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Protective potential of *Trachyspermum ammi* seeds in gentamicin-induced nephrotoxicity in rabbit model

[Potencial de protección de las semillas *Trachyspermum ammi* en la nefrotoxicidad inducida por la gentamicina en modelo de conejo]

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Abstract: Nephrotoxicity is one of the most important side effects and therapeutic limitations of aminoglycoside antibiotics, especially gentamicin. Gentamicin-induced nephrotoxicity involves free radical generation, reduction in antioxidant defense mechanism and renal dysfunction. A number of crude herbal extracts have potential to ameliorate gentamicin-induced nephrotoxicity due to presence of various antioxidant compounds. Therefore the goal of current study was to evaluate the protective activity of *T. ammi* seeds aqueous extract against gentamicin-induced nephrotoxicity in albino rabbits. The results showed that gentamicin caused severe alterations in serum biochemical parameters and kidney markers along with severe alterations in kidney tissues. However, *T. ammi* extract, when given along with gentamicin, reversed the severity of gentamicin-induced nephrotoxicity by normalizing the indicators of kidney function e.g. serum urea, creatinine, blood urea nitrogen, albumin and serum electrolyte parameters indicating the nephroprotective potential of *T. ammi*. Similarly the extract has ability to augment the endogenous antioxidant enzymatic machinery by increasing the activity of antioxidant enzyme catalase and by reducing the total oxidant status. Nephroprotective potential was further confirmed by the histopathological examination. Nephroprotective potential might be due to the presence of antioxidative polyphenolic compounds in aqueous extract of *T. ammi* seeds.

Keywords: Medicinal plants, Nephrotoxicity, Antioxidant enzymes

Resumen: La nefrotoxicidad es uno de los efectos secundarios más importantes limitaciones terapéuticas de los antibióticos aminoglucósidos, especialmente gentamicina. La nefrotoxicidad inducida por gentamicina implica generación de radicales libres, la reducción en el mecanismo de defensa antioxidante y la disfunción renal. Una serie de extractos de hierbas crudas tienen potencial para mejorar la nefrotoxicidad inducida por gentamicina debido a la presencia de varios compuestos antioxidantes. Por lo tanto, el objetivo del presente estudio fue evaluar la actividad protectora del extracto acuoso semillas de *T. ammi* contra la nefrotoxicidad inducida por gentamicina en conejos albinos. Los resultados mostraron que la gentamicina causó graves alteraciones en los parámetros bioquímicos séricos y los marcadores de riñón, junto con alteraciones severas en los tejidos renales. Sin embargo, el extracto de *T. ammi*, cuando se administra junto con la gentamicina, invierte la gravedad de la nefrotoxicidad inducida por gentamicina por la normalización de los indicadores de la función renal, por ejemplo, urea sérica, creatinina, nitrógeno ureico en sangre, albúmina y los parámetros de electrolitos séricos que indican el potencial nefroprotector de *T. ammi*. Del mismo modo, el extracto tiene la capacidad para aumentar la maquinaria enzimática antioxidante endógena mediante un aumento de la actividad de la enzima antioxidante catalasa y reduciendo el estado total de oxidante. El potencial nefroprotector fue confirmado por el examen histopatológico. El potencial nefroprotector podría ser debido a la presencia de compuestos polifenólicos antioxidantes en el extracto acuoso de semillas de *T. Ammi*.

Palabras clave: Plantas medicinales, nefrotoxicidad, enzimas antioxidantes

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INTRODUCTION

Kidney is an important target of the drug toxicity, oxidative stress and toxic materials. In addition, reactive oxygen species (ROS) derived from chemical materials cause renal necrosis through not well-defined mechanisms (Zhang *et al.,* 2013; El-Deeb *et al.,* 2014; Tiong *et al.,* 2014; Zhang *et al.,* 2014). Several antimicrobial agents cause up to 60% acute renal injury with significant morbidity and mortality rates (Dashti-Khavidaki *et al.,* 2013). Gentamicin is an effective antibiotic of aminoglycoside class against Gram-negative bacterial infections. However, it causes nephrotoxicity in 30% of treated patients at recommended therapeutic doses. The mechanism of gentamicin-induced nephrotoxicity involves inflammation and high oxidative stress resulting in accumulation of creatinine, urea, and other waste products (Khaliq *et al.,* 2015). The antioxidant enzymes including glutathione peroxidase and superoxide dismutase remove (Shahzad *et al.,* 2014). Several synthetic or natural compounds have been used to avoid or restore the gentamicin nephrotoxicity (Mohamed *et al.,* 2014).

Modern era is being focused on filtering the pharmacologically active phytoconstituents of plant owing to their safety and cost effectiveness so they can be used as neutraceuticals (Jafri *et al.,* 2011; Hashmi *et al.,* 2013). Plants are major source of antioxidants like polyphenols, terpenoids, carotenoids and tocopherol. Natural antioxidants present in medicinal and dietary plants might be helpful in preventing the damage induced by oxidative stress (Goswami & Chatterjee, 2014). Co-administration of various medicinal plants possessing antioxidant activity can cure various diseases caused by free radicals generation due to oxidative stress (Mishra *et al.,* 2014).

Trachyspermum ammi commonly known as 'Ajwain' belongs to family *Apiaceae* and is widely used for good flavor and aromatic nature in traditional and culinary applications (Ram *et al*., 2012). *T. ammi* seeds are cultivated in Pakistan, Afghanistan, India, Iran and Iraq, and possess antimicrobial, antioxidant, hepatoprotective, nematicidal, anthelmintic, and gastroprotective activities (Javed *et al.,* 2006; Ramaswamy *et al.,* 2010). However nephroprotective effect of *T. ammi* seeds has not yet been evaluated. Therefore, it was worthwhile to evaluate nephroprotective and free radical scavenging activity of *T. ammi* seeds against gentamicin-induced nephrotoxicity.

MATERIALS AND METHODS

Chemicals, plant material and extraction procedure

Gentamicin sulphate (Gentamicin sulphate® injectible solution 40 mg/ml) and Silymarin (Silliver® film coated tablet 200 mg) were purchased from Wuhan Grand Pharmaceutical Group Co. Ltd. China and Abbott Laboratories Ltd. Karachi Pakistan, respectively. Seeds of *T. ammi* were purchased and authenticated from University of Agriculture, Faisalabad (UAF). Seeds of *T. ammi* were washed thoroughly with distilled water, dried in shade and finally powdered. Two hundred gram of powdered *T. ammi* seeds were macerated with water for 48 hours long with occasional stirring at room temperature. After maceration, it was filtered through Whatman filter paper. Extract was concentrated using rotary evaporator under reduced pressure and the weight of resultant dry material recorded and percentage yield was calculated. The extract was stored at 4° C till further analysis.

Animals and study design

Healthy male albino rabbits were acclimatized for 7 days and experiment was conducted in accordance with international standards of animal welfare. The rabbits were divided into six equal groups, each including six animals. Group I: Control (CN) rabbits were administered normal saline/oral/day; Group II: Gentamicin treatment (GM) was injected intraperitoneally (i.p.) 80 mg/kg body wt./day; Group III: Silymarin+ Gentamicin treatment (S+GM) was administered with aqueous solution of silymarin tablets 200 mg/kg body wt./day and gentamicin sulfate injection (i.p.) 80 mg/kg body wt./day; Group IV: Extract+ gentamicin (low dose) treatment (T.A1+ GM) was administered aqueous extract of *T. ammi* 300 mg/kg body wt./day and gentamicin sulfate injection (i.p.) 80 mg/kg body wt./day; Group V: extract+ gentamicin (high dose) treatment (T.A2+ GM) was administered aqueous extract of *T. ammi* (T.A2) 600 mg/kg body wt./day and gentamicin sulfate injection (i.p.) 80 mg/kg body wt./day; Group VI: *T. ammi* extract only group (T.A2) was administered aqueous extract of *T. ammi* (T.A2) 600 mg/kg body wt./day.

Blood sampling and histopathological examination
At 15th day, animals were sacrificed and blood samples were collected. Serum was separated after centrifugation at 1507 g for 15 minutes. Pieces of kidney from each group were fixed immediately in 10% neutral formalin for histological examination.

Table 1
Renal function markers (Mean ± SE) in control and experimental groups

Parameters	Control (CN)	Gentamicin (GM)	Silymarin+ Gentamicin (S+GM)	*T. ammi* 1+ Gentamicin (T.A1+ GM)	*T. ammi* 2+ Gentamicin (T.A2+ GM)	*T. ammi* (T.A2)
Urea (mg/dl)	55.2±3.3^{BC}	89.5± 11.5^{A}	58.9±6.5^{B}	55.7±1.79 ^{BC}	47.7±3.75^{C}	51.7±4.7^{BC}
Creatinine (mg/dl)	0.7±0.02^{B}	0.9±0.09 ^{A}	0.8±0.04 ^{B}	0.78±0.03 ^{B}	0.77±0.052^{B}	0.8±0.013 ^{B}
BUN (mg/dl)	25.7±1.6 ^{BC}	42.0±5.43 ^{A}	27.7±3.01 ^{B}	26.2±0.85 ^{BC}	22.25±1.5 ^{C}	24.2±2.3 ^{BC}
Albumin (g/dl)	3.22±0.10 ^{A}	2.74±0.08 ^{B}	3.10±0.21 ^{A}	3.07±0.04 ^{A}	3.02±0.04 ^{A}	3.07±0.02 ^{A}
TOS (µmol/L)	2.42±0.06^{C}	4.42±0.58^{A}	2.30±0.07^{C}	3.47±0.13^{B}	2.70±0.17 ^{C}	2.27±0.06 ^{C}
Catalase (KU/L)	34.8±0.95 ^{B}	24.9±2.15^{C}	39.8±2.35 ^{A}	36.5±1.36 ^{AB}	39.8±1.36 ^{A}	34.0±0.82 ^{B}

Mean values with different superscripts in a row differ significantly at P ≤ 0.05. Control (CN): vehicle; Gentamicin (GM): 80 mg/kg/day; Gentamicin + silymarin (GM+S): 80 mg/kg/day + 200 mg/kg/day; Gentamicin + *T. ammi* 1(T.A1+GM): 80 mg/kg/day + 150 mg/kg/day; Gentamicin + *T. ammi* 2 (T.A2+GM): 80 mg/kg/day + 300 mg/kg/day; *T. ammi* (T.A2)

Renal function tests and oxidant status
Serum samples were analyzed for serum albumin, urea and creatinine by using colorimetric assay kits (Merck Private Ltd, Pakistan). The samples were analyzed for serum Na^+, Cl^- and K^+ levels using Prolyte Electrolyte Analyzer (K102959), Diamond Diagnostics, USA. Serum total oxidant status (TOS) and catalase activity were determined using the previously described methods (Erel, 2005; Goth, 1991).

Statistical analysis
The data were analyzed by one-way analysis of variance and presented as mean ± SE. The groups were analyzed by Duncan multiple range test for statistical differences. The statistical significance level was accepted at P ≤ 0.05 (Steel *et al*., 1997).

RESULTS
Renal function parameters
Urea, creatinine, blood urea nitrogen and albumin are the most important indicators of kidney function (Muhammad *et al.,* 2014). As predicted, in the present study, the serum levels of urea, creatinine, blood urea nitrogen (BUN) and albumin in gentamicin-treated group were significantly high (P ≤ 0.01), indicating nephrotoxic effects of gentamicin. This increased serum urea, creatinine and BUN levels by gentamicin were antagonized by *T. ammi* seeds aqueous extract in a dose-dependent manner as shown in Table 1.

Oxidative stress analysis:
Gentamicin causes renal damage by significant increase in serum total oxidant status (TOS) and decrease in endogenous antioxidant enzyme catalase. *T. ammi* aqueous extract along with gentamicin attenuated the nephrotoxicity induced by gentamicin by decreasing the total oxidant status and raising the serum catalase activity in dose dependent manner (Table 1).

Table 2
Serum electrolyte variation (Mean ± SE) in control and experimental groups
Renal histopathology
Mean values with different superscripts in row differ significantly at P ≤ 0.05.

Parameters	Control (CN)	Gentamicin (GM)	Silymarin + Gentamicin (S+GM)	*T. ammi* 1+ Gentamicin (T.A1+ GM)	*T. ammi* 2+ Gentamicin (T.A2+ GM)	*T. ammi* (T.A2)
Sodium (mEq/L)	138.3 ± 0.4^{A}	135.3 ± 0.4^{B}	139.0 ± 0.9^{A}	137.4 ± 1.2^{A}	138.4 ± 0.4^{A}	137.9 ± 0.3^{A}
Potassium (mEq/L)	5.6 ± 0.08^{ABC}	5.3 ± 0.14^{C}	5.5 ± 0.19^{BC}	5.7 ± 0.22^{ABC}	6.0 ± 0.3^{AB}	6.2 ± 0.33^{A}
Chloride (mEq/L)	106.9 ± 0.5^{AB}	104.6 ± 0.8^{B}	108.0 ± 0.4^{A}	107.6 ± 1.0^{A}	108.5 ± 1.2^{A}	106.7 ± 1.2^{AB}

Mean values in rows sharing similar alphabets do not differ significantly

ii. Serum electrolyte variation:

Gentamicin administration resulted in significant decrease in serum electrolytes *i.e.* sodium, potassium and chloride levels (as given in Table 2). Co-administration of *T. ammi* seeds aqueous extract restored the serum electrolytes levels towards normal values.

Results of the qualitative analysis of histopathological changes are presented in Table 3. Histopathological examination of kidney tissues also supported the biochemical results. Kidney tissues of gentamicin-intoxicated rabbits showed that there was massive tubular epithelial cell necrosis, with pyknosis, karyorrhexis *and* karyolytic changes. Kidneys tissues of rabbits that received silymarin along with gentamicin exhibited mild degenerative and necrotic changes in the tubular epithelium. However, kidney tissues from *T. ammi* low dose treatment group along with gentamicin, showed a slight decrease in the gentamicin-induced tubular necrosis. The tubular lumen was dilated along with mild degenerative changes in the proximal convoluted tubules. The treatment of rabbits with high dose of *T. ammi* along with gentamicin caused significant ameliorative changes on gentamicin-induced tubular necrosis. There was regeneration of tubular epithelium and intact nucleus along with mild congestion. Kidneys of rabbits that received *T. ammi* extract at high dose without gentamicin exhibited normal tubular epithelium with mild congestion as shown in Table 3.

DISCUSSION

The pathogenesis of gentamicin-induced nephrotoxicity is reported due to high level of reactive oxygen species (ROS) as gentamicin makes a complex with iron that acts as potent catalyst of free-radical formation and enhances the generation of reactive oxygen species. Abnormal production of ROS may result in cellular injury and necrosis through peroxidation of membrane lipids, protein denaturation and DNA damage (Noorani *et al*., 2011; Ali *et al.,* 2014; Gad & El-Maddawy, 2014). Oxidative stress is one of the main mechanisms involved in acute and chronic hepatic and renal pathologies (Suganya *et al*., 2011; Qin *et al*., 2014). Natural antioxidants have a variety of biochemical actions *i.e.* inhibition of reactive oxygen species production, scavenging of free radicals and modification of intracellular redox activity (Goswami & Chatterjee, 2014). It has been reported that polyphenolic bioactive compound such as tannins, flavonoids and phenols present in different medicinal plants exhibited significantly higher antioxidant and radical scavenging property (Hussain *et al.,* 2012).

In this study, gentamicin has significantly increased oxidative stress that was clear from increased total oxidant status in gentamicin-treated group. The administration of silymarin or plant extract along with gentamicin significantly reduced total oxidant status. The stimulating factors of gentamicin nephrotoxicity are increased level of hydrogen peroxide and hydroxyl radicals, and decreased amount of intracellular glutathione level.

The results showed the severe kidney damage by administration of gentamicin, most expected by ROS mediated mechanism as evident by decreased activity of catalase. The observed decrease in the level of catalase may be due to its increased utilization for scavenging gentamicin and/or oxygen derived radicals. The administration of aqueous extract of *T. ammi* seeds restored the catalase level to base line, indicating antioxidant activity of the plant extract

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Table 3
Histopathological features as seen in the kidneys of control and experimental groups

Parameters	Control (CN)	Gentamicin (GM)	Silymarin+ Gentamicin (S+GM)	Control (CN)	Gentamicin (GM)	Silymarin+ Gentamicin (S+GM)
Coagulative necrosis	-	+++	-	-	-	-
Necrosis	-	+++	+	+	-	-
Blood vessel congestion	-	+++	-	+	+	+
Pyknosis	-	++	-	-	-	-
Karryorehexis	-	++	-	-	-	-
Karryolysis	-	++	-	-	-	-
Hyperchromatic nuclei	-	++	-	-	-	-
Glomerular congestion	-	+++	-	+	+	+

(-): normal; (+): little effect; (++): appreciable effect; (+++): severe effect

Gentamicin caused a significant increase in creatinine and BUN (Reddy *et al.*, 2012). The mechanism behind elevated serum urea and creatinine might be that gentamicin increases the entry of Ca^{+2} in the mesengeal cells leading to reduced glomerular filteration rate (Stojiljkovic *et al*., 2008). There is an inverse relationship between the quantity of urea absorbed and the rate of tubular urine flow (Kaneko *et al*., 2008). The decreased level of albumin in gentamicin treatment is might be due to high level of ROS that might be alter the protein synthesis and catabolism. This mechanism may clarify the increase in BUN in the present study. The administration of graded doses of *T. ammi* seeds reduced the levels of raised serum urea and creatinine in a dose-dependent manner suggesting that the contents of *T. ammi* seeds protected the integrity of kidney tissue. Gentamicin administration is associated with severe necrosis, desquamation in proximal tubules and dysfunction of co-transport systems and channels, leading to decreased absorption of electrolytes (Chen *et al*., 2009). The results of present study indicated that serum electrolytes *i.e.*, sodium, potassium and chloride levels were significantly reduced in gentamicin-treated animals. The decrease in electrolyte level may be due to increased wasting or reduced absorption of electrolytes resulting from kidney damage. The administration of plant extract restored electrolytes levels, indicating nephroprotective activity.

Plants are a rich source of natural antioxidants (Nandave *et al*., 2009). Phytochemical constituents of *T. ammi* seeds are responsible for its antioxidant and free radical scavenging activity (Ramaswamy *et al.*, 2010). The potential antioxidant properties of this plant might be probably due to the active hydrogen donor ability of hydroxylated substitution of calotropogenin, calotropin and calotoxin. Similarly, high molecular weight compounds and the proximity of many aromatic rings of coroglaucegenin, glucofrugoside, calotroposide A and B could be more important for the free radical

scavenging activity of this plant extract. The bioactive compounds of *T. ammi* seeds might give the possible explanation about this plant to act as reactive oxygen species scavengers (Shimmi *et al.*, 2012).

The present study indicates that administration of gentamicin results in kidney damage, which is counteracted by co-administration of plant extract, showing nephroprotective activity. It has been shown that polyphenolic compounds prevent nephrotoxicity induced by oxidative stress and restored the renal markers level. Based on the above literature, we propose that the phytochemical constituents in *T. ammi* seeds might be responsible for its antioxidant activity and protective effects in gentamicin-induced nephrotoxicity. However, additional studies are necessary to explore other possible mechanisms.

CONCLUSIONS

Our results show that aqueous extract of *T. ammi* seeds possesses antioxidant properties and restored gentamicin-induced nephrotoxic effects as evidenced by various biochemical and renal function markers.

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