COMPARATIVE STUDY ON THE EFFECTS OF ERYNGIUM SP. EXTRACTS IN AN ACUTE INFLAMMATION MODEL IN RAT

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Summary

The aim of this paper was to evaluate the effect of the tinctures obtained by the aerial parts of three indigenous Eryngium species (Eryngium planum, E. campestre and E. maritimum) by an acute inflammation model in rats.

The anti-inflammatory properties of saponins, coumarins, flavonoids, caffeic acid and plant sterols are well-known.

Previous data on Eryngium species reported the anti-exudative effects of E. planum saponins and of the rhizome extract of E. maritimum suggesting their participation to the vascular response associated to the acute stage of inflammation. Another paper revealed that an E. campestre extract was able to inhibit cytokine-stimulated, iNOS-dependent synthesis of nitric oxide in murine cells.

The aerial parts of Eryngium planum, E. campestre and E. maritimum (Apiaceae) were extracted in ethanol 70º for 10 days to afford a 20% tincture (T). Experiments were carried out on 5 groups of 8 adult male Wistar rats (150-170g). The turpentine oil-induced acute inflammation model in rats was assessed. Turpentine oil was administrated to all animals (i.m., 6 mL/Kg.) in order to induce the inflammation. After 15 minutes, animals were treated intraperitoneally by groups: group P, C, M - 20% tincture of E. planum/E. campestre/E. maritimum (200 mg dried plant material/Kg) diluted in sterile saline, group I (inflammation control group) - sterile saline and group D - diclofenac (20 mg/Kg) used as a standard NSAID. Effects were evaluated comparatively by measuring serum nitrites and nitrates, total oxidative status (TOS), total antioxidant activity (TAR), index of oxidative stress (IOS), total leukocyte count and differential leukocyte count. All data were evaluated statistically by Anova test (P value <0.05 was considered as statistically significant).

All tested extracts decreased significantly the oxidative stress by increasing TAR. All extracts decreased significantly the total leukocytes count by reducing the neutrophils and monocytes. NO synthesis was decreased by all tested Eryngium extracts.

Among tested extracts, E. maritimum had the best anti-inflammatory, while Eryngium planum showed the best antioxidant activity.

Keywords. Eryngium, turpentine oil, inflammation, NO, leukocytes.

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Introduction

Genus Eryngium (Apiaceae/Saniculoideae) is represented in Romania’s flora by three species: Eryngium planum L. (speedwell), E. campestre L. (field eryngo) and E. maritimum L. (sea holly). Eryngii plani herba representing the aerial parts of Eryngium planum L is a well-known folk remedy that was introduced in modern phytotherapy as a remedy for whooping cough. Pater, Peyer, Manta and Weinrich (cited by Hiller) reported that the triterpene saponins were the active principles responsible for the diaphoretic, expectorant, depurative and diuretic effects (Hiller, 1966).

The other species, Eryngium campestre and E. maritimum were in the past popular medicinal herbs used in folk
medicine for the anti-scorbutic, diaphoretic, diuretic, expectorant, anti-inflammatory and aphrodisiac properties. Nowadays, European herbal medicine recommends them as diuretics for treating renal disorders (cystitis, urethritis, chronic prostatitis, painful urination) and for preventing kidney stones formation (Weiss, 2000).

Previous study conducted on several ethanolic and aqueous extracts obtained from either aerial parts or roots of eight Eryngium species growing in Turkey, results showed potent in vivo anti-inflammatory and antinociceptive activities. Aerial parts and roots of Eryngium maritimum and Eryngium kotschyi were found to possess most promising activities without including any apparent gastric damage (Küpeli et al., 2006). Another paper concluded that Eryngium maritimum presented relatively strong total antioxidant activities (Meot-Duros et al., 2008).

**Material and methods**

The aerial parts of Eryngium planum, E. campestre and E. maritimum (Apiaceae) were extracted in ethanol 70\(^0\) for 10 days to afford a 20% tincture (T). Experiments were carried out on 5 groups of 8 adult male Wistar rats (150-170g). Animals were housed in temperature-controlled room and received water and food ad libitum until used. The experimental protocol adhered to ethical standards (Zimmermann, 1983).

The turpentine oil-induced acute inflammation model in rats was assessed (Pleșca-Manea et al., 2002). Turpentine oil was administrated to all animals (i.m., 6 mL/Kg.) in order to induce the inflammation. After 15 minutes, animals were treated intraperitoneally by groups: group P – 20% tincture of E. planum, group C – 20% E. campestre, group M - E. maritimum. A dose of 200 mg plant material/Kg b.w.) was tested in animals. Group I (inflammation control group) - sterile saline and group D - diclofenac (20 mg/Kg) - a standard non-steroidal antiinflammatory drug.

After 24 h, blood samples were withdrawn retro-orbitally into 15 mL disposable centrifuge tubes (5U heparin/mL). Proteins were removed from serum by centrifugation through a 30 kDa filter at 14,000 rpm for 1.5 – 3 hours at 4\(^\circ\)C. Effects were evaluated comparatively by measuring: serum nitrites and nitrates, total oxidative status (TOS), total antioxidant activity (TAR), index of oxidative status (IOS), total leukocytes count and differential leukocytes count. All data were evaluated statistically by ANOVA test (P<0.05 was considered statistically significant).

**Total leukocyte count and differential leukocyte count (%)**

For total leukocyte count we assayed a blood sample diluted 1:10 in Türk solution. Count was performed with an optical microscope (OLYMPUS), using a Bürcker-Türk counting-chamber. Differential leukocyte count expressed as a percentage was carried out on May-Grünwald-Giemsa stained smears (Pleșca-Manea et al., 2002).

**Nitrite analysis**

Serum nitrite was quantified by the Griess reaction from a standard nitrite calibration curve, using a Cecil-CE 3021 diode – array spectrophotometer. The simplest and most frequently applied method employs colorimetric detection with Griess reagents followed by the measurement of the absorbance at 540 nm (Miranda et al., 2001). The Griess reaction is based on the formation of a chromophore from the diazotization of sulfanilamide by acidic nitrite followed by coupling with bicyclic amines such as \(N\-1\)-(naphthyl) ethylenediamine. The principle of this assay is reduction of nitrate by vanadium(III) combined with detection by the acidic Griess reaction (Miranda et al., 2001). Samples were assayed in duplicate. Results were expressed as mg nitrite/dL serum.
Total antioxidant response (TAR)

Total antioxidant response (TAR) was measured according by the method developed by Erel (Erel et al., 2004).

Because the measurement of different antioxidant molecules separately is not practical and antioxidant effects of them are additive, total antioxidant response (TAR) of a sample is measured. This is named as total antioxidant capacity, total antioxidant activity, total antioxidant power or total antioxidant status or TAR. Potent free radical reactions were initiated with the production of hydroxyl radical (OH\(^-\)) via Fenton reaction, and the rate of the reactions was monitored by following the absorbance of colored dianisidyl radicals. Ortho-dianisidine (10 mM) and ferrous ammonium sulfate (45 AM) were dissolved in KCl/HCl solution (75 mM, pH 1.8). This mixture was named as Reagent 1 and hydrogen peroxide solution (7.5 mM) as Reagent 2. The OH\(^-\) produced by mixing of R1 and R2, oxidized o-dianisidine molecules into dianisidyl radicals, leading to a bright yellow-brown color development within seconds. Absorbance was read at 444nm wavelength. Antioxidants, present in the sample, suppressed the color formation to a degree that is proportional to their concentrations.

Assay calibration. The suppression of the color formation is calibrated with Trolox, which is widely used as a traditional standard for TAR measurement assays, so the results in this assay are expressed as in millimolar Trolox equivalent per liter (mmol Trolox equiv./L).

Total oxidant status (TOS)

Measurement of the total oxidation status (TOS) was carried out according to a colorimetric method (Erel et al., 2005).

We evaluated the total oxidative status of plasma by measuring total peroxide level. The assay is based on the oxidation of ferrous ion to ferric ion in the presence of various oxidant species in acidic medium and the measurement of the ferric ion by xylene orange. Sample absorbance was read at 560nm.

The color intensity is related to the total amount of oxidant molecules present in the sample. The assay is calibrated with hydrogen peroxide and the results are expressed in terms of micromolar hydrogen peroxide equivalent per liter (µmol H\(_2\)O\(_2\) Equiv./L).

We evaluated the total oxidative status of plasma by measuring total peroxide level.

Oxidative stress index

The ratio of total peroxide to total antioxidant potential is named index of oxidative stress (IOS), an indicator of the degree of oxidative stress.

Results and discussion

All results were statistically analysed by ANOVA test. Data is presented in figures presented below (Fig.1 to Fig. 8).

All extracts decreased significantly the total leukocyte count by reducing the monocytes and neutrophils %. The effect on total leukocyte count was more intense in the group treated with Eryngium maritimum tincture (p<0.001 vs. diclofenac). E. planum had lowered most the neutrophils % (p<0.01 vs. diclofenac). Lymphocytes were increased in all groups treated with Eryngium tinctures. This effect was most intense in group treated with E. planum tincture (p<0.01 vs. control).

NO synthesis was decreased by all tested Eryngium extracts. Eryngium maritimum had the best effect on NO (p<0.001 vs. control). Comparable to diclofenac, effect on NO was more intense than diclofenac in group M (p<0.01), while E. campestre and E. planum had a similar effect to diclofenac on NO (p>0.05).

All tested Eryngium extracts decreased significantly the oxidative stress by increasing of TAR (p<0.001 vs. control). Index of oxidative stress (IOS) was significantly lowered by all extracts.
(p<0.001). IOS was decreased the most by E. planum tincture (p<0.001 vs control). Effect on IOS was better for diclofenac (p<0.01 vs. Eryngium extracts). Total antioxidant status (TAR) was increased by all Eryngium extracts (p<0.001 vs. control). Compared to diclofenac, the tincture of E. planum had the best effect on TAR thus the most promising antioxidative effect.

**Fig. 1 Effect on total leukocyte count**

**Fig. 2 Effect on monocytes**

**Fig. 3 Effect on neutrophils %**

**Fig. 4 Effect on lymphocytes**

**Fig. 5 Effect on NO synthesis**

**Fig. 6 Effect on total oxidant status**

**Fig. 7 Effect on total antioxidant response**

**Fig. 8 Effect on index of oxidative stress**

**Conclusions**

The anti-inflammatory properties of several bioactive compounds including saponins, cumarins, flavonoids, caffeic acid and plant sterols are well-known (Strzelecka et al., 2005). Previous papers on Eryngium sp., reported the anti-exudative effects of E. planum saponins (Jacker et al., 1976) and of a hydrophilic extract of E. maritimum rhizome (Lisciani et al., 1984) extract.
suggesting their participation to the vascular response associated to the acute stage of inflammation.

In another paper, the topically applied hexane extract containing phytosterols of *E. foetidum* reduced phorbol ester-induced acute and chronic auricular oedema in mouse (Garcia *et al*., 1999). Also, an *E. campestris* extract was able to inhibit cytokine-stimulated, iNOS-dependent synthesis of nitric oxide in murine cells (Stalinska *et al*., 2005).

Nitric oxide (NO) is an endogenous mediator involved in the regulation of many physiological functions and is involved in a variety of possible pathophysiological processes.

The signaling molecule NO is produced by the enzyme NO synthase (NOS) after activation. NOS is constitutively expressed in endothelial (ecNOS) and neuronal cells (neuronal NOS), while a third isoform (iNOS) is induced in response to inflammatory-like stimuli and is capable of sustained production of high levels of NO that is predominant in inflammation.

Under physiological conditions, NO released from the endothelium regulates vascular tone and maintains vessel patency by helping prevent platelet aggregation and down-regulating adhesion molecule expression. Mediators released during the acute phase of inflammation, including histamine, 5-hydroxytryptamine, bradykinin, platelet-activating factor, and substance P, evoke the release of endothelial NO, causing vasodilatation and vascular permeability, thus facilitating edema formation and trafficking of inflammatory cells (Korhonen *et al*., 2005).

Formation of NO from L-arginine is catalyzed by a diverse family of nitric oxide synthase (NOS) isoenzymes. Activation of the immune system can result in expression of inducible NOS (iNOS) in numerous cell types (e.g., macrophages, neutrophils, hepatocytes. NO levels may be useful markers of inflammation and disease pathogenesis.

NO has been found to be involved in a number of regulatory functions in inflammation. These include infection control, regulation of signaling cascades and transcription factors, regulation of vascular responses, and regulation of leukocyte rolling, migration, cytokine production, proliferation and apoptosis. Inhibitors of NO synthesis, especially selective iNOS inhibitors have been shown to be anti-inflammatory in various forms of experimentally induced inflammation, such as arthritis and colitis and selective iNOS inhibitors are under development for treatment of inflammatory diseases.

In addition to direct antimicrobial effects, NO also regulates neutrophil functions. In *in vitro* studies have been shown to inhibit degranulation, leukotriene production, superoxide anion generation and chemotactic movement in activated neutrophils.

NO has also shown to regulate leukocyte recruitment into the inflammatory focus. Endogenous NO as well as NO-releasing compounds attenuate leukocyte rolling and adhesion to activated endothelium. The underlying mechanism is not known, but it has been related to antioxidant mechanisms of NO and may be dependent on cGMP.

NO is also shown to down regulate adhesion molecules that mediate the interaction between leukocytes and endothelium (Korhonen *et al*., 2005). In the present study, results on NO synthesis showed that the tincture of *E. maritimum* had a better inhibitory activity than diclofenac (p<0.01).

The present study highlights that all tested extracts showed an anti-inflammatory action on bone marrow acute phase response by lowering the total leukocyte number by decreasing the neutrophils % and monocytes.
Also, all tested Eryngium extracts decreased significantly the oxidative stress by increasing of TAR (p<0.001 vs. control).

Although our results demonstrate promising anti-inflammatory effects of tested Eryngium extracts, further studies are required to confirm the pharmacological relevance of the findings.

References