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THE ALTERATIONS OF THE ENZYMATIC ANTIOXYDANT ACTIVITY BY ADDING ALKALINE WATER ON THE WHITE LABORATORY RATS AFTER HYPERTHERMIC STRESS

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Abstract

Alkaline water is in the focus of scientific interest over the last decade, due to its ability to alkalize the organism as well as its antioxidant effect. Due the hyperthermia, systemic and cellular changes occurs. Adding alkaline water (AW) and alkaline water with glutathione and vitamin C (Vit C) is expected the organism to act preventively to hyperthermic stress (HS). The aim of our research is to determine the influence of AW by adding enzymatic and non-enzymatic antioxidants, glutathione and Vit C during the HS. This treatment led to preventive action of the body from the influence of HS. All experiments were performed on female Wistar rats, divided into three groups of 15 individuals. Oxidative stress was caused by acute hyperthermic exposure. The first group is a control group, the second group is treated with AW, and the third group AW with added glutathione and Vit C. The duration of treatment lasted 21 days. In all rats, the body temperature (BT) was measured with a thermometer from 0-80 minutes, during exposure with a high ambient temperature of up to 41 ° C. The first group has a higher elevated BT of the other two groups. In the second group we have a BT increase, but with a lower value of the first group for 0.6 ° C and slightly higher than the third group for 0.4 ° C. In the third group we also have elevated BT values, but with lower values of the first group for 0.8 ° C and from the second group with lower values of BT for 0.4 ° C. Groups of rats treated AW and AW with added glutathione and Vit C react with slightly lower values of risen BT than the control group. We have received data that AW, glutathione and Vit C have an impact on the organism in the emergence of resistance to HS.

Keywords: Alkaline water, glutathione, hyperthermic stress, vitamin C, Wistar rats.

INTRODUCTION

Alkaline water often referred to as electrolyzed-reduced water (ERW) have been shown to exert a suppressive effect on free radical levels in living organisms, thereby resulting in disease prevention (Hanoka *et al.*, 2004).Various biological effects, such as antidiabetic and antioxidant actions (Jin *et al.,* 2006), DNA protecting effects (Shiharata *et al.,* 1997), and growth-stimulation activities (Watanabe *et al.,* 1995), were documented.Clinical data suggest that ERW improves the condition of diseases associated with oxidative stress such as cancer, diabetes, atherosclerosis and neurodegenerative diseases (Hayashi *et al.,* 2006), and it has also been proven that ERW neutralizes ROS and inhibits ROS-induced DNA damage *in vitro* (Shirahat *et al.,* 1997). Oxidative stress results with increased production of free radicals and reactive oxygen radicals, as well as reduction of antioxidant defenses, which in turn leads to damage to biological macromolecules and disturbance of normal metabolism and physiology

(Trevisan *et al.,* 2001). Oxidative stress usually occurs when free radicals are produced faster than can be neutralized by antioxidant mechanisms (Sies *et al.*, 1991). Increasing the temperature in the environment in which the organisms stay, and which leads to metabolic activation combined with elevated oxygen consumption, initiates the state of the so-called oxidative stress (Halliwel *et al.,* 1989). Even poor oxidative stress can have serious destructive effects and cause modification of many cellular functions that can end with cell death. In response to these problems, cells exposed to heat shock increase antioxidant defenses, particularly in the activity of antioxidant enzymes (Hermes *et al.*, 2004). Free oxygen radicals that are toxic to the cell can be produced in hyperthermia. After exposure to high temperatures, the levels of superoxide anions, hydrogen peroxide and nitrogen oxides increase, as well as an increase of the content of lipid peroxidation products in different cells, including tumor cells (Gorman *et al.*, 1999 – Matsumoto *et al.,* 1999). In conditions of hyperthermia, the formation of free radicals increases significantly (Hall *et al*., 1994).The different cells and tissues have varying degrees of metabolic activity and are characterized by different oxygen consumption. The levels of their antioxidants are also different, for example, glutathione and cystein are less common in the brain than in the liver, kidneys or muscle tissue. Examination of oxidative responses in different in vivo models suggests that complex organisms, such as mammals, tissues and organs, possess special antioxidant systems and this can be the basis for a varying degree of susceptibility to toxic influences on the external environment. Defensive mechanisms targeted against oxidative damage mediated with the action of free radicals, include: catalytic removal of free radicals and reactive forms helped by enzymes such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and thiol-specific antioxidants such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and thiol-specific antioxidants; binding of proteins (e.g., transferrin, metallothionin, haptoglobin, ceruloplasmin) for prooxidative metal ions such as iron and copper; protection from damage to macromolecules with the help of "stress" or "heat shock" proteins and reduction of free radicals by electron donors such as GSH, vitamin E (α-tocopherol), vitamin C (ascorbic acid), bilirubin and uric acid (Halliwell *et al.*, 1999).Among intracellular antioxidant molecules, reduced glutathione (GSH) is the most abundant intracellular non-protein thiol in cells. By keeping the cellular environment in a reduced state, GSH functions in the removal of potentially toxic electrophiles and metals, thereby protecting cells from toxic oxygen products (Anderson *et al.,* 1998). Furthermore, GSH exhibits a large panel of actions in controlling gene expression, apoptosis mechanisms, or membrane transport (Hammond *et al.*, 2001). Vitamin C is a powerful non-enzymatic antioxidant, capable of reacting with a wide range of biological oxidants, inhibits the formation of lipid and protein peroxidation and consequent DNA damage (Fraga *et al.,* 1991).

MATERIALS AND METHODS

Experimental model

The experiment was carried out on white laboratory rats of Wistar, a female sex, weighing 150-200 grams, which during the research were exposed to the standard food and water available *ad libidum* and stayed in a room after 12 hours of constant light regime and 12 hours dark regime, at a thermo-neutral temperature of 22 ˚C. The experiment lasted 21 days.

Experimental protocol

1. The first group of animals - who during the entire experimental period were under the above conditions and hereinafter referred to as the control group that does not receive any treatment
2. A second group of animals - who during the entire experimental period were under the above conditions and were treated intragastrically with alkaline water
3. Third group of animals - grown under the same experimental conditions and treated intragastrically with alkaline water enriched with glutathione and vitamin C

During the 21-day period, each animal from the three groups, on the 7th and 14th day, received 1.5 ml of blood from the tail for analysis of the parameters. On the last 21-st day during the hyperthermic exposure, the rats were individually placed in heated climate chambers, with maintained constant temperature of 41 ˚C and relative humidity of 40-50% air. During hyperthermic exposure, the rectal temperature of the animals was monitored using an electric thermometer every 10 minutes until the temperature of the chamber was reached, and afterward the monitoring was in every 2 minutes.

RESULTS

From the obtained results, we established statistical changes in BT in the three experimental groups. The changes were observed from 0-80 minute where the first group had a mean value BT of 37°C, the second group BT of 36.7°C and in the third group BT of 36.4°C. During the other measurements at different time intervals, BT was continuously varied between the three groups. The first group has a higher elevated BT of the other two groups. In the second group we have a BT increase, but with a lower value of the first group for 0.6 ° C and slightly higher than the third group for 0.4 ° C. In the third group we also have elevated BT values, but with lower values of the first group for 0.8 ° C and from the second group with lower values of BT for 0.4 ° C.

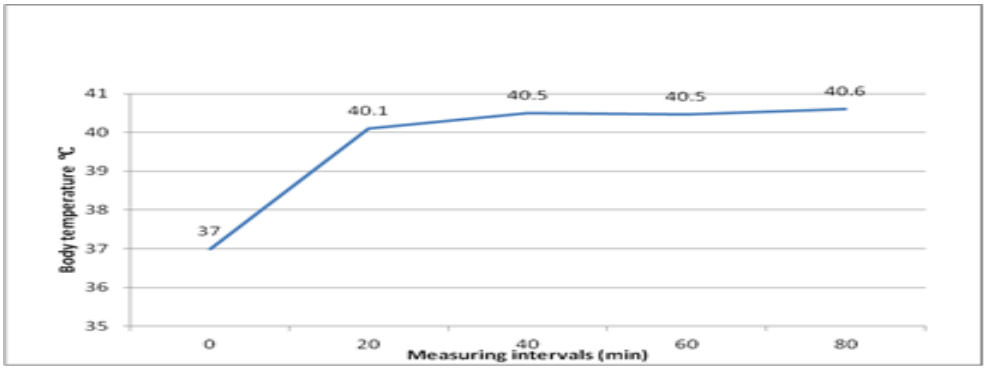

Figure 1. Change in body temperature when exposed to ambient temperature of 40°C - I group

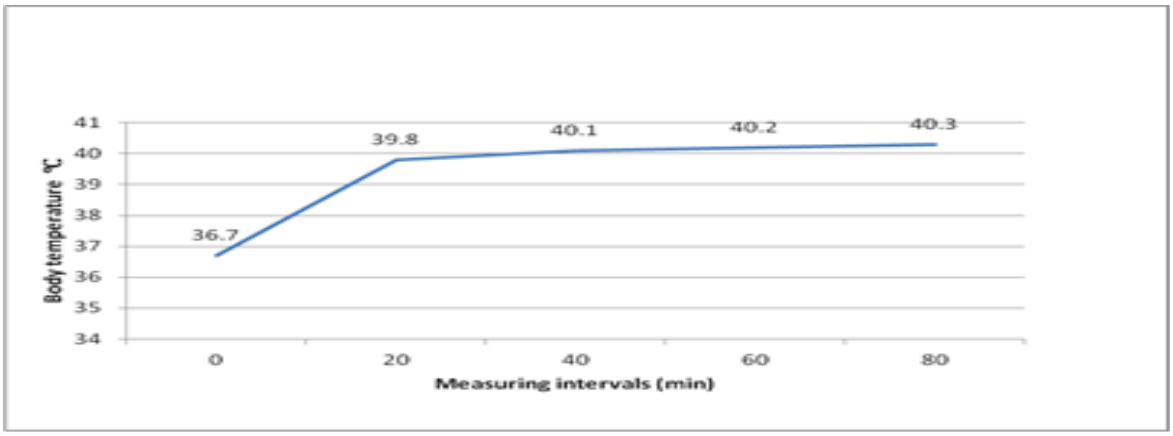

Figure 2. Change in body temperature when exposed to ambient temperature of 40°C - II group

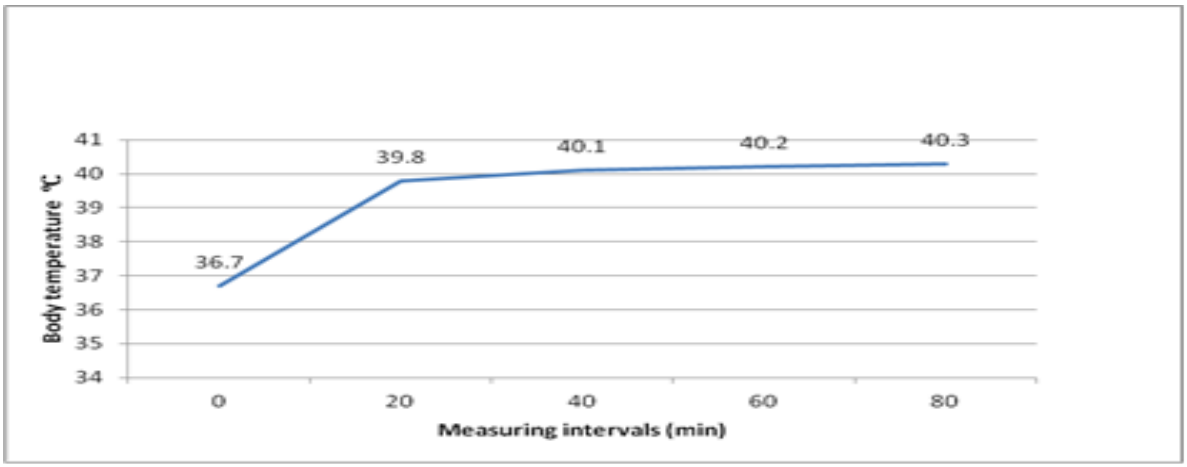

Figure 3. Change in body temperature when exposed to ambient temperature of 40°C - III group

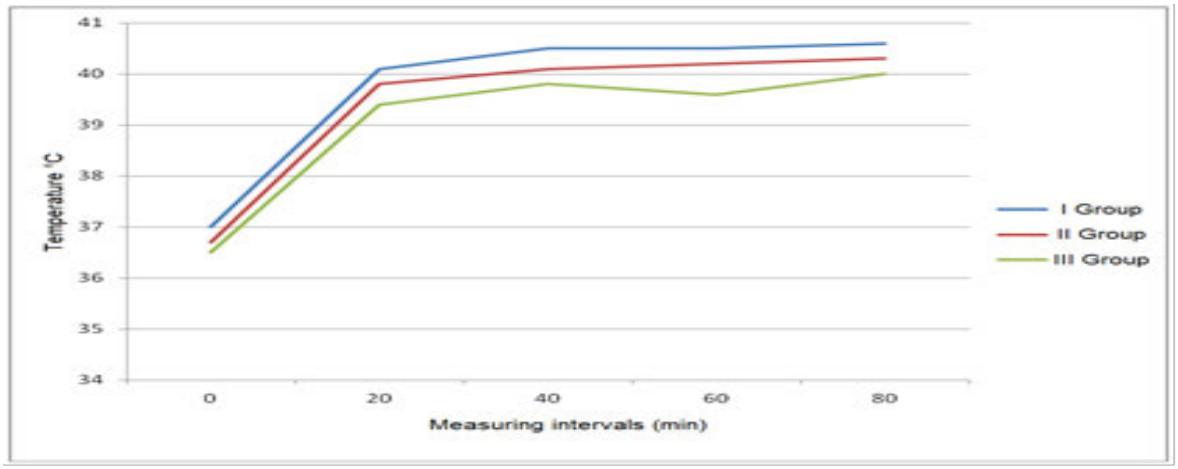

Figure 4. The temperature comparable in all three groups

DISCUSSION

In this present study, we evaluated the potential protective effects of AW and AW with added glutathione and Vit C against hyperthermic stress caused by hyperthermia in the female rats. Electrochemical activated water (reduced, alkaline water) is among natural agents that, among other effects, can enhance antioxidant defense of the organism. Electrochemically Reduced Water (ERW) is produced near the cathode and electrochemical oxidized water is obtained near the anode. The electrolysis of water creates strong reducing conditions near the cathode due to the production of hydrogen atoms (active hydrogen) and molecular hydrogen (Shirahata *et al.,* 2012).Active hydrogen in ERW can be considered an ideal 'scrubber' against ROS because it does not produce oxidized molecules after reduction, as is the case with organic antioxidants (vitamin C, vitamin E and polyphenols) (Li J *et al.,* 2003). As a result of exposure of rats to a high ambient temperature, a thermal gradient is created which results in the possibility of heat from the environment to absorb animals until the body temperature is equalized to the ambient temperature. The animals begin to increase their body temperature entering the state of hyperthermia. Additionally, higher body temperature leads to higher activity of the enzymes and intensification of metabolism resulting in produced metabolic heat that contributes to an additional elevation in body temperature. Heat stress is the initial state of hyperthermia, and in the event of a thermoregulatory collapse, conditions for progression towards heat shock are created, a condition in which the body temperature reaches over 40 ° C and ends with a multi-organ dysfunction syndrome. Exposure to acute temperature stress leads to the initiation of thermoregulatory mechanisms in rats in order to maintain temperature homeostasis. These adaptive responses to exposure to high ambient temperature include cellular and systemic responses. Changes in the cardiovascular, endocrine and nervous system are systemic responses that occur in all mammals at the beginning of hyperthermic exposure (Hutter *et al.,* 1996). It can be concluded that hypoxia, induced by acute temperature stress, leads to oxidative stress in the organism through the torsional mechanisms. It is interesting to point out that both reduced and increased cellular availability of O2 lead to the production of ROS (Maltepe *et al.,* 2009).The cell's antioxidative status is significant in hyperthermal 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 for better protection against ROS induced damage. A number of studies indicate the fact 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 needed to induce these enzymes (Wei *et al.,* 2002, Gechev *et al.,* 2002). Hydrogen molecules and active hydrogen can be new redox regulatory factors that can induce gene expression of antioxidant enzymes. H2 molecules can be converted to an active hydrogen with the catalytic action of metal nanoparticles that represent H - donors and contribute to the powerful ERW reduction (Shirahat *et al.*, 2012) Glutathione (GSH) reacts with other antioxidants (vitamin C, different nucleotides, etc.) to regenerate it. Thus, it regenerates the molecules that are important for improving the general immunological state of the organism. It is one of the most important molecules involved in reparation, expression, and synthesis of DNA, and it is responsible for the development of all other cells, hence glutathione indirectly affects the health of all cells. GSH

effectively inactivates free radicals directly or indirectly through enzymatic reactions. GSH is particularly important in mitochondria where catalase is absent and is critical in the defense of physiologically and pathologically generated oxidative stress (Fernandez *et al.*, 1997, Garcia *et al.*, 2006). Antioxidant properties of vitamin C are due to its ability to separate two electrons and therefore is a strong reducing agent. It acts as a cofactor of enzymes that catalyze biosynthetic pathways for the synthesis of collagen, carnitine and neurotransmitters (Arrigoni *et al.,* 2002), and also contributes to the synthesis of NO in the vascular endothelial cells and thus mediates vasodilatation (Nishikimi *et al.,* 1994).Vitamin C is a powerful non-enzymatic antioxidant, capable of reacting with a wide range of biological oxidants, inhibits the formation of lipid and protein peroxidation and consequent DNA damage (Fraga *et al.*, 1991).

CONCLUSIONS

Hyperthermic stress leads to increased production of oxidative stress indicators, and to the reduction of the activity of antioxidant enzymes. The exposure to high temperature leads to increased production of ROS, resulting in oxidative injuries

Groups of rats treated AW and AW with added glutathione and Vit C react with slightly lower values of risen BT than the control group. We have received data that AW, glutathione and Vit C have an impact on the organism in the emergence of resistance to HS.

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