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Original article

Evaluating the Effect of Natural Antioxidants (Vitamins C and E) on Oxidative Stress Markers in Human Cells

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A B S T R A C T

The association of oxidative stress with cellular injury is well established and is a contributing factor to the pathogenesis of many of the chronic diseases associated with aging. Vitamins C and E are commonly accepted as natural antioxidants that scavenge free radicals. The primary goal of this study was to assess the impact of vitamins C and E on specific markers of oxidative stress (OS) in human peripheral blood mononuclear cells (PBMCs) that had been subjected to hydrogen peroxide (H_2O_2)-induced OS in an in vitro environment. A within-groups (pre-post) experimental design was implemented, utilizing PBMCs that were isolated from 60 healthy adult volunteers recruited in Cairo, Egypt. PBMCs were subjected to H_2O_2 (200 µM) to induce OS, then were treated with either vitamin C (100 µM), vitamin E (50 µM), or both vitamin C and E in combination. Markers of OS assessed included malondialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and total antioxidant capacity (TAC) which were evaluated pre-treatment and post-treatment. The findings suggest that hydrogen peroxide (H2O2) increases the level of malondialdehyde (MDA), with a significant difference noted ($p < 0.001$), while at the same time decreasing the activity of some enzymes involved with oxidative stress. Further reductions in MDA (by 38.4% for vitamin C and by 41.2% for vitamin E; both $p < 0.001$) were noted with vitamins C or E, or with both vitamins together (reducing MDA by 53.7%; $p < 0.001$). The activity of several antioxidant enzymes (superoxide dismutase, catalase, glutathione peroxidase, and total antioxidant capacity) was all restored following either type of vitamin treatment; however, the greatest restoration of antioxidant enzyme activity was seen when both vitamins were used in combination. In conclusion, vitamins C and E have a potentiates the ability to reduce oxidative stress to human cells when tested in vitro; their combined use provides a synergistic effect to protect the cellular antioxidant defense system. Therefore, these two vitamins may also be effective in helping to reduce elevated levels of oxidative stress in disease states.

Introduction

Oxidative stress is a disruption in the balance between the production of reactive oxygen species (ROS) and the ability of living things to neutralize reactive or otherwise harmful species, or fix the damage that occurs as a result of exposure to them [1]. ROS refers to a number of different molecules produced in the body on a cellular level; generally, three types of ROS are included, i.e., superoxide ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2), and highly reactive OH radical ($^{\cdot}OH$); each of these ROS does damage to lipids, proteins, sugars, and DNA within the cell [2]. The body has invested a considerable amount of effort in providing itself with a variety of antioxidant solutions that will negate or lessen the negative effects of ROS. There are many enzymes, including superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx), as well as non-enzymatic compounds, including glutathione, vitamins C and E, and carotenoids [3]. If an organism's reactive oxygen defenses become overwhelmed or unable to keep up with ROS generation, oxidative damage will build up, leading a cell to become abnormal and make a contribution to the (ongoing) development of various chronic diseases such as cardiovascular disease, diabetes, neurodegeneration, and cancer [4].

Vitamin C (L-ascorbic Acid) is an essential nutrient and a powerful water-soluble antioxidant that serves as a strong free radical scavenger within the aqueous environment of cells. As a result of donating electrons to neutralise reactive oxygen species (ROS), it is also responsible for regenerating oxidised vitamin E, supporting the lipid-soluble antioxidant system [5]. In contrast, vitamin E is a chain-breaking fat-soluble antioxidant. It prevents the peroxidation (destruction) of cell membranes caused by free radicals by terminating the propagation phase of the free radical chain reaction, thus preventing further damage [6].

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Many researchers have become interested in the synergistic relationship between vitamin C and vitamin E. Specifically, when vitamin C regenerates vitamin E (in its oxidised form known as tocopheroxyl radical) back to active form, it enhances the cellular membrane antioxidant protection provided by vitamin E [7]. This evidence of biochemical cooperation between the two vitamins suggests that they may provide a greater benefit when consumed together compared to either one alone.

Several clinical trials and some in vitro studies have shown that vitamins C and E, when taken together, can significantly reduce the levels of oxidative stress markers in the body. In Moabedi and Milajerdi's systematic review and meta-analysis [7] that included a total of 17 randomized controlled trials with 965 participants, there were statistically significant reductions in malondialdehyde (MDA), WMD – 0.38 ug/L and lipid peroxides; there were also statistically significant increases in total antioxidant capacity (TAC), and glutathione peroxidase (GPx) activity after taking both vitamins. The potential for synergy is present because the combined effect of the two vitamins exceeds the individual effects when these two vitamins are combined. One of the documented biochemical mechanisms by which antioxidant protection is enhanced when these two vitamins are taken together is that vitamin C regenerates vitamin E within biological membranes [7]. Hidayatik et al. [9] also reported that in a stress model, significant increases were observed in superoxide dismutase (SOD), GPx, and catalase (CAT) activities, and a corresponding decrease was noted in MDA after administering vitamins C and E together. Additionally, several in vitro studies using human red blood cells [10] and human erythrocytes [11] have demonstrated that the combination of vitamins C and E provides protection against oxidative damage caused by hydrogen peroxide (H_2O_2) in a greater extent than that caused by either vitamin alone.

Malondialdehyde (MDA), a by-product of lipid peroxidation, is one of the most widely utilised markers for assessing oxidative stress levels [8]. Enzymatically-mediated antioxidant function (SOD, CAT, GPx and TAC) reflects overall cellular protection. Determining levels of markers before and after antioxidant treatment is a useful method for evaluating the protective effectiveness of antioxidant interventions in scientific experiments. The limited number of in vitro studies performed on human primary cells in the Egyptian context contrasts with the well-established antioxidant activity of vitamins C and E. A better understanding of how these vitamins modulate oxidative stress markers in human PBMCs (=peripheral blood mononuclear cells) will have clinical implications because PBMCs are critical components in mediating immune function and are more vulnerable to oxidative damage. This study was therefore designed to assess the individual and synergistic effects of vitamins C and E on oxidative stress markers (MDA, SOD, CAT, GPx, & TAC) in H_2O_2-induced oxidative stress of human PBMCs using a pre/post-experimental design.

Methods

Study Design and Ethical Approval

This study was an experiment conducted in the Biochemistry Department at Cairo University in Egypt between January and June 2022. The study was approved by the Institutional Ethics Committee at Cairo University (IRB-CU-2023-007). All subjects gave written informed consent to participate in the study.

Participants and Inclusion Criteria

Sixty (30 males, 30 females) healthy adult volunteers aged 18 to 45 years were enrolled. The following were the exclusion criteria from this study: any history of chronic disease (e.g. diabetes, cardiovascular disease); current use of antioxidant supplements or anti-inflammatory medications in the past three months; current smoking; and any acute infection at the time of enrollment.

PBMC Isolation

Peripheral blood (20 mL) was collected by venipuncture from each subject into a tube containing EDTA. Peripheral blood mononuclear cells (PBMCs) were isolated from whole blood using Ficoll-Hypaque density-gradient centrifugation (400 × g for 30 minutes at 20° C). The isolated PBMCs were washed two times with phosphate-buffered saline (PBS) and resuspended in RPMI-1640 tissue-culture medium (containing 10% fetal bovine serum, 100 IU/mL penicillin and 100 µg/mL streptomycin). Cell viability was determined by trypan blue exclusion assays and consistently found to be greater than 95%.

Induction of Oxidative Stress and Antioxidant Treatment

For this experiment, cells were cultured at a density of one million cells per milliliter in 24-well plates and divided into four groups:

Group 1 (control): Cells cultured in RPMI with no treatment.

Group 2 (H2O2 group): Cells were treated with hydrogen peroxide at 200 um for 2 hours to induce oxidative stress.

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Group 3 (vitamin C): After exposure to H2O2, cells were treated with ascorbic acid (100 um) for four hours.
Group 4 (vitamin E): After exposure to H2O2, Cells were treated with alpha tocopherol for 4 hours.
Group 5 (vitamin C plus vitamin E combined): After exposure to H2O2, Cells were treated with 100 um of ascorbic acid and 50 um of α-tocopherol for 4 hours.

Biochemical Analyses

MDA levels were measured via Ohkawa's method of forming thiobarbituric acid-reactive substances (TBARS). SOD activity was assessed via a commercially available colorimetric kit and the xanthine oxidase method (Randox, United Kingdom). CAT activity was determined using spectrophotometric techniques to follow the decomposition of hydrogen peroxide at 240 nm. GPx activity was assessed via a coupled reaction with glutathione reductase. TAC was determined using the ferric reducing ability of plasma (FRAP) assay. The Bradford method was performed to quantify the total amounts of protein for normalization purposes at each sample location.

Statistical Analysis

Data are presented as mean ± SD and were analysed by SPSS version 26.0. Analysis of differences between groups was performed using one-way ANOVA with Tukey's post hoc test. Paired t-tests were performed to compare pre and post-values within each group. The level of significance was $p < 0.05$.

Results

The sample consisted of 60 participants whose mean age was 28.3 ± 6.4 years. All four groups were homogeneous ($p > 0.05$) based on their baseline MDA, SOD, CAT, GPx, and TAC prior to treatment ($p > 0.05$). After 200 µM H_2O_2 exposure for 2 hours, levels of oxidative stress were significantly elevated compared to PBMC controls, indicated by a 127% increase in MDA ($p < 0.01$). SOD (43%), CAT (38%), GPx (47%), and TAC (41%) were all found to be at lower levels than those measured in the corresponding control groups ($p< 0.001$). Administration of vitamin C (100 µM) after exposure to H_2O_2 significantly decreased MDA levels by 38.4% compared to the H_2O_2 group ($p < 0.001$). In addition, vitamin C treatment significantly increased SOD (31.2% increase, $p < 0.001$), CAT (27.8% increase, $p < 0.001$), GPx (34.5% increase, $p < 0.001$), and TAC (29.3% increase, $p < 0.001$) compared to the H_2O_2-only group.
Vitamin E treatment at 50 µM produced similar effects as vitamin C treatment with decreases of 41.2% ($p < 0.001$) for MDA and increases of 28.6% ($p < 0.001$) for SOD, 30.4% ($p < 0.001$) for CAT, 31.9% ($p < 0.001$) for GPx and 33.1% ($p < 0.001$) for TAC, in comparison to the H2O2 only group. The combined treatment group had the largest dose-dependent antioxidant response. MDA decreased by 53.7% compared to the H2O2-alone group ($p < 0.001$) and this decrease was significantly greater than either vitamin treatment alone ($p < 0.05$ for both comparisons). SOD, CAT, GPx and TAC were restored to 47.3%, 43.8%, 52.4% and 48.7%, respectively. Although none of the combined treatment group's values returned to the baseline control values, all values were significantly improved in comparison with both individual treatment groups.

Table 1. Oxidative stress markers across study groups (Mean ± SD)

Parameter	Control	H_2O_2 Group	Vit. C	Vit. E	Combined
MDA (nmol/mg protein)	2.14 ± 0.31	4.87 ± 0.52*	3.00 ± 0.38†	2.86 ± 0.41†	2.25 ± 0.29‡
SOD (U/mg protein)	42.6 ± 4.1	24.3 ± 3.8*	31.9 ± 4.3†	30.2 ± 3.9†	35.8 ± 4.0‡
CAT (U/mg protein)	38.4 ± 3.7	23.8 ± 3.2*	30.4 ± 3.6†	31.0 ± 3.8†	34.2 ± 3.5‡
GPx (mU/mg protein)	29.7 ± 3.2	15.7 ± 2.9*	21.1 ± 3.1†	20.7 ± 3.0†	23.9 ± 2.8‡
TAC (mmol/L)	1.82 ± 0.18	1.07 ± 0.14*	1.38 ± 0.17†	1.43 ± 0.16†	1.60 ± 0.15‡

** $p < 0.001$ vs. Control; † $p < 0.001$ vs. H_2O_2 Group; ‡ $p < 0.05$ vs. individual vitamin groups. MDA = malondialdehyde; SOD = superoxide dismutase; CAT = catalase; GPx = glutathione peroxidase; TAC = total antioxidant capacity.*

Discussion

Our research indicates that both Vitamins E and C are capable of reducing oxidative stress caused by hydrogen peroxide (H_2O_2) in human peripheral blood mononuclear cells (PBMCs); moreover, when these antioxidants are combined, they provide greater protection from oxidative damage compared to either alone. The findings of this study are supported by many other studies demonstrating the ability of Vitamins E and C to act as antioxidants. The increase in malondialdehyde (MDA) after treatment with H_2O_2 demonstrates that we have created a model for lipid peroxidation in human PBMCs, similar to several previous studies in this area [12]. The reduction in superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (Gpx), and total antioxidant capacity (TAC) following exposure to H_2O_2 suggests a depletion of the cells' ability to

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neutralize ROS. This supports the idea that the cells were overwhelmed with excess ROS [3]. The use of vitamin C resulted in a decrease in MDA levels by 38.4% and also caused a significant increase in antioxidant enzyme activity levels. This effect agrees with the role of ascorbic acid being an effective direct scavenger of superoxide, hydroxyl radicals, and singlet oxygen found within the aqueous phase of cellular compartments [5]. The increase in activity for SOD and CAT enzymes may also indicate that vitamin C can potentially upregulate or maintain these antioxidant enzymes in an indirect manner, possibly due to the reduction of ROS levels that typically result in the inactivation of these enzymatic antioxidants.

Vitamin E led to a significant decrease of 41.2% in MDA, along with comparable improvements in antioxidant enzyme activity. This reflects the roles of α-tocopherol as a major chain-breaking antioxidant protecting against lipid peroxidation of cellular membranes [6]. It is worth noting the slightly greater ability of vitamin E to decrease MDA, compared to vitamin C; this difference may be due to the lipid-phase activity of tocopherol being more effectively able to inhibit membrane-peroxidation caused by exposure to H_2O_2. Co-treatment with vitamins C and E resulted in a considerably larger reduction (by 53.7%) of MDA (malondialdehyde), as well as completely restoring all the antioxidant markers better than either vitamin alone. Mechanistically, the synergistic interaction between ascorbate and tocopherol (vitamins C and E) is explained by the changes in electron transfer, in which the ascorbate donates electrons to convert the tocopheroxyl radical back to active α-tocopherol. This regeneration allows vitamin E to have a more extended antioxidant capacity throughout biological membranes, while ascorbate helps to provide its own independent antioxidant protection in the aqueous phase. The cooperative antioxidant activity of vitamins C and E also was supported by the findings of Anastasiadi et al. [10], who showed that the combined use of both vitamins totally prohibited the hemolytic actions of H_2O_2 on red blood cells at the highest dosages studied.

The greatest elevations in TAC (total antioxidant capacity) across all treatment groups indicate that both vitamins contribute to the total non-enzymatic antioxidant pool. The greatest increase (48.7%) in TAC was seen in the combined group, providing additional support for the notion of cooperative antioxidant protection. These results were consistent with the meta-analysis completed by Moabedi and Milajerdi [7], which demonstrated a significant cumulative increase in TAC following combined supplementation of vitamins E and C (WMD: 0.09, 95% CI: 0.05-0.13 mmol/L, $p < 0.001$). The improved mean MDA for the combined treatment group was not entirely equivalent to the baseline control MDA, however, the degree of improvement would be considered clinically meaningful and statistically better than the individual vitamin treatments. This observation correlated with the dose-dependent effects of antioxidants on protection from oxidative damage, implying that a higher dose or longer duration of use may produce higher degrees of restoration of oxidative homeostasis.

One limitation to this study was that it was carried out in vitro so the direct extrapolation to a physiological human condition (i.e. in vivo) cannot be made. Also, this study was limited by the focus on short-term treatment responses. Longer treatment durations or repeated treatments may produce different treatment response outcomes. Future studies need to look at the dose-response of vitamin C and E in relation to other antioxidants when treating human primary cells.

Conclusion

Researchers have shown that both vitamin C and vitamin E are able to reduce oxidative stress caused by H_2O_2 in human PBMCs. This conclusion is based on measurable decreases in MDA as well as increases in SOD, CAT, GPx, and TAC activity levels after taking these vitamins. The administration of vitamin C and vitamin E together has a greater antioxidant ability than either vitamin alone. This supports the conclusion that these vitamins work together through ascorbate-mediated recycling of tocopherol. The information here provides a chemical basis for the potential use of vitamin C and vitamin E together as a form of antioxidant therapy in settings where high levels of oxidative stress are present. Further studies conducted in vivo are needed to support these results and establish optimal dosing regimens.

Conflict of interest. Nil

References

1. Sies H. Oxidative stress: concept and some practical aspects. *Antioxidants*. 2020;9(9):852. doi:10.3390/antiox9090852.
2. Li D, Ding Z, Du K, Ye X, Cheng S. Reactive oxygen species as a link between antioxidant pathways and autophagy. *Oxid Med Cell Longev*. 2021;2021:5583215. doi:10.1155/2021/5583215.
3. Alhojaily SM. From imbalance to impairment: the central role of reactive oxygen species in oxidative stress-induced disorders and therapeutic exploration. *Front Pharmacol*. 2023;14:1269581. doi:10.3389/fphar.2023.1269581.

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4. Dubois-Deruy E, Peugnet V, Turkieh A, Pinet F. Oxidative stress in cardiovascular diseases. *Antioxidants*. 2020;9(9):864. doi:10.3390/antiox9090864.
5. Carr AC, Maggini S. Vitamin C and immune function. *Nutrients*. 2017;9(11):1211. doi:10.3390/nu9111211.
6. Azzi A. Molecular mechanism of α-tocopherol action. *Free Radic Biol Med*. 2007;43(1):16-21. doi:10.1016/j.freeradbiomed.2007.03.013.
7. Moabedi M, Milajerdi A. The effect of co-administration of vitamin E and C supplements on plasma oxidative stress biomarkers and antioxidant capacity: a GRADE-assessed systematic review and meta-analysis of randomized controlled trials with meta-regression. *Front Immunol*. 2025;16:1547888. doi:10.3389/fimmu.2025.1547888.
8. Dharmajaya R, Sari DK. Malondialdehyde value as radical oxidative marker and endogenous antioxidant value analysis in brain tumor. *Ann Med Surg*. 2022;76:103231. doi:10.1016/j.amsu.2021.103231.
9. Hidayatik N, Purnomo A, Fikri F, Purnama MTE. Amelioration of oxidative stress, testosterone, and cortisol levels after administration of vitamins C and E in albino rats with chronic variable stress. *Vet World*. 2021;14(1):137-43. doi:10.14202/vetworld.2021.137-143.
10. Anastasiadi AT, Stamoulis K, Kriebardis AG, Tzounakas VL. Synergistic effects of a novel combination of natural compounds prevent H_2O_2-induced oxidative stress in red blood cells. *Front Physiol*. 2025;15:1499308. doi:10.3389/fphys.2024.1499308.
11. Jain V, Vani RS. Synergistic activity of vitamin C and vitamin E to ameliorate the efficacy of stored erythrocytes. *Transfus Clin Biol*. 2023;30(1):87-95. doi:10.1016/j.tracli.2022.09.002.
12. Xie D, et al. Vitamin supplementation protects against nanomaterial-induced oxidative stress and inflammation damages: a meta-analysis of in vitro and in vivo studies. *Nutrients*. 2022;14(11):2214. doi:10.3390/nu14112214.
13. Ji LL, Yeo D. Oxidative stress: an evolving definition. *Fac Rev*. 2021;10:13. doi:10.12703/r/10-13.
14. Azzi A. Oxidative stress: what is it? Can it be measured? Where is it located? Can it be good or bad? Can it be prevented? Can it be cured? *Antioxidants*. 2022;11(8):1431. doi:10.3390/antiox11081431.
15. Bagheri Hosseinabadi M, et al. Investigating the effects of vitamins E and C on oxidative stress and hematological parameters among power plant workers: a double-blind randomized controlled clinical trial. *Toxicol Ind Health*. 2020;36(2):99-109. doi:10.1177/0748233720908993.
16. Anastasiadi AT, Stamoulis K, Kriebardis AG, Tzounakas VL. Erythrocyte oxidative status in people with obesity: relation to tissue losses, glucose levels, and weight reduction. *Antioxidants*. 2024;13(8):960. doi:10.3390/antiox13080960.
17. Patel TA, et al. Role of nanoparticle-conjugates and nanotheranostics in abrogating oxidative stress and ameliorating neuroinflammation. *Antioxidants*. 2023;12(10):1877. doi:10.3390/antiox12101877.

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