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# SCIENTIFIC WORK OF RADOSLAV V. ŽIKIĆ: ANTIOXIDANT DEFENSE SYSTEM

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**ABSTRACT**. This article is dedicated to the memory of Radoslav V. Žikić, Professor of General Physiology and Ecotoxicology at Faculty of Science, University of Kragujevac, who died in October 2008. The article consists of two parts. In the first part, a brief review of Žikić's scientific work is presented. The second part is the list of major scientific papers published during his life. In this way, we want to express our appreciation for his contribution to the development of the physiology of antioxidant defense system in Serbia, as well as his contribution to the development of Faculty of Science in Kragujevac.

## 1. REVIEW OF THE SCIENTIFIC WORK OF DR. RADOSLAV V. ŽIKIĆ*

In his first research, Dr. Radoslav V. Žikić was concerned with investigation of esterase polymorphism in Adriatic sardine (*Sardina pilchardus* Walb.), where he found four zones of esterase activity on starch gel. Polymorphism was observed in three zones, but it was not possible to give a genetic interpretation for all variations. Analysis of vertebral number of the sardine showed small differences, which indicated homogenous population of sardine in the investigated area (1, 2).

In 1986 Dr. Žikić begun with the investigations of effects of some pesticides in lower vertebrates (3) He established that pesticide fenithrotion selectively inhibited some esterase fractions in liver of frog (*Rana esculenta* L.), and that the degree of inhibition was expressed after the longer treatment.

After these investigations, the interest of Dr. Žikić was focused on antioxidative defense system in different vertebrate and invertebrate organisms, and in different tissues (liver, heart, kidney, intestine, brown adipose tissue, erythrocytes). He exposed organisms to manganese and cadmium and investigated their effects on superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px). In these experiments, he showed that manganese increased activity of antioxidative enzymes (4). He also showed that hormone dexametasone increased the activity of catalase and superoxide dismutase (5).

The effect of cadmium was investigated in series of studies. Chronic dietary intake of cadmium caused hematological disturbances (6) and significant reduction of mass of interscapular brown adipose tissue (7). In the liver of rats, cadmium inhibited the activity of multiple enzymes of the antioxidant defense system (SOD, CAT, GSH-Px). By exposure of rats to high concentrations of selenium, the activities of all enzymes of the antioxidant defense system increased (8).

After exposure of goldfish (*Carassius auratus gibelio* Bloch.) to cadmium, the activities of superoxide dismutase and catalase significantly decreased. At the same time, the liver ascorbic acid (AsA) content increased (9,16). Acute exposure of carps (*Cyprinus carpio* L.) to high concentrations of cadmium seriously disturbed carbohydrate and protein metabolism (10).

Cadmium accumulation in the kidneys of rats, due to chronic dietary intake of cadmium, was associated with marked alterations of antioxidant defense system. Cadmium induced injury was not prevented with simultaneous intake of selenium, which *per se* induced significant improvement of the kidney antioxidant defense system (11). Cadmium accumulation in the hearts of adult rats was associated with marked changes in the activities of antioxidant enzyme defense system. Cadmium-induced injury was not completely prevented by simultaneous intake of selenium (12).

In several experiments, the possible protective role of coenzyme $Q_{10}$ on antioxidant defense system was investigated in rats. By quenching of the free oxygen radicals and by inhibiting lipid peroxidation coenzyme $Q_{10}$ could improve the antioxidant defense enzyme activities in blood of rats (15). Coenzyme $Q_{10}$ administration in rats chronically exposed to exogenous cadmium exerted beneficial effects on the non-enzymatic components of antioxidative defense system, such as ascorbic acid and vitamin E, which resulted in a decreased concentration of lipid peroxide in blood (17). Coenzyme $Q_{10}$ exerted beneficial effects on antioxidant defense system in skeletal muscle of rats treated with cadmium, especially on non-enzymatic components, such as ascorbic acid, vitamin E, and coenzyme $Q_{10}$. At the same time, $CoQ_{10}$ diminished the toxic effects of cadmium on protein synthesis (18). A possible protective role of coenzyme $Q_{10}$ was investigated in the heart of rats treated with cadmium. As in previous experiments Dr. Žikić showed that coenzyme $Q_{10}$ exerted beneficial effects on antioxidative defense system in heart of rats (21), and that coenzyme $Q_{10}$ acted as a potent antioxidant in combination with vitamin E in protection of rat heart against oxidative stress induced by cadmium. Furthermore, Dr. Žikić investigated the protective influence of vitamin E on antioxidant defense system in the blood of rats treated with cadmium and found that cadmium induced oxidative damage in erythrocytes leading to anemia, and that vitamin E expressed protective role against toxic influence of cadmium on antioxidative defense system (20). Nutritional antioxidant selenium ameliorated oxidative stress and loss of cellular antioxidants, suggesting that selenium efficiently protected liver and kidneys from cadmium-induced oxidative damage (32).

Dr. Žikić paid much attention on Adriatic Sea protection. Therefore, he investigated the antioxidative defense system in some marine organisms. He found that concentrations of antioxidant compounds (lipid peroxides, vitamin E, and vitamin C) in the tissues of red mullet (*Mullus barbatus* L.) were good markers for pollution biomonitoring and follow-up analysis of environmental condition (27, 33). In the liver of gray mullet (*Liza ramanda* Risso) Dr. Žikić found that climate induced changes occurred in the organization of its antioxidant defense system and that information of its antioxidant defense system could be taken into account in biomonitoring of marine fish species (22). Another research showed that concentrations of antioxidant compounds (vitamin C and vitamin E) and lipid peroxidation in the white muscle of hake (*Merluccius merluccius* L.) were good markers for pollution biomonitoring of environment (34).

The activity of antioxidative defense enzymes was also investigated in marine invertebrates and Dr. Žikić found that seasonal variation should be incorporated into interpretation of biomonitoring studies in mussel (*Mytilus galloprovincialis*) (23) and Mediterranean sea shrimp (*Parapenaeus longirostris*) (28). Dr. Žikić also investigated the impact of spiny cheek crayfish (*Orconectes limosus*) on native fauna in the river Danube and found that there was a potential risk for the expansion of *Ortonectes limosus* in other regional waters in the future (29).

Dr. Žikić also investigated energy metabolism and redox status of rat reticulocytes and erythrocytes, and effects of nitric oxide donor. He found that isosorbide dinitrate inhibited total and coupled respiration and stimulated encoupled respiration, enhanced glycolysis, decreased ATP production, and prolonged ATP turnover time in rat reticulocytes. These effects were mediated by nitric oxide as an effector molecule (13). Similar effects were observed in rat erythrocytes (14) and in rat reticulocytes (21), when other nitric oxide donors were applied such as nitroglycerine, isosorbide dinitrate, molsidomin, and sodium nitropruside.

In the research concerned with effects of nitroglycerine on the redox status of rat erythrocytes and reticulocytes, Dr. Žikić found that nitroglycerine biotransformation was primarily connected with hemoglobin in erythrocytes. Nitroglycerine-induced oxidation of hemoglobin resulted in methemoglobin formation and $O_2^{\cdot -}$ generation, which caused lipid peroxidation. These results indicated the significant role of mitochondria in the enzymatic biotransformation of nitroglycerine (26).

In another research, it was found that there were two metabolic pathways for molsidomine biotransformation and that 3-morpholinosydnonimine induced oxidative damage in erythrocytes. These biochemical effects might account for the clinical effectiveness of molsidomine in coronary heart disease (30).

The last research of Dr. Radoslav V. Žikić was concerned with oxidative stress and changes in antioxidative defense system in erythrocytes of preeclampsia in women. He found that concentration of markers of oxidative stress, such as $H_2O_2$, $ONOO^-$ and GST, increased in erythrocytes of preeclamptic women and positively correlated with mean arterial pressure. The increased concentration of lipid peroxide was an indicator of oxidative injury of erythrocytes in preeclampsia.

Professor Žikić was the organizer and chairman at two scientific meetings “Oxidative Stress and Mechanisms of Protection“, held at Faculty of Science in Kragujevac in 2004 and 2006.

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Complete scientific work of Radoslav V. Žikić includes more 48 scientific papers published in national scientific journals and 171 scientific papers presented at international or national scientific meetings.