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Original article

Vitamin E reduces oxidative stress in brains of male albino male rats undergoing immobilization

Noorah Saleh Al-Sowayan [a,*], Reem Abdullah Almarzougi [b]

[a] Department of Biology, College of Science, Qassim University, Buraydah 53277, Saudi Arabia
[b] Master Student and Biology Department, College of Science, Qassim University, Buraydah, Saudi Arabia

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ABSTRACT

Stress can lead to various health problems. Exposure to stress is linked to several diseases including cancer, hypertension, diabetes, mental disorders, and heart attacks. Stress stimulates several pathways that produce free radicals, which increases oxidative stress. This results in functional and structural damage to organs, such as the brain, which is highly sensitive to oxidative stress Vitamin E is a naturally occurring potent antioxidant used for various purposes. The main purpose of the current study was to evaluate how vitamin E protects the brain and to what extent it affects antioxidant levels in rats subjected to two hours per day of immobilization stress, the form of stress with the strongest effect. The rats were immobilized by folding their limbs in and wrapping them in netting to prevent movement, while allowing their tails to be extended. The rats were then hung upside down. The effect of vitamin E was tested by intraperitoneally injecting rats with 40 mg/kg of vitamin E daily. Oxidative stress parameters were determined at the completion of the experiment. A dramatic decrease in malondialdehyde (MDA) levels and an increase in catalase (CAT), including glutathione peroxidase (GSH-Px) and superoxide dismutase (SOD), has been shown by vitamin E in the brain. The effects of vitamin E were significantly higher in the acute and chronic stress groups than in the control group. Vitamin E can decrease oxidative stress in stressed rats, indicating that it exerts therapeutic and protective effects owing to its antioxidant properties.

1. Introduction

Stress is a normal factor in everyday vernacular and a fundamental aspect of the human condition. Millions of missed working days are attributed to stress, which is acknowledged globally as the main contributor to chronic illnesses (e.g., Am. Psychol. Assoc. 2019, UK Health Saf. Executive 2019). It has been reported that stress affects every aspect of life. Furthermore, stress is known to have both direct and indirect negative effects on health via changes in neuroendocrine and autonomic functions (O'Connor et al., 2021).

Activation of the HPA axis, which causes an increase in corticosterone levels in the brain, is a key mechanism linked to stress-induced behavioral problems. Rapid depolarization caused by elevated corticosterone levels causes glutamate to be released into limbic and cortical areas. An increase in metabolic rate and mitochondrial dysfunction may result from glutamate overproduction. An increase in the metabolic rate results in a conflict between the generation of ROS and antioxidant system activity, which causes the generation of more free radicals. Nucleic acids, proteins, and lipids are just a few cellular components that oxidatively damage these free radical species oxidatively damage (Samarghandian et al., 2017).

Free radicals are associated with oxidative stress. Free radicals are highly reactive with unpaired electrons and atoms. An increase in the levels of antioxidants and free radicals can lead to oxidative stress.

Various diseases can develop because of organ and tissue damage. Several reactive species, including reactive nitrogen species (RNS), reactive sulfur species (RSS), reactive oxygen species (ROS), and reactive carbonyl species (RCS), have been identified in mammals. Se, Cl, and Br are biologically critical reactive species (Murphy et al., 2011). One helpful method for accurately simulating oxidative stress is the application of immobilization stress. The effectiveness of immobilization stress as an acute and chronic stressor in rats has been extensively studied (Iwa et al., 2006).

GSH-Px, CAT, and CAT are the main enzymes in the antioxidant

Abbreviations: **SOD**, Superoxide Dismutase; MDA, Malondialdehyde; GSH-Px, Glutathione Peroxidase; CAT, Catalase; HPA, Hypothalamic-Hypophyseal-Adrenal; ROS, Reactive-Oxygen-Species.

* Corresponding author.
E-mail addresses: nsaoiean@qu.edu.sa (N.S. Al-Sowayan), 411200047@qu.edu.sa (R. Abdullah Almarzougi).

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system. SOD is a metalloenzyme that rapidly converts superoxide into hydrogen peroxide (H2O2). When the conditions and concentrations are correct, SOD protects NO from superoxide inactivation and induces the discharge of biologically active NO. CAT and GSH-Px convert H2O2 to water (Rodrigo et al., 2007). Lupien et al. (2009) stated that stress can cause structural alterations in the human brain, owing to its influence on the neurological system. Because the brain comprises a large amount of readily oxidizable fats, consumes large amounts of oxygen (accounting for approximately 20 % of the total oxygen consumption in humans), and has low antioxidant levels, it is highly susceptible to oxidative stress.

Studies have linked neurodegenerative conditions, such as depression and cognitive impairment, to elevated ROS levels and insufficient antioxidant levels (Samarghandian et al., 2015). High levels of polyunsaturated fatty acids in the membranes and low levels of antioxidant and non-enzymatic enzymes contribute to oxidative damage (Zaidi & Banu, 2004).

The main antioxidant enzymes were CAT, SOD) and GSH-Px. Exogenous antioxidants such as vitamins E and C are found in cell membranes both intracellularly and extracellularly. The main role of vitamin E is to prevent membrane damage to membranes (Rodrigo et al., 2007).

Vitamin E, which was first discovered in the 1920s, is an essential nutrient. There have been studies on vitamin E for almost a century, including studies on its antioxidative properties. Vitamin E, a lipid-soluble vitamin, regulates redox balance in the body and is found everywhere, including in the cell membranes and lipoproteins. Vitamin E has eight isomers: four tocopherols (α-, β-, γ-, and δ-tocopherol), and four tocotrienols (α-, β-, γ-, and δ-tocotrienol). Tocotrienols and tocopherols are different from each other based on the location and number of methyl groups attached to the chromanol rings (Miyazawa et al., 2019). While tocotrienols have three double bonds and are saturated forms of tocopherols, each form of vitamin E has a side chain and a chromanol ring with 16 carbons; the difference is whether there are CH3 or H groups at the five and seven positions of the chromanol ring. In nature, tocopherols have an RRR configuration at positions 2, 4′, and 8,' and tocotrienols have an R configuration at these positions (Jiang, 2014).

Generally, the total number of methyl groups connected to the chromanol ring determines the antioxidative activity of various vitamin E isomers. There are three methyl groups in TocH, whereas there is only one methyl group in tocopherols. It is commonly known that the order of vitamin E isomers in terms of the strength of antioxidative activity is $\alpha > \beta > \gamma > \delta$, and that tocopherols have stronger antioxidative activity than tocotrienols. According to previous studies, tocotrienols have stronger antioxidative effects than tocopherols.

Tocopherols effectively attach to lipid free radicals to transfer hydrogen atoms, resulting in the matching of non-radical lipid products and tocopheroxyl radicals. Tocopherols are among the most biologically active and common forms of vitamin E in vivo. When alphatocopheroxyl radicals interact with other free radicals or with themselves, a non-radical product is formed. Therefore, tocopherol consumes two lipid free radicals per molecule, thereby stopping the chain reaction of free radical production (Yamauchi, 1997). Studies have revealed that vitamin E has various potential health benefits, including anti-allergic effects (Sato et al., 1993), anti-thermogenic and anticancer effects (Meydani et al., 1990), anti-cardiovascular disease effects (Wu & Meydani, 2014), antidiabetic, antilipidemic, and anti-hypertensive effects (Azzi et al., 2016), anti-inflammatory and anti-obesity effects (Mindlen et al., 1996), neuroprotective effects; and telomerase-modulating effects (Miyazawa et al., 2019).

2. Experimental procedure

2.1. Experimental animals

Seventy healthy male Wistar rats, procured from King Saud University's College of Pharmacy, weighing 200–250 g, were used in this study. The animals were housed under normal laboratory conditions (22–28 °C, 12 h of light and dark with 50–60 % relative humidity) in stainless-steel cages measuring 55 × 39 × 28. Food (commercial rat pellets) and water were provided *ad libitum*. Before testing, the animals were acclimatized to the laboratory atmosphere for a month.

2.2. Chemicals

Pure liquid vitamin E was obtained from Merck and injected intraperitoneally at a dose of 40 mg/kg body weight intraperitoneally (Al-Sowayan, 2020). Reagents for the GSH, MDA, SOD, and CAT assays were acquired from NWLSSTM.

Vitamin E is a pure liquid form of synthetic vitamin E obtained from Merck (alpha-tocopherol) and is the most potent antioxidant in the vitamin E group. This indicated that vitamin E was administered via injection into the peritoneal cavity. The peritoneal cavity is the area of the abdomen that contains the stomach, liver, and intestine. Administering compounds through the peritoneal cavity is a common method in animal studies because it allows for the direct delivery of the substance into the abdominal space, facilitating absorption and distribution.

2.3. Exposure to immobilization stress

Immobilization stress was applied to the animals by folding their limbs in and wrapping them in netting to prevent movement, while allowing their tails to be extended. The rats were suspended in their tails by using a spring. No food or water was administered to the rats during this process (Al-Sowayan, 2020).

2.4. Experimental design and treatment groups

Animals were randomly divided into two groups according to how long they had been exposed to stress. Four subgroups were created for each category, each containing ten rats.

In the first group, rats underwent **acute stress** for two consecutive hours.

1- Rats in the control subgroup received no treatment and no exposure to stress.
2- Rats in one subgroup were subjected to acute stress by immobilization for at least two consecutive hours.
3- Rats in another subgroup were administered a dose of vitamin E via intraperitoneal injection one hour before exposure to immobilization stress for at least two consecutive hours.

The rats in the second group were exposed to **chronic stress** for 02 h/day for 10 consecutive days.

1- Rats in the control subgroup received no treatment and no exposure to stress.
2- Rats in each subgroup were subjected to chronic immobilization stress for at least 02 h/day for 10 consecutive days.
3- Rats in another subgroup were administered a dose of vitamin E via intraperitoneal injection one hour before exposure to immobilization stress for at least 02 h/day for 10 consecutive days.
4- Rats in another subgroup were administered vitamin E via intraperitoneal injection one hour after exposure to immobilization stress for at least 02 h/day for 10 consecutive days.

2.5. Sample collection

After the rats were euthanized at the end of the experiment, blood samples were collected from the rat eye orbit. The brains of the animals were immediately removed to determine the level of oxidative stress in the brain using GSH, MDA, CAT, and SOD assays.

2.6. Biochemical analysis

2.6.1. Measurement of oxidative stress indicators

The solution used was phosphate-buffered saline (PBS; pH 7.4) with 0.16 mg/mL heparin to wash the brain and liver to remove clots and red blood cells. A refrigerated centrifuge (4000 rpm at 4 °C for 10 min) was used to centrifuge the samples after they were homogenized in cold phosphate buffer using an ultrasonic homogenizer. The supernatant was removed and stored at 20 °C for the purpose of conducting antioxidant tests (Al-Musa & Al-Hashem, 2013).

2.6.2. Measurement of glutathione (GSH-Px) levels

5–5′-Dithiobis [2-nitrobenzoic acid] reacts with GSH to form the 412 nm chromophore 5-thionitrobenzoic acid (TNB) and GS-TNB. After GS-TNB is reduced by glutathione reductase and NADPH, a second TNB molecule is released and GSH is recycled, thus magnifying the reaction. The reaction mixture initially contained or formed GSSG, which was rapidly reduced to GSH, and mixed disulfide reactions between GSH and GS-TNB were observed (Al-Musa & Al-Hashem, 2013).

2.6.3. Measurement of superoxide dismutase levels

A SOD assay kit from NWLSSTM was used for hematoxylin autooxidation, as previously described by Martin, with modifications to improve the reliability and robustness of the results. The level of superoxide dismutase (SOD) within a particular range is linearly related to the percentage of autooxidation inhibited by SOD (Lykkesfeldt, 2001). The autooxidation rate and inhibition of autooxidation were measured to determine the SOD activity.

2.6.4. Measurement of malondialdehyde levels

Malondialdehyde (MDA) and thiobarbituric acid (TBA) were mixed to form an adduct that absorbed light at 532 nm. The TBA method is the most common technique used for measuring MDA levels in biological materials (Lykkesfeldt, 2001).

2.6.5. Measurement of catalase levels

All aerobic cells contain catalase, an endogenous antioxidant enzyme that helps remove hydrogen peroxide. The four equally sized subunits of the enzyme contain heme-active sites that promote H2O2 decomposition into water and oxygen. Catalase activity in purified samples was estimated using an assay kit from NWLSSTM. The absorbance of H2O2 at 240 nm was used to measure catalase enzyme activity (Lykkesfeldt, 2001).

2.7. Statistical analysis

SPSS (version 23) was used for the statistical analysis. We entered the data obtained from the aforementioned experiments into a computer, and the data were displayed as the mean ± standard error of the mean. ANOVA was used to compare the data. Post-hoc comparisons of the means were performed using the least significant difference test. Each group consisted of 10 rats (n), with a significance level of $p \leq 0.05$.

3. Results

In this investigation, 27 rats died (4 were died due to acute stress while 23 were died because of chronic stress), which was approximately 38 % of the total rats.

3.1. The effect of intraperitoneal injection of vitamin E before and after stress exposure on MDA levels

In comparison with the control group, the mean serum and MDA levels in both the group exposed to acute stress before vitamin E treatment and the group exposed to chronic stress and untreated with vitamin E were significantly higher, and we found the same change in MDA levels in brain tissue homogenates. Furthermore, the levels of MDA in serum and brain tissue homogenates were considerably reduced in both groups exposed to acute stress after vitamin E treatment and the group exposed to acute stress before vitamin E treatment compared to the group exposed to acute stress and not administered vitamin E treatment. There was a significant increase in the mean serum level of MDA among the groups exposed to chronic stress before and after vitamin E treatment and the control group; however, there was a substantial decline in MDA levels in the group receiving vitamin E after being subjected to ongoing stress and the group that was chronically stressed before receiving vitamin E treatment, as opposed to the group that was chronically stressed and did not receive vitamin E treatment. However, there was no noticeable difference in the MDA levels in brain tissue homogenates among the groups receiving vitamin E before and after chronic exposure to stress, whereas the group that experienced prolonged stress did not receive vitamin E treatment.

3.2. The effect of intraperitoneal injection of vitamin E before and after stress exposure on glutathione (GSH-Px) levels

As shown in Tables 1 and 2, rats in the acute stress groups exhibited significantly lower glutathione levels in the serum and brain tissue homogenates than those in the control group after being subjected to immobilization stress. Vitamin E affected glutathione levels in the brain tissue and serum in the acute stress groups (treated with vitamin E before or after stress exposure). As shown in Table 1, the mean serum level of glutathione was significantly higher in the group exposed to chronic stress after vitamin E treatment and the group exposed to chronic stress before vitamin E treatment than in the group exposed to chronic stress and not treated with vitamin E However, as shown in Table 2, no noticeable differences in glutathione levels in brain tissue homogenates were observed in the groups under control and chronic stress or between the groups of chronic stress (given vitamin E treatment either before or after being under stress) and the group exposed to chronic stress and not treated with vitamin E.

3.3. The effect of intraperitoneal injection of vitamin E before and after stress exposure on superoxide dismutase levels

There was a significant increase in serum superoxide dismutase levels after exposure to both acute and chronic immobilization stress in comparison to the control group. Table 1 shows a comparative study of patients exposed to chronic or acute stress without vitamin E treatment. There was a substantial shift in the serum levels of superoxide dismutase in the groups treated with vitamin E either before or after stress exposure.

A substantial reduction in superoxide dismutase levels was seen in brain tissue homogenates after acute and chronic immobilization stress exposure compared to the control group. As shown in Table 2, relative to the group that was subjected to chronic or acute stress and not treated with vitamin E, superoxide dismutase levels were significantly altered in the groups subjected to chronic or acute stress while being treated with vitamin E either before or after stress exposure.

3.4. The effect of intraperitoneal injection of vitamin E before and after stress exposure on catalase levels

There was a substantial decrease in brain tissue homogenates and catalase levels in the serum after exposure to both acute and chronic immobilization stress compared with the control group. Furthermore, catalase levels were substantially changed in the groups exposed to chronic or acute stress and treated with vitamin E before or after stress exposure compared to the group without vitamin E treatment and experiencing either acute or chronic stress.

Table 1
The effect of intraperitoneal injection of vitamin E before and after acute and chronic immobilization stress exposure on serum antioxidant levels among the different groups.

Variable	Control group	Acute stress group	Acute stress group treated with vitamin E before stress exposure	Acute stress group treated with vitamin E after stress exposure	Chronic stress group	Chronic stress group treated with vitamin E before stress exposure	Chronic stress group treated with vitamin E after stress exposure
MDA level (μmol/L)	1.24 ± 0.02	3.90 ± 0.57 [a]	2.17 ± 0.43[b]	2.00 ± 0.53[b]	5.50 ± 0.87[a]	3.18 ± 1.33[a,b]	3.00 ± 1.63 [a, b]
GSH-Px level (μmol/L)	2.76 ± 0.36	1.18 ± 0.76[a]	2.63 ± 0.33[b]	2.85 ± 0.17[b]	1.15 ± 0.56[a]	1.92 ± 0.38[b]	2.94 ± 0.16[b]
SOD level (U/mL)	6.81 ± 0.08	10.01 ± 1.86[a]	6.72 ± 0.38[b]	7.53 ± 1.06[b]	12.03 ± 2.79[a]	7.23 ± 1.39[b]	8.52 ± 1.47[b]
CAT level (mM/L)	0.36 ± 0.02	0.02 ± 0.05[a]	0.29 ± 0.12[b]	0.30 ± 0.54[b]	0.11 ± 0.04[a]	0.29 ± 0.02[b]	0.31 ± 0.04[b]

The results are presented as the mean ± SE of 10 rats.
Values with different superscript letters in a single column are significantly different ($p < 0.05$).
[a] Substantial difference compared to the control group.
[b] Substantial difference compared to the acute stress group.

Table 2
The effect of intraperitoneal injection of vitamin E before and after acute and chronic immobilization stress exposure on antioxidant levels in brain tissue homogenates among the different groups.

Variable	Control group	Acute stress group	Acute stress group treated with vitamin E before stress exposure	Acute stress group treated with vitamin E after stress exposure	Chronic stress group	Chronic stress group treated with vitamin E before stress exposure	Chronic stress group treated with vitamin E after stress exposure
MDA level (nmol/g tissue)	1.65 ± 1.33	3.12 ± 1.43[a]	1.82 ± 2.22[b]	1.92 ± 1.25[b]	2.75 ± 2.1[a]	2.23 ± 1.98	2.56 ± 1.92
GSH-Px level (μmol/g tissue)	1.56 ± 0.17	0.76 ± 0.45[a]	1.92 ± 0.31[b]	1.93 ± 0.17[b]	1.45 ± 0.97	1.46 ± 0.29	1.43 ± 0.15
SOD level **(UI/mgp)**	26.94 ± 03.85	14.01 ± 2.67[a]	19.88 ± 4.14[b]	21.48 ± 3.86[b]	17.67 ± 3.49[a]	22.07 ± 1.58[b]	24.59 ± 2.15[b]
CAT level **(μmol/mgp)**	0.75 ± 0.47	0.27 ± 0.09[a]	0.61 ± 0.04[b]	0.65 ± 0.24[b]	0.25 ± 0.27[a]	0.62 ± 0.0.42[b]	0. 59 ± 0.27[b]

The results are presented as the mean ± SE of 10 rats.
Values with different superscript letters in a single column are significantly different ($p < 0.05$).
[a] Substantial difference compared to the control group.
[b] Substantial difference compared to the acute stress group.

4. Discussion

Research on oxidative stress in rats can be conducted by exposing laboratory animals to both acute and chronic stress. In this study, we examined the antioxidant balance in various tissues of rats exposed to acute and chronic stress and obtained varying results. These differences may have been due to variations in the chronic stress model as well as differences in the duration of stress exposure. A wide variety of stressors are used for chronic stress modeling, including prolonged exposure to cold, immobilization, and social isolation.

Stress causes the production of glucocorticoids (GCs) and increases the serum corticosterone levels. GCs promote anabolic and catabolic processes and increase metabolic rate and ROS generation. The liver plays a key role in the response to stress and metabolic capacity and represents a key peripheral target of GCs. Owing to the high oxygen consumption, abundance of lipids, and relative lack of antioxidant enzymes, the central nervous system can suffer severe damage when free radical overproduction occurs. The lack of antioxidant defense mechanisms increases the vulnerability of the brain to free-radical damage, leading to molecular and cellular dysfunction (Kalaz et al., 2012).

Glucocorticoids are well-known stress response hormones in vertebrates that are discharged from the adrenal cortex. Glucocorticoids induce several physiological changes in the response to stress. Glucocorticoids have several effects including stimulating triglyceride hydrolysis in adipocytes and increasing blood glucose levels (Grégoire et al., 1991).

An earlier study showed a substantial decline in glutathione levels in different tissues after stress exposure (Kalaz et al., 2012). According to our data, GSH levels decreased by 18 % in rats exposed to stress, particularly acute stress. This finding has also been reported in previous studies. The stressed group exhibited higher levels of MDA. Following exposure to stress, brain and serum MDA levels increase, whereas CAT and glutathione activities decrease (Ohta et al., 2015). Aşir et al. (2021) reported that MDA levels in stressed groups were substantially greater than those in control groups. Lata et al. (2004) found that on the 7th day of immobilization, the serum MDA level was more than double that of the control value. Based on a study by Zaidi et al. (2005), immobilization stress for six consecutive hours decreased brain glutathione, SOD, and CAT levels but increased MDA levels (Iwa et al., 2006).

Zafir and Banu (2009) also reported that restraint stress significantly decreased serum levels of glutathione and catalase, which is closely related to our findings. However, Sepidarkish et al. (2020) found no significant differences in the activities of glutathione, SOD, and catalase following stress. However, the MDA levels were significantly reduced.

Vitamin E has antioxidant properties because of its capacity to scavenge lipid radicals and minimize oxidative stress, which can harm the cardiovascular system (Alshiek et al., 2017). According to Sakr et al. (2015), vitamin E analogs may be advantageous to neuronal cells.

Vitamin E alleviated oxidative stress-mediated toxicity both in vitro and in vivo, and exerted shielding effects against neurodegenerative diseases. Accordingly, vitamin E may protect lipid-rich structures such as the brain from free radical damage. Vitamin E is also thought to significantly decrease MDA levels and improve CAT and SOD activity. In addition, it increases glutathione levels in the brain tissue. Prasad et al. (2012) found that vitamin E reduced MDA levels in all specified organs and serum. Vitamin E administration in stressed rats significantly increases oxidative stress, decreases brain MDA levels, and increases SOD activity (Patel et al., 2015). MDA serum levels were reduced with the treatment of vitamin E, while SOD levels were highly increased with vitamin E (Pan et al., 2013). The results of the aforementioned studies are similar to our findings, except for those related to SOD serum levels.

5. Conclusion

Levels of the antioxidants GSH, SOD, and CAT in the brain were significantly higher in the vitamin E group than in the acute and chronic stress groups (controls). Vitamin E also significantly reduced MDA levels and oxidative stress in stressed rats. These findings suggest that vitamin E can prevent and relieve stress.

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Ethical approval

The study protocol was approved by the Qassim University Committee for Scientific Research. (protocol code: 21–24-03; approval date: 31–7-2023).

Informed consent

Before the study began, legally appointed representatives provided written informed consent.

Author contributions

Conceptualization, R.M. and N.S.; methodology, N.S.; formal analysis, R.M.; investigation, R.M.; resources, N.S.; data curation, R.M.; prepared the original draft, R.M.; writing, reviewing, and editing the work, N.S.; oversaw it, N.S. After reading the published version of the article, all authors have approved the manuscript.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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