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MODIFICATIONS OF SUPEROXIDE DISMUTASE, CATALASE AND ISOFERMENTATIVE FORMS UNDER THE INFLUENCE OF POLYPHENOLS EXTRACTED FROM DANDELION (*Taraxacum officinale*)

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Abstract

As a result of the vital activity of living organisms through the metabolism of energy substances, the formation and accumulation of reactive oxygen species (ROS) occurs, regardless of the state of bio-objects, that is, with the physiological or pathological course of these metabolic processes. At the same time, living organisms have formed antioxidant protection systems against these invasive and toxic substances. Given that these toxic substances influence negatively on endocellular, exocellular metabolic processes and in general the body`s tissues, they can cause various pathological conditions and disturbances in the stable functioning of the body. In this paper are analyzed some researches on the enzymatic activity of superoxide dismutase, isofermentative forms of this and catalase. Superoxide dismutases (SOD) possess the ability to convert superoxide into hydrogen peroxide, which is then removed by catalase. Analyzing the obtained results of these researches about the role of these enzymes from the first line of antioxidant protection we will obtain information that will demonstrate their activity under the influence of dandelion polyphenols.

Key words*: catalase, enzymes, oxidative stress, reactive oxygen species, superoxide dismutase.*

INTRODUCTION

In biology, but especially in physiology and biochemistry, the problem of oxidative stress represents one of the most important problems in the regulation of cellular metabolic processes, having an important role in the detoxification of cells and tissues by eliminating free radicals, being focused on deepening the knowledge about the mechanisms of cellular self-control, which allow us to improve the course of metabolic processes in bio-objects and prevent the occurrence of multiple disturbances in the stable functioning of living organisms, where oxidative stress is a component of many metabolic and energetic dysfunctions in the cells and tissues of the living organism.

During research in this field, researchers have determined four lines of antioxidant defense of bio-objects. These lines include fermentative enzymes such as: superoxide dismutase, catalase, and glutathione peroxidase. They very quickly neutralize any molecule with the potential to transform into a free radical or any free radical with the ability to cause the production of other radicals. In addition to fermentative activity, these lines also possess metal ion blockers, such as transferrin and ceruloplasmin, which chelate or sequester iron and copper respectively, thus preventing their formation as free radicals. Non-enzymatic antioxidant compounds with low molecular weight include glutathione, vitamins C and E, β-carotene, polyphenols and uric acid.

SOD catalyzes the dismutation of superoxide to H_2O_2. Zn-SOD, Mn-SOD is most abundant in mitochondria, while Cu, Zn-SOD predominates in the cytoplasm (Fridovich, 1995).

Manganese superoxide dismutase is located in the mitochondrial matrix of the cell. Manganese (Mn) is a metal cofactor that is used to catalyze the conversion of superoxide to type 2 SOD. The third type of superoxide dismutase in mammals is a superoxide dismutase bound with Cu and Zn, it is

extracellular and called type 3 SOD, which activates in the tissues of the extracellular matrix and on the surface of cells (Fukai & Ushio-Fukai, 2011).

Catalase is an important antioxidant enzyme and catalyzes the reduction of H_2O_2 to H_2O. Polyphenols, regularly ingested, become a large family of natural organic compounds characterized by multiple phenolic hydroxyl units, with a polyphenolic structure (multiple hydroxyl groups on aromatic rings), including four main classes: phenolic acids, flavonoids, stilbenes (Brglez et al., 2016).

Under unfavorable conditions, in the presence of high amounts of ROS, catalase becomes the most important scavenger of H_2O_2 in the cytosol. In mitochondria this can be even more severe, as observed in *in vitro* studies using monoamines (Marcocci et al., 2002), flavones (Zanger & Schwab, 2013), isoflavones (Salvi et al., 2002) and cyclic triterpenes (Fiore et al., 2004). By interacting with the transition metals of the respiratory chain, these compounds produce H_2O_2 and the highly toxic hydroxyl radical. These species, in addition to causing peroxidation of the phospholipid bilayer with subsequent severe damage to membrane integrity (Grünberg, 2014), can also induce the transition of mitochondrial permeability (Dong & Surh, 2008; Fiore et al., 2004; Salvi et al., 2002) with the release of cytochrome C and activation of the proapoptotic cascade. As a result of energy metabolism, mitochondria become a main endogenous source of reactive oxygen species, where ROS are localized on the inner membrane of mitochondria and as a result appears superoxide anion that favors the generation of much more reactive oxygen species, which will be able to damage cells. The superoxide anion radical occurs as a result of cellular metabolism, during the transfer of electrons in the mitochondrial electron transport chain, being also generated by enzymes such as NADPH oxidase and xanthine oxidase. This superoxide anion is involved in various pathological conditions, including oxidative stress, inflammation and cell damage.

Due to physiological conditions, most of the molecular oxygen is transformed into water via mitochondrial complex IV and only a small part is transformed into reactive species with an intracellular signaling role, whose harmful action is blocked by superoxide dismutase enzymes (Turrens, 2003). Under unfavorable conditions increases the generation of mitochondrial free radicals by means of various enzyme complexes (Chandel & Budinger, 2007). The presence of superoxide dismutase and catalase is of major importance for the conversion of superoxide radicals and the subsequent catalysis of H2O2, thus protecting the components of the organism from the harmful effects of ROS (Bai & Cederbaum, 2001).

MATERIALS AND METHODS

The research was conducted on two groups of roosters, of five roosters in each group, the control and experimental group, maintained in vivarium conditions in accordance with the prescriptions of the regulation of the maintenance of laboratory animals. The roosters were distributed in individual pens and were adapted to the maintenance conditions. The animals from the experimental group were administered hydroalcoholic extract of polyphenols, obtained from dandelion (*Taraxacum officinale*), *per os* 1 ml of extract with a total antioxidant activity of 29.3 mg gallic acid equivalent/100 g. The extract was administered in a dose of one milliliter per animal. To exclude the irritation of the digestive tract, the extract was diluted with distilled water in a ratio of 1 to 4. The administration was carried out with an automatic device for oral administration of medicinal remedies to animals.

Testing for the content of superoxide dismutase (SOD) is based on SOD-mediated inhibition of the rate of reduction of nitroblue tetrazolium in blue formazane at alkaline pH. The optimized SOD test is performed in 50 mM NaOH glycine buffer, pH 9.5, at 25°C.

The SOD concentration is determined from the V/v ratio of the velocities measured in the absence (V) or presence (v) of SOD. One unit of SOD was defined as the concentration that reduced the rate by 50% (V/v = 2) (Nagi et al., 1995). The method for determining catalase is based on the reaction of non-compound hydrogen peroxide with ammonium molybdate, which produces a yellowish color with maximum absorption at 374 nm. The method is

characterized by the addition of a correction factor to eliminate interference arising from the presence of amino acids and proteins in the serum. The analysis acts to eliminate interference caused by measuring absorbance at inappropriate wavelengths (Hussein & Hussein, 2015).

RESULTS AND DISCUSSIONS

In these researches were monitored the changes of superoxide dismutase and catalase in the blood serum of roosters under the influence of polyphenols extracted from dandelion (*Taraxacum officinale*). The research results are presented in Table 1.

Table 1. Superoxide dismutase and catalase changes in the blood serum of roosters under the influence of polyphenols extracted from dandelion (*Taraxacum officinale*)

Groups	SOD, u/c (min/L)	Catalase, (μM/L)
Experimental	177.0±0.7	29.6±0.47
Reference values	110.93±0.30	26.23±0.37

From the results obtained and indicated in Table 1, we observe a change in the amount of superoxide dismutase in the experimental group, compared to the reference group, corresponding to mathematical values of 177.0±0.7 u/c (min/L) and 110.93±0.30 μM/L. From the results of the scientific literature, it is well known that this antioxidant enzyme is of particular importance in the capture and neutralization reactions of reactive oxygen species. Reactive species produced during normal cellular metabolism can chemically react with cellular biomolecules such as nucleic acids, proteins and lipids, thereby causing them to undergo oxidative modifications that lead to changes in their composition and can alter their cellular activity.

Superoxide dismutase (SOD) is an antioxidant metalloenzyme involved in the antioxidant protection process, also requires the participation of a number of metals being as cofactors in the neutralization of reactive oxygen species. SOD is produced in the cells and tissues of living organisms, but it can decrease with age. Several classes of SOD are known: intracellular - the cytosolic form copper-zinc SOD (Cu-Zn SOD) that neutralizes free radicals (FR) produced as a result of metabolic activity in the cytoplasm, and a mitochondrial form manganese SOD (Mn SOD or SOD2) which neutralizes FR produced in mitochondria as a result of cellular energy creation; extracellular (EC SOD) - is similar to Cu-Zn SOD in that it contains copper in its active place (Fukai & Ushio-Fukai, 2011; Vouldoukis et al., 2004). The SOD antiradical system is one of the first line systems of the body's antioxidant defense and plays an important role in reducing oxidative stress.

Catalase, in turn, being one of the most important antioxidant enzymes, contributes to the decomposition of cellular hydrogen peroxide to produce water and oxygen. From the results obtained and indicated in Table 1, we observe an increase in the catalase level in the experimental group, which indicates us a value of 29.6±0.47 μM/L, compared to the reference group with a value of 26.23± 0.37 μM/L.

It is established that catalase insufficiency or dysfunction is associated with the pathogenesis of many degenerative disorders related to age, nutrition, metabolism, etc.

In this experiment were also studied changes in the content of metals under the influence of polyphenols obtained from dandelion (*Taraxacum officinale*). The research results are presented in Table 2.

Table 2. Changes in the content of metals under the influence of polyphenols obtained from dandelion (*Taraxacum officinale*)

Groups	Phosphorus, mM/L	Magnesium, mM/L	Zinc, μM/L	Ferum, μM/L
Experimental	1.56±0.10	0.771±0.055	37.42±0.62	14.53±0.46
Reference values	1.45±0.11	0.674±0.033	31.32±0.45	12.15±0.31

Phosphorus (P) is an indispensable element for animal health, participating in a number of important metabolic processes, such as ensuring the structure and strength of bones, cell walls and phosphate buffer systems. From the results obtained and indicated in Table 2, we observe a clear difference in the mathematical values between the groups, namely for the experimental group we have a value of 1.56±0.10 mM/L, and for the reference group a value of 1.45±0.11 mM/L, obtaining an increase in phosphorus by about 8% for the experimental group. With an increase in phosphorus levels, the activity of SOD and CAT enzymes increased significantly.

Magnesium also increases the activity of these two extremely important antioxidants found in the mammalian organisms - catalase and superoxide dismutase. Mg is the bivalent intracellular cation in mammalian cells, second in abundance after potassium (K), it is essential for many biological processes, participating in oxidative phosphorylation, energy production, glycolysis, protein and nucleic acid synthesis (Pethő et al., 2024).

Decreased Mg concentration may contribute to decreased expression and activity of antioxidant enzymes (such as Gpx, SOD and CAT), leading to decreased antioxidant concentrations in cells and tissues and increased production of ROS, hydrogen peroxide and superoxide anion by inflammatory cells (Morais et al., 2017). In the researches carried out in our laboratory we observe a change in the magnesium values, namely for the experimental group we have a value of 0.771±0.055 mM/L and correspondingly for the reference group 0.674±0.033 mM/L. This minor difference may be influenced by Mg metabolism and its involvement in about 300 enzymatic reactions and its participation in the body's detoxification through its inclusion in ROS blocking reactions.

In the specialized literature, it is mentioned that zinc is a structural component of the enzyme superoxide dismutase, presenting as a cofactor for superoxide dismutase, participating in the regulation of glutathione peroxidase and in the expression of metallothionein. Moreover, zinc competes with iron and copper in the cell membrane, inhibits the NADPH enzyme, oxidase and reduces chronic inflammation and hyperglycemia (Cruz & Oliveira, 2015).

Superoxide dismutase has an active center with a copper ion and a zinc ion, promoting the conversion of two superoxide radicals into hydrogen peroxide and molecular oxygen, reducing ROS toxicity (Cruz & Soares, 2011). Thus, maintaining optimal zinc concentrations in the extra - and intracellular spaces is necessary for the proper functioning of the antioxidant defense system. The results of the researches demonstrate that zinc, like other elements, undergoes changes as a result of the researches performed, namely for the experimental group the following value was obtained 37.42±0.62 μM/L, and correspondingly for the reference group 31.32±0.45 μM/L, which demonstrates to us an increase in the value of the experimental group and proves a beneficial influence of the dandelion extract (*Taraxacum officinale*) on the studied indices.

Iron is a metal with essential functions, mainly involved in the biosynthesis of heme for proteins such as hemoglobin, which participates in oxygen transport. Both iron deficiency and iron excess can have harmful effects on health (Gozzelino & Arosio, 2016). Due to this important vital function, iron metabolism is strictly regulated to cover the need for hemoglobin biosynthesis while avoiding its toxicity (Muckenthaler et al., 2017; Papanikolaou & Pantopoulos, 2017). Important features of iron homeostasis are its distinctive mechanisms of systemic and cellular regulation of iron absorption, transport, use and storage, on the one hand, but the absence of a clear mechanism of excretion, on the other hand (Katsarou & Pantopoulos, 2020). A tightly balanced iron homeostasis is a physiological necessity, as unbound free iron is a key contributor to toxic potential as a source of oxidative stress through the Fenton reaction and its role in ferroptosis, an iron-dependent form of regulated cell death. Iron is a potentially toxic molecule because it can donate and accept electrons. Numerous endogenous antioxidants maintain the redox state of the cell and prevent the harmful effects of oxidative stress. These antioxidants include superoxide dismutase (SOD), catalase, glutathione (GSH), thioredoxin (Trx) and

ferritin. As is known, superoxide (O_2^-) is the first reactive radical formed, and this radical can be neutralized by SOD. From the data of Table 2, we observe that the difference in iron values for the experimental group is 14.53±0.46 μM/L, and for the reference group is 12.15±0.31 μM/L. This is possibly related to the regulation of metabolism through proteins (ferritin) and antioxidant systems, which control the level of iron in the body's intra- and extracellular space.

CONCLUSIONS

Under the influence of internal and external factors during ontogenesis, living organisms have become a complex network, which as a result of functioning and energy metabolism produce a wide range of reactive oxygen species, in turn, living organisms have formed antioxidant defense systems.

Antioxidants act collectively against free radicals to counteract their damaging effects on vital biomolecules and body tissues.

The role of superoxide dismutase (SOD) and catalase (CAT) is significant in antioxidant protection, especially with regard to the superoxide anion radical (O2) which is constantly generated in the normal metabolism of the organism, especially through the mitochondrial energy production pathway.

Catalase is an essential antioxidant enzyme that plays an important role in breaking down hydrogen peroxide and maintaining cellular redox homeostasis.

Multiple dysfunctions of living organisms, which can be associated with micro- and macroelement homeostasis are also associated with the prevention of oxidative stress, and a multitude of bioactive antioxidants and other plant-derived phytochemicals can simultaneously regulate the homeostasis of antioxidant systems, reduce the consequences of oxidative stress and maintain the stable functioning of the body's cells and tissues.

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REFERENCES

Bai, J., & Cederbaum, A.I. (2001). Mitochondrial catalase and oxidative injury. *Biological signals and receptors, 10*(3-4), 189-199.

Brglez, M.E., Knez, H.M., Škerget, M., Knez, Ž., & Bren, U. (2016). Polyphenols: Extraction methods, antioxidative action, bioavailability and anticarcinogenic effects. *Molecules*, *21*, 901.

Chandel, N., & Budinger, S. (2007). The cellular basis for diverse responses to oxygen. *Free Radical Biology & Medicine, 42*, 165-174.

Cruz, K.J.C., & Oliveira, A.R.S. (2015). Antioxidant role of zinc in diabetes mellitus. *World J. Diabetes, 6,* 333-337.

Cruz, J.B.F., & Soares, H.F. (2011). Uma revisão sobre o zinco. *Ensaios Ciência Ciências Biológicas Agrárias Saúde, 15*(1), 207-222.

Dong, Z., & Surh, Y.J. (2008). *Dietary modulation of cell signaling pathways.* Florida, USA: CRC Press, Boca Raton.

Fiore, C., Salvi, M., Palermo, M., Sinigaglia, G., Armanini, D., & Toninello, A. (2004). On the mechanism of mitochondrial permeability transition induction by glycyrrhetinic acid. *Biochim. Biophys. Acta., 1658,* 195-201.

Fridovich, I. (1995). Superoxide radical and superoxide dismutases. *Annu. Rev. Biochem., 64,* 97-112.

Fukai, T., & Ushio-Fukai, M. (2011). Superoxide dismutases: role in redox signaling, vascular function, and diseases. *Antioxid Redox Signal, 15,* 1583-1606.

Gozzelino, R., & Arosio, P. (2016). Iron homeostasis in health and disease. *Int. J. Mol. Sci., 17*(1), 130-144.

Grünberg, W. (2014). Treatment of phosphorus balance disorders. *Veterinary Clinics of North America: Food Animal Practice, 30,* 383-408.

Hussein, M.H., & Hussein, N.A. (2015). Data supporting the spectrophotometric method for the estimation of catalase activity. *Data Brief., 6,* 194-199.

Katsarou, A., & Pantopoulos, K. (2020). Basics and principles of cellular and systemic iron homeostasis. *Mol. Asp. Med.*, *75*, 100866.

Marcocci, L., De Marky, U., Salivi, M., Milella, Z.G., Nocera, S., Agostinelli, E., Mondovi, B., Toninello, A. (2002). Tyramine and monoamine oxidaseinhibitors as modulators of the mitochondrial membrane permeability transition. *J. Membr. Biol.*, *188,* 23-31.

Morais, J.B.S., Severo, J.S., dos Santos, L.R., de Sousa Melo, S.R., de Oliveira Santos, R., de Oliveira, A.R.S., Cruz, K.J.C., do Nascimento Marreiro, D. (2017). Role of magnesium in oxidative stress in individuals with obesity. *Biol. Trace Elem. Res., 176,* 20-26.

Muckenthaler, M.U., Rivella, S., Hentze, M.W., Galy, B. (2017). A red carpet for iron metabolism. *Cell., 168,* 344-361.

Nagi, M.N., al-Bekairi, A.M., al-Sawaf, H.A. (1995). Spectrophotometric assay for superoxide dismutase based on the nitroblue tetrazolium reduction by glucose-glucose oxidase. *Biochem. Mol. Biol., 36*(3), 633-638.

Papanikolaou, G., & Pantopoulos, K. (2017). Systemic iron homeostasis and erythropoiesis. *IUBMB Life, 69,* 399-413.

Pethő, Á.G., Fülöp, T., Orosz, P., Tapolyai, M. (2024). Magnesium is a vital ion in the body - it is time to consider its supplementation on a routine basis. *Clin. Pract., 14,* 521-535.

Salvi, M., Brunati, A.M., Clari, G., Toninello, A. (2002). Interaction of genistein with the mitochondrial electron transport chain results in opening of the membrane transition pore. *Biochim. Biophys. Acta., 1556,* 187-196.

Turrens, J. (2003). Mitochondrial formation of reactive oxygen species. *The Journal of physiology, 552*, 335-344.

Vouldoukis, I., Conti, M., Krauss, P. et al. (2004). Supplementation with gliadin-combined plant superoxide dismutase extract promotes antioxidant defences and protects against oxidative stress. *Phytother. Res., 18*(12), 957-962.

Zanger, U.M., & Schwab, M. (2013). Cytochrome P450 enzymes in drug metabolism: Regulation of gene expression, enzyme activities, and impact of genetic variation. *Pharmacology & Therapeutics*, *138,* 103-141.