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# A histopathological study on the effects of atorvastatin against gentamicin-induced renal injury

**Elham Emami[1], Ali Hasanpour Dehkordi[2], Ahmadreza Maghsoudi[3*], Hamid Nasri[3,4,5*], Alireza Vahedi[6]**

[1]Pediatric Nephrology Research Center (PNRC), Faculty of Medicine, Shahid Beheshti University of Medical Sciences, Emam Hosein Hospital, Tehran, Iran
[2]Social Determinants of Health, Research Center, School of Allied Medical Sciences, Shahrekord University of Medical Sciences, Shahrekord, Iran
[3]Department of Internal Medicine, Isfahan University of Medical Sciences, Isfahan, Iran
[4]Department of Pathology, Nickan Research Institute, Isfahan, Iran
[5]Eurasian Nephropathology Society (ENPS), Moscow, Russia
[6]Breast Cancer Research Center, Motemed Cancer Institute, Tehran, Iran

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**ABSTRACT**

**Introduction:** Gentamicin is an aminoglycoside antibiotic that is widely administered to treat infections caused by gram-negative bacteria. Gentamicin may cause renal injury in patients after seven days of administration. Atorvastatin is a cholesterol-lowering statin that acts through the mevalonate pathway.

**Objectives:** In this study, we investigated the histopathological effects of atorvastatin against gentamicin-induced renal injury.

**Materials and Methods:** Twenty male Wistar rats were randomly assigned into five groups and treated as the following; group 1 (normal group, no drug), group 2 [gentamicin group, daily 80 mg/kg, intra-peritoneal (i.p.) for 7 days], groups 3 to 5 (gentamicin 80 mg/kg + atorvastatin at doses of 5, 25 and 75 mg/kg, respectively). Kidney sections were examined for histopathological parameters including vacuolization of the kidney tubular cells, degeneration, necrosis, flattening of the tubular cells and debris in the tubular lumen.

**Results:** Compared to the normal group, gentamicin significantly exacerbated the histopathological parameters. Treatment with atorvastatin significantly decreased vacuolization, degeneration, necrosis and debris in the nephrotoxic rats.

**Conclusion:** The findings of this research indicated that atorvastatin therapy can ameliorate histopathological renal injury following gentamicin injection.

*Implication for health policy/practice/research/medical education:*
In a study on 20 male Wistar rats, we found atorvastatin therapy can ameliorate histopathological renal injury following gentamicin injection.

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## Introduction

Kidney injury may be acute (a sudden decrease in kidney function) or chronic (continuous structural and functional kidney dysfunction) and can be diagnosed by renal function markers such as the serum blood urea nitrogen (BUN) and creatinine levels. Since these markers are not very sensitive, other biomarkers have recently been employed to assess premature kidney damage (1).

Gentamicin is an aminoglycoside antibiotic that is widely utilized to treat infections caused by gram-negative bacteria (2). In addition to its clinically beneficial effects, gentamicin has been indicated to cause renal toxicity in 10-20% of patients after seven days of administration (3-6). Despite the development of new antibiotics, gentamicin is still administered due to its effectiveness against lactam-resistant microorganisms, low levels of resistance to Enterobacteriaceae and low-cost (7).

The exact mechanism of gentamicin that causes renal toxicity has not been established. However, one of the significant mediators of renal toxicity is reactive oxygen species (ROS) (8-10). gentamicin-induced nephrotoxicity is associated with increased ROS, such as superoxide

***Corresponding authors**: Ahmadreza Maghsoudi, Email: ahmaghsoudi@med.mui.ac.ir, dr_maghsoudi_md@ yahoo.com and Hamid Nasri, Email: hamidnasri@med.mui.ac.ir



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anion ($O2^{\bullet-}$), hydrogen peroxide ($H_2O_2$), and hydroxyl radicals, released by the renal cortical mitochondria (11, 12). In vitro and in vivo studies have demonstrated that ROS metabolites lead to cell damage and necrosis through various mechanisms such as membrane lipid peroxidation, protein oxidation and DNA damage (13,14). In addition, gentamicin can inactivate phospholipases and alter lysosomal membranes (15). According to recent studies, redox-sensitive transcription factors, mitogen-activated protein kinase (MAPK), and nuclear factor-kappa β (NF-κB) are involved in gentamicin-induced renal toxicity (16,17).

Statins are distinctive drugs that are administered in the treatment of hypercholesterolemia, DNA damage, reduced lipoprotein oxidation and free radical damage (18,19). Furthermore, studies have proved the antioxidant, anti-inflammatory and anti-apoptotic effects of statins (20). Statins can affect various signaling pathways, including inflammatory, proliferative and cell death responses in the kidney in addition to improving chronic or acute renal failure (21-23).

Atorvastatin is a cholesterol-lowering statin that acts through the mevalonate pathway by inhibiting 3-hydroxy-3-methylglutaryl coenzyme reductase, a rate-limiting enzyme in cholesterol biosynthesis (20). Atorvastatin has antioxidant activity against hydroxyl and peroxyl radicals and reduces the oxidation of lipoprotein in some oxidative systems (19). Various studies have revealed that atorvastatin has the ability to metabolize and reduce intracellular ROS (24,25). Atorvastatin is also able to scavenge all free radicals and block MAPK and NF-κB (26,27).

## Objectives

In this study, we investigated the histopathological effects of atorvastatin against gentamicin-induced renal injury.

## Materials and Methods

### *Animals and study design*

This study was conducted on 20 male Wistar rats weighing 200-250 g. The animals were kept in the research center of medicinal plants, Shahrekord university of medical sciences in a standard environment (temperature 21-25°C and 12-hour cycle of darkness and light) with access to water and food.

The rats were divided into five groups (n = 4); the first group, as the normal group, received no drug. The second group received gentamicin at a dose of 80 mg/kg. The third to fifth groups received gentamicin at a dose of 80 mg/kg and then one-hour interval gentamicin at doses of 5, 25, and 75 mg/kg, respectively. The injections were administered intraperitoneally for seven days.

### *Histopathological investigations*

Eventually, the kidneys of the rats were isolated, kept in formalin for 12 hours, and processed for histopathological investigations. The 3 µm-thick paraffin tissues were stained with hematoxylin and eosin (H&E) and evaluated for the severity of renal injury (vacuolization, degeneration of the tubular renal cells, flattening of kidney tubular cells and tubular cell necrosis and also debris in the tubular lumen).

### *Statistical analysis*

All data were evaluated using one-way ANOVA. Later, a post hoc Tukey test using GraphPad Prism version 4.03 was applied and expressed as the mean ± standard error (SE). $P < 0.05$ was considered statistically significant.

## Results

### *Vacuolization of the tubular renal cells*

According to the results of Figure 1, vacuolization in the groups that received gentamicin and gentamicin plus atorvastatin at doses of 5 and 25 mg/kg (groups 2 to 4) increased significantly compared to the normal group ($P < 0.001$). Moreover, the groups that received atorvastatin at doses of 25 and 75 mg/kg (groups 4 and 5) showed a significant decrease in vacuolization compared to the gentamicin group ($P < 0.001$).

### *Flattening of the tubular cells*

The results of flattening are shown in Figure 2. Analyses illustrated that flattening in the groups that received gentamicin and gentamicin plus atorvastatin at a dose of 5 mg/kg (groups 2 and 3) incremented significantly compared to the normal group ($P < 0.001$ and $P < 0.01$, respectively). Flattening in the group that received atorvastatin at a dose of 75 mg/kg (group 5) showed a significant decrease compared to the gentamicin group ($P < 0.01$).

### *Degeneration in kidney tissue*

According to the results of Figure 3, degeneration in the groups that received gentamicin and gentamicin plus atorvastatin at a dose of 5 mg/kg (groups 2 and 3) increased significantly compared to the normal group

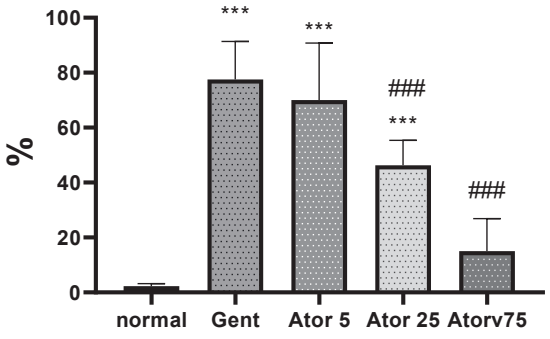


**Figure 1.** Comparison of vacuolization of the tubular renal cells among the study groups (atorvastatin in 5 mg/kg, 25 mg/ kg and 75 mg/kg), *** $P<0.001$ compared to the normal group. ### $P<0.001$ compared to the Gent group. Gent: Gentamicin. Ator: Atorvastatin.

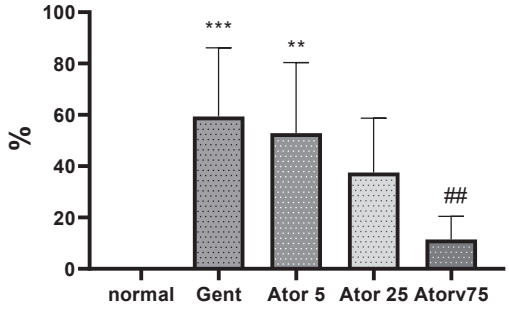


**Figure 2.** Comparison of flattening of the tubular cells among the study groups (atorvastatin in 5 mg/kg, 25 mg/kg and 75 mg/kg), ** $P<0.01$ and *** $P<0.001$ compared to the normal group. ## $P<0.01$ compared to the Gent group. Gent: Gentamicin. Ator: Atorvastatin

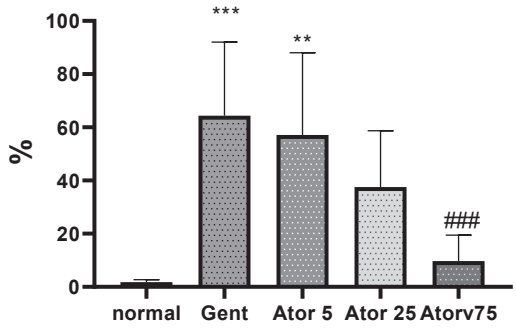


**Figure 3.** Comparison of degeneration among the study groups. ** $P<0.01$ and *** $P<0.001$ compared to the normal group (atorvastatin in 5 mg/kg, 25 mg/kg and 75 mg/kg), ### $P<0.001$ compared to the Gent group. Gent: Gentamicin. Ator: Atorvastatin

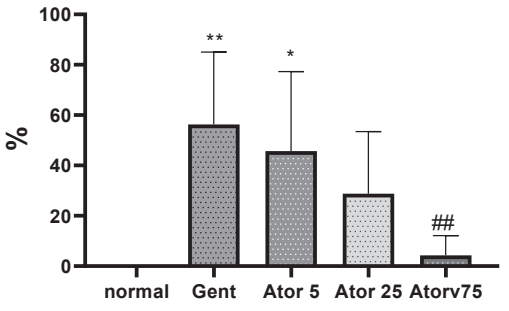


**Figure 4.** Comparison of the necrosis among the study groups. * $P<0.05$ and ** $P<0.01$ compared to the normal group (atorvastatin in 5 mg/kg, 25 mg/kg and 75 mg/kg), ## $P<0.01$ compared to the Gent group. Gent: Gentamicin. Ator: Atorvastatin

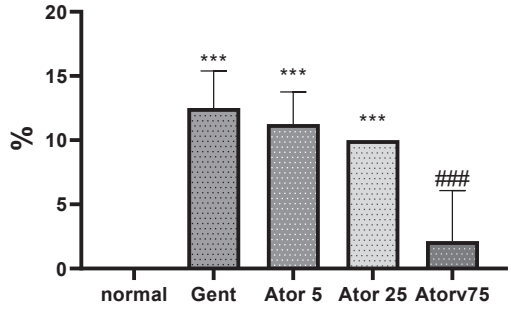


**Figure 5.** Comparison of the debris parameter among the study groups. *** P <0.001 compared to the normal group (atorvastatin in 5 mg/kg, 25 mg/kg and 75 mg/kg), ### $P<0.001$ compared to Gent group. Gent: Gentamicin. Ator: Atorvastatin

($P<0.001$ and $P<0.01$, respectively). In the group that received gentamicin plus atorvastatin at the dose of 75 mg/kg (group 5), degeneration was significantly reduced compared to the gentamicin group ($P<0.001$).

### *Necrosis in the study groups*

As shown in Figure 4, necrosis in the groups that received gentamicin and gentamicin plus atorvastatin at a dose of 5 mg/kg (groups 2 and 3) increased significantly compared to the normal group ($P<0.01$ and $P<0.05$, respectively). The group that received gentamicin plus atorvastatin at a dose of 75 mg/kg (group 5) demonstrated a significant decrease in necrosis compared to the gentamicin group ($P<0.01$).

### *Debris parameter*

The results of Figure 5 exhibit that debris was considerably enhanced in the groups that received gentamicin and also gentamicin plus atorvastatin at doses of 5 and 25 mg/kg (groups 2 to 4) compared to the normal group ($P<0.001$). Furthermore, this parameter was reduced significantly in the group of gentamicin plus atorvastatin at a dose of 75 mg/kg (group 5) in comparison to the gentamicin group ($P<0.001$).

## Discussion

Comparison of the effects of gentamicin (80 mg/kg) alone and concomitant administration of gentamicin and atorvastatin at different doses of 5, 25, and 75 mg/kg showed that atorvastatin could effectively reduce the histopathological changes induced by gentamicin.

Patients with renal failure have been shown to suffer from hyperlipidemia (28). Hyperlipidemia, or more precisely, an increase in total serum cholesterol and a decrease in high-density lipoprotein, leads to renal failure. Therefore, the administration of anti-hypercholesterolemic drugs such as statins can be effective in improving cholesterol levels and inhibiting renal dysfunction (29-31).

Gentamicin is administered for treating aerobic gram-negative bacteria. Accumulation of gentamicin in the proximal renal tubular cells causes renal toxicity resulting in injury to the brush border network (32). Production of free radicals in the kidney tissue, attenuation of antioxidant defense mechanisms, and acute renal tubular necrosis induce renal toxicity which results in the abolition of creatinine clearance and renal dysfunction (32).

Other pathological mechanisms involved in nephrotoxicity include increased endothelin-1, reset of growth factor-β, a significant increase in monocyte/macrophage infiltration

into the renal cortex and medulla, increased oxidative stress and finally, apoptosis resulting in necrosis. In this study, in line with other studies, gentamicin disrupted kidney function and structure compared to the normal group (30-33).

Statins reduce the oxidation of lipoproteins and improve free radical damage. Atorvastatin appears to have significant antioxidant activity against hydroxyl and peroxyl radicals (24-26). In addition, the synthesis of inflammatory mediators such as tumor necrosis factor-α (TNF-α) is suppressed by statins. Studies have shown that simvastatin diminishes TNF-α and α-myeloperoxidase through mevalonate-independent pathways (23,33).

In our study, administration of atorvastatin at the dosages of 5, 25 and 75 mg/kg in a dose-dependent manner reduced renal impairment induced by gentamicin, of which the dose of 75 mg/kg showed a significant improvement. Atorvastatin has been shown to have a protective effect against renal toxicity through its antioxidant, antimicrobial, anti-inflammatory and anti-apoptotic mechanisms. This study also confirmed or our pervious investigation on this subject (32). These studies suggest that atorvastatin ameliorates renal toxicity through the inhibition of free radical production, MAPK, and NF-κB pathway, in addition to the expression of nitric oxide synthesis (24-26).

## Conclusion

In summary, gentamicin injection leads to pathological renal damage and induces renal toxicity which can be improved with atorvastatin. Although the exact mechanism is not known, it appears that gentamicin-induced renal toxicity reduces with ameliorating oxidative stress conditions and antioxidant activity. We suggest further studies to clarify the mechanism of this drug.

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## Authors' contribution

Primary idea; HN. Animal lab supervision and headlining; AHD. Primary draft by ARM, AV and EE. ARM and HN both are corresponding authors who edited and finalized the paper equally. All authors read and signed the final paper.

## Conflicts of interest

The authors declare no conflict of interest.

## Ethical issues

This study was also approved by the Ethics Committee of NIMAD (National Institute for Medical Research Development; in Iran) (Ethical code#IR.NIMAD.REC.1399.024). This study as a part of a larger study was supported by NIMAD (Grant#972048 ). This investigation was also conducted according to the regulations of the Research Ethics Committee of Iranian Ethical Guidelines for using animals in research. Furthermore, all animal experiments were in accordance with the protocols approved by the United States National Institutes of Health (NIH, 1978). Besides, ethical issues (including plagiarism, data fabrication and double publication) have been completely observed by the authors.

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