

Effect of Zinc supplementation on acute and chronic heat stress induced antioxidant changes in Wistar albino rats

Madhuri BA¹, Anthony David M²

¹Assistant Professor, ²Professor & HOD, Department of Physiology, RVM Institute of Medical Sciences and Research Center, Mulugu, Laxmakkapally, Siddipet, Telangana State.

Abstract

Heat stress is a major health burden with potential health outcomes. Oxidative stress is a metabolic dysfunction that carries oxidative damage to cells and tissues. Zinc is a co-factor against oxidative stress. The present study was done to evaluate the Supplementation of zinc in acute and chronic heat stress on antioxidant changes in Wistar albino rats. **Methodology:** 30 Wistar albino rats were selected for the study. They were divided into five groups. Group 1 Control, Group 2 Acute heat stress, Group 3 Acute heat stress + pretreated with zinc, Group 4 Chronic stress, Group 5 Chronic stress + pretreated with zinc. Zinc in the form of zinc sulfate was administered orally at a dose of 50µgm/kg of body weight. The enzymatic and nonenzymatic activity were estimated in serum. **Results:** After acute and chronic heat stress, i), there was a significant increase ($P < 0.001$) in lipid peroxidation activity. ii) Enzymatic and Non-enzymatic antioxidants were reduced ($P < 0.001$) iii) Rats pretreated with zinc showed a decrease ($P < 0.001$) in lipid peroxidation and increase ($P < 0.001$) in enzymatic antioxidants ($P < 0.001$) and Non-enzymatic antioxidants. **Conclusion:** Supplementation of zinc showed a significant effect on antioxidants in heat-stressed rats. The level of antioxidants of heat stress was attenuated by zinc, Zinc supplementation to heat-stressed rats, stabilized the increased activities of antioxidative enzymes. So Zinc could be a potential protective agent.

Keywords: Antioxidants; Heat stress; Wistar albino rats.

Introduction

Stress is a complex multidimensional phenomenon promoted by several noxious or unpredictable stimuli (stressors) that causes a physiological response aimed to maintain or to recover the body homeostasis [1]. Heat stress causes physiological and psychological discomforts, deteriorates performance and productivity, increases incident rates, and even threatens survival [2]. Animals can dissipate heat to the surrounding environment. If they are subjected to greater heat, then they are set to suffer from heat stress. This is a stressor and elicits a response from the animal. To elicit a distress response, a stressor must be perceived as a critical factor by the animal, causing deleterious effects.

Two significant types of heat stress, “acute heat stress” and “chronic heat stress” [3], have classified as acute, sequential, episodic, chronically intermittent, sustained or anticipated. Once the central nervous system perceives a threat, it develops a response that consists of some combination of the four-general biological defense responses: behavioral, autonomic nervous system, neuroendocrine and immune [4].

Micronutrient zinc is essential to all living organisms and participates in numerous biochemical pathways in human cells [5, 6]. Zinc is a nutritional trace mineral that is nontoxic, as was elucidated by experimental studies [7]. Zinc deficient led to a 30% loss of the body’s zinc. It was proved that zinc has a critical role in maintaining human health in terms of antioxidant stress

[6]. Zinc has a vital role in maintaining human health, especially in terms of anti-oxidative stress [8, 9]. Excessive production of reactive oxygen species (ROS) and a decrease in its removal by the antioxidant defense system causes a disequilibrium between oxidants and antioxidants [10-12]. When the production of free radicals is high, cells are unable to detoxify, causing an increase in oxidative damage to DNA, proteins and lipids. It is termed oxidative stress [13].

Materials and Methods

The present study was an experimental animal-based study. The study was carried out after the approval of the Institutional animal ethics committee. A total of 30 adult male Wistar rats weighing 150-180g were taken for this study. Rats showing abnormal behavior were excluded. The rats were maintained (3rats/cage) under 12h: 12h light and dark cycle and were provided with food and water *ad libitum*.

Methodology:

Grouping: The rats were divided into five groups (n=6 in each group)

Group 1: Control

Group 2: Acute heat stress

Group 3: Acute heat stress + pretreated with zinc

Group 4: Chronic heat stress rats

Group 5: Chronic heat stress + pretreated with zinc

Stress procedure: Control group rats were kept in a home cage without any disturbance.

Acute stress procedure: Acute stress group rats were exposed to biological oxygen demand incubator at 38°C for the duration of four hours for one single day between 08.00 AM to 12.00AM

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Correspondence: Dr. B.A. Madhuri, Assistant Professor, Professor & HOD, Department of Physiology, RVM Institute of Medical Sciences and Research Center, Siddipet, T.S. E-Mail: antonymadhuri@gmail.com

Table 1. Comparison of Control, Zinc Pretreated with Acute and Chronic Heat Stress rats in Wistar albino rats on antioxidant status.

Parameters	Control [n=6]	AHS [n=6]	AHS + Zn [n=6]	CHS [n=6]	CHS + Zn [n=6]
LPO					
nmoles of MDA/min/mg/ptn	84.98±4.25	110.5± 4.23	71.24±7.37	118.74±3.89	113.19±3.81
SOD					
(min/mg/ptn)	5.68±0.22	4.47±0.26	6.39±0.33	4.88±0.37	4.75±0.37
CAT					
(min/mg/ptn)	3.71±0.21	2.40±0.10	4.67±0.26	3.16±0.14	2.81 ± 0.5
GPx					
(min/mg/ptn)	6.8±0.14	5.17±0.06	7.45±0.26	5.18±0.18	5.56±0.09
Vitamin C					
(min/mg/ptn)	2.65±0.05	2.17±0.06	3.59±0.20	2.26±0.12	1.68±0.13
Vitamin E					
(min/mg/ptn)	2.46±0.63	2.28±0.06	3.8±0.18	2.2±0.15	1.37±0.29

Significance fixed at P<0.05* Highly significant P<0.001. Zn-Zinc, AHS- Acute heat stress, and CHS-Chronic heat stress.

*Control vs AHS, Control vs CHS, AHS+Zn/ CHS +Zn Vs respective control.

Chronic stress procedure: Chronic heat stress group rats were exposed to biological oxygen demand incubator at 38°C for the duration of one hour *i.e.*, 11.00 a.m to 12.00 p.m. for 21 days.

Zinc: Zinc in the form of zinc sulfate was freshly dissolved in distilled water and administered orally at a dose of 50 µgm/kg of body weight for 15 days pretreated in acute heat stress Wistar albino rats and 30 days pretreated with zinc in chronic heat stress Wistar albino rats. Zinc sulfate and other chemicals used for this study were purchased from Merck, India. (Sriram Enterprises, Chennai).

Antioxidant status estimation: After heat stress, blood samples were collected for estimation of antioxidant status. The enzymatic and nonenzymatic activity like Lipid peroxidase (LPO) [14], Superoxide dismutase (SOD) [15], Glutathione peroxidase (GPx) [16], Catalase [17], Vitamin C [18], Vitamin E [19].

Statistical analysis: All data were analyzed; data were expressed as the mean ± SE and by using students 't' test was used to test the significant difference between the mean values of different groups.

Results

After acute and chronic heat stress, there was a significant increase (P <0.001) in lipid peroxidation activity. Enzymatic and Non-enzymatic antioxidants were reduced (P < 0.001) Rats pretreated with zinc in acute and chronic heat stress showed a decrease (P< 0.001) in lipid peroxidation and increase (P < 0.001) in enzymatic antioxidants (P < 0.001) and Non-enzymatic antioxidants.

Discussion

Acute stress occurs consequent to a temporary negative situation, either physical, emotional or psychological, that generally allows quick and complete recovery of the physiological balance; thus, the final result is a complete adaptation [3]. Chronic stress is a state of ongoing physiological arousal [20]. This occurs when the body experiences so many stressors or repeated exposure to the same acute stress or continuous stress that the autonomic nervous system rarely has a chance to activate the relaxation response.

In heat stress, i) Lipid peroxidation increases yielding a wide range of cytotoxic products. ii) enzymatic activity decreases providing defense against oxidative stress. iii) Non-enzymatic antioxidants are elevated. Aldehydes, hydrocarbons and isoprostanes are lipid peroxidation products. The serum plasma and tissue levels of aldehydes, hydrocarbons and Isoprostenes and protein carbonyls are taken as an index of oxidative stress. There is a difference in the elevation of MDA between acute and chronic heat stress [21].

The severity and duration of heat stress decide how the antioxidant system and its associated system and it's associated react. After acute heat stress, antioxidant enzymes like catalase, glutathione peroxidase and superoxide dismutase have sharply increased activity as they protect the cells against excess superoxide formation. Results in case of chronic heat stress, do not indicate this. When the concentration of zinc increases, the concentration of MTF-1 also increases. This zinc dependent transcription factor causes the expression of two genes metallothioneine and zinc transporter gene. So, MTF -1 cells protect cells from oxidative stress [22].

It also causes the expression of the selenoprotein-1 (sepw1) gene. This gene encodes glutathione binding protein which is an antioxidant scavenging free radicals [23]. The deficiency of zinc causes oxidative damage as well as endothelial dysfunction. In the condition of stress, zinc is released from the metallothioneine complex. Zinc is also a prooxidant. Whenever it is deficient, are in excess. In such circumstances, zinc promotes inflammation and apoptosis [24].

Conclusion

Current evidence strongly suggests that heat stress induces oxidative stress. The supplementation of zinc showed a significant effect on antioxidants in heat-stressed rats. The level of antioxidants of heat stress was attenuated by zinc, Zinc supplementation to heat-stressed rats, stabilized the increased activities of antioxidative enzymes. So Zinc could be a potential protective agent. Therefore, further studies may elucidate the role of this nutrient in protecting against oxidative stress.

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