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Research Article

Effects of Tartrazine Subchronic Ingestion on Brush Border Membrane Enzymes of Female Swiss Albino Mice Intestine

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ABSTRACT

Background: Tartrazine is a food colour that possesses adverse health effect to human such as hyperactivity in children, allergy and asthma. However, its effect on intestinal enzymes activity is not been established. Materials and methods: Tartrazine was administered to female swiss albino mice in drinking water at doses of 0.45% and 1% for 13 weeks. Mucosal scrape samples of the jejunum were collected for disaccharidases and dipeptidases activity analyses. Results: Maltase, sucrase and lactase specific activities were lower particularly in groups treated with 1% Tartrazine. Similarly, total and specific activities of L-leucyl-L-alanine, L-alanyl-L-proline and L-glycyl-L-proline dipeptidase are decreased in both treated groups. Villus height and crypt depth were decreased also in the same groups. Conclusion: the subchronic ingestion of Tartrazine at 0.45% and 1% in mice modifies disaccharidases and dipeptidases activities as well as intestine histological structure, thus it affects intestine function.

Keywords: Tartrazine, disaccharidases, dipeptidases, intestinal structure, mice.

INTRODUCTION

Artificial colorants are found in thousands of food products[1-6]. In particular breakfast cereals, candy, snacks, beverages and other products aimed at children are colored with dyes. Synthetic colorants are approved by the Food and Drug Administration (FDA) for use in foods, pharmaceuticals and cosmetic preparations.

Some of artificial colorants like Tartrazine pose a potential risk to human health, especially if they are excessively consumed[7-22].The acceptable daily intake (ADI) for human is 0-7.5 mg kg-1 body weight [23] but almost children exceed their ADI[24,25,26].

Several experimental studies have been conducted on Tartrazine demonstrated that among the biochemical systems which are likely to be affected by the ingestion of this food color is the oxidative stress because of its role in tissue damage. The mechanisms might be attributed to promoting lipid peroxidation products and reactive oxygen species, inhibiting endogenous antioxidant defense enzymes[12,15,19,27]. Oxidative stress is believed to be one important cause of gastrointestinal inflammation, ulcer and colitis[28].

The brush border (BB) membrane lining the epithelial cells of small intestine is one of the most important cellular membranes owing to its role in the digestion and absorption of nutrients. Due to this dual function the membrane contains a number of hydrolytic enzymes and transport system[29].The BB enzymes are crucial for the functioning of the intestine. It is well documented that dietary modification is correlated with significant changes in histology and biochemistry of the small intestine[30]. The study of Guendouz *et al.*[31] demonstrated that subchronic ingestion of Tartrazine at doses of 0.45% and 1% induced villous atrophy and inflammation of the intestine. Moreover, BB enzymes activities may be affected. Thus the aim of this study is to investigate the disaccharidase and dipeptidase activities following subchronic Tartrazine ingestion.

MATERIALS AND METHODS

Chemicals

The chemical name of the colorant is Tartrazine also known as (FD & C Yellow No. 5, C.I. No. 19140, and Food Yellow No. 4). The purity of this azo dye is 86.6%, it was provided from Dyechem, Morroco. The bovine serum albumine was purchased from Biochem (UK). Glucose oxydase, peroxydase, glucose, glycine, L- Leucyl-Alanine, L- Alanyl- Proline L- Glycyl- Proline dipeptides, lactose and sucrose were purchased from Sigma (USA). Alanine, maltose, Folin reagent and ninhydrin were purchased from Merck (Germany).

Animals and treatments

All animals were used following the instructions of Oran University guidelines for animal use.Thirty swiss albino female mice aged of 4 weeks old and weighing (12.85±0.26) g obtained from Pasteur institute, Algiers, Algeria, were kept under proper conditions of ambient temperature and adequate humidity. Tartrazine was diluted in water. Mice were divided into three groups, each one including 10 females. The first group was given drinking water as a control group, the second group was given drinking water containing 0.45% of Tartrazine (presents

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the no observed adverse effect levels(NOAEL))[32,33] and the third one was given drinking water containing 1% of Tartrazine (high dose) for a period of 13 weeks. Standard food pellets diet and water were given *ad libitum* for the duration of the experiment. Food and liquid consumption were measured daily and mean daily intake of tartrazine (mg/kg/day) during the administration period was calculated. Body weight was measured weekly.

After 13 weeks of administration, the animals were fasted for 18 hours and sacrificed on the day of necropsy by cervical dislocation. The small intestine was removed and its contents were flushed thoroughly with cold saline. The jejunum was then divided into two segments; one was excised for biochemical assays and the second for histological assessment.

Samples preparation

The mucosa was scraped with two glass slides onto an ice-chilled glass plate and then weighed. After, the mucosa was homogenized in cold Ringer (1000 µL/30 mg tissue) with a Potter-Elvehjem glass on ice for 3 minutes. The homogenate was used for enzyme determinations and protein content.

Protein determination

The protein concentration was determined according to the method of Lowry *et al.*[34] using Bovine serum albumin as standard.

Disaccharidases activities

Lactase (EC 3.2.1.23), maltase (EC 3.2.1.20) and sucrase (EC 3.2.1.48) activities were determined using the method of Dahlqvist[35]. Homogenate was diluted with cold Ringer solution. Diluted samples of homogenate (20µl) were pipetited into glass tubes for measurement disaccharidase activities with addition of an appropriate substrate (20µl) diluted in a maleate buffer (pH 6.0).The mixture was incubated at 37°C for 60 min. The content of released glucose was determined using the enzymatic glucose oxydase by adding 600µl of solution (glucose oxidase, peroxidase, triton x100, O-dianisidine, tris buffer pH 7.0). The mixture was incubated at 37°C for 35 min. Absorbance was determined using a spectrophotometer (UV/vis; JASCO V-530**,** Japan) set at 340-480 nm. The results were expressed as µmol glucose/min/g of tissue. The liberated glucose is measured, along with the total protein concentration of the homogenate. The specific activities of each enzyme are reported in units of µmol/min/g protein.

Dipeptidases activities

L- glycyl- L- proline, L-alanyl- L- proline and L-Leucyl-L-Alanine dipeptidases activities were measured by Doi *et al.*[36] method in homogenized jejuna mucosa. Dipeptidases were determined using appropriate substrates and the amino acid released was determined using the ninhydrin colorimetric method. The results were expressed as µmol of amino acids/min/g of tissue and specifics activities of each enzyme are reported in units of µmol/min/g protein.

Histological assessment

Small jejunum segments of 3 cm were removed and fixed in 10% formalin overnight. Paraffin-embedded, samples were sliced to approximately 4µm with a microtome, mounted on slides, and stained with hematoxylin and eosin. Approximately 40 Villi heights and crypt depths of each animal were measured at 10× magnification using a Nikon microscope and Optiphot-2 software.

Statistical analysis

The data was expressed as mean ± SE. Statistical test one way ANOVA was applied to find significant difference between values of various parameters recorded for control and treated animals. *P* values less than 0.05 were considered statistically significant.

RESULTS

Body weights, food and liquid consumption

The average of the final body weight gain, daily Food and liquid consumption through the administration period are shown in table 1.

No significant changes occur in final body weight gain and food consumption in experimental groups compared to controls. However, liquid consumption values were significantly increased at all experimental groups compared to controls ($p<0.05$).

Disaccharidase activities

Total activities of maltase and sucrase in the jejunum were not significantly reduced in treated groups compared to control group (figure 1A and figure 3A), but their specific activities were significantly decreased in 1% tartrazine treated groups ($p<0.01$) (figure 1B and figure 3B). In addition, the specific activity of sucrase was also significantly reduced in 0.45% tartrazine treated groups compared to controls ($p<0.05$) (figure 3B).

Jejunal total activity of lactase was significantly reduced in mice treated with 1% of tartrazine ($p<0.01$) (figure 2A). Whereas, its specific activity was significantly decreased in 0.45% ($p<0.01$) and 1% tartrazine treated groups ($p<0.01$) compared to controls (figure 2B).

Dipeptidase activities

The total and specific activities of all dipeptidases studied were depressed in the jejunum of treated mice with different doses of tartrazine.

Regarding both total and specific activities of L-leucyl-L-alanine and L-alanyl-L-proline dipeptidases were significantly decreased in all treated groups ($p<0.01$) compared to the control groups (figure 4 and figure 6). Similarly, total and specific activities of L-glycyl-L-proline dipeptidase were significantly decreased in 0.45% and 1% treated groups ($p<0.05$) and ($p<0.01$) respectively compared to controls (figure 5).

Villus height and crypt depth

Histological analysis of the jejunum in mice treated with tartrazine showed that increasing dietary tartrazine concentrations alter villus height and crypt depth (table 2). Shorter villi were observed in the jejunum of mice treated with tartrazine compared to controls. In the same time, crypt depth was also decreased significantly in both treated groups compared to control group ($p<0.01$).

DISCUSSION

The objective of this study was to measure the disaccharidase and dipeptidase activities following subchronic Tartrazine ingestion at dose of 0.45% and 1%.

Table 1: Effect of tartrazine ingestion on body weight gain.

Groups (g)	Initial BW(g)	Final BW(g)	Gain BW(g)
Controls	12.95±0.58	34.48±0.88	21.55±0.39
0.45%	12.82±0.35	34.11±0.82	21.29±0.86
1%	12.80±0.38	33.78±0.72	20.98±0.40^{NS}

Values represent mean ±SE of ten mice (n = 10).
NS: no significantly different (ANOVA test).

Table 2: Daily intake of fluid containing tartrazine and food for 13 weeks.

Groups	Food intake(g)	Fluid intake(ml)	Daily intake of tartrazine mg/kg/day
Controls	5.88±0.39	4.46±0.20	000.00
0.45%	5.94±0.42	5.75±0.42^{a}	839.42
1%	6.47±0.46	5.25±0.35^{a}	1625.99

Values represent mean ±SE of ten mice (n = 10).
Means which have letters are significantly different, P<0.05 (ANOVA test).

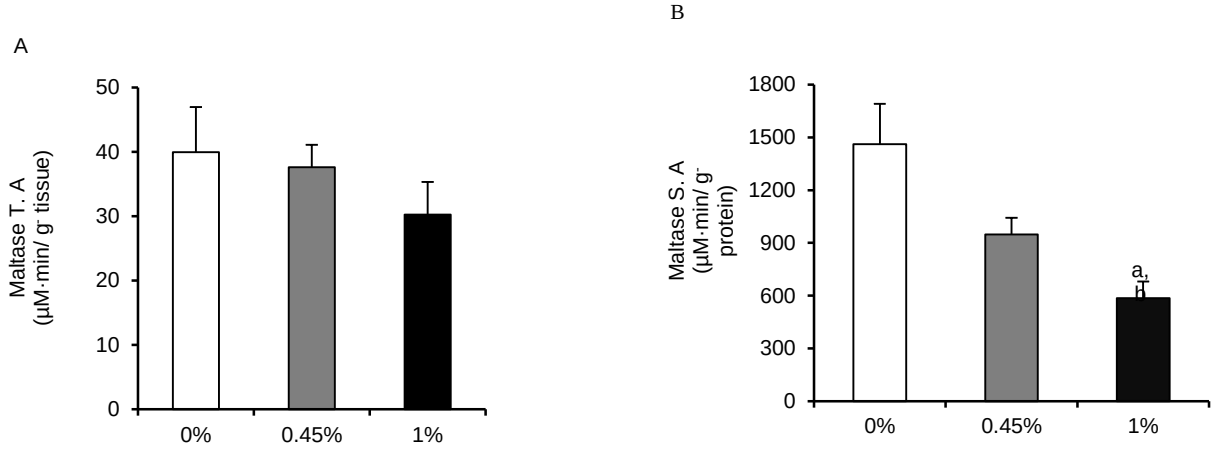

Figure 1: Effect of tartrazine on maltase activities in mice. Values represent mean ±SE of ten mice (n = 10). Means which have letters are significantly different, (a) significant versus control (P<0.01).

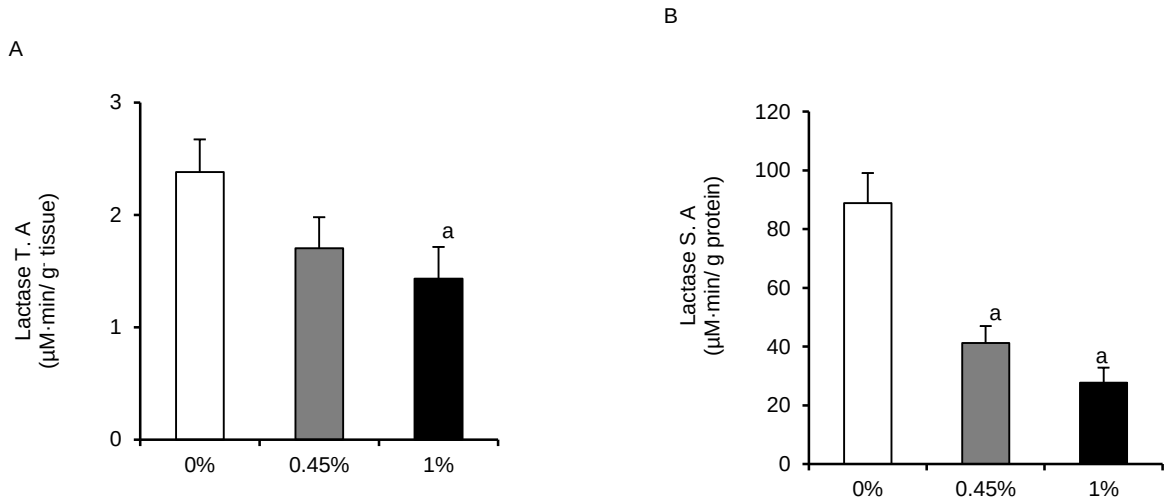

Figure 2: Effect of tartrazine on lactase activities in mice. Values represent mean ±SE of ten mice (n = 10). Means which have letters are significantly different, (a) significant versus control (P<0.05) and (P<0.01) respectively.

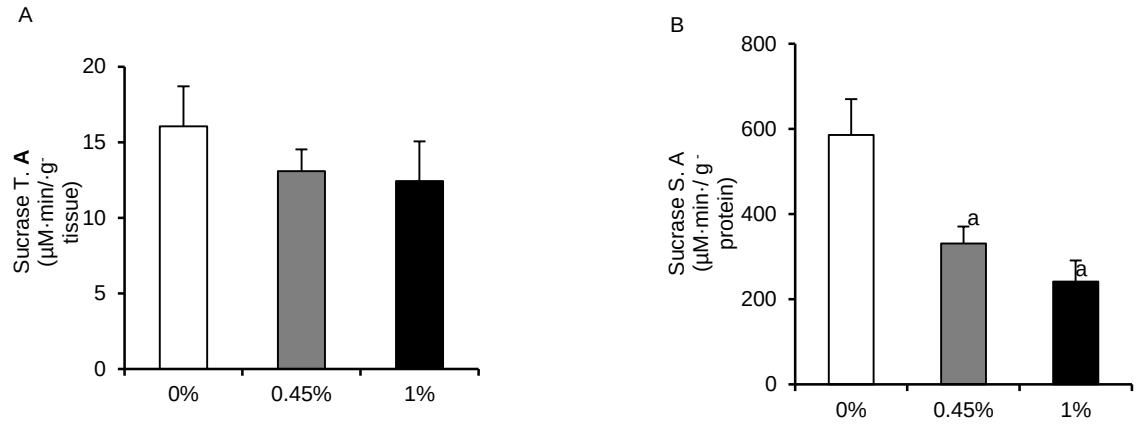

Figure 3**:** Effect of tartrazine on sucrase activities in mice. Values represent mean ±SE of ten mice (n = 10). Means which have letters are significantly different, (a) significant versus control (P<0.05) and (P<0.01) respectively.

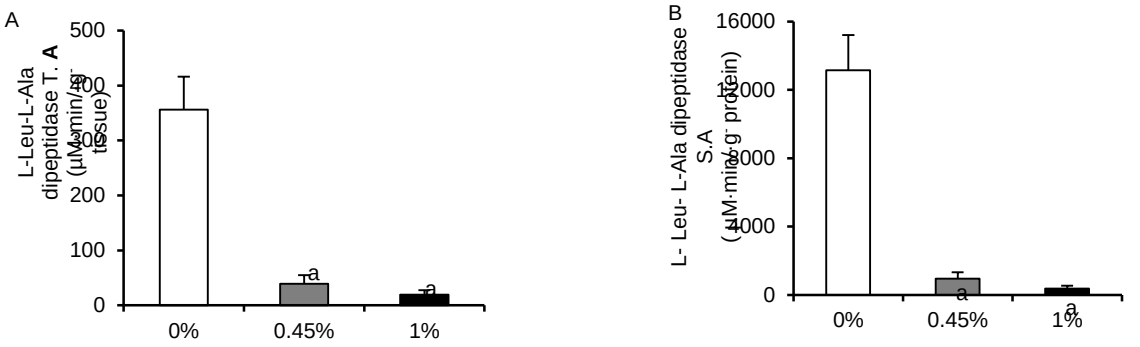

Figure 4: Effect of tartrazine on L-Leu-Ala dipeptidase activities in mice. Values represent mean ±SE of ten mice (n = 10). Means which have letters are significantly different, (a) significant versus control (P<0.01).

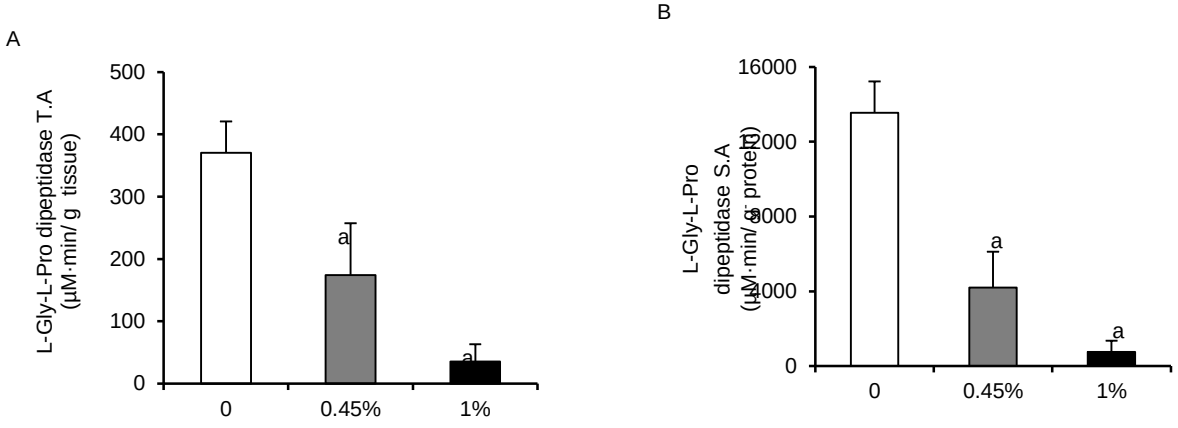

Figure 5: Effect of tartrazine on L-Gly-Pro dipeptidase activities in mice. Values represent mean ±SE of ten mice (n = 10). Means which have letters are significantly different, (a) significant versus control (P<0.05) and (P<0.01) respectively, (b) significant versus 0.45% (P<0.01).

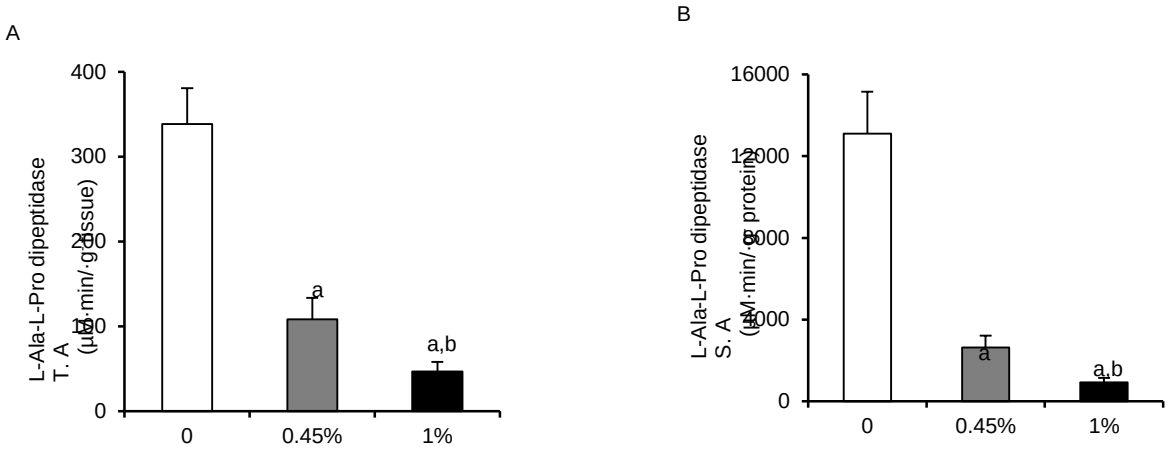

Figure 6: Effect of tartrazine on L-Ala-L-Pro dipeptidases activities in mice. Values represent mean ±SE of ten mice (n = 10). Means which have letters are significantly different, (a) significant versus control (P<0.01), (b) significant versus 0.45% (P<0.05).

The body weight gain decreases in treated groups in spite of high food intakes in 1% tartrazine groups. These results are in accordance with the study of Guendouz *et al.*[31] and the study of Amin *et al.*[12] which showed that oral administration of high (500 mg/kg bw) or low (15 mg/kg bw) doses of Tartrazine decreased significantly the body weight gain in young male rats. Fluid intake is higher in both experimental groups. Different studies showed an increased consumption of tartrazine in diet by rats or mice[37,38,11]. It seems that tartrazine increases the appetency, could it possibly addiction.

Disaccharidases such maltase, sucrase and lactase localize in the BB membrane of small intestinal mucosa, but not in the digestive fluid. The hydrolyzing activity is highest at the proximal jejunum and decreases toward the distal ileum[39]. The hydrolyzing activities are measured extensively in humans and experimental animals[40-46].

In the present study, we have shown that intestinal disaccharidase specific activities are lower in groups treated with 0.45% and 1% Tartrazine. However, maltase specific activity was only decreased in group treated with 1%. In under normal conditions, this level of activity is controlled by the abundance of the mRNA encoding the proteins[47,48]. In situ hybridation analysis of rat jejunum revealed that sucrase mRNA levels were maximal in lower and mild-villus cells with lower levels observed in the villus tip[49]. Lactase mRNA levels were also detected in the villus cells of rat jejunum[50].

The BB disaccharidases, lactase and sucrase are considered accurate markers of enterocyte maturity and functional capacity[51,52]. It has been reported that dietary modification is associated with altered activity of the BB disaccharidases[30], such as Zn deficiency[53,54,44], iron deficiency[55],and vitamin A deficiency[56]. Zn and iron deficiencies have been reported by Ward *et al.*[57] (1990) and Ward[58] in hyperactive children consuming food additives such Tartrazine. Further researches are needed to determine the mineral status of animals exposed to Tartrazine.

Dipeptidases are also a membrane bound enzymes, anchored to the membrane of the enterocytes, their activities may also be compromised and may have occasioned their observed low activities in jejunum of Tartrazine treated mice. The effect of Tartrazine on dipeptidases in the proximal end of the small intestine would severely compromise protein digestion and thus its avaibility.

The measurements of villus height and crypt depth provide an indication of the maturity and the functional capacity of small intestinal enterocytes. Our results have demonstrated that Tartrazine ingestion markedly decreased villus height and crypt depth in mice treated with 0.45% and 1%. Similar results were obtained by Guendouz *et al.*[31].

The BB lining the surface of the small intestinal epithelium provides the major interface for nutrient absorption. Reduction in the surface area of the microvilli is linked with impaired levels of disaccharidase enzymes such as sucrase and lactase that are essential for proper digestion and absorption of sugars[29].

Disaccharidase deficiency can result from numerous situations such as intestinal infection or inflammation. Jejunal inflammation (immune cell hyperplasia) associated with villus atrophy in mice treated with 0.45% and 1% tartrazine were noted in the study of Guendouz *et al.*[31]. They attributed inflammatory cell infiltration to an active chronic jejunitis. This lesion destroys the mucous membrane and can even stretch the mucosa layer[59]. Both in *vitro* and in *vivo* investigations show that uncontrolled synthesis of pro-inflammatory cytokines can have a strong influence on gut integrity and epithelial functions including permeability to macromolecules and transport of nutrients and ions[60].

In our study, the decrease of disaccharidase and dipeptidase activities is correlated with the decrease of villus height. Our results are consistent with previous reports describing a strong relation with low level of dipeptidase activity and villus atrophy[61,42]. Our data suggest that the decrease is likely due to a decline in enzyme protein rather than an inactivation of the enzyme

Table 3: Villus height and Crypt depth of jejunum in mice 13 weeks.

Groups	Villus height (μm)	Crypt depth (μm)
Controls	46.70±0.40	12.44±0.60
0.45%	24.92±1.31[a]	8.02±0.75[a]
1%	16.04±0.67[a,b]	6.00±0.90[a,b]

Values represent mean ±SE of ten mice (n = 10).
Means which have letters are significantly different, P<0.01 (ANOVA test).

activity. Further studies are necessary for determining the origin of these intestinal alterations.

In conclusion, our results suggest that subchronic ingestion of Tartrazine at 0.45% (equivalents to 839 mg/kg/day) and at high dose 1% (equivalents to 1626 mg/kg/day) decreases markedly jejunal disaccharidases particularly sucrase and lactase and dipeptidases as well as alters intestine structure and the body weight loss. Indeed, the excessive consumption of food products containing Tartrazine may contribute to affect the intestine function and integrity.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

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REFERENCES

1. Ates E, Mittendorf K, Senyuva H. LC/MS method using cloud point extraction for the determination of permitted and illegal food colors in liquid, semi liquid, and solid food matrixes: single-laboratory validation. J AOAC Int 2011; 94: 1853-62.
2. Feng F, Zhao Y, Yong W, Sun L, Jiang G, Chu X. Highly sensitive and accurate screening of 40 dyes in soft drinks by liquid chromatography-electrospray tandem mass spectrometry. J Chromatogr B Analyt Technol Biomed Life Sci 2011; 879: 1813-8.
3. Yang X, Qin H, Gao M, Zhang H. Simultaneous detection of Ponceau 4R and tartrazine in food using adsorptive stripping voltammetry on an acetylene black nanoparticle-modified electrode. J Sci Food Agric 2011; 91: 2821-5.
4. Medeiros RA, Lourencao BC, Rocha-Filho RC, Fatibello-Filho O. Flow injection simultaneous determination of synthetic colorants in food using multiple pulse amperometric detection with a boron-doped diamond electrode. Talanta 2012;15: 883-9.
5. Uhlemann U, Strelau KK, Weber K, Da Costa Filho PA, Rösch P, Popp J. Raman spectroscopic determination of norbixin and tartrazine in sugar. Food Addit. Contam. Part A Chem. Anal Control Expo Risk Assess 2012; 29: 1244-55.
6. Abu Shawish HM, Ghalwa NA, Saadeh SM, El Harazeen H. Development of novel potentiometric sensors for determination of tartrazine dye concentration in food stuff products. Food Chem 2013; 1:126-32.
7. Bateman B, Warner JO, Hutchinson E, Dean T, Rowlandson P, Gant C, Grundy J, Fitzgerald C, Stevenson J. The effects of a double blind, placebo controlled, artificial food colourings and benzoate preservative challenge on hyperactivity in a general population sample of preschool children. Arch Dis Child 2004; 89: 506-511.
8. McCann D, Barrett A, Cooper A. Food additives and hyperactive behaviour in 3-year old and 8/9-year-old children in the community: a randomised, double-blinded, placebo controlled trial. Lancet 2007; 370: 1560-1567.
9. Hannuksela M, Haahtela T. Food additive hypersenisivity--near myth]. Duodecim 2009; 125: 527-32.
10. Park M, Park HR, Kim SJ Kim MS, Kong KH, Kim HS, Gong EJ, Kim ME, Kim HS, Lee BM, Lee J. Risk assessment for the combinational effects of food color additives: neural progenitor cells and hippocampal neurogenesis. J Toxicol Environ Health A 2009; 72: 1412-23.
11. Mehedi N, Ainad-Tabet S, Mokrane N, Addou S, Zaoui C, Kheroua O, Saidi D. Reproductive Toxicology of Tartrazine (FD and C Yellow No. 5) in Swiss Albino Mice. Am J Pharma and Toxicol 2009; 4: 128-133.
12. Amin KA, Abdel Hameid H, Abd Elsttar AH. Effect of food azo dyes tartrazine and carmoisine on biochemical parameters related to renal, hepatic function and oxidative stress biomarkers in young male rats. Food Chem Toxicol 2010; 48: 2994-9.
13. Mpountoukas P, Pantazaki A, Kostareli E, Christodoulou P, Kareli D, Poliliou S, Mourelatos C, Lambropoulou V, Lialiaris T. Cytogenetic evaluation and DNA interaction studies of the food colorants amaranth, erythrosine and tartrazine. Food Chem Toxicol 2010; 48: 2934-44.
14. Kashanian S, Zeidali SH. DNA binding studies of tartrazine food additive. DNA Cell Biol 2011; 30: 499-505.
15. Gao Y, Li C, Shen J, Yin H, An X, Jin H. Effect of food azo dye tartrazine on learning and memory functions in mice and rats, and the possible mechanisms involved. J. Food Sci 2011; 76: T125-9.
16. Arnold LE, Lofthouse N, Hurt E. Artificial food colors and attention-deficit/hyperactivity symptoms: conclusions to dye for. Neurotherapeutics 2012; 9: 599-609.
17. Axon A, May FE, Gaughan LE, Williams FM, Blain PG, Wright MC. Tartrazine and sunset yellow are xenoestrogens in a new screening assay to identify modulators of human oestrogen receptor transcriptional activity. Toxicology 2012; 298: 40-51.
18. Wang J, Liu R, Qin P. Toxic interaction between acid yellow 23 and trypsin: spectroscopic methods coupled with molecular docking. J Biochem Mol Toxicol 2012; 26: 360-7.

19. El-Wahab H M, El-Deen Moram GS. Toxic effects of some synthetic food colorants and/or flavor additives on male rats. Toxicol Ind Health 2013; 29(2):224-32.
20. Stevens LJ, Burgess JR, Stochelski MA, Kuczek T. Amounts of artificial food colors in commonly consumed beverages and potential behavioral implications for consumption in children. Clin Pediatr (Phila) 2014; 53(2):133-40.
21. Amchova P, Kotolova H, Ruda-Kucerova J. Health safety issues of synthetic food colorants. Regul Toxicol Pharmacol 2015; Sep 25. pii: S0273-2300(15)30075-1.
22. Vojdani A, Vojdani C. Immune reactivity to food coloring. Altern Ther Health Med 2015; 21 Suppl 1:52-62.
23. Joint FAO/WHO Expert Committee on Food Additives [JECFA]. Summary of evaluations performed by the joint FAO/WHO expert committee on food additives (JECFA) 1956–1995 (first through 44th meetings); International Life Sciences Institute (ILSI) Press, Washington DC, 1996, T-3.
24. Husain A, Sawaya W, Al-Omair A, Al-Zenki S, Al-Amiri H, Ahmed N, Al-Sinan M. Estimates of dietary exposure of children to artificial food colours in Kuwait; Food Addit Contam 2006; 23, 245–251.
25. Dixit S, Purshottam SK, Khanna SK, Das M. Usage pattern of synthetic food colours in different states of India and exposure assessment through commodities preferentially consumed by children. Food Addit Contam Part A Chem Anal Control Expo Risk Assess 2011; 28(8):996-1005.
26. Stevens LJ, Burgess JR, Stochelski MA, Kuczek T. Amounts of artificial food dyes and added sugars in foods and sweets commonly consumed by children. Clin Pediatr (Phila) 2015; 54(4):309-21.
27. Saxena B, Sharma S. Food Color Induced Hepatotoxicity in Swiss Albino Rats, Rattus norvegicus. Toxicol Int 2015; 22(1):152-7.
28. Blau S, Rubinstein A, Bass P, Singaram C, Kohen R. Differences in the reducing power along the rat GI tract: lower antioxidant capacity of the colon. Mol Cell Biochem 1999; 194: 185-191.
29. Farthing MJ. The molecular pathogenesis of giardiasis. J Pediatr Gastroenterol Nutr 1997; 24: 79-88.
30. Henning SJ. Biochemistry of intestinal development. Environ Health Perspect 1979; 33: 9-16.
31. Guendouz M, Mehedi N, Zaoui C, Saidi D, Khéroua O. Immune response after Tartrazine subchronic ingestion in Swiss albino mice. Int J Pharm and Pharm Sci 2013; 5: 584-592.
32. Tanaka T. Reproductive and neurobehavioural toxicity study of tartrazine administered to mice in the diet. Food Chem Toxicol 2006; 44, 179-187.
33. Tanaka T, Takahashi O, Oishi S, Ogata A. Effects of tartrazine on exploratory behavior in a three-generation toxicity study in mice. Reprod Tox 2008; 10-1016.
34. Lowry OH, Rosenbrough NJ, Farr A, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem 1951; 193: 265- 275.
35. Dahlqvist A. Assay of intestinal disaccharidases. Anal Biochem 1968; 22: 99-107.
36. Doi E, Shibata D, Matoba T. Modified colorimetric ninhydrin methods for peptidase assay Anal Biochem 1981; 118: 173-184.
37. Borzelleca JF, Hallagan JB.Chronic toxicity/carcinogenicity studies of FD&C Yellow No. 5 (tartrazine) in rats. Food Chem Toxicol 1988a; 26: 179-187.
38. Borzelleca JF, Hallagan JB. A chronic toxicity/carcinogenicity study of FD&C Yellow No. 5 (tartrazine) in mice. Food ChemToxicol 1988b; 26:1891-94.
39. Semenza G, Auricchio S. Small-intestinal disaccharidases. In: Seriver CR, Beauset AL, Sly WS, Valk D. (Eds), Metabolic basis of inherited disease. 6th ed. New York, NY: McGraw- Hill, 1989, pp. 2975-2997.
40. Huang CC, Chen JR, Liu CC, Chen KT, Shieh MJ, Yang SC. Effects of long-term ethanol consumption on jejunal lipase and disaccharidase activities in male and female rats. World J Gastroenterol 2005; 11: 2603-2608.
41. Enattah NS, Kuokkanen M, Forsblom C, Natah S, Oksanen A, Järvelä I, Peltonen L, Savilahti E. Correlation of intestinal disaccharidase activities with the C/T_{-13910} variant and age. World J Gastroenterol 2007; 13: 3508-3512.
42. Missouri M, Chekroun A, Aboubeker N, Kheroua O. Evaluation des activités des disaccharidases et des dipeptidases dans la muqueuse jéjunale de sujets cœliaques. Médecine et Nutrition 2009; 45: 1-11.
43. Oku T, Tanabe K, Ogawa S, Sadamoria N, Nakamura S. Similarity of hydrolyzing activity of human and rat small intestinal disaccharidase. Clin Exp Gastroenterol 2011; 4:155-161.
44. Tran CD, Cool J, Xian CJ. Dietary zinc and metallothionein on small intestinal disaccharidases activity in mice. World J Gastroenterol 2011; 21: 354-360.
45. Solaymani-Mohammadi S, Singer SM. Host immunity and pathogen strain contribute of intestinal disaccharidase impairment following gut infection. J immunol 2011; 187: 3769-3775.
46. Varga J, Tóth SJr, Tóth S, Tomečková V, Gregová k, Veselá J. The relation between morphology and disaccharidase activity in ischemia-reperfusion injured intestine. Acta Biochimica Polonica 2012; 59: 631-638.
47. Goda T, Yasutake H, Tanaka T, Takase S. Lactase-Phlorizin hydrolase and Sucrase-Isomaltase genes are expressed differently along the villus-crypt axis of rat jejunum. J Nutr1999; 129:1107-1113.
48. Traber PG, Yu L, Wu GD, Judge TA. Sucrase-isomaltase gene expression along crypt-villus axis of human small intestine is regulated at level of mRNA abundance. Am J Physiol 1992; 262: G123-30.
49. Traber PG. Regulation of sucraseisomaltase gene expression along the crypt-villus axis of rat small intestine. Biochim Biophys Res Commun 1990; 173: 765–773.

50. Duluc I, Jost B, Freund JN,. Multiple levels of the stage and region specific expression of rat intestinal lactase. J Cell Biol 1993; 123: 1577-1586.
51. Pluske JR, Thompson MJ, Atwood CS, Bird PH, Williams IH, Hartmann PE. Maintenance of villus height and crypt depth, and enhancement of disaccharide digestion and monosaccharide absorption, in piglets fed on cow's whole milk after weaning. Br J Nutr 1996; 76: 409-422.
52. Peulen O, Denis G, Defresne MP, Dandrifosse G. Spermine-induced alteration of small intestine in suckling rat: involvement of apoptosis or Zn^{2+} enzymes? Dig Dis Sci 2001; 46: 2490–2498.
53. Gebhard RL, Karouani R, Prigge WF, Mc Clain CJ. The effect of severe zinc deficiency on activity of intestinal disaccharidases and 3-hydroxy-3-methyglutaryl coenzyme A reductase in the rat. J Nutr 1983; 113: 855-859.
54. Duff M, Ettarh RR. Crypt cell production rate in the small intestine of the zinc-supplemented mouse. Cells Tissues Organs 2002; 72: 21-28.
55. West AR, Oates PS. Decreased sucrose and lactase activity in iron deficiency is accompanied by reduced gene expression and upregulation of the transcriptional repression PDX-1. Am J Physiol Gastr 2005; 289: G1108- G1114.
56. Reifen R, Zaiger G, Uni Z. Effect of vitamin A on small intestinal brush border enzymes in a rat. Int. J Vit Nutr Res 1998; 68: 281- 286.
57. Ward NI, Soulsbury K, Zettel VH, Colquhoun ID, Bunday S, Barnes B. The influence of the chemical additive tartrazine on the zinc status of hyperactive children-a double-blind placebo-controlled study. J Nutr Med 1990; 1: 51-57.
58. Ward NI. Assessment of chemical factors in relation to child hyperactivity. J. Nutr Environ Med 1997; 7: 333-342.
59. Ross JS, Wilson K, Waugh A, Grant A. Anomalie et physiologie normale et pathologique. 10th ed. 2007, Paris: Masson.
60. McKay DM, Baird AW. Cytokine regulation of epithelial permeability and ion transport. Gut 1999; 44: 283-289.
61. Brasseur D, Goyens PH, Vis HL. Enzymes and histology of intestinal mucosa breast-fed African infants. Annales de Pédiatrie 1992; 39: 87-93.