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EFFECTS OF FAR INFRARED RAYS ON HYDROGEN PEROXIDE-SCAVENGING CAPACITY

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ABSTRACT

Far infrared rays (FIRs) have several proven effects on the human body and are generally considered to be biologically beneficial. In this study, we determined the effect of FIRs on hydrogen peroxide (H_2O_2)-scavenging activity, which was directly increased by 10.26% after FIR application. Even in the indirect use of FIRs accompanying carrot extract, FIRs still contributed to a 5.48% increase in H_2O_2-scavenging activity. We further proved that additional FIR treatment resulted in about 23.02% and 18.77% viability increases of osteoblast cells in the 200 and 800 μM H_2O_2, respectively; and about 25.67% and 47.16% viability increases of fibroblast cells in the 25 and 50 μM H_2O_2, respectively. Finally, FIR treatment also delayed senescence of detached *Railway Beggarticks* leaves in H_2O_2 solution with the concentrations of 10, 100, and 1000 μM. By reviewing past articles related to the effects of oxidative stress from metabolically produced H_2O_2, we discuss possible benefits of FIRs for plants and animals.

Keywords: Far infrared ray; FIR; Hydrogen peroxide; H_2O_2 scavenging; Carrot; H_2O_2-mediated oxidative stress; Osteoblast cells; Fibroblast cells; Leaf senescence.

INTRODUCTION

Far infrared ray (FIR) is an electromagnetic wave with wavelengths ranging within the IR spectrum. The FIR especially at 4–14 μm has many biological effects including, accelerated wound healing via fibroblast proliferation, enhanced immunity from leukocyte strengthening, and sleep promotion.[1–7] Specific discoveries include the report by Shimokawa *et al.*[8] that

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FIR-treated water increased the number of free tetrahedral water molecules or smaller-sized clusters. Further, FIR absorption causes the collapse of water clusters, and the energy transfer may have been converted into molecular vibrations. Also, by measuring scavenging activity against hydrogen peroxide (H_2O_2), Jeon *et al.*[9] showed that the antioxidant effects of rice hull extracts can be enhanced by thermal FIR irradiation. The beneficial value of FIR was proven by an experimental model of H_2O_2-induced DNA damage in human lymphocytes, in which the rice hull extract decreased DNA strand breakage.

It is known that H_2O_2 is a byproduct of normal oxygen metabolism in the aerobic cells of animals and plants. All organisms possess peroxidases or enzymes to decompose low concentration H_2O_2 into water and oxygen. However, the continuous production of H_2O_2 contributes to increased concentrations of reactive oxygen species within both the mitochondrial matrix and cytosol. This potentially causes damage to mitochondrial components and initiates degradation. Therefore, the continuous generation of H_2O_2 during aerobic metabolism is harmful, and acts as a burden to living systems.[10]

Although FIR has several proven clinical effects on the human body and is generally considered to be biologically beneficial, it still lacks precise biomolecular evidence to support the observations. Following our previous finding in increasing intracellular levels of nitric oxide[6] and calmodulin,[7] this study is aimed to further evaluate the H_2O_2-scavenging activity of the FIR-emitting ceramic material at room temperature. Based on the results of this study, we deduce and discuss the possible beneficial effects of FIR for both plants and animals in counteracting metabolically accumulated H_2O_2 and oxidative stress.

MATERIAL AND METHODS

FIR Ceramic Powder

This FIR-emitting ceramic powder consists of micro sized particles produced from several ingredients, mainly mineral oxides such as aluminum oxide, ferric oxide, zinc oxide, and magnesium oxide.[6,7] The average emissivity of ceramic powder is over 0.9 at wavelengths of 6–14 μm, which was proven by the Industrial Technology Research Institute, Taiwan. This emissivity represents an extremely high ratio of FIR radiation intensity. Equal amounts of 100 gm of FIR-emitting ceramic powder were enclosed in different plastic bags (10 × 20 cm) as the FIR irradiation source.

Direct Scavenging of H_2O_2 with FIR

An H_2O_2 solution (Sigma, St. Louis, MD, USA) with 1 M concentration was prepared and equal amounts of 9 ml H_2O_2 solutions were added to test tubes made of polypropylene. The H_2O_2 solution was categorized into two groups, the control and FIR. For the FIR group, test tubes were incubated at room temperature and externally covered by plastic bags filled with 100 gm FIR ceramic powder for 3 h of irradiation. The control group was treated similarly, with the exception of FIR treatment. After the incubation period, 7.5 mM phenol red (Sigma, St. Louis, MD, USA) and 5 mg/ml horseradish peroxidase (Sigma, St. Louis, MD, USA) were added to each test tube. The mixture was allowed to react for 10 min and absorbance was observed at 610 nm by an enzyme-linked immunosorbent assay (ELISA) reader (Gemini XPS Molecular Devices, Sunnyvale, CA, USA), with lower absorbance values representing higher H_2O_2-scavenging ability.

Indirect Scavenging of H_2O_2 with Carrot Extract and FIR

Similar to the direct scavenging experiment method, H_2O_2 solution was separated into the control and FIR groups. As much as 1 ml carrot extract solution (obtained by grinding fresh carrots) was added to each test tube containing 9 ml of H_2O_2 solution for both the control and FIR groups. This mixture was allowed to react for 3 h at room temperature. After the incubation period, phenol red (7.5 mM) and horseradish peroxidase (5 mg/ml) were reacted with H_2O_2. The mixture was treated and measured using the same methods as the direct scavenging experiment.

Cells Treated with FIR Under H_2O_2-Mediated Oxidative Stress

Murine calvaria-derived MC3T3-E1 osteoblast-like cells and NIH 3T3 fibroblast cells (Bioresource Collection and Research Center, Hsinchu, Taiwan) were cultured in ascorbic acid-free α-minimum essential medium (Gibco, Grand Island, NY, USA) containing 10% fetal calf serum (Biological Industries, Kibbitz Beit Haemek, Israel), 100 units/ml penicillin G sodium, 100 μg/ml streptomycin sulfate, and 0.25 μg/ml amphotericin B (Gibco) in a 5% CO_2-humidified atmosphere. Cells were seeded in 6-well tissue culture plates at a density of 4×10^5 cells per well. After 16 h of culturing, the medium was changed and various concentrations of H_2O_2 were added. For FIR groups, enclosed FIR ceramic powder was distributed uniformly in plastic

bags, which had been inserted beneath the tissue culture plates. Under these circumstances, the cells received FIR irradiation evenly and steadily. The control group received H_2O_2 treatment without any FIR interaction. After 24 h of treatment, the culture medium was replaced with a 600 μl medium containing MTT and incubated for 4 h. The medium was removed and formazan was dissolved in 600 μl DMSO and read at a wavelength of 550 nm by an ELISA reader (Power WaveX 340, Bio-TEK instrument Inc., Winooski, VT, USA).

Leaves Treated with FIR Under H_2O_2-Mediated Senescence

The fresh *Railway Beggarticks* leaves were excised from the same plant and then selected by pairs according to similar sizes and thickness, which were divided as control and FIR groups. Leaves were immersed in three different concentrations of H_2O_2 solutions (10, 100, and 1000 μM). Like the method in cell experiments, we inserted enclosed FIR ceramic powder beneath the H_2O_2 solution container in FIR groups, but not in control groups.

Statistical Analysis

After H_2O_2 degradation and cell viability were measured, the statistical relationship between groups was determined using the t-test method, with p values smaller than 0.05 considered significant.

RESULTS

Direct Scavenging of H_2O_2 with FIR

Figure 1 shows that the mean absorbances of the control and FIR groups were 0.156 and 0.140, respectively ($n = 31$). The extent of H_2O_2 disappearance in the FIR group is larger than that in the control group with a 10.26% decrease. The p value in the t-test is 8.79×10^{-8} with a significant difference. This result confirms that H_2O_2 can be scavenged directly by FIR treatment.

Indirect Scavenging of H_2O_2 with Carrot Extract and FIR

Figure 2 compares H_2O_2 content in indirect H_2O_2 scavenging with carrot extract and FIR. Results show that the FIR group also has less H_2O_2 when interacting with carrot extract. The means of the control and FIR groups are 0.073 and 0.069, respectively ($n = 36$). These two groups also show a significant difference with $p =$ 0.02713. This result indicates that more H_2O_2 was scavenged in the FIR group than the control group, with a 5.48% increase. Figures 1 and 2 demonstrate how direct application of FIR yielded greater H_2O_2-scavenging changes between the experimental and control groups compared to indirect use. Moreover, the figures also show the effectiveness and strength of carrot extract as an antioxidant.

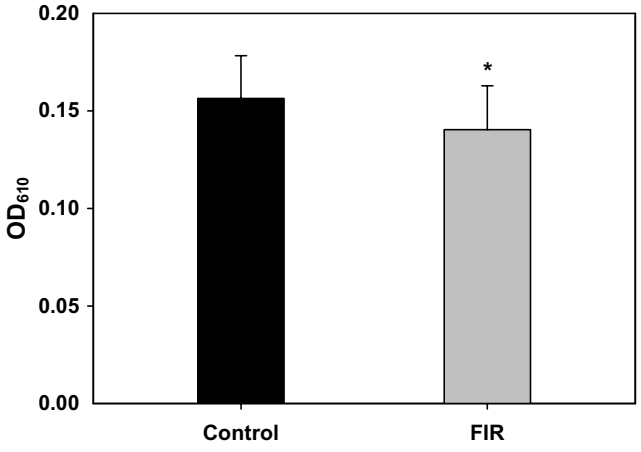


Fig. 1 Comparison of H_2O_2 content in direct H_2O_2 scavenging with FIR (FIR group: treatment with FIR ray ceramic powder; Control group: treatment without FIR ray ceramic powder; and OD_{610}: optical density at 610 nm).

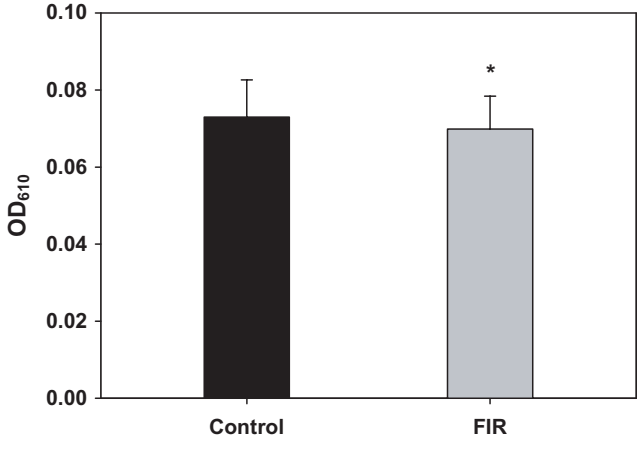


Fig. 2 Comparison of H_2O_2 content in indirect H_2O_2 scavenging with carrot extract and FIR.

The Effect of FIR on Cells Under H_2O_2-Mediated Oxidative Stress

Results (Figs. 3 and 4) reveal that the viability of cells treated with FIR under H_2O_2-mediated oxidative stress is higher than the control counterparts which received H_2O_2 treatment without FIR. In fact, additional FIR treatment resulted in about 23.02% and

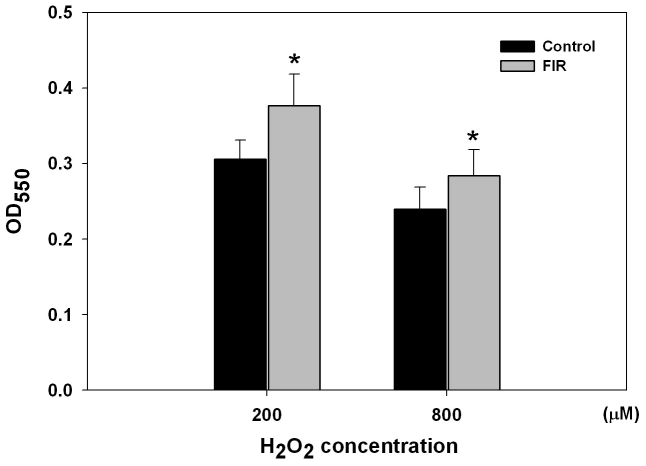


Fig. 3 Effect of FIR on osteoblast cell viability in H_2O_2-induced cytotoxicity.

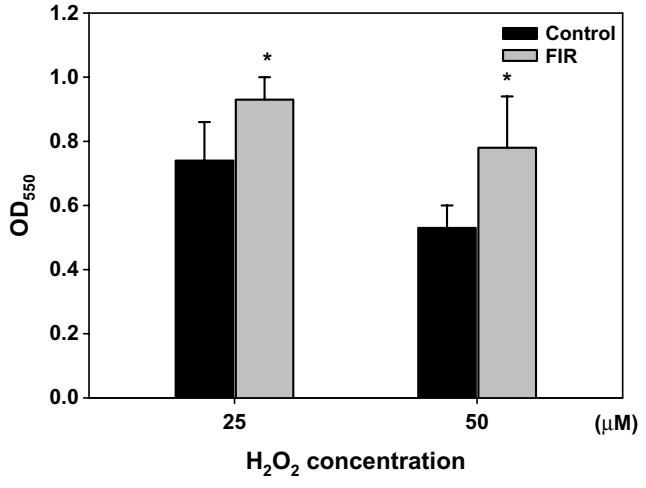
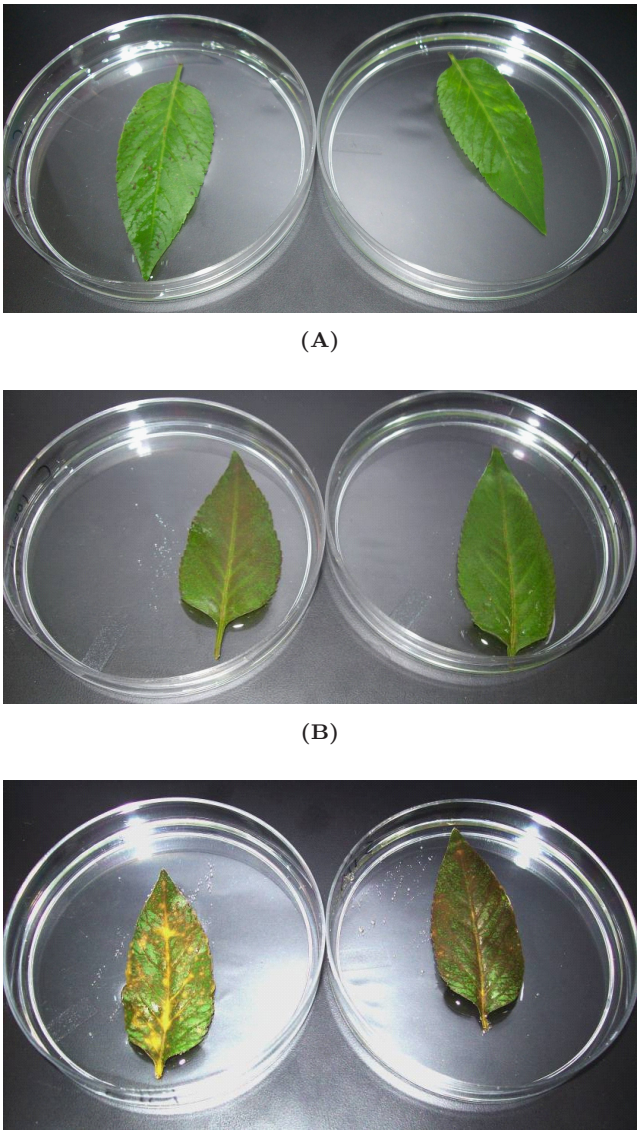


Fig. 4 Effect of FIR on fibroblast cell viability in H_2O_2-induced cytotoxicity.

18.77% osteoblast cell viability increases in the 200 μM and 800 μM H_2O_2 concentration groups, respectively. As for the fibroblast cells, there were about 25.67% and 47.16% cell viability increases in the 25 μM and 50 μM H_2O_2 concentration groups, respectively. The t-test ($n = 28$) further confirms these findings and suggests that FIR treatment may reduce cytotoxicity from H_2O_2-induced oxidative stress.

The Effect of FIR on Detached Leaves Under H_2O_2-Mediated Senescence

Figure 5 shows the photographs of detached *Railway Beggarticks* leaves immersed in different H_2O_2 solutions (Fig. 5(A): 10 μM, Fig. 5(B): 100 μM, and Fig. 5(C): 1000 μM) for 20 h. Results reveal that the degree of



(A)

(B)

(C)

Fig. 5 Effect of FIR on senescence of *Railway Beggarticks* leaves immersed in **(A)** 10 μM, **(B)** 100 μM, and **(C)** 1000 μM H_2O_2 solutions. In each photograph, the right leaf is the FIR group and the left one is the control.

senescence increases with the H_2O_2 concentration in both control and FIR groups. In the low 10 μM concentration, there were pigmentation spots on this green leaf in the control group but absent in FIR group (Fig. 5(A)). As the concentration increased from 10 μM to 1000 μM, there were increased diversity in spots and yellow change in control groups compared to FIR groups (Figs. 5(B) and 5(C)). Therefore, the leaves in FIR groups show delay of senescence by observations in the leaf-yellowing process. These results reflect that there is less chlorophyll breakdown of leaves in FIR groups than the control groups.

DISCUSSION

We know that FIR induces many biological activities[1–12] and studies have demonstrated that thermal FIR treatment has the capability of cleaving covalent bonds to liberate more antioxidant compounds, e.g., carotene and polyphenols from rice hulls and medicinal plants.[13,14] Therefore, the increased heat transfer efficiency of FIR-emitting ceramic material explains the direct and indirect effects on H_2O_2-scavenging capacity. Additionally, FIR can break hydrogen bonds (H–O bonds) by exciting "stretching or bending" vibrations in water clusters[8] and decrease the size of water clusters. Hydrogen bonds are a factor that decrease the volatility of any liquid possessing hydrogen bonds, and are related to the reduction rate of H_2O_2 which exist in cluster form in general conditions, and not as a single molecule. Therefore, the weakening of hydrogen bonds by FIR may also explain why FIR accelerates H_2O_2 transformation and releases H_2O and O_2 from H_2O_2 molecular clusters.

It is important to emphasize the beneficial biological effects of H_2O_2-scavenging capacity using FIR-emitting ceramic material. Basically, H_2O_2 is continuously produced in oxidation-redox centers in animal and plant aerobic respiratory systems. Cumulative increases in H_2O_2 and superoxide radicals can potentially damage cells including proteins, lipids, and DNA leading to proven augmented mutation rates.[15] Reactive oxygen radicals of H_2O_2 are an important factor of oxidative stress related to the pathogenesis of many important diseases in both animals and plants.[16,17] In general, oxidative stress is responsible for the postmenopausal and aging process in osteoporosis.[18,19] It was further found that H_2O_2-mediated oxidative stress is a major cause of cellular damage and osteoblast death, which significantly decreases bone formation and results in net bone loss. In normal or pathological aging, osteoblasts become more vulnerable to oxidative stress, which could explain why postmenopausal women and older people are more susceptible to suffering bone fractures. Hence, H_2O_2-induced oxidative stress may result in many bone diseases such as postmenopausal and senile osteoporosis.[18–21]

Fujimori *et al.*[22] reported that ultraviolet (UV) ray produced superoxide anion radicals that spontaneously convert to H_2O_2 in turn to suppress cell viability of human dermal fibroblasts. The decrease in fibroblast proliferation is a major factor of aging skin, showing a decrease in collagen production. The phenomenon of aging skin includes wrinkling, dryness, roughness, and pigmentation.[22,23] The protection against H_2O_2-induced oxidative damage of fibroblasts gives the support to the wound-healing process,[24] since it was known that the administration of anti oxidants or free radical scavengers is helpful in burns and thermal trauma to enhance the healing process.[25] The property of FIR as the H_2O_2 scavenger will be utilized not only in the field of cosmetics, but also in wound-repairing promotion.[22,23]

Moreover, H_2O_2 was found at significantly higher levels in the breathing secretions of asthmatic patients and patients with chronic obstructive pulmonary disease (COPD) compared to normal subjects.[26] The H_2O_2 concentration in the exhaled air condensate of asthmatic patients was significantly higher than the healthy control groups. Lower levels were also found in asthmatic patients who received anti-inflammatory treatment. It has been suggested that airway inflammation is responsible for H_2O_2 exhalation.[27] Pathologically, H_2O_2 released from luminal phagocytes in the airway can potentially injure the epithelium. Oxygen radical scavengers must, therefore, exist to prevent damage.[28] On the other hand, Friedreich's ataxia, a kind of neurodegenerative disorder, is due to a pathological impairment of antioxidant enzymes with increased oxidative stress as a consequence of free radical cytotoxicity.[29] Clinically, the capacity to scavenge H_2O_2 was found to successfully suppress the disease severity of Friedreich's ataxia.[30,31]

In plants, the aerobic environment is also continuously generating toxic reactive oxygen species of H_2O_2 and superoxide radicals. These reactive oxygen species are proven to degrade intracellular components, including membrane lipids, proteins, and DNA.[32] Further, H_2O_2 promotes senescence in detached rice leaves, and this leaf-aging process is correlated with free radical-induced lipid peroxidation.[33] Environmental stresses are known to induce H_2O_2 production in plant cellular compartments; as a result, leaf aging is accelerated through lipid peroxidation and other oxidative damage.[33,34] The accumulation of H_2O_2 and oxidative stress may disrupt metabolic function and endanger cellular integrity.[35]

CONCLUSION

This paper is the first to examine the H_2O_2-scavenging effect of FIR-emitting ceramic material at room temperature. Results demonstrated that FIR significantly increased H_2O_2-scavenging capacity by both direct and

indirect treatment. We further proved that FIR under somatothermal conditions effectively increased cell viability against H_2O_2-mediated toxicity. However, these *in vitro* experiments are not a perfect model reflecting the influence of FIR-emitting material on *in vivo* H_2O_2 production. In the future, a more precise measurement method to detect tiny differences in the cellular H_2O_2-scavenging capacity needs to be developed.

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