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Ginger and Atorvastatin Attenuates Diazinon Induced Nephrotoxicity

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Abstract

Diazinon (DZ) is an organophosphorus class II pesticide that is broadly used in agricultural fields and household environments. Ginger (GE) and Atorvastatin (AT) have been shown to have potent anti-oxidative, anti-inflammatory and anti-apoptotic properties in some animal models of toxicity. Here, we investigated the protective properties of GE and AT on DZ-induced renal damage in rats. Total of seven rat groups (7 rats/group) received saline solution (control), ginger (GE), Atorvastatin (AT), Diazinon (DZ), DZ/GE, DZ/AT, and DZ/GE/AT through gavage once per day for 30 days respectively. Blood were collected and sera were separated for analysis of kidney failure parameters (Urea and Creatinine). Anti-oxidation parameters were also measured in all the kidneys. Histopathology and IHC of cleaved caspase 3 were also performed on the renal tissues of different groups. Our results showed that GE and AT attenuated DZ-induced renal damage by reduction of malondialdehyde (MDA) and apoptotic (cleaved caspase-3) markers and improving the level of antioxidants and histological picture. Overall, the protective effects of GE and ATR on DZ-induced nephrotoxicity was prominent in DZ intoxicated rats co-treated with one or both compounds and this could be attributed to their potent antioxidative, anti-inflammatory and antiapoptotic properties.

Keywords: Diazinon; Ginger; Atorvastatin; Renal; Histopathology; Caspase 3; Antioxidants.

1-Introduction

Diazinon (DZ, 0, 0-diethyl-0-[2-isopropyl-6-methyl-pyrimidin-yl] phosphorothionate), is a class II organophosphate compound associated with high risk on communities. It is widely used as a pesticide throughout the world to eradicate insect populations and boost agricultural output [1]. The residues of this pesticide on fruits and vegetables have a negative impact on human health [2, 3]. The poisoning of organophosphorus (OP) causes many deaths among people in many nations [4]. It has been shown that DZ induces damage in liver and cardiovascular, renal, and nervous systems [5, 6]. Despite the fact that inhibition of acetylcholinesterase is the primary mechanism of OP toxicity, some other studies pointed to the significance of oxidative stress (OS) in DZN induced toxicity. In one study, DZN was proven to increase the lipid peroxidation as a sign of OS in some vital organs [7]. DZN and its metabolites are mainly excreted throughout kidneys and often lead to moderate to severe renal damage. DZN poisoning is always associated with an elevation in the levels of urea and creatinine (Cr) [8]. DZN was also found to enhance lipid peroxidation and decrease the activity of the renal antioxidant enzymes [9, 10]. Overall, OS have a negative impact on all renal functions, such as filtration of blood, fluid and electrolytes balance, hemodynamics control, etcs [11]. OS also modulates gene expression and enhance apoptosis and necrosis [11]. Based on all these findings, there is a great demand for antioxidants to counter the effect of DZN on the renal system.

Statins are compounds that inhibit the action of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase which has a vital role in cholesterol synthesis, thus reducing the endogenous level of cholesterol significantly [12]. The beneficial effect of cholesterol reduction by statins have been documented in some studies where the treatment decreased the rates of morbidity and mortality due to coronary heart disease [13, 14]. Recently, statins were shown to have nephroprotection role in several models of renal damage, such as diabetic nephropathy. Statins are currently being investigated as a renal protective agent in some clinical trials [15, 16]. Atorvastatin (ATR) is one of the strong antioxidant members of statins. It has been shown that ATR has potent antithrombotic, anti-inflammatory and antioxidant properties in some experimental animal models of diseases [17-19]. However, the potential nephroprotection of ATR against pesticide toxicity has not been studied yet.

GE (Zingiber officinale Roscoe, Zingiberacae) is considered one of the popular spices and medicinal plants worldwide. It has been shown that GE have beneficial effects on gastrointestinal and cardiovascular systems and has anti-inflammatory and antioxidant activities [20, 21]. Prior studies have shown that GE also have hypoglycemic, antipyretic, antibacterial, antimigraine, hepatoprotective, diuretic and hypocholesterolemia effects [22-24]. The nephroprotection of GE have also been shown in rat models of carbon tetrachloride, ischemia/reperfusion, and alcohol induced kidney injuries [25-29]. However,

the potency of GE against DZ induced renal damage remains unknown. In this study, the renal protection of ATR and GE was evaluated in rat model of DZ induced renal damage by using biochemical and histopathological parameters besides evaluation the extent of oxidative stress.

2. MATERIALS AND METHODS

2.1. Chemicals

Diazinon (DZ)-60® was bought from Drug Pharmaceutical company located in Cairo, Egypt. GE tablets (400 mg/tablet) was also purchased from MEPACO Company that is located in Abu Sultan, Ismailia, Egypt. ATR tablets (40 mg/tablet) was obtained from Delta Pharma Company, Egypt.

2.2. Experimental design

Forty-nine Wister Albino male rats with average weight of 185 - 200 grams were purchased from the Egyptian Organization for Biological Products and Vaccines. All rats were acclimated for 1-week period and housed at a temperature of 25°C, with a 12:12 h light/dark cycle and free access to water. The rats were fed with commercial pellets. Experimental rats were subdivided into 7 groups with total of seven rats in each group. Group (1) rats only received saline solution (5 ml/kg orally). Group (2) rats received GE (100 mg/kg/day; [30]). Group (3) rats were supplemented with ATR (20 mg/kg/day; [31]). Group (4) rats were intoxicated with DZ (20 mg/ kg/day; [32]) to induce renal injury. Group (5) rats received DZ (20 mg/ kg/day) + GE (100 mg/kg/day). Group (6) rats received doses of DZ (20 mg/ kg/day) + ATR (20 mg/kg/day) while Group (7) rats received DZ (20 mg/ kg/day) + GE (100 mg/kg/day) + ATR (20 mg/kg/day). All treatments were administered by oral gavage, once daily for 30 days. The study plan was approved by Institutional Ethical Committee, Faculty of Veterinary Medicine, Benha University (Approval No BUFVTM 05-08-22).

2.3. Blood sampling and processing

All the rats were anaesthetized briefly with isoflurane after 24 hrs of the last treatment. Blood samples were mainly collected from the retro-orbital blood plexus in serum vacutainer collecting tubes and serum was separated by centrifugation of samples at 1200 g for 15 min. The collected sera were kept at -20°C for further biochemical analysis.

2.4. Serum biochemical analysis

The concentration of serum urea and creatinine in all rats were calculated based on previous studies [33, 34].

2.5 Renal antioxidants analysis

The oxidative stress markers in kidneys were measured in all groups. The markers included MDA [35] , CAT [36], SOD [37], and GSH [38].

2.6. Histopathological studies

Renal specimens were harvested from the rats in all groups and fixed in 10% neutral buffered formalin for 72 hrs. The fixed specimens were then dehydrated using ascending grades of ethyl alcohol (70-100%), cleared using xylene, and embedded in a mold of paraffin wax. 4-5 µm tissue sections were cut and stained with hematoxylin and eosin (H&E) stain [39].

2.7. Immunohistochemical assessment of caspase 3

Renal sections were warmed at 65°C for 1 hr, deparaffinized, rehydrated, and incubated with antigen retrieval solution in 10 mM citrate buffer (pH 6.0) using a steamer for 50 min, followed by slow cooling. The tissue slides were incubated with 3% hydrogen peroxide (H_2O_2), washed with water, and incubated with the blocking antibody. Renal sections were incubated overnight at 4°C with rabbit anti rat cleaved caspase-3 antibody (Santa Cruz Biotechnology Inc., Dallas, TX, USA, 1:100 dilutions) for 2 hrs. at room temperature. Secondary biotinylated antibodies were incubated with the tissue sections on day 2 for 30 min at room temperature. Diaminobenzidine (DAB) was then added on tissue sections for 10 min and counterstained with hematoxylin stain. Tissue sections were dehydrated through ascending grades of ethanol. Brown coloration of cytoplasmic and or nuclear staining (DAB) was considered as positive staining.

2.8. Statistical analysis data

The data collected from the experiment were expressed as mean±SEM. GraphPad prism was used to create graphs of parameters in different groups. One-way ANOVA was used to analyze the variance in data between groups. Differences were regarded statistically significant at ≤P 0.05.

3. RESULTS

3.1 Serum biochemical analysis:

No significant elevation in the serum level of urea was noticed in control, GE and ATR treated rats (Figure 1). On the other hand, there was marked increase in urea level in the sera of DZ intoxicated rats. Compared to DZ treated group, there was marked decline in serum urea level in GE, and ATR treated rats. In intoxicated rats co-treated with either GE and or ATR, there was also a significant drop in urea level to a nearly normal level. The serum level of creatinine was nearly normal in control rats. There was a statistical increase in creatinine level in DZ treated rats compared to control rats. All treated rats (ATR, GE) and DZ co-treated ones (DZ/ATR, DZ/GE and DZ/ATR/GE) showed low creatinine blood level compared to DZ intoxicated rats (Figure 1).

3.2 Renal antioxidants analysis:

There was no evidence of oxidation stress in the kidneys of control, GE and ATR treated rats as demonstrated with normal low level of MDA and high level of SOD, CAT and GSH (Figure 2). DZ

intoxicated rats on the other hand showed significant higher level of MDA and lower level of CAT, SOD and GSH compared to control rats. Treatment of DZ intoxicated rats with GE, ATR or both improved the renal antioxidant parameters evidenced by sharp decrease in MDA and marked increase in CAT, SOD and GSH (Figure 2).

3.2 Histopathology:

In control, GE and ATR groups, most kidneys had apparently normal glomeruli and renal tubules (Figure 3A-C). Mild congested glomerular tuft and intertubular blood vessels were seen in few rats in ATR treated group (Figure 3C). In DZ treated rats, renal tubular degeneration and necrosis were more pronounced and extensive compared to other groups. Glomeruli were congested with variable degree of degenerated to necrotic podocytes. Most cortical renal tubules showed vacuolar degeneration with presence of intraluminal basophilic hyaline cast (Figure 3D-E). Other renal tubules had necrotic epithelial lining with pyknotic or karyolitic nuclei. Multifocal extensive intratubular oedema and hemorrhage were seen in several rats in this group (Figure 3F). Vacuolation of tunica media of some intertubular blood vessels was also evident in some rats in this group (Figure 3G). Multifocal intertubular inflammatory mononuclear cellular infiltration was also evident in many kidneys in DZ group (Figure 3H). In DZ intoxicated rats co-treated with GE or ATR, most kidneys showed normal glomeruli and null to mild degenerated renal tubules (Figure 3I-K). The rats intoxicated with DZ and co-treated with ATR had less ameliorative effect compared to DZ co-treated with GE group where moderate tubular degeneration with intraluminal necrotic cellular debris were seen in some rats in this group (Figure 3J). There was only mild congestion of interstitial blood vessels with occasional mild tubular degeneration in intoxicated rats co-treated with GE and ATR (Figure 3K).

3.1 Immunohistochemistry:

There was no detectable level of CC-3 protein in the renal sections of control, GE and ATR treated rats (Figure 4A-C). In DZ intoxicated rats, diffuse, and punctuate strong cleaved caspase 3 staining was prominent mainly in the glomerular tuft and renal tubular epithelia (specially distal convoluted tubules) (Figure 4D-F). A marked reduction in renal tubular CC-3 protein expression was noticed in intoxicated rats co-treated with either GE or ATR or both (Figure 4G-I). Compared to DZ treated rats, there was significant reduction in CC-3 positivity in control, GE and ATR treated group. In addition, the percentage of CC-3 positive renal cells were significantly decreased in groups co-treated with either GE, ATR or both.

4. DISCUSSION

DZN is an organophosphorus hazardous compound that has a negative impact on human and animal health. DZN has many adverse effects on different body systems such as cardiovascular, nervous system, and renal system [40]. DZ is mainly excreted through kidneys causing damage to their cells [41]. The damage induced by DZ is mainly attributed to the generation of reactive oxygen species and lipid peroxidation processes that enhance the apoptosis and necrosis of cells [7]. In the last decade, many efforts were made to find a better antioxidant that could prevent DZN induced renal toxification. Prior studies using ATR and GE as anti-oxidants showed promising results in prevention of kidney failure in some animal models of toxicity [18-20, 23, 24], however the efficiency of these compounds have not been evaluated in prevention of DZ induce renal toxicity model and this was our primary goal in this study.

Our results demonstrated that dosing DZN for 1 month resulted in an elevation in urea and creatinine thus reflecting impairment in renal function and nephrotoxicity. Consistent with these findings, the blood urea nitrogen and serum creatinine showed a dose-dependent increase in their levels upon treatment with different concentration of DZ [9]. Histopathological findings of DZN intoxicated rats in our study showed severe degeneration of renal tubular epithelia and podocytes with vascular abnormalities in form of congestion, edema, and hemorrhage. Overall, these findings are in accordance with other studies that reported that DZ intoxication induced obliteration in the space in Bowman's capsule with tubular epithelial degeneration/necrosis and congestion of blood vessels. It has been also shown that the process of apoptosis is mediated in several tissues of the body due to the activation of caspase dependent pathways resulting in cell death [42]. In this study, DZ induced upregulation of cleaved caspase 3 in in the glomerular tuft and renal tubular epithelia thus reflecting the necrotic effect of DZ on kidneys. Prior studies indicated that DZ induced lipid peroxidation evidenced by high level of MDA and markedly reducted in the levels of antioxidants i.e catalase in renal tissues [9]. Similar findings were reported in our study.

GE as a natural product has also been documented as strong antioxidant against many harmful oxygen species released in many drugs-induced stress conditions [43-45]. GE was examined as a potential nephroprotective agent in this study to prevent renal damage induced by DZ. GE improved the kidney functions in DZ intoxicated co-treated rats evidenced by the decrease in the level of Urea and Creatinine. It also mitigated the damage effect of DZ on kidneys and preserved the histological architecture and morphology

of kidneys in rats. This protection by GE could be attributed to its enhancement of anti-oxidants enzymes in kidneys and impeding the lipid peroxidation induced by DZ. In addition, the absence of cleaved caspase 3 staining in renal cells in DZ intoxicated rats co-treated with GE indicates the inhibition of cleaved caspase 3 in the renal tissue thus reflecting anti-apoptotic properties of GE. Similar to our findings, GE extracts mitigated CCl4 induced renal damage via scavenging free oxygen species, promotion of kidney functions, inhibition of inflammatory cytokines, and normalizing renal histopathological architect [25].

ATR (AT) is one of the strong antioxidant members of statins, however their nephroprotection against OP poisoning remain unclear. Conflicting data from previous studies have been reported about the renoprotection capability of AT. While some studies reported that AT is actually nephrotoxic to the kidneys, others found it to be useful and supportive against nephrotoxic agents [46, 47]. AT has been shown to protect kidneys against CdCl2 poisoning, downregulate endogenous antioxidant enzymes and improve biochemical parameters and histological morphology of kidneys [19, 48]. On contrast, one study reported that AT induced nephrotoxicity and damage in kidneys while Arjunolic acid and vitamin C protected kidneys from AT-induced nephrotoxicity [18]. In our experiment, AT inhibited the nephrotoxicity of DZ and improved the kidney function as demonstrated by low serum level of urea and creatinine as well as reduced renal histopathological abnormalities in DZ intoxicated co-treated rats. In addition, cleaved caspase 3 expression was null to minimal in DZ co-treated rats with AT. AT also reduced the level of MDA and upregulated antioxidant markers. All these findings reflect the protection of AT against DZ induced renal damage.

5. CONCLUSION

Our findings indicate that DZ is very nephrotoxic and causes programmed cell death (apoptosis) of renal tubular cells. The administration of GE or AT or both could mitigate the severity of DZ toxicity on kidneys by their antioxidant capabilities and free radical scavenging properties. However, it is important to note that more research is needed to fully understand the mechanisms underlying this protective effect and to determine the potential benefits and risks of using GE and AT as protective agents in clinical practice.

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Figure Legends

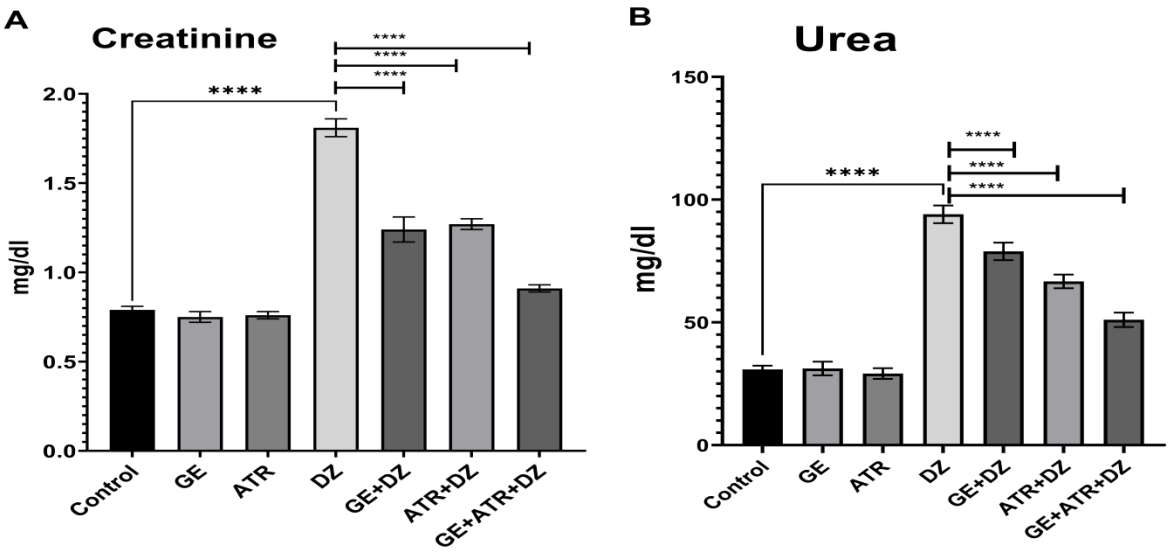
Figure (1) The effect of GE and ATR on the levels of urea and creatinine in the blood of DZ intoxicated rats (n=7)

Figure (2) The Effect of GE and ATR on renal antioxidants parameters in DZ-intoxicated rats (n=7).

Figure (3) Renal histopathological photomicrographs show; A-B) nearly normal renal tubules and glomeruli in control (A) and GE (B) treated rats (200X). C) Mild congestion (arrowhead) of intertubular and periglomerular blood vessels in ATR treated rats (200X). D-H) renal tubular degeneration (D)(arrowhead), intraluminal basophilic cast (E) (arrowhead), intratubular hemorrhage (F)(arrowhead), vacuolation of T. media of an intertubular blood vessels (G)(arrowhead) and focal intertubular mononuclear inflammatory cellular infiltration (arrowhead)(H) in the kidneys of DZ treated rats (200X). I-K) normal glomeruli and mild degenerated renal tubules in GE+DZ (I), ATR+DZ (J) and GE/ATR/DZ treated rats (200X).

Figure (4) Immunohistochemical staining of cleaved caspase of renal sections show; (A–C) No cleaved caspase protein expression in the glomeruli and renal tubules in control (A), GE (B) and ATR (C) treated groups (100X). (D-F) strong cytoplasmic staining of cleaved caspase 3 in the glomeruli and renal tubular epithelial cells of DZ intoxicated rats (D) (100X) (E-F)(200X). (G-I) No cytoplasmic cleaved caspase 3 expression in GE+DZ (G), ATR+DZ (H) and GE+ATR+DZ (I) treated rats (100X).

Figure 1:



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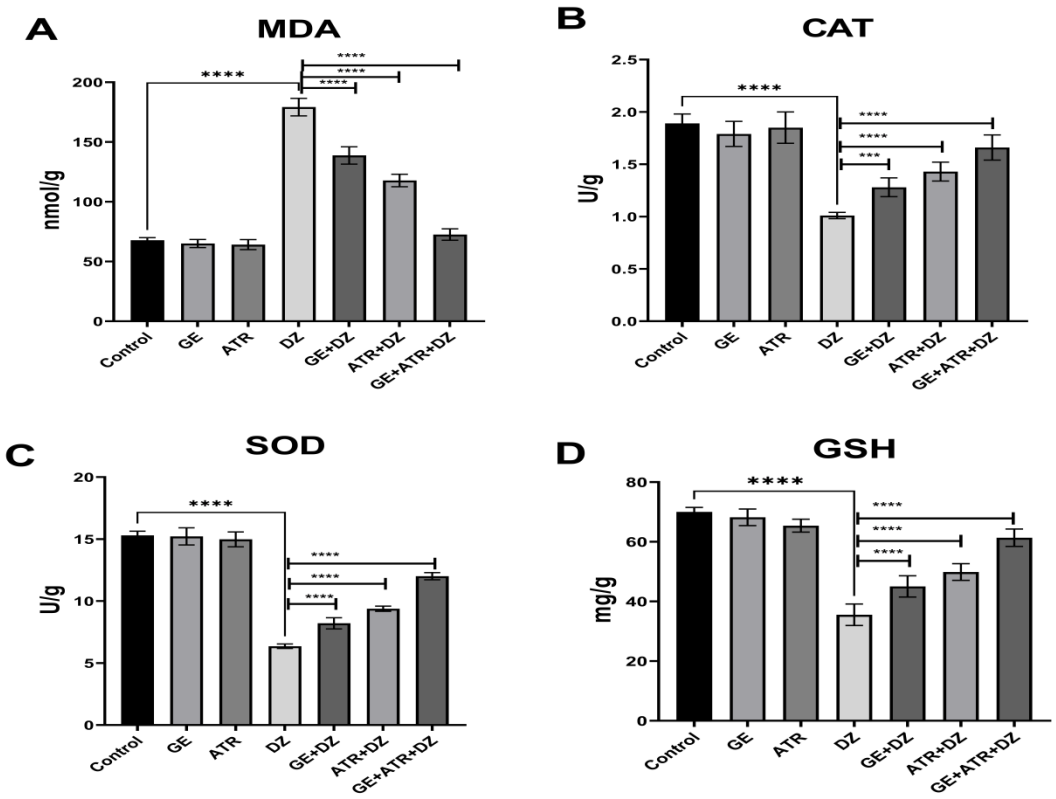

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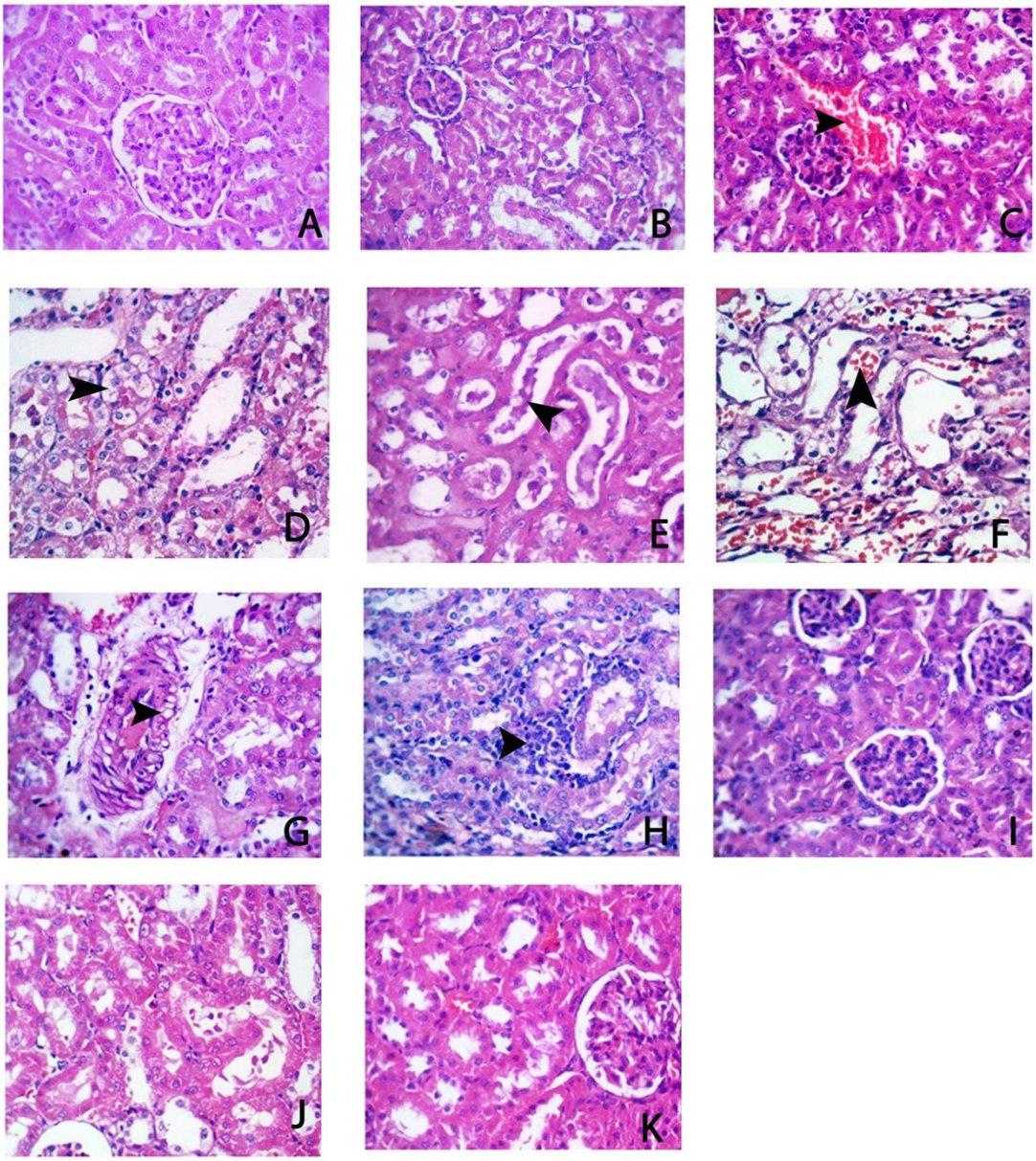

Figure 4: