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The protective effect of gallic acid on tartrazine-induced renotoxicity: Redox potential and morphological study

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ABSTRACT

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Tartrazine (Tz) is a commonly used artificial food dye which adversely impacts the health status. A broad spectrum of literatures indicates that gallic acid (GA) exert antioxidant and cytoprotective effects. Therefore, this study aimed to estimate the effect of four fold permitted dose of Tz as well as protective effects of GA on Tz-induced renotoxicity in adult male Wistar rats. Tz was administered daily at a dose of 30 mg/kg body weight alone orally, in combination with GA (TG) and a group of Tz intoxicated rats was allowed to be recovered, the experiment continued for 30 days. GA administration decreased the levels of creatinine, urea and uric acid. Furthermore, it increased catalase and superoxide dismutase activities, glutathione, nitric oxide and blood glucose while it decreased lipid peroxide compared with Tz treated group. Whereas, Tz recovery group (TR) enhanced some of toxic manifestations on kidney function caused by Tz. Histological investigation revealed that Tz induced many areas of inflammation around congested blood vessels and renal tubules, hemorrhage between renal tubules and necrosis of many tubular cells. Moreover, it increased the amounts of collagen fibers and negative PAS reaction in many tubular cells in Tz group. On the other hand, TG and the recovery groups reduced the deteriorations in histo-architecture and cellular structure caused by Tz exposure. In conclusion, Tz induces many extensive biochemical and histological changes meanwhile GA and recovery period could abolish or improve these changes.

INTRODUCTION

Food colorants are utilized in many commercial purposes for coloring, preserving, improvement the color of foods, and for many commercial purposes [1].

The most common artificial food colors were azo dyes that included the aromatic azo compounds, such as tartrazine (Tz) [2]. Tartrazine is a synthesized azo dye extracted from coal tar [3] and it is powder and orange in color recognized as synthetic lemon yellow and used as food color additives worldwide to color many foods, cosmetics and drugs, [4].

Tartrazine produced in many products as soft drinks, foods cotton candy, and cereals (cornflakes, muesli, etc.). Also, present in flavored chips as doritos, nachos, etc., ice cream, candy, jam and jelly. In addition, could be found in medical preparations like vitamins [5]. The daily intake (ADI) for tartrazine was established by FAO/WHO of 0–7.5 mg/kg b.w/day [6].

In animal intestine, the intestinal microflora can make metabolic reduction to Tz producing two metabolites, aminopyrazolone and sulfanilic acid [7]. These metabolites can form reactive oxygen species (ROS), producing oxidative stress, and causes hepatic and renal architectures disturbance and biochemical profiles disorder [8].

Previous study demonstrated that exposure to food colorants depleted the percentage of glutathione secretion and superoxide dismutase. Meanwhile, creatinine, lipid peroxidase, total cholesterol and plasma urea and were significantly increased. Furthermore, they increased activities of acid phosphatase, alkaline phosphatase, and lactate dehydrogenase [9]. It was found that tartrazine at higher doses, increased blood sugar and insulin levels as compared to that of control [10].

Oxidative stress can disorder the antioxidant-oxidant balance by the expulsion of free radicals/reactive oxygen species that have harmful effects on physical macromolecules (i.e., DNA, lipids, and proteins) and induced cellular damage [11]. Interestingly, oxidative stress induced damage can be lessened using certain substances known as antioxidants [12].

Gallic acid (GA) is a polyphenol compound extracted from plants. The antioxidant potential of GA has been well established against many toxicants induced oxidative stress [13]. GA has received significant interest for its antioxidant, anti-inflammatory, and antimicrobial properties that support the maintenance of intestinal health [14]. It founds richly in fruits like mango, grapes, different berries, and other

fruits and also in tea [15]. GA used extensively in the food and pharmaceutical industries application [16].

There is no available literature highlighted the ameliorating effect of GA against Tz renotoxicity. Therefore, the present study attempted to investigate the protective effects of GA against tartrazine-induced toxicity in kidney tissues and also the withdrawal effects after Tz exposure.

MATERIALS AND METHODS

1. Materials:

1.1 Chemicals

Tartrazine was purchased from Marine Chemicals Company, India and gallic acid was purchased from Sd Fine Chem. Limited (SDFCL) Company, India. Kits used for estimation of creatinine, urea and uric acid were obtained from Company of Spectrum Diagnostics, Egypt. All other chemicals and reagents were of the highest purity commercially available.

1.2 Animals

Male rats of Wistar strain (n = 40) weighing (180 ± 30 g) were used in this study. Animals were bought from the animal house of the Faculty of Pharmacy, Cairo University, Cairo, Egypt. All the experimental procedures were applied according to the animal care guidelines of the National Institutes of Health Assiut University. Under light/dark cycles consisting of 12 h of light and 12 h of dark, animals were housed in stainless steel cages at room temperature. They were fed a standard commercial pellet diet and water throughout the experiment period .

2. Methods

2.1. Experimental design

Rats were divided randomly into four groups (10 animals each) as follows :

G I: Served as a control group and designated as (cont).

G II: Received tartrazine (30 mg/kg body weight) dissolved in distilled water [5] and designated as (Tz).

G III: Received the same dose of Tz followed by subsequent daily oral administration with GA (200 mg/kg body weight) dissolved in carboxy methyl cellulose for 30 days [17] and this dose showed therapeutic effect on bisphenol induced toxicity in rats [18] and designated as (TG).

G IV: Tz recovery group received the same dose of Tz and was allowed to be recovered for another 30 days and designated as (TR).

2.2. Sample collection

The experiment was terminated at the end of 30 days for control and Tz treated groups and left for another 30 days for Tz recovery group. After an overnight fast, blood samples were taken from retro-orbital vein of rats and immediately collected into EDTA tubes then centrifuged at 4000 rpm for 10 min at 4 °C for preparation of plasma samples .

For the subsequent biochemical assays, 0.5 g of each tissue was homogenized in 5 ml (0.1 M) phosphate buffer (pH 7.4) using homogenizer (IKA Yellow line DI 18 Disperser, Germany). The homogenates were centrifuged at 6,000 rpm for 1 hour at 4 °C and the supernatant cytosols were kept frozen at -20 °C until use.

2.3. Measurement of renal damage biomarkers

Creatinine, urea, uric acid and glucose were determined in the plasma by commercial kits expressed as mg/dl (Spectrum Company).

2.4. Antioxidant/oxidant assays

Catalase (CAT) activity in tissue cytosol was determined based on its ability to decompose hydrogen peroxide [19] Superoxide dismutase activity in tissue cytosols was determined according to its ability to inhibit the autoxidation of epinephrine at alkaline medium according to the method of [20]. Additionally, glutathione (GSH) was determined using the method of [21] Nitric oxide (NO) was assayed by Griess reagent according to the method of [22]. Total protein was measured as described by [23]. Lipid peroxide (LPO) was measured according to the method of [24]. Total peroxide was measured according to [25]

2.5. Tissue preparation for general histological and histochemical analysis under light microscope.

At the end of the experiment period, the rats were sacrificed under ether anesthesia, the abdomen was dissected, and kidney specimens were removed and fixed in formol acetic alcohol for 24 h and then processed using a standard histological procedure. Sections 6µm thick were cut and stained with hematoxylin and eosin (H&E) for general histology and Milligan's trichrome stain (MTC) for collagen fibers according to **El-Maddawy and El-Sayed [26]**. To investigate mucopolysaccharides condensation, the sections were stained using the periodic acid-Schiff stain (PAS) according to the procedure of **Drury and Wallington [27]**. These sections were observed by light microscopy, focusing on the kidney tissue, and photographed.

2.6. Statistical analysis

Data were expressed as the mean \pm standard error of the mean (SEM). Statistical differences between groups were identified by one-way analysis of variance (ANOVA) followed by the Duncan post-test. All statistical analyses were carried out using SPSS for Windows software, version 16.0. (SPSS, Inc., Chicago, IL, USA). A probability (p) value of < 0.05 was considered statistically significant.

RESULTS

1. Biochemical study

1.1. Effects of different treatments on renal damage biomarkers:

Data obtained from Fig. 1 (A-D) indicated that the levels of renal damage biomarkers; creatinine, urea and uric acid in addition to glucose increased significantly in the plasma of rats treated with Tz alone compared with control group. In addition, treatment with GA restored all these levels as a control. Nevertheless, urea only elevated in Tz recovery group as control.

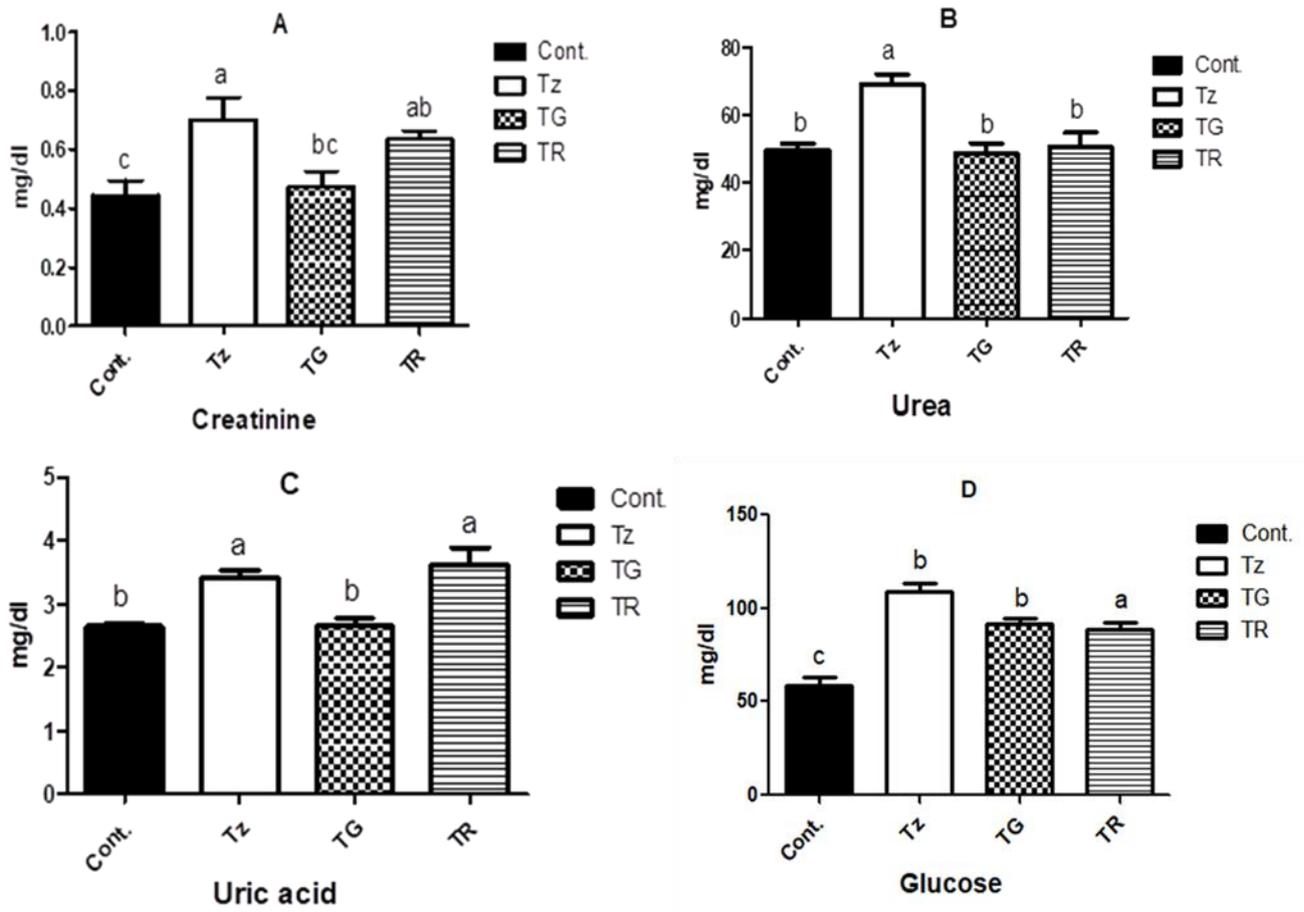


Fig. (1): Effect of different treatments on renal damage biomarkers and glucose of different experimental groups in plasma of different experimental groups. Data are represented as means \pm SE. $n = 10$, variance (ANOVA) followed by the Duncan post-test. a, b, c different letters indicate significant difference at $P < 0.05$. Cont.: Control, Tz: tartrazine; TG: tartrazine+gallic acid; TR: tartrazine recovery group.

1.2. Catalase (CAT)

Fig. 2A showed a significant decrease in CAT activity in kidney tissues of rats intoxicated with Tz compared with the control group. Treatment of Tz intoxicated rats with gallic acid significantly increased CAT activity in comparison with the untreated group. However, there was a non-significant increase in TR group compared with Tz group. Noticeably, GA treatment and TR recover CAT activity nearly as the control.

1.3. Superoxide dismutase activity (SOD)

The kidney SOD activity decreased significantly in Tz group compared with the control group as indicated in **Fig. 2B**. Treatment of Tz intoxicated rats with gallic acid significantly increased the activity of SOD. However, there wasn't any significant change in SOD activity of GA treated group and Tz recovery group compared with control .

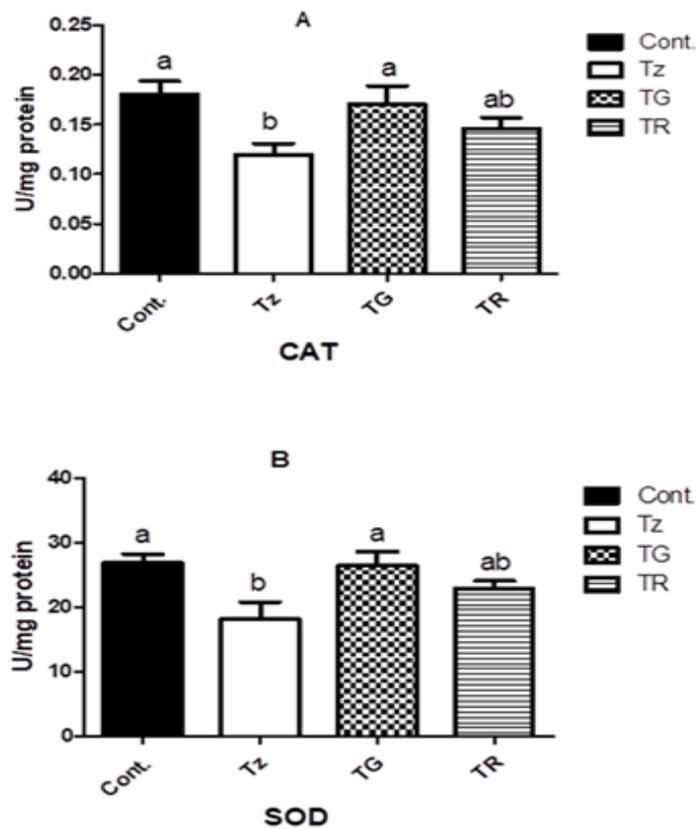


Fig. (2): Effect of different treatments on enzymatic antioxidant biomarkers in kidney tissues of different experimental groups **A**; catalase, **B**; superoxide dismutase. Data are represented as means \pm SE. n = 10, variance (ANOVA) followed by the Duncan post-test. ^{a, b, c} different letters indicate significant difference at P < 0.05. Cont.: Control, Tz: tartrazine; TG: tartrazine + gallic acid; TR: tartrazine recovery group.

1.4. Glutathione (GSH)

As shown in **Fig. 3A**, GSH content in kidney tissue was significantly decreased in Tz group in comparison with the control group. On the other hand, treatment of gallic acid in combination with Tz or Tz recovery group increased

significantly GSH concentration compared to the Tz group and the same as control group .

1.5. Nitric oxide (NO)

Fig. 3B indicated that Tz administration significantly decreased NO content in plasma compared with control group. Treatment of Tz intoxicated rats with gallic acid caused a significant increase in NO content in comparison with Tz group. In addition, NO level significantly restored in GA and TR groups nearly similar to the normal value observed in the control.

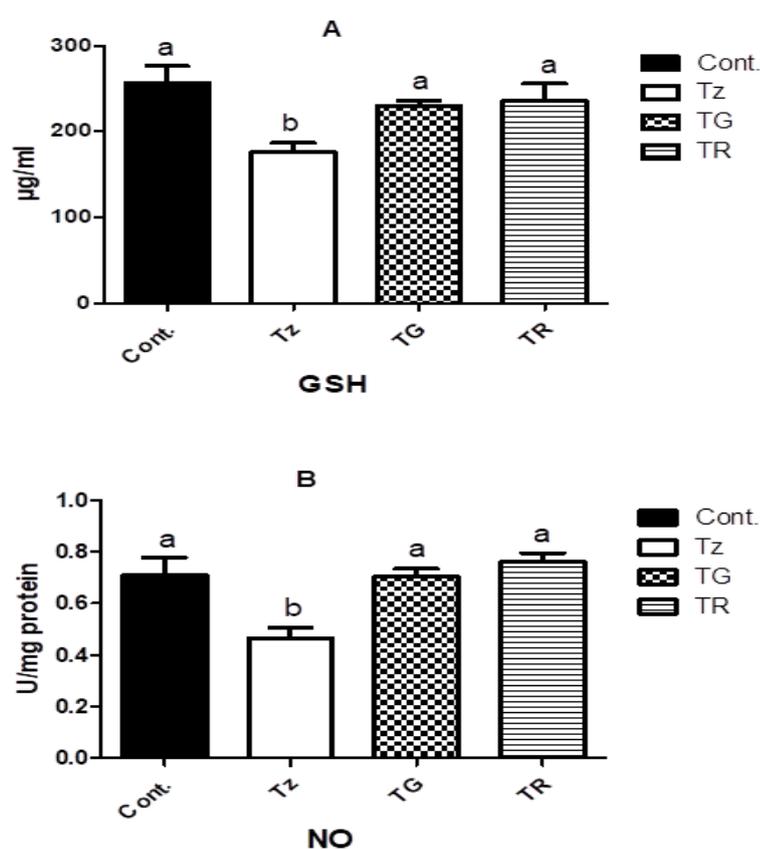


Fig. (3): Effect of different treatments on non-enzymatic antioxidant markers **A**; GSH (Reduced glutathione) in kidney tissues and **B**; NO (Nitric oxide) in plasma of different experimental groups. Data are represented as mean \pm SE, n = 10, Data are represented as means \pm SE. n = 10, variance (ANOVA) followed by the Duncan post-test. ^{a, b, c} different letters indicate significant difference at P < 0.05. Cont.: Control, Tz: tartrazine; TG: tartrazine + gallic acid; TR: tartrazine recovery group.

1.6. Lipid peroxidation (LPO)

As compared to the control group, the concentration of LPO significantly increased in the kidney of rats intoxicated with Tz. However, LPO concentration decreased significantly in both Tz group supplemented with gallic acid and Tz recovery group as control group (Fig. 4A).

1.7. Total peroxide

Fig. 4B demonstrated that Tz raised the level of total peroxide significantly in kidney tissue compared with control. Nevertheless, gallic acid supplementation numerically decreased the total peroxide without reaching the significant level in comparison with Tz group. On the other hand, there wasn't significant change in this level in Tz recovery group compared with Tz treated group.

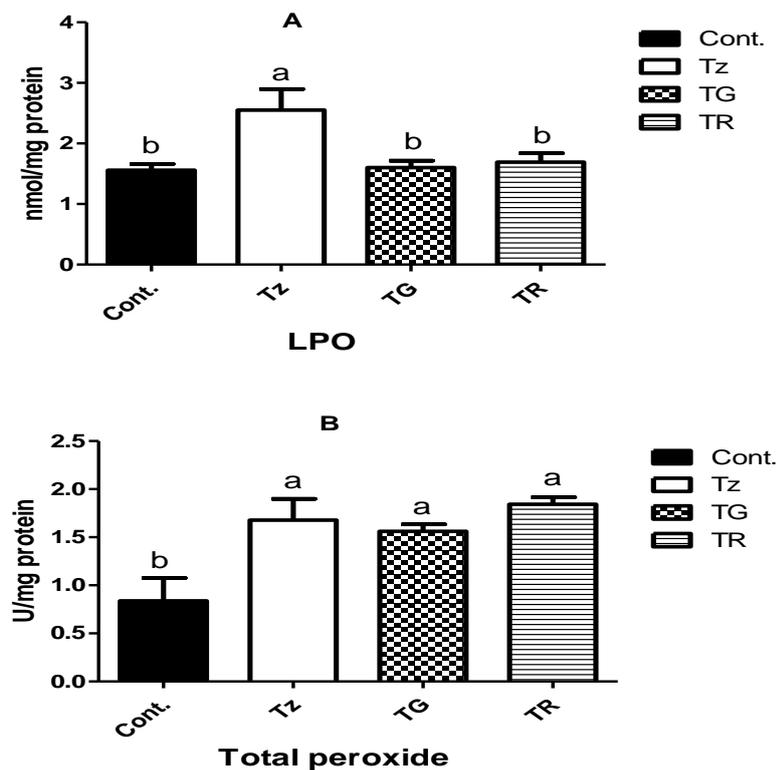


Fig. (4): Effect of different treatments on stress markers in kidney tissues of different experimental groups. **A;** LPO (Lipid peroxidation) and **B;** Total peroxide. Data are represented as Mean \pm SE, n = 10, Data are represented as means \pm SE. n = 10, variance (ANOVA) followed by the Duncan post-test. ^{a, b, c} different letters indicate significant difference at P < 0.05. Cont.: Control, Tz: tartrazine; TG: tartrazine + gallic acid; TR: tartrazine recovery group.

2. Morphology

2.1. H&E stain

In **Fig. 5a** showed the normal kidney cortex structure. Examination of the kidney section after administration of tartrazine to rats showed many marks of histopathological alternation. These alternations represented by lost the architecture of kidney structure, large extensive necrosis and vacuolation of most renal tubular cells (**Fig. 5b**). Many areas of leukocytic infiltrations around congested blood vessels and renal tubules and damage of glomeruli of Malpighian corpuscle were prominent (**Fig. 5c**). In other sections, diffusion of hemorrhage between renal tubules and necrosis of most tubular cells were observed (**Fig. 5d**). Meanwhile many tubular cells showed necrosis leading to widening of their lumens (**Fig. 6a**). In addition, damage of most apical part of renal tubular cells and others appeared completely destructed. Congestion of blood vessels and the glomeruli of renal corpuscle were also observed (**Fig. 6b**). In the TG group, most of the kidney tissue appeared more or less similar to control while very few of renal tubules still affected (**Fig. 6c**). The recovery group (TR) revealed normal structure of renal tubules and Malpighian corpuscles but their glomeruli still congested (**Fig. 6d**).

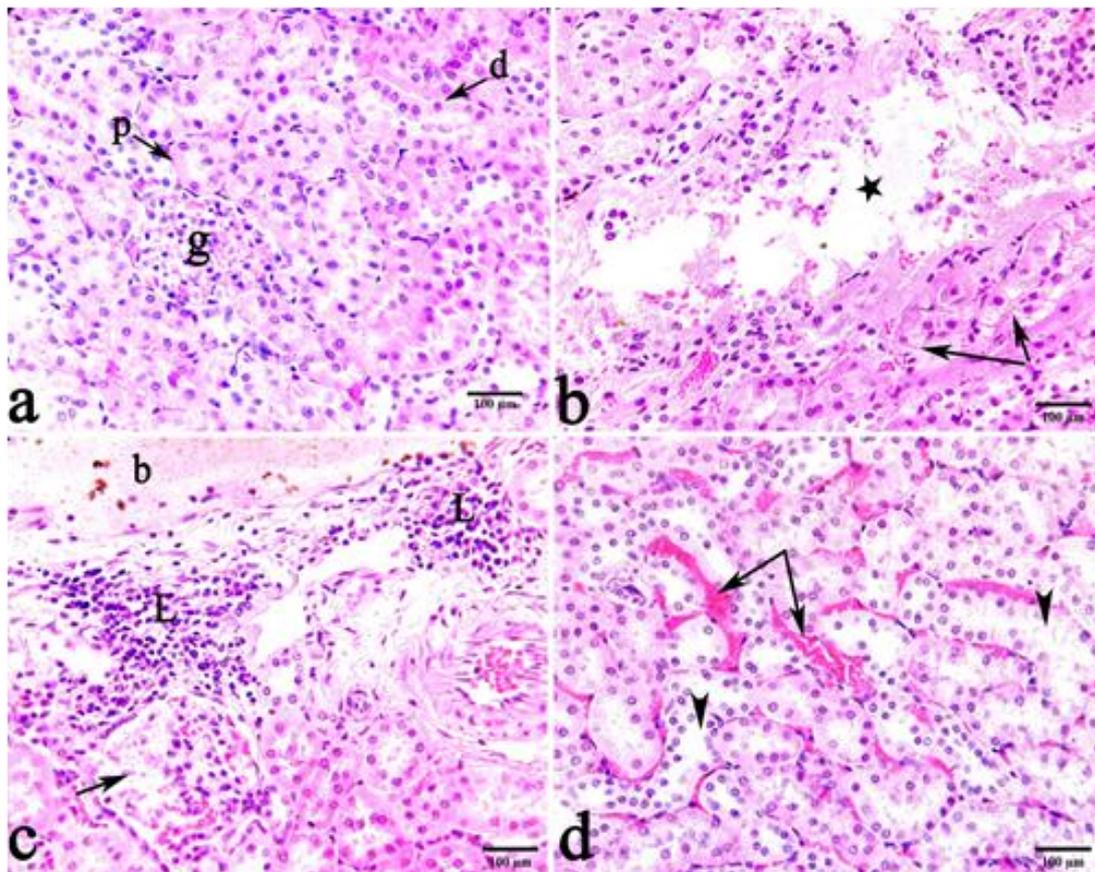


Fig. (5): Photomicrographs of histopathological changes (using hematoxylin and eosin stain) on the kidney cortex of adult male rat showing: **a) Control rats (cont):** normal appearance of Malpighian corpuscle (g), distal (d) and proximal convoluted tubules (p). **b-d) Tartrazine treated group (Tz):** **b)** lost the architecture of kidney structure, large extensive necrosis (star) and vacuolation of most renal tubular cells (arrows). **c)** many areas of leukocytic infiltration (L), damage of the glomeruli of Malpighian corpuscle (arrow) and congested blood vessel (b). **d)** diffusion of hemorrhage between renal tubules (arrows) and necrosis of most tubular cells (arrowheads)

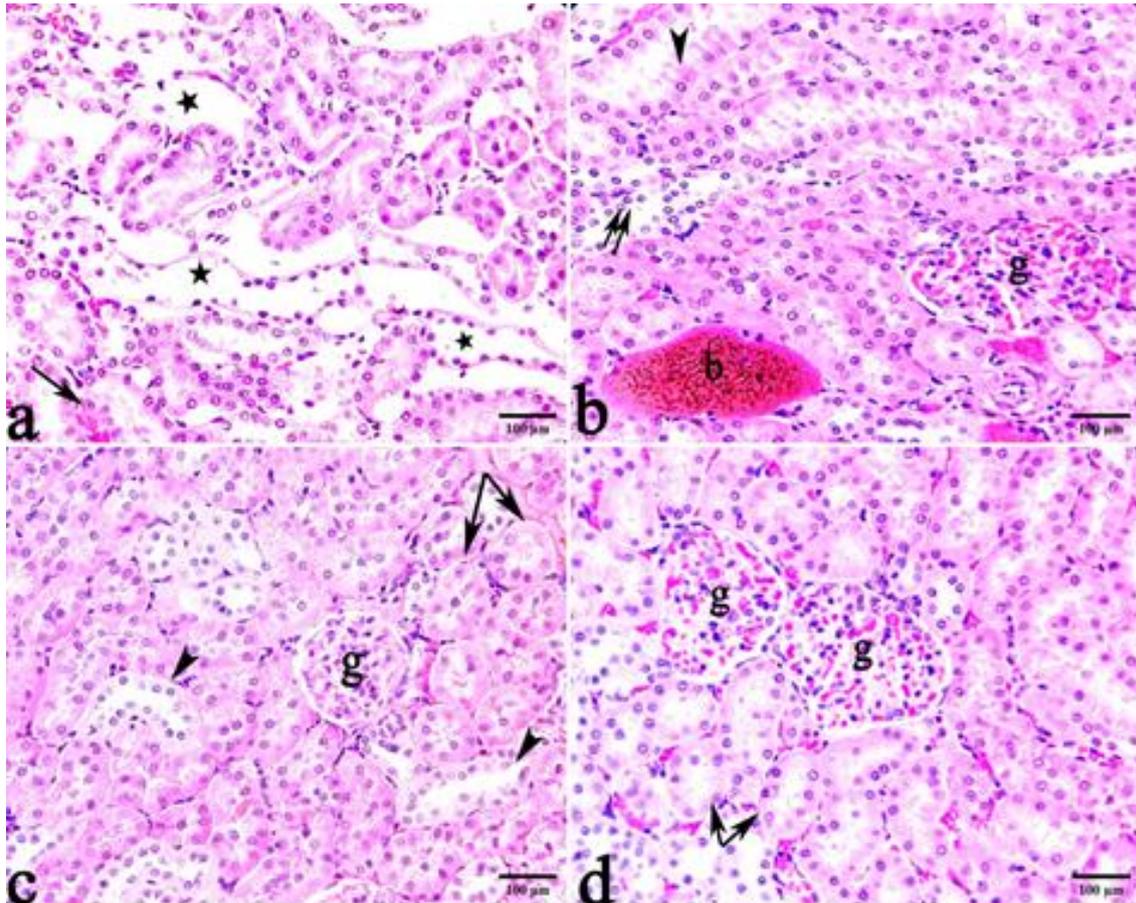


Fig. (6): Photomicrographs of histopathological changes (using hematoxylin and eosin stain) on the kidney cortex of adult male rat showing: **a-b) Tartrazine treated group (Tz):** **a)** necrosis of many tubular cells leading to widening of its lumen (stars) and hemorrhage between tubules (arrow). **b)** congested blood vessels (b) and the glomeruli of renal corpuscle (g). Damage of most apical part of renal tubular cells (arrowhead) and others appeared completely destroyed (two arrows). **c) Tartrazine + gallic acid treated group (TG):** normal structure of Malpighian corpuscle (g) and most of renal tubules (arrows). Few of renal tubules still affected (arrowheads). **d) Recovery group (TR):** normal structure of renal tubules (arrows) and Malpighian corpuscles but their glomeruli still congested (g).

2.2. Milligan's trichrome stain for collagen fibers

Milligan's trichrome stain of kidney sections of the control group, TG and TR groups revealed very few collagen fibers around Malpighian corpuscle and renal tubules (**Figs. 7a, d and e**). There was a large amount of collagenous fibers in the Tz treated group around dilated artery and renal tubules (**Fig. 7b**) and also fibrosis between renal tubules was also prominent (**Fig. 7c**).

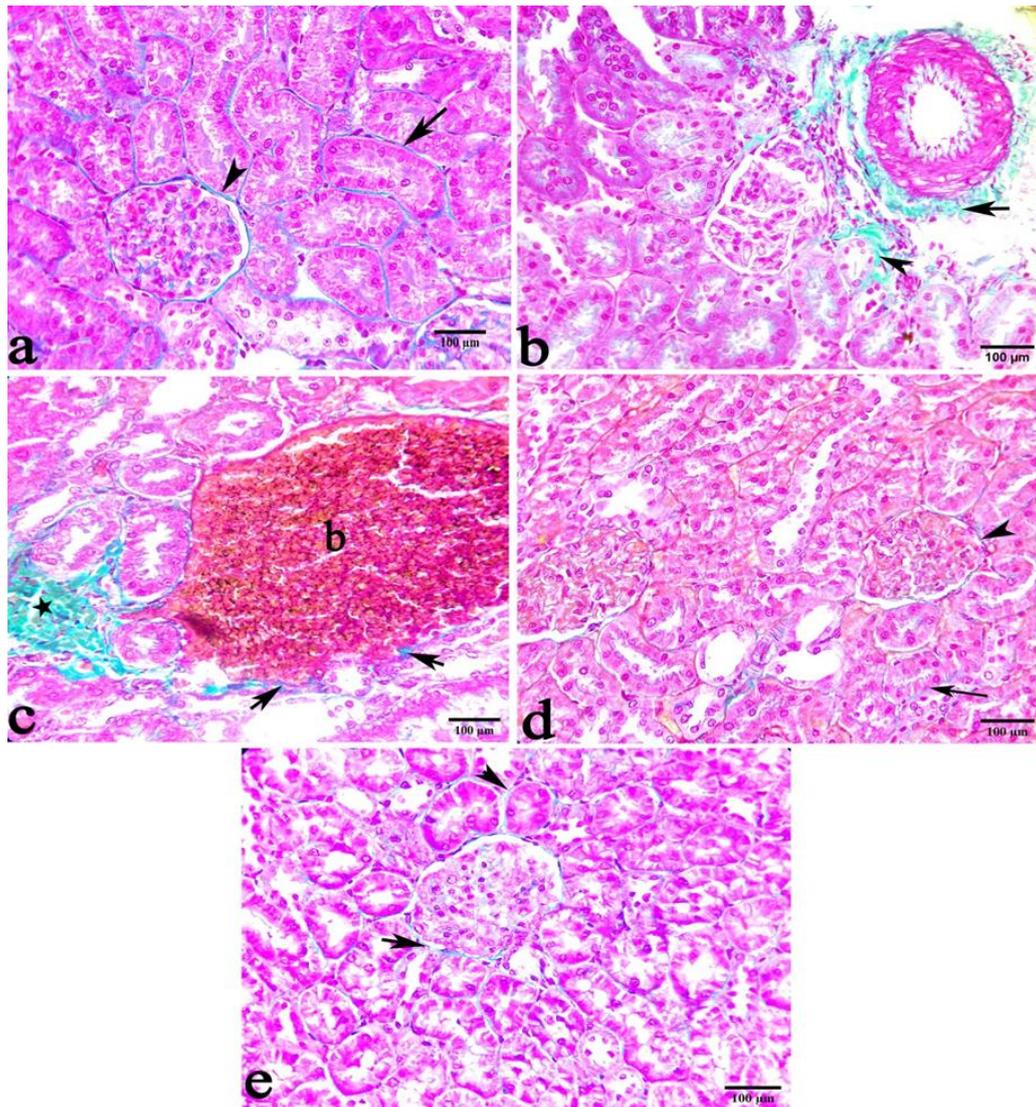


Fig. (7): Photomicrographs of histopathological changes (using Milligan's trichrome stain) on the kidney cortex of adult male rat showing; **a) Control group (cont):** very few amount of collagen fibers around renal capsule (arrowhead) and around renal tubules (arrow). **b and c)Tartrazine treated group (Tz):** **b)** large amount of collagen fibers around dilated artery (arrow) and renal tubules (arrowhead). **c)** fibrosis between renal tubules (star) and few collagen fibers (arrow) around dilated congested blood vessels (b). **d) Tartrazine + gallic acid treated group (TG):** very

few collagen fibers around Malpighian corpuscle (arrowhead) and renal tubules (arrow). **e) Recovery group (TR):** few collagen fibers around Malpighian corpuscle (arrow) and renal tubules (arrowhead).

2.3. Periodic acid-Schiff stain for mucopolysaccharides (PAS reaction)

A close examination of the PAS reaction of the control kidney revealed intense positive PAS reaction in the brush border and basement membrane of renal tubules, in the Bowman's capsule and the glomerular capillaries (**Fig. 8a**). In Tz treated group, many tubules showed negative PAS reaction may be due to their degeneration and destruction while some renal tubules and glomeruli showed positive PAS reaction (**Fig. 8b**). While in TG group and TR group revealed strong positive PAS reaction in brush border of tubular cells, glomeruli and basement membrane of renal tubules. Meanwhile, very few of renal tubules showed negative PAS reaction (**Figs. 8c & d**).

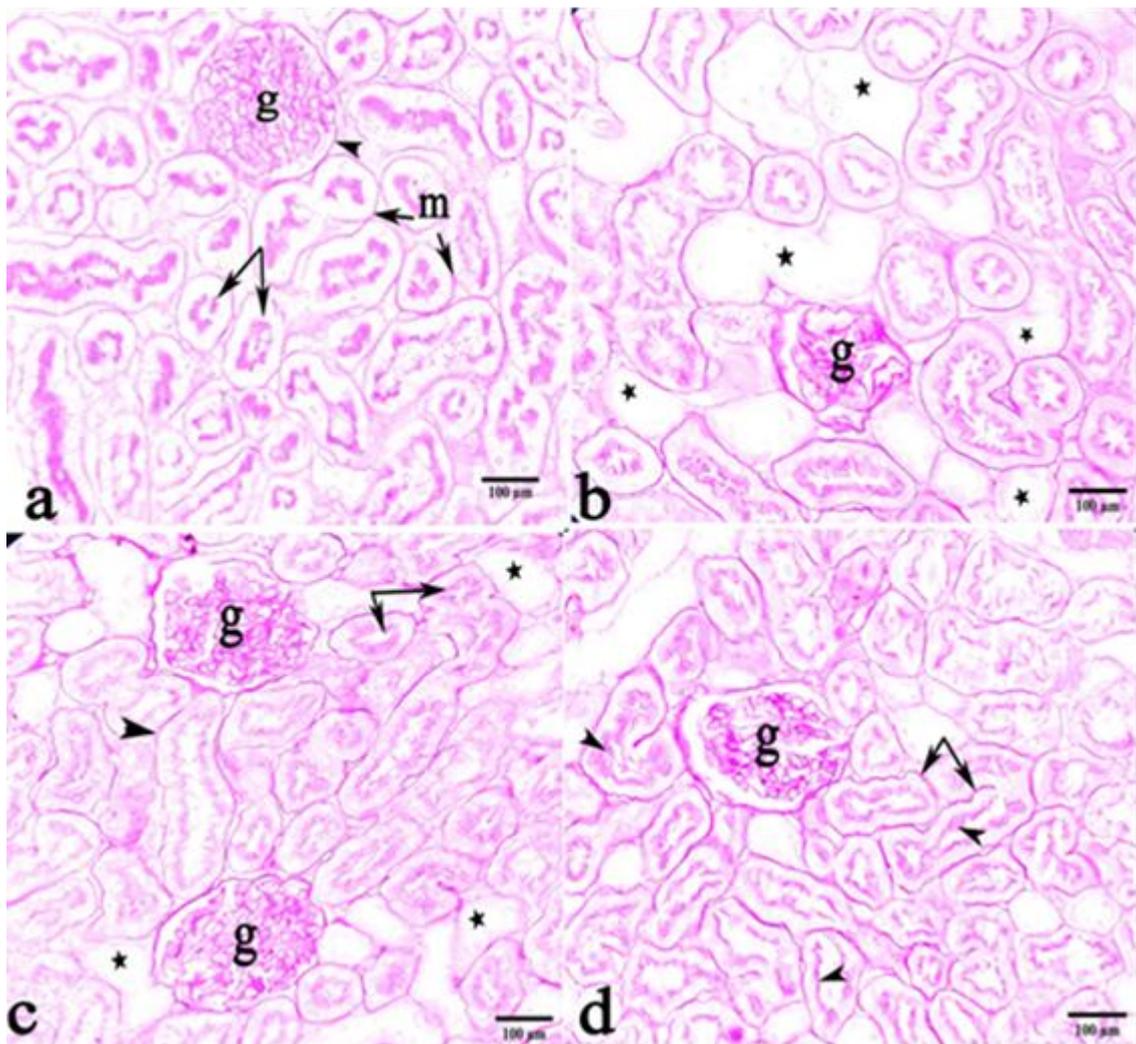


Fig. (8): Photomicrographs of histochemical changes (using Periodic acid-Schiff stain) on the kidney cortex of adult male rat showing; **a) Control group (cont):** intense +ve PAS reaction in the brush border (arrows) and basement membrane (m) of proximal tubules, in the Bowman's capsule (arrowhead) and the glomerular capillaries (g). **b) Tartrazine treated group (Tz):** many tubules showed -ve PAS reaction (stars) while some renal tubules showed +ve PAS reaction and the glomeruli (g). **c) Tartrazine + gallic acid (TG):** strong +ve PAS reaction in brush border of tubular cells (arrows), glomeruli (g) and basement membrane of renal tubules (arrowhead). Few of renal tubules showed -ve PAS reaction (stars). **d) Recovery group (TR):** strong +ve PAS reaction in most basement membrane of renal tubules (arrows), brush border of tubular cells (arrowheads) and Malpighian capsule and their glomeruli (g).

DISCUSSION

Previous studies demonstrated that various food additives as colorants have been reported to causes tissue inflammation and sensitization and are potential risk factors for the progression of numerous chronic diseases [28, 29]

In the current study, tartrazine (Tz) administration significantly increased the levels of plasma creatinine, urea and uric acid compared to control. This result is in accordance with previous studies which reported that Tz induced significant elevation in renal function tests [30, 31]. Also our results indicated that Tz supplementation increased glucose level in comparison with control group. The one of the important factors that causes evaluation of LPO, ROS, and depletion in the antioxidant defense status was hyperglycemia [32]. Also, it was found after exposure to sodium benzoate, MSG and tartrazine a highly significant elevation in fasting blood glucose level in rats [33], [34]. Hyperglycemia deteriorate endothelium-dependent vasodilation in healthy humans, [35] increased levels of blood glucose led to pathophysiological changes like the glycosylation of collagen basement membrane of renal tubules [36].

Statistical analysis of the present work indicated a marked rise in LPO and total peroxide. Furthermore, the antioxidant defense mechanism of the renal tissue including SOD, CAT, GSH and NO was significantly depleted during counteracting ROS production in rats-treated with Tz compared to control. The former results were in line with **Erdemlia et al** [37] who found statistical decreased in GSH, SOD and CAT levels in Tz group compared to all other groups. **Himri et al** [38] noted that Tz induced renal oxidative stress in rats and also, **Medehi et al.** found an elevation in

malondialdehyde (MDA) level and glutathione-S-transferase (GST) activity in Tz group which were in coincide with the present results [39]. The MDA level increased due to the reaction of ROS on lipids of cellular membrane [5].

Interestingly, gallic acid (GA) described as a strong chelating agent, has the ability to protect cells or tissues against oxidative stress, this is related to its anti-inflammatory and anti-oxidant effects (biological activities) [40]. In the current results, GA restored the kidney function parameters and redox potential near to control. In harmony with our findings, GA significantly curtails the elevation of kidney function markers such as urea and creatinine in Bisphenol A-treated rats showing ameliorative effect [41]. Moreover, gallic acid showed significant improvement in SOD activity of kidney and spleen in infected mice [42]. **Hilleman et al.** added that GA have protective effects on pathological changes occurred in liver and kidney exposed to manganese were related to the inhibition of reactive oxygen and nitrogen species, LPO, and inflammation and also an improvement in antioxidant status in the treated rats [43]. Recent study confirmed that due to the protective function of GA, it has the ability to inhibit ROS cellular damage, restore glutathione peroxidase expression, and alleviate the presence of free radicals [44].

Yang et al demonstrated in stressed puppies, GA administration mitigates the inflammatory response and oxidative stress via causing beneficial shifts on metabolites and gut microbiota [45]. Accordingly, the current work suggests that the protective effect of GA against Tz toxicity may be attributed to its antioxidant effect and probably due to its positive role on gastrointestinal microflora which metabolize Tz leading to generating of reactive oxygen species.

Histological results of present study revealed loss of the architecture of kidney structure, large extensive necrosis and vacuolition of most renal tubular cells in Tz group compared to control. These changes are in consistency with the reporting of **Khayyat et al** who demonstrated that kidney tubules lost their solidity, huge vacuoles in many areas, the injury of apical surfaces membranes in tubular cells and degeneration in the basal membrane of tubular cells [46]. Also, Erdemli et al found spacious epithelial cells damage in kidney tubules at different prevalence [37].

Some previous studies illustrated the apoptosis of cells and/or the histopathological lesions caused by tartrazine. **Reyes et al** mention to the reason apoptosis that synthetic color food agents like Tz prevent the respiration process and disrupt the membranes of mitochondrial in liver and kidney rat, which is very

important for integrity of mitochondrial functions and causing cellular apoptosis [47]. Meanwhile, **Chung *et al.*** pointed out that renal and hepatic architectures and histopathological alternations caused by ROS results from tartrazine harmful metabolites [7]. The biological membrane was very sensitive to the effect of ROS which cause oxidative stress and leads to peroxidation of lipid in cell membrane leads to lowering the fluidity and disturb the integrity of membrane and their function which is involved in sever pathologies and tissue damage [48].

Many leukocytic infiltrations around congested blood vessels and renal tubules were observed in the histological results of present study of Tz group. This alternation was in accordance with results recorded by **Erdemli *et al.*** and **Abd-Elhakim *et al.*** [37], [28], who reported many inflammation and blood vessels congestion in interstitial tissues (peritubular) and marked hemorrhage. **Yousef *et al.*** elucidate that the percentage of leucocytic cells increased may give an indication of presence of inflammation in tissue, activation of immune system and defense mechanism processes. [50] explained that congestion and hemorrhage in acute infiltration caused by toxin destroyed the wall of all capillaries and large number of red cells escaped into the tissue spaces and forms a prominent component of the cellular exudates; the inflammatory lesion appears hemorrhagic.

Phytochemicals (as gallic acid) have many scientific reports on their biological and pharmacological activities. These activities resembled in their antioxidant, antimicrobial, anti-inflammatory, anticancer, and neuroprotective effects [51]

Examination of kidneys tissue sections after gallic acid administered to tartrazine (TG group) showed improvement in histological structure of renal tissue compared to tartrazine treated group alone and appears more or less similar to control. These previous observation was in line with **Nair and Nair and Canbek *et al.*** who found that GA can partially balance the substance-induced toxicity due to antioxidant activity in the liver and neural system [52, 53].

In present study, no leukocytic infiltrations were observed in renal tissue sections of TG group. **Owumi *et al.*** illustrated that GA protected against liver and kidney toxicity because it prevent the inflammation by many variable mechanisms relating to decreasing renal and hepatic myeloperoxidase (MPO) activity similarly as in the levels of NO, IL-1 β , and TNF- α by scavenging ROS in the treated rats [54].

Hilleman *et al.* added that the gallic acid have a protective effects on hepatorenal damage due to manganese exposure are related to the restoration of antioxidant status, the inhibition of reactive oxygen and nitrogen species (RONS), LPO, and inflammation in the treated rats [43].

Finally, after withdrawal period, clear improvement was observed in the renal tubules while few cells were still affected. The former observation indicated the ability of kidney tissue to regenerated and restore the toxicity and alternations induced by tartrazine and may be need extra time to reach to the full normal structure appearance.

CONCLUSION

In conclusion, Tz induction of biochemical and histological changes may be associated with the disturbances in glucose metabolism and generating oxidative stress by its metabolites which result from metabolic reduction of Tz by the intestinal microflora. GA and TR could abolish or improve these changes. However, GA gives better improvement than recovery as observed in biochemical results of present study.

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