

## PHARMACOLOGY AND TOXICOLOGY

### Correction of Adjuvant Arthritis with Delphinium Extracts and Alkaloids

Yu. V. Nesterova, T. N. Povetieva, Yu. G. Nagornyak\*,  
N. I. Suslov, T. V. Vetoshkina, and T. I. Andreeva\*

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Antiinflammatory effects of water-alcohol extracts and sum of alkaloids from the aerial part of delphinium under conditions of immune inflammation caused by Freund's complete adjuvant were comparable to activity of orthophen, a classical antiinflammatory drug. Normalizing effects of the studied extracts on hematological and biochemical parameters were demonstrated.

**Key Words:** *immune inflammation; delphinium; alkaloids; antiexudative effect; hematological and biochemical parameters*

Inflammation and its control remain a pressing problem, especially in diseases of the locomotor system [6]. Experimental adjuvant arthritis is an informative model for the evaluation of antiinflammatory activity of prospective compounds in articular diseases. This model reflects immune mechanisms in the pathogenesis of rheumatoid arthritis and, in contrast to acute inflammation models, reproduces intricate complex of structural and metabolic shifts observed in collagenoses, and therefore it is most suitable for the evaluation of the efficiency of new antiinflammatory drugs [9].

Nonsteroidal antiinflammatory drugs (NSAID) are most often used for the treatment of articular diseases. However, serious side effects of modern NSAID [3,10] often limit their use, especially in chronic diseases, which necessitates the search for new effective drugs with low toxicity. Drugs of plant origin have certain advantages over synthetic drugs, because bioactive substances of plants are characterized by milder effect, better biological availability, and absence of side effects [5].

Laboratory of Phytopharmacology, Institute of Pharmacology, Tomsk Research Center, Siberian Division of the Russian Academy of Medical Sciences; \*Tomsk Military Medical Institute, Russia. **Address for correspondence:** ilz@pharm.tsu.ru. Yu. V. Nesterova

Antiexudative activity of complex extracts and individual substances from the aerial part of delphinium was previously demonstrated on the models of acute inflammation induced with carragenin, acetic acid, histamine, and serotonin [7].

Here we compared phlogolythic activities of delphinium extracts and NAID on the model of immune inflammation induced by Freund's adjuvant.

### MATERIALS AND METHODS

Experiments were carried out on 35 outbred male rats (200-250 g) from collection of Laboratory of Experimental Biosimulation, Institute of Pharmacology. Tincture of delphinium aerial parts, extract from delphinium aerial parts in 40% ethanol prepared by re-percolation after Chulkov, and sum of alkaloids extracted from delphinium grass by the common method [8] were studied. The tinctures were prepared in 40% ethanol in accordance with the requirements of the Pharmacopoeia. The delphinium tincture and extract were standardized by the dry residue.

Chronic immune inflammation was induced by subplantar injection of 0.1 ml complete Freund's adju-

vant (BCG suspension in mineral oil) [9] in the right hind paw. Symptoms of local (right paw edema) and generalized (left paw edema) reactions to adjuvant induction were recorded. The size of inflamed joint was measured with a plethysmometer by measuring the volume of fluid replaced by plunged limb; the percent of edema increment was calculated [11]. Antiinflammatory activity was also evaluated by the severity of hyperemia (expressed in arbitrary units): 0.5: local mild hyperemia of the inflamed limb; 1: mild (pink) hyperemia of the entire limb; 2: moderate (red) hyperemia of the entire limb; 3: severe (dark red) hyperemia of the entire limb.

Edema and hyperemia were evaluated over the course of inflammation every other day. Intragastric treatment with the studied extracts was started 24 h before adjuvant injection and continued throughout the development of the inflammatory process (14 days). Animals of the reference group received orthophen according to the same protocol. Controls received distilled water in an equivalent volume.

Hyperalgesia was evaluated on days 9, 12, and 14 of the experiment by the number of vocalizations during passive flexion of the inflamed joint [1]. Vocalization index was calculated by the formula:  $V=(N/5) \times 100\%$ , where N was the number of vocalizations.

Total serum protein was evaluated by the biuret method, seromucoids by turbidimetry, ALT activity by the method of Writeman—Frenkel [4]. Standard kits (Cormay, Technology Standard, Vital Diagnostics) were used.

Hematological parameters were evaluated by standard methods.

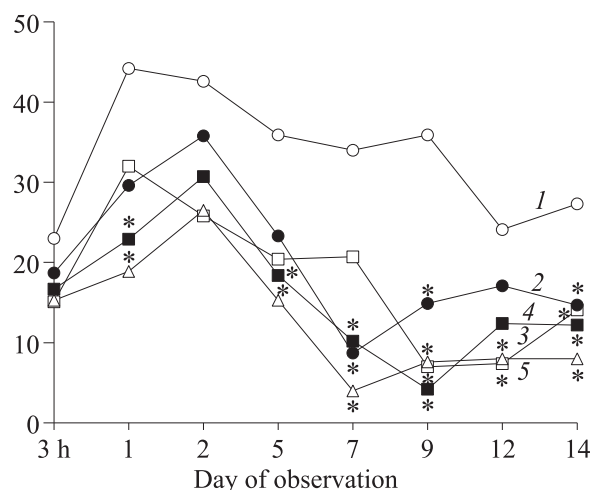
The results were statistically processed using parametric (Student's *t* test) and nonparametric (Mann—Whitney test) methods. The means ( $\bar{X}$ ) and mean square deviation ( $m$ ) were calculated. The significance of differences in the results expressed in percent was evaluated using Fisher's angular transformation. The differences were considered significant at Pt, Pu, Pf<0.05.

## RESULTS

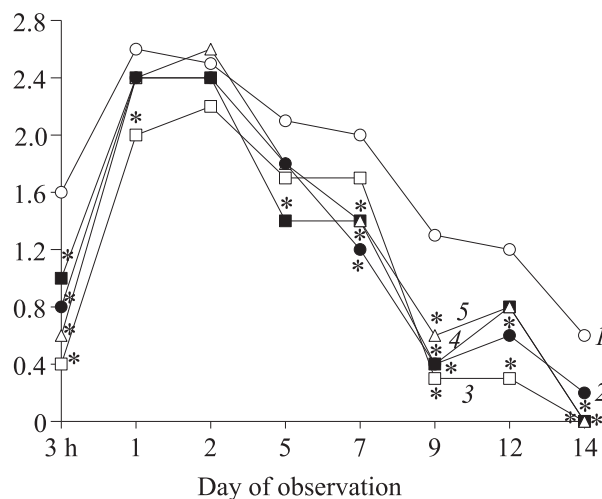
Injection of Freund's adjuvant led to the development of a complex of changes characteristic of chronic immune inflammation. The volume of the injected limb increased in the group of untreated rats as early as 3 h postinjection. The severity of the articular syndrome peaked on the next day, which was characterized by severe edema and hyperemia of the damaged paw (Figs. 1, 2). Generalized reaction (edema of the intact paw) in controls was more pronounced during week 2 of the experiment (Fig. 3). Treatment with the studied extracts alleviated the inflammatory reaction on days 1-14 (Fig. 1) and reduced hyperemia after 3 h and on

days 5-14 (Fig. 2). Delphinium extract and tincture exhibited maximum antiinflammatory activity. Treatment with both preparations led to at least 2-fold reduction of exudative reaction on days 1-5, the maximum suppression (by 8.5 times) of edema in comparison with controls was observed on days 7 and 9 (Fig. 1). Delphinium extract and sum of alkaloid most markedly inhibited the secondary reaction (Fig. 3). Orthophen (reference drug) inhibited edema throughout the entire observation period (by 2-5-fold compared to untreated rats, Fig. 1) and reduced limb hyperemia after 3 h and on days 9 and 12 of the experiment (Fig. 2).

The pain syndrome forms during the development of any inflammatory process and is its obligatory component. We studied analgesic activity of delphinium



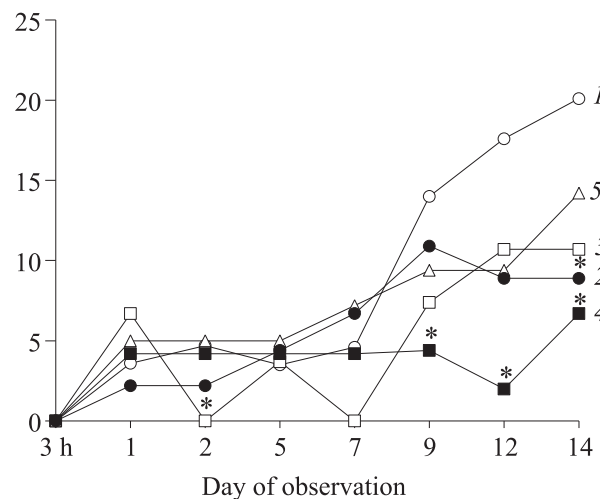
**Fig. 1.** Effects of delphinium extracts on the development of edema in the limb injected with Freund's adjuvant in outbred male rats. Ordinate: increment of edema of injected limb. Here and in Figs. 2, 3: 1) control; 2) alkaloid sum (0.05 mg/kg); 3) orthophen (10 mg/kg); 4) tincture (0.5 ml/kg); 5) extract (0.12 ml/kg). \**p*<0.05 compared to the control.



**Fig. 2.** Effects of delphinium extracts on the development of hyperemia in the limb injected with Freund's adjuvant in outbred male rats. Ordinate: severity of hyperemia in arbitrary units.

extracts in adjuvant arthritis by recording the vocalization index during flexion of the involved joint. On day 9 the vocalization index in experimental groups was 2.8-4.3 times lower than in the control. In addition, on day 12 the rats treated with the tincture and on day 14 those treated with the sum of delphinium alkaloids felt no pain during bending of involved joint (Table 1). Treatment with the reference drug provided the analgesic effect on days 9 and 12 of the study, vocalization index being 5 and 4-fold lower than in the control, respectively (Table 1). The analgesic effect of the studied delphinium extracts can be due to its effects on the peripheral and central components of pain sensitivity. Due to antiexudative effect (Fig. 1) and to elimination of the effects of endogenous algogens (histamine, serotonin) [7], delphinium water-alcohol extracts and sum of alkaloids modified the peripheral component of analgesia. As for the central effects, let us note that diterpene alkaloids (tertiary amines) easily penetrate through the blood-brain barrier; into the CNS, and interact with cholinoreceptors directly involved in the formation of pain sensation [2].

The blood system is one of the main indicators of homeostasis; it realizes the compensatory adaptive functions after exposure to adverse factors [12]. Adjuvant arthritis promoted the formation of changes in the peripheral blood characteristic of chronic immune inflammation. In controls, an increase in total neutrophil count (neutrophilic leukocytosis) and appearance of stab granulocytes (formation of the so-called "shift to the left" at the expense of incomplete maturation of neutrophils in the bone marrow) and basophils in the blood was noted against the background of reduced eosinophil count (Table 2). Treatment with complex extracts and individual delphinium alkaloids prevented the increase in total leukocyte count, while the hemogram values were at the basal level (Table 2). Treatment with the reference drug caused in fact no significant shifts in quantitative characteristics of the



**Fig. 3.** Effects of delphinium extracts on the development of edema in the intact limb after injection of Freund's adjuvant to outbred male rats. Ordinate: increment of edema in intact limb, %.

hemogram in comparison with the control, the total leukocyte count was elevated at the expense of high count of monocytes (Table 2).

The adjuvant disease is often associated with the development of parenchymatous hepatitis with relevant metabolic shifts characteristic of this process. We therefore studied some biochemical parameters of the blood under conditions of immune inflammation development for evaluation of the severity of pathological shifts and the efficiency of their correction with delphinium extracts. Serum levels of seromucoids and ALT increased significantly in rats with arthritis (Table 3). Treatment with delphinium water-alcohol extracts and alkaloid sum improved blood biochemistry in rats, presumably as a result of hepatocyte membrane stabilization with inhibition of enzyme diffusion. Seromucoid concentrations approached the level in intact group under the effect of delphinium tincture, while treatment with delphinium extract led to a 29% reduction of this parameter in comparison with the control.

**TABLE 1.** Effects of Delphinium Extracts on Inflammatory Hyperalgesia in Outbred Male Rats, Caused by Freund's Adjuvant

Group	Dose, mg/kg, ml/kg	Vocalization index, %		
		day after injection of Freund's adjuvant		
		9	12	14
Control	—	34.0±6.0	26.0±7.3	22.0±9.2
Orthophen	10	6.7±2.7*	6.7±1.7*	13.3±8.3
Tincture	0.25	8.0±2.9*	0*	8.0±4.9
Extract	0.12	8±1*	12.0±4.9	16.0±7.8
Alkaloid sum	0.025	12.3*	4±2*	0*

**Note.** Here and in Tables 2, 3: \* $p < 0.05$  compare to the control.

**TABLE 2.** Effects of Delphinium Extracts on Peripheral Blood Parameters ( $\times 10^9/\text{liter}$ ) in Outbred Male Rats on Day 14 after Injection of Freund's Adjuvant ( $X \pm m$ )

Group	Dose, mg/kg, ml/kg	Neutrophilic granulocytes		Eosino- phils	Baso- phils	Lympho- cytes	Mono- cytes
		stab	segmented				
Intact	—	0	2.59 $\pm$ 0.54	0.20 $\pm$ 0.03	0	10.61 $\pm$ 1.12	0.83 $\pm$ 0.14
Control	—	0.06 $\pm$ 0.02 <sup>+</sup>	5.09 $\pm$ 0.49 <sup>+</sup>	0.09 $\pm$ 0.04 <sup>+</sup>	0.08 $\pm$ 0.03 <sup>+</sup>	9.52 $\pm$ 0.46	0.91 $\pm$ 0.14
Orthophen	10	0*	4.83 $\pm$ 0.40 <sup>+</sup>	0.55 $\pm$ 0.28	0.28 $\pm$ 0.14	11.55 $\pm$ 2.03	1.63 $\pm$ 0.19**
Tincture	0.25	0*	3.04 $\pm$ 0.21*	0.20 $\pm$ 0.03*	0.03 $\pm$ 0.03	10.56 $\pm$ 1.25	0.78 $\pm$ 0.16
Extract	0.12	0*	2.43 $\pm$ 0.12*	0.12 $\pm$ 0.05	0*	9.31 $\pm$ 1.00	0.59 $\pm$ 0.20
Alkaloid sum	0.025	0*	3.05 $\pm$ 0.34*	0.23 $\pm$ 0.05*	0*	7.83 $\pm$ 0.57*	0.61 $\pm$ 0.10*

**Note.** Here and in Table 3: \* $p < 0.05$  vs. intact animals.

**TABLE 3.** Effects of Delphinium Extracts on Blood Biochemistry in Outbred Male Rats on Day 14 after Injection of Freund's Adjuvant ( $X \pm m$ )

Group	Dose, mg/kg, ml/kg	Protein, g/liter	ALT, $\mu\text{cat/liter}$	Seromucoids, arb. units
Intact	—	56.50 $\pm$ 1.20	1.15 $\pm$ 0.03	0.24 $\pm$ 0.01
Control	—	56.96 $\pm$ 0.65	1.27 $\pm$ 0.04 <sup>+</sup>	0.31 $\pm$ 0.02 <sup>+</sup>
Orthophen	10	53.70 $\pm$ 2.17	1.11 $\pm$ 0.05*	0.25 $\pm$ 0.02*
Tincture	0.25	56.22 $\pm$ 0.82	1.12 $\pm$ 0.04*	0.28 $\pm$ 0.01
Extract	0.12	58.08 $\pm$ 1.79	1.18 $\pm$ 0.06	0.22 $\pm$ 0.01*
Alkaloid sum	0.025	56.88 $\pm$ 1.16	1.11 $\pm$ 0.05*	0.25 $\pm$ 0.02*

The sum of alkaloids exhibited the best therapeutic effect. Treatment with this preparation supported all biochemical values at the level of normal animals. In the orthophen group the activity of ALT and seromucoid level corresponded to normal values (Table 3).

Hence, our results indicate that complex extracts and sum of alkaloids from the aerial part of delphinium exhibit pronounced antiexudative effect comparable to that of the reference drug. The studied extracts reduced inflammation and hyperemia of the damaged joint and prevented generalization of the process reducing edema of the intact limb. In addition, the studied extracts exhibited an analgesic effect, prevented changes in biochemical parameters of the blood, and, in contrast to classical antiphlogistic drug orthophen, normalized the main parameters of the hemogram.

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