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REGULAR PAPER

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White rice ethanol extract is qualitatively, but not quantitatively, equivalent to that of brown rice as an antioxidant source

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ABSTRACT

The purpose of this study is to compare the potentials to exhibit biologically active antioxidant actions between white rice (WR) and brown rice (BR) in *in vitro* assays and a cellular model. The Trolox equivalent (TE) per 1 mg ethanol extract of WR for the 1,1-diphenyl-2-picrylhydrazyl assay was slightly higher than that of BR, whereas the TE per 1 g whole WR was much lower than that for BR. This tendency was very comparable to those for the oxygen radical absorbance capacity and total polyphenol content. Both of the ethanol extracts also similarly suppressed the hydrogen peroxide-induced cytotoxicity and enhanced the gene expression of drug-metabolizing enzymes. Based on the α-tocopherol quantity, its contribution to the cytoprotective effect of the rice extracts is very limited. Taken together, the ethanol extract of WR might be a qualitatively, but not quantitatively, equivalent source of antioxidative phytochemicals to that of BR.

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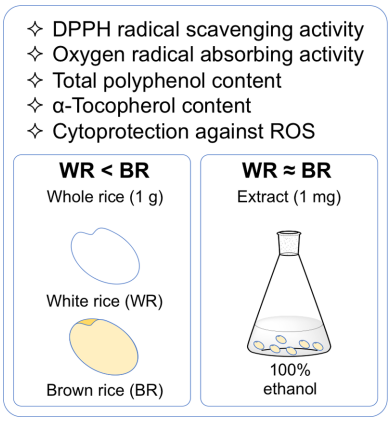
Graphical Abstract



Ethanol extracts of white rice and brown rice showed the equivalent antioxidative potentials not only in the *in vitro* assays but also the cellular assay.

Keywords: white rice, antioxidant, cytoprotection, phase 2 drug-metabolizing enzymes, α-tocopherol

Abbreviations: BR: brown rice; WR: white rice; αT: α-tocopherol; DPPH: 1,1-diphenyl-2-picrylhydrazyl; ORAC: oxygen radical absorbance capacity; TPC: total phenolic content; TE: Trolox equivalent; GAE: gallic acid equivalent; HO-1: heme oxygenase-1; NQO1: NAD(P)H: quinone oxidoreductase 1 (NAD(P)H dehydrogenase [quinone] 1); RT-PCR: reverse transcription-polymerase chain reaction; MTT: 3-(4,5-dimethylthiazol, 2-yl)-2,5-diphenyl tetrazolium bromide

Rice (*Oryza sativa*) is one of the most principal crops in the world, feeding nearly half of the world's population (Gong *et al.* 2017). In addition to meeting the essential energy requirements, it also serves as a good source of radical scavenging type antioxidants with beneficial functions for humans, such as vitamin E derivatives, phenolic acids, and γ-oryzanols (Goufo and Trindade 2014). Since these radical scavengers are highly concentrated on the rice bran layer and germ portion, brown rice (BR) with a lower degree of milling is regarded to possess higher biologically active potentials for disease prevention than fine milled white rice (WR) (Moongngarm, Daomukda and Khumpika 2012). BR or its extract has been reported to show a wide range of pharmacological effects, such as antidiabetic, anti-hyperlipidemic, and cardioprotective (Ravichanthiran *et al.* 2018). For example, in obese mice induced by a high-fat diet, the intake of germinated BR methanol extracts markedly reduced body weight and hepatic lipid accumulation (Ho *et al.* 2012). In the rabbit model of myocardial infarction, the oral administration of germinated BR recovered cardiac function as well as lowered blood glucose levels (Petchdee, Laosripaiboon and Jarussophon 2020). In people who suffered from high levels of total cholesterol, supplementation of BR extracts for 1 month reduced the cholesterol levels of the serum (Murata *et al.* 2007). On the other hand, to the best of our knowledge, the difference in antioxidative and cytoprotective potentials between WR and BR remains to be clarified, even though WR is the predominant type of rice consumed worldwide (Hu *et al.* 2012).

Vitamin E derivatives are famous lipophilic antioxidants and rich in BR, accounting for approximately 6.0 mg/100 g (Goufo and Trindade 2014). Among them, α-tocopherol (αT) is the most commonly detected form of vitamin E analogues, contributing to 10%-30% of total vitamin E amounts in BR (Lin and Lai 2011; Goufo and Trindade 2014). In the human body, αT tends to be maintained in the largest amounts because of the highest binding affinity of αT transfer protein (Galli *et al.* 2017). Of all the beneficial biological properties of αT, the antioxidative capability is reckoned as one of the most applicable mechanisms to interpret the prevention for chronic diseases. In contrast, αT inhibits the reactive oxygen species (ROS)-induced cancer cell invasion, possibly through the suppression of protein kinase C activation, but not by inhibiting peroxide production in the cytoplasm (Yoshida *et al.* 2011). Moreover, in a mouse depressive disorder model, αT administration normalized several antioxidative enzyme activities as well as the inflammatory factor levels (Herbet *et al.* 2018). These findings led us to a hypothesis that αT would exert a cytoprotective effect on the ROS-induced toxicity not only by directly scavenging the detrimental oxidants, but also through intracellular signaling pathway-mediated gene expression.

In the present study, we qualitatively and quantitatively compared the *in vitro* antioxidative activities of the ethanol extracts between WR and BR. To reassess the WR extract as a potential source of biologically active antioxidants, we examined whether the pretreatment of the rice extracts showed protective effects on the hydrogen peroxide-induced cytotoxicity in mouse hepatoma Hepa1c1c7 cells. The present results suggested that both of the ethanol extracts of WR and BR equally suppressed the cytotoxicity, possibly through the enhanced expression of the antioxidant enzyme genes. In addition, we evaluated the inhibitory effect of αT on cellular oxidative damage and discussed its contribution to the cytoprotective effect of the rice extract.

Materials and methods

Materials and chemicals

WR and BR, produced in Miyazaki prefecture, Japan, were provided by the Satake Corporation (Hiroshima, Japan). WR was obtained by grinding BR by 10% less than initial weight using a

rice milling machine (MC-250, Satake Corporation). D-αT was purchased from TCI Chemicals Company (Tokyo, Japan). Other chemicals were obtained from Wako Pure Chemicals Corporation (Osaka, Japan) or Nacalai Tesque (Kyoto, Japan).

Preparation of the rice extract

Five grams of a rice sample was ground using a grinder. One gram of the ground rice powder was extracted in 10 mL of ethanol, followed by incubation in an electrical shaker (BR-23FP, TAITEC, Koshigaya, Japan) at 1.67 rps and 60 °C for 90 min. The extract was filtered through a filter paper, followed by a 0.45 µm membrane filter. The filtrates were pooled, and the solvent was removed under reduced pressure in a rotary evaporator (CVE-3110 and UT-2000, EYELA, Tokyo, Japan). After drying, the resulting residual was stored at −20 °C until used.

DPPH assay

The 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity was assayed by a previously described method with minor modifications (Nakamura *et al.* 2003). Briefly, 0.6 mL of various concentrations of the rice ethanol extract dissolved and diluted in ethanol were mixed with 0.6 mL of a 0.1 mM DPPH ethanol solution, then the mixture was kept for 30 min at room temperature in the dark. The absorbance of the reaction solution was recorded at 520 nm. Trolox was employed as a standard and the radical scavenging capacity of the rice extract was calculated as µmol Trolox equivalents (TE)/g rice or nmol TE/mg extract.

ORAC assay

The oxygen radical absorbance capacity (ORAC) assay was performed according to the protocol by Watanabe *et al.* (2016) with the following modifications. Briefly, 35 µL of various concentrations of the rice ethanol extracts or Trolox standard were mixed with 115 µL of fluorescein working solution (77.5 nM) in a 96-well plate. The initial fluorescence was recorded as ($f_{0\,min}$). During the following 120 min, the fluorescence was recorded every 2 min after the addition of 50 µL of 2,2′-azobis(2-methylpropionamidine) dihydrochloride (AAPH, 82.4 mM). The area under the curve (AUC) was calculated for each sample by integrating the relative fluorescence curve. The net AUC of the sample was calculated by subtracting the AUC of the blank. The final results were converted as µmol TE/g rice or nmol TE/mg extract.

Determination of the total phenolic content

The total phenolic content (TPC) of the rice extract was determined using the Folin–Ciocalteu assay as reported previously (Iqbal, Bhanger and Anwar 2005) with slight modifications. Briefly, the reaction mixture consisted of 50 µL of diluted rice extract, 800 µL of distilled, deionized water (DDW), 50 µL of Folin–Ciocalteu reagent, and 100 µL of 10% sodium carbonate. The mixed solutions were subsequently kept for 30 min in the dark for the reaction. The absorbance was determined at 760 nm. Gallic acid was used as a standard and the results were calculated as µmol gallic acid equivalents (GAE)/g rice or nmol GAE/mg extract.

Cell cultures

The mouse hepatoma cell line Hepa1c1c7 was purchased from the American Type Culture Collection. The cells were cultured in the minimum essential medium-α (MEMα, Gibco) supplemented with 10% fetal bovine serum, 100 U/mL penicillin, and 100 µg/mL streptomycin at 37 °C in a 5% CO_2 containing atmosphere. The αT concentration of the cell culture media with 10% fetal bovine serum was below the detection limit.

MTT assay for cell viability determination

Hepa1c1c7 cells were seeded in a 96-well plate at 2×10^4 cells per well. After the 24-h preculture, the cells were pretreated with the rice extracts, αT or ethanol as a vehicle (final concentration, 0.1%) for the indicated time points. The cells were washed once with phosphate-buffered saline (PBS), followed by the exposure to hydrogen peroxide at 100 µM for 6 h. After the oxidative challenge, the cells were incubated with 0.5 mg/mL of an MTT solution for 2 h. The insoluble formazan crystals were dissolved in 2-propanol, then the absorbance was measured at 570 nm by a microplate reader (Benchmarkplus, Bio-Rad Laboratories, Hercules, CA, USA). The cell viability values were expressed as the percentages over the corresponding controls.

RNA extraction and reverse transcription-PCR analysis

Hepa1c1c7 cells (5.0×10^5) were precultured in a 60 mm dish for 24 h, then treated with or without the different concentrations of rice extracts or αT for 24 h. Total RNA was extracted using the Trizol reagent (Thermo Fisher Scientific, Waltham, MA, USA) according to the manufacturer's manual. The total RNA (5 µg) was reverse transcribed to cDNA using the ReverTra Ace (Toyobo, Osaka, Japan), and PCR amplification was then performed with Taq polymerase and specific primers for heme oxygenase-1 (HO-1) and NAD(P)H: quinone oxidoreductase 1 (NQO1, NAD(P)H dehydrogenase [quinone] 1). The primer sequences and the expected PCR product sizes are as follows; mHO-1, (F) 5′-ACATCGACAGCCCCACCAAGTTCAA-3′ and (R) 5′-CTGACGAAGTGACGCCATCTGTGAG-3′ (22 cycles, product size 203 bp); mNQO1, (F) 5′-TCGAAGA ACTTTCAGTATCC-3′ and (R) 5′-TGAAGAGAGTACATGGAGCC-3′ (23 cycles, product size 290 bp); mβ-actin, (F) 5′-GCTCTTTTCCAGCCTTCCTT-3′ and (R) 5′-CTTCTGCATCCTGTCAGCAA-3′ (16 cycles, product size 455 bp). The amplified PCR products were separated on an agarose gel (2%), stained with ethidium bromide, and visualized with an LAS3000 image analyzer (FujiFilm, Tokyo, Japan). The relative densities of bands were measured using the Image J Software Program.

HPLC analysis of αT

The concentrations of αT in the rice samples were analyzed by reverse-phase high-performance liquid chromatography (HPLC) with a fluorescence detector (Ex. 295 nm, Em. 325 nm) as previously reported (Bando, Yamanishi and Terao 2003) with some modifications. Briefly, the HPLC separation was done with isocratic elution (water:methanol = 7:93) using an Inertsil C8 (4.6 × 150 mm) column (GL Sciences, Tokyo, Japan) at the flow rate of 1.0 mL/min with a column oven temperature of 40 °C. The sample injection volume was 10 µL. The minimal limit of detection for αT was 10 nM.

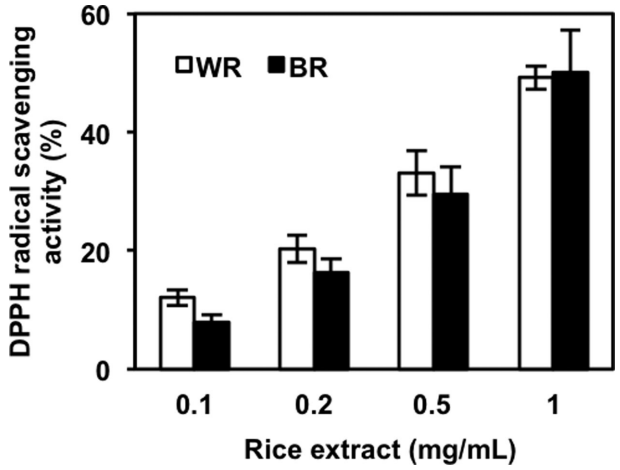


Figure 1. DPPH radical scavenging capacities of ethanol extracts of white and brown rice. The tested rice ethanol extracts were incubated with 0.1 mM DPPH for 30 min at room temperature in the dark. The DPPH radical scavenging activity was evaluated by the absorbance at 520 nm. All values were expressed as means ± SD of 4 separate experiments.

Statistical analysis

All values are expressed as the mean of at least 3 independent experiments ±SD. Statistical significance was determined by Student's paired two-tailed *t*-test or one-way ANOVA followed by Tukey's HSD using SPSS version 16.0 (IBM, Chicago, USA). A *P*-value <.05 was considered significant in all comparisons.

Results

Comparison of *in vitro* antioxidative potentials of ethanol extract between white and brown rice

The solvent extraction technique has received considerable interest in the food industry due to its feasibility to recover or separate the bioactive compounds in the food materials. A previous report showed that the yield of the plant constituents and antioxidant activity of the food-grade extraction using organic solvents with low polarities, such as ethanol, were higher than that of the water extract (Lapornik, Prošek and Wondra 2005). Considering that ethanol is a frequently used solvent to recover lipophilic compounds from food matrices, we applied 100% ethanol to extract our WR and BR samples. The yield of the ethanol extracts of WR (5.36 ± 1.71 mg/g rice) was about one-fourth less than that of BR (23.7 ± 4.51 mg/g rice). As shown in Figure 1, both of the ethanol extracts of WR and BR dose-dependently scavenged DPPH free radicals from 10.0% to 49.7%. Their scavenging effects were not significantly different when compared at each concentration. The TE value in the 1 mg WR extract (20.66 ± 0.68 nmol TE/mg extract) was slightly but significantly higher than that in the BR extract (15.50 ± 1.69 nmol TE/mg extract), though the TE value in the 1 g whole WR (0.11 ± 0.01 µmol TE/g rice) was about one-third less than that of BR (0.36 ± 0.09 µmol TE/g rice, Table 1). To compare the peroxyl radical scavenging capabilities of both rice extracts, we next employed the ORAC assay, which is more similar to the actual *in vivo* reaction. A similar tendency was observed; the TE in the 1 mg WR extract (279.57 ± 23.00 nmol TE/mg extract) presented a slightly higher ORAC value than BR (220.22 ± 12.60 nmol TE/mg extract), whereas that in the 1 g whole WR (1.55 ± 0.33 µmol TE/g rice) was about one-third less than that of BR (5.11 ± 0.42 µmol TE/g rice, Table 1). Consistently, the TPC of WR (60.39 ± 5.11 nmol GAE/mg extract) was slightly higher than that of BR (56.36 ± 2.44 nmol GAE/mg extract), though the TPC of the whole WR (0.31 ± 0.05 µmol GAE/g rice) was only one-fourth of that of the whole BR (1.36 ± 0.12 µmol GAE/g rice, Table 1). These results suggested that BR is a whole grain that contained much higher amounts of antioxidant compounds, thus presented a stronger antioxidative capacity than the whole WR. However, when compared between their ethanol extracts at the same amount, the WR ethanol extract might have at least an equivalent antioxidative potential to that of BR.

Cytoprotective effect of rice ethanol extracts on the hydrogen peroxide-induced cytotoxicity

Since the ethanol extracts of BR and WR have significant antioxidative potentials in the *in vitro* assays, we further investigated their protective effects on the hydrogen peroxide-induced oxidative damage in mouse hepatoma Hepa1c1c7 cells. Since both rice ethanol extracts at concentrations up to 1 mg/mL showed no obvious cytotoxicity toward Hepa1c1c7 cells (data not shown), these concentrations were used for the following experiments. The treatment of hydrogen peroxide (100 µM) significantly induced oxidative damage on the cells, reducing the cell viability to 71% (Figure 2), as previously reported (Tang *et al.* 2016). Next, after each rice extract was pretreated with Hepa1c1c7 cells for 24 h and washed out, the cells were challenged by hydrogen peroxide. The pretreatment with both of the WR and BR extracts significantly alleviated the hydrogen peroxide-induced cytotoxicity. The viabilities of the cells treated with 1 mg/mL of the WR and BR extracts were 85% and 82%, respectively. These results suggested that both ethanol extracts have the same potential to protect the cells from oxidative stress, possibly through the gene expression of the antioxidant-related enzymes, in addition to direct radical scavenging effects.

Since phase 2 drug-metabolizing enzymes play a critical role in maintaining redox homeostasis against oxidative and electrophilic damages (Nakamura and Miyoshi 2010), we next investigated the modulating effects of the rice ethanol extracts on the representative phase 2 drug-metabolizing enzymes, HO-1 and NQO1. Upon exposure to the WR extract, the mRNA level of HO-1 was increased to 1.54-fold of the control at the concentration of 1 mg/mL, while the enhanced expression of NQO1 was 1.33-fold at the same concentration (Figure 3b and c). A similar increasing pattern was observed for HO-1 and NQO1 with BR

Table 1. Free radical scavenging capacity and total phenolic content of various rice ethanol extract and whole rice[a]

Varieties	DPPH (nmol TE/mg extract)	DPPH (µmol TE/g rice)	ORAC (nmol TE/mg extract)	ORAC (µmol TE/g rice)	TPC (nmol GAE/mg extract)	TPC (µmol GAE/g rice)
WR	20.66 ± 0.68[b]	0.11 ± 0.01[b]	279.57 ± 23.00[b]	1.55 ± 0.33[b]	60.39 ± 5.11[b]	0.31 ± 0.05[b]
BR	15.50 ± 1.69[c]	0.36 ± 0.09[c]	220.22 ± 12.60[c]	5.11 ± 0.42[c]	56.36 ± 2.44[b]	1.36 ± 0.12[c]

[a]Results are expressed as mean ± SD (n = 3-4).
[b,c]Values with different letters in a column indicate statistical significance at P < .05.

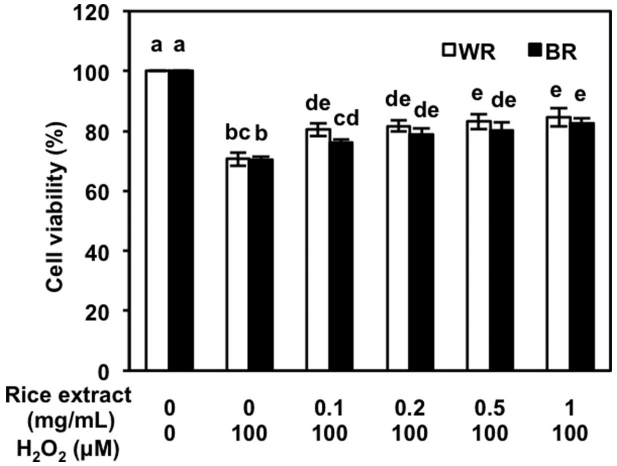


Figure 2. Protective effect of rice extracts on the hydrogen peroxide-induced cytotoxicity in Hepa1c1c7 cells. Hepa1c1c7 cells were pretreated with the ethanol extract of BR or WR for 24 h, followed by treatment with 100 μM of hydrogen peroxide for 6 h. Cell viability was measured using an MTT assay. Values are means ± SD of 4 independent experiments. Different letters above the bars indicate significant differences among the treatments for each condition ($P < .05$).

extract treatment (Figure 3d and e). Taken together, the enhanced expression of the phase 2 drug-metabolizing enzymes might, at least in part, contribute to the protective effect of both extracts.

Cytoprotective potential of α-tocopherol, one of the predominant antioxidants in rice extract

Given that αT is one of the representative lipophilic antioxidants in rice (Goufo and Trindade 2014), we then verified whether αT is the primary component responsible for the cytoprotective effect of the rice extracts. Since αT at the concentrations up to 100 μM was not toxic to cell growth (data not shown), these concentrations were used for the following evaluation. As shown in Figure 4a, the 24-h pretreatment with αT slightly but dose-dependently reversed the cytotoxicity caused by hydrogen peroxide. The significant protection of αT against hydrogen peroxide was observed at concentrations ranging from 25 to 100 μM. Furthermore, 100 μM of αT alleviated the cytotoxicity in a time-dependent manner with the significant protection observed in the cells with its 9-h incubation (Figure 4b). These results suggested that a long-term preculture is prerequisite for αT to exhibit the cytoprotection. Therefore, we examined whether αT modulates the gene expression of the phase 2 drug-metabolizing enzymes. As shown in Figure 5, the treatment of Hepa1c1c7 cells with αT for 24 h exhibited a significant and dose-dependent enhancement in the mRNA level of HO-1, whereas no changes were observed in that of NQO1. Taken together, these results indicated that αT also protects the cells from oxidative damage, possibly through upregulation of the HO-1 gene expression.

Contribution of α-tocopherol to antioxidative potentials of rice extracts

We determined the quantity of αT in both rice samples by HPLC analysis. The average concentration of αT in 1 mg of the WR extract (0.54 ± 0.07 nmol/mg extract) was slightly higher than that of BR (0.47 ± 0.06 nmol/mg extract). Based on these data, we next estimated the contribution of αT to the antioxidant capacity by comparing the αT quantity with TE of the rice extracts or whole grains. The % contributions of αT to each rice extract were quite similar (2.6% and 3.0% for the WR and BR extracts, respectively) (Table 2). However, the concentration of αT in 1g of the whole WR (3.16 ± 0.07 nmol/g WR) was only one-fourth of that of BR (11.12 ± 1.73 nmol/g BR), contributing to 2.9% and 3.1% of the antioxidant capacity of 1 g of the whole WR and BR, respectively (Table 2). These estimations indicated that αT might, at least in part, contribute to the *in vitro* antioxidative potentials of both WR and BR. Furthermore, the minimal concentration of the WR extract required to induce HO-1 gene expression was 0.5 mg/mL (Figure 3b), which contains approximately 0.25 nmol/mL of αT. However, more than a 100-fold higher concentration of αT (25 μM = 25 nmol/mL) was required for the significant enhancement of the same gene expression (Figure 5b). Thus, when compared to the minimal concentrations required for cytoprotection between αT and the rice extracts, the contribution of αT to the cytoprotective effect of the rice extract was quite limited.

Table 2. αT content and % contribution to the antioxidant capacity of various rice ethanol extract and whole rice[a]

Varieties	αT (nmol/mg extract) (percent contribution to antioxidant capacity of rice extract)	αT (nmol/g rice) (percent contribution to antioxidant capacity of rice extract)
WR	0.54 ± 0.07 (2.63 ± 0.35%)	3.16 ± 0.07 (2.87 ± 0.06%)
BR	0.47 ± 0.06 (3.03 ± 0.42%)	11.12 ± 1.73 (3.10 ± 0.48%)

[a]Results are expressed as mean ± SD ($n = 3$-4).

Discussion

In the present study, we qualitatively and quantitatively compared the *in vitro* antioxidative activities of the ethanol extracts between WR and BR. We demonstrated that the WR ethanol extract exerted a similar DPPH radical scavenging activity to that of BR at the same concentrations (Figure 1). The reproducibility of this tendency was confirmed using the rice samples produced in the different areas (unpublished data). Simultaneously, the TE value in 1 mg of the WR extract for the DPPH assay was comparable to or higher than that in the BR extract, the parallel tendency of which was observed in both the ORAC and Folin–Ciocalteu assays (Table 1). Conversely, the TE value in 1 g of the whole WR was much lower than that for BR (Table 1), supporting the idea that the whole BR contains more abundant amounts of antioxidants and herein exhibited a much stronger antioxidative capacity than the whole WR (Park *et al.* 2018). Collectively, these results indicated that the quantity, not the quality, of phytochemicals in the whole grain determines the difference in the antioxidant capacity between WR and BR. In other words, when compared between their ethanol extracts at the same amount or concentration, the WR ethanol extract might be qualitatively equivalent to that of BR as the antioxidant source.

In this study, not only the BR extract, but also the WR extract, was identified as one of the potential sources of biologically active antioxidants. This idea was supported by the finding that the minimal concentration of the rice extracts required for the enhanced expression of NQO1 as well as the cytoprotection (100 μg/mL, Figures 2 and 3) is equivalent to those of broccoli, celery, and parsley as previously reported (Hashimoto *et al.* 2002). It has been reported that rice extract upregulated the antioxidant

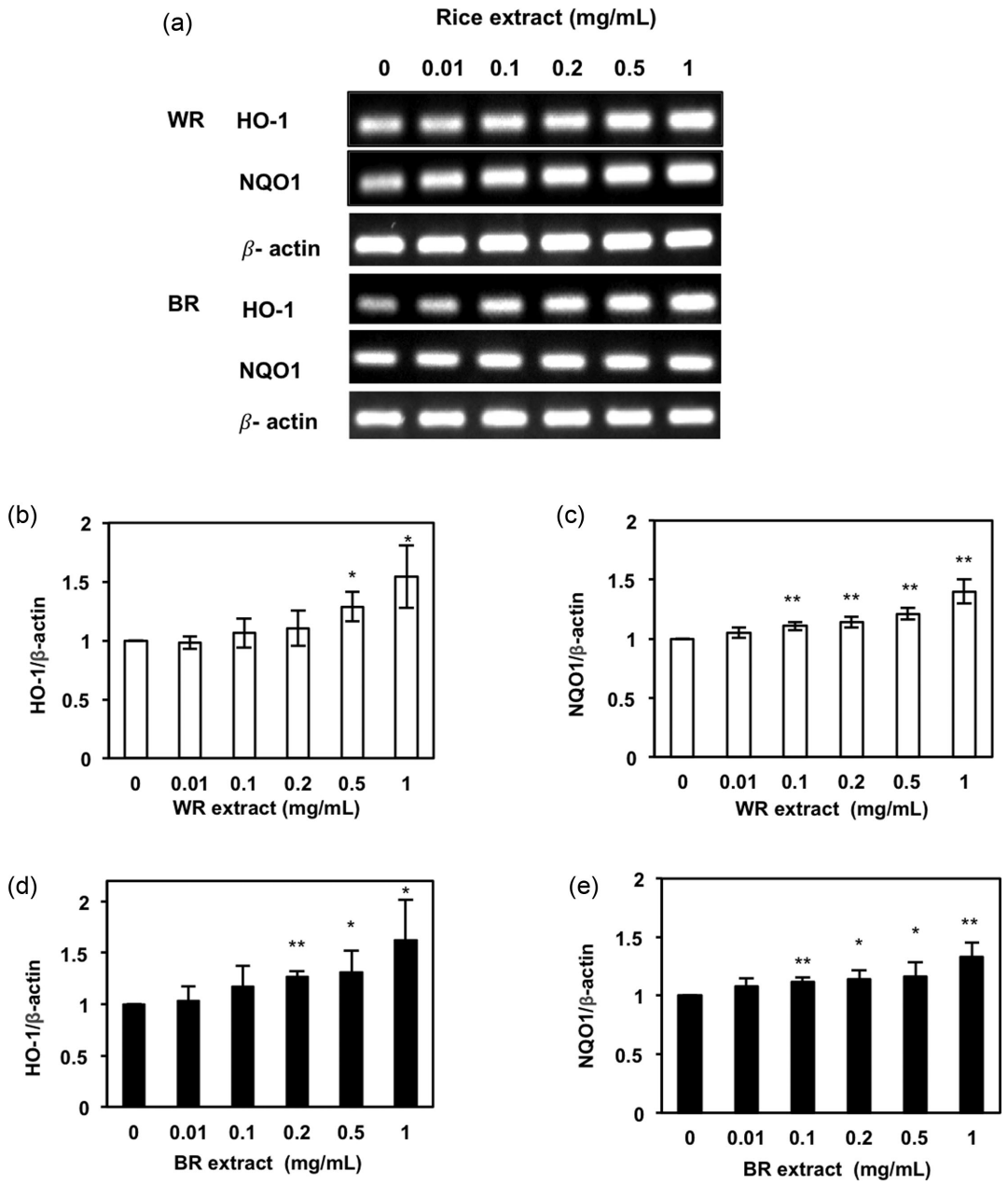


Figure 3. Enhancing effect of rice extracts on the gene expression of phase 2 drug-metabolizing enzymes. Hepa1c1c7 cells were treated with the ethanol extract of BR or WR at the indicated concentrations for 24 h and the mRNA levels of HO-1 and NQO1 were analyzed by RT-PCR. All values are expressed as means ± SD of 3 separate experiments. $^{**}P < .01$; $^{*}P < .05$ vs control.

enzymes, including superoxide dismutase and catalase, and downregulated the apoptosis-related transcriptional factors, such as p53 and nuclear factor-kappa β (NF-$\kappa\beta$), to potentiate an intracellular defense (Azmi *et al.* 2013). Another report indicated that the rice extract could prevent cell cycle arrest together with repression of early and late apoptosis (Ismail *et al.* 2012). In addition, the phase 2 drug-metabolizing enzymes, transcriptionally regulated by nuclear factor (erythroid-derived 2)-like 2 (Nrf2), are also plausible to play an important role in detoxifying oxidants as well as electrophiles (Nakamura and Miyoshi 2010). Previous studies suggested that food-derived extracts, such as the Lycii fructus extract (Xu *et al.* 2018) and *Olea europaea* leaf extract (ALHaithloul *et al.* 2019), might exert a protective potential against oxidative stress through the Nrf2/antioxidant response element (ARE)-dependent pathways. In the present study, we found, for the first time, both the BR and WR ethanol extracts significantly upregulated the gene expression of HO-1 and NQO1 (Figure 3). These findings led us to speculate that the Nrf2/ARE-dependent pathway might be involved in the cytoprotection by the rice extract, which will be investigated in a future study.

The pretreatment of αT, the predominant lipophilic antioxidant of rice, also showed the cytoprotective effect in Hepa1c1c7 cells (Figure 4a). The pretreatment time required for this effect was at least 9 h (Figure 4b), suggesting that the direct radical scavenging effect could be ruled out in the cytoprotective mechanisms. Even though the activation of the Nrf2/ARE-dependent pathway might be involved in the αT-induced cytoprotection in human retinal pigment epithelial cells (Feng *et al.*

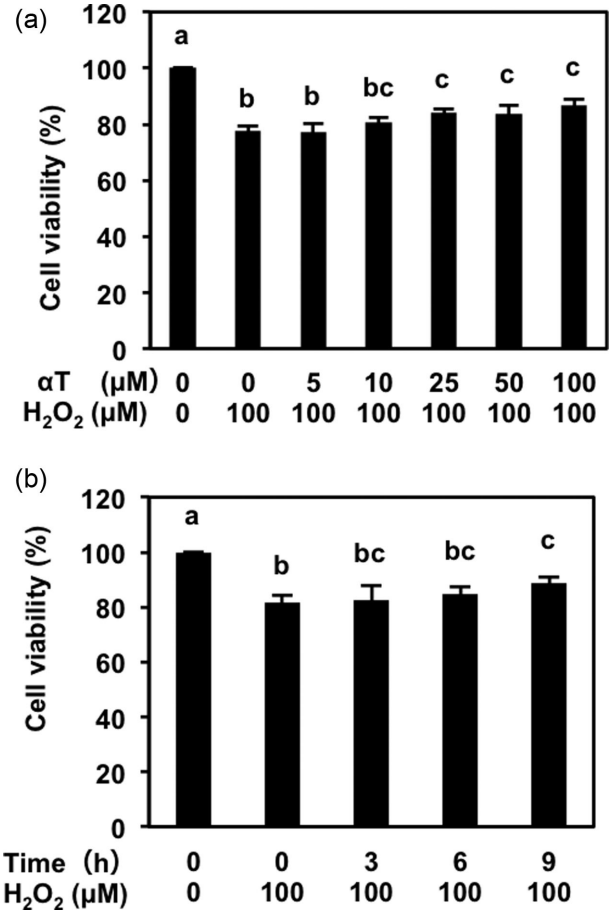


Figure 4. Protective effect of αT on the hydrogen peroxide-induced cytotoxicity in Hepa1c1c7 cells. Hepa1c1c7 cells were pretreated with αT at the indicated concentrations for 24 h (a) or pretreatment with 100 μM of αT for the indicated time point (b), followed by treatment with 100 μM of hydrogen peroxide for 6 h. Cell viability was measured using an MTT assay. Values are means ± SD of 3 independent experiments. Different letters above the bars indicate significant differences among the treatments for each condition ($P < .05$).

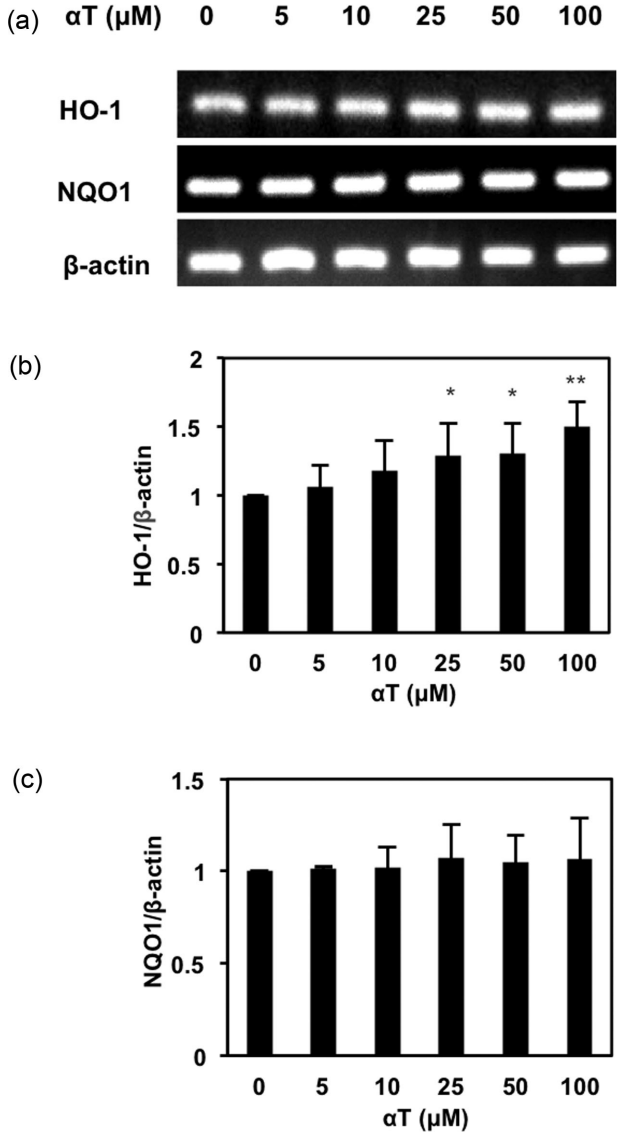


Figure 5. Enhancing effect of αT on the gene expression of phase 2 drug-metabolizing enzymes. Hepa1c1c7 cells were treated with αT at the indicated concentrations for 24 h and the mRNA levels of HO-1 and NQO1 were analyzed by RT-PCR. All values were expressed as means ± SD of 5 separate experiments. $^{**}P < .01$; $^{*}P < .05$ vs control.

2010), αT did not significantly enhance the gene expression of NQO1 in mouse hepatoma Hepa1c1c7 cells (Figure 5). Indeed, a recent study using rat renal cells suggested that αT induces HO-1, possibly through the extracellular signal-regulated kinase (ERK) or protein kinase A (PKA)/cAMP-response element (CRE)-dependent pathway, but not by the Nrf2/ARE axis (Reed, Hall and Arany 2015). Therefore, we could not exclude the possibility that αT shows the cytoprotective effect mainly through the HO-1 induction via the Nrf2-independent pathway. In any case, based on the different pattern of the phase 2 enzyme induction, αT is not fully responsible for the rice extract-induced cytoprotection in Hepa1c1c7 cells.

In the present study, we quantified the αT content as follows: 0.54 nmol/mg in the WR extract and 0.47 nmol/mg in the BR extract, similar to those previously reported (Goufo and Trindade 2014; Shammugasamy *et al.* 2015). Comparing them with the TE values, the contributions of αT to the antioxidant capacity of the WR and BR extracts were estimated to be 2.6% and 3.0%, respectively (Table 2). These data suggested that the WR ethanol extract is an equivalent source of αT to that of BR. Also, the αT content in the extract might be insufficient but partly accountable for the total antioxidant capacity of the rice extracts. On the other hand, the contribution of αT to the cytoprotective action of rice extract was estimated to be, more or less, just 1%. Therefore, the contribution of phytochemicals other than αT, such as γ-oryzanol and ferulic acid (Goufo and Trindade 2014), should be taken into account when evaluating the biologically active antioxidant property of the rice ethanol extracts. The distinction of effective concentrations for cytoprotection between the rice extracts and single αT treatment may consolidate a dietary strategy that whole food supplementation is more effective than the singular nutrient intake, because the interaction of diverse components amplifies their health-promoting benefits (Xi and Liu 2016).

In conclusion, the present study provided biological evidence that the WR ethanol extract might be a qualitatively equivalent source of biologically active antioxidants to that of BR. WR as well as BR induces cytoprotection against hydrogen peroxide, possibly through the enhanced expression of the phase 2 drug metabolizing enzyme genes. Since WR is the prevalent type of rice consumed worldwide, its extract has some advantages for

application as a food material with a high safety. Future efforts will be related to further understanding the involvement of the Nrf2-related signaling pathway in the enzyme induction as well as cytoprotection. In addition, the synergistic or additive functions of other phytochemicals in rice other than αT would be necessary to clarify.

Data availability

The data will be shared on reasonable request to the corresponding author.

Author contribution

H.W., T.N., and Y.N. designed the research, conceived the experiments, and drafted the article. H.W., T.N., Y.G., and M.H. performed the experiments. T.N., G.Z., S.M., Y.M., and Y.N. assisted with the experiments and contributed to the discussions. A.F. provided the materials. Y.N. supervised and edited the paper. All authors read and approved the final manuscript.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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