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Protective Effects of Vitamin C on Tartrazine and Allura Red-Induced Toxicity in Male Albino Rats

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ABSTRACT

Background: The food industry relies heavily on the incorporation of artificial pigments as additives, which significantly influences market demand.

Objective: To investigate the adverse effects on some hematological, immunological, biochemical, and antioxidant parameters induced by the administration of tartrazine and Allura red to male albino rats and how vitamin C protects against these effects.

Materials and Methods: 36 adult male albino rats were divided into 6 groups, each with six rats, and group 1 was administered saline orally (1 ml/rat); group 2 was administered 200 mg/kg of vitamin C; group 3 was administered 75 mg/kg of tartrazine; group 4 was administered 70 mg/kg of Allura red; group 5 was administered both tartrazine and vitamin C; and group 6 was administered Allura red and vitamin C.

Results: After six weeks, our results revealed a significant decline in RBC numbers accompanied with decreasing hemoglobin concentrations in the tartrazine or Allura red groups. Oxidative stress was also detected through significant increasing levels of lipid peroxidation (MDA) and nitric oxide (NO) activities and significant decreasing levels of reduced glutathione, catalase, and superoxide dismutase in the liver. Tartrazine or Allura red significantly increased the serum inflammatory cytokines INF-γ, TNF-α, IL-1β, and IL-6. Serum ALT, AST, ALP, GGT, AFP, total bilirubin, urea, creatinine, total lipids, triglycerides, total cholesterol, LDL, and HDL were significantly elevated in the tartrazine and Allura red groups. On the other hand, combining vitamin C with tartrazine or Allura red significantly modulated all those previous parameters.

Conclusion: We could conclude that administration of tartrazine or Allura red in rats resulted in significant changes in hematological, immunological, biochemical, and antioxidant parameters, indicating tartrazine and Allura red toxicity at the given dose level. Vitamin C had a protective role against adverse effects induced by tartrazine and Allura red.

Key words: Tartrazine, Allura red, Vitamin C, Oxidative stress, Inflammatory cytokines, Liver damage.

INTRODUCTION

Good food and nutrition are essential for good health and wellbeing. On the other hand, many manufactured foods may be high in artificial ingredients, which may cause a variety of health harms. Synthetic coloring agents play a significant role in the food industry to stay ahead in the market game. Approximately 60 to 70% of the synthetic colorants utilized within the food industry consist of azo dyes. Food colorings are available in two forms: either as naturally derived or artificially produced substances. They can be used in making food more attracting for consumers and to enhance food appearance, or restore colors lost during processing. Food colorants serve a dual purpose in the food industry, being utilized both in mass food production and household culinary activities [(1)].

Over the past five decades, the consumption of artificial food coloring has risen due to food manufacturers' preference for them over natural counterparts, resulting in a more vivid and striking coloration. Various colored dyes are included in the composition of cosmetic products, medications, and beverages and edibles. Over the recent years, there has been a significant surge in the utilization of artificial food dyes within Egypt, especially in foods targeted towards children, with no regulation or oversight. Several studies demonstrated that food dyes can cause several risks as irritability, allergies, depression, and aggressiveness and azo dyes have genotoxic, mutagenic and carcinogenic properties [(2)].

Tartrazine is an artificial azo dye, documented as E102, that can provide a lemon-yellow color and can be used in drugs, cosmetics, and foods, including dietary supplements, beverages, powder mixes, gelatin products, baked goods and dairy products. It has been suggested that tartrazine could cause many different adverse effects as nervousness, pathological lesions in the brain, asthma attacks, hazy vision, eczema, skin rashes and allergic responses in individuals [(3-5)].

Inside the body, tartrazine is transformed into sulfanilic acid, which is categorised as an aromatic amine. The resultant metabolite may produce reactive oxygen species, which could cause oxidative stress. Additionally, it can increase lipid peroxidation and restrict the body's naturally existing antioxidant defence enzymes from performing their normal activities. This could lead to an accelerated state of oxidative stress, which could impair a number of critical cellular components and ultimately cause cell death [(6,7)]. It was reported that overdoses of tartrazine may induce hepatic and kidney pathological changes [(8)].

Allura red is also known as FD and C red 40. This is a food dye with E129 as its E number. E129 is used to color gelatins, desserts, dairy products, sweets, drinks, condiments and numerous other commodities [(9)].

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It has been found that Allura red consumption causes release of aromatic amine that can cause some disorders and it could prompt allergic reactions in persons with preexisting sensible asthma [10]. Also, it was demonstrated that Allura red could change azo-reductase enzymes in the intestinal bacteria and in liver cells and cause frequent headaches in adults and hyperactivity in children [11].

Ascorbic acid, also known as vitamin C, serves as a relevant enzyme cofactor with a low molecular weight and is soluble in water. This vitamin is crucial in managing oxidative stress due to its antioxidant great efficacy, as it possesses the capacity to easily offer electrons, thereby safeguarding crucial biomolecules such as proteins, lipids, carbohydrates, and nucleic acids from harm by oxidants produced during cellular metabolism. Vitamin C also reduced inflammatory responses that can influence numerous vital biological reactions and contribute to its immune-modulating effects [12,13].

We conducted this study to showcase the potential adverse consequences of Allura red and tartrazine by examining the impacts these substances had on the immune, blood, and biochemical systems of male albino rats and to examine whether consuming vitamin C could act as a safeguard against any harmful consequences resulting from Allura red and tartrazine ingestion.

MATERIALS AND METHODS

Chemicals and Biochemical kits:

Tartrazine (CAS 1934-21-0, Purity 86.7%) and Allura red (CAS 25956-17-6; 80% purity) were bought in the form of powder from Elsafa Medical Company; EL-Minia, Egypt. Vitamin C (Ascorbic acid) was purchased from the pharmacy as a product of Unipharma (Chem. Co., Egypt). Elabscience Chemical Company was the source of the rat cytokine serum, TNF-α, INF-γ, IL-6, IL-1β, and AFP kits. Bio-diagnostic supplies were used to purchase kits for evaluating lipid profile, liver and kidney function, as well as antioxidant enzymes.

Animals

We used 36 male white rats in our study. They weighed around 150-160 grams. The rats were put in a room where the temperature was 25 degrees Celsius, the air was slightly wet (60% humidity), and the light was turned on for 12 hours and then turned off for another 12 hours. Rats were fed on commercial rodent pellets and water under good hygienic laboratory conditions. Animals were kept for two weeks before the experiments were conducted for adaptation.

Experimental Design:

Following a period of adaptation, the rats were segregated into 6 groups, consisting of a control and five treated groups, each comprising six rats. Daily oral administration of the treatment spanned over a period of six weeks in which **Group i:** considered as normal control group in which rats were administered saline solution (1 ml/rat). **Group ii:** rats received vitamin C in a dose of (200 mg/Kg) [14]. **Group iii:** rats received tartrazine (75 mg/Kg) [15]. **Group iv:** rats received Allura red (70 mg/Kg) daily [5]. **Group v:** rats received both tartrazine (75 mg/Kg) and vitamin C (200 mg/Kg). **Group vi:** rats received both Allura red (70 mg/Kg) and vitamin C (200 mg/Kg).

The creatures of all clusters were anesthetized and killed after the trial, and two blood specimens were taken from each rodent. One of the samples was allowed to coagulate for a duration of 20 minutes. The sample was subjected to a temperature of 37 degrees Celsius and then spun at 4000 revolutions per minute for 15 minutes. Afterward, the serum was isolated and preserved at a temperature of -20℃ in order to conduct biochemical and immunological investigations. To determine the hematological aspects, the other blood sample was gathered in tubes containing heparin. The liver was promptly collected from every animal following dissection, cleaned with saline, and blended in 5 mL of 50 mM phosphate buffer (pH 7. 8). After centrifuging the homogenate at 4,000g for 20 minutes, the resulting supernatants were preserved at -80C for subsequent assessment of indicators for assessing oxidative stress.

Hematological studies:

Blood samples containing heparin were analyzed to determine the number of red and white blood cells (RBCs and WBCs), hemoglobin concentration (Hb), Packed cell volume (PCV), and the distribution of WBCs based on established techniques utilizing an Animal Blood Counter.

Immunological studies:

Serum rodent INF-γ, IL-1β, TNF-α, IL-6 ELISA packs were based on a sandwich-ELISA guideline in which all plates were pre-coated with particular counteracting agents to rodent cytokines. Guidelines and tests were included for wells combined with a particular counteracting agent. Biotinylated location counteracting agents, particularly for rodent cytokines and Avidin-Horseradish Peroxidase (HRP) conjugate were included progressively in each plate well and cleared out to be hatched. Free components were washed away. The substrate arrangement was included in each well and the plate showed up blue. The enzyme-substrate response was ended by the expansion of the halt arrangement and the color turned to yellow. At wavelength 450 nm optical density (OD) was measured.

Biochemical assays:

Markers of oxidative stress, including malondialdehyde (MDA) and nitric oxide (NO), were examined in liver homogenate using bio-diagnostic assay kits as directed by the manufacturer. The

antioxidant enzymes reduced glutathione (GSH), super-oxide dismutase (SOD), and catalase were also analyzed in the same manner. Bio-diagnostic kits were used to analyze various biomarkers including serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), gamma-glutamyl transferase (GGT), total protein, albumin, urea, creatinine, total lipids, triglycerides, total cholesterol, low-density lipoprotein, and high-density lipoprotein via color-metric measurement.

Ethical considerations:

All the experimental procedures were carried out according to the principles and guidelines of the Ethics Committee of the of Faculty of Science, Minia University, Egypt, which conformed to "Guide for the care and use of Laboratory Animals" for the use and welfare of experimental animals, published by the US National Institutes of Health (NIH publication No. 85–23, 1996).

Statistical Analysis

The data collected from every group was presented as an average with Standard Error (SE), and then analyzed with SPSS program version 16. 0 from Chicago, IL, USA. The groups were compared using one way ANOVA and then further analyzed for differences using the post-hoc least significant difference method (LSD). A significant result was considered to be achieved when the P-value was equal to or less than 0.05**.**

RESULTS

Hematological parameters:

The present study revealed that significant decrease in RBCs count associated with a reduction in hemoglobin quantity was observed in rats treated with tartrazine or Allura red when compared to control group. However, no significant changes were observed in PCV, MCH, MCV, and MCHC.

Notable elevations in both overall white blood cell count and percentage of lymphocytes, along with a noteworthy drop in neutrophil percentage, were detected among subjects who received tartrazine or Allura Red. Only the group that received Allura red showed a significant increase in the percentage of monocytes. Alternatively, when vitamin C was administered together with tartrazine or Allura red, there was a notable enhancement in the number of red blood cells and hemoglobin levels. Additionally, the alteration in white blood cell index was regulated, as evidenced in table 1.

Table (1): Hematological changes in control, vitamin C, tartrazine, Allura red, tartrazine and vitamin C and Allura red and vitamin C rat groups

Groups / Parameter	Control	Vitamin C	Tartrazine	Allura red	Tartrazine and Vit C	Allura red and Vit C
RBCs ($x10^6$ µl)	7.60 ± 0.3	7.94 ±0.12	5.98 ±0.02 ***	5.87 ± 0.27 ***	7.06 ±0.03 #	7.16 ±0.34 ##
Hb (g/dl)	13.54 ±0.96	15.13±0.27	10.08 ±0.49**	10.32 ±0.37 **	14.01 ± 1.39 ###	13.73 ± 0.94 ##
PCV %	44.76 ±3.35	49.45 ±0.71	45.13 ±1.67	43.12 ±1.66	45.68 ± 3.37	46.26 ±3.06
MCV (fl)	58.76 ±2.62	62.33 ±1.36	64.67 ±6.10	78.46 ±5.45	62.66 ±5.53	64.84 ±5.41
MCH (pg)	17.8 ±0.95	19.08 ±0.56	19.24 ±1.74	18.03 ±1.69	19.05 ±1.15	20.28 ±0.9
MCHC (g/dl)	30.47 ±2.84	30.63 ±0.89	30.75 ± 5.11	22.67 ±3.41	30.75 ±3.27	28.98 ±2.05
WBCs ($x10^3$ µl)	6.71 ±0.34	6.48 ± 0.19	9.02 ±0.32 ***	9.74 ±0.14 ***	7.54 ±0.39 ##	7.39 ± 0.24 ###
Lymphocyte %	44.83 ±3.78	40.01 ±0.27	53.45 ±2.20 **	54.04 ±4.60 **	45.5 ±3.29 ##	41.6 ±3.6 ##
Neutrophil %	48.26 ±5.15	50.29 ± 1.67	40.88 ±2.39 *	36.20 ±4.61**	48.28 ±1.16 #	47.13 ±4.82 ##
Acidophile %	0.6 ±0.15	1.5 ±0.3	0.53 ±0.12	1.58 ±0.32	0.48 ±0.11	2 ±0.3
Basophil %	0.03 ±0.006	0.3 ±0.006	0.08 ±0.02	0.22±0.004	0.03 ±0.007	0.66 ±0.015*
Monocyte %	5.66 ±1.12	7.48 ±1.37	5.16 ±0.83	7.15 ±0.64*	5.71 ±1.06	7.6 ±1.4

Data are presented as mean ±standard error, * P <0.05, ** P <0.01, *** P <0.001 with respect to control rats. #P <0.05, ## P <0.01, ###P <0.001 with respect to rats administered tartrazine or Allura red.

Antioxidant enzymes and oxidative stress markers

Results in table (2) showed that administration of tartrazine or Allura red caused a significant increase in MDA and NO activities and a significant decrease in catalase, GSH and SOD compared to the control group. While, addition of vitamin C had the ability to reduce the increased concentrations of MDA and NO and increase the reduced concentration of GSH, SOD and catalase compared to tartrazine or Allura red groups in liver homogenate.

Table (2): Liver antioxidants in control, vitamin C, tartrazine, Allura red, tartrazine and vitamin C and Allura red and vitamin C rat groups

Groups / Parameter	Control	Vitamin C	Tartrazine	Allura red	Tartrazine and Vit C	Allura red and Vit C
MDA (nmol/g)	5.97 ±0.18	6.24 ±0.24	15.04 ±0.17 ***	14.91 ±0.34 ***	7.10 ±0.21 ###	8.05 ±0.66 *, ###
NO (µmol/g)	74.84 ±2.10	81.32. ±5.81	145.09 ±5.04 ***	103.40 ±2.01 ***	83.1±2.33 ###	82.75 ±7.24 #
GSH (mg/ g)	120. 86 ±2.37	111.45 ±0.06	68.37 ±3.31 ***	65.64 ±1.98 ***	89.94 ±4.71 *** ##	100.7 ±10.76 *, ###
Catalase (U/g)	2.19 ±0.17	1.97 ±0.02	0.95 ±0.04 ***	0.93 ±0.03 ***	1.90 ±0.05 ###	1.81 ±0.13 *, ###
SOD (U/g)	9.92 ±0.14	10.58 ±0.51	5.61 ±0.51 ***	7.27 ±0.55 ***	9.58 ±0.59 ###	9.92 ±0.17 ###

Data are presented as mean ±standard error, * P <0.05, *** P <0.001 with respect to control rats. #P <0.05, ## P <0.01, ###P <0.001 with respect to rats administered tartrazine or Allura red.

Inflammatory cytokines measurements

The present study demonstrated that rats consumed tartrazine or Allura red exhibited a significant increase in serum INF-γ, TNF-α, IL-1β, and IL-6 in comparison to the control group. Combining vitamin C with tartrazine or Allura red showed a significant modulation in those inflammatory cytokines (Table 3)

Table (3): Concentrations of some inflammatory cytokines in control, vitamin C, tartrazine, Allura red, tartrazine and vitamin C and Allura red and vitamin C rat groups

Parameter / Groups	INF-γ (pg/ml)	TNF- α (pg/ml)	Il-1β (pg/ml)	IL- 6 (pg/ml)
Control	0.65 ±0.03	39.12 ±1.81	59.74 ±5.65	55.92 ±1.85
Vitamin C	0.76 ±0.08	40.20 ±1.42	58.45 ±2.45	54.88 ±2.12
Tartrazine	1.24 ±0.14*	132.33 ±9.1***	99.20 ±7.22***	83.54 ±2.22***
Allura red	1.16 ±0.03*	138.47±10.13***	91.60 ±3.92***	83.23 ±3.10***
Tartrazine and Vit C	0.71 ±0.05#	80.47±2.19***, ###	70.89 ±4.61###	71.22 ±3.89**, #
Allura red and Vit C	0.75 ±0.03	85.64±2.81***, ###	71.59 ±1.50##	69.79 ±1.27**, ##

Data are presented as mean ±standard error, * P <0.05, ** P <0.01, *** P <0.001 with respect to control rats. #P <0.05, ## P <0.01, ###P <0.001 with respect to rats administered tartrazine or Allura red.

Liver and kidney functions measurements

Administration of tartrazine or Allura red significantly (P ≤0.001) resulted in increasing levels of ALT, AST, ALP, GGT, total bilirubin, and AFP. Serum albumin was decreased significantly in both tartrazine and Allura red groups while total protein was decreased only in the tartrazine group. Serum urea and creatinine were significantly increased in rats administrated with tartrazine or Allura red. Rats treated with vitamin C significantly improved liver and kidney function parameters by ameliorating all those previous parameters (Table 4).

Table (4): Liver and kidney function parameters in control, vitamin C, tartrazine, Allura red, tartrazine and vitamin C and Allura red and vitamin C groups

Groups / Parameter	Control	Vitamin C	Tartrazine	Allura red	Tartrazine and Vit C	Allura red and Vit C
ALT (U/ml)	49.56 ±10.63	46.33 ±3.58	80.53 ±8.18 ***	89.98 ±2.38 ***	53.21 ±3.58 ###	60.43± 6.69 ###
AST (U/ml)	46.86 ±3.57	55.77 ±3.03	95.57 ±4.81 ***	74.80 ±5.28 ***	65.36 ±5.80 ***, ###	62.83 ±1.78 **, #
GGT (U/L)	165.60 ± 8.53	167.26 ±7.03	331.06 ±45.82 **	395.68 ±40.93 ***	295.62 ±11.16	216.33 ±4.97 ###
ALP (IU/L)	75.62 ±7.36	73.95 ±4.36	133.72 ±7.47 ***	124.39 ±6.98 ***	87.73 ±1.89 ##	91.58 ±5.07 ###
ALF (pg/ml)	22.07 ±1.41	23.05 ±1.59	44.92 ±4.56 ***	42.64 ±3.77 ***	29.86 ±1.01 ###	28.5 ±2.14 ###
Total bilirubin (mg/dl)	0.47 ±0.11	0.29 ±0.02	1.25 ±0.29 **	1.01 ± 0.07 *	0.72 ±0.07 #	0.76 ±0.06
Albumin (g/dl)	5.42 ±0.63	6.04 ±0.11	3.19 ± 0.07 ***	3.18 ±0.08 ***	4.97 ±0.45 ###	4.36 ±0.41 *, ##
T. Protein (g/dl)	9.03 ±1.21	8.77 ±0.28	7.23 ±0.31 *	7.55 ±0.23	8.50 ±0.48	8.85 ±0.24
Urea	59.69 ± 5.05	66.37 ±4.05	146.5 ±6.39 ***	133.82 ±8.57 ***	88.08 ±1.27 ***, ###	91.08 ±4.72 ***, ###
Creatinine	1.14 ±0.12	1.13 ±0.08	1.56 ±0.13 **	1.79 ±0.05 ***	0.86 ±0.07 ***, ###	0.81 ±0.06 *, ###

Data are presented as mean ±standard error, * P <0.05, ** P <0.01, *** P <0.001 with respect to control rats. #P <0.05, ## P <0.01, ###P <0.001 with respect to rats administered Tartrazine or Allura red.

Lipid profile parameters

The present study demonstrated that rats administered with tartrazine or Allura red significantly resulted in a disturbance in lipid profile parameters in which serum total lipids, triglycerides, total cholesterol, LDL and HDL cholesterol were increased. Combining tartrazine or Allura red with vitamin C significantly reduced those increase in lipid profile parameters (Table 5).

Table (5): Lipid profiles concentrations in control, vitamin C, tartrazine, Allura red, tartrazine and vitamin C and Allura red and vitamin C groups

Parameter / Groups	Total Lipid	Triglycerides	T. Cholesterol	LDL	HDL
Control	160.60 ±9.65	66.42 ±3.72	60.28 ±2.15	35.32 ±3.94	31.49 ±5.10
Vitamin C	150.4 ±3.98	66.78±3.08	63.23± 2.11	36.85±2.51	35.23 ±3.84
Tartrazine	347.8 ±12.32***	108.54±4.75***	162.06±5.0***	87.01±2.82***	76.06 ±6.82 ***
Allura red	300.30 ±4.58 ***	108.06±3.43***	146.67±4.20***	81.36 ±3.23***	72.51± 4.27***
Tartrazine and Vit C	190.6 ±1.9 ###	66.95±3.52###	98.36±2.97 ###	67.91 ±1.12***	49.34±3.07**, ###
Allura red and Vit C	196.0±4.78 **, ###	66.41±3.61###	99.89±8.32**, ###	62.02 ±3.0***	54.99±4.80***, ##

Data are presented as mean ±standard error, * P <0.05, ** P <0.01, *** P <0.001 with respect to control rats. #P <0.05, ## P <0.01, ###P <0.001 with respect to rats administered Tartrazine or Allura red.

DISCUSSION

Exposure to repeated or high doses of food dyes has been reported to result in a lot of risks to human health. Studies involving laboratory animals suggest that frequent and long-term exposure to tartrazine harms gut health and promotes inflammation. Also, a recent study suggests that inflammatory bowel diseases (IBDs), Crohn's disease and ulcerative colitis could be kickstarted by consuming Allura red food dye over a long period of time. So, considerable attention from consumers, nutritionists, and toxicologists must be augmented [16,17]. Vitamin C offers various benefits including functioning as an antioxidant and anti-inflammatory agent. Our recent study discovered that its immune-modulating, antioxidant, and anti-inflammatory traits can help in reducing the toxicity brought by plant growth regulators in rats [18].

Tartrazine is reduced inside the body into sulfanilic acid [6], this metabolite can generate ROS which may cause oxidative stress. Additionally, tartrazine has the potential to stimulate the degradation of lipids and suppress the activities of enzymes that naturally protect against oxidative stress. As a result, this can hasten the occurrence of harmful oxidative stress, which can adversely affect various cellular constituents and ultimately result in cellular death [7,19]. It has been found that Allura red resulted in releasing of aromatic amine that can cause some health harms. Also, Allura red could change azo-reductase enzymes in the intestinal bacteria and in liver cells and cause toxic effects. The N2 group plays a crucial role in the formation of azo dye as it enables the combination of two dissimilar aryl/alkyl radicals or analogous radicals. The poisonous impact does not come directly from the original coloring substance, but rather from harmful by-products that are created during the breakdown process, including naphthalene, benzidine, toluene, and aniline [10,11].

Hematological parameters are appreciated tools for evaluating damages that are caused by any ingredients. Our study showed a marked lessening in RBCs count associated with a diminution in hemoglobin content in rats treated with tartrazine or Allura red compared to saline group along with leukocytosis, lymphocytosis, and decreased neutrophil percentage. These results came in accordance with **Alsolami** [9] and **Abd-Elhakim *et al.*** [15] who reported that oral administration of tartrazine resulted in severe anemia, leukocytosis and spleen abnormalities including red pulp hemorrhages and white pulp enlargement. The hematological values of rats were improved when they received an additional supply of vitamin C. The positive impact of vitamin C on hematological factors seen in this study could be attributed to its antioxidant properties, which safeguard the red blood cell membranes against oxidative harm. The positive effect of vitamin C on whole and differential counts of leukocytes may be because of its immunomodulation effect [20].

In the current study, increasing levels of malondialdehyde (MDA), which is the end product of lipid peroxidation and nitric oxide (NO), and reducing activities of redox enzymes (GSH, SOD, and CAT), indicates oxidative stress induction in both tartrazine and Allura red treated animals. The results were in agreement with the data reported by **Bawazir** [3] and **Abdel-Aziz *et al.*** [21]**.** These results indicate that administration of food dyes could result in increasing levels of free radicals, ROS production, dropping of antioxidant mechanisms [22]. Also, the present results indicated significant improvements in the levels of antioxidant enzymes and oxidative stress parameters as a result of vitamin C co-administration with food dyes. Vitamin C has the ability to function as a remover of ROS and could potentially be used to fight against oxidation-related harm in situations where oxygen concentration and apoptosis are on the rise. It was proved that vitamin C had the ability to increase the antioxidant parameters and reduce oxidative stress parameters in rats intoxicated with lead [23].

The present results demonstrated that both tartrazine and Allura red strangely increased serum TNFα, IFNγ, IL1β, and IL6, which are inflammatory cytokines. These results agreed with the findings of **Iheanyichukwu *et al.*** [24] who suggested that ingestion of tartrazine can increase expression of pro-inflammatory cytokines. Inflammatory diseases such as atherosclerosis, cancer, renal dysfunction, and neurological disease may be linked to the overproduction of cytokines with inflammatory properties [25], so, food dyes included in the present study could contribute to these diseases. The improvement in the levels of pro-inflammatory cytokines as a result of vitamin C administration in the present study came in accordance with **El-Senousey *et al.*** [26] who observed that ascorbic acid can reduce cytokines and mRNA expression of inflammatory markers in chickens under oxidative stress when added to their feed.

It was reported that overdoses of tartrazine may induce hepatic and kidney pathological changes [8]. Our study revealed a significant increases in liver enzymes as ALT, AST, ALP, GGT and bilirubin levels were observed in groups administrated with tartrazine or Allura red. Increasing activities of liver enzymes, in particular, aminotransferases in serum may be due to tissue damage particularly in liver, kidney and heart and the elevation in serum ALP and GGT may be an evidence of obstructive damage in the liver of rats administrated with tartrazine or Allura red. Hepatocellular damage may be due to changes in hepatocytes membrane permeability that lead to leaking cytoplasmic enzymes into the circulation and their actions in the serum increased and our result aligned with **Alsolami** [9]**, Khayyat *et al.*** [16] **and Hashem *et al.*** [27].

This study revealed that tartrazine or Allura red consumption caused a significant rise in alpha-

fetoprotein levels in adult male albino rats, but this effect was countered by vitamin C's antioxidant properties. Alpha-fetoprotein is a protein that emerges during fetal development and continues to be present in adulthood. Elevated alpha-fetoprotein levels detected in the bloodstream indicate potential liver malignancy, damage, or dysfunction resulting from the consumption of food dyes employed in the current investigation [(28)].

The present data indicated significant improving effect of vitamin C on liver toxicity and alteration in liver enzymes activities. In general, vitamin C has shown tremendous protective effect against drugs, chemicals, heavy metals, carbon tetrachloride, and food additives that induced hepatotoxicity. Administration of vitamin C with food azo dyes in the present study could modulate liver functions parameters by reducing the oxidative damage, lipid peroxidation and/or by preserving the antioxidant defence system [(29, 30)].

The classification of food colorants as harmful agents can be determined by their capacity to generate free radicals via metabolic activation, as evidenced by kidney biochemical markers [(10,15)]. Significant elevations of creatinine and urea were observed in tartrazine and Allura red treated rats compared to control group in the present study. The rise in these levels reveals the status of the kidney function and can increase different forms of kidney impairment indicating that tartrazine and Allura red could induce renal toxicity and kidney dysfunction and injury. The significant improving effect of vitamin C on kidney functions in our study may be due to its antioxidant capacity as it probably acts to remove ROS and limit products exposed to oxidation, which cause damage to the kidneys. Vitamin C pretreatment improved renal function with decreases in urea, creatinine, and malondialdehyde levels [(31)].

Administration of tartrazine or Allura red to rats in the present study caused significant alteration in lipids profile represented in increase in total lipids, triglycerides, total cholesterol, LDL and HDL cholesterol levels, when compared to control rats and these results agreed with **Tawfek *et al.*** [(32)] these changes might be attributed to the mobilization of free fatty acids from the adipose tissue to the blood stream and increase level of acetyl CoA leading to increase in the synthesis of cholesterol or due to peroxidation of cell membrane lipids. Also, increasing cholesterol concentration in the present study might be an indication of membrane structure and function disruption, thus influence its fluidity, permeability, activity of associated enzymes and transport system or may be due to the inability of the diseased liver to remove cholesterol from circulation [(33)].

The co-administration of vitamin C with tartrazine or Allura red showed a notable improvement in the lipid profile imbalance compared to food dyes rats. A previous investigation revealed that 12 weeks of 250 mg daily vitamin C supplementation yielded beneficial results in hemodialysis patients, including increased serum vitamin C levels, reduced MDA levels, and an improvement in lipid profiles [(34)]. Administration vitamin C could reduce the development of hypercholesterolemia in rabbits through its antioxidant property [(35)].

CONCLUSION

Tartrazine and Allura red are considered as the most mutual and approved food colorants used today and unfortunately our results indicated that these agents caused adverse effects in hematological, immunological, biochemical and antioxidant parameters and are toxic to kidney and liver functions in albino rats at the given doses and vitamin C had the ability to reduce most of the side effects caused by these food dyes because of its antioxidant and anti-inflammatory properties.

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