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Article

The Protective Antioxidant Effects of Sulforaphane on Germinating Radish Seeds Treated with Hydrogen Peroxide

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SUMMARY

Free radical chain reactions result when atoms containing unpaired electrons bind with biomolecules and alter their biological functions, contributing to the progression of diseases such as atherosclerosis, cancer, and diabetes. Antioxidants, such as vitamin E and sulforaphane, are effective neutralizers of free radicals and prevent cellular damage. This present study is conducted to determine the relative effectiveness of sulforaphane against free radicals generated by hydrogen peroxide (H_2O_2) compared with the known antioxidant vitamin E. In this experiment, H_2O_2 is a source of free radicals and germinating radish seeds are models to test the protective effects of antioxidants against free radicals. Generally, H_2O_2 diffuses into the cell and dissociates to form hydroxyl radicals through Fenton reactions. Based on current literature, the hypothesis is that sulforaphane is more effective than vitamin E in inhibiting free radicals from 1% H_2O_2 on germinating radish seeds due to its activation of different antioxidant pathways compared to vitamin E's direct neutralization of free radicals. The 1% H_2O_2-treated radish seeds show lower germination rates than seeds treated with sulforaphane and vitamin E with 1% H_2O_2. Furthermore, the germination rate is higher in sulforaphane with 1% H_2O_2-treated seeds compared to vitamin E and 1% H_2O_2 treated seeds. In summary, these experiments show sulforaphane is more effective than vitamin E in neutralizing the free radical effects of H_2O_2 on radish seed germination. These results point to sulforaphane's potential use as a dietary supplement to counteract free radical effects on cellular levels, helping to prevent the progression of certain diseases.

INTRODUCTION

Free radicals are atoms or molecules that are hazardous to cell health and are the root cause of several degenerative diseases (1, 2). Free radicals have an unpaired electron in their outer shell configuration that makes them highly reactive (3, 4). To achieve a stable electron configuration, the free radicals attracting electrons from biomolecules such as proteins, lipids, or nucleic acids in the cell (5). Because of the removal of an electron, the biomolecules thus become unstable. As a result, this instability results in a cascade of chain reactions that alter their biological function, eventually leading to either cell degeneration or death (6). Some sources of free radicals in our system come from burned or smoked food, alcohol, pre-manufactured goods, ionizing radiation, radioactive substances, and various types of machine exhaust (7). Free radical reactions are implicated in many degenerative diseases, including atherosclerosis, cancer, inflammatory joint disease, asthma, diabetes, senile dementia, and degenerative eye disease, by damaging genetic material within cells (8, 9).

Thus, many living organisms have evolved with certain molecules or enzyme systems that largely counteract the damage arising from free radicals. Nevertheless, naturally occurring compounds in our diet called antioxidants also play a role in neutralizing the free radicals (10). Antioxidant molecules either directly neutralize free radicals at the source by donating an electron through an ionic bond or by indirectly activating antioxidant enzymes that can neutralize free radical reactions (11), thereby protecting the biological molecules.

Antioxidant molecules vary in their neutralizing properties to counteract free radicals. vitamin E is well-known as an effective antioxidant against free radicals and one of the most abundant and significant lipophilic radical-scavenging antioxidant *in vivo* that forms a-tocopheroxyl radicals as the intermediate in its free radical scavenging reaction (12, 13). Recent studies demonstrate phytochemical ingredient molecules like Sulforaphane, richly found in cruciferous vegetables such as broccoli, brussels sprouts, and cabbage, can be cytoprotective and act as an antioxidant molecule, acting through the activation of various antioxidant enzymes in response to free radical stress (14). However, the relative effectiveness of various antioxidant molecules against free radicals is not known.

The model of action through which antioxidants neutralize free radicals differs in cellular systems (3). The antioxidant molecule vitamin E neutralizes free radicals at the source by donating an electron through an ionic bond (15), whereas sulforaphane has been shown to activate a cascade of antioxidant enzymes in response to free radical stress in the cellular system (14). However, both actions help protect biological molecules. These differences in the mode of antioxidant action between sulforaphane and vitamin E could be a reason for the observed differences in their effectiveness in counteracting free radical effects on germinating radish seeds. However, the mechanisms of action of antioxidants need further investigation to explore the reasons for the relative differences in their effectiveness as an antioxidant.

Our study aims to investigate the relative effectiveness of sulforaphane as an antioxidant molecule to counteract the effects of free radicals generated by 1% H_2O_2 in comparison with another known antioxidant, vitamin E (16, 17). We

hypothesize sulforaphane is more effective than vitamin E in inhibiting the effects of free radicals on the germinating radish seeds treated with 1% H_2O_2 based on existing research suggesting that vitamin E upregulates antioxidant defenses as an exogenous antioxidant while sulforaphane activates endogenous defenses instead to neutralize free radicals (18). Specifically, evidence shows sulforaphane controls the expression of genes involved in the neutralization and elimination of reactive oxidants via the activation of nuclear factor erythroid 2-related factor 2 (NRF2) pathway, although the sulforaphane's mechanisms are not completely understood at this time (19). In this experiment, H_2O_2 is used as a source of free radicals and the germinating radish seeds are used as models to test the protective effects of antioxidants in neutralizing free radicals. Previously, H_2O_2 was determined to be detrimental to the seed germination process as a toxic molecule and this observation is consistent with the results of this experiment; however, H_2O_2 in very small amounts can be conducive to seed germination as a signaling molecule (20). In the current study, H_2O_2 is used at concentrations that negatively affect the seed germination process.

RESULTS

After three days of incubating radish seeds in the presence of 1% H_2O_2 with or without antioxidants, seed germination rates are recorded and analyzed. A representative picture of germinating radish seeds with different treatments is shown in **Figure 1**. **Table 1** displays the summary of the radish seed germination results obtained with four different individual experiments. In all, the experiment derived significant results based on a one-way ANOVA test with $p<0.05$.

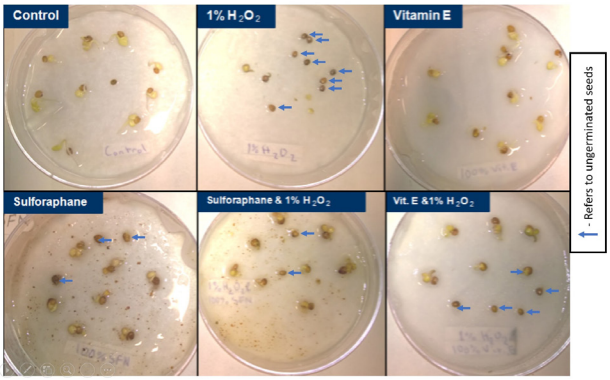

Figure 1. Representative images of radish seed germination exposed to H_2O_2 and antioxidants. As described in the methods section, radish seeds were treated either with 1% H_2O_2 antioxidants alone, or 1% H_2O_2 in combination with either of the antioxidants, Sulforaphane and Vitamin E. The radish seeds were incubated for 72 hours, images of the petri dishes were taken, and the germination rates were analyzed.

H_2O_2 is used as a source of free radical generation in this study. An optimum concentration of H_2O_2 for the seed germination experiments is initially determined by testing the seed germination rate using different concentrations of H_2O_2. 1% H_2O_2 concentration is found to be an optimum concentration for the germination experiments as the rate of radish seed germination was definitely 0% when used at a concentration of 3%. The rate of germination of radish seeds is about 12% when H_2O_2 is used at a concentration of 1%, which is sufficient to vastly inhibit seed germination.

The radish seeds from the control group show an average germination rate of 97.5% (**Figure 1; Table 1**). However, the seeds treated with 1% H_2O_2 had a 12.5% germination rate, which is the lowest of all the treatments and point towards the strong inhibition of H_2O_2 on seed germination. Furthermore, the treatment of radish seeds with either sulforaphane or vitamin E did not have significant effects on seed germination, resulting in a germination rate of 90% and 97.5% respectively when compared to the seeds treated with water alone (**Table 1; Figure 2**).

Experimental Results of Seed Germination

	Control	Vitamin E	Sulforaphane	Vit. E + 1% H2O2	SFN + 1% H2O2	1% H2O2
Experiment 1	10	10	9	4	10	0
Experiment 2	10	9	10	4	8	0
Experiment 3	10	10	7	5	8	3
Experiment 4	9	10	10	8	9	2
Average	9.75	9.75	9	5.25	8.75	1.25
STDEV	0.500	0.500	1.414	1.893	0.957	1.500
SEM	0.250	0.250	0.707	0.946	0.479	0.750
t-test		1.000000	0.355918	0.003705	0.113532	3.83E-05

ANOVA Single Factor Test Results based on above table

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	231.2083	5.0000	46.2417	29.9946	3.95E-08	2.7729
Within Groups	27.7500	18.0000	1.5417			
Total	258.9583	23.0000				

Table 1. A single factor ANOVA test for a robust comparison between the test groups was performed to determine the overall significance of the results. Statistical student *t*-test analysis was then performed on four independent sets of 10 healthy radish seed germination experiments, comparing each of the treatments to the control group containing only radish seeds and water. The germination rate of radish seeds treated with H_2O_2/antioxidants alone or in combination of both were recorded, and the results were analyzed using Student's t-test to determine the significant differences in the germination rate between the treatment groups. Significant differences in seed germination rate were observed in treatment groups treated with 1% H_2O_2 and Vitamin E+ 1% H_2O_2 when compared with control treated group but not with any other groups, suggesting Vitamin E is less effective than sulforaphane in neutralizing H_2O_2's free radical toxicity and its inhibition of germination.

The seeds group treated with vitamin E plus 1% H_2O_2 display an average seed germination rate of 52.5%, and the germination rate was significantly lower when compared to the control group with $p<0.05$. The radish seeds treated with sulforaphane and 1% H_2O_2 yielded a seed germination rate of 87.5%, which is higher compared to the rate of germination of seeds in the presence of vitamin E with 1% H_2O_2 but not significant (p-value>0.05) when compared to the control group (**Table 1; Figure 2**). The vitamin E with 1% H_2O_2 group is thus significantly different from the control

group with a $p<0.05$ and the sulforaphane with 1% H_2O_2 group is not significantly different from the control group treated with a $p=0.1135$, suggesting sulforaphane is relatively more potent in acting against free radical stress caused by H_2O_2 on germinating radish seeds when compared with vitamin E. In all, the statistical tests performed demonstrate the sulforaphane with 1% H_2O_2 group have a similar germination pattern as the control group while the vitamin E with 1% H_2O_2 did not, thus suggesting vitamin E is less effective than sulforaphane in neutralizing H_2O_2's free radical toxicity and its inhibition of germination.

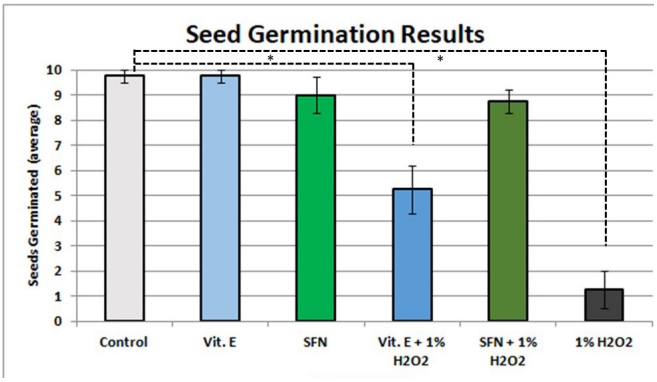

Figure 2. Sulforaphane maintained seed germination rates in the presence of H_2O_2 more effectively than vitamin E. Bar graph below gives a summary of the radish seed germination results from four different experiments. The error bars represent the standard error of the mean (SEM) between individual experiments. The difference between treatment groups is considered significant (*) when the *p*-value is $p<0.05$ based on student *t*-test results. Significant differences in seed germination rate were observed in treatment groups treated with 1% H_2O_2 and Vitamin E and 1% H_2O_2 when compared with control-treated group but not with any other groups indicating the sulforaphane-rich broccoli extracts are effective in neutralizing the effects of 1% H_2O_2.

DISCUSSION

This study was designed to determine the effectiveness of sulforaphane (from broccoli sprout extracts) in preventing the effects of free radicals on germinating radish seeds. Our experimental results support that sulforaphane-rich broccoli extract is more effective in neutralizing the free radical effects of H_2O_2 on germinating radish seeds when compared to the antioxidant, vitamin E.

The control group containing seeds treated with water alone exhibited an average germination rate of 97%, suggesting that the conditions used such as water, seed bed, temperature, water, and light were optimal for the germination of the radish seeds. The seeds treated with H_2O_2 plus sulforaphane alone showed a similar germination rate to the control group, indicating that sulforaphane is effective in preventing the free radical effects on germinating radish seeds under the experimental seeds. In contrast, the seeds treated with vitamin E and H_2O_2 showed an average germination rate of 50%, which indicates that vitamin E is less effective in neutralizing H_2O_2 effects when compared to sulforaphane.

The results from the present study show that sulforaphane rich-broccoli extract can act as an effective antioxidant and suggest that this extract is more potent than vitamin E in counteracting free radical effects. In effect, this study is helpful to further expand on existing research regarding sulforaphane and its potential effects and uses as an endogenous antioxidant. By demonstrating the higher effectiveness of sulforaphane over vitamin E in the context of H_2O_2 free radical toxicity and inhibition of germination, this research highlights the untapped therapeutic potential of sulforaphane as a potent antioxidant. By demonstrating these statistically significant results using radish seeds as an experimental model, this study will encourage other researchers to further investigate the mechanisms of how sulforaphane activates the NRF2 pathway as well as which enzymes are central to its function. We hope to open the path for the thorough investigation of sulforaphane including, but not limited to, its definite model of action in addition to its beneficial effects in mammalian cells and humans overall.

By using this research to demonstrate sulforaphane is effective in combating the free radical effects of H_2O_2, we can say that it would be beneficial for individuals to incorporate cruciferous vegetables, known to be an excellent source of sulforaphane, into their diets. Thus, this study has real-life applications as people could develop healthier eating habits by including sulforaphane rich-broccoli and other foods containing sulforaphane in their diet. As a natural antioxidant, sulforaphane can work as a defense against the free radical effects in our body with implications for preventing or delaying the underlying degenerative disease such as atherosclerosis, cancer, inflammatory joint disease, asthma, diabetes, senile dementia, and eye disease (8, 21, 22).

MATERIALS AND METHODS

Solution Preparations and Treatment Group Setup

The solutions for each of the experimental groups were prepared using a graduated cylinder. For this experiment, there were 6 petri dishes (100mm by 15mm; RGF) with 10 radish seeds in each (Seeds of Change) that had different 10 mL treatments added to each one (see **Figure 1**). First, coffee filter disks were placed in each petri dish at the bottom to produce a foundation for seed germination to occur. Ten mL of distilled water was added to the control group and 10 mL of 1% H_2O_2, diluted from 3% H_2O_2, was added to the comparative control group. Next, the contents of a broccoli extracts rich with sulforaphane pill (400 mcg per 20:1 broccoli sprout extract capsule; Swanson, Green Foods) were added to 10 mL of water, stirred well, filtered, and added to the petri dish labelled as SFN. This step was repeated for the vitamin E pill (400 IU per dose; Spring Valley) and the solution was added to the vitamin E petri dish. The concentration of the sulforaphane-related treatment was 400 mcg per 10 mL of its corresponding solution per experimental group and is comparable to the 400 IU or 20,000 mcg concentration of

vitamin E per 10 mL of its corresponding solution as both are the recommended daily dose of the antioxidant as established by dietary guidelines. Finally, for the experimental groups, the vitamin E pill and Sulforaphane pill were each added into a 10 mL solution of 1% H_2O_2. The solutions were stirred well, filtered, and added into their respective petri dishes labeled as "vitamin E + 1% H_2O_2" and "SFN + 1% H_2O_2". The petri dishes were then kept 3 feet away from a tube light at a temperature of around 73°F for 72 hours. Finally, pictures were taken and the seeds that had germinated from each petri dish were counted (see **Table 1**). This experiment was repeated four independent times.

Data Analysis

The average number of seeds germinated and the SEM (standard error of the mean) per experimental condition were calculated using Microsoft Excel. The significance (*p*-value) of the effectiveness of the treatment, compared to control treatment, on germinating radish seeds was calculated using a one-way ANOVA test to determine significance and a *t*-test to compare the means of each experimental group and the control group (see **Table 1 & Figure 2**).

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