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Quantitative Analysis of Antioxidant Protection Against Oxidative Stress: A Model of Cellular Damage in *Saccharomyces cerevisiae*

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ABSTRACT

Reactive oxygen species (ROS) play an important role in myocardial ischemia-reperfusion injury, which can cause further damage to cardiac cells after blood flow is restored following a myocardial infarction. Compounds capable of reducing oxidative damage are therefore of great cardiovascular interest. This study used *Saccharomyces cerevisiae* (yeast) to test whether two antioxidants, ascorbic acid (vitamin C) and green tea extract (GTE), could reduce cellular damage caused by hydrogen peroxide (H_2O_2). Twenty-four experimental samples were divided into four groups (Control; Stress: 3% H_2O_2 stock solution; Stress + Vitamin C; Stress + GTE; n = 6 replicates/group). Samples were analyzed at four time points (0, 15, 30, and 60 minutes) using methylene blue viability staining, budding rate analysis, and cellular morphology measurements with ImageJ. I also developed a composite Oxidative Damage Index (ODI) using ImageJ measurements of circularity, solidity, and aspect ratio. Higher ODI scores indicated greater structural damage to the cells.

At 60 minutes, the untreated stress group showed 92.39% cell death, compared with 36.45% for the vitamin C group and 39.17% for the GTE group. One-way ANOVA showed significant differences between groups in percent cell death, budding rate, circularity, and ODI (all $p < 0.001$). Tukey HSD analysis showed that both antioxidant groups had significantly less cell death than the untreated stress group ($p < 0.001$). The difference between the vitamin C and GTE groups was also statistically significant ($p = 0.030$). Compared with the untreated stress group, vitamin C reduced ODI by 57.5%, while GTE reduced it by 51.8%. Pearson correlation analysis also showed a strong positive relationship between the length of H_2O_2 exposure and percent cell death ($r = 0.966$, $p < 0.001$).

These findings show that both vitamin C and green tea extract reduced H_2O_2-induced cellular damage in yeast. Although this experiment did not directly model myocardial ischemia-reperfusion injury, it demonstrated how antioxidants can protect cells against oxidative stress. Further studies using cardiac cells would be needed to determine whether these findings could have applications in preventing cardiovascular injury.

INTRODUCTION

Cardiovascular disease is still the number one cause of death worldwide, responsible for almost 17.9 million deaths per year (1). An important process mediating acute myocardial injury is the abnormal build-up of reactive oxygen species (ROS) that exceeds the capacity of the natural antioxidant defenses. ROS accumulation is a hallmark of myocardial ischemia-reperfusion injury (IRI), which paradoxically occurs when coronary blood flow is restored after treatment of acute myocardial infarction (2). After reperfusion, dysfunctional mitochondria and activated NADPH oxidases generate a burst of superoxide anion (O2-) and hydrogen peroxide (H2O2), which damages mitochondrial membranes, oxidizes proteins and lipids, activates pro-apoptotic cascades, and significantly contributes to infarct size and negative cardiac remodeling (3).

Despite decades of research, there are no fully effective pharmacological strategies to limit oxidative injury mediated by reperfusion. Interest has been shown in the cardioprotective effects of antioxidant compounds. Vitamin C, ascorbic acid, is a potent water-soluble direct ROS scavenger and can also regenerate membrane-bound vitamin E (4). Epigallocatechin gallate (EGCG), one of the major catechins in green tea extract, modulates the activity of redox-sensitive transcription factors such as Nrf2 and NF-κB and upregulates the expression of endogenous antioxidant genes (5). Both compounds have demonstrated cardioprotective effects in preclinical models and observational clinical data, but mechanistic controlled studies are still limited (4, 5).

Saccharomyces cerevisiae is a common eukaryotic model organism in oxidative stress research. Core ROS-sensing and detoxification pathways are conserved between yeast and human cardiomyocytes, including superoxide dismutase (SOD1/SOD2), glutathione peroxidase, thioredoxin reductase, and the MAPK Hog1 stress cascade (6, 7). Exogenous H2O2 treatment can induce controlled and reproducible oxidative stress in yeast with dose- and time-dependent effects on cellular viability and morphology (8).
Previous studies mostly looked at H2O2 toxicity in yeast by colony-forming unit assays and biochemical markers of oxidative damage (9, 10). The present study expands this approach to a multidimensional evaluation including methylene blue viability staining, budding rate quantification, and ImageJ-based morphometric analysis. I further developed a composite Oxidative Damage Index (ODI) based on ImageJ-derived morphological measurements to provide an integrated measure of structural oxidative injury.

I hypothesized that antioxidant preconditioning with Vitamin C or green tea extract would significantly reduce H2O2-induced cell death, budding inhibition, and morphological deformation in S. cerevisiae over a 60 min exposure window.

2. METHODS

2.1 Yeast Preparation

Active dry Saccharomyces cerevisiae (Fleischmann's Active Dry Yeast) was dissolved in distilled water at a concentration of 1g per 10 mL at a water temperature of 37–43°C and allowed 10 minutes to activate. Yeast activation was confirmed by the presence of visible CO_2 production via bubbling and its accompanying fresh aroma. All experiments were conducted within 2 hours to ensure consistency with yeast viability.

2.2 Experimental Design

Twenty Four experimental tubes (n=6 replications per condition) were prepared across four treatment groups: (1) Control – no H_2O_2, no antioxidant; (2) Stress – 10 mL of 3% H_2O_2, no antioxidant; (3) Stress + Vitamin C - 10 mL of 3% H_2O_2 with ascorbic acid pretreatment; (4) Stress + Green Tea Extract - 10 mL of 3% H_2O_2 with GTE pre-treatment. Each tube received 18 mL of yeast as a base volume.

2.3 Antioxidant Preparation and Pretreatment

Vitamin C solution (28.4mM) was prepared by dissolving 10 mg of Vitamin C powder in 2 mL of distilled water. GTE stock solution was prepared at 5 mg/mL by dissolving 10 mg of extract in 2 mL of water. Each Stress + Vitamin C and Stress + GTE tube received 1.5 mL of the respective antioxidant solution 15 minutes prior to H_2O_2 being added, giving it time for cellular uptake. Control and stress tubes received 1.5 ml of distilled water as a substitute for the antioxidant.

2.4 Oxidative Stress Treatment

15 minutes after the antioxidant pre-treatment, all stress-group tubes received 10mL of 3% H_2O_2, while the control tubes received 10 mL of distilled water. The final tube volume was 29.5 mL. Tubes were incubated at room temperature. Timing commenced after H_2O_2 implementation.

2.5 Cell Viability Assessment

Samples of 0.5 mL were taken from each tube at 0, 15, 30, and 60 min after H_2O_2 treatment and mixed with 0.5 mL of methylene blue solution. Samples were mounted on glass slides and coverslipped, and images were taken using bright-field microscopy at 400x. Photos taken with a smartphone accessory. Dead cells were blue-stained, and live cells were not blue-stained because of active dye exclusion (11); one example of the procedure is presented in Figure 1. At least 100 cells were counted for each slide. Active cellular proliferation was determined by counting the number of budding cells (cells with visible daughter protrusions).

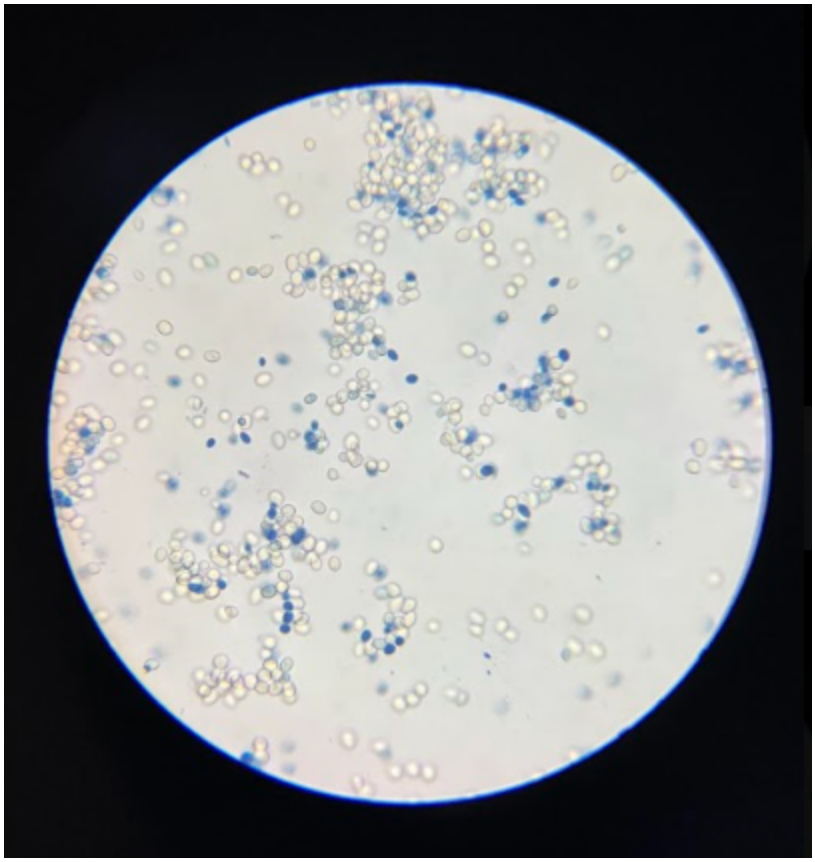


Figure 1. Light micrograph of yeast suspension stained with methylene blue (400×). Blue-stained cells indicate non-viable yeast; colorless cells indicate viable yeast with intact metabolic activity.

2.6 ImageJ Morphological Analysis

After all images were collected, they were processed utilizing ImageJ (National Institutes of Health, Bethesda, MD, USA, v.1.53) to quantify cell morphology. Images were converted to 8-bit greyscale, Watershed to isolate cell boundaries (separating adjacent cells), and analyzed using the Analyze Particle function (size boundary: 50-1000 px2). Four parameters were extracted: mean cell area (px2), circularity, aspect ratio, and solidity. The circular microscope field border was excluded by manual cropping before image analysis (Figure 2).

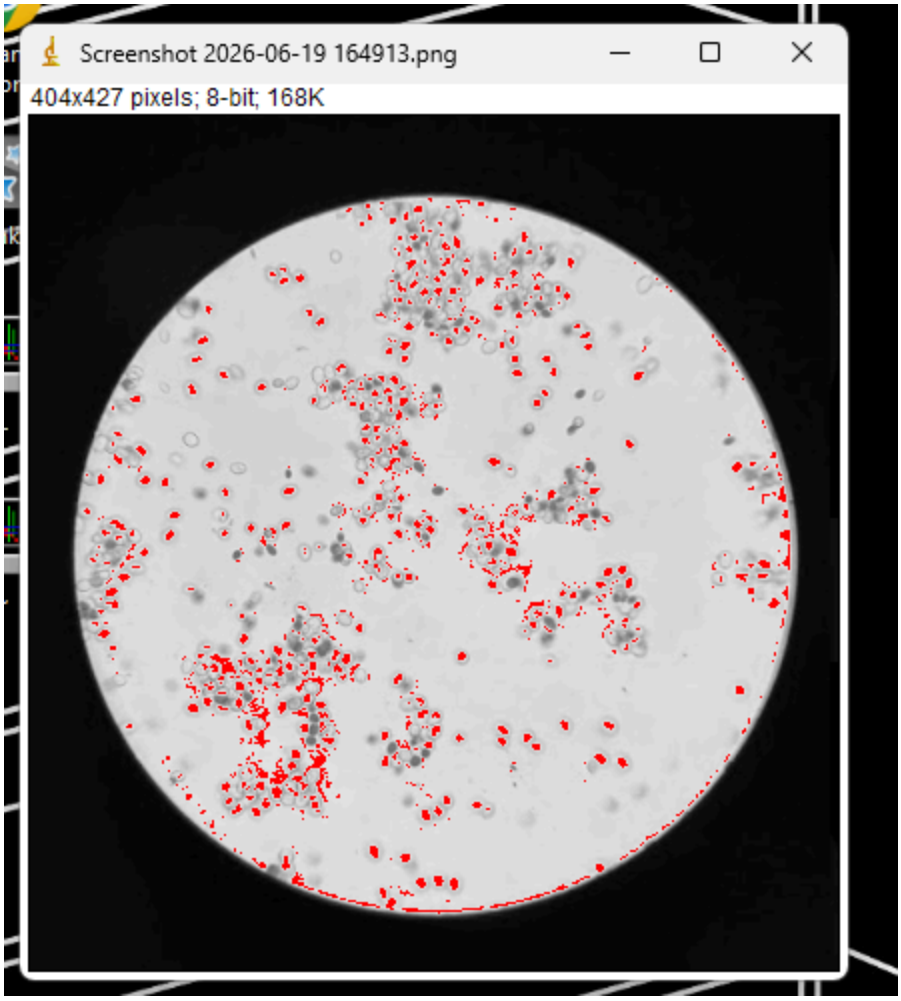


Figure 2. Brightfield micrograph of methylene blue-stained yeast cells (400×) following manual threshold application in ImageJ. Red-highlighted cells represent non-viable yeast identified through manual intensity thresholding for viability quantification.

2.7 Oxidative Damage Index

To provide an objective measurement of oxidative injury, Oxidative Damage Index was calculated using ImageJ-derived data of cell morphology (Eq. 1). ODI integrates three data points captured from ImageJ: circularity (C), solidity (S), aspect ratio (AR), and , and are baseline control means.

ODI =

$$100 \times \left[\tfrac{1}{3}\left(1 - \frac{C}{C^0}\right) + \tfrac{1}{3}\left(1 - \frac{S}{S^0}\right) + \tfrac{1}{3}\left(\frac{AR}{AR^0} - 1\right)\right]$$

(Eq. 1)

At baseline, ODI values were similar among all groups (Table 5), showing equivalent starting morphology. However, following H2O2 treatment, the Stress group showed a marked increase in ODI, indicating structural degradation. Both antioxidant groups mitigated ODI progression throughout the experiment.

2.8 Statistical Analysis

All data are reported as mean ± standard deviation (SD) across six biological replicates. One-way analysis of variance (ANOVA) was performed at the 60-minute time point for all primary endpoints (% dead, % budding, circularity, ODI). Tukey's Honest Significant Difference (HSD) post-hoc test was applied to identify pairwise differences between groups. Pearson's correlation coefficient (r) was calculated between H_2O_2 exposure duration and % dead cells in the stress group.

3. RESULTS AND DISCUSSION

3.1 Baseline Viability Confirms Equivalent Starting Conditions

At t = 0 minutes, all four groups showed low and comparable levels of cell death, ranging from 6.34% ± 0.84% (Control) to 8.97% ± 0.68% (Stress + No antioxidant), confirming equivalent starting viability before H2O2 challenge (Table 1). Baseline budding rates were comparable, ranging from 24.61% to 28.92% across groups. ODI scores at baseline ranged from 0.28 ± 2.04 to 1.67 ± 2.43 across all groups (Table 5), further confirming equivalent starting conditions.

3.2 Antioxidant Pretreatment Significantly Mitigates Cell Death

Following H_2O_2 addition, the Stress group exhibited rapid and progressive cell death: 46.00% ± 2.09% at 15 minutes, 73.96% ± 1.90% at 30 minutes, and 92.39% ± 1.26% at 60 minutes—a net increase of 83.42 percentage points from baseline. The Control group remained stable throughout, reaching only 9.15% ± 0.45% at 60 minutes, confirming that H_2O_2, rather than time alone, drove the observed damage (Table 1).

However, both antioxidant groups showed slowed cell death. The Vitamin C group 36.45% ± 2.53% and the Green Tea group reached 39.17% ± 1.19% at 60 minutes, representing reductions of 55.94 and 53.22 percentage points relative to the Stress group, respectively. One-way ANOVA confirmed highly significant differences among groups ($F(3, 20) = 3020.92$, $p < 0.001$). Tukey HSD analysis showed that all comparisons using the Stress group were highly significant (Table 2). The Vitamin C and Green Tea groups did differ significantly ($\Delta = 2.72\%$, $p = 0.030$), suggesting equivalent overall protective capacity at the concentrations tested.

These results are consistent with known cardio-protective mechanisms of both compounds. Ascorbic acid acts as a direct electron donor to ROS, converting superoxide and hydroxyl radicals into less reactive species and regenerating endogenous antioxidants (4). Clinical analysis also supports the use of Ascorbic Acid: Vitamin C administration during cardiac surgery has been associated with reduced reperfusion-associated oxidative stress biomarkers (13). Green tea modulates Nrf2-mediated antioxidant gene expression and has demonstrated protective effects in rodent models of myocardial IRI (5). The

equivalent protective efficacy of both compounds in this study suggests complementary mechanisms converging on similar cytoprotective outcomes under H2O2 challenge.

Table 1. Mean percent cell death (± SD) by group and time point.

Group	***0 min***	***15 min***	***30 min***	***60 min***
Control	6.34 ± 0.84%	7.20 ± 0.84%	8.15 ± 0.72%	9.15 ± 0.45%
Stress	8.97 ± 0.68%	46.00 ± 2.03%	73.96 ± 1.95%	92.39 ± 1.26%
Stress + Vit C	6.55 ± 0.73%	17.30 ± 1.05%	26.64 ± 1.14%	36.45 ± 2.53%
Stress + Green Tea	7.63 ± 0.85%	21.77 ± 1.38%	31.45 ± 1.39%	39.17 ± 1.19%

n= 6 biological replicates per group. Values represent mean ± SD.

Figure 3.

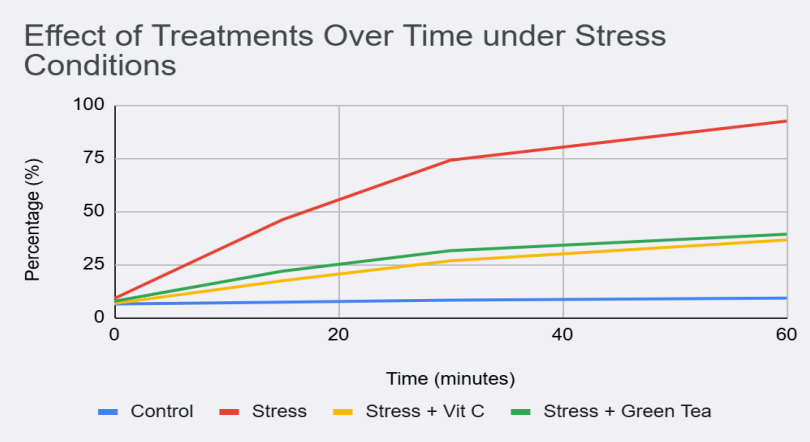


Table 2. Tukey HSD post-hoc pairwise comparisons of percent cell death at 60 minutes.

Comparison	***Mean 1 (%)***	***Mean 2 (%)***	***Difference (%)***	***p-value***	***Significance***
Control vs. Stress	9.15	92.39	83.24	< 0.001	***
Control vs. Stress + Vit C	9.15	36.45	27.30	< 0.001	***
Control vs. Stress + Green Tea	9.15	39.17	30.02	< 0.001	***
Stress vs. Stress + Vit C	92.39	36.45	55.94	< 0.001	***
Stress vs. Stress + Green Tea	92.39	39.17	53.22	< 0.001	***
Stress + Vit C vs. Stress + Green Tea	36.45	39.17	2.72	0.030	*

**** $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; ns = not significant ($p \geq 0.05$). One-way ANOVA: $p < 0.001$.*

3.3 Budding Rate Confirms Metabolic Suppression and Partial Rescue

Control group budding rates remained stable throughout (27.50% ± 1.43% to 30.52% ± 0.78%), reflecting uninterrupted cell division. The Stress group showed budding suppression, declining from 24.61% ± 2.82% at baseline to 0.55% ± 0.61% at 60 minutes, which is a near-complete cessation of cell division (Table 3). Antioxidant groups maintained significantly higher budding rates: 12.82% ± 1.26% (Vitamin C) and 11.81% ± 0.68% (Green Tea) at 60 minutes. One-way ANOVA confirmed significant group differences (1217.59, $p < 0.001$). The preservation of budding activity in antioxidant-treated groups indicates that, beyond preventing cell death, both antioxidant compounds partially maintained cellular metabolic processes under oxidative challenge.

Table 3. Mean percent budding rate (± SD) by group and time point.

Group	***0 min***	***15 min***	***30 min***	***60 min***
Control	28.92 ± 0.94%	28.30 ± 1.89%	27.50 ± 1.43%	30.52 ± 0.78%
Stress	24.61 ± 2.82%	11.18 ± 0.89%	4.78 ± 0.73%	0.55 ± 0.61%
Stress + Vit C	25.93 ± 1.03%	21.55 ± 0.85%	17.68 ± 0.97%	12.82 ± 1.26%
Stress + Green Tea	26.80 ± 1.02%	21.20 ± 1.66%	16.47 ± 1.80%	11.81 ± 0.68%

n = 6 biological replicates per group. Values represent mean ± SD. One-way ANOVA at 60 min: 1217.59, $p < 0.001$.

3.4 Morphological Analysis Reveals Progressive Cellular Deformation

ImageJ particle analysis of Stress group images showed progressive morphological deterioration across timepoints (Table 4). Mean circularity declined from 0.897 ± 0.015 at baseline to 0.283 ± 0.051 at 60 minutes, reflecting substantial cellular deformation under sustained oxidative stress. Mean cell area also decreased from 94.7 ± 1.8 px² at baseline to 72.3 ± 1.8 px² at 60 minutes, consistent with severe structural damage and loss of normal cellular morphology. In contrast, Control cells maintained relatively stable morphology throughout the experiment, with circularity values ranging from 0.795 ± 0.016 to 0.893 ± 0.013. Antioxidant-treated groups exhibited substantially higher circularity at 60 minutes (Vitamin C: 0.723 ± 0.022; Green Tea: 0.717 ± 0.021) than the untreated Stress group, indicating significant preservation of cellular structure. One-way ANOVA at 60 minutes confirmed significant group differences in circularity ($F(3,20) = 345.38$, $p < 0.001$). These findings suggest that both antioxidants mitigated oxidative stress–induced morphological deterioration.

Table 4. ImageJ morphological analysis of the Stress group (real experimental data, n = 6 per time point).

Tube	***Timepoint***	***Avg Area (px²)***	***Circularity***	***Solidity***
S 1-6	0 min	94.7 ± 1.8	0.897 ± 0.015	0.930 ± 0.016
S 1-6	15 min	90.3 ± 1.9	0.732 ± 0.055	0.828 ± 0.015
S 1-6	30 min	84.5 ± 2.4	0.378 ± 0.039	0.700 ± 0.092
S 1-6	60 min	72.3 ± 1.8	0.283 ± 0.051	0.547 ± 0.071

Real ImageJ output from experimental microscope images. Mean ± SD rows represent group averages in Area, Circularity, and Solidity.

3.5 Oxidative Damage Index Consolidates Multi-Metric Findings

The ODI (Eq. 1) provided a measure of oxidative damage by integrating changes in cell morphology (Table 5). At baseline, all groups exhibited comparable ODI values near zero (−0.84 to 0.45), indicating similar initial cellular morphology before oxidative stress exposure. Following H_2O_2 treatment, the Stress group demonstrated a progressive increase in ODI, rising to 17.26 at 15 minutes, 43.54 at 30 minutes, and 59.84 at 60 minutes. In contrast, the Control group remained relatively stable, reaching only 6.97 at 60 minutes, confirming that incubation alone did not substantially affect cellular morphology.

Antioxidant treatment attenuated oxidative damage relative to the Stress group. The Vitamin C group reached an ODI of 25.44 at 60 minutes, representing a 57.5% reduction compared with the Stress group, while the Green Tea group reached an ODI of 28.86, corresponding to a 51.8% reduction. These findings indicate that both antioxidants substantially preserved cellular morphology under oxidative stress conditions, with Vitamin C providing slightly greater protection than Green Tea.

The ODI mirrors the logic of composite endpoints commonly used in cardiovascular clinical research, where no single biomarker fully captures the multidimensional nature of ischemia-reperfusion injury (IRI) (14). By integrating multiple morphology-based indicators of cellular injury, including circularity, solidity, and aspect ratio, the ODI reduces reliance on any single measurement and provides a more robust summary of oxidative damage severity and antioxidant protection. Statistical analysis demonstrated significant differences among experimental groups (one-way ANOVA, 526.04, $p < 0.001$ at 60 minutes), supporting the conclusion that antioxidant treatment significantly mitigated H_2O_2-induced cellular damage.

Table 5. Mean Oxidative Damage Index (ODI) by group and time point, calculated using Eq. 1.

Group	***0 min***	***15 min***	***30 min***	***60 min***	***Δ (0→60 min)***
Control	0.00	2.55	4.34	6.97	+6.97
Stress	0.45	17.26	43.54	59.84	+59.39
Stress + Vit C	-0.84	9.12	19.33	25.44	+26.28
Stress + Green Tea	-0.49	7.19	20.69	28.86	+29.35

ODI was calculated using normalized ImageJ-derived circularity, solidity, and aspect ratio measurements. Higher ODI values indicate greater cumulative structural oxidative damage.

3.6 Pearson Correlation Confirms Duration-Dependent Oxidative Injury

Correlation analysis of the Stress group revealed a robust positive correlation between H2O2 exposure duration and percent cell death ($r = 0.966$, $p < 0.001$) supporting the notion that oxidative injury accumulated in a duration-dependent manner consistent with published H2O2 kinetics in yeast (8). On the other hand, the exposure duration was highly negatively correlated with the mean circularity ($r = -0.934$, $p < 0.001$) suggesting that longer exposure to oxidative stress led to a more irregular and distorted cell morphology.

The findings are taken as evidence of progressive exogenous oxidative stress rather than ischemia-reperfusion injury. Oxygen deprivation, metabolic ischemia, and controlled reoxygenation were not part of the experimental protocol. Thus, the model captures oxidative damage produced by H2O2 but does not reproduce the broader physiological processes associated with myocardial ischemia-reperfusion injury.

REFERENCES

3.7 Cardiovascular Relevance and Translational Implications

The present study demonstrates that Vitamin C and green tea extract reduce cellular damage following H_2O_2 exposure in S. cerevisiae. As yeast exhibits conserved cellular responses to oxidative stress, this model serves as a valuable preliminary platform for investigating antioxidant activity. However, these findings do not constitute direct evidence of cardioprotection.

Myocardial ischemia-reperfusion injury involves numerous interacting mechanisms, including ATP depletion, calcium overload, mitochondrial dysfunction, mitochondrial permeability transition, inflammatory signaling, endothelial dysfunction, and microvascular injury. None of these processes were directly modeled in the present experiment. In particular, the study did not include an ischemic phase or a subsequent restoration of oxygen. H_2O_2 was instead applied directly under normoxic conditions, making the experimental system an acute oxidative stress model rather than an ischemia-reperfusion model.

The antioxidant treatments were administered 15 minutes before H_2O_2 exposure and therefore represent antioxidant pretreatment or experimental preconditioning rather than treatment during or after reperfusion. The study did not compare multiple pretreatment durations. The 15-minute interval was selected as a standardized pretreatment period to ensure the antioxidants were present before the oxidative challenge while maintaining a practical and consistent experimental protocol. Future experiments should directly compare shorter and longer pretreatment intervals, including several-hour or overnight exposures, to determine whether treatment duration alters the magnitude of protection.

The antioxidant concentrations used in this study should also be interpreted cautiously. Although concentrations were selected for the yeast experimental system, intracellular antioxidant concentrations were not directly measured, and no pharmacokinetic comparison with concentrations achievable in human plasma or cardiac tissue was performed. Consequently, the present results cannot establish that the concentrations used are physiologically attainable in humans. Future studies should quantify antioxidant concentrations experimentally and investigate dose-response relationships before making translational comparisons.

3.8 Limitations

The Oxidative Damage Index (ODI) was created as a descriptive composite of the ImageJ-derived circularity, solidity, and aspect ratio. Subsequently, higher ODI values were associated with higher levels of accumulated morphological abnormality. The composite nature of the ODI, which utilizes multiple morphological parameters to describe cellular shape irregularity, is scientifically justified, but the ODI has not been validated as a metric of oxidative injury.

The calculation of the ODI utilizes an equal mathematical weighting of the three parameters. The choice of equal weighting was appropriate for the purpose of creating a descriptive composite index, but the ODI's sensitivity to differential weighting has not been assessed, and there is no formal justification for equal contribution of individual parameters to the final ODI. As such, the ODI should be considered a descriptive morphology index, and the Oxidative Damage Index should be validated as a biomarker of oxidative injury in future studies that assess its ability to track with standard biochemical markers of oxidative stress (e.g., ROS, lipid peroxidation, protein carbonylation, glutathione, mitochondrial membrane potential). The ODI's relationship to these variables could be assessed using principal component analysis or regression.

Other limitations of the current study include the following: First, the concentrations of individual antioxidants were not verified by independent biochemical assays (e.g., spectrophotometry or HPLC), and variability in the actual concentrations of the antioxidant preparations could confound the interpretation of the results. Second, cell imaging was performed using a smartphone-mounted microscope, which is associated with significant variability in image quality and consistency, compared to a standard research-grade microscope. Dedicated microscopy and image segmentation would improve the consistency of such assays. Third, the current study only tested a single concentration of H_2O_2. A dose-finding experiment would be necessary to identify the concentration of H_2O_2 that leads to consistent morphological abnormalities. Such an experiment would also identify the IC50 for the tested antioxidants. Fourth, the current study had a small sample size (n = 6). Larger sample sizes would be necessary to detect significant differences between groups. Fifth, the current study did not directly measure intracellular ROS or mitochondrial function.

Finally, the model system used in the current study does not recapitulate the complex pathophysiology of myocardial ischemia-reperfusion injury. The current study did not subject cells to oxygen deprivation, reoxygenation, ATP depletion, calcium overload, or inflammatory challenge, and thus, the model does not reflect the complex interactions that occur in the setting of myocardial infarction. As such, the current study demonstrated that Vitamin C and green tea extract are capable of reducing oxidative damage in a cellular model of oxidative stress, but the results of the current study cannot be extrapolated to the clinical setting of myocardial ischemia-reperfusion injury.

3.9 Future Directions

The Oxidative Damage Index (ODI) is a descriptive composite score based on ImageJ calculated circularity, solidity, and aspect ratio. Increased ODI reflects the total morphological abnormality. The composite score is useful to standardize reporting of morphological observations from multiple shape parameters, but the ODI needs biological and clinical validation as a measure of oxidative injury.
Currently, the three shape parameters are equally weighted. The rationale for this choice was to report a simple composite score, but it cannot be assumed that each parameter contributes equally to the total morphological abnormality, and the contribution of each shape parameter to oxidative damage was not tested. At this time, the ODI should be considered a provisional morphology composite score, rather than a quantitative oxidative injury index.

Future studies should validate the ODI against known measures of oxidative injury and test if equal weighting provides the best estimate of total morphological abnormality. The composite score could be validated using principal component analysis or regression to explore the relationships between the individual shape parameters. The ODI should undergo rigorous biological validation, including determining its association with standard assays of oxidative injury, including, but not limited to, intracellular reactive oxygen species (ROS), lipid peroxidation, protein carbonyl content, glutathione, and mitochondrial membrane potential.

Other limitations should be considered. First, antioxidant concentrations were not validated by spectrophotometry or high-performance liquid chromatography (HPLC). Therefore, the actual concentrations of administered vitamin C and green tea might differ from our estimates. Second, we used a smartphone to visualize and segment cell images in ImageJ, and future studies should aim to use standard microscope and automated image segmentation. Third, we tested only one hydrogen peroxide (H_2O_2) concentration. Ideally, a dose-finding experiment should have been performed to identify the sub-lethal concentration of H_2O_2 needed to observe antioxidant protection. Fourth, each group of experiments contained only n = 6 replicate samples. Lastly, we did not measure intracellular ROS or mitochondrial function to support our hypothesis.

Finally, this study used a model that poorly recapitulates the complexity of myocardial ischemia-reperfusion injury. Our experiments did not involve oxygen deprivation, reperfusion, ATP depletion, calcium homeostasis disruption, inflammatory cell infiltration, and other pathophysiological processes that occur during a myocardial infarction. Therefore, our results should not be extrapolated to understand the role of vitamin C or green tea extract in treating or preventing myocardial ischemia-reperfusion injury. Our experiments showed that vitamin C or green tea extract can reduce oxidative stress in yeast.

4. CONCLUSION

This study proved that Vitamin C and green tea extract decreased cellular damage caused by H_2O_2-induced stress in Saccharomyces cerevisiae . Specifically, treatment with Vitamin C reduced Oxidative Damage Index (ODI) by 57.5%, while green tea extract reduced ODI by 51.8% compared to the stressed control group. Significant differences were found between the tested groups (one-way ANOVA, $p < 0.001$), which showed that both anti-oxidant treatments have a notable protective effect against oxidative stress. At the same time, it was found that Vitamin C treatment resulted in slightly lower ODI values than treatment with green tea extract, although the protective effect of both substances was comparable.

Pearson's correlation analysis revealed that the damage from H_2O_2 is directly related to the time of exposure. Within the Stress group, the longer exposure time significantly increased cell mortality ($r = 0.966$, $p < 0.001$), while circularity decreased ($r = -0.934$, $p < 0.001$). In general, these results demonstrate that the effects of oxidative stress increase with time.

The ODI developed in this study is a preliminary attempt to combine multiple morphological measurements from ImageJ into one index. However, further studies are needed before it can be recommended as a quantitative biomarker of oxidative damage. It is necessary to confirm its correlation with specific biochemical and molecular changes that occur when cells are exposed to oxidative stress.
It is also important to note that this study did not replicate the clinically relevant model of myocardial ischemia-reperfusion injury. In contrast, the experiment only utilized exogenous H_2O_2 treatment under room air conditions to induce oxidative stress. Thus, it can be stated that this experiment showed the

anti-oxidant properties of Vitamin C and green tea extract in S. cerevisiae . However, further experimentation is needed to demonstrate the cardioprotective effects of these substances in the context of ischemia-reperfusion injury.

It is recommended that future studies examine the effects of different H_2O_2 concentrations as well as separate biochemical markers of oxidative damage on the cells. Moreover, experiments should test how the concentration and duration of anti-oxidant pretreatment affect the observed outcomes. Finally, the most promising studies would involve subjecting S. cerevisiae to hypoxia-reoxygenation injury and analyzing the changes to cardiomyocytes in more detail. It is only after such experiments that researchers would be able to conclude whether the anti-oxidant effects of Vitamin C and green tea extract observed in this study have the potential to be extrapolated onto mammals. In this regard, the current experiment represents only a starting point in research on oxidative stress cardiomyopathy.

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REFERENCES

(1) World Health Organization. Cardiovascular diseases (CVDs). WHO Fact Sheet. https://www.who.int/news-room/fact-sheets/detail/cardiovascular-diseases-(cvds), 2021.

(2) Hausenloy DJ, Yellon DM. Myocardial ischemia-reperfusion injury: a neglected therapeutic target. J Clin Invest, 123: 92-100, 2013. https://doi.org/10.1172/JCI62927

(3) Cadenas E, Davies KJA. Mitochondrial free radical generation, oxidative stress, and aging. Free Radic Biol Med, 29: 222-230, 2000. https://doi.org/10.1016/S0891-5849(00)00317-8

(4) Carr AC, Frei B. Toward a new recommended dietary allowance for vitamin C based on antioxidant and health effects in humans. Am J Clin Nutr, 69: 1086-1107, 1999. https://doi.org/10.1093/ajcn/69.6.1086

(5) Babu PVA, Liu D. Green tea catechins and cardiovascular health: an update. Curr Med Chem, 15: 1840-1850, 2008. https://doi.org/10.2174/092986708785132979

(6) Morano KA, Grant CM, Moye-Rowley WS. The response to heat shock and oxidative stress in Saccharomyces cerevisiae. Genetics, 190: 1157-1195, 2012. https://doi.org/10.1534/genetics.111.128033

(7) Temple MD, Perrone GG, Dawes IW. Complex cellular responses to reactive oxygen species. Trends Cell Biol, 15: 319-326, 2005. https://doi.org/10.1016/j.tcb.2005.04.003

(8) Lushchak VI. Oxidative stress in yeast. Biochemistry (Moscow), 75: 281-296, 2010. https://doi.org/10.1134/S0006297910030041

(9) Herrero E, Ros J, Belli G, Cabiscol E. Redox control and oxidative stress in yeast cells. Biochim Biophys Acta, 1780: 1217-1235, 2008. https://doi.org/10.1016/j.bbagen.2007.12.004

(10) Lushchak OV, Kubetsky S, Lushchak VI. Hydrogen peroxide induced cell death in Saccharomyces cerevisiae: dose-response analysis and recovery. J Biochem Mol Biol, 38: 565-571, 2005. https://doi.org/10.5483/BMBRep.2005.38.5.565

(11) Nebe-von-Caron G, Stephens PJ, Hewitt CJ, Powell JR, Badley RA. Analysis of bacterial function by multi-colour fluorescence flow cytometry and single-cell sorting. J Microbiol Methods, 42: 97-114, 2000. https://doi.org/10.1016/S0167-7012(00)00181-0

(12) Schneider CA, Rasband WS, Eliceiri KW. NIH Image to ImageJ: 25 years of image analysis. Nat Methods, 9: 671-675, 2012. https://doi.org/10.1038/nmeth.2089

(13) Demarchi AC, de Almeida Brandao CM, Thomazini M, Auler JOC. Perioperative vitamin C supplementation reduces myocardial oxidative stress: a meta-analysis of randomized controlled trials. J Cardiovasc Surg, 60: 390-397, 2019. https://doi.org/10.23736/S0021-9509.18.10448-8

(14) Thygesen K, Alpert JS, Jaffe AS, Chaitman BR, Bax JJ, Morrow DA, et al. Fourth universal definition of myocardial infarction. Eur Heart J, 40: 237-269, 2018. https://doi.org/10.1093/eurheartj/ehy462