THE IMPACT OF IONIZED WATER SUPPLEMENTED WITH GLUTATION AND VITAMIN C DURING ACUTE HYPERTHERMIC EXPOSURE ON THE ACTIVITY OF THE SUPEROXID DISMUTASE IN THE BLOOD PLASMA, LIVER AND KIDNEYS AT WHITE LABORATORY RATS

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Abstract: Heat stress is among the most critical variables that might increase the generation of reactive oxygen species (ROS). Increased quantities of reactive oxygen and nitrogen species (ROS and RNS) have been shown to have negative consequences by affecting the cellular redox state. Several studies have shown that ionized water or Electrolyzed Reduced Water (ERW) can act as a scavenger free radical, which is produced by hydrogen molecules with a strong reducing capacity and may play a role in cellular redox regulation. The goal of our study is to see how ionized water affects the activity of superoxide dismutase (SOD) in blood plasma, liver, and kidneys during hyperthermic stress by adding enzymatic and non-enzymatic antioxidants, glutathione and vitamin C. White laboratory Wistar rats, female sex, weighing 180-220 g, young rats, separated into three groups of 15 individuals, were used in the experiment. Acute hyperthermic exposure at 41°C generated oxidative damage. The first group was given tap water as a control (KPM), the second was given ionized water (TAM), and the third was given ionized water with additional glutathione and vitamin C (TAD). The treatment lasted a total of 21 days. When compared to results obtained for the same enzyme activity on the 14th and 7th days of treatment, acute hyperthermic exposure on the 21st day significantly ($p<0.001$) reduced SOD activity in the KPM and TAM groups. The group treated with enhanced ionized water deviates from this general finding by showing a significant change in SOD activity between days 7 and 21, compared to a statistically significant difference ($p<0.01$) in the same group only from the 14th to the 21st day of treatment. In both groups KPM and TAD, the treatment given adequately during the period of hyperthermic exposure resulted in a substantial change in SOD enzyme activity in the blood plasma and liver. Acute hyperthermic exposure on day 21 did not result in a significant difference in renal SOD activity in the KPM and TAM groups.
Keywords: Ionized water, hyperthermic stress, glutathione, vitamin C, superoxide dismutase

1. INTRUDUCTION

Heat stress is a physical stressor that can cause protein structural alterations and cell death. Heat stress increases the generation of reactive oxygen species (ROS), which lowers cell viability and proliferation and causes apoptosis. Furthermore, heat stress causes excessive ROS generation, which lowers the activity of antioxidant defense systems, resulting in increased oxidative damage (Ibtisham, F., et al.,2018). Because of the similarities in responses found following heat stress and those happening in the state of oxidative stress, heat stress is assumed to be an environmental factor for increasing reactive oxygen species (ROS) generation (Ilievska, J., et al., 2016). Oxidative stress is a condition that results from a balance of oxidative and reductive processes that occur constantly in every cell during the complicated biochemical changes of physiological metabolism. Many physiological and pathological processes are regulated by free radicals. ROS and RNS have been demonstrated to act as signal messengers in a variety of biological responses, including proliferation, immunity, apoptosis, aging, and gene expression, at physiologically low levels. It's also acknowledged that high concentrations of these compounds might cause toxicity by changing the cellular redox state (Franceschelli, S., et al.,2016). Electrochemically activated water with a pH greater than 7 is known as ionized or reduced water (ERW). Based on its physical, chemical, and physiological properties, ERW is also known as ionized water, alkaline water for electrolysis, alkaline ion water, alkaline cathode water, and alkaline ionized water. ERW has an alkaline pH, is dense in hydrogen molecules, and has a negative redox potential, which means it can eliminate and purify ROS. When the water does not include electrolytes, one of the mechanisms of ERW formation (Shirahata, S., et al., 2018) proposes the necessity for a high applied voltage between the anode and cathode (in the range of 110 to 250 volts). In vitro, ERW with a high pH and a significant negative redox potential (RP) was found to have SOD-like activity and catalase-like activity, scavenging active oxygen species and protecting DNA from oxygen radical damage. The antioxidant activity of ERW is its bioactivity. By scavenging ROS, ERW mimics the activities of antioxidant enzymes like SOD and catalase (CAT) (Ridwan R. D., et al., 2017). A series of antioxidant enzymes and non-enzymatic antioxidants make up the natural antioxidant system. The most important antioxidants are superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase

(GPx), vitamins A, C, and E, and glutathione (Shim, S-Y. and Kim, H-S., 2013). One of the most significant antioxidants in intracellular division is glutathione (GSH). Detoxification, signal transmission, DNA and protein synthesis, cell proliferation and death, mitochondrial function, and neurodegeneration are just a few of the metabolic roles it performs. As a result, it could be involved in the development of a variety of diseases, including neurological disorders, organ fibrosis, cardiovascular disease, diabetes, and liver disease (Polonikov, A. 2020). GSH is an intracellular redox buffer, and its major function, whether free or associated with proteins, is tightly linked to redox reactions, primarily serving as a reductant against oxygen and the reactive species that arise (Gaucher, C., et al., 2018). For decades, vitamin C has been known. The available evidence suggests that it has multidirectional cellular actions. Vitamin C, which belongs to the antioxidant family that scavenges free radicals, also has a pro-oxidative function (Kazmierczak-Baranska, J. et al., 2020). Vitamin C (Vit. C) is a potent antioxidant as well as a cofactor for a number of biosynthetic and genetic regulatory enzymes. It helps the immune system by supporting a number of cellular activities as well as the immune system itself (Carr & Maggini., 2017). ERW can preserve cellular redox balance, lowering the risk of inflammation and other disorders associated with disturbed cell homeostasis (Franceschelli, S., et al., 2016). The high negative electrochemical potential of activated water is linked to its antioxidant properties, and thus its potential for improving human health (Mircevski, V., 2016). Superoxide dismutase (SOD) is an important antioxidant that protects the organism from oxidative stress. The enzyme is an effective treatment for illnesses caused by reactive oxygen species (ROS) (Younus, H., 2018).

2. OBJECTIVES

Our research objectives stemmed from the premise that drinking ionized or electrochemically reduced water (ERW) would enrich the body's alkaline reserve. We highlighted this ability of ERW in the context of organism exposure to high external temperatures as a stress factor. Our assumptions were based on the concept that ERW will boost the body's thermotolerance to superoxide dismutase (SOD) activity in blood plasma, liver, and kidney by acting as an antioxidant.

3. MATERIAL AND METHODS

Experimental model

An experimental paradigm was employed to apply appropriate treatment to white laboratory rats of the Wistar breed, female 180-220 g, separated into three groups (15 animals, n = 45). The animals were housed at room temperature (20°C) in light mode for 12 hours during the experiment. All of the animals in the study were allowed free access to regular laboratory food and water .

The experimental groups of 15 animals were labeled and categorized as follows:

1. The first group of animals (CPM) - which were subjected to the same conditions for the duration of the experiment and will be referred to as a control group receiving only water.
2. Second group of animals (TAD) - which for the full study period were under the above conditions and were treated with ionized water
3. The third group of animals (TAM) - raised under the same experimental circumstances and given ionized water with glutathione and vitamin C supplementation.

Experimental protocol

For a total of 21 days, the three groups of rats in the experiment were given adequately modified natural water in the morning. During the time period mentioned, the control group received only natural water. Ionized water (ERW, alkaline water) and ionized water with additional glutathione and vitamin C were given to the other two groups, respectively. Water was administered intragastrically in 2 ml increments. On the 7th, 14th, and 21st days of therapy, samples were obtained for study of specified parameters. On days 7 and 14, blood was obtained from the rat tail and collected in correctly designated ependorphs for analysis. After 5 minutes of centrifugation at 1500 rpm, blood serum was collected for examination and stored at -80 °C for the needed assays. Animals in the relevant groups were exposed to a hyperthermic environment for five hours after receiving adequate therapy on day 21, until they reached a stage of secondary hyperthermia (body temperature of 43 C). Individual exposures were done in air chambers at 40 ± 1 ˚C for 80 minutes. During hyperthermic exposure, the rectal temperature was also recorded.

Superoxide dismutase (SOD) activity measurement

The enzyme superoxide dismutase catalyzes the dismutation of the superoxide anion O2 - into hydrogen peroxide and oxygen as part of the antioxidant defense against free radicals.

The method's principle

Marklund S. and Marklund G. describe a method for detecting superoxide dismutase activity (1974). This approach is based on the enzyme's capacity to prevent pyrogallol autooxidation. In an alkaline solution with a pH of 8.2, pyrogallol autooxidation is assessed by linearly raising the absorbance to 420 nm over several minutes. This

approach is based on the rivalry between O_2—induced pyrogallol autooxidation and SOD-induced dismutation. SOD inhibits pyrogallol autooxidation by converting O_2- to H_2O_2 and O_2 and preventing the increase in absorbance to 420 nm.

Procedure for testing

50 mM Tris-HCl, pH = 8.2, 1 mM diethyleneetriamine pentaacetic acid, and an adequate blood serum sample made up the reaction mixture. The reaction is started by the addition of pyrogallol (concentration 0.2 mM). In the presence of SOD, pyrogallone autoxidation is inhibited, and absorbance is measured at a wavelength of 420 nm for 3 minutes at a temperature of 25 °C. A Tris buffer blind test is read by the spectrophotometer. Pipette the control and test samples according to the following procedure:

Reagents	Test (µl)	Control (µl)
serum	50	-
Tris buffer	1000	1000
DW	-	50
pirogalol	1000	1000

SOD activity is measured in units of U/mL. The amount of enzyme required to block pirogalol autooxidation by 50% is defined as one unit of SOD activity.

SOD activity calculation

% inhibition of pirogalol autoxidation = ΔAtest / ΔAcontrol x 100%

SOD activity (U / mL) =% inhibition of pyrogallol autoxidation / 50%

Analytical statistics

With the statistical tool InStat, the results acquired during the experiment were statistically processed. The data is presented as mean values with standard deviation (SEM). Using one-way analysis of variance, the effect of individual treatment with alkaline water, as well as vitamin C and GSH supplementation in the same, in combination with hyperthermic exposure of the experimental model, was identified (ANOVA). A statistically significant difference was found both within a single group and when comparing three different groups. Repeated measures ANOVA was used to determine the significance of differences between groups of rats as a function of time, whereas Ordinary ANOVA was used to compare groups of animals. The values of p 0.001 showed significant changes.

4. RESULTS

Graph No.1 illustrates the findings of our study on the effect of ionized water treatment on SOD activity in blood plasma, black liver, and kidneys, both with and without the inclusion of suitable antioxidants, as well as the acute hyperthermic exposure introduced on the 21st day of treatment.

Graph 1. Superoxide dismutase activity in blood plasma, liver and kidney in rats

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

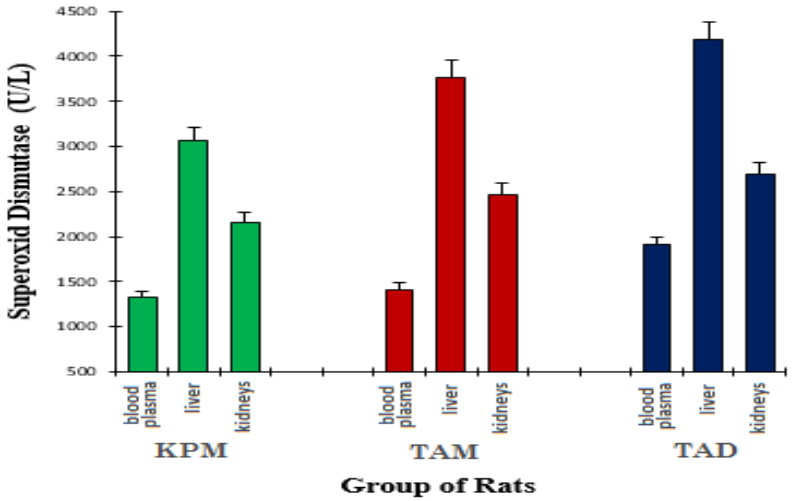

Table 1 shows the results of a statistical examination of SOD activity in blood plasma, liver, and kidney.

Superoxide dismutase activity - statistical analysis				
Compared groups			**Results**	
KPM b.plasma	vs	TAM b.plasma	p > 0,05	Ns
KPM b.plasma	vs	TAD b.plasma	p < 0,01	**
TAM b.plasma	vs	TAD b.plasma	p < 0,01	**
KPM liver	vs	TAM liver	p < 0,01	**
KPM liver	vs	TAD liver	p < 0,001	***
TAM liver	vs	TAD liver	p < 0,01	**
KPM kidney	vs	TAM kidney	p > 0,05	Ns
KPM kidney	vs	TAD kidney	p > 0,05	Ns
TAM kidney	vs	TAD kidney	p > 0,05	Ns

Treatment given to each group during the hyperthermic exposure period resulted in a significant difference ($p<0.001$) in SOD enzyme activity in blood plasma and liver in both groups. In the CPM and TAD groups, acute hyperthermic exposure on day 21 shows a statistically significant difference ($p<0.01$) in blood plasma compared to the CPM and TAM groups, where there is no statistically significant change ($p> 0.05$). Acute hyperthermic exposure on day 21 for SOD activity in the CPM and TAM groups for liver has a statistically significant difference ($p<0.001$); there is also a significant difference ($p<0.001$) between the groups CPM and TAD, and especially in the group treated with ionized water with added glutathione and vitamin C has increased SOD activity in the liver. SOD activity in the kidney is reduced after hyperthermic exposure. Acute hyperthermic exposure on day 21 did not create a significant difference in renal SOD activity in the CPM and TAM groups ($p> 0.05$); similarly, SOD activity in the kidney did not generate a significant difference in the TAM and TAD groups ($p> 0.05$).

5. DISCUSSION

The liver is the main source of GSH, as it is only capable of synthesizing cysteine, the precursor of GSH, from endogenous sources via the transulfurization process or obtaining it from protein and dietary breakdown. GSH availability in hepatocytes is determined by cysteine, cystine, and methionine membrane transfer. In fact, methionine insufficiency has been linked to a reduction in GSH availability, particularly in the liver. As a result, by exporting almost all of the produced GSH to plasma and bile, the liver plays a critical role in maintaining interorganic GSH homeostasis; in fact, the liver has the largest potential for GSH efflux through its basolateral and apical poles. As a result, changes in the ability of the liver to synthesis or export GSH have an impact on systemic GSH homeostasis (Vairetti, M. et al., 2021). During heat stress, erythrocytes may be a major source of circulatory GSH, according to our hypothesis (de Melo-Marins, D., et al., 2021). In the body, ionized water works as a strong antioxidant with SOD-like activity. The processes by which ERW functions as an antioxidant enzyme to neutralize ROS are assumed to be complex. Hydrogen molecules and active hydrogen could be new redox regulatory factors that drive antioxidant enzyme gene expression. Metal nanoparticles that are H-donors can catalyze the conversion of H2 molecules to active hydrogen, contributing to the ERW's high reductivity (Shirahata, S., et al., 2012). The findings of Franceschelli (Franceschelli, S., et al. 2016) are congruent with those of Shirahata, who found that ERW may neutralize ROS in a manner comparable to that of SOD enzymes. Our findings suggest that the values are similar. We believe that because of the antioxidant qualities of ERW as an enzyme with similar activity to SOD, we discovered greater SOD activity in the TAM group on the 7th, 14th, and 21st days compared to the control group. In the absence of hyperthermic exposure, the therapy given to each group had no effect on raising or lowering SOD enzyme activity. Animals exposed to high ambient temperatures, on the other hand, showed a substantial difference in SOD activity in all three groups compared to the period when it was absent (Graph 1). Acute hyperthermic exposure on day 21 significantly (<p 0.001) reduced SOD activity in the CPM and TAM groups compared to data obtained for the same enzyme activity on days 14 and 7 of treatment. The group treated with enriched ionized showed a modest difference in SOD activity between the 7th and 21st days, as opposed to a statistically significant difference ($p<0.01$) in the same group only between the 14th and 21st days of treatment (Table 1). The findings of the analyses performed on the seventh day of the study show statistically negligible changes in SOD activity when comparing two different combinations of the three groups that were included in the study. The comparison of SOD

activity between the CPM - TAM groups on the 14th day and CPM - TAM groups on the 21st day of therapy is identical. On the 14th and 21st days, an analogous comparison of the remaining group pairs reveals a statistically significant difference in SOD activity between the compared groups.

6. CONCLUSION

Acute hyperthermia increases the generation of oxidative stress markers while decreasing the activity of antioxidant enzymes. High temperatures cause an increase in ROS production, which causes oxidative damage. The treatment given to each group during the hyperthermic exposure period induced a substantial difference in SOD enzyme activity in the blood plasma and liver in both the CPM and TAD groups. The effect of acute hyperthermia on renal SOD activity in the CPM and TAM groups on day 21 was not significant.

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