Effect of caraway on gentamicin-induced oxidative stress, inflammation and nephrotoxicity in rats

Hoda Erjaee,[1] Fatemeh Azma,[2] Saeed Nazifi[2]

[1]Department of Basic Science, [2]Department of Clinical Studies, School of Veterinary Medicine, Shiraz University, Shiraz, Iran

Abstract

Different potentially therapeutic approaches to prevent or attenuate gentamicin (GEM) induced nephrotoxicity have been proposed. The aim of the present study was to investigate the possible protective effects of caraway seed oil against GEM-induced nephrotoxicity in rats. Rats (24) were randomly assigned into four equal groups: i) normal control group, ii) treated with GEM, iii) pretreated with orally caraway seed oil 10 (mg kg^{-1}) plus GEM and iv) treated with GEM and caraway seed oil 10 mg kg^{-1}. Biochemical examinations were utilized for evaluation of the oxidative stress and renal nephrotoxicity. Creatinine, blood urea nitrogen (BUN), plasma malondialdehyde (MDA) levels, catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) activities were determined. Administration of gentamicin to rats induced a marked renal failure, characterized by a significant increase in plasma creatinine and BUN concentrations. The animals treated with gentamicin alone showed a significantly higher plasma MDA level andlower SOD, GSH-Px and CAT activities when compared with the control group. Treatment and simultaneous treatment with caraway seed oil produced amelioration in MDA and increased the activity of antioxidant enzymes SOD, GSH-Px and CAT when compared with the gentamicin treated group. In addition, GEM nephrotoxicity increased renal inflammatory cytokines (TNF-α, IL-6 and IFN-γ). Pro-inflammatory cytokines were significantly decreased ($P<0.05$) in the test groups administered caraway seed oil. These findings suggest that caraway seed oil treatment attenuates renal dysfunction and structural damage through the reduction of oxidative stress and inflammation in rats.

Introduction

Aminoglycoside antibiotics are widely used for treatment of severe gram negative infections. One of the most aminoglycoside antibiotics which is applied in human clinical practice and veterinary medicine is gentamicin (GEM). Aminoglycosides induce dose-dependent nephrotoxicity and ototoxicity which limits their clinical use.[1,2] GEM nephrotoxicity is described as direct tubular necrosis, without morphological changes in glomerular structures.[3] The mechanisms involved in GEM-induced nephrotoxicity are not completely understood; however, reactive oxygen species (ROS) are involved in this progress and are one of the most important mediators.[4] ROS may damage some macromolecules which induces cellular injury and necrosis. Peroxidation of membrane lipids, protein denaturation and DNA damage are pathways which increase cellular injuries caused by ROS. Experimental evidence suggests the role of ROS is associated with increased lipid peroxide formation.[5,6] In this regard, malondialdehyde (MAD) which is formed during oxidative degeneration is accepted as an indicator of lipid peroxidation.[7] Furthermore, activity of antioxidant enzymes is altered during GEM-induced nephrotoxicity. The most important antioxidant enzymes are catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px). Accordingly, these enzymes protect cells against ROS damage.[8] An increased or unbalanced ROS production and oxidative stress mediate the inflammatory response unleashed by GEM. Hydrogen peroxides and superoxide anions activate NF-κB.[9,10] NF-κB is a key mediator for several inflammatory pathways and induces the expression of pro-inflammatory cytokines such as tumor necrosis factor alpha (TNF-α), interleukin 6 (IL-6) and interferon gamma (IFN-γ).[11-13]

Many researchers reported that renal damage induced by GEM, ameliorates by dietary supplement of antioxidants and nowadays attention has been focused on herbal medicines which have antioxidant activities.[2,14] *Carumcarvi* (caraway), belonging to the family Apaiaceae, have been used in herbal medicine since prehistoric times for various indications in traditional healing systems in wide geographical areas. Caraway is a highly efficient antioxidant with a singlet-oxygen and free radical scavenging capacity.[15] The intrinsic antioxidant activity of caraway is due to the presence of monoterpene alcohols, linalool, carvacrol, anethole and estragol, flavonoids, and other polyphenolic compounds.[16-20] The antioxidant activity of caraway is responsible for its various pharmacological properties such as antimicrobial, antidiabetic, anticarcinogenic/antimutagenic, antistress, and antiulcerogenic.[21] The present study was therefore designed to evaluate the possible protective or ameliorate effects of caraway seed oil on oxidative stress, lipid peroxidation and inflammation in GEM-induced nephrotoxicity in rats. For this purpose, antioxidant enzymes SOD, GSH-Px, CAT, the level of plasma MDA, TNF-α, IL-6 and IFN-γ were measured.

Correspondence: Saeed Nazifi, Department of Clinical Studies, School of Veterinary Medicine, Shiraz University, Shiraz, P.O. Box: 1731-71345, Iran.
Tel.: +98-.711.2286940 - Fax: +98.711.2286950.
E-mail: nazifi@shirazu.ac.ir

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Materials and Methods

Animals

In this investigation, 24 healthy adult male albino Wistar rats weighing 200-250 g were obtained from Razi Institute (Shiraz, Iran). The animals were housed under standard laboratory conditions (12 h light/12 h dark) in a room with controlled temperature (23±1°C) during the experimental period. All experimental procedures were conducted in accordance with the guide to the care and use of laboratory animals. The rats were provided tap water *ad libitum* and standard diet.

Animal ethics

This experiment was accomplished under the approval of the State Committee on Animal Ethics, Shiraz University, Shiraz, Iran. The recommendations of European Council Directive (86/609/EC) of November 24, 1986 regarding the standards in the protection of animals used for experimental purposes were also followed.

Preparation of caraway seed oil

Essential oil of caraway for this experiment was prepared by grinding the seeds, and the resulting powder was hydrodistillated for 3 h.[22] In aqueous and solvent derived seed extracts, different kind of flavonoids, isoflavonoids, flavonoid glycosides, monoterpenoid glucosides, lignins and alkaloids and other phenolic compounds have been found.[23] Caraway seed oil also includes several nutrients (vitamins, amino acids, protein, and minerals), starch, sugars and other carbohydrates and dietary fiber components (Table 1).[24]

Experimental design

The animals were randomly divided into four groups containing six rats each (Table 2). Nephrotoxicity was induced by GEM (Sigma, Aldrich, USA) in rats with an intraperitoneal dose of 100 mg k^{-1}, for six consecutive days.[1,25,26] Dose of caraway seed oil used in this study was selected on the basis of the previous studies.[27]

Experimental groups

Group I (Control)*:* controls received a daily intraperitoneal injection of normal saline (0.5 mL).

Group II (GEM): received GEM alone for six successive days plus the administration of 0.5 mL normal saline for 10 days.

Group III (GEM-S10): received caraway seed oil (10 mg kg^{-1}) during 10 consecutive days, after 10 days injection of GEM was initiated and lasted in daily manner for a 6 consecutive days.

Group IV (GEM-T10): received GEM for six successive days plus 10 days of caraway seed oil (10 mg kg^{-1}) treatment.

The animals in all groups were decapitated 24 h after the last application. Blood samples were collected into EDTA tubes. The samples were centrifuged at 750*g* for 20 min, and then, the plasma was pipetted into different aliquots.

Biochemical analysis

Blood urea nitrogen and serum creatinine

Urea nitrogen and creatinine were measured by commercial kits (Pars Azmoon Co., Tehran, Iran). Biochemical analyses were measured using a standard autoanalyser with veterinary software (Cobas-Mira, ABX-Diagnostics, Japan).

Antioxidant enzymes activities

SOD activity was measured with a commercial kit (RANSOD kit, Randox Com, UK). This method employs xanthine and xanthine oxidase (XOD) to generate superoxide radicals, which react with 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium chloride (INT) toform a red formazan dye. The enzyme activity was then determined by the degree of reaction inhibition, as one unit of SOD corresponded to 50% inhibition of INT reduction under assay condition. GPX activity was measured by a commercial kit (RANSEL kit, Randox Com, UK) based on the method of Paglia and Valentine.[28] GPX catalyses the oxidation of glutathione (GSH) by cumene hydroperoxide. In the presence of glutathione reductase and NADPH, the oxidized glutathione is immediately converted to the reduced form with a concomitant oxidation of NADPH to NADP+. The decrease in absorbance was measured at 340 nm. The values of both enzymes were expressed as units/gr of hemoglobin. The activity of catalase was determined with the commercial catalase assay kit (OxfordBiomedical Research, Inc., USA) based on the colorimetric method described by Slaughter and O'Brien[29] and the activities of the enzymes were expressed as U/g of hemoglobin. Hemoglobin concentration was measured by Cyanmethaemoglobin method.

Lipid peroxidation of red blood cells

Malondiadehyde (MDA), an end product of polyunsaturated fatty acid oxygenation, is a reliable and commonly used biomarker for assessing lipid peroxidation.[30] The lipid peroxidation level of the RBC membrane was evaluated by means of a modified HPLC method with UV-Visible spectrophotometry according to Lykkesfeldt.[31] The measurement was based on MDA reactions with thiobarbituric acid (TBA) to form a colored MDA-TBA adduct, and the values were expressed as U/gHb of MDA.

Assay of pro-inflammatory cytokines

IL-6 was assayed in serum using a rat Interleukin-6 ELISA kit (CUSABIO®, Wuhan, China) which employs the quantitative sandwich enzyme immunoassay technique, and expressed as pg/mL. The concentrations of IFN-γ and TNF-α were measured by a solid phase sandwich ELISA (AbC 606 and AbC 607, respectively; Votrefournisseur AbCysS.A. Paris, France) and expressed as pg/dL and ng/mL, respectively.

Statistical analysis

The data were expressed as means±SEM differences between groups. Means were esti-

Table 1. Essential oil composition of *Carum carvi* L.

Compound	%
a-Pinene	0.57
b-Pinene	4.68
Myrcene	0.4
p-Cymene	7.99
Limonene	1.48
c-Terpinene	17.86
p-Cymen-8-ol	0.94
Cuminaldehyde	22.08
a-Terpinene-7-al	2.88
Bornyl acetate	1.12
c-Terpinene-7-al	15.41
Cuminyl acetate	3.78
Myristicin	1.87
Elemicine	3.54
Germacrene B	1.58
Dillapiol	1.39
Total	87.57

Table 2. Treatment schedule of male Wistar rats exposed to gentamicin (GEM) and caraway seed oil (n=24).

Group/days	1 to 6		7 to 10		11 to 16	
	GEM, mg/kg	Caraway seed oil, mg/kg	GEM, mg/kg	Caraway seed oil, mg/kg	GEM, mg/kg	Caraway seed oil, mg/kg
Control	0	0	0	0	0	0
GEM	100	0	0	0	0	0
GEM- S 10	0	10	0	10	100	0
GEM- T 10	100	0	10	0	10	0

Control group: healthy rats, received the normal saline as placebo, (0.5 mL/kg) daily; GEM group: Received GEM alone for six successive days plus the administration of 0.5 mL normal saline for 10 days; GEM-S10 group: Received caraway seed oil (10 mg kg^{-1}) during 10 consecutive days, after 10 days injection of GEM was initiated and lasted in daily manner for a 6 consecutive days; GEM-T10 group: Received GEM for six successive days plus 10 days of caraway seed oil (10 mg kg^{-1}) treatment.

mated using one-way analysis of variance followed by Duncan's multiple range test using SPSS the software package, version 18. Results were considered statistically significant at P value ≤0.05.

Results

The effect of treatments on plasma creatinine and BUN levels is shown in Table 3. Plasma creatinine and BUN levels (P<0.05) were significantly higher at the end of administration of GEM for six successive days when compared to the control group. However, creatinine and BUN levels (P<0.05) in caraway seed oil treated groups (GEM-S10 and GEM-T10) were lower than the group treated with GEM alone.

The data in Table 4 indicate the effect of treatments on plasma levels of MDA and activities of SOD,GSH-Px and CAT. GEM treated group had significantly higher levels of plasma MDA (P<0.05), while having significantly lower GSH-Px (P<0.05), SOD (P<0.05) and CAT (P<0.05) activities when compared with the control group. Treatment with caraway seed oil (GEM-S10 and GEM-T10) caused significant reduction in MDA levels (P<0.05) when compared with the GEM treated group. However, treatment with caraway seed oil provided a significant increase in CAT levels (P<0.05), SOD levels (P<0.05) and GSH-Px activities (P<0.05). Activity of antioxidant enzymes SOD, GSH-Px and CAT was higher in GEM-S10 group (P<0.05) than GEM-T10 (P<0.05) and GEM (P<0.05) treated group but was lower than the control group.

Pro-inflammatory cytokines IL-6, TNF-α and IFN-γ levels are shown in Table 5. GEM administration produced a significant (P<0.05) elevation of IL-6, TNF-α and IFN-γ levels when compared to control rats. Caraway treatment in GEM-S10 group significantly (P<0.05) decreased the IL-6, TNF-α and IFN-γ levels when compared with GEM group. IL-6, TNF-α and IFN-γ levels were significantly (P<0.05) decreased in GEM-T10 group compared to GEM alone treated rats. In GEM-S10 group caraway treatment caused more reduction in mean levels of pro-inflammatory cytokines in comparison with GEM-T10 group.

Discussion and Conclusions

GEM is a typical aminoglycoside and it is widely used as a bacterial agent for treatment of severe gram-negative bacterial infections.[2] Nephrotoxicity is a major complication of GEM administration and is the limiting factor for GEM clinical usage.[32] A slight increase in GEM blood concentration is accompanied by a significant, incommensurate increase in the amount of the drug in renal cortex.[1,32,33] Several investigators reported that treatments with GEM produce nephrotoxicity and cause reduction in renal functions, which is characterized by an increase in serum creatinine and serum urea level accompanied by impairment in glomerular functions.[34,35] Serum creatinine level is more significant than the urea levels in the earlier phase of the renal damage. In this study, intraperitoneal injection of GEM caused nephrotoxicity in rats, which was correlated with increased creatinine and BUN levels (Table 2). These observations indicated that GEM-induced nephrotoxicity and the results are in accordance with otherresearchers.[8,36,37] Increase in BUN and creatinine levels, induced by GEM was ameliorated by oral treatment of caraway seed oil. In this regard, various antioxidant agents have been shown to reduce GEM-induced renal injury, including vitamin C, *Ginkgo biloba* extract, Grape Seed Extract, green tea extract and *Bauhinia purpurea*.[38-42]

The exact mechanism by which GEM induces nephrotoxicity is unknown; however, several investigators reported that ROS are the causative factors for the renal side effects of this drug.[43,44] Under normal conditions, ROS, which are generated during cellular functions, are eliminated by intrinsic antioxidant enzyme systems like SOD, CAT and GSH-Px.[45] On the other hand, lipid peroxidation, mediated by oxygen-free radicals, is believed to be an important cause of destruction and damage to cell membrane and MDA which is formed during oxidative degeneration is accepted as an indicator of lipid peroxidation.[7,46] Several agents that scavenge or interfere with the production of ROS have been used successfully to ameliorate GEM nephropathy.[32] In this study, the role of ROS in GEM-induced nephrotoxicity was assessed by the usage of antioxidantagent, caraway seed oil, and further evaluation of alterations in the biochemical indicators of oxidative stress mainly SOD,CAT and GSH-Px activities and MDA plasma levels.

In the present study, GEM caused depletion inactivities of antioxidant enzyme SOD, CAT and GSH-Px in blood and elevated MDA plasma

Table 3. Effect of caraway seed oil on blood urea nitrogen (BUN) and creatinine in rats.

Groups (n=6)	Treatment	Parameters	
		BUN, mg/dL	Creatinine, mg/dL
I	Control	11.6±0.3	1.1±0.4
II	GEM	52.0±2.3*	3.7±0.1*
II	GEM-S10	15.9±0.4**	1.5±0.0**
IV	GEM-T10	39.4±1.4**	3.1±0.2**

Values are mean ± standard mean error. *P<0.05 when compared to the normal control. **P<0.05 when compared to gentamicin induced non treated disease control.

Table 4. Effect of caraway seed oil on catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), and malondialdehyde (MDA) in rats.

Groups (n=6)	Treatment	Parameters			
		SOD, U/mL	GSH-Px, U/mL	CAT, U/L	MDA, U/gHb
I	Control	448.4±42.2	527.5±4.0	0.5±0.0	5.1±0.0
II	GEM	237.0±7.4*	276.6±5.7*	0.2±0.0*	5.8±0.0*
II	GEM-S10	319.5±1.6**	322.6±5.1**	0.4±0.0**	5.0±0.0**
IV	GEM-T10	279.2±4.7**	434.8±5.2**	0.3±0.0**	4.7±0.0**

Values are mean ± standard mean error. *P<0.05 when compared to the normal control. **P<0.05 when compared to gentamicin induced non treated disease control.

Table 5. Effect of caraway seed oil on TNF-α, INF-γ and IL-6 in rats.

Groups (n=6)	Treatment	Parameters		
		TNF-α, pg/mL	INF-γ, pg/L	IL-6, pg/mL
I	Control	380.8±17.2	11.9±0.3	18900.1±592.4
II	GEM	941.0±22.1*	45.4±3.1*	48223.5±1267.4*
II	GEMS-10	439.0±14.4**	14.6±0.9**	21942.8±1324.1**
IV	GEMT-10	772.5±26.4**	34.4±2.4**	41143.1±784.5**

Values are mean ± standard mean error. *P<0.05 when compared to the normal control. **P<0.05 when compared to gentamicin induced non treated disease control.

levels. Other investigators also reported that GEM induced nephrotoxicity is associated with low activity of GSH-Px, CAT and SOD in the renal cortex and high levels of MDA.[42,47] These decreases in renal antioxidant enzymatic protection could aggravate the oxidative damage. Furthermore, the increased production of ROS in GEM-induced nephrotoxicity may cause inactivation of antioxidant enzymes such as GSH-Px, CAT and SOD. In this study, SOD, GSH-Px and CAT were increased in blood of rats inGEM-S10 and GEM-P10groups compared to GEM group. On the other hand, treatment with caraway seed oil decreased the elevated MDA. These results could be in accord with several other researches, which reported that, compounds with antioxidant properties like garlic, lycopene and diallyl sulfide inhibited the reduced antioxidant enzymes in GEM-induced rats.[1,4,48] The antioxidant activity of caraway may be due to the presence of monoterpene alcohols, linalool, carvacrol, anethole and estragol, flavonoids, and other polyphenoliccompounds.[21] Many researchers have studied flavonoids and their major role in reducing ROS.[49] Abdel-Raheem *et al.*'s[45] investigation showed that quercetin, a flavonoid, increases antioxidant enzymes (SOD, GSH-Px, and catalase) activity and reduces tissue thiobarbituric acid reactive substance (TBARS) as an index of lipid peroxidation in GEM-induced rats. It has been reported that, quercetin exerts its antioxidant effects by scavenging free superoxide and hydroxyl radicals on one hand and by inhibiting xanthine oxidase activity and lipid peroxidation on the other. Harlalka *et al.*[50] suggested that aqueous extract of *Kalanchoe pinnata*, which has a notable amount of flavonol glycosides, has protective effect on GEM-induced nephrotoxicity. Sahu *et al*.[47] have reported that treatment with naringin, a major and active flavanone glycoside, leads to increase antioxidant enzymes GSH-Pxand CAT in GEM-induced nephrotoxicity. The renoprotective effect of *Cuminum cyminum,* which has related compounds similar to caraway, was studied by Burkenet *et al*.;[51] they showed that treatment of cisplatin-induced rats with different doses of aqueous extract of *Cuminum cyminum* significantly alters serum urea, creatinine, lipid peroxidation and antioxidant enzyme levels near to normal rats. As a result, the significant antioxidant and renoprotective activity of *C. carvi* is probably related to the presence of flavonoids.

In response to various inflammatory conditions, white blood cells release pro-inflammatory cytokines. In this regard, Balakumar *et al*.[5] reported that GEM-induced nephrotoxicity stimulates inflammatory events at the site of injury and enhances the migration of monocytes and macrophages to the site of tissue damage. The key factors in renal inflammatory process is activation and nuclear translocation of NF-κB, in response to oxidative stress which is regulated by gene expression of cytokines, chemokines and adhesionmolecules.[9,10]In the present study, addition of caraway seed oil (10 mg kg^{-1}) treatment along with gentamicin (GEM-T10 group) significantly decreased pro-inflammatory cytokines(IL-6, TNF-α and IFN-γ) compared to GEM alone treated rats. Earlier studies also demonstrated that an increase in NF-κB activation by GEM nephrotoxicity is followed by an increase in the concentration of inflammatory cytokines like TNF-α and IL-6.[36,47,52] Numerous studies have shown that GEM induces renal damage by free radical generation. Hence antioxidants and free radical scavengers of natural and synthetic origin have been studied by many researchers to provide nephro-protection in GEM-induced renal injury.[53] Among many antioxidants, consumption of flavonoid containing foods and beverages has been proposed as a useful practice to limit oxidative damage in the body.[54]

In conclusion, the present study indicated that caraway seed oil can provide protective effect against GEM induced oxidative stress and nephrotoxicity. However, further investigations are essential to elucidate the exact mechanism of protection and potential usefulness of caraway seed oil as a protective agent against drugs or xenobiotic toxicity in clinical trials.

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