

INDUCTION OF ORGANISM'S PROTECTIVE RESOURCES TO CHEMICAL AND BACTERIAL TOXINS

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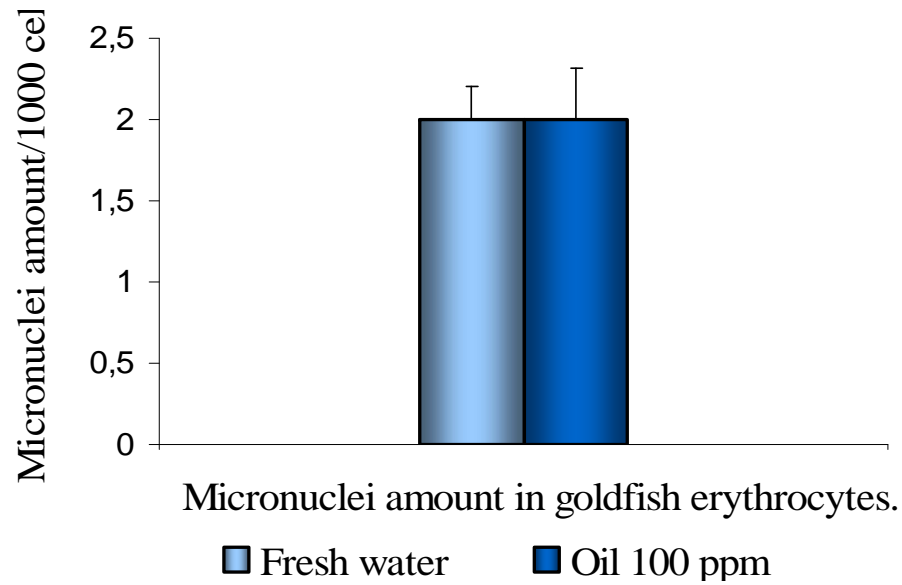
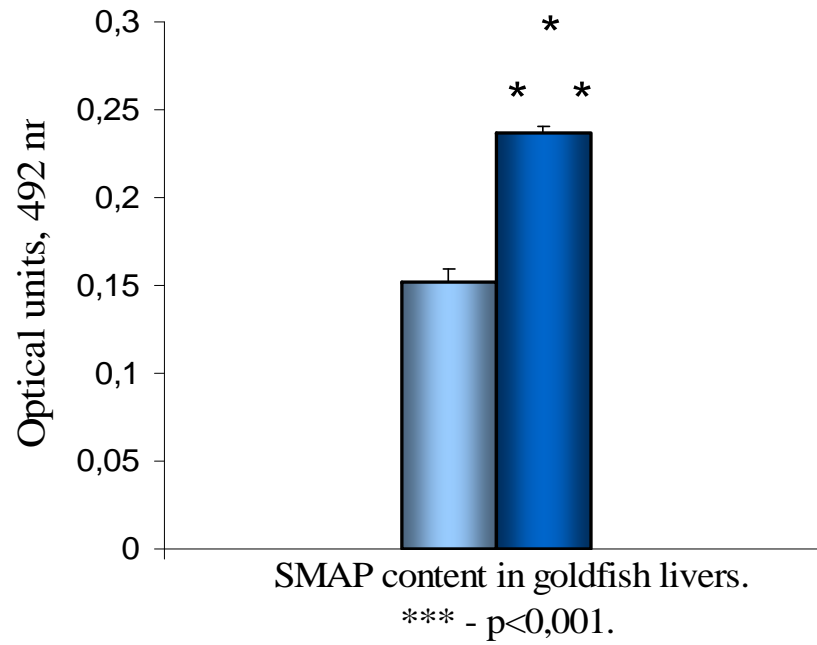
Partner Project P-302 of BTEP and STCU

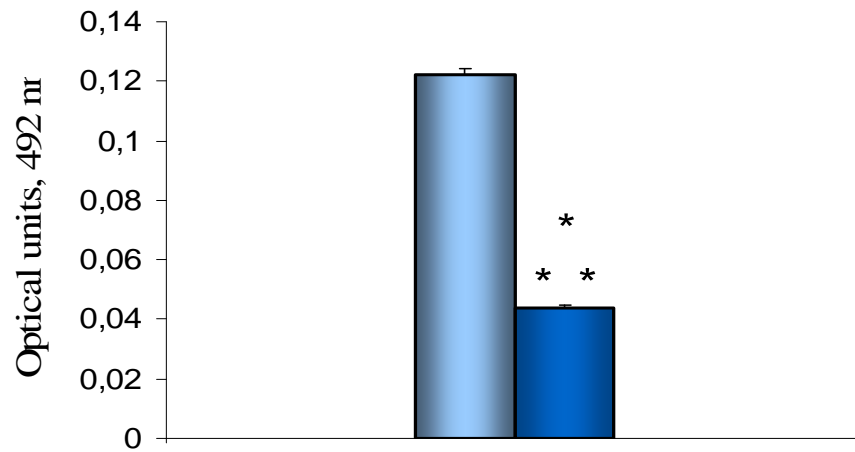
- Nowadays physicians are faced with the serious problem of ineffectiveness of a wide range of antibiotics and other pharmacological antibacterial remedies in treatment of bacterial infections. Such ineffectiveness of conventionally applied drugs is related to a high mutation rate of the bacterial species and, as a result, to formation of bacterial species which are insensitive even to relatively novel anti-bacterial drugs. This leads to poor recovery and serious complications of infectious diseases in patients and even to their death. Additionally, patients who have severe infections which are successfully treated with antibiotics sometimes experience life-threatening complications (toxic shock) due to the toxic and inflammatory effects of bacterial components such as lipopolysaccharides.

- It has been well-established that cells possess with their own biochemical defensive systems for protecting them from various adverse environmental factors. Much of the work in this area has focused on the studies of heat shock proteins (HSP), particularly on HSP70 and its related molecules. Numerous studies have shown that increased synthesis of HSP70 can play a protective role during cell exposure to high temperatures, anoxia, low and high pH values, X-ray irradiation, contaminations with heavy metals, PAHs, and epileptic seizures (Sharp et al.,1999). HSP70 is highly evolutionary conserved and present in many species. One interesting aspect in this phenomenon is the lack of specificity of HSP70 toward the nature of the adverse factors: no matter which factor induced increased HSP70 synthesis, these proteins protect cells from multiple adverse environmental conditions (Agayev,1993). The exact mechanism of these proteins' protective activity is still unclear. How-ever, the most widely accepted idea about the mechanism of HSPs' protective action is that they function as chaperon proteins recovering and maintaining natural structure and functional activity of proteins adversely affected by the stress situation.

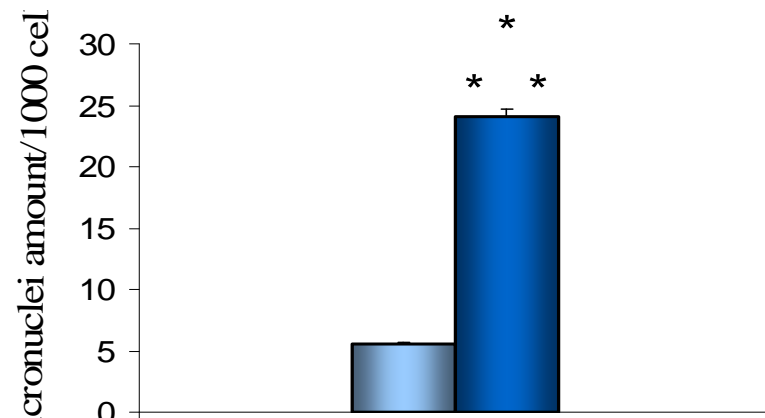
- We conducted our own studies to determine impact of adverse factors, particularly oil-contaminated water, on the serotonergic system, participating in organism's response to stress reactions of different origin (psychological, chemical, physical), and on HSP70. Due to the fact that serotonin mediates its activities through regulating expression of certain genes and synthesis of specific proteins (Barziali et al., 1989), activity of serotonergic system was evaluated by the ELISA-test measuring of the level of a novel serotonin-modulating anticonsolidation protein (SMAP) that had been identified and purified from the rat brains for the first time in our Department of Ecological Physiology & Toxicology. This protein consists of two subunits with molecular mass of 60 and 126 kDa, is tissue non-specific (Mekhtiev, 2000) and, as showed biochemical studies with serotonin application on the rat brain cortex and electrophysiological studies on identified command neurons of molluscs, is being in linear relationship with serotonin level (Gasarov & Mekhtiev, 1991; Mekhtiev et al., 2003).

- As a result of the studies under-taken, it was revealed that goldfish's exposure to water containing oil contamination at a concentration of 100 ppm for 5 days leads to significant increase ($p < 0.01$) of the SMAP level in the liver protein extracts. The mutagenesis level was evaluated by micronucleus test in fish erythrocytes (Schmid, 1975). The micronucleus test on the erythrocytes of the same animals, did not reveal differences in micronuclei amount between animals of the first experimental and control groups. Along with it, the same studies were conducted on the gobies, residential fish strictly attached to their habitats, caught from the polluted and clean zones of the Caspian Sea. The animals from the polluted zones had significantly lower SMAP protein content relatively to the values of fish from the clean zone ($p < 0.001$). The micronuclei amount in the fish from the polluted zone was noticeably higher than in the fish from the clean zone ($p < 0.001$). So, the results gave grounds to infer a conjecture concerning antitoxic and mutation-protecting activity of serotonergic system: when its resources are enough, cells are protected from mutations; in opposite, when its resources are exhausted, this system can not maintain further its protective function that issues in mutations formation.





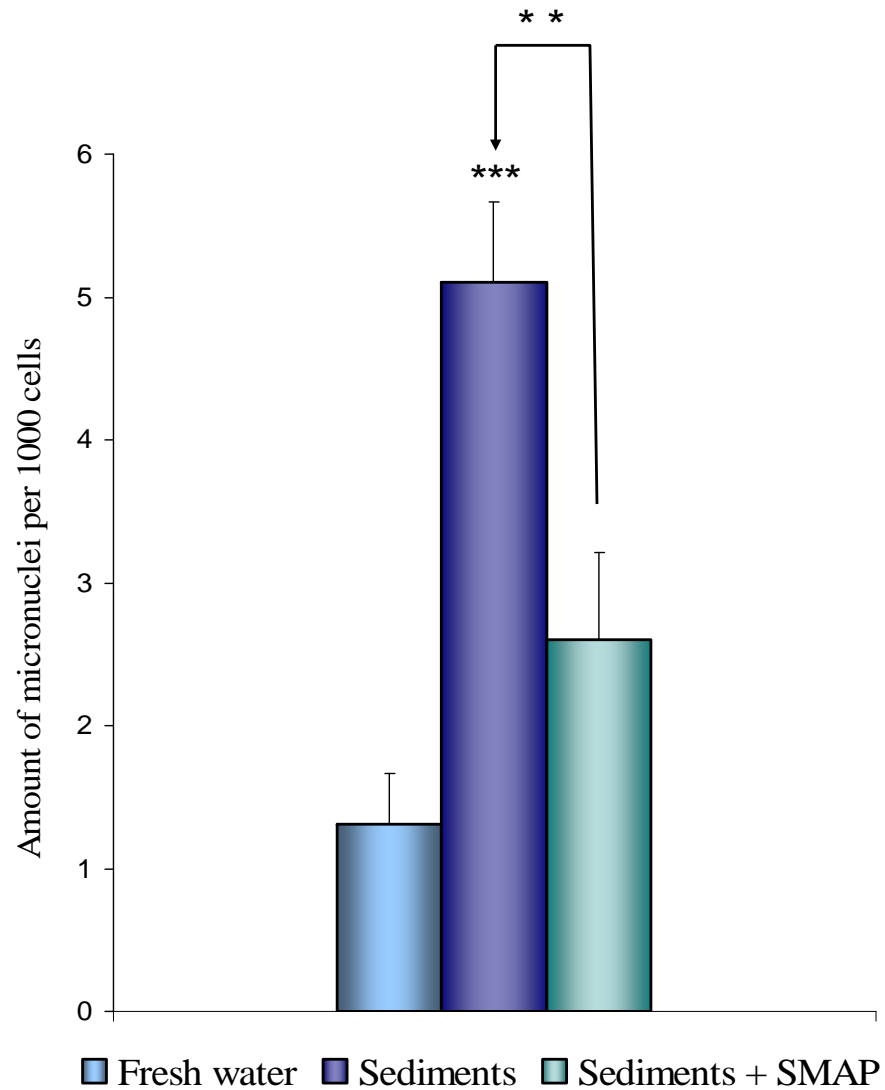
SMAP content in the livers of gobies from the polluted zone of the Caspian Sea. *** - $p < 0,001$.



Micronuclei amount in the erythrocytes of gobies from the polluted zone of the Caspian Sea. *** - $p < 0.001$

■ Clean zone ■ Polluted zone

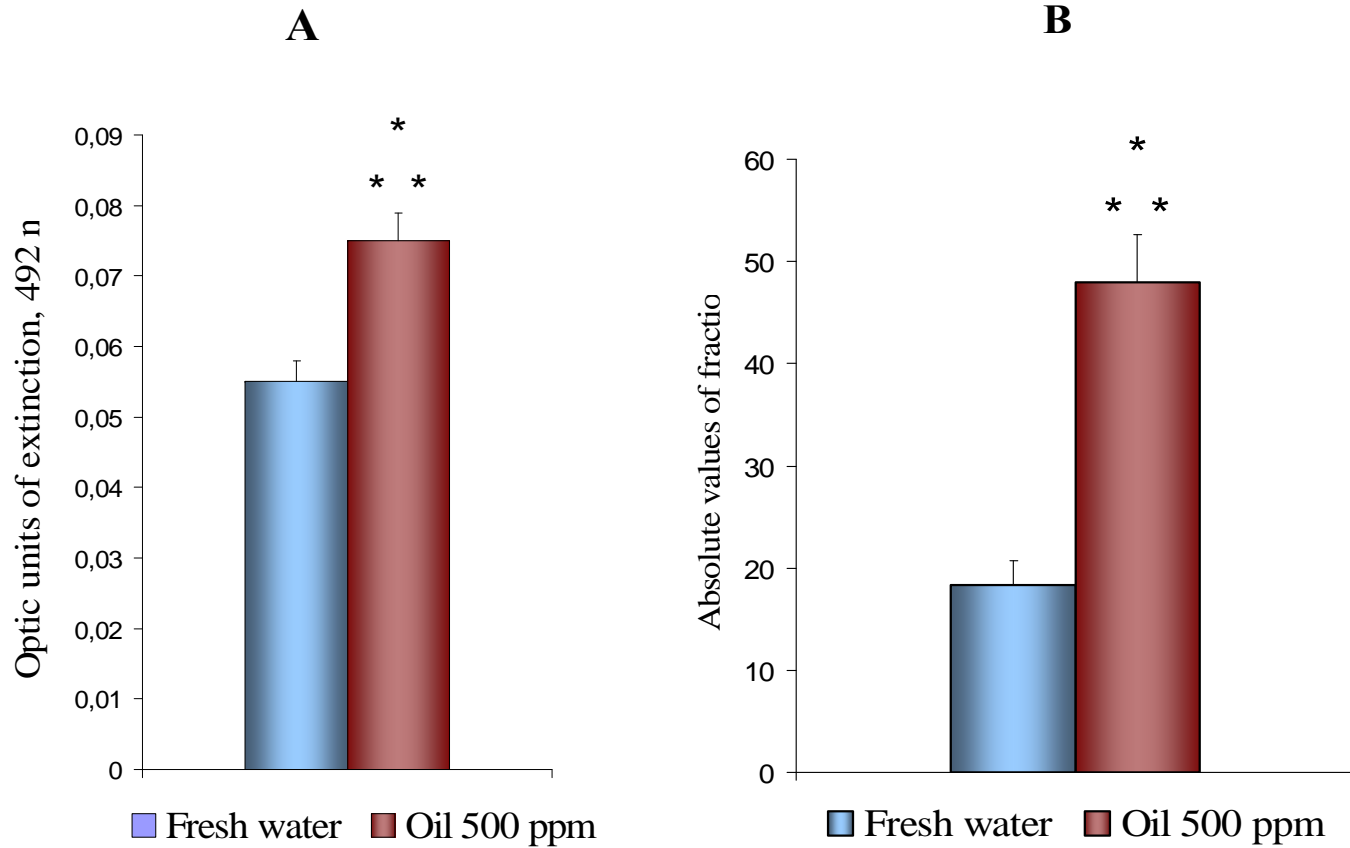
- Another studies were conducted over one-year-old sturgeon juveniles. Animals were culled into three groups: 1) control group – animals were kept in fresh water; 2) 1st experimental group – animals were kept in the water contaminated with sediments from Baku Harbor containing high level of heavy metals and PAHs, for 7 days and thereafter were put into pure water; 3) 2nd experimental group – animals were administered intramuscularly with SMAP protein (1.5 mg) prior to their exposure to sediment-contaminated water for 7 days, and injected again with SMAP before putting them into the pure water. The results showed that animals from the 1st experimental group have high level of micronuclei ($p < 0.001$), whereas intramuscular injection of the animals from the 2nd experimental group with SMAP protein leads to a significant decrease in mutagenicity relatively to the animals from the 1st experimental group ($p < 0.01$). So, artificially increasing the serotonergic system activity by administration of SMAP protein prevented and inhibited organism's intoxication and formation of mutations induced by industrial-polluted sediments.



Impact of sediments and SMAP on micronuclei level in sturgeon erythrocytes.

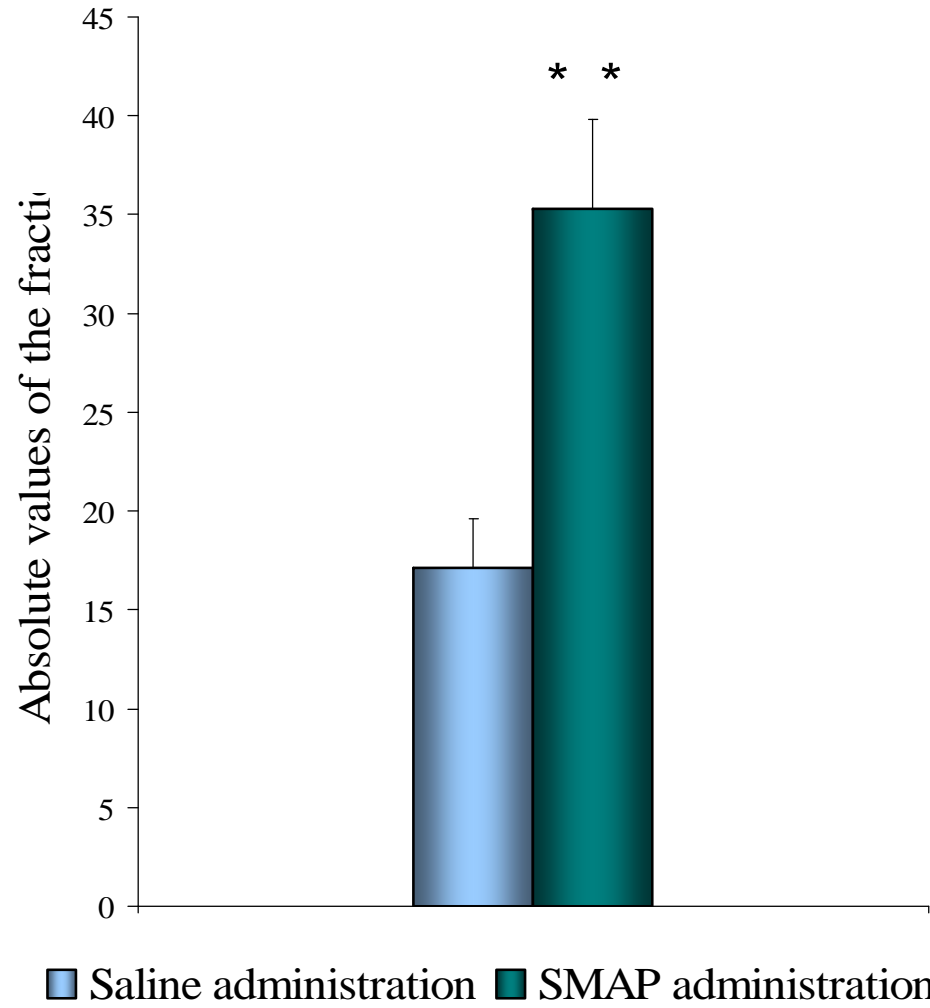
** - $p < 0,01$; *** - $p < 0,001$.

- For the purpose of analyzing the underlying mechanisms of antitoxic activity of SMAP protein, studies of impact of the oil-polluted water (500 ppm, 3 days) on SMAP level, determined by the ELISA-test, and HSP70 content, analyzed by polyacrylamide gel electrophoresis in the samples taken from the same goldfish brains were accomplished. The results showed that this impact leads to significant increasing of SMAP content and to parallel 2.6 times upregulation of HSP70 in the goldfish brains ($p < 0.001$). This data indicates to the existence of a certain relationship between SMAP protein and HSP70 synthesis.



Changes of SMAP level (A) and HSP70 amount (B) under oil contamination. *** - $p < 0.001$

- To clarify the character of relationship between SMAP protein and HSP70 metabolism, the studies of SMAP protein effect on water-soluble proteins' profiles of goldfish brains were performed. As a result of conducted studies it was revealed that single SMAP administration leads after 3 h to a sharp increase of the protein fraction ($p < 0.01$), possessing with molecular mass of 90 kDa and being, probably, HSP90. So, from the obtained results one can draw a conclusion that activation of serotonergic system through SMAP protein administration induces an increase in the HSP90 levels. According to the literature (Sharp et al., 1999), under adverse environmental factors, dissociation of HSP90 from its complex with proteins of heat shock factor leads to upregulation of HSP70.



Effect of SMAP on HSP90 upregulation.

** - $p < 0,01$.

- There are certain literature data available presently and supporting protective effects of HSP70 against bacterial toxins and bacterial infections:

Lau S.S., Griffin T.M., Mestral R. Protection against endotoxemia by HSP70 in rodent cardiomyocytes.//**Am. J. Physiol. Heart Circ. Physiol.** 2000; 278(5):1439-1445.

Sung Y.Y., Pineda C., Macrae T.H., Sorgeloos P., Bossier P. Exposure of gnotobiotic *Artemia franciscana* larvae to abiotic stress promotes heat shock protein 70 synthesis and enhances resistance to pathogenic *Vibrio campbellii*.// **Cell Stress Chaperones.** 2008;13(1): 59-66.

- Along with the proposed way of SMAP protein's antitoxic activity toward bacterial toxins, we admit the possibility of existence of another parallel way of antibacterial effect. Such effect might be realized through direct inhibitory impact of SMAP protein on bacterial genome disturbing bacterial cells proliferation.

So, taking to consideration the presented publications of other authors and our own experimental data on antitoxic activity of SMAP protein toward PAHs contamination, we are enthusiastic in the perspectives of SMAP protein application for the purpose of elimination of bacterial intoxication in the affected organism and treatment of infectious disease through launching internal protective resources of the organism in the form of HSP upregulation and disturbing bacterial cells proliferation.

**Thank you for your tolerance and
attention!**