DATA ON THE ACTIVITY OF SUPEROXIDE-DISMUTASE AND CATALASE IN TWO SUMMER-OLD *CTENOPHARYNGODON IDELLA* SPECIES

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Abstract: Research targeted the comparative study of some enzymes involved in oxidative stress in the two summer-old individuals of the *Ctenopharyngodon idella* species, derived from a systematic arrangement. Fresh samples were collected from five representatives of the grass carp, from the liver tissue, muscle and intestine. Superoxide-dismutase activity was determined by the Winterbourne, Hawkins, Brian and Carrell method, adapted by Vlad Artenie, catalase activity by the Sinha method, and protein by the Bradford method, and results being expressed in U / mg protein. The analysis of obtained results shows that there are significant differences, first from one enzyme to another, and secondly depending on the tissue analyzed, the maximum activity occurring in the liver tissue for catalase and in the intestinal tissue for superoxide-dismutase.

INTRODUCTION

Oxidative stress refers to the continuous pressure of substances that, by initiating oxidation reactions, carry an unfavorable action on cell membranes. Deterioration occurs in the cell walls from oxidative stress, with a disturbance of tissue metabolism, a process that leads to serious disorders. There are many compounds that maintain oxidative stress, the best known of which are free radicals. Antioxidants oppose oxidative-acting factors (prooxidants).
In the case of oxidative stress, the relationship between prooxidants and antioxidants balance in favor of the first (Cross, 2000).

Catalase is an enzyme with heteroproteic structure, being a hemoprotein containing as prosthetic group hemin or ferri-protoporphyrin IX, and the action of this enzyme is coupled with many biochemical reactions of lipid, carbohydrate and protein metabolism, which explains its wide distribution in the living world.

Superoxide-dismutase (SOD) is a component of the antioxidant system, together with glutathione-peroxidase and vitamin E, having the role to protect living cells against harmful effects of superoxide radicals which are one of the most harmful forms of reactive oxygen species. The superoxide radical is generated through univalent reduction of molecular oxygen during various enzymatic reactions or under the action of ionizing radiation. Because the enzyme is present in all aerobic organisms is considered that it plays a central role in protection against oxidative stress which is associated with production of reactive oxygen species (Artenie *et al*., 2008).

Data from specialty literature (Witas *et al*., 1984) suggests that superoxide-dismutase seems to be more involved in protection against destruction caused by oxygen free radicals, compared with catalase.

At the same time, superoxide-dismutase and catalase activities may be decisively influenced by a number of environmental factors such as temperature, salinity, season and feeding habitats, age, sex (Fahimi and Cajaraville, 1995; Rocha *et al*., 2003), water oxygen level (Janssens *et al*., 2000; Braun *et al*., 2008), and by the presence of chemicals such as aluminum, cadmium, uranium, phenanthrene, endosulfan, phenol-carboxylic acids, petroleum etc. (in case of catalase activity from different tissues: blood, liver, kidneys and gills) (Varanka *et al*., 1999; Sóle *et al*., 2000; Ikić *et al*., 2001; Pandey *et al*., 2001; Jena *et al*., 2002; Achuba and Osakwe, 2003; Buet *et al*., 2005; Gulcin *et al*., 2005; Lima *et al*., 2006; Sun *et al*., 2006) or lead, copper, zinc, cadmium, mercury etc., in the case of superoxide-dismutase (Diaconescu *et al*., 2008).

A number of other studies show that superoxide-dismutase activity differs from one species to another, from one tissue to another, from freshwater to marine environment, revealing higher values in marine species (Otto and Moon, 1996; Mila-Kierzenkowska *et al*., 2005; Lesser, 2006), but depending on the degree of water pollution as well, fish being able to retain large amounts of pollutants in their tissues, either directly from the water by breathing or through their diet (Velkova-Jordanoska *et al*., 2008).

This study aims to highlight the possible differences or similarities which exist between the first two oxidoreductases activities, in two summer-old *Ctenopharyngodon idella*, and secondly between the three tissue types analyzed.

MATERIALS AND METHODS

Biochemical determinations were made on five representatives of two summer-old *Ctenopharyngodon idella*, from Tiganasi farm, Iasi county. Biological material was processed in laboratory; and as a result, samples of liver, muscular and intestinal tissues were obtained, for each enzyme in hand, three parallel samples being made. Thus, superoxide-dismutase activity was determined by Winterbourne, Hawkins, Brian and Carrell method, adapted by Vlad Artenie, catalase by Sinha method and to calculate specific activity (results being expressed as U/mg protein) it has been determined the proteins by Bradford method (Cojocaru, 2005; Artenie *et al*., 2008; Cojocaru *et al*., 2009).

For a rigorous interpretation of the data obtained, statistical processing was used calculating a series of statistical indicators such as average, median, error and standard deviation, confidence index, average variation and precision coefficient, as well as confidence intervals within which each parameter varies (Dragomirescu, 1998; Gomoiu and Skolka, 2001; Varvara *et al*., 2001; Zamfirescu and Zamfirescu, 2008).

RESULTS AND DISCUSSIONS

As a result of chemical reactions involving oxygen, "one of the most curious cases of science" (Olinescu, 1982), harmful substances are released underlying the onset of major imbalances in the organism, due probably to the special electronic configuration of molecular oxygen which has a relative chemical inertia that can be defeated after activation under certain conditions and production of highly reactive species. Even under optimum conditions, following chemical reactions within the body, involving oxygen, harmful products are produced, highly reactive -so-called free radicals that can interact with other molecules (amino acids, proteins, lipids), that they can distort. There is therefore a very delicate balance between the formation of free radicals and blocking or neutralizing their action, free radicals can affect even more, through the process of oxidative stress in the event of an imbalance, molecules and cell life.

Reactive oxygen species (ROS) are generated in particular physiological and pathological conditions, both insufficient antioxidant protection and excess production of free radicals causing oxidative distruction (Neves *et al*., 2000). Their physiological and pathological effects are determined by the balance between oxidant and antioxidant enzyme systems, imbalance of pro-oxidant / antioxidant defining oxidative stress. The most important enzymes involved in defending the organism against oxidative stress are superoxide-dismutase, catalase and peroxidase (Bâldea, 2010).

Superoxide-dismutase (SOD) is an antioxidant enzyme that reduces the O_2^- radical to hydrogen peroxide, while catalase is an enzyme that reduces hydrogen peroxide to water, thereby removing the toxic effect of hydrogen peroxide that arises from redox processes at the cellular level (Zakaryan *et al*., 2002).

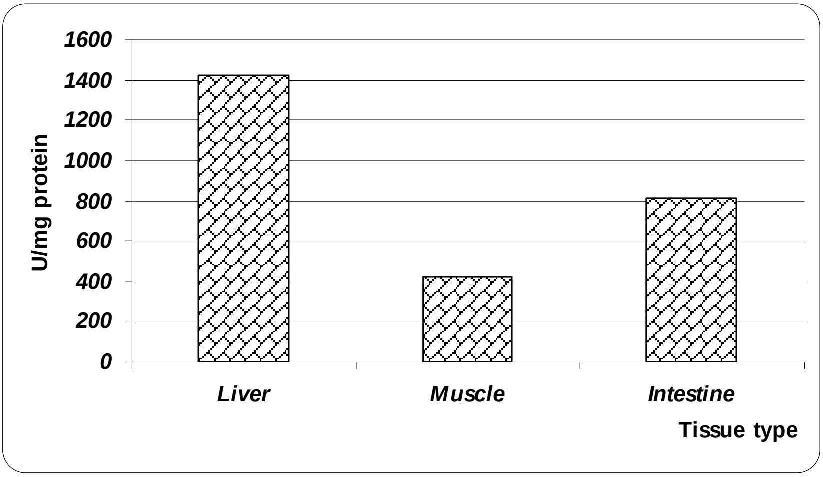

Fig.1. Dynamics of catalase activity in different tissues at two summer-old *Ctenopharyngodon idella* species

A first objective of this study was to determine catalase activity in liver, muscle and intestine in two summer-old *Ctenopharyngodon idella* representatives. As stated in the graphical representation (Fig. 1), the enzyme has a higher specific activity in the liver (1425.35 U/mg protein), followed by the intestine (812.74 U/mg protein) and muscle (425.79 U/mg protein). The results concords with those obtained from literature data, indicating elevated liver catalase activity in freshwater fish species (Ciornea *et al*., 2009).

After statistical processing of obtained results a series of statistical indicators were calculated, based on average values and standard deviation, subsequently drawing confidence interval limits in which catalase activity varies for each tissue. Thus, with a 95% probability, catalase activity range between 1367.18-1480.18 U/mg protein for liver tissue, from 781.09 to 844.38 U/mg protein in intestine, respectively, from 412.87 to 438.71 U/mg protein for muscular tissue (Fig. 2).

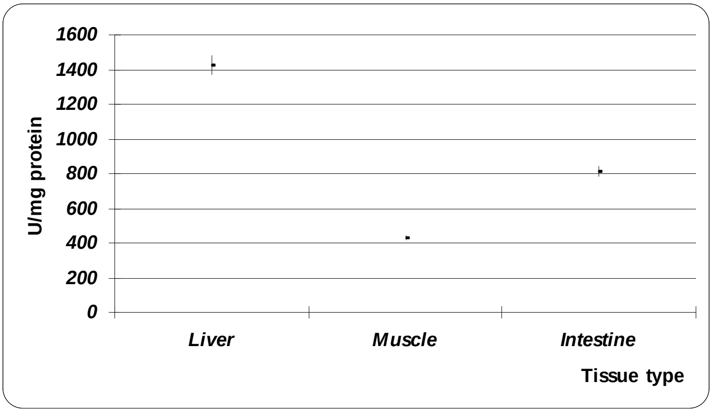

Fig.2. Confidence interval limits of catalase activity in different tissues

at two summers-old *Ctenopharyngodon idella* species

The next step in our study was the determination of superoxide-dismutase activity in the five representatives of grass carp, knowing that this enzyme would have a higher importance than catalase in the prevention of harmful action of free radicals that can damage cells, destroying cell membranes and cellular genetic apparatus, having harmful effects on DNA, particularly in the mitochondria, limiting their operation, blocking enzymes and disturbing physiological functions if left uncontrolled.

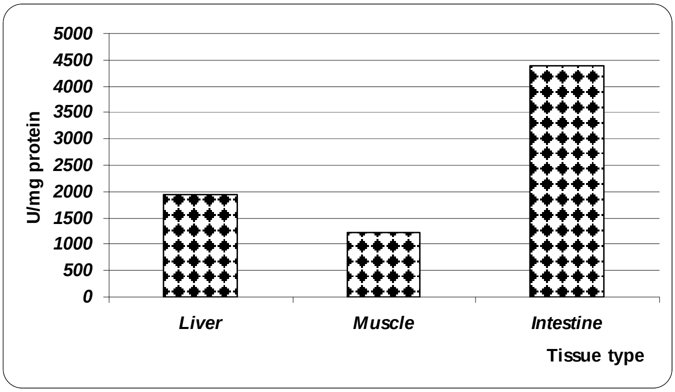

Fig.3. Dynamics of superoxide-dismutase activity in different tissues at two summer-old Ctenophar yngodon idella species

As noted in Figure 3, superoxide-dismutase has a significantly higher activity in intestinal tissue compared with the other two types of tissues analyzed, being 2.2 times higher than that detected in liver and 3.6 times higher than that recorded in muscle tissue.

Regarding the limits of confidence intervals, as can be seen in the graphical representation (Fig. 4), are small for all samples analyzed, higher values being recorded in muscular tissue (1160-1210. 88 U/mg protein).

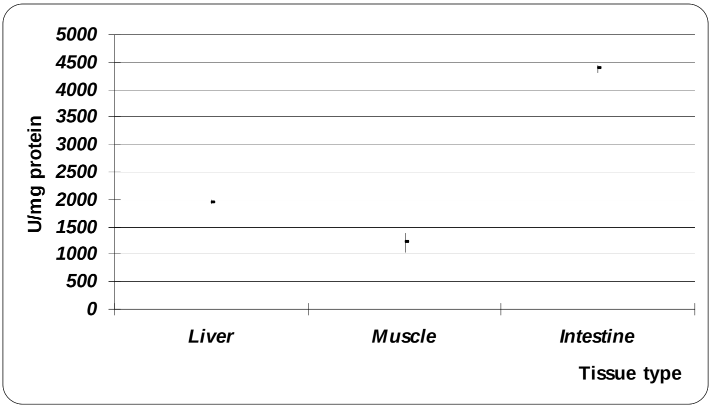

Fig.4. Confidence interval limits of superoxid-dismutase activity in different tissues at two summers-old *Ctenopharyngodon idella* species

CONCLUSIONS

The results obtained allowed us draw the following conclusions:

1. After a comparative analysis of the activity of catalase and superoxide-dismutase, we can note that there are significant differences from one enzyme to another, but also from a tissue to another, in the case of liver recording a higher activity, because at this level intense catabolic degradation processes take place, which are aerobic processes generating primarily hydrogen peroxide first, and then superoxide anions.

2. Regarding the two oxidoreductases activity in the intestine, the situation is reversed, closely related to the role of absorption of this organ, many nutrients cross intestinal wall by active transport and from this process can result superreactive oxygen species.

3. In muscular tissue both enzymes have lower values of activity, due to the fact that muscles contractions prevail at this level, that occur with consumption of ATP and catabolic processes are less extensive.

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