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CATALASE ACTIVITY IN INTERACTION WITH IONIZED WATER AND OTHER ANTIOXIDANTS IN BLOOD PLASMA, LIVER, AND KIDNEY OF THE RAT DURING HYPERTHERMIC STRESS

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Abstract: The similarity between the responses seen after heat stress and those happening in the condition of oxidative stress suggests that heat stress is an environmental element that stimulates the generation of reactive oxygen species (ROS). Alkaline water, also known as ionized or reduced water (ERW), is water that has undergone electrochemical activation and has a pH greater than 7. The ERW also has excellent redox properties and other reducing features. ERW mimics the activity of the antioxidant enzyme, such as catalase (CAT) by scavenging ROS. The aim of this study was to examine the catalase activity in interaction with ERW under hyperthermic stress by including non-enzymatic antioxidants, glutathione, and vitamin C. White laboratory Young female Wistar rats weighing 180–220 g were divided into three groups of 15 for the experiment. Oxidative stress was caused by 41°C acute hyperthermic exposure. The first group is referred to as the control group (CPM), the second group is referred to as the ionized water treatment (TAM), and the third group is referred to as the ionized water treatment with added glutathione and vitamin C (TAD). The treatment period lasted 21 days. The treatment applied respectively to each group during the period of hyperthermic exposure caused a significant difference in CAT activity in blood plasma among the three groups. Liver CAT activity was increased in all three groups. Treatment for 21 days in all three groups led to a decrease in CAT activity in the blood plasma and in the kidneys. Acute hyperthermic exposure on the 21st day in the CPM and TAD groups for blood plasma has a statistically significant difference ($p < 0.01$). Also, in both the TAM and TAD groups there is a statistically significant difference ($p < 0.01$) in CAT activity, which is in contrast to the difference in the liver and kidney CAT activity between the remaining compared groups, which was shown to be statistically insignificant.

Keywords: *catalase, ionized water, blood plasma, liver, kidney*
Field: Medical sciences and health

INTRODUCTION

Heat stress is assumed to be an environmental element for promoting the generation of reactive oxygen species (ROS) due to the similarities between the reactions seen after heat stress and those that occur when under oxidative stress (Ilievska, J. et al., 2016). A wide range of changes in cellular shape, biochemistry, and function is brought about by hyperthermia (HT) in mammalian cells. A significant function as an intracellular mediator of HT-induced cell death, including apoptosis, has been established for one of these HT-induced modifications, oxidative stress, which has been linked to an increase in reactive oxygen species (ROS) generation (Tabuchi, Y., et al., 2016). One of the body's many multifactorial processes for maintaining homeostasis is the antioxidant defense system. A network of enzymes that regulates the processes of free radical oxidation started by active oxygen species is the primary part of it (Boriskin, P., et al., 2019). A system of specialized enzymes that prevent and eliminate free radicals is one of the enzymatic defenses against ROS. These enzymes are connected to one another and take part in a series of actions that neutralize free radicals. Superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GR), and glutathione S-transferase are some of the most crucial antioxidant enzymes (GST). These enzymes work in concert to directly neutralize free radicals, prevent lipid peroxidation, activate non-enzymatic antioxidant defense components, repair damaged molecules, and degrade structures that cannot be repaired. (Palma, J. M., and Seiquer, I. 2020). Hydrogen peroxide will build up in the cytosol and mitochondria under pathological circumstances that accelerate the pace of its synthesis. Catalase, which is only found in peroxisomes, safely disposes of hydrogen peroxide (Sapojnikova, N., et al., 2012). Cells have developed a number of antioxidant defense mechanisms (including metabolites, vitamins, and enzymes) to counteract or lessen the negative effects of reactive

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species and/or their byproducts. “Oxidative stress” is a physiological condition that occurs from any disruption in the equilibrium between the levels of antioxidants and reactive species. By oxidizing cellular hydrogen peroxide to produce water and oxygen, catalase is one of the essential antioxidant enzymes that significantly reduces oxidative stress (Nandi, A., et al., 2019). The oxidation-reduction potential of electrolyzed reduced water (ERW) is extremely negative (Ridwan, R. D., et al., 2017). According to a number of research, electrolyzed reduced water (ERW), which is made up of hydrogen molecules with a strong reducing ability and may take part in the redox control of cellular function, has the capacity to scavenge free radicals (Franceschelli, S., et al., 2017). To safeguard itself from harm brought on by the abundance of reactive species, the cellular environment has to have optimal GSH storage. A naturally occurring low molecular weight thiol molecule that is not a protein is glutathione. Because it possesses an antioxidant potential, the sulfhydryl (SH) group’s presence has given it special significance. It shields the cells from free radicals and other oxidizing agents that are comparable (Khan, S., et al., 2022). Antioxidant glutathione is found in practically all bodily cells and aids in the detoxification of xenobiotics and pharmaceuticals. Additionally, hydrogen peroxide detoxification uses reduced glutathione (GSH) as a hydrogen donor (Weschawalit, S., et al., 2017). Along with serving as a cofactor for various enzymes, ascorbic acid’s primary biological role is to shield cell components from free radicals, which are frequently produced during metabolism. One of the hydrophilic antioxidants that build up in the aqueous phase of the cell is ascorbate (Kazmierczak-Baranska, J., et al., 2020). In our research, we linked the rat plasma, liver, and kidney catalase activity increase to the oxidative damage that resulted from heat stress.

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 rats’ 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 impact on the body’s tolerance to catalase (CAT) activity in the blood plasma, liver, and kidneys.

MATERIAL AND METHODS

Experimental model

With the aid of an experimental paradigm, three groups of white Wistar laboratory rats weighing 180–220 g were each given the appropriate care (15 animals in total; n = 45). The animals were kept in the light mode for the duration of the experiment for 12 hours at room temperature (20°C). Standard laboratory food and water were made available to all of the study’s animals without restriction.

The labels and groupings applied to the 15 experimental animal groups were as follows:

1. For the duration of the experiment, identical conditions were applied to the first group of animals (CPM), which will be referred to as the control group and received only water.
2. A second set of animals (TAD) were subjected to the aforementioned circumstances and were given ionized water treatment throughout the course of the trial.
3. Animals in the third group (TAM) were raised under the same experimental circumstances and were given ionized water with glutathione and vitamin C supplementation.

Experimental protocol

For a total of 21 days, the three rat groups in the experiment were given adequately modified natural water in the morning. Only natural water was given to the control group within the allotted time. The ionized water (ERW, alkaline water) and ionized water with additional glutathione and vitamin C were given to the other two groups, respectively. 2 ml dosages of water were injected into the stomach. On the seventh, fourteenth, and twenty-first days of treatment, samples were collected for the analysis of specific parameters. On days 7 and 14, blood samples were taken from the rat tail and preserved for analysis in ependorphs with the relevant labels. After being centrifuged for five minutes at a speed of 1500 rpm, blood serum was collected for examination and maintained at -80 °C for the necessary tests. The animals in the relevant groups received therapy as usual on day 21, then spent five hours in a hyperthermic setting until they developed secondary hyperthermia (body temperature of 43 ˚C). In air chambers, individual exposures were carried out for 80 minutes at 40 1 ˚C. When subjected to hyperthermic exposure, the rectus temperature was also recorded.

Catalase (CAT) activity measurement

An antioxidant enzyme known as catalase helps hydrogen peroxide break down into water and oxygen. Blood plasma and tissue homogenates were used to gauge its activity.

The method's principle

This technique relies on the spectrophotometric measurement of hydrogen peroxide as a result of the stable interaction between H2O2 and ammonium molybdate, which results in the production of a complex with a yellow hue (Góth, 1991). Take a 405 nm reading of the absorbance.

Procedure for testing

1.0 ml of the substrate (65 mol/ml H2O2 in 60 mmol/l sodium-potassium-phosphate buffer, pH 7.4) is incubated with 0.2 ml of plasma at 37 °C for 60 seconds. With 1.0 ml of 32.4 mmol/L ammonium molybdate ((NH4)6 Mo7O24 4 H2O), the enzyme activity was stopped. The yellow-colored complex's absorbance was then measured at 405 nm in comparison to 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

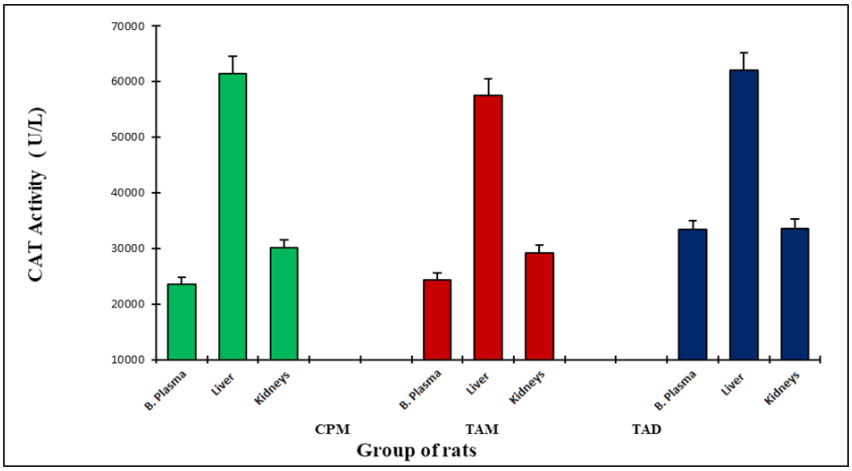
At 37 °C, 1 mol of H2O2 is broken down by 1 unit of catalase in 1 minute.

Analytical statistics

The statistical tool InStat was used to statistically examine the experiment's outcomes. Standard deviation and mean numbers are used to display the data (SEM). One-way analysis of variance was used to assess the effects of individual alkaline water treatment, vitamin C addition, and GSH addition in the same, along with the experimental model's exposure to hyperthermia (ANOVA). A statistically significant difference was found, both when comparing three different groups and within a single group. In contrast to repeated measures ANOVA, which was used to evaluate the significance of differences between rat groups differences as a function of time, conventional ANOVA was used to compare groups of animals. Changes with p 0.001 values were considered significant.

RESULTS

The results of our study on the impact of ionized water treatment on CAT activity in blood plasma, liver, and kidneys in rats, 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 plasma, liver, and kidneys 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. Results from the statistical analysis of data on the activity of CAT in blood plasma, liver, and kidneys

CAT activity – Statistical analysis				
Compared groups			Results	
CPM B.pasma	vs	TAM B.pasma	p > 0,05	Ns
CPM B.plasma	vs	TAD B.pasma	p < 0,01	**
TAM B.plasma	vs	TAD B.pasma	p < 0,01	**
CPM Liver	vs	TAM Liver	p > 0,05	Ns
CPM Liver	vs	TAD Liver	p > 0,05	Ns
TAM Liver	vs	TAD Liver	p > 0,05	Ns
CPM Kidneys	vs	TAM Kidneys	p > 0,05	Ns
CPM Kidneys	vs	TAD Kidneys	p > 0,05	Ns
TAM Kidneys	vs	TAD Kidneys	p > 0,05	Ns

DISCUSSION

Different organ systems may be harmed by hyperthermia brought on by heat stress. One of the most significant elements that can increase the generation of reactive oxygen species (ROS) is heat stress. (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 the antioxidant enzyme of catalase as in our experiment. The 240-kDa homotetrameric heme-containing protein known as CAT is widely distributed in the liver, lungs, and kidneys and is mostly found in the peroxisome. In the proximal tubules of the juxtamedullary cortex of the kidney, CAT is mostly distributed, whereas it is less expressed in the proximal tubules of the superficial cortex. On the other hand, glomeruli, distal tubules, the loop of Henle, or collecting ducts do not contain CAT. The overexpression of mitochondrial ROS and functional mitochondrial impairment are caused by CAT deficiency. The H2O2 produced by SOD is converted into oxygen and water by CAT. CAT is extremely effective in reducing H2O2, but because it is mostly found in the peroxisome, it may not play a significant role in modulating H2O2 (Hong, Y.A. and Park, C. W. 2021). One of the theories for how ERW forms when there are no electrolytes in the water suggests that a high applied voltage between the anode and cathode is required (in the range of 110 to 250 volts). SOD-like activity and catalase-like activity were discovered in ERW with a high pH and a strong negative redox potential (RP), which scavenges active oxygen molecules and guards DNA against oxidative damage. ERW's bioactivity is its antioxidant capacity (Shirahata, S., et al., 2018). Our findings are consistent with Shirahata and his team's in vitro research trials, which showed that ERW neutralizes ROS in a manner that is strikingly comparable to the actions of SOD and CAT enzymes (Franceschelli, S., et al., 2016). The liver plays a crucial role in the biotransformation and detoxification of toxic endogenous and exogenous substances. Toxin overload, viral infection, metabolic dysfunction, immunological assault, and ischemia injury are among the major causes of acute liver failure. Free radical overdoses are also thought to be the root cause of a number of chronic disorders (Nguyen Q.V., et al., 2021). Normally the tissue damage caused by oxidants in the body is controlled by enzymatic and nonenzymatic antioxidant defense systems (Sulaiman, S. H., et al., 2021). In the enterocytes and in the proximal tubular cells of the kidney, the enzyme γ-glutamyl transpeptidase plays an important role in GSH homeostasis (Moine, L., et al., 2018). In our case, the most important antioxidant enzyme is CAT; among the nonenzymatic antioxidants as glutathione and ascorbic acid (vitamin C). The majority of the blood's glutathione is made up of erythrocytes, which make up the majority of blood cells. One indicator of oxidative stress is the oxidation of GSH to glutathione disulfide (GSSG). According to scientific studies, a number of diseases are at an increased risk when there are highly reactive oxygen species (ROS) levels and low GSH levels (Grucza, K., et al., 2019). The primary source of GSH exported into the blood is the liver. Both GSH and GSSG are used to nourish other organs, mainly the kidney, and are circulated in the body. GSH functions are connected to both the liver's export and production (Lushchak, V. I., 2012).

It can be concluded that hypoxia, induced by acute temperature stress, leads to oxidative stress. Acute heat stress intensifies metabolic processes, ie there is a state of increased consumption of O2, which is followed by an increased flux of O2 in the mitochondrial respiratory chain, which again leads to the creation of ROS. At the whole-cell level, heat stress produces oxygen radicals as a result of increased

oxygen flow through the mitochondrial electron transport chain. We believe that in our research, the above-mentioned mechanisms for the temperature stress-oxidative stress pathway were also at work in the rats, and we can say with certainty that the rats on the 21st day of the treatment when they were subjected to acute temperature stress, were consequently also exposed to strong oxidative stress. The treatment applied respectively to each group during the period of hyperthermic exposure caused a significant difference in CAT activity in blood plasma among the three groups. Liver CAT activity was increased in all three groups. Treatment for 21 days in all three groups led to a decrease in CAT activity in the blood plasma and in the kidneys. Acute hyperthermic exposure on the 21st day in the CPM and TAD groups for blood plasma has a statistically significant difference ($p < 0.01$). Also, in both the TAM and TAD groups there is a statistically significant difference ($p < 0.01$) in CAT activity, which is in contrast to the difference in the liver and kidney CAT activity between the remaining compared groups, which was shown to be statistically insignificant.

CONCLUSION

It can be concluded that acute heat stress, leads to oxidative stress. 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. Hyperthermic exposure in all three groups led to a decrease in CAT activity in the blood plasma and in the kidneys. Liver CAT activity was increased in all three groups during hyperthermic exposure.

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