J Nephropharmacol. 2023; 12(1): e10379.

http://www.jnephropharmacology.com DOI: 10.34172/npj.2022.10379

Journal of Nephropharmacology

The nephroprotective effect of *Eryngium caucasicum* extract alone and in combination with metformin in adult male diabetic rats

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ARTICLE INFO

Article Type:
Original

Article History:
Received: 2 November 2020
Accepted: 23 December 2020
Published online: 13 January 2021

Keywords:
Diabetes mellitus
Eryngium caucasicum
Metformin
Renal tubular cells

ABSTRACT

Introduction: Diabetes mellitus (DM) is a metabolic disease associated with the disorders in the metabolism of carbohydrates, proteins, and lipids that affect insulin action.

Objectives: The present study was designed to evaluate the nephroprotective effect of *Eryngium caucasicum* (Eryngo) extract alone and in combination with metformin (MET) in adult male diabetic rats.

Materials and Methods: Thirty male Wistar rats randomly were designated into five groups (n = 6) including; group I (Control); rats received normal saline by gavage for 15 days. Group II; rats received a single injection of STZ at a dose of 60 mg/kg intraperitoneally. Group III; rats, after STZ injection, received 30 mg/kg of MET by gavage for 15 days. Group IV; rats, after STZ injection, received 30 ml/kg of Eryngo extract by gavage for 15 days. Group V; rats, after STZ injection, received the combination of MET and Eryngo extract at a dose of 30 mg/kg by gavage for 15 days. The kidneys were removed immediately after sacrificing and prepared for morphological examination. Kidney sections were examined for the intensity of kidney damage (vacuolization, flattening, degeneration, and necrosis).

Results: Significant differences were observed in types of morphologic injury to renal tubular cells between groups ($P<0.05$). Eryngo extract had more protective effect against kidney damage due to DM compared to MET and the combination of MET+Eryngo. Additionally, in pairwise comparisons of groups, the relationship between group II (DM group) and group IV (DM + Eryngo) was significant ($P<0.05$).

Conclusion: The administration of MET and Eryngo extract alone and their combination ameliorate types of morphologic injury to renal tubular cells in diabetic rats, however, the renoprotective effect of Eryngo extract alone is more remarkable.

Implication for health policy/practice/research/medical education:
In an experimental study on 30 male Wistar rats, we found the administration of metformin and *Eryngium caucasicum* extract alone and their combination ameliorate types of morphologic injury to renal tubular cells in diabetic rats; however, the renoprotective effect of *E. caucasicum* extract alone is more remarkable.

Please cite this paper as: Golestaneh E, Hasanpour Dehkordi A, Yalameha B, Noorshargh P, Nasri P, Baradaran A. The nephroprotective effect of *Eryngium caucasicum* extract alone and in combination with metformin in adult male diabetic rats. J Nephropharmacol. 2023;12(1):e10379. DOI: 10.34172/npj.2022.10379.

Introduction

Diabetes mellitus (DM) is a metabolic disease associated with the disorders in the metabolism of carbohydrates, proteins, and lipids that affect insulin action (1). Diabetes is recognized as one of the major public health-threatening factors, therefore it was estimated the global prevalence of diabetes to be 2.8% in 2000 to 5.4% in 2025 (2). The morbidity and mortality of diabetes are results of microvascular (neuropathy, nephropathy, and retinopathy) and macrovascular complications (3). Herbal drugs have been extensively used since ancient times for treating diseases and nowadays the attention

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of many researchers and physicians has been attracted due to their fewer side effects and relatively low costs (4,5). Ethnobotanical studies have demonstrated that approximately 800 plants are effective to control and management of DM. In fact, effective compounds of the plants such as antioxidants provide a therapy option for DM (6). Recently, it has been demonstrated that the genus *Eryngium* has antidiabetic property. *Eryngium caucasicum* (Eryngo) is one of 250 species of *Eryngium* from Apiaceae family that commonly applied for nutrition and therapeutic purposes in some areas of the world. The substantial therapeutic properties have been considered for this plant that can be referred to as diaphoretic, stimulant, stone inhibitor, diuretic, anthelmintic, and also anti-diabetes (7-9). The various parts of this plant possess rich sources of bioactive compounds. Base on previous reports, phenols and flavonoids extracted from Eryngo possess reno-protective, antioxidant activities, and free radicals scavenging (10, 11). The studies have been shown that methanolic extracts of the plant species (*E. creticum* and *E. caeruleum*) are related to remarkable antidiabetic activities (12).

Hyperglycemia is a major drawback of metabolic disorders and complications related to diabetes leading to oxidative stress and lipid oxidation (13). One of the available drugs to lower high blood glucose levels in the treatment of diabetes is metformin (MET) (14). MET, as a known biguanide, is applied in patients with DM via its antihyperglycaemic impact. The drug ameliorates lipoprotein profiles, sensitivity to insulin and blood pressure (15) Additionally, MET induces usage of peripheral glucose by the intestine cells, especially by non-oxidative metabolism (16). The administration of MET has revealed significant protective effects against oxidative stress in DM by decreasing malondialdehyde and glycation end-products levels (17). Also, the findings have indicated that MET is associated with lower side effects (18,19). The biguanide can use as monotherapy or in combination with other medicine including herbal drugs (20,21). The use of herbal drugs with antioxidant features in combination with synthetic medicine applied in some trials and suggest to intensify their antioxidant properties. Although, it requires more investigation by performance studies. Thus, the present study aimed at evaluating this ambiguity.

Objectives

The present investigation was designed to evaluate the nephroprotective effect of *E. caucasicum* extract alone and in combination with MET in adult male diabetic rats.

Materials and Methods

Plant material and extraction

Dried *E. caucasicum* were prepared from a local herb market in Shahrekord and were identified by a local herbalist of the center. To prepare the extract of the *E. caucasicum*, at first it powdered and then 250 g of *E. caucasicum* power macerated in aqueous ethanol 70% for 72 hours at room temperature with intermittent shaking. The macerated mixture was filtered through Whatman filter paper. Afterward, mixture concentrated using rotary evaporator (RE 100 Bibby, Stone Staffordshire England, ST15 OSA) at 50˚C and dried in a laminar hood to remove residual moisture.

Animals

Thirty male Wistar rats with a mean bodyweight of 200-250 g in the Medical Plants Research Center in Shahrekord University of Medical Sciences were studied. All animals were kept in normal laboratory condition (temperature; 21-25˚C and light cycle; 12 h dark-12 h light).

Induction of diabetes

To induce diabetes, 60 mg/kg streptozotocin (STZ) (Sigma-Aldrich Co., St Louis, MO, USA) was dissolved in 0.1 M citrate buffer and injected into the rats after a fasting night intraperitoneally. Then, 72 hours after STZ injection, blood glucose was determined by glucometer and rats with blood glucose levels above 250 mg/dL were considered as diabetes.

Study design

1. Rats randomly assigned into five groups, 6 rats for each;
2. Group I (control; non-diabetic); rats received normal saline by gavage for 15 days.
3. Group II (DM); rats received single injection of STZ at a dose of 60 mg/kg intraperitoneally and were also given isotonic saline orally for 15 days.
4. Group III (DM+ MET); rats, after STZ injection, received 30 mg/kg of MET by gavage for 15 days.
5. Group IV (DM+ Eryngo); rats, after STZ injection, received 30 ml/kg of Eryngo extract by gavage for 15 days.
6. Group V (DM+MET+ Eryngo); rats, after STZ injection, received the combination of MET and Eryngo extract at a dose of 30 mg/kg by gavage for 15 days.

Histopathological study

For histopathological examination, kidney tissues were removed immediately after sacrificing and fixing with 10% formalin for morphological study. Then, the 2-3 µm-thick sections of renal tissues were prepared and stained with hematoxylin and eosin (H&E) for pathological evaluation. Kidney sections were examined by a light microscope for intensity of kidney damage by examination for degeneration, flattening and necrosis of renal tubular cells and also dilatation of tubular lumen. For statistical analysis and comparing among the groups we used a total

of mean percent of four morphological variables, including vacuolization, flattening, degeneration, and necrosis.

Statistical analysis

All parameters were summarized with mean and standard deviation (SD) and categorical variables are presented as percentage. One-way analysis of variance (ANOVA) and post-hoc tests (Bonferroni test) were applied for comparison of mean values between groups. To calculate sample size and data analysis, SPSS version 21.0 software was used. Accordingly, *P* values of less than 0.05 were assumed to be significant.

Results

As indicated in Table 1, significant differences were observed in types of morphologic injury to renal tubular cells (vacuolization, flattening, degeneration, and necrosis) between groups ($P<0.05$). The results of the comparison of the morphological variable of vacuolization have been indicated between groups in Table 2. There were remarkable differences between the control group with groups of II (DM group) and V (DM+MET+ Eryngo) (P <0.05). Additionally, remarkable differences were seen between group II (DM group) and groups of III (DM +MET) and IV (DM+ Eryngo) ($P<0.05$). Table 3 shows the comparison of the morphological variables of flattening between groups. The findings were illustrated that there were signification differences between the control group and group II (DM group), also between group II (DM group) and groups of III (DM +MET) and IV (DM+ Eryngo) ($P<0.05$). According to the comparison of degeneration between groups in Table 4, the only difference of the control group with group II (DM group) was significant ($P<0.05$). The findings obtained from the comparison of necrosis between groups were revealed in Table 5. A significant relationship was observed between the control group and group II (DM group), as well as between group II (DM group) and group IV (DM+ Eryngo) ($P<0.05$).

Table 2. Comparison of morphological variable of vacuolization between groups

Between groups comparison	Mean Difference(I-J) ± SE	*P* value
I vs. II	-34.67 ± 5.97	0.002*
I vs. III	-13.0 ± 5.97	0.544
I vs. IV	-11.33 ± 5.97	0.867
I vs. V	-24.67 ± 5.97	0.020*
II vs. III	21.67 ± 5.97	0.047*
II vs. IV	23.33 ± 5.97	0.030*
II vs. V	10.0 ± 5.97	0.999
III vs. IV	1.67 ± 5.97	0.999
III vs. V	-11.67 ± 5.97	0.795
IV vs. V	-13.33 ± 5.97	0.494

* The significance level for *P* value is less than 0.05.

Table 3. Comparison of morphological variable of flattening between groups

Between groups comparison	Mean Difference(I-J) ± SE	*P* value
I vs. II	-26.67 ± 4.08	0.001*
I vs. III	-8.33 ± 4.08	0.685
I vs. IV	-5.00 ± 4.08	0.999
I vs. V	-13.33 ± 4.08	0.085
II vs. III	18.33 ± 4.08	0.012*
II vs. IV	21.67 ± 4.08	0.003*
II vs. V	13.33 ± 4.08	0.085
III vs. IV	3.33 ± 4.08	0.999
III vs. V	-5.0 ± 4.08	0.999
IV vs. V	-8.33 ± 4.08	0.685

* The significance level for *P* value is less than 0.05.

Discussion

The present study evaluated the nephroprotective feature of *E. caucasicum* and MET alone and their combination in diabetic rats. The findings showed a significant difference in types of morphologic injury to renal tubular cells including vacuolization, flattening, degeneration, and necrosis between groups. Eryngo extract had more protective effect against kidney damage due to DM compared to MET and the combination of MET+Eryngo. Additionally, in pairwise comparisons of groups, the relationship between group II (DM group) and group IV (DM + Eryngo) was significant.

The results of studies revealed that the Eryngo extract is associated with the renoprotective efficacy through

Table 1. Mean ± SD of vacuolization, flattening, degeneration, and necrosis of renal tubular cells

Groups	Injury			
	Vacuolization	Flattening	Degeneration	Necrosis
Control	0.33 ± 0.55	0.00 ± 0.00	1.0 ± 1.0	0.00 ± 0.00
DM	35.0 ± 13.23	26.00 ± 10.41	33.33 ± 16.07	33.33 ± 20.82
DM + MET	13.33 ± 2.89	8.33 ± 2.89	21.67 ± 5.77	6.67 ± 2.89
DM + Eryngo	11.67 ± 2.89	5.0 ± 0.0	8.33 ±5.77	1.67 ± 2.89
DM + MET + Eryngo	25.0 ± 8.66	13.33 ± 2.89	20.0 ± 10.0	11.67 ± 2.89
P value	0.002*	0.001*	0.013*	0.010*

* The significance level for *P* value is less than 0.05.

Table 4. Comparison of morphological variable of degeneration between groups

Between groups comparison	Mean Difference(I-J) ± SE	*P* value
I vs. II	-32.33 ± 7.54	0.016*
I vs. III	-20.67 ± 7.54	0.208
I vs. IV	-7.33 ± 7.54	0.999
I vs. V	-19.00 ± 7.54	0.303
II vs. III	11.67 ± 7.54	0.999
II vs. IV	25.00 ± 7.54	0.078
II vs. V	13.33 ± 7.54	0.999
III vs. IV	13.33 ± 7.54	0.999
III vs. V	1.67 ± 7.54	0.999
IV vs. V	-11.67 ± 7.54	0.999

* The significance level for *P* value is less than 0.05.

Table 5. Comparison of morphological variable of necrosis between groups

Between groups comparison	Mean Difference(I-J) ± SE	*P* value
I vs. II	-33.33 ± 7.82	0.017*
I vs. III	-6.67 ± 7.82	0.999
I vs. IV	-1.67 ± 7.82	0.999
I vs. V	-11.67 ± 7.82	0.999
II vs. III	26.67 ± 7.82	0.066
II vs. IV	31.67 ± 7.82	*0.023
II vs. V	21.67 ± 7.82	0.197
III vs. IV	5.00 ± 7.82	0.999
III vs. V	-5.00 ± 7.82	0.999
IV vs. V	-10.00 ± 7.82	0.999

* The significance level for *P* value is less than 0.05.

reducing blood urea nitrogen and serum creatinine (22). Recently, hepato-protective impacts of this plant have been proved in under-treated diabetic rats (23). Peña-Montes et al investigated anti-diabetic effect of the *Eryngium carlinae* extract, another species of *Eryngium* (24). Their study indicated that 30 mg/kg of *E. carlinae* in addition to reduced lipid peroxidation and blood glucose levels improves ROS production in the kidney, liver, and brain of diabetic rats (24). Besides, the results of a study presented that the utilization of E. *carlinae* extract, as an adjuvant in the treatment of DM, reduced the levels of triglycerides, cholesterol, uric acid, and creatinine without the hypoglycemic effect. They offered that the plant extract administration can alleviate cardiovascular complications and hyperlipidemia in diabetic rats (25). The study on hydro-alcoholic extract impact of *Eryngium billardieri* in hypercholesterolemic rats illustrated that the medical plant can relieve liver function markers (ALT, AST, and ALP) as well as lipid profile (cholesterol, triglycerides, and LDL) because of its antioxidants property. In addition, different doses of the *E. billardieri* extract caused no side effects on renal function (26).

Recent studies reported that *Eryngium* species possess antioxidant and anti-inflammatory activities via phenolic and flavonoids compounds. Flavonoids extracted from Eryngo may prevent the development of over 60 diseases. In fact, these compounds, as free radicals scavenging, reduce complications due to oxidative stress (27). It has been displayed that oxidative stress plays a vital role in the occurrence of DM complications including diabetic nephropathy (28). This result showed the progression of hyperglycemia as a result of STZ injection. High glucose in DM contributes to the ROS production from different pathways such as auto-oxidation, glucose metabolism, and formation of the advanced glycation end product and subsequent alterations of redox state (29). Redox state alterations affect renal tissue and cause renal fibrosis because of the mesangial cells accumulation, the thickening of the glomerular and tubular membranes, apoptosis, and disorder in podocytes function (30). Therefore, it seems Eryngo extract with its antioxidant property can improve the injury of renal tubular cells caused by oxidative stress in diabetic rats.

In this study, the diabetic rat's treatment with MET alone and in combination with Eryngo extract showed a remarkable difference compared to untreated diabetic rats in healing kidney damage. MET possesses a lucrative role in glycaemic control through suppressing gluconeogenesis by phosphorylation of AMP-activated kinase (AMPK) in hepatic cells. It has been demonstrated that MET inhibits functional and structural damage of renal cells and hypoxia using AMPK activation, in rats with renal ischemia-reperfusion. Actually, the presence of hyperglycemic conditions prevent activation of defense mechanisms in renal cells such as autophagy and AMPK and induces oxidative stress and hypoxia (31, 32). Thus, it offers that MET contributes to improving oxidative stress condition, and prevents diabetic renal injury.

Conclusion

The results of the present study indicated that the administration of MET and Eryngo extract alone and their combination ameliorate types of morphologic injury to renal tubular cells in diabetic rats, however, the renoprotective effect of Eryngo extract alone is more remarkable.

Authors' contribution

EG and AB designed the research. AHD conducted the animal study. AB and PaN supervised the study. BY prepared the final draft of the article. PeN analyzed the data. AB studied pathology matters. AB edited the manuscript. All authors read and signed the final paper.

Conflicts of interest

The authors declare that they have no competing interests.

Ethical issues

All experimental protocols were conducted in compliance with the regulations of the Research Ethics Committee of the University and Iranian Ethical Guidelines for the use of animals in research. Additionally, all animal experiments were in accordance with protocols approved by the United States National Institutes of Health (NIH, 1978). This study was also approved and supported by Ethics Committee of NIMAD (http://nimad.ac.ir; national institute for medical research development) in Iran (ethical code; IR.NIMAD.REC.1397.146). Besides, ethical issues (including plagiarism, misconduct, data fabrication, falsification, double publication or submission, redundancy) have been completely observed by the authors. This study was conducted in the animal lab of Shahrekord University of Medical Sciences and supervised by AHD.

Funding/Support

This study was supported by the Ethics Committee of NIMAD (national institute for medical research development in Iran (Grant #963554).

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