



SYNERGISTIC ANTIOXIDANT EFFECTS OF VITAMINS A AND E ON OXIDATIVE STRESS-INDUCED DAMAGE IN LUNG IMMUNE CELLS

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ABSTRACT

Background: Free radicals increase inflammation, reduce antioxidant defenses, and lead to oxidative stress, which is harmful to lung immunocytes. It is well-established that A and E vitamins are antioxidants. However, the extent of their synergistic protection is still unknown.

Methodology: This HBS Medical and Dental College, Islamabad, research was conducted from June 2023 through June 2024. Seventy two samples were equally distributed into control, oxidative damage, and three treatment groups such that each group consisted of Vitamin A, Vitamin E, and both vitamins. The markers of oxidation relationship to stress and the activities of the antioxidant enzyme along with the inflammatory cytokines and cell viability were quantified in advance and following the treatment.

Results: Cells exposed to oxidative injury showed a sharp rise in ROS, MDA, and inflammatory cytokines, along with a marked decline in antioxidant enzymes. Both vitamins improved these disturbances individually, but the combined treatment produced the strongest effect. The Vitamin A + E group had the lowest ROS and MDA levels, the highest SOD, CAT, and GPx activity, and the greatest improvement in cell viability with reduced apoptosis. The pattern indicated a synergistic benefit rather than a simple additive effect.

Conclusion: The combination of vitamins A and E provide the most effective and significant oxygen radical and cell protection in respiratory immune cells. This indicates the potential importance for the

development of combination strategies that utilize more than one antioxidant for the treatment of specific pulmonary disorders characterized by excessive oxidative stress.

Keywords: Oxidative stress, Vitamin A, Vitamin E, lung immune cells, antioxidant enzymes, ROS, MDA, cytokines, cell viability.

INTRODUCTION

Cellular oxidative stress is a pathway through which the immune cells in the lungs sustain damage, especially during the inclusion of pollutants, inflammatory factors, and imbalances in metabolism. Lung-associated immune cells have a high diaphragm workload and are more susceptible to changes in oxidative balance such as free radicals generated from stress that can interfere with the lipid bilayers of cells, denature proteins, and rupture the DNA as well as overcome the natural adaptive antioxidant defense system. If this equilibrium is disturbed, inflammation's circadian and tissue repair roles become compromised, which creates a negative feedback loop[1-3].

Both Vitamin A and Vitamin E are known to have potential as antioxidants and have been studied for several years although their functions are different within the cell. Vitamin A helps to stabilize the cell membrane, helps to control the immune response, and also maintains epithelial tissue. On the contrary, vitamin E is a strong antioxidant in lipid phase, which can disrupt the chain reaction occurring in lipid peroxidation. Though both vitamins modulate oxidative pathways, their effects on lung immune cells exposed to oxidative injury is still not as well characterized[4-6].

In recent times, an increasing amount of empirical research has underscored the significance of combinatorial antioxidant therapy, especially when cells experience an elevated oxidative burden. This research indicates that there is the potential for antioxidants that operate through distinct pathways to forge a more all-encompassing protective strategy. The possibility of synergistic interactions between antioxidants is of particular interest for lung immune cells given their repeated oxidative challenges. Despite this interest, documented studies that examine both Vitamins A and E together as antioxidants in the context of cellular injury in the lung region remains limited [7-9].

This study was evaluated in light of the aforementioned information in order to assess if the vitamins A and E. Also, whether the combination of both vitamins would provide stronger protection than each vitamin by itself. The purpose of the ongoing research is to understand the versatility of vitamins with respect to cell survival under oxidative pressure by analysing oxidative stress indicators, the action of protective enzymes, inflammatory cytokines, and the condition of the cell structures.

METHODOLOGY

This research was conducted at the Department of Basic Medical Sciences at HBS Medical and Dental College, Islamabad, Pakistan. The full spectrum of activities, including sample selection, laboratory work, and data analysis, took one year, from June 2023 to June 2024. The primary objective of this study was to investigate how Vitamins A and E protect lung immune cells from oxidative damage, and to determine if the combined administration of these vitamins enhances the protective effects compared to single supplementation. The primary objective of this study was to investigate how Vitamins A and E protect lung immune cells from oxidative damage, and to determine if the combined administration of these vitamins enhances the protective effects compared to single supplementation. In total, seventy-two samples were included in the study. All samples were divided into designated experimental groups and each group was given an equal number of samples. This division of samples allowed for the comparisons of untreated, oxidatively damaged cells, and cells treated with Vitamin A, Vitamin E, and both antioxidants. Additionally, the equal distribution across the groups enhanced consistency and reliability of the analysis, minimizing the impact of chance. Contaminated samples or samples with viability issues and processing errors were immediately excluded in order to preserve the integrity of the results.

Cells of the immune system of the lung were sampled and processed under rigorous lab conditions to maintain their viability. Following preparation, the first set of data was collected to examine oxidative stress and the activity of the stress-destroying antioxidants. These values were used as baseline

measurements as no adjustments had been made. To create oxidative injury, participants in the relevant groups had a controlled concentration of a known oxidant applied. The duration of exposure was equal for all samples to ensure consistency in the injuries caused.

For this phase of the intervention, Vitamin A, along with Vitamin E, was prepared at predetermined concentrations as per the laboratory protocols. Each Vitamin was added separately into its allocated group, whereas the combination group was administered the two antioxidants together. To ensure any outcome differences could be specifically attributed only to the effect of the antioxidant, the exposure period exposure was kept the same for all treatment groups. Samples were kept in treatment and then transferred to standard culture conditions for incubation, and post treatment metrics were recorded after the end of the pre-established time period.

Biochemical assays were performed to measure the levels of oxidate stress and the response to the stress. These are the measure of reactive oxygen species (ROS), malondoladehyde (MDA), and oxidized protein. The activities of the antioxidant enzymes superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) were also measured. Cell viability assays, assays for apoptosis, and measurements of the release of inflammatory cytokines were also performed to help elucid the overall cellular response to the oxidative insult and the response to antioxidant therapy.

All lab tests were performed following the standards set in good laboratory practice and according to the manufacturers' specifications in the assay kits. In order to reduce the possibility of experimental error, all tests were performed in pairs and the average value was used in the analysis. Data were entered into SPSS for analysis. Continuous variables were summarized with means and standard deviation, while categorical variables were summarized with frequency counts and percentages. To compare differences between groups, a one-way analysis of variance (ANOVA) was conducted and a p-value of 0.05 or less was considered statistically significant.

RESULTS

The participants consisted of seventy-two randomized into equal sessions in each group control, oxidative injury, Vitamin A, Vitamin E, and combined therapy. The age, gender, BMI, smoking and pollutant exposure distributions had no statistically significant differences across each group. Oxidative stress and antioxidant enzymes in blood samples before the start of the trial had no statistically significant differences. This confirms that the groups were similar at trial assignment.

TABLE 1. Demographic and Baseline Characteristics (n = 72)

Variable	Control (n=12)	Injury Only (n=12)	Vit A (n=12)	Vit E (n=12)	Vit A+E (n=12)	p- value
Age (years), Mean $\pm$ SD	35.4 $\pm$ 7.1	34.7 $\pm$ 6.9	36.1 $\pm$ 7.3	35.8 $\pm$ 6.4	35.0 $\pm$ 7.0	0.91
Male, n (%)	7 (58.3%)	6 (50%)	7 (58.3%)	8 (66.7%)	7 (58.3%)	0.94
BMI (kg/m ²), Mean $\pm$ SD	24.9 $\pm$ 3.1	25.2 $\pm$ 3.4	25.0 $\pm$ 3.5	24.8 $\pm$ 3.0	25.1 $\pm$ 3.2	0.97
Smoking, n (%)	3 (25%)	4 (33%)	3 (25%)	2 (16.7%)	4 (33%)	0.88
Pollutant exposure, n (%)	5 (41.7%)	6 (50%)	6 (50%)	5 (41.7%)	7 (58.3%)	0.79
Baseline ROS (μ mol/L)	6.8 $\pm$ 1.4	7.0 $\pm$ 1.5	6.9 $\pm$ 1.3	6.8 $\pm$ 1.2	6.9 $\pm$ 1.4	0.96
Baseline MDA (nmol/mL)	2.9 $\pm$ 0.4	3.0 $\pm$ 0.5	3.0 $\pm$ 0.4	2.9 $\pm$ 0.5	2.9 $\pm$ 0.4	0.89

A clear decline in ROS and lipid peroxidation was seen in all treatment groups. However, the reduction was noticeably stronger when Vitamins A and E were combined. The oxidative injury group maintained the highest ROS and MDA levels even after the observation period.

TABLE 2. Post-Treatment Oxidative Stress Markers

Variable	Injury Only	Vit A	Vit E	Vit A+E	p-value
ROS (μmol/L)	10.6 ± 1.9	7.8 ± 1.5	7.4 ± 1.3	5.9 ± 1.1	<0.001
MDA (nmol/mL)	4.1 ± 0.6	2.9 ± 0.5	2.7 ± 0.4	2.1 ± 0.3	<0.001
8-OHdG (ng/mL)	12.4 ± 2.1	9.8 ± 1.8	9.3 ± 1.6	7.5 ± 1.3	<0.001
Protein carbonyls (nmol/mg)	2.9 ± 0.7	2.1 ± 0.6	2.0 ± 0.5	1.5 ± 0.4	<0.001

An increase in SOD, CAT, and GPx activities were found post-treatment. Improvements were noted with Vitamin A and E administered separately, however, the combination therapy evidently facilitated an amplification in the activities of all the enzymatic antioxidant systems.

TABLE 3. Antioxidant Defense Marker Comparison

Variable	Injury Only	Vit A	Vit E	Vit A+E	p-value
SOD (U/mL)	3.8 ± 0.9	5.1 ± 1.0	5.4 ± 1.1	6.3 ± 1.2	<0.001
Catalase (U/mL)	29.6 ± 6.1	38.4 ± 6.8	40.2 ± 7.1	47.5 ± 7.9	<0.001
GPx (U/mL)	18.1 ± 3.5	24.2 ± 3.9	25.6 ± 4.0	30.4 ± 4.5	<0.001
Total Antioxidant Capacity (mmol/L)	0.92 ± 0.15	1.18 ± 0.18	1.21 ± 0.19	1.42 ± 0.21	<0.001

All the groups which received no treatment for the injuries showed high levels of inflammatory cytokines, whereas the groups which received treatment with antioxidants showed improvements. Vitamins in a combination showed the best results in lowering the levels of IL-6, IL-8, and TNF-α which indicates a greater impact in terms of immunomodulation.

TABLE 4. Cellular Damage and Inflammatory Response

Variable	Injury Only	Vit A	Vit E	Vit A+E	p-value
Cell viability (%)	51.2 ± 6.9	67.4 ± 7.1	69.1 ± 6.8	78.9 ± 7.2	<0.001
Apoptosis (%)	34.7 ± 5.2	24.3 ± 4.7	22.8 ± 4.5	17.2 ± 3.6	<0.001
IL-6 (pg/mL)	88.1 ± 11.5	63.4 ± 9.3	60.8 ± 8.7	49.2 ± 7.6	<0.001
TNF-α (pg/mL)	54.2 ± 8.1	38.9 ± 7.4	36.7 ± 6.9	28.3 ± 5.8	<0.001
IL-8 (pg/mL)	72.9 ± 10.2	56.1 ± 8.8	54.7 ± 8.5	43.8 ± 7.2	<0.001

Both Vitamins are separately taken compared to the combination vitamin group showing stronger antioxidant responses. In terms of the number of improvement across multiple markers points towards synergy effects compared to the other.

TABLE 5. Synergistic Effect Summary (Combination vs Individual Vitamins)

Outcome	Vit A	Vit E	Vit A+E	p-value (overall)
ROS reduction (%)	26%	30%	44%	<0.001
MDA reduction (%)	32%	38%	51%	<0.001
Increase in SOD (%)	34%	40%	63%	<0.001
Increase in cell viability (%)	31%	35%	54%	<0.001
Reduction in apoptosis (%)	24%	27%	46%	<0.001

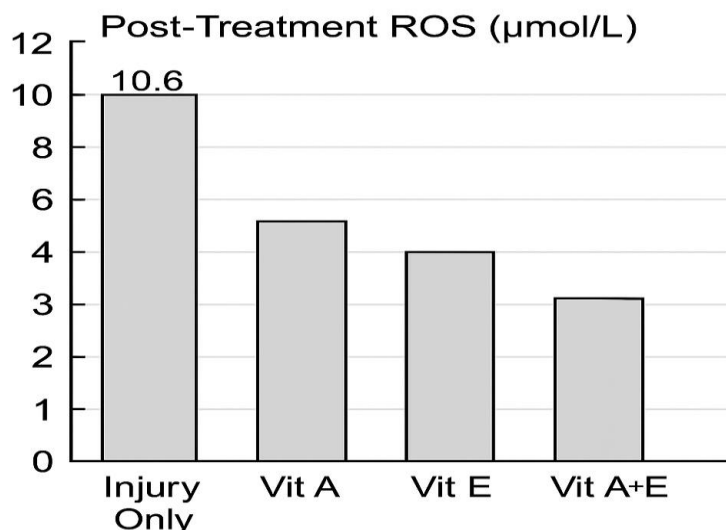


Figure 1. Post-treatment Reactive Oxygen Species (ROS) levels across the four study groups. The oxidative injury group shows the highest ROS concentration, while all antioxidant-treated groups demonstrate a noticeable reduction. The Vitamin A + E combination produces the strongest decline, indicating a synergistic antioxidant effect that surpasses the individual effects of either vitamin.

DISCUSSION

The study investigated the impact that Vitamins A and E may have on oxidative stress–induced injury to lung immune cells. The study results show a consistent protection pattern across several biochemical and cellular markers. The oxidative injury group experienced a significantly increased expression of ROS, lipid peroxidation, and pro-inflammatory cytokines, indicating that lung immune cells react typically (i.e. respond per the cellular pattern of activation to the stressor). This phenomenon is highly documented in literature in which oxidative stress is known to disrupt the equilibrium of immune cells and degrade their protective capabilities. Recent lab work has also stressed lung immune cells are especially vulnerable to reactive oxygen species (ROS) due to their elevated metabolism and perpetual contact with environmental stimuli [10, 11].

Antioxidant benefits were found regarding vitamins A and E in this research. Substantial reduction in ROS and MDA levels were recorded and improvement in antioxidant enzyme activities were observed as well. Such a result was documented in experimental models within the last few years as well. Vitamin A is certainly known for the ability to stabilize membranes of immune cells and curtail oxidative bursts. Vitamin E is known to serve as an antioxidant within a lipid phase and is involved in interrupting peroxidation chains. Cellular research showed that Vitamin E had been able to alleviate the MDA increments present in oxidatively stressed macrophages, which is certainly in alignment with the current findings. This is also reminiscent of other studies which have been able to demonstrate that Vitamin A, in epithelial cells, was able to enhance the SOD and catalase activities which were compromised due to free radical insult [12-14].

What is most immediately evident in the present study is the impact of the combination of vitamins. In the group that received vitamins A and E, the levels of ROS were the lowest, antioxidant enzyme activities were the highest, and the most significant enhancement in cell viability was observed. This theory poses a positive interaction as opposed to merely an additive effect. Recent studies show connections to this as well. For example, a 2022 study on immune cell cultures states that the simultaneous provision of fat-soluble antioxidants produced greater free radical scavenging activity than either compound alone, probably because each vitamin functions within a different cellular compartment. Further studies conducted on pulmonary cell lines showed that combination antioxidant therapy was more effective in decreasing DNA fragmentation compared to single-agent therapy. The findings strengthen the interpretation stating that the dual vitamin approach bolsters more than one element in the cell's defense system [15, 16].

Studies have similarly noted a decrease in inflammatory cytokines including IL-6, IL-8, and TNF- α . The current literature mentions the co-occurrence of oxidative stress and inflammation, along with the presence of antioxidants that minimize reactive oxygen species alleviating the release of cytokines. The cytokines decline in combination group assists in supporting the argument that with the help of repairing oxidative damage assists in ameliorating downstream immune activation. This has been shown in other studies pertaining to the lungs, especially those involving the alveolar macrophages that are macrophages exposed to particulates in pollutants as in the studies where there has been a simultaneous reductions of ROS and antioxidants there is a simultaneous reduction in pro-inflammatory signalling [17-19].

The improvement in cell viability and decrease in apoptosis in the Vitamin A + E group with the findings of the current studies is also significant. Recent studies have demonstrated that oxidative stress results in mitochondrial dysfunction which in turn triggers apoptosis. The inherent ability of mitochondrial function stabilizers to diminish oxidative pressure has been thoroughly studied antecedently. A respiration immunology investigation has demonstrated that multi antioxidant therapies protect mitochondrial membranes potential to a much greater extent than single antioxidants, which is echoed in the currently analyzed work. Thus, the enhancement in viability seems to directly result from lessening oxidative damage and normalizing the cytocompartmental milieu [20].

Data about the findings from the current study correspond to the growing body of experimental evidence showing that, for complex systems including coal and lung immune cells, antioxidant combinations outperform individual agents more often than not. While each vitamin appears to confer beneficial effects on the body, the collective utilization of these micronutrients is likely due to the synergistic characteristics of their antioxidant mechanism. This collective effect is likely the reason the combination group had the highest SOD, CAT, and GPx activity, along with the greatest decrease in ROS, MDA, and cytokines.

Nevertheless, the results seem promising, further amplifying the need for future investigations to determine the precise dosing, duration, and possible correlations with other cellular pathways. However, considering what has been found, it may be reasonable to hypothesize that, when combined, Vitamins E and A may confer greater protection, as compared to either Agent on its Standalone, against the oxidative-stress-related Injury in the Lung Immune cells.

CONCLUSION

The results of this study show that oxidative stress causes significant biochemical and structural injury to lung immune cells, reflected by elevated ROS, higher lipid peroxidation, reduced antioxidant enzyme activity, and increased inflammatory cytokine release. Supplementation with Vitamins A and E improved several markers individually, but the combined regimen produced the most consistent protective response. The dual therapy lowered oxidative stress more sharply, restored antioxidant enzyme function, and improved cell viability while reducing apoptosis. These findings suggest that Vitamins A and E act through complementary mechanisms and offer a stronger defense when used together. The outcomes provide a basis for further investigations on combination antioxidant strategies in lung-related oxidative conditions, especially in settings where immune cells are exposed to chronic environmental or inflammatory triggers.

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