

Original Article

Anti-obesity potential of glutinous black rice bran extract:
Anti-adipogenesis and lipolysis induction
in 3T3-L1 adipocyte modelWarathorn Chumchoochar^{1,2} and Khaetthareeya Sutthanut^{1*}¹ Department of Pharmaceutical Chemistry, Faculty of Pharmaceutical Sciences,
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Abstract

An ethanolic glutinous black rice bran extract (RBE) was prepared and used in the experiments to determine the phenolic and flavonoid contents, antioxidation using DPPH and TBARS assay, anti-adipogenesis, and lipolysis induction in a 3T3-L1 pre-adipocyte model to discover its anti-obesity potential. The results showed that RBE was composed of phenolics, flavonoids, and anthocyanins and showed antioxidative activities (8.58 ± 0.14 mg Vit C eq/g extract and 6.06 ± 0.49 mg BHT eq/g extract). Interestingly, its anti-obesity potential was demonstrated in the interruption of the adipocyte life cycle, i.e. a significant anti-adipogenesis effect in the reduction of lipid accumulation (50.86 ± 3.26 to $97.06 \pm 8.09\%$ of the negative control, and triglyceride content in 3T3-L1 pre-adipocytes (36.89 ± 2.08 to $90.56 \pm 0.67\%$ of the negative control), and lipolysis induction (relative glycerol content 117.62 ± 1.44 and $163.72 \pm 10.18\%$ of control). Therefore, implementation into obesity therapy requires further research.

Keywords: anti-obesity, anti-adipogenesis, lipolysis induction, antioxidation, glutinous black rice bran

1. Introduction

Obesity is one of the most common worldwide health problems that is associated with comorbidities including metabolic syndrome, cardiovascular diseases, muscular-skeleton syndromes, diabetes, hypercholesterolemia, hypertension, atherosclerosis, cancer, muscle weakness, heart failure, and mortality. Therefore, obesity prevention and treatment are global community needs. Medication is a choice in addition to behavioral modification, exercise, and surgery. However, serious central nervous system (CNS) and cardiovascular system (CVS) side effects occur from the use of medications. Therefore, anti-obesity agents from natural

sources are interesting alternatives. The expected advantages of natural sources for obesity therapy include safe, economic, and long-term use.

An increased number of adipocytes can contribute to developing obesity. This is regulated by a concert of multiple factors such as genetic, metabolic, and nutritional factors (de Ferranti & Mozaffarian, 2008) as well as oxidative stress (Albuali, 2014). Therefore, interruption of the adipocyte life cycle via the inhibition of adipocyte differentiation (adipogenesis), reduction of lipid accumulation as well as oxidative stress, and the induction of lipolysis are believed to be strategies to prevent obesity (Abdul Rahman *et al.*, 2017; Mohamed, Ibrahim, Elkhayat, & El Dine, 2014; Rayalam, Della-Fera, & Baile, 2008).

Interestingly, phenolics, flavonoids, and anthocyanins ubiquitously found in plants have been widely accepted for their health benefits. Apigenin, genistein, catechin,

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quercetin, and anthocyanins were reported to be anti-obesity agents by regulating the life cycle of adipocytes (Ahn, Lee, Kim, Park, & Ha, 2008). Moreover, they also offer health benefits as antioxidants.

Rice bran from glutinous black rice (*Oryza sativa L.*) consists of high amounts of cyanidin-3-glucoside, caffeic acid, and ferulic acid (Phetpornpaisan, Tippayawat, Jay, & Sutthanut, 2014). It has become an interesting natural source for anti-obesity application. It is postulated that the anti-obesity potential is due to the anti-adipogenesis contributed by phenolics such as gallic acid and ferulic acid (Chaiittianan, Chayopas, Rattanathongkom, Tippayawat, & Sutthanut, 2016). Furthermore, anti-obesity potential may be the result of lipase inhibition contributed by γ -oryzanol (Minatel *et al.*, 2013), anthocyanins, catechin (Ahn *et al.*, 2008), and its derivative (epigallocatechin-3-gallate) (Sergent, Vanderstraeten, Winand, Beguin, & Schneider, 2012). However, reports on the anti-obesity activity of glutinous black rice bran have been limited. This study aimed to investigate the anti-obesity effect of the ethanolic extract of glutinous black rice bran focusing on lipogenesis (adipogenesis) and lipolysis.

2. Materials and Methods

2.1 Chemicals and reagents

Mouse 3T3-L1 pre-adipocytes (ATCC, Manassas, VA, USA); gallic acid, quercetin, cyanidin-3-glucoside, dimethyl sulphoxide (DMSO), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), 3-isobutyl-1-methylxanthine (IBMX), dexamethasone (DEX), insulin, biotin, Oil Red O solution, triacylglycerol assay kit, free glycerol reagent, isopropyl alcohol (IPA), Triton X-100, ethylenediaminetetraacetic acid (EDTA), tris(hydroxymethyl) aminomethane (Tris), egg yolk, isopropanol, 2, 2-Diphenyl-1-picrylhydrazyl (DPP-H), ascorbic acid (vitamin C), 2-thiobarbituric acid (TBA), butanol, butylated hydroxytoluene (BHT), and Folin-Ciocalteu's reagent were purchased from Sigma (St. Louis, MO, USA). Ethanol and formaldehyde were purchased from RCI Labscan Ltd. (Bangkok, Thailand). Methanol was purchased from Fisher Scientific UK Ltd. (Bishop Meadow, Loughborough, UK). Dulbecco's modified Eagle's medium (DMEM) and antibiotic-antimycotic were purchased from Gibco by Life Technologies (Grand Island, NY, USA). Fetal calf serum (FCS), phenol red-free Dulbecco's modified eagle's medium (phenol red-free DMEM), and fetal bovine serum (FBS) were purchased from Invitrogen Co. (Carlsbad, CA, USA). Potassium chloride (KCl), potassium dihydrogen orthophosphate, sodium chloride (NaCl), sodium hydroxide (NaOH), di-sodium hydrogen orthophosphate anhydrous (Na_2HPO_4), sodium carbonate anhydrous, aluminum chloride hydrated (AlCl_3), sodium acetate (CH_3COONa), ferrous sulphate (FeSO_4), acetic acid (CH_3COOH), sodium lauryl sulfate (SLS), and hydrochloric acid were purchased from Ajax Finechem Pty Ltd. (Albany, Auckland, N.Z.).

2.2 Plant materials

The glutinous black rice bran material was derived from the milling process of a variety of Thai glutinous black rice (*Oryza sativa L.*) cultivated in the Baanhad community of Khon Kaen, Thailand. Extraction was performed by the mace-

ration technique in 50% ethanol solvent for 24 h. After filtration, the extract was filtered and concentrated by rotary evaporator and lyophilized using a freeze-dryer (EYELA FDU-1200, Tokyo, Japan). Finally, the rice bran extract (RBE) was obtained with a yield of 10.42% (w/w) of rice bran and kept at -20°C until use.

2.3 Total phenolics determination

Total phenolics content was determined by Folin-Ciocalteu colorimetric assay (Singleton & Rossi, 1965). The RBE solution was prepared at various concentrations of 62.5–1000 $\mu\text{g/mL}$. Gallic acid (0–100 $\mu\text{g/mL}$) was used as a reference compound. Briefly, a mixture of 50 μL of the sample solution, 125 μL of 20% (w/v) Na_2CO_3 , and 25 μL of 50% (v/v) of Folin-Ciocalteu reagent was constituted and incubated at 25°C for 40 min prior to absorbance measurement of the mixture at a wavelength of 700 nm using a microplate reader (Varioskan™ Flash Multimode Reader, Thermo Scientific®, Waltham, MA, USA). The total phenolics content was expressed in terms of mg gallic acid equivalent per gram of extract (mg GA eq/g extract).

2.4 Total flavonoids determination

Total flavonoids content was determined by AlCl_3 colorimetric assay (Chang, Zuo, Harrison, & Chow, 2002). The RBE solution was prepared at various concentrations of 62.5–1000 $\mu\text{g/mL}$. Quercetin (0–100 $\mu\text{g/mL}$) was used as a reference compound. Briefly, a mixture of 100 μL of the sample solution and 200 μL of 5% AlCl_3 was constituted and incubated at 25°C for 30 min. Then, the optical absorbance was measured at a wavelength of 437 nm using a microplate reader. The total phenolics content was expressed in terms of mg quercetin equivalent per gram of extract (mg Q eq/g extract).

2.5 Total anthocyanins determination

Total anthocyanins content was determined by the pH differential method (Shafazila, Lee, & Lee Kong, 2010). The RBE solution was prepared at a final concentration of 500 $\mu\text{g/mL}$. Cyanidin-3-glucoside (200 $\mu\text{g/mL}$) was used as a reference compound. Briefly, the RBE solution was prepared in a KCl solution pH 1.0 and in CH_3COONa pH 4.5 and incubated at 25°C for 30 min. The optical absorbance was measured at 520 nm and 700 nm for the calculation to obtain total anthocyanin content. The total anthocyanins content was expressed in terms of mg cyanidin-3-glucoside equivalent per gram of extract (mg C3G eq/g extract).

$$A = (A_{520} - A_{700})_{\text{pH } 1.0} - (A_{520} - A_{700})_{\text{pH } 4.5}$$

$$\text{Total anthocyanin content} = \frac{(A \times \text{MW} \times \text{dilution factor} \times 100)}{(\epsilon)}$$

$$\text{MW} = 449.2 \text{ g mol}^{-1}, \epsilon = 226900 \text{ (mol.cm)}^{-1}$$

2.6 Antioxidation activity by DPPH assay

Free radical-scavenging activity was determined using a modified DPPH assay (Chu, Chang, & Hsu, 2000).

The RBE solution was prepared at various concentrations of 125–2000 µg/mL. Vitamin C (1.625–50 µg/mL) was used as a reference antioxidant. Briefly, the mixture of 150 µL of the sample solution and 50 µL of DPPH solution (0.1 mM in methanol) was constituted and incubated at 25 °C for 15 min. Then, optical absorbance was measured at a wavelength of 570 nm using a microplate reader. The antioxidative effect was expressed in terms of 50% inhibitory concentration (IC₅₀) and vitamin C equivalent per gram of extract (mg Vit C eq/g extract).

2.7 Antioxidation activity by TBARS assay

The RBE solution was prepared at various concentrations of 125–2000 µg/mL. BHT (0.5–10 µg/mL) was used as a reference antioxidant. Briefly, the mixture of 90 µL of the sample solution, 90 µL of 30% (w/v) egg yolk in 1.5% (w/v) KCl, and 60 µL of 1 mM FeSO₄ was constituted and incubated at 37 °C for 1 h prior to the addition of 300 µL of 20% (v/v) CH₃COOH and 300 µL of 0.8% (w/v) TBA solution (dissolved in 1.1% w/v SLS solution). After 1-h incubation at 90 °C, 500 µL of butanol was added and mixed well. The supernatant was collected and the optical absorbance was measured at a wavelength of 532 nm using a microplate reader. The antioxidative effect was expressed in terms of 50% inhibitory concentration (IC₅₀) and BHT equivalent per gram of extract (mg BHT eq/g extract).

2.8 Cell culture and differentiation

Mouse 3T3-L1 pre-adipocytes were maintained at 37 °C in a humidified atmosphere of 5% CO₂ in pre-adipocyte medium (PM: a mixture of high-glucose Dulbecco's modified Eagle medium [DMEM] supplemented with 10% fetal calf serum [FCS], and 1% penicillin/streptomycin mixture) until 90% confluence was reached. In all experiments, cells were seeded into a 24-well plate at a density of 30,000 cells/well and cultured for 2 days until 90% confluence was reached. The cells were then induced to differentiate by differentiation media (DM) treatments: DM I (a mixture of DMEM, 10% FCS, 1 µM dexamethasone, 500 µM IBMX, and 10 µg/mL insulin) and DM II (dexamethasone and IBMX-free DM I). The cells were then maintained in adipocyte medium (a mixture of DMEM supplemented with 10% FBS) with sub-culturing every 2 days until use.

2.9 Pre-adipocyte cell viability determination

Mouse 3T3-L1 pre-adipocytes were seeded at a density of 20,000 cells/well and maintained in a 96-well plate until 90% confluence. After 24-h preconditioning, the cells were treated with RBE at various concentrations (62.5, 125, 250, 500, 1000, and 2000 µg/mL). PM medium was used as the control. The cells were maintained at 37 °C in 5% CO₂ humidified atmosphere for 24 h. Cell viability was examined by MTT 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay (Mosmann, 1983). Briefly, cells with 50 µL MTT solution (5 mg/mL in phosphate-buffered saline [PBS]) were added into the well followed by 4-h incubation at 37 °C. The MTT solution, was then removed and 150 µL DMSO was added and incubated at ambient temperature for 30 min. The

optical absorbance was determined at 540 nm using a microplate reader. Compared to the control, the results were demonstrated as pre-adipocyte viability (% of control).

2.10 Anti-adipogenesis evaluated by Oil Red O staining and triacylglycerol assay kit

To evaluate the effect of RBE on differentiation (adipogenesis), the cells were treated with different culturing vehicles: PM as the positive control, or DM as the negative control, or the RBE solutions at various concentrations of 62.5, 125, 250, 500, 1000, and 2000 µg/mL with periodical replacement of fresh culturing medium every 2 days until differentiation was complete. The cells were then used for Oil Red O staining and triacylglycerol determination.

2.10.1 Oil Red O staining assay

The differentiated adipocytes were washed with PBS, pH 7.4, and fixed with 4% (v/v) formaldehyde for 30 min and washed with 60% (v/v) isopropanol. The adipocytes were stained with freshly prepared 0.5% (w/v) Oil Red O solution at 37 °C in a humidified atmosphere of 5% CO₂ for 1 h. The stained cells were photographed using an inverted microscope (Axi Vert.A1 FL-LED, ZEISS®, Jena Germany). In addition, lipid accumulation content was determined. The retained dye in the adipocytes was extracted with 100% isopropanol and the optical absorbance was then measured at 510 nm using the microplate reader.

2.10.2 Triacylglycerol determination assay

The differentiated adipocytes were washed with ice-cold PBS and scraped into 300 µL of lysis buffer (a mixture of 0.15 M NaCl, 10 mM EDTA, 0.1% Triton-X, 50 mM Tris buffer, pH 7.4) and followed by 10-min sonication (ultrasonicator, Witeg Labortechnik GmbH, Germany). The supernatant was then separated by centrifugation at 4 °C at 12,000 rpm for 20 min. The triglyceride content in the supernatant of each sample was determined using the triacylglycerol assay kit. The optical absorbance was measured at 540 nm using a microplate reader. The results are expressed as relative triglyceride content (% of negative control).

2.11 Lipolysis inductive effects by using glycerol assay

To examine the effect of RBE on cell lipolysis, the adipocytes were seeded into a 24-well plate at 24,000 cells/well, incubated overnight in DMEM with 0.5% FBS at 37 °C in 5% CO₂ humidified atmosphere. The adipocytes were then treated with phenol red-free DMEM (as negative control) or IBMX (as positive control) or the various concentrations of RBE (125, 250, 500, 1000, and 2000 µg/mL) followed by 24-h incubation. Afterward, the released glycerol was quantified using a test kit to determine the free glycerol concentration (Sigma, St. Louis, MO, U.S.A.) to indicate lipolysis activity. Briefly, the supernatant of each sample was heated at 70 °C for 10 min to inactivate the released enzymes; thereafter, it was mixed with the free glycerol reagent at 1:1 (v/v) ratio. Following 15-min incubation, the optical absorbance was

determined at 540 nm using a microplate reader. The results were expressed as relative glycerol content (% of control).

2.12 Statistical analysis

Results are expressed as mean±standard deviation (SD) of values obtained from triplicate determination (n=3). Statistical analysis was performed using one-way ANOVA followed by Bonferroni's multiple comparison of SPSS version 17.0 (Chicago, IL, USA). Significance was considered at P values less than 0.05 (P<0.05).

3. Results and Discussion

3.1 Phytochemical contents and antioxidation activity

Phenolics, flavonoids, and anthocyanins in RBE were extracted by 50% ethanol (Table 1). With respect to the "like dissolves like" principle, 50% ethanol as the extractive solvent is considered the factor which resulted in high amounts of extracted phenolics and anthocyanins which are polar compounds in RBE. The phytochemical compounds in the rice bran extract were cyanidin-3-glucoside, caffeic acid, *p*-coumaric acid, ferulic acid, gallic acid, vanillic acid, protocatechuic acid, synaptic acid, syringic acid, and peonidin-3-glucoside. Although, previous studies reported on the wide spectrum of biological activities of phenolics, flavonoids, and anthocyanins including antioxidation and anti-obesity, the differences among them have also been widely reported (Ahn *et al.*, 2008; Chaiittianan *et al.*, 2016; Minatel *et al.*, 2013; Sergent *et al.*, 2012). Interestingly, the multiplicative benefits for prevention and treatment of obesity between antioxidative and anti-obesity effects were evidenced by the reduction of oxidative stress-induced obesity and complications (Alcalá *et al.*, 2015; Calzadilla *et al.*, 2011), attributed by overproduction of reactive oxygen species which leads to cell function impairment (Halliwell, Gutteridge, & Cross, 1992) and induction of lipid accumulation (Kim & Lee, 2017; Lee *et al.*, 2016). From this, various antioxidants, i.e. vitamin C, green tea, green coffee, cinnamon, lipoic acid, and L-carnitine, have been used to prevent and treat obesity (Abdali, Samson, & Grover, 2015) as well as in oxidative stress related chronic diseases. Interestingly, RBE expressed antioxidative effects in both the hydrophilic and hydrophobic condition. In the hydrophilic condition (DPPH assay), RBE had IC₅₀ values of 1686.70±22.75 µg/mL and 8.58±0.14 mg Vit C eq/g extract which was much lower than vitamin C (IC₅₀ 14.46±0.33 µg/mL). In the hydrophobic condition (TBARS assay), RBE showed IC₅₀ values of 2853.10±31.34 µg/mL and 6.06±0.49 mg BHT eq/g extract compared to BHT (IC₅₀ 6.98±0.03 µg/mL) (Table 2). The results suggested that RBE is a potential natural extract for further research to evaluate the possibility in applications for health and anti-obesity.

3.2 Cytotoxicity of RBE on pre-adipocytes by MTT assay

The very low cytotoxicity of RBE to pre-adipocytes was demonstrated with high viability of cells which was more than 80% cell viability compared to the control, which ranged

from 96.58±5.17% to 104.26±6.86% of the control at all of the tested concentrations (62.5–2000 µg/mL). Therefore, this concentration range is logically safe for further experiments (Figure 1).

3.3 Anti-adipogenesis by Oil Red O staining & triacylglycerol determination assay

Adipogenesis or adipocyte differentiation is the process in which pre-adipocytes transform to mature adipocytes via intercellular adipogenesis. For example, lipids are produced and accumulate in the form of triglycerides. This commonly results in enlargement and coalescence of lipid droplets and a morphological change to bigger and round-shape cells (Niemelä, Miettinen, Sarkanen, & Ashammakhi, 2008). The Oil Red O staining technique demonstrated anti-adipogenesis of RBE at concentrations from 125 to 2000 µg/mL in a dose dependent trend that was indicated by the reduction of oil droplets in size and number compared to the negative control (Figure 2A). In addition, the lipid contents were significantly reduced at RBE concentrations of 500–2000 µg/mL with a lipid content that ranged from 50.86±3.26% to 84.57±1.70%

Table 1. Phytochemical contents of glutinous black rice bran extract (RBE): phenolics, flavonoids, anthocyanins content and equivalent (eq) amount to reference compounds (mg/g extract) as gallic acid (GA) for phenolics, quercetin (Q) for flavonoids and cyanidin-3-glucoside (C3G) for anthocyanins. The content was obtained by extrapolation from linear equation (regression coefficient, r²) of the corresponding standard compound calibration curve: y=0.0052x (r²=0.9875) for gallic acid, y=0.0396x (r²=0.9888) for quercetin.

| Phytochemical content | |
|------------------------------------|-------------|
| Phenolics (mg GA eq/g extract) | 180.67±7.88 |
| Flavonoids (mg Q eq/g extract) | 0.43±0.34 |
| Anthocyanins (mg C3G eq/g extract) | 2.01±0.03 |

Table 2. Antioxidative effects of glutinous black rice bran extract (RBE): 50% inhibitory concentration (IC₅₀) and equivalent (eq) amount to reference compounds (mg/g extract) as vitamin C (Vit C) or butyl hydroxyl toluene (BHT) for antioxidant per gram extract. The content was obtained by extrapolation from linear equation (regression coefficient, r²) of the corresponding standard compound calibration curve: y=3.4883x (r²=0.9945) for vitamin C, y=9.151x - 14.09 (r²=0.9915) for BHT.

| Antioxidation | IC ₅₀ (µg/mL) | Equivalent (eq) amount to reference compounds |
|---|--------------------------|---|
| DPPH assay | 1686.70±22.75 | 8.58±0.14 |
| Vitamin C showed IC ₅₀ of 14.46±0.33 µg/mL | | mg Vit C eq/g extract |
| TBARS assay | 2853.10±31.34 | 6.06±0.49 |
| BHT showed IC ₅₀ of 6.98±0.03 µg/mL | | mg BHT eq/g extract |

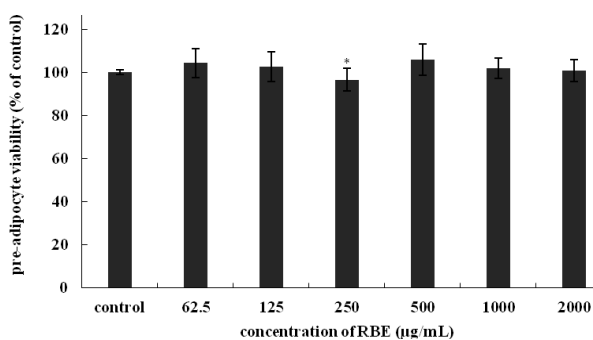
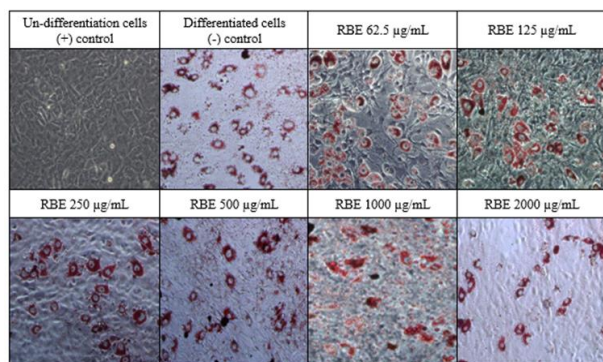
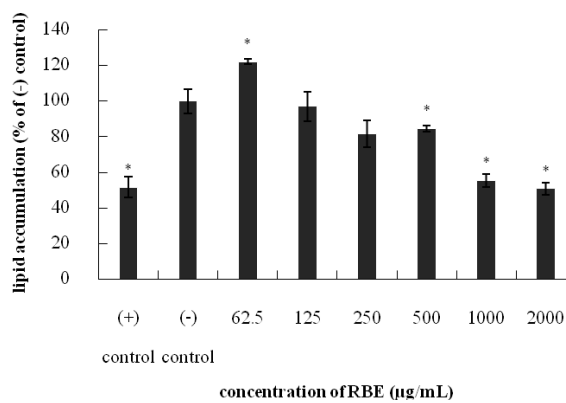


Figure 1. Effects on 3T3-L1 pre-adipocyte viability expressed as relative viability (% of control) of glutinous black rice bran extract (RBE) in concentrations of 62.5–2000 µg/mL. The viability ranged from 96.58±5.17% to 104.26±6.86% of control which were more than 80% of control. This implied the high safety level for further experiments. However, the difference was statistically significant at the concentration of 250 µg/mL (96.58±5.17% of control) and the P-value was less than 0.05 compared to the control (*).



A.



B.

Figure 2. Anti-adipogenesis expressed reduction of lipid accumulation reflected by (A) Oil Red O staining after treatment of glutinous black rice bran extract (RBE) at various concentrations (62.5–2000 µg/mL). The reduction in size and number of oil droplets was detected in a dose dependent manner compared to differentiated cells and by (B) relative lipid accumulation (% of negative control). Significance was found at concentrations of 500–2000 µg/mL with a P-value less than 0.05 compared to the negative control (differentiated cells) (*).

of the negative control (Figure 2B). Accordingly, the reduction of lipid accumulation was exhibited by significant diminishing of the triglyceride content in a dose dependent manner in which the triglyceride content ranged from 36.89±2.08% to 90.56±0.67% of the negative control (Figure 3). This is supported by the reports of anti-obesity potential of other natural extracts which could reduce or inhibit both adipogenesis and accumulation (Buerger *et al.*, 2017; Niemelä *et al.*, 2008; Walter & Marchesan, 2011). Remarkably, the results showed a slight increase in lipid accumulation but a reduction in the triglyceride content at a low concentration (62.5 µg/mL) of RBE which was possibly due to some phytochemicals contained in the RBE which led to lipid accumulation. As supporting evidence, cell proliferative induction and total lipid accumulation in adipocytes was found due to cyanidin-3-glucoside (Roh & Jung, 2012). From our results, the anti-obesity effects of RBE were manifested. As supporting evidence, rice bran is promoted as a rich source of beneficial health agents, such as γ -oryzanol, gallic acid, catechin, chlorogenic acid, caffeic acid, and ferulic acid, owing to the merits of anti-oxidation and a wide spectrum of biological activities (Furu-yashiki *et al.*, 2004; González-Castejón, García-Carrasco, Fernández-Dacosta, Dávalos, & Rodríguez-Casado, 2014; Kim *et al.*, 2012; Otavio *et al.*, 2016; Park *et al.*, 2014; Seo *et al.*, 2015). In addition, the possibility in applications of RBE for health promotion and obesity prevention and treatment was enhanced by a report on its safety in an *in vivo* model (Lee *et al.*, 2013). However, to achieve rational implementation for health and clinical applications focusing on anti-obesity, more results from studies need to be obtained. The needed information includes an evaluation of the efficacy in anti-adipogenesis and lipolysis induction in *in vivo* models and in clinical studies. Also, the bioactive compounds need to be identified along with their biomolecular mechanisms and targets.

3.4 Lipolysis inductive effect

Adipocyte lipolysis is a catabolic process leading to the breakdown of triglycerides stored in fat cells into fatty acids and glycerols. Lipolysis is a hormone-sensitive lipase

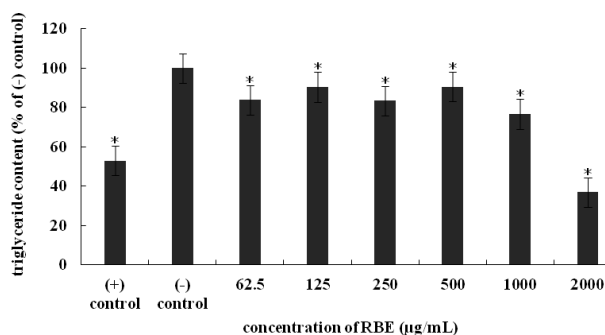


Figure 3. Anti-adipogenesis expressed as reduction of adipocyte intracellular triglyceride content of glutinous black rice bran extract (RBE) at a concentration of 62.5–2000 µg/mL. Significance was found at all tested concentrations with P-values less than 0.05 compared to the negative control (differentiated cells) (*). Remarkably, RBE at the high concentration of 2000 µg/mL was lower than the positive control (un-differentiated cells).

dependent event, in which hormone-sensitive lipase expression is regulated by cyclic adenosine monophosphate (cAMP) (Duncan, Ahmadian, Jaworski, Sarkadi-Nagy, & Sul, 2007; Greenberg *et al.*, 2001; Langin *et al.*, 2005). Phosphodiesterases are regulatory feedback enzymes that catalyze the conversion of cAMP to 5'AMP which is an important signal-transducing molecule in regulating intracellular concentrations and biological actions of these signal-transducing molecules (Moorthy, Gao, & Anand, 2011). Thus, phosphodiesterase inhibitors, including the IBMX which was used as the positive control, exert lipolysis stimulation involving the prevention of cAMP breakdown (Robidoux, Martin, & Collins, 2004). Similar to IBMX (relative glycerol content $235.42 \pm 9.56\%$ of the control), RBE exhibited the lipolysis induction by the increase in released glycerol content in a dose dependent manner with significant results observed at 1000 and 2000 $\mu\text{g/mL}$ (Figure 4A-B). The 3T3-L1 lipolysis induction effect of RBE may be attributed to the phenolics and flavonoids, such as anthocyanidin, proanthocyanidin, and quercetin which were reported to

increase intracellular cAMP levels in 3T3-L1 adipocytes (Pinent, Bladé, Salvadó, Arola, & Ardévol, 2005a; Pinent *et al.*, 2005b). Based on the results, RBE shows evidence to be a potential natural anti-obesity agent that targets adipocytes which should support the application for weight control and obesity treatment. However, further *in vivo* studies are required to confirm the safety and efficacy of action.

4. Conclusions

Anti-obesity potential of glutinous black rice bran extract (RBE) was significantly demonstrated in dose-dependent anti-adipogenesis and in lipolysis induction in adipocytes. This finding suggests that RBE is an alternative natural agent of interest for applications in both the prevention and treatment of obesity. However, further studies to evaluate the efficacy of action and identification of the biological markers in *in vivo* models need to be performed.

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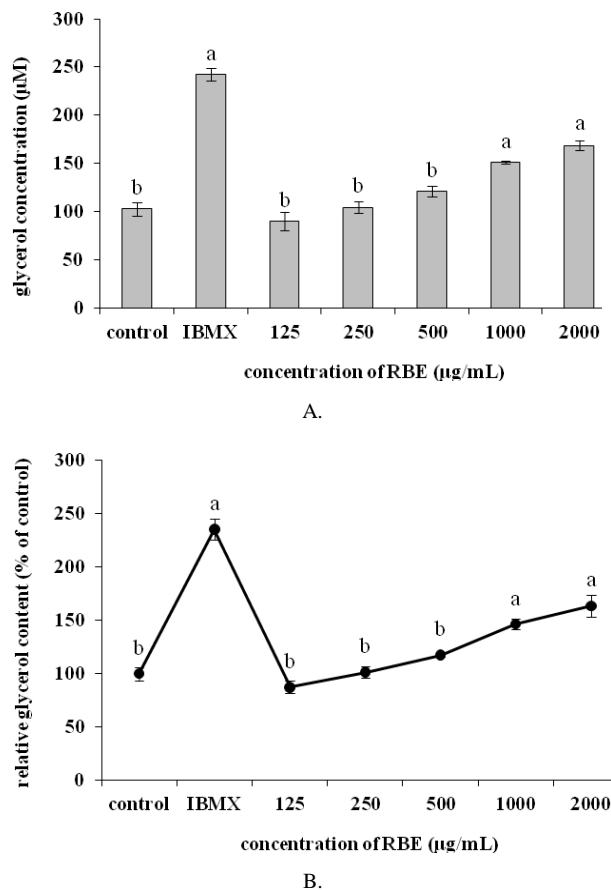


Figure 4. 3T3-L1 adipocyte lipolysis induction of the glutinous black rice bran extract (RBE) was expressed as an increase of (A) glyceride concentration (bar graph) and (B) relative glycerol content (—■—, % of control) to the differentiated group (negative control) as similar to the 3-isobutyl-1-methylxanthine treated group (positive control, IBMX). Significance was found at RBE concentrations of 1000 and 2000 $\mu\text{g/mL}$ with P-values less than 0.05 compared to control (a) or IBMX (b).

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