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Impact of tartrazine and curcumin on mineral status, and thyroid and reproductive hormones disruption *in vivo*

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Abstract

Endocrine disrupters (EDCs) are naturally occurring or man-made substances that either mimic or obstruct the functions of oestrogens and androgens, thyroid hormones, as well as microminerals in the body. The present work aimed to evaluate the effects of oral administration of tartrazine and curcumin, synthetic and natural dyes, respectively, on thyroid hormones (T_3, T_4, and TSH), female reproductive hormones (oestrogen, progesterone, LH, and FSH), and minerals (iron, copper, zinc, sodium, potassium, and chloride) in plasma, liver, and kidney of female rats after 15, 30, and 45 d of treatment. The rats were treated with admissible daily intake (ADI) and 10× ADI (9.6 and 96 mg/kg/body weight for tartrazine, 3.85 and 38.5 mg/kg/body weight for curcumin, respectively). Results showed significant changes in thyroid and female reproductive hormones, especially, in the tartrazine-treated groups as compared to the control. Low and high doses of tartrazine and curcumin significantly ($p < 0.05$) decreased iron, copper, and zinc concentrations in plasma, whereas, the concentrations of sodium and copper in liver and kidney increased. Both tartrazine and curcumin, at ADI and 10× ADI, resulted in lower LH levels after 30 and 45 d of treatment. After 30 d, low and high dose of tartrazine significantly decreased T_4, oestrogen, and FSH levels; whereas, progesterone level increased. The results demonstrated that hormone secretion and mineral content in tissues are severely affected at ADI and higher concentrations of tartrazine and curcumin. These observations suggested that lower doses of these dyes might be a safer option for their usage in foods and pharmaceuticals.

Introduction

Food colorants (FCs) are used in the food industry to make food appear appealing to consumers. Their usage is also for the sake of variety in food and non-food products. Taste, appearance, texture, and microbiological safety must be maintained in foodstuff for the longest period of time. Although considerable improvements have been achieved in terms of food additives, yet few are still controversial. The lack of uniformity in worldwide laws regarding additives, along with conflicting results of many studies, contributes to perpetuating the controversy (Carocho *et al.,* 2015; Andreozzi *et al.,* 2019).

Generally, natural dyes extracted from plant materials, *e.g.,* caramel, saffron, and curcumin, in addition to pharmaceutical applications, are preferred in foods due to their familiarity and acceptance among consumers. However, the use of natural dyes has been replaced by synthetic dyes due to their low cost, stability, and more attractive colours (Sharma, 2015). Numerous side effects *i.e.,* allergic reactions, behavioural and neurocognitive effects, and toxicity have been related to both medium and long-terms use of food colorants (Martins *et al.,* 2016). Chequer *et al.* (2011) reported that the synthetic dye ‘‘azo’’ was condensed through aromatic amines via intestinal microflora, and possibly by mammalian azoreductase in liver, or in intestinal wall following ingestion by

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rat. The aromatic amines are then converted into N-hydroxy derivatives by the actions of cytochrome P450 enzymes. This biotransformation occurs in several mammalian species, as well as in humans (Chequer *et al.,* 2011).

Tartrazine, also known as E102 or FD and C Yellow 5 or C.I. 19140, is a synthetic lemon-yellow azo dye made by coal tar, and used as a food colorant. Tartrazine [trisodium 5-hydroxy-1-(4-sulfonatophenyl)-4-(4-sulfonatophenylazo) diazenyl-H-pyrazol-3-carboxylate] is water soluble. Amin *et al.* (2010) reported that tartrazine can negatively affect biochemical markers such as antioxidant enzymes in vital organs of rats. It is well established that these antioxidant enzymes, *e.g.,* superoxide dismutase (SOD), catalase, glutathione peroxidase, and dietary antioxidants such as β-carotene, melatonin, and vitamins A, C, and E play an important protective role against oxidative stress (El-Habit *et al.,* 2000; Canter *et al.,* 2007; Kharwar and Haldar, 2012).

Curcumin (E100) is a yellow-orange pigment, and generally obtained from turmeric (*Curcuma longa*). Turmeric is a common constituent in gravy spices, and has been used in Asian medicine for treatment of various disorders such as obesity, hypertension, cancer, and skin related diseases for many years. Experimental studies have demonstrated that although concentrations of curcumin induce antioxidant effects, yet higher concentrations of this compound increase the cellular levels of ROS (Fang and Holmgren, 2005; López-Lázaro, 2008).

The general health and wellbeing of humans are coordinated by complex interactions and functions of various biological and chemical components. Several minerals are required to maintain homeostasis and physiological functions of the body such as redox reactions, cell membranes, stability of biological molecules, and control of biological processes (Cemek *et al.,* 2014). Thyroid hormones play crucial roles in the maintenance and regulation of metabolic functions, growth, and development of normal sexual characteristics (Gyamfi *et al.,* 2009; Bursuk *et al.,* 2010). Several factors have been reported to affect the thyroid functions such as drugs, chemicals, and food additives (including food colorants). Reproduction is an event which requires the coordination of peripheral organs with the nervous system to ensure that the internal and external environments are optimal for successful procreation of the species (Christensen *et al.,* 2012). Anterior pituitary gonadotropes are responsible for the regulation and release of follicle stimulating hormone (FSH) and luteinising hormone (LH). These hormones might assist in reproduction regulation including egg maturation, oestrogen and progesterone production, and successful pregnancy (Nakamura *et al.,* 2005).

There is limited information available about the effects of food colorants on minerals, thyroid, and reproductive hormones in biological systems. Therefore, the objective of the present work was to explore the effects of two widely used food colorants, *e.g.,* tartrazine (synthetic) and curcumin (natural), on reproductive hormone levels, and concentrations of minerals [iron (Fe), copper (Cu), zinc (Zn), sodium (Na), potassium (K), and chloride (Cl] in plasma, liver, and kidney of female adult rats after 15, 30, and 45 d of treatment.

Materials and methods

Tartrazine (CAS 1934-21-0, purity of 86.7%) was purchased in the form of powder from Sigma Aldrich (USA). Its chemical formula is trisodium salt is trisodium 5-hydroxy-1-(4-sulfonatophenyl)-4-(E)-(4 sulfonatophenyl) diazenyl-1H-pyrazole-3-carboxylate, its molecular formula is $C_{16}H_9N_4Na_3O_9S_2$, and its formula weight is 534.3 g/mol.

Curcumin (CAS 458-37-7, purity of 95%) was purchased in the form of powder from Carbosynth (UK). Its chemical formula is 1,7-bis (4-hydroxy-3methoxyphenyl)-1E,6E-heptadiene-3,5-dione, its molecular formula is $C_{21}H_{20}O_6$, and its formula weight is 368.4 g/mol.

Dose calculation

The animal equivalent dose (AED) is calculated on the basis of body surface area, either dividing or multiplying the human dose (mg/kg) by Km ratio provided by the literature (FDA, 2005; Nair and Jacob, 2016). In the present work, the AED was calculated using Eq. 1.

$$AED = HED \times Km \quad \text{(Eq. 1)}$$

where, AED = animal equivalent dose (mg/kg); HED = human equivalent dose [for tartrazine = 7.5 mg/kg body weight/day, and for curcumin = 3 mg/kg body weight/day]; Km = body weight (kg)/body surface area (m^2), The Km ratio for rat used in the present work was 6.2 or 0.162 (FDA, 2005).

The animal grouping and doses are shown in Figure 1.

Animal ethics

The animal ethics for the present work was

approved by the Institute of Pharmacy, Physiology and Pharmacology, University of Agriculture (Faisalabad) Ethics Committee (Punjab-Pakistan) with the application number of 1502, dated 4th July, 2017.

Animal selection

Female subjects are underrepresented in animal research across different disciplines; therefore, the lack of research on female subjects has likely resulted in poorer treatment outcomes for women (Klein *et al.,* 2015). In 2014, noting the potential human health consequences of research bias, the NIH instituted policies to encourage the use of female animal research subjects, considering sex as a biological variable; similarly, female rats could also be important to be included in researches. Klein *et al.* (2015) also reported that the inclusion of both sexes in animal studies derived novel discoveries in both basic and clinically relevant researches. There is deficient literature regarding the effect of food colorants on adult female rats.

On the basis of the above discussion, 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 of 27 ± 5°C, and under good ventilation.

Body weight, feed, and water intake

Body weights of animals were recorded before and after the experiment using a weighing scale. The percentage of gained weight was expressed as [(final weight – start weight) / start weight] × 100 (El-Malky *et al.,* 2014; Shakoor *et al.,* 2020). Animals (under experiment) were provided with 30 - 35 g/oral feed and 30 - 35 mL/water; according to the composition of experimental diets by Kim *et al.* (2005). Each group consisted of six rats, categorised 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 206.65 ± 3.20 g for tartrazine group, and 206.95 ± 5.40 g for curcumin group.

Sampling

Sampling was done within 15, 30, and 45 d to check for acute, sub-acute, and post-acute toxicity. By the end of each experimental periods, 4 - 5 mL blood samples were collected through cardiac puncture of the rats by using 23G needle under general anaesthesia (diethyl ether; 4 - 5 mL/rat) (Plate *et al.,* 2005; JHU, 2018). Blood was collected in BD Vacutainer® heparin tubes, and then rats were immediately euthanised through cervical dislocation. The blood was centrifuged (LYC; Centrifuge 80-2, Jiangsu, China) for 15 min at 1,795 *g* at room temperature to collect plasma, which was transferred to the Eppendorf tubes (1.5 mL), and stored at -20°C until further analysis (within four weeks).

Determination of mineral contents

Wet digestion method was used to determine the concentrations of iron (Fe), copper (Cu), zinc (Zn), sodium (Na), potassium (K), and chloride (Cl) by adopting the method of Licata *et al.* (2004). Briefly, 1 mL of plasma or 1 g of organ tissue was subsampled and used for the analysis. Next, 10 mL of nitric acid was added to the sample in a digestion

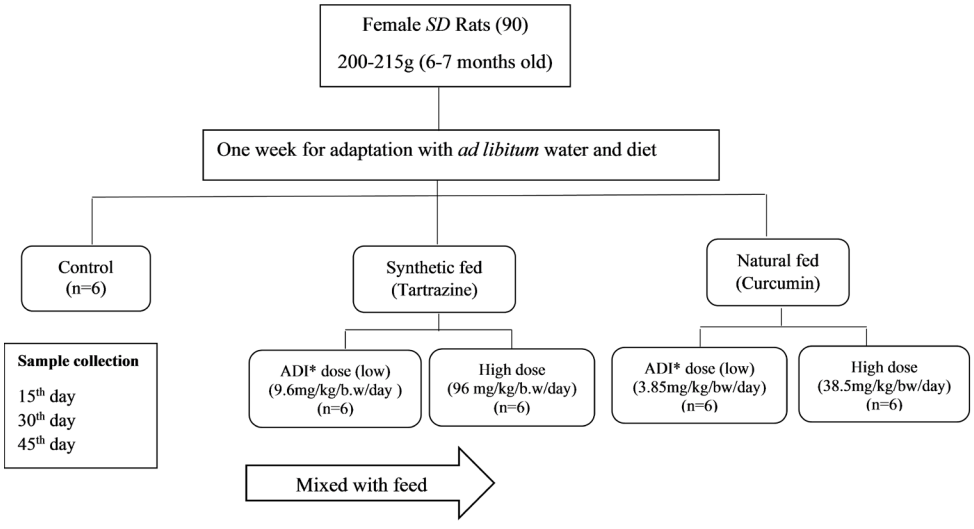

Figure 1. Experimental animals and study protocol. n = number of rats/group, SD = Sprague Dawley.

flask, mixed, and heated at 60 - 70°C, and was allowed to evaporate to a point where 2 - 3 mL of the mixture was left in the digestion flask. Later, 5 mL of perchloric acid was added to this mixture, and heated again at 60 - 70°C; it was allowed to evaporate until 1 - 2 mL of colourless material was left in the flask. The remaining mixture was reconstituted to 25 mL with deionised water, filtered, and stored in clean screw-capped containers at room temperature, until the mineral analysis was performed. The estimation for copper, zinc, and iron was done by automatic biochemistry analyser (TC200; Tecom Science Corporation, China). Sodium, potassium, and chloride were measured by flame photometer (Jenway Clinical PFP7, UK).

Determination of thyroid hormones

Immunoassay EIA kits (BioVision, CA, USA) were used for the determination of triiodothyronine (T_3) (cat# E0453Ge-96), thyroxin (T_4) (cat# E0452Ge-96), and thyroid stimulating hormone (TSH) (cat# E0463r-96) in plasma, following the manufacturer's guidelines.

Determination of reproductive hormones

Following the manufacturer's guidelines, ELISA kits were used to measure the level of oestrogen (Cusabio; cat# CSB-E07279r), progesterone (cat# K7416-100), luteinising hormone (LH, EIAab: cat # E1174r), and follicle stimulating hormones (FSH, EIAab: cat # E0830r). The kits were purchased from BioVision, USA.

Statistical analysis

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

Results and discussion

Feed intake, water intake, and body weight parameters

The body weight gain of treated group (low tartrazine, high tartrazine, low curcumin, and high curcumin) significantly increased in 30 and 45 d as compared to control (Table 1). The water intake significantly increased in LT30, HT30, and LT45. The feed intake significantly decreased after 15, 30, and 45 d (Table 1). The gain of b.w. was about 20 to 25% from the initial b.w. These results agree with Sharma (2015) who described an increment in the b.w. Increment over 20% of the mean b.w. is considered as obesity. Golli (2016) described opposite results as at the end of their study, tartrazine-treated rats indicated a significant decrease in b.w.; therefore, the difference in b.w. reported by various studies might represent the first marker of adverse effect.

Compounds of colour may reach to the gut through oral ingestion, through parenteral administration, or through the bile. These could be subjected to the digestive enzymes, the action of gastric acids, and microbiota (Amin *et al.,* 2010).

Water and feed intakes decreased after 30 and 45 d of treatment. The findings partially agree with Mehedi *et al.* (2009) who confirmed that feed intake decreased in experimental animals, but water intake showed different results from the present work. The decrease in feed and water intakes could possibly be due to the incorporation of tartrazine and curcumin. It has been hypothesised that the digestion of diet or feed may be inhibited in a certain manner by the supplementation of synthetic colorants or synthetic flavours. Changes in the food intake were not parallel to the growth and feed efficiency of the different colorants.

Mineral contents in plasma, liver, and kidney

It is well known that diet is the main source of nutritional and toxic minerals. Various factors such as the chemical form and bioavailability of elements, interaction between elements, as well as the animals' homeostatic mechanisms can have a strong impact on the diet-mineral content relationship (Suttle, 2010).

Although various studies were conducted to explore the oxidative effect caused by tartrazine within many organs such as kidney, liver, and brain (Amin *et al.,* 2010; Cemek *et al.,* 2014), there is still a lack of information concerning the mineral contents, disturbance induced by tartrazine, and curcumin consumption in plasma, liver, and kidney. In the present work, the effects of tartrazine and curcumin on mineral contents in plasma, liver, and kidney were evaluated with exposure to the food colorants at a dose of 9.6 and 96 mg/kg b.w. for tartrazine and 3.85 and 38.5 mg/kg b.w. for curcumin which were given daily up to 15, 30, and 45 d. The present work concluded that oral administration of tartrazine and curcumin significantly decreased the iron level of plasma in HT30, HC30, LT45, HT45, LC45, and HC45 groups as compared to control (Table 2).

For mineral levels in kidney, it was observed

Table 1. Effects of tartrazine and curcumin on body weight gain, and water and feed intakes of rats after 15, 30 and 45 d of treatment.

Day	Food colour exposure	Body weight gain (%)	Water intake (mL)	Feed intake (g)
15	Control	0.99 ± 0.49^{e}	161.20 ± 4.34^{a}	118.20 ± 6.81^{a}
	LT	0.68 ± 0.30^{e}	153.26 ± 9.26^{abcde}	103.20 ± 2.52^{e}
	HT	0.84 ± 0.58^{e}	157.33 ± 4.23^{ab}	106.73 ± 9.25^{bcde}
	LC	1.68 ± 0.59^{e}	156.93 ± 5.63^{ab}	107.93 ± 4.47^{bcde}
	HC	0.66 ± 0.76^{e}	154.13 ± 6.56^{abcd}	104.53 ± 3.02^{cde}
30	Control	5.81 ± 1.27^{de}	154.26 ± 10.59^{abc}	120.30 ± 6.90^{a}
	LT	16.28 ± 2.44^{abc}	135.80 ± 19.57^{fg}	109.96 ± 5.49^{bc}
	HT	13.69 ± 3.13^{bcd}	137.6 ± 19.31^{efg}	106.00 ± 5.72^{bcde}
	LC	13.35 ± 5.74^{bcd}	140.43 ± 21.90^{cdefg}	109.80 ± 5.81^{bcd}
	HC	16.57 ± 1.59^{ab}	143.10 ± 15.48^{bcdef}	110.73 ± 6.37^{b}
45	Control	7.85 ± 1.65^{cde}	151.42 ± 9.55^{abcdef}	111.88 ± 8.00^{b}
	LT	11.48 ± 6.08^{bcd}	133.35 ± 22.18^{g}	106.88 ± 6.00^{bcde}
	HT	16.09 ± 2.33^{abc}	137.82 ± 18.25^{defg}	102.97 ± 6.85^{e}
	LC	17.45 ± 3.06^{ab}	138.24 ± 19.2^{cdefg}	106.57 ± 6.56^{bcde}
	HC	23.44 ± 3.70^{a}	136.08 ± 19.08^{fg}	103.71 ± 5.25^{de}

Values are mean ± SD of six rats ($n = 6$). Means in each column followed by different lowercase superscripts are significantly different ($p \leq 0.05$). LT = low tartrazine, HT = high tartrazine, LC = low curcumin, and HC = high curcumin (Adopted from Shakoor, 2019)

that oral intake of curcumin did not have a significant increase in iron level of kidney for LC15 and HC15; whereas, iron non-significantly decreased in the HC30 and HC45. On the other hand, tartrazine non-significantly decreased the kidney iron level of LT30, HT30, and HT45 groups as compared to control (Table 3).

For mineral levels in liver, it was observed that iron level of liver significantly decreased following the administration of tartrazine and curcumin amongst the treated group of 30 and 45 d as compared to control. Iron level of liver was significantly high in LT30 as compared to HT30 (Table 4). Collectively, these results demonstrated that iron concentrations in plasma, kidney, and liver were reduced by the food colorants. Therefore, the ingestion of foods containing food colorants for a long time may cause iron deficiency and anaemia. These findings are in line with the results of Cemek *et al.* (2014) who observed that iron decreased in liver and kidney tissues of rats treated with 2.5 mg/kg b.w. of carmoisine (azorubine; red dye) and 15 mg/kg b.w. of tartrazine for 15 d.

When iron deposition in the body exceeds, the storage and detoxification capacity of ferritin and transferrin is fully saturated, then the free iron starts to accumulate in blood and body tissues (Shazia *et al.,* 2012). This free iron can cause the formation of harmful molecular species such as hydroxyl radical (•OH). The hydroxyl radicals are highly reactive entities, and attack biomolecules in close vicinity by causing oxidative stress (Raghuveer and Vidya, 2009).

Copper level in plasma was significantly high in LT15, HT15, and LT30 groups (Table 2), non-significantly low in LT45 and HT45 groups, as compared to control, and significantly in HC45. Copper level significantly decreased in HT15, HT30, HT45, HC30, and HC45 groups. Similar result was shown by Cemek *et al.* (2014) that Cu concentrations significantly increased in rats' kidneys treated with 15 mg/kg b.w. of tartrazine for 15 d. In the present work, as compared to control, copper level was significantly high in the liver of HT45, LC45, and HC45 groups (Table 4). Copper accumulation in body leads to Wilson's disease and cirrhosis of liver (Stevens *et al.,* 2013). Copper is a key constituent of haemoglobin, which is the protein responsible for oxygen transportation in blood cells. Deficiency in copper will lead to anaemia, neutropenia, growth

Table 2. Effects of tartrazine and curcumin on iron, copper, zinc, sodium, potassium, and chloride concentrations in rat plasma after 15, 30 and 45 d of treatment.

Day	Food colour exposure	Iron (µg/dL)	Copper (µmol/L)	Zinc (µmol/L)	Sodium (mEq/L)	Potassium (mEq/L)	Chloride (mEq/L)
15	Control	124.33 ± 33.63^{a}	20.66 ± 16.32^{f}	18.5 ± 4.92^{b}	24 ± 10.39^{bcd}	0.47 ± 0.17^{b}	33.67 ± 2.33^{bc}
	LT	115.66 ± 22.02^{a}	52.16 ± 3.06^{abc}	18.66 ± 2.65^{b}	19.83 ± 8.75^{d}	0.33 ± 0.24^{b}	37.83 ± 2.42^{ab}
	HT	134 ± 16.81^{a}	48.83 ± 2.71^{abcd}	18.33 ± 3.50^{b}	20.83 ± 5.07^{d}	0.45 ± 0.26^{b}	35.67 ± 1.72^{abc}
	LC	129.66 ± 25.55^{a}	41.33 ± 7.5^{bcdef}	19 ± 1.41^{b}	32.5 ± 6.25^{abcd}	0.35 ± 0.25^{b}	36.67 ± 1.96^{abc}
	HC	105.83 ± 46.20^{ab}	40.5 ± 5.16^{bcdef}	21.16 ± 1.16^{ab}	20.83 ± 6.04^{cd}	1.05 ± 0.53^{ab}	34.83 ± 2.31^{abc}
30	Control	138 ± 23.38^{a}	28.33 ± 14.92^{def}	34.66 ± 4.50^{a}	35.66 ± 13.45^{abcd}	0.75 ± 0.48^{b}	36.83 ± 3.48^{ab}
	LT	107.16 ± 14.83^{ab}	47.66 ± 15.98^{abc}	28.83 ± 4.35^{ab}	38 ± 10.23^{abcd}	0.38 ± 0.30^{b}	35.17 ± 4.53^{abc}
	HT	43.5 ± 11.00^{c}	50.17 ± 7.16^{abcd}	23 ± 7.56^{ab}	44 ± 9.50^{ab}	0.43 ± 0.23^{b}	40.17 ± 6.52^{a}
	LC	131.33 ± 25.29^{a}	25 ± 12.5^{ef}	29.5 ± 9.00^{ab}	37.33 ± 12.75^{abcd}	1.06 ± 0.22^{ab}	34.5 ± 1.64^{abc}
	HC	34.16 ± 8.35^{c}	38.5 ± 20.20^{cdef}	24.66 ± 10.89^{ab}	49 ± 20.46^{a}	1.67 ± 0.75^{a}	38.33 ± 2.33^{ab}
45	Control	134.66 ± 21.42^{a}	66.67 ± 5.57^{a}	30.00 ± 7.48^{ab}	29.5 ± 7.50^{abcd}	0.47 ± 0.33^{b}	34.17 ± 3.06^{bc}
	LT	57.83 ± 23.81^{bc}	47.16 ± 14.42^{abcde}	24.5 ± 11.92^{ab}	28.67 ± 8.14^{abcd}	0.7 ± 0.40^{b}	33.83 ± 1.16^{c}
	HT	30.66 ± 16.58^{c}	47.83 ± 8.95^{abcd}	20.68 ± 7.99^{b}	43.34 ± 18.56^{abc}	0.72 ± 0.33^{b}	30.8 ± 2.78^{bc}
	LC	55.83 ± 34.32^{c}	61.83 ± 3.12^{ab}	26.66 ± 9.33^{ab}	35.66 ± 6.65^{abcd}	0.67 ± 0.35^{b}	34.33 ± 0.51^{abc}
	HC	40.00 ± 15.40^{c}	30.66 ± 6.18^{cdef}	24.34 ± 3.93^{ab}	48.00 ± 13.14^{a}	1.08 ± 0.72^{ab}	33.84 ± 0.75^{bc}

Values are mean ± SD of six rats (n = 6). Means in each column followed by different lowercase superscripts are significantly different ($p \leq 0.05$). LT = low tartrazine, HT = high tartrazine, LC = low curcumin, and HC = high curcumin.

Table 3. Effects of tartrazine and curcumin on iron, copper, zinc, sodium, potassium, and chloride concentrations in rat kidney after 15, 30, and 45 d of treatment.

Day	Food colour exposure	Iron (µg/dL)	Copper (µmol/L)	Zinc (µmol/L)	Sodium (mEq/L)	Potassium (mEq/L)	Chloride (mEq/L)
15	Control	75.83 ± 20.34^{bc}	40.5 ± 11.58^{abc}	20.33 ± 2.42^{de}	40 ± 14.19^{a}	1.95 ± 0.50^{bc}	33.83 ± 3.25^{a}
	LT	73.17 ± 19.15^{bc}	30.5 ± 12.95^{cd}	16 ± 3.34^{e}	48 ± 12.44^{a}	2.03 ± 0.35^{bc}	36.5 ± 1.37^{a}
	HT	70.5 ± 22.49^{c}	16.33 ± 3.38^{d}	15.67 ± 3.14^{e}	43.33 ± 15.17^{a}	2.05 ± 0.37^{a}	33.5 ± 2.94^{a}
	LC	92.33 ± 3.72^{abc}	24.17 ± 8.79^{cd}	20 ± 1.78^{de}	30.83 ± 2.13^{a}	2.68 ± 0.54^{b}	36.83 ± 1.72^{a}
	HC	87.83 ± 9.53^{abc}	20 ± 14.79^{d}	19.83 ± 2.31^{de}	36 ± 5.72^{a}	1.85 ± 0.32^{bc}	34 ± 0.89^{a}
30	Control	101 ± 10.03^{a}	33.67 ± 12.29^{bcd}	17.33 ± 2.80^{de}	31.67 ± 15.48^{a}	2.23 ± 0.37^{bc}	36.66 ± 2.16^{a}
	LT	87.33 ± 11.27^{abc}	53 ± 3.03^{ab}	21.83 ± 5.23^{cde}	30.66 ± 5.85^{a}	2.03 ± 0.90^{bc}	35.5 ± 1.51^{a}
	HT	83.5 ± 4.32^{abc}	55.33 ± 2.25^{a}	28.33 ± 5.39^{cde}	32.17 ± 10.99^{a}	2.56 ± 0.61^{bc}	35.5 ± 1.97^{a}
	LC	97.5 ± 2.50^{ab}	41.83 ± 19.27^{abc}	38.5 ± 2.88^{abc}	43.83 ± 9.34^{a}	1.917 ± 0.16^{a}	34.5 ± 1.22^{a}
	HC	80.33 ± 3.44^{abc}	55.67 ± 7.60^{a}	46.83 ± 3.86^{ab}	34.17 ± 2.99^{a}	1.37 ± 0.36^{c}	37 ± 3.94^{a}
45	Control	94.83 ± 6.17^{abc}	19.83 ± 5.07^{d}	32 ± 15.82^{bcde}	41.5 ± 5.16^{a}	2.9 ± 1.56^{ab}	33.66 ± 1.96^{a}
	LT	85.5 ± 12.34^{abc}	25 ± 6.06^{cd}	50.5 ± 10.67^{a}	37.83 ± 6.70^{a}	1.98 ± 0.32^{bc}	34.67 ± 1.86^{a}
	HT	81.33 ± 7.65^{abc}	40.17 ± 7.60^{abc}	46.83 ± 9.34^{ab}	34.83 ± 6.21^{a}	1.61 ± 0.61^{bc}	33.5 ± 1.04^{a}
	LC	87.5 ± 12.16^{abc}	40.17 ± 7.60^{abc}	22.83 ± 10.53^{cde}	28 ± 16.70^{a}	4.11 ± 0.87^{a}	33.5 ± 0.83^{a}
	HC	75.33 ± 15.09^{bc}	52 ± 9.71^{ab}	34.67 ± 22.75^{bcd}	30.16 ± 2.31^{a}	2.35 ± 0.30^{bc}	37.16 ± 3.48^{a}

Values are mean ± SD of six rats (n = 6). Means in each column followed by different lowercase superscripts are significantly different ($p \leq 0.05$). LT = low tartrazine, HT = high tartrazine, LC = low curcumin, and HC = high curcumin.

Table 4. Effects of tartrazine and curcumin on iron, copper, zinc, sodium, potassium, and chloride concentrations in rat liver after 15, 30 and 45 d of treatment.

Day	Food colour exposure	Iron (µg/dL)	Copper (µmol/L)	Zinc (µmol/L)	Sodium (mEq/L)	Potassium (mEq/L)	Chloride (mEq/L)
15	Control	125.5 ± 21.72^{a}	32 ± 6.81^{ef}	20.5 ± 2.07^{de}	19.17 ± 3.31^{c}	3.16 ± 0.46^{b}	36.17 ± 2.04^{b}
	LT	109.67 ± 20.33^{a}	34.83 ± 19.66^{adef}	24 ± 6.78^{cde}	31.33 ± 18.34^{bc}	3.08 ± 1.10^{b}	36.33 ± 2.25^{b}
	HT	132.17 ± 17.54^{a}	39.5 ± 17.81^{cdef}	20 ± 2.00^{de}	33.66 ± 6.31^{abc}	3.2 ± 0.97^{b}	36.33 ± 1.63^{b}
	LC	126.67 ± 18.12^{a}	48.83 ± 3.48^{bcde}	25.83 ± 3.25^{bcde}	30.33 ± 8.04^{bc}	3.85 ± 0.71^{b}	37.17 ± 1.32^{ab}
	HC	106.33 ± 47.85^{ab}	42.66 ± 5.92^{bcde}	22.66 ± 2.33^{cde}	43.34 ± 8.43^{ab}	3.67 ± 0.80^{b}	34.33 ± 2.94^{b}
30	Control	142 ± 24.16^{a}	54 ± 2.00^{abc}	27.17 ± 4.99^{bcde}	31.83 ± 8.79^{bc}	2.93 ± 0.18^{b}	34 ± 2.28^{b}
	LT	105.5 ± 8.09^{ab}	51.33 ± 2.65^{bcd}	21.83 ± 3.18^{de}	25.83 ± 1.16^{bc}	3.02 ± 0.76^{b}	34.5 ± 0.83^{b}
	HT	45.5 ± 9.79^{c}	52.16 ± 4.70^{abcd}	40.16 ± 12.12^{ab}	45 ± 17.22^{ab}	3.6 ± 0.66^{b}	39.33 ± 1.50^{ab}
	LC	126 ± 22.42^{a}	69.17 ± 4.16^{a}	38.33 ± 5.08^{abc}	44.5 ± 5.00^{ab}	3.12 ± 0.98^{b}	44 ± 10.86^{a}
	HC	34.16 ± 8.35^{c}	69.16 ± 4.16^{a}	31.83 ± 11.5^{bcd}	43 ± 10.44^{ab}	4.6 ± 0.74^{ab}	37.33 ± 3.72^{ab}
45	Control	135.5 ± 26.96^{a}	23.67 ± 2.42^{f}	48.33 ± 10.50^{a}	45.16 ± 18.53^{ab}	3.1 ± 0.17^{b}	33 ± 1.78^{b}
	LT	63 ± 22.33^{bc}	39.83 ± 9.21^{cdef}	26.5 ± 16.65^{bcde}	37.83 ± 9.02^{abc}	2.78 ± 1.67^{b}	34 ± 2.36^{b}
	HT	35.83 ± 11.70^{c}	44.67 ± 1.86^{bcde}	34.33 ± 9.02^{bcd}	27.5 ± 4.32^{bc}	2.86 ± 0.35^{b}	33.16 ± 1.94^{b}
	LC	58.5 ± 28.94^{c}	59.33 ± 7.31^{ab}	14.83 ± 2.04^{e}	52.33 ± 3.38^{a}	5.87 ± 1.38^{a}	34 ± 2.60^{b}
	HC	40.33 ± 12.43^{c}	58 ± 11.67^{ab}	22.5 ± 7.36^{cde}	27 ± 5.93^{bc}	4.53 ± 1.52^{ab}	36.67 ± 3.88^{ab}

Values are mean ± SD of six rats (n = 6). Means in each column followed by different lowercase superscripts are significantly different ($p \leq 0.05$). LT = low tartrazine, HT = high tartrazine, LC = low curcumin, and HC = high curcumin.

impairment, abnormalities in glucose and cholesterol metabolisms, and increased rate of infections (Mahyar *et al.,* 2010).

High doses of curcumin and tartrazine non-significantly decreased the plasma zinc level in HT30, HC30, and HT45 groups as compared to control (Table 1). On the other hand, higher level of zinc in kidney was observed in HT30, LC30, HC30, LT45, and HT45 groups as compared to control (Table 3). Disturbance was observed in the zinc level of liver where curcumin and tartrazine significantly decreased the zinc level in all treated groups of 45 d as compared to control (Table 3). This is in agreement with literature that zinc decreased within liver and kidney tissues during 15 d of rats' treatment with 2.5 mg/kg b.w. of carmoisine and 15 mg/kg b.w of tartrazine (Cemek *et al.,* 2014). Research in hyperactive children suggested that Yellow # 5 and Yellow # 6, given in a single dose, may chelate zinc, and therefore, long-term consumption of these dyes may cause chronic zinc depletion (Stevens *et al.,* 2013).

Zinc has important antioxidant properties, and protects the cells from damages caused by free radicals. Zinc is absorbed from small intestine, and found in the blood bound to albumin. Hyperactivity and various behavioural disorders might be induced by the deficiency in zinc (Stevens *et al.,* 2013). It is known that the xenobiotics are transferred to the liver by blood, and detoxified in the hepatocytes. Biological membranes are particularly prone to the ROS effect; where peroxidation of unsaturated fatty acids in biological membranes leads to a deficiency in membrane fluidity, and disruption of membrane integrity and functions (Amin *et al.,* 2010). While low dose of tartrazine is known to reduce liver zinc content, high dose tartrazine can cause the same effect in kidney (Cemek *et al.,* 2014).

Oral administration of tartrazine significantly increased the sodium level in liver of HC15 and HC45 groups as compared to LC15 and LC45 (Table 4); on the other hand, there was a significant decrease in HT45 as compared to control and LT45. Oral intake of tartrazine increased the kidney potassium level in LT15, LT45, HT45, and LC45 groups; while decreased that in LC30 and HC30 as compared to control. Similarly, plasma chloride was not significantly higher in HT30 and HT45 groups, as compared to control, LT30, and LT45 groups (Table 2). On the other hand, chloride level was high in liver among the low curcumin treated group, during 30 d of treatment as compared to control (Table 4). Similarly, Jokanovi *et al.* (2013) concluded that liver and kidney accumulate higher concentrations of these elements, and may pose health risks to humans as compared to muscle mineral level (which are generally low).

It is well known that micro- (Fe, Zn, Cu, Mn, Se, Cr, Co, and Ni) and macro-elements (Na, K, Mg, and Ca) are vital for the normal functioning of various biochemical and enzymatic processes in the body. Minerals are required at certain range for normal growth and good health throughout life (Lombardi-Boccia *et al.,* 2005). Results obtained in the present work are in accordance with Cemek *et al.* (2014) who determined some significant changes about levels of elements in rats' liver and kidney tissues exposed to 15 mg/kg b.w. of tartrazine and 2.5 mg/kg b.w. of carmoisine.

Thyroid hormones

The present work revealed that concentration of triiodothyronine (T_3) was affected by tartrazine and curcumin. T_3 was non-significantly ($p \geq 0.05$) high in HT30 and HC30 groups as compared to LT30 and HC30 groups. Oral administration of tartrazine and curcumin significantly ($p < 0.05$) increased the T_4 level in HT15; while this was considerably low in HT30, LT30, HT30, and HT45 groups as compared to control. The level of thyroid stimulating hormone (TSH) in all treatment groups of 15, 30, and 45 d were considerably different (Table 5).

Similar results have been reported by Abdel-Aziz *et al.* (2019) in male rats which were orally exposed to tartrazine for 30 d; they found a significant increase in T_3 and T_4, and a decrease in TSH of treated groups. On the other hand, high dose of curcumin increased the level of TSH at 30 and 45 d of treatment. Our findings are in contrast with Abdel-Aziz *et al.* (2019) who observed a non-significant increase in TSH in curcumin-treated group for 30 d with 100 mg/kg b.w. dose. Elekima and Ollar (2016) concluded that oral administration of carmoisine for four weeks induced a dose-dependent increase in the plasma level of T_3 and T_4, as well as TSH of albino rats. Results obtained in the present work are also in accordance with those reported by Elekima *et al.* (2017) that daily intake of tartrazine (0.14 g and 0.18 g/kg) for 30 d stimulated dose-dependent (treated male rats) increase of T_3 and T_4, respectively. The significant ($p < 0.05$) decrease in TSH may suggest that oxidative stress might have occurred due to the intake of tartrazine and curcumin. Sayari *et al.* (2018) reported heavy presence of ROS or free radicals on the follicular cells of the thyroid gland; whereas, T_3 and T_4 which are stored, could decrease TSH. The increase in T_3

Table 5. Effects of tartrazine and curcumin on thyroid and reproductive hormones in rats after 15, 30, and 45 days of treatment.

Day	Thyroid hormone				Reproductive hormone			
	Food colour exposure	T_3 (ng/m L)	T_4 (µg/dL)	TSH (mIU/L)	Oestrogen (pg/m L)	Progesterone (pg/m L)	FSH (mIU/mL)	LH (ng/m L)
15	Control	1.14 ± 0.29 ^{ab}	7.38 ± 0.53 ^{a}	2.51 ± 0.27 ^{abcd}	96.81 ± 6.84 ^{abcde}	99.82 ± 56.79 ^{ab}	2.8 ± 1.30 ^{ab}	3.4 ± 0.96 ^{abc}
	LT	0.98 ± 0.23 ^{abc}	6.09 ± 0.41 ^{abc}	2.37 ± 0.26 ^{bcd}	79.19 ± 37.39 ^{cde}	118.81 ± 18.91 ^{ab}	3.03 ± 1.67 ^{ab}	3.59 ± 0.66 ^{abc}
	HT	0.98 ± 0.13 ^{abc}	5.71 ± 0.71 ^{bc}	2.69 ± 0.30 ^{abc}	65.49 ± 31.67 ^{de}	122.81 ± 26.33 ^{ab}	3.15 ± 1.69 ^{ab}	4.35 ± 0.36 ^{a}
	LC	1.12 ± 0.27 ^{ab}	6.2 ± 0.50 ^{abc}	2.39 ± 0.37 ^{bcd}	90.49 ± 28.19 ^{abcde}	117.99 ± 22.18 ^{ab}	1.35 ± 0.98 ^{b}	2.57 ± 0.45 ^{cde}
	HC	1.09 ± 0.48 ^{abc}	5.93 ± 0.59 ^{abc}	2.19 ± 0.30 ^{cd}	88.03 ± 12.96 ^{bcde}	153.14 ± 33.96 ^{ab}	1.39 ± 0.65 ^{b}	2.36 ± 0.95 ^{cde}
30	Control	0.9 ± 0.15 ^{abc}	6.68 ± 0.80 ^{abc}	2.35 ± 0.27 ^{bcd}	115.39 ± 26.30 ^{abcd}	92.89 ± 17.78 ^{ab}	3.35 ± 0.85 ^{ab}	3.13 ± 0.72 ^{abcd}
	LT	1.05 ± 0.08 ^{abc}	5.61 ± 0.19 ^{bc}	1.95 ± 0.57 ^{cd}	68.24 ± 16.90 ^{de}	150.05 ± 28.29 ^{ab}	1.2 ± 0.39 ^{ab}	2.42 ± 0.77 ^{cde}
	HT	1.26 ± 0.13 ^{a}	5.41 ± 0.47 ^{c}	2.61 ± 0.44 ^{abc}	61.41 ± 15.71 ^{e}	157.02 ± 53.37 ^{a}	1.11 ± 0.56 ^{b}	1.38 ± 0.25 ^{e}
	LC	0.98 ± 0.41 ^{abc}	6.49 ± 1.81 ^{abc}	2.33 ± 0.29 ^{bcd}	108.05 ± 16.17 ^{abcde}	114.08 ± 19.35 ^{ab}	4.28 ± 1.98 ^{a}	2.28 ± 0.40 ^{cde}
	HC	1.21 ± 0.40 ^{a}	7.12 ± 0.65 ^{ab}	3.00 ± 0.13 ^{ab}	59.2 ± 24.58 ^{e}	125.85 ± 71.41 ^{ab}	5.07 ± 1.74 ^{a}	1.94 ± 0.38 ^{de}
45	Control	0.86 ± 0.06 ^{abc}	7.05 ± 0.68 ^{ab}	2.45 ± 0.24 ^{abcd}	99.85 ± 12.12 ^{abcde}	118.29 ± 15.24 ^{ab}	2.97 ± 0.86 ^{ab}	4.015 ± 0.84 ^{ab}
	LT	0.63 ± 0.19 ^{bc}	6.69 ± 0.81 ^{abc}	1.79 ± 0.55 ^{d}	132.47 ± 44.25 ^{ab}	100.4 ± 10.11 ^{ab}	1.32 ± 0.73 ^{b}	2.64 ± 0.38 ^{cde}

Values are mean ± SD of six rats (n = 6). Means in each column followed by different lowercase superscripts are significantly different ($p \leq 0.05$). LT = low tartrazine, HT = high tartrazine, LC = low curcumin, HC = high curcumin, T_3 = triiodothyronine, T_4 = thyroxine, TSH = thyroid stimulating hormone, FSH = follicle stimulating hormone, and LH = luteinising hormone.

could also be as a result of peripheral conversion of T_4 to T_3 vis–a-vis increase presence of 5'deiodinase due to morphological changes of the parenchymal cells of the liver. This increase is due to the presence of ROS during the metabolism of the dye. The present work indicated the increased level of T_3 in HT30 and HC30 groups; whereas TSH was significantly higher in HC30, HT45, and HC45 groups as compared to control. Low and high levels of T_3 and TSH were dose-dependent, which could be the reason why the significant increases were seen when the doses were also increased (Crook, 2007). The increased level of TSH cause hyper stimulation of the thyroid gland, thus resulting in high levels of T_3 and T_4; however, due to negative feedback mechanism, increased level of T_4 and T_3 stimulates reduced production of TSH from the pituitary (Sayari *et al.,* 2018).

Among female mice, ingestion of diets containing turmeric oleoresin (curcumin) was associated with an increased incidence of thyroid gland follicular cell hyperplasia (NTP, 1993); histopathological changes in thyroid gland have been recorded by Shakoor (2019) in the high tartrazine and curcumin treated groups.

Reproductive hormones

Oral administration of tartrazine significantly ($p \leq 0.05$) decreased the concentration of oestrogen in HT30 and HC30 groups, but led to non-significantly high concentration in all groups of 45 d (Table 5). The concentration of progesterone in all treatment groups of 15, 30, and 45 d was found non-significant. Low concentrations of FSH were found in LT30, HT30, LT45, and HT45 treatment groups as compared to control. LT vs HT and LC vs HC at 15, 30, and 45 d were non-significantly different.

The decrease in the FSH level may suggest gonadal dysfunction which delays maturation of ovarian follicles in the pre-ovulatory phase. The increase in the FSH level exerts stimulatory effect on the anterior pituitary or hypothalamus because the secretion of the hormone is regulated by the gonadotropic releasing hormone of the hypothalamus (El-Kashoury *et al.,* 2010).

Oral administration of tartrazine and curcumin has decreased the level of LH in all treated groups, except LT15, as compared to control (Table 5). After 30 d, significant ($p \leq 0.05$) decrease in FSH and LH levels was found in female albino rats after oral administration of 250 and 300 mg doses of ethanolic extract of *Curcuma longa* (curcumin), while the amount of oestrogen in curcumin-treated animals were found to increase (Thakur *et al.,* 2009). Previous results from Faraidoon (2018) identified that the levels of LH, FSH, progesterone, and oestrogen hormones decreased significantly ($p \leq 0.05$) in groups of Sprague Dawley where the female rats were orally treated with low (5 mg/kg b.w.), mild (10 mg/kg b.w.), and high (20 mg/kg b.w.) doses of azorubine. Tartrazine and curcumin might obstruct female hormonal functions, which could lead to side effects on the reproductive system through the disruption of the hormonal balance required for proper functioning (Eweka, 2009).

Abbas and Al-Hamadawi (2019) demonstrated significant decrease ($p \leq 0.05$) in the levels of FSH, LH, GnRH, and testosterone hormones among male albino rats which were treated with chocolate brown dye at doses of 200 and 400 mg/kg b.w. for eight weeks as compared to control. These results also agree with those of Khatun *et al.* (2017) who noted the low concentration of LH, FSH, and estradiol with increased dose of brown chocolate dye. This could impact the fertility since chocolate dye is involved in the inhibition of hypothalamic pituitary-testis axis function by producing free radicals. Furthermore, Mahmoud (2006) reported that the treatment of blue-dye on rats caused the reduction in the serum which led to the atrophy of Leydig cells and reduced testosterone level.

Similar finding was reported by Vlassara (2002) that caramel colouring has been associated with adverse reproductive health effect in animal models. A more recent study found that caramel colouring is associated with elevated estradiol level (Schliep *et al.,* 2013). Boussada *et al.* (2002) reported that tartrazine oral consumption (dose of 300 mg/kg b.w.) for 30 d significantly ($p \leq 0.05$) decreased the testosterone level in male Wistar rats. Kesari powder (which contains synthetic colorants) was found to cause imbalance in the oestrogen/progesterone ratio (Sharma, 2015). The dye exerted direct influence on the ovary or indirectly through the hypothalamus and pituitary. The ovarian synthesis of estradiol is a carefully regulated system that is coordinated by feedback mechanisms among the hypothalamus, anterior pituitary, and ovaries. It is this estradiol signal which regulates the neural network; either inhibiting or facilitating the release of GnRH to act on the pituitary, thus causing the synthesis and release of FSH or LH, ovulation, and reproductive behaviours (Christensen *et al.,* 2012).

Both carmoisine and curcumin treatments induced significant down-regulation of follicle stimulating hormone receptor (FSHR) gene

(Montaser *et al.,* 2019). These effects were time- and dose-dependents. After 15, 30, and 45 d, the expression level of FSHR gene decreased significantly ($p \leq 0.05$) as compared to control (Montaser *et al.,* 2019).

Conclusion

The present work concluded that tartrazine and curcumin could cause various changes in the treatment groups. Frequent or increased intake (high dose) of tartrazine and curcumin could affect the thyroid and reproductive hormones either directly or indirectly. Results of thyroid and reproductive hormones showed significant changes, especially among the tartrazine-treated groups. However, curcumin, as a natural food colour, showed minimum side effects on thyroid and reproductive hormones as compared to tartrazine. It can also be concluded that the use of food colorants increases the chances of free radical production, thus leading to the development of oxidative stress in the body and rendering it prone to other serious illnesses. Therefore, food colorants should be used with great caution in the food products. The proper doses and permissible limits should be adhered to as recommended by the WHO, FDA, and other regulatory agencies. It is worth mentioning that the present work demonstrated the effects of food colorants on healthy animals; the impact might be worse on unhealthy animals.

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