KJPP

Original Article

Protective effects of lutein against vancomycin-induced acute renal injury in mice *via* upregulation of peroxisome proliferator-activated receptor gamma/nuclear factor erythroid 2-related factor 2 and inhibition nuclear factor-kappaB/caspase 3

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ABSTRACT Vancomycin, an antibiotic used occasionally as a last line of treatment for methicillin-resistant *Staphylococcus aureus*, is reportedly associated with nephrotoxicity. This study aimed at evaluating the protective effects of lutein against vancomycin-induced acute renal injury. Peroxisome proliferator-activated receptor gamma (PPARγ) and its associated role in renoprotection by lutein was also examined. Male BALB/c mice were divided into six treatment groups: control with normal saline, lutein (200 mg/kg), vancomycin (250 mg/kg), vancomycin (500 mg/kg), vancomycin (250 mg/kg) with lutein, and vancomycin (500 mg/kg) with lutein groups; they were euthanized after 7 days of treatment. Thereafter, samples of blood, urine, and kidney tissue of the mice were analyzed, followed by the determination of levels of N-acetyl-β-D-glucosaminidase (NAG) in the urine, renal creatine kinase; protein carbonyl, malondialdehyde, and caspase-3 in the kidney; and the expression of PPARγ, nuclear factor erythroid 2-related factor 2 (Nrf2), and nuclear factor-kappaB (NF-κB) in renal tissue. Results showed that the levels of protein carbonyl and malondialdehyde, and the activity of NAG, creatine kinase and caspase-3, were significantly increased in the vancomycin-treatment groups. Moreover, the levels of Nrf2 significantly decreased, while NF-κB expression increased. Lutein ameliorated these effects, and significantly increased PPARγ expression. Furthermore, it attenuated vancomycin-induced histological alterations such as, tissue necrosis and hypertrophy. Therefore, we conclude that lutein protects against vancomycin-induced renal injury by potentially upregulating PPARγ/Nrf2 expression in the renal tissues, and consequently downregulating the pathways: inflammation by NF-κB and apoptosis by caspase-3.

INTRODUCTION

Kidneys are the main excretory organ for many drugs, and could easily be exposed to toxins. Nephrotoxicity has been reported to contribute to approximately 8%–40% of all cases of acute renal injury. Since its introduction, vancomycin, a glycopeptide antibiotic often used as the last line of defense against drug resistant gram-positive bacteria, has been associated with acute renal injury [1,2]. Vancomycin is commonly used to treat hospital-acquired methicillin-resistant *Staphylococcus aureus* (MRSA)

infections [1]. Due to treatment failures, clinicians have been reported to be using higher doses of vancomycin [3]. Although the use of high-dose regimens of vancomycin is not approved by regulatory authorities, it is often administered to achieve a trough of 15–20 mg/L due to the emerging drug resistance; it is believed to be clinically effective against drug-resistant bacteria [4]. The results of cohort and retrospective studies have reported rising rates of nephrotoxicity due to the increased use of high-dose vancomycin [4,5]. An earlier report indicated that vancomycin-induced nephrotoxicity is associated with oxidative stress [6]; thereby, establishing that high-dose vancomycin leads to the generation of reactive oxygen species that disrupt antioxidant activity [7]. Production of oxyradicals in the state of oxidative stress can cause cellular damage that can lead to renal injury and cell death [8]. Studies show that, the molecular components for the function and regulation of cell-signaling, which are susceptible to oxidative stress, are altered under these conditions [9]. Despite the evidence implicating oxidative stress in vancomycin-induced renal injury, the underlying mechanism of the process is still unclear. A better understanding of this mechanism would help in planning a better therapeutic management, as vancomycin is still the first-line drug for both hospital- and community-acquired MRSA infections [5].

Lutein is a carotenoid that has been reported to modulate pro-inflammatory mediators, such as the peroxisome proliferator-activated receptors (PPAR), which is known to generate oxyradicals that alter signaling transduction pathways [10]. PPARγ, a transcription factor and a member of the PPAR family, has been found to be expressed in renal tissues, and is implicated in the regulation of redox hemostasis in the kidney [11]. PPARγ activity is said to be well-regulated during various cellular processes; indeed, nuclear factor-kappaB (NF-κB) stimulation has shown to often suppress PPARγ activity [12]. PPARγ agonists reportedly decrease NF-κB activity in many tissues during inflammation [11]. Activation of PPARγ could be useful against renal damage induced by oxidative stress through the inhibition of NF-κB protein expression, possibly *via* multiple mechanisms. Lutein was shown to enhance PPARγ levels, when administered to enhance anti-inflammatory responses in chickens [13]. Agents that target PPARγ have been shown to mediate inflammatory activities in both animals and humans [14]. Studies have shown that lutein activates the antioxidant response element transcription system, which might explain the anti-cancer properties of carotenoids that has been reported [10,15]. However, the mechanism of antioxidant effect by lutein in renal injury is still debatable and could be multifactorial. Here, we hypothesized that lutein could activate PPARγ in the kidney, through which it could attenuate the oxidative effects of oxyradicals. Therefore, the objectives of this study were to: (a) evaluate the protective effects of lutein in vancomycin-induced acute renal injury in mice, and (b) examine PPARγ expression and the associated signaling pathways in mice with vancomycin-induced renal injury, using Western blotting and real-time polymerase chain reaction (PCR).

METHODS

Chemicals, assay kits, and antibodies

Vancomycin was supplied by Julphar (Ras Al Khaimah, UAE), and lutein was supplied by Extrasynthese (Genay, France). Assay kits for N-acetyl-β-D-glucosaminidase (NAG), protein carbonyl content, and caspase-3 were purchased from Sigma-Aldrich (St. Louis, MO, USA). Primary antibodies against PPARγ, nuclear factor erythroid 2-related factor 2 (Nrf2), and NF-κB p65 were purchased from Santa Cruz Biotechnology (Dallas, TX, USA). Gels for Western blotting were procured from Bio-Rad (Hercules, CA, USA), and primers for real-time PCR of PPARγ were procured from Gibco, Thermo Fisher Scientific Inc. (Waltham, MA, USA).

Animals

Male BALB (Bagg Albino)/c mice with an average weight of 29.5 g, were obtained from the Department of Biological Sciences, College of Science, King Faisal University, Saudi Arabia. The mice were maintained in groups of six per cage in controlled environmental conditions according to the specified standards, with 12 h dark and 12 h light cycle, at 23 ± 1°C. They were allowed access to food and water throughout the study. Animal care and experimental procedures were carried out according to the guidelines of the Research Ethics Committee at King Faisal University (140237/27/2013) and that of the National Committee of Bioethics (NCBE), King Abdulaziz City for Science and Technology (KACST), Saudi Arabia.

Experimental protocol

The mice were divided into six groups (six mice per group) as follows: control with normal saline, lutein (L: lutein) 200 mg/kg (dissolved in 0.2% dimethyl sulfoxide [DMSO]), vancomycin 250 mg/kg (low-dose vancomycin: VL), 500 mg/kg vancomycin (high-dose vancomycin: VH), L + VL, and L + VH. Both the drugs were administered intraperitoneally and consecutively for 7 days, according to the modified methods described by Qu *et al.* [16], Nakamura *et al.* [17], and Vasudeva *et al.* [18]. Vancomycin was given 30 min after lutein administration. At the end of the study, mice were euthanized by cervical dislocation under mild anesthesia. The blood samples, 24-h urine samples, and harvested kidneys were collected at the same time, and stored at –85°C for analysis. The collected samples were used for estimation of biochemical markers of renal injury, such as NAG, creatinine kinase, protein carbonyl, and caspase-3. Western blotting was performed to analyze the expression of PPARγ, Nfr2, and NF-κB p65. Real-time PCR was also used to analyze the expression of PPARγ in the harvested kidneys from all the groups.

Urinary assay of NAG

NAG activity in the urine samples was evaluated using an enzymatic assay, according to the manufacturer's instructions. As reported by Horak *et al.* [19], the assay was based on the hydrolysis of 4-nitrophenyl N-acetyl-β-D-glucosaminide by NAG that releases p-nitrophenol with a basic pH, leading to the formation of the yellow-colored p-nitrophenylate ion, which was then measured colorimetrically at 405 nm.

Creatine kinase activity

Creatine kinase activity was evaluated according to the method described by Hughes [20]. The tissue homogenates of the kidney was incubated in 50 mM Tris buffer (pH 7.5), 7.5 mM MgSO$_4$, 7 mM phosphocreatine, and 3.2 mM ADP medium for 10 min at 37°C. Then, 15 μl of 1 mM p-hydroxymercuribenzoic acid was added after the reaction was stopped for 10 min. To analyze the reaction colorimetrically, 2% α-naphthol and 1% diacetyl were added; the reaction mixture was allowed to develop color for 15 min at 37°C, and then measured at 540 nm.

Protein carbonyl content

Protein carbonyl content, which is a marker of oxidative damage, was measured using a previously reported method [21]. The oxidized proteins produced protein carbonyl, which was then reacted with 2,4-dinitrophenylhydrazine to produce 2,4-dinitrophenylhydrazone, which was measured spectrophotometrically at 375 nm.

Malondialdehyde (MDA) level

Lipid peroxidation (assessed based on MDA production) in the tissue homogenates of the kidney was measured by determining the levels of thiobarbituric acid-reactive substances, as described by Ohkawa *et al.* [22]. Phosphoric acid solution (1%) and a known volume of thiobarbituric acid was added to each homogenate sample, and then incubated at 95°C for 1 h. The concentration of the subsequent thiobarbituric acid-reactive substances was then measured spectrophotometrically at 535 nm.

Measurement of caspase-3 activity

Caspase-3 activity was measured using a colorimetric assay, according to the manufacturer's instructions. The kidney tissues were homogenized in 3 ml of 10 mM phosphate buffer (pH 7.4) [23]. Detection of caspase-3 activity was predicated on the hydrolysis of the peptide substrate acetyl-Asp-Glu-Val-Asp p-nitroanilide by caspase-3. The reaction led to the consequent release of the p-nitroaniline component, which was then measured spectrophotometrically at 405 nm.

Western blotting

The protein expression of PPARγ, Nrf2, and NF-κB p65 in the kidney tissues were determined through Western blotting using specific antibodies, according to the method described by Ahn *et al.* [24]. The harvested tissues were homogenized with radio-immunoprecipitation assay lysis buffer to isolate and extract the content of whole protein. The nuclear extracts were isolated and used for the determination of PPARγ, Nrf2, and NF-κB p65 expression. Approximately 50 μg of protein samples were separated using 10% Sodium dodecyl sulfate polyacrylamide gel electrophoresis gels. These were later transferred on to polyvinylidene fluoride membranes, and 5% non-fat dried milk was used to block the non-specific sites at 20–22°C for 1 h. This was followed by washing of the membranes with Tris-buffered saline and 0.1% Tween 20 (TBST). The membranes were then incubated overnight at 4°C with primary mouse monoclonal antibodies against PPARγ, Nrf2, and NF-κB p65 in a ratio of 1:1,000. Thereafter, the membranes were washed with TBST, and incubated with secondary peroxidase-conjugated goat anti-mouse IgG in a ratio of 1:5,000 for 1 h. The resulting bands were visualized according to the manufacturer's instructions, using an enhanced chemiluminescence system. ImageJ software (freeware; rsbweb.nih.gov/ij) was used to perform the densitometric analyses of the bands.

Real-time PCR

Quantitative real-time PCR was performed for expression analysis of PPARγ in the kidney tissues. cDNA was synthesized from the samples by using a previously reported method [25]. In brief, total RNA was isolated from kidney tissues using the TRIzol method (Gibco; Thermo Fisher Scientific), according to the manufacturer's instructions, and stored at –85°C for later use. The oligonucleotide primers for PCR were as follows: PPARγ, forward 5′-CGGTTTCAGAAGTGCCTTG-3′, reverse 5′-GGTTCAGCTGGTCGATATCAC-3′; and β-actin forward 5′-AGCTATGAGCTGCCTGACGG-3′, reverse 5′-CCAGACAGCACTGTGTTGG-3′. Using kinetic analysis of PCR amplification, the amounts of each template of PPARγ/β-actin mRNA were calculated. The PPARγ mRNA expression was standardized with that of β-actin mRNA, which was used for comparison with each sample. The relative expression of PPARγ was represented as fold change and determined by the relative quantification algorithm with the $2^{-\Delta\Delta CT}$ method [26].

Histological examination

The kidney tissues were fixed in 10% formaldehyde in phosphate-buffered saline. Later, the tissues were embedded in paraffin for light microscopic evaluation; they were sectioned and stained with hematoxylin and eosin. Slide preparation and a double-blinded evaluation were performed by a histopathologist.

The results of pathology were indicated as follows: (–) absence of pathology, (+) mild pathology, (++) moderate pathology, (+++) severe pathology, and (±) recovery.

Data analysis

The results obtained were expressed as mean ± SD; they were analyzed using the GraphPad Prism software version 8.2 (San Diego, CA, USA). Comparisons between all the treatment and control groups were made using the one-way analysis of variance, and the differences between the groups were measured using the Tukey's multiple comparison test. $p < 0.05$ was considered statistically significant.

RESULTS

Effects of lutein on NAG, creatine kinase, protein carbonyl, MDA, and caspase-3 activities

Mice treated with VL and VH showed a significant elevation in NAG activity, which was significantly attenuated by co-admin-istration with lutein (Fig. 1A). In Fig. 1B, the activity of creatine kinase was significantly higher in VL- and VH-treatment groups, than that in the control group. In L + VL or L + VH groups, creatine kinase had reduced significantly. As shown in Fig. 1C, the protein carbonyl content in VL and VH groups were significantly higher than those in the control group, and co-administration with lutein significantly reduced the protein carbonyl content in these treatment groups. MDA levels had increased significantly in the vancomycin-treatment groups than those in the control group. However, following co-administration with lutein, these levels had also decreased significantly (Fig. 1D). Caspase-3 activity in the kidney tissues had increased significantly in the VL- and VH-treatment groups than that in the control group. The activity had reduced significantly again, with the co-treatment of vancomycin with lutein in both the treatment groups (Fig. 1E). Our results indicate that lutein effectively increases the antioxidant activity in the kidney tissues. Subsequently, co-administration with lutein attenuated the injurious effects due to the administration of VL and VH, respectively.
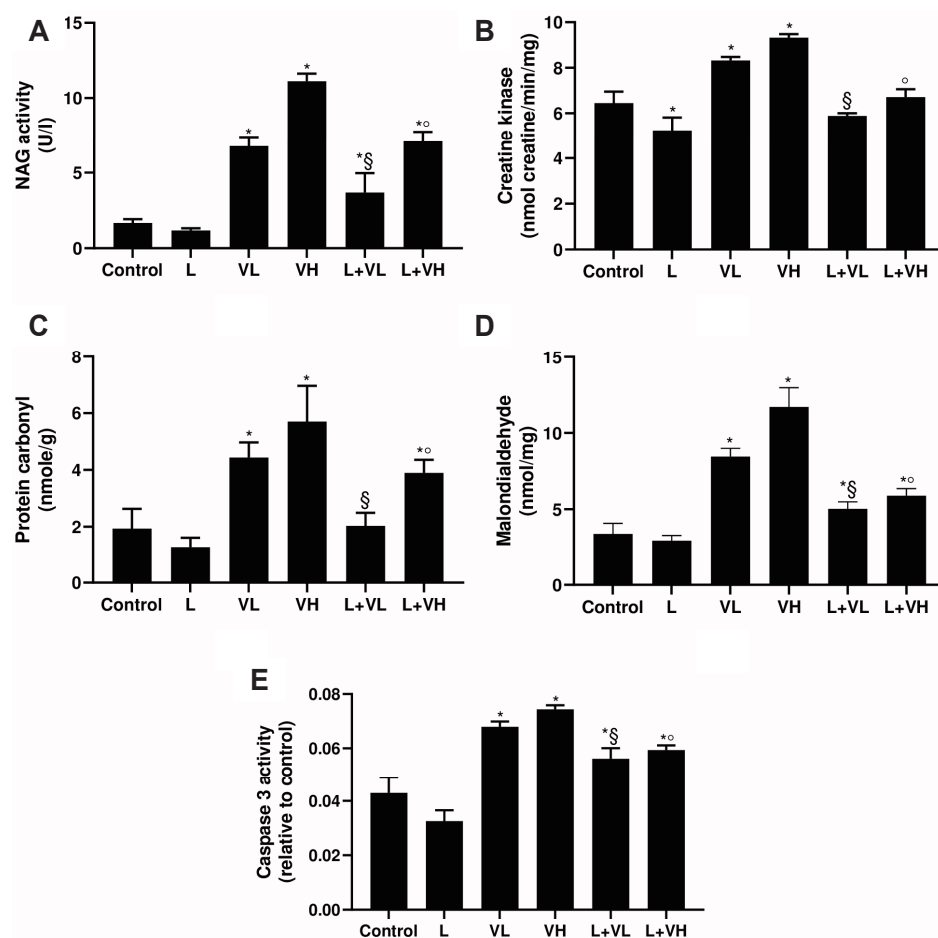

Fig. 1. Effect of lutein on (A) N-acetyl-β-D-glucosaminidase (NAG), (B) creatine kinase, (C) protein carbonyl, (D) malondialdehyde, and (E) caspase-3, in low (250 mg/kg) and high (500 mg/kg) dose vancomycin-induced renal injury in mice. Results are mean ± SD. L, lutein 200 mg/kg; VL, vancomycin 250 mg/kg; VH, vancomycin 500 mg/kg. *Shows significant different ($p < 0.05$) from control, while §indicates significant different ($p < 0.05$) from VL-treatment, and VH-treatment groups, respectively.

Effect of lutein on histopathological changes induced by vancomycin

In the control group and groups treated with lutein, the glomeruli and renal tubules showed no signs of cellular damage (Fig. 2A, B). In addition, there was no infiltration of inflammatory cells, and renal architecture was normal (Table 1). In the VL-treatment group, we observed abnormal dilation of renal tubules that were filled with leukocytes, in addition to significant infiltration of inflammatory cells into the renal parenchyma (Fig. 2C). However, in the L + VL-treatment group, there were reductions in infiltration of inflammatory cells, and recovery of the renal parenchyma (Fig. 2D and Table 1). Administration of 500 mg/kg vancomycin produced significant renal damage such as epithelial necrosis of the renal tubules, and complete obliteration of the tubules in most cases (Fig. 2E). Lutein administration to this group, significantly attenuated the injurious effects of vancomycin, indicating recovery of the renal tubules (Fig. 2F).

Effects of lutein on PPARγ (protein and mRNA) and Nrf2 expressions in vancomycin induced changes

The expression levels of PPARγ, (protein and mRNA) and Nrf2, determined in the control and treatment groups, are shown in Fig. 3A–C. Western blotting analysis showed a significantly increased PPARγ expression in the VL- and VH-treatment groups than that in the control group. In addition, co-administration with lutein in both VL- and VH-treatment groups further increased PPARγ
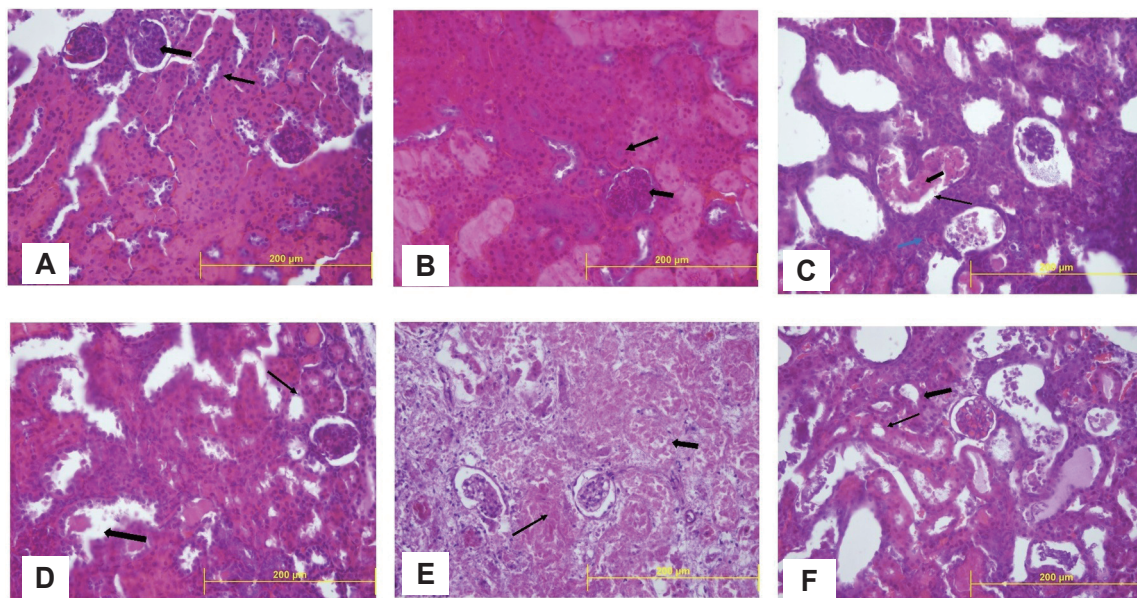

Fig. 2. Effect of lutein on histopathological changes in low (250 mg/kg) and high (500 mg/kg) dose vancomycin-induced renal injury in mice (A–F: H&E ×40). In (A, B) thick black arrow: normal glomerulus, thin black arrow: normal renal tubules. (C) Thick black arrow: abnormal dilatation of the renal tubules, thin black arrow: renal tubules filled with leukocytes, blue arrow: infiltration of inflammatory cells in the renal parenchyma. (D) Showing recovery of the parenchyma of the kidney (thick arrow and thin black arrows). (E) Thick black arrow: obliteration of renal tubules filled with necrotic cells, thin black arrow: necrosis of the epithelium of renal tubules. (F) Thick and thin black arrows: showing recovery of the parenchyma of kidney with no observed necrosis of epithial lining of renal tubules.

Table 1. Histopathological scoring of the effect of lutein on low (250 mg/kg) and high (500 mg/kg) dose vancomycin-induced renal injury in mice

Group	Abnormal dilatation of the renal tubules	Renal tubular obliteration	Tubular necrosis	Renal tubules filled with leukocytes	Inflammatory cell infiltration in parenchyma
Control	–	–	–	–	–
Lutein	–	–	–	–	–
Vancomycin 250 mg/kg	+	–	–	+	+
Lutein + vancomycin 250 mg/kg	±	–	–	–	±
Vancomycin 500 mg/kg	–	+++	+++	–	–
Lutein + vancomycin 500 mg/kg	+	–	–	+	+

–, absence of pathology; +, mild pathology; ++, moderate pathology; +++, severe pathology; ±, recovery.
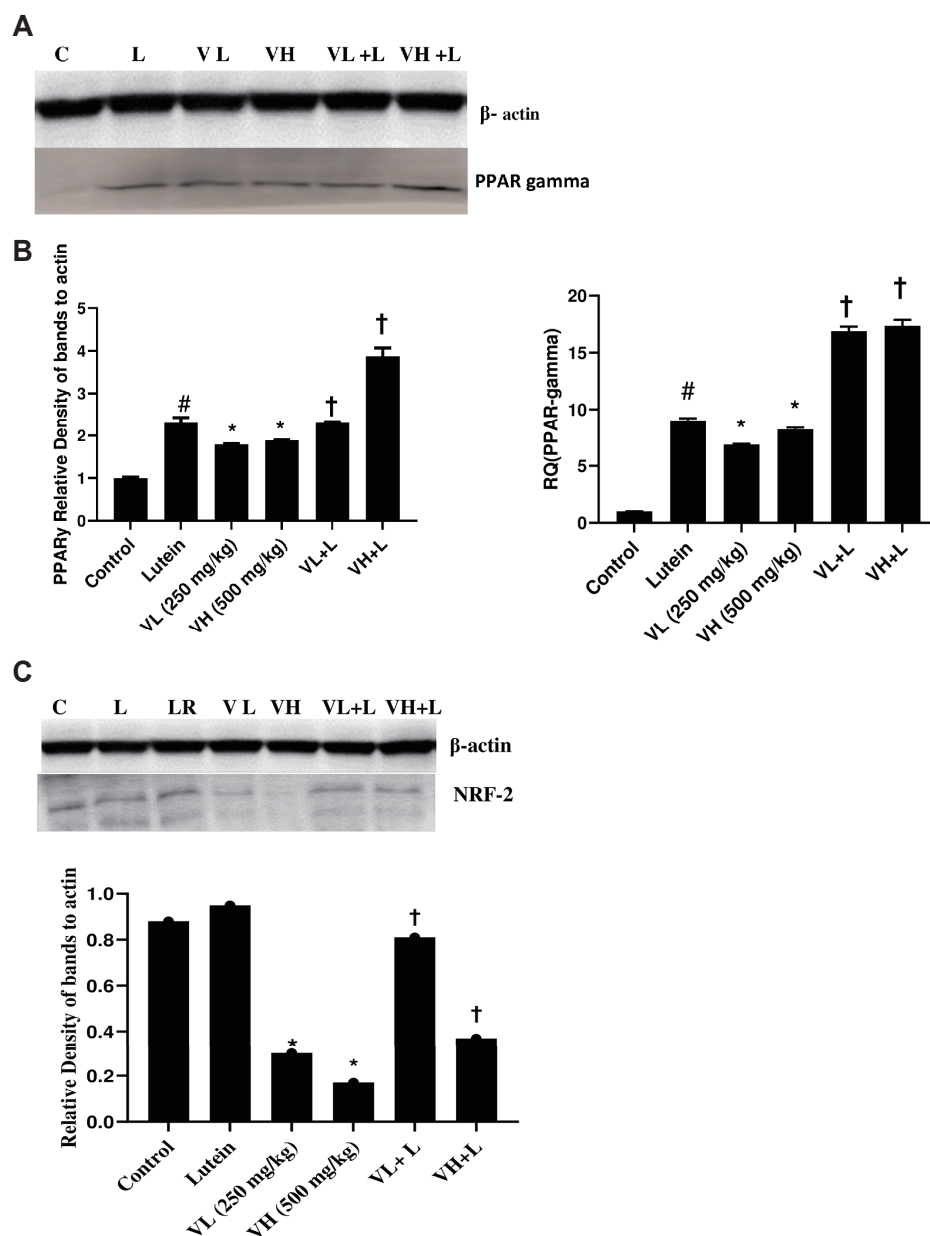
A

B

C

Fig. 3. Effect of lutein on (A) peroxisome proliferator-activated receptor gamma (PPARγ), (B) peroxisome proliferator-activated receptor gamma (PPARγ) mRNA and (C), nuclear factor erythroid 2-related factor 2 (Nrf2) expressions respectively, in low (250 mg/kg) and high (500 mg/kg) dose vancomycin-induced renal injury in mice. Results are represented as mean ± SD. L, lutein; VL, 250 mg/kg vancomycin; VH, 500 mg/kg vancomycin; VL + L, 250 mg/kg + lutein; VH + L, 500 mg/kg + lutein. *Represent significant difference (p < 0.05) between control and vancomycin treated groups, while †represents significant difference (p < 0.05) between vancomycin treated and lutein plus vancomycin treatment groups. #Represents significant difference (p < 0.05) between control and lutein.

expression significantly, compared to that in the control group (Fig. 3A). In the mRNA expression analysis across the different treatment groups, the mRNA levels of PPARγ were found to be increased in the VL- and VH-treatment groups than those in the control group. Co-administration of lutein with either VL or VH significantly increased the mRNA expression of PPARγ in these renal tissues (Fig. 3B). Therefore, we conclude that lutein elevates the mRNA levels of PPARγ in the vancomycin-treatment groups. Western blotting analysis also showed a significantly reduced Nrf2 expression in both VL- and VH-treatment groups; however, in both the groups, the expression was restored with lutein treatment (Fig. 3C).

Effect of lutein on NF-κB p65 expressions in vancomycin induced changes

Results showed that NF-κB p65expression was significantly elevated in both VL- and VH-treatment groups compared to that in the control group; however, co-administration with lutein reduced NF-κB p65 expression in both the groups (Fig. 4).

DISCUSSION

Vancomycin is an antibiotic used clinically to treat drug resistant bacterial infections, particularly by the gram-positive bacteria, MRSA. Reports indicate that, for clinical effectiveness, the daily area under the curve values of vancomycin should achieve
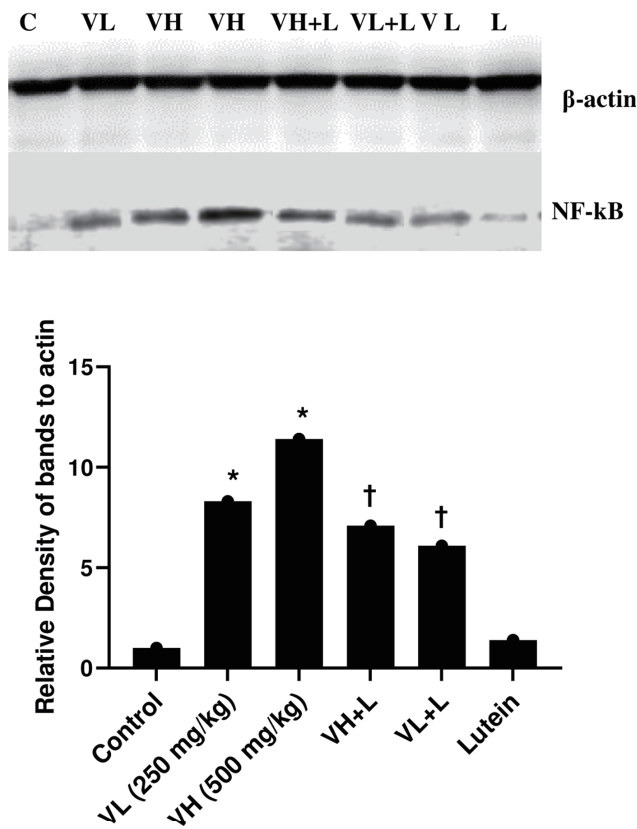
Fig. 4. Effect of lutein on nuclear factor-kappaB (NF-κB) expression in low (250 mg/kg) and high (500 mg/kg) dose vancomycin-induced renal injury in mice. Results are represented as mean ± SD. L, lutein; VL, 250 mg/kg vancomycin; VH, 500 mg/kg vancomycin; VL + L, 250 mg/kg + lutein; VH + L = 500 mg/kg + lutein. *Represent significant difference (p < 0.05) between control and vancomycin treated groups, while †represents significant difference (p < 0.05) between vancomycin treated and lutein plus vancomycin treatment groups.

plasma concentrations of 400 and 600 mg × h/L, respectively, *via* dose escalation [27]. As a result, there is a high concentration of free drug in the plasma, and dose escalation poses a cumulative risk for renal injury. Studies have shown various mechanisms for vancomycin-induced renal injury, suggesting that it could indeed be multifactorial in nature, involving several signaling transduction pathways [2,16]. However, no consensus on the mechanism has been reached. Improving the clinical outcome of vancomycin treatment is predicated on mitigating its associated nephrotoxicity.

In this study, we examined vancomycin-induced acute renal injury and its underlying mechanism. Moreover, we examined various signaling pathways involved in the generation of oxyradicals by vancomycin, and the inhibition of these oxyradicals by lutein—a natural antioxidant. Lutein has been reported to have a variety of biological effects related to its antioxidative properties [28]. The present findings indicate that the administration of low-dose and high-dose vancomycin in mice induces oxyradicals, leading to a significant reduction in the antioxidant activity in the renal tissues. In addition, co-administration of lutein with

vancomycin restored the antioxidant levels, and consequently reduced the generation of cellular oxyradicals. Our data also showed that lutein administration led to a significant reduction in NAG excretion that was observed to increase after vancomycin treatment. The significantly increased NAG levels usually indicate cellular damage in the kidney, and this can be a sensitive biological marker for glycopeptide-antibiotic–induced renal toxicity [29]. Our findings are corroborated by an earlier study by Qu *et al.* [16] that showed increased NAG excretion in rats upon vancomycin administration (200 mg/kg). Lutein was found to reduce NAG levels and mitigate vancomycin-induced renal injury. In this study, a significant increase in creatine kinase activity was observed in the vancomycin-treatment groups, in a dose-dependent manner. Increased creatine kinase activity suggests muscle damage; therefore, is a marker for renal injury. In the present study, lutein co-administered with vancomycin, was observed to significantly decrease the levels of creatine kinase. Lutein has previously shown to decrease creatine kinase levels by 85% in C_2C_{12} mice myocytes [30]. Ouyang *et al.* [31] also documented similar findings, wherein lutein administration lowered creatine kinase levels that were increased by isoproterenol administration in rats. According to Cheng *et al.* [32], lutein treatment significantly decreased the oxidative stress by reducing reactive oxygen species, protein carbonylation and sulphydryls, lipid peroxidation. Also, some studies indicated that low serum/plasma carotenoid levels are independently associated with sarcopenia and related muscle dysfunctions [33].

Furthermore, during renal injury, protein carbonyl content is known to usually increase. This can be used as another biomarker for cellular injury. Protein carbonyl content has been reported to be consistent in protein oxidative damage [34]. In this study, we observed that vancomycin treatment leads to elevated protein carbonyl content in a dose-dependent manner. However, our findings also show that lutein administration mitigated these effects, indicating that it has a protective role, in accordance with the findings of Cheng *et al.* [32]. Lipid peroxidation is a consequence of oxidative stress, and vancomycin treatment has been reported to significantly increase MDA levels in the renal tissues [16]. Our study also reports similar observations, although we show that lutein significantly reverses these effects. Evidence show that several drugs and diseases may affect blood flow and promote cell death *via* apoptosis, in the renal tissues [35]. Oxyradicals are reported to play a crucial role in renal injury, and consequently cell death via several pathways. In the present study, caspase-3 activity was evaluated as they are one of the main executors of apoptosis [36]. We found that administration of vancomycin at 250 and 500 mg/kg increases caspase-3 protease activity in the renal tissues. These observations are similar to the findings of Yang *et al.* [36]. We also showed that lutein administration significantly reverses these effects. Our results are consistent with the findings of Ouyang *et al.* [31] and Nataraj *et al.* [37].

Through the histopathology examination of photomicrographs

of renal tissues, this study confirms that different doses of vanco-mycin cause nephrotoxicity. We observed various hallmarks of renal injury, including inflammatory cells, leukocytes infiltration, necrosis, renal hypertrophy, and obliteration of renal tubules. However, upon lutein treatment, these effects were considerably reversed. This further goes to confirm that lutein has potent an-tioxidant and anti-inflammatory properties against oxyradicals. These findings are also consistent with other studies documented by Ouyang et al. [31] and Fung et al. [38].

It is well established that oxyradical generators like vancomy-cin induce oxidative stress [16]. Studies have shown that NF-κB, a transcriptional mediator of the inflammatory process, plays a key role in cellular injury and damage during this process [39].

In addition, NF-κB has been tagged the "holy grail" of inflam-matory processes, activating multiple pathways during cellular oxidative stress [40]. In a study conducted by Xiao et al. [41], indi-cated that NF-κB pathway is induced during oxidative stress, pro-ducing inflammatory cytokines, chemokines, adhesive molecules and upregulating apoptotic signalling. This consequently leads to renal cell programmed death. Abundance evidence show that it causes DNA fragmentation and hence apoptosis via the activation of caspase 3 [42].

We also show that, vancomycin treatment leads to elevated expression of NF-κB p65 in a dose-dependent manner, and this correlates with tissue damage. These findings are similar to those reported by Qu et al. [16]. In the present study, lutein adminis-tration led to a dramatic reduction in NF-κB p65 expression, as indicated by the recovery of the parenchyma of the renal tubules.

Therefore, this study found that lutein supressed NF-κB p65 activation, thereby inhibiting its regulated gene products such as cytokines and caspase 3. This finding is consistent with the re-ports of Buhrmann et al. [43], who used curcumin in same fash-ion.

Accumulative evidence shows that PPARγ plays a vital role in redox homeostasis by regulating several signaling pathways [11]. Therefore, the activation of PPARγ in the renal tissues could be critical for kidney functioning [44]. According to documented re-ports, the activation of PPARγ mitigates inflammation in various tissues; thereby, preventing cellular injury [11,44].

Numerous studies have shown that PPARγ agonists can be renoprotective by ameliorating inflammation [45]. Similarly, it has also been documented that, lutein has antioxidant proper-ties, which suppress inflammatory responses, possibly through PPARγ activation [25]. The increased PPARγ expression upon vancomycin administration, in comparison to that in the control, can be explained as a compensatory measure by renal cells to overcome oxyradical-induced cellular damage. In this study, we also observed that the PPARγ expression increased with lutein administration, mitigating the effects of vancomycin-induced renal injury. These findings appear to be in agreement with those of Diep and Schiffrin [46]; they suggested that increased PPARγ expression might play a role in cell remodeling under stressful

conditions. Kvandova et al. [47] also reported similar findings in spontaneously hypertensive rats, which evoked renal oxidative stress, but was attenuated by a PPARγ agonist. The activation of PPARγ under conditions of oxidative stress is linked to its ability to interact with the Nrf2 signaling pathway [48]. In this study, our findings show that vancomycin treatment led to the down-regulation of Nrf2, which induced renal injury similar to that in previously reported studies [32]. However, lutein administration restored Nrf2 expression. There is abundant evidence show-ing that lutein acts by activating Nrf2, in addition to serving as a direct antioxidant. This suggests that there is a link between Nrf2 activation and PPARγ activity, which has been documented previously [48]. It has been reported that Nrf2-deficient mice lack protection from oxidants because Nrf2 modulates PPARγ [48].

However, another body of evidence show that PPARγ activa-tion, in fact, regulates the Nrf2 pathway [49], owing to the evi-dence of reduced Nrf2 expression in PPARγ-deficient mice [50]. Furthermore, the aforementioned studies strengthen our obser-vation in the present study that lutein, an activator of PPARγ and Nrf2, attenuates oxidative stress by increasing the expression of both. This view has been supported by many studies, indicating that co-activation of these pathways suppresses inflammation and cellular oxidative stress [51]. Moreover the attenuation of cel-lular expression of NF-κB by PPARγ, and the suppression of pro-inflammatory mediators, is well documented [48]. Our study also showed a reduction in the NF-κB expression on co-administra-tion of lutein. This indicates that lutein may have a role in the me-diation of antioxidant and anti-inflammatory activities observed in this study. Based on these results, we suggest that lutein upreg-ulates PPARγ/Nrf2 signaling pathways and downregulates NF-κB signaling pathway. This finding was also corroborated by Ma-karov [52]. Jin et al. [53] clearly suggests that lutein inhibits NF-κB expression through the activation of PPARγ/Nrf2 signaling pathways, which in turn mediates the expression of inflamma-tory genes [52]. These observations puts lutein in the category of PPAR gamma ligand. The effect of lutein is similar to the effects of PPAR gamma agonist. Pioglitazone, PPAR gamma agonist was found to significantly decrease acute renal injury induced by cis-platin model in mice [54]. Ibrahim et al. [55], also demonstrated this mediation in MTX-induced nephrotoxicity in rats. However, the combination of lutein and PPAR gamma agonist has not re-ceived much attention. Abundant evidence reveal that lutein acts by increasing the expression of PPAR gamma and hence could have a synergistic effect therapeutically as documented by Rafi et al. [56]. Observing that lutein showed mild decrease in cancer cell proliferation that improved in combination treatment with PPAR gamma agonists.

This study has highlighted the potential of lutein in protecting against vancomycin-induced renal injury, a major drawback of vancomycin treatment. In addition, this study confirms the anti-inflammatory properties of lutein. The antioxidant activity of lutein is crucial in attenuating the effects of oxyradicals generated

by vancomycin treatment; thereby, reducing oxidative stress, and consequently, mitigating renal cellular injury in mice. Lutein's renoprotective effects seem to be associated with increased PPARγ expression, and subsequent co-activation of Nrf2; hence, suppressing renal NF-κB expression thereby inhibiting the activation of apoptotic process. Based on these observations, we suggest that the molecular mechanism of vancomycin-induced renal injury could be associated with the inhibition of PPARγ/Nfr2 signaling pathways. We show that lutein can reactivate PPARγ/Nfr2 signaling to attenuate the harmful effects of oxyradicals generated from the use of escalated doses of vancomycin.

ACKNOWLEDGEMENTS

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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