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Renal Protective Effect of Sulbutiamine, Thiamine, Riboflavin and their Combinations on Vancomycin-Induced Acute Renal Failure in Male Rats

Dayan K. Jabbar [*,1], **Ghaith A. Jasim**[2] and **Muthanna I. Al-Ezzi**[1]

[1]Pharmacology and Toxicology Department, College of Pharmacy, Mustansiriyah University, Baghdad, Iraq.

[2] College of Health and Medical Technologies, AL-Bayan University, Baghdad, Iraq.

[*]**Corresponding author**

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Abstract

The main mechanisms thought to be involved in the pathophysiology of acute renal failure with vancomycin treatment are oxidative stress, inflammation and apoptosis. This study aimed to evaluate the potential effects of sulbutiamine, thiamine, riboflavin and their combinations on vancomycin-induced acute renal failure as well as the underlying mechanisms. A model of vancomycin-induced acute renal failure was performed on male rats. Forty-two rats were divided randomly into seven groups: group 1 (a control group), group 2 (vancomycin induction group) given 200mg/kg/twice daily of vancomycin in third week of the study, group 3 (sulbutiamine + vancomycin), group 4 (thiamine + vancomycin), group 5 (riboflavin + vancomycin), group 6 (sulbutiamine+ riboflavin + vancomycin) and group 7 (thiamine+ riboflavin + vancomycin). In rats' model with vancomycin-induced acute renal failure, sulbutiamine, thiamine, riboflavin and their combinations could protect against vancomycin-induced acute kidney injury which included histological damage, renal dysfunction, increased oxidative stress, elevated creatinine and blood urea nitrogen levels. Furthermore, increased immune-expression of kidney injury molecule-1 and tumor necrosis factor-alpha resulted from vancomycin treatment also ameliorated by sulbutiamine, thiamine, riboflavin and their combinations. The findings showed that sulbutiamine, thiamine, riboflavin and their combinations protected against nephrotoxicity caused by vancomycin by relieving renal functions and reducing the level of expression of kidney injury molecule-1, tumor necrosis factor-alpha and attenuating histopathological alterations through their anti-oxidative and anti-inflammatory characteristics. Sulbutiamine and its combination with riboflavin giving the best results in attenuating vancomycin-induced acute renal failure. The research also revealed an additional effect of sulbutiamine with riboflavin.

Keywords: Vancomycin, Sulbutiamine, Thiamine, Riboflavin, KIM-1

Introduction

Vancomycin is a glycopeptide antibiotic used to treat severe infections brought on by gram-positive bacteria resistant to methicillin, particularly methicillin-resistant Staphylococcus aureus [(1)]. It has been found that 5-25% of patients who received VCM have nephrotoxicity [(2)]. Acute renal failure caused by VCM is a complicated process involving numerous factors and signaling pathways. Reactive oxygen species (ROS) and associated metabolic processes were the basis of the mechanism causing nephrotoxicity in VCM therapy, according to earlier investigations [(3),(4)]. Reactive oxygen species (ROS) that are indirectly produced and linked to inflammatory processes may be the cause. ROS can damage cells in a number of ways, including by peroxiding membrane lipids, denaturing proteins, and damaging DNA [(5)]. Additionally, Oxidative stress can also impact intracellular signaling pathways, including mitotically activated protein kinases (MAPKs), which are linked to apoptosis and caspase activation in the VCM-induced acute renal failure [(6)]. Given the part that oxidative stress plays in the pathophysiology of VCM-induced acute renal failure, combining oxidative stress with antioxidants may help to mitigate or avoid this side effect [(7)]. **Thiamine** is vital water-soluble vitamin (is a crucial to human body's cellular metabolism) [(8)]. Additionally, it gives cells some protection against oxidative stress [(9)]. It's essential for aerobic metabolism that serves as a cofactor for pyruvate dehydrogenase, metabolism of certain reactive oxygen species (ROS) and ATP production [(10)]. Pyruvate cannot enter the Krebs cycle without thiamine, which results in lactate being formed instead of acetyl-coenzyme A from pyruvate. Therefore, thiamine shortage results in a switch from aerobic to anaerobic metabolism, which leads to increased blood lactate levels, cellular apoptosis, organ destruction (including renal failure), and potentially fatal outcomes [(11),(12)]. **Sulbutiamine** is a synthesized lipophilic thiamine derivative which have a high bioavailability and may easily diffuse through plasma membranes [(13)]. Inside the cells, the

sulbutiamine converted to thiamine [14]. It's interesting to note that sulbutiamine and other thiol-containing substances have been demonstrated to increase GSH [15], which may decrease oxidative stress in the cells. The methods of action, however, are still unclear. According to experimental and clinical research, sulbutiamine appears to have a number of effects on human physiology, including the capability to distribute thiamine to harder-to-reach tissues, increase antioxidant capacity, and regulate protein function [16]. **Riboflavin** is one of the water-soluble vitamins [17] (a crucial component of mitochondrial energy metabolism and ion absorption) [18]. A health risk is presented with low dietary riboflavin consumption. At this time, riboflavin insufficiency can result in gastrointestinal problems, brain irregularities, skin disorder, and metabolic illnesses [19]. Additionally, riboflavin deficiency may result in higher levels of apoptosis [20],[21],[22]. riboflavin is naturally occurring vitamin that serve as a precursor to the coenzymes flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD), which are essential for a number of metabolic activities, including the electron transport chain, fatty acid oxidation, and antioxidant systems [23]. By scavenging free radicals, riboflavin supplementation reduced oxidative stress and tissue ischemia-reperfusion damage [24]. According to recent research, riboflavin mitigated the liver and kidney damage brought on by toxic substances including cisplatin, potassium bromide, and lipopolysaccharide (LPS) [25], [26].

Aim of study:

To evaluate the protective effect of sulbutiamine, thiamine, riboflavin and their combination on vancomycin-induced acute renal failure in male rats.

Methodology

Animals:

Forty-two male Wister rats weighing between 160-200 grams were employed in this research. The rats were obtained from the Iraqi Center for Cancer Research, and placed in big comfortable cages there. For one week, the rats were allowed to acclimatize in a controlled environment., including temperature (24°C), humidity (40-50%) and a light schedule of 12 hours' light-dark cycles. They had unlimited access to food and drink [27]. In this study, the rats were pretreated with sulbutiamine, thiamine, and riboflavin followed by induction with vancomycin (as described in the experimental design), scarification of the rats, followed by evaluation of many markers such as serum creatinine, BUN, immunohistochemistry of TNF-alpha, KIM-1 and evaluation of histopathological alterations.

Experimental design:

The rats were randomly separated into seven groups, each of it contain six rats. The groups were classified as follows: Group 1 (n=6): Control (negative) group, rats received the (PBS) orally by using oral gavage for 2 weeks and intraperitoneal injection of normal saline at third week. Group 2 (n=6): Induction (positive) group, rats received the (PBS) orally for 2 weeks, additionally to intraperitoneal injection of vancomycin (200 mg/kg/ twice daily) at third week of the experiment. Group 3 (n=6): Treatment group of oral sulbutiamine suspension at a dose of 50 mg/kg daily for 3 weeks, additionally to vancomycin injection (200 mg/kg/ twice daily) I.P. at 3rd week of the experiment. Group 4 (n=6): Treatment group of oral thiamine suspension at a dose of 100 mg/kg daily for three weeks, additionally to vancomycin injection (200 mg/kg/ twice daily) I.P. at 3rd week of the experiment. Group 5 (n=6): Treatment group of oral riboflavin at a dose of 100 mg/kg daily for three weeks, additionally to intraperitoneal injection of vancomycin (200 mg/kg/ twice daily) at 3rd week of the experiment. Group 5 (n=6): combination group of oral sulbutiamine and riboflavin suspension at a dose of 50 mg/kg and 100 mg/kg, respectively daily for three weeks, additionally to intraperitoneal injection of vancomycin (200 mg/kg/ twice daily) at 3rd week of the experiment. Group 7 (n=6): combination group of oral thiamine and riboflavin suspension at a dose of 100 mg/kg for both treatments daily for three weeks, additionally to intraperitoneal injection of vancomycin (200 mg/kg/ twice daily) at 3rd week of the experiment.

Serum biochemical analysis:

Colorimetric method was used to determine blood urea and serum creatinine which are carried out by using (linear chemicals S.L Joaquim Costa 18 2ª planta, SPAIN) according to the manufacturer's instructions [28].

Renal tissue biochemical analysis:

Renal tissues were homogenized in 1.5 ml extraction buffer (which contain 10 mMTris pH 7.4, 150 mM NaCl, 1% Triton X-100) using a glass homogenizer. The homogenate was transported to the Eppendorf tubes, centrifuged at 13,000 xg for 10 min at 4C, and the supernatant was stored -80C until analyzed. Then the latter was used for biochemical analysis [29]. Renal oxidative stress marker (malondialdehyde) was determined by sandwich ELISA kits obtained from (Mybiosource, USA).

Immunohistochemical analysis

Renal sections have been cut into five-millimeter pieces, which were then placed on slides with an +ve charge. With a preheated retrieval solution, all slides had been put into the PT-linker for automatic HIER (Heat-induced Epitope retrieval), and then they were all allowed to cool until the temperature dropped to 65 °C. Each slide was drained, plotted, and placed in the most suitable location in the Auto-Stainer to prepare it for automatic immunostaining. Hydrogen peroxide was

put to each slide and allowed to set for 15 minutes before being twice rinsed with washing buffer. KIM-1 mAB and TNF- alpha were used directly. After 25 minutes of incubation, Envision FLEX/HRP was applied to each slide before being rinsed with washing buffer (Phosphate buffer saline (PBS)). DAB was subsequently applied to the slides after 10 minutes. Each tissue section got Mayer's Hematoxylin after two PBS rinsing on each slide. After that, each slide was cleaned with distilled water, dried with alcohol in descending grades, clarified by xylene, and mounted by DPX. Reviewing, scoring, and taking photos of the slides was done. By using a single Light microscope, all images were taken [30]. For every slide, six microscopic fields were examined, and a semi-quantitative recording was made for both the number of positively stained cells (mainly from glomeruli, tubules, and proinflammatory cells) and the intensity of the immunostaining as follows: The number of positive cells was evaluated with a score of 0 to 6: (0= no positive cells, 1= 1-5 positive cells, 2= 6-10 positive cells, 3= 11-50 positive cells, 4= 51-100 positive cells, 5= 101-150 positive cells, 6= more than 150 positive cells). The intensity of immunostaining was graded on a scale from 0 to 3: (0= no staining, 1= mild staining, 2= moderate intensity, 3= maximum intensity). The score for positive cell number was multiplied by the corresponding value for immunostaining intensity to determine the overall score of TNF-alpha for each microscopic field [31]. Additionally, the scoring of KIM-1 was calculated semi-quantitatively through counting the stained tubules as follows: Grade 0= 0 tubules, Grade 1= 1–5 tubules, Grade 2= 6–10 tubules and Grade 3= more than 10 tubules [32].

Histopathological analysis

To avoid autolysis, the kidney tissues should be directly fixed in 10% formalin. After that different concentrations of alcohol were used to remove water from kidney tissue. Following that the tissue was cleaned with xylene and set in paraffin wax. A rotary microtome was used for cutting the tissue into 5 micrometer slices, and the slices were then immersed in a warm water bath. Moreover, the slices were placed on microscopic slides and heated for about 15 minutes to facilitate their adherence to the slide. The slides were completely cleared from paraffin wax by first immersing them in a xylene jar, then in different concentrations of alcohol, and finally washed by distilled water. Hematoxylin staining was subsequently applied for 10 minutes. After that, 30 seconds of 1% Eosin staining was performed. Finally, a light microscope was used to examine the slides. The observed histopathological alterations were scored as follows: an inflammation score ranged from 0 to 6 according to severity (0 = no sign of inflammation, 1-2 = mild inflammation, 3- 4 = moderate inflammation, 5- 6 = severe inflammation). Additionally, a scale from 0 to 4 was used to evaluate vancomycin associated cast formation VTC: (0= none, 1= 1- 3/ HPF, 2= 4-10/ HPF, 3= more than 10/ HPF) and tubular necrosis as well (0= none, 1= 1- 3/ HPF, 2= 4-10/ HPF, 3= more than 10/ HPF) [33,34].

Statistical analysis

The data were expressed as mean+SD by using Statistical Package for the Social Science-16 program. Data were subjected to Shapiro test to identify their normality. One way ANOVA and least significant difference post hoc test was used to assess the significant differences among means. $P \leq 0.05$ is considered as a significant.

Results

The effect of sulbutiamine, thiamine, riboflavin and their combinations on the renal function test (serum creatinine and blood urea):

The effect of sulbutiamine, thiamine, riboflavin and their combinations on the renal function test in vancomycin-induced acute renal failure in male rats was be shown in table 1.

Table 1. The effect of sulbutiamine, thiamine, riboflavin and their combinations on parameters of renal function (serum creatinine and urea)

Groups	Creatinine (mg/dl)	Urea (mg/dl)
Group 1 (Control group)	0.46±0.05cd	24.71±2.05bc
Group 2 (Induction group)	0.97±0.02a	46.74±1.95a
Group 3 (Sulbutiamine group)	0.38±0.05d	25.70±1.66b
Group 4 (Thiamine group)	0.60±0.03bc	29.87±0.59b
Group 5 (Riboflavin group)	0.68±0.03b	30.28±1.45b
Group 6 (Sulbutiamine+ Riboflavin)	0.31±0.04d	17.71±1.71c
Group 7 (Thiamin + Riboflavin)	0.56±0.09bc	27.96±6.03b

Data are represented as means+SD (standard deviations).Different lowercase letters (a,b,c,d) referred to significant differences among groups.Following vancomycin-induced acute renal failure in male rats, the mean blood urea and serum creatinine in the induction group were significantly higher $p<0.05$ as compared to the negative group. Whereas group 3, group 4, group 5, group 6 and group 7 showed significant decrease in the mean blood urea and serum creatinine compared to the induction group. According to the mean of serum creatinine level, the group 3 and group 7 were showed non-significant differences as compared with group 1 and group 5 but were showed significant differences as compared with group 3 and group 6. Group 3 and group 6 showed best significant decrease in the mean of serum creatinine as compared with other groups and showed non-significant differences as compared with group 1. On the other hand, according to the mean level of blood urea, the group 3, group 4, group 5 and group 7 were showed non-significant differences as compared with group 1. While group 6 showed best significant decrease in mean of blood urea as compared with other groups and non-significant differences $p>0.05$ as compared with group 1.

The effect of sulbutiamine, thiamine, riboflavin and their combinations oxidative stress biomarker (malondialdehyde)

The effect of sulbutiamine, thiamine, riboflavin and their combinations on the oxidative stress biomarker in vancomycin-induced acute renal failure in male rats was be shown in table 2.

Table 2. The effect of sulbutiamine, thiamine, riboflavin and their combinations oxidative stress biomarker (malondialdehyde).

Groups	MDA nmol/ml
Group 1 (Control group)	2.80±0.02b
Group 2 (Induction group)	4.63±0.04a
Group 3 (Sulbutiamine group)	1.89±0.13c
Group 4 (Thiamine group)	2.83±0.01b
Group 5 (Riboflavin group)	2.70±0.01b
Group 6 (Sulbutiamine+ Riboflavin)	0.٩9±0.02d
Group 7 (Thiamin + Riboflavin)	1.76±0.01c

Data are represented as means+SD (standard deviations)
Different lowercase letters (a,b,c,d,e) referred to significant differences among groups.
After the induction of acute renal failure with vancomycin in male rats, the mean tissue concentration of MDA was significantly higher $p<0.05$ in group 2 as compared with group 1. Whereas group 3, group 4, group 5, group 6, and group 7 showed significant decrease $p<0.05$ in mean tissue concentration of MDA.

The effect of sulbutiamine, thiamine, riboflavin and their combinations on immunohistochemical expression of TNF-alpha:

Renal expression of TNF-alpha was higher in group 2 as compared with group 1. Both quantity of TNF-positive cells and the degree of immunostaining were higher in group 2 (score 5 of TNF-alpha immunostaining and grade 3 maximum intensity). In contrast to the group 2, group 6 showed a significant decrease in TNF-alpha positive staining, there was only slight staining, mostly in the proximal tube, and there was less expression in the glomerulus which was close to the group 1. Among the other groups, different distributions of these cells were seen. In group 1, normal expression for TNF-alpha within renal tissue. While in group 3, there was minimal TNF cytoplasmic expression. Moreover, in group 4 there was a brown staining in the cortical region of the kidney. Group 5 showed exhibited TNF-alpha immune-cytoplasmic staining in the glomerulus that ranged from moderate to intense. Finally, in group 7 there was intense with mild expression is still noticed as a cytoplasmic expression. As shown in figure 1, table 3.

Table 3. The effect of different treatment in vancomycin-induced acute renal failure on immune-expression of tumor necrosis factor-alpha.

Groups	Score of TNF-alpha	Grading	Overall scoring
Group 1 (Control group)	0	0	0
Group 2 (Induction group)	5	3	15
Group 3 (Sulbutiamine group)	3	2	6
Group 4 (Thiamine group)	3	3	9
Group 5 (Riboflavin group)	4	2	8
Group 6 (Sulbutiamine+ Riboflavin)	2	1	2
Group 7 (Thiamin + Riboflavin)	3	2	6

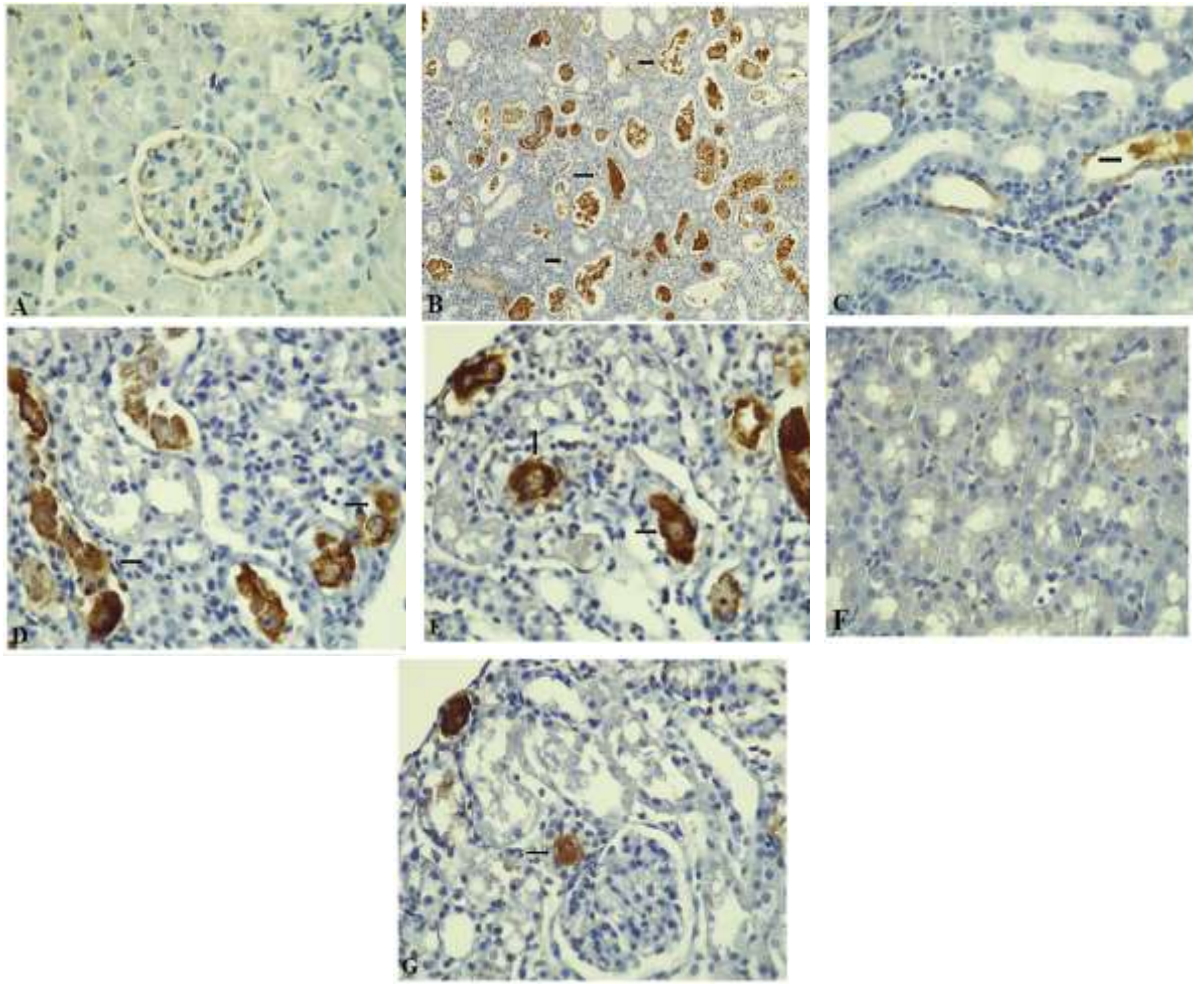

Figure 1. The immune-expression of TNF-alpha (represented by black arrows) in kidney sections in seven animal groups (A: group 1, B: group 2, C: group 3, D: group 4, E: group 5, F: group 6 and G: group7)

The effect of sulbutiamine, thiamine, riboflavin and their combinations on immunohistochemical expression of KIM-1.

The highest expression of KIM-1 (grade 3) was observed among group 2 (the vancomycin-induced acute renal failure). On the other hand, no expression of KIM-1 (grade 0) was seen in group 1, while the lowest expression of KIM-1 (grade 1 near to normal) was seen in group 3 and group 6. Moreover, the tissue section from the kidney of male rats (from group 7) demonstrating grade 2 expression of KIM-1 in the proximal tubules mainly. Group 4 and group 5 showed grade 3 immuno-expression of KIM-1 in the longitudinal section of male kidney rats. As shown in table 4 and figure2

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Table 4. The effect of different treatment in vancomycin-induced acute renal failure on immune-expression of kidney injury molecule -1 (grading of KIM-1)

Groups	Grade
Group 1 (Control group)	0
Group 2 (Induction group)	3
Group 3 (Sulbutiamine group)	1
Group 4 (Thiamine group)	3
Group 5 (Riboflavin group)	3
Group 6 (Sulbutiamine+ Riboflavin)	1
Group 7 (Thiamin + Riboflavin)	2

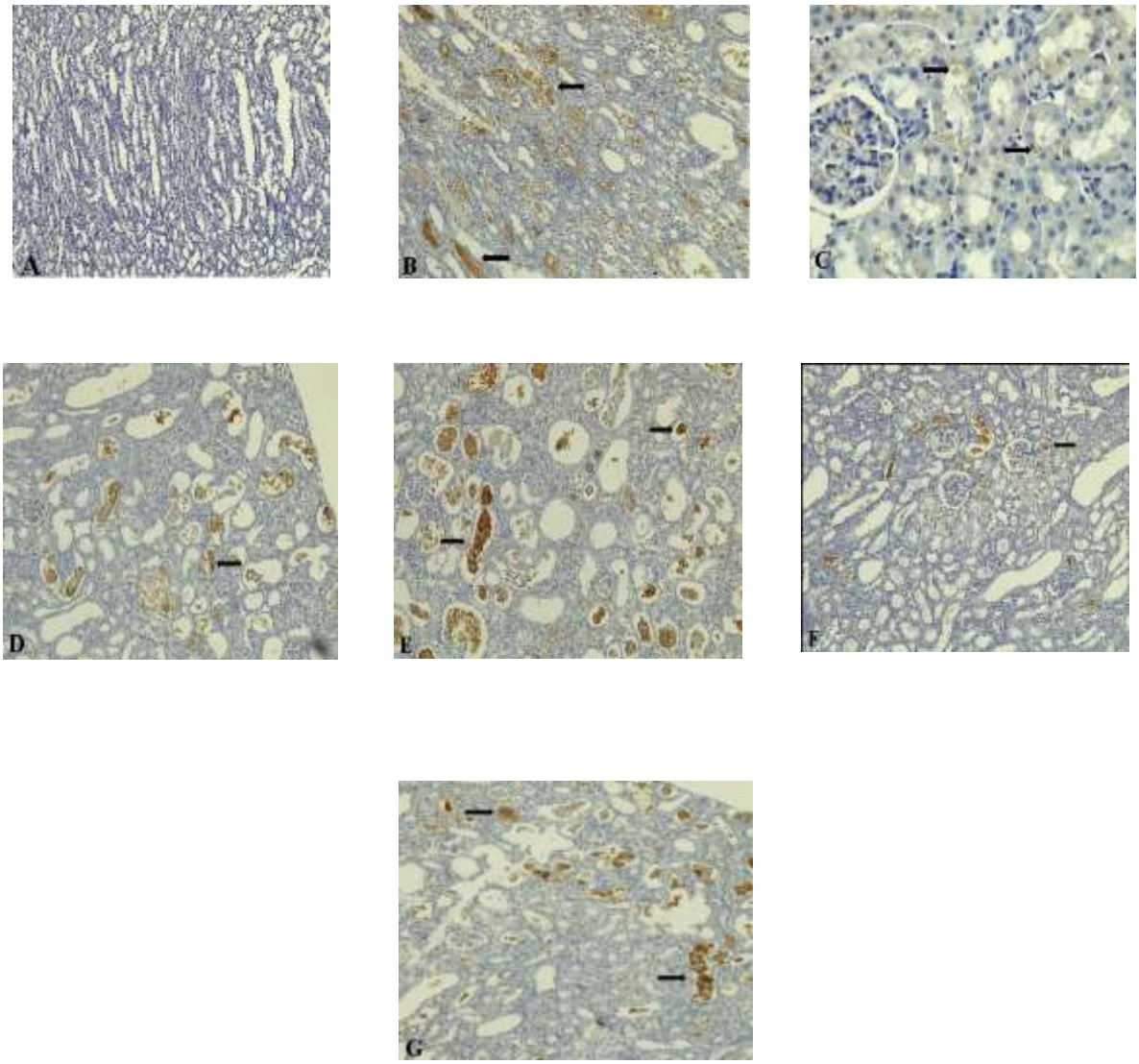

Figure 2. The immune-expression of KIM-1 (represented by black arrows) in kidney sections in seven animal groups (A: group 1, B: group 2 (vancomycin group), C: group 3, D: group 4, E: group 5, F: group 6 and G: group 7)

Result of Histopathology

As shown in figure 3, group 1 demonstrating normal histological picture. While group 2 manifested AKI and Vancomycin nephrotoxicity features. The vancomycin treated group revealed significant damage, as seen by a cast, tubular dilatation, tubular necrosis, and tubular degeneration as well as interstitial edema and infiltration of inflammatory cells compared with group 1. The Vancomycin effect was countered by sulbutiamine, which also maintained the kidney's normal architecture as shown in figure (3) that demonstrates normal medullary renal tissue with intact collecting tubules. Moreover, group 6 showed significant improvement in renal tissue that close to the group 1 and group 3. Necrotic tissue and VTC were not seen. Very few proinflammatory cells were seen, and there was no tissue bleeding. Group 4 showed large bleeding regions in the kidneys' cortex and medulla. In the renal tissue of this group of rats, no vancomycin-tubulin casts were visible. Additionally, group 5 showed the VTC was not visible. However, it was discovered that the glomerulus' subcapsular area had dilated and contained vacuoles in the distal convoluted tubules with symptoms of atrophy. Finally, the effect of vancomycin was also reversed in group 7 (thiamine+riboflavin) although a few VTC were still visible. Atrophy of the glomerulus and an abnormal configuration of the glomeruli, some of which have extensive subcapsular spaces. As shown in figure 3 and table 5.

Table 5. Inflammatory indexes of Acute Kidney Injury (AKI) classification and scoring

Group	Loss of brush border in renal tubules	Vancomycin associated Cast formation (VTC)	Tubular necrosis
Group 1 (Control group)	none	None	None
Group 2 (Induction group)	severe	3	3
Group 3 (Sulbutiamine group)	Mild	1	1
Group 4 (Thiamine group)	Moderate	2	2
Group 5 (Riboflavin group)	Moderate	2	2
Group 6 (Sulbutiamine+ Riboflavin)	Mild	1	1
Group 7 (Thiamin + Riboflavin)	moderate	2	2

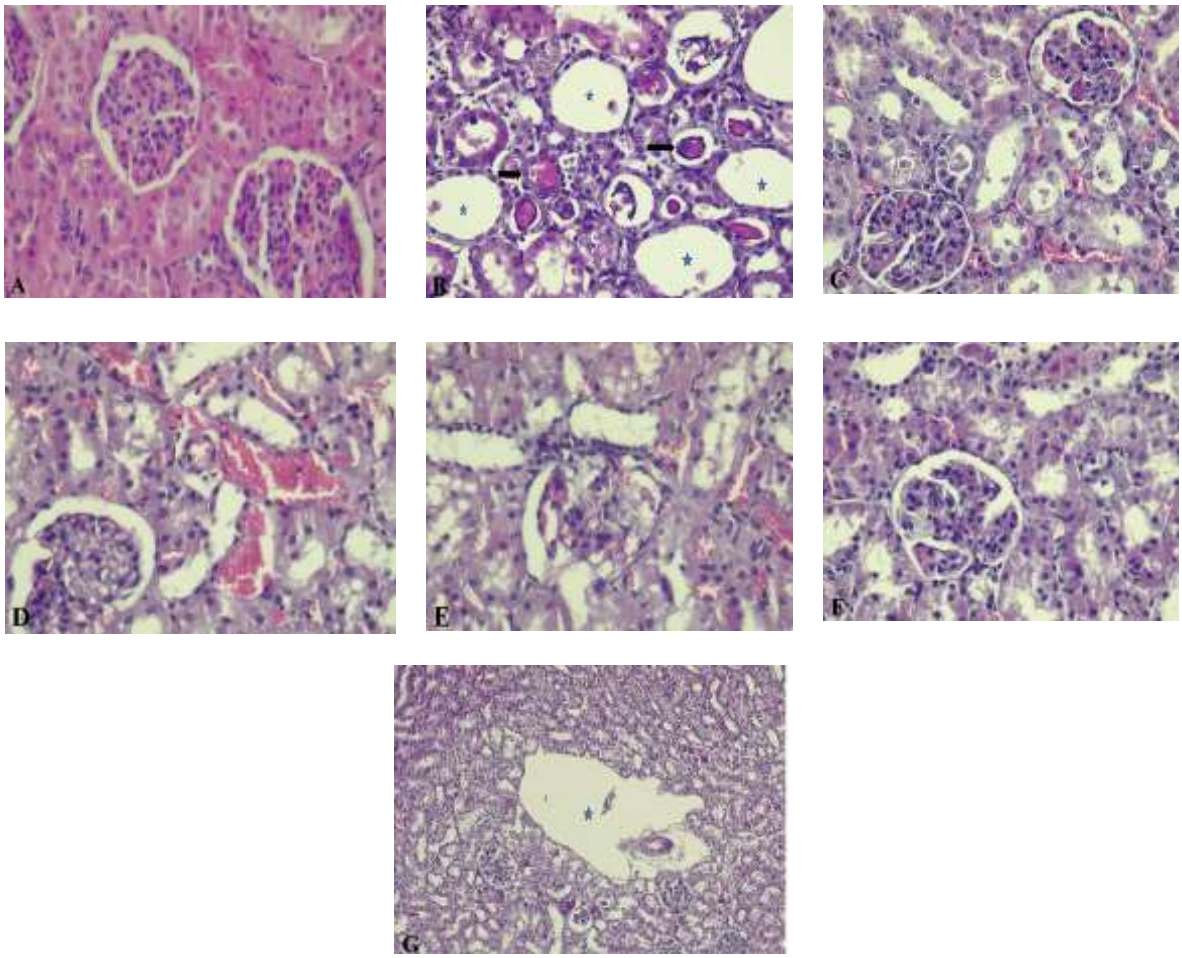

Figure 3. Representative pictures of kidney sections A: group 1, B: group 2 that showed Renal tubules are dilated (★), manifesting Acute- Tubular Injury/ necrosis (ATN). Some of the distal tubules are filled with pink casts (VTC) represented by black arrows. C: group 3, D: group 4, E: group 5, F: group 6 and G: group 7.

Discussion

VCM is a most popular clinical treatments for MRSA infections [35]. Meanwhile, nephrotoxic effects are a major problem during VCM therapy and are directly related to high amounts or extended durations of VCM administration, limiting its clinical uses [36]. VCM treatment also considerably increases serum creatinine and BUN levels, which represented essential biochemical indicators of renal failure [37]. Histopathological examination revealed that vancomycin was caused renal damage that characterized by tubular degeneration, atrophy, dilation, necrosis, cast formation, and interstitial edema [38]. In this study vancomycin-induced acute

renal failure in rats' model was demonstrated by increased level of serum creatinine, blood urea and increased MDA level together with higher level of KIM-1 and TNF-alpha accompanied by histopathological kidney damage. The research findings are as follows. To begin, the findings revealed that sulbutiamine, thiamine, riboflavin and their combinations might prevent or attenuate VCM-induced acute renal failure with elevated the level of serum creatinine and blood urea and histological kidney damage. Sulbutiamine, thiamine, riboflavin and their combinations significantly improved vancomycin-induced acute renal failure by inhibiting the production of reactive oxygen species. Additionally, increased immune-expression of TNF-alpha and KIM-1 that caused during vancomycin therapy was also ameliorated by sulbutiamine, thiamine, riboflavin and their combinations. In the present research, group 2 showed significant increase in serum creatinine and blood urea as compared with group 1 (apparently healthy group) and this is consistent with previous study [7]. In group 3, group 4, group 5, group 6 and group 7, the results showed significant decreased in the level of serum creatinine and blood urea as compared with group 2. Group 4 showed significant decreased in the level of blood urea and serum creatinine as compared with induction group and these results were consistent with other studies [39],[40]. Group 5 also viewed significant decrease in BUN and serum creatinine as compared with group 2 and these results was agreed with previous research [41]. In group 7, there was be significant decrease in the urea and creatinine levels as compared with group 2 and non-significant differences as compared with group 1. According to studies, sulbutiamine, a thiamine derivative with a high lipophilicity [42], is ten times more efficient than thiamine at raising intracellular thiamine concentrations. However, the increases in intracellular thiamine diphosphate and triphosphate concentrations were hardly noticeable after 4 hours of administration, suggesting that sulbutiamine is hydrolyzed and quickly reduced to thiamine once it enters the cells, which can then be incorporated in thiamine phosphate derivatives [43]. Therefore, after administration of sulbutiamine, thiamin has been found to be the most prevalent chemical in the renal cortex, the medulla oblongata (including the pons), the cortex (telencephalon), the cerebellum, and the hippocampus [44]. In present study, group 3 (sulbutiamine group) viewed significant decrease in the blood urea and serum creatinine as compared with group 2. The best results were shown with group 6 (sulbutiamine+riboflavin group) in decreased blood urea and serum creatinine, the sulbutiamine together with riboflavin were showed best results in improvement of renal function biomarker after vancomycin induction.The findings of this study showed significant increase in the MDA level in group 2 (vancomycin group) as compared with control group and this result consistent with previous study [45]. While group 3, group 4 and group 5 showed significant decrease in the level of MDA and this is agreed with other studies [46][47][48]. Best results were seen in combination groups (group 6 and group 7) due to the dual action oftreatment.

The TNF-alpha is largely produced by macrophages, although a variety of other cell types, such as lymphoid cells, mast cells, fibroblasts, endothelial cells, and neuronal cells, can also produce it [49]. The increased TNF-alpha level is thought to be related to the fact that oxidative damage appears to promote leukocyte aggregation in the tissues involved, exacerbating tissue damage indirectly through neutrophil stimulation. Stimulated neutrophils are thought to release enzymes like (TNF-alpha, myeloperoxidase (MPO), elastase, and proteases as well as oxygen free radicals), which are thought to exacerbate tissue damage. Free radicals also directly damage these tissues [50]. In this experiment, vancomycin treated rats showed significant increase in the expression of TNF-alpha as compared to the apparently healthy rats and this is consistent with other studies [51],[37]. Group 3, group 4, group 5 and group 7 showed significant decreased in the expression of TNF-alpha as compared with group 2 and these results are consisting with other study findings [52],[53]. Group 6 showed a best significant decreased in the immune-expression of TNF-alpha compared to the other group and this result was close to the group 1. Moreover, this study showed significant increase in the immune-expression of KIM-1 in group 2 as compared to the group 1 and this result was agreed with another research [54]. Groups 4 and 5 showed significant decreased in the immune-expression of KIM-1 compared to the vancomycin group and this outcome agreed with other study [55]. While group 7 showed significant decreased in the expression of KIM-1 compared to group 4 and group 5, this may be connected to the thiamine and riboflavin additive action. On the other hand, group 3 and group 6 showed best significant decreased in the immuno-expression of KIM-1 compared to the vancomycin group and this result is very close to the group 1. In the current experiment, comparing group 2 to the group 1, the group 2 (vancomycin-treated group) had considerable damage, as evidenced by a cast, tubular dilatation, tubular necrosis, and tubular degeneration as well as interstitial swelling and the glomeruli are sclerotic and the cortex is fibrous, and there were widespread interstitial acute inflammatory cell infiltrates. The primary histological patterns of vancomycin-induced acute kidney damage include tubulointerstitial nephritis and acute tubular necrosis. Lower healing rates and worse long-term renal outcomes could be associated with fibrosis in the presence of interstitial inflammation [56]. Sulbutiamine, thiamine, riboflavin

and their combination were able to attenuate histopathological feature of vancomycin-induced acute renal failure and this was agreed with other studies [57],[58]. The present study showed that group 3 and group 6 significantly relive histopathological feature. By significantly lowering the histopathological score in comparison with the group 2, sulbutiamine demonstrated a protective effect for the kidney against the nephrotoxic effects of vancomycin. According to the result of this research, sulbutiamine showed the highest nephroprotective effect among all the medications studied in this investigation. When compared to the vancomycin group, sulbutiamine caused a highly significant decrease in histopathological scores, demonstrating a nephroprotective effect. Sulbutiamine lowers the histopathological scores and multifocal regions of tubular abnormalities with cellular desquamation and necrosis, vacuolated transparent cytoplasm, and tubular dilatation. The nephroprotective effect could be attributed to powerful antioxidant and anti-inflammatory action [59]. Group 6 (sulbutiamine+riboflavin combination treated group) showed slight better result than sulbutiamine group in attenuating histopathological feature of vancomycin-induced acute renal failure and this was due to additive effect of sulbutiamine and riboflavin. Renal failure is commonly caused by drug-induced nephrotoxicity, which can have hazardous consequences [60]. Previous research investigations have mostly focused on examining the potential protective effects of anti-inflammatory and antioxidant compounds against nephrotoxicity [61],[62],[63].

Conclusion

Sulbutiamine, thiamine, and riboflavin all have a renal protective effect against vancomycin-induced nephrotoxicity in rats when the following parameters are corrected: kidney function parameter (serum creatinine and BUN), KIM-1, pro-inflammatory mediator (tumor necrosis factor-alpha), oxidative stress biomarker (MDA) and histopathological alteration. In this study, thiamine, riboflavin and their combination didn't give best results in the management of vancomycin induced acute renal failure, but a greater positive effect was found from combination of sulbutiamine with riboflavin in relieving vancomycin-induced acute renal failure.

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Conflicts of interest

There are no conflicts of interest.

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Ethics Statements

The ethical guidelines that originated with the Declaration of Helsinki were followed in the conduct of this study. The local ethics committee of the College of Pharmacy at Mustansiriyah University in Iraq examined and approved the study protocol. (Approval number:10 at 28/10/2022).

Author Contribution

The authors confirm contribution to the paper as follows: study conception and design: Dayan K. Jabbar; Ghaith A. Jasim; Muthana I. Al-Ezzi; data collection: Dayan K. Jabbar; analysis and interpretation of results: Dayan K. Jabbar; draft manuscript preparation: Dayan K. Jabbar. All authors reviewed the results and approved the final version of the manuscript.

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تأثير السلبيوتامين والثيامين والريبوفلافين ومزيجهما على الفشل الكلوي الحاد المستحث بالفانكومايسين في ذكور الجرذان

ديان كريم جبار[١]، غيث علي جاسم[٢] و مثنى ابراهيم العزي[١]

[١] فرع الادوية والسموم، كلية الصيدلة، جامعة المستنصرية، بغداد، العراق
[٢] كلية التقنيات الصحية والطبية، جامعة البيان، بغداد، العراق

الخلاصة

يعتقد إن الإجهاد التأكسدي والالتهاب وموت الخلايا المبرمج هي الآليات الرئيسية التي تشارك في الفيزيولوجيا المرضية للفشل الكلوي الحاد مع علاج الفانكومايسين(VCM) ، لذلك، تم في هذه الدراسة تقييم التأثيرات المحتملة للسلبيوتامين والثيامين والريبوفلافين ومجموعاتها على الفشل الكلوي الحاد المستحث بالفانكومايسين وكذلك الآليات المحتملة .تم إستحداث نموذج للفشل الكلوي الحاد على ذكور الجرذان بواسطة الفانكومايسين. تم تقسيم إثنان وأربعون جرذاً عشوائياً إلى سبع مجموعات: المجموعة-١ مجموعة السيطرة، مجموعة-٢ تحريض الفانكومايسين أعطيت ٢٠٠ ملغم/كغم/مرتان يوميا من الفانكومايسين في الاسبوع الثالث من الدراسة، مجموعة-٣ أعطيت (سلبيوتامين + فانكومايسين)، مجموعة-٤ أعطيت (ثيامين + فانكومايسين)، مجموعة-٥ أعطيت (ريبوفلافين + فانكومايسين)، مجموعة-٦ أعطيت (سلبيوتامين + ريبوفلافين + فانكومايسين) ومجموعة-٧ أعطيت (ثيامين + ريبوفلافين + فانكومايسين) .في نموذج الجرذان المصابة بالفشل الكلوي الحاد المستحث بالفانكومايسين، يمكن للسلبيوتامين والثيامين والريبوفلافين ومجموعاتها أن تحمي من إصابة الكلى الحادة الناجمة عن VCM والذي يؤدي الى الضرر النسيجي والخلل الكلوي وزيادة الجهد التأكسدي وارتفاع مستويات اليوريا والكرياتينين. علاوة على ذلك، فإن زيادة التعبير المناعي لـ KIM-1 و TNF-alphaنتجت عن علاج الفانكومايسين الذي تم تحسينه أيضًا بواسطة السلبيوتامين والثيامين والريبوفلافين ومجموعاتهم. أظهرت النتائج أن السلبيوتامين، الثيامين، الريبوفلافين ومجموعاتها تحمي من السمية الكلوية الناجمة عن VCM عن طريق استعادة وظائف الكلى وتقليل مستوىKIM-1، TNF-alpha وتخفيف التغيرات النسيجية المرضية من خلال خصائصها المضادة للأكسدة والمضادة للالتهابات. أعطى مركب السلبيوتامين مع الريبوفلافين أفضل النتائج في تخفيف ARFالناجم عن .VCM وكشف البحث أيضاً عن تأثير إضافي للسلبيوتامين مع الريبوفلافين.

الكلمات المفتاحية: فانكومايسين، سلبيوتامين، الثيامين، الريبوفلافين، كيم-١