Research Article

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Effect of food azo-dye tartrazine on physiological functions of pancreas and glucose homeostasis

Gıda azo-boyaar maddesi tartrazinin pankreas ve glukoz homeostazının fizyolojik fonksiyonları üzerine etkisi

<https://doi.org/10.1515/tjb-2017-0296>

Received November 7, 2017; accepted March 20, 2018; previously published online August 8, 2018

Abstract

Background: Food industry is extensively using tartrazine however, influence of tartrazine-exposure on pancreas remains to be elucidated.

Materials and methods: This study was designed to evaluate the effect of tartrazine on pancreas and glucose homeostasis in rats. Albino rats were divided into three groups. Group I was control, group II and III were assigned as low and high doses of tartrazine-exposed groups respectively. Blood samples were collected to estimate the glucose homeostasis and insulin, amylase and lipase activity, and glucose tolerance along with morphology of pancreas.

Results: The results showed that tartrazine at higher doses, increased blood sugar (230.830 ± 0.940 mg/dL) and insulin (0.395 ± 0.012 ng/mL) levels as compared to that of control. HOMA-IR value of tartrazine-exposed rats was significantly high (1.450 ± 0.090) as compared to that of control. Moreover, the serum levels of amylase and lipase were also increased significantly in tartrazine-exposed rats. Correspondingly, morphology of pancreas was also found to be changed in tartrazine-exposed rats.

Conclusion: These results demonstrated that tartrazine has a critical influence on glucose homeostasis. This evidently reveals that tartrazine has damaging effects on pancreas and enduring the exposure of tartrazine could possibly result in the disturbance of normal endocrine functioning of pancreas.

Keywords: Food azo-dye; Tartrazine; Lipase; Amylase; Insulin; Glucose tolerance.

Öz

Amaç: Gıda endüstrisi yaygın olarak tartrazin kullanıyor, ancak tartrazine maruziyetinin pankreas üzerindeki etkisi açıklığa kavuşmuş durumda.

Gereç ve Yöntem: Bu çalışma, tartrazinin farelerde pankreas ve glukoz homeostazisi üzerindeki etkisini değerlendirmek için tasarlanmıştır. Albino sıçanları 3 gruba ayrıldı. Grup I kontrol grubuydu, grup II ve III sırasıyla düşük ve yüksek dozlarda tartrazine maruz bırakıldı. Kan örnekleri glikoz homeostazisini ve insülin, amilaz ve lipaz aktivitesini ve glikoz toleransını ve pankreas morfolojisini tahmin etmek için toplandı.

Bulgular: Sonuçlar daha yüksek dozlarda tartrazinin, kontrol grubuna kıyasla kan şekerini (230.830 ± 0.940 mg/dL) ve insülini (0.395 ± 0.012 ng/mL) artırdığını göstermiştir. Tartrazine maruz kalan sıçanların HOMA-IR değeri, kontrolüne kıyasla anlamlı derecede yüksekti (1.450 ± 0.090). Ayrıca tartrazine maruz bırakılan sıçanlarda serum amilaz ve lipaz seviyeleri de önemli ölçüde artmıştır. Buna karşılık olarak, tartrazine maruz bırakılan sıçanlarda pankreas morfolojisinin de değiştiği bulunmuştur.

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Sonuç: Bu sonuçlar, tartrazinin glukoz homeostazı üzerinde kritik bir etkiye sahip olduğunu göstermiştir. Bu, tartrazinin pankreas üzerinde zararlı etkilere sahip olduğunu ve tartrazin maruziyetini sürdürdüğünün, muhtemelen pankreasın normal endokrin fonksiyonunun bozulmasına neden olabileceğini ortaya koymaktadır.

Anahtar Kelimeler: Gıda azo-boyası; Tartrazin; lipaz; amilaz; insülin; glukoz toleransı

Introduction

Currently, food additives including food azo-dyes are most commonly used to enhance the color and taste of the food items and are considered as an important item of the food industry [1]. More than 2500 food additives have been reported to be used as colorants and preservatives [2]. Generally, food additives are classified into five major types; among which, coloring agents are an essential class that are mostly obtained from the animal and/or plant sources [3]. Since 1995, natural and synthetic coloring agents have been widely used in majority of food and pharmaceutical industries [4]. Pharmaceutical products and medicines such as antacids, vitamins, shells of medicated capsules and other prescription drugs are known to contain coloring agents [5].

There are number of coloring agents that are commonly used in pharmaceutical and food industries among which tartrazine is most commonly used. Tartrazine is a yellow to orange colored synthetic azo-dye which is also known as FD&C Yellow 5 and/or E102. Tartrazine is a water soluble colorant that is used in many food products like cotton candy, corn flakes, cake, soft drinks, chips, sauces, jelly, ice creams, sauces, snacks, mustard, cheeses, foodstuffs, pickles, chewing gum and drugs [5, 6]. The chemical name of tartrazine is trisodium-5 hydroxy-1-(4-sulfonatophenyl)-4-(4-sulfonatophenylazo)-H-pyrazole-3-carboxylate (Figure 1) and contains azo linkage ($-N=N-$) in its structure that is known to be metabolized into sulfanilic acid and aminopyrazolone by intestinal bacteria. Whereas, aminopyrazolone is further cleaved into sulfanilic acid by the action of intestinal bacteria [7].

Figure 1: Structural formula of tartrazine.

However, excess intake of tartrazine has shown various adverse effects such as urticaria, migraine, rashes and gastro-intestinal disturbances [8]. World Health Organization (WHO) expert committee on food additives has recommended 0–7.5 mg/kg body weight of tartrazine as a maximal daily acceptable intake [7, 9]. Several studies have reported variable results about deleterious effects of tartrazine. Tartrazine has known to show chromosomal aberration in somatic cells of rats [10]. Moreover, 10 mg/kg dose of tartrazine has depicted carcinogenic effects on colon which is a dose that is very close to the daily acceptable intake of tartrazine recommended by WHO [7]. Despite of such toxic effects observed on tartrazine-exposure, this azo-dye is still cheaply available worldwide and has been observed to be used as an alternative for β -carotene which is considered to be more expensive than tartrazine [11].

Pre-diabetes is a condition that has high risk for diabetes and is characterized by high blood sugar level as compared to that of the normal, but lower than the level found after onset and progression of diabetes. However, interestingly, 5–10% pre-diabetic people have been recognized to develop diabetes mellitus (DM) every year. Experts have estimated that more than 470 million people might be in the range of pre-diabetes by the year 2030 globally. Pre-diabetes is correlated with β -cell's dysfunction and insulin resistance which is the inability of peripheral tissues to utilize insulin for metabolism of glucose [12, 13]. Studies have also shown that pre-diabetes can lead to the increased risk of neuropathy, nephropathy, retinopathy, kidney and macrovascular diseases. Thereby, it is essential to identify and eradicate unnecessary exposure to the risk factors that not only lead towards pre-diabetes but also increases the chances of prevalence of DM.

The growing literature provides a great deal of evidences regarding toxic compounds like tartrazine and their relation to human health particularly, has revealed the toxic effects of tartrazine on public health however, till now, a very limited work has been done on exploring the exposure of tartrazine on endocrine system and its association with endocrine dysfunction and metabolic disorders. Hence, we opted to evaluate the effect of tartrazine-exposure on the normal functioning of pancreas, an important organ of the body that regulates the glucose homeostasis using rats as an experimental animal model. Furthermore, we also investigated the effect of tartrazine on insulin sensitivity, glucose tolerance, status of anti-oxidant enzyme, serum levels of amylase and lipase and morphology of pancreas. We hypothesized that exposure to tartrazine might probably alter the normal morphology of pancreas and glucose homeostasis.

Materials and methods

Chemicals

Tartrazine labeled as food colorant was obtained from Standard manufacturing company Private limited Sheikh-pura Road, Lahore, Pakistan. Prior to use in experiment, tartrazine was dissolved in distilled water to prepare two different doses, low dose (10 mg/kg of body weight) and high dose (50 mg/kg of body weight). All other chemicals and reagents used in this study were at least analytical grade.

Animals and treatments

Total of 18 white albino rats of either sex were used in the present study. They were kept in the animal house of University of Agriculture, Faisalabad, Pakistan and were given standard basal diet. The rats were provided with water ad libitum and kept in individual cages at room temperature ($22 \pm 2^\circ\text{C}$) for 12 h light and 12 h dark cycle. After 7 days of acclimatization, rats were randomly divided into three groups ($n=6$) i.e. Group I: normal control on routine feed; Group II: low-dose group exposed to the low dose of tartrazine (10 mg/kg of body weight); Group III: high dose group exposed to the high dose of tartrazine (50 mg/kg of body weight). All methods used in this study involving rats as an animal model were performed according to the Bio-Safety Protocol for Human/Animal by the Institutional Bio-Safety Committee (IBC-3127), University of Agriculture Faisalabad for the use of laboratory animals.

Blood sampling

For biochemical analysis, blood was collected from the tail vein before the treatment (0 day) and on 15th and 30th days of experiment. The samples were allowed to clot for 20 min at room temperature. Then the samples were centrifuged at $3000 \times g$ for 14 min and serum was separated and stored at freezing temperature (-20°C) for biochemical analysis of pre-determined parameters.

Biochemical analysis

The separated serum was used to investigate the glycaemic status by measuring the serum levels of glucose

and insulin, carbohydrate metabolism by measuring the serum levels of amylase, fat metabolism through evaluating the serum levels of lipase, anti-oxidant enzyme capacity using serum level of superoxide dismutase (SOD). Further, the serum levels of calcium and magnesium were also investigated.

Estimation of insulin level

Insulin level in the serum samples of all experimental groups were measured using simple step ELISA (Enzyme-Linked Immunosorbent Assay) kit provided by abcam® for insulin detection.

Estimation of glucose level

To estimate the level of glucose in experimental rat groups, Optium xceed® glucometer was used to measure the blood glucose levels.

Estimation of amylase activity

Amylase assay kit (Colorimetric) (ab102523) provided by abcam® was used in this study to determine the serum level of amylase (U/L) in experimental animals. In this experiment, α -amylase first cleaved the substrate ethylidene-pNP-G7 to produce smaller fragments that were eventually modified by α -glucosidase, causing the release of a chromophore that can then be measured by spectrophotometer.

Estimation of lipase activity

To evaluate the impact of tartrazine-exposure on lipase in experimental animals, serum level of lipase (U/L) was measured quantitatively using assay kit (ab102524) provided by abcam® at predefined time points.

Estimation of antioxidant enzyme status

To estimate the impact of tartrazine-exposure on antioxidant enzyme status, the serum level of SOD (U/mg) was measured quantitatively at predefined time points using commercially available kit (ab65354) provided by abcam®.

Estimation of serum calcium and magnesium levels

Serum calcium level was determined by photometric test using cresol-phthaleincomplexone (CPC). Similarly, serum level of Magnesium was determined by colorimetric assay using relevant Magnesium assay kit provided by abcam®.

Estimation of glucose tolerance

Before the end of treatment period, we also performed oral glucose tolerance test (OGTT) by administering the glucose (2 g/kg body weight) in all rat groups after an overnight fasting. Blood samples were collected from the tail vein before (0 min) and after the administration of glucose (30, 60, 90 and 120 min).

Insulin sensitivity determination

We also investigated the effect of tartrazine-exposure on insulin sensitivity and/or resistance by homeostasis model assessment for insulin resistance (HOMA-IR) using the values of fasting blood glucose and serum level of fasting insulin.

Histopathological examination of pancreas

At the end of treatment, the rats were mercifully sacrificed and pancreas was separated. A section was separated from the separated pancreas and fixed in 10% buffered formalin for 3–5 days. The fixed tissue was washed and dehydrated by placing it in a series of changes in alcohol concentration. After dehydration, the tissue was placed in xylene to make the tissue clear. The clear tissue was infiltrated with melted paraffin for 2 h and embedded in embedding media having paraffin wax. After embedding, tissues were then cut into thin slices (5 μ m) with microtome. The sections were flattened by floating in the water bath at temperature 50–55°C. Sections were mounted directly on the slides using adhesive mixture of Mayer's egg albumin. Finally, the mounted sections were stained by Hematoxylin and Eosin (H&E) stains with DPX placed on the stained slide and covered the tissue section by putting the glass cover slip on it. Slides were examined by research microscope at 40X for histopathological studies.

Statistical analysis

The results were expressed as mean $\pm$ SD. Statistical analysis as conducted by one-way analysis of variance (ANOVA) followed by Duncan's multiple range test (DMR) at 5% level of significance ($p \leq 0.05$) using Statistical Package for Social Sciences (SPSS).

Results

Effect of tartrazine-exposure on glycemic status

In present study, we investigated the exposure of tartrazine on glycemic status of experimental animal model. Normal glucose homeostasis is indicated by the serum and/or blood levels of glucose and insulin. The present study showed that tartrazine-exposure exhibited its effects on the glucose homeostasis by altering the levels of blood glucose and serum insulin. At the end of treatment, both low and high dose of tartrazine significantly increased the levels of glucose (118.5 ± 1.68 mg/dL) and (130 ± 2.59 mg/dL), respectively as compared to the control group (Figure 2A). Similarly, at the end of treatment, we also investigated the impact of tartrazine-exposure on serum level of insulin. We found that both low and high dose of tartrazine significantly decreased the levels of insulin (0.468 ± 0.008 ng/mL) and (0.395 ± 0.012 ng/mL), respectively as compared to the control group (Figure 2B).

Effect of tartrazine-exposure on amylase activity

To investigate the effect of tartrazine-exposure on carbohydrate metabolism, we measured the serum level of amylase. The results mentioned in Figure 3 indicate that at the end of treatment, both low and high dose of tartrazine significantly increased the serum level of amylase (429.660 ± 6.940 U/L) and (646.830 ± 9.780 U/L) respectively as compared to that of control group. This indicate the disruption of normal function of pancreas by tartrazine due to which the production of carbohydrate metabolizing enzyme (amylase) was increased which in turn might have increased the production of glucose in GIT.

Figure 2: Effect of tartrazine-exposure on glycemic status: rats were divided into three groups having equal number of rats ($n=6$) in each group.

Group I was control group that only received normal saline, while group II and III received low dose (10 mg/kg body weight of rat) and high dose (50 mg/kg body weight of rat) of tartrazine respectively for 30 days. The time courses of serum levels of glucose (A) and insulin (B) at pre-treatment (0 day), during treatment (15th day) and post-treatment (30th day) have been expressed as mean $\pm$ SD. *, ** $p < 0.05$ when compared to control group.

Effect of tartrazine-exposure on lipase activity

We also assessed the exposure of tartrazine on fat metabolism by measuring the serum level of lipase (U/L). Lipase is an enzyme that plays active role to hydrolyze lipids and ester bonds in triglycerides, to form fatty acids and triglycerides. At the end of treatment, we found that tartrazine significantly increased the serum level of lipase enzyme in both tartrazine-exposed groups as compared to that control group (Figure 4).

Figure 3: Effect of tartrazine-exposure on amylase level: rats were divided into three groups having equal number of rats ($n=6$) in each group.

Group I was control group that only received normal saline, while group II and III received low dose (10 mg/kg body weight of rat) and high dose (50 mg/kg body weight of rat) of food azo-dye tartrazine respectively for 30 days. The time course of serum levels of amylase at pre-treatment (0 day), during treatment (15th day) and post-treatment (30th day) have been expressed as mean $\pm$ SD. *, ** $p < 0.05$ when compared to control group.

Figure 4: Effect of tartrazine-exposure on lipase level: rats were divided into three groups having equal number of rats ($n=6$) in each group.

Group I was control group that only received normal saline, while group II and III received low dose (10 mg/kg body weight of rat) and high dose (50 mg/kg body weight of rat) of food azo-dye tartrazine respectively for 30 days. The time course of serum levels of lipase at pre-treatment (0 day), during treatment (15th day) and post-treatment (30th day) have been expressed as mean $\pm$ SD. *, ** $p < 0.05$ when compared to control group.

Effect of tartrazine-exposure on calcium and magnesium level

We also evaluated the effect of tartrazine-exposure on the serum levels of calcium (mg/dL) and magnesium (mg/dL).

The results mentioned in Figure 5 indicate that the serum levels of calcium and magnesium were decreased in tartrazine-exposed groups as compared to that of control group.

Effect of tartrazine-exposure on antioxidant enzyme status

We also investigated the effect of tartrazine on antioxidant enzyme capacity by measuring the serum level of SOD. At

the end of treatment, we found that tartrazine-exposure significantly decreased the antioxidant enzyme capacity as the serum levels of SOD were found to be significantly decreased in tartrazine-exposed rat groups as compared to that of control (Figure 6).

Effect of tartrazine-exposure on glucose tolerance

Before the end of treatment period, we performed OGTT to investigate the effect of tartrazine-exposure on glucose tolerance in experimental animal model. We administered glucose at the rate of 2 g/kg body weight of the rat after an overnight fasting. We collected the blood from the tail of rats before (0 min) and after the administration of glucose (30, 60, 90 and 120 min). From the results mentioned Figure 7, we found that the maximum concentration of blood glucose was achieved at 30 min. Among the three rat groups, the concentration of blood glucose in rat group exposed with high dose of tartrazine (50 mg/kg body weight) was significantly high (230.830 ± 0.940 mg/dL) as compared to the control rat group treated with normal

Figure 5: Effect of tartrazine-exposure on calcium and magnesium level: rats were divided into three groups having equal number of rats ($n=6$) in each group.

Group I was control group that only received normal saline, while group II and III received low dose (10 mg/kg body weight of rat) and high dose (50 mg/kg body weight of rat) of food azo-dye tartrazine respectively for 30 days. The time courses of serum levels of calcium (A) and magnesium (B) at pre-treatment (0 day), during treatment (15th day) and post-treatment (30th day) have been expressed as mean $\pm$ SD. *, ** $p < 0.05$ when compared to control group.

Figure 6: Effect of tartrazine-exposure on antioxidant enzyme capacity of pancreas: rats were divided into three groups having equal number of rats ($n=6$) in each group. Group I was control group that only received normal saline, while group II and III received low dose (10 mg/kg body weight of rat) and high dose (50 mg/kg body weight of rat) of food azo-dye tartrazine respectively for 30 days. The time course of serum levels of superoxide dismutase (SOD) at pre-treatment (0 day), during treatment (15th day) and post-treatment (30th day) have been expressed as mean $\pm$ SD. *, ** $p < 0.05$ when compared to control group.

Figure 7: Effect of tartrazine-exposure on glucose tolerance: before the end of treatment period, oral glucose tolerance test (OGTT) was performed by administering glucose (2 g/kg body weight) to all rat groups (control, low dose and high dose groups) after an overnight fasting.

The time course of serum levels of glucose at 0 min, 30, 60, 90 and 120 min has been expressed as mean $\pm$ SD. *, ** $p < 0.05$ when compared to control group.

saline (195.830 ± 1.240 mg/dL). Moreover, the blood glucose level of rats exposed to high and low dose of tartrazine were also found to be significantly high at all-time points when compared with control rat group (Figure 7). Tartrazine significantly impaired the glucose tolerance due to which the ability of peripheral tissues to utilize the exogenously administered glucose, was impaired. During OGTT, no significant difference ($p < 0.05$) was observed in the blood glucose levels between the rat groups exposed to low dose (10 mg/kg body weight) and high dose (50 mg/kg body weight) of tartrazine at almost all time points after the administration of glucose. After 120 min of glucose administration, the blood glucose level in control group was 99.500 ± 0.760 mg/dL having non-significant difference with its basal blood glucose level (88.660 ± 2.450 mg/dL) whereas, the low and high dose of tartrazine exposed rat groups exhibited the higher levels of blood glucose (117.830 ± 0.940 mg/dL, 131.160 ± 1.440 mg/dL) as compared to their basal blood glucose levels (92.000 ± 0.570 mg/dL, 90.160 ± 0.600 mg/dL), respectively (Figure 7).

Effect of tartrazine-exposure on insulin sensitivity

During OGTT, we further elucidated the impact of tartrazine-exposure on insulin sensitivity and/or resistance using HOMA-IR model which was based on the fasting levels of insulin and glucose. While performing OGTT, we evaluated the levels of fasting insulin and glucose. Upon the evaluation of HOMA-IR, the values for low and high dose of tartrazine-exposed rat groups were found to

be significantly high (1.370 ± 0.120 , 1.450 ± 0.090 , respectively) as compared to that of the control. These high values indicated that tartrazine decreased the insulin sensitivity by increasing insulin resistance (Figure 8).

Effect of tartrazine-exposure on morphology of pancreas

H&E staining of pancreas was done at the end of treatment period. After histopathological examination, we found that control group treated with normal saline exhibited the normal endocrine cells that were properly arranged and lined up surrounded with plenty of capillaries (Figure 9A). Both low dose (10 mg/kg body weight) and high dose (50 mg/kg body weight) of tartrazine affected the pancreas massively. Tartrazine exhibited significant impact on the normal morphology of pancreas (Figure 9B and C, respectively). Moreover, it was observed that the endocrine cells were smaller in size due to their proliferation.

Discussion

In the last many years, considerable interest has been paid to evaluate the toxicological evaluation of food additives and colorants. Food colorant and/or additive is any

Figure 8: Effect of tartrazine-exposure insulin sensitivity: insulin sensitivity/resistance was assessed using HOMA-IR (homeostasis model assessment for insulin resistance).

HOMA-IR was calculated using the values of serum levels of fasting glucose and fasting insulin.

Figure 9: Effect of tartrazine-exposure on pancreas morphology: histopathology of pancreas was performed at the end of treatment period. (A) Control group showing normal ratio and structure of pancreatic beta islet cells (IC) and acinar cells (AC), (B) low dose exposed group showing reduced number of islet cells (IC) and sloughed acinar cells (AC) and (C) high dose exposed group depicting moderate to severe damage of islet cells (IC) and acinar cells (AC) along with vacuolated cells (arrows).

pigment or dye that exhibits its color when added into the soft drink, food and/or any non-food item such as drugs or pharmaceuticals [14, 15]. Among the five classes of food colorants, tartrazine is considered as very toxic to the human beings if consumed in excess amount [16–19]. Toxicological studies conducted on human beings exhibit that ingestion of tartrazine may cause several behavioral changes, sleep disturbance and endocrine disruptions in children [20] whereas, toxicological studies conducted on experimental animals indicate that tartrazine (both higher and lower doses) alters various biochemical markers of vital organs [5, 21]. More recent studies have found that tartrazine has the ability to bind with albumin and cease the normal physiological functions of this protein [22–24].

In present study, we investigated the impact of tartrazine-exposure on pancreatic function of experimental animal model. Pancreatitis is a condition in which the inflammation of pancreas occurs. Pancreas is one of the main organ of the endocrine system that consists of alpha (α) and beta (β) cells. The α -cells are known to secrete the hormone glucagon and β -cells are known to secrete insulin hormone. Insulin mainly responsible to regulate the glucose homeostasis within the body. When the level of glucose in blood exceeds the normal limit, it triggers the β -cells of pancreatic islets to secrete insulin which in return metabolizes the excess glucose accordingly, but if the β -cells of pancreatic islets are not functioning properly, insulin secretion is impaired due to which the levels of glucose in blood becomes high [12, 13, 25]. In our study, we found that tartrazine significantly impaired the normal functioning of pancreas due to which the secretion of insulin in response of glucose was impaired (Figure 2B). Moreover, due to impaired insulin secretion, the levels of glucose in blood were also increased exponentially (Figure 2A). Production of oxidative stress in

pancreatic islets disturbs the balance between antioxidative enzymes and reactive oxygen species (ROS) which leads to further increase in the production of oxidative stress and as results, normal functioning of the pancreatic islets is disrupted. Tartrazine exhibited its toxic effects in dose-dependent manner by potentiating the mechanism of oxidative stress [5, 26] which was further verified by measuring the levels of SOD (Figure 6). The suppressed expression of SOD indicated that tartrazine induced oxidative stress in β -cells of pancreatic islets due to which the impaired insulin secretion occurred (Figure 2B). Moreover, tartrazine also induced the oxidative stress in peripheral tissues due to which impaired glucose tolerance (Figure 7) and insulin resistance (Figure 8) occurred. Further, we verified our results by applying HOMA-IR model to assess the ability of peripheral tissues to utilize insulin for the metabolism of glucose. We found that insulin resistance in tartrazine exposed animal groups was very high (Figure 8) as compared to the control group which indicated that tartrazine induced the insulin resistance (Figure 8) by generating the mechanism of oxidative stress.

Carbohydrates and fats are the main sources of energy in our daily diet. Once ingested, these are metabolized into glucose and fatty acids by enzymes namely amylase and lipase, respectively. Acute pancreatitis is a condition in which the pancreas is inflamed. Pancreas is one of the main endocrine glands that secretes variety of enzymes and hormones in feed-back mechanism. In case of acute pancreatitis, the secretion of these enzymes in response to feed-back mechanism is affected. Likewise, amylase and lipase are metabolizing enzymes that are also secreted from the pancreas. In acute pancreatitis, the secretion of these enzymes is also altered. In our study, we also measured the levels of these two enzymes to assess the impact of tartrazine on pancreatic function. We found that the

levels of amylase (Figure 3) and lipase (Figure 4) were significantly increased in the groups exposed to tartrazine. The increased levels of amylase and lipase in blood indicate that tartrazine exhibited its toxic effects on pancreas by inducing the oxidative stress in pancreas due to which the inflammation in pancreas occurred and altered the normal functioning of pancreas.

Calcium and magnesium are known to play their significant role in the proteins synthesis of pancreatic enzymes and in acute pancreatitis, the levels of calcium and magnesium are decreased due to which hypocalcemia and hypomagnesemia occur [27–29]. In our present study, we found that tartrazine significantly decreased the levels of calcium and magnesium (Figure 5) which indicated that tartrazine impaired the normal functioning of pancreas by inducing the oxidative stress in pancreas. From several studies, it has been reported that deficiency of calcium and magnesium is strongly associated with the development of diabetes mellitus [30–32]. Tartrazine is a food colorant that belongs to group of azo dye. It is metabolized into aromatic amine by intestinal bacteria and the resultant aromatic amines will generate ROS due to their metabolism by interaction of nitrite or nitrate containing foods with these amino groups [5]. ROS such as hydroxyl radical, superoxide anion and hydrogen peroxide could be produced due to the metabolism of nitrosamines and increase oxidative stress. Due to ROS formation, the anti-oxidant defensive mechanism of the cells starts to become activated and compromised to prevent the cell death by toxic radicals. Therefore, SOD levels in the serum are lowered [5]. In our study, we also assessed the anti-oxidant enzyme capacity of pancreas by measuring the serum levels of SOD. Tartrazine decreased the serum levels of SOD (Figure 6) in tartrazine exposed experimental rats. The results of our study were inconsistent with already published report [5]. Once oxidative stress is produced, it exhibits its drastic effects on surrounding cells and tissues. We also investigated the hazardous effects of tartrazine on the normal morphology of pancreas by performing the H&E staining of pancreas. The results exhibited in Figure 9 indicate that tartrazine affected the pancreas massively (Figure 9C) and endocrine cells were smaller in size due to their proliferation. Due to alteration in the morphology of pancreas, it could not secrete sufficient amount of insulin to metabolize the glucose.

Our study may have some limitations as we only focused on the impact of tartrazine on pancreatic functions and glycemic status of experimental animal model. Therefore, we propose that further studies may be required to investigate the impact of tartrazine on endocrine systems and its association with the causative

factors of DM. Another limitation of our study is that we only focused the impact of tartrazine-exposure on the pancreatic function and glycemic status of experimental animal model, therefore, based on significant impact of tartrazine-exposure on pancreatic function, further studies may also be required to compare the association of tartrazine-exposure and development of DM in diabetic patients.

Conclusion

This study provides scientific evidence that exposure of tartrazine even in small doses may exhibit its potential toxic effects on endocrine system due to which the normal functioning of endocrine system alters which may lead to interfere the glucose homeostasis, carbohydrate and fat metabolism, and glucose tolerance. Moreover, tartrazine also alters the normal physiology of pancreas due to which the impaired insulin secretion from β -cells of pancreatic islets occurred. The results of present study also highlight the unexpected toxic effects of tartrazine and urge the attention of regulatory authorities for use of colorant to ensure the public health. Furthermore, there should be proper labeling on tartrazine containing food items so that, the patients that are at high risk of diabetes should not consume these products.

Conflict of interest: Authors have no conflict of interest.

References

1. Himri I, Bellahcen S, Fatima F, Aziz B, Bnouham M, Zoheir J, et al. A 90 day oral toxicity study of tartrazine, a synthetic food dye, in wistar rats. *Int J Pharm Pharm Sci* 2011;3:159–69.
2. Moutinho IL, Bertges LC, Assis RV. Prolonged use of the food dye tartrazine (fd&c yellow no 5) and its effects on the gastric mucosa of wistar rats. *Braz J Biol* 2007;67:141–5.
3. Abdel-Reheim ES, Abdel-Hafeez HA, Mahmoud BM, Abd-Allah EN. Effect of food additives (monosodium glutamate and sodium nitrite) on some biochemical parameters in albino rats. *Int J Bioassays* 2014;3:3260–73.
4. Helal EG, Zaahkhouk SA, Mekawy HA. Effect of some food colorants (synthetic and natural products) on young albino rats. *Egypt J Hosp Med* 2000;1:103–13.
5. Amin KA, Abdel Hameid H, 2nd, 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.
6. Mehedi N, Ainad-Tabet S, Mokrane N, Addou S, Zaoui C, Kheroua O, et al. Reproductive toxicology of tartrazine (fd and c yellow no. 5) in Swiss albino mice. *Am J Pharmacol Toxicol* 2009;4:130–5.

7. Sasaki YF, Kawaguchi S, Kamaya A, Ohshita M, Kabasawa K, Iwama K, et al. The comet assay with 8 mouse organs: results with 39 currently used food additives. *Mutat Res* 2002;519:103–19.
8. Tanaka T. Reproductive and neurobehavioural toxicity study of tartrazine administered to mice in the diet. *Food Chem Toxicol* 2006;44:179–87.
9. Tanaka T, Takahashi O, Oishi S, Ogata A. Effects of tartrazine on exploratory behavior in a three-generation toxicity study in mice. *Reprod Toxicol* 2008;26:156–63.
10. Elhkim MO, Heraud F, Bemrah N, Gauchard F, Lorino T, Lambré C, et al. New considerations regarding the risk assessment on tartrazine an update toxicological assessment, intolerance reactions and maximum theoretical daily intake in France. *Regul Toxicol Pharmacol* 2007;47:308–16.
11. Hallagan JB, Allen DC, Borzelleca JF. The safety and regulatory status of food, drug and cosmetics colour additives exempt from certification. *Food Chem Toxicol* 1995;33:515–28.
12. Akash MS, Rehman K, Sun H, Chen S. Interleukin-1 receptor antagonist improves normoglycemia and insulin sensitivity in diabetic Goto-Kakizaki-rats. *Eur J Pharmacol* 2013;701:87–95.
13. Akash MS, Shen Q, Rehman K, Chen S. Interleukin-1 receptor antagonist: a new therapy for type 2 diabetes mellitus. *J Pharm Sci* 2012;101:1647–58.
14. de Boer L. Biotechnological production of colorants. *Adv Biochem Eng Biotechnol* 2014;143:51–89.
15. Newsome AG, Culver CA, van Breemen RB. Nature's palette: the search for natural blue colorants. *J Agric Food Chem* 2014;62:6498–511.
16. Al-Degs YS. Determination of three dyes in commercial soft drinks using HPLC/GO and liquid chromatography. *Food Chem* 2009;117:485–90.
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. Mpountoukas P, Pantazaki A, Kostareli E, Christodoulou P, Kareli D, Poliliou S, et al. Cytogenetic evaluation and DNA interaction studies of the food colorants amaranth, erythrosine and tartrazine. *Food Chem Toxicol* 2010;48:2934–44.
19. Ngah WS, Ariff NF, Hanafiah MA. Preparation, characterization, and environmental application of crosslinked chitosan-coated bentonite for tartrazine adsorption from aqueous solutions. *Water Air Soil Pollut* 2010;206:225–36.
20. Ward NI. Assessment of chemical factors in relation to child hyperactivity. *J Nutr Environ Med* 1997;7:333–42.
21. Soares BM, Araujo TM, Ramos JA, Pinto LC, Khayat BM, De Oliveira Bahia M, et al. Effects on DNA repair in human lymphocytes exposed to the food dye tartrazine yellow. *Anticancer Res* 2015;35:1465–74.
22. Basu A, Kumar GS. Thermodynamics of the interaction of the food additive tartrazine with serum albumins: a microcalorimetric investigation. *Food Chem* 2015;175:137–42.
23. Masone D, Chanforan C. Study on the interaction of artificial and natural food colorants with human serum albumin: a computational point of view. *Comput Biol Chem* 2015;56:152–8.
24. Pan X, Qin P, Liu R, Wang J. Characterizing the interaction between tartrazine and two serum albumins by a hybrid spectroscopic approach. *J Agric Food Chem* 2011;59:6650–6.
25. Akash MS, Rehman K, Chen S. Role of inflammatory mechanisms in pathogenesis of type 2 diabetes mellitus. *J Cell Biochem* 2013;114:525–31.
26. 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.
27. Logsdon CD, Ji B. The role of protein synthesis and digestive enzymes in acinar cell injury. *Nat Rev Gastroenterol Hepatol* 2013;10:362–70.
28. Perkins PS, Park JH, Pandol SJ. The role of calcium in the regulation of protein synthesis in the exocrine pancreas. *Pancreas* 1997;14:133–41.
29. Weir GC, Lesser PB, Drop LJ, Fischer JE, Warshaw AL. The hypocalcemia of acute pancreatitis. *Ann Intern Med* 1975;83:185–9.
30. Barbagallo M, Dominguez LJ. Magnesium and type 2 diabetes. *World J Diabetes* 2015;6:1152–7.
31. Dasgupta A, Sarma D, Saikia UK. Hypomagnesemia in type 2 diabetes mellitus. *Indian J Endocrinol Metab* 2012;16:1000–3.
32. Pittas AG, Dawson-Hughes B, Li T, Van Dam RM, Willett WC, Manson JE, et al. Vitamin D and calcium intake in relation to type 2 diabetes in women. *Diabetes Care* 2006;29:650–6.