METHODS

Study of Pharmacological Properties of New Drugs for External Use by the Method of Cell Monolayer Bioindication

M. V. Seregina and L. P. Mikhailova

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The effects of new plant extracts on phagocytosis-stimulating, antioxidant, antiinflammatory, and mitotic activities were studied by the method of bioindication on cultures of normal human leukocytes, RH cells, and blood neutrophils. The preparations showed dose-dependent antiinflammatory activity and produced wound-healing, antioxidant, and moderate phagocytosis-stimulating effects.

Key Words: *plant extracts; antioxidant activity; phagocytosis; cell monolayer; bioindication*

Antioxidants produce a protective effects on human organism exposed to unfavorable (exo- and endogenous) and extreme factors (stress, heavy physical work, hyperthermia, hypoxia, *etc.*) [1,6].

New drugs tested in this work were plant extracts. They contain hundreds of organic compounds chosen with consideration for cell vital activity, which is particularly important under conditions of impaired function of the organism. These preparations are applied on the skin and their effects on the organism are in principle the same, because they differ only by the method of application, quantity, and composition of volatile oils. These preparations promote neutralization of pathogenic acids not absorbed by the organism and elimination of pathogenic microorganisms and their acid products. These preparations are sources of vitamins, essential and trace elements. They correct uncompensated acidosis caused by extreme exo- and endogenous factors.

Common methods of pharmacological studies are inadequate for evaluation of the efficiency of these preparations [5], and therefore we analyzed their effects at the cellular level, in order to obtain information important at the first stage of the investigation [2,4].

V. V. Karavaev Foundation, Moscow; Laboratory of Biophysics, Research Center for Clinical and Experimental Medicine, Siberian Division of the Russian Academy of Medical Sciences, Novosibirsk

MATERIALS AND METHODS

Plant extracts of the following composition were studied: oil extracts from spearmint, wormwood, thyme, marigold, yarrow, St. John wort, celandine, pine buds with volatile oils and oil extract from camomile, dog rose fruit, fennel, and caraway; water extracts of the same plants with water extract of licorice, volatile oils, and aqueous solution of ammonium.

Phagocytosis-stimulating activity was evaluated on blood neutrophils by NBT test. The method consists in the following: blood phagocytes bind and internalize dye particles, the number of captured particles serves as the measure of phagocytic activity. The preparations stimulating phagocytosis were referred to immunotropic drugs modulating the nonspecific resistance of the organism.

Neutrophils were obtained from donor blood collected from the ulnar vein. Blood was stabilized with heparin, NBT test was carried out within 1 h after collection. Inductors of phagocytosis prodigiosan and lysozyme were used as reference preparations.

Heparinized blood (0.05 ml) was incubated in an immunological plate with Hanks' solution (0.025 ml,

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test for spontaneous activity), reference preparations (prodigiosan and lysozyme, 0.025 ml each), oil extract in concentrations of 0.08 and 0.16% in medium 199, or aqueous extract in concentrations of 0.1, 0.5, and 1.0% in Hanks' saline. Medium 199 (0.025 ml) was added in one well (solvent control).

Antioxidant activity was studied on normal human blood neutrophils. We evaluated the inhibition of zymosan-induced oxidative burst by test drug. Chemiluminescence is the most informative method; it is recommended for the development of new drugs. The preparations inhibiting induced oxidative burst possess antioxidant and antiinflammatory (antiexudative) activity.

Whole blood from the ulnar vein was used. Heparinized blood (0.1 ml) was mixed luminol and zymosane (0.1 ml each) and oxidative burst was evaluated by chemiluminescence intensity. At the peak of oxidative burst the preparation in a final concentration of 0.5% was added and chemiluminescence was recorded. Blood without preparations served as the control.

Mitotic activity of water and oil extracts was evaluated by bioindication in cultured RH cells.

RH cells (8×10^4/ml culture medium) were grown on coverslips in penicillin flasks. Medium 199 containing 10% bovine serum was used. After attaining confluence the medium was replaced with medium 199 containing the test preparation. Oil extract was used in a concentration of 0.16%, water extract in a concentration of 0.5%. Cell culture without preparations served as the control.

The flasks were incubated in a thermostat at 37°C for 48 h; every 24 h preparations for morphological analysis were prepared routinely and mitotic cells were counted using a Stefanov morphometrical grid [7]. The mitotic index was calculated as the ratio of mitotic to total cell number in the monolayer (per 1000 cells).

RESULTS

Experiments showed that all test preparations stimulated phagocytosis (Table 1). Phagocytic activity increased 2.73-3.03 times in experiments with water extract and 2.3 times in the presence of oil extracts in comparison with the control; however, the phagocytosis-simulating effect of test drugs was 1.3 times lower than that of reference preparations (Table 1).

These findings suggest that the test preparations moderately stimulated phagocytosis and can be used in the therapy of states associated with impaired cell immunity. They also stimulate regeneration of damaged tissues.

Water extract virtually completely inhibited the formation of active oxygen forms involved in LPO and inflammatory reactions (Fig. 1). Background luminescence of the serum is negligible and does not affect the result. The results indicate that the test preparations are characterized by antioxidant and antiinflammatory (antiexudative) activities.

TABLE 1. Phagocytosis-Stimulating Activity of Extracts from Medicinal Plants

Experimental series	*M*±*m*	Standard deviation	Range of values
Control	10.8±0.5	1.0	10-12
Medium 199	14.5±0.9	1.7	12-16
Lysozyme	58.5±2.3	4.7	53-64
Prodigiosan	63.5±0.5	1.0	62-64
Oil extract, %			
0.08	23.0±1.2	2.4	20-25
0.16	22.8±1.4	2.9	19-25
Water extract, %			
0.1	37.5±2.5	5.0	30-40
0.5	42.0±1.2	2.4	40-45
1.0	42.5±1.7	3.3	40-47

TABLE 2. Mitotic Activity (Mitotic Index) of Cell Monolayer in the Presence of Oil and Water Extracts

Series No.	Control	Extract	
		oil	water
1	22.0	47.0	49.0
2	20.0	49.0	50.0
3	21.0	49.0	48.0
4	20.0	48.0	47.0
5	21.0	48.0	49.0
Mean	20.8	48.7	48.6

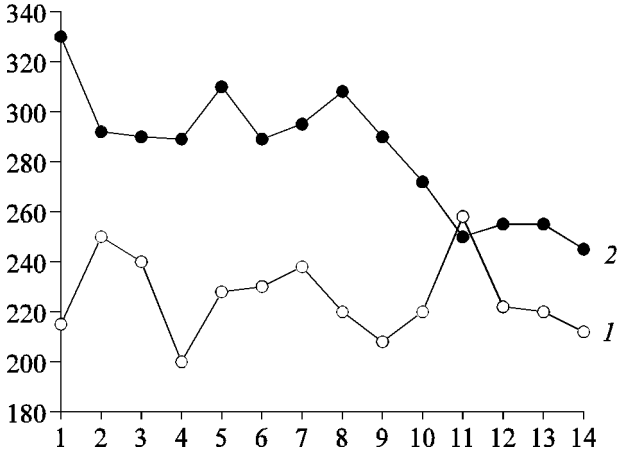

Fig. 1. Chemiluminescence intensity of the blood serum (*1*) and medicinal plant extract (*2*).

The test preparations increased mitotic activity of cells in comparison with the control culture (Table 2). According to published data, mitotic index for RH cells is 20.5. Comparative analysis showed that oil and water extracts increased mitotic activity 2.32 and 2.34 times, respectively. No pathological mitoses were observed. The studied drugs are believed to improve reparative processes via stimulation of mitotic activity in damaged tissue and stimulate wound healing.

Hence, the test extracts from medicinal plants moderately stimulate phagocytosis, possess immunotropic activity, and can be used in the therapy of states associated with impaired cell immunity.

Since water extracts exhibit pronounced antioxidant activity, they can be used in extreme situations: for prevention of hypoxia and correction of its consequences, and as antiinflammatory (antiexudative) agents in fever or at high environmental temperature. These preparations can be used as reparative means in various states and diseases.

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