

# In Vivo Antioxidant And Kidney Protective Potential Of Atorvastatin In Rat Cadmium-Induced Toxicity

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## Research Article

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# Abstract

**Purpose:** Environmental and occupational exposure to cadmium chloride is known to cause nephrotoxicity linked with oxidative stress in humans and animals. This study used Atorvastatin to examine its effect on cadmium chloride-induced nephrotoxicity in rat model using biochemical and histological methodologies.

**Methods:** Experiments were performed on 56 adult male Wistar rats ( $200 \pm 20$  g), randomly assigned to eight groups. Atorvastatin was administered by oral for 15 days at 20 mg/kg/day, started 7 days before cadmium chloride intraperitoneal administration (1, 2, and 3 mg/kg) for eight days. On day 16, blood samples were collected, and kidneys were excised to evaluate the biochemical and histopathological changes. Cadmium chloride significantly increased malondialdehyde (MDA), serum creatinine (Cr), blood urea nitrogen (BUN), and decreased superoxide dismutase (SOD), glutathione (GSH), and glutathione peroxidase (GPx) levels.

**Results:** Administration of Atorvastatin (20 mg/kg) significantly improved lipid peroxidation, glutathione and activities of antioxidant enzymes and significantly decreased BUN and Creatinine. Atorvastatin clearly improved the histological changes, demonstrating its protective role against Cadmium chloride-induced kidney injury.

**Conclusion:** Treatment with Atorvastatin significantly improves all biochemical parameters and suggests a protecting role against cadmium chloride-induced oxidative stress and histological changes in rat kidney.

## Introduction

Cadmium (Cd) is frequently used in various industrial processes and considered as one of toxic metals to environment. Occupational and environmental exposures to Cd are linked to industrial activities. (Mezynska and Brzóska, 2018) The kidney is known as the main target organ for chronic Cd exposure. (Rana et al., 2018) The biological half-life of Cd in humans reported 10 to 30 years in the kidney cortex which may account for a higher occurrence of nephrotoxicity. (Moitra et al., 2014) In the kidney, the accumulation of 50  $\mu\text{g}$  Cd/g, wet tissue weight resulted in renal dysfunction (Satarug et al., 2000).

Evidence suggests that Cd absorption may result in oxidative stress. Cd-induced oxidative injury includes a difference between production and removal of reactive oxygen species (ROS) in kidney. Furthermore, the production and accumulation of ROS are associated with hydrogen peroxide ( $\text{H}_2\text{O}_2$ ), hydroxyl radical ( $\text{OH}^-$ ), superoxide anions, singlet oxygen, lipid hydroperoxides and phospholipid hydroperoxides, (Patra et al., 2011) causing inflammation and injury in cell membrane, enzymatic pathways, and connective tissue structures. (Gabr et al., 2019) Cd-induced nephrotoxicity may be related to toxic intermediates generated in the kidney. (Talebpour Amiri et al., 2018) Moreover, decrease in antioxidant activity may have a pathological role in biological reactions and Cd-induced nephrotoxicity. (Şişman et al., 2003) Cd is filtered

by the glomeruli and is then reabsorbed by the epithelial cells of the proximal tubule, possessing a potent toxic effect on the kidney.(Rana et al., 2018; Yang and Shu, 2015)

Cd is known to cause physiological and biochemical effects in animals and humans.(Bernhoft, 2013; Patra et al., 2011; Renugadevi and Prabu, 2009) Cd administration in rats resulted in significant decrease of antioxidant indicators such as superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase (CAT), and glutathione (GSH), and a rise in malondialdehyde (MDA) peroxidation marker in kidney. (Andjelkovic et al., 2019; Evcimen et al., 2020; Hormozi et al., 2018; Kim et al., 2018) Chronic exposure to Cd may attribute to some morphological and metabolic alterations, causing an increased dose-dependent in blood urea nitrogen (BUN) and Creatinine in rats.(Renugadevi and Prabu, 2009) Meanwhile, Long-term Cd exposure can develop tissue necrosis in rats.(Erboga et al., 2016; Kim et al., 2018)

Atorvastatin (AT) is the leading antioxidant of the class of statins and has been shown to possess anti-inflammatory, antioxidant (10 mg/kg, for 10 days),(Pal et al., 2015) antithrombotic (10 mg/kg/day), (Ozbek et al., 2009) antiplatelet, immunomodulatory (40 mg for 4 weeks),(Fuentes-Orozco et al., 2018) and antitumor effects in experimental animal models.(Garjani et al., 2012) Doses of Statin compounds at 20mg/day have reduced inflammation by HMG-CoA reductase inhibitors, lowered low-density lipoprotein (LDL) cholesterol in plasma, and promoted antioxidant effects by suppressing oxidation pathways. (Goodarzi et al., 2016; Wang et al., 2017) AT is a potent HMG-CoA reductase inhibitor and effective in lipid profiles, lipid peroxidation, and antioxidant systems.(Yan et al., 2020) It has been reported that Atorvastatin ameliorated renal dysfunction,(El-Moselhy and El-Sheikh, 2014) morphological changes,(Yan et al., 2020) and oxidative stress injuries in rats.(Wu et al., 2014)

Overall, the widespread emission of Cd has increased exposure in working and general population and demanded further research to prevent health-related hazards. However, few experimental studies have investigated and reported the protective role of atorvastatin on toxic metals in animal model with renal failure. As per our knowledge, the protective role of AT on Cadmium-induced nephrotoxicity in rats has not been demonstrated. Therefore, the main goal of the current experiment was to investigate in vivo biochemical and histological changes induced by CdCl<sub>2</sub> on the kidney of adult rat model. We evaluated the possible role of Atorvastatin in protecting CdCl<sub>2</sub>-induced renal toxicity in rats and in ameliorating the renal oxidative stress biomarkers and histological changes in rats.

## **Materials And Methods**

### **2.1. Experimental Animals**

Adult Wistar male rats (n = 56) weighing between 200 and 220 g were used. The animals were housed in metal cages (7 rats in each cage) under hygienic conditions and maintained at 22 ± 2°C and 12-hour light-dark cycles with free access to food and water. Rats were acclimatized for one week before treatment commenced.

### **2.2. Ethics**

Prior to the experiment, we reviewed and ensured that the protocols were in accordance with the guidelines of animal acts proposed by the institutional Ethical Review Board of Semnan University of Medical Sciences (IR.SEMUMS.REC.1395.177).

## 2.3. Drug and Chemicals

AT was purchased from Tehran Chemie pharmaceutical Co. Cadmium chloride ( $\text{CdCl}_2$ ) was obtained from Merck (Darmstadt, Germany). Biochemical markers were assessed using Rat SOD, GPx, MDA, GSH ELISA Kits from ZellBio, GmbH, (Germany) as instructed by manufacturer. BUN and Creatinine in the kidney tissues were detected as instructed by kits obtained from Pars Azmun Pharmaceutical (Iran). Purchased Chemicals were of standard grade and purity for performing experimental tests.

## 2.4. Rat treatments

Rats pretreated with AT (20-mg/kg),(Goodarzi et al., 2020; Sun et al., 2009) dissolved in saline solution and gastric gavage at a dose of 4 ml/kg. Doses of  $\text{CdCl}_2$  (1, 2, and 3 mg/kg),(Golbaghi et al., 2019) was dissolved in distilled water and was administered at a dose of 2-ml/kg intraperitoneally. Cd or its vehicle (normal saline) was used 30 minutes after gavage administration of AT or its vehicle. Rats were treated with intra-gastric gavage of saline, AT, or vehicle of AT for 15 days, which started 7 days before intraperitoneal injection of cadmium chloride or its vehicle. On day 16 samples were taken for biochemical and histological analyses. Period of experimental treatments is shown in Fig. 1.

## 2.5. Experimental groups

The wistar rats were randomly assigned into eight groups of seven rats in each group. The first group of rats received physiologic saline. The second group treated with oral gavages a dose of 20 mg/ kg /day AT for 15 days. The third, fifth, and seventh groups received intraperitoneal (i.p.)  $\text{CdCl}_2$  with doses of 1, 2 and 3 mg/kg, while the fourth, sixth, and eighth groups pretreated with oral gavages containing 20 mg AT/kg body weight, 30 minutes prior to the intraperitoneal (i.p.) administration of  $\text{CdCl}_2$  at 1, 2 and 3 mg/kg. All animals received  $\text{CdCl}_2$  from day 8 to day 15. After 24 hours of the last administration blood samples were taken from the heart under anesthesia with sodium pentobarbital (50 mg/kg). Excised Kidneys were washed thoroughly with saline solution. Obtained parts of kidney tissues were fixed and examined by light microscopy, using hematoxylin and eosin (H&E) staining technique and a 0.5·0.5-cm<sup>2</sup> slices of additional half was cut for the measurement of GSH, SOD, MDA and GPx.

## 2.6. Histological analyses

Renal tissues were fixed in buffered formalin (10%) for 48 hours and embedded in paraffin wax. Slices of 10  $\mu\text{m}$  were cut and placed on glass slides. After making and drying kidney tissue slides, they were stained with hematoxylin and eosin (H&E) method. Kidney cell injury was examined based on dilated nuclei, loss of staining capacity and swelling of kidney tubular cells. Five fields of each slide were randomly selected and photographed under a magnification of x400 and evaluated by a pathologist and a histologist.

## 2.7. Biochemical analyses

The samples of kidney tissue were washed and homogenized with phosphate buffered saline. The supernatants were prepared by centrifuging the homogenates at 800× g for 10 min at 4°C, and preserved the samples in -80°C. The activities of GSH, MDA, GPx, and SOD were measured in the obtained supernatants,(Yang and Shu, 2015) Serum was separated from blood samples by centrifugation at 3000 rpm for 20 minutes and stored at - 20°C until the measurement of parameters. Serum levels of BUN, CR and the renal tissues levels of GSH, MDA, GPx, and SOD were measured as instructed by relevant kits. Serum level of BUN was assayed using colorimetric kit according to the method described. Serum level of Cr was measured by Jaffe's method.(Brouwers et al., 2013) The Concentrations of GSH, MDA, GPx, and SOD in kidney tissues were measured as per the instructions of kits from ZellBio GmbH (Ulm, Germany).

Thiobarbituric acid reaction with MDA Assay kit estimated the level of lipid peroxidation. Adding thiobarbituric acid to MDA results in the formation of red complex product that was measured at 532 nm by a NanoDrope Spectrophotometer.. The detection limit of MDA was 0.1 µM.(Pirmoradi et al., 2019) An assay kit (Zellbio Co) was used to measure the activity of SOD in kidney tissue. Measurement of SOD was based on an enzyme reacting with superoxide anion to produce oxygen and hydrogen peroxide. GSH and GPx were quantified by colorimetric method at 412 nm, using chemical assay kits ZellBio GmbH, (Ulm, Germany) with a 0.1 mM detection limit.(Sheikh, 2016) The content of renal Gpx was assessed by measuring NADH catalyzed one micromole GSH per minute to Oxidized glutathione (GSSG). The protein content of supernatant was assayed according to method described by Bradford method using standard bovine serum albumin at 560 nm.(Verdi et al., 2005)

## 2.8. Statistical analyses

Statistical analyses were performed using the Graphpad Prism 8 software. Experimental data were processed to present the Standard Error Means. Data were analyzed by one-way analysis of variance (ANOVA), and Tukey's multiple comparisons tests were performed to compare the means of all groups to the mean of every other group. Differences were statistically considered significant at  $P < 0.05$ .

## Results

### 3 – 1. Effects of Cd and Atorvastatin on kidney MDA level

MDA level in the rat kidney homogenates increased significantly in the group treated with CdCl<sub>2</sub> compared to control group (Cd 3 mg/kg/day, \*\*P = .0011 and Cd 2 mg/kg/day \*P = 0.0428). Atorvastatin at dose of 20mg/kg/day significantly decreased MDA level in CdCl<sub>2</sub> treated rats (Cd 3 mg/kg/day + AT 20mg/kg/day, \*P = 0.0268) as illustrated in Fig. 2.

The activities of non-enzymatic antioxidants (GSH) and, enzymatic (SOD and GPx) in the kidney of rats are shown in Figures 3-a, 3-b, and 3-c.

#### 3.2.1. Glutathione GSH

The effect of CdCl<sub>2</sub> on GSH levels and treatment with a combination of CdCl<sub>2</sub> plus AT on GSH concentration of rat renal tissues is depicted in Fig. 3-a. The administration of CdCl<sub>2</sub> significantly decreased GSH concentration compared to control values in kidney (Cd 3 mg/kg/day, \*\*P = .0078). The combination of CdCl<sub>2</sub> with AT significantly altered kidney GSH content compared to CdCl<sub>2</sub> treated rats (Cd 2 mg/kg/day + AT 20mg/kg/day, \*P .0401 and Cd 3 mg/kg/day + AT 20mg/kg/day, \*P = .0390).

### **3.2.2. Superoxide dismutase (SOD)**

In rats given CdCl<sub>2</sub> at a dose of 3 mg/kg, SOD activity significantly lowered compared to control group (\*\*P = 0.0056). Equally, pretreatment of AT (20 mg/kg/day) significantly increased SOD enzymatic activity (\*P = 0.0323) compared to CdCl<sub>2</sub>-treated group (1 mg/kg/day) as depicted in Fig. 3-b.

The effect of CdCl<sub>2</sub> on rat kidney tissues GPx activity and treatment with a combination of CdCl<sub>2</sub> plus AT on the rat tissue GPx activity is depicted in Fig. 3-c. Administration of CdCl<sub>2</sub> significantly reduced GPx concentration in kidney compared to control values (Cd 2 mg/kg/day, \*P = 0.0138 and Cd 3 mg/kg/day, \*\*P < 0.0078). The combination of CdCl<sub>2</sub> plus AT increased kidney GPx content compared to CdCl<sub>2</sub> treated rats, but the difference was not significant.

### **3-3. Effects of treatments on the serum level of BUN**

A dose of 3-mg/kg CdCl<sub>2</sub> significantly increased BUN compared to the rats treated with saline (\*\*P < 0.01) but not significantly at dose of 2 mg as shown in Fig. 4. Pretreatment with AT (20 mg/kg/day) significantly decreased BUN level in Cd-induced changes of 1 and 3 mg/kg (\*P < 0.05) and control group (\*\*P < 0.01) but not significantly at Cd dose of 2 mg/kg.

### **3-4. Effects of CdCl<sub>2</sub> and AT on the serum level of Creatinine**

Figure 5 shows the status of creatinine serum level of control group and experimental group. CdCl<sub>2</sub> administration (3 mg/kg/day) significantly increased creatinine in the serum as compared to control rats (\*\*P = 0.0029). Increased levels of creatinine due to CdCl<sub>2</sub> challenge were significantly decreased at doses of 1, 2, and 3 mg/kg/day upon the pre-treatment with Atorvastatin 20 mg/kg/day (Cd1 + AT \*\*P = 0.0072, Cd2 + AT \*\*P = 0.0048, Cd3 + AT \*P = .0155).

### **3-5. Histological changes in the rat kidney:**

The Histological examination on the renal tissues of the control rats demonstrated normal architecture and regularly arranged kidney tissue cells in both lateral cortex and medullary segments, respectively. In the central part of the tissue, normal epithelial cells were arranged in the collecting duct. The appearance of epithelial cells and glomerular size were observed normal in the proximal convoluted tubule and the distal convoluted tubule (Fig. 6-a).

The kidney tissue of CdCl<sub>2</sub>-treated rats group demonstrated severe histological damages, where renal glomerular size was reduced compared to normal tissue and hemorrhage was observed inside the

Bowman's capsule. Lymphocytic cells increased in the renal tissues and the death of epithelial cells in the wall of Bowman's capsule was observed in glomeruli. Disrupted epithelial cells were deposited in the collector duct. In the central part of the tissue, the number of renal cells in the proximal and distal convoluted tubules was indistinguishable, indicating histopathological alterations in the cellular structure. Further, in rats treated with CdCl<sub>2</sub>, the aggregation of interstitial lymphocyte infiltration was increased in renal tubular cells, and vacuolated cytoplasm was observed, which ultimately led to cellular death (Fig. 6-b). In the central part of the tissue, abnormal epithelial cells were placed in the collection duct.

In contrast, these histopathological changes were reduced in the kidney of rats following administration of CdCl<sub>2</sub> and AT (20mg/kg/day). The results of this study in the rat groups treated with CdCl<sub>2</sub> and AT 20 mg/kg/day showed that Bowman's capsule wall epithelial cells in the kidney glomeruli had a normal appearance. Bowman space is slightly larger in the distance between the epithelial cells and the vessel wall thicker than in the control groups. The percentage of lymphocyte cells in tissue was normal and interstitial hemorrhage was not observed. In the cortical tissue, the entire complex tubes were seen around and near the epithelial wall and the cells were aligned. The epithelial cells near the convoluted tubules and the surrounding tubules were normal (Fig. 6-c).

## Discussion

This in-vivo experimental study examined the potential effects of AT on CdCl<sub>2</sub>-induced kidney toxicity in rat. Our results showed that administration of CdCl<sub>2</sub> significantly increased MDA levels, and decreased the enzymatic and non-enzymatic antioxidants in the kidney of rats. Similarly, Renugadevi et al. (Renugadevi and Prabu, 2009) suggested oxidative stress as the main mechanism of acute Cd toxicity. Prior studies reported significant increased MDA levels in the kidney from CdCl<sub>2</sub> -administered rats. (Gabr et al., 2019; Mohammed and Hashem, 2019) In addition, Mohammed et al. (Mohammed and Hashem, 2019) reported that CdCl<sub>2</sub> (5 mg/kg/day) orally for 30 days significantly increased MDA, lowered activity of GSH and histopathological changes in the rat. Our results suggest that CdCl<sub>2</sub> administration resulted in the reduction of renal GSH concentration as compared to control group, which is in agreement with the previous results obtained by Xiao, (Xiao et al., 2002) and Messaoudi et al. (Messaoudi et al., 2009) Indeed, these findings agreed with previous study on cadmium in male jewelry manufacturing workers, indicated decrease in plasma antioxidant enzymes, and increase in MDA and erythrocyte instability. (Moitra et al., 2014)

Our results showed the administration of CdCl<sub>2</sub> significantly decreased GPx (doses of 2 and 3 mg/kg), SOD, and GSH (dose of 3 mg/kg) activities in the kidney compared to a control group and resulted in oxidative stress in the rat kidney that was reflected by the renal histopathological and biochemical changes. Our results were in agreement with a rat experimental study by Adi et al. (Adi et al., 2016) concluded that Cd exposure (20 mg/kg bodyweight for 30 days) meaningfully reduced CAT, GR, SOD, and GPx activities and improved LPx and GST activities. Also, Hormozi et al. (Hormozi et al., 2018) demonstrated that concurrent workplace exposure to lead and cadmium in tile industry might result in a

remarkable increase in lipid peroxidation, and altered antioxidant enzymes (CAT, SOD, and GPx) and oxidative stress.

In our study, the functional nephrotoxicity was indexed through BUN and creatinine levels, which were increased in CdCl<sub>2</sub>-treated rats as matched to control rats. Our results confirm the study by Wallin et al. (Wallin et al., 2014) revealed a link between Cd levels in urine and blood with exposure to Cd and chronic kidney disease. Our findings also correlated with the results of Andjelkovic et al. (Andjelkovic et al., 2019) that suggested Cd (15 and 30 mg/kg BW) significantly increased BUN and Cr compared to a control group CdCl<sub>2</sub>- induced oxidative stress in rat kidney. Another study showed that BUN and serum creatinine levels significantly reduced following administration of CdCl<sub>2</sub> (25 mg/kg, orally for 7 days) in rat. (Kim et al., 2018) Previous studies have shown severe histological changes in the kidney of CdCl<sub>2</sub>-treated rats. (Gabr et al., 2019; Mohammed and Hashem, 2019) Besides, CdCl<sub>2</sub> exposure induced toxic injuries to the renal and declined glomerular function and progressive renal failure in the kidney, as seen by histopathological examination of the current study and as reported before by Gabr, (Gabr et al., 2019) Renugadevi, (Renugadevi and Prabu, 2009) and Mohammed et al. (Mohammed and Hashem, 2019)

Our experiment revealed that the pretreatment of AT before CdCl<sub>2</sub> poisoning improved biochemical parameters. Previous study showed protective effect of AT on antioxidant enzymes and inhibits the reduction of endogenous antioxidant enzymes. (Ozbek et al., 2009) In this study, taking AT (20 mg/kg/day) plus CdCl<sub>2</sub> did have much biochemical and pathological impact in rat kidney. However, conflicting results have been reported regarding the effect of AT on renal tissue. Some studies find the effect toxic and others find it useful and supportive. (Mehrzadi et al., 2016; Nasri et al., 2016) Mehany et al. (Mehany et al., 2013) reported the effect of pretreatment with AT and vitamin E on rat's kidney. They suggested that in potassium dichromate-induced nephrotoxicity (15 mg/kg) in rat, pretreatment with vitamin E (200 mg/k) and Atorvastatin (10 mg/kg/day for 14 days) resulted in lowered toxicity and as well as improvement of kidney histopathological changes. In another study, Talebpour et al. (Talebpour Amiri et al., 2018) reported that AT administration produced a significant protective effect against radiation-induced nephrotoxicity.

A study reported opposite results showed that administration of AT (30 mg/kg/day for 8 weeks) induced adverse effect in renal tissues and a post-treatment of Arjunolic acid (20 mg/kg for 4 days) and vitamin C might protect kidney from AT-induced severe tissue toxicity. (Pal et al., 2015) Three major differences between our findings and the results of Pal et al. (Pal et al., 2015) could be due to the discrepancy between dose, exposure time, and animals. In the current study, rats were exposed to 20 mg/kg AT for 15 days, while in Pal et al. (Pal et al., 2015) study mice were given 30 mg/kg/day AT for 8 weeks.

Our results highlighted that the administration of CdCl<sub>2</sub> in rat model may lead to nephrotoxicity. Histological examination showed many alterations in renal tissue structure following exposure to CdCl<sub>2</sub> (Fig. 6b). These results consistent with those of Mohammad et al. (Mohammed and Hashem, 2019) which described CdCl<sub>2</sub> (5 mg/kg b.w, orally for 4 weeks) induced glomerular injury, acute dilatation of Bauman's capsule, congestion of the renal blood vessels, and injury to glomerular epithelial in rat.

A previous report concluded that CdCl<sub>2</sub> administration (5 mg/kg for 30 days) indicated adverse effect on cortical blood flow and renal parenchyma replacement with numerous lymphocytes infiltrates, and dilation of glomeruli in rat.(Gabr et al., 2019) Also, El-Sokkary et al.(El-Sokkary et al., 2010) who reported that CdCl<sub>2</sub> (5-mg /kg b.w for 22 days) are associated with spaces separating the cortical tissue, attributed to the cellular degeneration and interstitial edema, mesangial cell proliferative glomerulonephritis in rat, and dilation of Bowman's spaces.

Our results indicated that in cadmium-induced nephrotoxicity pretreatment of AT (20 mg/kg/day) significantly improved concentrations of SOD, GSH and GPx in rat kidney and significantly decreased MDA, BUN, and Creatinine contents. The results of this study confirm previous studies, suggesting the antioxidant effect of AT. (Ghelani et al., 2019; Ozbek et al., 2009) Additionally, our findings suggest that the administration of AT might reduce cadmium-induced tissue damage. Similar to our findings El-Moselhy et al.(El-Moselhy and El-Sheikh, 2014) showed AT intake (10 mg/kg for 10 days) produced a renal protective effect against doxorubicin (15mg/kg for 5 days) induced nephrotoxicity.

## Conclusion

This study indicated that varying doses of CdCl<sub>2</sub> caused oxidative stress and accounted for decreasing enzymatic activity in neutralizing free radicals and histological changes. Our results highlighted AT might protect rats against CdCl<sub>2</sub>-induced oxidative stress. Workers must be informed about the potential health effects related to Cd exposure. We proposed that AT might be clinically relevant to CdCl<sub>2</sub>-induced renal disorders due to its potential therapeutic use in industrial workers.

## Declarations

**Ethical approval:** The methodology for this study was approved by the Research Ethics committee of the Semnan University of Medical (Ethics approval number: (IR.SEMUMS.REC.1395.177).

**Consent to participate:** Animal studies: Animal experiments were performed in accordance with guidelines for animal acts established and proposed by the institutional Ethical Review Board of Semnan University of Medical Sciences.

**Consent to Publish:** Not applicable

**Authors Contributions:** AD, EK, ZG contributed to conception, design, drafting and revising of the study. AG, ARB, SY conducted experiments, AD, EK, ZG, AG, ARB performed data analyses and interpretations. AD, EK, ZG contributed to writing and revising manuscript. AD supervised the study and final approval of the version to be published. The authors declare that all data were generated in-house and that no paper mill was used.

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**Competing Interests:** The authors declared no potential discord of interest with respect to the research, authorship, and publication of this article.

**Availability of data and materials:** supplemental original source data files

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## Figures

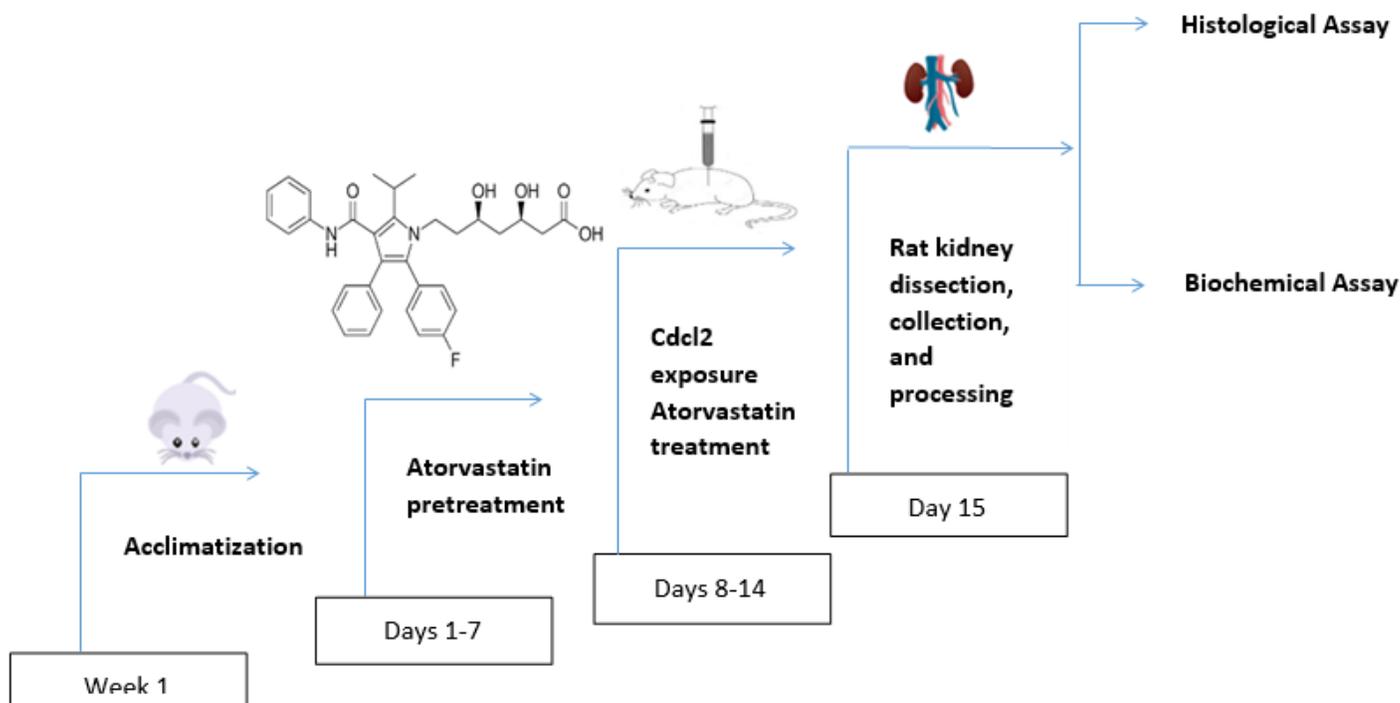


Figure 1

The time schedule for experimental rat treatments

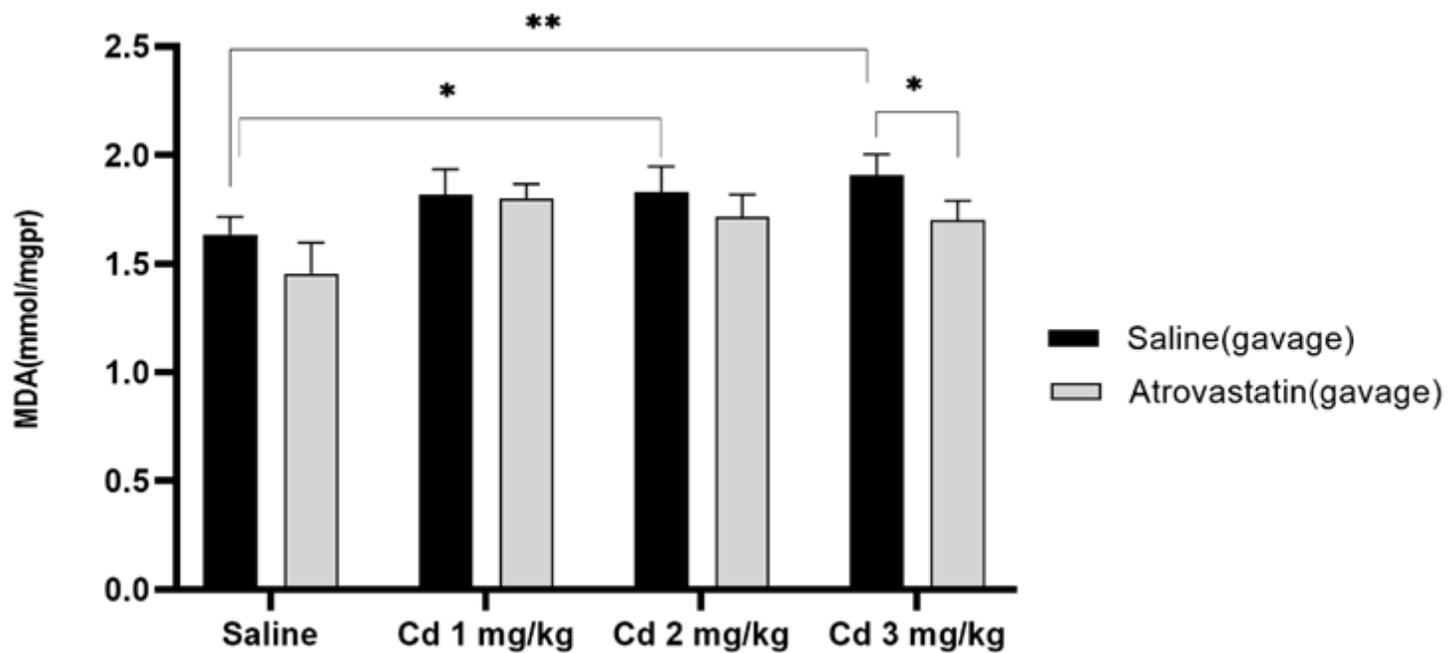
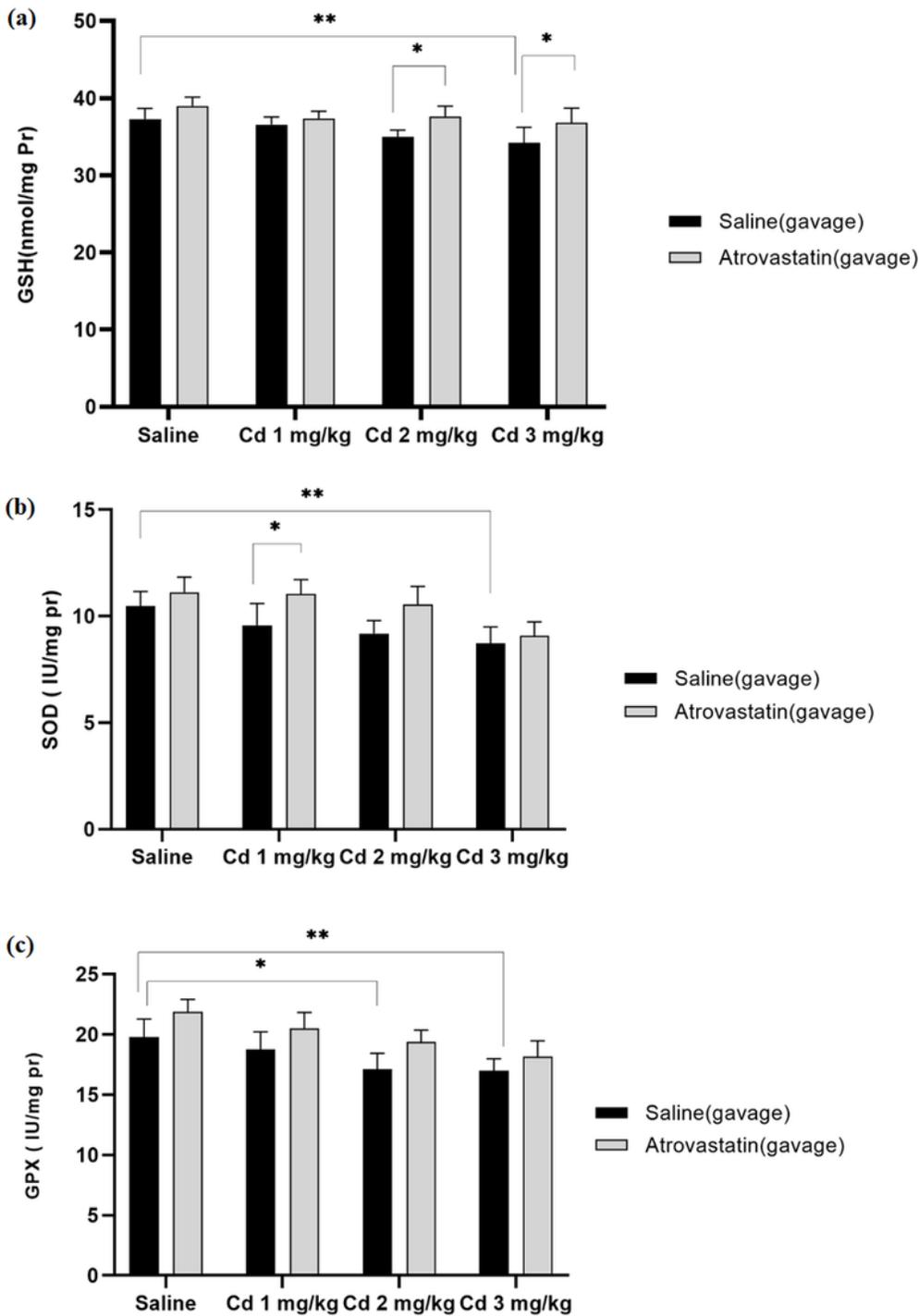


Figure 2

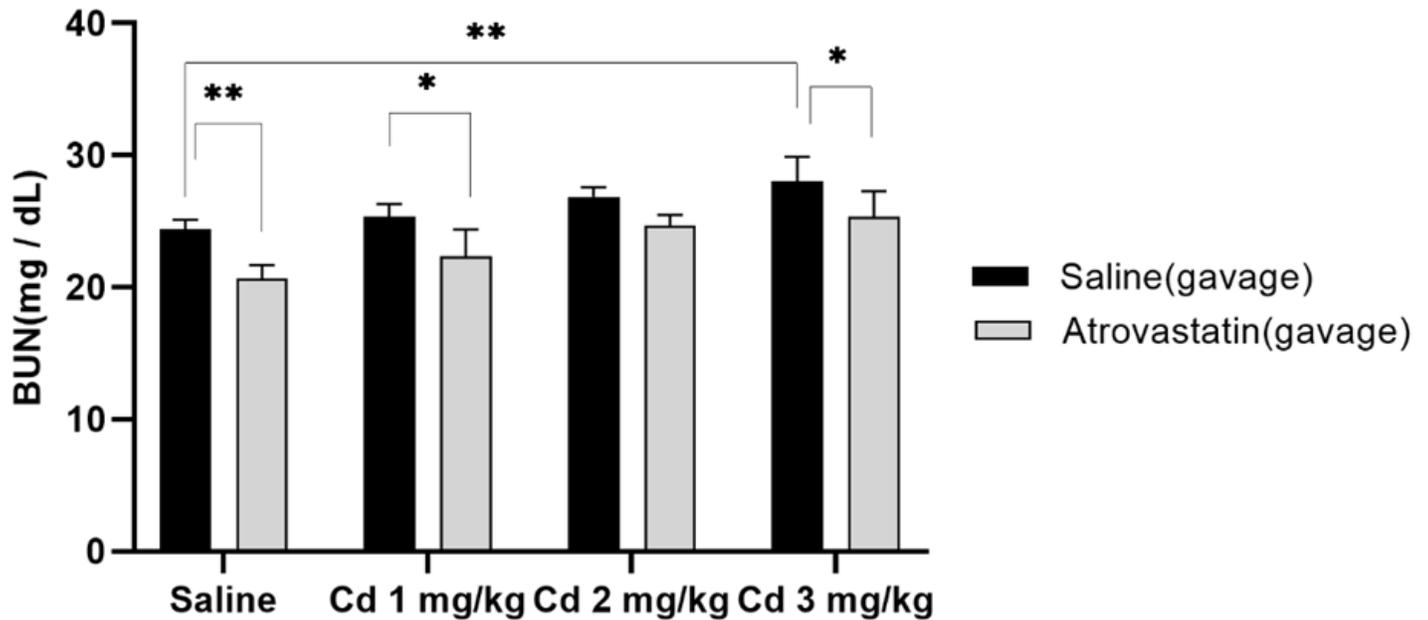
Effects of AT on MDA levels in kidney tissues of rats treated with CdCl<sub>2</sub> at doses of 1, 2, and 3 mg/kg. Administration of CdCl<sub>2</sub> (2 and 3 mg/kg) significantly increased levels of MDA in serum compared to the rats received saline. Pretreatment with AT decreased the effect of CdCl<sub>2</sub> (3 mg/kg). Presented figures are mean  $\pm$  S.E.M (n=7) 0.05999. \*P <0.05, \*\*P <0.01.



**Figure 3**

a. Effects of AT on GSH levels in kidney of rats received CdCl<sub>2</sub> (1, 2 and 3 mg/kg). Administration of CdCl<sub>2</sub> (3 mg/kg) significantly lowered the content of GSH. Pretreatment of AT significantly suppressed the CdCl<sub>2</sub>-induced effects of 2 and 3 mg/kg doses. Values are mean ± S.E.M (n=7) 0.7959. \*P < 0.05, \*\*P < 0.01. b. Effects of AT on SOD activity in the kidney tissues of rats received CdCl<sub>2</sub> (1, 2, and 3 mg/kg). CdCl<sub>2</sub> (3 mg/kg) significantly decreased the content of SOD compared to the saline-treated rats. AT

pretreatment improved SOD at the level of control group and significantly suppressed the effect of CdCl<sub>2</sub> (1 mg/kg). Results are mean ± S.E.M (n=7) 0.4352. \*P <0.05, \*\*P <0.01. c. Effects of AT on GPx activity in renal tissues of rats received CdCl<sub>2</sub> with doses of 1, 2, and 3 mg/kg. Data showed no significant difference of GPx levels between rats pretreated with AT and the rats received the various doses of CdCl<sub>2</sub>. Results are mean ± S.E.M (n=7) 0.7233. \*\*P =0.0078, \*P =0.0138.



**Figure 4**

Effects of AT on BUN concentrations in the rats exposed to CdCl<sub>2</sub> with doses 1,2, and 3mg/kg. Administration of CdCl<sub>2</sub> (3 mg/kg) significantly increased the level of BUN. AT pretreatment significantly decreased BUN compared to saline and CdCl<sub>2</sub>-treated rats (1 and 3 mg/kg). Figures are mean ± S.E.M (n=7) (0.8043). Saline and AT \*\*\*P=.0009, Cd3 and saline \*\*P=.0015, Cd1 and AT+Cd1 \*P=.0123, Cd3 and AT+Cd3 \*P=.0378.

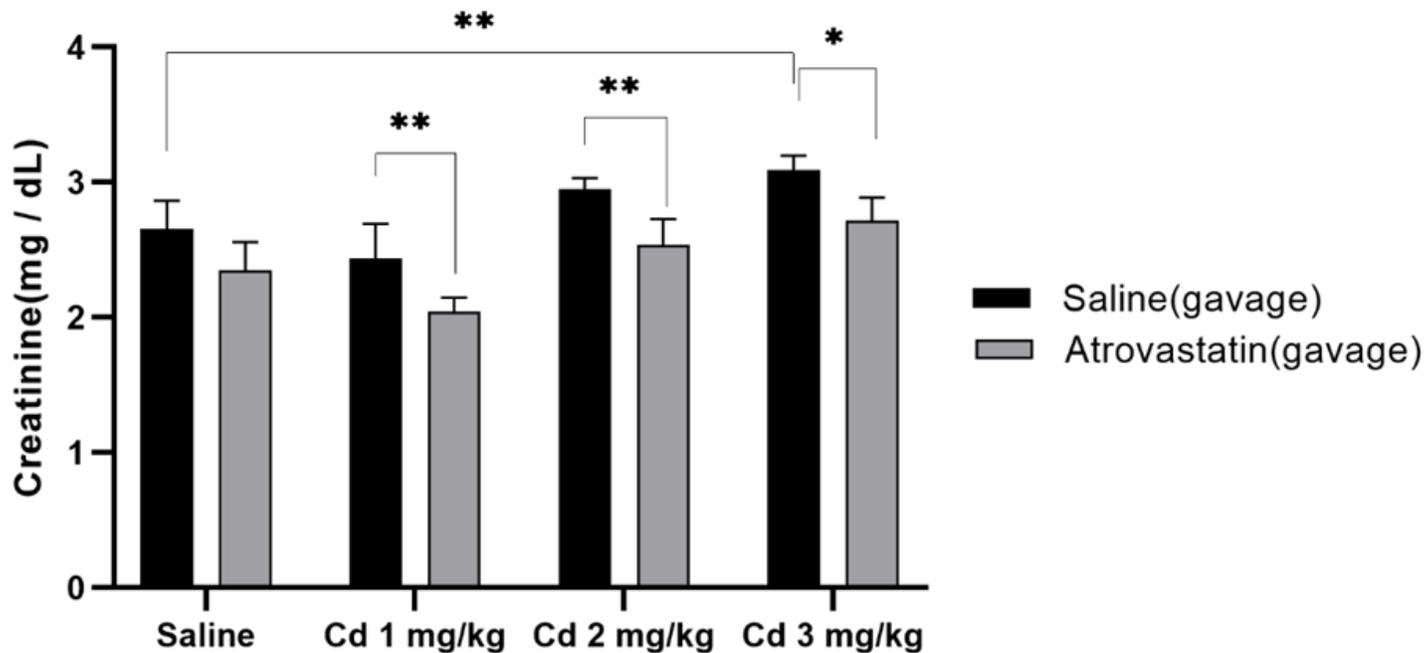
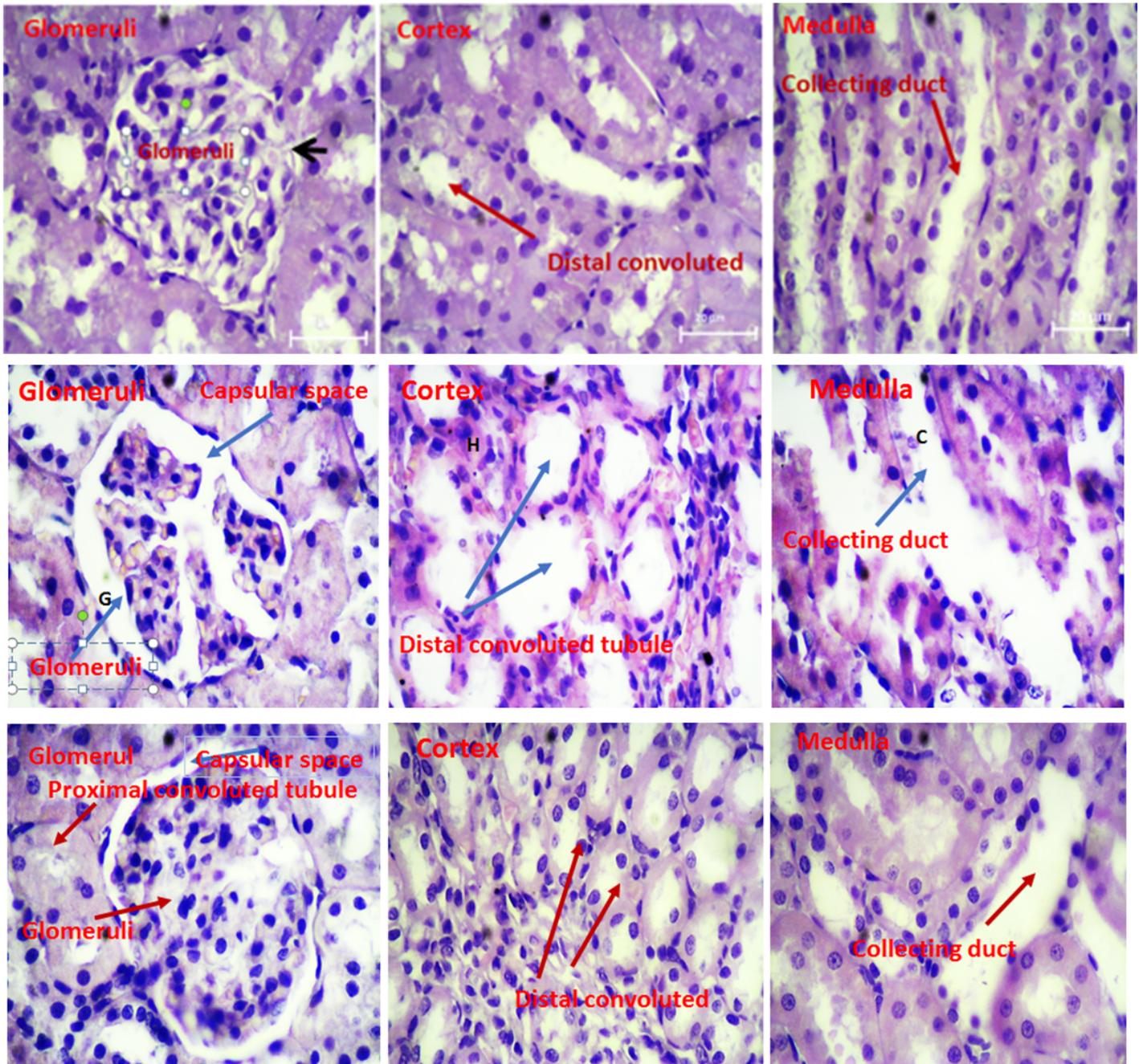


Figure 5

Effects of AT on creatinine in rat kidney tissues exposed to CdCl<sub>2</sub>. Administration of CdCl<sub>2</sub> (3 mg/kg), induced significant increase in creatinine level compared to the rats treated with saline and AT pretreatment significantly decreased creatinine and the effect of CdCl<sub>2</sub> (1, 2 and 3 mg/kg) . Results are presented as mean ± S.E.M (n=7) 0.1018. \*P <0.02, \*\*P <0.01.



**Figure 6**

a. Light microscopy of rat renal tissue of the control group under normal saline treatment illustrating the healthy architecture of Bowman's capsule (black arrow), glomeruli, distal tubules, and collector duct. b. Light microscopy of rat kidney received CdCl<sub>2</sub> displaying renal damages: degeneration of glomeruli (G), hemorrhage (H), deposited epithelial cells in collector duct (C). c. Light microscopy of rat kidney structure following treatment of AT (20 mg/kg) and 30 min before administration of CdCl<sub>2</sub>. AT protective effect on injury in the kidney tissue, exhibiting normal kidney tissue structure with glomeruli, renal distal convoluted, and collecting duct.

## Supplementary Files

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