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Curcumin protects against tartrazine-mediated oxidative stress and hepatotoxicity in male rats

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Abstract. – OBJECTIVE: Synthetic dyes have been reported to exert detrimental effects on the health of humans. This study evaluated the effects of a diet containing tartrazine (Tz) on rats which included: i) biochemical parameters including hepatic enzymes, kidney functions and profiles of lipids; ii) markers of oxidative stress in cells by measuring concentrations of malondialdehyde (MDA) and glutathione (GSH); iii) activities of selected, key hepatic antioxidant enzymes including catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GPx); iv) pathologies of liver. Also, protective effects of three doses of curcumin (CUR), a natural food coloring agent, on these parameters in rats that had been co-exposed to Tz.

MATERIALS AND METHODS: Fifty Wistar male albino rats were randomly divided into five groups: Group I, control, where rats were fed a normal diet; Group II, rats were fed normal diets containing 7.5 mg Tz/kg diet, dry mass (dm); In Groups III, IV and V, rats were fed diets containing Tz plus 1.0, 2.0 or 4.0 g CUR/kg diet, dm, respectively. Whole blood was collected after 90 d of exposure, homogenates of liver were prepared and the above analyses were conducted.

RESULTS: Exposure to Tz in the diet caused statistically significant ($p<0.05$) greater concentrations of lipids, hepatic enzymes, and kidney function parameters as well as the indicator of oxidative stress MDA. Alternatively, activities of several antioxidant enzymes (i.e. CAT, SOD and GPx) and concentration of the substrate GSH, an indicator of non-enzymatic antioxidant capability, were significantly ($p<0.05$) less than those in control rats not exposed to Tz. Tz caused various histopathological changes in livers of rats, which were characterized by hemorrhage and dilatation of the central vein and sinusoids, hepatocyte necrosis, intracellular vacuolization. Co-administration of 2.0 (Group IV) or 4.0 g CUR/kg diet (Group V) with Tz significantly mitigated effects on functions of liver and kidney and the profile of relative concentrations of lipids. CUR significantly ($p<0.05$), and almost completely, reversed effects on enzymatic and non-enzymatic antioxidant and indicators of oxidative stress about rats fed Tz (Group II) to values in control rats. However, co-administration of 1.0 g CUR with Tz (Group III) exhibited a negligible effect on those parameters. The results of this study suggest benefits of the use of CUR, as a promising natural food additive to counteract oxidative stress caused by dietary exposure to the synthetic dye Tz due to potent protective antioxidant activity.

CONCLUSIONS: Blending some natural food additives, such as CUR with diets containing synthetic dyes, could moderate potential effects of these artificial dyes. Decreasing or removing toxins in food is an essential step for the amelioration of human health status and decreasing risk of onset or progression of degenerative diseases.

Key words:
Azo dyes, Lipid profile, Antioxidant enzymes, Liver functions, Kidney functions, Histopathology.

Introduction

Colors in food constitute an essential part of our life. Several years ago, the technology for processing food changed completely. Use of synthetic

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dyes, either separately or mixed for coloring foods, became more prevalent. Burned ash of minerals and plants were first used as dyes for purposes of appearance[1]. Colors in foods can be divided into dyes or pigments. Pigments are classified into organic or inorganic materials. These pigments are less soluble in aqueous media of food, while synthetic dyes are more soluble in processed food[2]. Synthetic dyes are classified into five categories: 1) azo dyes such as tartrazine (Tz) and sunset yellow; 2) chemophthalene such as Quinoline Yellow; 3) triaryl methane; 4) xanthenes and 5) indigo color. Annually, approximately 800 tons of synthetic dyes are manufactured globally, 60-70% of which are azo dyes. During processing of food, approximately 10-15% of dyes produced are introduced into the environment, which results in contamination of the ambient environment[3]. Yellow lemon azo dyes, such as Tz, are manufactured from coal tar. In 1996, Japan and the USA manufactured approximately $7.1x10^1$ and $9.9x10^2$ metric tons of Tz, respectively[4]. The chemical formula of the hydrophilic azo dye Tz is Trisodium 5-hydroxy-1-(4-sulfonatophenyl)-4-(E)-(4-sulfonatophenyl)diazenyl-1H-pyrazole-3-carboxylate.

Synthetic dyes have controversial effects on the health of humans. The results of some of these studies[5] have indicated that synthetic dyes exhibit their detrimental effects on human lymphocytes via direct interactions with genetic material like DNA. However, results of other studies have suggested that these dyes are not hazardous to humans and are not carcinogenic[6,7]. Therefore, the World Health Organization (WHO) and the Food and Drug Administration (FDA) of the United States of America (USA) regularly evaluate the safety and revise ADIs for dyes used to color food. Azo dyes, such as Tz, have been reported to cause adverse effects in animals, including inflammation of the lining of the stomach, increased lymphocyte and eosinophil counts when consumed by rats for extended periods[8]. In humans, detrimental effects of azo dyes, such as cancer of the bladder, have been reported for workers exposed occupationally to benzidine-based dyes[9,10]. The acceptable (admissible) daily intake (ADI) of Tz is 0-7.5 mg/kg body mass (bm)[11]. The main products of catabolism of azo dyes by mammalian gut microflora are aromatic amines, which might potentially be carcinogenic or mutagenic[12-14]. It has been previously reported that different doses of Tz induce hepatic and kidney pathological changes, which are primarily dependent factors such as: concentration, genetic predisposition, age, nutrition and duration of exposure to a particular dose[15,16]. In spite of their risks to the health of humans, Tz and sunset yellow comprise the majority of synthetic dyes used for coloring a variety of food products. A variety of consumer goods including: food products, such as potato chips, jelly, cereal products, candy and soft drinks; non-food products, such as shampoo and soaps; drugs, such as vitamins and capsules, colored with Tz are found on the market. However, due to their mutagenicity or carcinogenicity, some synthetic food colors have been discontinued. Recently, in the USA, only nine dyes have been licensed for use in foods, drugs or cosmetics[17]. Limited amounts of these dyes can be consumed and recommended amounts are given as admissible daily intakes (ADI)[18]. A limited concentration policy for azo dyes in foods, drugs and cosmetics has been emphasized and specified[19,20]. The use of natural colors with or without synthetic colors as food coloring agents is considered essential for control of oxidative stress in processed foods whereby promoting health status and minimizing risks of adverse effects in consumers. Widespread usage of natural colors in the food industry occurred due to mounting demands by consumers for safer foods with natural additives. Currently, 13 natural food colors are approved for coloring foods one of which is curcumin (CUR). CUR is found in the natural food spice turmeric, which has been extensively used because of its medicinal characteristics such as anti-inflammatory and antiseptic effects[21]. CUR is a yellow-colored, polyphenol compound that has been shown to protect against reactive oxygen species (ROS)-mediated oxidative damage to cellular components and it has been reported to exhibit some anticancer, antiviral (HIV) and antibacterial activities and accelerates detoxification in liver[21]. Mixing the natural food color, CUR with diets containing Tz fed to rats might reduce oxidative stress and toxicity of Tz.

This study was conducted to assess oxidative stress associated with exposure to artificial, azo dye, Tz by analyzing: i) the product of lipid peroxidation malondialdehyde (MDA) and the antioxidant enzymatic system (GPx, CAT and SOD); ii) liver function enzymes (ALT, AST, ALP and LDH); iii) kidney function parameters (creatinine, urea, uric acid); iv) histological changes in liver and v) the dose-dependent ameliorating capacity of CUR on these parameters in rats co-exposed to Tz.

Materials and Methods

Chemicals

Tartrazine (Tz) (C.I. 19140 CAS No. 1934-21-0, Mw 534,37; Synonyms: E 102, Food yellow 4, FD &

C yellow 5) is an azo dye with the chemical formula for trisodium salt: Trisodium 5-hydroxy-1-(4-sulfonatophenyl)-4-(E)-(4-sulfonatophenyl)diazenyl-1H-pyrazole-3-carboxylate, which was purchased from Sigma-Aldrich Corp. (St. Louis, MO, USA). The manufacturer assured purity of 86.7%.

Experimental Design of Animals

Animals

Male, albino, Wistar rats between 180 and 200 g, were obtained from the Animal House, Faculty of Pharmacy, King Saud University, Riyadh, Saudi Arabia. Rats were housed throughout the experiment in polypropylene cages (eight animals housed per cage) and allowed to acclimatize to the laboratory environment for seven days before the beginning of experiments. The animals were maintained under controlled conditions of temperature (23±1°C), humidity (50±15%) and normal photoperiod (12 to 12 h light-dark cycles). Rats were provided ad libitum, standard dry pellet diet and water. This study was conducted in the Zoology Department, Faculty of Science, King Saud University, Saudi Arabia. The care and handling of experimental animals were performed according to the Animal Ethical Committee of the College of Pharmacy, King Saud University.

Experimental Design

Rats were divided randomly into five groups. Tz and curcumin (CUR) were purchased from local markets and dissolved in deionized water and subsequently added to pellet diet and fed to rats daily for 90 d. Tz daily-administered dose of 7.5 mg/kg, dm diet was chosen based on a previous study[22], which is representative of subchronic oral toxicity. The experimental groups were: Group I (G I): control fed normal pellet diet and water ad libitum. Group II (G II): fed Tz (7.5 mg/kg, dm diet). Groups III (G III), 4 (G IV) and 5 (G V) rats were administered blends of 7.5 mg/kg, dm Tz plus either 1.0 g CUR/kg, dm diet, (G III), 2.0 g CUR)/kg, dm diet (G IV) or 4.0 g CUR/kg, dm diet (G V), respectively. Water was given to all groups *ad libitum*.

Collection of Samples

After 90 d, blood was drawn from the orbital sinus of fasting rats by use of a glass capillary. To obtain serum, whole blood was transferred into non-heparinized glass centrifuge tubes and permitted to clot at room temperature followed by centrifugation at 3.500 g for 15 m. Blood serum was employed for measurement of all biochemical parameters including functions of liver and kidney, total protein and profiles of relative concentrations of lipids. Rats were euthanized after having been anesthetized under mild anesthesia with diethyl ether. The liver was removed, rinsed in cold saline and processed for histological study and biochemical analysis. Masses of individual livers were determined. Relative liver masses were calculated based on body masses measured on the day of sacrifice. Homogenates of liver were prepared by transferring small sections of known masses to test tubes containing physiological saline and homogenized for 15 min and centrifuged 10 min at 3000 g. The resulting clear supernatant was decanted and directly used for quantification of biomarkers of oxidative stress, GSH and MDA. A portion of liver was fixed in 10% neutral buffered formalin for histopathology, and was further processed using standard histological fixing and staining. Sections of 5 μm thickness were stained with hematoxylin-eosin and the prepared slides were examined under light microscope for histopathological examination.

Clinical Biochemistry

Liver Function

Activities of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and lactate dehydrogenase (LDH) in serum were determined using a Technicon RA-XT auto-analyzer (Albany, NY, USA). Concentrations of bilirubin in serum were measured by use of previously described methods[23].

Kidney Function

Concentrations of creatinine, uric acid and urea in serum were measured calorimetrically using standard protocols[24,25].

Lipid Profile

Concentrations of lipids TC, TG, HDL-C and LDL-C in serum were measured by use of a Chemistry Analyzer (Dimension, RXL, Newark, NJ, USA)[26].

Protein Estimation

Total concentrations of protein in serum were quantified by use of the Biuret[27] and Bradford[28] procedures employing bovine serum albumin (BSA) as a standard.

Superoxide Dismutase

The activity of SOD was quantified by use of previously described methods[29] and by use of nitroblue tetrazolium as a chromogenic agent.

Catalase

The activity of CAT was quantified by use of hydrogen peroxide (H_2O_2) as the substrate[30].

Glutathione Peroxidase

The activity of GPx was quantified by use of the standard procedure of Paglia and Valentine's[31].

Thiobarbituric acid reactive substances (TBARS) and reduced glutathione

GSH was determined using the procedure of Beuther and co-workers[32], whereas TBARS indicative for lipid peroxidation in the liver was quantified using the procedure of Presuss[33].

Statistical Analysis

All statistical analyses were carried out using SPSS 17.0 software (SPSS, Inc., Chicago, IL, USA). Mean and standard error were descriptive measures of quantitative data using the analysis of variance test (ANOVA) for independent samples, followed by the post hoc Tukey-HSD test for multiple comparisons of the means according as previously described by us[34]. All determinations have been done in triplicate, p-values < 0.05 were considered significant.

Results

Body Mass and Relative Liver Mass

No mortality was observed during the experiment. Body mass and relative masses of livers of rats exposed to Tz were significantly (p<0.05) less than decreases those of controls. No significant differences were observed among body masses or relative liver masses of any of the rats fed mixtures of CUR and Tz and controls (Table I). Body masses and relative masses of livers of rats fed mixtures of CUR and Tz (Groups III, IV and V) were greater than those of rats fed only Tz (Group II).

Effects of Curcumin on Oxidative Stress in Rats Exposed to Tartrazine

While exposure to Tz resulted in significantly (p<0.05) greater concentrations of both MDA and total protein, it resulted in statistically significant (p<0.05) lesser concentrations of reduced glutathione (GSH) (Table II). Exposure to 1.0, 2.0, or 4.0 g CUR (Groups III, IV or V) resulted in statistically significant greater concentrations of GSH, which were rescued to values near that of the control group (I). Exposure to all concentrations of CUR resulted in significantly lesser concentrations of MDA and total protein in blood serum compared to concentrations in rats fed Tz in the absence of CUR (Group II) (Table II).

Effects of Curcumin on Activities of Antioxidant Enzymes in Rats Exposed to Tartrazine

All concentrations of CUR utilized in this study exhibited significant protection from Tz-mediated inhibition of activities of antioxidant enzymes. Significantly (p<0.05) lesser activities of CAT, SOD and GPx were observed in rats fed Tz in the absence of CUR (Group II) when compared with controls exposed to neither Tz nor CUR (Group I) (Table III). Supplementation of diets of rats fed Tz with 1.0, 2.0, or 4.0 g CUR (Groups III, IV and V) resulted in significantly (p<0.05) greater activities of antioxidant enzymes about rats fed Tz in the absence of CUR (Group II). The greatest concentration of CUR rescued activities to almost normal levels observed in controls (Group I). Maximum ameliorating effects on activities of enzymatic were observed when diets were supplemented with 2.0 g of CUR (Group IV) compared to rats exposed to Tz alone (Group II). Significant correlations (p<0.05) between activities

Table I. Body mass and relative liver masses of experimental rats.

Groups Parameters	I	II	III	IV	V
Initial body mass (g)	243.2±1.9	245.2±2.8**	245.0±2.5	245.2±3.1	244.8±3.4
Final body mass (g)	274.0±12.2	208.0±18.3**	283.6±21.0	249.0±16.8	231.2±8.3
Gain of body mass in 90 days	30.8±10.3	-37.2±15.5**	38.6±18.5	3.8±0.8	-13.6±4.9
Liver mass (g)	4.3±0.2	2.7±0.11**	3.9±0.1	4.1±0.2	3.2±0.1
Ratio of Liver-to-body mass	0.016±0.002	0.013±0.002**	0.016±0.001	0.15±0.002	0.014±0.001

Values are expressed as mean ± S.E. **p<0.01 relative to Group I. Group I – Control, Group II – tartrazine, Group III – tartrazine + 1.0 g curcumin. Group IV – tartrazine + 2.0 g of curcumin. Group V – tartrazine + 4.0 g curcumin.

Table II. Effects of curcumin on total protein in serum, reduced glutathione (GSH) and malonyl dialdehyde (MDA) in livers of tartrazine-fed rats.

Group	I	II	III	IV	V
GSH (nmole/100 mg)	72.9±1.4[a]	67.37±2.73[b]	70.38±1.36[a]	71.82±1.8[a]	71.9±1.45[a]
MDA (nmol/h)	4.82±0.41[a]	6.63±0.16[b]	4.99±0.73[a]	4.86±1.87[a]	4.63±1.76[a]
Total protien (g/dl)	5.63±0.23[a]	6.76±0.12[b]	5.97±0.21[a]	5.56±0.32[a]	5.44±0.13[a]

± SE. Number of animals is ten for each group. A mean that shares the same letter are not significantly different. Means, which have different letters, are significantly different (p<0.05).

Table III. Effects of various concentrations of curcumin on antioxidant enzymes of tartrazine treated rats.

Group	I	II	III	IV	V
SOD	2.96[a]±0.05	1.45[d]± 0.04	2.0[c]±0.06	2.87[a]+0.09	2.53[b]±0.16
GPX	37.56[a]±0.	23.25[d]±0.62	26.78±1.01	33.34b±0.31	32.41[b]±0.49
CAT	266.66±1.52	232.52±1.5	246.30[c]±0.80	256.44[b]±0.40	257.03±0.52

Same letters mean there is no significant at p<0.05. Different letters indicate significance at p<0.05.

Table IV. Effects of various concentrations of curcumin on liver function enzymes of rats fed tartrazine.

Group	I	II	III	IV	V
ALP	105.68c±1.56	139.70a±0.83	116.72b±1.53	107.77c±0.86	106.07c±1.61
LDH	158.34c±1.03	180.50a±1.43	163.93b±0.90	161.05b±0.76	159.36b,c±0.66
SGOT	36.5d±2.66	58.26a±1.23	46.29b±0.92	39.67c±0.44	41.26c±0.42
SGPT	42.50d±0.76	66.95a±0.78	48.77b±0.94	44.93c±0.89	45.34c±0.71

Same letters means there is no significant at p < 0.05: Different letters means there is significant at p<0.05.

of antioxidant enzymes concentrations of MDA were observed. These results indicate that peroxidation of lipids was caused by exposure of rats to Tz in their diet. Toxicity of Tz was inversely proportional to activities of antioxidant enzymes. These results suggest the importance of the dye CUR as an antioxidant for protecting against reactive oxygen species (ROS), which are produced as a result of Tz in the diet (Table III).

The Ameliorating Effect of Curcumin on Liver Functions

Exposure of rats to Tz (Group II) resulted in significantly (p<0.01) greater activities of several enzymes in the liver. Activities of ALP, LDH, AST and ALT were 32.2, 14.0, 59.4 and 57.4% greater, respectively, than those of the control group (Group I) (Table IV). Administration of 1 g CUR (Group III) resulted in significantly (p<0.05) lesser activities of these enzymes by 16, 14, 20.6, and 57.0 %, respectively, compared with rats fed tartrazine in the absence of CUR (Group II). Similarly, activities of these enzymes in rats fed a diet supplemented with 2.0 (Group IV) or 4 (Group V) g CUR concurrently with Tz exhibited significantly (p<0.05) lesser activities about rats exposed to Tz without CUR (Group II). However, no significant differences in activities of ALP were observed between Groups IV or V and the control (Group I), or activity of LDH in Group V compared to the control (Group I). Supplementing diets of rats with the two greatest doses of CUR (Groups IV and V) successfully restored liver function activities of AST and ALT enzymes back to control group levels (Group I).

The Ameliorating effect of Curcumin Concentrations on Lipid Profile

Concentrations of serum TC, LDL-C, and TG of rats exposed to Tz (Group II) were significantly (p<0.05) greater by 65.2%, 95.2% and 34.2%, respectively, compared to the controls (Group I), but there were no significant differences between concentrations of HDL-C and those of the controls (Table V). While, co-exposure to Tz and 1.0 g CUR (Group III) resulted in significantly lesser concentrations of TC, LDL-C and TG in blood serum by 19.6, 35.7, 18.8%, respectively, compared with rats exposed to Tz without CUR (Group

Table V. Effects of various concentrations of curcumin on lipid profile of rats fed tartrazine.

Group	I	II	III	IV	V
TC	$51.95^{d}\pm0.90$	$85.79^{a}\pm1.68$	$68.94^{c}\pm1.26$	$53.96^{c,d}\pm1.14$	$56.52^{c}\pm1.47$
LDL-C	$28.68^{c}\pm0.80$	$55.97^{a}\pm0.63$	$36.38^{b}\pm0.60$	$26.79^{c}\pm0.56$	$28.41^{c}\pm0.79$
HDL-C	$22.68^{d}\pm1.16$	$23.67^{c,d}\pm0.57$	$31.50^{a}\pm0.79$	$26.06^{b,c}\pm0.79$	$27.18^{b}\pm0.86$
TG	$67.45^{c}\pm0.90$	$90.51^{a}\pm0.68$	$73.45^{c}\pm0.98$	$64.04^{d}\pm1.63$	$63.08^{d}\pm0.94$

Same letters mean there is no significant at $p<0.05$; Different letters mean there is significant at $p<0.05$.

Table VI. Effects of various concentrations of curcumin on kidney function of rats fed tartrazine.

Group	I	II	III	IV	V
Creatinine	$0.19^{d}\pm0.02$	$0.45^{a}\pm0.01$	$0.32^{b}\pm0.03$	$0.24^{c}\pm0.01$	$0.21^{c,d}\pm0.01$
Urea	$41.9^{d}\pm1.29$	$55.52^{a}\pm0.35$	$49.10^{b}\pm0.63$	$46.18^{c}\pm1.01$	$45.43^{c}\pm1.10$
Uric acid	$2.52^{d}\pm0.18$	$4.23^{a}\pm0.11$	$3.43^{b}\pm0.14$	$2.92^{c,d}\pm0.10$	$3.16^{b,c}\pm0.19$

Same letters means there is no significant at $p < 0.05$: Different letters means there is significant at $p < 0.05$.

II), concentrations of TC and LDL-C in serum were 32.7%, and 26.8%, respectively, compared with Group I, and the concentration of TG was nearly equivalent to that of the controls. The mean concentration of HDL-C in rats fed 1.0 g CUR along with Tz were significantly ($p<0.05$) greater than that in rats fed Tz alone (Group II) or the unexposed control (Group I) by 33.1% and 38.9%, respectively. Supplementation of diets of rats with 2 or 4 g of CUR (Groups IV and V) significantly attenuated effects on concentrations of both TC and LDL-C, rescuing concentrations to values near to those of the controls (Group I). In contrast, concentrations of HDL-C were significantly ($p<0.05$) greater in Groups IV and V in relation to both Groups I and II, whereas concentrations of TG were significantly less.

Ameliorating Effect of Curcumin on Functions of Kidney

Rats that had been fed with Tz in their diet exhibited significantly ($p<0.05$) greater concentrations of creatinine, urea, and uric acid in blood serum compared to the controls (Group I; Table VI). Supplementation of diets with 1.0, 2.0, or 4.0 g CUR/kg diet (Groups III, IV and V, respectively) resulted in significant decrease ($p<0.05$) in concentrations of creatinine, urea and uric acids in blood serum about those in blood of individuals in Group II, but remained significantly greater than those of Group I. Concentrations of uric acid in Group IV and creatinine in Group V, were recovered to quasi-normal values relative to Group I (Table VI).

Discussion

Body mass and relative liver masses are good general, integrative indicators of toxicity[35]. The significantly lesser body mass and relative liver masses of rats fed Tz relative to control might be due to Tz in the diet reducing the palatability of food or otherwise resulting in avoidance. Furthermore, Tz might result in generation of free radicals, which resulted in oxidative stress that caused metabolic disorders and general losses of body mass. The addition of the natural food color, CUR, to diets containing Tz might have improved metabolism by increasing capacities of antioxidants or acting directly as a scavenger of free radicals. These ameliorating effects of CUR on body mass and liver/body mass ratio caused by Tz in diets of rats are consistent with results of previous studies[36]. Previous reports have suggested that lesser body mass of rats fed Tz was due to the lesser caloric intake. The rate of gain of body mass of female mice was significantly ($p<0.05$) less in three groups that were fed various doses of Tz, whereas in male mice lesser gain in body mass was significant only at the greatest dose. A similar trend was reported in an independent but related study which observed lesser gain in body mass in male rats exposed for 30 d to lesser and greater oral doses of Tz[37]. Therefore, body mass has been adopted as a sensitive and reliable indicator of toxic effects of Tz to rodents[38-41].

In the study, the results of which are reported here, concentrations of total protein and MDA concentrations were significantly greater in rats

fed Tz without (Group II) relative to control group (Group I). These results are consistent with those of recent reports[15,42,43] where significantly greater concentrations of total protein and MDA were observed in rats fed synthetic dyes, colors A and B, used to color chocolate.

The fact that concentrations of GSH and activities of selected antioxidant enzymes in blood serum of rats exposed to Tz (Group II) are significantly less than those of the control has been suggested to be due to metabolism of azo dyes in the small intestine to produce genotoxic compounds. These ROS, such as hydrogen peroxide, superoxide anion and hydroxyl radical can, if not scavenged, result in oxidative stress, whereby leading to peroxidation of lipids in membranes[44-47]. Consequently, if the intracellular antioxidant defense system, both enzymatic and non-enzymatic is exhausted particularly the liver, residual ROS can cause damage. When protection by these antioxidant defense systems is overwhelmed, the resulting ROS-mediated oxidative damage results in greater concentrations of MDA. Reactive oxygen species have been reported to be an essential causative factor for histopathological alterations of the liver[48]. Membranes are particularly prone to effects of ROS, peroxidation of unsaturated fatty acids in biological membranes leads to a decrease of fluidity and disruption of membrane integrity and function, which is implicated in serious pathologies[49,50], including lesions in liver (Figure 1B). As a consequence of direct damage of ROS on plasma membranes, the permeability of hepatic cells increase, which results in leakage of enzymes into circulating blood. In the current work, greater activities of enzymes associated with functions of the liver, observed in serum were observed in rats fed with Tz (Table IV), which is indicative of damage to hepatocytes and subsequent permeability. Both GPT and ALP are localized in the cytoplasm, whereas more soluble enzymes like LDH and AST are primarily localized in or-

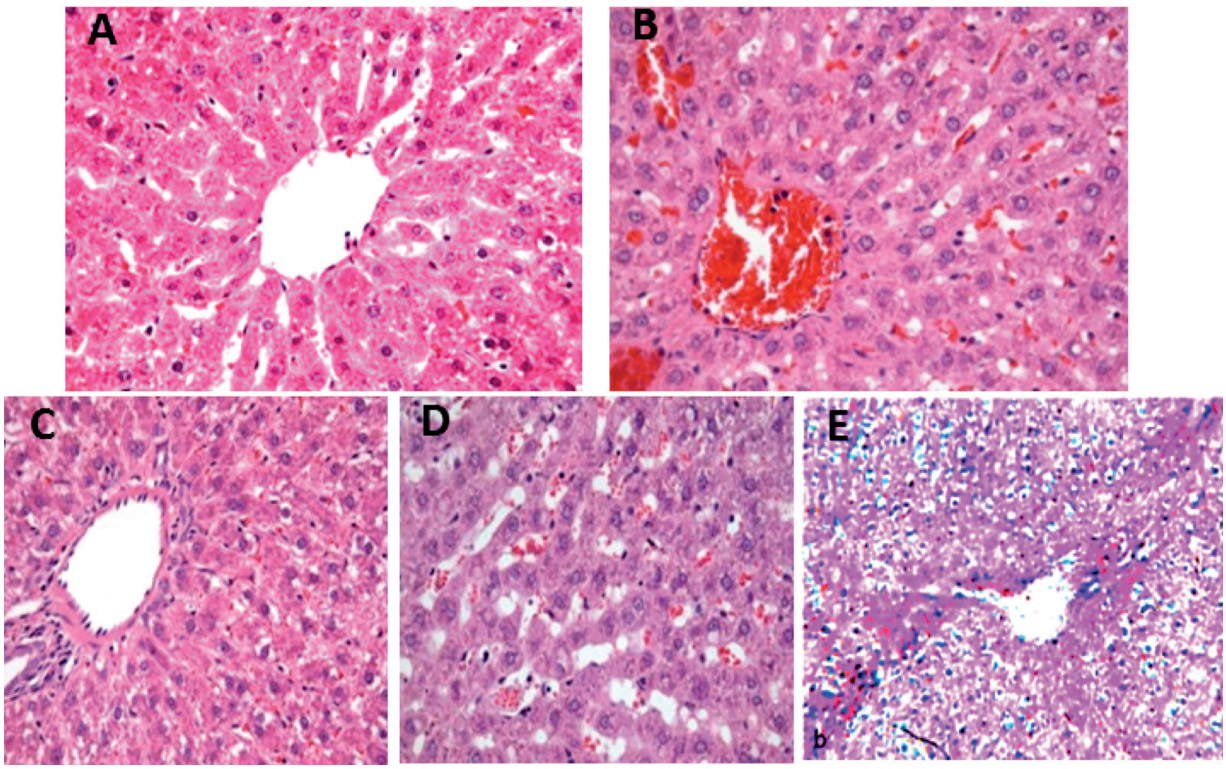

Figure 1. Histopathology in livers of rats fed tartrazine (Tz) and ameliorative effects several doses of curcumin (CUR). ***A)*** Normal structure of liver tissue of control showing the central vein, normal arrangement of hepatic cords, normal blood sinusoids(s) and hepatocytes, HE, X 400; ***B)*** Liver tissue of rats exposed to Tz showing dilation of blood sinusoids, and central vein with hemorrhage and necrosis (*), HE, X 400; ***C)*** Liver tissue of rats fed Tz in combination with 1.0 g/ kg dry mass (dm) diet of CUR, showing less necrosis and moderate degenerative changes compared to the control and rats fed Tz alone (N), HE, X 400; ***D)*** Liver tissue of rats fed a diet containing Tz in combination with 2 g/ kg, dm diet of CUR, showing little necrosis (N) compared to the controls or rats exposed to lesser amounts of CUR. HE, X 400; E) Liver tissue of rats fed a diet containing Tz supplemented with 4.0 g/ kg, dm diet of CUR, showing little necrosis (N), H&E, X 400.

Table VII. Histopathological changes in livers of rats, based on scoring severity of effects of various concentrations of curcumin on histological changes in livers of rats fed tartrazine.

Group	I	II	III	IV	V
Score average	0	3	2	1	1
Severity	Normal	Sever	Moderate	Mild	Mild

Scores in terms of numerical values are detailed in histopathological studies.

ganelles such as mitochondria[51]. It can be inferred that the observed activities of these enzymes were results of damage by Tz to membranes of hepatocytes as well as mitochondrial membranes. This conclusion is supported by histopathological findings (Figure 1B; Table VII) indicating degenerative changes in livers of rats exposed to Tz. These results are consistent with those of other studies, in which Tz resulted in significantly greater activities of ALP and transaminases in blood of mice[52]. Similarly, subchronic exposure to 500 mg Tz/kg, bw for 30 d resulted in statistically significant greater activities of ALT, AST and ALP in serum[37,38]. The results of the study, which are presented here, agree with other studies, where it was found that synthetic azo dyes, such as Tz and carmoisine of resulted in significantly greater activities of enzymes associated with functions of the liver in rats fed colors containing Tz used in chocolate[15,43]. Curcumin (CUR) successfully normalized activities enzymes, used as markers for damage to membranes in liver, to quasi-normal values in a dose-dependent fashion (Table IV). This finding is conforming to previous reports on the hepatoprotective effects of CUR against heavy metals and diethyl nitrosamine-induced toxicity[53,54]. It has been shown that CUR exerts its protective effects against severe oxidative damage via: (1) its powerful antioxidant property, whereby scavenges oxygen free radicals, and (2) its ability to increase intracellular GSH levels, which consequently lead to the efficient control of levels of lipid peroxidation[54].

Altered profiles of lipids in blood plasma observed in rats fed with Tz (Group II) during this study agree with other studies[43]. Cholesterol is a soft waxy substance found in blood as well as membranes. Significantly greater concentrations of TC and TG has been reported to occur in response to exposure to fast green, which is another azo dye used as to color food, when administered orally to rats over a period of 35 d[55-57]. In the current study, when CUR was added to the diet at several doses it successfully normalizes concentrations of lipids, which indicates that it was able to block the adverse effects of ROS generated by metabolism of Tz (Table V).

Tz resulted in significantly greater concentrations of creatinine, urea and uric acid in blood plasma, about control (Group I). These indications of damage to kidney function agree with other researches[37]. However, the mechanism for this observation remains to be elucidated. The fact that administration of CUR resulted in a significant reduction in concentrations of creatinine, urea, and uric acid and reverting it back to quasi-normal in a concentration-dependent manner can be ascribed to CUR modulating peroxidation of lipids in membranes[54].

Deposition of pigment in portal tracts and infiltration of Kupffer cells, congested blood vessels with various areas of hemorrhage (Group II; Figure 1B) compared to control group (Group I, Figure 1A) was histological evidence of damage to the liver by exposure to Tz (Table VII). With progression of cellular damage, hepatocyte swallowing follows and vacuolization occurs pushing the nucleus aside and rendering the typical signet ring shaped hepatocyte morphology. Identical histopathological alterations have been observed in hepatocytes of guinea pigs exposed for 3 weeks to Tz in drinking water[58]. In the current study, CUR effectively restored normal morphological features of hepatocytes in a concentration-dependent fashion (Figure 1C-E). Hepatoprotective and therapeutic properties of CUR against Tz-mediated toxicity has been ascribed to its ability to scavenge free radicals, whereby it moderated progression of the oxidative stress cascade to recover a healthy antioxidant status and chelate metal ions, thereby preventing the Fenton reaction to occur[59,60]. CUR has been suggested as a potential inducer of de novo synthesis of GSH[61]. A support for the hypothesis that CUR exhibits a significant ameliorating effect on Tz-mediated toxicity in rats by minimizing AFB_1-induced hepatotoxicity in rats, has been recently published[62]. These CUR-mediated protective effects against AFB_1 toxicity have been postulated to take place via modulation of

lipid peroxidation via enhancement of the antioxidant defense system[59]. The distinctive protective effect of CUR is thought to be via expression of a gene subset since it has been shown to regulate gene expression of insulin-like growth factor, B-cell CLL/lymphoma 2 and antioxidant enzymes in streptozotocin-induced diabetic rats[63].

Conclusions

We provided evidence for the potential of CUR, as a natural food coloring agent, to minimize or prevent peroxidation of lipids, commonly taking place due to consumption of various potentially hazardous foods and pharmaceuticals by humans. CUR can thus delay the onset of formation of potentially damaging ROS, eventually leading to a better capacity in maintaining nutritional food quality and more efficient cellular redox state homeostasis.

Conflict of interest

The authors declare no conflicts of interest.

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