# NEW MEDICAL PREPARATIONS

# Pine Resin and Biopin Ointment: Immunotoxic and Allergenic Activity

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We studied properties of ointment Biopin containing pine resin and used for the therapy of burns, wounds, and purulent and inflammatory diseases. Long-term treatment with this preparation in clinical doses had no effect on the nonspecific, but modulated the specific immune response. Biopin ointment inhibited humoral, but stimulated cell immunity. Neither local irritation, nor allergic reactions were found after long-term epicutaneous application of the preparation.

**Key Words:** *pine resin; Biopin ointment; nonspecific immunity; specific immunity; allergic effect*

Galipot from various coniferous tress (pine, fir, larch, and cedar) is widely used in traditional medicine. Now galipot is a component of various medicinal preparations (ointments) [5]. Biopin ointment (BO) contains beeswax and pine resin and is effective during the therapy of burns, wounds (phase I of wound process), and purulent-and-inflammatory diseases of the skin and subcutaneous fat [4]. Here we studied immunotoxic properties of BO. The effects of BO on phagocytic activity (PA) of macrophages and humoral (hemagglutination) and cell immunity (delayed-type hypersensitivity, DTH) were evaluated. Possible irritating and allergic effects of BO were also tested.

## MATERIALS AND METHODS

Immunotoxicity of BO was studied in 3 experimental series. Each series was performed on 18 male and 18 female CBA mice weighing 16-18 g and obtained from the Rappolovo nursery (Russian Academy of Sciences). Experimental mice (2 groups of males and 2 groups of females, 6 mice each) were daily treated with 70 and 700 mg/kg BO (1 and 10 clinical doses, respectively) for 30 days. The preparation was applied to shaven skin on the back (4 $cm^2$). Control males ($n$=6) and females ($n$=6) received water.

The effects of BO on PA of peritoneal macrophages were evaluated colorimetrically [2]. Peritoneal cells obtained after decapitation were placed in a 96-well microtitration plate (200 μl, $1.5\times10^6$ cells/ml, in medium 199 with 20% bovine serum), centrifuged, and incubated at 37°C for 2 h (100% humidity and 5% $CO_2$). Neutral red dissolved in medium 199 (200 μl 0.1% solution) was added to each well, the plate was incubated at 37°C for 2 h, centrifuged, and washed with physiological saline. Lysing solution (200 μl) was added to each well and optical density was measured on a Multi-Scan spectrophotometer (Pharmacia) at 540 nm (against wells with the dye and without cells).

The reaction of hemagglutination was performed at the peak of the immune response. The mice were intraperitoneally immunized with sheep erythrocytes (SE) in a dose of $2\times10^8$ cells in 0.2 ml physiological saline after treatment with BO. Hemagglutination was performed at the peak of immune response (day 7). Mouse serum was inactivated by heating at 56°C for

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**TABLE 1.** Parameters of Cell and Humoral Immunity and PA of Peritoneal Macrophages after 30 Daily Applications of BO ($M \pm m$, $n$=6)

| Parameter | Control | | BO, mg/kg | | | |
|---|---|---|---|---|---|---|
| | | | 70 | | 700 | |
| | males | females | males | females | males | females |
| PA of peritoneal macrophages, $10^3$ optical density units | 47±3 | 55±4 | 48±4 | 58±5 | 12±2* | 20±3* |
| Plasma hemagglutinin titer, $\log_2$N | | | | | | |
| IgG+IgM | 8.58±0.31 | 9.33±0.26 | 6.83±0.27* | 9.04±0.18 | 6.58±0.45* | 6.33±0.51* |
| IgG | 8.20±0.23 | 8.99±0.33 | 6.83±0.27* | 8.47±0.26 | 6.58±0.45* | 6.33±0.51* |
| DTH index, % | 20.6±4.8 | 14.1±5.8 | 14.5±2.9 | 47.2±2.3* | 29.8±5.6 | 15.0±2.9 |

**Note.** *$p$<0.05 compared to the control. N: highest dilution yielding pronounced reactions.

30 min and diluted 5-fold. 2-Mercaptoethanol (0.2 M) was added to some plasma samples followed by incubation at 37°C for 30 min (IgG titer); other samples were not treated with 2-mercaptoethanol (total IgG+ IgM titer). The plasma was transferred into plates (180 μl/well) with subsequent 2-fold serial dilution with physiological saline. SE suspension was added to each well (1%, 50 μl/well) and the plates were incubated at 24°C for 2 h. The highest dilution yielding a pronounced reaction was determined for each mouse [3].

In experiments with DTH, the mice were subcutaneously immunized with SE ($10^7$ per 0.1 ml physiological saline) after treatment with BO. DTH was tested after 5 days. SE ($10^7$ cells in 20 ml physiological saline) were administered subaponeurotically into the limb. Physiological saline (20 ml) was injected into the contralateral limb. The degree of local reactions was estimated by comparing the volume of edemas in treated ($V_T$) and control limbs ($V_C$): $(V_T-V_C)/V_C$.

The observed mean and dispersion were calculated for each group. The differences between the control and experimental groups were evaluated by Student's $t$ test (probability 0.95).

The local irritating and allergic effects of BO were studied on 36 guinea pigs weighing 200-250 g (Rappolovo nursery, Russian Academy of Sciences). The scheme of treatment and doses of preparations were similar to those in experiments on mice. BO was applied to the left side for 20 days. Skin reactions were scored daily according to the Suvorov scale [6]. We evaluated whether BO produces a sensitizing effect. Ten days after the first course of treatment, BO was daily applied in the same doses to the right side for 7 days.

## RESULTS

Long-term treatment with BO in a dose of 70 mg/kg had no effect on PA of peritoneal macrophages (in females treated with BO a slight tendency towards its stimulation was found, Table 1). BO in a dose of 700 mg/kg significantly inhibited PA of peritoneal macrophages, especially in male mice. BO in a dose of 70 mg/kg suppressed the humoral immune response in males. In a dose of 700 mg/kg the preparation inhibited the humoral immune response in male and female mice (Table 1). BO stimulated cell immunity in females (70 mg/kg) and males (700 mg/kg, insignificantly, Table 1).

Studies of the local irritating effect showed that BO did not cause contact nonallergic dermatitis. No signs of sensitization were found after repetitive daily application of BO.

Our results show that long-term treatment with BO in clinical doses has no effect on the nonspecific, but modulates the specific immunity. The preparation inhibits humoral, but stimulates cell immunity. Neither local irritation nor allergic reactions were observed after long-term epicutaneous application of BO.

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