetic colorants on tested male albino rats

The impact of the oral administration of caramel IV, sunset yellow and both of (tartrazine + brilliant blue) in doses equal to ADI and 5 times ADI on some biochemical markers of tested male rats are presented in table (4). From the current data, it could be noticed that there were no significant (*P≤0.05*) difference in the level of aspartate amino transferase (AST), alanine amino transferase (ALT) and alkaline phosphatase (ALP), urea and creatinine activity in treated rats with both low and high dose of caramel and that treated with low dose of sunset yellow compared to control group. On the other hand, the level of AST, ALT, ALP, urea and creatinine were significantly increased in rat's administration of high dose of sunset yellow (and both low and high dose of (tartrazine + brilliant blue) when compared to control animals. Liver functions enzymes such as ALT, AST and ALP are not secreted into the blood; any increase of their activities in blood is resulted from leakage of liver damage cells and dysfunctions in liver functional enzymes (Attia and Nasr, 2009). (Abdel-Rahim *et al.*, 1989) reported that any elevation in aminotransferase activity may be attributed to the changes in liver function and hepatocellular impairment which subsequently caused the release of greater than normal levels of intracellular enzymes into the blood.

Table (4): Alterations in biochemical parameters of tested rats after 28 days of oral administration with caramel IV, sunset yellow and (tartrazine+brilliant blue).

Groups	Dose•	Biomarker (Mean±SD)				
		AST (IU/L)	ALT (IU/L)	ALP (IU/L)	Urea (mg/dl)	Creatinine (mg/dl)
Control	---	36.04±0.93^{d}	24.81±0.72^{d}	86.34±0.89^{c}	45.26±1.23^{d}	0.64 ±0.02^{d}
Caramel	Low	35.73±0.75^{d}	26.31±0.88^{d}	85.20±1.15^{c}	45.90±1.85^{d}	0.62± 0.05^{d}
	High	36.21±1.17^{d}	26.18±2.05^{d}	87.11±1.22^{c}	46.18±0.94^{d}	0.63± 0.01^{d}
Sunset yellow	Low	38.68±0.94^{d}	24.95±1.46^{d}	85.94± 0.91^{c}	47.21±0.68^{d}	0.64±0.09^{d}
	High	45.77±1.32^{c}	41.55 ±1.15^{c}	103.76±2.06 ^{b}	69.75 ±1.43 ^{c}	0.78±0.06^{c}
Tartrazine + Brilliant blue	Low	52.60±1.14^{b}	49.33±0.93^{b}	104.55±1.84 ^{b}	126.13±2.16^{b}	0.85±0.02^{b}
	High	68.82±1.56^{a}	62.10±1.28^{a}	118.29±1.39^{a}	165.22±1.72^{a}	1.13±0.04^{a}

Mean ± Standard deviation for biochemical parameters; the means having significant different between control and caramel treated groups *(P≤0.05)*; Low dose: equal ADI and High dose: equal 5 times ADI; AST: aspartate transaminase; ALT: alanine transaminase; ALP: alkaline phosphatase.

Also, elevation of urea and creatinine in serum of treated male albino rats may be attributed to a reduction in glomerular filtration in the kidney and also reflect dysfunction of the kidney tubules (Al-Hashem *et al.*, 2009). Our results are approximately similar to data reported by Mahmoud (2006) who reported that rats treated with a single high dose (0.4 g/kg diet) of synthetic brilliant blue dye caused an increase in AST, ALT, ALP activities. Tawfek *et al.* (2015) who found that rats administrated with 20 mg/kg tartrazine and 200 mg/kg sunset yellow had a highly significant increase in ALT, AST, ALP, total bilirubin, urea and creatinine.

Khayyat *et al.* (2018) who indicated that an increase in the biochemical markers of hepatic and renal function including AST, ALT, urea, uric acid and creatinine in rats administrated with 2.5 mg/kg b.w from sunset yellow and 7.0 mg/kg b.w. from allura red.

3.5 Histopathological alterations

Results indicated that, there were no histopathological changes in the livers and kidneys of the control group (Figures A1 and B1) and treated groups with low dose and high dose of caramel IV (Figures A2 and A3) and (Figures B2 and B3); respectively. Also, no histopathological alterations were found in the livers and kidneys of treated groups with low dose of sunset yellow (Figures A4 & B4). On the other side, some histopathological alterations were found in the liver of rats treated with high dose of sunset yellow (Figure A5) and both of low and high doses of (tartrazine + brilliant blue) (Figures A6, A7 and A8) including pyknosis, inflammatory infiltration, cellular infiltration, disarray in hepatic cords, congested blood vessel and thickened wall bile duct.

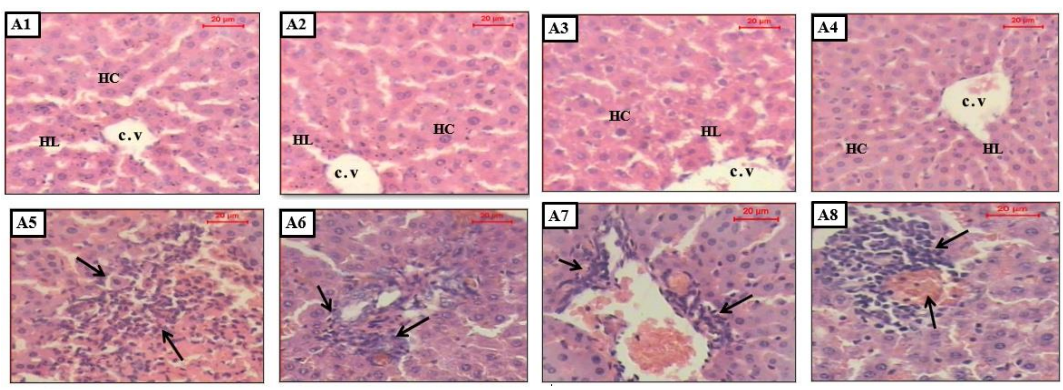

Figure (A): Histopathological examination photomicrographs for liver sections of male albino rats as affected by oral administration of caramel IV, sunset yellow and (tartrazine+brilliant blue) for 28 days. A1: control rats group; A2: rats group treated with oral low dose of caramel IV; A3: rats group treated with oral high dose of caramel IV and A4: rats group treated with oral low dose of sunset yellow showing normal hepatocytes and blood sinusoids. A5: rats group treated with oral high dose of sunset yellow showing inflammatory infiltration, disarray in hepatic cords and congested blood vessel. A6: rats group treated with oral low dose of (tartrazine+brilliant blue) showing pyknosis, cellular infiltration, blood congested and thickened wall bile duct. (A7 and A8): rats group treated with oral high dose of (tartrazine + brilliant blue) showing pyknosis, cellular infiltration and congested blood vessel (H & E stain. scale bar: 20µm).

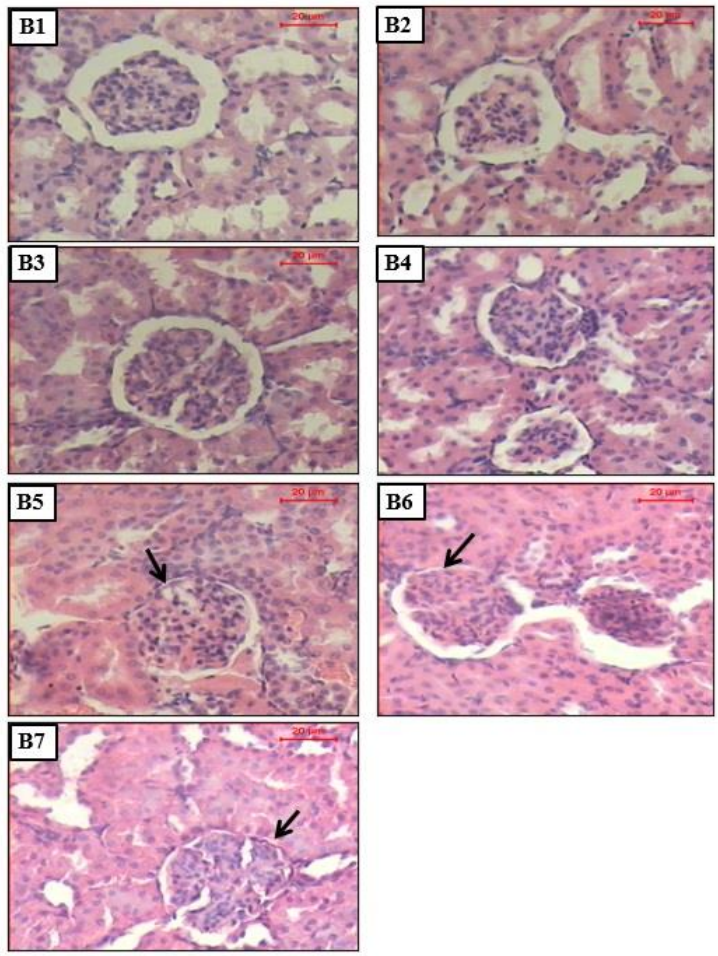

Figure (B): Histopathological examination photomicrographs for kidney sections of male albino rats as affected by oral administration of caramel IV, sunset yellow and (tartrazine + brilliant blue) for 28 days. B1: control rats group; B2: rats group treated with oral low dose of caramel IV; B3: rats group treated with oral high dose of caramel IV and B4: rats group treated with oral low dose of sunset yellow showing normal structure of glomerulus and renal tubules. B5: rats group treated with oral high dose of sunset yellow showing hypercellularity of the glomeruli and narrowing of the urinary space. B6: rats group treated with oral low dose of (tartrazine + brilliant blue) showing infract glomerulus, cellular infiltration and hypotrophy of the glomerulus. B7: rats group treated with oral high dose of (tartrazine + brilliant blue) showing lobulated glomsuluy with cellular infiltration. (H & E stain. scale bar: 20µm).

Similarly, kidneys of rats treated with high dose of sunset yellow (Figure B5) and both of low and high doses of (tartrazine + brilliant blue) (Figures B6 and B7) showed that some alterations including hypercellularity of the glomerular, narrowing of the urinary space, infract glomerulus, cellular

infiltration and hypotrophy of the glomerulus. Similar histopathological effects were found in other studies by AL-Dahhan *et al.* (2014); Ali *et al.* (2016); Yadav *et al.* (2017); Mohamed *et al.* (2019) and Marins *et al.* (2020).

4. Conclusion

The present study indicated that, caramel color IV in doses equal to (ADI and 5 times ADI) and sunset yellow in dose equal to ADI did not cause any toxic effects on tested male albino rats. But the high dose of sunset yellow equal to 5 times ADI and mixture of (tartrazine + brilliant blue) at low (equal to ADI) and high doses (equal to 5 times ADI) caused significant changes in relative organs weights, antioxidant defense enzymes and biochemical parameters, in addition to inducing some histopathological alterations in the liver and kidneys of tested rats.

References

Aebi H (1984), "Catalase *in vitro*", Methods Enzymol, Vol. 105, pp. 121–126.

A.O.A.C. (2008), *Official Methods of Analysis*, 16th ed, Association of

Official Agriculture, Washington DC, Analytical Chemists International Arligton, Virginia, USA.

Abdel-Wahhab, H. M. F. and Moram, G. S. (2012), "Toxic effects of some synthetic food colorants and/or flavor additives on male rats", *Toxicology and Industrial Health*, Vol. 14 No. 5, pp. 1–9.

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", *Menofia Journal of Agricultural Research*, Vol. 14 No. 1, pp. 557.

Al-Dahhan, M. A. H., Al-Samawy, E. R. M., Al-Kaisei, B. I. and Jarad, A.S. (2014), "Effect of synthetic colorants (Sunset yellow and Ponceau 4R) in some biochemical and histopathological parameters", *Al-Qadisiyah Journal of Veterinary Medicine Sciences*, Vol. 13 No 1, pp. 80–84.

Ali, F. A., Abdelgayed, S. A., El-Tawil, S. O. and Baker, M. A. (2016), "Toxicological and histopathological studies on the effect of tartrazine in male albino rats", *International Journal of Pharmacological and Pharmaceutical Sciences*, Vol. 10 No.3, pp. 527–532.

Al-Hashem, F, Mohammed, D., Bashir, N., Mohammed, A., Riyadh, E., Mohammed, K. and Al-Khateeb, M. (2009), "Camels milk protects against cadmium chloride induced toxicity in white albino rats", *American Journal of Pharmacology and Toxicology*, Vol. 4 No. 3, pp. 107–117.

Al-Shinnawy, M. S. (2009), "Physiological Effect of a food

additive on some haematological and biochemical parameters of male albino rats", *Egyptian Academic Journal of Biological Sciences*, Vol. 2 No. 1, pp. 143–151.

Amin, A. A. and Al-Shehri, F. S. (2018), "Toxicological and safety assessment of tartrazine as a synthetic food additive on health biomarkers: A review", *African Journal of Biotechnology*, Vol. 17 No. 6, pp. 139–149.

Amin, K. A., Hameid, H. A. and Abd Elsttar, A. H. (2010), "Effect of food Azo ayes tartrazine and carmoisine on biochemical parameters related to renal, hepatic function and oxidative stress biomarkers in young male rats", *Food and Chemical Toxicology*, Vol. 48 No. 10, pp. 2994–2999.

Armitage, P., Berry, G. and Matthews, J. N. S. (1971), *Statistical Methods in Medical Research, Fourth Edition*, Wiley-Blackwell, USA.

Attia, A. M. and Nasr, H. M. (2009), "Dimethoate-induced changes in biochemical parameters of experimental rat serum and its neutralization by black seed oil", *Slovak Journal Animals Science*, Vol. 42 No. 2, pp. 87–94.

Balta, I., Sevastre, B., Mireşan, V., Taulescu, M., Raducu, C., Longodor, A. L., Marchiş, Z., Mariş, C. S. and Coroian, A. (2019), "Protective effect of blackthorn fruits (*Prunus spinosa*) against tartrazine toxicity development in albino Wistar rats", *BMC Chemistry*, Vol. 13, pp. 104.

Bawazir, A. E. (2016), "Impact of mix food colors with Barley Water (Talbina) on some neurotransmitters in different brain region biochemical and histological structure of liver male albino rats", *Journal of American Science*, Vol. 12, No. 11, pp. 94–103.

Belfield, A. and Goldberg, D. M. (1971), A colorimetric method for determination of alkaline phosphatase in serum, *Enzyme,* Vol. 12, pp. 561–565.

Bu, F., Li, T., Ding, Y., Sun, L., Tu, T., Zhou, Z., Qi, W., Jiang, X., Fang, J., Hu, J., Zhu, W. and Sun, X. (2015), "Cytotoxic effects of 4-methylimidazole on bone marrow mesenchymal stem cells *in vitro*", *American Journal of Translational Research*, Vol. 7 No. 10, pp. 1736–1746.

Chen, H. and Gu, A. (2014), "Effect of ascorbic acid on the properties of ammonia caramel colorant additives and acrylamide formation", *Journal of Food Science*, Vol. 79 No. 9, pp.1678–1682.

Delgado-Vargas, F. and Paredes-Lo´pez, O. (2003), *Natural Colorants for Food and Nutraceuticals Uses*, Chapter 4, CRC Press LLC, New York, USA.

Demirkol, O., Zhang, H. 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", *Journal für Verbraucherschutz und Lebensmittelsicherheit*, Vol. 8, No. 31, pp. 1–12.

Drury, R. A. and Wallington, E. A. (1980), *Carleton's Histological Technique*, 5th Edition, Oxford University Press, New York, USA.

EFSA (2009), "Scientific opinion on the re-evaluation of sunset yellow FCF (E 110) as food additives", *European Food Safety Authority Journal*, Vol. 7 No. 11, pp. 1330.

EFSA (2010), "Scientific opinion on the re-evaluation of brilliant blue FCF (E 133) as food additives", *European Food Safety Authority Journal*, Vol. 8 No. 11., pp. 1853.

EFSA (2011), "Scientific opinion on the re-evaluation of caramel colors (E 150 a, b, c, d) as food additives, EFSA panel on Food additives and nutrient sources added to food (ANS)", *European Food Safety Authority Journal*, Vol. 9, No. 3.

Faulkner, W. R. and King, J. W. (1976), "Renal function", In Tietz N. (ed.), *Fundamentals of clinical chemistry*, W. B. Saunders Co., Philadelphia, Pennsylvania, USA, pp. 975–1014.

Ferreira, L. G. B., Faria, R. X., Ferreira, N. C. D. and Soares-Bezerra, R. J. (2017), "Brilliant blue dyes in daily food: How could Purinergic system be affected", *International Journal of Food Science*, Vol. 2016 Article ID 7548498, pp. 13.

IARC (2013), "International Agency for Research on Cancer. "IARC monographs on the evaluation of carcinogenic risks to humans, 4-Methylimidazole", In: *Some chemicals present in industrial and consumer products, food and drinking water*, Vol. 101, pp. 447–459.

Ighodaro, O. M. and Akinloye, O. A. (2017), "First line defense antioxidants-superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX): Their fundamental role in the entire antioxidant defence grid", *Alexandria Journal of Medicine*, Vol. 54 No. 4, pp. 287–293.

Jacobson, M. F. (2013), "Carcinogenicity and regulation of caramel colorings", *International Journal of Occupational Medicine and Environmental Health*, Vol. 18 No. 3, pp. 254–263.

JECFA, (1992), *Joint FAO/WHO Expert Committee on Food Additives*, Compendium for Caramel Colour International Technical Caramel Association, Washington, DC, USA.

Hashem, M., Atta, A. H, Arbid, M. S., Nada, S. A., Mouneir, S. M. and Asaad, G. F. (2014), "Toxicological impact of amaranth, sunset yellow and curcumin as food coloring agents in albino rats", *Journal of Pakistan Medical Students*, Vol. 1 No. 2, pp.

43–51.

Himri, S., Bellahcen, S., Souna, F., Belmekki, F., Aziz, M., Bnouham, M., Arzohheir, J., Berkia, Z., Emekhfi, H. and Saalaoui, E. A. (2011), "Day Oral Toxicity Study of Tartrazine, as synthetic Food dye, in wistar rats", *International Journal of Pharmacy and Pharmaceutical Sciences*, Vol. 3 No. 3, pp. 0975–1491.

Kahoun, D., Řezková, S. and Královský, J. (2017), "Effect of heat treatment and storage conditions on mead composition", *Food Chemistry*, Vol. 219, pp 357–363.

Khayyat, L. I., Essawy, A. E., Sorour, J. M. and Soffar, A. (2018), "Sunset Yellow and Allura Red modulate Bcl2 and COX2 expression levels and confer oxidative stress-mediated renal and hepatic toxicity in male rats", *Peer Journal*, Vol. 6, Article ID e5689.

Khalil, M. I., Sulaiman, S. A. and Gan, S. H. (2010), "High 5-hydroxymethylfurfural concentrations are found in Malaysian honey samples stored for more than one year", *Food Chemistry and Toxicology*, Vol. 48, pp. 2388–2392.

Mackenzie, K. M., Boysen, B. G., Field, W. E. and Petsel, R. W. (1992), "Toxicity and carcinogenicity studies of caramel color IV in F344 rats and B6c3f mice", *Food Chemical and Toxicology*, Vol. 30 No. 5, pp. 431–443.

Mahmoud, N. H. (2006), "Toxic effects of the synthetic food dye Brilliant blue on liver, kidney and testes functions in rats", *Journal of the Egyptian Society of Toxicology*, Vol. 34, pp. 77–84.

Marins, E., Spanhol, J., Silva, D., Pasinato1, Z., da-Rosa, P. C., de-Oliveira, V. A., Moretto, J. G., Vargas1, J. L., Soares, F. A. A. and Barcelos, R. P. (2020), "Caramel dye IV increases hepatic and renal oxidative stress injuries", *Integrative Food, Nutrition and Metabolism*, Vol. 7, pp. 1–6.

Mohamed, E. G., Abo-laila, I. M. and Masoud, M. A. (2019), "Fortification role of curcumin against renal and testicular toxicity of synthetic food dye brilliant blue in rats", *Journal of Drug Delivery & Therapeutics*, Vol. 9, No. 2, pp. 1–8.

Ohkawa, H., Ohishi, N. and Yagi, K. (1979), "Assay of lipid peroxides in animal tissues by thiobarbituric acid reaction", *Annals of Biochemistry*, Vol. 95, pp. 351–358.

Paglia, D. E. and Valentine, W. N. (1967), "Studies on quantitative and qualitative characterization of erythrocyte glutathione peroxidase", *Journal of Laboratory and Clinical Medicine*, Vol. 70, pp. 158–169.

Patton, C. J. and Crouch, S. R. (1977), "Spectrophotometric and kinetics investigation of the Berthelot

reaction for the determination of ammonia", *Analytical Chemistry*, Vol. 49, pp. 464–469.

Pereira, V., Albuquerque, F. M., Ferreira, A. C., Cacho, J. and Marques, J. C. (2011), "Evolution of 5-hydroxymethylfurfural (HMF) and furfural (F) in fortified wines submitted to overheating conditions", *Food Research International*, Vol. 44, pp. 71–76.

Ping, X. U., Xing-Guo, Z., You-Ming, L. I., Chao-Hui, Y. U., Lei, X. U. and Gen-Yun, X. U. (2006), "Research on the protection effect of pioglitazone for non-alcoholic fatty liver disease (NAFLD) in rats", *Journal of Zhejiang University Science*, Vol. B No. 8, pp. 627–633.

Ridnour, L. A., Winters, R. A., Ercal, N. and Spitz, D. R. (1999), "Measurement of glutathione disulfide, and other thiols in mammalian cell and tissue homogenates using high-performance liquid chromatography separation of N-(1-pyrenyl) maleimide derivatives", *Methods in Enzymology*, Vol. 299, pp. 258–267.

Sadar, P., Dande, P., Kulkarni, N. S. and Pachori, R. R. (2017), "Evaluation of toxicity of synthetic food colors on human normal flora and yeast", *International Journal of Health Sciences and Research*, Vol. 7 No. 8, pp. 110–114.

Saxena, B. and Sharma, S. (2015), "Food color induced hepatotoxicity in Swiss albino rats", *Toxicological International*, Vol. 22 No. 1, pp. 152–157.

Schmidt, E. and Schmidt, F. W. (1963), "Colorimetric method for determination of transaminases; GOT & GPT", *Enzymologia Biologica et Clinica*, Vol. 3, pp. 1.

Tawfek, N. S., Amin, H. M., Abdalla, A. A. and Faragali, S. H. M. (2015), "Adverse effects of some food additives in adult male albino rats", *Current Science International*, Vol. 4 No. 4, pp. 525–537.

USFDA (2012), *21 CFR 73.85 - Caramel*, United States Food and Drug Administration, available at: http://www.fda.gov/ForIndustry/ColorAdditives/ColorAdditiveInventories/ucm106626.htm, Accessed 2012 July 23.

Wang, L., Ren, B., Liu, Y. and Lu, Y. (2015), "2- Acetyl-4-tetrahydroxybutylimidazol and 4-methylimidazole in caramel colors, vinegar and beverges in China", *Food Additives and Contaminants: Part B Surveillance*, Vol. 8, No. 3, pp. 163–168.

Xu, X. B., Liu, D. B., Yu, S. J., Yu, P. and Zhao, Z. G. (2015), "Separation and determination of 4-mehtylimidazole, 2-methylimidazole and 5-hydroxymethyfurural in beverages by amino trap column coupled with pulsed amperometric detection", *Food Chemistry*, Vol. 169, pp. 224–229.

Yadav, S., Kalra, S. and Wadhwa, S. (2017), "Destructive effects of 4-methylimidazol on histomorphology of liver", *Journal of morphological sciences*, Vol. 34 No. 4, pp. 232–235.