Protective Effect of Gallic Acid Against Thioacetamide Induced Metabolic Dysfunction of Lipids and Hepatic and Renal Toxicity

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Research Article

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Abstract

Background

Gallic acid (GA) has a potential antioxidant bio-activity and inhibits diet-induced hypertriglyceridemia with reducing the size of adipocytes. GA also was found to increase the uptake of glucose by cell.

Methods

The present research studied the influence of gallic acid (GA) (100mg, 200 mg/Kg orally) on the liver and kidney injuries motivated by thioacetamide (TAA; 100 mg/Kg IP). The treatment of TAA was carried out three times weekly for eight weeks, while gallic acid was given daily.

Results

GA relieved the decrease of hepatic or renal reduced glutathione (GSH) or increase of malondialdehyde (MDA, an indicator for lipid peroxidation) induced by TAA. TAA treatment led to a significant increase in plasma inflammatory markers (TNF-α, CRP), liver enzymes (Gamma-glutamyltransferase (GGT), Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), alkaline phosphatase (ALP) and kidney function parameters (creatinine, urea, uric acid). However, these parameters were reduced after treatment with GA. Moreover, GA reduced the significant decline in plasma protein induced by TAA. In addition, the hepatic fibrosis or histopathological changes of the liver and kidney were lowered by GA.

Conclusion

Our results suggested that GA may attenuate TAA induced liver and kidney toxicity via suppression of oxidative stress.

Introduction

Thioacetamide (TAA) is frequently used in the food, leather processing, laboratory, beverage, textile, and paper and motor fuel industries [1]. TAA is classified as a human carcinogen [2]. TAA is a well-known liver toxin that requires oxidative bioactivation to activate its hepatotoxic impact, which alters amine-lipids and proteins [3]. A single dosage from TAA causes centrilobular necrosis in liver cells and elevations in plasma transaminases and bilirubin leads to acute hepatic damage, whilst chronic exposure causes hepatic cirrhosis and formation of liver tumors and cytomegaly [4, 5]. Treating rats with TAA leads to changes in the structure of renal corpuscles, including Bowman's capsule and glomeruli degeneration [6]. The effects of TAA on the rat’s kidney are through DNA damage, oxidative stress, cytokine release and renal function [7]. TAA damages the proximal renal tubules end by triggering cell death. When TAA is bioactivated, it produces thioacetamide S-oxide, which produces peroxide radicals, which produces reactive oxygen species (ROS). The free radicals are then dispersed throughout the body organs [3]. Humans are exposed to TAA in two ways: by inhaling/ingesting harmful fumes or by absorbing them through the skin [7].

Oxidative stress, which is caused by the overproduction and accumulation of free radicals, is the major cause of various degenerative diseases such as atherosclerosis, cancer, aging, cardiovascular, and inflammatory disorders. Natural products are potent antioxidants attributed to the management of several diseases, such as diabetes [8, 9]. Impaired diabetic wound healing [10, 11], renal toxicity [12, 13]. hepatotoxicity [14] and nephrotoxicity [15], and can increase the activities of antioxidant enzymes by scavenging ROS. Polyphenols, which are naturally occurring antioxidants, have a wide range of biological actions, including antibacterial, anticancer, antiviral, antifungal, anticholesterol [16]. Gallic acid (GA) (3, 4, 5-trihydroxybenzoic acid), a low molecular triphenolic molecule, is highly antioxidant and has been found to be an effective inducer of apoptosis [17]. GA is a phenolic molecule that protects many tissues from oxidative stress-induced damage [18]. GA compounds with strong antioxidant activity and suitable hydrophobicity are more efficient in reducing oxidative stress-induced damage in neurodegenerative disorders [19]. GA protects against hepatotoxicity and nephrotoxicity by lowering the impact of oxidative damage on tissues [20]. GA has antioxidant and hepatoprotective properties in rats with TAA-induced liver fibrosis [21]. GA protects diabetic rats' livers from oxidative stress-induced damage by preventing a reduction in catalase and glutathione S-transferase activity. In hepatic tissue, it reduces the number of nuclei and increases the area of the core, whereas, in renal tissue, it increases the glomerular area [22]. In acute nephrotoxicity caused by cisplatin in rats, the pure GA nanoparticles reduces oxidative stress, inflammation, and mitochondrial dysfunction [23]. GA antioxidant and anti-inflammatory effects are thought to be responsible for this protective impact [24]. In renal tissues, sodium fluoride produced nephrotoxicity and oxidative stress, while daily treatment of GA for 1 week before intoxication prevented toxicity and oxidative stress [25].

The aim of this study is to investigate the influence of Gallic acid against TAA-induced liver and kidney damage, hyperlipidemia and oxidative stress in male rats. It was assessed using liver and kidney function tests, lipid profile, inflammatory markers and oxidative stress parameters.

Materials And Methods

Chemicals

Gallic acid (GA) and thioacetamide (TAA) were purchased from Sigma. Other chemicals used in these experiments were analytical grade from commercial sources.

Animals

Adult male Wistar rats (150-160 g) were chosen from the animal house of NRC, Egypt They were fed standard pellet diet and water ad libitum and keep at adjusted temperature (22 ± 2 °C) with 12 h light-dark cycle. Animal handling was carried followed the recommendations of the National Institutes of Health Guide for Care and Use of Laboratory Animals [26].

Grouping

Twenty-four rats were sorted equally into four groups.

Group 1: It was a control group and administered orally the same volume of a vehicle.

Group II: Rats administered IP of TAA100 mg /kg [27].

Group III: Rats pre-treated with 100mg/kg gallic acid orally [28] followed by treatment with TAA IP after a half hour. Both gallic acid and TAA were dissolved in water.

Group IV: Rats pre-treated with 200 mg/kg GA orally [28] followed by treatment with TAA IP after a half hour.

The rats were treated by TAA three times weekly, while they treated with GA daily for 8 weeks.

Samples collection

The blood (3ml) was gathered after 8 weeks in heparinized tubes from the retro- orbital plexus under local anesthesia by diethyl ether. The animals were killed by cervical dislocation under ether anesthesia. Plasma was isolated by centrifugation at 3000 g for 15 min. Liver and kidney were rapidly removed and washed in ice-cooled saline. A weighed part of each tissue was homogenized with ice-cooled saline (0.9% NaCl) to prepare homogenate. The homogenate was then centrifuged at 3000 rpm for 10 min. at 5°C using a cooling centrifuge (Laborzentrifugen, Sigma, Germany). The supernatant was used for various analysis. The remaining portion of liver or kidney was fixed immediately in 10% neutral buffered formalin.

Biochemical analysis in plasma

All routine bio kits for liver, kidney functions and lipid profile were produced by Egyptian company for biotechnology.

Plasma inflammatory markers

Tumor necrosis factor-alpha (TNF-α) and C-reactive protein (CRP) were assayed by enzyme-immunoassay using kit manufactured in R&D Systems, USA.

Hepatic and renal MDA and GSH

MDA and GSH were determined colorimetrically in liver and kidney homogenates using kits of Biodiagnostic kits, Egypt.

Histological analysis

The testis was fixed in 10% formal saline for one week. The samples were subjected to routine histology and stained with hematoxylin and eosin.

Statistical analysis

Statistical analysis of the data was performed using SPSS program. One-way analysis of variance with Tukey's test. * Indicates the statistical significance compared with the control. # Indicates the significance compared with the TAA group. Data are presented as the mean ± SEM, and significance was set at $P < 0.05$, 0.005, and 0.001.

Results

Liver function tests

The results presented in fig.1, indicated a significant increase ($P \leq 0.05$) in plasma liver enzymes (ST, ALT, GGT, ALP) of rats treated with TAA. The liver enzymes were significantly stored to values close to the normal in rats treated with GA+TAA (low and high doses) as compared to TAA group. The effect of GA on liver enzymes was clearly dose dependent.

Kidney function tests

Statistical analysis showed a significant increase in plasma urea, uric acid and creatinine concentrations in the rats treated with TAA. Interestingly, these parameters tended to be recovered to the normal values after treatment with GA in GA+TAA rat group in a dose dependent manner as compared to TAA group (Fig. 2).

Lipid profile and protein

Fig.3 demonstrated that plasma cholesterol and triglycerides values of TAA-treated rats were significantly ($P \leq 0.05$) higher than those of control rats. Injection of TAA and GA in combination caused a significant decrease in cholesterol or triglycerides when compared with TAA group. Moreover, GA was found to significantly prevent the decrease of plasma protein produced by TAA.

Inflammatory markers

Both plasma CRP and TNF-α have been significantly elevated by TAA in TAA-given rat group as compared with control rats (Fig. 4). GA was found to significantly reduce the effect of TAA on previous two parameters, namely CRP and TNF-α.

Oxidative stress parameters

The data presented in fig.5 showed a significant increase in content of hepatic or renal MDA of TAA treated group, while it lowered significantly in the rats given GA +TAA as compared to TAA group. On the other hand, GA prevented the decrease of hepatic or renal GSH level (Fig.5) observed in TAA group

Histopathological results

Liver

Stained sections of the control group revealed normal histological hepatic architecture. Liver parenchyma is composed of small lobules of a roughly hexagonal shape. Inside the lobules, the hepatocytes are arranged as cords of cells radiating from the central veins to form hepatocytes and separated by blood sinusoids. Blood sinusoids lined by kupffer cells and endothelial cells (Fig. 6A). Histopathological alterations of liver tissues treated with TAA only showed loss of normal architecture, with deleterious effect of hepatocytes and formation cirrhotic nodules. Cirrhosis is defined as the histological development of regenerative or abnormal nodules surrounded by fibrotic bands and response to chronic liver injury. Dense fibrous bands surrounded the central vein and around portal vein exhibiting numerous small proliferated bile duct. Hyperchromatic nuclei with dense chromatin clumping and some apoptotic cells (acidophilia) note the condensation and dark eosinophilic of the cytoplasm, absence of nucleus and few inflammatory infiltrates in fibrous tissue were clearly observed. Hepatocellular, karyomegaly and multiple nuclei are present. The blood vessel was obviously dilated. Ballooning degeneration these form of cell death, accumulation of fatty changes (steatosis) and blood sinusoids appeared narrow or obliterated (Fig. 6. B&C and D). The histological sections in rats treated with TAA and subjected to low dose of GA showed some improvement in changes induced by TAA in the form of no thick bands fibrous tissue, no apoptotic cells, no hyperchromatic cells. The liver tissues still suffer from pathological changes in the form of hepatocytes regenerative nodules separated by thin fibrous bands, the fibrous bands surrounded the nodules and around the dilated, congested blood vessels and cellular infiltration around. Dilated, congested portal vein and bile duct hyperplasia associated with deposition of thin collagen fibers mixed with few inflammatory cells around portal tract. Red blood vessel in dilated blood sinusoidal space could be observed (Fig. 7A &B). In the case of rats treated with TAA and subjected to high dose of GA revealed more decrease in degenerative changes and hepatic lesions (such as cirrhosis, fibrosis and degeneration of hepatocyte or cell death), the liver lobules regained their regular architecture, although pyknotic cells, dilated, congested portal vein, thickened portal vein vascular wall and bile duct hyperplasia. Congested central vein and red blood cells in sinusoidal space still present (Fig. 7C&D).

kidney

Kidney sections showed normal histological structure in the form of normal tubules. It consists of tuft of capillaries located within Bowman's capsule, glomerular capillaries, responsible for plasma filtration. Mesangium is a supporting tissue consisting of mesangial cells and matrix (Fig. 8 A). While kidney of group treated with TAA only revealed extensive tubular dilatation with epithelial flattening, cloudy swelling of some renal tubules with renal casts in lumen and desquamation of some tubular epithelial cells. Sings of degeneration of lining epithelial cells in the form of pyknosis, necrosis and cytoplasmic vacuolation is characterized by the presence of vacuoles in renal tubular epithelium. Glomerular degeneration with intraglomerular hemorrhage, increase wide space of Bowmans capsule and hemorrhage were detected in the interstitial tissue (Fig. 8 B&C&D). light microscopic examination, the liver of rats treated TAA and subjected to gallic acid at dose level of (low dose), revealed no improvement in pathological changes, the liver tissues still suffer from the deleterious effect induced by TAA in the form of hyaline casts are present in the modularly of some tubules, desquamation in tubular epithelial cells, few inflammatory infiltrate and hemorrhage in interstitial tissue. Glomerular lobulation, interglomerular hemorrhage and hydropic

degeneration of some tubular epithelial cells (Fig. 9 A&B). Histological examination in kidney tissues of rats treated with TAA and gallic acid at dose level of (high dose) exhibited improved in pathological changes in comparing with the group treated with TAA only in the form of no hyaline cast in the lumen of tubules, no tubular dilatation although glomerular shrinkage, cell debris in lumen of some tubules and cloudy swelling of the tubules still present, some tubules appeared normal (Fig. 9 C&D).

Discussion

A complex antioxidant system has been developed in mammals to relieve oxidative stress. However, excessive reactive species lead to oxidative damage. Oxidative stress, a result of an overproduction and accumulation of free radicals, is the leading cause of several degenerative diseases. Oxidative stress has been considered as a conjoint pathological mechanism, and it contributes to initiation and progression of liver and kidney injuries [29, 30]. Thioacetamide is familiar hepatotoxic producing free radicals and oxidative stress through its S-oxide metabolite (thioacetamide-S dioxide), an unstable and reactive metabolite [31]. Present study clarified the decrease of oxidative stress results from TAA by gallic acid as evidenced by a decrease of hepatic or renal MDA content and enhancement of GSH levels. MDA is an indicator for lipid peroxidation that represent another route for the propagation of reactive species and their deleterious effects. It is an uncontrolled reaction that elevates the level of lipid hydroperoxides in cellular and subcellular membranes. These highly reactive cytotoxic species can then disrupt a number of cellular components [32]. GA protect peroxidation of lipids, using free radical scavenging activity and lipid peroxidation inhibitory activity. This characteristic has been supported by a study in which GA scavenges the DPPH radical by a hydrogen donating mechanism and are more effective than Vitamin E itself [33].

Even though they exhibit strong inhibition against lipid peroxidation, they do not scavenge ROS. Similar anti-lipid-peroxidative effect with no antiradical potential has been reported for a flavanol compound as well [33]. Reduced glutathione (GSH) has a multiple role as an antioxidant agent. It functions as a scavenger of ROS, including hydroxyl radicals and singlet oxygen [34]. However, restoration of GSH levels in the pretreated rats suggests the antioxidant and hepatorenal protective properties of the gallic acid.

TAA is active hepatorenal toxic agent which manifested by histopathological changes and biochemical analysis of the present work. Our results demonstrated a significant increase in plasma liver enzymes (GGT, ALT, AST, ALP) due to TAA treatment. The increase in plasma enzymatic activities is linked to liver damage and change of membrane characteristics result in the leakage of enzymes and therefore its level elevated in the plasma. The increase in liver enzymes may indicate hepatocytes necrosis [35]. Also, plasma creatinine, urea, and uric acid elevated significantly in TAA group which may indicate renal insufficiency and tubular injury [36]. The pathological changes observed in liver and kidney of TAA group can be attributed to increase of tissue MDA, an indicator of lipid peroxidation which alters the physiological functions of cell membranes and plays an important role in cellular membrane damage through free radical chain reaction mechanism [37]. It was reported that gallic acid treatment alleviate the hepatic and renal toxicities induced by tramadol via enhancement of reduced glutathione and antioxidant enzymes [38]. The decrease of pathological changes here in GA + TAA groups is likely due to the decrease

of lipid peroxidation, and preserve the tissue GSH. Also, GA administration prior to cisplatin administration reduced histological renal damage and suppressed the generation of ROS, lipid peroxidation, and oxidative stress in kidney tissues [39]. Here, GA reduce hepatic fibrosis due to TAA treatment as indicated from histological study. It was found that GA counteracted the progression of hepatic fibrosis through reduction of hepatic stellate cells proliferation/activation [40]. Moreover, GA lowered plasma triglycerides or cholesterol and hepatic steatosis as observed in liver sections. The patients with fatty liver have high plasma triglycerides and low levels of high-density lipoprotein [41]. It is reported that GA alleviate lipid accumulation via the upregulation of β-oxidation and ketogenesis [42]. The present investigation demonstrated a significant decrease in plasma protein level in the group of rats treated with TAA only. It was suggested that the increase in lipid peroxidation affects protein synthesis [43]. GA may reduce protein decline in TAA treated rats by decrease hepatic MDA or liver pathological changes since reduction in protein synthesis is linked with increase cellular damage [44].

Moreover, GA here mitigate the increase of IL-6 and TNF-α stimulated by TAA. TNF is involved in the pathogenesis of various inflammatory liver diseases [45]. Interlukin-6 was strongly associated with fatty liver disease and it was highly specific in a diagnose of non-alcoholic steatohepatitis [46]. We can suggest that GA may protect hepatic or renal damage induced by TAA via reducing levels of IL-6 and lipid peroxidation.

Conclusion

In conclusion, it is probable that GA can inhibit hepatic or renal toxicity induced by TAA through lowering oxidative stress and inflammatory markers and improved the antioxidant status of the hepatic and renal tissues.

Declarations

Ethics statement

This experiment was carried out in according to recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health (NIH publication No. 85–23, revised 1996) and under regulations of Animal Care and Use of National Research Centre in Egypt with ethical approval No. 18576.

Consent for publication

All authors are consent for publication

Availability of data and materials

Not applicable for that section

Competing interests

The authors confirm that this article does not include any Competing of interest.

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Authors' contributions

SAEB conceived the research idea and performed the experiments. HE drafted the manuscript and analyzed the results. FAM described the histology results. JAT prepared the figures and performed the statistical analysis of the generated experimental data. IMA arranged the funds. HE and JAT finalized the manuscript in the communicable format after checking the language and for plagiarism. All of the authors have approved the final version of the manuscript.

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Figures

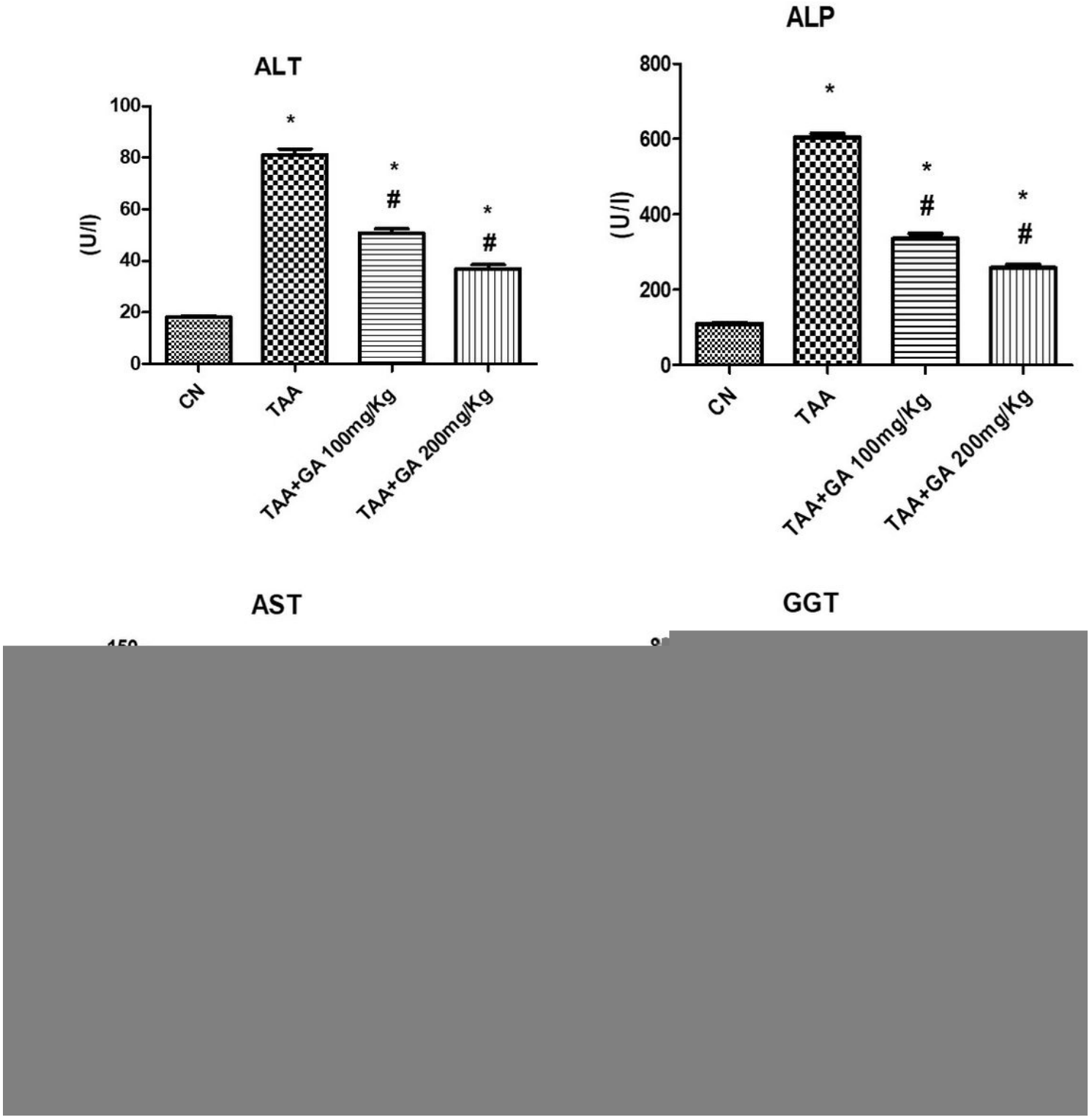

Figure 1

The levels of liver functions enzymes (ALT, ALP, AST, GGT) in plasma from different rat groups. * Indicates the statistical significance compared with the control. # Indicates the significance compared with the TAA group. Values in the histogram are the mean ± SEM, and significance was set at $P < 0.05$, 0.005, and 0.001.

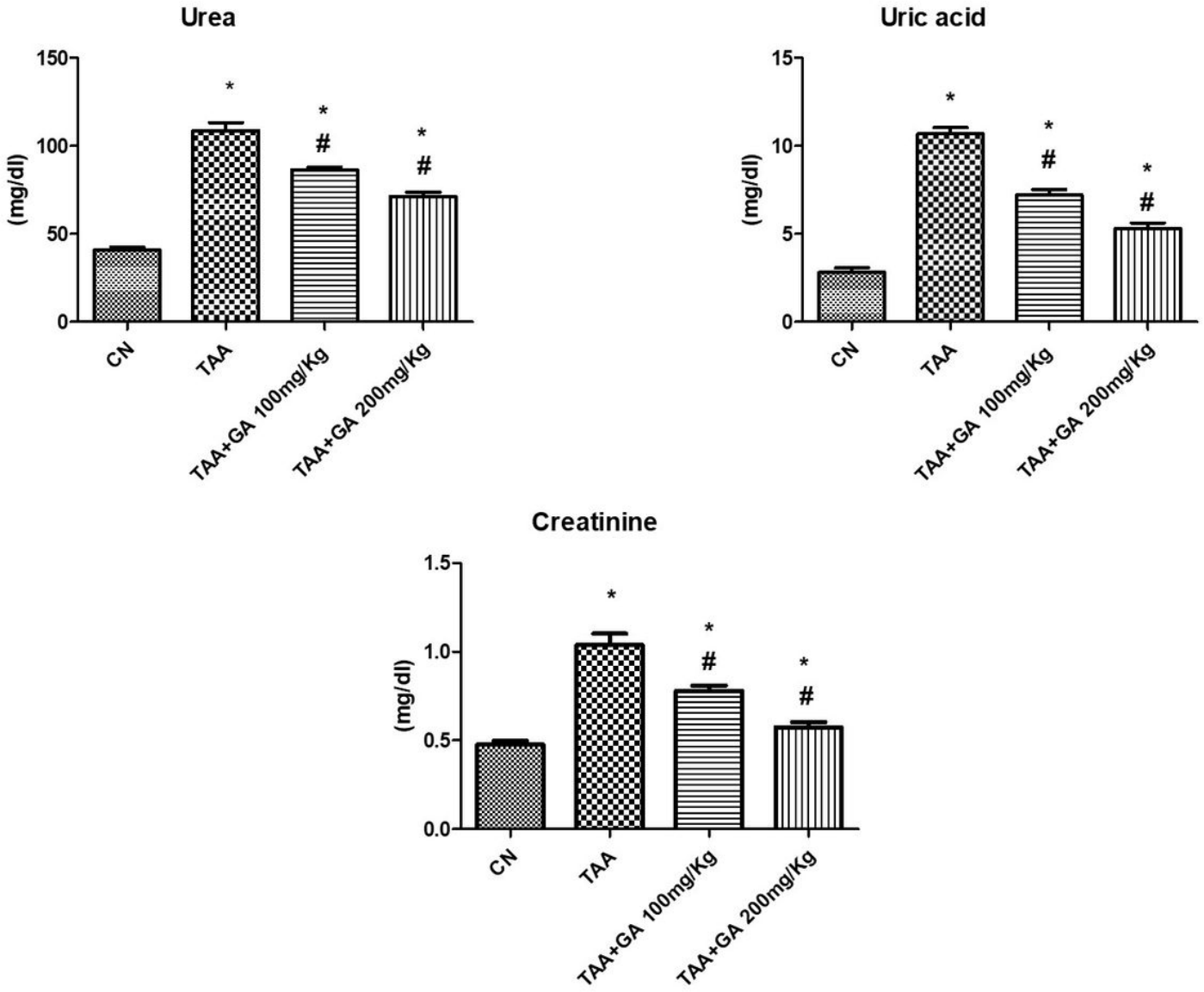

Figure 2

The levels of kidney functions parameters (urea, uric acid, creatinine) in plasma from different rat groups. * Indicates the statistical significance compared with the control. # Indicates the significance compared with the TAA group. Values in the histogram are the mean ± SEM, and significance was set at $P < 0.05$, 0.005, and 0.001.

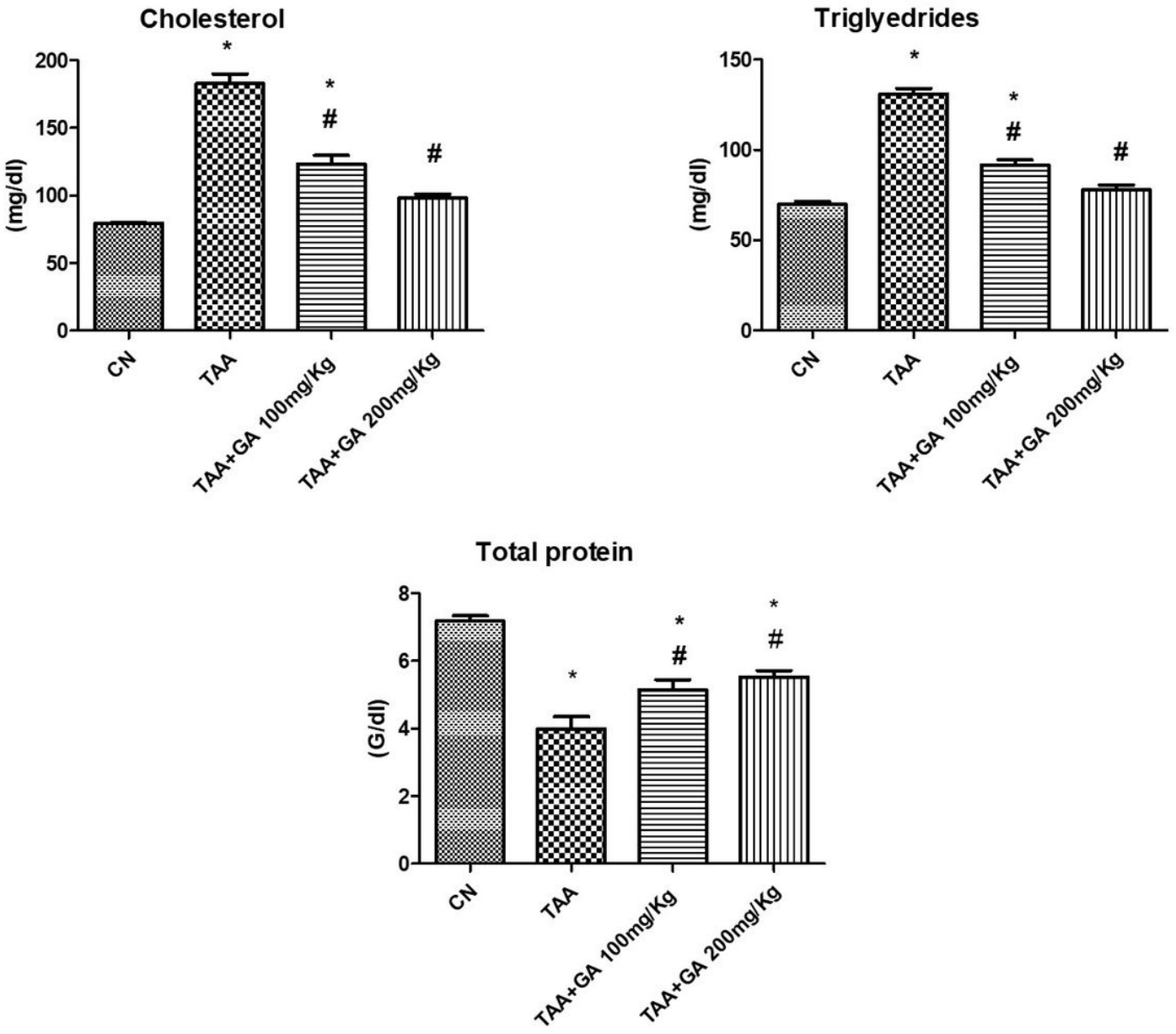

Figure 3

The concentrations of lipids (cholesterol, triglycerides) and plasma proteins from different rat groups. * Indicates the statistical significance compared with the control. # Indicates the significance compared with the TAA group. Values in the histogram are the mean ± SEM, and significance was set at $P < 0.05$, 0.005, and 0.001.

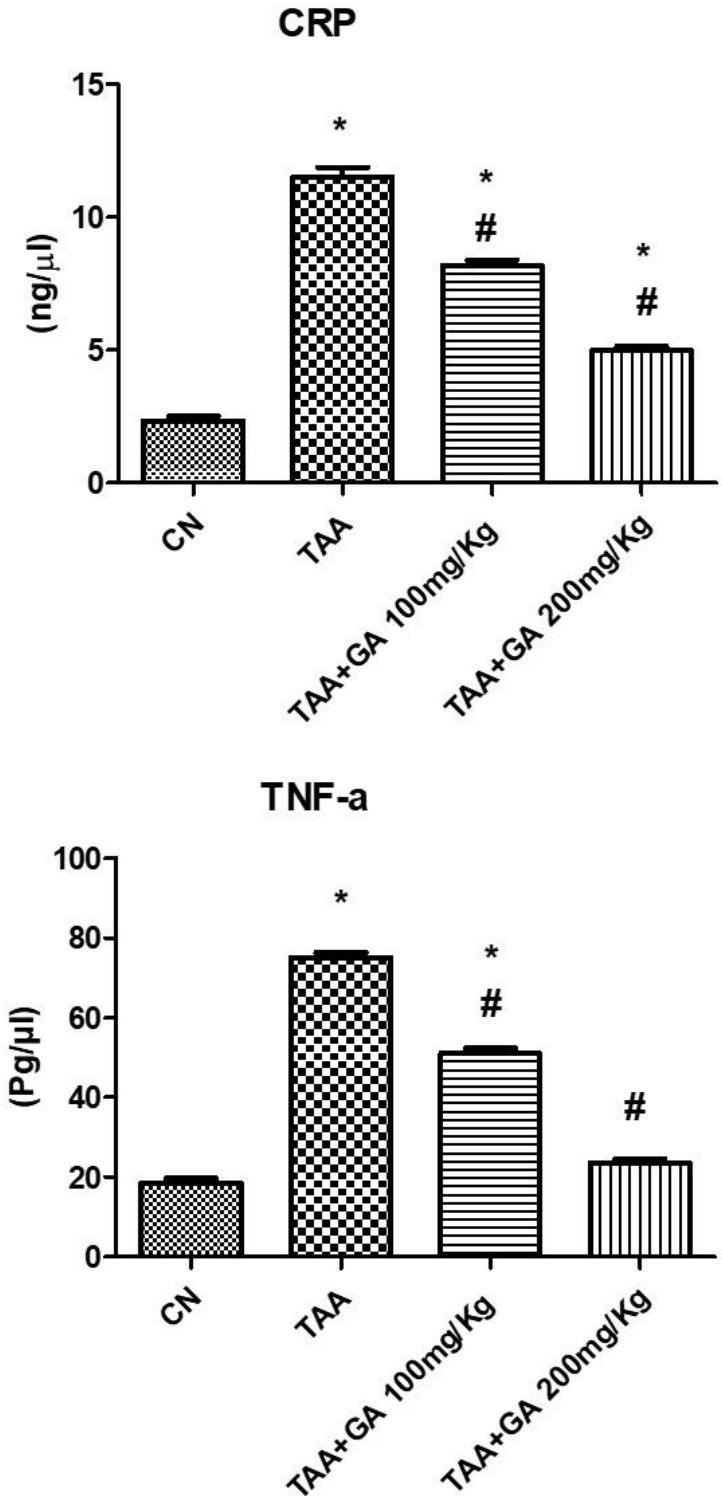

Figure 4

The levels of both plasma CRP and TNF-α in plasma from different rat groups. * Indicates the statistical significance compared with the control. # Indicates the significance compared with the TAA group. Values in the histogram are the mean ± SEM, and significance was set at $P < 0.05$, 0.005, and 0.001.

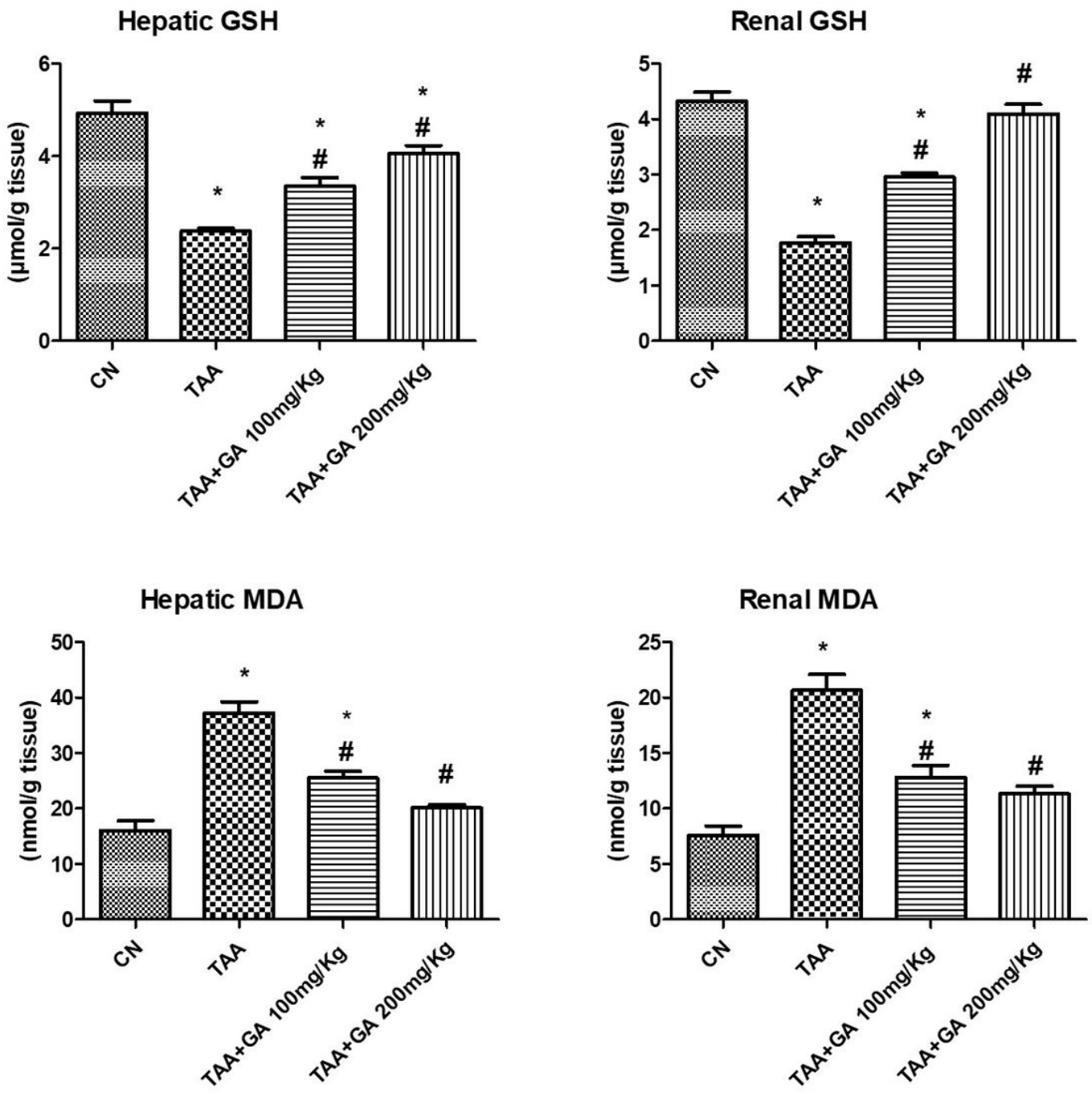

Figure 5

The concentrations of reduced glutathione (GSH) in and malondialdehyde (MDA) in both renal and hepatic tissues from different studied rat groups. * Indicates the statistical significance compared with the control. # Indicates the significance compared with the TAA group. Values in the histogram are the mean ± SEM, and significance was set at $P < 0.05$, 0.005, and 0.001.

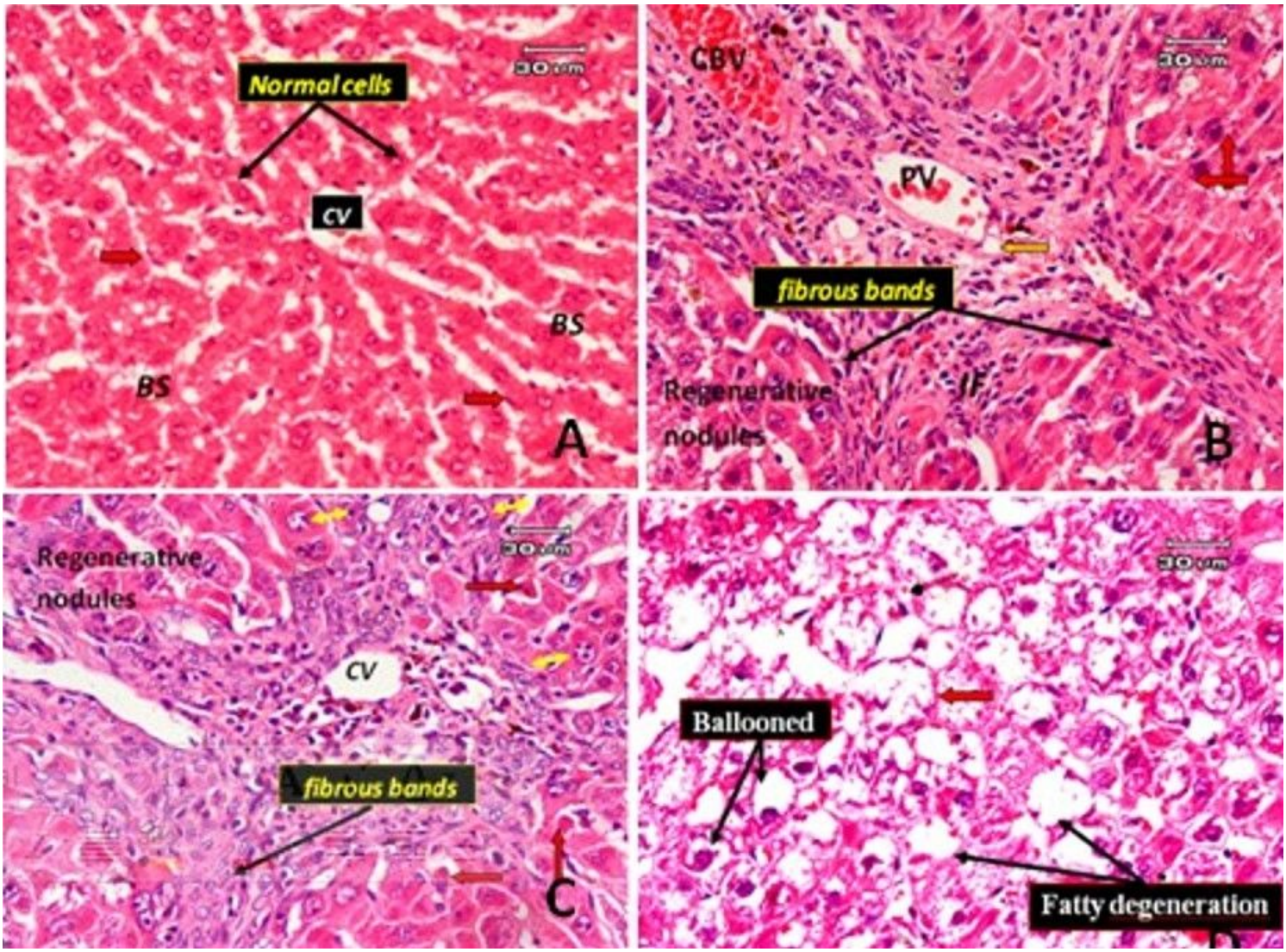

Figure 6

Photomicrographs of rat liver sections. A: control section showing normal hepatic with classic hepatic lobules containing central vein (CV) and separated by blood sinusoids (BS). The cords of hepatocytes are lined by flattened endothelial cells (arrowhead) and Kupffer cells (red arrow). B: liver section of rat treated with TAA only showing cirrhotic nodules. Cirrhosis is characterized by fibrotic bands with loss of architecture and nodule formation. The bands intervene between the regenerative hepatocytes nodules, around portal vein (PV), around congested blood vessels (CBV) and numerous small proliferated bile duct (orange arrow). Hyperchomatic nuclei with clumping chromatin (red arrow), few inflammatory cells are present in fibrous tissue (IF). C: liver section of rat treated with TAA only (another filed) showing dense fibrous bands surrounded the central vein and around perisinusoidal spaces and eventually will progress to cirrhosis, some hepatocyte appeared apoptosis with cells shrinkage and chromatin margination (red arrow), some enlarged hepatocytes with karyomegaly and multiple nuclei are present (Yellow arrow). D: liver section of rat treated with TAA only showing ballooning degeneration form of cell death, accumulation of fatty changes (steatosis) and blood sinusoids appeared narrow or obliterated (red arrow).

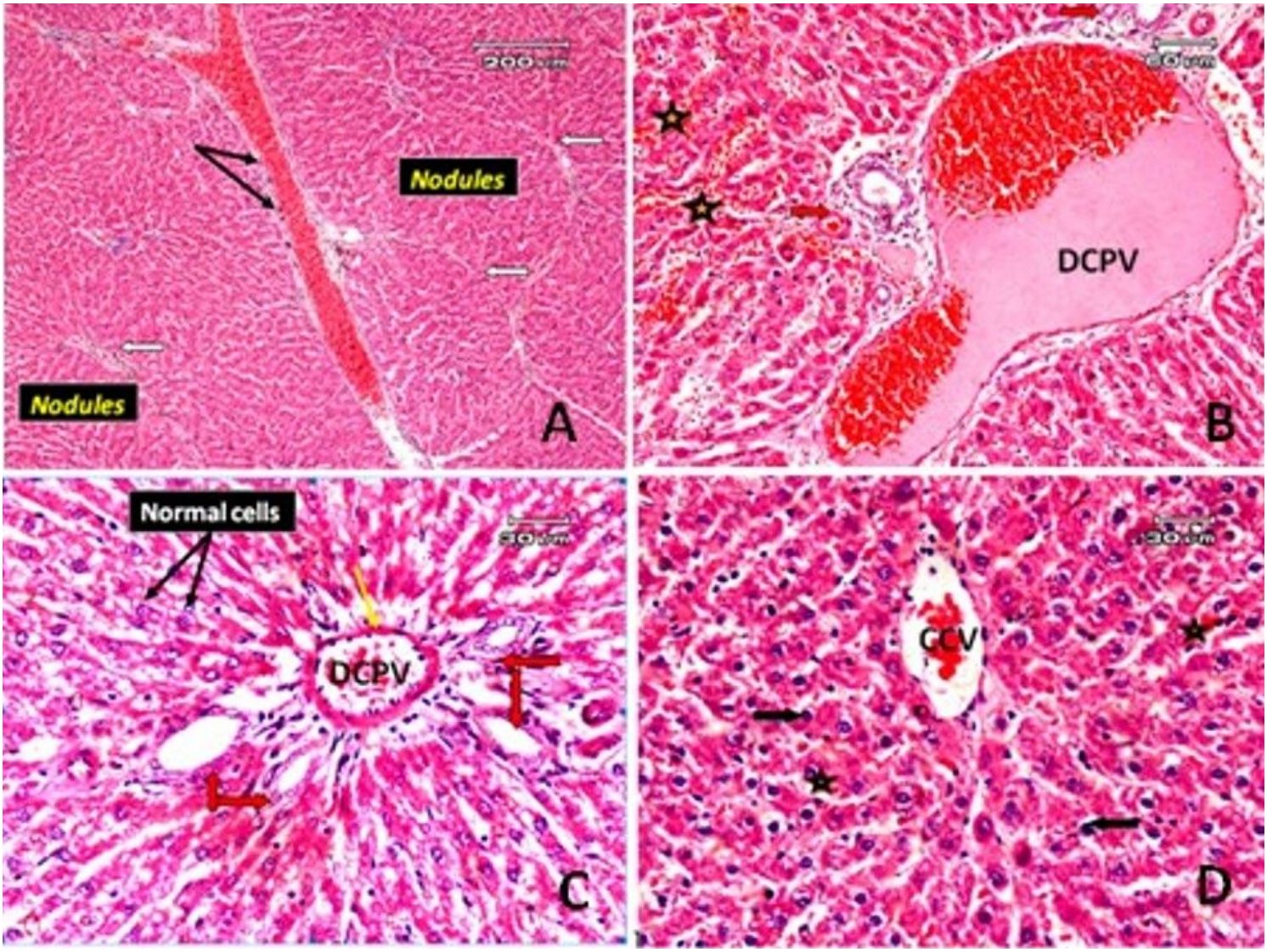

Figure 7

Photomicrographs of sections of rat liver. A: liver section of rat treated with TAA and subjected to gallic acid at dose level of 100mg/kg (low dose) showing micronodular liver cirrhosis, regenerative nodules separated by thin fibrous bands, fibrous bands (white arrow) surrounded the nodules and around the dilated congested blood vessels and cellular infiltration around (black arrow). B: liver section of rat treated with TAA and subjected to gallic acid (low dose) (another filed) showing dilated congested portal vein (DCPV) and bile duct hyperplasia (red arrow), deposition of thin collagen fibers mixed with few inflammatory cells in the portal area. Red blood vessel in dilated blood sinusoids could be observed (star). C: liver section of rat treated with TAA along with gallic acid at dose level of 200mg/kg (high dose), showed some hepatic cells more or less appeared normal (black arrow), but mild dilated and congested portal vein (DCPV), a thickened portal vein vascular wall (yellow arrow), hyperplasia of bile duct (red arrow). D: liver section of rat treated with TAA along with gallic acid at high dose (another filed), showed congested central vein (CCV), some hepatocytes appeared pyknotic (black arrow), red blood cells in blood sinusoidal space (star).

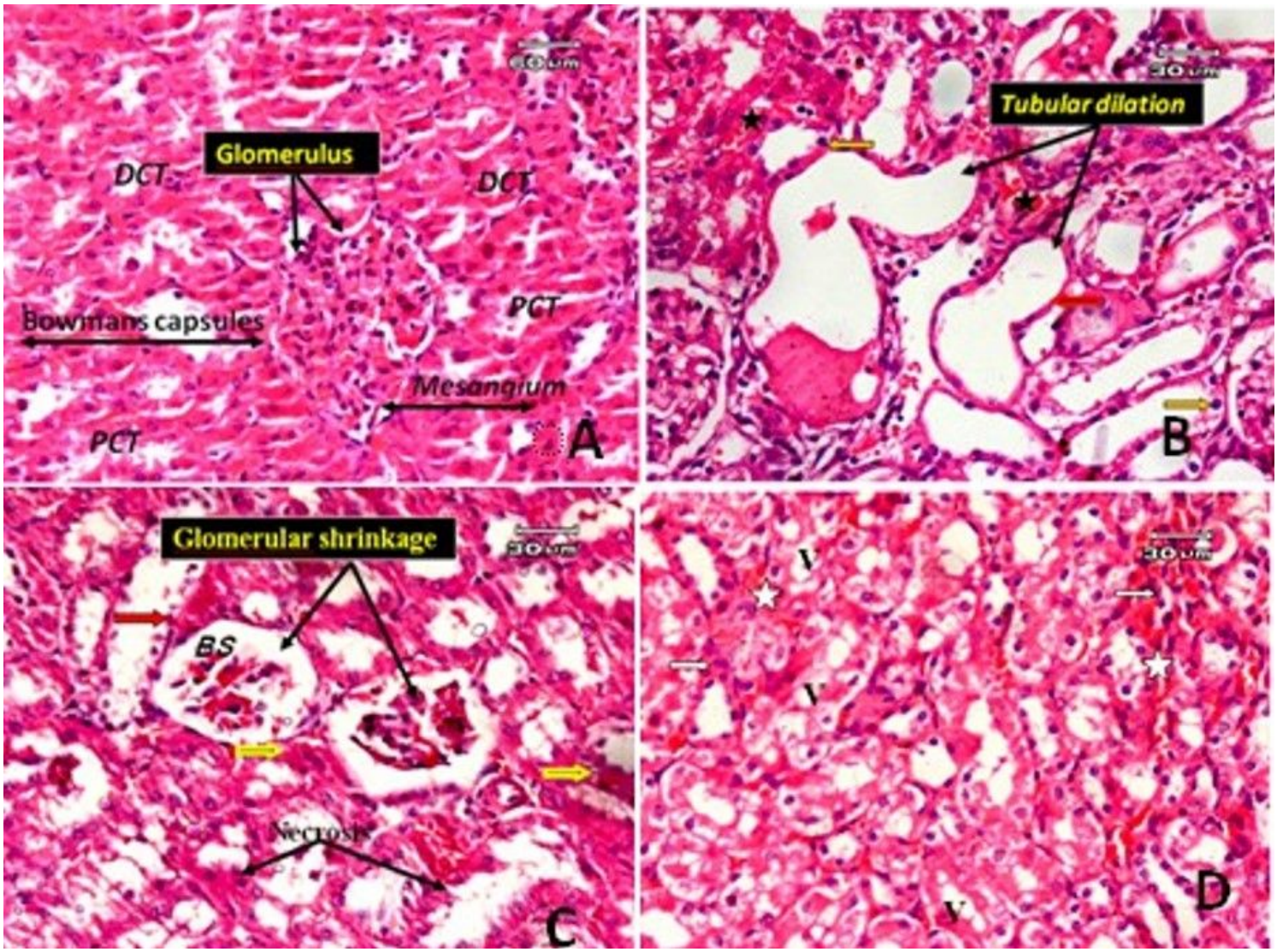

Figure 8

Photomicrographs of rat kidney. A: Normal control showing proximal convoluted tubules (PCT), distal convoluted tubules (DCT), the renal corpuscle consists of Bowman's capsule and glomerular capillaries, mesangium. B: kidney section of rat treated with TAA only showing extensive tubular dilatation, epithelial flattening, (red arrow), hemorrhage in interstitial tissue (star), pyknotic cells (orange arrow). C: kidney section of rat treated with TAA only (another filed) showing glomerular shrinkage with wide urinary space of Bowman's capsule l (BS), cloudy swelling of some tubular epithelium (yellow arrow), sings of degeneration in the form of necrosis (black arrow) and desquamation of some cells (red arrow). D: kidney section of rat treated with TAA only (another filed) showing vacuoles in renal tubular epithelial cells (v), hemorrhage in interstitial tissue (star).

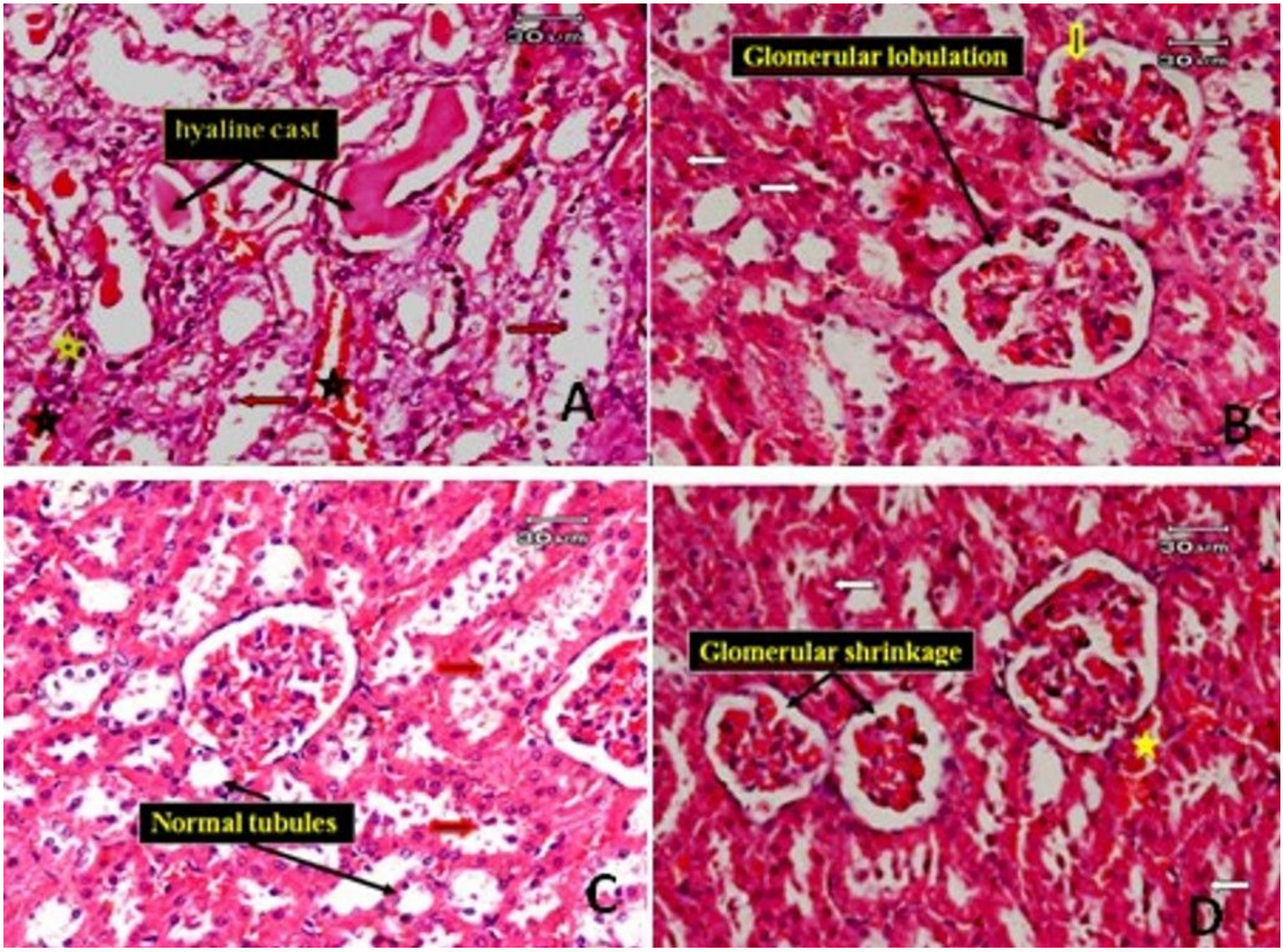

Figure 9

Photomicrographs of rat kidney. A: kidney section rat treated with TAA and subjected to gallic acid at dose level of 100mg/kg (low dose), showed no improvement in pathological changes, the kidney tissue showed hyaline casts (black arrow) present in the modularly of some tubules, desquamation in tubular epithelial cells (red arrow), and some degenerated tubules, few inflammatory infiltrates (yellow star), hemorrhage in interstitial tissue (black star). B: kidney section of rat treated with TAA and subjected to with gallic acid at low dose (another filed) showed glomerular lobulation (black arrow) interglomerular hemorrhage (yellow arrow) and cloudy swelling of some renal tubules (white arrow). C: kidney section of rat treated with TAA and subjected to gallic acid at high dose showing some improvement in pathological changes in the form of no hylain cast with no dilatation of tubules, although cell debris in lumen of some tubules (red arrow), some normal tubule could be observed. D: kidney section of rat treated with TAA and subjected to gallic acid at high dose (another filed) showing glomerular shrinkage, cloudy swelling of some tubules (red arrow) and hemorrhage in interstitial tissue (star).