Histological Changes of Selected Westar Rat Tissues Following the Ingestion of Tartrazine With Special Emphasis on the Protective Effect of Royal Jelly and Cod Liver Oil

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Abstract

The present investigation was carried out on thirty six mature Westar rats from laboratory animal unit in the faculty of veterinary medicine, Zagazig University, Egypt. The animals were divided into six groups of six animals for each. Group I was considered as a control group. Group II was treated with tartrazine only in a dose 500 mg/kg body weight by oral gavage for 30 day. Group III was treated with the same dose of tartrazine with 300 mg/kg body weight Royal jelly. Group IV was treated with tartrazine and Code liver oil in a dose 0.4 mg/kg body weight. While the last two groups, V and VI were treated with Code liver oil and Royal jelly separately by the above mentioned doses for 30 days. At the end of experiment, cervical dislocation of rats and for the histological studies, the liver, kidney, testis and brain tissues in addition to stomach were separated and small pieces from them were taken, fixed in neutral buffered formalin 10%, dehydrated, cleared and processed for paraffin blocks and 5 micron sections were obtained, mounted on a glass slides and stained histologically with Hematoxylin and Eosin (H&E) and Blue Masson’s Trichrome. Groups I, V, VI showed normal histological structure of all selected tissues without any abnormalities. Group II showed severe steatosis, diffuse degeneration, necrosis of hepatic tissues, fibrous tissue proliferation with anti-inflammatory cells infiltration in the portal areas and moderate disorganization of hepatic cords. Furthermore, hyperplasia of the interstitial connective tissue and Leydig cells with marked vacuolations were recognized. Moreover, slightly vacuolations in brain tissues were recognized specially within the white matter as well as degenerative changes in stomach mucosa. And also, hyaline degeneration in renal tubules and vacuolations of glomeruli were observed. The present investigation concluded that the serious effect of tartrazine toxicity was more pronounced in liver then kidney, stomach, testis and brain tissues respectively. Moreover, the curative protective effect of both royal jelly and cod liver oil are non-significant against tartrazine toxicity.

Keywords: Tartrazine; Histoarchitecture; Westar Rat; Steatosis; Hyperplasia; Vacuolation; Necrosis; Degeneration

Introduction

Food additives play a vital role in food supply industries, they allow growing population of the world to enjoy a variety of safe, wholesome, and tasty foods. Food cookers are materials of natural origin have been used to provide color in food, drugs and cosmetics for a few thousands of years. By the early 1995, natural and synthetic colors additives have been used extensively to color the foods, drugs and cosmetics. Tartrazine is known as E102 or FD and C, yellow 5 and characterized by its lemon yellow azo dye, and water soluble. Many products contain tartrazine like foods cotton candy, soft drinks, flavored chips, cereals, cakes, soups, sauces, some ice cream, candy, chewing gum, marzipan, jam and jelly, some of non-food products include tartrazine such as soaps, cosmetics, shampoo and other hair products, also some medical preparations contain tartrazine such as vitamins, antacids, medicinal capsules and certain prescription drugs. The acceptable daily intake (ADI) for humans is 7.5 mg/kg body weight/day [2]. Tartrazine has been implicated as the food additive which is most often responsible for allergic reactions in specific human. When the Tartrazine reaches the intestine, it can undergo metabolic reduction by intestinal micro flora [3], and the reductive cleavage products are rapidly absorbed. Following reductive cleavage of the azo linkage by intestinal bacteria, Sulfanilic acid and aminopyrazolone are produced. The pyrazolone fragment is further degraded by intestinal bacteria to yield a second molecule of Sulfanilic acid [4].

Royal jelly (RJ) is a part of diet of honey bee larvae of great antioxidant and immunostimulant properties, and it contains many important compounds with biological activity, such as free amino acids, proteins, sugars, fatty acids (mainly 10-hydroxy-2-decanonic acid; 10-HDA), minerals (mainly iron and calcium) and vitamins (mainly thiamine, niacin, riboflavin) [5,6]. And also, cod liver oil (CLO) has a potent antioxidant effect against much oxidative stress as diabetes and toxicity [7].

Aim of Work

The present study was performed to investigate the sub-chronic toxicity of tartrazine as well as the protective effects of both royal jelly and cod liver oil in some visceral organs in Westar rats.

Materials and Methods

Chemicals

Tartrazine (CAS 1934-21-0, Purity 86.7%), was purchased from

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Received June 06, 2015; Accepted July 10, 2015; Published July 12, 2015.


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Alfa Aesar, Sigma (Germany). Royal jelly produced by honey bees as well as cod liver oil was purchased from pharmacy in Zagazig, Egypt.

**Animals and housing**

Westar rats weighing between 170 and 200 g were housed in a controlled room with suitable conditions as normal temperature and light dark cycle. They were kept in transparent polypropylene cages with free access to water and dry rat pellets feeds.

**Experimental design**

The animals were divided into six groups of six animals each. Group I was considered as a control group, food and water only. Group II was obtained a tartrazine only in a dose 500 mg/kg body weight by oral gavage for 30 day. Group III was obtained the same dose of tartrazine with 300 mg/kg body weight Royal jelly. Group IV was obtained tartrazine and Cod liver oil in a dose 0.4mg/kg body weight while the last two groups; V and VI were obtained Cod liver oil and Royal jelly separately by the above mentioned doses for 30 days.

At the end of experiment, cervical dislocation of rats and for histological studies, the liver, kidney, testis and brain tissues in addition to stomach were separately and small pieces from them were taken, fixed in neutral buffered formalin10%, dehydrated, cleared and paraffin ionized for paraffin blocks and 5 micron sections were obtained, mounted on a glass slides and stained with Hematoxylin and Eosin (H&E) and Blue Masson’s Trichrome according to Bancroft and Gamble [8].

**Results**

The results of histological examination in the control group as well as in groups V and VI showed no any histoarchitectures changes in the liver, kidney, testis, brain and stomach, suggesting that these animals were healthy and the conditions under which the experiment was conducted were proper (Figure 1). The liver parenchyma of the control group was observed very homogenous and consisting of numerous hepatic lobules that were difficult demarcated from each other by a very thin connective tissue septa or trabeculae in between, so the hepatic lobulations were not clear (Figures 1a and 1b). Furthermore, they appeared hexagonal in shape and had central veins in their center. The hepatic cords were dorsally radiating from the central vein towards the periphery, the portal areas (Figures 1b and 1c). The major compartment of each hepatic lobule were the hepatocytes that represent about 80% of its structure and appeared irregular polygonal or polyhedral shaped cells typically with single, central, large vesicular nucleus with fine dispersed chromatin in most cases, however, some of them appeared occasionally bi-nucleated (Figure 1c). Most of hepatocytes nuclei had a central position but other located peripherally specially toward with triangular area, portal area or portal canal. The portal area is a fibro elastic connective tissue that contained branches of hepatic artery, portal vein, lymph vessels, bile duct and nerve. Moreover, the hepatic sinusoids were observed distributing in between the hepatic cords supplying the hepatocytes while bile canaliculi are located between hepatocytes rows.

Tissues sections of liver from group II, III, as well as group IV showed that, tartrazine induced sever degenerative changes which were evident in numerous hepatocytes (Figure 1). The hepatocytes were enlarged, had light and foamy cytoplasm filled with vacuoles of variable size that tended to form cystic degeneration and mostly central nuclei with proliferation of Kupffer cells (Figures 1j-1l and 2f-2i). Hepatocytes necrotic changes were evident; small, pyknotic cellular nuclei with condensed chromatin, lack of nucleolus and acidophilic cytoplasm were observed (Figures 2a, 2b and 2i). Mononuclear cell infiltrates were also noted in-between hepatocytes and the trabecular structure of the lobules was blurred. (Figures 1i-1k). The portal area was thickened and contained dilated vessels (Figures 1e, 1f, 1h, 1i and 2d) and thickened hyperplastic bile duct (Figures 2b and 2c). Most of portal veins in the portal areas were congested and overfilled with erythrocytes (Figures 1e-1h), leukocytes and in particular lymphocytes (Figure 1g). In numerous liver sections of the tartrazine group, the necrotic cells were evident after exposure to tartrazine alone with an increased density of nuclear chromatin and a very compact nuclear structure. And also, thickening in the central vein was noticed (Figure 2a). Moreover, dense collagenous connective tissue fiber proliferation and thickening around the blood vessels and bile ducts of the portal area were observed.

![Figure 1:](image-url)
III and IV the protective effect of cod liver oil and royal jelly were non-significant as in liver. While group V and VI were as normal

Sections of the tests of control group showed normal architecture of the seminiferous tubules and the interstitial tissues including Leydig cells. The seminiferous tubules appeared rounded or oval in shape and were surrounded by a thin basal Lamina (Figures 4a and 4b). The stratified germlinal epithelium that lined the seminiferous tubules, consists of two distinct populations of cells; the proliferating, highly dividing numerous spermatogenic cells and non-dividing fewer Sertoli cells (Figures 4c and 4d).

Complete set of the germinal epithelial cells was present in the tests of control group. In between the tubules, the interstitial tissue had blood vessels with clusters of cells of ovoid or polygonal shape and spherical nuclei representing the leydig cells were observed as well as flat, squamous cells with flat nuclei representing the myoid cells were also noticed (Figure 4d).

All the tissue sections obtained from the tests of experimental Westar rats were not different from the control animals tissue with exception of moderate changes in the interstitial tissue of some examined sections of group II, mainly in the interstitial connective tissue not in the interstitial Leydig cells in the form of vacuolation of variable sizes, moderate edema that we can say that all the sections were essentially normal without any histological changes (Figures 4e-4l).

( Figures 1e, 1f, 1h, 1II, 2b, 2c and 2d). Regarding the group III, and IV, the present investigation showed that the curative effect of both royal jelly and cod liver oil were non-significant against tartrazine toxicity. Furthermore, small foci of homogenous acidophilic hepatocytes were distributed in between wide area of necrotic cells with pale vacuolated cytoplasm and pyknotic nucleus (Figures 2e-2i).

The kidney of the control group showed no any histological changes in both cortex and medulla where, there was no degeneration of renal tubules as well as in the glomerulus (Figures 3a and 3b). While there were changes occurred in the kidney of group II, where the examined sections showed degenerative changes in the renal tubules and glomeruli (Figure 3c). An enlargement of renal glomeruli, with separation and vacuolation of its lining epithelial cells as well as congestion of its blood capillaries, was observed (Figure 3d). Furthermore, hypertrophy and degeneration of the lining epithelial cells of the renal tubules (Figure 3e), with hyperemia of the medullary and cortical parts with mononuclear cell infiltrates as well as hyaline degenerative changes were found in renal tubules in all animals treated with tartrazine (Figure 3f). Mononuclear cell infiltrates were observed in some places of the medullary part of the kidney with fibrous tissue proliferation (Figure 3g). The most advanced change after tartrazine exposure was the blood vessels dilatation that were congested and overfilled with erythrocytes in between the renal tubules (Figures 3h and 3i). Generally, the histological changes in kidney cortex and medulla of group II were more serious but in group

\textbf{Figure 2:} (a): Section of mature Westar rats liver of exposed group to tartrazine (II) showing thickening of the wall of central vein (arrow) and was surrounded with wide area of necrotic hepatocytes with pyknotic nuclei (arrow head). H&E Obj.x20 : 10X. (b-d): Section of mature Westar rats liver of exposed group to tartrazine (II) showing fibrous connective tissue proliferation in the portal areas (arrow). (c): showing thickening of bile duct (arrow), fibrous connective tissue proliferation around bile duct (double arrow head) and leukocytes infiltrations (single arrow head). (d): showing congested blood vessels (arrow), fibrous connective tissue proliferation around blood vessels (double arrow head) and leukocytes infiltrations (single arrow head). (b-d): Blue Masson’s Trichrome Obj.x20 : 10X. (e-f): Section of mature Westar rats liver of exposed group to tartrazine with royal jelly (III) (e): showing small foci of normal homogenous, acidophilic hepatic tissue (arrow head) beside pale, necrotic and degenerated hepatic parenchyma (arrow). (f): higher magnification of Figure e showing the same. (e-f) H&E e) 4X : 10X f) Obj.x20 : 10X. (g-i): Section of mature Westar rats liver of exposed group to tartrazine with cod liver oil (IV) showing the same as in group III. (g): showing foci of normal acidophilic hepatic tissue (arrow) in between necrotic and degenerated pale hepatic parenchyma (arrow head). (h): higher magnification of Figure g showing the same. (i): showing enlarged, vacuolated, pale acidophilic necrotic hepatocytes with pyknotic nucleus (arrow head). (g-i) Blue Masson’s Trichrome. g) 4X : 10X (h,i) 4X0 : 10X.

\textbf{Figure 3:} (a,b): Section of mature Westar rats kidney of control group (I). (a): showing normal, intact renal tubules as well as renal glomeruli. (b): higher magnification of Figure 1 showing the same. (a,b) H&E a) 10X b) 4X0 : 10X. (c): Section of mature Westar rats kidney of exposed group to tartrazine (II) showing an enlargement of renal glomeruli with vacuolations (single arrow head) and separation of its lining and epithelial cells (double arrow head) as well as congestion of its blood capillaries (arrow). H&E 4X0: 10X. (e): Section of mature Westar rats kidney of exposed group to tartrazine (II) showing hypertrophy and degeneration of the lining epithelial cells of the renal tubules. H&E Obj.x20 Oc.x15. (f): Section of mature Westar rats kidney of exposed group to tartrazine (II) showing hyaline degenerative changes in the renal tubules (arrow head) and glomeruli (arrow). Blue Masson’s Trichrome Obj.x20. 10X. (d): Section of mature Westar rats kidney of exposed group to tartrazine (II) showing degenerative changes in the renal tubules (arrow head) and glomeruli (arrow). Blue Masson’s Trichrome Obj.x20. 10X. (g): Section of mature Westar rats kidney of exposed group to tartrazine (II) showing fibrous connective tissue proliferation with leukocytes infiltration in between renal tubules (arrow) and hyaline degenerative changes in the renal tubules (arrow head). Blue Masson’s Trichrome Obj.x20. 10X. (h): Section of mature Westar rats kidney of exposed group to tartrazine (II) showing blood vessels dilatation and were congested and overfilled with erythrocytes in between the renal tubules (arrow). (h,i) Blue Masson’s Trichrome (h,i) Obj.x20. 10X.
The cerebellar sections obtained from control group of Westar rats showed normal architecture in both cortex and medulla where three layers of cerebellar cortex, the molecular, purkinje and granular layers, appeared clear and distinct without any changes in their cells, any inflammatory cells or gliosis (Figures 5a-5c).

The main effect of tartrazine was observed in the white matter of both cerebrum and cerebellum especially medulla where the myelin sheath was more affected. Extensive myelin vacuolation within the cerebellar white matter of rats was observed. These vacuolar clfts are typically empty and represent the classic pattern of “myelin sheath splitting”. Some of these vacuoles contain small amounts of poorly stained material (Figures 5d and 5f). While the gray matter was little affected as a very few vacuolations were observed within the cerebellar gray matter (Figure 5e).

The cerebral sections obtained from control group of Westar rats showed normal architecture in both cortex and medulla without any changes in their cells without any inflammatory cells or gliosis (Figure 6a).

The effect of tartrazine on cerebrum was typically similar to its effect on cerebellum where the main effect was observed in the white matter. Extensive myelin vacuolation within the cerebral white matter of rats was observed (Figures 6c and 6d). While the gray matter was little affected as few vacuolations were observed within the cerebral gray matter (Figure 6b).

However, the oligodendrocyte nuclei were microscopically normal. The oligodendrocytes with prominent perinuclear haloes, producing the typical “fried egg” were present in the cerebral gray matter. In cerebral cortex from a rat treated with tartrazine, oligodendrocytes had small, round, relatively dark nuclei and, were often in close proximity to neurons and have no any histological changes (Figure 6e). Regarding the neurons, some neurons have a large nucleus which appeared from deeply basophilic to pyknotic, and the cytoplasm became acidophilic, this means that the neurons in this way become necrotic (Figure 6e). The pervious results were relatively the same in group III and IV, no significant changes could be observed.

Sections from stomach of the control group as well as groups V and VI showed normal histological patterns of all tunics. The tunica mucosa was normal in its three subtonics, no degenerative changes, no leukocytic infiltration and no dilated blood vessels (Figures 7a-7c). Regarding the histological changes in the gastric mucosa of group II, we found lymphocytes infiltration in the gastric mucosa, in addition to disrupted and degenerated arrangement and architecture of the gastric mucosa, with haemorrhage in the propria and dilated and congested blood vessels (Figures 7d-7f). All tissues sections from group III and IV showed moderate and non-significant protective effects against tartrazine.

**Discussion**

The present study revealed that all tissue sections obtained from the stomach of experimental rats of group V and VI were not different from the control animal tissues. These results are in agreement with data reported by Amin et al. [9] who revealed that no changes were recorded in the stomach tissue of rat fed on tartrazine with Cod liver oil. Our results are also in coincidence with Moutinho et al. [10] who obtained an increase in the number of lymphocytes and eosinophils of the gastric antrum mucosa when 7.5 mg/kg bw of Tartrazine was offered in drinking water *ad libitum* to Westar rats from weaning to the age of twelve months. Our results are going hand to hand with data reported by Sasaki et al. [11] who studied the genotoxicity of 39 chemicals currently...
Red, New Coccine, and Tartrazine induced DNA damage in the colon hours after treatment. He added that, tartrazine induced dose related liver, kidney, urinary bladder, lung, brain, and bone marrow, 3 and 24 mg/kg bw) and performed Comet assays on glandular stomach, colon, orally with each additive at up to half its LD 50 or the limit dose (2000 mg/kg bw).

A marked severe histological changes in the liver; congested blood vessels and areas of hemorrhage were recorded. In the early phase of fatty liver degeneration [14], vacuoles appear in the cytoplasm around the nucleus, because their lipid content is dissolved in the course of embedding. The vacuoles appear empty. As the damage to the cells progresses, the hepatocytes become swollen and a single large vacuole will occupy their entire cytoplasm, pushing aside the nucleus and making the hepatocyte signet-ring shaped are in consistency with the reporting of Rus et al. [13]. The toxic irritant substances brought to the liver by circulatory blood cause degenerative changes in the hepatic tissues, this was in line with Cameron [15]. The present study showed tubular degeneration, dilatation and vacuolization of the glomerular capillaries, intercapillary sclerosis, and atrophy of glomeruli in kidney sections from groups (II, III and IV). These changes are in consistency with the reporting of Rus et al. [13] who described changes in the kidney when guinea pigs received Tartrazine in drinking water in concentrations of 1, 2 and 3% for 3 weeks. The changes in the kidney were somewhat comparable to those of liver, meaning that there is congestion of different intensities and perivascular edema. In addition to these, abnormal glomerular filtration and glomerular or tubular stasis were observed. Renal corpuscles atrophy is present in both groups, for the group treated with Tartrazine having in addition several corpuscles and tubular necrosis. Tartrazine is transformed into aromatic amine sulfanilic acid after being metabolized by the gastrointestinal microflora [10]. The formed aromatic amines can generate reactive oxygen species as part of their metabolism by interaction of these amino groups with nitrite. Reproductive studies show that tartrazine does not have effects on rats or rabbits and no adverse effects on reproductive parameters were recorded in one-generation studies at doses up to 2% in the diet [16,17]. So our results support the results mentioned by the pervious authors.

As reported by Mehedi et al. [18] in mice, a gialle cell hyperplasia with congestion of blood vessels was observed in 2.5% tartrazine groups. A sub cortical edema in the brain was observed in mice treated with 1 and 2.5% tartrazine doses. Gao et al. [19] showed a decline in the activities of catalase, glutathione peroxidase (GSH-Px) and superoxide dismutase (SOD) as well as a rise in the level of malonaldehyde (MDA) activities of catalase, glutathione peroxidase (GSH-Px) and superoxide dismutase (SOD) as well as a rise in the level of malonaldehyde (MDA) concentrations of 1, 2 and 3% for 3 weeks. The changes in the kidney were somewhat comparable to those of liver, meaning that there is congestion of different intensities and perivascular edema. In addition to these, abnormal glomerular filtration and glomerular or tubular stasis were observed. Renal corpuscles atrophy is present in both groups, for the group treated with Tartrazine having in addition several corpuscles and tubular necrosis. Tartrazine is transformed into aromatic amine sulfanilic acid after being metabolized by the gastrointestinal microflora [10]. The formed aromatic amines can generate reactive oxygen species as part of their metabolism by interaction of these amino groups with nitrite. Reproductive studies show that tartrazine does not have effects on rats or rabbits and no adverse effects on reproductive parameters were recorded in one-generation studies at doses up to 2% in the diet [16,17]. So our results support the results mentioned by the pervious authors.
promoting lipid peroxidation products and reactive oxygen species, inhibiting endogenous antioxidant defense enzymes and then cause brain tissue damage. Our results on brain tissues of sections from group II, III, IV were in agreement with Mehedi et al. [18] in mice and confirmed by that of Gao et al. [19]. Our results revealed that, the protective effect of both royal jelly and cod liver oil against oxidative effect of tartrazine was non-significant although amongst them [5,6] and Hünka et al. [7] investigated the antioxidant and immunostimulant effect of both cod liver oil and royal jelly. This might be due to the high dose of tartrazine in our work.

Conclusion

From our results, we can concluded that the serious effect of tartrazine toxicity are more pronounced in liver then kidney, stomach, testis and brain tissues respectively. Moreover, the curative protective effect of both royal jelly and cod liver oil are non-significant against tartrazine toxicity.

References

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