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Original Article

Rice extracts (KDML105 and PL2 cultivars) modulate lipid accumulation, oxidative status, and inflammation in oxidative stress-induced 3T3-L1 adipocytes

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Abstract

High reactive oxygen species (ROS) level accumulations are coupled with alterations of pro- and anti-inflammatory cytokines, and antioxidant system abnormalities, and they contribute in adipocytes with developing obesity. This study aimed to investigate the effects of rice extracts from KDML105 and PL2 cultivars on lipid accumulation, oxidative status, and inflammatory gene and protein expression in 3T3-L1 adipocytes. Cells were treated with rice extracts (GBR, BR, and WR). GBR and BR extracts significantly decreased intracellular ROS levels and lipid accumulation by DCFH-DA assay and oil-red O staining. Further, qPCR also showed a decrease in the mRNA expression of *TNF-α and IL-6*. Conversely, the rice extracts increased the expressions of *antioxidants* (*Nrf2, HO-1, GPx4, CAT*), and the *adiponectin* gene, and the expression of the adiponectin SOD2 and GPx4 proteins. These results demonstrated an inhibitory effect of both the GBR and BR extracts on lipid accumulation and regulated the expression of oxidative stress, inflammatory genes, and the proteins in H_2O_2-induced 3T3-L1 adipocytes. Due to anti-adipogenesis and the oxidative status of GABA and γ-oryzanol, the germinated rice extracts can be served as a beneficial supplement for the prevention and/or treatment of obesity, and oxidative-related diseases.

Keywords: rice extracts, lipid accumulation, oxidative stress, obesity

1. Introduction

Obesity is a chronic disease that is caused by several diverse factors including environmental factors, and lifestyle, which are associated with type 2 diabetes mellitus and cardiovascular diseases (Ferrarezi, Cheurfa, Reis, Fumeron, & Velho, 2007). Mature adipocytes accumulate more fat, while the differentiated cells have an excessive lipid accumulation that leads to abnormalities of the insulin receptor, antioxidant system, and inflammation, which are all observed in obesity (Dulloo, 2007; Han *et al*., 2011).

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Physiological levels of reactive oxygen species (ROS), maintained by the efficient detoxification system, can induce insulin-mimicking effects of H_2O_2 and promote preadipocyte differentiation. In contrast, high ROS or inappropriate redox balance in intracellular ROS, will have detrimental effects on insulin signaling, and induce pro-inflammatory cytokines while reducing adiponectin in obese adipocytes that are exposed to high levels of ROS (Le Lay, Simard, Martinez, & Andriantsiohaina, 2014). Excess lipid accumulation in adipocytes, is primarily involved in increased oxidative stress that is associated with obesity (Zhao, Jiang, Zhang, & Yu, 2019). Adipose tissue can produce ROS via several pathways, such as the electron transport chain (ETC) in the mitochondria, the NADPH oxidase 4 (NOX4) enzyme, and endothelial NOS (eNOS) (Zhao *et al*., 2019). High ROS

levels can imbalance oxidative stress and antioxidant systems, such as the antioxidant enzymes, superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) (Amirkhizi *et al*., 2007), which may cause lipid peroxidation, protein oxidation, and cellular damage (Schröder, 2019).

Additionally, ROS has the ability to directly cause an inflammatory state, which leads to increased cytokine levels, such as pro-inflammatory cytokines produced by adipocytes (e.g. tumor necrosis factor alpha (TNF-α) and interleukin-6 (IL-6)), while high levels of ROS can reduce the levels of anti-inflammatory cytokines (e.g. adiponectin) (Amirkhizi *et al*., 2007). ROS can potentiate oxidative stress and inflammation, which can impair insulin sensitivity. It has been reported that the increase of hypertrophic adipocytes, was associated with glucose intolerance observed in mice fed with a high-fat diet (HFD) (Riant, Waget, Cogo, Arnal, & Burcelin, 2009). While intracellular ROS can promote inflammatory cytokines that contribute to the development of obesity (Fernández-Sánchez *et al*., 2011).

Rice extracts have metabolically beneficial effects. Recent studies have shown that rice extracts prevent obesity and type 2 diabetes (Sun, Spiegelman, & Van Dam, 2012) by improving glucose uptake. Germinated brown rice (GBR) extracts exhibited anti-obesity effects through the suppression of body weight gain and food intake, the improvement of lipid profiles, the reduction of leptin levels, and white adipose tissue mass, in obese rats fed with a high-fat diet (Lim, Goh, Mohtarrudin, & Loh, 2016). However, the mechanistic effects of rice extracts on oxidative stress-induced 3T3-L1 adipocytes, have not yet been fully examined. In this study, we examined the metabolic effects of rice extracts (GBR, BR, and WR) from KDML105 and PL2 cultivars on lipid accumulation, oxidative status, and inflammation in oxidative stress-induced 3T3-L1 adipocytes.

2. Materials and Methods

2.1 Rice extracts preparation

The PL2 and KDML105 rice cultivars were obtained from Phitsanulok Rice Research Center and Lopburi Rice Research Center, respectively. There were three types of rice extracts: germinated brown rice (GBR), brown rice (BR), and white rice (WR). The GBR rice was germinated using the method modified by Patil & Khan, 2011. The dehydrated GBR, BR, and WR were powdered in a blender, then extracted using a procedure modified from Ho, Son, Lim, & Cho, 2013. Firstly, 50 g of each of the GBR, BR, and WR powder were placed in 250-ml flasks containing 100 ml of 70% (v/v) methanol solution, then the mixture was shaken at 180 rpm for 20 hours at 37 °C twice, then 150 ml of supernatant was collected and filtered through a 1-micron filter paper (Whatman, USA) and evaporated using a rotary evaporator (BUCHI, Switzerland). The crude rice extracts were weighed and dissolved in dimethyl sulfoxide (DMSO) (final concentration 0.1%) as the stock solution.

2.2 3T3-L1 cell culture and treatments

3T3-L1 preadipocytes (#CL-173, ATCC) were grown in DMEM containing 10% FBS and 1% antibiotic-antifungal basal medium (BM) at 37 °C in a 5% CO_2 incubator. After two days post-confluent, the cells ("day 0") were then differentiated for three days in differentiation medium 1 (DM1) with components BM, 1 mM IBMX and 2 µM DEX ("day 3") and for a further two days in differentiation medium 2 (DM2) with components BM and 1 µg/ml insulin ("day 5"). After differentiation completion, the cells were maintained in BM which was replaced with fresh BM on day 7 and 9. The oxidative stress-induced cells were treated with H_2O_2 for 48 hours soon after the completion of differentiation at day 9. The rice extracts at concentrations of 0.1, 0.5, and 1 mg/ml were then treated for 24 hours.

2.3 Cell viability measurement by 3-(4, 5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay

After the completion of differentiation, the 3T3-L1 preadipocytes were treated with rice extracts and H_2O_2 for 24 hours. The control cells received the same amount of DMSO (0.1% v/v), and culture supernatants were then removed and replaced by 180 µl of fresh free-serum BM, containing 20 µl of MTT (5 mg/ml) and incubated for 2 hours at 37 °C, then the solution was discarded, and 100 µl of DMSO was added to each well. The absorbance was measured at 595 nm using a microplate reader (Labsystems, Finland).

2.4 2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) assay

The $ABTS^{\bullet+}$ scavenging ability of rice bran was measured according to the method determined by Re *et al*. (1999). $ABTS^{\bullet+}$ was prepared by mixing 7 mM of ABTS stock solution in water with 2.45 mM potassium persulfate, then allowed to stand for 12-16 hours at room temperature in the dark. For the analysis, 240 µl of the $ABTS^{\bullet+}$ solution and 10 µl of rice extract were mixed and the absorbance was determined at 734 nm. The $ABTS^{\bullet+}$ scavenging activity was expressed in milligram Trolox equivalent per gram of sample (mg TE/g of sample).

2.5 Intracellular ROS measurement by DCFH-DA assay

3T3-L1 adipocytes, after differentiation completion, the cells were induced oxidative stress with 100 µM of H_2O_2 for 48 hours and then treated with rice extracts for 24 hours. After incubation, the cells were washed with PBS and incubated with dichlorofluorescein diacetate100 µl (DCFH-DA) (100 µM) in medium (BM) for 30 minutes at 37 °C in the dark. After being washed several times with PBS, the fluorescence was measured by a microplate reader (Labsystems, Finland), using 485 nm excitation and 530 nm emission wavelengths.

2.6 Intracellular lipid accumulation measurement by Oil-red O staining

After the cells were treated with 100 µM of H_2O_2 for 48 hours, they were then treated with rice extracts for a further 24 hours, and at day 12 of differentiation, oil-red O staining was carried out. The cells were then washed with PBS and fixed with 10% formaldehyde for one hour,

incubated with 60% isopropanol and stained with 0.5% (w/v) Oil-red O solution, and then washed with PBS. The stained lipid droplets in the cells were dissolved in 100% isopropanol and measured using a microplate reader at 510 nm (Labsystems, Finland).

2.7 mRNA expression measurement by quantitative real-time PCR (qPCR)

Total RNA was extracted from the cells using Ribozol reagent (Amresco, USA), and reverse transcription was performed using 1000 ng of total RNA which was subjected to first-strand cDNA synthesis with random primers and using Superscript II reverse transcriptase (Promega, USA), 2 µl of cDNA was added into a 10 µl PCR reaction containing 10 µM of each primer and 1x Master SYBR Green 1 mix (RBC Bioscience, Taiwan). Samples were incubated for an initial denaturation at 95°C for 10 minutes; 40 cycles of denaturation at 94 °C for 30 seconds, annealing at 58 °C for 30 seconds; and a final extension at 72 °C for 30 seconds, respectively (ESCO Swift^{TM}. Real-Time PCR Thermal Cyclers, Singapore). Relative changes in gene expression were determined using the $2^{-\Delta\Delta Ct}$ method. *β-actin* was used as an internal control. The Primer sequences are shown in Table 1.

2.8 Protein expression measurement by Western Blot

Protein concentration was measured using a bicinchoninic acid assay kit (Thermo Scientific, Rockford, IL, USA). Proteins were separated in 15% SDS gels and transferred onto PVDF membranes. After incubation for one hour at room temperature in a blocking solution (5% skim milk), the membranes were incubated overnight at 4 °C in primary antibodies (1:1,000 dilution). Membranes were then washed with 1X-Tris-buffered saline with Tween-20 (TBST), and incubated in horseradish peroxidase conjugated secondary antibody (1:1,000) for one hour at 4 °C. Protein bands were detected using enhanced chemiluminescence (Millipore, USA) and chemiluminescence imager instrument (Image Quant LAS 500, GE Healthcare Life Sciences, Sweden). The antibodies are shown in Table 2.

2.9 Statistical analyses

Data were presented as mean ± S.D for at the least biological triplicate experiment. The comparison between the groups was performed using ANOVA analysis, and Tukey's HSD multiple comparisons and the differences of $P<0.05$ were considered to be statistically significant.

3. Results and Discussion

3.1 Antioxidant potentials of rice extracts

ABTS assays were used to measure the antioxidant activity of the GBR, BR, and WR extracts from the KDML105 and PL2 cultivars at concentrations of 0.1-10 mg/ml. The GBR extracts showed a greater antioxidant capacity than BR, with WR showing the least. The antioxidant activities of the GBR, BR, and WR extracts from KDML105 and PL2 cultivars were expressed as Trolox Equivalent Antioxidant Capacity (TEAC) (mM/g samples) values (Table 3).

3.2 Cell viability

After 3T3-L1 adipocytes were treated with GBR, BR, and WR extracts from both cultivars at various concentrations (0.1, 0.5, and 1 mg/ml), there were no toxicity effects on the cell viability (Figure 1A-1B). H_2O_2 had no cytotoxic effect at 100-200 µM in both undifferentiated cells (UD) and differentiated cells (D) (Figure 1C).

Table 1. Sequences of primers used in qPCR

Genes	Forward primer (5'→3')	Reverse primer (5'→3')
TNF-α	GATTTGCTATCTCATACCAGGAGAA	AAGTCTAAGTACTTGGGCAGATTGA
IL-6	AGGCTTAATTACACATGTTCTCTGG	TTATATCCAGTTTGGTAGCATCCAT
Adiponectin	CAGGTCTTCTTGGTCCTAAGGG	GTCCACATTCTTTTCCTGATACTG
Nrf2	CGAGATATACGCAGGAGAGGTAAGA	GCTCGACAATGTTCTCCAGCTT
HO-1	GACATGGCCTTCTTGTATGG	ACCCAGGTAGCGGGTATATG
SOD2	AAAGATGGTGTGGCCGATGT	TTCCAGCGTTTCCCGTCTTT
GPx4	TCCTCATCGACAAGAACGGC	CACGCTGGATTTTCGGGTCT
β-actin	ATTTATCCCGGTGTACTGTG	CGGAAGTTCAGAGAGGCGTAGTAG

Table 2. Antibodies used in Western Blot.

Proteins	Primary antibody	Secondary antibody
Adiponectin	Rabbit mAb (Cat. NO: DF7000) (Affinity, USA)	Horseradish peroxidase (HRP) conjugate anti rabbit (MILLIPORE, USA)
SOD2	Rabbit mAb (Cat. NO: D3X8F) (Cell Signaling Technology, USA)	
GPx4	Rabbit mAb (Cat. NO: PB9625) (BOSTER Biological technology, USA)	
β-actin	Mouse mAb (Cat.NO. 8H10D10) (Cell Signaling Technology, USA)	

Table 3. Trolox Equivalent Antioxidant Capacity; TEAC (mM/g of rice extract) of the GBR, BR, and WR extracts from KDML105 and PL2 cultivars at concentrations of 0.1-1 mg/ml and ABTS assay; (N=8)

Type of rice extracts	mg TE/g of GBR	mg TE/g of BR	mg TE/g of WR
KDML105	7.84±0.96	6.33±1.30	3.75±1.11
PL2	7.01±1.41	5.54±0.91	3.58±0.80

3.3 Intracellular ROS levels

Intracellular ROS was first induced by treating the 3T3-L1 adipocytes with H_2O_2 for 24 hours. The ROS levels were then measured using a DCFH-DA assay. As shown in Figure 2A, the intracellular ROS levels significantly increased (P<0.05) in H_2O_2-induced 3T3-L1 adipocytes in the comparison with both normal undifferentiated (UD) and differentiated adipocytes (D). However, after treated with GBR and BR extracts (0.5 and 1 mg/ml) from both cultivars, the ROS levels significantly decreased (P<0.05) compared to the H_2O_2-induced 3T3-L1 adipocytes (DH) (Figure 2B and 2C).

3.4 Intracellular lipid accumulation

The result showed that the lipid accumulation was significantly increased (P<0.05) in the H_2O_2-induced 3T3-L1 adipocytes (DH). 3T3-L1 cells were then induced with H_2O_2 for 48 hours and then treated with rice extracts (0.5 and 1 mg/ml) from KDML105 and PL2 cultivars for 24 hours, before the induction of oxidative stress. Oxidative stress caused significantly reduced (P<0.05) lipid accumulation in 3T3-L1 adipocytes as compared to the control group shown in (Figure 3).

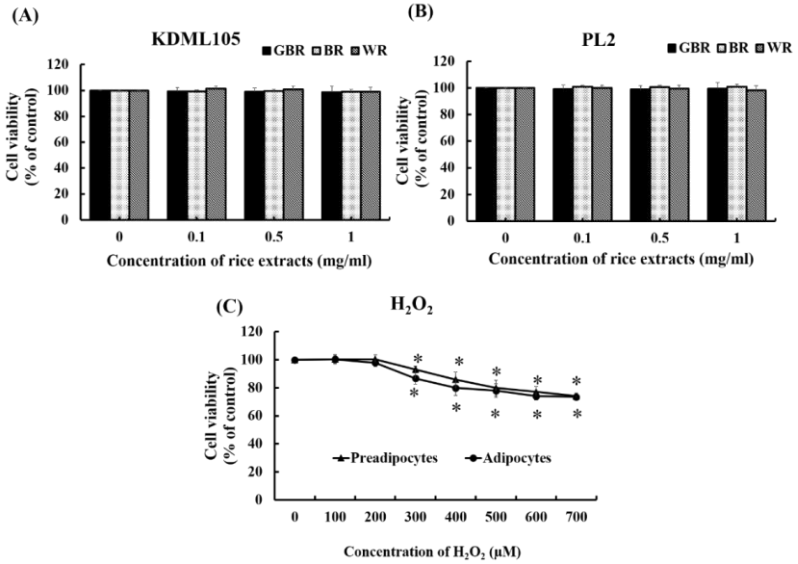

Figure 1. Effects of rice extracts from (A) KDML105 and (B) PL2 cultivars and (C) H_2O_2 on cell viability in 3T3-L1 adipocytes. *P<0.05, compared to control. Data represented as mean ± S.D. of 16 replicates per treatment.

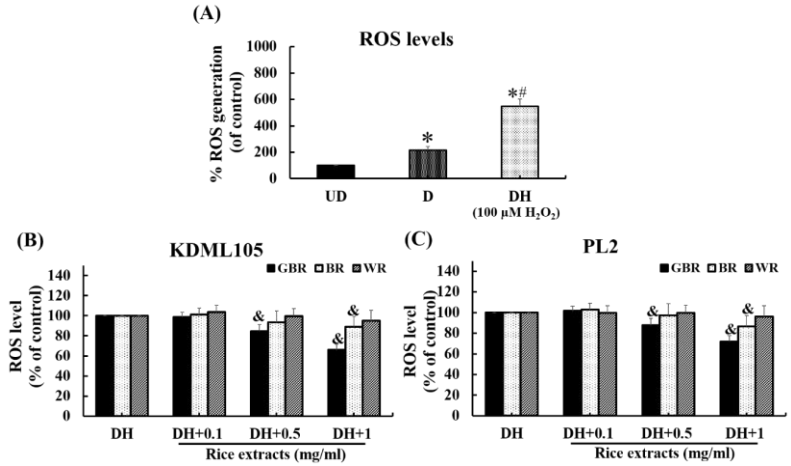

Figure 2. Effects of (A) 100 μM of H_2O_2 and rice extracts (GBR, BR, and WR) from (B) KDML105 and (C) PL2 on ROS levels in H_2O_2-induced 3T3-L1 adipocytes. *P<0.05, compared to UD and $^{\#}P$<0.05, compared to D. $^{\&}P$<0.05, compared to DH. Data represented as mean ± S.D. of two replicates per treatment (N=16).

3.5 Gene and protein expressions

3.5.1 Adipocytokines

GBR and BR extracts (0.5 and 1 mg/ml) of both cultivars significantly decreased (P<0.05) mRNA expression of *TNF-α* and *IL6* compared to the differentiated cells (D) and H_2O_2-induced 3T3-L1 adipocytes (DH) (Figure 4A and 4B). In contrast, H_2O_2-induced 3T3-L1 adipocytes were treated with GBR and BR extracts (0.5 and 1 mg/ml) led to increased

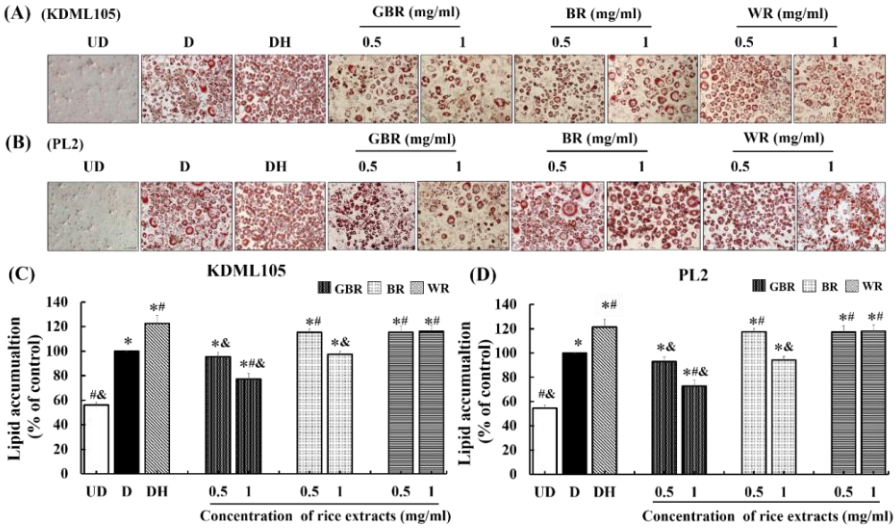

Figure 3. Effects of rice extracts from (A and C) KDML105 and (B and D) PL2 cultivars on lipid accumulation in H_2O_2-induced 3T3-L1 adipocytes. The lipid accumulation was examined by Oil Red O staining and the absorbance was measured at 510 nm. *P<0.05, compared to UD and $^{\#}P$<0.05, compared to D. $^{\&}P$<0.05, compared to DH. Data represented as mean ± S.D. of two replicates per treatment (N=8).

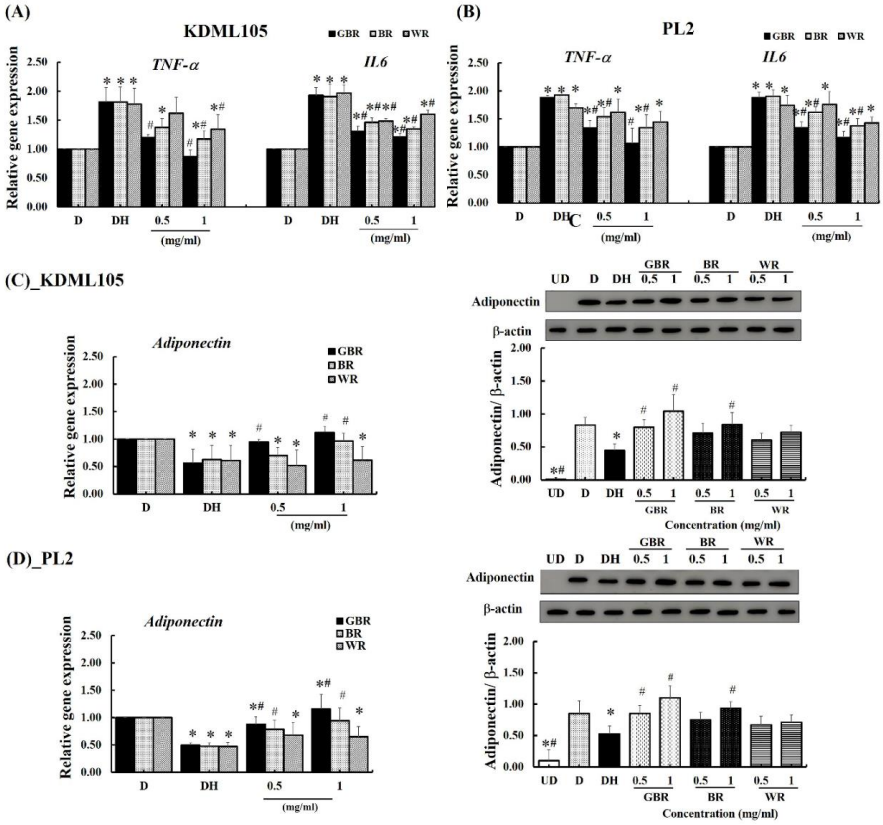

Figure 4. Effects of rice extracts from (A and C) KDML105 and (B and D) PL2 cultivars on mRNA and protein expression of *TNF-α*, *IL6*, and Adiponectin in H_2O_2-induced 3T3-L1 adipocytes. The mRNA and protein expression was analyzed by qPCR and Western blotting. β-actin was used as an internal control. *P<0.05, compared to D and $^{\#}P$<0.05, compared to DH. Data represented as mean ± S.D. of two replicates per treatment (N=5).

adiponectin expression in a dose-dependent manner (P<0.05) compared to the differentiated cells (D) and H_2O_2-induced 3T3-L1 adipocytes (DH) (Figure 4C and 4D).

3.5.2 Antioxidants

GBR and BR extracts from both cultivars on the mRNA expression levels of *Nrf2* and the downstream genes *HO-1*, *SOD2*, *GPx4*, and *CAT* in oxidative stress-induced cells were significantly decreased (P<0.05) compared to differentiated cells (D) (Figure 5A and 5B). However, after being treated with the GBR and BR extracts at concentrations of 0.5 and 1 mg/ml, their expressions were significantly increased (P<0.05) in a dose-dependent manner compared to the differentiated cells and H_2O_2-induced 3T3-L1 adipocytes (DH). We also found that the protein expression of SOD2 and GPx4 significantly decreased (P<0.05) in the H_2O_2-induced 3T3-L1 adipocytes compared to the differentiated cells (D). As expected, the treatment of the GBR and BR extracts (0.5 and 1 mg/ml) significantly up-regulated the expression of SOD2 and GPx4 protein in the H_2O_2-induced 3T3-L1 adipocytes (DH) in a dose-dependent manner compared to the differentiated cells (D) and the H_2O_2-induced 3T3-L1 adipocytes (DH) (Figure 5).

3.6 Discussion

The antioxidant activities of the GBR, BR, and WR extracts from KDML105 and PL2 cultivars were determined using the ABTS radical cation assay, as shown in Table 3. GBR and BR showed greater antioxidant activity than WR. This was in agreement with a previous study (Mohd Esa, Abdul Kadir, Amom, & Azlan, 2013). Moreover, Azmi, Ismail, & Norsharina (2013) showed that the ethyl acetate GBR extract had higher antioxidant activity than BR.

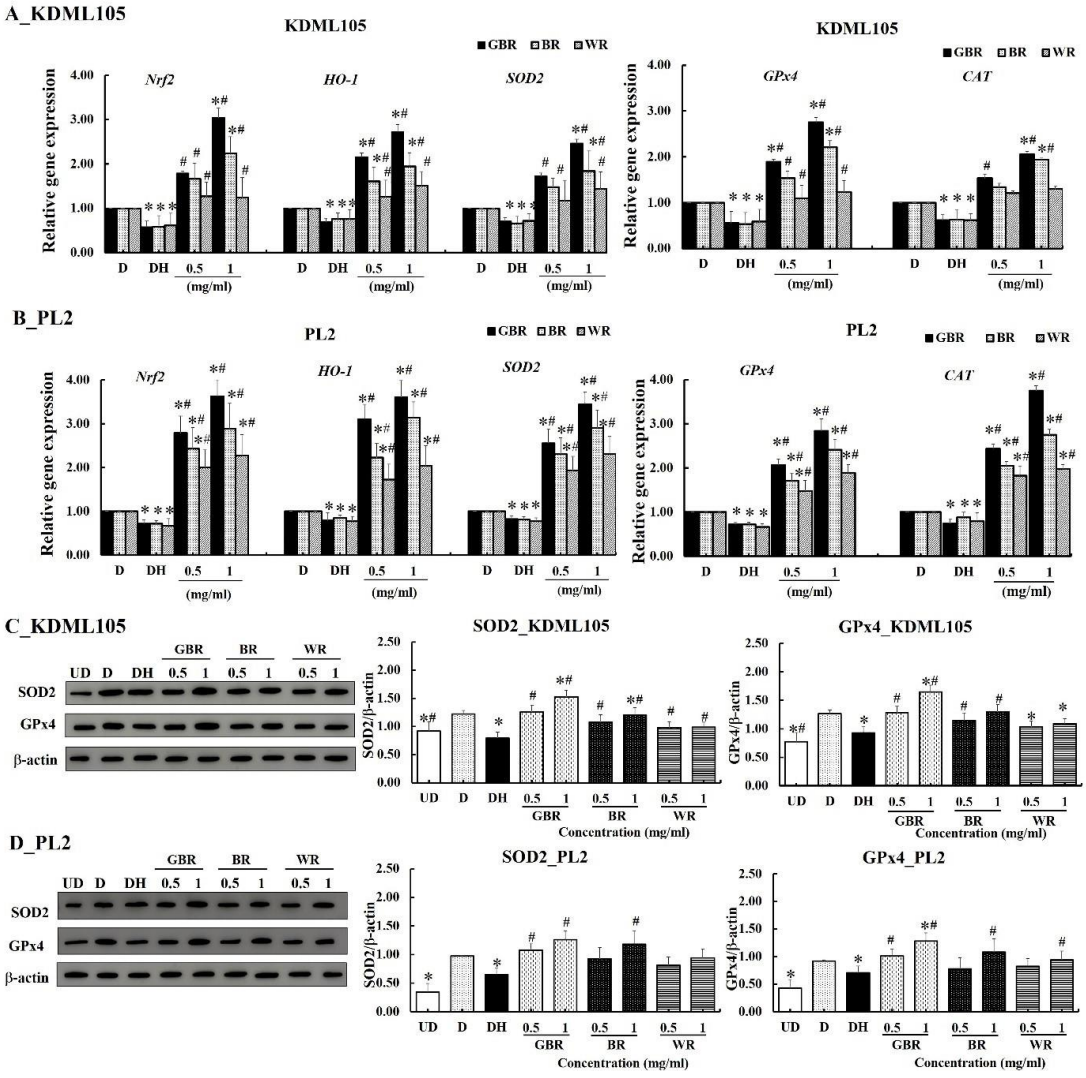

Figure 5. Effects of rice extracts from (A and C) KDML105 and (B and D) PL2 cultivars on mRNA and protein expression of *Nrf2*, *HO-1*, SOD2, GPx4, and *CAT* in H_2O_2-induced 3T3-L1 adipocytes. The mRNA and protein expression was analyzed by qPCR and Western blotting. β-actin was used as an internal control. *P<0.05, compared to D and $^{\#}P$<0.05, compared to DH. Data represented as mean ± S.D. of two replicates per treatment (N=5).

To create an oxidative stress cell model, mature 3T3-L1 adipocytes were treated with 100 µM of H_2O_2 for 48 hours, which significantly increased the intracellular ROS levels and lipid accumulation, compared to undifferentiated and differentiated adipocytes, resulting in lipid peroxidation and cell membrane damage (Hauck, Huang, Hertzel, & Bernlohr, 2019). In addition, Lee, Choi, Ko, & Kim (2009) demonstrated that H_2O_2 treatment can induce the G2/M phase leading to increase adipocyte differentiation. Additionally, increasing H_2O_2 and decreasing the antioxidant system will increase lipid storage and adipocyte secretion, leading to an imbalance of energy homeostasis, insulin resistance, and type 2 diabetes (Masschelin, Cox, Chernis, & Harti, 2020). The present study demonstrated that H_2O_2-induced 3T3-L1 adipocytes treated with 0.5 and 1 mg/ml of GBR and BR extracts from both cultivars reduced intracellular ROS levels and lipid contents in a dose-dependent manner. This result corresponds with a previous study, which reported that GBR extracts can reduce lipid contents in 3T3-L1 adipocytes (Ho, Son, & Lim, 2012). In addition, it was found that high-fat diet-induced obese rats fed by fermented brown rice extract can reduce body weight gain, improve lipid profiles, and white adipose tissue mass (Lim *et al*., 2016).

From the results of changes in gene expression, it was shown that the mRNA levels of antioxidants *Nrf2*, *HO-1*, *SOD2*, *GPX4*, and *CAT* were significantly down-regulated the mRNA levels in H_2O_2-induced 3T3-L1 adipocytes. However, after treating with the GBR and BR extracts, the mRNA level of *Nrf2*, which is upstream of antioxidant enzymes such as *HO-1*, *SOD2*, *GPX4*, and *CAT* (Furukawa *et al*., 2004) was up-regulated., *HO-1*, *SOD2*, *GPX4*, and *CAT*. In addition, it was found that the GBR and BR extracts can induce SOD2 and GPX4 protein expression in a dose-dependent manner in H_2O_2-induced 3T3-L1 adipocytes. This result corresponds with previous studies that H_2O_2 was associated with the Nrf2-ARE antioxidant pathway (Schneider & Chan, 2013). Activation of Nrf-2 by the rice extracts will up-regulate the expression of downstream phase II detoxification enzyme, e.g., HO-1, SOD, CAT, and GPx, which may protect the cells from oxidative stress, inflammation, and insulin resistance (Buendia *et al*., 2016).

High oxidative stress-induced adipocytes can promote inflammation by producing pro-inflammatory cytokines (TNF-α and IL-6), which cause insulin resistance (Hotamisligil, 2017). In addition, oxidative stress can induce IKKβ activation leading to NF-κB translocation and increasing the expression of potential mediators of inflammation that can cause insulin resistance (Shoelson, Lee, & Goldfine, 2006). This study demonstrated that the GBR and BR extract reduced mRNA expression of *TNF-α* and *IL6* in H_2O_2-induced 3T3-L1 adipocytes. On the other hand, anti-inflammatory (adiponectin) plays a role in the uptake of glucose and lipid metabolism (Matsuura, Oku, Koseki, Sandoval, & Yuasa-Kawase, 2007). As previously described, γ-oryzanol was presented in the GBR extract. It can directly influence lipid accumulation and triglyceride synthesis via inhibiting adipogenic-related genes e.g. PPARγ and C/EBPs (Ho *et al*., 2013). Minatel, Francisqueti, Correa, & Lima, 2016) also demonstrated that γ-oryzanol can activate adiponectin production in adipocytes, which will modulate the hepatic cells by activating peroxisome proliferator-activated receptors (PPAR-α) via AdipoR2 and thereby stimulating fatty-acid oxidation and decreasing triglyceride contents in liver tissue. In addition, γ-oryzanol can induce insulin production in pancreatic cells, which will then directly activate AMPK via AdipoR1 in hepatic cells. After AMPK activation, it will inhibit phosphoenolpyruvate carboxykinase (PEPCK) and Glucose 6-phosphatase (G6Pase), resulting in reducing gluconeogenesis in hepatic cells. Activated AMPK also induces β-isoform of coenzyme A carboxylase (ACC-β) phosphorylation, which inhibits acetyl coenzyme A carboxylase (ACC) and results in increased fatty acid oxidation in liver tissue. It suggests that the ability to activate adiponectin production in adipocytes, together with the high amount of γ-oryzanol content in the GBR and BR extracts can show a potential effect for the improvement of insulin sensitivity.

4. Conclusions

In this study, the two Thai rice extracts from PL2 and KDML105 cultivars display antioxidant activity in GBR and BR greater than WR extracts. GBR and BR extracts have the potential greater than WR extract to reduce ROS levels in oxidative stress-induced 3T3-L1 adipocytes by H_2O_2. The underlying mechanistic effects occur via the decrease of the expression of genes and proteins involved in inflammation (*TNFα* and *IL-6*) as well as an increase anti-inflammatory cytokine (*adiponectin*), antioxidant system (*Nrf2*, *HO-1*, *SOD2*, *CAT*, and *GPx4*). These results revealed that the GBR and BR extracts exhibited anti-adipogenesis and antioxidative capacity. Particularly, it suggests that the GBR extract, which is obtained from the germination process, seems to produce a high amount of bioactive compounds e.g., GABA and γ-oryzanol, which are important for regulating oxidative status in adipocytes. Thus, this study reveals the possibility of using the GBR and BR extracts as a supplement for the prevention or treatment of obesity or obesity-linked disease such as type 2 diabetes.

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