CATALASE ACTIVITY IN RAT BLOOD SERUM UNDER THE IMPACT OF IONIZED WATER SUPPLEMENTED WITH GLUTATHIONE AND VITAMIN C DURING HYPERTHERMIC STRESS

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Abstract: Heat stress is thought to be a factor in the environment that increases the production of reactive oxygen species (ROS) due to the similarities between the responses that follow heat stress and those that occur during oxidative stress. Alkaline water, commonly referred to as ionized or reduced water (ERW), is water that has been electrochemically activated and has a pH higher than 7. Otherwise, the ERW possesses exceptional redox characteristics and has an alkaline pH and many reducing features. Our study's objective was to examine the effects of ERW on catalase activity under hyperthermic stress by including enzymatic and non-enzymatic antioxidants, glutathione, and vitamin C. White laboratory Wistar rats of the female sex, weighing 180–220 g, young rats, separated into three groups of 15, were used in the experiment. Acute hyperthermic exposure at 41°C led to the development of oxidative stress. The first group, known as the control group (CPM), is given tap water treatment, the second group, ionized water treatment (TAM), and the third group, ionized water treatment with additional glutathione and vitamin C (TAD). The treatment lasted for 21 days. All three groups, concerning the respective treatment that each of them individually received and the time of its application, do not show a statistically significant difference in CAT activity, in the period 7-14 days, when the rats were not exposed to high ambient temperature. Acute hyperthermic exposure caused a significant increase ($p < 0.001$) in CAT activity in all three groups. From the statistical analysis of the compared groups on the 7th day of treatment, it is observed that there is no significant difference in the enzyme mentioned above activity. This finding is identical to the comparison made concerning the CPM and TAM groups on the 14th and 21st days of the experiment, which is contrary to the difference in CAT activity between the remaining groups compared during those days, which was shown to be statistically significant. Treatment with ERW, without added antioxidants or with their combination, did not lead to significant changes in the activity of CAT in the blood serum during the absence of high ambient temperature. Moderate levels of free radicals induce increased expression of genes for the synthesis of antioxidant enzymes as a compensatory mechanism to better protect against ROS-induced damage. This data explains the higher activity of CAT in the period of hyperthermic stress in the blood serum.
Keywords: catalase, ionized water, hyperthermic stress, glutathione, vitamin C

1. INTRODUCTION

Mammalian and non-mammalian aerobic cells, which have a cytochrome system, are invariably present with catalase (EC 1.11.1.6), the most prevalent protein in peroxisomes. This enzyme is crucial for clearing the cell of harmful hydrogen peroxide. A heme is found at the catalytic core of the dumbbell-shaped tetramer of four identical subunits that makes up catalase (Chen, Q. et al, 2015). The enzyme catalase, which oxidizes low molecular alcohols and nitrites involved in cellular respiration and does not require energy for activation, is one of the body's natural defense mechanisms. It accelerates the breakdown of hydrogen peroxide that is constantly formed into its final products while also oxidizing these molecules. A sign of metabolic problems is the presence of catalase in the blood and tissues (Boriskin, P. et al., 2019). The molecular mechanisms controlling the expression of catalase, the first and oldest antioxidant enzyme identified, are not well understood. Catalases were categorized into three groups based on their structure and function as a result of the identification of unique homologies with the growing number of full sequences available. Catalase-peroxidases and typical or real catalases are found in the first and second groups, respectively, while manganese catalases, which do not include heme, are found in the third category (Glorieux, C. and Calderon, P. B., 2017). Large amounts of catalase are produced in the cytoplasm and organelles (peroxisomes) of cells. This oxidoreductase class member is highly prevalent in the liver, kidneys, and red blood cells of mammals (Boriskin, P et al., 2020). Inflammation and oxidative stress can both be treated medically using catalase. There is also proof that certain inhibitors can specifically target the catalase found in malignant cells (Vitolo, M., 2021). The antioxidant system of animals is made up of the antioxidant enzyme system and non-antioxidant system (containing various antioxidants), which can eliminate free radicals created in the body. CAT is a crucial enzyme in the antioxidant enzyme system, which is extensively found in microbes, animals, and plants and has anti-inflammatory and antioxidant properties (Tang, M et al, 2022). Since antioxidants have the capacity to suppress oxidation, they are essential agents in the battle against free radicals and oxidative stress. In situations where the body's natural defense

mechanism, which primarily consists of enzymes, is unable to do its essential job, antioxidants are a vital tool for countering reactive oxidative species. The antioxidant vitamin C helps the cells fight oxidative stress and inflammatory processes by donating an electron and neutralizing free radicals. The creation of enzymes including collagen, carnitine, and neurotransmitters is one of vitamin C's secondary roles as a cofactor (Kumašin, A. et al., 2022). All tissues contain glutathione (GSH), which serves as the "master antioxidant." The high concentration of the reduced form (millimolar) emphasizes its critical role in the regulation of numerous activities, including detoxification, protein folding, antiviral defense, and immunological response (Silvagno, F., 2020). GSH is an intracellular redox buffer, and whether it is free or attached to proteins, its primary function is closely linked to redox reactions. It primarily acts as a reductant against oxygen and the reactive species that result (Gaucher, C., et al., 2018). Ionized or reduced water is electrochemically activated water with a pH greater than 7 (ERW). ERW can remove and purify ROS because it has an alkaline pH, is rich in hydrogen molecules, and has a negative redox potential. The bioactivity of ERW is its antioxidant activity. ERW imitates the actions of antioxidant enzymes like SOD and catalase (CAT) by scavenging ROS (Ridwan R. D., et al., 2017). Physical stressors like heat stress can affect the structural makeup of proteins and lead to cell death. Reactive oxygen species (ROS) are produced more frequently under conditions of heat stress, which reduces cell viability and proliferation and results in apoptosis. In addition, high levels of ROS production brought on by heat stress reduce the activity of antioxidant defense mechanisms, increasing the risk of oxidative damage (Ibtisham, F., et al.,2018).

2. OBJECTIVES

Our study's goals were based on the idea that consuming electrochemically reduced water (ERW), also known as ionized water, would increase the body's alkaline reserve. In the context of organism exposure to high external temperatures as a stress factor, we highlighted this capacity of ERW. Our presumptions were founded on the idea that ERW, which functions as an antioxidant, will increase the body's tolerance to catalase (CAT) activity in blood serum.

3. MATERIAL AND METHODS

Experimental model

White laboratory rats of the Wistar breed, weighing 180–220 g, were divided into three groups and given the proper care using an experimental paradigm (15 animals total; n = 45). Throughout the experiment, the animals were kept in the light mode for 12 hours at room temperature (20°C). All of the study's animals were given unrestricted access to standard laboratory food and water.

Following were the labels and classifications used for the 15 experimental groups of animals:

1. The first group of animals (CPM), which will be referred to as the control group and received only water, was kept under the same conditions for the length of the experiment.
2. A second group of animals (TAD) that underwent the preceding conditions and received ionized water treatment for the duration of the study
3. The third group of animals (TAM) was grown in identical experimental conditions and was given ionized water with glutathione and vitamin C supplements.

Experimental protocol

The three rat groups in the experiment received appropriately modified natural water in the morning for a total of 21 days. The control group received only natural water within the specified time period. The other two groups received ionized water (ERW, alkaline water) and ionized water with extra glutathione and vitamin C, respectively. Water was injected into the stomach in 2 ml doses. Samples were taken for the study of particular parameters on the seventh, fourteenth, and twenty-first days of therapy. Blood was drawn from the rat tail on days 7 and 14 and stored for analysis in appropriately labeled ependorphs. Blood serum was taken for analysis and kept at -80 °C for the required tests after five minutes of centrifugation at 1500 rpm. After obtaining proper treatment on day 21, the animals in the pertinent groups were subjected to a hyperthermic environment for five hours until they reached a state of secondary hyperthermia (body temperature of 43 C). Individual exposures were conducted for 80 minutes at 40 1 C in air chambers. Rectal temperature was also taken when under hyperthermic exposure.

Catalase (CAT) activity measurement

Catalase is an antioxidant enzyme that catalyzes the breakdown of hydrogen peroxide into water and oxygen. Its activity was determined from blood plasma and tissue homogenates.

The method's principle

This method is based on the spectrophotometric determination of hydrogen peroxide as a result of the formation of a stable complex with a yellow color between H2O2 and ammonium molybdate (Góth, 1991). Measure the absorbance at 405 nm.

Procedure for testing

0.2 ml of plasma is incubated with 1.0 ml of the substrate (65 μmol/ml H_2O_2 in 60 mmol/l sodium-potassium-phosphate buffer, pH 7.4) at 37 °C for 60 seconds. The enzyme reaction was stopped with 1.0 ml of 32.4 mmol/L ammonium molybdate ($(NH4)6\ Mo7O24 \cdot 4\ H_2O$) and the absorbance of the yellow-colored complex was measured at 405 nm against blank 3.

Reagents are added to the blank samples according to the table:

	Blind trial 1	**Blind trial 2**	**Blind trial 3**
Substrate	1,0 ml	1,0 ml	/
Blood serum	0,2 ml	/	/
Molybdate	1,0 ml	1,0 ml	1,0 ml
Puffer	/	0,2 ml	1,2 ml

One unit of catalase breaks down 1 μmol of H2O2 in 1 minute at 37 °C.

Analytical statistics

The results obtained from the experiment were statistically analyzed using the statistical program InStat. The information is displayed using mean values and standard deviation (SEM). The impact of individual alkaline water treatment, as well as vitamin C and GSH addition in the same, in conjunction with the experimental model's hyperthermic exposure, was determined using one-way analysis of variance (ANOVA). Both within a single group and when contrasting three different groups, a statistically significant difference was discovered. While ordinary ANOVA was used to compare groups of animals, repeated measures ANOVA was employed to assess the significance of differences between rat groups differences as a function of time. Changes that were significant were those with p 0.001 values.

4. RESULTS

The results of our study on the impact of ionized water treatment on CAT activity in blood serum, both with and without the addition of appropriate antioxidants, as well as the acute hyperthermic exposure introduced on the 21st day of treatment, are shown in Graph No. 1.

Graph 1. Catalase activity in blood serum in rats

Legend: CPM - control group treated with natural water; TAM - group treated with ionized water; TAD - group treated with ionized water with added glutathione and vitamin C.

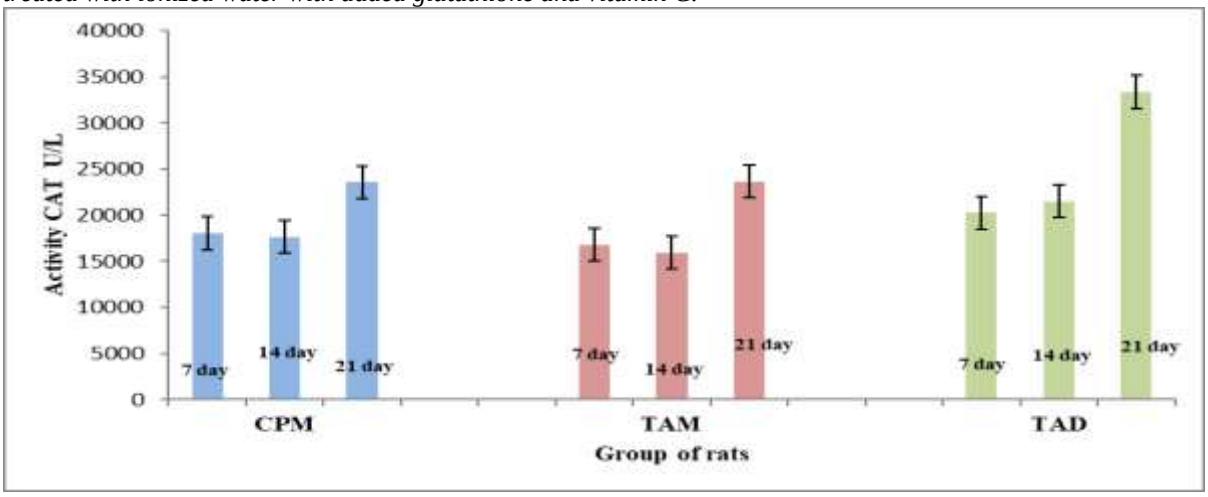

Table 1 shows the results of the statistical analysis of data on the activity of CAT in blood serum

Statistical analysis – CAT activity				
Compared groups			Results	
CPM 7	vs	CPM 14	$p > 0,05$	Ns
CPM 7	vs	CPM 21	$p < 0,001$	***
CPM 14	vs	CPM 21	$p < 0,001$	***
TAM 7	vs	TAM 14	$p > 0,05$	Ns
TAM 7	vs	TAM 21	$p < 0,001$	***
TAM 14	vs	TAM 21	$p < 0,001$	***
TAD 7	vs	TAD 14	$p > 0,05$	Ns
TAD 7	vs	TAD 21	$p < 0,001$	***
TAD 14	vs	TAD 21	$p < 0,001$	***
CPM 7	vs	TAM 7	$p > 0,05$	Ns
CPM 7	vs	TAD 7	$p > 0,05$	Ns
TAM 7	vs	TAD 7	$p > 0,05$	Ns
CPM 14	vs	TAM 14	$p > 0,05$	Ns
CPM 14	vs	TAD 14	$p < 0,01$	**
TAM 14	vs	TAD 14	$p < 0,001$	***
CPM 21	vs	TAM 21	$p > 0,05$	Ns
CPM 21	vs	TAD 21	$p < 0,001$	***
TAM 21	vs	TAD 21	$p < 0,001$	***

All three groups, in relation to the respective treatment that each of them individually received and the time of its application, do not show a statistically significant difference in CAT activity, in the period 7-14 days, when the rats were not exposed to high ambient temperature (table 1). Acute hyperthermic exposure caused a significant increase ($p < 0.001$) in CAT activity in all three groups. From the statistical analysis of the compared groups on the 7th day of treatment, it is observed that there is no significant difference in the aforementioned enzyme activity. This finding is identical for the comparison made in relation to the KPM and TAM groups on the 14th and 21st days of the experiment, which is contrary to the difference in CAT activity between the remaining groups compared during those days, which was shown to be statistically significant.

5. DISCUSSION

Heat stress is one of the most important factors capable of enhancing reactive oxygen species (ROS) production (Li L., et al. 2017) The antioxidant status of the cell is significant in hyperthermic conditions, when the production of free radicals is intensified. When cells are exposed to oxidative stress, they increase the expression and activity of antioxidant enzymes as a compensatory mechanism to better protect against ROS-induced damage. A number of studies indicate that moderate levels of toxic reactive radicals induce the expression of genes responsible for the synthesis of antioxidant enzymes and their activity, while very high levels reduce the same enzyme activity as a result of damage to the molecular machinery required for the induction of these enzymes (Wei & Lee, 2002; Gechev et al., 2002). Electrolyzed reduced water (ERW) has a high redox potential. It has been revealed that ERW with a high pH and a significant negative redox potential (RP) have superoxide dismutase (SOD)- and catalase-like activities, respectively. As a result, these substances scavenge active oxygen species and shield DNA from oxygen radical damage in vitro. The bioactivity of ERW is its antioxidant activity. ERW mimics the activity of antioxidant enzymes such as SOD and catalase (CAT) by scavenging ROS (Ridwan R. D., et al., 2017). Almost all of the body's cells contain the antioxidant glutathione, which aids in the body's process of eliminating toxins such as xenobiotics and pharmaceuticals. Additionally, hydrogen peroxide is detoxified using reduced glutathione (GSH), which also functions as a hydrogen donor (Weschawalit, S., et al.,2017). For decades, vitamin C has been understood. Its multiple cellular impacts are indicated by the studies currently available. Vitamin C, an antioxidant that scavenges free radicals, also has a "second face" in the form of a pro-oxidative component (Kazmierczak-Baranska, J., et al., 2020).

At the end of our experiment, in all groups, we observed an increasing trend and a significantly higher difference in CAT activity on day 21, when rats were exposed to hyperthermic stress, compared to the period when there were no stressful conditions. Acute hyperthermic exposure caused a significant elevation of CAT activity in all three groups. A possible mechanism for reducing the activity of antioxidant enzymes is the inactivation caused by excess free radicals (Schmatz et al., 2012). Furthermore, hydrogen peroxide can be detoxified via CAT, and GRx or enter the

Fenton reaction to generate OH. The alternative fate for the decomposition of H_2O_2 leads to the conclusion that it does not consume only CAT for its neutralization, which is why a higher concentration of CAT is registered than of SOD as the only enzyme for the dismutation of O^-_2. A study by Jaeschke and Benzick (Jaeschke & Benzick, 1992) shows that catalase contributes only 20% to the neutralization of H_2O_2. The results of our research show similar values. We are of the opinion that such properties of ERW as an antioxidant enzyme with similar activity of CAT in the TAM group on the 7th, 14th, and 21st day show a higher activity of the same enzymes compared to the control group. In the TAD group, we observed the highest activity of CAT compared to the previous two groups, because in this group, in addition to the presence of ERW, we also introduced high temperature as a stressful factor, which, according to the above explanations, indirectly, through oxidative stress, can to induce transcription of genes responsible for the synthesis of antioxidant enzymes. CAT recognizes only H_2O_2 as a substrate and has a very low affinity. Thus, it is activated only when H_2O_2 is in a higher concentration than physiological, and these conditions can be met when there is oxidative stress.

6. CONCLUSION

While antioxidant enzyme activity increases during acute hyperthermia, oxidative stress indicators are produced at a higher rate. Increased ROS generation, which results in oxidative damage, is brought on by high temperatures. Treatment with ERW, without added antioxidants or with their combination, did not lead to significant changes in the activity of CAT in the blood serum during the absence of high ambient temperature. Moderate levels of free radicals induce increased expression of genes for the synthesis of antioxidant enzymes as a compensatory mechanism to better protect against ROS-induced damage. This data explains the higher activity of CAT in the period of hyperthermic stress in the blood serum.

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