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Effect of Food Colorants Supplementation on Reactive Oxygen Species, Antioxidant Vitamins Level and DNA Damage

(Kesan Tambahan Pewarna Makanan pada Spesies Oksigen Reaktif, Tahap Antioksidan Vitamin dan Kerosakan DNA)

SADAF SHAKOOR*, AMIN ISMAIL, ZIA-UR-RAHMAN, MOHD REDZWAN SABRAN & NORHAFIZAH MOHTARRUDIN

ABSTRACT

There are various undesirable products generated from endogenous aerobic metabolism such as reactive oxygen species (ROS). Physiological and biochemical lesions are caused by ROS and which give rise to oxidative damages towards DNA, proteins and lipids which ultimately lead to cell death. This study was aimed to examine the effect of oral administration of food colorants (tartrazine and curcumin) on the oxidants and antioxidants level in blood and fecal of rats after 15, 30, and 45 days. Two doses were used based on the admissible daily intake (ADI) of 9.6 and 96 (high) mg/kg/body weight for tartrazine, 3.85 and 38.5 6 mg/kg/body weight for curcumin. The results showed that oral administration of tartrazine had significantly increased the total oxidant level, arylesterase, glutathione reductase, and MDA whereas there was significantly decreased the total antioxidants level, catalase, glutathione peroxidase in plasma and fecal after 30 and 45 days. Vitamin E and C were decreased in plasma. Fecal showed high level of vitamin A. High dose of tartrazine caused alteration in the aldehyde reactive probe (ARP) sites of DNA showing the DNA damage. After 45 day, significant increment was observed in the level of AST in low and high curcumin treated group. Whereas, elevations of arylestrase were seen in high curcumin group after 45 day. High dose of curcumin significantly (P≤ 0.05) decreased the concentration of vitamin C after 45 days of treatment and increased the level of vitamin E in plasma of treated groups after 30 and 45 days of treatment. The present study showed that the ADI and doses up to 10 times higher than ADI showed negative effects on antioxidant level and demonstrated the importance of using appropriate doses of food colorants such as tartrazine and curcumin in different processed food products.

Keywords: Antioxidants; arylesterase; food colorants; glutathione; lipid peroxidation; ROS

ABSTRAK

Terdapat pelbagai produk yang tidak diingini yang dijanakan daripada metabolisme aerobik endogen seperti spesies oksigen reaktif (ROS). Lesi fisiologikal dan biokimia disebabkan oleh ROS yang mengakibatkan peningkatan kepada kerosakan oksidatif terhadap DNA, protein dan lipid yang akhirnya membawa kepada kematian sel. Kajian ini bertujuan untuk meneliti kesan pelaksanaan oral pewarna makanan (tartrazin dan kurkumin) ke atas tahap oksidan dan antioksidan dalam darah dan najis tikus selepas 15, 30 dan 45 hari. Dua dos telah digunakan berdasarkan pengambilan harian yang dibenarkan (ADI) iaitu 9.6 dan 96 (tinggi) mg/kg/berat badan bagi tartrazin, 3.85 dan 38.5 6 mg/kg/berat badan bagi curcumin. Keputusan menunjukkan bahawa pelaksanaan oral tartrazin telah meningkat secara signifikan total tahap oksidan, arilestras, glutation reduktase dan MDA manakala terdapat penurunan yang signifikan total tahap oksidan, katalase, glutation peroksidase dalam plasma dan najis selepas 30 dan 45 hari. Vitamin E dan C telah menurun dalam plasma. Najis menunjukkan tahap vitamin A yang tinggi. Dos tartrazin yang tinggi yang menyebabkan pengubahan dalam tapak prob reaktif aldehid (ARP) DNA menunjukkan kerosakan DNA. Selepas 45 hari, kenaikan yang signifikan telah dikesan dalam tahap AST dalam kumpulan terawat rendah dan tinggi kurkumin. Manakala, elevasi arilestras telah kelihatan dalam kumpulan tinggi kurkumin selepas 45 hari. Dos tinggi kurkumin secara signifikan (P≤ 0.05) telah menurunkan kepekatan vitamin C selepas 45 hari rawatan dan meningkatkan tahap vitamin E dalam plasma kumpulan kawalan selepas 30 dan 45 hari rawatan. Kajian ini menunjukkan bahawa ADI dan dos sehingga 10 kali lebih tinggi daripada ADI menunjukkan kesan negatif ke atas tahap antioksidan dan ia memaparkan kepentingan untuk menggunakan dos pewarna makanan yang sesuai seperti tartrazin dan kurkumin dalam produk makanan diproses yang berbeza.

Kata kunci: Arilesteras; glutation; pewarna makanan; pemperoksidaan lipid; ROS

Introduction

Recent argument and critique about the safety of food colorants have been coordinated towards the synthetic colorants. The natural colorants have been relatively free of disapproval may be due to the conviction that most are inferred from food sources that have been expended for numerous years (Francis 1995).

The application of natural food colorants are limited and they have a impediments compared with the synthetic colorants, in spite of the fact that they are moderately secure in terms of toxicological check and contradiction responses (Abd El-Galeel 2002). However, after using for a long time, many of them are toxic even on admissible dose and causes health complications such as; hormonal disturbance, oxidative stress production and DNA damage (Ashida et al. 2000).

In developing countries, food colorants were used, above from the ADI during various occasions which would cause severe health complications and human health hazards (Rao et al. 2006). The effect of DNA-damaging (mutagenic) of tartrazine has been reported based on animal studies (Sasaki et al. 2002). ROS can be generated from the tartrazine metabolite and accelerate the oxidative stress (Bansal et al. 2005). At high doses, it leads to the modification of liver and kidney biochemical profiles and becomes risk as it forms free radicals and induces oxidative stress on tissue (Himri et al. 2011). In order to protect against tissue or cellular damage mediated from free radicals, antioxidant is required therefore, to prevent damage to immune cells (Mourad & Noor 2011).

Oxidative stress can also be viewed as an imbalance between the oxidants and antioxidants in the body (Rahal et al. 2014). Endogenous formation of ROS and free radicals occur due to aerobic metabolism (Sahu 2002). Free radical generation is an important characteristic of normal function of cells (Khadija et al. 2009).

Tartrazine (FD and C Yellow No. 5) is a yellow orange-colored azo dye widely used in food products, drugs, and cosmetics. This color has a potential toxicological risk (Mehedi et al. 2009). These food colorants might induced DNA damage in the gastrointestinal organs (Sasaki et al. 2002). Tartrazine is transformed into aromatic amine sulfanilic acid after being metabolized by the gastrointestinal microflora and the formed aromatic amines can generate ROS by interaction of these amino groups with nitrite or nitrate-containing foods or in the stomach, leading to organ injuries (Bansal et al. 2005; Moutinho et al. 2007). Interestingly, there are studies that indicated tartrazine to be a potential inducing agent for oxidative stress by disturbing antioxidant and prooxidant balance and forming ROS in rat brain and liver (Amin et al. 2010; El-Wahab & Moram 2013).

On the other hand, curcumin is yellow odorless powder and used as natural food colorants. It has anti-inflammatory, antifungal, antimicrobial, virucidal, antimutagenic and antioxidant properties (Rajesh et al. 2013). Literature is full of studies regarding the beneficial effect of cucumin, but there is few publications provided evidence that curcumin induced DNA damage in human lymphocytes and gastric mucosa cells *in vitro* (Blasiak et al. 1999). Another publication by Antunes et al. (1999) substantiates this finding in that curcumin was found to induce DNA-damage in CHO cells at a concentration of 10 μM. Whereas oral administration of curcumin in its low and high doses (15.75 and 157.5 mg/kg bw) for 2 months decreased hepatic lipid peroxide concentration in male albino rats.

Glutathione (GSH) is the most abundant antioxidant in all aerobic cells, presenting with high-concentrations in body fluids and tissue. GSH using enzymatic reduction, removes most of the free radicals whereas the removal of hydrogen peroxides requires catalysis of enzymes (Owen & Butterfield 2010). During this process GSH becomes oxidized glutathione (GSSG). The nicotinamide adenine dinucleotide phosphate (NADPH)-dependent catalysis of the flavoenzyme GSH reductase effectively decreased to GSH (Aquilano et al. 2014). Aryl esterase, also known as paraoxonase (PON1), is a high-density lipoprotein (HDL) linked enzyme which hydrolyzes phenyl acetate to phenol and acetate. It is a calcium-dependent esterase. PON1's natural physiological function happens to be toxic oxidized lipids metabolism of both low-density lipoprotein (LDL) particles and HDL particles (Durrington et al. 2001). Studies have showed PON1's ability to protect against atherosclerosis by hydrolyzing particular derivatives of oxidized cholesterol and/or phospholipids in atherosclerotic lesions and in oxidized low density lipoprotein. It may also be a major modulator of risk of cardiovascular disease (Bergmeier & Siekmeier 2004).

The enzymatic mechanism of antioxidants includes enzymes, such as glutathione peroxidase, SOD and catalase involved enzymes in recycling glutathione reductase (Halliwell 1992). Vitamin A, C, E, β- carotene, and melatonin have vital part in protecting against induced oxidative stress in various models of experiments (Kharwar & Haldar 2012).

Food colorants are commonly used in packed food and confectionaries to gave attractive look and these food colorants might produced several health problems in human. Low and high dose of tartrazine and curcumin

might trigger the formation of reactive oxygen species, thereby ultimately playing a role in the development of various health issues. Significant investigation of the current study is that it has been experimented among adult animals which was not focused in previous studies.

As mentioned by Borzelleca and Hallagan (1988) that a serious draw - back of food colors studies conducted on animal studies is that infant animals are likely to be more susceptible to toxic or carcinogenic effects than older animals. Several studies are available regarding the adverse effect of different food colorants in children, male, and infant animals. Previous studies lacking the data related to antioxidants vitamins and ROS in females plasma with reference to consumption of food colorants. No previous study has evaluated the effect of different doses of tartrazine and curcumin on fecal total oxidant, total antioxidant level and antioxidants vitamins in adult female rats. Keeping in view of these aforementioned information, this study has been designed to examine the oxidative effects of curcumin and tartrazine (popular food colorants), on adult female albino rats by measuring oxidative stress parameters, including glutathione, malondialdehyde, glutathione peroxidase activity, catalase activity, arylesterase, antioxidants vitamins (A, C and E) and DNA damage.

Materials and Methods

Food colorants used in the experiment

Tartrazine

Tartrazine ($C_{16}H_9N_4Na_3O_9S_2$, Trisodium5-hydroxy-1-(4-sulfonatophenyl)-4-(E)-(4- sulfonatophenyl) diazenyl-1H-pyrazole-3-carboxylate, MW = 534.3 g/mol, CAS 1934-21-0, powder with purity of 86.7%) is synthetic food colorant and was purchased from Sigma Aldrich (Germany).

Curcumin

Curcumin ($C_{21}H_{20}O_6$, 1,7-bis (4-hydroxy-3methoxyphenyl)-1E,6E-heptadiene-3,5-dione, MW = 368.4 g/mol, CAS 458-37-7, powder with purity of 95%) is commercially available natural food colorant and was purchased from Carbosynth (Campton, United Kingdom).

Calculation of dose

The animal equivalent dose (AED) can be calculated on the basis of body surface area by either dividing or multiplying the human dose (mg/kg) by the Km ratio provided by literature (Anroop & Jacobin 2016; FDA 2005). AED is calculated using (1):

$$AED = HED \times K_{m\,(1)} \quad (1)$$

where AED is animal equivalent dose (mg/kg); HED is human dose for tartrazine and curcumin, which is 7.5 and 3 mg/kg body weight/day, respectively; and K_m is body weight (kg)/body surface area (m^2).

K_m ratio for rat used in this study is 6.2 or 0.162 (either dividing human dose with 6.2 K_m ratio or multiplying the human dose by 0.162 (mg/kg) K_m ratio, result dose will be same (FDA 2005)). The animals grouping and dose is mentioned in Figure 1.

Animals ethics

The animal ethic of this study was approved by the Institute of Pharmacy, Physiology and Pharmacology, University of Agriculture, Faisalabad Ethics Committee (Punjab, Pakistan) application number 1502, dated: 04/07/17.

Selection of animals

Ninety (n-90) non pregnant female *Sprague Dawley* rats, 6 - 7 months old (From animal house of University of Agriculture Faisalabad, Pakistan) were selected and housed in stainless steel cages individually at normal temperature 27 ± 5 ºC and under good ventilation. Animals were fed on *ad libitum* standard basal diet with addition of ADI and high dose of food colorants daily. The experimental diets were prepared according to Kim et al. (2005). Each group consist of six rats, categoriezed by two level of dosage i.e. low and high dose as mentioned in Figure 1. The average rat weight at the start of the trial was 200.65 ± 15.20.

Sampling

Sampling was done at 15, 30, and 45 days for control and treated groups (Control= C: C15, C30, C45, Low Tartrazine= LT: LT15, LT30, LT45, High Tartrazine= HT: HT15, HT30, HT45, Low Curcumin= LC: LC15, LC30, LC45, High Curcumin= HC: HC15, HC30, HC45) to check acute, sub-acute and post-acute toxicity. At the completion of each trial periods, 4-5 mL blood samples were collected through cardiac puncture of the rats by using 23G needle under general anaesthesia. Blood was collected in BD Vacutainer® heparin tubes. Then, rats were immediately euthanized through cervical dislocation. The blood was centrifuged using centrifugation (LYC;

Centrifuge 80-2, Jiangsu, China) for 15 min at 1795×g and room temperature to collect plasma, which was transferred to the Eppendorf tubes and stored at -20 °C until further analysis (within 4 weeks).

At the end of each experimental period, fresh fecal samples were collected immediately after defecation over a period of 3 days; a subsample of approximately 2 g was mixed with 10-mL phosphate-buffered saline (PBS) using an electric mixer. The resulting mix was centrifuged at 3420×g for 10 min to obtain a supernatant free of solid cellular debris. This supernatant was kept until further analysis at -80 °C to avoid any major deviation of composition and biological variation during long term storage (Clauss et al. 2008).

DETERMINATION OF REACTIVE OXYGEN SPECIES IN PLASMA AND FECAL

Total Antioxidants (TAC; mmol trolox Equiv./L) were determined by the applied calorimetric method was (Erel 2004). Measured the absorbance of the sample before mixing of reagent 1 and reagent 2 into sample, this will be sample blank solution. Mixed 5 µL of plasma, 200 µL of Reagent 1 (Dissolved 2.46 g of CH_3COONa in 1 L of deionized water. Dilute glacial acetic acid (1.705 mL) into 1000 mL of deionized distilled water. 75 mL of dodium acetate solution mixed 925 mL of acetic acid solution by using pH meter. The pH of acetic acid solution is 3.6. Then, 278 µL of H_2O_2 solution (35%) was diluted to 1000 mL with buffer solution. Then, 0.549 g of ABTS was dissolved in 100 mL of prepared solution (final concentration:10 mmol/L). Incubate it for one hour at room temperature. The color of ABTS -1 appeared. The colored reagent was stable for at least 6 months at 4 °C) and 20 µL of reagent 2 (Dissolved 32.8 g of CH_3COONa in 1000 mL of deionized water. Dilute reagent glacial acetic acid to 1000 mL with deionized water. About 940 mL of sodium acetate solution was mixed with 60 mL of acetic acid solution by using pH meter. The pH of acetic sodium acetate buffer was 5.8. Stable the buffer for at least 6 months at 4 °C. Then, after mixing incubate the assay solution for five min and measured the absorbance at 660 nm. Reduction in absorbance is directly proportional to the concentration of antioxidants. Determination of catalase Activity (KU/L) was done by following the procedure of Goth (1991). Standard curve was plotted to conclude the results of catalase level in plasma and fecal. Glutathione Reductase (GR; U/mL) was determined by adopting the method of Bergmeyer (1965). Measured the absorbance at 340 nm. The GR activity will be calculated by (2):

$$U/mL = \Delta A340nm/min \times 3 \times Df/ (6.22) (0.1) \quad (2)$$

Determination of Glutathione Peroxidase (GPX; U/L) was done by the protocol of Alam et al. (2013). Calculated the resulted by comparing with standard curve. GPx activity was measured at 340 nm. For the determination of Total Oxidants (TOS; µmol H_2O_2 Equiv./L), colorimetric method was used (Erel 2005). Mixed 225 µL of reagent 1 ((Xylenol orange (150 µM), NaCl (140 mM), Glycerol (1.35M), pH1.75)) with 35 µL of the sample. After mixing measured first absorbance at biochromatic length (560 and 800 nm). Then, mixed 11 µL of reagent 2 (Ferrous ammonium sulfate 5 mM, O-dianisidine dihydrochloride 10 mM) with the solution containing reagent 1 and the sample. Then, measured the final absorbance after 4 min of reagent 2 mixing. Measured the absorbance from serial dilution to draw a standard curve between absorbance and concentration of hydrogen peroxide. Then, measured the absorbance at main wavelength 560 nm and at secondary wavelength 800 nm (Bichromatic). Used the delta change in the absorbance to calculate the actual concentrations (Final Absorbance - First Absorbance = ΔAbsorbance) from the standard curve of hydrogen peroxide in terms of micromolar hydrogen peroxide equivalent per liter (µM H_2O_2 Equiv/L). The arylesterase's activity of enzymes was evaluated through spectrophotometric by using (Biosystem, BTS-330) using the process of Juretic et al. (2006). Arylestrase activity was measured using substrate, phenylacetate. 0.1 mL of diluted plasma was taken from 200 times diluted serum in 0.1 mol L-1 Tris-HCL buffer (pH8.0) in eppendorfs and 2.7 mL buffer was added and mixed well. Then, 20 mmol L-1 phenylacetate was added and after incubation of 5 min take reading as R2 at 270 nm at 37 ºC on spectrophotometer by following the increase of phenol concentration. The arylestrase activity was calculated by following formula and was expressed in international units can be refer in (3). An international unit is amount of hydrolyzed substrate in µmol per min.

$$\text{Arylesterase activity } \left(\frac{U}{L}\right) = \frac{\text{Absorbance}/5}{0.017} x200 \quad (3)$$

Lipid peroxidation was evidenced by measuring the formation of malondialdehyde (MDA) using the method of Tüközkan et al. (2006). About 0.5 mL of plasma was centrifuged at 1795×g for 10 min with 2.5 mL of 20% trichloroacetic acid (TCA) in a 10 mL centrifuge

tube. Then, 1 mL of 0.67% thiobarbituric acid (TBA) was added to the mixture and warmed for thirty min in a boiling water bath followed by rapid cooling. Then, it was added into a 4 mL of n-butyl alcohol followed by centrifugation at 1795×g for 15 min and organic layer separated and malondialdehyde (MDA) content in the plasma was determined from the absorbance at 532 nm by spectrophotometer against butanol. The standard of 20 nmol/mL tetraethoxypropane (TEP) was used. The results were expressed as nmol/mL plasma.

ANTIOXIDANT VITAMINS IN PLASMA AND FECAL

DETERMINATION OF VITAMIN A

Vitamin A was determined by the method of Rutkowski et al. (2006). One mL of the sample was taken into the test-tube I (centrifugal) with a tight stopper and added 1 mL of the KOH solution, plugged the tube and shake vigorously for 1 min - heat the tube in a water bath (60 °C, 20 min), then, cool it down in cold water. Added 1 mL of xylene, plug the tube and shake vigorously again for 1 min. Centrifuged the tube (1500×g, 10 min), collected the whole of the separated extract (upper layer) and transfer it to the test tube II made of 'soft' (sodium) glass measured the absorbance A of the obtained extract at 335 nm against xylene. Irradiated the extract in the test tube II to the UV light for 30 min, then measured the absorbance A2 – calculated the concentration c1 of vitamin A (μM) in the analyzed liquid, using (4):

$$Cx = (A1\text{-}A2) \times 22.23 \quad (4)$$

where 22.23 is multiplier received on basis of the absorption coefficient of 1% solution of vitamin A (as the retinol form) in xylene at 335 nm in a measuring cuvette about thickness =1 cm. xylene (a/a); 1 M solution of potassium hydroxide in 90% ethanol.

DETERMINATION OF VITAMIN C

Determination of vitamin C was carried out by adopting the method of Rutkowski and Grzegorczyk (1998). One mL of sample was measured into the centrifugal test-tube, added 1 mL of the phosphotungstate reagent (PR), thoroughly mixed and left in a room temperature for 30 min. Centrifuged the tube (7000×g, 10 min), and collected the whole of the separated supernatant with a pipette the supernatant is a test sample for spectrophotometric measurements. Standard sample was prepared as mentioned (using 1 mL of the standard solution instead of the analysed liquid), without centrifugation. Measured the absorbance of the test sample Ax and of the standard sample A at 700 nm against the mixture PR: 50 mM solution of oxalic acid = 1:1 (v/v) as a reference sample of vitamin C (μM). The concentration Cx of vitamin C (μM) in the analysed liquid sample, using (5).

$$Cx = ({}^{A_x}/_{A_s}) \times C_s \quad (5)$$

where C_x is the concentration of the sample solution; C_s is the concentration of the standard solution; A_x is the absorbance of test sample; and A_s is the absorbance of standard sample.

Phosphotungstate reagent (PR), prepared periodically, as it's used up (suspension of 150 g sodium tungstate *molybdenium-free* and 60 g sodium hydrogen phosphate anhydrous in 240 mL DI water, mixed with heating to dissolve and slowly added 145 mL 3.7 M Sulphuric acid (VI); Solution was heated for 2 h with reflux condenser not allowing it to boiling; after cooling the solution down, pH was adjusted to 1.0 by adding dropwise concentrated sulphuric acid (VI); the reagent should be light greenish-yellow, a darker one is useless); 56.8 μM vitamin C (L-ascorbic acid) standard solution made with use 50 mM solution of oxalic acid as a solvent.

DETERMINATION OF VITAMIN E

Vitamin E was determined by using the method of Rutkowski et al. (2005). About 0.5 mL of the sample was measured into the test-tube I (centrifugal) with a tight stopper. 0.5 mL of anhydrous ethanol was added and shake vigorously the plugged test tube for 1 min. Added 3 mL xylene, plugged the test tube and shake vigorously for another 1 min. Centrifuged the tube to separate the extract (1500×g, 10 min); simultaneously measure 0.25 mL solution of batophenanthroline into a usual test-tube II. 1.5 mL of the extract (upper layer) was collected and transferred to the test-tube II and mix the content. About 0.25 mL of $FeCl_3$ solution was added into the test tube II, mixed it. Added 0.25 mL of H_3PO_4 solution and mix again; this way a test sample is obtained for spectrophotometric measurements. Then, prepared the standard sample (0.5 mL of the standard solution instead of the analysed liquid):using Trolox; prepare as the test sample, using a-tocopherol; add 0.5 mL of DI water instead of anhydrous ethanol at the beginning of the analysis; do not centrifuge this sample - measured the absorbance of the test sample Ax and of the standard sample A at 539 nm against the blank test (preparation; as the test sample but using water instead of the analysed liquid) of vitamin E (μM) in the analysed liquid, using the a/a formula.

Anhydrous ethanol; xylene (mixture of isomers); 6.02 mM solution of batophenanthroline (stable for three weeks in a fridge), 0.98 mM solution of anhydrous iron chloride (III) (stable for one week in a fridge) and 40 mM solution of crystalline orthophosphoric acid - all in anhydrous ethanol; 23.2 µM standard solution of vitamin E (as substance Trolox - in DI water, or as a-tocopherol - in anhydrous ethanol).

Dietary antioxidant compounds are normally poorly digested and absorbed in the small intestine, and then enters the colon (Bianchi et al. 2010). Hence, the present experiment was performed with the prime intention of studying whether the curcumin and tartrazine modifies digestion of antioxidants vitamins and unabsorbed dietary antioxidants reaches the colon or not. For determination of antioxidants vitamin, A, C and E in fecal, same methods were used as plasma.

DNA DAMAGE

DNA was extracted from blood sample of tartrazine and curcumin control and treated groups by using the extraction kit (Catalog #:10503027, DNAzol™ Reagent; Thermo Fisher Scientific, USA). DNA damage was quantified by quantification colorimetric kit (Catalog #K253-25; BioVision Incorporated, 155 S. Milpitas Boulevard, Milpitas, CA 95035 USA).

STATISTICAL ANALYSIS

The statistical analysis was done by using SPSS software (version, 25). The data were analysed using one-way analysis of variance (ANOVA) followed by Tukey-Kramer (TK) multiple comparisons post-test. Mean values were separated at $p \leq 0.05$. The data are reported in tables as mean ± standard deviation (SD).

RESULTS

REACTIVE OXYGEN SPECIES (ROS) IN PLASMA AND FECAL

Oral administration of curcumin and tartrazine significantly increased the total oxidants level in HT in treatment groups of 30 days. Whereas, significantly decreased the total antioxidants level in HT 45 treatment (Table 1). Catalase was significantly decreased in LC15 but increased in LC30 and LC45 compared to control, HC30 and HC45. Current findings showed that glutathione reductase was lower in LT30, HT30, HC30, LT45, and HT45 while significantly increased in LC45 and HC45 compared to control. Whereas, elevation of arylestrase was seen in treated group of HT15, LT45, HT45 and HC45 compared to control shown in Table 1. Oral administration of low curcumin significantly decreased the MDA level in LC30 and LC45 treated groups, respectively.

This study finds out that, alterations were seen in the fecal of treated rats with high and low dose of curcumin and tartrazine. Tartrazine and curcumin administration was significantly ($p \leq 0.05$) decreased the total antioxidants level in HT30, HC30 and HC45. Level of catalase was high in all treated groups of 15 days, LT, LC and HC groups of 30 days; HT, LC an HC of 45 days compared to control (Table 2). Whereas, glutathione reductase was significantly ($p \leq 0.05$) decreased in HT15, LC15, LT30, LC30 and increased in HT30, LC30, LT45, LC45 and HC45 compared to control mentioned in Table 2. Regarding arylestrase, curcumin treated group (LC30) showed significant ($p \leq 0.05$) increased level whereas low and high tartrazine treated group depicted higher level arylesterase after 45 days. MDA level was significantly low in LT15, LC15 and HC15 compared to control.

ANTIOXIDANTS VITAMINS IN PLASMA AND FECAL

The present study showed that low and high doses of curcumin and tartrazine significantly ($p \leq 0.05$) decreased the concentration of vitamin C in all groups of 30 days, HT45 and HC45. Concentration of vitamin C have been lower in low and high doses of curcumin and tartrazine group of 15 days compared to control. Oral administration of tartrazine and curcumin significantly ($p \leq 0.05$) increased the level of vitamin E in HT30, HC30, LT45 and HC45 (Table 3).

Oral administration of tartrazine significantly increases the level of vitamin A in fecal of HT45 and HC45. Vitamin C was significantly high in fecal after 45 days of treatment with high curcumin dose as compared to control. Regarding vitamin E, it was significant increment in HT30, HC30, LT45 and HC45 as compared to control group (Table 3).

DNA DAMAGE

Current finding showed that oral administration of curcumin and tartrazine did not caused alteration in the ARP sites of DNA showing the DNA damage in treated group compared to control after 30 and 45 days of treatment as mentioned in Table 4.

TABLE 1. Effects of tartrazine and curcumin on reactive oxygen species in plasma after 15, 30 and 45 days of treatment

Days	Food color exposure	TOS (µmol/l)	TAS (mmol/l)	CAT (KU/L)	GP (U/L)	GSH (U/mL)	ARY (U/L)	MDA (nMol/mL)
15	Control	17.86±2.38 de	5.01±0.69 ab	19.46±6.3 de	50.49±5.80 abcde	413.49±49.64 ab	39.13±0.62 b	0.84±0.12 c
	LT	18.76±3.07 de	4.11±0.65 ab	14.66±2.83 de	29.52±20.73 de	373.73±91.99 abcd	38.89±0.18 b	1.16±0.44 abc
	HT	19.09±3.27 de	3.93±0.56 ab	25.48±6.37 bcde	22.26±9.24 e	365.63±79.18 abcd	47.72±5.61 a	0.66±0.26 abc
	LC	16.38±3.82 e	4.29±0.83 ab	11.84±2 e	45.82±16.61 abcde	392.38±42.78 abc	39.51±0.93 b	0.89±0.06 abc
	HC	18.09±1.77 de	4.15±0.56 ab	19.14±5.63 de	43.27±8.32 bcde	331.98±51.86 bcd	38.95±0.55 a\b	1.19±0.85 abc
30	Control	18.48±3.69 de	5.1±1.77 ab	20.08±1.78 cde	62.74±4.00 abc	330.08±15.88 bcd	39.308±0.53 a\b	1.07±0.29 abc
	LT	38.91±13.07 abcd	3.70±1.05 ab	17.28±6.80 de	68.84±12.24 ab	229.65±49.67 cde	49.39±0.75 a	1.15±0.35 abc
	HT	59.08±27.61 a	3.47±0.59 b	15.98±2.5 de	46.11±7.067 abcde	239.03±64.17 cde	45.15±4.07 ab	1.82±0.05 abc
	LC	23.12±1.68 cde	4.99±0.58 ab	39.72±18.66 ab	64.45±22.81 abc	413.53±43.04 ab	38.93±0.34 b	0.75±0.12 c
	HC	27.34±10.04 cde	4.78±0.78 ab	27.72±7.19 bcd	49.45±28.62 abcde	211.90±142.78 de	44.58±7.52 ab	1.28±0.42 abc
45	Control	36.02±13.23 bcde	6.04±1.28 a	34.08±6.76 abc	55.78±10.92 abcd	243.83±38.37 bcde	39.01±0.45 b	1.36±0.39 abc
	LT	48.19±10.77 ab	4.15±0.75 ab	23.45±8.08 cde	39.21±4.89 bcde	101.83±31.52 e	49.85±6.14 a	2.02±0.66 ab
	HT	52.53±19.72 ab	3.30±0.47 b	22.97±3.710 cde	37.47±5.75 cde	153.23±35.35 e	51.47±5.25 a	2.05±0.61 ab
	LC	40.05±9.93 abcd	4.82±0.90 ab	45.08±4.86 a	73.87±28.00 a	520.88±81.10 a	39.64±0.79 b	0.85±0.14 c
	HC	42.36±6.38 abc	5.06±3.11 ab	27.55±3.81 bcd	49.28±7.41 bcde	531.46±24.51 a	47.71±7.49 a	2.15±1.52 a

Values shown are mean (± SD) from n = 6 rats/group at each timepoint. Values in each column followed by different letters are significantly different ($p \leq 0.05$).
C =Control, LT= Low Tartrazine, HT= High Tartrazine, LC= Low Curcumin, HC= High Curcumin at 15, 30 and 45, TOS= Total oxidants status; TAS= Total Antioxidants Status; CAT= Catalase; GP= Glutathione peroxidase; GSH= Glutathione; ARY= Arylesterase; MDA= Malondialdehyde

TABLE 2. Effects of tartrazine and curcumin on reactive oxygen species in fecal after 15, 30 and 45 days of treatment

Days	Food color exposure	TOS (µmol/l)	TAS (mmol/l)	CAT (KU/L)	GP (U/L)	GR (U/mL)	ARY (U/L)	MDA (nMol/mL)
15	Control	16.08±3.49 c	3.18±0.22 abc	77.51±3.01 de	304.18±3.66 abc	558.60±3.29 bc	44.27±0.77 c	0.87±0.06 ab
	LT	17.06±4.70 c	2.37±0.34 abc	176.14±66.81 abc	196.42±4.77 abc	592.95±4.07 b	44.90±0.77 c	0.64±0.10 b
	HT	15.26±3.20 c	2.26±0.59 bc	213.18±12.99 ab	160.41±6.61 c	478.95±25.21 de	46.22±0.36 c	1.35±0.52 a
	LC	16.45±0.88 c	2.43±0.42 abc	180.22±11.11 abc	170.62±0.81 bc	383.10±40.18 f	45.18±0.83 c	0.64±0.09 b
	HC	16.48±0.78 c	2.49±0.39 abc	164.58±5.00 bc	270.71±0.59 abc	548.80±50.23 bcd	43.13±0.61 c	0.65±0.11 b
30	Control	19.44±6.01 bc	3.98±1.18 ab	230.90±1.90 a	386.57±3.86 abc	596.72±5.38 b	46.93±6.36 c	0.67±0.13 b
	LT	39.83±7.07 abc	2.01±0.50 bc	132.23±3.35 cd	486.23±12.34 a	211.99±1.71 g	44.40±1.83 c	0.52±0.05 b
	HT	38.74±13.71 abc	1.69±0.45 c	222.03±7.58 a	177.44±3.48 bc	683.05±2.85 a	46.48±2.35 c	0.69±0.20 b
	LC	46.94±18.93 a	2.65±0.43 abc	88.79±7.35 de	255.49±5.94 abc	511.91±2.74 cde	59.72±1.27 a	0.60±0.15 b
	HC	44.61±13.16 ab	2.14±0.56 bc	75.85±3.14 e	149.18±1.43 c	594.48±4.23 b	42.00±1.30 c	0.55±0.06 b
45	Control	30.97±13.82 abc	4.27±0.43 a	180.24±1.72 abc	486.18±85.64 a	592.14±4.23 b	42.43±2.65 c	0.61±0.11 b
	LT	41.84±8.99 abc	2.31±0.26 abc	180.96±2.36 abc	242.70±59.30 abc	702.92±15.20 a	45.55±2.28 b	0.59±0.07 b
	HT	50.19±1.15 a	2.67±1.59 abc	50.96±8.96 e	321.74±67.07 abc	707.94±2.61 a	46.44±2.02 b	0.56±0.06 b
	LC	47.35±4.28 a	2.64±0.49 abc	44.16±1.47 e\	242.64±67.54 abc	455.04±6.83 ef	42.56±0.80 c	0.58±0.08 b
	HC	55.24±5.68 a	2.27±0.51 bc	37.20±0.99 e	466.26±68.88 ab	597.17±63.07 b	42.02±0.06 c	0.62±0.10 b

Values shown are mean (± SD) from n = 6 rats/group at each timepoint. Values in each column followed by different letters are significantly different ($p \leq 0.05$).
TOS= Total oxidants status; TAS= Total Antioxidants Status; CAT= Catalase; GP= Glutathione peroxidase; GSH= Glutathione; ARY= Aylesterase; MDA= Malondialdehyde

TABLE 3. Effects of tartrazine and curcumin antioxidants vitamin in plasma and fecal samples after 15, 30 and 45 days of treatment

Days	Food color exposure	Plasma			Fecal		
		Vitamin A (µM/L)	Vitamin C (µM/L)	Vitamin E (µM/L)	Vitamin A (µM/L)	Vitamin C (µM/L)	Vitamin E (µM/L)
15	Control	4.518±2.08 ^{a}	15.29±4.14 ^{abc}	16.55±3.34 ^{a}	3.05±0.65 ^{bc}	9.30±1.19 ^{ab}	2.53±0.66 ^{c}
	LT	3.851±1.34 ^{a}	11.64±4.88 ^{bcdef}	14.57±3.70 ^{abc}	3.88±1.79 ^{abc}	8.76±0.32 ^{b}	2.21±0.96 ^{c}
	HT	2.456±1.27 ^{a}	11.51±4.16 ^{bcdef}	10.00±2.62 ^{bcd}	4.96±0.65 ^{abc}	9.77±2.28 ^{ab}	2.54±1.20 ^{c}
	LC	3.458±1.12 ^{a}	14.22±1.50 ^{abcd}	16.47±4.77 ^{a}	2.92±0.44 ^{c}	9.13±0.76 ^{ab}	2.33±0.96 ^{c}
	HC	3.28±1.46 ^{a}	12.74±3.45 ^{abcde}	8.44±1.34 ^{de}	3.16±1.78 ^{bc}	9.3±1.95 ^{ab}	2.18±0.85 ^{c}
30	Control	3.85±1.96 ^{a}	18.67±2.49 ^{a}	14.79±2.21 ^{ab}	2.97±1.23 ^{bc}	7.71±1.59 ^{b}	2.22±0.96 ^{c}
	LT	2.681±0.70 ^{a}	9.09±0.34 ^{cdef}	9.67±2.05 ^{bcd}	4.22±0.30 ^{abc}	9.12±0.99 ^{ab}	3.18±0.93 ^{bc}
	HT	2.631±1.89 ^{a}	6.11±1.69 ^{f}	9.39±2.70 ^{cd}	5.60±1.00 ^{abc}	9.24±1.20 ^{ab}	5.07±0.41 ^{ab}
	LC	3.494±3.06 ^{a}	11.63±4.95 ^{bcdef}	13.53±1.83 ^{abcd}	3.96±0.68 ^{abc}	6.99±0.32 ^{b}	1.97±0.32 ^{c}
	HC	2.841±0.79 ^{a}	8.05±2.18 ^{def}	11.54±2.03 ^{abcd}	5.75±0.17 ^{ab}	8.88±0.87 ^{b}	5.7±0.03 ^{a}
45	Control	4.23±1.25 ^{a}	17.03±2.74 ^{ab}	11.47±2.91 ^{abcd}	3.29±0.17 ^{bc}	8.56±1.29 ^{b}	3.31±0.88 ^{bc}
	LT	5.13±1.78 ^{a}	11.09±4.26 ^{bcdef}	3.85±2.62 ^{ef}	4.98±0.63 ^{abc}	10.16±0.65 ^{ab}	5.69±0.36 ^{a}
	HT	2.68±2.70 ^{a}	8.06±1.79 ^{def}	1.03±0.88 ^{f}	6.17±1.14 ^{a}	10.38±0.06 ^{ab}	1.35±0.82 ^{c}
	LC	3.43±0.88 ^{a}	13.29±2.31 ^{abcde}	2.30±1.39 ^{f}	5.03±0.68 ^{abc}	9.45±0.54 ^{ab}	2.28±0.96 ^{c}
	HC	2.24±1.99 ^{a}	7.42±1.85 ^{ef}	0.82±0.31 ^{f}	6.28±0.51 ^{a}	12.87±2.23 ^{a}	5.82±0.35 ^{a}

Values shown are mean (± SD) from n = 6 rats/group at each timepoint. Values in each column followed by different letters are significantly different ($p \leq 0.05$)

TABLE 4. Effects of tartrazine and curcumin on ARP Site of DNA for DNA damage

Days	Food color exposure	DNA damage
30	Control	8.99±4.15 ^{a}
	LT	5.29±2.68 ^{a}
	HT	5.66±1.44 ^{a}
	LC	8.74±6.54 ^{a}
	HC	8.49±3.08 ^{a}
45	Control	4.35±1.71 ^{a}
	LT	8.65±2.07 ^{a}
	HT	19.39±15.9 ^{a}
	LC	5.96±2.59 ^{a}
	HC	8.08±1.89 ^{a}

Values shown are mean (± SD) from n = 6 rats/group at each timepoint. Values in each column followed by different letters are significantly different ($p \leq 0.05$).

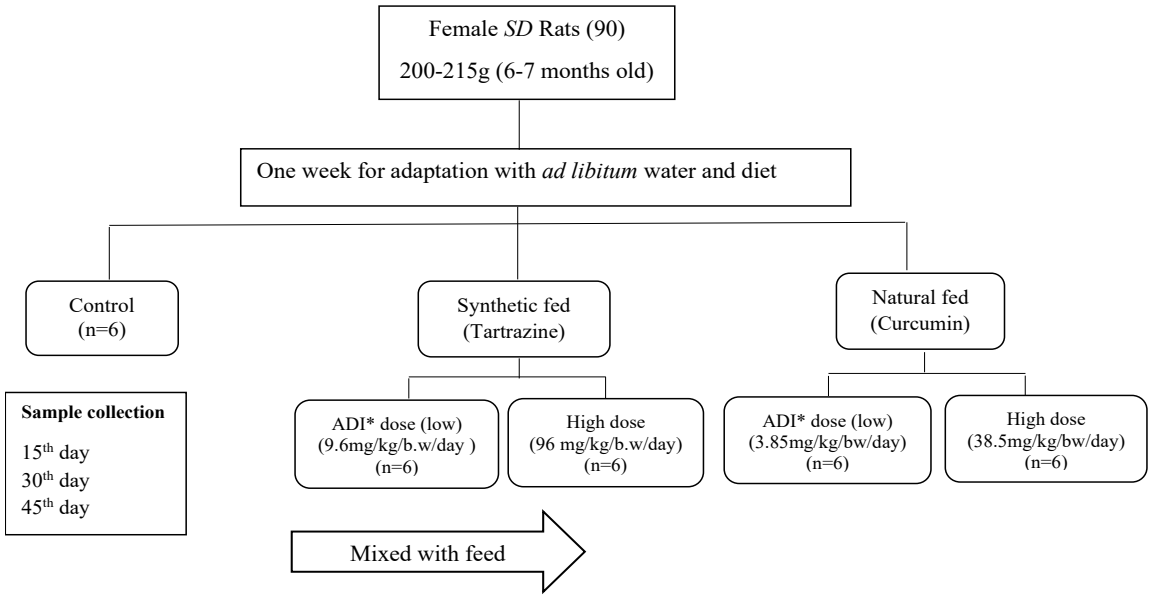

FIGURE 1. Experimental animals and study protocol

DISCUSSION

Results are in agreement with Amin et al. (2010) who check the effect of tartrazine and carmoisine synthetic food colorants: El-Wahab and Moram (2013) who found reduction in antioxidant level and high level of oxidants/ reactive oxygen species (ROS) after administration of brilliant blue, carmoisine, and tartrazine in male rats. Whereas, curcumin did not affect total oxidant and total antioxidants level of treated group after 15, 30, and 45 days, but in previous studies curcumin was found to have a strong antioxidant effect (Amani et al. 2010). A decrease in the production of antioxidants, mechanism of antioxidants and accelerated production of ROS are some of the reasons behind the occurrence of oxidative stress (Rahal et al. 2013). In this study, the reduction of total antioxidant level in the plasma of tartrazine-treated animals may be associated to enhanced generation of free radical generation because of administering tartrazine and/or impairing machinery of antioxidant resulting in enhanced oxidative stress.

Pincemail et al. (2002) stated that production of ROS is accurately regulated because the antioxidants have the appropriate capacity. The risk of chronic diseases from oxidative stress is decreased when antioxidants are applied because they reduce oxidation of lipid and protein by scavenging species of oxygen (Farombi et al. 2007).

These results of catalase are in line with Amin et al. (2010) who stated that high dose of tartrazine treated male rats indicated particular reduction in the level of catalase enzymes. Similar results were found by Golli et al. (2016) who resulted that oral administration of tartrazine decreased the level of catalase in liver, whereas increased in kidney tissue of treated rats. Catalase accelerates the degradation of hydrogen peroxide in water molecules and oxygen to prevent the formation of hydroxyl radicals, acting as the first line of defensive antioxidant enzyme (Kaur & Sandhir 2006). Futhermore, Abdel-Aziz et al. (2019) also concluded that 300 mg/kg b.wt. dose of tartrazine given with orogastric tube to wister rats for 30 days significantly decreased the catalase level. Whereas, low curcumin dose after 30 day and high dose after 45 days elevated the catalase level in treated as compared to control. It is confirmed from previous studies that curcumin was found to have a strong antioxidant effect (Amani et al.

2010). Results are different from Abdel-aziz et al. (2019) who stated that cucurmin have non-significant effect on catalase level of treated rats after 30 days.

The results of glutathione are in accordance with Amin et al. (2010) who found decreased level of GSH in tartrazine treated rats. Similar results were found by Golli et al. (2016) who conclude that 300 mg/kg b.wt. of tartrazine significantly decreased the hepatic GSH level. The subsequent reduction in hepatic GSH activity is suggestive of impaired hepatic detoxifying capacity. In a same way, El Wahab and Moram (2012) who noticed decrease in the GSH content in the blood of rats receiving tartrazine, carmosine and brilliant blue and it might be related to the increased utilization of GSH in conjugation with foreign compounds entering the body (Murray 1996; Thomas 1994).

Low and high doses of curcumin did not change the level of glutathione peroxidase. These results are in line with Sharad et al. (1999) who stated that oral administration of curcumin (15.7 and 157 mg/kg b.wt.) daily for 2 months did not affect either hepatic or renal glutathione as compared to the control group. Abdel-Aziz et al. (2019) further confirmed that curcumin have no effect on glutathione level in treated group after 30 days. Results of glutathione reductase are in accordance with Mehedi et al. (2009) who studied the toxic effects of tartrazine in mature male albino rats and observed a specific increase in glutathione reductase (GR) concentration. The data from the previous studies have shown that the administration of these tartrazine, carmosine and brilliant blue causes a significant decrease in the hepatic GSH level with a subsequent reduction in hepatic GST activity, which is suggestive of impaired hepatic detoxifying capacity (Murray 1996). One possible consequence of this event is the increased amount of the food additive reactive metabolite irreversibly bound to hepatic protein sulfur nucleophiles (Halliwell et al. 1997; Liu et al. 1999).

When a strong oxidative stress is exposed to mammalian cells, this condition may need both increased action of GSH for maintaining redox status, but also boosted the precursors and supply of energy for increasing content of GSH and pass it to the required areas. Moreover, when there is prolonged oxidative stress when the cells are not capable anymore to defend themselves from oxidative stress, the GSH/GSSG ratio will decrease as a result of reduced free GSH level (Aquilano et al. 2014). Superoxide anion, hydrogen peroxide and hydroxyl radical could enhance oxidative stress and could be formed by the nitrosamine metabolism (Bansal 2005).

Regarding the arylesterase, results are different from the Saima (2011) who concluded that tartrazine have non-significant effect on arylesterase level in treated rabbit after 15 day. Differences in results might be due to species, dose of administration of tartrazine and duration of study. The current study stated the increased malondialdehyde (MDA, end product of lipid peroxidation) levels to be causing oxidative stress in the rats treated with tartrazine. Similar results were found by Amin et al. (2010) who resulted that low and high dose of carmosine and tartrazine showed significantly increased in liver MDA level of male rats. Current results are also in line with Golli et al. (2016) who concluded that 300 mg/kg b.wt. dose of tartrazine increased the MDA level in liver and kidney of treated rats after 30 days. Furthermore, Abdel-Aziz et al. (2019) also concluded that 300 mg/kg b.wt. dose of tartrazine given with orogastric tube to wister rats for 30 days significantly increased the MDA level. These results are also related to the study by Demirkol et al. (2012), who examined the oxidative effects of tartrazine and other azo dyes on Chinese hamsters and concluded that tartrazine increased the MDA level in treated group. Tartrazine administration may attribute increased peroxidation of lipid which can lead to the formation of ROS.

On the other hand, high curcumin dose significantly elevated the MDA level in treated animal after 45 days. Results are different from Kalpana and Menon (2004) who found that oral administration of curcumin (15.7 or 157 mg/kg b.wt.) daily for 2 months decreased hepatic lipid peroxide concentration as compared to the control group. Another study also concluded that curcumin have no significant effect on MDA level of treated group (Abdel-Aziz et al. 2019).

Current study is accordance with Orozco et al. (2010) who observed a specific enhancement in production of fecal ROS by 36% after iron supplementation ($p = 0.026$). Babbs (1990) and Erhardt et al. (1997) gave out hypothesis that fecal matrix can be involved in the formation of ROS and it may be important generating colorectal cancer. The generation of reactive species of O and N in the intestine are contributed by endogenously produced metabolites, intestinal microflora, and dietary factors. The demonstration to produce feces dilution to form hydroxyl radicals has been done by Babbs (1990). It is due to the catalytic activity of the available Fe and oxidative metabolism of the bacteria. Free radicals in the large bowel can be generated through minor inflammatory reactions as well. ROS is elaborated by activated macrophages and neutrophils upon migration to injured tissues which can cause cell damage through inflammation (Babior 1978).

Furthermore, phospholipid breakdown can be induced in the membrane of colonic cells by bile acids affecting the gut oxidative status (Craven et al. 1986). Various important biological products can be damaged by free radicals, such as; lipids, proteins, and DNA. Additionally, aromatic hydroxylation is accomplished by the hydroxyl radical (Grootveld & Halliwell 1986); this is specifically harmful in the colon where a number of harmful chemicals from foods, additives, constituents and drugs, can be easily converted into carcinogen mechanism. The production of free radical in the colon can be contributed by different dietary intakes. But pro-oxidants only contribute to oxidative stress. Oral administration of rutoside in rats, ameliorated inflammatory diseases, through preventing depletion of glutathione (Cruz et al. 1998).

Previous study discussed about the positive impact of tartrazine as iron chelators on fecal antioxidant status in regard to the assumptions; simultaneously, the antioxidant status of human feces has positive correlation with the polyphenolic-containing beverages consumption (Garsetti et al. 2000). In contrast, diets having high pellets more supplemented vitamins (Vitamin E) and iron enhanced levels of dietary vitamin E have positive influence on the status of fecal antioxidant (Stone et al. 2002).

Antioxidant vitamins (A, C and E) level of control and treated experimental group are presented in Table 3. Result are contrast with the Cemek et al. (2014) who did not observed any statistically significant changes in antioxidant vitamin levels in serum of rats treated with 15 mg/kg b.wt. of tartrazine. Vitamin E (mostly α-tocopherols, lipid soluble antioxidant) and Vitamin A (retinol and β-carotene) can take part in the mechanism of redox of the cell and neutralize ROS (Sayed et al. 2012).

Any xenobiotic or endobiotic that produces oxidative stress by generating ROS or inhibiting antioxidant systems is called pro-oxidant. All kinds of molecules and cellular tissues that have free radicals are included in it. Literatures dealing with this subject is lacking, in this regards not been able to find a studies targeting antioxidant vitamin in plasma and fecal. It is difficult to compare results with the literature. Thus, the study is a unique work to present relationship between antioxidant vitamins and synthetic and natural food colorants. Present work is a major reference of which results certainly have great potential to be translated into important advances in public health.

Current study showed that administration of curcumin and tartrazine did not cause any significant alterations in ARP sites of DNA. Concerning the genotoxicity of synthetic colorants, results are contradicted with Himri et al. (2011) who showed that DNA damage in leucocytes was caused by tartrazine as detected by comet assay. This genotoxic effect is because of the direct contact of tartrazine with nuclear DNA. Data pertaining to the tartrazine's genotoxic effect with positive results are available. Mpountoukas et al. (2010) investigated the toxic effect of tartrazine at 0.02 - 8 mM in *in vitro* human peripheral blood cells. Additionally, chromosomal aberrations in fibroblast cells of Muntiacus muntjac were induced by tartrazine (Chung et al. 1981). Results were opposite from Hassan (2010) who showed that regular dose of tartrazine administration (7.5 and 15 mg/kg b.wt.) for seven weeks leads to kidney and liver damage of DNA. Induction of tartrazine-induced DNA damage in comet assays was observed in cells from the colon of mice at a low dose (10 or 100 mg/kg) (Sasaki et al. 2002) that is slightly higher than the recommended regular intake of humans approved by the Joint FAO/WHO Expert Committee on Food Additives (FAO/WHO 1999).

Conclusion

Based on the results mentioned before, it can be stated that tartrazine is capable to form ROS, thus, accelerate oxidative stress, changing the plasma antioxidants vitamins profiles which in turn, decrease the level of antioxidant. Curcumin, as a natural food color have minimum side effect on the total antioxidant level. From the present study it was concluded that use of food colorants in food items increases the chances of different diseases in the body i.e. free radical production leading to development of oxidative stress in the body and prone the body to other serious ill effects. Therefore, tartrazine consumption control is necessary for health and minimizing the usage of tartrazine as well as high dose of curcumin, especially in foods on daily basis, is highly advisable.

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Sadaf Shakoor*, Amin Ismail & Mohd Redzwan Sabran
Department of Nutrition
Faculty of Medicine and Health Sciences
Universiti Putra Malaysia
43400 UPM Serdang, Selangor Darul Ehsan
Malaysia

Sadaf Shakoor*
Sub Campus Burewala-Vehari
University of Agriculture Faisalabad
Punjab
Pakistan

Amin Ismail
Research Centre of Excellence
Nutrition and Non-Communicable Diseases (RCOE NNCD)
Faculty of Medicine and Health Sciences
Universiti Putra Malaysia
43400 UPM Serdang, Selangor Darul Ehsan
Malaysia

Zia-Ur-Rahman
Institute of Pharmacy
Pharmacology, and Physiology
University of Agriculture Faisalabad
Punjab
Pakistan

Norhafizah Mohtarrudin
Department of Pathology
Faculty of Medicine and Health Sciences
Universiti Putra Malaysia
43400 UPM Serdang, Selangor Darul Ehsan
Malaysia

*Corresponding author; email: gs45177@student.upm.edu.my

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