

# The impact of humus water on inflammatory cytokines of endothelial cells cultured under conditions of hyperglycemia

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**Introduction:** Diabetes has become an epidemic and is a risk factor of cardiovascular disease. Hyperglycemia in diabetes is responsible for damaging of the endothelium and increases inflammation on the surface of the vascular lining. The inflammatory process in diabetes is associated with the secretion of inflammatory cytokines by endothelial cells, e.g., tumor necrosis factor-alpha (TNF- $\alpha$ ) and interleukin 6 (IL-6) and by a reduction of cell proliferation.

Humus waters (HW) containing humus acids with sources in Poland are originally bacteriologically pure, physically and chemically stable. Because of the physicochemical properties of humus acids of adsorption, complexation and ion exchange they present numerous therapeutic activities including anti-inflammatory. Data regarding its molecular mechanisms underlying these phenomena are scarce. The present study reveals that humus water exerts important beneficial effects on endothelial cells under conditions of hyperglycemia.

**Material and methods:** Endothelial cells (HUVEC line) were derived from human umbilical veins using the enzymatic method with collagenase. Cells were cultured in M199 media in accordance the standard method. The experiment was repeated three times with three independent cells isolations. The study was conducted in four groups: 1- control group – culture medium without impact of glucose and humus water; 2 - an appropriate volume of humus water was added to obtain its 1% solution in the culture medium; 3- (30 mM/L) glucose added to the culture medium to imitate hyperglycemic condition; 4 - glucose, humus water in the medium. At the end of experiment, conditioned medium from each well of culture plates was collected and the concentration of TNF- $\alpha$  and IL-6 in the supernatant was measured by ELISA test (Diacclone) according to the manufacturer's instructions.

The remaining cells on the bottom of each well were harvested by using trypsin and counted by Buerker hemocytometry. The results of the concentration of the parameters in the supernatant from each well of culture plates were analyzed per number of cells in each well.

**Results:** The number of HUVECs was highest in group 2 (with added humus water) ( $4.44 \times 10^5$ ), and slightly lower in control group ( $4.21 \times 10^5$ ). The lowest number was observed in group 3 ( $3.39 \times 10^5$ ) cultured under hyperglycemic conditions. While the number of cells in group 4 ( $4.24 \times 10^5$ ) with glucose and humus water reached the level similar to the control group. The difference in relation to the group 3 was statistically significant. The level of TNF- $\alpha$  in the group 1 (control group) was 1.65 pg/105 cells, in the group 2 (humus water) was 1.91 pg/105. Its concentration was notably higher (2.51 pg/105 cells) in the group 3 containing glucose in the medium. The TNF- $\alpha$  level in group 4 was notably lower (1.67 pg/105) when compared to the group 3 result. The concentration of IL-6 increased after the addition of glucose to the culture medium in group 3 (56.22 pg/105 cells). Adding of humus water to the culture medium with glucose causes decrease level of IL-6 in group 4 (39.9 pg/105) to the level similar to control group and group with humus water (41.74 and 43.81 pg/105 cells).

**Conclusions:** The lowest number of endothelial cells cultured under hyperglycemic condition indicates the negative impact of high glucose concentration on the cells culture. It appears that the adverse effects of hyperglycemia on vascular endothelial cells may be reduced by adding of humus water which enhance of the cell proliferation. Adding of humus water also led to a significant decrease of inflammatory cytokines such as TNF  $\alpha$  and IL-6. The present study reveals that humus water exerts important beneficial effects including anti-inflammatory on endothelial cells under conditions of hyperglycemia.