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The Effect of *Olive Leaf* Extract and α-Tocopherol on Nephroprotective Activity in Rats

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Abstract

The present study aimed to evaluate the nephroprotective effects of different doses of aqueous extract of olive leaf (OLE), vitamin E and their combination on gentamicin induced nephrotoxicity in albino rats. 54 adult healthy male rats were divided into nine groups comprising 6 animals each. Group 1 served as normal control group. All other groups (group 2 to group 9) were administered gentamicin sulphate (GS) intraperitoneal at dose 70 mg/kg body weight for 4 week daily. Group 3, 4, 5 and 6 received olive leaf extracts at doses 20, 40, 80 and 160 mg/Kg/oral respectively. Group 7 received vitamin E 250 mg/Kg, whereas group 8 and 9 received vitamin E and olive leaf extracts (20 mg/Kg/oral and 40 mg/Kg/oral respectively). All the treatments were continued for 30 days.

The results of the study indicated that olive leaf significantly protected the nephrotoxicity induced by Gentamisin sulphate. Serum creatinine, urea, malonaldhyde levels significantly increased as a result of nephrotoxicity in group 2. The renal antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) activities were decreased; level of malondialdehyde (MDA) was elevated in group 2. Remarkably treatment with olive leaf extracts and vitamin E increased levels of antioxidant enzymes and decrease levels of MDA, serum creatinine and urea in all treated groups. Sodium levels were significantly reduced and elevated potassium levels in G2.

According to the biochemical findings, were supported by histopathological evidences, administration of olive leaf extracts with vit. E more was effective protection than their individual dose. Administration of olive leaf extract (20 mg/kg, 40 mg/kg) plus vitamin E (250 mg/kg) indicated better protection against GS-induced nephrotoxicity. This study concluded that olive leaf acts in the kidney as a potent scavenger of free radicals to prevent the toxic effects of GS both in the biochemical and histopathological parameters. Germinated barley extract with OLE (20 mg, 40 mg) + vitamin E was showed good antioxidant properties.

Keywords: Olive leaf; Gentamicin; Rat; Nephrotoxicity; Vitamin E; Antioxidant

Introduction

Kidneys are the organ that has numerous biological roles. Their primary role is to maintain the homeostatic balance of body fluids by filtering and secreting metabolites (such as urea) and minerals from the blood and excreting the nitrogenous wastes along with water, as urine [1]. Nephrotoxicity is a poisonous effect of some substances, both toxic chemicals and medication (nephrotoxins are chemicals displaying nephrotoxicity) on the kidneys [2]. Nephrotoxicity is an inherent adverse effect of certain antibiotics, anticancer drugs and other synthetic molecules.

Gentamicin sulphate (GS) is considered as one of the aminoglycoside antibiotics used to treat infection in human and animals especially against Gram-negative bacteria (*Pseudomonas, proteus* and Serratia) [3,4]. However, a major complication of therapeutic doses of GS is nephrotoxicity. GS is known as one of the most common causes of acute renal failure, which occurs in about 10% - 30% of patients receiving the drug [5]. Gentamicin induced nephrotoxicity is characterized by an increase in plasma creatinine and urea levels and severe proximal renal tubular necrosis, followed by deterioration and renal failure [6,7].

GS increases the generation of reactive oxygen species (ROS) such as superoxide anions [8], hydroxyl radicals, hydrogen peroxide and reactive nitrogen species in the kidney. ROS also activate nuclear factor kappa β that plays a key role in the induction of inflammatory process [9]. Abnormal production of ROS may damage some macromolecules to induce cellular injury and necrosis via several mechanisms including peroxidation of membrane lipids, protein denaturation and DNA damage [10,11]. GS reduces the activity of renal antioxidant enzymes like catalase (CAT), glutathione peroxidase (GPx) and the glutathione content [8,12]. Gentamicin also acts as an iron chelator and the iron-gentamicin complex is a potent catalyst of radical generation.

Several natural antioxidants such as vitamin E, vitamin C and phenolic compounds have been used to protect the liver injury and nephrotoxicity induced by drugs [13,14]. Herbs and spices are generally considered safe and proved to be effective against various human ailments and their medicinal uses have been gradually increasing in developed countries. Olive tree (*Olea europaea* L.) leaves have been widely used in traditional remedies in European and Mediterranean countries.

They have been used in the human diet as extracts, herbal teas, and powder and contain several potentially bioactive compounds that may have antioxidant, antihypertensive, antiatherogenic, anti-inflammatory, hypoglycemic, and hypocholesterolemic properties [15]. However, the olive leaves also contain phenolic compounds; the oleuropein, hydroxytyrosol, verbascoside, apigenin- 7-glucoside and luteolin-7-glucoside are the most abundant already identified in olive leaf extracts [16]. Oleuropein, a phenolic secoiridoide, is used as a well-known compound of extracts and its concentration is significantly high in leaves and fruit. Oleuropein has a high antioxidant activity *in vitro*, scavenges superoxide anions, hydroxyl radicals, and hypochlorous acid-derived radicals [17].

The current study was designed to evaluate the protective effect of olive leaf extract (OLE), which has antioxidant activity, alone and in combination with vitamin E against GS-induced nephrotoxicity in rats.

Materials and Methods

Chemicals

Gentamicin sulphate was purchased from Sigma-Aldrich Chemicals (St. Louis, MO, USA). Vitamin E was obtained from sigma chemical Co. DPPH (2,2-diphenyl-1-picrylhydrazyl), and other reagents were supplied from Sigma (St. Louis, MO, USA) or Merck (Darmstadt, Germany). The used reagents were analytical grades. All biochemical assay kits were purchased from Randox Laboratories Ltd, Diamond Road Crumlin, Co, Antrim, United Kingdom, BT294QY.

Preparation of olive leaf extracts (OLE)

The olive leaves (*Olea europaea*) were collected from the region of Ismailia, Egypt, in October 2013. 200 g of olive leave were washed and air-dried. Leaves were grinded into fine powder. The powder was extracted with 1 L of 80% ethanol for 24 h. The collective ethanol extract was filtered, and concentrated to dryness under low pressure in a rotary evaporator, after that the product was freeze-dried [18].

Animals

In this experimental study, 54 healthy adult male albino rats weighting between 128 g and 143 g were used. The rats were kept under normal health laboratory conditions and fed on basal diet for one week as adaptation period.

Water and basal diet [19] were provided ad libitum during the experimental period (30 days). All experimental procedures were conducted in accordance with the guide to the care and use of laboratory animals, and carried out in Ophthalmology Research Institute, Giza, Egypt. Body weight changes were recorded at regular intervals throughout the experimental period.

Determination of nephroprotection of olive leaves

After acclimatization, rats were randomly divided into 9 groups (n = 6) as follows: group 1 as negative control intraperitoneal (IP) saline injection, 0.5 mL/d, group 2-9 received daily GS (70 mg/kg, IP), for thirty consecutive days, which is well known to cause significant nephrotoxicity in rats [6,20]. Group 2 as positive control, Group 3 to 6 were treated with OLE 20, 40, 80, 160 mg/Kg b.w/d, plus GS.

Group 7 was treated with vitamin E (α-tocopherol) (250 mg/kg) plus GS. Group 8 and 9 was treated with 20 mg/kg and 40 mg/kg extract of olive leaves and vitamin E plus GS. The extract of olive leaves and vitamin E were administrated orally by gavage every day, 1 h before gentamicin injection.

Sample collection and biochemical assays

After 30 days treatment, all animals were fasted for 12 h, and then blood samples were collected from the animal's eye plexus under diethyl ether anesthesia. Samples were allowed to clot for 20 min at room temperature and then centrifuged at 3000 rpm for 10 min for serum separation for determination creatinine, urea, MDA, Na, and K. After the collections of blood samples, animals were sacrificed and the kidney of each animal was dissected. Kidneys were washed and weighed thoroughly with ice-cold normal saline (10%, w/v) and homogenates were prepared in phosphate buffer saline (50 mmol/l, pH 7).

Homogenates were centrifuged at 10000 rpm (4°C) for 10 min, to determine superoxide dismutase (SOD), catalase (CAT), malondialdehyde (MDA), and glutathione peroxidase (GPx) activities. SOD was determined as the volume of homogenate that is required to scavenge 50% of the superoxide anion generated from the photo illumination of riboflavin in the presence of EDTA (1 unit of SOD activity) [21]. GPx activity was determined by measuring the decrease in GSH content after incubating the sample in the presence of H_2O_2 and sodium azide (NaN_3) [22].

CAT activity was determined by measuring the decomposition of hydrogen peroxide at 240 nm, according to the method of Aebi [23]. Activities of enzymes were expressed as U/mg protein. The concentrations of MDA as proceeding of lipid peroxidation were determined according to a modified method of Ohkawa *et al.,* [24] based on the reaction with thiobarbituric acid, and were expressed as nmol g protein^{-1} .Protein concentrations were measured according to Lowry *et al.,* [25]. Lipid peroxidation in serum was estimated by the method of Ledwozyw *et al.,* [26]. Creatinine, and urea were determined by using the methods described by Larsen [27] and Orsonneau *et al.,* [28], sodium and potassium in serum was measured by the method of Mazzachi *et al.,* [29].

Histopathological examinations

Another kidney was excised after sacrifice. The tissues were fixed in 10% formalin, embedded in paraffin, sectioned at 4 μ thick and stained with hematoxylene-Eosin [30]. Histopathological examination was done by the histopathological laboratory of Veterinary Medicine Faculty, Cairo University.

Preparation of germinated barley

Barley seeds were washed and soaked in water for 6 h at room temperature. Soaked seeds were evenly spread on wet cotton and covered by it in aluminium baskets, which was kept moist for 48 h. The germinated seeds were dried at 55°C for 6 h [31].

Preparation drinks from germinated barley and olive leaf extract

1 - 12 g (one spoon) of germinated seeds were mixed with 100 mL of water and boiled for 10 min at 100 °C. Add powder (20 mg OLE + Vit E 250 mg or 40 mg OLE + Vit E 250 mg, respectively).

2 - 12 g of germinated seeds were mixed with 100 mL of water and add powder (20 mg OLE + Vit E 250 mg or 40 mg OLE + Vit E 250 mg, respectively), the mixture was boiled for 10 min at 100°C. All the mixture was determinate antioxidant activity by DPPH radical scavenging.

Antioxidant activity by DPPH radical scavenging

The antioxidant activities of the plant extracts were measured by scavenging of stable DPPH radical according to the method of Hatano *et al.,* [32]. Briefly 0.25 mM solution of DPPH radical (0.5 ml) was added to the sample solution in ethanol (1 ml) at different concentrations (25 µg/ml – 200 µg/ml) of aqueous extracts. The mixture was shaken vigorously and left to stand for 30 minutes in the dark, and the absorbance was measured at 517 nm.

The capacity to scavenge the DPPH radical was calculated using the following equation: (%) scavenging = [(Ao - A1) / Ao)] × 100, Where, Ao is the absorbance of the control reaction and A1 is the absorbance of the sample itself. All determinations were carried out in triplicate.

Statistical analysis

The results were represented as mean ± SE with 3 replicates. These results were interpreted following one way ANOVA using SPSS version 16.0. Results were considered statistically significant at $p < 0.05$ [33].

Results

The results are summarized in Tables 1-3. Acute nephrotoxicity occurred in G2 due to GS injections during 30 days and according to this, values of Table 1 showed that serum creatinine and urea levels were significantly high ($p < 0.05$) when compared with G1. Also, there were significant decreases ($p < 0.05$) in serum creatinine and urea levels in group treatment of olive leaf extracts, Vit. E and olive leaves extract with Vit. E when compared with the GS group (G2). Serum urea was low in the group treated with Gentamicin + 20 mg olive leaf extract + Vit. E (G8) value was 42 mg/dl.

However, differences in serum creatinine levels were not statistically significant ($p < 0.05$) in group treated with Gentamicin + 80 mg olive leaf extract (G5) and Gentamicin + 40 mg olive leaf extract + Vit. E (G9) when compared with the control group (G1). GS treated rats (G2) significantly low in sodium level and high in potassium level when compared with control group (G1). Administration of 80 mg olive leaf extract (G5) and 40 mg olive leaf extract with vitamin E (G9) restored the normal level of sodium and potassium in gentamicin treated rats (Table 1).

Serum MDA levels increased significantly ($p < 0.05$) in G2 when compared with the control group (G1). Administration of 80 mg olive leaf extract (G5) or 40 mg olive leaf extract + vitamin E (G9) significantly decreases of the serum MDA levels were observed. The data in Table 2 indicates the effect of treatments on kidney levels of MDA and activities of antioxidants enzyme activities (CAT, GPx, and SOD). GS administration (G2) had significantly higher levels of MDA ($p < 0.05$) in kidney tissue, while having significantly lower values of CAT, GPx, and SOD activities, when compared with the control group (G1).

Groups	Urea (mg/dl)	Creatinine (mg/dl)	Na (mmol/l)	K (mmol/l)	Serum MDA (nmol/ml)
Control Negative (G1)	44.333 ± 2.603^{f}	0.55 ± 0.029^{d}	164.67 ± 0.882^{bc}	4.433 ± 0.186^{de}	2.867 ± 0.088^{e}
Control Positive (G2)	154 ± 2.082^{a}	1.8333 ± 0.033^{a}	148.33 ± 3.756^{a}	5.267 ± 0.145^{a}	5.333 ± 0.088^{a}
Gentamicin + OLE 20 mg/Kg (G3)	94 ± 2.646^{bc}	1.2333 ± 0.120^{b}	145.67 ± 2.333^{a}	5 ± 0.058^{ab}	3.567 ± 0.176^{cd}
Gentamicin + OLE 40 mg/Kg (G4)	73 ± 1.732^{e}	0.9333 ± 0.088^{c}	168 ± 1.528^{c}	4.9 ± 0.058^{abc}	3.3 ± 0.116^{d}
Gentamicin + OLE 80 mg/Kg (G5)	74.333 ± 2.333^{de}	0.7667 ± 0.088^{cd}	164.67 ± 2.906^{bc}	4.667 ± 0.120^{bcd}	2.9 ± 0.058^{e}

Table 1: Effect of olive leaf extracts (OLE), vitamin E (Vit E), and their combination on serum urea, creatinine, Na, K, and MDA levels in gentamicin induced nephrotoxic rats. [Values represented as Mean ± SE, The mean difference is significant ($P < 0.05$) when compared with the positive control group (gentamicin-induced nephrotoxicity)].

Groups	Catalse (CAT) (U/g protein)	Glutathione Peroxidase (GPx) (U/g protein)	Superoxide Peroxidase (SOD) (U/g protein)	Tissues MDA (nmol/g protein)
Control Negative (G1)	66.978 ± 3.296g	91.244 ± 3.62e	910.06 ± 18.66de	77.081 ± 2.412g
Control Positive (G2)	10.325 ± 0.934a	48.174 ± 1.787a	503.69 ± 10.92a	127.20 ± 1.714a
Gentamicin + OLE 20 mg/Kg (G3)	31.615 ± 1.914b	63.901 ± 2.105b	753.12 ± 14.41b	107.25 ± 4.676b
Gentamicin + OLE 40 mg/Kg (G4)	43.725 ± 1.439cd	72.898 ± 1.355d	843.93 ± 33.84c	94.395 ± 2.093cd

Gentamicin + OLE 80 mg/Kg (G5)	46.132 ± 2.439^{de}	71.153 ± 2.136cd	840.15 ± 11.91c	85.948 ± 1.76ef
Gentamicin + OLE 160 mg/Kg (G6)	38.599 ± 1.358c	65.125 ± 2.57bc	886.84 ± 8.62cd	99.622 ± 0.694c
Gentamicin + Vit. E (G7)	41.483 ± 1.524cd	73.541 ± 1.51d	853.24 ± 5.56c	95.513 ± 2.69c
Gentamicin + OLE 20 mg/Kg + Vit. E (G8)	50.147 ± 1.275e	88.036 ± 1.109e	917.17 ± 4.58de	87.811 ± 2.114de
Gentamicin + OLE 40 mg/Kg + Vit. E (G9)	59.326 ± 1.87f	87.804 ± 1.962e	955.46 ± 8.26e	80.048 ± 1.45fg

Table 2: Effect of olive leaf extracts, vitamin E, and their combination on renal CAT, GPx, SOD activities and MDA in gentamicin induced nephrotoxic rats. Values represented as Mean ± SE, The mean difference is significant ($P < 0.05$) when compared with the positive control group (gentamicin-induced nephrotoxicity).

All extract, vitamin E, and extract with vitamin E administration resulted in a significant decrease in MDA and an increase in CAT, GPx, and SOD activities when compared with GS administration alone. In group 9, treatment with Gentamicin + 40 mg olive leaf extract + vitamin E daily, could inhibit the decrease in kidney MDA compared to GS treated group (G2), but could not restore its level to the level of the control group (G1).

Olive leaf extract in G9 cause a significantly increase renal catalase activity compared with G2. There were no significant differences in GPx and SOD activities between the control group, G8 and G9.

The changes of body weights in the experimental animals are presented in Table 3. GS decreased the percentage of changes in body weights compared to the control group ($P < 0.05$). Treatment with extracts or vitamin E or their mixture did not make significant difference in % body weight when compared to G1. In group 8 and 9 could inhibit the loss of % body weight in comparison with G2 but could not restore it to that of the control group (G1).

Groups	Initi (g)	Fin. (g)	Body weight change (%)
Control Negative (G1)	141 ± 1.528^{a}	147.67 ± 2.33^{b}	4.717 ± 0.587^{b}
Control Positive (G2)	138.67 ± 2.33^{a}	132.67 ± 2.67^{a}	-4.332 ± 0.735^{a}
Gentamicin + OLE 20 mg/Kg (G3)	139 ± 2.082^{a}	134 ± 2.08^{a}	-3.599 ± 0.054^{a}
Gentamicin + OLE 40 mg/Kg (G4)	140 ± 1.155^{a}	136.67 ± 1.76^{a}	-2.388 ± 0.4899^{a}
Gentamicin + OLE 80 mg/Kg (G5)	139 ± 1.155^{a}	134.33 ± 1.45^{a}	-3.361 ± 0.263^{a}
Gentamicin + OLE 160 mg/Kg (G6)	139 ± 1^{a}	134 ± 1.53^{a}	-3.598 ± 0.825^{a}
Gentamicin + Vit. E (G7)	137 ± 1.73^{a}	133.67 ± 0.88^{a}	-2.418 ± 0.611^{a}
Gentamicin OLE 20 mg/Kg + Vit. E (G8)	135.67 ± 1.453^{a}	133.33 ± 0.33^{a}	-1.698 ± 1.052^{a}
Gentamicin + OLE 40 mg/Kg + Vit. E (G9)	137.67 ± 1.202^{a}	135.67 ± 2.33^{a}	-1.427 ± 2.184^{a}

Table 3: Effect of olive leaf extracts, vitamin E, and their combination on animal body weight and % body weight changes in GS-induced nephrotoxic rats. Values represented as Mean ± SE, The mean difference is significant ($P < 0.05$) when compared with the positive control group (gentamicin-induced nephrotoxicity).

The histological changes in kidney sections from various treatment groups are shown in Figures 1 to 9. Histological examination of the kidneys from rats in the control group revealed, as expected, entirely normal histological features, illustrated in Figure 1. However, there was marked a hypertrophy of glomerular tufts and vacuolations of epithelial liming renal tubules in kidney tissues from rats in the GS treated group (Figure 2).

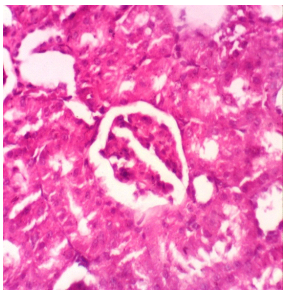

Figure 1: kidney of rat from G1 showing no histological changes.

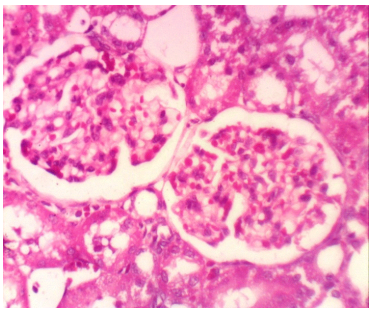

Figure 2: Kidney of rat from G2 showing hypertrophy of glomerular tufts and vacuolations of epithelial liming renal tubules.

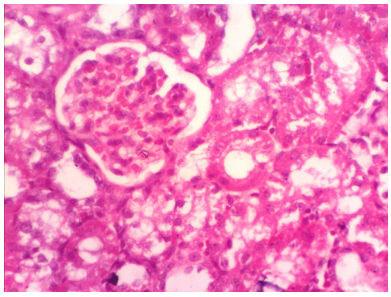

Figure 3: Kidney of rat from G3 showing vacuolation of epithelial liming renal tubules.

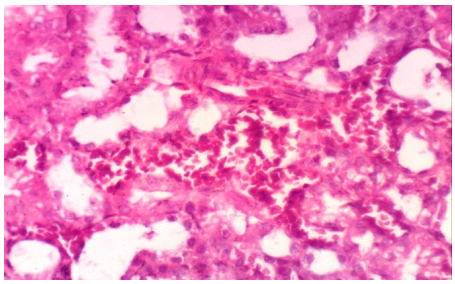

Figure 4: Kidney of rat from G4 showing congestion of intertubular blood vessels.

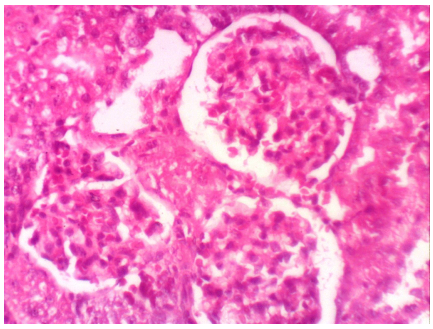

Figure 5: Kidney of rat from G5 showing no histopathologicl changes.

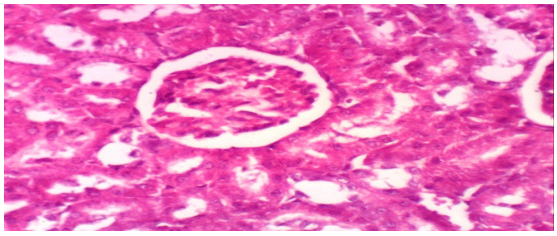

Figure 6: Kidney of rat from G6 showing no histopathological changes.

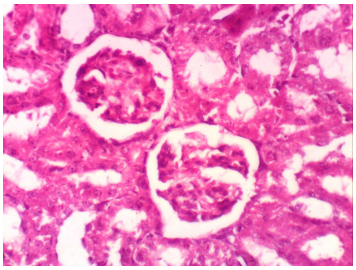

Figure 7: Kidney of rat from G7 showing no histopathological changes.

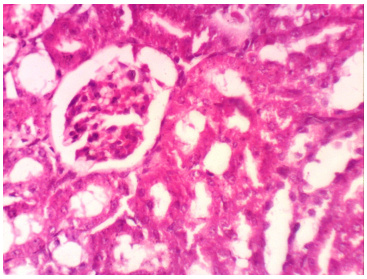

Figure 8: Kidney of rat from G8 showing no histopathological changes.

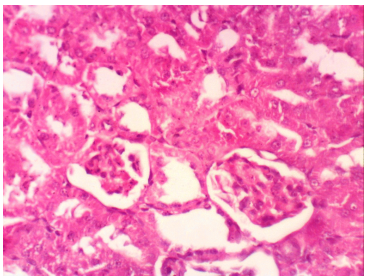

Figure 9: Kidney of rat from G9 showing no histopathological changes.

In the GS + 20 mg olive leaf extract (G3) treated group, tubular changes were observed. In this group, the affected renal tubules showed vacuolation of epithelial liming, seen in Figure 3. There were also some changes in the kidney tissues of rats treated with GS + 40 mg olive leaf extract (G4) such as congestion of intertubular blood vessels (Figure 4). There were no differences in kidney tissues between control group and other groups (G5 to G9).

The free radical ability of extracts to scavenge DPPH radical was measured. DPPH radical has been widely used in assessment of radical scavenging activity because of its ease and convenience. All the samples showed their abilities to scavenge the DPPH radical, the results in Figure 10 showed the effect of adding 20 mg or 40 mg OLE to boiling germinated barley before and after boiling on DPPH scavenging activity.

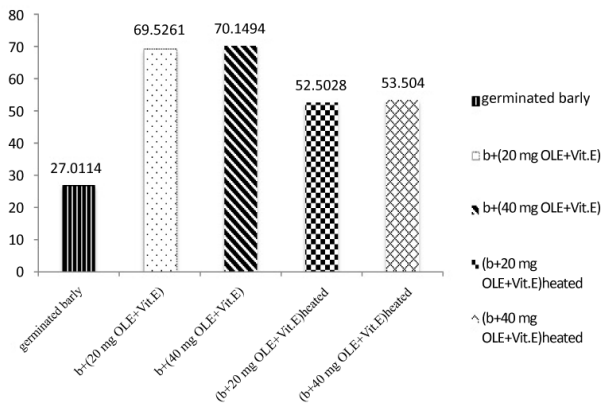

Figure 10: DPPH scavenging activity (% inhibation).

Discussion

The purpose of the current study was to assess the antioxidant effect of oral administration of olive leaf aqueous extract alone, vit. E, and in combination together on lipid peroxidation and tissue antioxidant enzymes kidney of gentamicin induced nephrotoxic rats. Our results indicated that gentamicin induced rat's demonstrated lower activities of antioxidant enzyme. An appreciable increase in the levels of lipid peroxidation, serum creatinine and urea was noted in the group treated with GS (G2). Remarkably, following treatment with olive leaf extracts and vitamin E, we found increase levels of antioxidant enzymes, and decrease levels of MDA, serum creatinine and urea in all treated groups. The yield of 200 g olive leaf was 2.3 g of OLE.

Aminoglycoside antibiotics are well recognized to cause nephrotoxicity, therefore their clinical uses are limited [6,7]. The onset of damaging renal function induced by aminoglycosides for example GS occurs after 5-7 days treatments injection intraperitoneally between of 80 mg/kg and 150 mg/kg. In this study, GS was injected intraperitoneally at the dose of 80 mg/kg, for four successive weeks, which is well known to cause significant nephrotoxicity in rats [20]. Many researchers have reported that oxygen-free radicals are considered to be important mediators of GS-induced acute renal failure [34].

GS enhance the generation of reactive oxygen species (ROS), abnormal production of ROS may result in cellular injury and necrosis through peroxidation of membrane lipids, protein denaturation and DNA damage), and also oxidative stress by overproduction of reactive oxygen species is one of the main mechanisms involved in acute and chronic renal pathologies. In addition, specify of GS renal toxicity may be related its accumulation in the renal proximal tubules. In brief, the fundamental role of gentamicin-induced nephrotoxicity is oxidative stress and inflammation [35].

Urea is the nitrogen containing end product of protein catabolism. The concentration of urea is elevated when glomerular filtration rate (GFR) is markedly decreased in renal apathies. Moreover, urea concentration begins to rise only after parenchymal tissue damage [36]. Creatinine derives from endogenous sources by tissue creatinine breakdown and its clearance enables a quite good estimation of the GFR.

Many investigators [34,37,38] observed that treatments with GS produces nephrotoxicity, evident by the reduction in renal functions which is characterized by Elevation of urea and creatinine levels in serum accompanied by impairment in glomerular functions. Ozbek *et al.,* [39] suggested that there are some experimental data about nephrotoxic drugs may change levels of MDA, GSH-Px, CAT, SOD, GSH, creatinine and urea, which are commonly used to monitor the development and extent of renal tubular damage due to oxidative stress.

The results of this study indicate that aqueous extracts of olive leaf alone and in combination with vitamin E significant protection against gentamicin induced nephrotoxicity which was evident from the lowered serum urea and creatinine levels. All the doses of olive leaf extracts significantly protected the elevation of GS-induced serum creatinine and urea levels. However the concentration of urea and creatinine in the extracts of olive leaf treated group was not normalized. Treatment of vitamin E at 250 mg/kg was not effective like olive leaf in preventing the elevated serum urea (40 mg/kg and 80 mg/kg) and creatinine levels (80 mg/kg).

However their combination treatment could partially protect the elevation of urea and creatinine compared to their individual treatments. The results of present study are in line with those reported previously [7,34,40,41]. The mechanism behind elevated serum urea and creatinine might be that the gentamicin increases the entry of Ca^{+2} in the mesangial cells leading to reduced glomerular filtration rate [42].

Gentamicin administration is associated with severe necrosis, desquamation in proximal tubules and dysfunction of co-transport systems and channels, leading to decrease absorption of electrolytes [43]. The results of present study indicated that serum electrolytes, i.e., sodium levels were significantly reduced and elevated potassium levels in gentamicin-treated rat (G2). The decrease in sodium levels may be due to increased wasting or reduced absorption of electrolytes resulting from kidney damage [44].

The administration of olive leaf extracts and vitamin E restored sodium and potassium levels, indicating nephroprotective activity. Acid, bases and salts are collectively called electrolytes. Electrolyte imbalance can leads to serious consequences as it affects the homeostasis of the body. Homeostasis is the process by which the body cells maintain their internal balance in spite of changes in the external environment commonly measured electrolytes are sodium, potassium, calcium, chloride bicarbonate etc., which are good indicators of kidneys function [45].

Controversially, in accordance with the several investigations [37,46], has been confirmed a relationship between nephrotoxicity and oxidative stress in experimental models. In the present study, GS administration caused severe damage to renal tissues most likely by ROS mediated mechanism as evident by decreased activities of antioxidant enzymes (GPx, CAT and SOD) and increased of MDA levels. These observations are in agreement with those reported earlier [34,40,47]. This decrease in activity of enzyme may either be due to loss of copper and zinc, which are essential for the activity of enzyme, or due to ROS-induced inactivation of enzyme [48]. The oxidant-antioxidant system is in equilibrium in normal conditions.

An intracellular antioxidant enzyme, SOD, rapidly and specifically reduces O^{2-} to hydrogen peroxide (H_2O_2). The other endogenous antioxidant enzyme, CAT is a key component of the antioxidant defense system and catalyses the reaction of hydrogen peroxide into

water [49]. GPx is a seleno-enzyme two third of which is present in the cytosol and one-third in the mitochondria. It catalyse the reaction hydroperoxides with reduced glutathione to form glutathione disulphide (GSSG) and the reduction product of the hydroperoxide [50]. Moreover the decreased CAT and GPx activities in the GS treated group in turn increased the H_2O_2 concentration and enhanced the lipid peroxidation. It has been proposed that oxidative stress may be responsible for tubular damage. It is well known that the production of ROS causes cell damage due to cytotoxic action of oxygen and nitrogen derived free radical species. Lipid peroxidation has a relationship with the release of lysosomal enzymes. Hence, Lipid peroxidation activates the phospholipases and removes the peroxidized lipid from the membrane [50].

The oxidation of unsaturated fatty acids in biological membranes by free radical leads to a decrease in membrane fluidity and disruption of membrane structure and function [51]. The present results treatment with olive leaf extract prevents the lipid peroxidation by enhancing the renal SOD, CAT, and GPx activities. GS causes renal phospholipidosis via inhibition of lysosomal hydroxylase, such as sphingomylinase and phospholipidase in conjunction with oxidative stress leading to nephrotoxicity [40]. An antioxidant has been defined as "any substance that when present at low concentrations compared with those of oxidizable substrates significantly delays or prevent oxidation of that substrate. When reactive oxygen / nitrogen species (ROS / RNS) are generated in view, their actions are opposed by intricate and coordinated antioxidant lines of defense systems.

This includes enzymatic and non-enzymatic antioxidant that keeps in check ROS / RNS level and repair oxidative cellular damage [50]. Enzymatic antioxidants such as SOD, GPx and Catalse are free radical scavengers. Their synergestic action in scavenging oxygen-derived free radicals is well documented [52]. Accordingly, our results were consistent with this finding as administration of GS alone (G2) the significant progressive weight losses are remarkable. Olive leaf and Vitamin E aided in prevention of significant reduction in weight loss. Oral administration of olive leaf extracts 20 mg/kg and 40 mg/kg with vitamin E (G8 and G9) had a protective potential against weight loss induced by gentamicin. It is reasonable to conclude that the use of antioxidant effect of OLE was responsible for the inhibited weight loss recorded in this study. This result is support by Adeneye and Benebo [53]; Tavafi *et al.,* [18,41] studies.

Histopathological assessment of kidney in group 1 shows normal architecture of tubules (Figure 1). In contrast, the gentamicin treated rats (G2) produced hypertrophy of glomerular tufts and vacuolations of epithelial liming renal tubules (Figure 2). These findings are consistent with the results of earliest studies De la Cruz Rodríguez *et al.,* [54]; Negrette-Guzmán *et al.,* [55] and Ghaffar *et al.,* [56]. Administration of Vitamin E, aqueous extracts of olive leaf (80 mg/kg and 160 mg/kg) alone and in combination with vitamin E kidney sections showed architecture similar to normal tubules.

The experimental results also reveal that the nephroprotective activity of the extracts is comparable to that of vitamin E. The activity elicited by the extract might be due to its ability to activate antioxidant enzymes. According to our biochemical findings which were supported by histopathological evidence administration of olive leaf for induced GS, these findings were in accord with the other experimental studies [18,57].

Herbs are recognized as sources of natural antioxidants and thus play an important role in the chemoprevention of diseases resulting from lipid peroxidation. In the olive leaf, the oleuropein, hydroxytyrosol, tyrosol, and caffeic acid were identified as the major active components.Furthermore, olive leaves contain p-coumaric acid, vanillic acid, vanillin, luteolin, diosmetin, rutin, luteolin-7-glucoside, apigenin-7-glucoside and diosmetin-7-glucoside have been identified as therapeutic agents delaying the progression of advanced glycation end products-mediated inflammatory diseases such as diabetes [58]. Additionally, oleuropein and tannins in olive leaves are reported to act as a-glucosidase inhibitors, reducing the absorption of carbohydrates in the gut [59]. Komaki *et al.,* [60] showed that olive leaf extract have an inhibitory effect on the postprandial blood increase in glucose in diabetic rats.

And also, in humans treated with olive leaf extract, blood glucose was significantly decreased after cooked rice loading compared with untreated controls. The total content of flavonoids and polyphenols of olive leaves was determined as 2.058 mg GAE (gallic acid equivalent) per 100 g and 858 mg CTE (catechin equivalent) per 100 g, values similar to a red grape [61]. Vogel *et al.,* [62] and Benavente-Garcia *et al.,* [16] suggests that the olive phenolics compounds exhibit a synergistic behavior in the capacity of elimination of free radical when mixed in the form of extract, superior to the antioxidant capacity of the vitamin C and E. The findings of this study strongly indicate that olive leaf is important in protecting the kidney from GS induced injury. Recent studies have tested the beneficial effects of olive leaf in the prevention of diabetic complications in several tissues, including kidney [63]. In this study shows combination of olive leaf (20 mg/Kg and 40 mg/Kg) with vitamin E (250 mg/Kg), having strong antioxidant and cellular anti-inflammatory properties improved the oxidant status. The nephroprotective activity of olive leaf may be due to the presence of phytochemicals like flavonoids, also its ability of anti-inflammatory activity and antioxidant status.

It is well acknowledged that many phenolic compounds exert powerful antioxidant effects. Therefore, they could inhibit lipid peroxidation by scavenging ROS. Treatment of extract of Olive leaf (40 mg/kg) plus vitamin E (250 mg/kg) showed a better protection when compared to the all extracts of olive leaf or vitamin E alone treated groups, which was evident from the renal SOD, CAT, and GPx activities. In this study, concentrations of OLE (20, 40 mg + Vit E) exhibited a strong DPPH• scavenging activities with boiling germinated barley as shown in Figure 10.

Conclusion

The present study showed that aqueous extract of olive leaf, Vitamin E, and their mixture protected against GS-induced nephrotoxicity by inhibiting lipid peroxidation and improving renal antioxidant enzyme activities. According to the biochemical findings, were supported by histopathological evidences, administration of olive leaf extract with vit E more effective protection than their individual dose. 100 mL of boiling germinated barley with OLE (20, 40 mg) + vitamin E was showed good antioxidant properties. The nephroprotective activity of olive leaf may be due to the presence of phenolic compounds and flavonoids. Phenolic compound from olive leaf may be more effective for scavengers of reactive oxygen species. In addition, administration of olive leaf extract (20 mg/kg, 40 mg/kg) plus vitamin E (250 mg/kg) indicated better protection against GS-induced nephrotoxicity.

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