Azhar Int J Pharm Med Sci 2025; Vol 5 (1): 223- 235
Research Article

Open Acces

Print ISSN: 2735-4598
Online ISSN: 2735-4601

Carvacrol amended Vancomycin-induced nephrotoxicity in rats via regulating Nrf2/HO-1, IKBβ /NF-κB and Bax/Bacl2 pathways

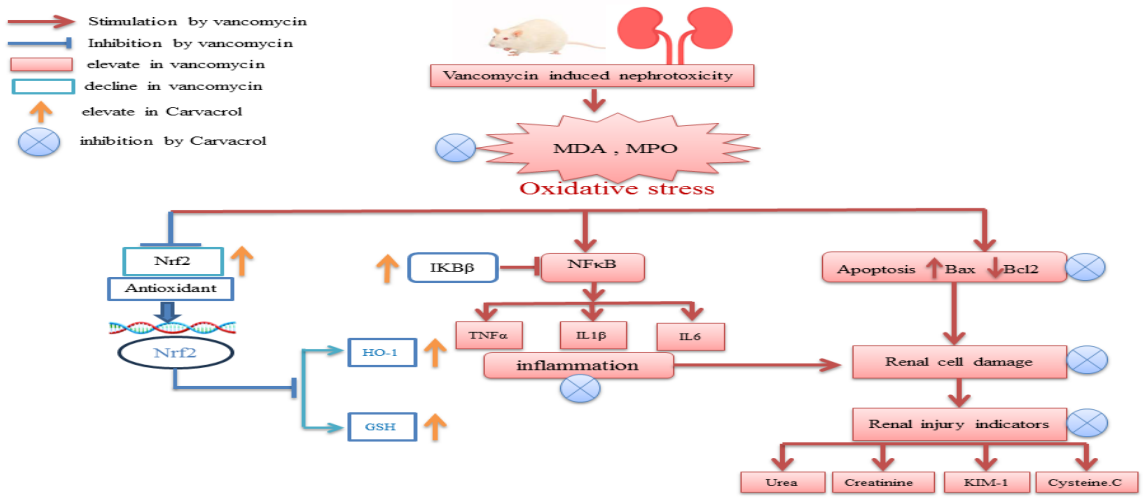
Alzahraa A. Elhemiely[1]*, Rania Yahia[1] and Hayat A. Abd EL Aal[1]

[1] Department of Pharmacology, Egyptian Drug Authority, EDA, Formerly NODCAR, Giza, Egypt
* Correspondence: zahraa_elhimeily123@yahoo.com

Article history: Received 2024-02-04 **Revised 2024-06-09** **Accepted 2024-07-11**

Abstract: Drug- induced kidney injury is considered as a dangerous condition caused morbidity and mortality and most notably Vancomycin induced nephrotoxicity via oxidative stress, inflammatory insult and apoptosis. Our investigation was applied to assess the potential ameliorative effect of Carvacrol against vancomycin-triggered nephrotoxicity. Male Sprague-Dawley rats were allocated into 5 groups: Normal control group, DMSO; a vehicle for carvacrol group, Group of animals were given carvacrol 50 mg/kg/day orally, Group of rats were challenged with intraperitoneal vancomycin 200mg/kg/day and group co-treated with carvacrol and vancomycin as previously mentioned, and all treatments were applied for 7 continuous days. Vancomycin caused nephrotoxicity which revealed as elevated levels of Urea, Creatinine, kidney injury molecule.1 (KIM-1) and Cystatin C and confirmed histologically by degenerative changes in renal histo-architecture. Vancomycin enhanced protein expression of some inflammatory markers as tumor necrosis factor alpha (TNF-α), interleukin-1 beta (IL-1β), interleukin-6 (IL-6) and nuclear factor kappa B (NF-κB) along with down-regulation of protein expression of nuclear factor kappa B inhibitory protein (IKBβ). Likewise, it caused oxidative stress via down-regulation of nuclear factor erythroid 2–related factor 2 (Nrf2), heamoxygenase-1(HO-1) and glutathione (GSH) but increased Malondialdehyde (MDA) and Myeloperoxidase (MPO) levels. Also, vancomycin caused apoptosis by increasing Bax gene expression together with decreasing Bcl2 gene expression. On contrast, Carvacrol mitigated vancomycin induced kidney injury by restoration of kidney architecture and modulation of the inflammation, oxidative stress and apoptosis. This study discloses that carvacrol suppresses vancomycin-induced nephrotoxicity through its anti-inflammatory, anti-oxidative stress and anti-apoptotic actions via Nrf2/HO-1, IKBβ/ NF-κB and Bax -Bcl2 pathways.

Keywords: Carvacrol; Vancomycin; Nephrotoxicity; Oxidative stress; Inflammation; Apoptosis.



Cite this article: Elhemiely AA., Raia Yahia R., Abd EL Aal1 HA. Carvacrol amended Vancomycin -induced nephrotoxicity in rats via regulating Nrf2/HO-1, IKBβ /NF-κB and Bax/Bacl2 pathways. Azhar International Journal of Pharmaceutical and Medical Sciences, 2025; 5(1): 223- 235. doi: 10.21608/aijpms.2024.267591.1253

1. INTRODUCTION

Vancomycin (VCM) is a broad-spectrum glycopeptide antibiotic used to treat many infectious diseases especially against methicillin-resistant Staphylococcus aureus. Clinically, it used for more than 50 years ago but duo to its toxicity; it is substituted with other antibiotics which are less toxic[1-3]. The most common vancomycin-triggered health hazard is the nephrotoxicity as it accumulates in kidney tissues and also its elimination occurs unchanged via kidney causing histo-pathological lesions and alteration in the kidney function indices [1,2,4].

It is previously mentioned that the mechanisms by which VCM caused nephrotoxicity are its ability to exert oxidative stress damage, inflammatory events and apoptosis [5]. It provokes oxidative stress through inhibition of Nrf2/ HO-1 pathway causing depletion of Glutathione (GSH) and heme oxygenase-1 (HO 1) which considered important cellular antioxidants [6]. This oxidative stress is mediated by raised malondialdehyde and depleted antioxidant enzymes so using of antioxidants has protective effects against vancomycin-mediated nephrotoxicity [7]. Additionally, oxidative damage triggered by vancomycin associated with inflammatory insult which may be correlated to NF-κB pathway and in the same context, down-regulation of NF-κB inhibitory protein (IkBβ) by vancomycin leading to activation of the NF-κB causing up-regulation of the pro-inflammatory cytokines TNF-α, IL-1β and IL-6 [6,8].

Regarding renal cells apoptosis as evidenced by He et al and Darwish et al [8,9] vancomycin stimulated oxidative stress thus activated NF-κB causing renal damage by apoptosis through activation of Bax/Bcl2 pathway.

Carvacrol (CAR) is a phenolic monoterpenoid obtained from pepperwort, thyme, oregano, wild bergamot and other plants essential oils. Carvacrol has antimicrobial, antioxidant and anticancer properties [10]. It acts as antioxidant and can amend oxidative stress induced nephrotoxicity and protects biomolecules such as membrane lipids against oxidative stress damage. these functions correlated to activation of Nrf2 which is a master regulator of many antioxidants downstream as HO.1; a cyto-protective enzyme and GSH which considered as a powerful free radical scavenger and related to abrogation of the lipid peroxidation which estimated by reduced MDA Level [11-14]. Specifically, Carvacrol attenuated deleterious effects caused by oxidative stress via mechanism related to HO-1 pathway therefore it is deemed as HO.1 agonist [12].

Likewise, it has anti-inflammatory capacity through inhibition of the pro-inflammatory action of H2O2 by downregulating the transcription factor NF-κB so targeting the NF-κB pathway has therapeutic benefits attributed to its role in activation of pro-inflammatory cytokines such as IL-1β, IL-6, TNF-α [12,15].

Thus, the purpose of this work was to evaluate the effect of carvacrol on VCM-evoked nephrotoxicity and to give an overview about the underlying mechanisms by estimation of different parameters of the oxidative stress, inflammatory and apoptotic signals.

.2. METHODS

2.1. Animals

Adult male Sprague-Dawley rats (150-200 g) body weights were used in our study. Source of animals is the breeding colony of Egyptian Drug Authority (EDA) and kept in its animal house. During experiment, rats were given adequate amounts of food and water. 2-weeks adaption period given to animals prior to starting of the experiment and we must supply them with 40%–60% relative humidity, room temperature 21–24°C and a 12-h light–dark cycle. The ethical standards for laboratory animal research were applied according to the protocols of the EDA's standard operating procedures. We obtained (Approval number NODCAR/I/23/2021) in handling the experimental animals and corresponds to the Guide for Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996).

2.2. Chemicals

Carvacrol and Vancomycin were obtained from (Sigma-Aldrich Chemistry, St. Louis, MO, USA) and (Xi'an Tian Guangyuan Biotech Co.,Ltd) respectively, Carvacrol dissolved in 50% dimethyl sulfoxide (DMSO) and given by 50 mg/kg/day per os [14] but Vancomycin dissolved in distilled water and administered intrapreotenially by 200 mg/kg/day [15] . All used chemicals were of analytical grade.

2.3. Experimental Design

Animals were divided into five groups with six rats per treatment. All treatments were applied for 7 continuous days.

Group I (normal control) animals were given 1ml of distilled water intrapreotenially.

Group II Animals were received 50% dimethyl sulfoxide (DMSO) orally.

Group III Rats were orally given carvacrol 50 mg/kg/day [14] per os.

Group IV animals were given intrapreotenially vancomycin 200 mg/kg/day [15].
Group V Rats were co-treated with vancomycin and carvacrol as previously mentioned. vancomycin given one hour after the administration of carvacrol.

2.4. Sampling of Blood and Tissue

At 8th day, we collected the blood from the orbital venous plexus and then centrifugation occurs at 3000 rpm for 15 minutes to collect serum. After that, Rats were euthanized by cervical dislocation and then kidneys were taken off and washed with ice-cold saline. For histopathological evaluation, right kidney stored in 10% formalin solution and the left one was stored at -80. part of the left kidneys homogenized in PBS (10%, w/v) (10 mmol/l, pH 7.4) and subsequently we performed centrifugation for this homogenate at 10000 ×g for 10 min at 4°C to obtain supernatant and the other part kept for western blotting. Both serum and supernatant were stored at −20°C until biochemical investigation.

2.5. Biochemical Analysis

2.5.1. Estimation of nephrotoxicity markers

Colorimetric assay kits (BioMed, Egypt) were used for estimation of Urea and Creatinine levels and (MyBiosource USA; ELISA kit Catalog No: MBS355395 and Catalog No: MBS763996) were used to detect KIM-1 and Cystatin C levels respectively, following the manufacturer's roles.

2.5.2. Detection of oxidative stress markers

Kidney homogenate were used for estimation of GSH activity and MDA content using assay kits (Biodiagnostic, Egypt). In addition, (Reddot biotech ELISA Kit Catalog No: RDR-MPO-Ra) utilized for determination of MPO activity and (MyBiosource USA; ELISA Kit Catalog No: MBS752046 and Catalog No: MBS764989) were used to estimate Nrf2 and HO-1 expression respectively, following the manufacturer's guidelines.

2.5.3. Inspection for inflammatory markers

TNF-α, IL-1β and IL-6 expression were measured in kidney homogenate using (Cusabio ELISA Kit Catalog No: CSB-E11987r), (MyBiosource ELISA Kit Catalog No: MBS825017) and (Quantikine ELISA kit Catalog No: R6000B) respectively, in accordance with manufacturers' standards.

2.6. Immunoblotting of IKBβ and NF-κB

We evaluated IκBβ and NF-κB protein expression in renal tissue using immunoblotting protocol. ice-cold lysis solution (Tris-HCL pH 8.0, 1% NP40 (1% v/v), 0.1% SDS, and 0.5% sodium deoxycholate; provided with phenylmethylsulfonylfluoride (PMSF)) were used for kidney homogenization. The lysates centrifuged at 10,000× g obtaining supernatants. Protein extract (50 μg) were resolved with SDS-PAGE then proteins transferred with a semi-dry electro-blotter into a nitrocellulose membrane. Membrane blockade for 1 h occurred using 5% non-fat milk/TBST and then membrane incubated with the primary antibody overnight in a fridge using specific antibodies against IκBβ (IκBβ Polyclonal Antibody ,1:500, Catalog NO PA1-32136, thermos scientific, USA) and NF-κB (NF-κB Polyclonal Antibody, eBioscience™ 1:1000, Catalog NO: 14-6731-81). Membrane washing was occurred, and secondary antibody incubation was carried out for 2 h at room temperature (Cell Signaling Technology, Beverly, MA, USA; dilution 1: 10,000). Finally, chemiluminescence kit (Cat. no. RPN2132, ECL plus; GE Healthcare, Buckinghamshire, UK) used to detect the protein bands which quantified using Molecular Analyst Software (Bio-Rad, Hercules, CA, USA).

2.7. mRNA Extraction and Quantitative Real Time-PCR

Extraction of total RNA from tissue homogenate was applied using SV Total RNA Isolation system (Thermo Scientific, USA) and then synthesis of first strand complementary DNA (cDNA) form this RNA by using cDNA reverse transcription kit (Thermo Fisher Scientific, USA, catalog NO: K4374966) and this process called reverse transcription (RT). After that polymerase chain reaction (PCR) carried out using Jena Bioscience PCR-101 Taq Master Mix (Jena bioscience, Germany) In accordance with the manufacturer's protocol. After that (StepOne™, USA) was used for amplification and analysis of Real-time qPCR and the used primer pairs were listed in (Table 1). Initial denaturation step performed for 10 min at 95°C and then 40 cycles of amplification for Bcl2 and Bax primer pair were carried out. The expression levels of Bcl2 and Bax genes were normalized to the level of GAPDH gene expression in each sample.

2.8. Histopathological Evaluation

Autopsy samples were taken from the kidney of rats in different groups and histological preparation and examination occurred as described by Banchroft et al [16].

Table 1. Characteristics of primers were used in the real-time PCR assay.

Rat-Bcl2-F	**5′-TGTGGATGACTGACTACCTGAACC3′**
Rat-Bcl2-R	5′CAGCCAGGAGAAATCAAACAGAGG3′
Rat-Bax-F	5′CGGCGAATTGGAGATGAACTGG3′
Rat-Bax-R	5′CTAGCAAAGTAGAAGAGGGCAACC3′

2.9. Statistical Analysis

Prism version 5 (Graph Pad Software Inc., San Diego, USA) was used to carry out statistical analysis. Results were evaluated using one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test and data in form of means ± SE. For all statistical tests, the level of significance was provided at $p < 0.05$.

3. RESULTS

3.1. Carvacrol Can Ameliorate Alterations in Kidney Function Biomarkers in Vancomycin Treated Rats

Alterations in kidney function biomarkers like Urea, Creatinine, and KIM1and Cystatin C considered as most important sign of nephrotoxicity. As illustrated in Figure 1, Vancomycin intoxication caused renal injury which displayed as elevated serum levels of kidney function indices Urea, Creatinine, KIM1and Cystatin C by 189.9, 916.6, 180.5 and 279.7% respectively, when compared to control animals and this regarded as first sign of renal dysfunction. Carvacrol administration to vancomycin challenged animals ameliorated these alterations by marked reduction in the levels of theses biomarkers by 51.3, 66.3, 48.4 and 42.8% respectively, versus vancomycin treated rats. Carvacrol alone did not cause any significant alternations in these indicators when compared with the control animals and these results explain the ability of Carvacrol to overcome renal injury evoked by vancomycin which firstly demonstrated by altered serum biomarkers.

3.2. Carvacrol Can Abrogate Vancomycin Induced Oxidative Stress and Activate Nrf2 / HO.1 Pathway in Kidney of Animals

Oxidative stress considered as a main cause of many renal diseases as reactive oxygen species causes lipid peroxidation leading to up-regulation of MDA and MPO meanwhile Nrf2 is a master regulator for many antioxidants as HO.1 and GSH. Vancomycin caused oxidative stress in renal tissue which evidenced by significant elevation of renal MDA and MPO levels by nearly 4-folds and 105.8% respectively, in comparison with normal control rats alongside considerable decline in GSH level by 67.11% as compared to the control rats. To confirm these findings, we assessed Nrf2 and HO.1 expression as Vancomycin caused marked down-regulation of Nrf2 and HO.1 protein expression by 72.5 and 76.0 % respectively, in comparison with the control animals. On the other hand, Carvacrol administration for Vancomycin intoxicated rats halted this oxidative stress damage by decreasing levels of MDA and MPO by 58.6 and 42.3 % respectively, together with significant enhancing of GSH level by 120 % when compared with vancomycin intoxicated animals. In the same context, Nrf2 and HO.1 protein expression significantly increased by 126 and 220 % respectively, versus vancomycin treated rats. The group solely treated with carvacrol exhibited normal range of these biomarkers' levels similar to the control group as illustrated in Figure 2. These findings give an idea about the role of oxidative stress amelioration and activation of Nrf2 / HO.1 pathway by carvacrol in mitigating vancomycin-induced renal dysfunction.

3.3. Carvacrol Administration Enhances Vancomycin-Induced Inflammation in Rat Renal Tissue.

One of the important mechanisms by which vancomycin exerts its toxicity is evoking inflammatory insult which thought to be related to NF-κB signaling pathway as when activated causing overly release of many pro-inflammatory biomarkers. As demonstrated in Figure 3, transcriptional factor NF-κB protein expression in kidney of vancomycin intoxicated rats was significantly increased by nearly 6-folds along with considerable decrease in its inhibitory protein IKBβ by 69.3% in comparison with normal control rats which confirmed by significant increase of the pro-inflammatory mediators TNFα, IL-1 β and IL-6 by 252.9 %, nearly 5–folds and 283.3 % respectively, versus normal control animals.

Co-treatment with Carvacrol significantly declined the protein expression of NF-κB by 54.1 % along with up-regulation of IKBβ protein expression by 119.3 % in vancomycin-challenged rats. In the same line, expression of TNFα, IL-1 β and IL-6 were significantly declined by 53.5, 57.7 and 56.0 % respectively; versus vancomycin intoxicated animals as explained in Figure 4.

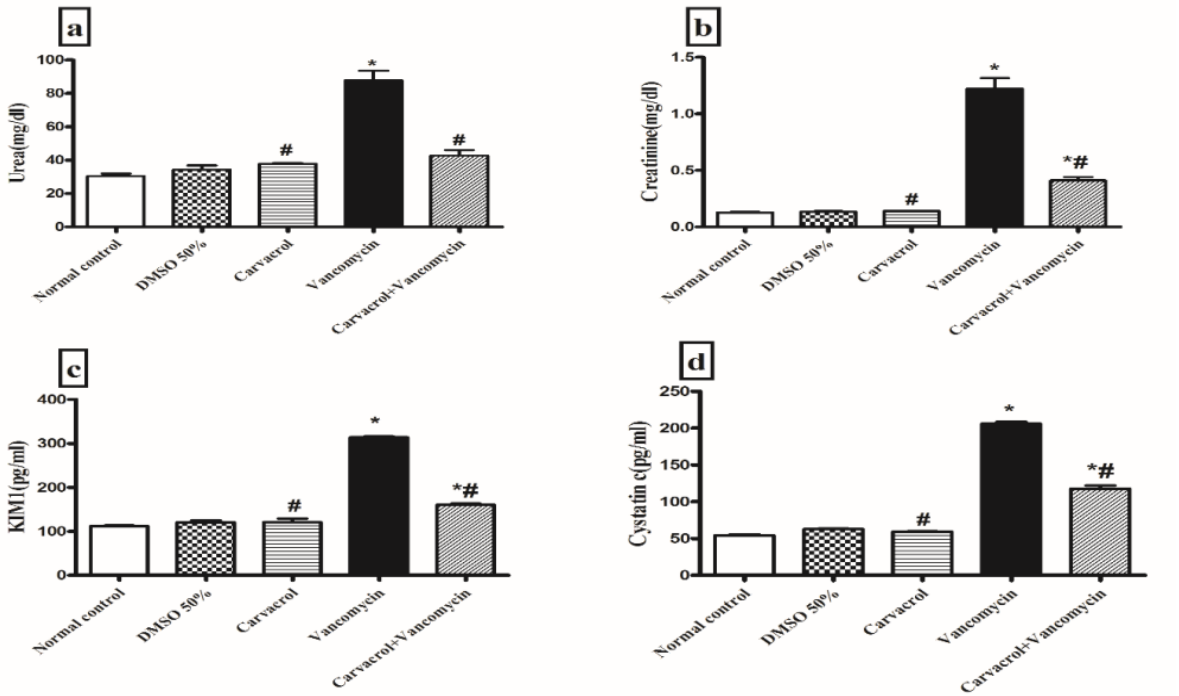


Figure 1. Effect of carvacrol administration on vancomycin induced alteration in nephrotoxicity markers (a) Urea, (b) Creatinine,(c) KIM1 and (d) Cystatine. Values are illustrated as mean ± SE. using (ANOVA) followed by Tukey-Kramer as a post-hoc test. * or # statistically significant from control or vancomycin group, respectively.

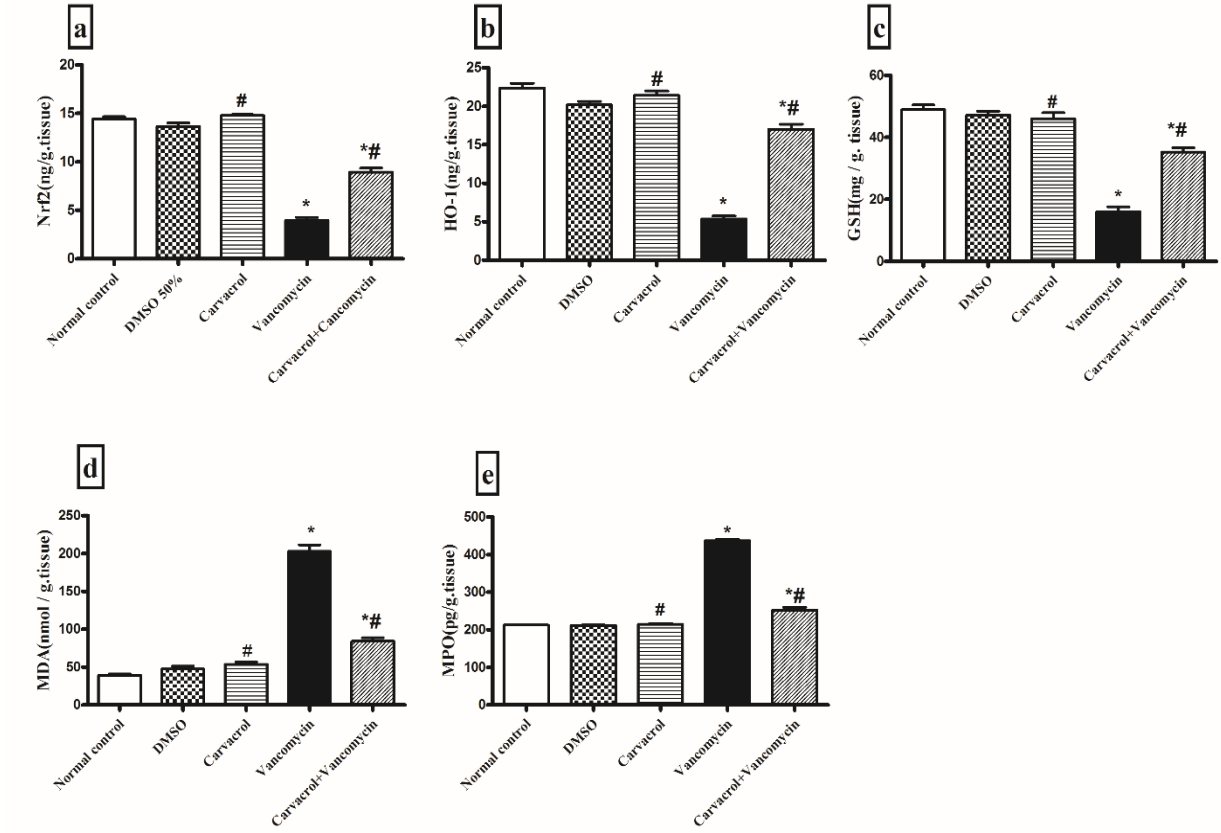


Figure 2. Effect of carvacrol administration on vancomycin induced oxidative stress (a) Nrf2, (b) HO.1, (c) GSH, (d) MDA and (e) MPO. Values are represented as mean ± SE. using (ANOVA) followed by Tukey-Kramer as a post-hoc test * or # statistically significant from control or vancomycin group, respectively.

Unlike these results carvacrol given alone did not alter these transcriptional factors or pro-inflammatory mediators in renal tissue when compared with those of control animals. These results revealed the importance of inflammation suppression by carvacrol in the abrogation of vancomycin-evoked renal damage.

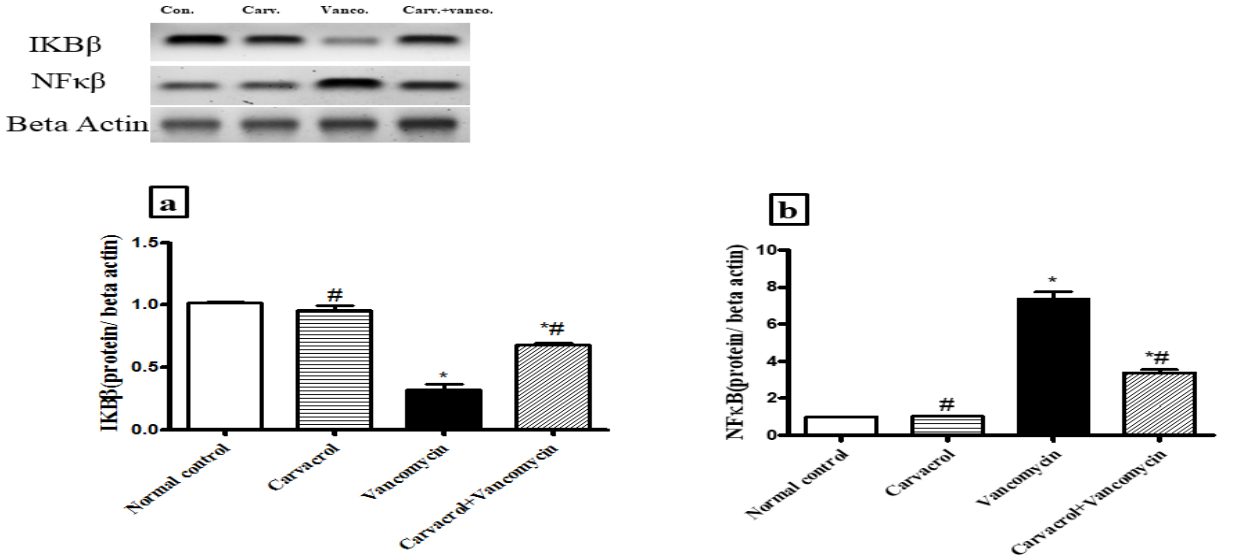


Figure 3. Effect of carvacrol administration on (a) IKBβ and (b) NFκB in vancomycin treated rats .Values are represented as mean ± SE. using (ANOVA) followed by Tukey-Kramer as a post-hoc test * or # statistically significant from control or vancomycin group, respectively.

3.4. Carvacrol Alleviates Vancomycin-Evoked Apoptosis in Rat's Kidney.

Apoptosis is programmed cell death and one of the most vital pathways by which vancomycin causes renal toxicity. As demonstrated in Figure 5, Apoptotic status in kidney tissue was determined by analyzing gene expression of Bax and Bcl-2. In vancomycin treated rats, the expression of Bax mRNA, a pro-apoptotic marker in renal tissue was significantly increased by nearly 5-folds but in contrast, Bcl-2 mRNA, the anti-apoptotic marker was down-regulated by 77% in renal tissue as compared to normal control animals. carvacrol exhibited anti-apoptotic properties by lowering Bax mRNA expression by 60% and significantly elevated mRNA expression of Bcl-2 by 300% as compared to vancomycin-treated rats. However, carvacrol alone did not cause any variation from normal control. These data gave an overview about the anti-apoptotic properties of carvacrol which overcome vancomycin-triggered intoxication.

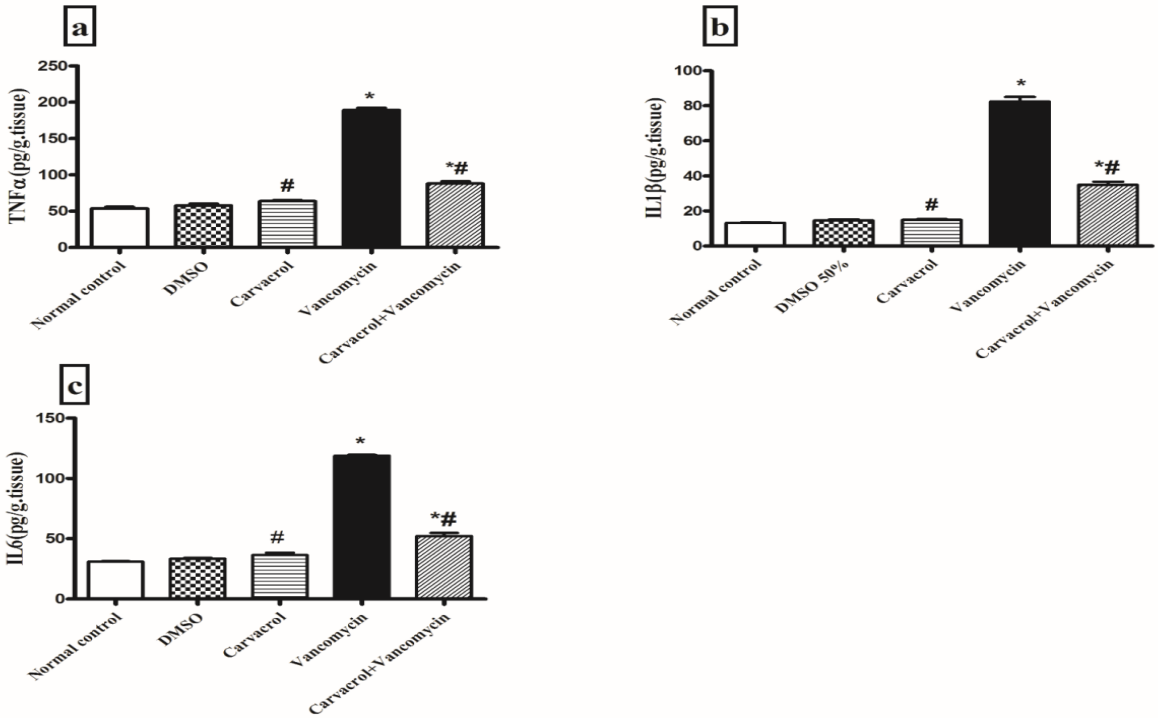


Figure 4. Effect of carvacrol administration on (a) TNFα, (b) IL1β and (c) IL6 in vancomycin treated rats .Values are represented as mean ± SE. using (ANOVA) followed by Tukey-Kramer as a post-hoc test * or # statistically significant from control or vancomycin group, respectively.

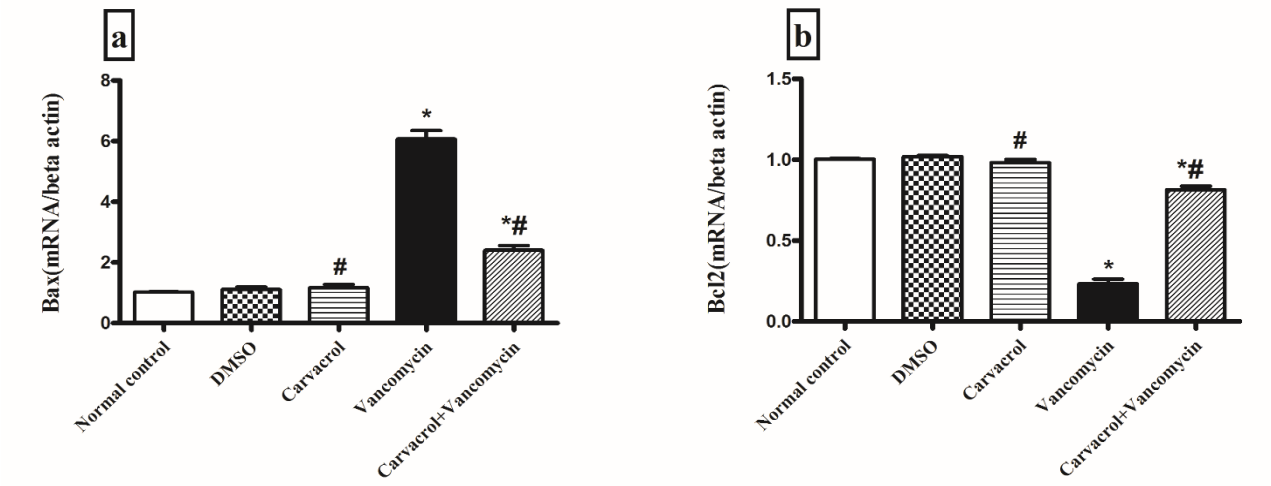


Figure 5. Effect of carvacrol administration on vancomycin induced apoptosis (a) Bax mRNA transcript level and (b) Bcl2 mRNA transcript level measured by RT-PCR method. Values are demonstrated as mean ± SE. using (ANOVA) followed by Tukey-Kramer as a post-hoc test * or # statistically significant from control or vancomycin group, respectively.

3.5. Carvacrol Curtails Vancomycin-Evoked Histological Lesions in Rat's Kidney

Our biochemical results are confirmed by histological findings as kidney histopathological examination showed that carvacrol partially repairs VCM-induced tissue injury as illustrated in Figure 6.

Renal tissue of normal control and DMSO treated rats revealed no histopathological alteration with normal histological structure of the glomeruli and tubules at the cortex. But the kidney tissue of rats challenged with vancomycin showed extensive damage in form of focal inflammatory cells infiltration in between the tubules accompanied with vacuolar degeneration in the tubular lining epithelium at the cortex.

Carvacrol markedly attenuated vancomycin -induced tissue damage and showed degree of improvement which evidenced by decreasing tubular degeneration and focal inflammatory cells infiltration in between the tubules. Carvacrol alone did not cause any histopathological changes in the treated animals and their renal histology resembled that of control animals.

Table 2 presented a scoring system for Focal inflammatory reaction in between the tubules, Tubular degeneration and Renal cast formation. The scoring system employed symbols such as (-) for normal, (+) for mild, (++) for moderate and (+++) Sever

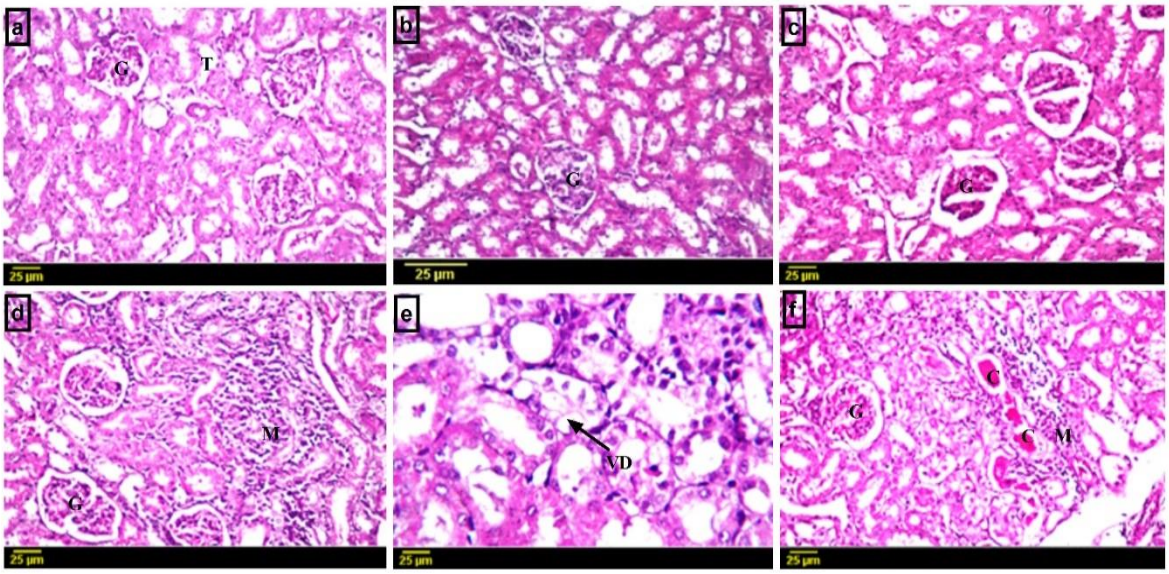


Figure 6. Effect of carvacrol administration on vancomycin induced histological alterations in the renal tissue (H & E, X40).(a, b and c) kidney tissue of rat in normal control , DMSO and carvacrol groups respectively showing normal histological structure of glomeruli and tubules in the cortex,(d and e) kidney tissue of vancomycin-challenged rat showing Focal inflammatory cells infiltration(m) in between the tubules at the cortex and vacuolar degeneration in the tubular lining epithelium at the cortex (arrow vd) and (f) kidney tissue of rat treated with vancomycin along with carvacrol showing few focal inflammatory cells infiltration(m) in between the tubules with eosinophilic casts(c) in the lumen in some few tubules.

Table 2. Scoring System for kidney Histopathological Parameters.

	Control group	DMSO group	Vancomycin group	Carvacrol group	Vancomycin+ carvacrol group
Focal inflammatory reaction in between the tubules	-	-	+++	-	++
Tubular degeneration	-	-	+++	-	+
Renal cast formation	-	-	-	-	+

+++ Sever ++Moderate +Mild _Nil

4. DISCUSSION

The goal of current investigation is to provide evidence about carvacrol protection against vancomycin induced kidney injury. Vancomycin is an effective antibiotic used commonly against gram-positive bacteria, but its nephrotoxic effects limit its efficacy [17]. Using Carvacrol can ameliorate kidney damage by suppressing oxidative stress and apoptosis as a result of its antioxidant and anti-apoptotic properties [11, 18].

Urea, Creatinine, KIM1 and Cystatine C levels are the first indicators for renal disorder as Creatinine and Cystatine C deemed as indicators of glomerular function and revealed the decreased glomerular filtration rate of the kidney and also KIM-1 regarded as vital biomarker of vancomycin-triggered renal histopathologic damage [6,11,19,20]. Herein, vancomycin caused nephrotoxicity which evidenced as elevated levels of Urea, Creatinine, KIM1 and Cystatine C and these findings are in concurrent with previous studies [8,20,21] elevation of the concentration of these indices indicated that the kidneys will collapse within few days of vancomycin administration [2,22].

Concerning Carvacrol administration, it attenuated these injury signs, so it considered as agent which have an ameliorative effect against kidney injury [18, 19].

Oxidative stress occurs when reactive oxygen species surpass antioxidants contributing to pathogenesis of many kidney diseases [23, 24]. attributed to the fact that the kidney is a highly metabolic organ, many oxidation reactions occur thus it is highly susceptible to oxidative stress which hasten kidney disease progression specially vancomycin-triggered renal damage [21,25] so evaluation of oxidative stress biomarkers is valuable method for diagnosis of different diseases [26].

Nrf2 is a transcription factor which considered the master regulator of endogenous antioxidant defense via control of expression of many detoxifying and antioxidant genes [27, 28]. Once it stimulated, it activates the transcription of cyto-protective HO-1 ; the enzyme that promotes the destruction of pro-oxidant heme to biliverdin, carbon monoxide and irons so HO-1 and these by-products have antioxidant and anti-inflammatory capacities therefore the expression of HO-1 have an important role in maintaining balance between antioxidant system and oxidative condition during kidney injury. For that reason, inhibition of HO-1 promotes structural and functional damage in the proximal renal tubules [29-32].

Under oxidative stress status, Free radicals provoke lipid peroxidation and the more important end product is Malondialdehyde (MDA) therefore it is used as oxidative stress indicator and also MPO is a member of heme peroxidases which up-regulated by oxidative stress [33,34]. Concerning kidney diseases, production of myeloperoxidase and formation of MPO-derived reactive species (reactive/chlorinating intermediate products and hypochlorous acid) cause inflammation and oxidative damage to renal cells [35]. On the other hand, Glutathione is a vital antioxidant can protect cells against oxidative stress-caused tissue damage [19, 36].

Depending on these facts, vancomycin induced renal damage via oxidative stress by inhibiting Nrf2 / HO-1 signaling and altering level of GSH which regarded as a crucial free radical scavenger in kidney tissue together with increasing levels of MDA and MPO and these come into agreement with previously proved results [6,15, 32, 37,38].

Considering this oxidative reaction as a main cause of renal damage in patients treated with vancomycin, carvacrol abrogated it by its antioxidant action via elevation of GSH level which confirmed by up-regulation of Nrf2 and HO-1 along with decreasing MDA and MPO levels [11,39,40].

NF-κB is a transcription factor which normally located in the cytoplasm in inactive form via physical association with inhibitory proteins which called IκBs which inhibits NF-κB. under effect of oxidative stress, NF-κB become activated and move to the nucleus to regulate the inflammatory insult as its pathway is the main intracellular pathway responsible for inflammatory response in injured kidneys [41-45] and this contributed to its action as regulatory factor that has binding sites in the promoter regions of many pro-inflammatory

cytokines therefore activation of NF-κB enhances pro-inflammatory mediators expression specially TNF-α [46, 47]. The increased production of pro-inflammatory cytokines as TNF-α, IL-1β and IL-6 are responsible for initiation of inflammation in some renal disorders. particularly, IL-1β and IL-6 included in the progression of many disorders as chronic kidney disease thus they used as therapeutic target in many renal injuries as Anti-IL-6 antibodies appear to have clinical therapeutic value in some chronic kidney disease [48- 51].

In our study, Vancomycin caused inflammation via over-expression NF-κB protein which attributed to down-regulation of its inhibitory protein (IkBβ) protein expression as mentioned by khalaf et al [6]. Activated NF-κB augmented levels of the pro-inflammatory mediators IL-1β, IL-6 and TNF-α as proved by He et al [8]. These results indicated that kidney dysfunction by vancomycin caused by oxidative stress which was in relation with augmentation of transcription factors and pro-inflammatory genes causing inflammation [23]. On the other hand, carvacrol administration increased IkBβ protein expression leading to inactivation of NF-kB and pro-inflammatory cytokines [6,52] thus carvacrol administration significantly lowered TNF-α, IL6 and IL-1β expression so inhibit inflammation in renal tissue [19, 53, 54].

One of the critical effects of oxidative stress is helping in the progression of apoptosis which is the programmed cell death. Bcl-2, an endogenously produced protein prevents cells from dying by an anti-oxidative mechanism [55 55,56]. Concerning renal injury, apoptosis causes parenchymal cell loss [57]. In our study, vancomycin increased the pro-apoptotic marker (Bax) and decreased the anti-apoptotic marker (Bcl2) gene expressions as supported by Kandemir et al [15].

On contrast, carvacrol amended this apoptosis by up-regulation of Bcl-2 and decreasing Bax gene expressions18,52 this effect may be attributed to its antioxidant effect which can block or delay apoptosis [55].

Finally, Histopathological results supported all previous results as we found that vancomycin caused renal tissue damage and these lesions may be attributed to oxidative stress which evoke lipid peroxidation and collapse both cells and cytoplasmic membrane and our findings agreed with those mentioned by Yu et al [21]. By carvacrol administration, Remarkable improvement in renal architecture occurred and this effect may be attributed to its antioxidant effect [14,39, 58,59] as oxidative reactions are the major cause of kidney diseases in patients treated with vancomycin so it is clear that antioxidants can abrogate this damage by counteracting the oxidative stress [60].

Nonetheless, there are major limitations in this study that could be addressed in future research which is studying the expression of Caspase-3 due to its role in and Bax /Bcl2 pathway.

5. CONCLUSION

It is concluded that the ability of carvacrol to halt vancomycin-induced renal damage is contributed to its anti-inflammatory, anti-oxidative stress and anti-apoptotic actions as it is clear that the protective impact of carvacrol on the kidney tissue could be attributed to its capability to neutralize the free radicals, its property as Nrf2, HO-1 and IKBβ activator, its action as IL1β and IL-6 inhibitor so it plays this ameliorative effect via Nrf2/HO.1, IKBβ / NFκB and Bax /Bcl2 pathways.

Funding: No funding was received for conducting this study.

Acknowledgments: The authors like to thank Prof. Adel Bakear, Faculty of Veterinary Medicine, Pathology Department, Cairo University, for his effort in the histopathological examination.

Conflicts of Interest: The authors declare that they have no conflict of interest.

Ethical Statement: The investigation follows the Guide for Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85–23, revised 1996) and was approved by the Ethics Committee for Animal Experimentation at Egyptian drug authority (EDA) with the ethical approval number of (NODCAR/I/23/2021) by 3/11/2021.

Author Contribution: E.A: Conceptualization, Validation, Formal analysis, Writing - review & editing, Visualization, Y.R: Conceptualization, Validation, Formal analysis, Visualization, A.H: Methodology, Validation, Formal analysis, Writing - review & editing, Visualization.

REFERENCES

1. Uckuna Z, Guzelb S, Canacankatanc N, Yalazad C, Kibare D, Yilmaze B C. Potential protective effects of naringenin against vancomycin-induced nephrotoxicity via reduction on apoptotic and oxidative stress markers in rats. Drug and Chem. Tox.2020; 43(1): 104 –111.

2. Uhuo E N, Egba S I, Nwuke P N, Odinamadu, H. Renoprotective effects of Adansonia digitata leaf extract on renal function and histopathological changes in

vancomycin induced nephrotoxicity in Wister rats. Comp. Clin.Path.2022; 31:229–242.

3. Levine D P. Vancomycin: A History. Clin. Infect. Dis., 2006; 42(1): S5–S12.

4. Filippone E J, Kraft W K, Farber JL. The Nephrotoxicity of Vancomycin. Clin. Pharmacol. Therap. 2017; 102 (3): 459-469.

5. Humanes B, Jado J C, Camano S, López-Parra V, Torres A, Alvarez-Sala L A et al. Protective Effects of Cilastatin against Vancomycin-Induced Nephrotoxicity. Bio. Med. Res. Intern. 2015; 1-12.

6. Khalaf M M, Hassan S M, Sayed A M, Abo-Youssef A M. Carvacrol mitigates vancomycin-induced nephrotoxicity via regulation of IkBα/p38MAPK and Keap1/Nrf2 signaling pathways: an experimental study within silico evidence. Eur.Rev. Med. Pharmacol. Sci. 2022; 26 (23): 8738-8755

7. Celik I, Cihangiroglu M, Ilhan N, Akpolat N, Akbulut H H. Protective effects of different antioxidants and amrinone on vancomycin-induced nephrotoxicity. Basic. Clin Pharmacol Toxico. 2005; l97: 325–332.

8. He J, Xu W, Zheng X, Zhao, B, Ni T, Yu P, et al. Vitamin C reduces vancomycin-related nephrotoxicity through the inhibition of oxidative stress, apoptosis and inflammation in mice. Ann. Translational Med. 2021; 9(16):1319-1329.

9. Darwish S F, Mahmoud A M A, Abdel Mageed S S , Sallam A M ,Oraby M A. Dapagliflozin improves early acute kidney injury induced by vancomycin in rats: Insights on activin A/miRNA-21 signaling and FOXO3a expression. Eur. J. Pharmacol. 2023; 955.

10. Sharifi-Rad M, Varoni E M, Iriti M, Martorell M, Setzer W N, Contreras M, et al. Carvacrol and human health: A comprehensive review. Phytotherapy Research. 2018; 32 (11), 1675–1687.

11. Gunes S, Ayhanci A, Sahinturk V, Altay D U, and Uyar R. Carvacrol attenuates cyclophosphamide-induced oxidative stress in rat kidney. Anadian J. Physio. Pharmacol. 2017; 95(7), 1-30.

12. Chenet A L, Duarte A R, de Almeida F J S, Andrade C M B, de Oliveira M R. Carvacrol Depends on Heme Oxygenase-1 (HO-1) to Exert Antioxidant, Anti-inflammatory, and Mitochondria-Related Protection in the Human Neuroblastoma SH-SY5Y Cells Line Exposed to Hydrogen Peroxide. Neurochem. Res. 2019; 44: 884–896.

13. Naeem K, Al Kury LT, Faiza N, Alattar A, Alshaman., Shah FA, et al. Natural Dietary Supplement, Carvacrol, Alleviates LPS-Induced Oxidative Stress, Neurodegeneration, and Depressive-Like Behaviors via the Nrf2/HO-1 Pathway. J. Inflamm. Res. 2021; 14: 1313–1329.

14. Khalaf A A, Elhady M A, Hassanen E I, Azouz A A, Ibrahim M A, Galal M K, et al. Antioxidant Role of Carvacrol Against Hepatotoxicity and Nephrotoxicity Induced by Propiconazole in Rats. Rev. Brasil. Farmaco. 2021;31: 67–74.

15. Kandemir FM, Yildirim S, Kucukler S, Caglayan C, Mahamadu A, Dortbudak M B. Therapeutic efficacy of zingerone against vancomycin-induced oxidative stress, inflammation, apoptosis and aquaporin 1 permeability in rat kidney. Biomed. Pharmaco.2018; 105: 981-991.

16. Banchroft JD, Stevens A, Turner DR. Theory and practice of Histological Technique Fourth Ed., Churchil Livingstone, New York, London, San Francisco, Tokyo.1996.

17. Qu S, Dai C, Hao Z, Tang Q, Wang H, Wang J, et al. Chlorogenic acid prevents vancomycin-induced nephrotoxicity without compromising vancomycin antibacterial properties. Phytotherapy Res. 2020; 34:3189–3199.

18. Najafizadeh A, Kaeidi A, Rahmani M R, Hakimizadehjalal E, Hassanshahi,J. The Protective Effect of Carvacrol on Acetaminophen-Induced Renal Damage in

Male Rats. Mol. Bio. Re. 2022; 49: 1763–1771.

19. Nouri A, Izak-Shirian F, Fanaei V, Dastan M, Abolfathi M, Moradi A, Khaledi M, Mirshekari-Jahangiri H. Carvacrol exerts nephroprotective effect in rat model of diclofenac-inducedrenal injury through regulation of oxidative stress and suppression of inflammatory response. Heliyon. 2021; 7 (11): 1-7.

20. Pais G M, Avedissian S N, O'Donnell N, Rhodes N J, Lodise,T P, Prozialeck W C, et al . Comparative Performance of Urinary Biomarkers forVancomycin-Induced Kidney Injury According to Timeline of Injury. Antimicrob. Agent. Chemoth. .2019; 63 (7).

21. Yu P, Luo J, Song H, Qian T, He X, Fang J, et al. N-acetylcysteine Ameliorates Vancomycin-induced Nephrotoxicity by Inhibiting Oxidative Stress and Apoptosis in the in vivo and in vitro Models. Intern. J. Medi. Sci.2022;19 (4): 740-752.

22. Sadeghi H, Karimizadeh E, Sadeghi H, Kokhdan E P, Mansourian M, Abbaszadeh-Goudarzi K, et al. Protective Effects of Hydroalcoholic Extract of Rosa canina Fruit on Vancomycin-Induced Nephrotoxicity in Rats. J. Toxicol. 2021.

23. Chatterjee S. Oxidative Stress, Inflammation, and Disease. Oxid. Stress. Biomat. 2016; 35-58.

24. Gyuraszova M, Gurecka R, Babickova J., Tothova L. Oxidative Stress in the Pathophysiology of Kidney Disease: Implications for Noninvasive Monitoring and Identification of Biomarkers. Hindawi Oxi. Med. Cell. Long .2020.

25. Daenen K, Andries A, Mekahli D, Schepdael A V, Jouret F, Bammens B. Oxidative stress in chronic kidney disease. Pedi. Nephro.2019; 34:975–991.

26. Krata N, Zagozdzon R, Foroncewicz B, Mucha K. Oxidative Stress in Kidney Diseases: The Cause or the Consequence? Arch. Immuno. Therap. Experiment.2018; 66 :211–220.

27. Tanaka Y, Aleksunes LM, Goedken M J, Chen C, Reisman S A, Manautou J E. Coordinated Induction of Nrf2 Target Genes Protects Against Iron Nitrilotriacetate (FeNTA)-Induced Nephrotoxicity. Toxicol. Appl. Pharmacol. 2008; 231(3): 364–373.

28. Zhang R, Xu M, Wang Yu, Xie F, Zhang G, Qin X. Nrf2: a promising therapeutic target for defensing against oxidative stress in stroke. Mol. Neurobiol.2017;54:6006–6017.

29. Bolisetty S, Traylor A, Joseph R, Zarjou A. , Agarwal A. Proximal tubule-targeted heme oxygenase-1 in cisplatin-induced acute kidney injury. Am. J. Physiol. Renal. Physiol. 2016; 310(5): 385-394.

30. Loboda A, Damulewicz M, Pyza E, Jozkowicz A, Dulak J. Role of Nrf2/HO-1 system in development, oxidative stress response and diseases: an evolutionarily conserved mechanism. Cell. Mol. Life Sci. 2016; 73:3221–3247.

31. Younis N N, Elsherbiny N M, Shaheen M A, Elseweidy M M. Modulation of NADPH oxidase and Nrf2/HO-1 pathway by vanillin in cisplatin-induced nephrotoxicity in rats. J. Pharma. Pharmaco. 2020; 72(11): 1546–1555.

32. ZhuY, Jin H, Huo X, Meng Q, Wang C, Sun P, et al Protective effect of Rhein against vancomycin-induced nephrotoxicity through regulating renal transporters and Nrf2pathway. Phytotherap. Res.2022; 1–19.

33. Gaweł S, Wardas M, Niedworok E, Wardas P. Malondialdehyde (MDA) as a lipid peroxidation marker. Wiadomosci Lekarskie .2004; 57(9-10):453-455.

34. Ndrepepa G. Myeloperoxidase – A bridge linking inflammation and oxidative stress with cardiovascular disease. Clinica. Chimica. Acta. 2019; 493:36-51.

35. Kisic B, Miric D, Dragojevic I, Rasic J, Popovic L. Role of Myeloperoxidase in Patients with Chronic Kidney Disease. Oxi. Med. Cell. Long. 2016;1–10.

36. Schmitt B, Vicenza M, Garrel C, Denis F M. Effects of N-acetylcysteine, oral glutathione (GSH) and a novel sublingual form of GSH on oxidative stress markers:

A comparative crossover study. Redox Bio.2015; 6: 198-205.

37. Qu S, Dai C, Lang F, Hu L, Tang Q, Wang H, et al. Rutin Attenuates Vancomycin-Induced Nephrotoxicity by Ameliorating Oxidative Stress, Apoptosis, and Inflammation in Rats. Antimicrob. Agents. Chemother. 2019; 63(1).

38. Kocaturk H, Bedir F, Sener E, Aktas M, Akyuz S, Dabanlioglu B, et al. The effect of lutein on vancomycin-induced oxidative kidney damage in rats. Ann. Med. Res. 2021; 28(9):1643-8.

39. Samarghandian S, Farkhondeh T, Samini F, Borji A. Protective Effects of Carvacrol against Oxidative Stress Induced by Chronic Stress in Rat's Brain, Liver, and Kidney. Biochem. Res. Internation. 2016.

40. Carvalho F O, Silva E R, Nunes P S, Felipe F A, Ramos K P P, Ferreira L A S, et al Effects of the solid lipid nanoparticle of carvacrol on rodents with lung injury from smoke inhalation. Naunyn-Schmiedeberg's Arch. Pharmaco.2020;393:445–455.

41. Zhang H, Sun S. NF-κB in inflammation and renal diseases. Cell. Biosci .2015; 5(63)

42. Mulero M, C., Huxford T, Ghosh G. NF-κB, IκB, and IKK: Integral Components of Immune System Signaling. Struct. Immuno. 2019;207-226

43. Sanz A B, Sanchez-Nin M D, Ramos A M, Moreno J A, Santamaria B, Ruiz-Ortega M, et al. NF-_B in Renal Inflammation. Am. Soc. Nephrol. 2010; 21(8), 1254–1262.

44. Siomek A. NF-κB signaling pathway and free radical impact. Acta Biochimica Polonica. 2012; 59(3): 323–331.

45. Liu N, Zhang G, Niu Y, Wang Q, Zheng J, Yang J, et al. Anti-inflammatory and analgesic activities of indigo through regulating the IKKβ/IκB/NF-κB pathway in mice. Food. Funct. 2020; 11: 8537.

46. Abraham E. NF-κB activation. Critical Care Med.2000; 28(4): 100-104.l

47. Kucukler S, Benzer F, Yildirim, S, Gur C, Kandemir F M, Bengu A S, et al. Protective Effects of Chrysin against Oxidative Stress and Inflammation Induced by Lead Acetate in Rat Kidneys: a Biochemical and Histopathological Approach. Bio. Trace Elem. Res.2021; 199:1501–1514.

48. Ren Q, Guo F, Tao S, Huang R, Ma L, Fu P. Flavonoid fisetin alleviates kidney inflammation and apoptosis via inhibiting Src-mediated NF-κB p65 and MAPK signaling pathways in septic AKI mice. Biomed. Pharmaco.2020;122.

49. Godarzi S M, Gorji A V, Gholizadeh B, Mard S A , Mansouri E. Antioxidant effect of p-coumaric acid on interleukin 1-β and tumor necrosis factor-α in rats with renal ischemic reperfusion. Nefrologia, 2020; 40(3): 311-319.

50. Lei Y, Devarapu S K, Motrapu M, Cohen C D, Lindenmeyer M T, Moll S, et al. Interleukin-1b Inhibition for Chronic Kidney Disease in Obese Mice with Type 2 Diabetes. Frontiers in Immuno. 2019; 10.

51. Kreiner F F, Kraaijenhof J M, Herrath M V, Hovingh K K G, Scholten B J. VInterleukin 6 in diabetes, chronic kidney disease, and cardiovascular disease: mechanisms and therapeutic perspectives. Expert Rev. Clin.Immuno.2022; 18(4): 377–389.

52. Yesildag K, Gur C, Ileriturk M, Kandemir F M. Evaluation of Oxidative Stress, Inflammation, Apoptosis, Oxidative DNA Damage and Metalloproteinases in the Lungs of Rats Treated with Cadmium and Carvacrol. Mol. Bio. Rep. 2022; 49:1201–1211.

53. Ragab T I M, Zoheir K M A, Mohamed N A, El Gendy A G, Abd-ElGawad A M, Abdelhameed M F, et al. Cytoprotective potentialities of carvacrol and its nanoemulsion against cisplatin-induced nephrotoxicity in rats: development of nano-encasulation form. Heliyon. 2022;8.

54. Mortazavi A, Hosseini M, Beheshti F, Hakimi Z, Vaezi G H, Kargar H M P. The Effect of Carvacrol on IL-1β and Nitric Oxide Levels on

Lipopolysaccharide-induced Acute Renal Injury in Male Rats. J. Chem. Health R. 2023; 13.

55. Kannan K, Jain S K. Oxidative stress and apoptosis. Pathophysio. 2000; 7 (3): 153–163.

56. Obeng E. Apoptosis (programmed cell death) and its signals - A review. Braz. J. Biol. 2021;81(4) :1133-1143.

57. Priante G, Gianesello L, Ceol M, Prete D D , Anglani F. Cell Death in the Kidney. Int. J. Mol. Sci. 2019; 20: 3598.

58. Bozkurt M, Serda E, Oktayoglu P, Turkcu G, Yuksel H, Sariyildız, M, et al. Carvacrol prevents methotrexate induced renal oxidative injury and renal damage in rats. Clin. In. est. Med. 2014; 37(1).

59. Kokhdan E P, Sadeghi H, Kazemi S, Doustimotlagh A H. Nephroprotective Effects of Zataria multiflora Boiss Hydroalcoholic Extract, Carvacrol, and Thymol on Kidney Toxicity Induced by Cisplatin in Rats. Evid.Based Complement. Alternative Med. 2021.

60. Soltani R, Khorvash F, Meidani M, Badri S, Alaei1 S, Taheri S. Vitamin E in the prevention of vancomycin-induced nephrotoxicity, Res. Pharmaceut. Sci. 2020; 15(2): 137-143.