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Original article

Possible modulation of nervous tension-induced oxidative stress by vitamin E

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ABSTRACT

Stress is an unavoidable part of human life that affects a majority of people: In 2018, 55% of Americans reported experiencing stress (Gallup Global Emotions, 2019). Various factors contribute to the emergence of nervous stress among individuals, including environmental, physical, and psychological stimuli. Physical and psychological issues arise as a result of stress, which is the subject of our research study, giving it significant practical value. Here, we have tested the possible correlation between increase in oxidation species and severe psychological issues at a community level. To understand any possible connections between these two parameters, tests were conducted on 200 rats that were divided into three general groups based on the duration of stress exposure. Each group was further divided into five smaller groups with 10–20 rats. Treatments were setup with or without vitamin E with periods of stress immobilization. Samples were then collected to conduct necessary analyses from control, experimental, and treatment groups. Immobilization stress types, i.e., acute and chronic stress, caused noticeably different physiological changes, especially with respect to nature and severity of response. Chronic stress induced different responses depending on the exposure period as well. Furthermore, vitamin E appeared to have a protective role due to its antioxidant nature, which highlights the need for investigations on oxidative stress-related disease treatment and prevention.

1. Introduction

Everyday life of the modern person is replete with tension and high levels of stress (Can et al., 2019). While stress is itself of diverse types, its influence varies in terms of psychological and physiological manifestations as well (Tian et al., 2018). Recent studies provide a link between stress among adults and the risk of cardiac diseases, including coronary heart artery disease and arteriosclerosis; stressed individuals may, in particular, be prone to developing high blood pressure and diabetes (Keltikangas-Jarvinen et al., 1998). Latest research has provided evidence of a causal relationship between oxidative stress and various cognitive disorders, such as Parkinson's illness, dementia, and amyotrophic horizontal sclerosis (Baek et al., 2020).

Continued states of nervous tension have been reported to induce increased oxidative stress, in conjunction with the release of catecholamines. This, in turn, may produce free oxygen radicals, causing functional and structural damage in cells, as well as tissue damage (Hu et al., 2000; Ainsah et al., 1999; Hu et al., 2000). Increased levels of lipid peroxidation in the heart muscles have been reported in rats after periods of immobilization (Davydov and Shvets, 2001).

Vitamin E (α-tocopherol) is an essential and potent antioxidant that is commonly found in nature (Sezer et al., 2020). Vitamin E increases the body's ability to fight against oxidation by regulating the level of antioxidants in tissues (Atalay et al., 2000). It is soluble in fat, and thus, is considered to provide primary protection to the cells against oxidative damage (Sezer et al., 2020). Vitamin E is an effective root cleanser for oxides produced by lipid peroxidation (Sezer et al., 2020). Due to these properties, vitamin E can neutralize the effect of free radicals, and thus, counter or delay the chronic physiological abnormalities caused by them (Sezer et al., 2020).

Vitamin E is involved in the production and multiplication of thiolate (Gokkusu et al., 2001) and glutathione. Furthermore, together with selenium, it protects biofilms from undergoing oxidation and the damage associated with it (Altayeb and Salem, 2017). Other effects of vitamin E include increased activity of superoxide dismutase (SOD), glutathione reductase (GR) (Hsu et al., 2002), and glutathione peroxidase (GPx) (Gokkusu et al.,

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2001), etc. Other than the aforementioned studies, Vitamin E has been reported to boost immunity and reduce oxidative stress in a multitude of studies referenced herein (Khatti et al., 2017, Chin and Nirwana, 2018; Tantavisutl et al., 2017).

The goal of our study was to define the antioxidant functioning of vitamin E during oxidative stress caused by nervous tension. We outline the effect of immobilization stress on the growth of adult male white rats, while differentiating between the effects of chronic and acute stress. We also discuss the differences in response to chronic stress over varying periods of time.

2. Materials and methods

2.1. Experimental animals

This study employed 200 adult white male rats (Wistar) with a weight range between 135 and 145 g. The animal house of the College of Pharmacy at King Saud University provided the rats. The rats were kept in suitable conditions, including proper ventilation and temperature levels; they were given a balanced diet and had a defined water intake regime, which helped the animals adapt to their environment. For acclimation, rats lived for two weeks in such conditions before the experiment was initiated.

2.2. Animals' exposure to stress

The animals were subjected to immobilization stress to simulate nervous pressure. This type of stress is regarded as the most severe nervous pressure and has been used in research as an adaptation of the methodology suggested by Yang et al., 2014. This was achieved using rat restraining units that were designed locally with the use of flexible metal wire that wrapped the rats with their tails remaining extended. The edges of the designed net were folded to paralyze the animals' movement and activity. The tails of rats were further held by a spring, which hanged the rat without support. Furthermore, during this procedure, the rats were provided no food or drinks.

2.3. Chemicals used

Vitamin E was obtained as a pure liquid from Merck Company and was diluted using olive oil acquired from the College of Pharmacy of King Saud University. The dose was calculated to be 40 mg/kg of body weight (Sano et al., 1998). Throughout the experiment, injections were performed via the peritoneal cavity of rats.

2.4. Experimental design

Three main groups of rats were organized according to the period of exposure to stress.

First group: Acute stress exposure, including:

1. A control group with rats given no treatment
2. A group that was subjected to acute stress by paralyzing movement once every two hours

Second group: Chronic stress exposure (duration of exposure = 4 days), including:

1. A control group of rats given no treatment
2. A control group given melted olive oil via injections into the peritoneum and vitamin E daily for four consecutive days
3. A group given vitamin E and olive oil via injections into the peritoneum for four days
4. A group subjected to neurotic pressure by paralyzing their movement for two hours per day for four consecutive days
5. A group given vitamin E dissolved in olive oil via injections into the peritoneum, followed by being subjected to stress by paralyzing their movement after half an hour of injection for two hours a day for four consecutive days

The third group: Chronic stress exposure (duration of exposure = 10 days), including:

The following groups were included:

1. A control group of rats given no treatment
2. A control group given olive oil solvent via injections into the peritoneum for 10 consecutive days
3. A group given vitamin E dissolved in olive oil via injections into the peritoneum for 10 consecutive days
4. A group subjected to neurotic pressure by paralyzing the movement for two hours a day for 10 consecutive days
5. A group that was given vitamin E dissolved in olive oil via injections into the peritoneum, followed by being subjected to stress by paralyzing their movement after half an hour of injection for two hours a day for 10 consecutive days

Groups that were given vitamin E through daily peritoneal cavity injections had a set dosage of 40 mg/kg (Sano et al., 1998). Exposure to stress in the corresponding groups lasted for two hours a day (from 8 to10 am).

2.5. Sample collection

After slaughtering the rats, blood samples were collected. Animals were dissected immediately to collect the liver. Serum and liver samples were sent to King Fahd Specialist Hospital in Buraidah to be analyzed for serum selenium concentration, along with estimation of glutathione and hydrosulfide content in the serum and liver.

2.6. Statistical analyses

The data was analyzed using SPSS Inc. (version 20), Chicago, Illinois, USA. Data has been reported as mean ± standard error (mean ± SE), where comparison was done using analysis of variance (ANOVA). Results were considered significant at $p < 0.05$.

3. Results

The concentration of serum selenium, along with glutathione and hydrosulfide content in the liver and serum, provide evidence for exposure to acute neurological pressure due to immobilization for two hours. Decline in the amount of selenium in the serum was statistically insignificant. On the other hand, exposure to chronic stress led to a considerable decline in serum selenium concentrations. A considerable degree of decrease was observed in both periods: i.e., four and 10 days of immobilization. The content of glutathione and total hydrosulfide groups in the serum and liver decreased insignificantly as a result of exposure to chronic stress by paralysis for two hours.

Exposure to chronic stress by paralysis for two hours for four consecutive days was found to result in a significant decrease in the content of glutathione and total hydrosulfide groups in the liver and serum. It must be noted that this decline in glutathione was observed in the liver only, while its level was not significantly altered in the serum.

Injection of vitamin E half an hour before exposure led to a full adjustment of rats to their new environment and the extrinsic stress incurred on them. This was observable in the levels of glutathione and hydrosulfide groups in the liver and serum in the 10-day-long exposure as well (see Tables 1–3).

Table 1
Effect of immobilization stress on serum selenium concentration in untreated and vitamin E-treated rats.

Animal groups	Selenium (ppm)		
	Acute stress	Chronic stress	
		4 days	10 days
	mean ± SE	mean ± SE	mean ± SE
Control I (normal)	0.92 ± 0.02	0.92 ± 0.02	0.92 ± 0.02
Control II (Vehicle (oil) treated)	–	0.96 ± 0.05	0.94 ± 0.04
Vitamin E	–	0.92 ± 0.06	0.89 ± 0.06
Stress	0.88 ± 0.05	0.77 ± 0.06[a*]	0.78 ± 0.01[a*]
Stress + vitamin E	–	0.92 ± 0.04[c*]	0.92 ± 0.06[c*]

Results are given as the mean ± SE for 10 rats.
a, b, c denote a significant change in comparison to the normal control, vehicle-treated control, or stress-exposed rats, respectively, at $p < 0.05$ significance*.

4. Discussion

The current study provides evidence for the correlation between decrease in the content of total hydrosulfide groups and glutathione in the liver and serum after exposure to stress using rats as models. However, a significant deficiency was observed only in case of chronic nervous pressure (except in case of glutathione during the 10-day exposure). Maximum reduction in hydrosulfide and glutathione content was observed as a result of exposure to prolonged stress for a duration of 10 days.

A comparison with the control group revealed that a decline in selenium content in the rats' serum was attributable to stress. However, this was observable only after exposure to chronic stress for four or 10 days.

Thiol groups break the chain of interactions that results in generation of free radicals, which is necessary to protect against harmful reactive oxygen species. Among these, glutathione is a significant antioxidant because it is produced inside the living cells (Lomaestro and Malone, 1995). Glutathione interacts either with free oxygen radicals (such as hydroxyl or superoxide radicals) directly or acts as a substrate for the reaction of glutathione peroxidase. It also maintains the balance between antioxidants and oxidants. Furthermore, glutathione plays a vital role in processing other antioxidants and preserving them in their active intracellular state (Lomaestro and Malone, 1995). Accordingly, the deficiency of glutathione and thiols (Patra et al., 2001) results in increased production of free radicals and further development of oxidative stress.

Selenium is known to increase an organism's antioxidative ability (Qin et al., 2015). Oxidative stress can be prevented by an increase in the selenium-dependent glutathione peroxidase enzyme levels in tissues. As the selenium levels in the plasma/serum are positively correlated with the balance of antioxidants and oxidants in an organism (Yang et al., 2010), reduction in selenium levels may be a sign of oxidative stress. Our research results demonstrated a decrease in the level of thiol and glutathione groups in the liver and serum of rats, along with a decrease in selenium levels in the serum, after exposure to stress by immobilization. Nervous pressure caused oxidative stress in rats, which provides evidence for the positive correlation between nervous tension and oxidative stress (Ainsah et al., 1999).

The role of glucocorticoids in the induction of free radicals during stress should also be considered. There is a proven link between oxidative brain damage in primates and long-term administration of glucocorticosteroids (Sapolsky et al., 1990). Our results prove that vitamin E treatment counters exposure to hypotensive neurotransmitters by enhancing total glutathione and hydrosulfide groups in the liver and serum and by increasing selenium content in serum. Vitamin E is an efficient chain-breaking antioxidant that can scavenge free radicals, and thus, prevent oxidative damage, i.e., it has protective abilities (Nadeem et al., 2005). Vitamin E improves an organism's oxidizing abilities by regulating antioxidant levels in tissues to counterbalance oxidants (Atalay et al., 2000). This process takes place as vitamin E

Table 2
Effect of immobilization stress on serum glutathione and total hydrosulfide groups content in normal and vitamin E-treated rats.

Animal groups	Glutathione (mg/100 ml)			Hydrosulfide groups (mmol/100 ml)		
	Acute stress	Chronic stress		Acute stress	Chronic stress	
		4 days	10 days		4 days	10 days
	mean ± SE	mean ± SE	mean ± SE	mean ± SE	mean ± SE	mean ± SE
Control I (normal)	1.44 ± 0.11	1.44 ± 0.11	1.44 ± 0.11	0.31 ± 0.02	0.31 ± 0.02	0.31 ± 0.02
Control II (Vehicle (oil) treated)	–	1.46 ± 0.1	1.45 ± 0.09	–	0.33 ± 0.01	0.32 ± 0.01
Vitamin E	–	1.43 ± 0.1	1.42 ± 0.09	–	0.31 ± 0.02	0.31 ± 0.02
Stress	1.35 ± 0.16	1.03 ± 0.16[a*]	1.38 ± 0.34	0.28 ± 0.01	0.21 ± 0.02[a*]	0.25 ± 0.02[a*]
Stress + vitamin E	–	1.36 ± 0.14[c*]	1.38 ± 0.13	–	0.31 ± 0.03[c*]	0.30 ± 0.01[c*]

Results are given as the mean ± SE for 10 rats.
a, b, and c denote a significant change in comparison to normal control, vehicle-treated control, or stress-exposed rats, respectively, at $p < 0.05$ significance*.

Table 3
Effect of immobilization stress on liver glutathione and total hydrosulfide groups content in normal and vitamin E-treated rats.

Animal groups	Glutathione (mg/100 ml)			Hydrosulfide groups (mmol/100 ml)		
	Acute stress	Chronic stress		Acute stress	Chronic stress	
		4 days	10 days		4 days	10 days
	mean ± SE	mean ± SE	mean ± SE	mean ± SE	mean ± SE	mean ± SE
Control I (normal)	16.5 ± 1.73	16.5 ± 1.73	16.5 ± 1.73	5.29 ± 0.30	5.29 ± 0.30	5.29 ± 0.30
Control II (Vehicle (oil) treated)	–	16.1 ± 1.65	16.3 ± 1.59	–	5.29 ± 0.39	5.28 ± 0.39
Vitamin E	–	15.8 ± 1.47	16.0 ± 1.41	–	5.23 ± 0.31	5.25 ± 0.31
Stress	15.8 ± 1.37	11.8 ± 0.68[a*]	11.4 ± 0.78 [a*]	4.94 ± 0.23	3.72 ± 0. 24[a*]	3.01 ± 0.28[a*]
Stress + vitamin E	–	15.2 ± 1.25[c*]	15.6 ± 1.27[c*]	–	5.29 ± 0.14[c*]	5.29 ± 0.14[c*]

Results are given as the mean ± SE for 10 rats.
a, b, and c denote a significant change in comparison to normal control, vehicle-treated control or stress-exposed rats, respectively, at $P < 0.05$ significance*.

increases glutathione (Sharma et al., 2000), selenium (Sobajic et al., 1998), and thiols (Gokkusu et al., 2001), while also working as the active substance of GPx, SOD (Gokkusu et al., 2001), and GR (Hsu et al., 2002). Furthermore, vitamin E may also have significant potential to function as an agent that prevents meta-oxidation of fats (Hsu et al., 2002).

5. Conclusion and recommendations

The current research provides evidence for increase in oxidative stress in rats that were exposed to chronic and acute stress, which manifested as a significant decline in the concentration of antioxidants, hydrosulfide groups, selenium, and glutathione in the serum and/or liver within a period of 4 and 10 days. Injections of vitamin E before being subjected to stress successfully modified and prevented pressure-induced oxidative stress among rats.

Accordingly, the following recommendations can be made:

- Our research has demonstrated a potentially beneficial role of vitamin E in addressing oxidative stress. Considering the correlation between stress and emergence of many widespread diseases, including high blood pressure, atherosclerosis, diabetes, coronary artery disease, cancer, cataracts, and Alzheimer's, using vitamin E as a food supplement may be vital in their prevention. This is especially vital for people at high risk of acquiring the aforementioned diseases, as well as for individuals who have been already diagnosed with them. In the latter case, including vitamin E in regular diet may mitigate the progression and complications of such health disorders.
- Vitamin E intake should be recommended to the elderly as oxidative stress may provoke early aging.
- Further studies should be conducted to define the most suitable dosage of vitamin E as done for subjects in the current research; however, the chosen dosage here did not address all stress-related effects.

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Further reading

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