PHARMACOLOGY AND TOXICOLOGY

Effects of Bioactive Substances from Tall Delphinium on the Development of Acute Inflammation of Different Genesis

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Effects of complex and individual bioactive substances extracted from tall delphinium on acute inflammation induced by carrageenin, acetic acid, serotonin, and histamine were studied. The studied plant substances produced a pronounced antiinflammatory effect comparable to that of nonsteroid antiinflammatory drugs.

Key Words: *acute inflammation; serotonin and histamine edema; antiexudative activity; alkaloids; flavonoids*

The search and development of new wide-spectrum antiinflammatory drugs is a perspective trend of research in modern medicine.

Extracts based on alkaloid-containing plants can serve as potential antiinflammatory drugs [4,5].

We studied antiinflammatory activity of complex (water/ethanol extracts) and isolated (flavonoids, alkaloids) substances from the stem and leaves of tall delphinium.

MATERIALS AND METHODS

The study was carried out on 1st category conventional inbred mice (20-25 g) from Breeding Center of Department of Experimental Biomedical Simulation of Institute of Pharmacology. Extracts from tall delphinium were studied on various models of experimental aseptic inflammation and their effects were compared with the effects of nonsteroid antiinflammatory drugs (NSAID).

Acute inflammatory reaction was reproduced by subaponeurotic (under paw aponeurosis) injection of 0.1 ml of 1% carrageenin. The intensity of inflammatory reaction was evaluated 3 h after inflammation induction by changes in the weight of the inflamed paw in comparison with the intact paw. Antiinflammatory effect was evaluated by the decrease of edema and expressed in percent of control [6].

Serotonin edema was induced by injection of 0.05 ml serotonin creatinine sulfate (Reanal) diluted in 0.9% NaCl to a concentration of 0.5 mg/ml. One hour after subaponeurotic injection of serotonin and injection of the same volume of 0.9% NaCl into the contralateral paw, talocrural exarticulation was carried out. The intensity of inflammatory edema was expressed in percent of paw weight increment after serotonin injection [1].

Histamine inflammation was induced similarly by injecting 0.1% histamine. Nimesulide (10 mg/kg) and sodium diclofenac (17 mg/kg) served as the reference drugs.

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Acute exudative reaction (peritonitis) was induced by intraperitoneal injection of 1% acetic acid. After 3 h the animals were sacrificed, the abdominal cavity was opened, and exudate was collected. Metamizol sodium (250 mg/kg) served as the reference drug for this experiment. The antiexudant effect was evaluated by reduction of exudate volume in percent of control. Antiinflammatory activity was expressed as the percent of edema suppression:

$$\text{edema suppression percent} = \frac{(V_c - V_e)}{V_c} \times 100,$$

where V_c is the mean increase of the volume of ascitic fluid in the control and V_e is the mean increase of the volume of ascitic fluid in the experimental group [6].

Plant agents were prescribed by preventive courses orally for 5 days, the last dose being administered 1 h before inflammation induction. The sum of alkaloids and aqueous fraction of flavonoids from tall delphinium stem and leaves were prepared by routine methods at Department of Pharmacy, Tomsk Military Medical Institute. The tinctures and extracts were standardized by the dry residue [2].

The results were processed by methods of variation statistics using Student's *t* test and Wilcoxon's paired comparison test.

RESULTS

Inflammation is associated with vascular changes and impairment of capillary permeability. Dilatation of capillaries and venules are caused by inflammation mediators (histamine, serotonin, kinins, and other antiinflammatory agents) [7]. The study of the effects of water ethanol extracts and individual substances from tall delphinium on the permeability of vascular/tissue barriers for the studied substances on the model of experimental peritonitis during the exudative phase of inflammation showed that all studied tinctures significantly reduced the volume of fluid in the abdominal cavity in comparison with the control. Suppression of inflammation after treatment with tincture and extract from tall delphinium constituted 29 and 31%, respectively. The use of delphinium alkaloids reduced the inflammatory reaction by 41%, and injection of aqueous flavonoid fraction promoted a 47% reduction of inflammation in comparison with the control (Fig. 1). The reference drug metamizol provided a stable antiexudant effect and suppressed edema by 27-45%. Delphinium flavonoids and alkaloids proved to be the most effective for this inflammation model, reflecting the effects of the studied extracts on membrane permeability. The majority of phenol compounds reduces permeability and improves the strength of capillary walls [3], while modification of the selectivity of sodium channels of cell membranes plays a certain role in the mechanism of action of diterpene alkaloids present in tall delphinium [9].

Subplantar injection of carrageenin led to development of acute inflammation in experimental animals, the weight increment in the inflamed paw in comparison with the contralateral paw reaching 42%. The inflammatory reaction was less pronounced in animals treated with the studied substances in comparison with the control group. Edema suppression in mice treated with delphinium tincture was 16%, in the group treated with the extract 20%,

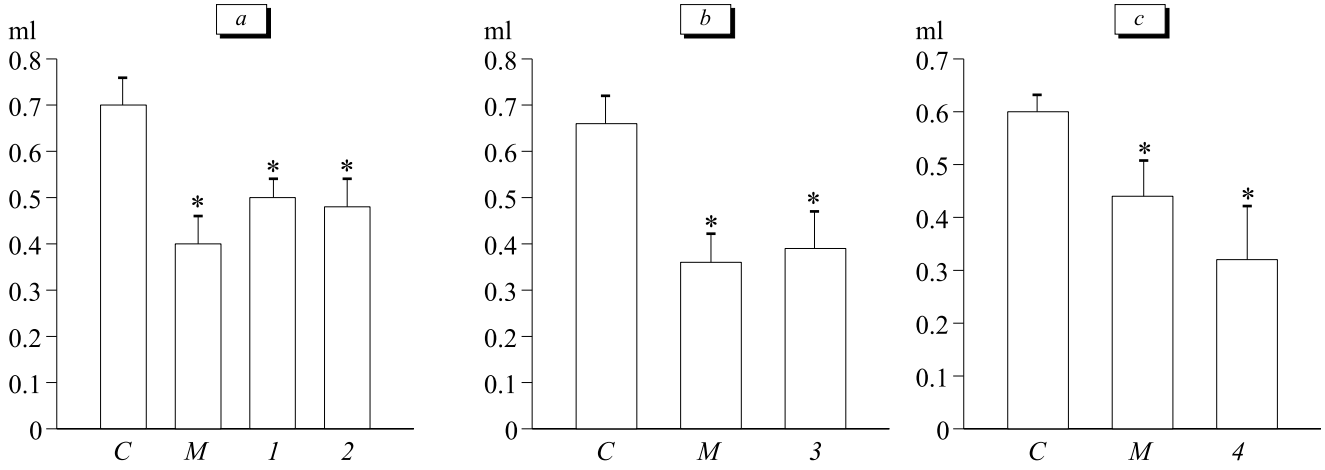

Fig. 1. Effects of complex and individual substances isolated from tall delphinium in acute inflammation induced by 1% acetic acid. *a*) antiexudant activity of delphinium tincture and extract; *b*) antiexudant activity of delphinium alkaloids; *c*) antiexudant activity of delphinium flavonoids. *C*: control; *M*: metamizol sodium. Here and in Fig. 2: *1*) tincture (0.6 ml/kg); *2*) extract (0.25 ml/kg); *3*) alkaloids (0.05 mg/kg); *4*) flavonoids (25.0 mg/kg). **p*<0.05 compared to the control.

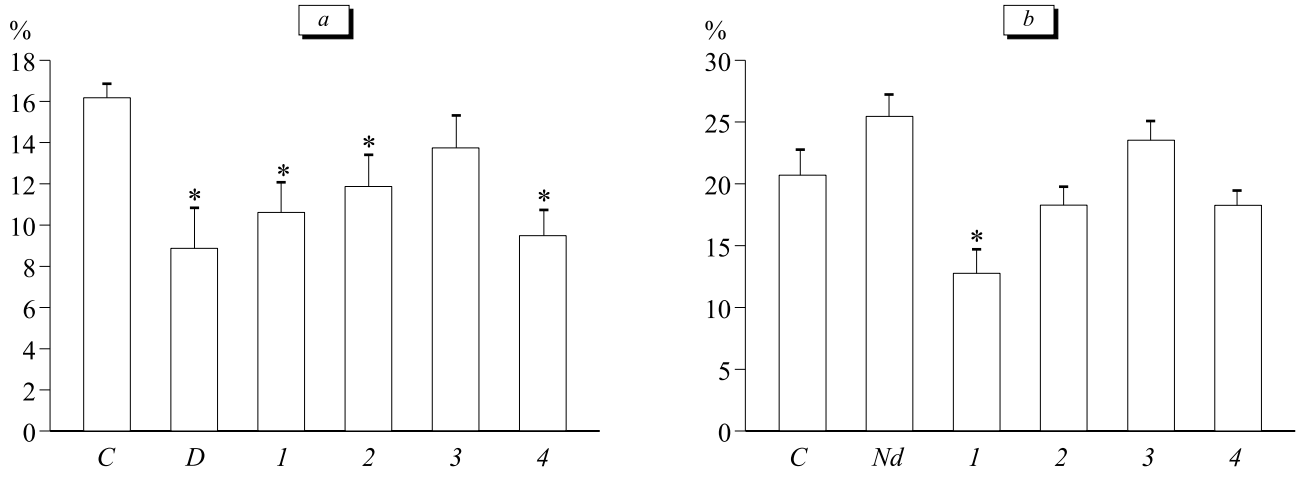

Fig. 2. Effects of complex and individual substances isolated from tall delphinium on paw weight increment in acute inflammation induced by histamine (*a*) and serotonin (*b*). *D*: diclofenac; *Nd*: nimesulide.

and in animals treated with alkaloids 23% in comparison with the control. Antiinflammatory activity of the reference drug (aspirin) was 26%. However, the antiinflammatory effect of the studied drugs was not significant on this model, but manifested just as a stable trend. Three phases of mediator release are observed in carrageenin edema: histamine is released during phase 1, kinins during phase 2, and prostaglandins (PG) during phase 3. Prostaglandins E_2 and F_2 are mediators of the late phase of carrageenin inflammation; after 3 h they potentiate the phlogogenic effects of other inflammation mediators, including lysosomal enzymes [7]. It is assumed that activation of inflammation phase 3 depends on activation of the complement system. Since measurements were carried out 3 h after the phlogogen injection (during stage 3 of carrageenin edema), the studied substances little stimulated the release of the mediator and more actively modified histamine and kinin metabolism.

Published data indicate that NSAID modify interactions of tissue receptors with histamine and serotonin and reduce the release of these substances from tissue basophils, modify the formation of these inflammation mediators, their degradation and conjugation. Hence, NSAID modify the metabolism of antiinflammatory amines (histamine and serotonin) [8]. We evaluated possible effects of the studied substances on inflammation mediator metabolism and compared their activities with those of NSAID on the models of histamine and serotonin edemas.

Acute inflammatory reaction was observed in experimental animals 1 h after injection of histamine (Fig. 2, *a*). Treatment with the test substances promoted suppression of histamine-induced inflammation. Treatment with delphinium tincture reduced paw edema by 31.5%, delphinium extract by 26.6%, aqueous fraction of flavonoids by 41.4%, and alkaloid sum by 15%. Edema was 45% suppressed in animals treated by diclofenac (voltaren; reference drug). Hence, diclofenac and tall delphinium flavonoids exhibited the best effects on the model of histamine edema.

Serotonin promoted the development of a more intense inflammatory reaction than histamine (Fig. 2, *b*). Preventive treatment with tall delphinium tincture significantly reduced the phlogogenic reaction in comparison with the control, edema suppression reached 38.4% (Fig. 2, *b*). The use of delphinium extract and alkaloid sum caused a trend to a reduction of inflammatory reaction, while aqueous fraction of flavonoids exhibited no antiinflammatory effect in serotonin-induced edema. Nimesulide, a new generation NSAID (selective blocker of cycloxygenase-2), served as the reference drug for this model of inflammation. No antiinflammatory effect was observed in the group of animals treated with nimesulide (Fig. 2, *b*).

Hence, our experiments demonstrated a pronounced antiexudant effect of complex tincture and extract and of individual substances (alkaloids, flavonoids) from tall delphinium, comparable to the effects of NSAID. The phlogolytic effect of complex extracts and phenol compounds of tall delphinium in histamine edema and the antiexudant activity of the tincture in serotonin inflammation indicate that these substances modulate the inflammation mediator metabolism, particularly histamine metabolism. Presumably, delphinium preparations compete with receptors or respective enzyme systems, involved in the synthesis, deposition, release, and transformation of these pro-inflammatory agents.

REFERENCES

1. I. A. Volchegorskii, I. I. Dolgushin, O. L. Kolesnikov, and V. E. Tseilikman, *Experimental Simulation and Laboratory Evaluation of Adaptive Reactions* [in Russian], Chelyabinsk (2000).
2. *State Pharmacopoeia of the USSR* [in Russian], Version 11, No. 2, Moscow (1989).
3. V. F. Korsun, S. A. Roizman, and T. V. Chuiko, *Phytotherapy of Cardiovascular Diseases* [in Russian], Moscow (2003).
4. V. G. Pashinskii, Yu. V. Nesterova, T. N. Poveteva, *et al.*, *Byull. Eksp. Biol. Med.*, Suppl. No. 1, 79-81 (2005).
5. T. N. Poveteva, N. N. Gaidamovich, V. G. Pashinskii, *et al.*, *Rast. Resursy*, No. 4, 99-105 (2005).
6. *Manual of Experimental (Preclinical) Studies of New Forms of Substances* [in Russian], Moscow (2000).
7. Ya. A. Sigidin, G. Ya. Schwartz, A. P. Arzamastsev, and S. S. Liberman, *Drug Therapy of Inflammatory Process* [in Russian], Moscow (1988).
8. F. P. Trinus, B. M. Klebanov, I. M. Gandzha, and R. D. Seifulla, *Pharmacological Regulation of Inflammation* [in Russian], Kiev (1987).
9. U. T. Gutser and J. Gleitz, *Neuropharmacology*, **37**, No. 9, 1139-1146 (1998).