EXTRACT OF LIMONIUM GMELINII ATTENUATES A|5- AND H_2O_2- INDUCED OXIDATIVE RESPONSE IN CEREBRAL ENDOTHELIAL CELLS

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Introduction. Alzheimer's disease (AD) is a chronic neurodegenerative disorder which affects approximately 10% of the population at age 65 and 40% of people over the age 80. Clinically, AD pathology is characterized by an increased deposition of amyloid-(3 peptide (A(3) in the brain, and a progressive impairment of cognition and memory of affected individuals. Blood Brain Barrier (BBB) dysfunction is observed in all of the stages of AD, and may even precede neuron degeneration in AD brains. During the early stages of AD, microvasculature deficiencies and hypertrophy of astrocytes are commonly observed. Numerous *in vivo* and *in vitro* studies have demonstrated that the vascular deposition of A(3 induces oxidative stress in cerebral endothelial cells (CECs). A(3-induced oxidative stress in cells, in turn, initiates a cascade of redox reactions leading to apoptosis and neurovascular inflammation. Consequently, antioxidants are considered as therapeutic agents in A(3-induced CECs damage.

It has been reported previously, that reach with polyphenols extract of *Limoniun gmelinii* (a plant widespread on the territory of Kazakhstan) exerts a wide range of therapeutic action. Here, we studied the effect of polyphenols extracted from roots, stems and leaves of *Limoniun gmelinii* on the H_2O_2- and A(342-induced oxidative responses in cerebral endothelial cells *in vitro*.

Materials and methods. Mouse bEnd3 line (from ATCC) of cerebral endothelial cells (CECs) was applied in this research as following: control; cells incubated with 5 |iM of A(342 for 2 hrs; cells incubated with 0.5 mM of H_2O_2 for 30 min; cells pretreated with extracts of *Limonium gmelinii* followed by A(342 or H_2O_2 exposure; cells treated with extracts of *Limonium gmelinii* only.

The cellular levels of ROS were measured with CM-H2DCF-DA (Invitrogen, Cat. No. C6827). The CM-H2DCF-DA stock solution (5 mM) was dissolved in DMSO and diluted in the culture medium to a final concentration of 1 just before addition to the cells. For ROS measurements, cells were starved for 12 hr, rinsed twice with warm phenol free DMEM, and incubated with CM-H2DCF-DA for 1 hr at 37°C in dark. The CM-H2DCF-DA fluorescence was measured on a plate reader Synergy H1 Hybrid Reader with excitation and emission wavelengths of 492 nm and 520 nm.

For cell imaging we applied DHE staining. DHE reacts with O_2 - to produce oxyethidium (oxy-E), a highly fluorescent product, which binds to DNA and causes an increased fluorescent intensity of the cell nuclei.

Results. We have shown that H_2O_2 increased CM-H2DCF-DA fluorescence by almost 100%, and A(342 - by 75% compared to the control. At the same time, H_2O_2 stimulated ROS production in CECs was attenuated by the pretreatment with all three extracts of *Limoniun gmelinii.* Similarly, A(342- induced oxidative response in cerebral endothelial cells was suppressed by *Limonium gmelinii* polyphenols as well. As a positive control, the ROS scavenger NAC also reduced A(342- stimulated ROS production. Furthermore, we observed significant decrease in CM-H2DCF-DA fluorescence when the cells were treated with extracts of *Limonium gmelinii* alone.

Conclusions. This study demonstrated that extracts of *Limonium gmelinii* could attenuate H_2O_2- and A(342- induced reactive oxygen species (ROS) generation in cerebral endothelial cells. Thus, their protective potential requires further investigations.