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Original Article

Nephroprotective Effect of *Anethum graveolens* in a Murine Model of Gentamicin induced Nephrotoxicity

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ABSTRACT

Background: Antioxidant rich herbs possess significant activity against various disease conditions induced by oxidative stress. *Anethum graveolens* is a rich source of bioactive compounds that possess varying pharmacological activities including antioxidant. **Objective:** To evaluate the nephroprotective effect of aqueous extract of *Anethum graveolens* seeds in a murine model of gentamicin induced renal damage. **Materials and Methods:** Wistar albino rats of either sex, weighing 150-200g were divided into 5 groups; normal saline, gentamicin (80 mg/kg, i.p), aqueous extract of *Anethum graveolens* seeds at 0.5, 1 and 2g/kg/ body wt., p.o, for 8 days, the extract being administered 3 days prior and concurrently with gentamicin for 5 days. Serum urea, creatinine, uric acid, blood urea nitrogen (BUN) analyses and histopathological examination of kidney were performed. **Results:** Gentamicin treatment caused nephrotoxicity as evidenced by marked elevation in Serum urea, creatinine, uric acid and BUN (107.5±16.92 mg/dl, 0.88±0.09 mg/dl,3.05±0.29 mg/dl and 47.80±9.07 mg/dl respectively) as compared to saline treated animals. Co-administration of aqueous extract of *Anethum graveolens* at doses 0.5, 1 and 2g/kg/ body wt decreased the rise in these parameters in a dose dependent manner. However statistical significance was obtained only with 1 and 2g/kg body wt doses employed, when compared to the gentamicin treated group. Histopathological analysis revealed epithelial loss with intense granular degeneration in gentamicin treated rats, whereas the test extract mitigated the severity of gentamicin induced renal damage. **Conclusion:** Our data suggests that aqueous extract of *Anethum graveolens* seeds exhibits renoprotective effect in gentamicin induced renal damage probably due to its antioxidant actions.

Key words: *Anethum graveolens*, Drug induced nephrotoxicity, Gentamicin, Nephroprotective.

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INTRODUCTION

Acute renal failure (ARF) is a major complication of kidney, encountered globally and 20 percent of cases can be directly attributed to drugs.[1] Drugs such as antibiotics became the major implicating factor in the acute kidney injury due to indigenous functions of kidney to excrete them. This acute renal injury often leads to renal failure which in turn is associated with other pathological manifestations such as sepsis, cardiovascular disorders and diabetes.[2]

Aminoglycosides have been one of the common causes of drug induced nephrotoxicity.[3] Gentamicin is an aminoglycoside antibiotic that is still commonly used in the treatment of life-threatening infections. The broad-spectrum activity against aerobic gram positive and gram negative organisms, their chemical stability and their rapid bactericidal action has made them the first-line drugs in a variety of clinical situations. However, higher concentrations of these antibiotics are nephrotoxic.[1] Gentamicin induced renal damage is widely used model for inducing nephrotoxicity in experimental animals.[4] It is characterized by direct tubular necrosis, which is localised mainly in the proximal tubules. It is a complex phenomenon characterized by an increase in plasma creatinine, urea levels, severe proximal tubular necrosis, followed by deterioration and renal failure. The toxicity of gentamicin is thought to be related to generation of reactive oxygen species (ROS) in the kidney. Because of the evidence of mediation of ROS in gentamicin induced renal damage, several compounds with antioxidant activity have been tested to ameliorate gentamicin nephrotoxicity.[3] Several studies have claimed antioxidant property of drugs for their nephroprotective effects in gentamicin induced renal damage.[5,6,7]

Anethum graveolens L. commonly known as dill belonging to the family Umbelliferae is an annual herb growing in the Mediterranean region, central and southern Asia.[8,9] It has been used in ayurvedic medicines since ancient times. *Anethum* graveolens is used as a common household remedy against a variety of gastrointestinal disorders, e.g. indigestion, flatulence, colic pain etc. It is also used as spices and condiments in foods for their flavour, aroma and preservation. Their dried ripe fruits and essential oils have aromatic, carminative, stomachic, diuretic, galac¬togogue properties. There are various volatile components of dill seeds and herb. Carvone and limonene are monoterpenes, which are present as main constituent of dill oil from fruits. α-phellandrene, dill ether and myristicin are the compounds, which form the important odour of dill herb. Other compounds isolated from seeds are coumarins, flavonoids, phenolic acids and steroids. The two flavonoids that have been isolated from *Anethum graveolens* L. seed, quercetin and isoharmentin, have shown antioxidant activity and could counteract with free radicals.[10] Essential oil of dill exhibited various biological activities such as antimicrobial, antifungal, anti-inflammatory, antispasmodic, antidiabetic, anticancer and anti-hypercholesterolemia, due to the presence of biologically active compounds.[8] Further to this it is reported that *Anethum graveolens* possess significant antioxidant activity.[11,12] It is known from literature search that antioxidant rich herbs possess significant activity

against various disease condition characterized by induced oxidative stress. In view of the above findings the purpose of this study was to evaluate the protective role of aqueous extract of *Anethum graveolens* seeds in gentamicin induced nephrotoxicity in rats.

MATERIALS AND METHODS

This study was conducted as per the guidelines set by the Committee for the Purpose of Control and Supervision of Experiments on Animals. The study was undertaken after obtaining the approval by the Institutional Animal Ethics Committee.

Experimental Animals

Adult Wistar albino rats of either sex, weighing 150-200g, inbred in the institutional animal house were used for the study. Animals were housed in clean polypropylene cages in a controlled environmental condition (22± 3^0C, 55 ± 5% humidity and a 12 h light/ dark cycle). The animals were fed with standard rodent diet and water *ad libitum*. They were allowed to acclimatize to these conditions for one week before the experimental procedure.

Drugs and chemicals

Gentamicin sulfate injection (Piramal Health Care Ltd) dissolved in normal saline was used to induce renal damage. Gentamicin and test drug were suspended in suitable vehicle and administered either oral (p.o.) or intraperitoneal (i.p.).

Plant material and preparation of extract

Anethum graveolens seeds were procured from the local market, identified and authenticated by a local botanist and a voucher specimen of the plant (AG/12-98) is being maintained in the herbarium of Department of Botany, St Aloysius, College, Mangalore. The seeds were cleaned, dried in shade and powdered using a mechanical grinder. For the preparation of aqueous extract, the seed powder (200 grams) was macerated in 600 ml of distilled water for 24 hrs. It was filtered and concentrated over the water bath and the extract was then dried in desiccator.[13]

Experimental procedure

After acclimatization, the animals were divided randomly into five groups of 6 animals each and placed in separate cages. The animals were grouped as follows. Group one, receiving normal saline (1ml/kg body weight) intraperitoneal, was used as normal control. The second group, received daily intraperitoneal injections of gentamicin (80 mg/ kg body weight) served as disease control.[14] The animals in group three, four and five received 80 mg/kg of gentamicin intraperitoneal and in addition also received the test drug, aqueous extract of seeds of *Anethum graveolens* orally at doses 0.5g, 1g and 2g/kg body weight respectively.[15] The drugs were administered for a total duration of 8 days. The test drug, the aqueous extract of *Anethum graveolens* seeds was started 3 days prior to the commencement of the study.

Blood Sample collection and Assessment of biochemical parameters

Twenty-four hours after the last injection the rats were anesthetized with ketamine (60mg/kg) and xylazine (5mg/kg) given intraperitoneally and blood samples were collected by cardiac puncture.[16] The blood sample collected, after a standing time of 30 min, was centrifuged at 2500 rpm for 10 min. The serum was rapidly separated and processed for determination of serum urea, creatinine, uric acid and blood urea nitrogen (BUN) as an indicator of kidney damage, using commercially available kits from Aspen Diagnostics Private Ltd (Liquid stable biochemistry kits). The animals were sacrificed and both kidneys were isolated. The kidneys from all the groups were weighed and processed for histopathological examination.

Histopathological examination

The kidneys from all the groups, fixed in 10% neutral buffered formalin were processed and embedded in paraffin wax and sections were taken using a microtome. The sections (5 microns) were then stained with haematoxylin and eosin and subjected to examination under light microscope. They were evaluated and assigned scores as follows:[14]

Score 0 = Normal

Score 1 = Areas of focal granulovacuolar epithelial cell degeneration and granular debris in tubular lumens with or without evidence of tubular epithelial cell desquamation of small foci (<1% of total tubule population).

Score 2 = Tubular epithelial necrosis and desquamation easily seen but involving less than half of cortical tubules.

Score 3 = More than half of proximal tubules showing desquamation of necrosis but involved tubules easily found.

Score 4 = Complete or almost complete tubular necrosis.

Statistical analysis

Data was expressed as mean ± standard error of mean (SEM). The differences among treated groups was analysed by one-way ANOVA followed by Tukey's test. A value of $p < 0.05$ was considered statistically significant.

RESULTS

Effect on biochemical parameters

In the present study, gentamicin (80mg/kg) when injected for eight consecutive days caused marked nephrotoxicity as is evident from Table 1, showing significant ($p < 0.05$) increase in serum urea (107.5±16.92mg/dl), creatinine (0.8±0.09mg/dl), uric acid (3.05±0.29mg/dl) and BUN(47.8±9.07 mg/dl) as compared to normal control animals. The test drug, aqueous extract of *Anethum graveolens* depicted protective effects at doses 0.5, 1 and 2g/kg body weight by reducing the levels of serum urea, creatinine, uric acid and BUN as compared to gentamicin treated group. There was a significant nephroprotective effect at doses 1 and 2g/kg body weight of the test drug as evidenced by a significant decrease in serum urea, creatinine and blood urea nitrogen ($p<0.05$) as compared to gentamicin treated group. Though there was a decrease in serum uric acid at all 3 dose of test drug, it was not significant when compared to gentamicin treated group.

Histopathological evaluation

The histological changes in the kidney of all the groups were graded and the results are expressed in Table 2. It was noted that the microscopic study of the kidney sections of the rats which were treated with normal saline appeared histologically normal with the score of 0. The kidney sections of the gentamicin treated group showed extensive tubular necrosis involving most of the renal cortex with an average score of 3. The observed changes included dilated tubules with denuded epithelium, intracytoplasmic vacuolation, blebs, interstitial and glomerular congestion, stromal inflammation and granular casts (Figure 1). The histomorphology of the kidney sections in rats treated with aqueous extract of *Anethum graveolens* showed moderate tubular epithelial degeneration and desquamation. Treatment with doses 0.5 and 1g/kg of aqueous extract of *Anethum graveolens* appeared to mitigate the severity of the gentamicin treatment-induced renal necrosis, preserving the normal histology with an average Score of 1.2 and 0.7 respectively (Figure 2). The dose at 2g/kg of *Anethum graveolens* showed protection against the gentamicin induced histological alteration and also showed signs of renal tubular regeneration with an average score of 0.2 (Figure 3) providing evidence of nephroprotection afforded by *Anethum graveolens.*

Table 1: Effect of gentamicin and *Anethum graveolens* on various biochemical parameters.

Group	Parameters			
	Urea (mg/dl)	Creatinine (mg/dl)	Uric acid (mg/dl)	BUN (mg/dl)
Normal control	53.83±18.6	0.55±0.62	1.71±0.34	25.09±8.69
Gentamicin (80mg/kg)	107.5±16.92*	0.88±0.09*	3.05±0.29*	47.80±9.07*
Anethum graveolens (0.5g/kg)	68.98±14.51	0.75±0.14	2.05±0.58	32.13±6.77
Anethum graveolens (1g/kg)	46.30±6.21**	0.48±0.04**	2.20±0.50	21.55±2.90**
Anethum graveolens (2g/kg)	43.95±2.11**	0.43±0.02**	2.55±0.16	20.45±0.97**

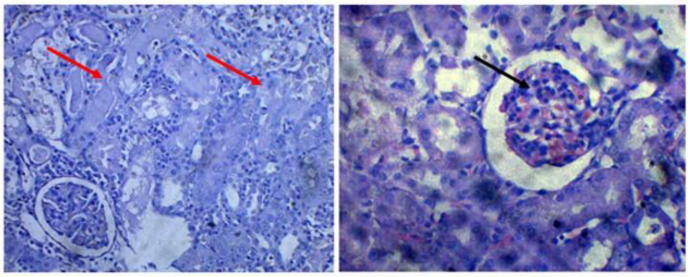
Values are expressed as mean ± SEM

*p < 0.05 when compared to normal control group

**p< 0.05 when compared to gentamicin treated group

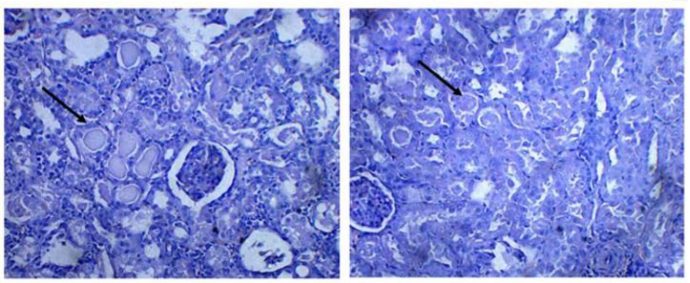
Table 2: Histological features and histopathological scoring in gentamicin and *Anethum graveolens* treated groups.

Histopathological Feature	Normal control	Gentamicin	*Anethum graveolens* (0.5g/kg)	*Anethum graveolens* (1g/kg)	*Anethum graveolens* (2g/kg)
Glomerular infiltration	_	+++	+++	++	_
Interstitial congestion	_	+++	++	+	_
Interstitial infiltration	_	+++	++	+	+ Sparse, scattered
Average score	0	3	1.2	0.7	0.2



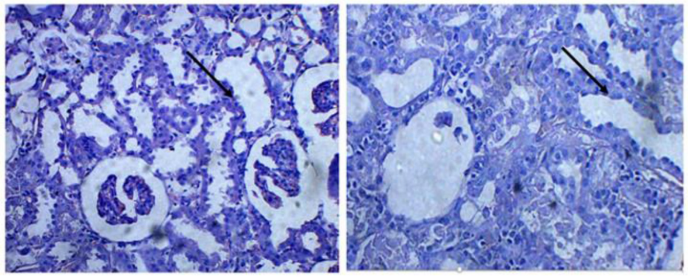
A B

Figure 1: Photomicrograph of kidney sections of only gentamicin treated group: showing extensive necrosis (red arrow), glomerular congestion (black arrow), interstitial congestion and inflammatory cell infiltration-score 3 (40 X, H &E).



A B

Figure 2: Photomicrograph of kidney sections treated with aqueous extract of *Anethum graveolens* at doses 0.5 and 1 g/kg: showing granular casts (black arrow) and degenerated tubular epithelium with interstitial inflammatory infiltration -score 1.2 & 0.7 respectively (40X, H &E).



A B

Figure 3: Photomicrograph of kidney sections treated with aqueous extract of *Anethum graveolens* at dose 2g/kg: showing dilated tubules with flattened epithelium in the regenerative phase after tubular injury (black arrow), shedding of necrotic cells and sparse interstitial infiltration –score 0.2 (40X, H &E).

DISCUSSION

Gentamicin being the most nephrotoxic antibiotic amongst all aminoglycosides, its over dosing ultimately leads to acute nephrotoxicity and consequently precipitating ARF in human as well as animal subjects. Hence, gentamicin-induced nephrotoxicity is a recognized experimental model of ARF caused by oxidative stress generated by free radicals. The intracellular metabolism of drugs leads to the formation of reactive metabolites, which are toxic for cell, as are free radicals. These ROS produce cellular injury and necrosis through several mechanisms including peroxidation of membrane lipids, protein denaturation and DNA damage resulting in dramatic modification of structure and function.[17,18] In the present study, we observed that gentamicin at a dose of 80mg/kg produces significant renal damage when compared to control group which is demonstrated by increase in serum urea, creatinine, uric acid, BUN(107.5±16.92 mg/dl, 0.88±0.09 mg/dl,3.05±0.29 mg/dl, 47.80±9.07 mg/dl respectively) and renal tubular necrosis as established with

previous reports.[5,6,7,18] Administration of aqueous extract of *Anethum graveolens* seeds provided marked functional and histological protection against acute renal damage in rats treated with gentamicin as evident by the changes in biochemical parameters observed and its significant role in maintaining tubular integrity.

Agents with significant free radical scavenging property can either inhibit or alleviate the renal damage induced by drugs. Antioxidants are capable of stabilizing or deactivating free radicals before they attack cells and have the ability to protect the body from damage caused by free radical induced oxidative stress. The importance of plants as natural antioxidants is well established and there is a growing interest because of their antioxidative properties in scientific research. These properties of herbs and plants can be attributed to the presence of numerous phytochemicals in them. It is shown that several phyto derived compounds because of their antioxidant properties have been experimentally used to reduce the gentamicin nephrotoxicity and confer cytoprotection against free radical induced damage.[4,11]

The phytochemical analysis of dill plant demonstrated the presence of alkaloids, flavonoids, tannins, saponins, cardiac glycosides, terpenoids, anthocyanin which are important for antioxidant and free radical scavenging properties.[19] The antioxidant properties of the above phytoconstituents are because of their redox activities, which allow them to have different activity such as hydrogen donors, reducing metabolites, reactive oxygen species quenchers and metal chelation.[20] It was reported that water extract fraction of dill had significant DPPH scavenging activity. The stable free radical of DPPH value is generally used to evaluate plant antioxidant ability by working as hydrogen donors or free radical scavengers. D-carvon and D-limonene, the active ingredients in *Anethum graveolens* have shown hepatoprotection in liver damage induced by carbon tetrachloride (CCl_4) in rats. The lipid peroxidative degradation of biomembranes is one of the principle causes of hepatotoxicity due to CCl_4. D-carvon and D-limonene afford protection by stabilizing the hepatocyte membrane, decreasing the production of free radicals and release of enzymes into the blood.[21] It is also reported from other studies that *Anethum graveolens* possesses significant antioxidant potential.[11,21,22] Because of the above properties dill seeds have the potential to protect biological molecules from oxidation, decreasing the rate of lipid peroxidation. Pre-treatment and simultaneous administration of aqueous extract of *Anethum graveolens* seeds with gentamicin provided nephroprotection as substantiated by improvement in biochemical parameters and renal histology. Though the underlying mechanism is not clear, it might be probably related to the presence of phytochemicals and their antioxidant potential. Further studies are required to explore and associate the antioxidant effect to nephroprotection afforded by *Anethum graveolens* which might help better characterize and ascertain its mechanism in attenuating gentamicin induced renal damage.

CONCLUSION

To conclude, this study provides scientific evidence of the nephroprotective effects of orally administered aqueous extract of *Anethum graveolens* seeds in gentamicin induced renal damage. It further suggests that the observed protective effects of *Anethum graveolens* in gentamicin nephrotoxicity could be attributed to its well-known antioxidant potential which needs to be established by future studies.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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