

ANTI-INFLAMMATORY EFFECTS OF *ERYNGIUM PLANUM* L. AND *E. MARITIMUM* L. (*APIACEAE*) EXTRACTS IN TURPENTINE-OIL INDUCED ACUTE INFLAMMATION IN RATS

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Abstract

The aim of this paper was to evaluate the effects of two 1:1 fluid extracts (FE) of *Eryngium planum* and *E. maritimum* aerial parts on acute inflammation induced by turpentine oil in rat. Results of 1:10 and 1:100 dilutions of the two extracts were compared to those from a control group treated only with sterile saline and to a group treated with diclofenac (a nonsteroidal anti-inflammatory drug – NSAID). Both tested extracts showed anti-inflammatory effects, because they lowered the circulating phagocytes proliferation and activation and decreased the nitrogen oxide (NO) synthesis. On bone marrow acute phase response, both extracts showed a very significant inhibitory action by reducing the total leukocyte number by lowering the neutrophils percentage (acute phase phagocytes).

Rezumat

Scopul acestui studiu a fost acela de a evalua efectele a două extracte fluide 1:1 obținute din *Eryngium planum* și *E. maritimum* asupra inflamației acute induse la șobolan cu ulei de terebentină. Rezultatele diluțiilor 1:10, respectiv 1:100 din cele două extracte au fost comparate cu cele ale unui lot martor tratat numai cu ser fiziologic și cu ale unui lot tratat cu diclofenac (un antiinflamator nesteroidian). Ambele extracte testate au demonstrat efecte antiinflamatoare deoarece au redus proliferarea și activarea fagocitelor circulante și au inhibat sinteza de monoxid de azot (NO). Asupra răspunsului medular de fază acută, ambele extracte au evidențiat un efect inhibitor semnificativ, ca urmare a reducerii numărului total de leucocite, datorită scăderii procentului de neutrofile (fagocite de fază acută).

Keywords: inflammation, phagocytosis, NO, *Eryngium planum*, *Eryngium maritimum*

Introduction

The aerial parts of *Eryngium planum* L. were introduced in phytotherapy as a remedy for whooping cough [4, 13]. Previous data reported that the triterpene saponins are the main active principles responsible for the expectorant, depurative, anti-exudative and diuretic effects [5, 10, 11, 12].

Eryngium maritimum was in the past a widely used medicinal herb and in modern phytotherapy it is considered a remedy in renal disorders [5, 12].

Previous papers reported the antiexudative effects of *Eryngium sp.* suggesting their participation to the vascular response associated to the acute stage of inflammation [5, 10].

Materials and Methods

The aerial parts of *Eryngium planum* and *E. maritimum* were collected in July 2004, from Topa Mică (Sălaj) and Eforie Nord (Constanța),

respectively. After identification and authentication by Professor Dr. Mircea Tămaș, a voucher specimen from each species was deposited at the Herbarium of the Department of Pharmaceutical Botany, "Iuliu Hațieganu" University of Medicine and Pharmacy, Cluj-Napoca, Romania.

The air-dried powdered aerial parts (1 Kg) were extracted with 70° ethanol (1L), for 10 days, at room temperature, by repeated percolation, to obtain 1L of 1:1 fluid extract (FE) [3].

The standardized content in crude saponins was determined by densitometry: 4% saponins in *E. planum* FE [11] and 10% saponins in *E. maritimum* FE [12]. Fluid extracts were diluted 1:10 and 1:100 in sterile saline.

Experiments were carried out on six groups, each constituted of six male Wistar rats (150 - 170 g). Animals were housed in a temperature-controlled room and received water and food *ad libitum*. Rats were treated in accordance with the guidelines of animal bioethics from the Act on Animal Experimentation and Animal Health and Welfare

from Romania and all procedures were in compliance with European Communities Council Directive 1986 (86/609/EEC) and were approved by the Ethics Committee of the "Iuliu Hațieganu" University of Medicine and Pharmacy Cluj-Napoca (Romania) [2].

Turpentine oil-induced acute inflammation model in rats was employed [7, 8]. Briefly, the experimental acute inflammation was induced by injecting turpentine oil (6 mL/Kg, i.m. body weight) into the rat's right hind paw. After 15 minutes, animals were administered i.p. as follows: group 1 - *E. planum* FE (1 g/Kg b.w.); group 2 - *E. planum* FE (100 mg/Kg b.w.); group 3 - *E. maritimum* FE (1 g/Kg b.w.); group 4 - *E. maritimum* FE (100 mg/Kg b.w.); group 5 (control) - sterile saline; group 6 - diclofenac (20 mg/Kg b.w.). After 24 h, blood samples were withdrawn via the retro bulbar vein puncture on EDTA solution in order to evaluate the phagocytosis properties of the blood and to count the white blood cells, and without EDTA for the biochemical parameters determination [6]. The blood was centrifuged at 3000 rpm for 10 minutes, to separate the serum in maximum 3 hours after collection, serum was separated immediately, brought into Eppendorf tubes and stored at -20°C until determinations [1, 6, 9].

Test for *in vitro* phagocytosis

Briefly, 20 µL of a suspension of *Escherichia coli* was incubated at 37°C with 0.2 mL blood taken in EDTA, for 20 min, and slowly rotated. The samples were used for smears, stained with May-Grünwald-Giemsa [7, 9].

Anti-inflammatory effects were quantified by measuring serum nitrite level, by assaying an *in vitro* phagocytosis test and by total leukocyte count and differential leukocyte count (%) [7].

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 Bürker-Türk counting-chamber. Differential leukocyte count expressed as a percentage was carried out on May-Grünwald-Giemsa stained smears [7, 9].

We counted the phagocytosis index (PI) = number of phagocytes/100 leukocytes; and the phagocytes activity (PA) = number of *E. coli* phagocytosed by 100 leukocytes [7].

Serum nitrite was quantified by the Griess reaction from a standard nitrite calibration curve, using a Cecil-CE 3021 diode – array spectrophotometer. Results were expressed as mg nitrite/dL [7].

The statistical analysis of data was carried out with SPSS Software 13.0. Results were expressed as mean ± SEM of 3 independent experiments. Unpaired Student *t*-test was employed.

Results and Discussion

Compared to the control group, FE of *E. planum* and *E. maritimum* in both dilutions reduced

significantly the leukocytes number ($p < 0.0001$) (Figure 1). These changes were caused by the decrease in neutrophils % ($p < 0.0001$) (Figure 2). The effect of the 1:100 dilution of the *E. planum* FE was better ($p < 0.05$). For *E. maritimum* FE, both dilutions had a similar effect on leukocyte count ($p > 0.05$) (Figure 1).

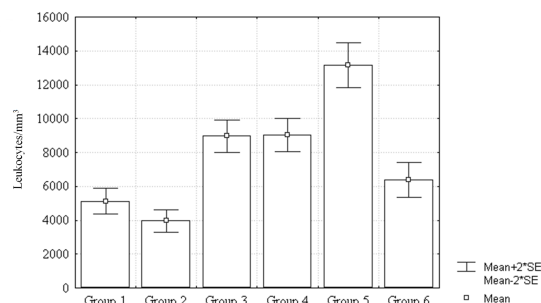


Figure 1.

Effect of tested *Eryngium* extracts (groups 1-4), control (group 5) and diclofenac (group 6, positive control) on total leukocyte count (no/mm³)

Effect of 1:10 dilution of *E. planum* FE was similar to diclofenac on the leukocyte count ($p > 0.05$) (Figure 1), while only the 1:100 dilution had a significantly better effect than diclofenac ($p < 0.01$) (Figure 1). Both tested dilutions of *E. maritimum* FE lowered significantly the leukocytes number by lowering the neutrophils percentage more than diclofenac ($p < 0.001$) (Figure 2).

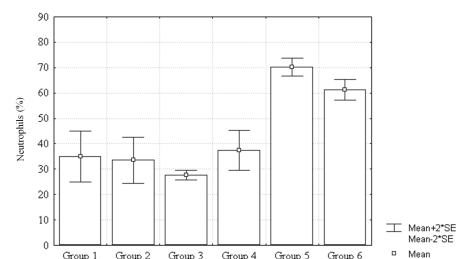


Figure 2.

Effect of tested *Eryngium* extracts (groups 1-4), control (group 5) and diclofenac (group 6, positive control) on neutrophils %

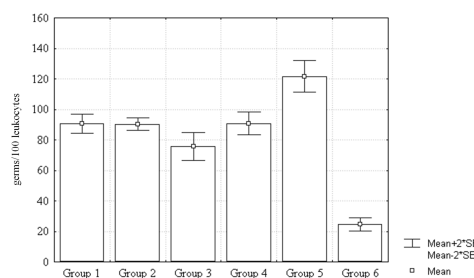


Figure 3.

Effect of tested *Eryngium* extracts (groups 1 - 4), control (group 5) and diclofenac (group 6) on phagocytosis activity (PA) %

Both extracts had a significant inhibitory effect on phagocytes activity (PA), compared to control ($p < 0.001$), but lower to diclofenac ($p < 0.001$) (Figure 3). 1:100 dilution of *E. planum* FE lowered moderately the phagocytosis index (PI) ($p < 0.05$). Compared to control, *E. maritimum* FE in both dilutions showed a significant inhibitory action of the phagocytosis index (PI) ($p < 0.001$). The effect of diclofenac on PI was higher than both *E. planum* and *E. maritimum* FE ($p < 0.001$) (Figure 4).

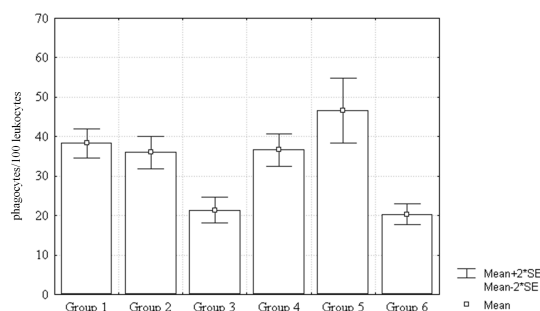


Figure 4.

Effect of tested *Eryngium* extracts (groups 1 - 4), control (group 5) and diclofenac (group 6) on phagocytosis index (PI)

Both dilutions of *E. planum* and *E. maritimum* FE reduced significantly the NO synthesis compared to control ($p < 0.001$). Compared to diclofenac, both 1:10 dilutions reduced more than diclofenac the NO synthesis, but *E. planum* FE had the best effect ($p < 0.001$) (Figure 5).

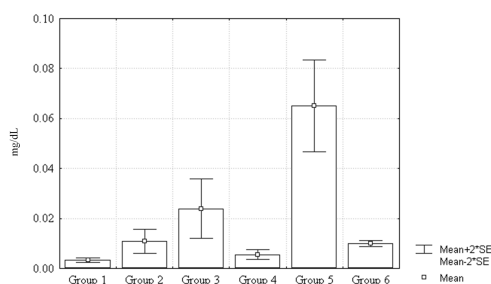


Figure 5.

Effect of tested *Eryngium* extracts (groups 1 - 4), control (group 5) and diclofenac (group 6) on NO synthesis (mg/dL)

Conclusions

The present study highlights that all tested extracts showed an anti-inflammatory action on bone marrow acute phase response by lowering the total leukocytes number by decreasing the neutrophils proliferation and activity. Compared to diclofenac, the effect of both tested *Eryngium* extracts on phagocytosis was lower for both phagocytosis activity and phagocytosis index. On NO synthesis,

1:10 dilution of the *E. planum* FE had a better inhibitory activity than diclofenac.

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