

# The effect of lutein on vancomycin-induced oxidative kidney damage in rats

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## Abstract

**Aim:** It has been reported that nephrotoxicity is observed in 5-25% of patients during vancomycin use. The part oxidative stress plays in the pathogenesis of vancomycin-induced nephrotoxicity has been demonstrated. Lutein shows antioxidant activity. The purpose of the present study was to evaluate effects experienced by lutein on oxidative kidney damage induced by vancomycin in rats biochemically an histopathologically.

**Materials and Methods:** Oral administration of lutein at a rate of 1mg per kg was performed for the lutein plus vancomycin group (L+VAN) (n=6), whereas saline was administered to the vancomycin (VAN) (n=6) and healthy control (C) (n=6) groups. Sixty minutes after administration of lutein and solvent, 200 mg/kg vancomycin was intraperitoneally injected twice a day to L+VAN and VAN groups. This procedure was repeated for 7 days. Then malondialdehyde (MDA), total glutathione (tGSH), total oxidant system (TOS) and total antioxidant system (TAS) levels have been assessed in kidney tissues; in addition, creatinine and BUN were measured in blood samples. Kidney tissues were also evaluated histopathologically.

**Results:** Significant histopathological damage could be seen in the kidney tissue of the VAN group that increasing MDA and TOS and creatinine and BUN. Histopathological damage was not very significant in the L+VAN group that decreasing MDA, TOS, BUN and creatinine levels.

**Conclusion:** This suggests that lutein may be effective for the treatment of the treatment of vancomycin-associated oxidative kidney damage.

**Keywords:** Kidney; lutein; oxidative damage; vancomycin

## INTRODUCTION

Vancomycin is a glycopeptide antibiotic commonly used to treat methicillin-resistant Gram-positive bacteria, especially *Staphylococcus aureus* (1). Vancomycin attaches to the terminal D-ala-D-ala sequence of peptides which create the cellular wall of gram-positive bacteria, inhibiting the transglycosylation reaction and peptidoglycan form. It inhibits cell wall synthesis and has a bactericidal effect on proliferating bacteria. In addition, it induces protoplast damage by changing the permeability of the cytoplasmic membrane. It also contributes to the antibacterial effect by influencing ribonucleic acid synthesis (2). Multiple antibacterial action mechanisms lead to lower development of resistance to vancomycin. However, it has been reported that 5-25% of patients

experience nephrotoxicity during vancomycin use (3). It has also been shown in animal studies that vancomycin is nephrotoxic (4). Vancomycin has been reported to increase serum creatinine levels, leading to loss of kidney tubule cells and ultimately tubular necrosis (5). The exact mechanism of vancomycin-induced nephrotoxicity is unknown, but the part oxidative stress plays in pathogenesis was discussed (6). The literature data support the idea that antioxidants may have a practical effect on the treatment of vancomycin-associated oxidative kidney damage (5). Lutein (C40H56O2) to be investigated in the present study for its effect against vancomycin-induced oxidative kidney damage is known as tetraterpenoids (7). It has been reported that lutein provides antioxidant activity by inhibiting lipid peroxidation and preventing GSH levels

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from decreasing (8). There were no studies evaluating the effect of lutein on oxidative kidney damage triggered by vancomycin in previous studies. Therefore, the purpose of the present research is to evaluate effect of lutein on oxidative kidney damage induced by vancomycin in rats biochemically and histopathologically.

## MATERIALS and METHODS

### Animals

The subjects of this study were 18 male albino wistar rats weighing 260-265 grams which were randomly selected. The subjects were obtained from the Medical Experimental Application and Research Center of Ataturk University. Approval for the research was received from the local animal welfare panel at Ataturk University. The subjects were kept and fed at normal room temperature as 22°C in groups prior to the experiment.

### Chemicals

Thiopental sodium has been obtained from IE Ulagay-Turkey, vancomycin was supplied from Edicin (Sandoz Pharmaceuticals, Istanbul, Turkey), and lutein was supplied from Solgar (America).

### Experimental Groups

The subjects of the experiment were categorized as healthy control (C), vancomycin administered (VAN), and lutein plus vancomycin administered groups (L+VAN).

### Experimental Procedure

Oral administration of lutein was provided to the L+VAN group (n-6) at a rate of 1 mg per kg. The same volume of normal saline (0.9% NaCl) was given to the VAN (n-6) and C (n-6) groups as a solvent. One hour after the administration of lutein and solvent, L + VAN and VAN groups were intraperitoneally, (ip) injected with 200 mg/kg vancomycin at an interval of 12 hours. This procedure was repeated 7 times. After the completion of these procedures, the subjects were humanely euthanized with a high dose of anesthesia (50mg/kg thiopental sodium) and their kidney tissues removed. These kidney tissues were sampled for levels of malondialdehyde (MDA), total glutathione (tGSH), total oxidant status (TOS) and total antioxidant status (TAS). Pre-mortem/Post-mortem blood samples were used to measure creatinine and blood urea nitrogen (BUN) levels. Kidney tissues were also evaluated histopathologically.

### Biochemical Analyses

#### Malondialdehyde (MDA) measurement

The method used by Ohkawa et al. was used as the foundation for MDA measurements in this study. This method involves spectrophotometrical measurement of the absorption of the complex created by thiobarbituric acid (TBA) and MDA (9).

#### Total glutathione (tGSH) measurement

The tGSH analysis described by Sedlak et al (10) was used in this study. In this type of analysis, when DTNB (5,5'-dithiobis 2-nitrobenzoic) acid was decreased to disulfide sulfhydryl groups, a yellowish compound is formed and measured at a wavelength of 412 nm.

### Measurements of Total Oxidant Status (TOS) and Total Antioxidant Status (TAS)

An innovative way of measuring TOS and TAS levels of tissue homogenates was used in addition to commercially available kits (Rel Assay Diagnostics, Turkey), both of which were developed by Erel (11,12).

### Creatinine measurement

A spectrophotometric method was used to perform quantitative determination of serum creatinine with the Roche brand cobas 8000 auto analyzer. The Jaffe method is the foundation for this type of kinetic colorimetric test.

### BUN measurement

A spectrophotometric method was used to perform quantitative determination of serum urea nitrogen level using the Roche brand cobas 8000 auto analyzer. BUN level was calculated with the formula  $BUN = UREA \times 0.48$ .

### Histopathological examination

After the renal tissue samples were stabilized in a 10% neutral buffered formalin solution for 24 hours, routine follow-up was performed. After that, sections having the thickness of 5 µm have been taken out of the prepared samples and stained with hematoxylin - eosin (H&E), and Periodic Acid-Schiff (PAS) dye. Histopathological examination was conducted using a light microscope (Olympus BX 51, Tokyo, Japan). A Zeiss Axiocam ICc 5 camera was used to photograph the samples. Renal sections of all three groups (healthy, damaged by vancomycin, and treated with lutein) were examined.

### Statistical Analyses

Results were shown as "mean value ± standard deviation" ( $\bar{x} \pm SD$ ). Significance levels of the differences among groups were established using the one-way ANOVA test. After that, Fisher's post-hoc Tukey was conducted. All statistical procedures have been performed on "SPSS for Windows, 18.0" and  $p < 0.05$  was accepted as significant.

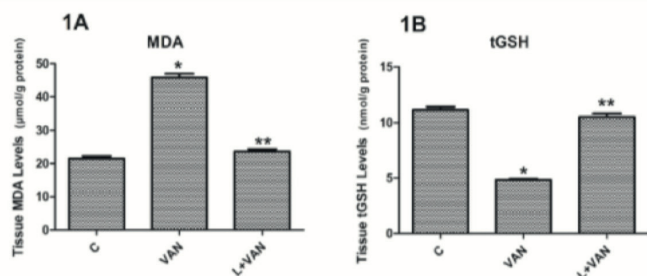
## RESULTS

### Biochemical analyses results

This results of this study revealed that the amount of MDA in kidney samples of the VAN group showed a comparative increase to the C group ( $P < 0.0001$ ). The levels of MDA decreased in the L+VAN group compared to VAN group ( $P < 0.0001$ ). In addition, the difference in MDA level between the C group and the L+VAN groups was considered insignificant ( $P > 0.05$ ) (Figure 1A). However, tGSH was significantly decreased in renal tissues of the VAN group when measured against the C group ( $P < 0.0001$ ). Levels of tGSH increased in the L+VAN group compared to the VAN group ( $P < 0.0001$ ). The difference in tGSH levels between the C group and the L+VAN groups was found to be insignificant ( $P > 0.05$ ) (Figure 1B).

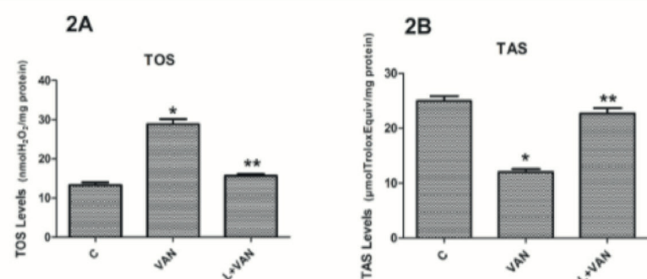
TOS values in kidney tissue of VAN group animals were higher than the C group ( $P < 0.0001$ ). Levels of TOS were found to be less in the L+VAN subjects when compared to the VAN group. Levels of TAS in the kidney tissue of the VAN group were higher than the C group ( $P < 0.0001$ ).

In the L+VAN group, TAS levels decreased when compared to the VAN group ( $P < 0.0001$ ). The difference in TOS and TAS levels between the C and L+VAN groups was not found to be significant ( $P > 0.05$ ) (Figure 2A-B).



C: Healthy control group, VAN: Vancomycin administered group, L+VAN: Lutein plus vancomycin administered group. \* $p < 0.0001$  according to C group, \*\* $p < 0.0001$  according to VAN group

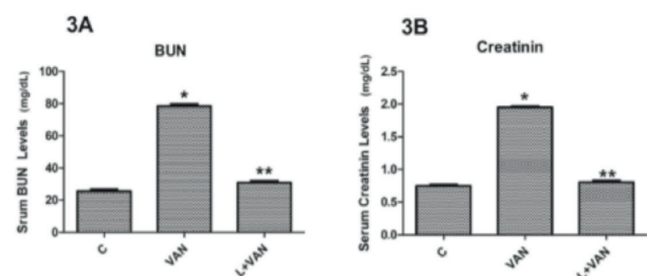
**Figure 1.** The levels of MDA and tGSH in kidney tissues of experimental groups



C: Healthy control group, VAN: Vancomycin administered group, L+VAN: Lutein plus vancomycin administered group. \* $p < 0.0001$  according to C group, \*\* $p < 0.0001$  according to VAN group

**Figure 2.** The levels of TOS and TAS in kidney tissues of experimental groups

Increased creatinine and BUN levels were found in the blood samples of the VAN group compared to the C group ( $P < 0.0001$ ), whereas decreased creatinine and BUN levels of L+VAN group were found when measured against the VAN group ( $P < 0.0001$ ). The difference in creatinine and BUN levels between the C group and L+VAN group was not found to be significant. ( $P > 0.05$ ) (Figure 3A-B).



C: Healthy control group, VAN: Vancomycin administered group, L+VAN: Lutein plus vancomycin administered group. \* $p < 0.0001$  according to C group, \*\* $p < 0.0001$  according to VAN group

**Figure 3.** The levels of BUN and Creatinin in kidney tissues of experimental groups

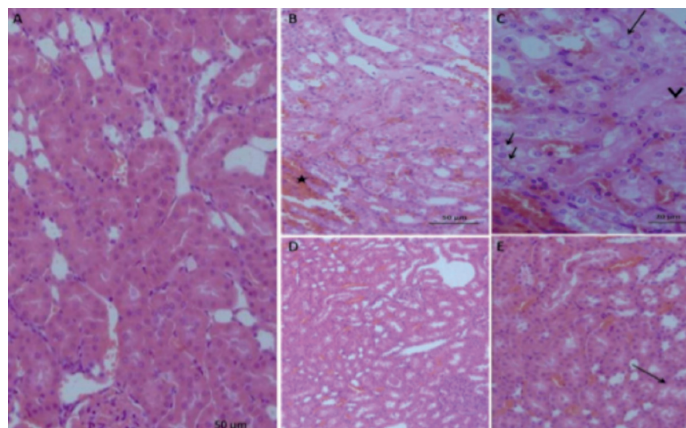


**Figure 4.** Kidney histological appearance in animal study groups H&E staining sections

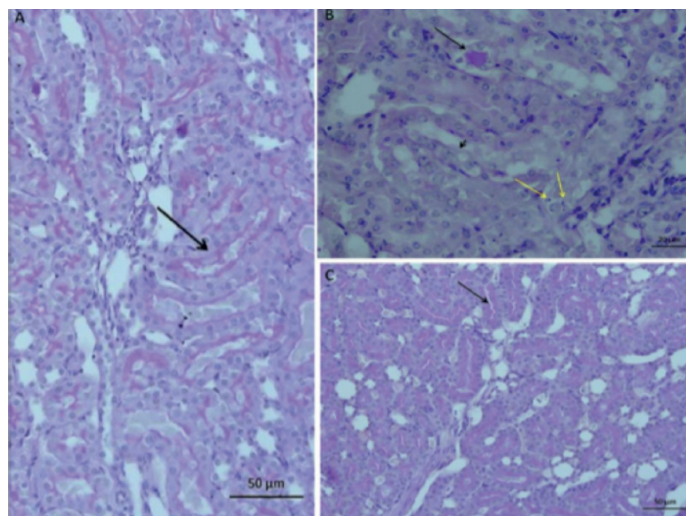


**Figure 5.** Kidney histological appearance in animal study groups PAS staining sections

### Histopathological findings

Normal histological structure is revealed by H&E staining in the renal tissue of the C group (Figure 4A). However, severe hemorrhage, necrosis in tubular epithelial cells, tubular vacuolization and reactive nuclear growth have been detected in the interstitial area of the VAN group (Figure 4B-C). Histopathological examinations for H&E staining revealed minimal damage to kidney tissue and

mild swelling in tubular epithelial cells in the L+VAN group (Figure 4D-E), Normal histological structure is revealed by PAS staining in kidney tissue of the C group (Figure 5A). Widespread tubular damage, such as intratubular cast, apoptotic bodies in tubular epithelial cells, and brushy margin loss in proximal tubules, were detected in examinations with PAS staining of the VAN group (Figure 5B), whereas PAS staining revealed that brushy margins were preserved in proximal epithelial cells in the L+VAN group (Figure 5C).

## DISCUSSION

In the present research, the role of lutein on vancomycin-induced oxidative kidney damage in rats was evaluated biochemically and histopathologically. It has been widely known that kidneys are one of the organs that metabolize chemicals to toxic intermediates. These products having toxicity cause an excess production of reactive oxygen species (ROS), including superoxide anion ( $O^-$ ), hydrogen peroxide ( $H_2O_2$ ), peroxynitrite ( $ONOO^-$ ), hydroxyl radical ( $OH^-$ ) and hypochloric acid ( $HOCL^-$ ) (13). As mentioned in previous studies, ROS-associated damage begins with cell membrane lipid peroxidation (LPO) (14). LPO is a chain of reactions that continues with auto catalytic reactions, causing damage in many organisms (15). Of the variety of aldehydes, the best known formed by LPO is MDA. By causing cross-linking and polymerization of membrane components, MDA causes serious damage to cells by inactivating membrane receptors and membrane-bound enzymes (16). It is clear due to the biochemical results of this study that an increase in the amount of MDA was observed in kidney tissue of the group treated with vancomycin. Literature data also show that vancomycin increases ROS production (17). ROS damage caused by vancomycin can be direct or indirect (4). Previous studies detected increased oxidant damage products in kidney tissue; in addition, the administration of ROS inhibitors showed a nephroprotective effect and the role of ROS in the pathogenesis of kidney damage was demonstrated (18). In the L+VAN group, investigated for its protective effect against vancomycin-related oxidative kidney damage, MDA level was found to be very close to that of the C group animals. As stated above, lutein has been reported to provide antioxidant activity by inhibiting LPO (8). Antioxidants inhibit LPO by inhibiting the peroxidation chain reaction and/or by collecting ROS. The main purpose of the antioxidant control mechanism is to prevent overproduction of ROS as well as the exacerbation of oxidative damage (19,20).

Consistent with the literature, a considerable decrease of tGSH level, an endogenous antioxidant, in the kidney tissue of the VAN group was observed in this study compared to that of the L+VAN and C groups. Bayomy et al. reported that vancomycin reduces GSH level in kidney tissue (21).

GSH is a tripeptide including of L-glutamate, L-cysteine and glycine available in high levels in most cells. The role of GSH in protecting cellular macromolecules from endogenous and exogenous reactive oxygen and nitrogen species is known. GSH directly clears ROS such as  $O^-$ ,

$H_2O_2$ , peroxynitrite ( $ONOO^-$ ),  $OH^-$  and hypochloric acid ( $HOCL^-$ ) (22). GSH is one of the crucial cellular antioxidants and its decrease expedites damage triggered by oxidative stress (23). The present research found that tGSH level in kidney tissues of lutein-administered animals was almost the same as that of the C group. These results indicate that lutein prevents the balance between oxidant antioxidant in kidney tissue from changing in favor of oxidants. It has been reported by Vasudeva V et al. that lutein protects blood cells from radiation damage by inhibiting the decrease in GSH and antioxidant enzyme levels and increase in MDA and lipid peroxidation (24). Sindhu et al. reported that lutein protects kidney tissue from cisplatin-induced oxidative damage by increasing GSH level and decreasing MDA production (25). In the present study, we have demonstrated by TOS and TAS measurements that vancomycin changes the oxidant antioxidant equity in favor of oxidants, and that lutein inhibits this change. It is known that TOS and TAS are used to ascertain the total oxidant and antioxidant capacity (11,12). In the previous study, it was documented that lutein, which prevents oxidant parameters from increasing and antioxidants from decreasing, also prevents equity between TOS and TAS from changing in favor of TOS (26). In our study, it was found that the levels of creatinine and BUN observed in blood serum of the VAN group, in which the equity between oxidants and antioxidants was obstructed in favor of oxidants, were increased compared to the C and L+VAN groups. Recent studies have also reported that serum creatinine and BUN levels are increased in vancomycin-associated oxidative kidney damage (27). In a previous study, it was emphasized that suppression of oxidative stress is an effective intervention to alleviate the nephrotoxicity of vancomycin (5). There were no studies evaluating the effect of lutein on serum creatinine and BUN increase due to vancomycin in previous studies. However, there is data showing that, at an antioxidant dose, lutein significantly suppresses the increase of creatinine and BUN levels due to cisplatin nephrotoxicity (25). It has also been reported that, through antioxidant activity, lutein protects kidney function and histological structure from oxidative damage caused by experimental burns (28).

Biochemical findings obtained in our study are consistent with histopathological findings. Severe hemorrhage, necrosis, apoptotic bodies, and tubular vacuolization in tubular epithelial cells, loss of brushy margin in proximal tubules, reticular reactive nuclear growth and intratubular cast formation were observed in the interstitial area of kidney tissue in the VAN group with high oxidant and low antioxidant levels. However, histopathological damage was much milder in the L+VAN group, in which the oxidant antioxidant equity was still favored antioxidants. In of the research of Cetin et al, pathological findings such as tubular necrosis, vacuolization, edema, and cast formation were found in vancomycin-related oxidative kidney damage (29). Sener et al. showed that apoptotic bodies are one of the histopathological findings of drug-induced oxidative kidney damage (30).

## CONCLUSION

This study has demonstrated that oxidative damage develops in the kidney tissue of rats treated biochemically and histopathologically with vancomycin. It has been found that oxidative damage in renal tissue of subjects underwent a treatment with lutein is much milder compared to the VAN group. In addition, lutein prevented a significant increase in creatinine and BUN levels through vancomycin, which indicates renal dysfunction. This appears to suggest lutein could have a major effect on the treatment for vancomycin-associated kidney toxicity.

*Competing Interests: The authors declare that they have no competing interest.*

*Financial Disclosure: There are no financial supports.*

*Ethical Approval: The study protocol (25.10.2019-75296309-050.01.04-E.1900304448) was approved by local animal ethics committee of Ataturk University Erzurum, Turkey.*

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