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Nephroprotective and Diuretic Effects of Three Medicinal Herbs Against Gentamicin-Induced Nephrotoxicity in Male Rats

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Abstract: The nephroprotective and diuretic effects of three medicinal herbs namely *Petroselinum sativum*, *Eruca sativa* and *Curcuma longa*, alone and in combination, against gentamicin (GM)-induced nephrotoxicity in rats were investigated. Forty two adult male Sprague Dawley rats were randomly distributed into 6 equal groups, each of 7 animals. The 1st group was injected intraperitoneally (i.p.) with saline solution (0.2 mL/rat). The 2nd group was i.p., injected with GM (80 mg/kg b.wt.) for 8 consecutive days. The other four groups were given orally aqueous infusion of the three herbs, alone and combined, (1 mL/rat, 150 mg/kg b.wt.) along with GM. Twenty four hours after the last administration, blood and urine samples were taken for biochemical analyses. Kidney specimens were taken for estimating oxidant/antioxidant parameters and for histopathology. The results showed that GM induced nephrotoxicity characterized by renal dysfunction as evident by biochemical and histopathological alterations, elevated lipid peroxidation and reduced activity of antioxidant enzymes in kidney tissues. Oral administration of aqueous infusion of *Petroselinum sativum*, *Eruca sativa* and *Curcuma longa* herbs caused a nephroprotective effect evident by significant decreases in the elevated serum urea, creatinine and ALP activity and normalized the decreased serum levels of Na⁺ and K⁺ electrolytes in GM-treated rats. It significantly increased urine output and urinary concentration of Na⁺ and K⁺; denoting a diuretic activity. It also ameliorated renal tubular necrosis in GM-treated rats. The nephroprotective of herbs could be due to the antioxidant effect of these herbs as evident by increasing activity of antioxidant enzymes. Conclusively, the study suggests that mixture of these three herbs may be useful for patients who suffer from renal diseases and those on GM therapy.

Key words: Medicinal herbs, gentamicin, nephroprotective, diuretic, antioxidant, biochemistry, histopathology, rats

INTRODUCTION

Nephrotoxicity induced by several synthetic drugs represents a major problem of modern age population. Gentamicin (GM) is one of amino glycoside antibiotics commonly used for the treatment of Gram negative bacterial infection in man. It is an effective drug against resistant bacterial strains to other antibiotics, but its nephrotoxic side effect has limited its therapeutic use (Salgado *et al.*, 2007). The incidence of amino glycosides-induced nephrotoxicity had increased and about 30% of patients treated with GM for more than 7 days showed signs of nephrotoxicity (Ademuyiwa *et al.*, 1990; Elliott *et al.*, 2000). Nephrotoxicity caused by GM seemed to be attributed to the oxidative stress caused by generation of reactive oxygen species (Cuzzocrea *et al.*, 2002; Tavafi *et al.*, 2012). However, Sha and Schacht (1999) suggested that amino glycoside antibiotics can stimulate formation of reactive oxygen species (ROS) and cause oxidative stress. In addition, ROS scavengers and antioxidants can be used to alleviate GM-induced nephrotoxicity (Mazzon *et al.*, 2001; Maldonado *et al.*, 2003). Moreover, gentamicin induced nephrotoxicity by inhibiting protein synthesis in

renal cells. This mechanism specifically induced necrosis of cells in the proximal tubule, resulting in acute tubular necrosis which can lead to acute renal failure (Sundin *et al.*, 2001).

Medicinal plants and herbs have played an important role in the treatment and prevention of renal diseases. In this concern, Kreydiyyeh and Usta (2002) reported that *Petroselinum sativum* (Parsley) aqueous seeds extract produced a diuretic effect in rats. The authors concluded that the mechanism of action of parsley seems to be mediated through an inhibition of the Na⁺/K⁺ pump that would lead to a reduction in Na⁺ and K⁺ reabsorption leading thus to an osmotic water flow into the lumen and diuresis. Moreover, *Petroselinum sativum* had a therapeutic effect on calcium oxalate stones in rats with nephrolithiasis and reduced the number of calcium oxalate deposits (Saeidi *et al.*, 2012). *Eruca sativa* is widely used in folklore medicine and has a good reputation as a remedy of renal ailments. Sarwar *et al.* (2007) reported that *Eruca sativa* produced potent antioxidant and renal protective activities and precluded oxidative damage inflicted to the kidney by mercuric chloride in rats. Curcumin, the active principle of turmeric

(*Curcuma longa*) ameliorated diabetic nephropathy in rats. The antioxidative activity is responsible for the nephroprotective action of curcumin (Sharma *et al.* 2006). The ethanol extract of *Curcuma comosa* exhibited an effective protection against cisplatin-induced nephrotoxicity in mice mediated through its antioxidant activity (Jariyawat *et al.*, 2009). *Curcuma longa* (turmeric) extract was found to possess multiple therapeutic activities that block the cardiac, hepatic and renal toxicities induced by doxorubicin and had as a free radical scavenger activity (Mohamad *et al.*, 2009). Recently, Zhong *et al.* (2011) concluded that curcumin might be potentially useful in some kidney diseases by preventing renal inflammation. The present study was therefore carried out to investigate the nephroprotective, diuretic and antioxidant effects of *Petroselinum sativum*, *Eruca sativa* and *Curcuma longa* herbs, alone and in combination, against gentamicin-induced nephrotoxicity in rats.

MATERIALS AND METHODS

Materials herbs: *Petroselinum sativum* (Parsley, Family *Apiaceae*) seeds; *Eruca sativa* (Rocket or Rucola, Family *Brassicaceae*) seeds and *Curcuma longa* (Turmeric, Family *Zingiberaceae*) rhizomes were purchased from the Agricultural Seeds, Herbs and Medicinal Plants Company, Cairo, Egypt. The dried seeds and rhizomes of the selected herbs were finely grinded into fine powders and kept for further use.

Gentamicin: Gentamicin (Garamycin® injection), an amino glycoside antibiotic, was obtained from Memphis Company for Pharmaceutical and Chemical Industries, Cairo, Egypt. It is dispensed in the form of ampoules, each containing 40 mg/mL of gentamicin sulphate. The injected dose of gentamicin (80 mg/kg b.wt to the rat was selected according to Bibu *et al.* (2010) to induce acute nephrotoxicity.

Rats: Forty two adult male rats of Sprague Dawley strain weighing 180-190 g b.wt and 12-14 weeks old were used in this study. The rats were purchased from the Laboratory Animal Colony, Helwan, Egypt. The animals were housed under hygienic conditions at a room temperature of 25±2°C with relative humidity of 50-60% and on 12 h light/12 h dark cycles in the Animal House of Agricultural Research Center, Giza, Egypt. Basal diet and water were allowed *ad libitum*.

METHODS

Preparation of basal diet: Basal diet was prepared according to Reeves *et al.* (1993). It consists of 20% protein, 10% sucrose, 4.7% corn oil, 2% choline chloride, 1% vitamin mixture, 3.5% salt mixture and 5% fibers. The remainder was corn starch up to 100%.

Preparation of herbal aqueous infusions: One hundred and fifty grams of fine powder of each herb were soaked in one liter of hot water (to obtain 15% concentration) at 80°C for 2 h and thereafter kept in a refrigerator with daily shaking for 5 days. The aqueous infusion was obtained in by filtration with double layers of gauze to get rid of herb debris. For preparing the herb mixture, fifty grams of each herb were thoroughly mixed together, soaked in one liter of hot water at 80°C for 2 h and processed as previously mentioned. The prepared aqueous infusions were kept in a refrigerator pending for further use.

Experiment and grouping of rats: Forty two adult male Sprague Dawley rats were randomly distributed into six equal groups, each of 7 animals. Group (1) was injected intraperitoneally (i.p.) with sterile normal saline (0.2 mL/day) and kept as normal control. Group (2) was injected i.p., with gentamicin in a dose 80 mg/kg b.wt./day for 8 consecutive days to induce acute nephrotoxicity (Bibu *et al.* 2010) and kept as nephrotoxic control. Groups (3), (4), (5) and (6) were given i.p., the same dose of gentamicin along with oral administration of the aqueous infusion of each of the 3 herbs and their mixture at 15% concentration (1 mL/rat), respectively. Twenty four hours after the last administration, animals were placed in separate metabolic cages for 24 h and total urinary volume was measured using a graduated cylinder. A drop of concentrated hydrochloric acid was added to urine before being stored at 4°C. Urine was analyzed for sodium and potassium levels. Blood samples were collected and used for serum separation, Serum samples were used for estimation of blood urea, uric acid, creatinine and alkaline phosphates as well as serum sodium and potassium levels. Kidney tissue specimens were collected and stored at -70°C for evaluation of oxidant/antioxidant parameters. The other kidney specimens were preserved in neutral 10% formalin for histopathology. The experiment was carried out according to rules and guidelines for animal experimentation which approved by the Institutional Animal Care and Use Committee, National Research Centre, Dokki, Egypt.

Serum and urine analyses: Concentrations of blood urea nitrogen; uric acid and creatinine were estimated as described by Patton and Crouch (1977), Fossati *et al.* (1980) and (Husdan and Rapoport, 1969), respectively using specific diagnostic kits (Sigma Aldrich, St. Louis, USA). The activity of serum alkaline phosphates (ALP) enzyme was estimated according to the method of Kind and King (1954) using standard reagent kits (Sigma Aldrich, St. Louis, USA). Serum and urine levels of sodium and potassium were determined using flame photometer (Model FP 20 seac, Seag Radim Company, Italy) with specific diagnostic kit (BioMérieux, France) as described by Ali (2010).

Preparation of kidney homogenate: One gram of kidney tissue was collected, washed in ice-cold 0.9% NaCl and homogenized in ice-cold 1.15% solution of potassium chloride and 50 mM potassium phosphate buffer solution (pH 7.4) to yield 10% homogenate (W/V). Homogenization was performed using ultrasonic homogenizer (Sonicator, model 4710, Cole-Parmer Instrument Company, USA). The homogenate was then centrifuged at 4000 rpm for 5 min at 4°C. The supernatant was collected for further use.

Determination of reduced glutathione (GSH): GSH content of kidney tissue was determined according to Ellman (1959). The assay is based on the reduction of 5, 5 dithiobis (2-nitrobenzoic acid) with glutathione producing a yellow compound. The reduced chromogen was directly proportional to GSH concentration and its absorbance was measured at wave length 412 nm. The concentration of GSH was expressed as nmol/min/mg protein.

Determination of lipid peroxidation product (LPx): LPx in renal tissue was measured according to Ohkawa *et al.* (1979). The technique is based on the reaction of thiobarbituric acid with lipid peroxides malondialdehyde (MDA) in acidic medium at 95°C for 45 min to form thiobarbituric acid reactive substance (TBARS). The resulting pink color was extracted with n-butanol and absorbance was determined spectrophotometrically at wave length 530 nm. The level of MDA was expressed as nmol/min/mg protein.

Determination of superoxide dismutase (SOD): The renal SOD activity was measured according to Nishikimi *et al.* (1972). This assay relies on the ability of the enzyme to inhibit the phenazine methosulphate-mediated reduction of nitroblue tetrazolium dye. The activity of SOD was expressed as Unit of activity/mg protein.

Determination of glutathione peroxidase (GPx): Renal GPx activity was measured by the method of Paglia and Valentine (1967). This assay was indirect measurement of the activity of GPx. The oxidized glutathione (GSSG), produced upon reduction of organic peroxide by GPx, was recycled to its reduced state by the enzyme glutathione reductase (GR). The reaction was initiated by the addition of hydrogen peroxide and the oxidation of NADPH to NADP⁺ is accompanied by a decrease in the absorbance at wave length 340 nm. The activity of GPx was expressed as nmol of GSH utilized/min/mg protein.

Determination of catalase (CAT): Renal CAT activity was measured in tissue homogenate according to Aebi (1984). The assay is based on that catalase reacts with a known quantity of hydrogen peroxide. This reaction is

stopped after exactly one minute with catalase inhibitor. In the presence of peroxidase, the remaining hydrogen peroxide reacts with 3,5-Dichloro-2-hydroxybenzenesulfonic acid and 4-amino-phenazone to form a chromophore with a colour intensity inversely proportional to the amount of catalase. The activity of CAT was expressed as nmol of H₂O₂ utilized/min/mg protein.

Histological procedure: Kidney specimens were taken and fixed in 10% neutral formalin solution. The fixed specimens were trimmed, dehydrated in ascending grades of alcohol, cleared in xylene. They were embedded in paraffin boxes, sectioned at 4-6 microns thickness and stained with Hematoxylen and Eosin (H and E). They were microscopically examined under light microscope as described by Carleton (1976).

Statistical analysis: Data are expressed as Mean± SD error (SE) of mean. Differences between the groups were tested for significance using one-way analysis of Variance (ANOVA) followed by Duncan 's multiple range test according to Snedecor and Cochran (1986). Statistical analyses were performed using the SPSS software (Statistical Package for the Social Sciences, version 15.0, Chicago, USA).

RESULTS

Serum and urine analyses: Intraperitoneal injection of gentamicin (GM) in a dose of 80 mg/kg/days for consecutive days to rats caused nephrotoxicity manifested by significant (p<0.05) increases in serum levels of blood urea nitrogen, creatinine and activity of alkaline phosphatase (ALP) enzyme when compared with the normal control group. Oral administration of aqueous infusions of *Petroselinum sativum*, *Eruca sativa* and *Curcuma longa* herbs, alone and in combination, along with GM induced significant (p<0.05) decreases in the elevated levels of blood urea nitrogen, creatinine and ALP in the serum when compared with GM-treated rats as shown in Table 1.

Serum sodium (Na⁺) and potassium (K⁺) levels: Data in Table 2 showed that daily intraperitoneal injection of GM to rats for 8 days caused significant (p<0.05) decreases in serum levels of sodium (Na⁺) and potassium (K⁺) electrolytes when compared with the normal control group. Oral administration of aqueous infusions of *Petroselinum sativum*, *Eruca sativa* and *Curcuma longa* herbs and their mixture concomitantly with GM normalized the decreased levels of Na⁺ and K⁺ electrolytes in the serum when compared with GM-treated rats.

Urine volume and urinary sodium (Na⁺) and potassium (K⁺) levels: As shown in Table 3 daily intraperitoneal injection of GM to rats for 8 days caused significant

Table 1: Serum urea, uric acid, creatinine and alkaline phosphatase enzyme (ALP) levels in gentamicin-nephrotoxic rats

Groups and treatments (mg/dL)	Urea (mg/dL)	Uric acid (mg/dL)	Creatinine (mg/dL)	ALP (U/L)
Group 1 Normal control	31.95±2.44 ^d	1.48±0.12	0.58±0.02 ^d	158.4±4.52 ₃
Group 2 Nephrotoxic control	70.46±3.54 ^a	1.49±0.17	0.97±0.04 ^a	177.6±4.21 ^a
Group 3 PS 15 %	39.35±2.10 ^b	1.52±0.15	0.68±0.02 ^b	165.4±5.32 ^b
Group 4 ES 15 %	40.42±2.24 ^b	1.50±0.13	0.65±0.01 ^b	164.8±4.22 ^b
Group 5 C; 15 %	38.63±3.30 ^b	1.51±0.16	0.67±0.03 ^b	163.5±5.03 ^b
Group 6 Herb mixture 15%	36.46±3.70 ^c	1.49±0.12	0.56±0.04 ^c	160.3±2.44 ^c

Means ± SD with different superscripts in the same column are significant at p<0.05 using one way ANOVA test

Table 2: Serum sodium (Na⁺) and potassium (K⁺) levels in gentamicin-nephrotoxic rats

Groups and treatments	Na ⁺ (MEq/L)	K ⁺ (MEq/L)
Group 1 Normal control	129.10±0.16 ^b	4.15±0.2 4 ^b
Group 2 Nephrotoxic control	110.55±0.27 ^a	2.12±0.27 ^a
Group 3 PS 15%	124.75±0.24 ^b	4.85±0.12 ^b
Group 4 ES 15%	125.79±0.14 ^b	4.76±0.23 ^b
Group 5 CL 15%	128.77±0.08 ^b	4.78±0.15 ^b
Group 6 Herb mixture 15%	127.95±0.04 ^b	4.88±0.23 ^b

Means ± SD with different superscripts in the same column are significant at p<0.05 using one way ANOVA test

Table 3: Urine volume and urinary sodium (Na⁺) and potassium (K⁺) levels in gentamicin-nephrotoxic rats

Groups and treatments	Urine volume (mL)	Na ⁺ (MEq/L)	K ⁺ (MEq/L)
Group 1 Normal control	3.75± 0.23 ^d	93.12±4.86 ^d	20.70±1.11 ^b
Group 2 Nephrotoxic control	3.20± 0.25 ^d	90.55±2.27 ^c	18.12±0.07 ^c
Group 3 PS 15%	6.30± 0.15 ^b	120.75±0.24 ^b	42.85±0.02 ^a
Group 4 ES 15%	7.30±0.15 ^b	155.79±0.14 ^b	41.76±0.03 ^a
Group 5 CL 15%	6.50±0.34 ^b	145.77±0.08 ^b	40.78±0.05 ^a
Group 6 Herb mixture 15%	8.50 ±0.24 ^a	167.95±0.04 ^a	40.88±0.03 ^a

Means ± SD with different superscripts in the same column are significant at p<0.05 using one way ANOVA test

(p<0.05) decreases in levels of urinary Na⁺ and K⁺ electrolytes when compared with the normal control group. Oral administration of aqueous infusions of *Petroselinum sativum*, *Eruca sativa* and *Curcuma longa* herbs and their mixture concomitantly with GM significantly (p<0.05) increased urine volume and urinary levels of Na⁺ and K⁺ electrolytes when compared with GM-treated rats.

Lipid peroxidation and antioxidant activity: Daily intraperitoneal injection of GM to rats for 8 days caused a significant (p<0.05) decrease in the content of reduced glutathione (GSH) and an increase in the level of lipid peroxidation product malondialdehyde (MDA) in kidney tissues when compared with the normal control group. Oral administration of aqueous infusions of *Petroselinum sativum*, *Eruca sativa* and *Curcuma longa* herbs and their mixture concomitantly with GM caused a significant (p<0.05) increase in GSH and decrease in MDA contents in renal tissue when compared with GM-treated rats (Table 4).

Activity of Superoxide dismutase(SOD), glutathione peroxidase (GPx) and catalase (CAT) enzymes in kidney tissue: It is clear from Table 5 that intraperitoneal injection of GM to rats for 8 consecutive days induced significant (p<0.05) decreases in the activity of tissue superoxide dismutase (SOD), glutathione peroxidase

(GPx) and catalase (CAT) enzymes when compared with the normal control group. Oral administration of aqueous infusions of *Petroselinum sativum*, *Eruca sativa* and *Curcuma longa* herbs and their mixture concomitantly with GM increased the activity of SOD, GPx and CAT enzymes when compared with GM-treated rats. The increases in the activity of SOD, GPx and CAT was significantly, except treated group with *Petroselinum sativum* and herbs mixture had no significant differences.

Histopathological findings: Histological examination of kidneys of normal rats showed normal histological structure of renal parenchyma (glomeruli and tubules) as illustrated in Fig. 1. Kidneys of rats injected with GM (80 mg/kg, i.p.) for 8 consecutive days marked necrosis of renal tubules associated with protein casts in their lumens as shown in Fig. 2. Examination of kidneys of rats given orally the aqueous infusion of *Petroselinum sativum* herb concomitantly with GM showed mild congestion of intertubular blood capillaries Fig. 3. In rats given the aqueous infusion of *Eruca sativa* herb concomitantly with GM, the examination of kidney showed vacuolations of epithelial lining of renal tubules Fig. 4. In rats received the aqueous infusion of *Curcuma longa* herb along with GM, the microscopic examination of kidneys revealed little peritubular leukocytes infiltration Fig. 5. Concomitant administration of the mixture of

Table 4: Kidney levels of GSH and MDA in gentamicin-nephrotoxic rats

Groups and treatments		GSH (nmol/min/mg protein)	MDA (nmol/min/mg protein)
Group 1	Normal control	34.57±1.89 ^a	0.32±0.16 ^b
Group 2	Nephrotoxic control	24.00±0.92 ^c	0.73±0.23 ^a
Group 3	PS 15%	22.36±0.61 ^b	0.21±0.20 ^c
Group 4	ES 15%	25.44±0.51 ^b	0.24±0.10 ^b
Group 5	CL 15%	30.57±0.79 ^b	0.28±0.19 ^b
Group 5	Herb mixture 15%	32.35±0.75 ^b	0.29±0.20 ^b

Means±SD with different superscripts in the same column are significant at p<0.05 using one way ANOVA test

Table 5: Activity of SOD, glutathione peroxidase GPx and catalase CAT enzymes in kidney tissue of gentamicin-nephrotoxic rats

Groups and treatments		SOD (U/mg protein)	Gpx (nmol/min/mg protein)	CAT (nmol/min/mg protein)
Group 1	Normal control	51.8±0.18 ^a	0.50±0.02 ^a	0.186±0.001 ^a
Group 2	Nephrotoxic control	36.00±2.3 ^c	0.13±0.03 ^c	0.144±0.003 ^c
Group 3	PS 15%	39.00±2.62 ^c	0.25±0.04 ^b	0.123±0.004 ^b
Group 4	ES 15%	42.25±2.42 ^b	0.28±0.01 ^b	0.135±0.005 ^b
Group 5	CL 15%	44.64±3.75 ^b	0.39±0.03 ^b	0.169±0.002 ^b
Group 6	Herb mixture 15%	48.77±2.43 ^c	0.44±0.02 ^b	0.177±0.001 ^b

Means±SD with different superscripts in the same column are significant at p<0.05 using one way ANOVA test

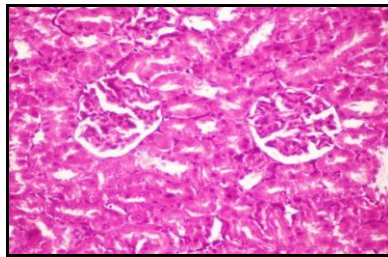


Fig. 1: Cross sections of kidney a normal control rats showing normal architecture of renal parenchyma (glomeruli and tubules). (H and E, X 400)

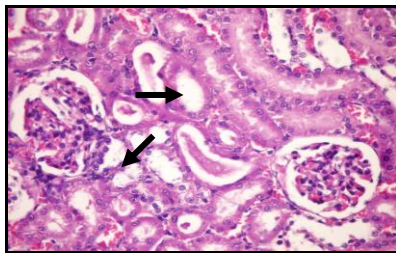


Fig. 2: Cross sections kidney of a gentamicin- treated rats showing marked necrosis of renal tubules (Arrow) with protein casts in their lumens (arrow). (H and E, X 400)

Petroselinum sativum, *Eruca sativa* and *Curcuma longa* herbs along with GM showed almost normal histological architecture of renal parenchyma Fig. 6.

DISCUSSION

In the current study, the nephroprotective, diuretic and antioxidant activities of *Petroselinum sativum*, *Eruca sativa* and *Curcuma longa* herbs, alone and in combination, against gentamicin- induced nephrotoxicity in rats were investigated. The obtained results revealed

that intraperitoneal injection of gentamicin (GM) to rats caused signs of nephrotoxicity manifested by significant increases in serum urea, creatinine and activity of ALP enzyme associated with decreases in serum levels of sodium and potassium. Urine analysis showed significant decreases of urinary excretion of sodium and potassium in GM-treated rats. In addition, lipid peroxidation in kidney tissues showed significant elevation of lipid peroxide malondelhyde (MDA) and the antioxidant enzymes were markedly decreased in GM-treated rats. Examination of kidney sections of GM-injected rats revealed a marked necrosis of renal tubules. These results were in agreement with the previously reported by Ademuyiwa *et al.* (1990), Elliott *et al.* (2000), Cuzzocrea *et al.* (2002), Salgado *et al.* (2007) and Tavafi *et al.* (2012) who concluded that GM induces nephrotoxicity manifested by biochemical and histological changes in rats.

The mechanism of nephrotoxicity caused by GM was attributed to stimulation of generation of reactive oxygen species (ROS) causing tissue oxidative stress (Sha and Schacht, 1999; Cuzzocrea *et al.*, 2002 and Tavafi *et al.*, 2012). GM- nephrotoxicity associated with decreases in serum levels of sodium and potassium suggested that the site of GM action is the distal convoluted tubules causing increased urinary excretion of sodium and potassium (Elliott *et al.*, 2000). In addition, it was previously reported that high serum alkaline phosphatase (ALP) concentrations might be a marker of renal inflammation (Damera *et al.*, 2011).

In the present work, oral administration of *Petroselinum sativum*, *Eruca sativa* and *Curcuma longa* herbs and their combination caused nephroprotective and diuretic effects as they reversed the biochemical and histological alterations induced by GM in rats. These herbs produced an antioxidant activity as evident by decreasing lipid peroxidation byproduct (MDA), increasing content of reduced glutathione and restoring activities of

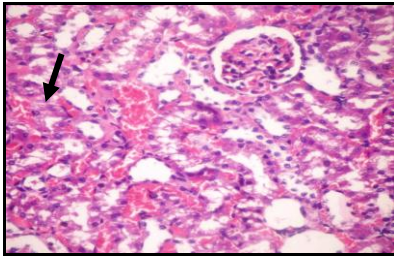


Fig. 3: Cross sections of kidney of a gentamicin-treated rats and given orally aqueous infusion of *Petroselinum sativum* herb showing mild congestion of intertubular blood capillaries (Arrow). (H and E, X 400)

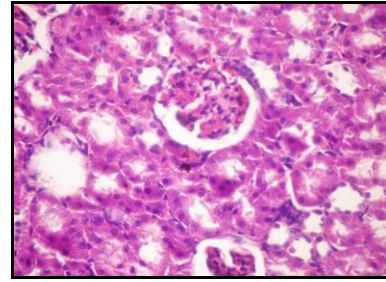


Fig. 6: Cross section of kidney of a gentamicin-treated rat and given orally aqueous infusion of mixture of the 3 herbs showing almost normal histological structure of renal parenchyma. (H and E, X 400)

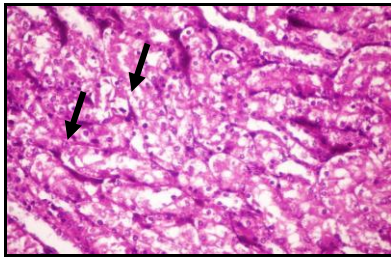


Fig. 4: Cross sections of kidney of a gentamicin-treated rats and given orally aqueous infusion of *Eruca sativa* herb showing vacuolations of epithelial lining of renal tubules (Arrows). (H and E, X 400)

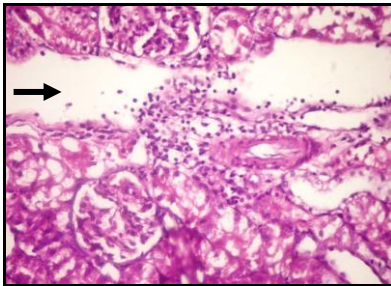


Fig. 5: Cross sections of kidney of a gentamicin-treated rats and given orally aqueous infusion of *Curcuma longa* herb showing peritubular leukocytes infiltration (Arrow). (H and E, X 400)

antioxidant (SOD, GPx and CAT) enzymes in renal tissue.

The nephroprotective effect of *Petroselinum sativum* herb, reported in the present study, was similar to that reported by Afzal *et al.* (2004) who found that a polyherbal formulation containing *Petroselinum sativum* produced a nephroprotective effect in rats. The nephroprotective effect of *Petroselinum sativum* was attributed to its *in vitro* antioxidant activity (free radical scavenger activity) due to its high content of flavonoids (Fejes *et al.*, 1998). This explanation was confirmed by

the reported *in vivo* antioxidant effect of *Petroselinum sativum* in the current study. The diuretic effect of *Petroselinum sativum* (parsley) was reported by Kreydiyyeh and Usta (2002) who found that parsley aqueous seeds extract produced a diuretic effect in rats. The authors concluded that the mechanism of action of parsley seems to be mediated through an inhibition of the Na^+/K^+ pump that would lead to a reduction in Na^+ and K^+ reabsorption leading thus to an osmotic water flow into the lumen and diuresis.

Concerning *Eruca sativa* herb, it is widely used in folkloric medicine and has a good reputation as a remedy of renal ailments. It was reported that *Eruca sativa* produced potent antioxidant and renal protective activities and precluded oxidative damage inflicted to the kidney by mercuric chloride in rats (Sarwar *et al.*, 2007). Moreover, Alqasoumi (2010) reported that *Eruca sativa* L. extract protected the liver against CCl_4 -induced hepatic injury through its potent antioxidant activity in rats.

Regarding *Curcuma longa* herb, Sharma *et al.* (2006) reported that active principle of turmeric (*Curcuma longa*) ameliorated diabetic nephropathy in rats and the anti-oxidative mechanism being responsible for the nephroprotective action of curcumin. The authors concluded that curcumin might be potentially useful in some kidney diseases by preventing renal inflammation. *Curcuma longa* (turmeric) extract was found to possess multiple therapeutic activities that block the cardiac, hepatic and renal toxicities induced by doxorubicin (Mohamad *et al.*, 2009) and by arsenic trioxide (Saxena *et al.*, 2009) and had as a free radical scavenger activity. It was suggested that curcumin might be potentially useful in some kidney diseases by preventing renal inflammation (Zhong *et al.*, 2011).

In the current study, the histopathological changes induced by GM in kidney of rats and the ameliorative effects by the tested herbs were parallel with the reported biochemical alterations. The amelioration of renal tubular necrosis caused by the tested herbs and

their combination in GM- treated rats was similar to those reported by Afzal *et al.*, (2004) for *Petroselinum sativum* and Sarwar *et al.*, (2007) for *Eruca sativa* and Kheradpezhohu *et al.* (2010) and Zhong *et al.* (2011) for *Curcuma longa*.

Conclusion: In conclusion, *Petroselinum sativum*, *Eruca sativa* and *Curcuma longa* herbs produce nephroprotective and diuretic effects in rats as their oral administration concomitantly with gentamicin (GM) induced significant reduction in biochemical and morphological renal alterations caused by GM. The nephroprotective effect could be due to the antioxidant activity of these herbs. Therefore, the study recommends that intake of aqueous infusion of these herbs and their mixture may be beneficial for patients who suffer from kidney diseases and those on GM therapy.

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