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Tadalafil shows greater renoprotective role than losartan, grape seed extract and ginko biloba against cisplatin-induced nephrotoxicity in rats

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ABSTRACT

Although cisplatin (Cis) is widely used in the treatment of cancers, clinical use of cisplatin is limited due to its nephrotoxicity. There is no specific treatment for Cis-induced renal dysfunction or injury. The present study was to investigate possible protective effect of Tadalafil (Tad), Losartan (Los), Grape seed (G.S) and Ginko biloba (G.B) on Cis -induced renal damages in rats by evaluating biochemical assay and histophathological examination of kidneys. The biochemical assays included the measurements of creatinine, urea, albumin, total protein (TPs), nitric oxide (NO), lactate dehydrogenase (LDH), malonaldehyde (MDA), reduced glutathione (GSH) in the serum and glutathione peroxidase (GPx), superoxide dismutase (SOD) and catalase (CAT) levels in kidney tissue homogenate. The biochemical assay showed that Tad was the best in reducing the levels of serum creatinine, LDH, MDA, phosphorus, potassium and aldosterone hormone and increasing the levels of GSH in the serum and NO levels in serum and kidney homogenate. In addition, Tad greatly reversed Cis-induced histopathological lesions in the kidneys better than the other treatments.

Key words: Biochemical parameters, cisplatin, histopathology, rats, tadalafil.

INTRODUCTION

Cisplatin (Cis) is an antitumor drug widely used in the treatment of several human cancers such as malignancy of testis, colon, ovarian, head, and neck (Kanotra et al., 2011; Saad et al., 2009; Stevens et al., 2008). However, Cis -induced nephrotoxicity is a major complication in the cancer therapy and had a dose-limiting toxicity (Kuhlmann et al., 1997). The wide clinical usage of Cis is limited by its role in inducing renal toxicity. Even a large amount of hydration has not been effective in eliminating toxicity and the use of diuretics may even complicate the electrolyte problems induced by Cis. Thus, there is a need to protect the kidney while administering effective chemotherapeutic agents, such as Cis.

Grabe seed (G.S) is a natural extract from the seed of *Vitis vinifera*. It is a rich source of one of the most beneficial groups of plant flavonoids, pro-anthocyanidins oligomers. These flavonoids exert many health-promoting effects (Singth et al., 2004), including the ability to increase intracellular vitamin C levels, decrease capillary permeability and fragility and scavenge oxidants and free radicals (Schwitters and Masquellier, 1993; Facino et al., 1994). The activity of pro-anthocyanidins oligomers is approximately 50 times greater than that of vitamin C and vitamin E, in term of antioxidant action (Shi et al., 2003).

Ginko biloba extract (G.B) has a broad spectrum of pharmacological activities, which allows it to be in adequacy to the numerous potential therapeutic applications, including conditions associated with cognitive impairment, depression problem, liver and kidney injury, cerebral vascular insufficiency, myocardial ischemia and peripheral arterial occlusive disease (Dubey et al., 2004; Zhang et al., 2004; Zhu et al., 2004; Deng et al., 2006; Welt et al., 2007; Naidu et al., 2000).

Losartan (Los) is a novel, orally active, non-peptide angiotensin II receptor antagonist that specifically blocks the angiotensin II receptor (Chiu et al., 1990; Wong et al., 1990). Early studies have shown that Los inhibits the angiotensin II–mediated presser response in healthy subjects (Christen et al., 1991). Los also reduces the blood pressure response to angiotensin II in healthy subjects with a plateau in effect at doses of

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80 mg and more (Munafo et al., 1992). A single-dose study in patients with heart failure demonstrated beneficial vasodilator and neurohormonal effects of Los in these patients (Gottlieb et al., 1993).

Tadalafil (Tad) is an inhibitor of PDE5, the other available PDE5 inhibitors are sildenafil citrate (Viagra) and vardenafil HCl (Levitra). Tadalafil differs from sildenafil and vardenafil by its pharmacokinetic profile, 17.5 h half-life, and maximum plasma concentration at 2 h and efficacy for up to 36 h after dosing (Rosen and Kostis, 2003). The present study was an attempt to screen the renoprotective effects of Los, Tad, G.S and G.B against Cis- induced renal damage in rats by evaluating the biochemical assays and histopathogical changes.

MATERIALS AND METHODS

Experimental animals and their management

A total of one hundred and eight healthy male albino rats initially weighting between 200 and 220 g were used in this study. The animals purchased from animal house in Helwan, and housed in Department of Physiology, Faculty of Veterinary medicine, Mansoura University Animals were left for one week to acclimatize the place. Rats were kept in cages in a rate of six rats per cage in a controlled environment, maintained under a 12 hours light:dark cycle, 24ºC (± 3ºC) and 50-70% humidity. Rats were provided with standard diet and water ad-libitum. All animal procedures were performed in accordance with the Ethics Committee of the National Research Centre, Egypt. Registration number (09/189).

Drugs and plant extracts and their administration

Cisplatin was purchased from Sigma Company (Sigma, St. Louis, Mo, USA): in the form of liquid 1mg/ml sterile concentrate and was injected intraperitoneally (IP) at a dose rate of 4 mg/Kg body weight (BW) once a week for 4 weeks (Carozzi et al., 2009).

Losartan (Losatan Potassium 50 mg) was purchased from Ameriyah Pharmaceuticals Industries (Alexandria, Egypt) and was administrated daily at a dose of 10 mg/kg BW IP (Deegan et al., 1995).

Tadalafil (Tad) tablet was purchased from ELI LILLY (American global pharmaceutical company) and was administrated daily at a dose of 0.4 mg/kg BW IP (Ali et al., 2011).

Grape seed proanthocyanidin extract (G.S) was obtained from Pharco Pharmaceuticals Company (Alexandria, Egypt) which is a standardized water–ethanol extract from grape seed. The extract was supplied in the form of standardized G.S extract (P 95.0–96.29% Oligomeric Proanthocyanidins) and was administrated by stomach tube at a dose of 200 mg/Kg BW (Yamakoshi et al., 2002).

Ginkgo Biloba was obtained from Pharco Pharmaceuticals Company (Alexandria, Egypt) and was administrated by stomach tube at a dose of 300 mg/Kg BW (Li et al., 2011).

Experimental design and sample collection

To evaluate renoprotective effects of Los, Tad, G.s and G.B, rats were randomly divided into six groups (n = 18); Control group received a single dose of 0.9% saline (IP) and three times of distilled water by oral gavage, Cis group received Cis besides three times of distilled water by oral gavage, Tad group received Tad and Cis, G.B group received G.B and Cis, G.S group received G.S and Cis and Los group received Los and Cis. The duration of the experiment was four weeks.

Blood samples were drawn via retro-orbital bleeding after 12 h fasting at the end of 1st, 2nd and 4th weeks of the experiment. Half amount of each blood sample was left in plain test tube at room temperature for 1 hour and then centrifuged for 10 min at 3000 rpm to obtain the serum. Serum samples were stored at −80°C for further analysis. At the end of 1st, 2nd and 4th weeks of the experiment, six rats were sacrificed from each group by decapitation. Right and left kidneys were quickly removed from all

rats. Right kidneys were washed with ice-cold normal saline and homogenates (10%, w/v) were prepared in PBS. A part of the homogenate was used for the estimation of reduced glutathione (GSH) and lipid peroxidation marker (MDA). The remaining homogenate was centrifuged at 5000 g for 10 min at 4°C and the supernatant was stored at −80°C until further analysis for superoxide dismutase (SOD), catalase (CAT), nitric oxide (NO) and glutathione peroxidase (GPx). Left kidneys were fixed in 10% neutral buffered formalin. Fixed specimens were processed for routine histopathological examination.

Biochemical analysis of serum and tissue homogenate:

Urea and creatinine were measured in the serum by a colorimetric method using commercial kit (Diamon, Egypt) according to the method described by Tietz (1990).

Total protein (TPs) and albumin were measured in the serum by UV- calorimetric spectrophotometric method using commercial kits (Vitro Scient, Egypt) according to the method of Grant *et al.* *(*1987). Antioxidant markers like lactate dehydrogenase, glutathione reductase, glutathione peroxidase, and catalase and superoxide dismutase were also determined.

Lactate dehydrogenase activity (LDH) was measured by a kinetic method using commercial kit (Egyptian company for biotechnology) according to Young (1990).

Reduced glutathione (GSH) was measured in serum and kidney tissue by a colorimetric method using commercial kits (Bio-diagnostic, Egypt) according to the method of (Beutler et al., 1963). Glutathione Peroxidase (GPx) was measured in kidney tissue by a colorimetric method using commercial kits (Bio-diagnostic,, Egypt) according to the method of *(*Paglia and Valentine, 1967). MDA was measured by a colorimetric spectrophotometric method (Stocks and Dormandy, 1971) using commercial kits (Bio-diagnostic, Egypt).

Catalase (CAT) was measured in kidneys by a colorimetric method using commercial kits (Bio-diagnostic, Egypt) according to the method of (Aebi, 1984). Superoxide dismutase (SOD) was measured in kidneys by a colorimetric method using commercial kits (Bio-diagnostic, Egypt) according to the method of (Nishikimi, 1972).

NO was assayed in the serum by a colorimetric method using the diazotization procedure according to Bartholomew (1984), while in the kidney homogenate, NO was estimated according to Montgomery and Dymock (1961).

Potassium, calcium and phosphorous were measured in serum by a colorimetric method using commercial kits (Vitro Scient, Egypt) according to the method of Thomas (1998).

Measurement of Aldosterone hormone

Aldosterone hormone was measured in the serum using BioAssay™ ELISA Kit according to previously described method (Carledge and Lowson, 2000) using immulite device at the Faculty of Medicine, Mansoura University, Egypt.

Histopathological examinations

Paraffin sections of 5µm thickness were cut and picked up on uncoated slides, dried, de-parafinized, rehydrated with graded alcohol, washed and stained with H&E according to Bancroft and Stevens. (1990). Histological changes were examined by light microscopy (binocular, Olympus) in a blinded fashion. Images were taken using Digital camera (Canon 5 mega pixels, 3.2x optical zoom).

Statistical analysis

Data were analyzed by analysis of variance (ANOVA) using the general liner model procedure of SAS (SAS Institute, 2004). F-test (Fisher least significant difference test) was used to compare between groups and T-test (student T-test) was used to compare between parameters at 1, 2 weeks and 4 weeks of treatments. The mean values were significant at $P<0.05$.

RESULTS

Serum levels of creatinine, urea, albumin and TPs

The biochemical findings revealed that, Cis significantly increased serum levels of creatinine and urea and decreased albumin and TPs when compared with the control group (Table 1).
After four weeks of treatment, Tad was superior in reducing serum creatinine, increasing serum albumin and TPs. Meanwhile, Los was superior in reducing serum urea over other treatments (Table 1).

Levels of antioxidants markers
Cis significantly increased levels of serum LDH and MDA in serum and kidneys. However, Cis significantly decreased serum and kidney´s GSH, kidney´s GPx, kidney´s CAT and SOD levels when compared to control group (Table 2&3).

Four weeks post treatment; Tad was superior in reducing levels of serum LDH and MDA in serum and kidneys and increasing kidney tissues' GSH levels. Both Tad and Los raised serum GSH values. Los was the best to significantly increase kidney´s GPx and CAT levels. Meanwhile, G.B extract was superior in raising kidney´s SOD better than the other treatments (Table 2&3).

Effects of selected drugs and/or plant extracts on NO levels
Serum and kidney´s NO levels were significantly ($P<0.0009$) decreased in Cis group when compared to control group. Four weeks post treatment, Tad was superior in increasing serum and kidney´s NO level more than the other treatments (Table 4).

Effects of selected drugs and/or plant extracts on levels of mineral ions
Cis significantly decreased serum calcium, potassium and magnesium levels but significantly increased serum phosphorus levels when compared with the control group (Table 5).
After four weeks of treatment, Los was superior in increasing serum calcium and magnesium. Tad was superior in reducing serum phosphorus and increasing serum potassium (Table 5).

Serum levels of Aldosterone hormone
Serum aldosterone levels were significantly ($P<0.0083$) increased in Cis group compared to control group. After four weeks of treatment, Tad was superior in decreasing serum aldosterone values (Table 6).

Histopathological examination of kidneys
In the control group, normal histological picture was seen in kidneys (Fig.1a). After the 1st week, kidneys of the Cis group showed tubular damage including tubular dilatation, hyaline casts, epithelial cells degeneration, tubular epithelial cell detachment from the basement membrane, desquamation into tubular lumina, tubular dilation in the corticomedullary junction and the basement membranes of the affected tubules appeared to be irregularly arranged or injured. Glomerular damage (including atrophy, shrinkage, collapse and sclerosis) were shown (Fig. 1b). Macrophages were seen inside lumen of the damaged renal tubules. Lipofuscin pigment was clearly visible inside the renal epithelium. Mild congestion, perivascular edema and interstitial edema were seen. In the kidneys from Tad group, hyaline casts were more prominent than in the Cis group (Fig. 1c). Epithelial cells degeneration in the form of moderate vacuolation was visible. Localized areas of tubular necrosis were recognized. Congestion, perivascular edema, interstitial edema and hemorrhage were also detected. The extent of glomerular damage was milder than in Cis group. In the G.B group, tubular dilation was more remarkable in the coticomedullary and medullary regions than in the Cis group. The dilated renal tubules were lined by regenerating, flattened epithelial cells with basophilic cytoplasm. The renal epithelium of some renal tubules had a swollen cytoplasm and enlarged nuclei with dispersed and segregated heterochromatin (Fig. 1d). Epithelial cells degeneration in the form of severe vacuolation was present. Hyaline casts were less frequent than in the Cis and Tad

groups. The extent of glomerular damage was less than in Cis group. In the G.S group, tubular vacuolation was marked. However, tubular dilation was milder than in G.B group (Fig. 1e). Necrotic tubules were absent. The extent of glomerular damage was milder than that found in Cis group. Peri-glomerular fibrous tissue deposition was detected accompanied with mononuclear cells infiltration. Kidneys from Los group showed focal tubular calcification and severe glomerular damage. Severe tubular dilation, epithelial vacuolation and moderate tubular hyaline casts were detected in the outer medulla (Fig. 1f).

After the 2nd week, kidneys from Cis group showed epithelial cells degeneration increased, besides, marked congestion, perivascular edema, interstitial edema and hemorrhage. Arteriolar hyalinization was observed in the renal tissues where the renal arterioles showed thickened wall and narrowed lumen. Fibrotic areas consisting of spindle-shaped fibroblastic cells and mononuclear cells began to be clearly developed around the affected tubules in the corticomedullary junction. Some renal tubules were lined by epithelium with basophilic cytoplasm. The extent of the glomerular damage increased (Fig. 2a). Polymorphnuclears leukocytes were rarely seen. Mononuclears cells infiltration were detected with perivascular edema and in the peri-renal adipose tissue. Dilated renal pelvis was observed in one animal. In the kidneys from Tad group, tubular damage was greatly relieved. Mild epithelial vacuolation was recorded in the renal tubules (Fig. 2b). Mild congestion and perivascular edema were seen. In the G.B group, the tubular damage was slightly reduced. Small necrotic areas were shown (Fig. 2c). The wall of the renal arterioles showed thickened wall and narrowed lumen. Congestion, perivascular edema, interstitial edema and hemorrhage were noticed. Perivascular few mononuclear cells infiltration was demonstrated. In kidneys from the G.S the extent of glomerular damage slightly increased more than in the 1st week. The dilated renal tubules at the corticomedullary junction were lined by severely vacuolated epithelium (Fig. 2d). Congestion, perivascular edema, interstitial edema and hemorrhage were also seen. Perivascular few mononuclear cells infiltration were demonstrated. The extent of the interstitial fibrosis was much more than in the other groups. Kidneys from Los showed focal areas of tubular necrosis and calcification accompanied with glomerular sclerosis (Fig. 2e). Tubular dilation, epithelial vacuolation and tubular casts were still seen inside outer medulla.

After the 4th week, kidneys from Cis group severe tubular necrosis and calcification were seen at the corticomedullary junction (Fig. 3a). The fibrotic areas became more evident especially around the variously dilated tubular lumina in the corticomedullary junction indicating progressive fibrosis. Adjacent to the areas of necrosis, dilated renal tubules were lined by regenerating, flattened epithelial cells with basophilic cytoplasm. Moreover, the extent of glomerular atrophy became severely increased. In the kidneys from Tad group, the basement membranes of the renal tubules appeared to be regularly arranged. Renal tubules were lined by normal columnar epithelium with eosinophilic cytoplasm (Fig.3b). The extent of glomerular damage after the 2nd and 4th weeks was similar to that found after the 1st week. In the G.B group, mild perivascular edema and hemorrhage were seen. Tubular damage was greatly ameliorated and tubular dilation disappeared. Renal tubules appeared regularly arranged lined with normal columnar epithelium with eosinophilic cytoplasm. The extent of glomerular damage was similar to that found after the 2nd week. Perivascular fibrosis was occasionally detected in the renal medulla were (Fig. 3c). In kidneys from the G.S, tubular damage was greatly relieved. Focal interstitial fibrosis, mild congestion, perivascular edema and hemorrhage were seen (Fig. 3d). Perivascular few mononuclear cells infiltration was still seen in some sections. The extent of damaged glomeruli was similar to that found at the 2nd week. In kidneys from Los, tubular damage was

greatly relieved. Tubular necrosis and calcification were disappeared. Mild tubular dilatation and casts was demonstrated associated with glomerular sclerosis (Fig. 3e). Mild perivascular edema and hemorrhage were obvious with few perivascular mononuclear cells infiltration. Interstitial fibrosis was not observed in Los group at any sacrifice.

DISCUSSION

Cis is a highly effective antineoplastic DNA alkylating agent used against a wide variety of cancers Although, higher doses of Cis are more efficacious for the treatment of cancer, many reversible and irreversible side-effects including nephrotoxicity often limit its utility and therapeutic profile (Lynch et al., 2005). There is a continuous search for agents which provide renoprotection against the renal impairment caused by drugs like Cis. Hence, the present study was an attempt to compare the renoprotective activity among Los, Tad, G.S and G.B. Several investigators Antunes et al. (2000); Naziroglu et al. (2004); Saleh et al. (2014) reported that the alterations induced by Cis in the kidney functions were characterized by signs of injury, such as increase of products of lipid peroxidation and changes in GSH levels in kidney tissue, creatinine and urea levels in plasma. In the present study, the biochemical analysis showed Tad was the best to reduce the levels of serum creatinine, blood glucose, LDH, MDA, phosphorus and potassium, increase serum TPs, GSH and NO levels in serum and kidney homogenate. Los was the best to decrease serum levels of urea and increase serum levels of GSH, GSH-Px, CAT, calcium and magnesium. G.B was superior to reduce serum levels of lipid profile and SOD. According to the number of the biochemical parameters had been normalized, Tad was superior to counteract the nephrotoxic effects of Cis. In a previous study in male rats, G.S reduced Cis-induced high levels of thiobarbituric acid-reactive substances in plasma, heart, kidney and liver, total lipids, cholesterol, urea and creatinine, and liver alanine aminotranferease and aspartate aminotransferase **(**Yousef et al., 2009). Moreover, G.S ameliorated Cis-induced decrease in the activities of antioxidant enzymes, and GSH, TPs and albumin. However, the present results showed that Tad exerted a more protective effect than the other tested agents. The treatment with Tad prevented the lipid peroxidation by reducing LDH and MDA and enhancing the serum and renal GSH activities. All the examined biochemical parameters were corrected after the 4th week indicating the need for at least 4 weeks to normalize these parameters.

Cis is believed to exert its damage on the different segments of the nephron. Proximal tubular dysfunction results in impaired sodium and water reabsorption. This leads to increased delivery of sodium chloride to the macula densa in the distal tubule, thus activating the tubuloglomerular feedback mechanism, which causes a decrease in the renal blood flow followed by a decrease in the glomerular filtration rate (Thurau and Boylan, 1976; Cornelison and Reed, 1993). The increased delivery of sodium and water to the distal nephron does not result in the expected increase in the reabsorption rate at that site, which demonstrates that Cis also damages the distal tubule (Daugaard et al., 1988; Daugaard and Abildgaard, 1989; Daugaard, 1990). The loop of Henle is also affected by Cis, where the loss of the countercurrent gradient induces a urinary concentrating defect and impairs water reabsorption (Isnard-Bagnis, 2003; Meyer and Madias, 1994). Finally, Cis has been reported to decrease the expression of the aquaporin water channels in the collecting tubules, leading to further impairment of water reabsorption (Kim et al., 2001). Renal salt wasting syndrome occurs when Cis damages the proximal tubules, the major site of sodium and water reabsorption, leading to an obligatory natriuresis, with an increase in urine output and urine sodium. Because of the resulting volume depletion, antidiuretic hormone is secreted as an appropriate response, Vassal et al. *(*1987) together with aldosterone, in an attempt to

retain salt and water and prevent intravascular volume depletion. Tad was the best to reduce the levels of aldosterone.

The above findings were well-correlated with the renal histological results. Cis-induced tubular damage including tubular casts, epithelial cells degeneration, tubular epithelial cell detachment from the basement membrane and desquamation into tubular lumina besides tubular dilation at the corticomedullary junction. Cis nephrotoxicity involves both inflammatory and oxidative stress processes (Koyner et al., 2008; Chirino and Pedraza-Chaverri, 2009). It has been reported that Cis induces (both *in vitro* and *in vivo*) necrosis in the tubular cells of the kidney by generating large quantities of hydroxyl radicals, followed by increased synthesis of TNF-α. A role for apoptosis after injection of Cis has also been suggested (Yano et al., 2007). Kidneys from Tad group at 1st and 2nd weeks showed epithelial cells vacuolation, hyaline casts and localized areas of tubular necrosis were recognized. Tad was superior in reducing Cis induced renal damage as these histopathological changes were greatly reversed at the end of the week 4. Tad is an inhibitor of the PDE5. PDE is a family of enzymes that regulate the cellular levels of second messengers, cAMP and cGMP (Reffelmann and Kloner, 2009). By inhibiting PDE5, Tad induces accumulation of cGMP, resulting in dilatation of blood vessels and decrease in blood pressure. PDE5 catalyzes the breakdown of the potent smooth muscle-relaxing agent cGMP, a second messenger of NO. Inhibition of PDE5 increases cGMP levels, reduces intracellular calcium and induces vasodilation (Aversa et al., 2004). So far, clinical studies have shown that PDE5 inhibitors are safe and do not increase cardiovascular risk in patients with coronary artery disease (Kloner, 2004; Jackson et al., 2004; Kloner and Padma-Nathan, 2005). A recent study Ali et al. (2011) has reported that when sildenafil (0.4 mg/kg/day, IP. for 5 days) was combined with Cis, there was a dramatic improvement in renal histopathology, reduction in *N*-acetyl-β-d-glucosaminidase and increase in renal blood flow. In the group of rats treated with Cis and sildenafil (0.4 mg/kg/day IP for 5 days), there were only few focal areas of degenerated vacuolated cells and acute tubular necrosis constituting about 20% of the total tissue fields examined and few apoptotic cells. However, sildenafil (10 mg/kg/day, subcutaneous for 5 days) did not affect CP nephrotoxicity, suggesting the importance of dose and route selection of sildenafil as a nephroprotectant. We concluded that Tad was effective than Los, G.B and G.S to relieve the nephrotoxic effects of Cis when administered at 0.4 mg/kg/BW IP daily for a period not less than 4 weeks.

CONCLUSION

Based on our study results we conclude that Tad is more effective than Los, G.B and G.S to relieve the nephrotoxic effects of Cis when administered at 0.4 mg/kg/BW IP daily for a period not less than 4 weeks.

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Table (1): Effect of Tad, G.B, G.S and Los on serum levels of creatinine, urea, albumin and total proteins in control and treated rats:

Group	1st Week	2nd Week	4th Week	F-test	
				F-value	P-value
		Creatinine (mg/dl)			
Control	0.670±0.37^{Fa}	0.62±0.04^{Ea}	0.70±0.01^{Ea}	1.33	0.331
Cis	2.91±0.12^{Ac}	3.78±0.09^{Ab}	5.09±0.08^{Aa}	107.10	0.0001
Tad	1.45±0.03^{Eb}	1.67±0.03^{Da}	1.03±0.04^{Dc}	71.40	0.0001
Los	1.71±0.05^{Db}	1.98±0.06^{Ca}	1.50±0.02^{Cc}	21.99	0.0017
G.B	1.96±0.01^{Cb}	2.60±0.04^{Ba}	1.99±0.06^{Bb}	61.23	0.0001
G.S	2.27±0.02^{Ba}	2.77±0.03^{Bb}	2.12±0.06^{Bb}	57.60	0.08
F value	153.42	344.86	792.86		
LSD	0.189	0.178	0.172		
		Urea (mg/dl)			
Control	32.33±2.33^{Da}	27.66±2.02^{Ea}	24.33±3.84^{Ca}	1.99	0.217
Cis	155.66±3.17^{Ab}	169.33±6.64^{Ab}	219.66±10.6^{Ab}	20.33	0.0021
Tad	106.00±7.77^{Ca}	105.33±4.84^{Ca}	32.00±2.89^{Cb}	58.92	0.0001
Los	99.66±8.88^{Ca}	90.33±3.84^{Da}	31.00±1.45^{Cb}	42.61	0.0003
G.B	125.00±5.03^{Ba}	111.00±2.08^{Cb}	54.33±2.9^{Bc}	110.22	0.0001
G.S	145.33±2.90^{Aa}	126.00±5.13^{Bb}	64.33±4.48^{Bc}	97.81	0.0001
F value	61.84	110.78	198.56		
LSD	17.26	13.613	16.26		
		Albumin (g/dl)			
Control	3.94±0.02^{Aa}	3.970±0.09^{Aa}	3.97±0.10^{Aa}	0.03	0.9738
Cis	2.40±0.01^{Ea}	2.11±0.06^{Eb}	1.94±0.03^{Dc}	29.82	0.0008
Tad	3.56±0.02^{Bb}	3.77±0.02^{Ba}	3.82±0.03^{Aa}	21.15	0.0019
Los	3.41±0.02^{Cb}	3.50±0.02^{CDb}	3.64±0.03^{Ba}	14.33	0.0052
G.B	3.47±0.02^{Cc}	3.65±0.03^{BCb}	3.80±0.01^{ABa}	45.50	0.0002
G.S	3.15±0.04^{Db}	3.38±0.03^{Da}	3.45±0.03^{Ca}	16.41	0.0037
F value	357.13	162.75	199.59		
LSD	0.0849	0.1605	0.1646		
		Total proteins (g/dl)			
Control	6.300±0.11^{Aa}	6.43±0.08^{Aa}	6.40±0.05^{Aa}	0.59	0.5831
Cis	4.53±0.03^{Ea}	4.30±0.05^{Eb}	4.13±0.06^{Eb}	13.63	0.0059
Tad	5.73±0.03^{Bc}	5.96±0.03^{Bb}	6.23±0.03^{Ba}	56.33	0.0001
Los	5.23±0.06^{Cc}	5.56±0.08^{Cb}	5.90±0.05^{Ca}	21.43	0.0019
G.B	5.63±0.03^{Bc}	5.90±0.05^{Bb}	6.13±0.03^{Ba}	33.80	0.0005
G.S	4.90±0.05^{Dc}	5.20±0.05^{Db}	5.43±0.03^{Da}	27.57	0.0009
F value	98.36	124.24	293.06		
LSD	0.1967	0.2054	0.1512		

-Values are mean ±S.E
-Values with the different capital letters in the same column are significantly different at (P< 0.05).
- Values with the different small letters in the same raw are significantly different at (P< 0.05).

Table (2): Effect of Tad, G.B, G.S and Los on levels of LDH, MDA and GSH in control and treated rats:

Group	1st Week	2nd Week	4th Week	F-test	
				F-value	P value
		Serum LDH (U/L)			
Control	62.33±6.74^{Fb}	82.66±3.93^{Fa}	70.66±4.26^{Fab}	3.97	0.0798
Cis	396.33±3.7^{Ac}	438.66±11.2^{Ab}	470.66±4.26^{Aa}	26.23	0.0011
Tad	299.33±3.47^{Ea}	215.33±2.84^{Eb}	103.33±5.04^{Ec}	635.21	0.0001
Los	337.33±3.1^{Da}	253.66±4.05^{Db}	127.00±4.62^{Dc}	702.51	0.0001
G.B	356.33±2.33^{Ca}	300.66±2.33^{Cb}	195.33±4.81^{Cc}	589.92	0.0001

G.S	379.33±3.4^{Ba}	327.00±2.51^{Bb}	280.00±4.36^{Bc}	197.06	0.0001
F value	924.20	477.07	1061.92		
LSD	12.53	16.80	14.07		
		Serum MDA (nmol/mL)			
Control	19.766±0.38^{Fa}	20.50±0.40^{Ea}	21.06±0.08^{Ea}	1.32	0.3349
Cis	44.53±0.40^{Ac}	50.10±0.60^{Ab}	57.46±1.26^{Aa}	58.85	0.0001
Tad	26.166±0.21^{Ea}	29.36±0.40^{Db}	24.10±0.52^{Dc}	43.81	0.0003
Los	29.43 ±0.43^{Da}	30.53±0.44^{Da}	25.10±0.54^{Db}	35.76	0.0005
G.B	33.80 ±0.24^{Ca}	34.86±0.75^{Ca}	30.33±0.49^{Cb}	19.32	0.0024
G.S	37.93 ±1.24^{Ba}	40.70±0.54^{Ba}	32.80±0.52^{Bb}	22.62	0.0016
F value	214.10	352.46	315.07		
LSD	1.849	1.67	2.304		
		Kidney MDA (nmol/g)			
Control	18.30±0.36^{Fa}	19.26±0.43^{Fa}	19.266±0.24^{Da}	2.49	0.1633
Cis	40.03±0.20^{Ac}	44.10±0.20^{Ab}	51.100±1.21^{Aa}	60.47	0.0001
Tad	26.60±0.31^{Ea}	25.16±0.17^{Eb}	21.86±0.44^{Cc}	52.67	0.0002
Los	30.30±0.40^{Da}	26.86±0.08^{Db}	22.83±0.20^{Cc}	197.45	0.0001
G.B	33.80±0.09^{Ca}	31.53±0.27^{Cb}	27.73±0.38^{Bc}	121.40	0.0001
G.S	35.20±0.37^{Ba}	32.70±0.37^{Bb}	28.06±0.63^{Bc}	56.87	0.0001
F value	584.05	877.81	347.72		
LSD	0.967	0.878	1.917		
		Serum GSH (mmol/L)			
Control	1.05±0.03^{Aa}	1.09±0.05^{Aa}	1.07±0.02^{Aa}	0.30	0.7545
Cis	0.30±0.006^{Da}	0.27±0.01^{Ea}	0.20±8.78^{Eb}	18.56	0.0027
Tad	0.65±0.03^{Bb}	0.89±8.68^{Ba}	0.95±8.78^{Ba}	59.55	0.0001
Los	0.65±0.04^{Bc}	0.78±0.03^{Cb}	0.90±0.01^{Ca}	12.83	0.0068
G.B	0.61±0.04^{Bb}	0.71±0.01^{CDa}	0.80±8.78^{Da}	12.83	0.0068
G.S	0.50±0.01^{Cc}	0.67±0.01^{Db}	0.78±0.01^{Da}	97.04	0.0001
F value	59.58	96.08	411.90		
LSD	0.0987	0.0858	0.0463		
		Kidney GSH (nmol/g)			
Control	2.033±0.03^{Aa}	2.02±0.06^{Aa}	1.97±0.05^{Aa}	0.35	0.7201
Cis	0.90±0.03^{Ea}	0.85±0.03^{Fa}	0.72±0.01^{Fb}	8.34	0.0185
Tad	1.38±0.03^{Bb}	1.51±0.02^{Bb}	1.82±0.05^{Ba}	30.26	0.0007
Los	1.21±0.01^{Cc}	1.39±6.64^{Cb}	1.64±0.03^{Ca}	94.61	0.0001
G.B	1.08±0.04^{Dc}	1.27±0.02^{Db}	1.40±0.01^{Da}	35.98	0.0005
G.S	1.01±0.02^{Db}	1.08±0.04^{Eab}	1.16±0.02^{Ea}	6.17	0.0350
F value	149.66	114.10	146.88		
LSD	0.103	0.1157	0.117		

-Values are mean ±S.E
-Values with the different capital letters in the same column are significantly different at (P< 0.05).
- Values with the different small letters in the same raw are significantly different at (P< 0.05).

Table (3): Effect of Tad, G.B, G.S and Los on levels of kidney GPx, CAT and SOD in control and treated rats:

Group	**1st Week**	**2nd Week**	**4th Week**	**F-test**	
				F-value	**P-value**
		GPx (U/g)			
Control	30.00±0.05^{Aa}	29.46±0.29^{Aab}	28.93±0.12^{Ab}	8.35	0.0185
Cis	19.03±0.14^{Fa}	17.73±0.17^{Eb}	16.83±0.23^{Ec}	34.41	0.0005
Tad	21.46±0.26^{Dc}	24.03±0.20^{Cb}	26.06±0.17^{Da}	113.87	0.0001
Los	23.63±0.20^{Bc}	25.80±0.17^{Bb}	27.83±0.27^{Ba}	90.92	0.0001
G.B	22.56±0.37^{Cc}	25.66±0.20^{Bb}	26.96±0.20^{Ca}	68.64	0.0001
G.S	20.16±0.27^{Ec}	22.50±0.47^{Db}	25.53±0.27^{Da}	58.36	0.0001
F value	259.64	205.32	391.87		
LSD	0.743	0.8449	0.6774		
		CAT (U/g)			
Control	50.43± 1.19^{Aa}	48.03±1.24^{Aab}	45.70±0.47^{Ab}	5.26	0.0478
Cis	33.66±0.67^{Ea}	30.43±0.59^{Eb}	24.90±0.79^{Ec}	41.07	0.0003

Tad	39.30±0.20^{BCc}	41.06±0.42^{BCb}	42.60±0.20^{Ba}	30.55	0.0007
Los	40.03±0.12^{Bc}	42.23±0.17^{Bb}	43.80±0.15^{Ba}	155.92	0.0001
G.B	37.83±0.34C^{Dc}	39.26±0.27^{CDb}	41.13±0.28^{Ca}	29.69	0.0008
G.S	36.63±0.27^{Dc}	38.13±0.20^{Db}	39.60±0.35^{Da}	27.63	0.0009
F value	93.01	89.37	301.26		
LSD	1.834	1.8785	1.3339		
		SOD (U/g)			
Control	22.26±0.27^{Aa}	21.70±0.15^{Aa}	21.46±0.33^{Aa}	2.39	0.1722
Cis	12.73±0.44^{Ea}	12.06±0.16^{Fa}	11.70±0.20^{Da}	3.02	0.1235
Tad	16.40±0.30^{Cc}	18.26±0.14^{Db}	19.43±0.17^{Ba}	49.38	0.0002
Los	16.80±0.17^{Cc}	19.10±0.17^{Cb}	20.03±0.17^{Ba}	91.18	0.0001
G.B	17.73±0.14^{Bc}	20.03±0.17^{Bb}	21.16±0.18^{Aa}	105.94	0.0001
G.S	14.73±0.21^{Dc}	17.06±0.26^{Eb}	18.63±0.24^{Ca}	66.66	0.0001
F value	133.63	329.20	250.01		
LSD	0.8573	0.5641	0.7029		

-Values are mean ±S.E
-Values with the different capital letters in the same column are significantly different at (P< 0.05).
- Values with the different small letters in the same raw are significantly different at (P< 0.05).

Table (4): Effect of Tad, G.B, G.S and Los on levels of NO in control and treated rats:

Group	**1st Week**	**2nd Week**	**4th Week**	**F-test**	
				F-value	**P-value**
		Serum NO (Umol/L)			
Control	25.40±0.36 ^{Aa}	25.10±0.15 ^{Aa}	25.33±0.68 ^{Aa}	0.12	0.8902
Cis	15.73±0.54 ^{Fa}	13.00±0.75 ^{Db}	9.90±0.20 C^{c}	28.25	0.0009
Tad	22.66±0.52 ^{Bb}	23.63± 0.27 ^{Bab}	24.00±0.11 ^{ABa}	3.93	0.0811
Los	20.00±0.17 ^{Cc}	22.00±0.17 ^{Cb}	24.13±0.43 ^{ABa}	51.04	0.0002
G.B	18.40±0.55^{Dc}	20.90±0.37 ^{Cb}	23.30±0.86 ^{ABa}	15.13	0.0045
G.S	17.06± 0.17^{Eb}	20.60±0.70 ^{Ca}	22.70±1.17B^{a}	12.70	0.0070
F value	73.74	80.27	70.22		
LSD	1.2992	1.4495	2.1257		
		Kidney NO (Umol/g)			
Control	43.86±0.14^{Aa}	43.46±0.31^{Aa}	44.03±0.14^{Aa}	1.78	0.2480
Cis	20.60± 0.34^{Fa}	17.20±0.23^{Fb}	13.70± 0.26^{Cc}	146.75	0.0001
Tad	32.56± 0.26^{Bc}	37.16±0.26$^{B\ b}$	42.33±0.42^{Aa}	226.17	0.0001
Los	29.76± 0.38^{Cc}	33.90±0.43^{Cb}	39.10±0.54^{ABa}	102.35	0.0001
G.B	26.80± 0.17^{Db}	30.33±0.31^{Db}	39.83± 3.77^{ABa}	9.51	0.0138
G.S	24.03± 0.08^{Eb}	27.86±1.14^{Eb}	35.40± 2.55^{Ba}	12.86	0.0068
F value	1010.62	264.53	35.34		
LSD	0.79	1.6945	5.8006		

- Values are mean ±S.E
-Values with the different capital letters in the same column are significantly different at (P< 0.05).
- Values with the different small letters in the same raw are significantly different at (P< 0.05).

Table (5): Effect of Tad, G.B, G.S and Los on serum levels of mineral ions in control and treated rats:

Group	**1st Week**	**2nd Week**	**4th Week**	**F-test**	
				F-value	**P-value**
		Calcium(mg/dl)			
Control	12.15±0.01 ^{Aab}	12.50±0.17^{Aa}	11.71±0.23^{Ab}	5.45	0.0448
Cis	8.23±0.10 ^{Ea}	7.19±0.17^{Db}	6.25±0.17^{Cc}	39.51	0.0040
Tad	10.24±0.04 ^{Bb}	10.81±0.16^{Ba}	11.24±0.16^{Aa}	13.53	0.0060
Los	10.36±0.06^{Bb}	10.90±0.17^{Bab}	11.48±0.23^{Aa}	10.63	0.0107
G.B	9.39±0.18^{Cb}	10.42±0.19^{Ba}	10.60±0.18^{Ba}	12.37	0.0074
G.S	9.03±0.04^{Db}	9.58±0.27^{Cab}	10.13±0.10^{Ba}	10.35	0.0114

F value	213.28	79.60	118.78		
LSD	0.2865	0.6109	0.5763		
		Potassium (meq/L)			
Control	5.83±0.07Aa	5.97±0.06Aa	5.95±0.04Aa	1.43	0.3113
Cis	4.29±0.02Da	4.15±0.03Eb	4.06±0.03Eb	12.26	0.0076
Tad	5.34±0.03Bc	5.57±0.03Bb	5.73±0.03Ba	30.11	0.0007
Los	5.27±0.01Bc	5.48±0.03Bb	5.63±0.05BCa	22.32	0.0017
G.B	5.05±0.02Cc	5.31±0.02Cb	5.51±0.02Ca	70.81	0.0001
G.S	4.93±0.03Cb	5.06±0.04Db	5.25±0.04Da	16.98	0.0034
F value	149.55	223.71	293.54		
LSD	0.1293	0.1275	0.1212		
		Phosphorus(mg/dl)			
Control	2.54±0.21^{Da}	2.43±0.13^{Da}	2.60±0.01^{Da}	0.23	0.7989
Cis	3.77±0.01^{Ab}	3.87±0.05^{Ab}	4.08±0.03^{Aa}	20.83	0.0020
Tad	3.10±0.02^{Ca}	2.98±0.11^{Ca}	2.73±0.21^{CDa}	1.74	0.2542
Los	3.22±0.02B^{Ca}	3.11±0.09^{BCa}	2.88±0.14^{BCDa}	2.82	0.1371
G.B	3.34±0.03B^{Ca}	3.23±0.03^{BCa}	3.04±0.06B^{Cb}	9.80	0.0129
G.S	3.46±0.03^{Ba}	3.30±0.02^{Bb}	3.19±0.02^{Bc}	22.49	0.0016
F value	19.51	29.38	16.64		
LSD	0.2864	0.2668	0.4005		
		Magnesium (mg/dl)			
Control	2.47±0.03^{Aa}	2.45±0.02^{Aa}	2.38±0.02^{Aa}	2.61	0.1531
Cis	1.02±0.03^{Ea}	0.72±0.02^{Fb}	0.36±0.02^{Fc}	115.47	0.0001
Tad	1.30±0.01^{Cc}	1.52±0.02^{Cb}	2.13±0.02^{Ca}	568.87	0.0001
Los	1.38± 0.02^{Bc}	1.73±0.03^{Bb}	2.24±0.02^{Ba}	228.72	0.0001
G.B	1.21 ±0.01^{Dc}	1.41±0.02^{Db}	1.97±0.01^{Da}	537.23	0.0001
G.S	1.17±0.01^{Dc}	1.30±0.01^{Eb}	1.75±0.02^{Ea}	250.49	0.0001
F value	474.96	544.22	909.89		
LSD	0.0743	0.075	0.0757		

- Values are mean ±S.E
-Values with the different capital letters in the same column are significantly different at (P< 0.05).
- Values with the different small letters in the same raw are significantly different at (P< 0.05).

Table (6): Effect of Tad, G.B, G.S and Los on the level of aldosterone hormone in control and treated rats (pg/ml):

Group	**1st Week**	**2nd Week**	**4th Week**	**F-test**	
				F-value	**P value**
Control	13.00±0.11^{Da}	13.30±0.30^{Ea}	13.63±0.20^{Da}	2.04	0.2112
Cis	18.50±0.50^{Ab}	19.43±0.32^{Ab}	21.00±0.20^{Aa}	11.84	0.0083
Tad	15.60±0.35^{Ca}	14.63±0.23^{Db}	14.10±0.15 ^{Db}	8.62	0.0172
Los	16.56±0.28^{BCa}	15.66±0.28^{Cb}	14.90±0.05^{Cb}	12.61	0.0071
G.B	16.70±0.58^{BCa}	15.83±0.14^{Cab}	15.16±0.20^{Cb}	4.37	0.0674
G.S	17.800±0.55^{ABa}	17.06±0.08^{ab}	16.16±0.31^{Bb}	4.79	0.0570
F value	19.93	72.95	170.36		
LSD	1.3339	0.7606	0.6331		

- Values are mean ±S.E
-Values with the different capital letters in the same column are significantly different at (P< 0.05).
- Values with the different small letters in the same raw are significantly different at (P< 0.05).

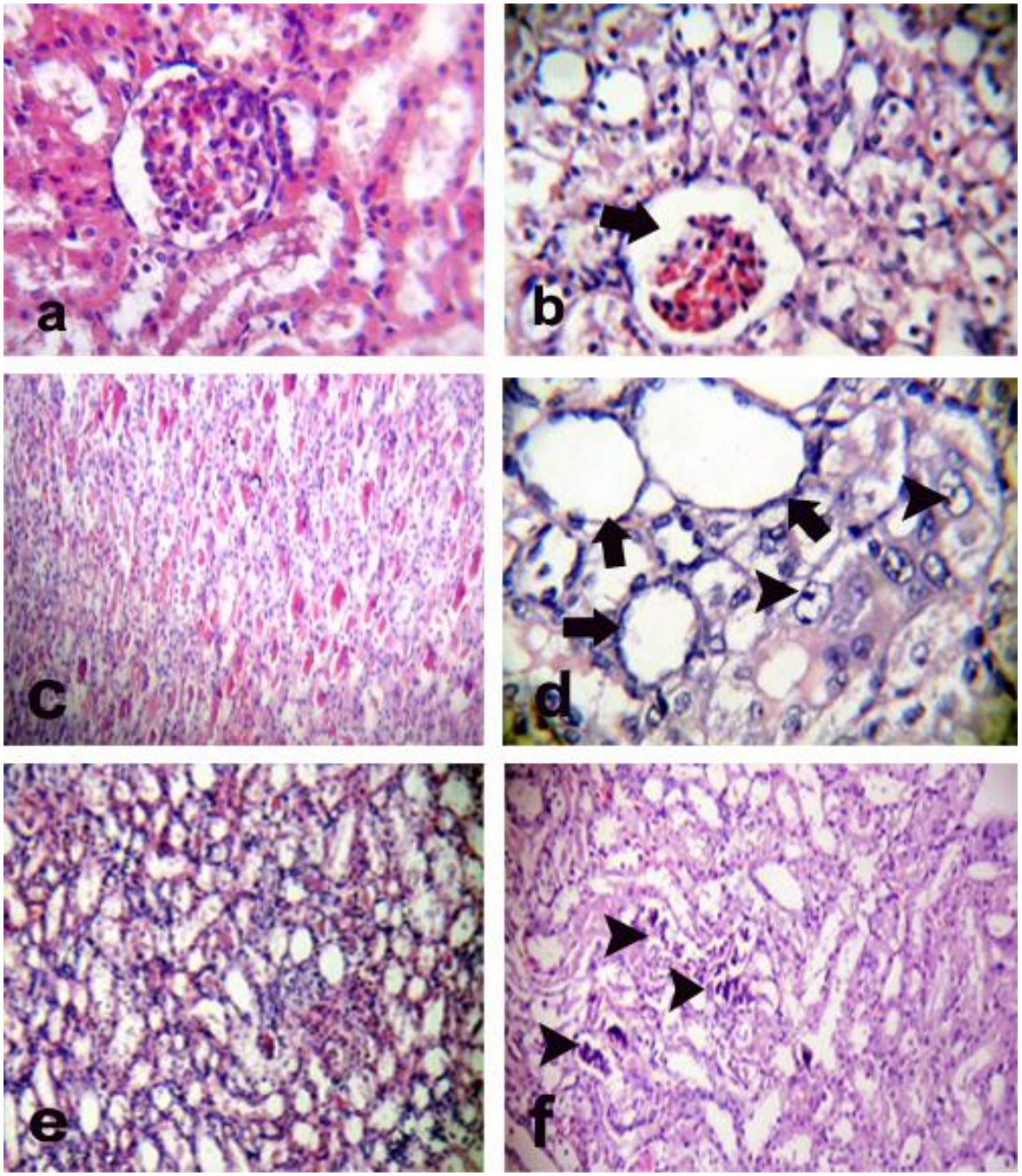

Fig. 1 (a-f): Kidneys after the 1st week shows: a, normal histological picture (Control group, H&E, x 200); b, glomerular shrinkage (arrow) and epithelial cells vacuolation (Cis group, H&E, x 200); c, prominent hyaline casts (Tad group, H&E, x 100), prominent tubular dilation in the coticomedullary and medullary regions. The dilated renal tubules are lined by regenerating, flattened epithelial cells with basophilic cytoplasm (arrows); d, the renal epithelium of adjacent renal tubules has a swollen cytoplasm and enlarged nuclei with dispersed and segregated heterochromatin (arrowheads) (G.B group, H&E, x 200); e, moderate tubular dilation (G.S group, H&E, x 100) and f, mild tubular calcification (arrowheads) (Los group, H&E, x 200).

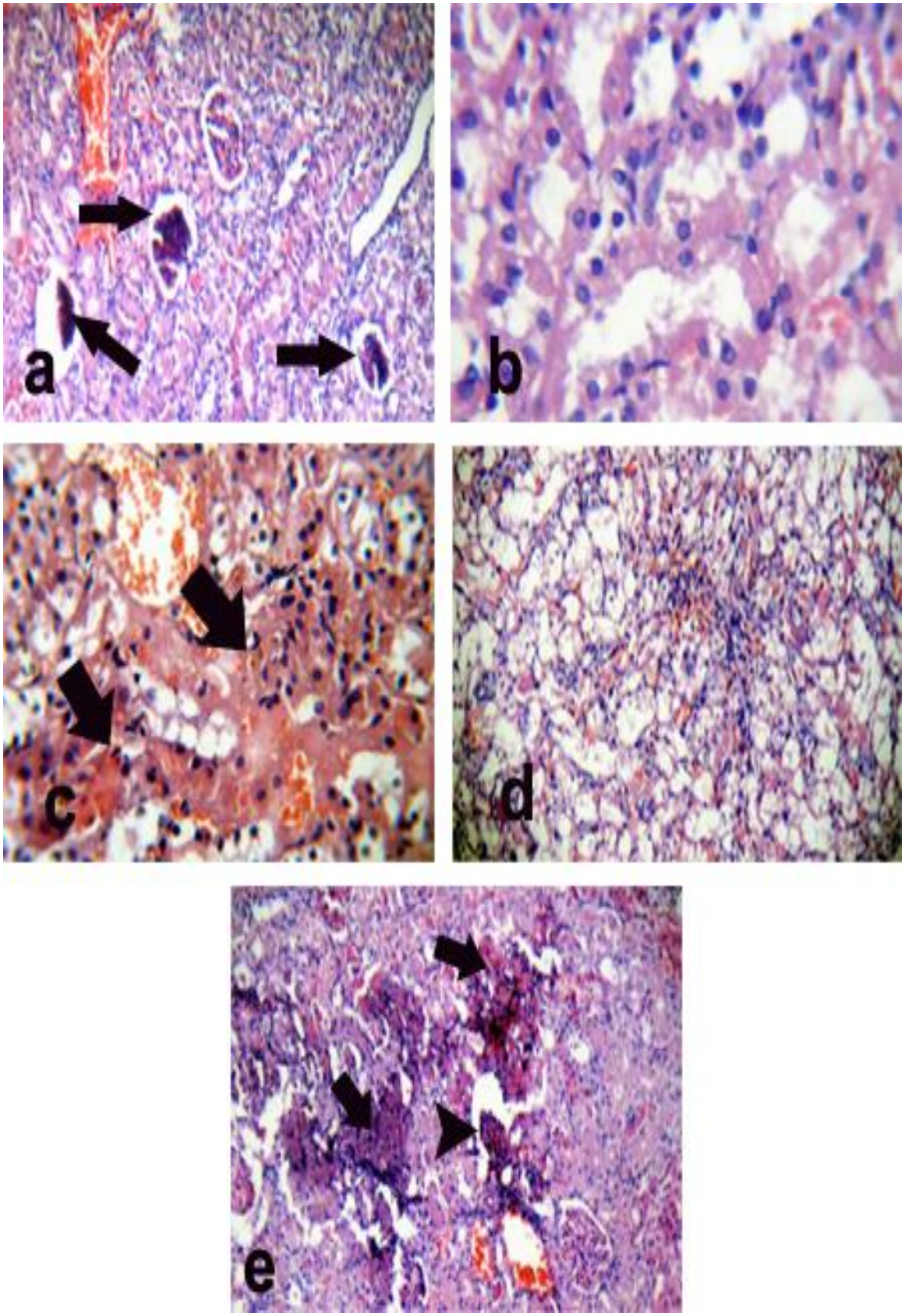

Fig. 2 (a-e): Kidneys after the 2nd week shows: a, severe glomerular sclerosis and atrophy (arrows) (Cis group, H&E, x 100); b, mild epithelial vacuolation (Tad group, H&E, x 200); c, focal necrotic area (arrows) (G.B group, H&E, x 200); d, severe tubular dilatation and epithelial vacuoation (G.S group, H&E, x 100) and e, focal areas of necrosis (arrows) associated with sclerotic glomerulus (arrowhead) (Los group, H&E, x 100).

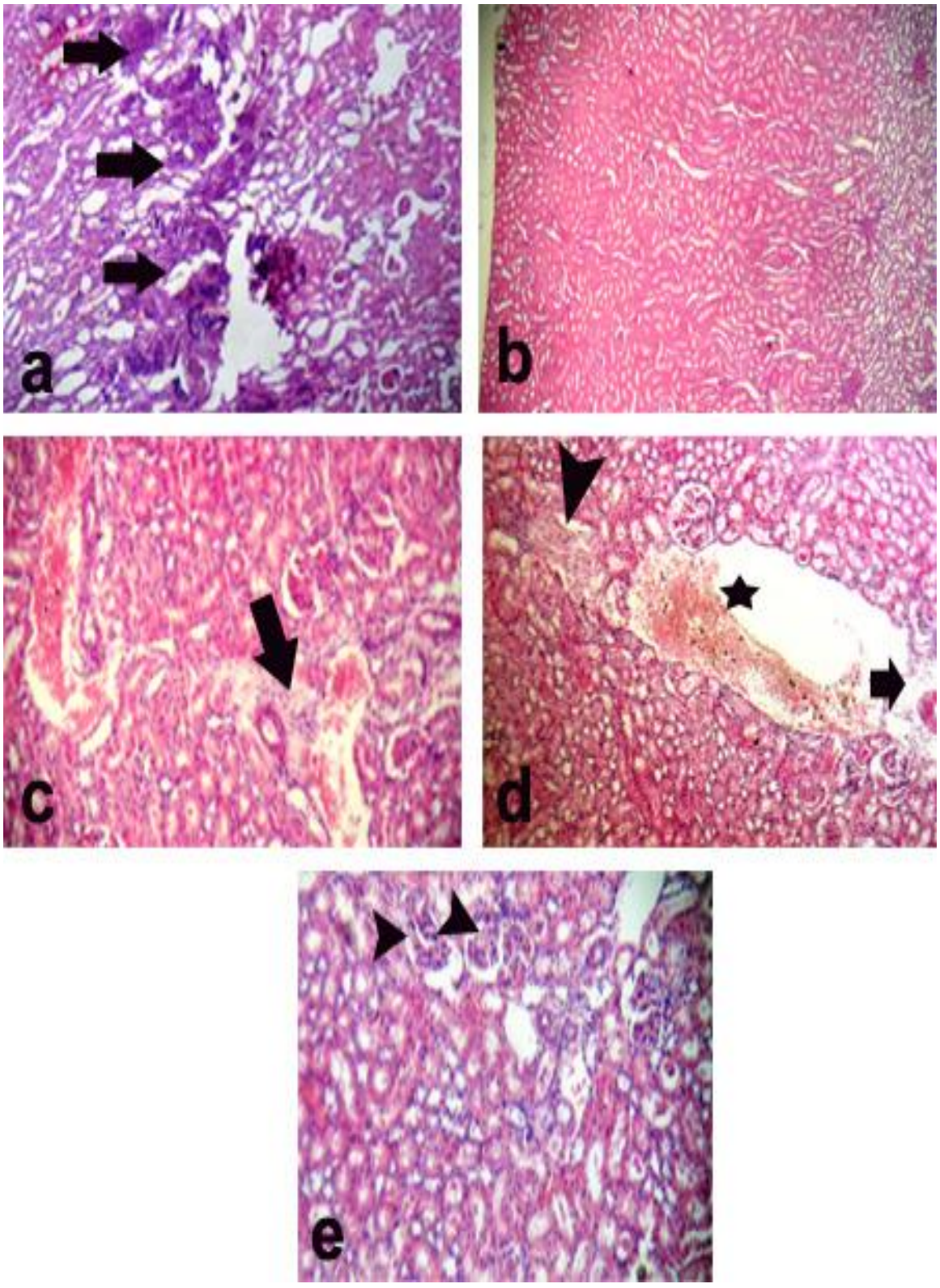

Fig. 3 (a-e): Kidneys after the 4th week shows: a, severe tubular necrosis and calcification at the corticomedullary junction (arrows) (Cis group, H&E, x 50); b, regularly arranged and the renal tubules lined by normal columnar epithelium with eosinophilic cytoplasm (Tad group, H&E, x 50); c, an occasional mild perivascular fibrosis (G.B group, H&E, x 100); d, focal interstitial fibrosis (arrowhead), congestion (asterisk) and perivascular edema (arrow) (G.S group, H&E, x 100) and e, mild tubular dilatation and casts associated with sclerotic glomeruli (arrow heads) (Los group, H&E, x 100).

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