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Research Article
Protective Effect of Celastrol on Gentamicin-induced Nephrotoxicity in Mice

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Abstract

Background and Objectives: Gentamicin is an aminoglycoside antibiotic used to cure serious bacterial infections, although its usefulness is restricted due to the risk of nephrotoxicity. Celastrol is an active component of the Chinese medicine *Tripterygium wilfordii*, which has been demonstrated to be a potent anticancer agent in preclinical studies of cancer and inflammatory disorders. This study was aimed to investigate the effect of celastrol on gentamicin nephrotoxicity in mice and to explore the underlying mechanisms involved. **Materials and Methods:** Serum creatinine and blood urea-nitrogen concentrations, oxidative stress, inflammatory cytokines and histopathological changes of kidney tissues were monitored. Nephrotoxicity was detected after 50 mg kg^{-1} daily dose of gentamicin intraperitoneal injection (IP) for 7 consecutive days. Mice were randomly divided into 5 groups. Group 1 received normal saline containing 5% dimethyl sulfoxide, IP, at the same volume as the drugs received by other groups. Group 2 received gentamicin (50 mg/kg/day, IP). Groups 3 and 4th received gentamicin (50 mg kg^{-1}, IP) with celastrol (1 or 5 mg/kg/day, IP, respectively), celastrol was given 30 min before the gentamicin injection. Finally, group-5 received celastrol only (5 mg kg^{-1}, IP). **Results:** Celastrol significantly ameliorated gentamicin-induced elevation of serum creatinine, blood urea nitrogen and uric acid. It significantly reduced renal tissue malondialdehyde and restored activity of renal glutathione and superoxide dismutase. Moreover, it reduced TNF-α, IL-6, IL-β1 and NF-κBp65 and partially recovered cellular damage, including distorted glomerular capillaries and tubular dilation with epithelial atrophy corresponding with dosage level. **Conclusion:** Celastrol could protect against gentamicin-induced acute kidney injury, possibly through suppressing oxidative stress and pro-inflammatory biomarkers and improving renal functional disturbances.

Key words: Celastrol, gentamicin, nephrotoxicity, oxidative stress, inflammatory cytokines, stress biomarkers, renal profile, acute kidney injury

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Aminoglycoside (AG) antibiotics are commonly prescribed antibacterial agents for treating patients with infections, but the associated adverse effects of oxidative stress and kidney injury limit long-term clinical use[1]. Gentamicin, which is a member of the AG class of antibiotics, is effective against gram-negative bacterial infections[2]. While often inducing nephrotoxicity, gentamicin is used clinically due to its wide spectrum of activities that act against gram-negative bacterial infections caused by pseudomonas, proteus and serratia[3]. It has been reported that up to 30% of people who receive a course of gentamicin treatment develop some symptoms of nephrotoxicity[4]. The renal injury induced by this drug is related to its preferential accumulation in the renal proximal convoluted tubules that leads to loss of brush border integrity[5]. The gentamicin nephrotoxicity involves renal free radical generation, reduction in antioxidant defense mechanisms, activation of inflammatory processes, acute tubular necrosis and glomerular congestion[6], resulting in diminished glomerular filtration rate and renal dysfunction. The pathological mechanisms pertaining to gentamicin-induced nephrotoxicity involve upregulation of transforming growth factor-beta (TGF-β), elevation of endothelin-1, marked increase in monocyte/macrophage infiltration into the renal cortex and medulla, induction of oxidative stress and apoptosis and necrosis[7]. Serum creatinine and blood urea nitrogen are characteristically increased 7-10 days after initiation of AG therapy[8]. Acute kidney injury is a relatively common complication of therapy with AG antibiotics, with a rise in the serum creatinine concentration of >0.5-1 mg dL^{-1} (44-88 μmol L^{-1}), or a 50% increase in serum creatinine concentration from baseline, occurring in 10-20% of patients[9]. A central aspect of gentamicin-induced nephrotoxicity is its tubular cytotoxicity. Apoptosis[10] and necrosis[11] of tubular epithelial cells and induction of several inflammatory mediators and reactive oxygen species production and enhanced oxidative stress have been observed in renal specimens of animals treated with gentamicin[11]. Given the effectiveness of AGs for the treatment of severe infections, continued research to improve their therapeutic indices and associated nephrotoxic adverse effects is often highly recommended.

Celastrol, a pentacyclic triterpene extracted from the roots of the *Tripterygium wilfordii* plant (a traditional Chinese herb), exerts various bioactivities, including anti-inflammatory, anti-diabetic, anti-obese and anticancer effects[12-15]. Research has found that celastrol can protect acute ischemic stroke-associated brain injury[16,17]. In addition, celastrol promotes Nur77 translocation from the nucleus to mitochondria to remove the damaged mitochondria to alleviate oxidative stress and inflammation[15,18,19]. Recent research has also reported that celastrol ameliorated acute kidney injury caused by ischemia-reperfusion, which was associated with the inhibition of NF-κB activation and inflammation[20,21]. Nevertheless, the effect of celastrol on gentamicin nephrotoxicity has not been fully investigated. The present study was designed to evaluate the protective effect of celastrol against gentamicin-induced nephrotoxicity in a mice model and to explore the underlying causal mechanisms.

MATERIALS AND METHODS

The study was carried out at King Fahd Medical Research Center (KFMRC), King Abdulaziz University, Jeddah, Saudi Arabia. The practical experiments, including the animal model and assessed parameters, were conducted from October, 2018 until March, 2019.

Reagents: Celastrol (>97% pure) was purchased from Paypay Technologies, Inc. (Shenzhen, China), dissolved in 0.9% sodium chloride containing 5% dimethyl sulfoxide at a concentration of 5 mg mL^{-1} and stored at -20°C. Gentamicin ampoules, each containing 20 mg mL^{-1} of gentamicin sulfate, were obtained from Jamjom Pharma Company (Jeddah, KSA). The solutions were diluted in normal saline immediately before each experiment to the desired final concentration. Kits for the determination of creatinine, urea and uric acid were purchased from My BioSource, Inc., (WH Laboratories, Houston, USA). Oxidative stress markers kits, including reduced glutathione, malondialdehyde and superoxide dismutase, were obtained from My BioSource, Inc., (WH Laboratories, Houston, USA). Inflammatory markers kits, including TNF-α, IL-6, IL-1β and NF-κBp65, were obtained from Abcam, Inc., (Cambridge, USA). All other chemicals and solvents used were of the highest grade commercially available. All ELISA protocols were based on companies' instructions.

Animals: Adult male Swiss albino mice, (weighing 30-32 g), were obtained from King Fahd medical research center, King Abdulaziz University, Jeddah, Kingdom of Saudi Arabia. Mice were adapted to the environment for a week before starting the experiment. The handling and experimentation on animals protocol was approved by the Bioethical Research Unit of the Faculty of Medicine, KAU (No: 193-18). Mice were housed in a temperature-controlled atmosphere (25±2°C) and kept on a standard diet and given water *ad libitum*.

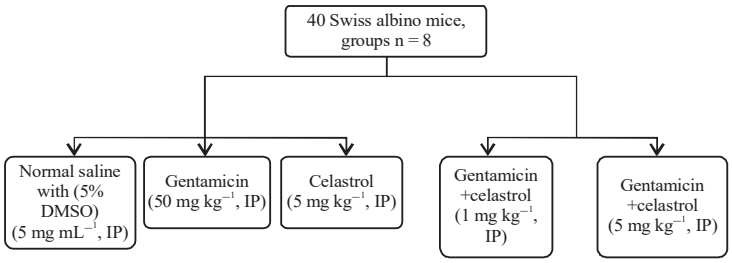

Fig. 1: Experimental schematic

Control groups: normal saline containing 5% DMSO (5 mg kg^{-1}, IP), gentamicin was dissolved in normal saline at a concentration of 50 mg mL^{-1} and administered at a dose of 50 mg kg^{-1}, IP, celastrol (5 mg kg^{-1}, IP) was administered IP, Treatment groups: Gentamicin plus celastrol (1 mg kg^{-1}, IP) or (5 mg kg^{-1}, IP)

Experimental design: Forty Swiss albino mice (30-32 g) were equally divided into 5 groups (8 mice/group) based on a study previously described by Chen *et al.*[22], as shown in Fig. 1. Group 1 received normal saline intraperitoneally (IP), containing 5% dimethyl sulfoxide, which served as a control group. Group 2 received IP gentamicin injection (50 mg kg^{-1}) while group 3 received IP gentamicin injection (50 mg kg^{-1}) and celastrol (1 mg kg^{-1}). Group 4 received IP gentamicin injection (50 mg kg^{-1}) and celastrol (5 mg kg^{-1}). Group 5 received celastrol (5 mg kg^{-1}) alone. The study was continued for 7 consecutive days and celastrol was injected 30 min before gentamicin in the relevant groups. Twenty-four hours after the last dose of gentamicin, the mice were euthanized with ether and blood samples were collected from the abdominal aorta. Sera were isolated and stored at -80°C for the determination of creatinine and urea-nitrogen concentrations. Following this, the right kidney tissue was removed and immediately frozen in liquid nitrogen for the evaluation of oxidative stress markers, such as lipid peroxidation markers (malondialdehyde), reduced glutathione content, superoxide dismutase activity and pro-inflammatory cytokines, such as TNF-α, IL-6, IL-1β and NF-κBp65. In addition, the left kidneys were removed and kept in formalin saline for histopathological examination.

Renal function parameters

Determination of serum creatinine: Serum creatinine was determined using a commercial ELISA kit (Cat. No. MBS763433), obtained from My BioSource, Inc., (WH Laboratories, Houston, USA). The kit uses the competitive technique between sample creatinine and known quantity of creatinine on the solid phase supporter for pre-coated creatinine biotinylated antibodies. Optical density was measured spectrophotometrically at $\lambda = 450$ nm.

Determination of blood urea nitrogen: Blood urea nitrogen (BUN) was determined using a commercial ELISA kit (Cat. No. MBS751125), obtained from My BioSource, Inc., (WH Laboratories, Houston, USA). The BUN ELISA kit uses the quantitative sandwich enzyme-linked immunoassay technique. The microtiter plate has been covered with horseradish peroxidase (HRP)-conjugated polyclonal antibodies specific for BUN. The samples were added to the microtiter plate. In the presence of BUN the samples will stick to the antibody pre-coated wells. To measure the quantitative amount of BUN in each sample, the HRP-conjugated polyclonal antibody specific for BUN was added to each well. The microtiter plate was incubated and the well was washed to eliminate any unbound components. Subsequently, the substrate was added to each well with the presence of the enzyme HRP. The substrate inside the incubator reacted in a short time, where the color changed only in the wells that contained BUN and the enzyme-conjugated antibodies. Sulfuric acid was added to terminate the reaction between the enzyme substrate once the color changed. Following this, the optical density was measured spectrophotometrically at $\lambda = 450$ nm.

Determination of uric acid: Uric acid was determined using a commercial ELISA kit (Cat. No. MBS9357219), obtained from My BioSource, Inc., (WH Laboratories, Houston, USA). The kit works using the sandwich ELISA technique as previously discussed. The optical density was measured spectrophotometrically at $\lambda = 450$ nm.

Oxidative stress markers: Reduced glutathione and malondialdehyde were determined using commercial ELISA kits (Cat. No. MBS724815 and MBS9389387, respectively) obtained from My BioSource Inc., (WH Laboratories, Houston,

USA). The kits work by a competitive ELISA technique; the optical density was measured spectrophotometrically at $\lambda = 450$ nm. In addition, the superoxide dismutase level was determined by the sandwich ELISA technique using a commercial ELISA kit (Cat. No. MBS034842) obtained from My BioSource, Inc., (WH Laboratories, Houston, USA).

Inflammatory markers: Inflammation was assessed by measurement of different cytokines, TNF-α, IL-6 and IL-1β, in addition to the NF-κBp65 subunit, using commercial ELISA kits purchased from Abcam, Inc., (Cambridge, USA), with Cat. No. ab100747, ab100713, ab197742 and ab176648; respectively. All kits work using the sandwich ELISA technique, with optical density at $\lambda = 450$ nm.

Histopathology examination: Right kidney samples were washed with saline and fixed in 10% formalin saline for 48 h and preprocessed for paraffin, sectioning at 5 µm thickness. The sections were mounted on glass slides and stained with hematoxylin and eosin. The pathological changes of kidney tissues were observed under a light microscope, following Aldahmash *et al.*[23].

Statistical analysis: Data were expressed as mean±standard deviation (SD). Statistical analysis was performed using Graph Pad Instat (Version 3). All graphs were sketched using Graph Pad Prism (Version 5). A one-way analysis of variance (ANOVA) was used, followed by Tukey's *post hoc* test for multiple comparisons. A p-value<0.05 was considered significant.

RESULTS

Effect of celastrol on gentamicin induced renal functional disturbances: As shown in Fig. 2a, gentamicin caused more than 3-fold increase in serum urea that reached (48.72±8.19) compared to the control value (14.38±2.28). Celastrol pretreatment at 1 and 5 mg/kg/day caused significant decrease in serum urea level by about 31 and 50% (33.17±6.71 and 24.43±4.97), respectively, compared to the gentamicin group 48.72±8.19. A similar pattern of activity was observed while assessing serum uric acid and creatinine levels (Fig. 2b and c): celastrol administration (5 mg/kg/day) significantly ameliorated the elevation of serum creatinine by 42% (0.91±0.13) and urea-nitrogen concentrations by 24% (5.68±0.75) in comparison with corresponding gentamicin groups (1.57±0.35 and 7.48±0.63), respectively.

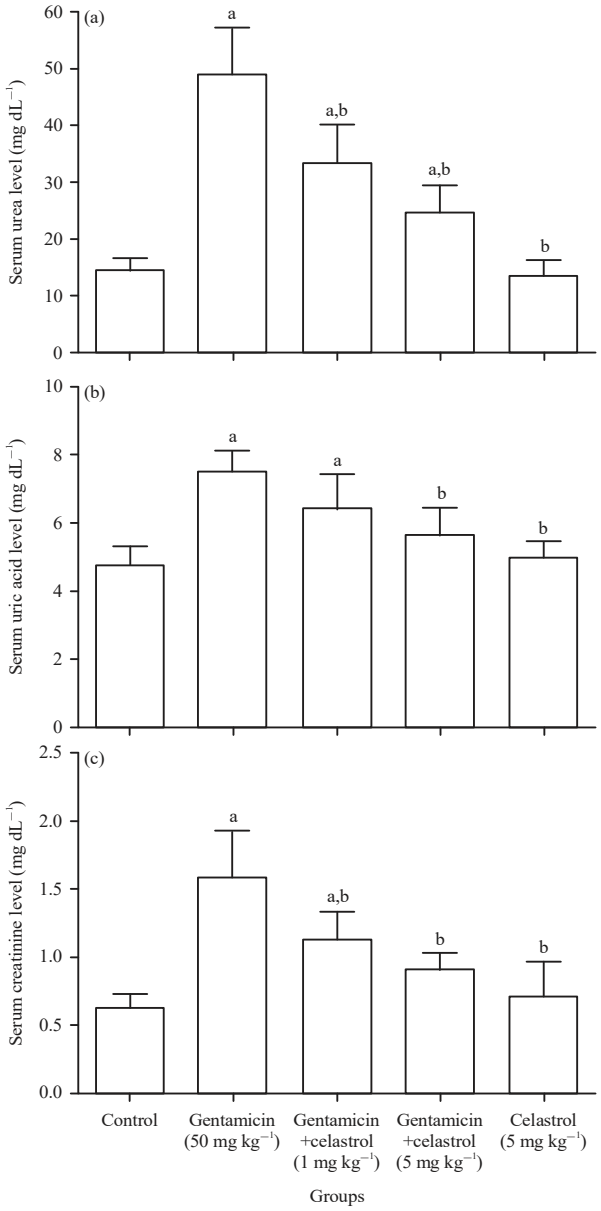

Fig. 2(a-c): Effect of celastrol administration for 7 days on (a) Serum urea, (b) Serum uric acid and (c) Serum creatinine in gentamicin-induced nephrotoxicity in mice

Data are presented as Mean±SD, n = 6, statistical analyses were carried out using a one-way ANOVA, followed by Tukey's *post-hoc* test, [a]Significantly different from the corresponding control at p<0.05, [b]Significantly different from the corresponding gentamicin-only group at p<0.05

Effect of celastrol on renal reduced glutathione content: An intraperitoneal injection of mice with gentamicin for 7 consecutive days at a dose of 50 mg kg^{-1} decreased the renal content of GSH significantly by about 69% (5.67±1.34), compared to the control group (17.33±3.61) (Fig. 3a).

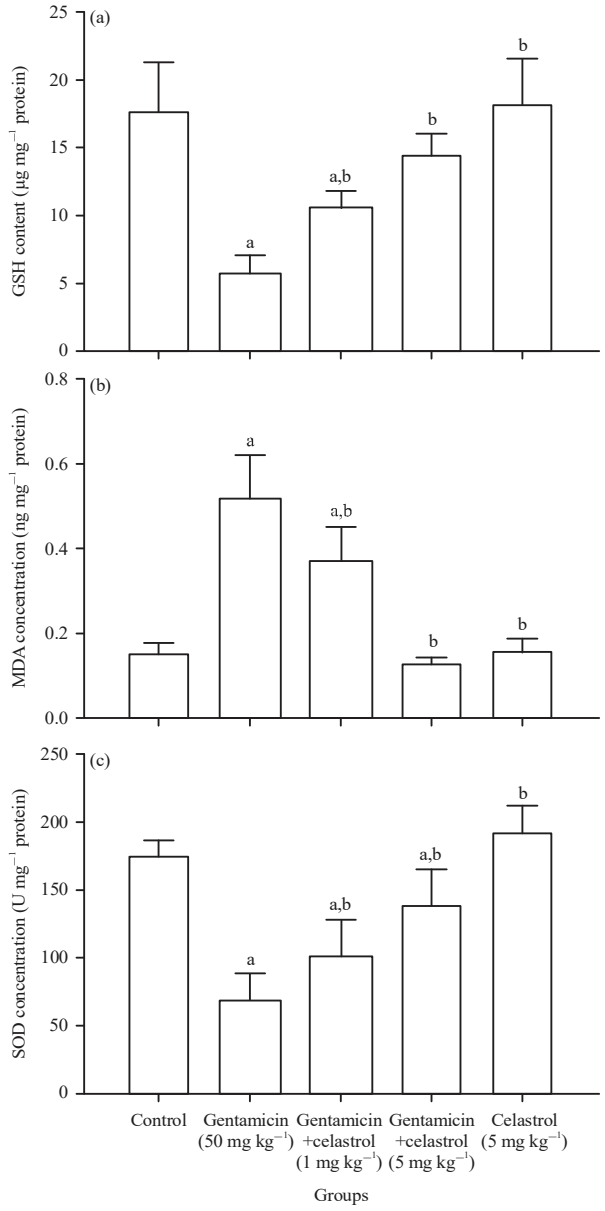

Fig. 3(a-c): Effect of celastrol pretreatment on renal, (a) GSH content, (b) MDA and (c) SOD concentrations in gentamicin-induced nephrotoxicity in mice

Data are presented as Mean±SD, n = 8, statistical analyses were carried out using a one-way ANOVA, followed by Tukey's *post hoc* test, [a]Significantly different from the corresponding control at $p<0.05$, [b]Significantly different from the corresponding gentamicin-only group at $p<0.05$

Moreover, pretreatment with celastrol (1 and 5 mg/kg/day) for 7 consecutive days, 30 min before gentamicin administration, caused significant 2 and 3-fold increases in renal glutathione content (10.6±1.27 and 14.5±1.56), respectively, compared with the gentamicin group (5.67±1.34).

Effect of celastrol on renal tissue malondialdehyde concentration: As shown in Fig. 3b, gentamicin injection (50 mg/kg/day) for 7 days caused significant elevation of renal lipid peroxidation, which manifested as an increase in malondialdehyde concentration by more than 2 folds (0.52±0.101), compared with the control group (0.148±0.27). Pretreatment with celastrol (1 and 5 mg/kg/day) for 7 days significantly reduced malondialdehyde concentration with respect to dosage by about 29 and 75% reaching (0.37±0.08 and 0.127±0.13), respectively, in comparison with the gentamicin group (0.52±0.101). Interestingly, no significant difference was found between the celastrol (5 mg/kg/day for 7 days) group and the control group.

Effect of celastrol on renal superoxide dismutase concentration: Oxidative stress induced by gentamicin in renal tissue was further confirmed by assessing the superoxide dismutase concentration, which demonstrated a significant decrease by about 61% (69.17±14.61), compared with the control group (176.3±12.65) (Fig. 3c). However, pretreatment with celastrol significantly enhanced superoxide dismutase concentration in a dose-dependent manner by about 48 and 103% (102.3±26.48 and 140.8±26.51, respectively), compared with the gentamicin group (69.17±14.61). Furthermore, celastrol-treated mice (5 mg/kg/day for 7 days) failed to show any significant difference in comparison with the control group.

Effect of celastrol on renal tumor necrosis factor (TNF-α) concentrations: As indicated in Fig. 4a, gentamicin administration (50 mg/kg/day) for 7 consecutive days caused a significant increase in TNF-α concentration by 75% to reach (23.95±3.81), compared with the control group (14.35±1.93). Also, celastrol pretreatment (1 and 5 mg/kg/day) was capable of reducing TNF-α concentration by about 23 and 39.4%, (18.43±3.19 and 14.52±2.04), respectively, compared with the gentamicin group (23.95±3.81).

Effect of celastrol on kidney interleukin 6 (IL-6) concentrations: A similar pattern of activity was obtained with IL-6. Injection of mice with gentamicin (50 mg kg^{-1}) for 7 consecutive days resulted in an increase of IL-6 by about two-folds to (16.15±2.97), compared with the control group (4.91±1.35) (Fig. 4b). Pretreatment with celastrol (1 and 5 mg/kg/day) for 7 days, 30 min before gentamicin, significantly decreased the renal concentration of IL-6 by approximately 40 and 51.7%, reaching (9.75±2.21 and 7.80±1.91), respectively, compared with the gentamicin group (16.15±2.97).

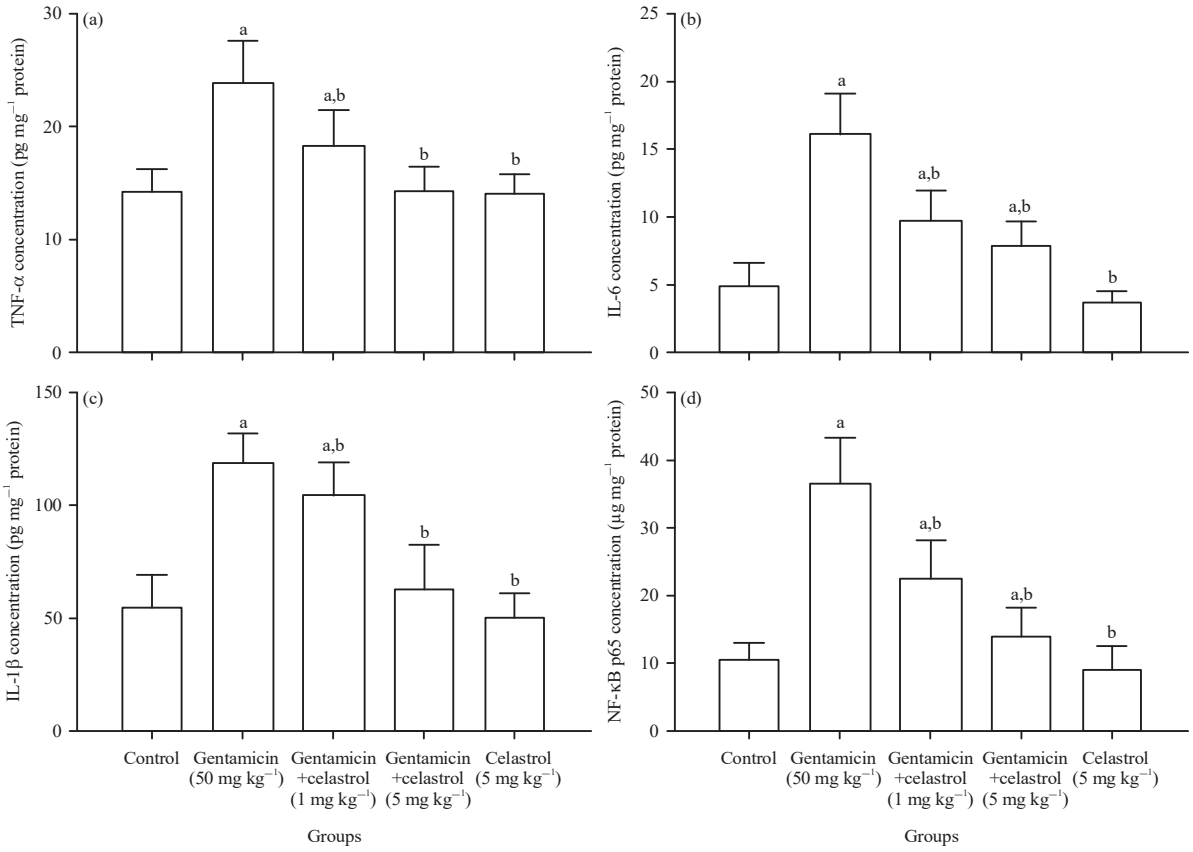

Fig. 4(a-d): Effect of celastrol pretreatment on renal, (a) TNF-α, (B) IL-6, (c) IL-1β and (d) NF-κB p65 concentrations in gentamicin-induced nephrotoxicity in mice

Data are presented as Mean±SD, n = 8, statistical analyses were carried out using a one-way ANOVA, followed by Tukey's *post hoc* test, [a]Significantly different from the corresponding control at p<0.05, [b]Significantly different from the corresponding gentamicin-only group at p<0.05

Effect of celastrol on renal interleukin 1β (IL-1β) concentrations: Regarding IL-1β assessment, mice exposed to gentamicin (50 mg/kg/day) for 7 consecutive days have shown a significant two-fold increase in IL-1β concentration to (118.8±13.08) compared to the control group (55.16±13.7) (Fig. 4c). Celastrol pretreatment at 1 mg/kg/day caused small but significant reduction by 11.3% of IL-1β (105.3±13.71) compared with the gentamicin group (118.8±13.08). On the other hand, celastrol (5 mg/kg/day) was able to markedly reduce IL-1β concentration by 47% (63.1±14.23) to the extent that no significant difference was detected when compared to the control group (55.16±13.7).

Effect of celastrol on kidney transcription factor (NF-κBp65): Mice exposed to gentamicin (50 mg/kg/day) for 7 days have shown (>3-fold) increase of NF-κBp65 concentration to (36.50±6.92) in correlation to the control group (10.72±2.45). Moreover, pretreatment with celastrol resulted in dose-related reduction by about 38% and 62 (22.50±5.86 and 13.96±3.18, respectively), compared with the gentamicin group (36.50±6.92) (Fig. 4d).

Histopathological examination: Histopathological examination of kidney tissue has shown intact glomeruli and tubular epithelial lining as shown in Fig. 5a. Seven days after gentamicin administration, glomerular capillaries were distorted with epithelial tubular atrophy compared to the saline group (Fig. 5b). Pretreatment with celastrol (1 or 5 mg/kg/day) could alleviate the damage in such a way that the total histopathologic score that had significantly increased by gentamicin was markedly decreased by celastrol to its level in the control group in a dose-dependent manner (Fig. 5c, d). In addition, the celastrol treated group (5 mg/kg/day) showed a normal renal histological architecture (Fig. 5e).

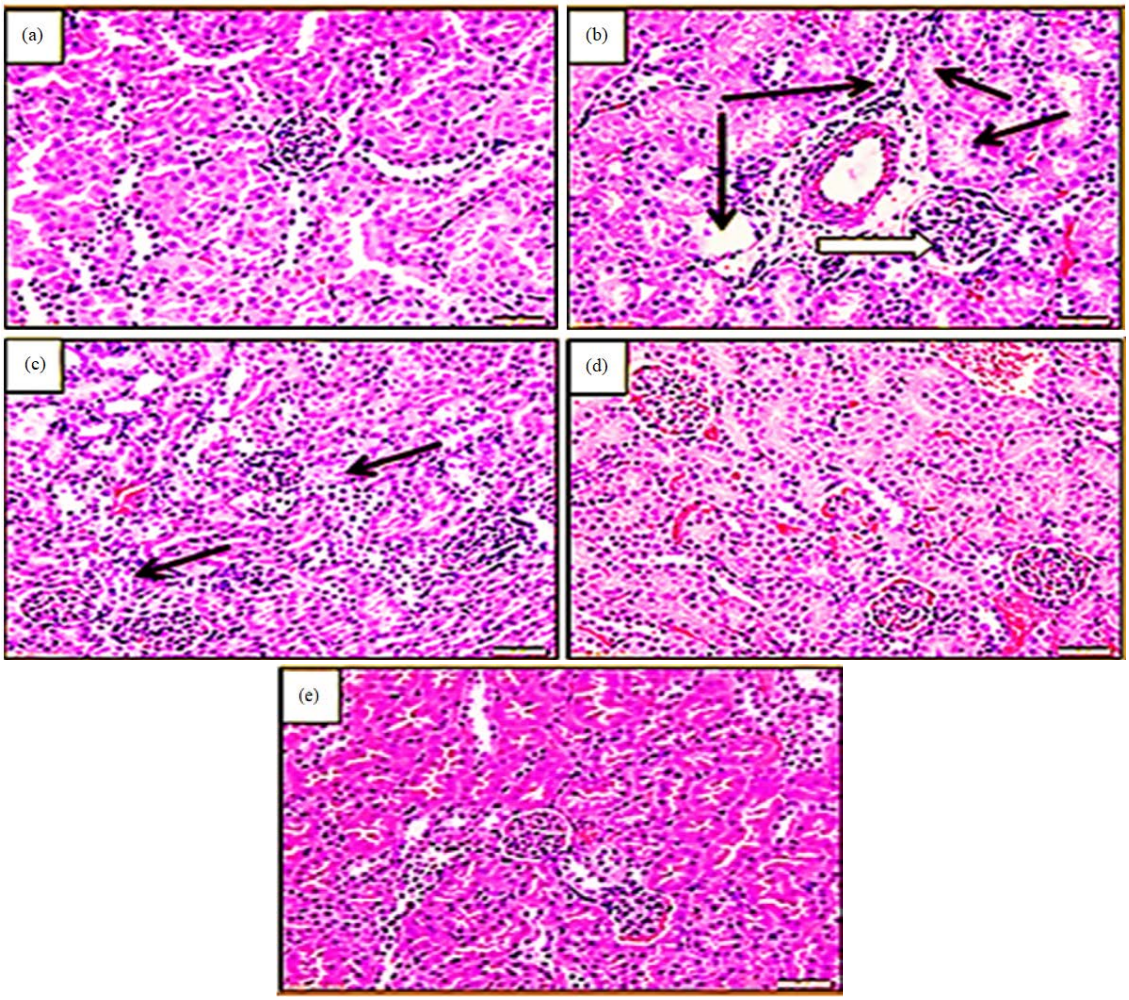

Fig. 5(a-e): Histopathological examination of kidney sections after celastrol administration for 7 days in gentamicin-induced nephrotoxicity in mice X400, (a) Control, showing normal histological architecture of renal tissue with normal glomeruli and tubules, (b) Gentamicin (50 mg/kg/day), showing distorted glomerular capillaries (white arrows) and tubular dilation with epithelial atrophy (black arrows), (c) Gentamicin+celastrol (1 mg/kg/day), showing mild changes in glomerular and tubular epithelial tissue, (d) Gentamicin+celastrol (5 mg/kg/day), showing marked improvement of renal tissues with respect to glomeruli and tubules and (e) Celastrol (5 mg/kg/day) showing a normal structure of renal tissue

DISCUSSION

In this study, the protective effects of celastrol were examined against gentamicin-induced renal functional disturbances, oxidative stress, inflammatory processes and tissue damage in mice. Gentamicin is a member of the AG antibiotics class and often used against gram-negative bacteria[24]. However, the use of gentamicin is frequently associated with nephrotoxicity, which has placed limits on its general use. Oxidative stress activation with inflammatory process constitutes a major part of gentamicin nephrotoxicity[25]. The results showed that administration of celastrol could improve gentamicin-induced nephrotoxicity in mice. Gentamicin administration caused distortion of glomerular capillaries with epithelial tubular atrophy. Several experimental studies have reported that gentamicin causes cell damage in the kidney by stimulating reactive oxygen species production[1]. This leads to a reduction in antioxidant defense mechanisms, causing cellular damage and necrosis that stimulate inflammatory cytokine production and adhesion molecule recruitment, which exacerbates migration of the leukocytes to the injured site[26]. It is possible that celastrol administration could relieve all cell damage induced by gentamicin. A number of studies have demonstrated

that celastrol improves kidney function in renal ischemia/reperfusion and greatly reverses the high renal parameter markers and lipid peroxidation level, healing renal tubular injury. It also decreases the expression of TNF-α, IL-1β and MCP-1, which are connected with inhibition of nuclear translocation of NF-κB[20]. Also, celastrol has been shown to improve renal functional and structural changes, both through metabolic and anti-inflammatory effects in the kidney, by the inhibition of NF-κB[27] and inhibition of the growth of renal cysts, maintaining kidney function[28], resulting in improved renal functional parameters.

In the current study, gentamicin administration resulted in a marked increase in serum creatinine, blood urea-nitrogen concentrations and uric acid, in agreement with the results of a number of previous studies[1,29,30]. Since serum creatinine concentration is inversely related to the glomerular filtration rate (GFR), it seems that increased serum creatinine concentration was due to a decrease in GFR. It has been demonstrated that gentamicin increases renal vascular resistance and tubular necrosis and decreasing glomerular ultrafiltration coefficient (Kf) reduces GFR[31].

The concurrent administration of celastrol with gentamicin markedly ameliorated the elevated levels of serum creatinine, blood urea nitrogen and uric acid. These results suggest that celastrol has an ability to prevent gentamicin nephrotoxicity in mice, in line with the results of a previous study[32] , which showed that the administration of celastrol to mice at various doses for 14 days could provide substantial protection against gentamicin-induced ototoxicity via heat shock protein[32].

It is worth noting that oxidative stress plays a major role in gentamicin-induced nephrotoxicity[33]. This was attributed to the generation of reactive oxygen species and enhanced lipid peroxidation, manifested as elevated malondialdehyde concentration with consequent diminution of glutathione content. Also, the decrease of superoxide dismutase expression or activity augments oxidative stress[34]. In the current study, renal malondialdehyde has been noted to increase in gentamicin-challenged mice, in addition to the reduction of renal glutathione content. These results suggest the induction of renal oxidative stress in mice upon gentamicin administration. However, pretreatment with celastrol markedly ameliorated renal oxidative stress by reducing renal malondialdehyde and restoring renal glutathione content and superoxide dismutase. Accordingly, it might be suggested that the antioxidant effect of celastrol may have played a key role in preventing the gentamicin nephrotoxicity. These findings are in accordance with Chu *et al.*[20], who concluded that single doses of celastrol (4-6 mg kg^{-1}, IP) 30 min before renal ischemia in rats were able to inhibit NF-κB activation and inflammation.

Another mechanism of gentamicin nephrotoxicity was attributed to the generation of pro-inflammatory markers, causing tissue inflammation such as IL-1β[35], TNF-α[36] and NF-κB[37]. In order to investigate the anti-inflammatory activity of celastrol, the pro-inflammatory biomarkers TNF-α, IL-6, IL-β1 and NF-κBp65 were assessed in renal tissue homogenates. The current findings revealed the ability of celastrol to markedly ameliorate these inflammatory cytokines induced by gentamicin, through reducing renal concentrations of TNF-α, IL-6, IL-1β and NF-κBp65. Accordingly, it could be that the anti-inflammatory effect of celastrol played a key role in preventing the gentamicin nephrotoxicity.

Notably, inflammation involves several changes which begin in the living tissue upon injury or through stimulation to the damaged living microcirculation. Such changes include altered blood flow, increased permeability of blood vessels, tissue destruction through the activation and migration of leukocytes with synthesis of reactive oxygen species and the synthesis of local inflammatory mediators, such as prostaglandins, leukotriene's, cytokines and platelet-activating factors induced by phospholipase A2, cyclooxygenases and lipoxygenases[38].

In many cases, inflammation is mediated by reactive oxygen species produced from phagocytic leukocytes that invade tissue. Indeed, reactive oxygen species promote cytotoxicity and may initiate inflammation through upregulation of some genes that code for pro-inflammatory cytokines, such as TNF-α, IL-6, IL-1β[39]. In addition, reactive oxygen species are included in the activation of NF-κB and nuclear translocation of its p65 subunit, which induces expression of inflammatory cytokines[40] and COX2. Hence, NF-κB represents an important linkage between oxidative stress and inflammation. Therefore, using antioxidants would diminish the effect of free radicals, inhibit formation of NF-κB and consequently prevent production of inflammatory mediators.

Examination of kidney sections of gentamicin-injected mice revealed a marked necrosis of renal tubules. These results were in agreement with previous reports that concluded that gentamicin induces nephrotoxicity manifested by biochemical and histological changes in mice[4,34]. Indeed, the histopathological changes induced by gentamicin in the kidneys of mice and the ameliorative effect of celastrol were parallel with the reported biochemical alterations in the present work.

It has been shown that celastrol has anti-oxidant and anti-inflammatory effects which are consistent with previous work[22]. By reducing oxidative stress and cellular damage, celastrol may weaken the GFR-reducing parameters and as a result improve plasma creatinine and urea-nitrogen concentrations.

CONCLUSION

Broadly, current results indicate that celastrol as a dietary phytochemical has a protective effect against gentamicin-induced nephrotoxicity in mice. This protective effect might be due to its antioxidant and anti-inflammatory properties. These findings demonstrate that celastrol may be effective as an adjuvant to conventional therapies and to minimize possible related adverse effects. Further studies using celastrol could therefore focus on defining the exact molecular mechanisms or other pathways underlying the nephroprotective effects against agents that trigger renal tissue damage.

SIGNIFICANCE STATEMENT

This research investigated the role of celastrol treatment in ameliorating gentamicin nephrotoxicity through decreasing the oxidative stress and inflammatory biomarkers toward improving renal functional disturbances. This introduces a potential avenue for researchers and clinicians to study other mechanisms which may be related to this beneficial effect. Moreover, it is suggested that a celastrol supplement may be a potential adjuvant agent.

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