# LYMPHOCYT-STIMULATING ACTIVITY OF HUMAN PERIPHERAL BLOOD MONONUCLEARS IN THE PRESENCE OF PHALLUS IMPUDICUS L EXTRACTS

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Ethanol extracts of Ph. impudicus exhibit immunomodulatory effects. In this regard, the effect of different concentrations of ethanol extracts and Ph. impudicus on the level of spontaneous and PHA-induced proliferation of PBMC was studied.

To assess the effect of the studied substances on the proliferative activity, the cells were prestained with CFSE fluorescent dye and cultivated for 6 days in the presence of various dilutions of the studied substances.

*Keywords:* Ph. impudicus, PBMC, viability, proliferative activity.

The immunobiological effects of most of the studied polysaccharides are carried out, first of all, through the activation of mononuclear phagocytes. It is suggested that one of the mechanisms of immunomodulation [1].

The action of polysaccharides is their contact with the surface of the membrane of immunocompetent cells.

By binding to the receptors of the membranes of lymphocytes and mononuclear phagocytes, polysaccharides can enhance the functional activity of these cells, which leads to an increase in the immune response to various antigens and an increase in the body's overall resistance [2, 3].

**The purpose of the study**

To assess the effect of extracts of Ph. impudicus on the viability and proliferative activity of donor peripheral blood mononuclear cells (PBMC) (n = 5).

**Materials and methods**

The initial 40% alcoholic extracts of Ph. impudicus were diafiltered with a sodium chloride 0.877% (Sigma, USA) on a Vivaflow 50/50R/200 apparatus (Sartorius, Germany) with a partition containing a membrane with an exclusion coefficient of 10,000 MWCO PES. The final alcohol concentration in the extracts is 0,3%.

PBMC was isolated by centrifugation on a Histopaque gradient density (p = 1,077 g/ml), followed by CFSE (7 mM). Stained PBMC (2x106 cells/ml) were cultivate (6 days) in the presence/absence of extracts of Ph. impudicus and PHA (2,5 mg/l) in RPMI-1640 medium containing 10% fetal calf serum, 2 mM L-glutamine, 1% antibiotic (Sigma, Germany). The results were recorded on a CytoFLEX flow cytometer (Beckman Coulter, USA) for 30,000 events per event.

**Results**

The addition of Ph. impudicus extracts to PBMC did not affect cell viability (88,8 (86,2 ÷ 90,1)% vs. 90,3 (87,6 ÷ 92,1)% in the absence of extracts, $p < 0,05$). The number of spontaneously recorded (CFSElow) PBMC was 29,6 (27,6 ÷ 31,7)% and decreased during cell cultivation with Ph. impudicus in the ratio of cells: extracts – 1:10, 1:20 and 1:50 (2,1 (1,9 ÷ 2,6)%, 2,0 (1,6 ÷ 2,4)%, 1,6 (1,3 ÷ 2,1)%, respectively, $p < 0,05$). The amount of PHA-stimulated CFSElow PBMC in the presence of extracts in a ratio of 1:10 was 26,9 (23,2 ÷ 30,2)%, 1:20 – 31,0 (24,6 ÷ 33,5), 1:50 – 28,6 (21,0 ÷ 32,1)% ($p < 0,05$, compared with the same indicator in the absence of extracts – 67,9 (60,4 ÷ 70,4)%).

**Conclusions**

The addition of Ph. impudicus extracts to PBMC in different ratios did not affect cell viability, while at the same level, statistically significantly suppressed both spontaneous and mitogen-induced proliferative activity of PBMC.

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