The Possible Protective Effect of Simultaneous Administration of Alcoholic *Ammi majus* Seeds' Extract Against Gentamicin-induced Nephrotoxicity in Rats

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الخلاصة

يعتقد إن أجناس الاوكسجين النشطة تلعب دورا هاما في التلف الخلوي والتليف الحاصل في السمية الكلوية لعقار الجنتمايسين وعن طريق العديد من الميكانيكيات المعقدة. إن نبات الخلة الشيطانية يعتبر مصدرا غنيا للعديد من المركبات التي تملك فعالية مضادة للاكسدة اضافة لغيرها من الفعاليات.

الهدف من هذه الدراسة هو تقييم التأثير الوقائي للمستخلص الكحولي لبذور نبات الخلة الشيطانية ضد السمية الكلوية لعقار الجنتمايسين عند اعطاءهما بصورة متزامنة.

تم تقسيم ثمانية عشر جرذا بصورة عشوائية الى ثلاثة مجاميع: المجموعة الاولى (مجموعة سيطرة) تلقت حقنة 0.5 مل من محلول ملحي متجانس داخل الصفاق لمدة ستة ايام متتالية. المجموعة الثانية تلقت جرعة جنتمايسين مقدارها 100 ملغ/كغم في اليوم داخل الصفاق ولمدة ستة ايام متتالية. المجموعة الثالثة تلقت نفس جرعة الجنتمايسين داخل الصفاق ومحلول مائي للمستخلص الكحولي لبذور نبات الخلة الشيطانية بجرعة مقدارها 128 ملغ/كغم في اليوم عن طريق الفم بصورة متزامنة ولمدة ستة ايام متتالية.

تم قياس مستوى اليوريا والكرياتين في مصل الدم و MDA نسيج الكلية وتركيز GSH لتقييم التأثير الوقائي للمستخلص الكحولي لبذور الخلة الشيطانية ضد السمية الكلوية للجنتمايسين عند إعطاءهما بصورة متزامنة.

أظهرت الدراسة إن مستويات اليوريا والكرياتين في مصل الدم في المجموعة التي أعطيت الخلة الشيطانية كانت اقل من تلك في المجموعة التي أعطيت الجنتمايسين لوحده ($P<0.05$). المعاملة المتزامنة مع نبات الخلة الشيطانية قللت مستويات MDA نسيج الكلية ($P<0.001$) عند مقارنتها مع المجموعة التي أعطيت الجنتمايسين فقط. مع ذلك قدمت هذه المعاملة المتزامنة زيادة معنوية في مستوى GSH الكلية ($P<0.05$).

بينت النتائج إن المستخلص الكحولي لبذور نبات الخلة الشيطانية لها تأثير وقائي ضد السمية الكلوية للجنتمايسين عند إعطاءهما بصورة متزامنة. هذا التأثير الوقائي قد يكون عن طريق النشاط المضاد للأكسدة لمكونات النبات الفعالة.

نوصي بدراسة أخرى لتقييم التأثير الوقائي للمعاملة المسبقة بنبات الخلة الشيطانية ضد السمية الكلوية للجنتمايسين.

Abstract

In gentamicin-induced nephrotoxicity, reactive oxygen species (ROS) is believed to play a pivotal role in cellular damage and necrosis via several complex mechanisms. *Ammi majus* L. (*Apiaceae*) is a rich source of many compounds which possess, among others, anti-oxidant activities.

The aim of this study is to assess the possible protective effect of the alcoholic extract of *Ammi majus* seeds against gentamicin-induced nephrotoxicity.

Eighteen rats were divided randomly into three groups: ***Group 1*** (control) received a daily intraperitoneal (ip.) injection of 0.5 ml isotonic saline for six successive days. G***roup 2*** received gentamicin dose of 100 mg $kg^{-1}day^{-1}$ (ip.), for six successive days. ***Group 3*** received simultaneously the gentamicin dose (ip.) and aqueous solution of *Ammi majus*seeds' alcoholic extract (orally), at a dose of 128 mg kg^{-1} day^{-1} for six successive days.

Serum urea, creatinin, tissue MDA and GSH concentrations were measured to assess the possible protective effects of *Ammi majus*.

Serum creatinine and urea levels in *Ammi majus* treated group were lower than the group treated with gentamicin alone ($P < 0.05$). Simultaneous treatment with *Ammi majus* normalized the kidney tissue MDA content ($P < 0.001$) when compared to gentamicin-treated group. However, simultaneous treatment with *Ammi majus* provided a significant increase in kidney GSH levels ($P < 0.05$).

In conclusion *Ammi majus* seeds' alcoholic extract has a protective effect against gentamicin-induced nephrotoxicity when given simultaneously. This protective effect may be mediated by the antioxidant activity of its active constituents.

Further study is needed to assess the effect of treatment with *Ammi majus* extract before the gentamicin-induced nephrotoxicity.

Key words: *Ammi majus,* Gentamicin, Nephrotoxicity, GSH, MDA.

Introduction:

Gentamicin, an aminoglycosidic antibiotic is widely used to treat various gram negative infections [1]. However, treatment schedule induces nephrotoxicity, which accounts for 10–20% cases of acute renal failure[2]. Gentamicin administration into rats provides an excellent model of acute renal failure for studying the therapeutic potential of different drugs[3]. In gentamicin-

induced nephrotoxicity, reactive oxygen species (ROS) is believed to play a pivotal role in cellular damage and necrosis via several complex mechanisms including peroxidation of membrane lipids, protein denaturation and DNA damage[4]. It was reported that gentamicin acts as an iron chelator, and that the iron–gentamicin complex is a potent catalyst of free radical formation[5]. Accordingly, the administration of several compounds with antioxidant activity has been successfully used to prevent or ameliorate gentamicin-induced nephrotoxicity[6,7].

Ammi majus L. (*Apiaceae*) seeds contain different active ingredients namely, xanthotoxin, bergapten,imperatorin, isoimperatorin, isopimpinellin, and marmesinin[8]. The pharmacological activity of *Ammimajus* has been known since the work of Schoberg and Sina[9] when it was shown that the therapeutically effective substances of these plants are furanocoumarins. The fruit of *Ammimajus* has been used in the Mediterranean and bordering regions in the treatment of leucoderma, *psoriasis*, v*itiligo* and the production of suntan lotion[10,11].

Coumarins and furanocoumarins possess, among others, anti-oxidant activities, probably due to their structural analogy with flavonoids and benzophenones[12].Indeed, this structure type could bind transition metal ions, such as Fe(III), and thus inhibit hydroxyl radical and hydrogen peroxide formation produced by Fenton's reactions. Furthermore, their hydroxyl functions are potent $H^{\cdot}$ donors for free radical acceptors, due to electron delocalization across the molecule[13]. Thus, coumarins and furanocoumarins could be potent ROS scavengers and metal chelating agents.

The aim of this study was to assess the possible protective effect of the simultaneously administered alcoholic *Ammi majus* seeds' extract against gentamicin-induced nephrotoxicity.

Materials and Methods:

Dried seeds of Iraqi *Ammi majus L*. was obtained from Department of Pharmacognosy and Medicinal Plants, College of Pharmacy, University of Baghdad after taxonomic identification performed by Iraqi National Herbarium. The alcoholic extract of *Ammi majus L*. seeds was prepared according to the method of Meier B[14].

Gentamicin sulphate (Megental®) was purchased from Menarini (Florence, Italy). 5,5ó-dithiobis-2-nitrobenzoic acid (DTNB),Sulfosalicylic acid, Thiobarbituric acid, Phosphotungustic acid, were supplied from BDH chemicals (Poole, England) .

Eighteen adult albino rats weighing 200-250gm were used in this study; they were obtained from and maintained in the Animal House of the Collage of Pharmacy; University of Baghdad, under the conditions of controlled

temperature. Animals were fed commercial pellet and tap water in free access *ad libitum.*

To study the possible protective effect of *Ammi majus* extract against gentamicin-induced nephrotoxicity, rats were randomly divided into 3 groups containing six rats in each one, as follows:

Group 1 (control): received a daily intraperitoneal (i.p) injection of 0.5 ml isotonic saline for six successive days.

Group 2: received gentamicin in a dose of 100 mg $kg^{-1}day^{-1}$ (i.p), for six successive days. This dose is well known to cause significant nephrotoxicity in rats [7,15- 17] .

Group 3: received simultaneously the gentamicin dose (i.p.) and aqueous solution of Ammi majusseeds' alcoholic extract (orally), at a dose of 128 mg kg^{-1} day^{-1} for six successive days. The dose of Ammi majus seeds' extract used was selected based on a previous study[18].

The animals in all groups were decapitated 24 h after the last treatment. Kidney was quickly excised, homogenized and utilized for the estimation of malonaldehyde (MDA) contents[19] and glutathione (GSH) levels[20]. Blood was collected by intra-cardiac puncture and then centrifuged at 3000 rpm for 15 minute to obtain serum, which was used for the estimation of urea and creatinine, using commercially available kits.

Statistical Analysis:

The data were expressed as Means±SEM. Differences between groups means were estimated using t-test and one way analysis of variance (ANOVA), using Microsoft office Excel for Windows. Results were considered statistically significant at $P < 0.05$.

Results:

Table-1showed thatserum creatinine and urea levels were significantly higher ($P < 0.001$) in gentamicin-treated rats when compared to the control group. Moreover, serum creatinine and urea levels in group of rats which received *Ammi majus*with gentamicin were significantly lower than in group treated with gentamicin alone ($P < 0.05$).

Groups	Serum Creatinine (mg/dl)	Serum Urea (mg/dl)
Control	0.49 ± 0.1	46 ± 1.8
Gentamicin alone, P1	1.21 ± 0.16**	107 ± 4.9**
Gentamicin + Ammi majusP2	0.69 ± 0.15*	81 ± 3.6*

Table-1: Effect of Gentamicin alone and its combination with Ammi majus on serum creatinine and urea levels of the study groups.
P1: compared to controls; P2: compared to gentamicin treated group; * p < 0.05; ** p < 0.001.

Table-2 showed that gentamicin-treated group had a significantly higher content of MDA ($p < 0.001$) in kidney tissue, but had no significant difference in GSHcontent when compared with the control group ($p > 0.05$).

Simultaneous administration of*Ammi majus*with gentamicin resulted in a significant decrease in the contents of MDA in renal tissue homogenate ($p < 0.001$) when compared to gentamicin treated group. However, simultaneous treatment of*Ammi majus* with gentamicin provided a significant increase in kidney GSH content ($p < 0.05$).

Groups	MDA(nmol g protein^{-1})	GSH(nmol g tissue^{-1})
Control	78.5 ± 4.1	11.7 ± 0.88
Gentamicin alone, P1	128.5 ± 5.1**	10.8 ± 0.92
Gentamicin + Ammi majusP2	52.4 ± 2.18**	16.3 ± 0.82*

Table-2: Effect of Gentamicin alone and its combination with Ammi majus on renal MDA, and GSH of the study groups.
P1: compared to controls; P2: compared to gentamicin treated group; *p < 0.05; **p < 0.001.

Discussion:

The clinical usefulness of gentamicin is limited due to its nephrotoxicity, manifested as acute tubular necrosis and impairment in renal function. In the present study, it was shown that administration ofgentamicin to rats caused a reduction in glomerular function which is characterized by increased serum creatinine andurea (table-1).

The impairment in glomerular function wasaccompanied by an increase in kidney tissue MDA contents.The observations manifested by this study are in accordance with those of other workers [7,21-24].

Co-administration of *Ammi majus* with gentamicin to rats resulted in normalization of the serum levels of creatinine and urea which may indicate an increase in the glomerular function.

The exact mechanism by which gentamicin induce nephrotoxicity is unknown, however, several investigators reported that aminoglycoside antibiotics are a class of drug capable of generation ROS which can be directly involved in gentamicin-induced damage. Malonaldehyde, the end product of lipid peroxidation in tissues, results in a decrease in polyunsaturated fatty acid content, which serves as substrate for free radicals. The interaction between cationic drugs such as aminoglycosides, with the anionic phospholipid is considered the first step for the development of gentamicin toxicity[17,25].

On the other hand, some authors reported that iron is important in models of tissue injury, because it is capable of catalyzing free-radical formation. Gentamicin and some antibiotics acts as an iron chelator and that have been

shown to cause release of iron from renal cortical mitochondria and iron-gentamicin complex is a potent catalyst of free-radical formation and enhance the generation of ROS[4,26].

Many studies showed that *Ammi majus* extract has strong antioxidant properties, produced by its many active constituents such as imperatorin[27,28], bergapten[29,30], isoimperatorin[29], and isopimpinellin[31].

The effect of *Ammi majus* extract on GSH level could be one of the important factors which prevent lipid peroxidation induced in rats by gentamicin.Reduced glutathione has powerful antioxidant properties through direct and indirect actions. The indirect effect through reduction of other antioxidants[32,33]. The main action of GSH is direct reducing action on hydroperoxides, which is catalyzed by the enzyme gluthathione peroxidase (GPX)[33], in addition to the enzymes Glutathione Reductase (GR) and glucose-6-phosphate dehyrogenase (G6PD) which catalyze and maintain the redox state of GSH[32]. The three enzymes GPX GR, and G6PD are known as glutathione peroxidase system which areresponsible for antioxidant activity through reducing and regeneration of GSH[34].

In conclusion *Ammi majus* extract has a protective effect against gentamicin-induced nephrotoxicity when given simultaneously. This protective effect may be mediated by the antioxidant activity of its active constituents.

Further study to assess the effect of treatment with *Ammi majus* extract before gentamicin-induced nephrotoxicity is recommended.

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