

EVALUATION OF THE ANTI-INFLAMMATORY ROLE OF A NATURAL PRODUCT WITH *ARNICA MONTANA* EXTRACT AND *BOSWELLIA SERRATA* RESIN

EVALUAREA ROLULUI ANTIINFLAMATOR AL UNUI PREPARAT NATURAL CU EXTRACT DE *ARNICA MONTANA* ȘI RĂȘINĂ DE *BOSWELLIA SERRATA*

Cristina DUMITRACHE^{1),2)},
Maria CRIVINEANU^{1),*}, Mihaela EREMIA²⁾,
Diana-Mihaela ALEXANDRU¹⁾

ABSTRACT | REZUMAT

Arnica montana and *Boswellia serrata* are plants with very important properties, among which the strong anti-inflammatory activity stands out. This study aimed to evaluate the anti-inflammatory activity of a mixture of the two compounds (*Arnica montana* and *Boswellia serrata*) in an experiment on rats (carrageenan-induced oedema, at the level of the paw) by comparing the effect obtained with a gel with extract of Arnica. Rats were treated with Arnica gel and with a mixture of Arnica and *Boswellia serrata* extract and a significant reduction in carrageenan-induced paw oedema was observed ($p < 0.05$). For the Arnica gel, an inhibition of edema appeared 5 hours after the carrageenan injection. The inhibitory effect of the natural mixture with *Arnica montana* and *Boswellia serrata* extract began 3 hours after the carrageenan injection, demonstrating that both compounds produce anti-inflammatory effects in the second phase of oedema, indicating the inhibition of prostaglandin synthesis. Both the reference product (Arnica gel) and the tested mixture showed anti-inflammatory action, but following the measurements, it could be concluded that the mixture was more effective in reducing oedema.

Keywords: anti-inflammatory, oedema, carrageenan, *Arnica montana*, *Boswellia serrata*

Arnica montana și *Boswellia serrata* sunt plante cu proprietăți foarte importante, printre care se remarcă activitatea antiinflamatorie puternică. Scopul acestui studiu a fost de a evalua activitatea antiinflamatorie a unei mixturi cu cei doi compuși (*Arnica montana* și *Boswellia serrata*) într-un experiment pe șobolani (edem indus de caragenină, la nivelul labei) prin compararea efectului obținut cu al unui gel cu extract de Arnica. Șobolanii au fost tratați cu gel cu Arnica și cu preparat natural cu extract de Arnica și *Boswellia serrata* și s-a observat o reducere semnificativă a edemului labei indus de caragenină ($p < 0,05$). Pentru gelul cu Arnica s-a constatat o inhibare a edemului la 5 ore după injecția cu caragenan. Efectul inhibitor al preparatului natural cu extract de *Arnica montana* și *Boswellia serrata* a început la 3 ore după injecția cu caragenan, demonstrând că ambii compuși produc efecte antiinflamatorii în a doua fază a edemului, indicând inhibarea sintezei prostaglandinelor. Atât produsul de referință (gel de Arnica) cât și mixtura testată au prezentat acțiune antiinflamatorie, însă în urma măsurărilor s-a putut concluziona că mixtura a avut o eficiență mai mare în reducerea edemului.

Cuvinte cheie: antiinflamator, edem, caragenan, *Arnica montana*, *Boswellia serrata*

Inflammation plays a major role in most chronic diseases, including neurodegenerative, cardiovascular, pulmonary, metabolic, autoimmune, and neoplastic diseases. The anti-inflammatory activity of *Arnica montana* extract and *Boswellia serrata* resin has been known for centuries, both compounds being used in ancient medicine for the treatment of inflammatory disorders (1, 5, 7).

Studies on their effects have revealed numerous pharmacological activities, including antioxidant and anti-inflammatory properties. These activities can be

attributed to lactone sesquiterpenes compounds (derivatives of helenalin and dihydrohelenalin) (3, 10). Micromolar concentrations of helenalin and dihydrohelenalin and their esters inhibit the activation of transcription factors - the nuclear factor of activated T cells (NF-AT), important mediators in inflammatory processes. As a result of the inhibition of the transcription factors, the cascade of triggered inflammatory reactions is also inhibited through the activation of NF- κ B, respectively the production of a variety of pro-inflammatory mediators (C-reactive protein, interleukins IL-1, IL-2, IL-6, IL-8) (2, 6, 9). Unlike other anti-inflammatories, the compounds of Arnica intervene in the initiation phase of the inflammatory process. Helenalin also contributes to the reduction of the inflamed area by stabilizing the lysosomal membranes. Interestingly, that its derivatives can work both anti-infla-

1) University of Agronomic Sciences and Veterinary Medicine, Faculty of Veterinary Medicine, Bucharest, Romania

2) National Institute for Chemical Pharmaceutical Research and Development, Bucharest, Romania

*) Corresponding author: maria_crivineanu@yahoo.com

mmatory and immunostimulant (8, 11).

In this study, the anti-inflammatory activity of the mixture of *Arnica montana* and *Boswellia serrata* was investigated in comparison with that of an Arnica gel, by testing the inhibitory effects of these compounds on paw oedema in rats. The purpose of the study was to provide evidence of the anti-inflammatory activity of Arnica extract and *Boswellia serrata* resin and to highlight its effectiveness in the prevention and treatment of various chronic pro-inflammatory diseases.

MATERIALS AND METHODS

The experiment was carried out in the Biobase of the National Chemical-Pharmaceutical Research and Development Institute in Bucharest and it was approved by the Institute's Ethics Committee.

For the study, 40 Wistar rats were used, with body weight between 180 - 200 g, individualized by coloured signs. The operating conditions were compliant, and the animals benefited from optimal housing conditions. The animals were kept at the constant temperature of the laboratory for at least 6 hours (19-21 °C) and they did not receive water 12 hours before the experiment (4). Four homogeneous groups of 10 animals each were formed:

Group 1 — control animals, which receive the phlogistic agent (carrageenan 1% solution administered subcutaneously in the right hind paw, on the interdigital surface between the hallux and the first phalanx, in a volume of 0.05 ml/animal);

Group 2 — animals receiving the phlogistic agent and the reference agent (Arnica gel). The reference product was applied locally to the rats and, after one hour, they were administered carrageenan (under the conditions of batch 1);

Group 3 — animals that receive the phlogistic agent and the product base (glycerine),

Group 4 — animals that receive the phlogistic agent and the product to be researched (the mixture of *Arnica montana* and *Boswellia serrata*). The research product was initially applied and after one hour they were administered carrageenan (under the conditions of batch 1).

In all animals, the initial volume of the right hind paw was measured by plethysmometric determination. Subsequently, plethysmometric determinations are made one hour, three hours, five hours, and 24 hours after the administration of the phlogistic agent (carrageenan). To interpret the results, we calculated: the averages in absolute value (mm³) of the initial volume of the right hind paw in the 4 groups, the averages obtained after the induction of oedema with carrageenan at one hour, three hours, five hours, and 24 hours, as well as the averages obtained after treatment with the research product.

The anti-inflammatory activity of the researched product was determined using a carrageenan-induced paw oedema assay according to the method of Winter et al., 1962. The test product was administered topically (massage of the right lower limb) to rats one hour before the subcutaneous injection of carrageenan (1%) at the plantar level of the right lower limb. The control group received an equivalent volume, and the positive control group received the reference substance (Arnica gel). After the carrageenan injection, paw volumes were measured at one hour, three, five, and 24 hours, using a plethysmometer Model 7150, UGO Basile, Italy. Oedema was expressed as the mean increase in paw volume relative to control animals. The percentage of oedema inhibition was calculated by the following equation:

% oedema inhibition = $100 (1 - V_t / V_c)$, where:

V_c = volume of oedema in the control group,

V_t = oedema volume in the tested group.

For the statistical analysis, the Excel program was used, and the data were presented as mean ± SD (standard deviation). Differences in mean values between groups were analysed by one-way analysis of variance (ANOVA), and statistical significance was assessed as $p < 0.05$.

RESULTS AND DISCUSSION

Carrageenan-induced paw oedema is a suitable experimental animal model for evaluating an anti-inflammatory effect. Oedema developed after carrageenan injection serves as an index of acute inflammatory changes and can be determined from differences in paw volume measured immediately after carrageenan injection and then at one hour, three hours, five hours, and 24 hours. Carrageenan-induced oedema is biphasic: the first phase (at one hour) involves the release of serotonin and histamine, and the second phase (over one hour) is mediated by prostaglandins, products of cyclooxygenase. Continuity between the two phases is ensured by kinins.

To demonstrate the validity of the carrageenan-induced paw oedema test, rats were given the reference product (Arnica gel) one hour before the carrageenan injection. As expected, the reference product (Arnica gel) significantly decreased paw oedema, three and five hours after the carrageenan injection. These results demonstrate that Arnica gel, a cyclooxygenase inhibitor, exerts an antioedematous effect during the second phase of paw oedema due to the reduction of prostaglandins, which are inflammatory mediators in the second phase.

The results of the experiment are also consistent with many other studies that demonstrate that Arnica gel strongly inhibits the second phase of oedema, without affecting the first phase.

Table 1
The dynamics of the inflammatory process in the four groups in the experiment during the 24 hours

Group / time	0 h	1 h	3 h	5 h	24 h
Group 1	1.32 ± 0.07	1.83 ± 0.36	2.04 ± 0.33	1.91 ± 0.32	1.65 ± 0.23
Group 2	1.26 ± 0.04	1.79 ± 0.33	2.00 ± 0.31	1.89 ± 0.35	1.56 ± 0.12
Group 3	1.32 ± 0.02	1.89 ± 0.25	2.07 ± 0.23	1.98 ± 0.22	1.66 ± 0.11
Group 4	1.23 ± 0.05	1.68 ± 0.20	2.02 ± 0.06	1.95 ± 0.04	1.73 ± 0.05

* Values are presented as mean ± DS (standard deviation); p<0.05

The next step was to examine the anti-inflammatory effect of the mixture of *Arnica montana* and *Boswellia serrata*. The rats were topically applied, one hour before the administration of carrageenan, the natural product with Arnica extract and Boswellia resin. Thus, it was observed that the tested mixture had a better anti-inflammatory effect, directly observed by the significant reduction of paw oedema. For example, at t_0 = the beginning of the experiment, its dynamics were the lowest, following that the maximum inflammation was recorded at t_3 = 3 hours (from 1.32 ± 0.07 to 2, 2.04 ± 0.33 for group 1, from 1.26 ± 0.04 to 2.00 ± 0.31 for group 2, from 1.32 ± 0.02 to 2.07 ± 0.23 for group 3 and from 1.23 ± 0.05 to 2.02 ± 0.06 for group 4) (Table 1).

It can be seen that the applied product has different effects for each individual batch. Thus, the most potent products are those used in batch 4 (a natural preparation with *Arnica montana* extract and *Boswellia serrata* resin), followed by the one from batch 2 (the reference preparation - Arnica gel). Thus, it can be concluded that the products from the other batches, respectively 1 (untreated control batch) and 3 (batch treated with product base - glycerine) had no anti-inflammatory reaction (Fig. 1).

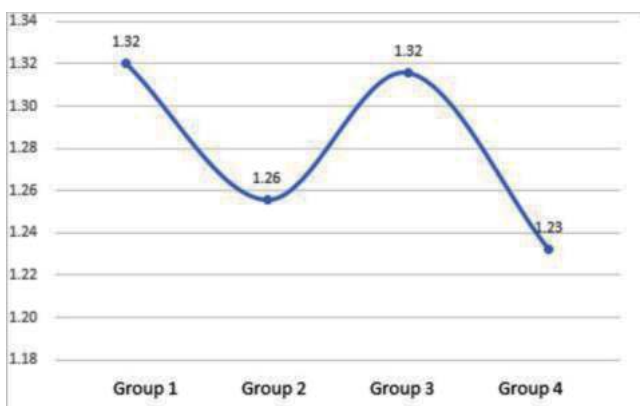


Fig. 1. The anti-inflammatory reaction of the 4 batches at t_0

In the recordings made after one hour, changes are observed in all the values of the anti-inflammatory reaction, highlighting batch 4 which shows the strongest

anti-inflammatory action (Fig. 2).

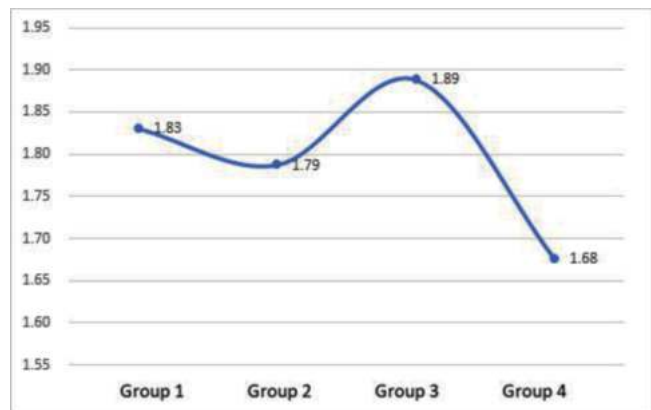


Fig. 2. The anti-inflammatory reaction of the 4 batches at 1 h

The measurements taken at 3 hours continue to indicate an increase in the value of the anti-inflammatory reactions, and at 5 hours it can be observed that there is a slight decrease in the anti-inflammatory reaction of the tested products.

CONCLUSIONS

The reference product (Arnica gel) and the tested mixture (a natural product containing *Arnica montana* extract and *Boswellia serrata* resin) have a good anti-inflammatory effect. However, the tested mixture proved to have a better ability to reduce the inflammation induced by carrageenan in the paw of rats and also that the anti-inflammatory effect appears faster than when using only the Arnica gel.

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