

Toxicological and Histopathological Studies on the Effect of Tartrazine in Male Albino Rats

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Abstract—Tartrazine is an organic azo dyes food additive widely used in foods, drugs, and cosmetics. The present study aimed to investigate the toxic effects of tartrazine on kidneys and liver biomarkers in addition to the investigation of oxidative stress and change of histopathological structure of liver and kidneys in 30 male rats. Tartrazine was orally administrated daily at dose 200 mg/ kg bw (1/ 10 LD₅₀) for sixty days. Serum and tissue samples were collected at the end of the experiment to investigate the underlying mechanism of tartrazine through assessment oxidative stress (Glutathione (GSH), Superoxide dismutase (SOD) and malondialdehyde (MDA) and biochemical markers (alanine aminotransferase (ALT), aspartate aminotransferase (AST), Total protein and Urea). Liver and kidneys tissue were collected and preserved in 10% formalin for histopathological examination. The obtained values were statistically analyzed by one way analysis of variance (ANOVA) followed by multiple comparison test. Biochemical analysis revealed that tartrazine induced significant increase in serum ALT, AST, total protein, urea level compared to control group. Tartrazine showed significant decrease in liver GSH and SOD where their values when compared to control group. Tartrazine induced increase in liver MDA compared to control group. Histopathology of the liver showed diffuse vacuolar degeneration in hepatic parenchyma, the portal area showed sever changes sever in hepatportal blood vessels and in the bile ducts. The kidneys showed degenerated tubules at the cortex together with mononuclear leucocytes inflammatory cells infiltration. There is perivascular edema with inflammatory cell infiltration surrounding the congested and hyalinized vascular wall of blood vessel. The present study indicates that the subchronic effects of tartrazine have a toxic effect on the liver and kidneys together with induction of oxidative stress by formation of free radicals. Therefore, people should avoid the hazards of consuming tartrazine.

Keywords—Albino rats, tartrazine, toxicity, pathology.

I. INTRODUCTION

FOOD additives are substances added to food to preserve flavor or enhance its taste and appearance. Some additives have been used for preserving food by pickling, salting, preserving sweets. More than three thousand additives and preservatives are available in the market used as antioxidants and anti-microbial agents [1]. Synthetic dyes are widely used due to their coloring properties, uniformity, stability and low cost. However, many of them become toxic after prolonged use, causing health problems such as indigestion, anemia and allergic reactions as asthma and urticaria, pathological lesions

in the brain, kidney, spleen and liver, tumors and cancer, paralysis, mental retardation, abnormalities in offspring, growth retardation and eye defects resulting in blindness [2], [22]. Tartrazine is a synthetic lemon yellow azo dye used as a food coloring. It is derived from coal tar [3]. Products containing tartrazine are foods, medications, pet foods, cosmetic products. The World Health Organization (WHO) assigned the acceptable daily intake for tartrazine to be 7.5 mg/kg/day [4]. Tartrazine oral toxicity has been confirmed in rodents and mice. The effects on body weight, blood picture, liver and kidney functions, blood glucose, serum and liver lipids, liver nucleic acids (DNA and RNA), thyroid hormones (T3 and T4), growth hormone and histopathological examinations of liver, kidney and stomach sections were evaluated [5]. Tartrazine was documented to induce vacuolation, swelling, necrosis and pyknosis of the liver cells [6]. The histopathological studies shown by [5] indicated a brown pigment deposition in the portal tracts and Van K  pffer cells of the liver, in addition congested blood vessels and hemorrhage areas. While histopathological findings of the kidneys were lumen compression in tubular cells with an interstitial lymphocytes cell infiltration, edema and glomerular damages [7], [8] showed that there were tubular dilatation, tubular degeneration, dilation of the glomerular capillaries, and inter-capillary sclerosis and atrophy of glomerulus.

A. Aim of Work

The present study aimed to evaluate the subchronic toxicity of tartrazine at a dose of 1/10 LD₅₀ (200mg/kg) on male rats.

II. MATERIALS AND METHODS

A. Chemicals

All chemicals were of analytical grade and were purchased from Sigma-Aldrich Co. (St. Louis, USA), and El-Nasr Co. (Egypt).

B. Animals

Thirty adult male Sprague Dawley rats (120-150 g) were obtained from Vacsera Co, Egypt. The animals were acclimatized for one week before start of the study in polyethylene plastic cages, at ambient temperature of 25 ± 2 °C, and a light- dark cycle of 12 hours. The rats were supplied with pelleted balanced diet, and tap water *ad libitum*. All animals received humane care in compliance with the regulations of the Ethics of Animal Use in Research Committee (EAURC) in Egypt. The rats were divided into two main groups. The first group kept as the control group. The second group was treated orally with tartrazine (200 mg/kg

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bw) dissolved in distilled water for 60 days. Serum and tissue samples from the liver and kidney were fixed in 10% neutral buffered formaline for histopathology. In addition to frozen liver specimens collected for assessing the oxidative stress enzymes.

C. Determination of Hepatic Function Tests

Serum ALT and AST were measured using a test reagent kit according to the method described by [9].

D. Estimation of Total Protein

Total protein was estimated in serum of all rats using a test kits according to [10].

E. Determination of Renal Function Tests

Serum urea was determined colorimetrically by spectrophotometer according to [11].

F. Preparation of Liver Tissue Homogenate for Measurement of GSH, SOD, and MDA

For GSH assay, liver tissue homogenization was done in 5-10 ml cold buffer (50 mM potassium phosphate, PH 7.5 and 1 mM EDTA) per gram hepatic tissue, using tissue homogenizer [12], while for SOD, liver tissue homogenization was done in 5-10 ml cold buffer (100 mM potassium phosphate, PH 7.0 containing 2 mM EDTA) per gram tissue [10]. On the other hand, homogenization of liver tissue was done in 5-10 ml cold buffer (50 mM potassium phosphate, PH 7.5) per gram tissue for MDA [14]. After that all homogenates were centrifuged at 4000 rpm for 15 minutes at 4 °C and the supernatants were aspirated for GSH, SOD and MDA.

G. Determination of Reduced GSH

Reduced GSH was assessed according to the method adapted by [12].

H. Determination of SOD

SOD was assessed according to the method described by [13].

I. Determination of MDA (Lipid Peroxidation)

The lipid peroxidation was estimated by monitoring the thiobarbituric acid reactive substance formation as described by [14].

J. Gross and Histopathological Studies

The rats were observed throughout the experimental period for any clinical signs. The rats were sacrificed and subjected to postmortem examination. Liver and kidneys tissue samples were fixed for 48 h in 10% neutral buffered formalin, dehydrated by passing successfully in ascending concentration of ethyl alcohol, cleared in xylene and embedded in paraffin. Sections (5-6 µm thick) were prepared and stained with H&E stain for microscopic examination [15].

K. Statistical Analysis

The obtained values were presented as means $\pm$ SE of the mean. Comparisons between different groups were carried out by one way ANOVA followed by a multiple comparison test.

The level of significance was set at $P < 0.05$ using SPSS software (version 16.0).

III. RESULTS

A. Biochemical Analysis

Table I revealed that tartrazine induced significant increase in serum ALT compared to control group. The values were 49.5 ± 2.8 , 27.02 ± 0.4 , respectively. The present study revealed that tartrazine induced significant increase in serum AST compared to the control group. The values were 48.65 ± 0.27 , 32.91 ± 0.6 , respectively (Table I). Table II showed that tartrazine induced a significant increase in serum total protein concentration compared to the control group. The values were 8.61 ± 0.4 , 6.55 ± 0.44 , respectively. Rat consumed tartrazine showed a significant increase in serum urea level compared to control group, 87.09 ± 0.29 , 6.55 ± 0.44 , respectively (Table II). Rat consumed tartrazine showed significant decrease in liver GSH and SOD where their values 23.40 ± 0.32 , 22.52 ± 2.01 , respectively when compared to the control group, where 34 ± 1.4 , 34.83 ± 0.16 , respectively (Table III). Tartrazine induced increase in liver MDA which was compared to the control group. The values were 40.26 ± 0.24 , 18.57 ± 2.0 , respectively (Table III).

TABLE I
EFFECTS OF TARTRAZINE ON LIVER FUNCTION OF RATS

Group	Parameter	ALT activity (RFU/ml)	AST activity (IU/l)
Control males		27.02 ± 0.4	32.91 ± 0.6
Treated males with tartrazine (60 days)		49.56 ± 2.8^a	48.65 ± 0.27^a

Values are expressed as mean $\pm$ SE // a: significance difference from control group

TABLE II
EFFECTS OF TARTRAZINE ON SERUM UREA AND TOTAL PROTEIN OF RATS

Group	Parameter	Serum Urea (mg/dl)	Serum Total Protein (g/dl)
Control males		27.79 ± 0.21	6.55 ± 0.44
Treated males with tartrazine (60 days)		87.09 ± 0.29^a	8.61 ± 0.40^a

Values are expressed as mean $\pm$ S// a: significance difference from control group

TABLE III
EFFECTS OF TARTRAZINE ON OXIDATIVE STRESS OF RATS

Group	Parameter	GSH	SOD	MDA
Control males		34 ± 1.4	34.83 ± 0.16	18.57 ± 2.01
Treated males with tartrazine (60 days)		23.40 ± 0.32^a	22.52 ± 2.01^a	40.26 ± 0.24^a

Values are expressed as mean $\pm$ SE // a: significance difference from control group

B. Histopathological Results

1. Gross Findings

There were no macroscopical alterations in both the control and experimental administrated groups of rat.

2. Histopathological Findings

i. Control Group

There were no histopathological changes in the liver and kidneys (Figs.1 and 2).

ii. In Group Treated with Tartrazine

The liver showed diffuse vacuolar degeneration in hepatic parenchyma (Fig. 3). The portal area showed sever congestion in hepatoportal blood vessels associated with hyperplasia in the lining epithelium of bile ducts and mild mononuclear leucocytes inflammatory cells infiltration (Fig. 4), Küpffer cells proliferation was observed in diffuse manner along the course of dilated and congested sinusoid (Fig. 5). Some individual hepatocytes showed necrobiosis (Fig. 6). The kidneys showed focal mononuclear leucocytes inflammatory cells infiltration was detected in between the degenerated tubules at the cortex (Fig. 7). There was perivascular edema with inflammatory cell infiltration surrounding the congested and hyalinized vascular wall of the blood vessel (Fig. 8). The glomeruli were congested and showed swelling in the endothelial cells lining (Fig. 9). Renal blood vessels showed congestion (Fig. 10), in addition to the presence of regenerated renal tubules (Fig. 11).

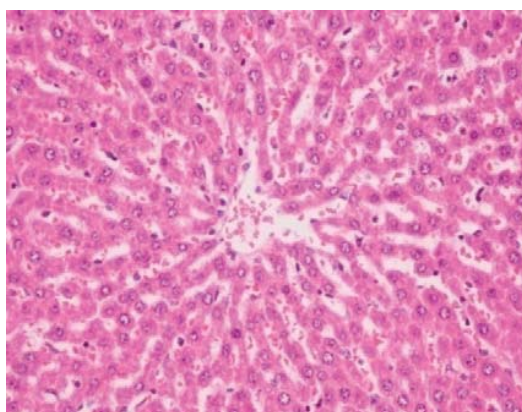


Fig. 1 Photomicrographs of Control rat liver showing normal hepatocytes and blood sinusoids (H&E X 200)

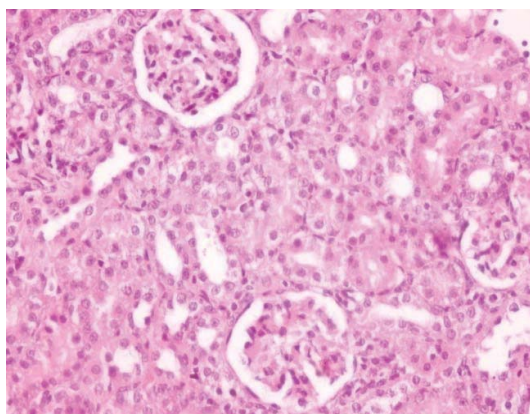


Fig. 2 Photomicrographs of Control rat kidneys showing normal renal glomeruli and renal tubules (H&E X 200)

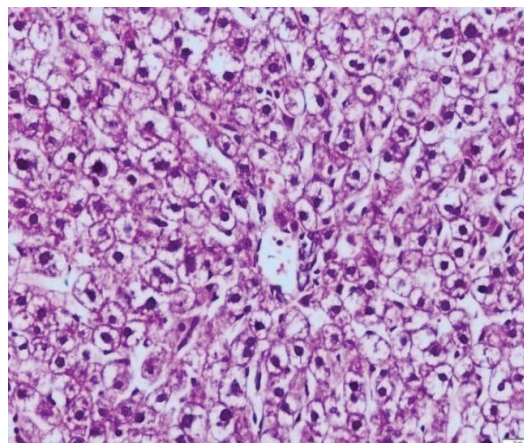


Fig. 3 Photomicrographs of Rat liver treated with tartrazine showing vacuolar degeneration (H&E X 400)



Fig. 4 Photomicrographs of Rat liver treated with tartrazine showing hepatoportal congestion and hyperplasia in the bile duct (H&E X 200)

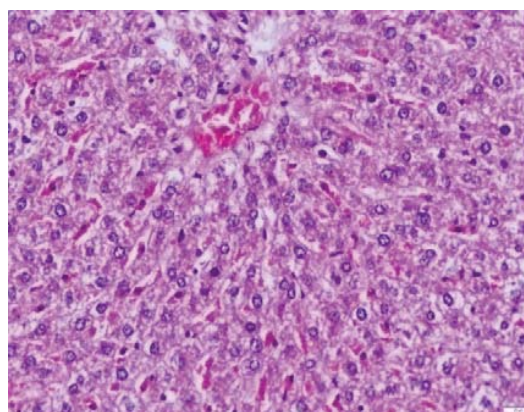


Fig. 5 Photomicrographs of Rat liver treated with tartrazine showing congestion of hepatic sinusoid (H&E X 400)

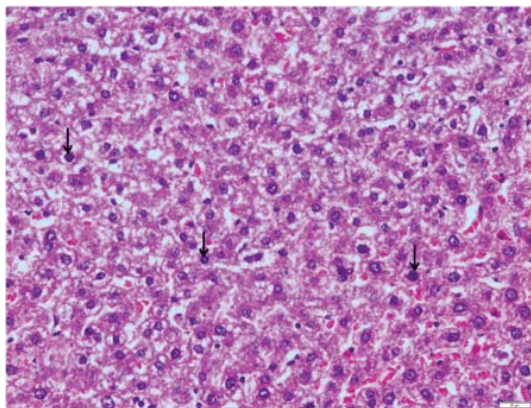


Fig. 6 Photomicrographs of Rat liver treated with tartrazine showing necrobiotic changes (H&E X 400)

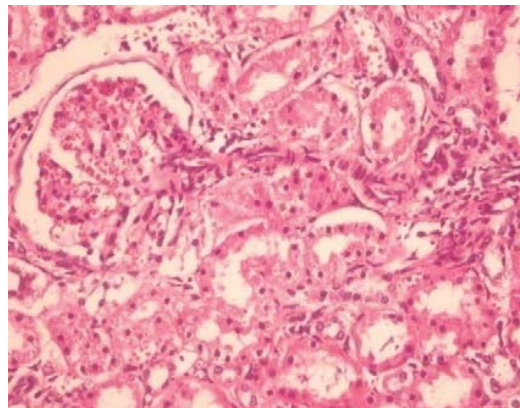


Fig. 9 Photomicrographs of Rat kidneys treated with tartrazine showing congestion and hypertrophy of the renal glomeruli (H&E X 200)

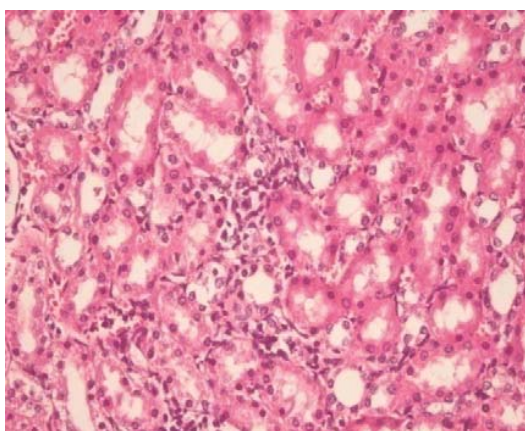


Fig. 7 Photomicrographs of Rat kidneys treated with tartrazine showing focal mononuclear cells infiltration (H&E X 400)

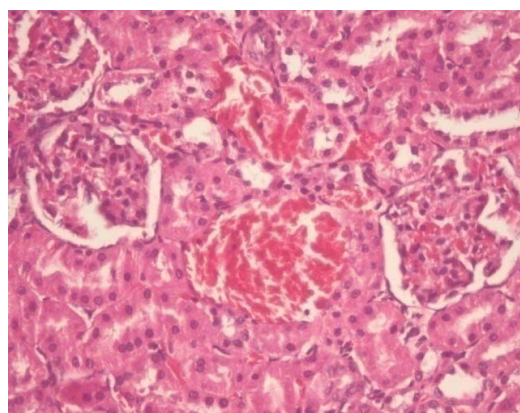


Fig. 10 Photomicrographs of Rat kidneys treated with tartrazine showing congestion of the renal blood vessel with necrobiotic changes (H&E X 400)

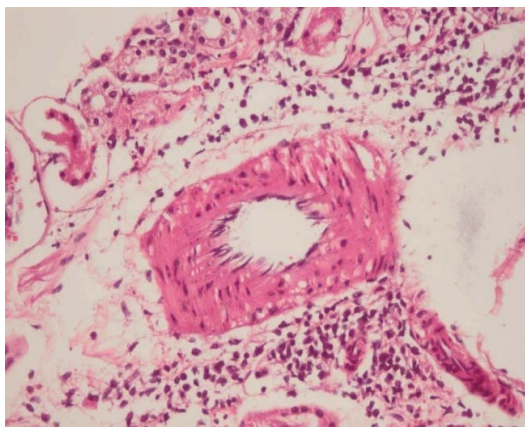


Fig. 8 Photomicrographs of Rat kidneys treated with tartrazine showing hyalinization of the renal blood vessel wall (H&E X 200)

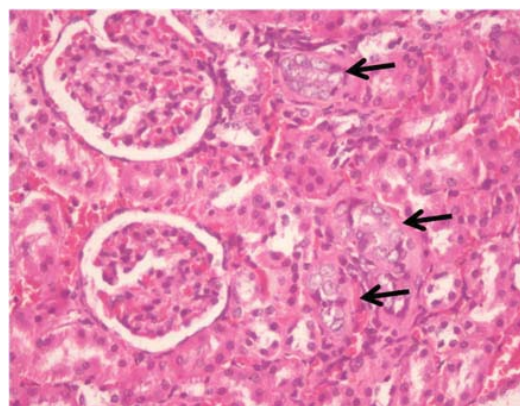


Fig. 11 Photomicrographs of Rat kidneys treated with tartrazine showing regenerated renal tubules (H&E X 400)

IV. DISCUSSION

The biochemical results concerning liver function revealed that daily intake of tartrazine for 60 days exhibits a significant increase in serum AST and ALT of the experimental group

when compared to the control group. These results are in accordance with data reported by [3], who revealed that rats which consumed a high dose of tartrazine exhibited a significant increase in serum ALT and AST when compared to the control rats. At the same time, these results are correlated with those reported by [16] who found an increase in both the serum ALT and AST of rats which were fed on brown food dyes for three months. These results are attributed to hepatocellular damage caused by the toxic effect of these synthetic dyes which were indicated by vacuolation and swelling of the liver, [17]. The elevation of aminotransferases activities in the serum may be due to tissue damage particularly in liver, kidney and heart, which was in harmony with [18]. And increase permeability of cell membrane or increased synthesis or decrease catabolism of transaminases may be involved, as described by [19]. We concluded that the release of abnormally high levels of specific tissue enzymes into the blood stream is dependent on both the degree and the type of damage exerted by the toxic compound administration, which is also attributed by [20]. Our study revealed that rats which consumed tartrazine showed a significant increase of serum total protein level when compared with the control group. These results are in accordance with [17], who found that a significant increase in serum total protein, and [21], who observed a significant increase of serum total protein and globulin in rats whose diets were supplemented with chocolate colors A and B. We explained this increased of serum total protein by the fact that, proteins are the fundamental components of all living cells and include many substances such as enzymes, hormones and antibodies that are necessary for the proper functioning of an organism, and increased release of enzymes by the damaged tissues and the antibodies to counter act the dye [21]. Regarding toxicological results, The group treated with tartrazine revealed a significant decrease in liver reduced GSH and liver SOD content, and also showed a significant increase in MDA content when compared with the control group. These results are correlated with [3] who revealed a significant decrease in liver GSH and liver SOD content and increase in MDA content in rats which were treated with tartrazine in both high and low doses. These results might be attributed to the fact that tartrazine is metabolized by the gastrointestinal tract and transformed into sulfanilic acid [22], which can interact with nitrate or nitrite containing food or in stomach and generate reactive oxygen species (ROS) as a part of their metabolism [23]. Moreover, as a result of ROS formation, SOD and GSH began to be consumed to prevent the cell death by these toxic radicals. On the other hand, the MDA level was increased due to the action of ROS on lipids of cellular membrane [3]. The biochemical results concerning the kidney function tests demonstrated that the daily intake for 60 day of tartrazine exhibited a significant increase in the serum urea level when compared with the control group. This is in agreement with [24] who found a significant increase in serum urea and creatinine in rats which consumed a synthetic or natural food colorants after 30 days of treatment, and [3] observed a significant elevation in the serum level of urea. We attributed these changes to a reduction

in the glomerular filtration rate as a result of an acute renal dysfunction. The serum level of this parameter depends largely on the glomerular filtration [25]. Our attribution was confirmed with [26] who found that synthetic food additives induced an acute kidney dysfunction manifested by a reduction in the renal blood flow and the glomerular filtration rate. Also they reported that food additives resulted in a substantial increase in the plasma levels of urea and creatinine indicating the development of an acute renal dysfunction in rats. Histopathological results of liver of the experimental group treated with tartrazine revealed congestion of the hepatic blood vessel, with vacuolation of hepatocytes which appear swollen with central and sometimes peripheral nuclei. Our results correlated with those of [17] who found that synthetic dyes (low or high doses) (Ponceau, Carmoisine, Erythrosine, Sunset Yellow, tartrazine, Fast Green, Indigotine, Brilliant Blue and Brilliant Black) had a toxic effect which appeared as hepatocellular damage indicated by vacuolation, swelling, necrosis and pyknosis of the liver cells. While [6] reported a mild hydropic degeneration of hepatocytes in rats treated with tartrazine. On the other hand, [5] reported a brown pigment deposition in the portal tracts and Van K  pffer cells of the liver, in addition to congested blood vessels. These hepatic lesions are attributed to auto-oxidation of the hepatic cells due to increased generation of ROS or free radical [27], and these lesions were confirmed with increased levels of AST and ALT suggesting damage of both hepatic cells and mitochondrial membrane in tartrazine administrated rats [3]. Histopathological results of kidneys revealed focal mononuclear cell infiltration with congestion of renal blood vessels, in addition to the presence of hyalinization in the wall of renal blood vessels with the presence of the perivascular edema. These changes are in a consistency with [28] who described changes in the kidneys of guinea pigs administered tartrazine in drinking water in concentrations of 1%, 2% and 3% for three weeks.

Kidney lesions were attributed to the fact that the glomerulus is the primary site of action of several chemicals and it may be injured by any toxic, metabolic and immunologic mechanism. Our explanation of kidneys lesions was confirmed with [8] who reported that the toxic irritant substances brought to the kidneys by circulatory blood cause degenerative changes in the kidney tissues. Our results of kidneys lesions were confirmed with an increased level of blood urea [29].

V. CONCLUSION

The present study indicates that subchronic effects of tartrazine not only cause changes in hepatic and renal parameters, but also they can induce oxidative stress by formation of free radicals. Therefore, it could be concluded that the public should be made aware of the hazards of consuming tartrazine.

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