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Research Article

ROLE OF SELENIUM SUPPLEMENTATION ON ANTIOXIDANT CHANGES IN WISTAR ALBINO RATS AFTER CHRONIC RESTRAINT STRESS

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ABSTRACT

Objective: Stress is a non-specific response of the body to any physical or physiological demand. Oxidative stress may occur due to imbalance between pro-oxidants and antioxidants. Restraint stress or immobilization has been used extensively as a stressor for the study of stress-related biological, biochemical, and physiological responses in animals. Nutritional treatment by exogenous supplementation of antioxidants like selenium reactivates which guard against the insult caused ROS during the repeated restraint stress. The objective of the study is to determine the role of selenium on antioxidant changes in Wistar albino rats after chronic restraint stress.

Methods: Adult male Wistar albino rats weighing about 180–200 g were taken for the study and were divided into three groups – the control group (n=6) chronic restraint stress group (n=6) and chronic stress treated with selenium (n=6). Restraint stress was given in wire mesh restrainers for 30 days (6 h/day), and the blood from the jugular vein was collected for estimation of antioxidant status (Superoxide dismutase, Glutathione peroxidase, CAT, Vit C, and Vit E) in rats.

Results: One-way analysis of variance statistical test was used to analyses the mean and SD among the groups. The rats pre-treated with selenium ($p<0.001$) showed a significant decrease in lipid peroxidation. In chronic restraint stress, albino rats administered with Selenium showed a significant increase ($p<0.001$) in enzymatic and non-enzymatic antioxidant activity when compared to controls.

Conclusion: The effect of the Selenium acts as a antidote to counteract the effects of restraint stress and has significant therapeutic application in counter acting oxidative damage on Wistar albino rats.

Keywords: Selenium, Restraint stress, Antioxidants, Wistar albino rats.

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INTRODUCTION

Stress has become an important part of the human condition worldwide and every individual is likely to face stressful situations in day-to-day life which seriously agitates physiological and psychological homeostasis. Restraint stress triggers a physiological response by increasing the metabolic rate which translates into oxidative stress, resulting in the etiopathogenesis of many diseases [1].

Chronic restraint stress stimulates many mechanisms leading to an increase in the production of oxygen-free radicals. One of the reasons for the stress-induced development of free radicals is very reactive species with one unpaired electron. This large group of molecules is represented mainly by the superoxide radical, peroxyl radical, hydroxyl radical, and nitric oxide. All of these molecules and their derivatives, such as hydrogen peroxide or hypochlorous acid, are referred to as reactive oxygen species (ROS) [2].

Overproduction of ROS results in an imbalance of pro-and antioxidative processes, which creates a phenomenon known as oxidative stress. This kind of oxidative stress is involved in many neuropsychiatric disorders, such as Alzheimer's disease, Parkinson's disease, Schizophrenia, Bipolar Disorder, and Major Depressive Disorder. Several antioxidants such as selenium, vitamins, and minerals can modulate the state of oxidative stress [3].

Selenium is known to play an important role in restraint stress in rats. Accumulated evidence has indicated that much of the beneficial effect of selenium is attributed to its antioxidant nature. Selenium supplementation has been reported to have a positive effect. Earlier studies have shown that selenium administration treatment decreased cell death and improved cell viability from induced restraint stress [4]. The positive effect of selenium could have been mediated through lowering ROS production and thus preserving mitochondrial membrane potential and mitochondrial function performance. Therefore, selenium pretreatment reduced oxidative DNA damage [5].

Substances, which enhance endurance for physical and metabolic activities and increase nonspecific resistance to stress during a prolonged stay in psychologically adverse habitants, are called "adaptogens." Selenium is well known for its anti-stress and adaptogenic properties [6].

This study was taken to find the effects of restraint or immobilization stress on the biochemical changes of rats and also to find the effect of selenium acting as an antidote to counteract the effects of restraint stress on Wistar albino rats.

METHODS

Adult male Wistar rats (18) weighing 180–200 g were taken for this study. The Institutional Animal Ethical Committee approval was obtained. The rats were maintained (three rats/cage) under 12 h: 12 h light and dark cycle and were provided with food and water ad libitum. The rats were divided into three groups. Control group (n=6), chronic restraint stress group (n=6), and chronic stress treated with selenium (n=6). The control groups were kept in a home cage without any disturbance, whereas rats in the stress group were restrained for 6 h/day for 30 days by placing each rat in a wire mesh cage [7]. Selenium in the form of sodium selenite (Merck, Germany) was administrated at a dose of 0.5 mg/kg of body weight. The sodium selenium was freshly dissolved in distilled water and administrated orally for about

30 days on chronic restraint stress. After 30 days of restraint stress, the blood samples were collected from the jugular vein for estimating the antioxidant status of animals.

Procedure for estimating the antioxidant levels

Parameter studied

Biochemical estimation

Lipid peroxidase (LPO) (OhKawa *et al*., 1979): Melondialdehyde (MDA) a secondary product of LPO reacts with Thiobarbituric acid to form a pink chromogen which was measured spectrophotometrically at 532 nm and expressed as n moles of MDA formed/min/mg/protein in tissue samples [8].

Antioxidants status

Enzymatic

Superoxide dismutase (SOD) (Marklund and Marklund - 1974): Pyrogallol auto-oxidizes rapidly in an aqueous solution at a faster rate with higher PH (8.0) to produce several intermediate products. The inhibition of auto-oxidation brought about by the addition of enzyme is evaluated at an early stage as an increase in absorbance at 420 nm and expressed as units/min/mg of tissue protein [9].

Glutathione peroxidase (GPx) (Rotruck et al., 1973): GBH is converted to GSSG in presence of GPx and the color developed was read at 412 nm using the spec and expressed as glutathione oxidized/min/mg/protein [10].

Catalase (Sinha 1972): Dichromate in acetic acid is reduced to chromic acetate when heated in the presence of H_2O_2 resulting in the formation of per chromic acid as an unstable intermediate. The chromic acetate was measured using spec at 570 nm and expressed as µmoles of H_2O_2 utilized/min/mg/protein [11].

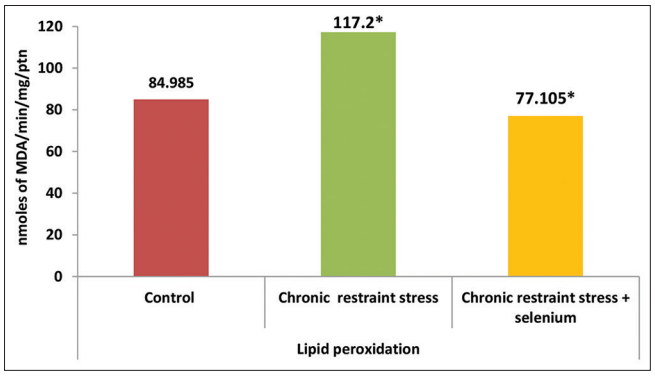

Fig. 1: Comparison of control, chronic restraint stress, and chronic restraint stress and selenium treated on LPO in Wistar albino rats

Non-enzymatic

Vit C (Omaye et al., 1979): Ascorbic acid is oxidized by Cu to form dehydroascorbic acid, and diketogluconic acid when with DNPH forms a derivative bis 2,4-dinitrophenyl hydrazone when combined with H2SO4 forms a yellowish orange produced which was measured at 520 nm and expressed as µg/mg protein [12].

Vit E (Desai 1984): Tocopherol in liver tissue extracted into Xylene and its content was measured using Emmeric-Eegelreaction. This reaction is based on the reduction of Fe3+ to Fe2+ in the presence of α, α1 dipyridyl forming red color and absorbance read at 530 nm and expressed as µg/mg/protein [13].

RESULTS

All data were analyzed and expressed as the mean±SE and using one-way analysis of variance was used to test the significant difference between the mean values of different groups.

In chronic restraint stress, there was a significant increase in LPO when compared to controls, whereas in chronic restraint stress, albino rats administered with Selenium showed a significant decrease ($p<0.001$) in LPO, when compared to controls which depicted in Fig. 1.

There was a significantly decrease in enzymatic and non-enzymatic antioxidant activity in chronic restraint stress when compared to control group, whereas chronic restraint stress albino rats administered with Selenium showed a significant increase ($P<0.001$) in enzymatic and non-enzymatic antioxidant activity when compared to controls which showed in Figs. 2 and 3 and Table 1.

DISCUSSION

Repeated stress daily may impair the antioxidant defenses in the body, leading to oxidative damage by changing the balance between oxidant and antioxidant factors [14]. Oxidative stress is a central feature of many diseases and stress-induced damage to these tissues may be the cause of severe stress disorders after repeated restraint stress exposure [15]. Restraint stress which is widely employed to provoke psychological stress has also been reported to be associated with oxidative damage in rats. The generation of ROS is a primary event under a variety of stress conditions and the consequence of ROS formation depends on the intensity of the stress. In our study, there was a significant increase in the level of LPO in chronic restraint stress-induced rats as compared to controls. This is in corroboration with an earlier investigation, which suggested that the high vulnerability of tissues to peroxidative damage is mainly due to a decline in the level of free radicals for scavengers, whereas in lipid peroxidation significantly decrease in selenium-treated rats as compared to controls. This could be due to the exquisite protective role that emanates from selenium's efficient breaking property in the ROS chain reactions. Similar studies have been reported by other workers as explained by Mukherjee *et al*., 1996 [16].

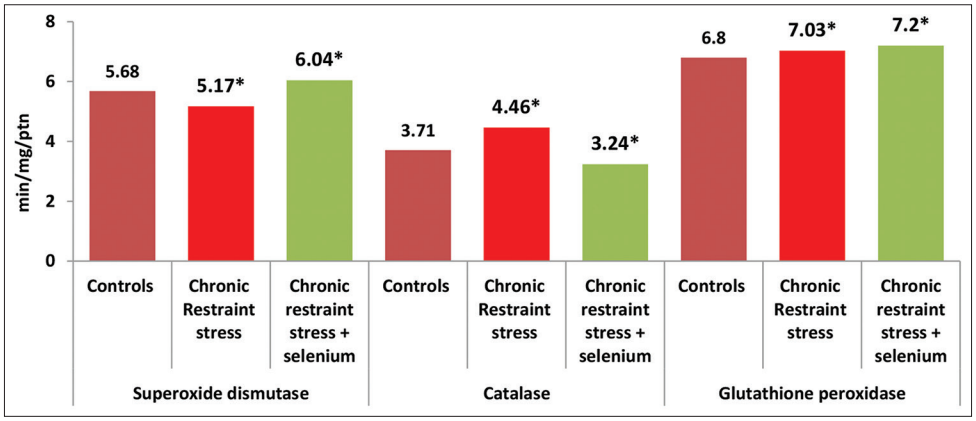

Fig. 2: Comparison of control, chronic restraint stress, and chronic restraint stress and selenium treated on enzymatic antioxidants in Wistar albino rats

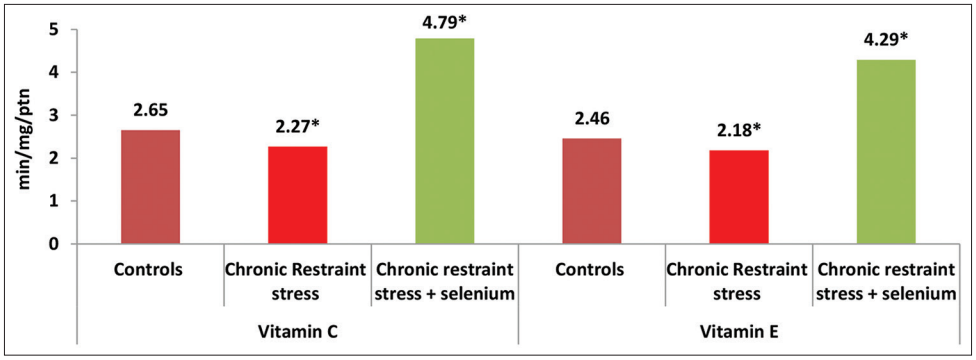

Fig. 3: Comparison of control, chronic restraint stress, and chronic restraint stress treated with selenium on non-enzymatic antioxidants in Wistar albino rats

Table 1: Comparison of control, chronic restraint stress, and chronic restraint stress and Selenium treated on LPO, enzymatic, and non-enzymatic antioxidants in Wistar albino rats

Groups	LPO (nmoles of MDA/min/mg/ptn)	SOD min/mg/ptn	CAT min/mg/ptn	GPx min/mg/ptn	VIT C µ/mg ptn	VIT E µ/mg ptn
Control	84.98±1.33	5.68±0.09	3.71±0.08	6.80±0.11	2.65±0.02	2.46±0.02
Chronic restraint stress	117.20±3.6	5.17±0.60	4.46±0.08	7.03±0.60	2.27±0.12	2.18±0.13
Chronic restraint stress+selenium	77.10±1.99	6.04±0.80	3.24±0.41	7.20±0.10	2.81±0.15	2.70±0.05

Values are means±standard error. Significance fixed at $p<0.05$*

The SOD converts superoxide radicals into H_2O_2. The ROS scavenging activity of SOD is effective only when its activity is followed by the actions of CAT and GPx, because H_2O_2 generated by SOD is further scavenged by CAT and GPx as explained by Halliwell *et al.*, 2001 [2]. Therefore, it is hypothesized that there can be an imbalance in the results of oxidative enzymes. In the present study, there was a significant decrease in enzymatic and non-enzymatic antioxidant activity in chronic restraint-stressed rats when compared to the control group. This decline could be due to the increased SOD activity in chronic restraint stress since SOD catalyzes the dismutation of superoxide radical anion into less noxious H_2O_2 into H_2O and O_2. The GPx enzyme was the first established selenoenzyme that could act as an antioxidant defense system for the removal of the ROS and excess hydrogen peroxide (H_2O_2) produced by restraint stress as reported by Zaidi *et al.*, 2005 [18].

Selenium is an essential component of several major metabolic pathways in the antioxidant defense system. Selenoproteins with known functions play an important role in a variety of biological processes and several of them are involved in antioxidant defense. Selenoprotein P has been associated with the oxidant defense properties of selenium. Selenoprotein P is an extracellular glycoprotein found in plasma and also associated with endothelial cells as explained by Hill *et al.*, 2003 [17]. The selenoenzymes that are found to have strong antioxidant activity include six groups of the GPx (catabolize hydroperoxides) that plays a significant role in protecting cells against oxidative damage from ROS and reactive nitrogen species.

There was a significant increase in enzymatic and non-enzymatic antioxidant activity in selenium-treated Wistar albino rats when compared to controls. This might be due to its function in the active sites of many antioxidant enzymes and a reduced GSH level plays a primary role in the balance of the redox status through the reduction of ROS and peroxides produced by restrained stress. Similar studies have been reported by other workers as highlighted by Bhat *et al.*, 2006 [19].

In rats, selenium has been shown to prevent the harmful effects of free radicals and may also reduce the formation of the reactive metabolites induced by restraint stress. The results support the general hypothesis of the importance of free radical formation in restraint stress and that selenium could act by increasing the cellular defense against free radicals. The administration of selenium on restrained stressed rats tends to increase the antioxidant enzymes and selenoenzymes play an essential role in improving cellular immune function and regulation of DNA synthesis has indicated that much of the beneficial effect of selenium is attributed to its antioxidant nature.

CONCLUSION

The effect of Selenium acts as an antidote to counteract the effects of restraint stress and has significant therapeutic application in counteracting oxidative damage in Wistar albino rats.

AUTHORS' CONTRIBUTIONS

Qairunnisa S and Purushothaman G. carried out the conception and design of this study, Mohanapriya P acquired the data and analysis part. Poornima Kumbakonam Nagarajan has drafted the article with intellectual content.

CONFLICTS OF INTEREST

The author declared "no conflicts of interest."

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