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Protective effect of the methanolic extract of *Eryngium caucasicum Trautv* on gentamicin-induced toxicity in mice: a histopathological investigation

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Abstract

The generation of reactive oxygen species and the resultant oxidative stress are significant contributors to the toxic effects of gentamicin. *Eryngium caucasicum Trautv*, a member of the Apiaceae family, is recognized for its strong antioxidant effect. This study aimed to evaluate the effect of the methanolic extract of this plant on gentamicin-induced toxicity, with a focus on histopathological alterations of kidney and liver. A total of 32 mice were randomly assigned to four equal groups and treated over a period of 10 days. The first group acted as the control and received no treatment. The second group was given gentamicin through intraperitoneal injection. The third group received the herbal extract orally, while the fourth group was administered the herbal extract orally alongside the intraperitoneal injection of gentamicin. The findings revealed that the oral administration of the herbal extract significantly reduced gentamicin-induced hemorrhage and inflammation in kidney. Furthermore, there was a decrease in gentamicin-induced hyperemia, congestion, degeneration, and necrosis in kidney, although these changes did not achieve statistical significance. The co-administration of the herbal extract also led to a reduction in gentamicin-induced hyperemia and congestion in liver tissue. The liver data did not demonstrate statistical significance in either gentamicin-induced hepatotoxicity or the hepatoprotective effects of the herbal extract. The results of this study suggest that the methanolic extract of *E. caucasicum Trautv* offers obvious protective benefits to kidney tissues against the toxic effects of gentamicin, while the antibiotic did not exhibit significant hepatotoxicity at the administered dosage.

Introduction

Gentamicin, an aminoglycoside antibiotic, is used extensively worldwide to treat severe human and animal infections caused by gram-negative bacteria (Ahmed and Mohamed, 2019). Despite the adverse effects associated with gentamicin, such as nephrotoxicity, ototoxicity, and hepatotoxicity, this antibiotic continues to be extensively employed in clinical practice (Arjinajarn*et al.,* 2017; Chen *et al.,* 2017; Kaplan *et al.,* 2017).The toxicity of aminoglycosides, including gentamicin, is thought to result from the production of reactive oxygen species (ROS) in kidney and liver tissue (Ali, 1995; Al-Majed*et al.,* 2002; Reiter *et al.,* 2002; Lesniak*et al*., 2005).

Even minimal quantities of antioxidants present in food can protect the body from various forms of oxidative damage induced by oxygen free radicals (Banerjee *et al.,* 2001; Stojiljkovic*et al.,* 2012). In this context, natural resources, particularly medicinal plants, provide a source of natural antioxidants that

could potentially serve as a therapeutic approach to alleviate the toxic effects resulting from drugs that induce oxidative stress.

As mentioned earlier, gentamicin-induced nephrotoxicity and hepatotoxicity have been attributed to its oxidative effects. Consequently, we opted to utilize an extract from an edible plant known for its confirmed antioxidant effects, as evidenced by previous research. This plant, scientifically classified as *Eryngium caucasicum Trautv*, belongs to the Apiaceae family. It is commonly found as a vegetable in the forests and gardens of northern Iran, where the young leaves are utilized to enhance the flavor of local cuisine (Nabavi*et al.,* 2008). Extracts from the genus "*Eryngium*" have shown effects such as destroying human tumor cells, anti-inflammatory properties, and effectiveness as antivenom in countering the toxins produced by snakes and scorpions, along with antibacterial, antifungal, antimalarial, antioxidant, and anti-diabetic activities. Research involving various species of *Eryngium* has revealed the presence of essential compounds, including terpenoids, saponins, flavonoids, coumarins, polyacetylenes, and steroids. The extracts and compounds derived from these *Eryngium* species have demonstrated their potential applications in both nutrition and medicinal practices (Wang *et al.,* 2012).

Considering the scarcity of research focused on the use of different extracts from *E. caucasicum* in the context of oxidative stress, this study seeks to investigate the histopathological effects of the methanolic extract of this plant on gentamicin-induced toxicity.

Materials and Methods

The plant was gathered from the forest of Mazandaran province in December, and sent to the Botanical Research Institute of Ferdowsi University of Mashhad for verification of its identity and scientific nomenclature. It was identified and confirmed as *E. caucasicum Trautv*. The plant leaves were dried at room temperature and gathered in a dark container. Dried and finely powdered plant leaves were mixed with 80% (v/v) methanol at a ratio of 5 ml/g and placed in a laboratory shaker for 72 hours. Following the filtration process and the removal of methanol through rotary distillation, the lyophilization method was employed to eliminate water. Gentamicin was purchased from Darupakhsh, Company (Teheran, Iran). The other chemicals were analytical grade and sourced from Sigma Company (St. Lewis, Missouri, USA) and Merck Company (Darmstadt, Germany).

A total of 32 healthy adult male mice were divided into four equal groups and kept under standard conditions. This research was conducted ethically in accordance with the Ethics Committee of the Ferdowsi University of Mashhad, Mashhad, Iran. All groups were provided with drinking water and a basic diet for duration of 10 days. The group number 1 served as the control group and received no treatment. The group number 2 was administered gentamicin intraperitoneally at a dosage of 80 mg/kg for 10 days. The group number 3 received the herbal extract via the gavage method at a dosage of 500 mg/kg for 10 days. Lastly, the group number 4 was given gentamicin at a dosage of 80 mg/kg intraperitoneally and the herbal extract at a dosage of 500 mg/kg via the gavage method for 10 days.

Upon completion of this period, the mice were euthanized using the cervical dislocation technique. In order to evaluate the histological changes in the kidney and liver, H&E staining was performed. The severity of histopathological alterations was assigned a score ranging from 0 to 3 which was subsequently expressed as mean ranks for statistical analysis.

Statistical analysis of the data was conducted using SPSS version 26. The results were presented as mean rank. The Kruskal–Wallis test was employed for data analysis. A p-value of less than 0.05 was deemed statistically significant.

Results

Kidney

Administration of gentamicin in group 2 resulted in a significant increase in hyperemia and congestion, degeneration and necrosis, as well as hemorrhage and inflammation within the kidney tissue when compared to the control group ($p < 0.05$) (Table 1).

The oral administration of the herbal extract in group 3 did not cause any significant histopathological alterations in the kidney tissue when compared to the control group ($p < 0.05$) (Table 1).

Co-administration of the herbal extract plus gentamicin in group 4 resulted in a significant reduction in hemorrhage and inflammation in kidney tissue relative to group 2 ($p < 0.05$) (Table 1). Additionally, the concurrent administration of the herbal extract and gentamicin mitigated the severity of hyperemia and congestion, as well as degeneration and necrosis, when compared to the second group;

however, this reduction did not reach statistical significance ($p < 0.05$) (Table 1 and Fig. 1).

Liver

The administration of gentamicin in group 2 led to a significant increase in hyperemia and congestion in the liver tissue in comparison to the control group. Microscopic examination of the liver tissue further indicated that gentamicin treatment was associated with increased degeneration, necrosis, and hemorrhage relative to the control group; nevertheless, this increase did not achieve statistical significance.

The administration of the herbal extract in group 3 did not result in any significant histopathological changes in the liver tissue when compared to the control group ($p < 0.05$) (Table 2).

Comparison between the groups 2 and 4 indicated that co-administration of the herbal extract and gentamicin resulted in a decrease in hyperemia and congestion within the liver tissue. However, this decrease did not reach statistical significance ($p < 0.05$) (Table 2). Furthermore, this co-administration did not produce any significant alterations in degeneration, necrosis, or hemorrhage when compared to group 2 ($p < 0.05$) (Table 2 and Fig. 2).

Table 1. Effect of extract on hyperemia and congestion, degeneration and necrosis, hemorrhage, and inflammation of kidney tissue.

Groups	Hyperemia and congestion	Degeneration and necrosis	Hemorrhage	Inflammation
1	9.71[a]	9.64[a]	10.43[a]	11.50[a]
2	23.29[b]	23.00[b]	23.29[b]	21.79[b]
3	12.50[ab]	12.57[ab]	13.86[ab]	13.21[ab]
4	12.50[ab]	12.79[ab]	10.43[a]	11.50[a]

Values were mean rank ($p < 0.05$)

Table 2. Effect of extract on hyperemia and congestion, degeneration and necrosis, and hemorrhage of liver tissue.

Groups	Hyperemia and congestion	Degeneration and necrosis	Hemorrhage
1	7.93[a]	9.50[ab]	13.36
2	23.36[b]	20.00[b]	17.93
3	9.07[a]	8.00[a]	13.36
4	17.64[ab]	19.36[b]	13.36

Values were mean rank ($p < 0.05$)

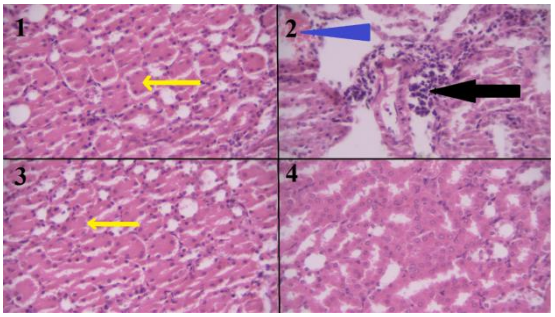

Fig. 1. The pathology images (magnification: 400X) of kidney tissues in experimental groups: (1) Group 1 or control group. (2) Group 2 or gentamicin group. (3) Group 3 or herbal extract. (4) Group 4 or co-administration of herbal extract and gentamicin. The yellow arrows indicate cell swelling and necrosis. The thick black arrow indicates inflammation. The blue triangle indicates hemorrhage.

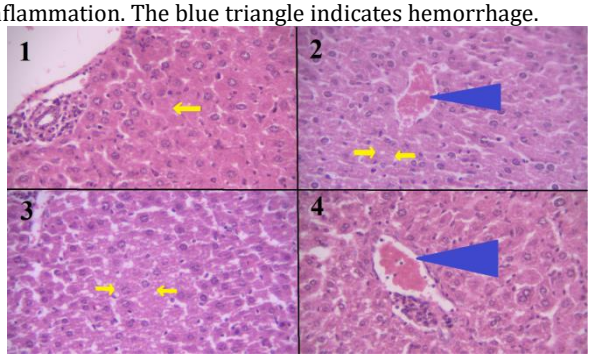

Fig. 2. The pathology images (magnification: 400X) of liver tissues in experimental groups: (1) Group 1 or control group. (2) Group 2 or gentamicin group. (3) Group 3 or herbal extract. (4) Group 4 or co-administration of herbal extract and gentamicin. The yellow arrows indicate hepatocyte vacuolation and necrosis. The blue triangles indicate hyperemia.

Discussion

The phenomenon of drug-induced nephrotoxicity is gaining recognition as a significant precursor to various kidney diseases. The nephrotoxicity associated with aminoglycosides, particularly gentamicin, is now widely acknowledged (Mingeot-Leclercq and Tulkens, 1999).

Agents mitigating aminoglycoside-induced nephrotoxicity would offer a distinct clinical advantage in therapeutics utilizing gentamicin. Given this, the present study aimed to assess the nephroprotective effects of the methanolic extract of *E. caucasicum Trautv* in gentamicin-induced toxicity in a murine model.

In this study, the present findings confirmed that gentamicin induced nephrotoxicity. These findings align with the results of prior research, including a

study carried out by Fauzi*et al.,* which in 2020 assessed the impact of three different doses of gentamicin in kidney histology. Their research findings indicated that all gentamicin-treated groups of rats experienced kidney damage and the group of rats administered higher doses of gentamicin exhibited a greater severity of histopathological alterations (Fauzi*et al*., 2020).

Another promising finding was that the oral administration of the specified dosage of this herbal extract does not result in any significant damage to the kidney and liver tissue or overall health of the mice, representing a significant finding for future research on this herbal extract. Furthermore, co-administration with this extract significantly reduced the gentamicin-induced kidney damage. A similar conclusion was reached by Eslami *et al.,* which in 2011 assessed the impact of a 400 mg/kg dosage of this herbal extract on gentamicin-induced nephrotoxicity, focusing on biochemical markers associated with kidney function. The protective effect observed in their study was ascribed to the antioxidant characteristics of the bioactive compounds present in the extract, which mitigated the oxidative stress induced by gentamicin administration (Eslami *et al.,* 2011). This interpretation is based on the established knowledge regarding oxidative stress induced by gentamicin, as evidenced by research findings, including those from the study conducted by Karatas *et al.* (2004) and the antioxidant effect of *E. caucasicum* which was reported in a prior study by Nabavi *et al.,* in 2008, which demonstrated that the extract of *E. caucasicum* has the ability to neutralize free radicals (Nabavi *et al.,* 2008).

Contrary to the study of Jafaripour *et al.* (2019) which indicated a gentamicin-induced liver dysfunction based on elevated levels of AST and ALT in rats, our study did not observe any histopathological evidence to support the hepatotoxic effects of gentamicin. The discrepancies in results may be attributed to the use of different gentamicin dosages, variations between the two animal species, and our emphasis on histopathological damage rather than on liver dysfunction.

It should be noted that many plant-derived compounds exhibit instability and limited bioavailability, which presents obstacles to their application in disease treatments. Consequently, advancements in nanotechnology have been extensively utilized to tackle these issues and enhance the solubility and bioavailability of natural antioxidants. For instance, synthesizing a nanocarrier for this herbal extract may lead to enhanced renoprotection (Myint *et al.,* 2021).

The main objective of this study was to complete those earlier studies that have explored the therapeutic benefits of medicinal plants in relation to various diseases. This investigation, alongside numerous preceding studies, aims to stimulate greater interest within the pharmaceutical industry regarding the potential of medicinal plants and traditional medicine.

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Conflict of Interest

The Submitters state that they are not in a conflict of interest.

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