UDC: 616.153.495.9:[616-008.82:546.21
Professional paper

THE IMPACT OF IONIZED WATER COMBINED WITH ADDITIONAL NON-ENZYMATIC ANTIOXIDANTS ON THE CONCENTRATION OF CREATININE IN RATS' SERUM DURING HYPERTHERMIC STRESS

Majlinda ADEMI[1]

1 Faculty of Medical Sciences, Study Program of General Medicine, University of Tetovo, Republic of N. Macedonia
**Corresponding Author: email: majlinda.ademi@unite.edu.mk*

Abstract

One of the most important factors that could promote the production of reactive oxygen species (ROS) is hyperthermic stress. The cellular redox state has been found to be negatively impacted by elevated levels of reactive oxygen species (ROS). Ionized water or Electrolyzed Reduced Water (ERW) has been proven in numerous studies to have the ability to scavenge free radicals that are formed by hydrogen molecules with a substantial reducing capacity and may be involved in the regulation of cellular redox. The goal of our study was to see the impact of ionized water on the concentration of creatinine in rats' serum during hyperthermic stress by adding other non-enzymatic antioxidants, glutathione, and vitamin C. White Wistar laboratory rats, female, weighing 180–220 g, young rats, separated into three groups of 15, were used for the experiment. Oxidative stress was induced by acute hyperthermic exposure to 41˚C. The first group was a control group (CPM) treated with natural water, the second group was treated with ionized water (TAM), and the third group was treated with ionized water with added glutathione and vitamin C (TAD). The duration of treatment lasted 21 days. Acute heat stress, except that it results in oxidative stress, conditions accelerated catabolic reactions in the body. The higher concentration of urea and creatinine in the period of hyperthermic exposure is due to the intensified breakdown of proteins. In such conditions, urea and creatinine concentrations do not represent a consequence of the applied treatment.

Keywords: Ionized water, hyperthermic stress, glutathione, vitamin C, creatinine

1. Introduction

One of the most significant elements that can increase the generation of reactive oxygen species (ROS) is heat stress (Li et al., 2017). The generation of ROS is induced by heat stress, and their excessive accumulation inhibits cell viability and proliferation and triggers apoptosis. Additionally, high ROS production brought on by heat stress decreases the effectiveness of antioxidant defense mechanisms, increasing oxidative damage. Heat stress is a physical stressor that may induce structural changes in proteins, leading to cell death (Ibtisham et al., 2018). Antioxidants are compounds that can shield cells from the negative effects of prescription medications, xenobiotics, carcinogens, and harmful radical molecules. It has been suggested that a number of natural substances have antioxidant properties. Electrolyzed reduced water (ERW) is one of these antioxidants (Franceschelli et al., 2016). Our data is in accordance with in vitro research experiments by Shirahata and his group (Shirahata et al., 2018), which made it evident that ERW neutralizes ROS, that is, a very similar process to the action of SOD and CAT enzymes (Shirahata et al., 2012). In essence, the H2 dissolved in ERW behaves as an antioxidant, scavenging free radicals. This is crucial when taking into account the fact that oxidative stress plays a role in the emergence of numerous inflammatory conditions (Franceschelli et al., 2016). Because the oxygen concentration in constricted muscles is higher during heating exposure, various organs, including the heart, kidneys, and other organs, experience hypoxia, and ischemia. This interferes with metabolism, and cell homeostasis also harms the tissue. The blood quickly returns to the kidneys after being exposed to heat. Furthermore, a significant amount of an oxidant is generated, which might harm kidney cells. The kidneys will suffer serious damage

as a result. Serum creatinine and urea levels can be used to clinically assess the progression of kidney disease. Naturally, the organism has a defense mechanism against ROS through an endogenous antioxidant system that consists of superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase (CAT). This enzyme is crucial as the first line of defense against the negative effects of ROS produced by numerous sources. However, endogenous antioxidants' abilities will be reduced when ROS production is significant. Exogenous antioxidant supplementation is therefore crucial to safeguard cells from the harmful effects of ROS. Those exogenous sources are non-enzymatic antioxidants such as ionized water, glutathione, and Vitamin C (Sinaga et al., 2019). The most typical cause of acute kidney injury (AKI) during heta exposure is hypoperfusion, which is caused by reduced blood flow to the kidneys. AKI is characterized by a loss of renal function that progresses quickly and causes harmful toxins of nitrogen metabolism and creatinine to build up in the blood. Due to the energy-intensive nature of solute reabsorption, renal tubule cells have an abundance of mitochondria. This makes kidney cells especially vulnerable to oxidative stress and damage (Gyuraszova et al., 2020). AKI has been described as a sudden (hours to days) reduction in kidney function (e.g., GFR), along with rising plasma concentrations of nitrogen metabolism products (e.g., creatinine, urea), which may also be accompanied by a decrease in urine output. (Chapman et al., 2021). According to previous studies, ERW shows antioxidative properties upon consumption due to the presence of H2 and negative oxidation-reduction potential ORP values, which protect the body from OS caused by free radicals. According to a different study, ERW increased ascorbic acid's antioxidant properties and prevented the oxidative cleavage of proteins. In signaling pathways and the defense against oxidative damage, glutathione (GSH) is crucial. Cellular redox status and functionality are indicated by the concentration of GSH, its oxidative state (GSSG), and it’s molar ratio. The most significant antioxidant in the human body is glutathione (Grucza et al., 2019). The liver is the primary source of GSH and has the extraordinary capacity to produce cysteine, a precursor to GSH, from endogenous sources using the trans-sulphuration process (Varietti et al., 2021). For many years, vitamin C has been known. The data so far show that it has cellular impacts in multiple directions. 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, et al., 2020).

Our study's goals were based on the idea that consuming ionized or electrochemically reduced water (ERW) would increase one's body's alkaline reserve. This capability of ERW was revealed in the context of organism stress caused by exposure to high external temperatures. Our assumptions were based on the concept that ERW will boost the body's thermotolerance to kidneys by acting as an antioxidant.

2. Material and Methods

2.1. Experimental model: An experimental paradigm was employed to apply the 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 the 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 15 animals in the experimental groups were labeled and categorized as follows:

1. The first group of animals (CPM) - was 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) - was raised under the same experimental circumstances and given ionized water with glutathione and vitamin C supplementation.

2.2. 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. The control group received only natural water within the specified time period. 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 the 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 During hyperthermic exposure, and the rectal temperature was also recorded in 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.

2.3. Analytical statistics: The experiment's results were statistically processed using the statistical program InStat. The data are 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.

2.4. Determination of creatinine concentration:

Principle of the method

Creatinine reacts with alkaline picrate, forming an orange-red complex that absorbs light at 492 nm. The intensity of the staining is proportional to the concentration of creatinine in the sample.

Test procedure

Mix 1 volume of 35 mmol/L picric acids with 1 volume of 0.32 mol/L sodium hydroxide. A series of dilutions are made from the standard solution in order to form a standard curve. It is pipetted according to the following scheme:

	Standard	Analysis
Working solution	1000 µl	1000 µl
Standard solution	100 µl	-
Sample	-	100 µl

For a blank test, a reading is taken against distilled water. After placing the reagents in the cuvette, mix and read the absorbance at 492 nm exactly after 20 seconds (A1). After 80 seconds from the first reading, A2 is measured.

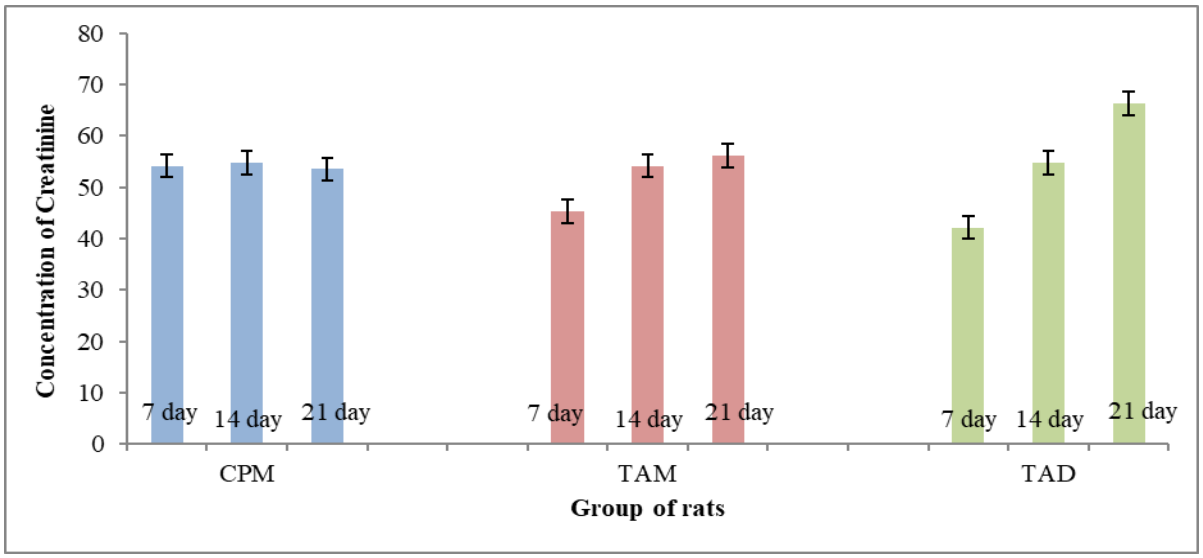
Calculation

ΔA = A1 – A2 is calculated

ΔA sample / ΔA standard x C standard = C sample

3. Results

The results obtained from our research on the influence of treatment with ionized water, without and with the addition of the appropriate antioxidants to it, as well as the acute hyperthermic exposure introduced on the 21st day of treatment on the creatinine level in blood serum are shown in Graph 1.



Graph 1. The concentration of creatinine in blood serum

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 statistical analysis of data on the concentration of creatinine in serum

Statistical analysis – concentration of creatinine in the blood serum				
Compared groups			Results	
CPM 7	vs	CPM 14	p > 0,05	Ns
CPM 7	vs	CPM 21	p > 0,05	Ns
CPM 14	vs	CPM 21	p > 0,05	Ns
TAM 7	vs	TAM 14	p > 0,05	Ns
TAM 7	vs	TAM 21	p > 0,05	Ns
TAM 14	vs	TAM 21	p > 0,05	Ns
TAD 7	vs	TAD 14	p > 0,05	Ns
TAD 7	vs	TAD 21	p > 0,01	**
TAD 14	vs	TAD 21	p > 0,05	*
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	*
CPM 14	vs	TAD 14	p > 0,05	Ns
TAM 14	vs	TAD 14	p > 0,05	Ns
CPM 21	vs	TAM 21	p > 0,05	Ns
CPM 21	vs	TAD 21	p > 0,05	Ns
TAM 21	vs	TAD 21	p > 0,05	Ns

The control group in terms of the time period of the research did not show a significant difference in terms of creatinine concentration. The two treated groups, TAM and TAD, manifested an increasing trend in the concentration of the aforementioned parameter, in relation to the treatment time, both in the period of absence and in the presence of the introduced hyperthermic stress. A statistically significant difference is observed in the TAM group between the 7th day as a starting point and the 14th and 21st days of treatment, while no significant difference was registered between the 14th and 21st days in the same group. A statistically significant difference with a value of $p < 0.001$ was found in the comparisons made within the TAD group between days of treatment taken for the analysis of all parameters. No significant difference in creatinine concentration between any two groups of the three groups included in the study was observed on day 14 of treatment. An identical case was registered in the concentration of the same analysis between the two treated groups on the 7th day, as well as between the KPM and TAM groups on the 21st day of treatment. The remaining possible comparisons between the given two groups on the 7th and 21st day of the study showed a statistically significant difference with values $p < 0.01$ and $p < 0.001$.

4. Discussion and Conclusion

One of the negative impacts of heat stress is a reduction of kidney function or acute kidney disease (Alayyannur et al., 2022). The most popular method for calculating glomerular filtration rate (GFR) is probably creatinine clearance. The non-enzymatic dehydration of muscle creatine results in a rather continuous release of creatinine into the blood. The majority of the body's creatine, which is generated in the liver, is kept in muscle and just 1.6% of it is converted to creatinine each day. Because creatinine is produced naturally and can be measured in the blood and urine using standard clinical procedures, using it as a marker of GFR has benefits (Chapman et al., 2021). Alkali or ERW has been shown to have a renoprotective impact in mouse studies, slowing renal functional decline or preventing structural abnormalities in a variety of kidney disease models. In contrast to the encouraging findings of previous single-center clinical trials, more recent multicenter, well-controlled clinical investigations have demonstrated no appreciable effect of alkali therapy to halt the course of kidney disease (O'Connor et al., 2022). Electrochemically activated water (reduced, alkaline water) is counted among the natural agents that can strengthen the body's antioxidant defense. A special type of electrolysis is one of the methods used to obtain functional water, which is activated water with the ability to perform specific and different functions compared to non-activated water. In the group of functional waters, electrolyzed water is the most studied. Electrochemically reduced water (ERW) is produced near the cathode and electrochemically oxidized water (EOW) is obtained near the anode (Shirahata et al., 2012). They suggest that the function of ERW similar to SOD and CAT is not due to dissolved molecular hydrogen, but to active atomic hydrogen, which possesses a higher reducing ability. 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.

These data from the relevant literature partially coincide with our experimental results. Creatinine is a degradation product of protein catabolism in the body. The intensified breakdown of proteins explains the statistically significant high concentration of creatinine on day 21 in the treated groups compared to the treatment period in the absence of hyperthermic stress. Acute hyperthermic exposure of experimental animals in relation to treatment with ionized water, with or without elements added to it, led to a significant difference in the concentration of urea compared to the period when the high temperature was not applied to them. The exception to this finding is the CPM group, in which the period of hyperthermic exposure did not lead to a significant difference in urea concentration. The two treated groups, TAM and TAD, showed a trend of increase in creatinine concentration, both in the absence and in the presence of the introduced

hyperthermic stress. A statistically significant difference is observed in the TAM group between the 7th day as a starting point and the 14th and 21st days of treatment, while no significant difference was registered between the 14th and 21st days in the same group. A statistically significant difference with a value of $p < 0.01$ was found in the comparisons made within the TAD group between days of treatment taken for the analysis of all parameters. On day 14 of treatment, no significant difference in creatinine concentration was observed between any two of the three study groups. Comparisons of the difference in the concentration of urea and creatinine made during the entire treatment in the control group did not show statistical significance. In conclusion, acute temperature stress, in addition to resulting in oxidative stress, conditions accelerated catabolic reactions in the body. The higher concentration of creatinine in the period of hyperthermic exposure is due to the intensified breakdown of proteins. In such conditions, creatinine concentration does not represent a consequence of the applied treatment.

References

[1]. Alayyannur, P. A. and Ramdhan, D. H. (2022) Relationship of heat stress with acute kidney disease and chronic kidney disease: A literature review. Journal of Public Health Research, Vol. 11(2), 1–7

[2]. Bajgai, J., Kim, Ch-S., Rahman Md. H., Jeong, E-S., Young Jang, H-Y., Kim, K-E., JaeHo Choi, J., Il-Young Cho, I-Y., Lee, K-J and Lee, M.(2022). Effects of Alkaline-Reduced Water on Gastrointestinal Diseases. Processes, 10, 87. https://doi.org/10.3390/pr10010087

[3]. Chapman, C. L., Johnson, B. D., Parker, M. D., Hostler, D., Pryor, R. R. and Schlade, Z. (2021). Kidney physiology and pathophysiology during heat stress and the modification by exercise, dehydration, heat acclimation, and aging. Temperature, vol. 8, no. 2, 108–159

[4]. Franceschelli, S., Pia Gatta, D. M., Pesce, M., Ferrone, A., Patruno, A., de Lutiis, M. A., Grilli, A., Felaco, M., Croce, F. and Speranza, L. (2016). New Approach in Translational Medicine: Effects of Electrolyzed Reduced Water (ERW) on NF-κB/iNOS Pathway in U937 Cell Line under Altered Redox State. Int. J. Mol. Sci. 2016, 17, 1461

[5]. Grucza, K., Chołbiński, C., Kwiatkowska, D. and Szutowski, M. (2019). Effects of Supplementation with Glutathione and its Precursors on Athlete Performance. Biomed J Sci & Tech Res Vol.12 (4).

[6]. Gyuraszova, M., Gurecka, R., 2 Babíčkova, J. and Tothova, L. (2020) Oxidative Stress in the Pathophysiology of Kidney Disease: Implications for Noninvasive Monitoring and Identification of Biomarkers Oxidative Medicine and Cellular. Longevity, Article ID 5478708, 11 pages.

[7]. Ibtisham ,F., Zhao, Y., Nawab, A., Liguang, H., Wu, J., Xiao, M., Zhao, Z. and An, L. (2018). The Effect of High Temperature on Viability, Proliferation, Apoptosis and Anti-oxidant Status of Chicken Embryonic Fibroblast Cells. Brazilian Journal of Poultry Science Vol. .20 (3) 463-470

[8]. Kazmierczak-Baranska, J., Boguszewska, K., Adamus-Grabicka, A. and Bolesław T. Karwowski, B. T. (2020). Two Faces of Vitamin C—Antioxidative and Pro-Oxidative Agent. Nutrients, 12, 1501; doi:10.3390/nu12051501

[9]. Li L, Tan H, Yang H, et al. (2017) Reactive oxygen species mediate heat stress-induced apoptosis via ERK dephosphorylation and Bcl-2 ubiquitination in human umbilical vein endothelial cells. Oncotarget 8(8): 12902–12916.

[10]. O'Connor, P. M., and Mannon, E. C. (2022). New mechanisms for the kidney-protective effect of alkali in chronic kidney disease. Clinical Science; 136 1433–1437

[11]. Shirahata, S., Hamasaki, T., Teruya, K. (2012). Advanced research on the health benefit of reduced water. Trends in Food Science & Technology, 23(2), 124–131

[12]. Shirahata, S., Hamasaki, T.,Teruya, K. (2018). Newest Research on the Health Benefit of Electrochemically Reduced Water. Department of Bioscience and Biotechnology, Faculty of Agriculture, Kyushu.

[13]. Sinaga, F. A., Harahap, U., Silalahi, J. and Sipahutar, H. (2019) Antioxidant Effect of Virgin Coconut Oil on Urea and Creatinine Levels on Maximum Physical Activity. Open Access Macedonian Journal of Medical Sciences; 7(22):3781-3785. https://doi.org/10.3889/oamjms .2019.503

[14]. Vairetti, M., Di Pasqua, L. G., Cagna, M., Richelmi, P., Andrea Ferrigno, A. and Berardo, C. (2021). Changes in Glutathione Content in Liver Diseases: An Update. Antioxidants: 10, 364