Marmara Pharmaceutical Journal 21/3: 564-569, 2017
DOI: 10.12991/marupj.319304

RESEARCH PAPER

Tartrazine induced changes in physiological and biochemical parameters in Swiss albino mice, *Mus musculus*

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ABSTRACT

Now-a-days synthetic food dyes are being used most commonly as food colorant in confectionaries, drugs and cosmetics. Present study was designed to evaluate the toxic effect of tartrazine, a widely used azo dye, on *Swiss Albino* mice. Experimental animals were treated with tested dye at a dose level 200mg/kg & 400mg/kg body weight along with normal diet. Various physiological and biochemical parameters were assessed to study the toxic effect of tartrazine. Our study revealed a highly noticeable decrease in the body weight gain of mice at 400mg/kg dose compared with the control group. A significant variation in the average weight of the major organs (heart, kidney and liver) was also observed. The average weights of the heart and kidney were increased whereas the average weight of liver was decreased significantly. Serum triglyceride, creatinine and bilirubin levels were significantly increased, in contrast cholesterol level was decreased in the groups of mice treated with tartrazine.

Keywords: Azo-Dye, tartrazine, Swiss Albino Mice, Physiological changes, Serum biochemistry.

1. INTRODUCTION

Color has become an essential part of food processing, as it can influence the perceived flavor of foods by the consumers. In order to make food more appealing and attractive, the practice of addition of color to foods has been being continued for centuries. Today consumers rely on the technological, aesthetic and convenient benefits provided by the color additives. Although these additives may come from both natural and synthetic origin, however 95% of those used now-a-days are synthetic because they are produced easily, cheaper and provide better coloration (1). Among the different dyes, Tartrazine, also known as FD&C yellow 5, is frequently used in foods, drugs, and cosmetics (2).

The use of artificial dye in wide ranges of food is being suspected to be toxic or harmful. Tartrazine has been reported to produce allergic reactions in atopic eczema and sensitive individuals (3-5). Moreover tartrazine has toxic potential for human lymphocyte (6), learning and memory functions (7) and behavior in children (8). But in case of carcinogenic effect, the role of tartrazine is ambiguous as different studies reported differently (9, 10). Different food dye like chocolate brown has been reported to alter the serum biochemistry (11). Although several toxic effects of

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Submitted / Gönderilme: 30.12.2016 **Revised / Düzeltme:** 03.02.2017
Accepted / Kabul: 06.02.2017

Tartrazine has been reported but alteration of biochemical and physiological functions with Tartrazine is not well understood. This current study was aimed to find out the physiological and biochemical changes in Swiss albino mice in relatively higher dose of tartrazine as a safety concern. The outcome of this study will help us to make decision in using of tartrazine as food dye.

2. MATERIALS AND METHODS

2.1 Materials

Tartrazine (C.I. 19140, CAS No 1934-21-0, Mw 534.37, synonyms: E 102, Food yellow 4, FD&C yellow No.5) was obtained from local market.

2.2 Animal's model and housing

Swiss Albino mice are widely used in toxicology research because they respond favorably. Healthy *Swiss albino* mice (25-30g) were collected from Department of Pharmacy, Jahangirnagar University, Savar, Dhaka, Bangladesh and were kept in polypropylene plastic cages having dimensions of 30 × 20 × 13 cm and soft wood shavings were employed as bedding in the cages. Feeding of animal was done along with standard laboratory pellet diet and water at *libitum*, exposing them to alternate cycle of 12hr dark and light, at temperature 25±20˚c and relative humidity 55±10%. All the mice were allowed to acclimatize for 7 days to the laboratory conditions before conducting the experiment.

2.3 Study protocol

Fifteen experimental healthy animals were randomly selected and divided into three groups with 5 animals in each group.

Group-1: Control group

Group-2: Mice treated with dye at 200mg/kg body weight (b.w.) dose

Group-3: Mice treated with dye at 400mg/kg b.w. dose

Tartrazine dye was administered orally, using an intra-gastric feeding syringe for an experimental period of 25 days. A daily record of body weight was maintained. At the end of the study, the animals were weighed and sacrificed by cervical dislocation. The body organs and the blood samples were collected to analyze the toxic effect caused by the tartrazine. This study protocol was approved from the institutional committee.

2.4 Monitoring of body weight

To observe the effect of chronic administration of the tartrazine on growth rates of the experimental animals, body weight of all of the experimental mice including the controlled group, were carefully monitored throughout the 25 days period of dye administration.

This following equation described previously (11,18) was used to calculate the percentage of body weight gain.

$$\% \text{ Body weight gain} = \frac{\text{Mean final weight} - \text{Mean initial weight}}{\text{Mean initial body weight}} \times 100$$

2.5 Biochemical analysis of blood

To collect the intended serum and to remove red blood cells, the collected samples of blood were centrifuged at 1000 g for 10 min using bench top centrifuge (MSE Minor, England). After separation, serum was collected carefully and stored in the refrigerator at -20˚c for analysis. The analysis of all the biochemical parameter was accomplished within 24 h of sample collection. All of the analyzes were conducted with respective analysis kit e.g Triglyceride kit (CAT# CS 611, Crescent Diagnostics, Soudia Arabia), Cholesterol kit (CAT# CH 200, Randox Laboratories, UK), Creatinine kit (CAT # CR510, Randox Laboratories, UK), Bilirubin kit (CAT# CS 601/602, Crescent Diagnostics, Soudia Arabia) according to the manufacturer's instructions. The absorbance was measured using a spectrophotometer JA.S.CO V-530 (UV/vis), Japan.

2.6 Pathological examination

After sacrificing the experimental animals, specific organs of interest were then separated and preserved in normal saline for 24 hours. After collecting the organs and drying, the weight of each organ of each mice was measured separately and the average weights were compared for statistical evaluation.

2.7 Statistical analysis

All the results obtained by *in-vitro* and *in-vivo* experiment are presented in the tables or figures as the mean ± SEM. The statistical significance of the differences between control and experimental groups was evaluated by paired *t*-test (using SPSS software, version-20), where $p<0.05$ was considered as statistically significant.

3. RESULTS

3.1 Clinical observation, Food intake, Body weight and Growth

Treatment with tartrazine did not affect mortality, clinical signs, food intake when compared to the control group.

As a function of growth, body weight of experimental animal was monitored. Our data showed a noticeable retardation in the body weight gain in mice treated with relatively large dose (400mg/kg b.w.) of tartrazine but at 200mg/kg b.w. did not affect the body weight gain significantly. Results are presented in Table 1.

3.2 Organ weight

With regard to the organ weight, average weight of heart of the mice has been increased significantly in tartrazine treated groups ($p = <0.05$). Statistically insignificant increase in the average weight of kidney was also reported in the tartrazine treated groups of animal but a significant decrease in the average weight of liver was noticed in case of tartrazine administration to mice. All the data has been presented in Table 2.

Table 1. Changes in the body weight gain of the mice during the 25 day period of study.

Treatment	Body Weight (g) (Mean ± S.E.M.)		% of Body Weight gain
	Initial	Final	
Group-1 (Control)	26.97± 0.53	33.54±0.84	6.57
Group-2 (200mg/kg b.w)	27.39± 0.44	35.01±0.86	7.62
Group- 3 (400mg/kg b.w)	27.68± 0.24	32.05±0.77*	4.40*

Values are represented as mean ± SEM (n=5); *p<0.05 versus control

Table 2. Effect of tartrazine on the average weight of heart, kidney and, liver of mice

Treatment	Heart (gm)	Kidney (gm)	Liver (gm)
Group-1 (Control)	0.184 ± 0.003	0.314 ± 0.036	1.931 ± 0.021
Group-2 (200mg/kg b.w)	0.275 ± 0.011*	0.397 ± 0.011	1.75 ± 0.03*
Group- 3 (400mg/kg b.w)	0.212 ± 0.015*	0.42 ± 0.029	1.76 ± 0.029*

Values are represented as mean ± SEM (n=5); *p<0.05 versus control

Table 3. Effect of tartrazine on Cholesterol, Triglyceride, Bilirubin, Creatinine

Treatment	Cholesterol (mg/dl)	Triglyceride (mg/dl)	Bilirubin (mg/dl)	Creatinine (mg/dl)
Group-1 (Control)	161.539 ± 2.29	232.22 ± 6.75	6.601 ± 0.28	0.79 ± 0.18
Group-2 (200 mg/kg b.w)	156.154 ± 5.26	207.41 ± 9.79	8.27 ± 0.23**	1.48 ± 0.21
Group-3 (400mg/kg b.w)	151.923 ± 4.81	288.97 ± 12.76*	8.36 ± 0.23**	1.78 ± 0.25**

Note: values are represented as mean ± SEM (n=5); *p<0.05, **p<0.01 versus control

3.3 Biochemical examination

Data presented in Table 3, showed the different biochemical parameter tested in this experiment. We found a statistically insignificant decrease in the cholesterol level in mice treated with tartrazine regardless of dose than that the control group. Whereas, the concentration of triglyceride was increased in mice treated with tartrazine. Dose at 400mg/kg b.w showed statistically significant ($p<0.05$) increase in triglyceride level but at lower dose 200mg/kg b.w this changes was insignificant.

Tartrazine was able to increase the serum bilirubin significantly ($p < 0.01$) in a dose dependent manner. Again serum creatinine level was also increased ($p< 0.01$) in both tartrazine treated group.

4. DISCUSSION

Toxic effects of tartrazine were evaluated in this experiment with a chronic administration of dye. The acceptable daily intake of tartrazine for human is 0-7.5 mg/kg-b.w (12). Poul *et al* (13) studied that tartrazine up to 1000mg/kg-b.w twice at 24 hours interval did not produce any genotoxic activity in mice. Whereas, Amin *et al* (21) reported the alteration of renal, and hepatic functions by tartrazine at 500 mg/kg-b.w dose repeated for 30 days in rats. Considering these findings and to assess the toxicity arising from chronic dose of tartrazine, a dose level of 200mg/kg-b.w and 400mg/kg-b.w with repeated administration up to 25 days, was evaluated in this study. During the whole period of treatment no death, no abnormal clinical signs of experimental mice were observed.

Monitoring of body weight is considered as proper growth of animal or human. Some researchers considered the loss of body weight is a reliable sensitive indicator for toxicity study (14, 15). In our study, a noticeable retardation in the body weight gain was found in mice treated with 400mg/kg b.w than that of control group. This observation was in similarity with the other works done where a significant decrease in body weight was found in the mice treated with tartrazine (16). Thus the result indicates the potentiality of tartrazine to alter the growth as a function of toxicity. Along with the role in growth, we studied the effect of tartrazine on different organ weight. Tatrazine was capable to increase the weight of vital organs i.e heart and kidney of mice effectively but average liver weight was decreased. It was reported that oral ingestion of synthetic dyes such Fast Green (12.5 mg/kg/day) and Sunset Yellow (5 mg/kg/day) for 30 days in mice increased the liver and kidney weight (17, 18). While Maekawa *et al.* (10) indicated that subchronic tartrazine ingestion in rats decreased the absolute and relative liver in 2% dose group. As the change of the absolute and relative organs weight is a sign of toxicity thus it is obvious that our experimental dye must have considerable toxicity on respective organ of the test animals.

Mehedi *et al.* (18) reported the intestinal lymphocyte infiltration, glomerular changes and edema in kidney after the administration of tartrazine, these pathophysiological changes may be attributed to our finding of elevated kidney weight. Furthermore, to investigate the function of kidney, we assessed the serum creatinine level and we found an elevated serum creatinine concentration in mice treated with tartrazine as predicted. Elevated serum creatinine is considered as renal dysfunction (19). Thus our result revealed that tartrazine has the potentiality to develop renal dysfunction.

In case of hepatoactivity, we found decreased liver weight and elevated serum total bilirubin in mice treated with tartazine. Both the doses applied here, were capable of doing the same function in dose dependent manner. Thus interpreting our study result, it can be said that tartrazine dye might cause in obstruction in liver function that increased the serum bilirubin level in the mice. This data is similar to our previous data the data where we observed a significant elevation in serum bilirubin level with other azo dyes like chocolate brown (11). Serum bilirubin concentration may be elevated due to acute hepatocellur injury, cholestatic injury or biliary obstruction. Moreover, the increased concentration of bilirubin may also be attributed to the hemolytic destruction of RBC as bilirubin is a waste product, that is primarily produced by the normal breakdown of heme, a substance found mainly in the protein hemoglobin in red blood cells (RBCs) (20).

To assess the effect of tartrazine on lipid profile, we examined the serum concentration of triglyceride and cholesterol. Our data showed that, tartrazine, regardless of dose, was capable to decrease cholesterol level insignificantly but in contrast, hypertriglyceridemia was observed in test animal when compared to the control group. The ability to elevate the triglyceride level was dose dependent manner. At higher dose, the effect was statistically significant. Our result agreed with the result of Amin *et al*. who observed a reduction in serum cholesterol level and an increase in triglyceride level in male rats treated with different azo dye (21). Although, other researchers showed the elevated level of triglyceride and cholesterol as an effect of azo dyes (15,19). This dissimilarity in results may be due to the differences of doses applied in

different studies. Moreover, elevation of serum triglyceride has been documented due to the deficit production of hepatic lipase in human (22, 23). Thus it might happen that damaged hepatocytes were unable to produce sufficient hepatic lipase to reduce the triglyceride in tartrazine treated test animal.

The wide use of tartrazine in foods, drinks, sweets, and cosmetics is a concern as a possibility of randomly exposure to this dye. Consumptions of tartrazine more than acceptable daily intake by children has been reported in different developing country like India (24), because the children are very fond of colored food. Chronic administration of tartrazine is associated with different toxic effect. Thus, exposure of excessive clororants like tartrazine in children may pose a health risk.

5. CONCLUSION

In conclusion, administration of azo dye tartrazine to mice causes many problems in the physiological and biochemical parameters particularly at higher dose. So ingestion of tartrazine containing food might not be free from harmful effects in the long run. Therefore awareness among people should be created about the hazardous effect of tartrazine consumption.

Conflict of interest

None

Swiss albino farelerde-*Mus musculus*, tartrazin'in neden olduğu fizyolojik ve biyokimyasal parametrelerindeki değişiklikler

ÖZ

Günümüzde sentetik gıda boyaları, şekerlemelerde, ilaçlarda ve kozmetiklerde renklendirici olarak sıklıkla kullanılmaktadır. Çalışmamızda, geniş kullanım alanı olan bir azo boyar madde-tartrazin'in *Swiss albino* farelerdeki toksik etkilerinin tespit edilmesi hedeflendi. Normal diyetle beslenen laboratuvar hayvanlarına tartrazin 200mg/kg ve 400mg/kg dozlarda uygulandı. Tartrazin'in toksik etkisini incelemek için çeşitli fizyolojik ve biyokimyasal parametreler değerlendirildi. Çalışmamızda tartrazin'in 400 mg / kg dozunda uygulandığı grupta yer alan farelerde kontrol grubuna kıyasla vücut ağırlığı artışında belirgin bir düşüş olduğu tespit edildi.

Kalp, böbrek ve karaciğer gibi hayatî organların ortalama ağırlığında önemli farklılıklar gözlendi. Kalbin ve böbreklerin ortalama ağırlıkları artarken, karaciğerin ortalama ağırlığı önemli derecede azaldığı tespit edildi. Tartrazin'in uygulandığı grupta yer alan farelerde serum trigliserid, kreatinin ve bilirubin düzeyleri anlamlı olarak artarken kolesterol düzeyinin azaldığı tespit edildi.

Keywords: Azo boyaları, Tartrazin, Swiss Albino fare, Fizyolojik değişiklikler, Serum biyokimyası

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