Research Article

Nephroprotective Effect of Vitamin E and *Origanum vulgare* Extracts against Vancomycin Induced Nephrotoxicity in Rats

Abdulkareem H. Abd[1], Ban Jumaa Qasim[2], Hayder B Sahib[3], Hanaa Raheem[1]
[1]Al-Nahrain University, College of Medicine, Pharmacology Department, Egypt.
[2]Al-Nahrain University, College of Medicine, Histology Department, Egypt.
[3]Al-Nahrain University, College of Pharmacy, Pharmacology Department, Egypt.
***Corresponding author's E-mail:** haider_bahaa@yahoo.com

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ABSTRACT

The study designed to investigate the nephroprotective effects of vitamin E and three different extracts of *Origanum vulgare* against vancomycin-induced nephrotoxicity in rats. The *Origanum vulgare* were grounded to fine powder, and extracted sequentially with chloroform, methanol and water. Forty eight male rats were divided randomly into six groups; and all groups were treated for 10 days. Group 1: control group received 5% dimethylsulphoxide (DMSO), Group 2: vancomycin group treated with (200 mg/kg/day, IP), Group 3:Vitamin E group received (200mg/kg) orally, vancomycin (200mg/kg/ IP) was administered at fourth day and both were continued till day 10, Group 3, 4 and 5 received Chloroform, methanol and water extract (CE) of *Origanum vulgare* respectively, (100mg/kg) orally, for 10 days then vancomycin (200mg/kg/ IP) was administered at fourth day and both were continued till day 10. Animals were sacrificed at day 11 and serum levels of creatinine, urea, albumin, total protein, Tumor Necrosis Factor-alpha (TNF-α) as well as tissue levels of malondialdehyde were measured. Free radical scavenging activity was measured by DPPH assay. Vitamin E and O*riganum vulgare* extracts produced significant reduction of serum levels of urea, creatinine, TNF-α and tissue MDA levels with significant elevation in the serum levels of albumin and total protein when compared to vancomycin group. Methanol extract showed significant effect on parameters above. Methanol extract had strong free radical scavenging activity with IC_{50} of (2.33µg/ml). *Origanum vulgare* extracts and vitamin E showed significant nephroprotective activity.

Keywords: Origanum vulgare, Nephrotoxicity, Vancomycin, Nephroprotective.

INTRODUCTION

Nephrotoxicity occurs when kidney-specific detoxification and excretion do not work properly due to the damage or destruction of kidney function by exogenous or endogenous toxicants. Nephrotoxicity is defined as a 0.5 mg/dL increase in serum creatinine, a 50% increase in baseline serum creatinine, or a 50% decrease in baseline creatinine clearance[1]. Vancomycin is a bactericidal tricyclic glycopeptides, it inhibits the cell wall synthesis of Gram-positive bacteria by the formation of stable complex murein pentapeptides, thus causing inhibition of further peptidoglycan formation[2]. Despite the recent availability of alternative agents, vancomycin still remains the drug of choice for serious MRSA infections including bacteremia, endocarditis, meningitis, pneumonia, cellulitis, and osteomyelitis, Oral vancomycin is not absorbed systemically and achieves high levels in the colon thus oral vancomycin formulation may be used (against *C. difficile* colitis)[3,4]. Although the exact mechanism of vancomycin-induced renal toxicity is not well defined, initial reports of vancomycin-induced nephrotoxicity were largely attributed to impurities in the original formulation. Following modern fermentation methods and purification, its purity has increased from 70 % to about 95 %, thereby reducing drastically the severity of its toxicities, particularly nephrotoxicity[3]. Other postulated mechanism that vancomycin causes the increased expression of the complement genes (C3 and C4b) and complement activation mediates tissue inflammation. Another postulated mechanism is that vancomycin results in increased expression of genes encoding lysosomal proteins such as cathepsins that degrade the worn out organelles such as mitochondria Animal data suggest that vancomycin has oxidative effects on cells of the proximal renal tubule and that the use of antioxidants can protect against vancomycin-induced kidney injury[2,5]. Vancomycin has been shown to be able to change the energy-dependent renal reabsorption function of the proximal tubule cells and alter mitochondrial function. Increased tubular cells oxygen consumption following short-term exposure to vancomycin has been reported, and proliferative responses observed following vancomycin treatment in renal proximal tubule epithelial cells. Based on the available animal data, renal tubular ischemia following oxidative effects of vancomycin is the main proposed mechanism of vancomycin-induced renal toxicity. Vancomycin-induced toxicity appears to be a consequence of the interaction of many factors that includes the concomitant nephrotoxic agents, high-dose regimen, high trough serum level, and duration of therapy, administration methods, and other risk factors of nephrotoxicity obesity, advanced age and critically ill patient or already have, a compromised renal function[3]. Vitamin E is a collective term that refers to all tocol and tocotrienol derivatives that exhibit the antioxidant activity of α-tocopherol. These antioxidants include four

tocopherols and four tocotrienols that share the chromanol ring structure but which differ by the number of methyl moieties present on the chromanol ring[6]. In addition to its activities as an antioxidant, vitamin E has many biological functions; the antioxidant function being the most important and best known. Vitamin E is involved in immune function, neurological functions, cell signaling, enzymatic activities, regulation of gene expression, and other metabolic processes[7]. α-tocopherol inhibits the activity of protein kinase C, an enzyme involved in cell proliferation and differentiation in smooth muscle cells, platelets, and monocytes[8]. Vitamin E is a naturally occurring, lipid-soluble, chain-breaking antioxidant that scavenges reactive oxygen species and lipid peroxyl radicals both *in vitro* and *in vivo*[9]. It is protects the integrity of membrane by inhibiting lipid peroxidation and augmenting the activity of antioxidant enzymes and its structure plays an essential role in the antioxidant defense system in the membrane[10]. As nephroprotective vitamin E was reported to be a protective agent against tissue damage after ischemia in isolated organs. Vitamin E treatment corrected the glomerular filtration deficiency and proteinuria by enhancing ATP synthesis during reperfusion status following ischemia Vitamin E maintains lysosomal membrane against lysosomal damage in renal injury may be one of the protective mechanisms[11]. By acting as indirect vasodilator, vitamin E preventing of nephrotoxicity through cytosolic up-regulation of phospholipid A2 and cyclooxygenase-1,which finally leads to the release of prostacyclin which is a potent vasodilator[12]. *Origanum vulgare* a medicinal and perennial plant, *Origanum* species have been used for thousands of years as spice[13]. Further, as a folk remedy, it is used against colic, cough, toothaches and irregular menstrual cycles[14-16].

MATERIALS AND METHODS

Plant Materials and Extraction

The dried leaves of *Origanum vulgare* were purchased from local market in Baghdad-Iraq and were identified by the National Iraqi Institute for Herbs. The dried powder of leaves (600 gm) was extracted using successive solvent extraction, the extraction process was performed using the following solvents in increasing order of polarity: chloroform(CE), methanol(ME) and distilled water(WE), respectively, by adding 100gm in each of the six closed flask with 400ml of chloroform ;with continuous shaking by using water bath for eight hours at 40°C, then filtration was done by using whatman filter paper (15 cm); the filtrate was kept for concentration with rotary evaporator while the residue was dried and extracted with methanol and water. Each extract was concentrated using a rotary-evaporator under vacuum and then dried. The lyophilized extract was then kept in desiccators at room temperature prior to the experiment[17-20].

Experimental Animals

All experimental protocols were approved by the ethics committee of the College of Medicine/AL Nahrain University. Forty eight albino male rats weighing (200-220) gm were used in this study. Before starting the study, the animals were left for 48 hours to acclimatize to the animal room conditions and were maintained on an environment of controlled temperature with a 12 hours light /dark cycle. All rats have free access to food and tap water. Rats were divided into 6 groups randomly, each group including 8 animals**. Group1** (Control): treated with 0.5 ml/kg body weight (BW) of dimethylsulfoxide (DMSO) orally once daily and continued till day 10. **Group 2** (Vancomycin) treated with daily dose of (200 mg/kg/IP)[21] for 7 days pretreated for 3 days with 0.5ml/kg BW/day DMSO orally and both were continued till day 10. **Group3** (Vitamin E+ vancomycin): treated with daily oral dose of vitamin E (200 mg/kg [22] dissolved in DMSO administered by gavage, for 10 days, vancomycin (200mg/kg/Ip) was administered at fourth day and both were continued till day 10. **Group 4** (CE of *Origanum vulgare*+ vancomycin): treated with daily oral dose of (100mg/kg)[23] dissolved in DMSO and administered by gavage, for 10 days, vancomycin (200mg/kg/IP) was administered at fourth day and both were continued till day 10. **Group 5** (ME of *Origanum vulgare*+ vancomycin): treated with daily oral dose of ME of (100 mg/kg)[(23)] dissolved in DMSO and administered by gavage, for 10days. Vancomycin (200mg/kg/IP) was administered at fourth day and both were continued till day 10. **Group 6** (WE of *Origanum vulgare*+ vancomycin): treated with daily oral dose of (100 mg/kg) dissolved in DMSO and administered by gavage, for 10 days. Vancomycin (200mg/kg/IP) was administered at fourth day and both were continued till day 10.

Sample Collection and Biochemical analysis

All animals were sacrificed at day 11. At the end of the experiment, the rats were subjected to blood collection under anesthesia by ether inhalation, the blood collected directly from the heart, centrifuged to get serum which used for the estimation of serum levels of creatinine, urea, albumin, total protein and tumor necrosis factor alpha levels in the tissue[24]. After scarification, the kidney tissue were excised, and was mobilized into the cooling box quickly to prevent autolysis and homogenization was done by rinsing the kidney piece with chilled phosphate buffer saline (PBS) (PH 7.4) at 4°C, blotted with filter paper and weighed. One gram of kidney tissue was homogenized in 10 ml of (PBS) utilizing tissue homogenizer for 1 minute at 4C, then after two freeze thaw cycles were performed to break the cell membranes, the homogenates were centrifuged for 5 minutes at 5000 xg at 2 - 8°C[25]. The supernatant was obtained and stored at -20°C for the assay of malondialdehyde.

DPPH Radical Scavenging Activity

The free radical scavenging activity of the *Origanum vulgare* ME were measured by 1, 1-diphenyl -2-picrylhydrazyl (DPPH) scavenging activity assay. One

milliliter of 0.1 mM solution of DPPH in methanol was added to 2 ml ME with the following concentrations (0.5, 0.25, 0.12, 0.062, 0.031, 0.015, and 0.007µg/ml); after 30min, absorbance was measured at 490 nm. All concentrations of ME were tested in triplicate. Percentage reduction of DPPH was calculated according to the formula below[26]

$$AA\% = 1 - \frac{\text{mean of sample absorbance} - \text{mean of blank}}{\text{mean of control} - \text{mean of blank}} x\ 100$$

Concentrations that inhibit 50% (IC_{50}) values describe the concentration of sample required to scavenge 50% of DPPH free radicals.

IC_{50} value was determined from the plotted graph of scavenging activity against the different concentrations, which is defined as the total antioxidant necessary to decrease the initial DPPH radical concentration by 50%.

The measurements were triplicates and their scavenging effect was calculated by percentage of DPPH scavenged[27].

RESULTS

Origanum vulgare crude extract yields

The percentage yield of the extract was determined gravimetrically using the dry weight of extracts and weight of powdered sample material, the extraction procedure yielded a highest percentage of 40 g (14%) of methanol extract and the lowest yield was for the chloroform extract was 12g (2%) while the water extract 31 g (5%).

Effect of treatments on serum levels of creatinine and urea

The serum levels of creatinine and urea were highly significantly elevated in vancomycin group (P < 0.001) in comparison with control group. In the groups treated with vitamin E, *Origanum vulgare* CE, ME and WE, the means of serum creatinine level and urea were highly significantly reduced in comparison with vancomycin group (P<0.001), In vitamin E, *Origanum vulgare* CE, ME and WE groups, means of serum creatinine level were reduced to near normal level, with non-significant difference compared to control group (p>0.05), however, *Origanum vulgare* ME group had the closest mean to the mean of control group as shown in the table1.

Effect of treatments on serum levels of albumin and total protein

The serum levels of albumin and total protein were highly significantly reduced in vancomycin group (P < 0.001) in comparison with control group. In the groups treated with ME and WE. The serum levels of albumin are highly significantly elevated in comparison with the vancomycin group (p<0.001), while in vitamin E and CE groups the means of serum albumin were significantly elevated in comparison with the vancomycin group (p<0.05), Although, the registered levels of serum albumin improved slightly in all treatment groups but failed to correct serum albumin level and stay highly significantly different from the control group (p<0.001) as shown in (table 2). In CE, ME and WE groups the means of total serum protein were highly significantly elevated in comparison with the vancomycin group (p<0.001)), and in vitamin E group, the mean of total serum protein was significantly elevated in comparison with the vancomycin group (p<0.05).

Treatment with CE, ME and WE, bring the levels of total serum protein near normal level with non-significant difference from the control group (p> 0.05), while total serum protein in vitamin E group was significantly lower than that in control group (p< 0.05), as shown in (table 2).

Effect of treatments on serum TNF-α and tissue MDA level

The mean of serum TNF-α level and mean of tissue MDA were highly significantly elevated in vancomycin group (p< 0.001) in comparison with control group. In the groups treated with vitamin E, CE, ME and WE, the means of serum TNF-α and tissue MDA level were showed significant reduction in comparison with the vancomycin group (p< 0.05).

In ME and WE groups, the means of serum TNF-α level reduced to near normal level, with non-significant difference compared to control group (p> 0.05), Methanol extract group caused the maximum reduction in serum TNF-α, which is even lower than that in control group. However, all treatment groups caused reduction of tissue MDA with non-significant difference from the control group (p> 0.05). The ME group had the closest mean to the mean of control group, as shown in the (table 3).

Table 1: Effect of Vancomycin and the combination of treatment groups with vancomycin on the serum creatinine and urea levels.

Groups (N=8)	Serum Creatinine (Mean ±SEM) (mg/ml)	Urea (Mean ±SEM) (mg/ml)
Control group	0.68±0.03	32.88±2.32
Vancomycin group	1.83±0.18^{a**}	123.88±17.94^{a**}
Vit. E group	0.92±0.02^{aNS,b**}	63.88±4.97^{a**,b**}
Origanum vulgare CE	0.79±0.04^{aNS,b**,cNS}	37.75±3.27^{aNS,b**,c**}
Origanum vulgare ME	0.69±0.05^{aNS,b**,c*,dNS}	33.38±3.64^{aNS,b**,c**,dNS}
Origanum vulgare WE	0.70±0.03^{aNS,b**,cNS,dNS,eNS}	57.75±3.54^{aNS,b**,c**,dNS,e*}

N=Number of animals; SEM = standard error of mean; CE= chloroform; ME= methanol; WE=water; a=Comparison with control group; b=Comparison with vancomycin group, c=Comparison with Vit. E group; d=Comparison with CE group; e=Comparison with ME group; NS= Not statistically significant (p>0.05); *= statistically significant (p<0.05); **= highly statistical significant (p<0.001). Effect of treatments on serum levels of albumin and total protein.

Table 2: Effect of vancomycin and the combination of treatments with vancomycin on the serum albumin and total protein levels.

Groups (N=8)	Total serum protein level (Mean ±SEM) (g/dl)	Serum albumin (Mean ±SEM) (g/dl)
Control group	7.08±0.11	3.74±0.09
Vancomycin group	6.43±0.15^{a**}	2.64±0.09 ^{a**}
Vit. E group	6.75±0.06 ^{a*,b*}	2.91±0.07 ^{a**,b*}
Origanum vulgare CE	6.90±0.12^{aNS,b**,cNS}	3.03±0.08^{a**,b*,cNS}
Origanum vulgare ME	7.10±0.08^{aNS,b**,c*,dNS}	3.09±0.04^{a**,b**,cNS,dNS}
Origanum vulgare WE	6.96±0.09^{aNS,b**,cNS,dNS,eNS}	3.03±0.04^{a**,b**,cNS,dNS,eNS}

N=Number of animals; SEM = standard error of mean; CE= chloroform; ME= methanol; WE=water; a=Comparison with control group; b=Comparison with vancomycin group, c=Comparison with Vit. E group; d=Comparison with CE group; e=Comparison with ME group; NS= Not statistically significant (p>0.05); *= statistically significant (p<0.05); **= highly statistical significant (p<0.001).

Table 3: Effect of vancomycin and the combination of treatments with vancomycin on the serum TNF-α and tissues MDA levels.

Groups N=8	Tissue MDAlevel (Mean ±SEM) (nmol/ml)	Serum TNF-α level (Mean ±SEM) (pg/ml)
Control group	1.48±0.24	72.42±5.06
Vancomycin group	2.51±0.17^{a**}	113.69±8.81^{a**}
Vit. E group	1.88±0.21^{aNS,b*}	98.33±2.97^{a*,b*}
chloroform extract group	1.78±0.24^{aNS,b*,cNS}	87.81±4.28^{a*,b*,c**}
methanol extract group	1.63±0.2^{aNS,b*,cNS,dNS}	67.86±2.03^{aNS,b*,c**,d*}
water extract group	1.72±0.22^{aNS,b*,cNS,dNS,eNS}	81.55±4.36^{aNS,b*,c**,d*,Ens}

N=Number of animals; SEM = standard error of mean; CE= chloroform; ME= methanol; WE=water; a=Comparison with control group; b=Comparison with vancomycin group, c=Comparison with Vit. E group; d=Comparison with CE group; e=Comparison with ME group; NS= Not statistically significant (p>0.05); *= statistically significant (p<0.05); **= highly statistical significant (p<0.001).

Table 4: The percentage of DPPH scavenging activity of the ME of *Origanum vulgare* and ascorbic acid

Concentrations (µg/ml)	Methanol	Ascorbic acid
500	94.9 ± 0.003	94.9 ± 0.005
250	94.66 ± 0.005	95.8 ± 0.003
125	94.9 ± 0.005	95.3 ± 0.004
62.5	93.8 ± 0.0005	93.97 ± 0.002
31.25	87.8 ± 0.0011	93.8 ± 0.003
15.525	63.7 ± 0.012	93.5 ± 0.002
7.81	52.6 ± 0.008	77.3 ± 0.025

DPPH scavenging activity Assay

The dose response curve of methanol extract of *Origanum vulgare* was measured using 2,2-diphenyl-2-picrylhydrazyl (DPPH) scavenging activity test, results are expressed as mean ±SD. Result shows the percentage of DPPH scavenging activity of ascorbic acid (positive control) and the ME extract of *Origanum vulgare* was dose related as shown in Table 4. Serial concentrations of 0.5, 0.25, 0.125, 0.0625, 0.03125, 0.0156 and 0.0078 µg/ml were used. IC50 of DPPH scavenging activities of the tested agents were calculated by the linear regression equations; Figure (1) show the dose response curves for the extracts and the positive control. The IC50 of DPPH scavenging activity was calculated by the liner regression equation of both tested agent by considering Y to be 50%.

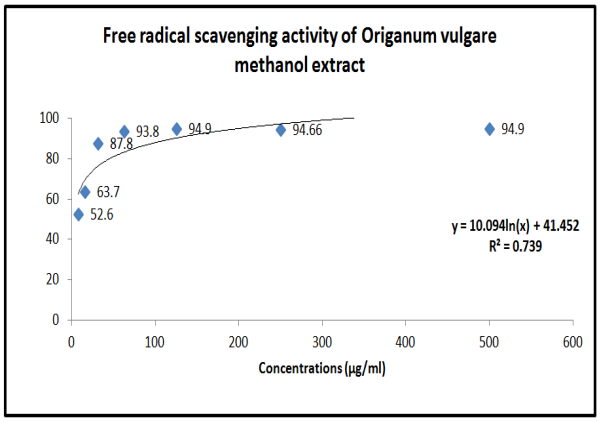

Figure 1: The dose response curve of serial dilutions of *Origanum vulgare* methanol leaves extract on DPPH free radical scavenging activity.

The IC50 of DPPH for Ascorbic acid was (0.00006µg/ml). IC50 of DPPH for methanol extract was 2.33µg/ml. The free radical scavenging activity results showed that the methanol extract had very strong activity in compared to ascorbic acid.

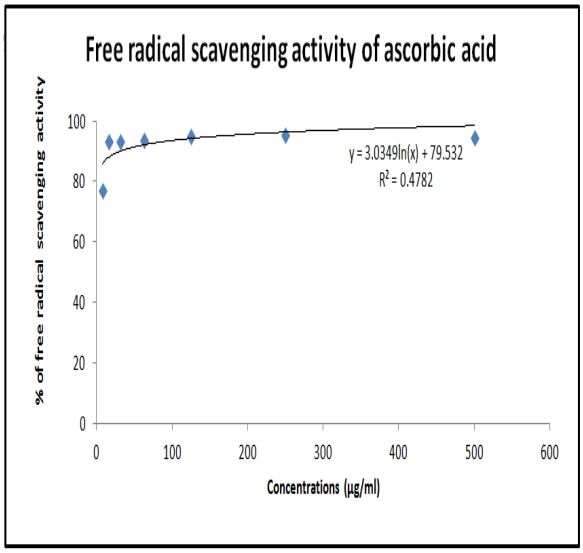

Figure 2: The dose response curve of serial dilutions of ascorbic acid on DPPH free radical scavenging activity.

DISCUSSION

Origanum vulgare crude extracts yield

In this study, the extraction process was done sequentially to ensure that the majority of the constituents had been extracted and isolated in accordance to their polarity to be ready for use in the experiments to test their pharmacological activity either they have nephroprotective effect or no. Extraction method used in this study was cold method or maceration method. The cold extraction method was used in order to avoid any loss or destruction to the compounds inside the leaves from exposure to high temperature[20].

Vancomycin-induced nephrotoxicity

Vancomycin-induced renal toxicity was reported in 10–20 % and 30–40 % of patients following conventional and high doses of vancomycin therapy, respectively. It has been reported that suppression of free radical scavenging function and the enhancement of reactive oxygen species (ROS), contribute to vancomycin induced oxidative stress and its associated toxic effects, in addition to the role of inflammatory mediators[3].

In this study, the levels of serum creatinine and urea were highly significantly elevated in vancomycin treated rats in comparison with the control group (tables1). Choi reported that oxidative stress can promote the formation of a variety of vasoactive mediators that can affect renal functions directly by causing renal vasoconstriction or decreasing the glomerular capillary ultra filtration coefficient and thus reduce glomerular filtration rate (GFR)[(28)], so this will lead to reduce creatinine and urea clearance that is associated with elevation of serum creatinine and urea this results are compatible with a study done by[21]. In the this study, vancomycin had induced highly significant reduction of serum albumin and total serum protein levels in comparison with the control group (tables 2). In normal conditions, the glomerulus restricts the migration of high molecular weight proteins from blood to nephron lumen by filtration. In some pathological states, however, high molecular weight proteins can be detected in the urine because the selective penetration through glomerulus is not functioning properly. High molecular weight proteins that can reveal kidney damage include albumin which can be used for early diagnosis of changed glomerular filtration. The reduction in serum albumin and total serum protein levels can be due to glomerular changes, which lead to loss of protein in urine with consequent reduction of serum protein[29]. In the present study, vancomycin had induced nephrotoxicity by causing highly significant elevation of tissue MDA level in comparison with the control group (table 3). It is well known that oxidative stress plays an important role in the tissue damage due to vancomycin[3]. Vancomycin induced oxidative stress was demonstrated by decreased activity of antioxidant enzyme levels such as superoxide dismutase(SOD), glutathione (GSH), catalase due to the direct binding of vancomycin to enzyme active sites, if it contains (SH) groups or by displacement of metal cofactors from active sites[30]. Impaired functioning of antioxidant enzymes by vancomycin would result in unopposed production of free radicals, this ROS results in destructive peroxidation of cell membrane lipids, leads to cell membrane damage and yields a wide variety of lipid peroxidation end products, including MDA, which is accepted as an indicator of lipid peroxidation[5]. In the present study, vancomycin had induced nephrotoxicity by causing highly significant elevation of serum TNF-α level in comparison with the control group (table 3). The elevated TNF-α levels are related to the fact that the free-radicals seem to trigger the accumulation of leukocytes in the tissues involved, and thus exacerbate tissue injury indirectly through the activation of neutrophils. It has been supposed that activated neutrophils secrete enzymes such as (TNF-α, myloperoxidase (MPO), elastase and proteases) and liberate oxygen radicals. Also free radicals have direct damaging effects on these tissues[31].

Effects of vitamin E against vancomycin induced nephrotoxicity

In the present research, vitamin E had highly significant reduced levels of serum creatinine and urea as compared to vancomycin group, but the levels were significantly higher than control group (tables1). Vitamin E prevents nephrotoxicity by acting as indirect vasodilator through cytosolic up-regulation of phospholipid A2 and cyclooxygenase-1, which eventually leads to the release of prostacyclin which is a potent vasodilator, which lead to improvement the GFR and correct the serum levels of creatinine and urea[12]. Vitamin E had significantly

enhanced levels of serum albumin and total serum protein as compared to vancomycin group (tables 2). Vitamin E enhances the serum levels of albumin and total protein. This result is in agreement with other findings[32,33]. In the present study, vitamin E had highly significant reduced level of tissues MDA as compared to vancomycin group (table 3) and normalized its level. The antioxidant property of vitamin E at renal tubules is probably mediated by enhances of antioxidant enzyme superoxide dismutase and glutathione peroxidase activity or even increases in catalase contents of kidney tissue[34]. Treatment with vitamin E has been proved to suppress lipid peroxidation pathway as effective as preventing the rise of MDA level[32]. Halliwell and Gutteridge (2002) suggested that treatment with vitamin E averted oxidative damage, probably through its capacity to quickly and efficiently scavenge lipid peroxide radicals before they attack the membrane lipids. This ability might be related to the fact that lipid peroxyl radicals react more rapidly (by four orders of magnitude) with vitamin E, than with membrane lipids[35].

In this work, vitamin E significantly decreased serum TNF-α level in comparison with vancomycin group (table 3), due to its direct antioxidant effect, in addition it may offer indirect protection by decreasing neutrophil recruitment[36]; and this result agrees with other studies[22,34].

Moreover, Azzi reported that vitamin E inhibits protein kinase C in various cell types, with the consequent inhibition of platelet aggregation, nitric oxide production in endothelial cells, and superoxide radical generation by neutrophils and macrophages[7].

Nephroprotective of *origanum vulgare* extracts

Origanum vulgare extracts in this study significantly lowered the elevated levels of creatinine and urea in serum indicating its nephroprotective effect (table 1). ME group show closest mean to the mean of control group in comparison with CE and WE. This nephroprotective effect verified the membrane stabilizing activity of *Origanum vulgare* extracts in addition to its antioxidant, renal epithelial cell protective and promoting increased the urine output, all these activities reported by[19]. Also, *Origanum vulgare* extracts prevented the decrease in the serum albumin levels and total serum protein (table 2), but ME group show closest mean to the mean of control group in comparison with CE and WE. This moreover signifies the curative nature of *Origanum vulgare* extract against vancomycin toxicity by restoring the functioning ability of the kidney by preventing proteins and albumin loss by the kidney through its nephroprotective effects as reported by[19]. Moreover, the current study reported that the administration of *Origanum vulgare* extracts with vancomycin exhibited a marked lowered the levels of the serum TNF-α and renal tissue MDA significantly as compared to vancomycin group. ME group show closest mean of MDA to the mean of control group and caused marked reduction of serum TNF-α level to even less than normal level in comparison with CE and WE. As proposed by Srihari it is feasible for the *Origanum vulgare* extract to mediate its antioxidant activities by enhancing the antioxidant defense enzymes SOD, CAT and GSH storage. Furthermore, *Origanum vulgare* extract which show anti-oxidant activity has an inhibitory effect on lipid peroxidation, which could decrease the strength of inflammatory response[37]. In this study, TNF-α level was reduced significantly in animals receiving Origa*num vulgare* extracts with vancomycin the effect which probably related to the carvacrol (CVL) that has anti-inflammatory effect[38]. Andreas reported CVL and thymol as dominant components of *Origanum vulgare* essential oil[39]. Furthermore the essential oils of *Origanum vulgare*, which mainly contain the antioxidant terpene compounds, thymol and carvacrol, could be demonstrated to impair the mRNA and the protein concentration of the pro-inflammatory cytokines interleukin 1 beta (IL-1) and interleukin6 (IL-6) in mice with 2,4,6-trinitrobenzol (TNBS-) induced colitis[40]. For instance, the essential oil of *Origanum vulgare*, containing high concentrations of the phenolic terpenes, carvacrol and thymol, has been shown to efficiently reduce the mRNA levels of the proinflammatory cytokines TNFα, IL-1, and IL-6 in human macrophages[41].

Free radical scavenging activity of *Origanum vulgare* ME (DPPH Assay)

DPPH assay is an easy, rapid and sensitive method for the antioxidant screening of plant extracts, DPPH is a stable free radical at room temperature. It is scavenged by antioxidants through the donation of a proton, forming the reduced DPPH[2]. Free radicals are atoms or molecules with an unpaired electron, and there are considerable evidence that free radicals induce oxidative damage to bio-molecules and play an important role in cardiovascular diseases, aging, cancer, inflammatory disease and a variety of other disorders[20]. Free radical scavenging activity for the methanol extract was important to be tested to understand the mechanism of action and the significant nephroprotective effect. The presence of phenols and terpenes, which show their very potent antioxidant properties[40], in addition to other non-phenolic components with potent antioxidant activity in *Origanum vulgare* extract may elucidate the nephroprotective mechanisms, as all these groups may show their pharmacological effect through antioxidant properties. This study has found that methanol extract of *Origanum vulgare* leaf extract gave the potent anti-oxidant activity in comparisons to positive control and it showed the highest nephroprotective activity, and this could be contributed to its strong antioxidant behavior, as shown in DPPH scavenging assay. This may result in a decrease in the free radicals present, and this due to the presence of significantly high phenolic and non-phenolic content with potent antioxidant activity in *Origanum vulgare* ME, which may possibly play an important role in contributing to its nephroprotective effect.

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