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Effects of *Eryngium caucasicum* extract on Testosterone, inflammation and oxidative status of Nicotinamide-Streptozotocin induced Type-2 Diabetes in male rats

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Objective Regarding the unfavorable side effects of anti-diabetic drugs including peripheral edema, gastrointestinal discomfort and hypoglycemia, using medicinal plants due to their useful contents such as flavonoids, alkaloids, glycopeptides, terpenoides, phenolic compounds and other constituents with antioxidant and anti-inflammatory effects, is encouraged in treatment of diabetes mellitus. Thus, the hypothesis was that *Eryngium caucasicum* extract will decrease inflammation and oxidative stress in nicotinamide-streptozotocin induced type 2 diabetes mellitus model in Wistar rats.

Methods In this study, sixty adult male Wistar rats (150–250 g) were randomly allocated into six groups (n = 10) including: (1) healthy control, (2) diabetic control, (3) diabetic rats which received Sitagliptine, (4–6) diabetic rats which received 100, 200 and 300 mg/kg of *E. caucasicum* extract oral gavages for 30 days. Eventually, total antioxidant capacity, vitamin B12, malondyaldehyde, interleukin-6, high sensitive C-reactive protein and testosterone serum levels were measured.

Results Administration of *E. caucasicum* in type 2 diabetes mellitus animal model did not change serum vitamin B12 and animal weight compared with control groups. Total antioxidant capacity and malondyaldehyde improved in all doses of *E. caucasicum*; in the highest doses, the total antioxidant capacity was higher than sitagliptine group. Interleukin-6 and high sensitive C-reactive protein both decreased in all doses of *E. caucasicum*. Administration of *E. caucasicum* extract improved testosterone serum level only in highest dose of *E. caucasicum* extract. Since the highest dose showed the highest antioxidant and anti-inflammatory effects; dose-responses of antioxidant and anti-inflammatory effects are suggested.

Conclusion In conclusion, we showed that administration of *E. caucasicum* in T2DM animal model has antioxidant and anti-inflammatory effects. However, in future studies other dose-escalating intervention must be performed. Also toxicity in diabetes must be elucidated in future studies.

Keywords *Eryngium caucasicum* extract, testosterone, inflammation, oxidative status

Introduction

Diabetes mellitus (DM) is a multi-systemic endocrine disorder with constant hyperglycemia due to either absolute or relative defective insulin secretion, insulin resistance or both.[1] The prevalence of DM is rising all over the world in both developed and developing countries. About 90% of all diabetic cases are type 2 diabetes mellitus (T2DM). According to WHO, the global prevalence of T2DM will increase to 366 million in 2030.[2] DM is reported to devote 7–13% of the healthcare budget of worldwide and a major challenge to healthcare systems.[3] Long-term hyperglycemia is contributed to many chronic end-organ microvascular and macrovascular damages in the eyes, kidneys and the brain. Also, T2DM-related cardiovascular diseases can increase morbidity and mortality among patients with DM.[4] Mild to moderate proinflammatory status has been implicated in DM. This setting is proposed as a link between disease progression and its complications. Mounting evidence showed increased cytokine level and immune cell infiltration in pancreatic cells in DM.[5] In addition epidemiological studies support this data that inflammatory markers like interleukin-6 (IL-6) are associated with development of DM.[6] IL-6 reported as a mediator which act between insulin-sensitive tissues, and pancreatic islets to adapt to changes in insulin demand.[7] Prolonged hyperglycemia along with proinflammatory settings, disturbs normal balance of oxidant-antioxidant capacity within the cells, which results in oxidative stress and injury, beta cell death and/or de-differentiation to glucagon-producing cells.[8] Despite current advances in anti-diabetic drugs, their use is complicated due to the unfavorable side effects like peripheral edema, gastrointestinal discomfort and hypoglycemia.[9] Recently the use of medicinal plants for the sufferers of DM is encouraged because of their perceived effectiveness, fewer side effects and relatively low costs. It has been estimated that more than 1000 medicinal plants are used as anti-diabetic remedies.[10] Several phytoconstituents in herbal remedies reported as useful agents against DM. These include flavonoids, alkaloids, glycopeptides, terpenoides and phenolic compounds and other constituents which show fasting blood glucose (FBS) lowering effects.[11]

The *Eryngium* genus, as the largest genus of the family Apiaceae, consists of more than 250 flowering species all over the world.[12] *Eryngium caucasicum* which is known as Eryngo has been cultivated in middle-east countries like Iran and Turkey.[13] In northern Iran it has been widely used as different foodstuffs like pickles. Apiaceae have several therapeutic uses as diuretic, stone inhibitor, expectorant and antinociceptive.[13] Other uses in Turkish folk medicine are against various inflammatory disorders, edema, sinusitis, urinary infections or inflammations etc.[14] In essence, *E. caucasicum* reported to

have numerous therapeutic properties comprising strengthening generative power, diuretic, lenitive and appetizer.[15] Antioxidant and potent free radical scavenging activity of *E. caucasicum* Trautv leaves have been reported. In addition it is reported to have antihypoxic and reno-protective effects.[16] Anti-inflammatory activities of *Eryngium* species growing in Turkey has been evaluated *in vivo*. Aerial parts and roots reported to possess many activities.[17] Several bioactive compounds, mainly phenolic compounds and terpenoids have been purified from *Eryngium* species which include triterpenoid saponins, flavonoids, coumarins and acetylenes.[18]

In this study we aimed to evaluate anti-inflammatory and anti-oxidative potential and possible effects of *E. caucasicum* extract on testosterone in nicotinamide-streptozotocin (NA-STZ) induced T2DM model in rats.

Materials and Methods

Plant Material and Extract Preparation

Fresh leaves of *E. caucasicum* were obtained from Mazandaran province, Iran and authenticated scientifically by the Botany Department of Ahvaz Jundishapur University of Medical Sciences (AJUMS), Ahvaz, Iran. Voucher specimens are deposited in the Herbarium Department of Biology, University of Mazandaran, Iran (No 1442). The plant leaves were desiccated in shade and then crushed and powdered by grinder.

A total of 300 g of the powder of *E. caucasicum* leaves were soaked in 1200 ml of an ethanol and distilled water mixture (70–30) and stocked for 72 h at room temperature. The mixture filtered through Whatman filter papers (No. 1) and then centrifuged at 3500 rpm for 20 min. Condensation conducted by rotary evaporator. Finally, supernatant was dried at 37°C, and the obtained semisolid mass was stocked at 4°C until injection.

Experimental Animals

Sixty adult male Wistar rats (150–250 g) were purchased from animal house of AJUMS. All experimental animals were kept in standard cages under approved conditions for animal procedures (temperature 22 ± 2°C with a 12–12 h light–dark cycle). Experimental animals were allowed to access normal commercial chow and tap water. Maintenance and care of experimental animals complied with the National Research Council of the National Academic.

Induction of Type 2 Diabetes Mellitus

In this study, type 2 diabetes was induced by intraperitoneal (IP) injection of NA (120 mg/kg body weight in normal saline) (Sigma-Aldrich, USA) 15 min before a single dose of STZ (55 mg/kg body weight; dissolved in citrate buffer (0.1M), pH 4.5) (Sigma-Aldrich, USA). Animals suffer from fasting for 8–12 h before T2DM induction. The development of T2DM was evaluated by FBS in animals. FBS was measured before and 72 h after injection to confirm diabetes induction. FBS more than 250 mg/dl were considered diabetic. Only those animals with confirmed diabetes were used for following experiments.

Experimental Protocol

After 2 weeks of adaptation, rats were divided into the following groups (n = 10 in each group):

- Group 1: Six healthy control (0.5 ml/kg normal saline oral gavage).
- Group 2: Six diabetic control group (0.5 ml/kg normal saline oral gavage).
- Group 3: Six T2DM + sitagliptine (10 mg/kg oral gavage).
- Group 4: Six T2DM + *E. caucasicum* extract (100 mg/kg oral gavage).
- Group 5: Six T2DM + *E. caucasicum* extract (200 mg/kg oral gavage).
- Group 6: Six T2DM + *E. caucasicum* extract (300 mg/kg oral gavage).

All groups treated once a day for 28 sequential days with sitagliptine or *E. caucasicum* extract. Sitagliptine was dissolved in distilled water. Body weight was recorded weekly during the experiment. At 24 h after the last injection, mice were kept fasted overnight. All animals were killed under ether anesthesia. Blood samples were obtained by heart puncture. Samples were poured into centrifuge tubes and centrifuged at 3500 rpm for 20 min. Serum samples were refrigerated at −70°C refrigerator until biochemical analysis.

Biochemical Assessments

Fasting blood glucose were assessed by an Elegance glucometer (CT-X10, Convergent Technologies, Germany) using the lateral tail vein of the animals on the first and last days of the experiment.

Total antioxidant capacity (TAC) was assessed by spectrophotometer and commercial kits (RANDOX, England). Also malondyaldehyde (MDA) serum level was assessed through thiobarbiturate method. Following factors were assessed by ELISA method: IL-6 (BehpadTeb, Iran), testosterone (Mono-Biol, USA). Circulating high sensitive C-reactive protein (hs-CRP) was evaluated by turbidimetry method (BioSystem, Barcelona, Spain). Serum vitamin B12 was evaluated by radioimmunoassay using gamma counter.

Statistical Analysis

All data were expressed as mean ± standard error of mean (SEM). Results were analyzed by one-way analysis of variance (ANOVA), followed by post-hoc test [least significant difference (LSD)]. All Statistical analysis were performed by SPSS Statistics V. 17.01 (SPSS Inc., Chicago, USA).

Results

Effects of Hydro-alcoholic Extract E. caucasicum on Body Weight, FBS and Serum Vitamin B12

Our results indicated that STZ-NA-induced diabetes did not change body weight in diabetic control group (P-value = 0.634). Administration of sitagliptin in diabetic rats decreased body weight significantly compared with diabetic control (P-value = 0.008) and healthy control group (P-value = 0.028). However, *E. caucasicum* administration in 300 mg/kg increased body weight significantly compared with Sitagliptin group (P-value = 0.002). Induction of diabetes significantly increased FBS in diabetic rats compared with healthy control group (P-value = 0.009). Sitagliptin also decreased FBS in diabetic rats significantly (P-value = 0.004). Furthermore, only high doses including 200 and 300 mg/kg decreased FBS in

distinct period (*P*-value = 0.038 and 0.004 respectively). Although a dose-dependent manner reported in reducing FBS following increasing doses of *E. caucasicum* extract as outlined in Table 1. Furthermore, serum vitamin B12 revealed no significant changes in any of experimental groups during intervention period.

Effects of Hydro-alcoholic Extract E. Caucasicum on Inflammatory Biomarkers

Induction of diabetes using STZ-NA increased serum hs-CRP in diabetic control group compared with healthy control group. Sitagliptine significantly lowered hs-CRP serum level (*P*-value = 0.001). Treatment with plant extract also lowered serum hs-CRP level in dose-dependent manner as shown in Table 2.

Interleukin-6 serum level increased significantly after diabetes induction. Administration of Sitagliptine significantly improved IL-6 serum level. *E. caucasicum* extract also lowered IL-6 in a dose-dependent manner as depicted in Table 2.

Effects of Hydro-alcoholic Extract E. caucasicum on TAC and MDA

Malondyaldehyde serum level increased after induction of diabetes in diabetic control group. Although sitagliptine improved MDA serum level, did not improve it as efficiently as *E. caucasicum* extract. Administration of herb extract lowered serum level of MDA in a dose-dependent manner. MDA also in 200 and 300 mg/kg were significantly lower than sitagliptine (*P*-value = 0.023 and 0.006 respectively).

Total antioxidant capacity also decreased after T2DM induction. However, administration of sitagliptine improved TAC significantly (*P*-value = 0.030). All three doses of *E. caucasicum* extract improved TAC but was not as efficient as sitagliptine (*P*-value for all doses <0.001).

Effects of Hydro-alcoholic Extract E. Caucasicum on Serum Testosterone Level

As shown in Figure 1, all experimental diabetic groups showed lower testosterone serum level compared with healthy control group. Sitagliptine treatment did not improve testosterone serum level in diabetic rats. But 300 mg/kg of plant extract increased serum testosterone level significantly. This observation showed dose-dependent effects of *E. caucasicum* extract as shown in figure.

Discussion

The results of this study indicate that *E. caucasicum* exerted beneficial effects on increasing TAC, decreasing MDA, IL-6, hs-CRP and improving testosterone serum level without any changes on serum vitamin B12 and animals' body weights in STZ-NA rats, T2DM animal model compared with control groups. TAC improved in all doses of *E. caucasicum* although it was higher than sitagliptine group in the highest dose. MDA also improved in all groups after administration of *E. caucasicum*. All of these observations showed the antioxidant effects of *E. caucasicum* in diabetic rats.

Mounting evidence has pointed out several pharmacological effects of the genus *Eryngium* including antioxidant, anti-hypoxic and free radical scavenging effects.[19] Similarly, Wang et al.[18] and Mirjana et al.[20] reported the same findings. Antioxidant activity of *Eryngium* genius contributed to its aerial parts, extracted oil, roots, fruits etc.[21] The antioxidant effects of *Eryngium* extracts were evaluated using 2,2-diphenyl-l-1-picrylhydrazil and ferric reducing antioxidant power assays which showed significant antioxidant activities.[22] In essence, *E. caucasicum* fractions demonstrated different levels of antioxidant and anti-hemolytic activities in different models. Hypoxia mediates the production of nitric oxide (NO) and its radicals and also ROS.[13] NO reported to modulate iron catalyzed oxidation reactions such as Fenton reaction, which generates potent oxidants such as the hydroxyl radical ($OH^{\cdot}$). NO scavenging may suggest likelihood to counteract hypoxia.[23] The mechanisms by which NO may block lipid peroxidation needed to be clarified, however, one plausible mechanism linked to the potential of NO to cease propagation of lipid peroxidation reactions.[24] *Eryngium*

Table 1. **Effect of hydro-alcoholic extract of *Eryngium caucasicm* (E.C.) on body weight, fasting blood glucose and serum vitamin B12 in nicotinamide-streptozotocin-induced diabetic Wistar rats**

	Healthy control	Diabetic control	Diabetes + sitagliptin	Diabetes + 100 mg/kg E.C.	Diabetes + 200 mg/kg E.C.	Diabetes + 300 mg/kg E.C.
Weight (g)	221.08 + 6.96	216.52 + 7.04	193.67 + 6.45[a,b]	240.28 + 6.87[c]	209.30 + 6.55	211.12 + 6.35
FBS (mg/dl)	107.50 + 34.5	157.28 + 59.00[a]	104.57 + 22.32[b]	130.16 + 18.49	120.42 + 16.25[b]	103.85 + 17.72[b]
Serum B12 (pg/ml)	119.60 + 17.17	129.12 + 26.85	128.13 + 18.88	127.93 + 19.07	134.16 + 20.70	133.46 + 20.53

[a]$p < 0.05$ compared with the control group. [b]$p < 0.05$ compared with the diabetic control group. [c]$p < 0.05$ compared with the diabetic animals received Sitagliptin. Results are expressed as mean + SEM (standard error of mean), one-way ANOVA and post-hoc LSD test. E.C.: *Eryngium caucasicm*; FBS: fasting blood glucose.

Table 2. **Effect of hydro-alcoholic extract of *Eryngium caucasicm* (E.C.) on inflammatory and oxidative stress markers in nicotinamide-streptozotocin-induced diabetic Wistar rats**

	Healthy control	Diabetic control	Diabetes + sitagliptin	Diabetes + 100 mg/kg E.C.	Diabetes + 200 mg/kg E.C.	Diabetes + 300 mg/kg E.C.
TAC (µmol/l)	0.931 + 0.135	0.371 + 0.101[a]	0.611 + 0.348[a,b]	0.776 + 0.127[b]	0.814 + 0.181[b]	0.872 + 0.172[b,c]
MDA (µmol/l)	1.57 + 0.65	3.98 + 1.02[a]	2.88 + 0.83[a,b]	1.97 + 0.71[b]	1.83 + 0.96[b]	1.56 + 0.71[b]
Il-6 (pg/ml)	66.68 + 11.80	124.59 + 32.09[a]	89.90 + 12.64[a,b]	101.47 + 6.16[a,b]	101.80 + 10.84[a,b]	90.25 + 11.45[a,b]
hs-CRP (µmol/l)	7047 + 1.29	14.62 + 2.49[a]	10.14 + 2.60[a,b]	11.24 + 2.79[a,b]	10.77 + 1.88[a,b]	8.94 + 1.96[b]

[a]$p < 0.05$ compared with the control group. [b]$p < 0.05$ compared with the diabetic control group. [c]$p < 0.05$ compared with the diabetic animals received Sitagliptin. Results are expressed as mean + SEM (standard error of mean), one-way ANOVA and post-hoc LSD test. E.C.: *Eryngium caucasicm*; FBS: fasting blood glucose; MDA: malondialdehyde; TAC: total antioxidant capacity; hs-CRP: high sensitivity C-reactive protein; IL-6: interleukin-6.

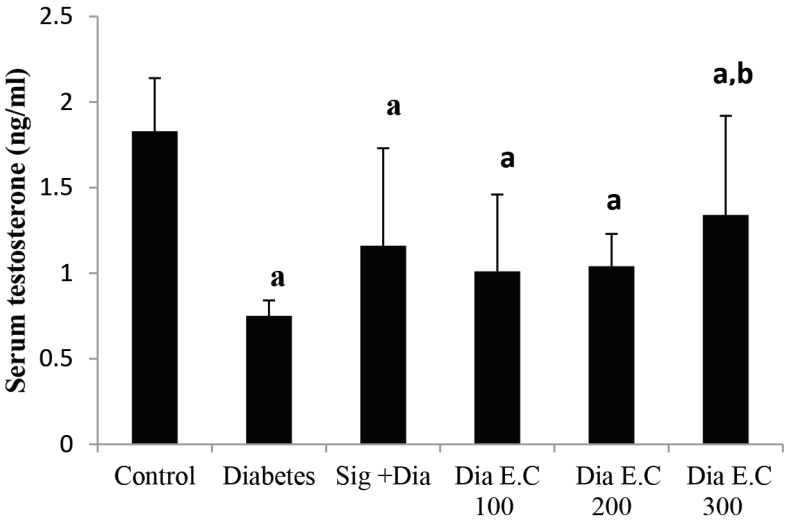

Fig. 1 **Effect of hydro-alcoholic extract of *Eryngium caucasicm* (E.C.) on testosterone serum level in nicotinamide-streptozotocin-induced diabetic Wistar rats. Values outlined as Mean (SEM; standard error of mean), One-way ANOVA and post-hoc LSD test. Dia: Diabetic, E.C.: *Eryngium caucasicm*, Sig: sitagliptin, 100: 100 mg/kg hydro-alcoholic extract E.C., 200: 200 mg/kg hydro-alcoholic extract E.C., 300: 300 mg/kg hydro-alcoholic extract E.C. $^{a}P < 0.05$ compared with the control group, $^{b}P < 0.05$ compared with the diabetic control group.**

species reported to contain several phenolic compounds, flavonoids and coumarins which their antioxidant activities have been documented.[25] Because of high polyphenols and flavonoids contents of *E. caucasicum* extract, their efficient NO scavenging activities have been reported.[23] Lipid peroxidation marker, MDA, in our study significantly decreased in dose-dependent manner; the effect on NO production could be one of possible mechanisms. The correlation between total phenolic contents and antioxidant activity has been widely studied. The important point is that the NO scavenging effect was mostly dose-dependent in previous studies.[16] We also found almost dose-dependent effect regarding antioxidant activity of *E. caucasicum* in diabetic rats.

We showed anti-inflammatory effects of *E. caucasicum* extract in diabetic rats. IL-6 and hs-CRP both decreased in all doses of *E. caucasicum*. These findings were in agreement with findings of Dawilai et al.[26] and Jaghabir[27] in which anti-inflammatory effects of *E. caucasicum* extract were reported.

Alteration in serum vitamin B12 could be due to hepatic injury in diabetic rats.[28] Previous studies in humans reported lowered serum B12 in diabetes;[29] although *E. caucasicum* due to its antioxidant effects could be hepato-protective, serum vitamin B12 could be lost via urine which has not examined in this study. Our study did not show any significant effect in serum vitamin B12.

Effects of *Eryngium* species (especially roots) against different inflammatory disorders are recognized in the traditional medicines worldwide; for instance, *Eryngium campestre* as anti-edema[30] or *Eryngium creticum* for inflammatory wounds[31] or against kidney and urinary tract inflammations and edema.[32] Despite widespread uses of *Eryngium* species in the treatment of inflammatory disorders worldwide, the number of scientific papers investigating the anti-inflammatory activity potential are scarse; like *Eryngium yuccifolium* aerial parts,[33] *Eryngium maritimum* rhizomes,[34] *Eryngium bilardieri* aerial parts and roots,[35] *Eryngium foetidum* leaves.[36] Ethanol and water extract of roots and aerial parts of eight *Eryngium* species were investigated for their inhibitory effects on several inflammatory models in mice. Results showed different degrees of anti-inflammatory activity for *Eryngium* species.[17] A significant anti-inflammatory activity was reported in the *n*-butanol extract *E. billardieri*, which mainly contains saponins.[35] The myeloperoxidase activity as a marker of polymorphonuclear cells migration to the inflamed tissues reported to be reduced strongly after *E. foetidum* extract administration.[37] NO is also implicated in inflammation[38] and as mentioned recently, *E. caucasicum* showed anti-scavenging effect against NO. Interestingly *E. foetidum* leaves extract showed a significant and dose-dependent anti-inflammatory activity, orally against carrageenan-induced rat paw edema.[39] Here, we showed dose-dependent anti-inflammatory effects of *E. caucasicum* in diabetic rats. Additionally, hs-CRP and IL-6 decreased significantly in a dose-dependent manner.

Administration of *E. caucasicum* extract improved testosterone serum level only in the highest doses of *E. caucasicum* extract. Reduced serum testosterone level reported in diabetic rats due to reduced amount and functioning of Leydig cells[40] and also absence of normal serum insulin levels because, insulin plays a positive role in Leydig cell function and testosterone production.[41] Furthermore, inhibition of steroidogenesis because of elevated inflammatory biomarkers and reduction in the activity of antioxidant enzymes also has been implicated.[42] We showed that *E. caucasicum* significantly improved serum testosterone level. Since insulin acts as an anti-apoptotic factor which is able to regulate testicular apoptosis; thus reduction in insulin serum levels leads to reduced testosterone serum level. Insulin secretary effects of *E. caucasicum* extract could improve testosterone levels in treated groups. Another probable mechanism is that increasing of ROS and oxidative stress can prevent production of androgens.[43] The protective effects of *E. caucasicum* extract could be due to antioxidant and anti-inflammatory effects. Some researchers also suggested that flavonoids and saponins have positive effects on androgen production and bioavailability. Saponins increase testosterone generation through influencing on pituitary leuteizing hormone secretion on Leydig cells.[44] Therefore, it can be inferred that observed effects of *E. caucasicum* extract on serum testosterone levels are related to the presence of these compounds.

Conclusion

In conclusion, we showed that administration of *E. caucasicum* in T2DM animal model has antioxidant and anti-inflammatory effects. *E. caucasicum* decreased serum IL-6, MDA and hs-CRP and also increased TAC. Highest doses of *E. caucasicum*

also improved testosterone serum level. On the whole, it seems that *E. caucasicum* influences on inflammation and oxidative stress in diabetes rat fellow a dose-dependent manner, although higher doses showed better effects. However, in future studies other dose-escalating intervention must be performed; in which higher doses evaluated to clarify the exact dose-response effects of eryngo. Also toxicity in diabetes must be elucidated in future studies.

Conflict of Interest

None.

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