

# Antihypoxic activities of *Eryngium caucasicum* and *Urtica dioica*

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**Abstract. – OBJECTIVE:** *Urtica dioica* and *Eryngium* spp. have been used in traditional medicine for many years. In spite of many works, nothing is known about their protective effect against hypoxia-induced lethality.

**MATERIALS AND METHODS:** Protective effects of *U. dioica* (UD) aerial parts and *E. caucasicum* (EC) inflorescence against hypoxia-induced lethality in mice were evaluated by three experimental models of hypoxia, asphyctic, haemic and circulatory.

**RESULTS:** Statistically significant protective activities were established in some doses of extracts in three models. Antihypoxic activity was especially pronounced in polyphenol fractions in asphyctic model. EC polyphenol fraction at 400 mg/kg prolonged survival time ( $48.80 \pm 4.86$ ,  $p < 0.001$ ) which was comparable with that of phenytoin ( $p > 0.05$ ). It was the most effective extract in circulatory model, too. It prolonged survival time significantly respect to control group ( $p < 0.001$ ). UD extracts protected the mice but the response was not dose-dependent. In haemic model, extracts of EP significantly and dose dependently prolonged survival time as compared to control group ( $p < 0.001$ ). At 600 mg/kg, EP was the most effective one, being capable of keeping the mice alive for  $12.71 \pm 0.75$  min. Only the concentration of 300 mg/kg of UD was effective ( $p < 0.001$ ).

**CONCLUSIONS:** Extracts showed remarkable antihypoxic effects. Pharmacological effects may be attributed to the presence of polyphenols in the extracts.

*Key Words:*

Antihypoxia; *Urtica dioica*; *Eryngium caucasicum*; Asphyctic hypoxia.

## Introduction

Hypoxia occurs especially in heart diseases, ischemia and heart attack, causing numerous deleterious effects and resulting in tissue destruction and death. Hypoxia mediates the production of nitric oxide and its radicals<sup>1</sup>. Therefore, nitric

oxide scavenging may offer a possibility to neutralize hypoxia. Hypoxia induces the production of oxygen reactive species as well<sup>3</sup>. It has proven that compounds with antioxidant activity are able to exhibit antihypoxia property. There are increasing interests for use of natural antioxidants.

*Urtica dioica* (UD), is widely used in traditional medicine in many countries. There are many reports indicating the benefits of using this plant for the use in different conditions such as prostatic hyperplasia, inflammation and hypertension<sup>3</sup>. It possesses many therapeutic effects such as cardiovascular activity<sup>3</sup>. Antioxidant activities and inhibition of nitric oxide production of UD have been reported<sup>4,5</sup>.

Literature on possible activities of *Eryngium* spp. highlights anti-inflammatory and antinociceptive properties<sup>6</sup>. *E. caucasicum* founds in cultivation in Northern Iran and has reported recently. Antioxidant activities and significant antidepressant, antiinflammatory and antinociceptive activities of EC have been reported recently<sup>7</sup>. Because of high polyphenol and flavonoids contents and their good nitric oxide scavenging activities, these two medicinal plants were nominated for assay of antihypoxic activities.

## Materials and Methods

### Animals

Male Swiss albino mice ( $20 \pm 2$  g) were randomly housed in groups of 8 in polypropylene cages at an ambient temperature,  $25 \pm 1^\circ\text{C}$  and 45-55% relative humidity, with a 12 h light: 12 h dark cycle (lights on at 7 a.m.). The animals had free access to standard pellet and water and libitum. Experiments were conducted between 8:00 and 14:00 h. All the experimental procedures were conducted in accordance with the NIH guidelines of the Care and Use of Laboratory Animals.

### Plant Materials and Preparation of Freeze-Dried Extract

*U. dioica* aerial parts and *E. caucasicum* inflorescence were collected from Mazandaran, Iran in April, 2012 and confirmed by Dr Bahman Es-lami. A voucher specimen has been deposited in university Herbarium (1442 and 1443). Materials were dried and coarsely ground before extraction. Materials were extracted by percolation using methanol. The resulting extract was concentrated over a rotary vacuum and then freeze-dried (methanol extracts).

### Preparation of Polyphenol Fraction

Extractions were performed at 20°C in a shaking incubator. Extracting solvent was methanol/acetone/water (3.5/3.5/3) containing 1% formic acid. Extracts were collected and evaporated under vacuum to remove organic solvents. Lipophilic pigments were then eliminated from the aqueous phase by extraction with petroleum ether. The aqueous phase was further extracted three times by ethyl acetate. Organic phases were collected and concentrated over a rotary vacuum and then freeze-dried and used as polyphenol (PP) fraction.

### Maximum Non-Fatal Dose

Different doses of each extracts were injected to separated groups of four mice to find maximum non-fatal dose. After 48 h, induction of any mortality was considered as the maximum non-fatal dose.

## Antihypoxic Activity

### Asphyctic Hypoxia

Animals were subjected to hypoxia by putting them individually in a tightly closed 300 ml glass container which was placed under water in an aquarium of 25°C. The animals had convulsions and died from hypoxia. The latencies for death were recorded. Mice received single i.p. injections of extracts (200 and 400 for PP fractions and 300 and 600 mg/kg for methanol extracts) and phenytoin (50 mg/kg) as 30 min before they were subjected to hypoxia. Control group was treated with normal saline<sup>8</sup>.

### Haemic Hypoxia

Seventy two mice were divided into nine groups each containing eight mice. Control group was treated with normal saline. Thirty

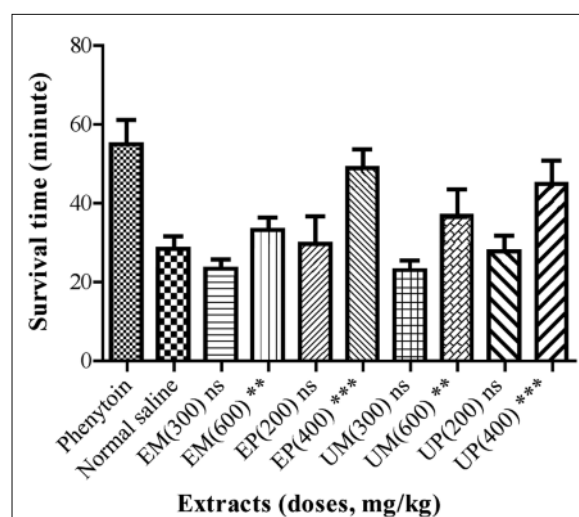
minutes after i.p. administration of different doses of extracts (200 and 400 for PP fractions and 300 and 600 mg/kg for methanol extracts), NaNO<sub>2</sub> (360 mg/kg) was applied i.p. to mice and antihypoxic activity was estimated as the latent time of evidence of hypoxia in minutes<sup>9</sup>.

### Circulatory Hypoxia

Seventy two mice were divided into nine groups each containing eight mice. Groups were treated with normal saline. Thirty minutes after i.p. administration of extracts (200 and 400 for PP fractions and 300 and 600 mg/kg for methanol extracts), NaF (150 mg/kg) was applied i.p. to mice and antihypoxic activity was estimated in minutes as the latent time of evidence of hypoxia<sup>10</sup>.

## Results and Discussion

The maximum non-fatal doses for methanol extract of UD and PP fraction of EC were 1 g/kg, and for PP fraction of UD and methanolic extract of EC were 2 g/kg. At these doses no mortality were observed after 48 hrs. Statistically significant antihypoxic activities were established in some doses of extracts in the experimental model of hypoxia in mice. The effects were mostly dose-dependent. Extracts showed significant antihypoxic activity in asphyctic model (Figure 1). EC methanolic extract at 600 mg/kg prolonged



**Figure 1.** Antihypoxic activities of *E. caucasicum* (EC) and *U. dioica* (UD) in asphyctic hypoxia. Data are Mean  $\pm$  SD (n = 8), (ns: not significant; \*\* $p < 0.01$ , \*\*\* $p < 0.001$ , as compared to control).

latency for death ( $33.20 \pm 3.11$  vs.  $28.20 \pm 3.27$  min for control groups,  $p < 0.01$ ). PP fraction at 400 mg/kg also prolonged latency for death ( $48.80 \pm 4.86$  min,  $p < 0.001$ ). Its effect was comparable with that of phenytoin ( $55.00 \pm 6.05$  min,  $p > 0.05$ ). PP fractions were more active than methanol extract. UD methanol extract at 600 mg/kg prolonged latency for death ( $36.86 \pm 6.76$  min,  $p < 0.01$ ). Its PP fraction at 400 mg/kg also prolonged latency for death ( $44.80 \pm 5.84$  min,  $p < 0.001$ ), but its effect was not comparable with that of phenytoin ( $p < 0.01$ ). For both extracts, PP fractions were more active than methanol extract. There are literature data that administration of sodium fluoride increases the blood histamine content and decreases the oxygen carrying capacity<sup>8</sup>. The results of circulatory hypoxia are shown in Table I. The effects of EC were dose-dependent. PP fraction of EC at 400 mg/kg was the most effective extract. It prolonged latency for death significantly respect to control group ( $23.80 \pm 3.82$  vs.  $9.29 \pm 0.95$  min for control groups,  $p < 0.001$ ). UD extracts protected the mice but the response was not dose-dependent. At 300 mg/kg, methanol extract prolonged survival time ( $19.50 \pm 3.62$  min,  $p < 0.001$ ). Its PP fraction at 200 mg/kg also kept mice alive for  $15.20 \pm 1.30$  min. This effect was statistically significant from control ( $p < 0.01$ ). In contrast, the mean survival time was decreased by increasing extract dose. It seems some components in higher concentrations, antagonizes the hypoxic effect. Toxicity of extracts is another possibly for this observation.

Injection of sodium nitrite (360 mg/kg i.p.), reduces the oxygen-carrying capacity of the blood by converting hemoglobin to methemoglobin. This lethal dose is injected 30 min after extract treatment and the time between injection of NaNO<sub>2</sub> and cessation of respiration is recorded<sup>8</sup>. The results of haemic hypoxia are shown in Table I. Extracts of EC (300, 400 and 600 mg/kg) significantly ( $p < 0.001$ ) and dose dependently prolonged survival time as compared to control group ( $7.01 \pm 0.85$  min). EC methanol extract at 600 mg/kg was the most effective one, being capable of keeping the mice alive for  $12.71 \pm 0.75$  min. Only the concentration of 300 mg/kg of UD was effective. This extract kept mice alive for  $10.00 \pm 1.73$  min ( $p < 0.001$ ). A significant protective effect on hypoxia has been reported by Ginkgo biloba that contains flavonoids<sup>10</sup>. Our findings may be supported by other literature data that flavonoids increase cerebral blood flow

**Table I.** Antihypoxic activities of *E. caucasicum* (EC) and *U. Dioica* in (UD) haemic and circulatory hypoxia in mice.

Groups (extract)	Dose (mg/kg)	Haemic hypoxia (min)	Circulatory hypoxia (min)
Control	–	$7.01 \pm 0.85$	$9.29 \pm 0.95$
EC (M)	300	$10.50 \pm 1.50^{***}$	$12.60 \pm 2.70^{ns}$
EC (M)	600	$12.71 \pm 0.76^{***}$	$14.50 \pm 1.52^*$
EC (PP)	200	$7.86 \pm 0.69^{ns}$	$12.00 \pm 1.41^{ns}$
EC(PP)	400	$10.57 \pm 0.98^{***}$	$23.80 \pm 3.82^{***}$
UD (M)	300	$10.00 \pm 1.73^{***}$	$19.50 \pm 3.62^{***}$
UD (M)	600	$8.66 \pm 0.51^{ns}$	$11.20 \pm 1.31^{ns}$
UD (PP)	200	$8.14 \pm 0.69^{ns}$	$15.20 \pm 1.30^{**}$
UD (PP)	400	$8.71 \pm 1.11^{ns}$	$10.50 \pm 0.57^{ns}$
Phenytoin	–	–	$55.00 \pm 6.05^{***}$

<sup>a</sup>Data are expressed as mean  $\pm$  SD (n = 8), (\*\* $p < 0.01$ , \*\*\* $p < 0.001$ , compared to control). M: methanol extract; PP: polyphenol fraction.

and possess antihypoxic activity. The mechanism of this protective action may be due in part to the antioxidant activity of polyphenols<sup>9</sup>. This relationship is in agreement with our recently published papers which improved good antihypoxic activities of some medicinal plants with good antioxidant activity<sup>8,9</sup>.

## Conclusions

PP fractions of EC and UD are valuable sources of antihypoxic activity. Extracts showed remarkable antihypoxic effects. Pharmacological effects may be attributed to the presence of polyphenols in the extracts.

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## Conflict of Interest

The Authors declare that there are no conflicts of interest.

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