Anti-obesity potential of glutinous black rice bran extract: anti-adipogenesis and lipolysis induction in 3T3-L1 adipocyte model

<table>
<thead>
<tr>
<th>Journal:</th>
<th>Songklanakarin Journal of Science and Technology</th>
</tr>
</thead>
<tbody>
<tr>
<td>Manuscript ID</td>
<td>SJST-2018-0269.R2</td>
</tr>
<tr>
<td>Manuscript Type:</td>
<td>Original Article</td>
</tr>
<tr>
<td>Date Submitted by the Author:</td>
<td>14-Nov-2018</td>
</tr>
<tr>
<td>Complete List of Authors:</td>
<td>Chumchoochart, Warathorn; Khon Kaen University Faculty of Pharmaceutical Sciences, Department of Pharmaceutical Chemistry Sutthanut, Khaetthareeya; Khon Kaen University Faculty of Pharmaceutical Sciences, Department of Pharmaceutical Chemistry</td>
</tr>
<tr>
<td>Keyword:</td>
<td>anti-obesity, anti-adipogenesis, lipolysis induction, glutinous black rice bran, antioxidation</td>
</tr>
</tbody>
</table>

Note: The following files were submitted by the author for peer review, but cannot be converted to PDF. You must view these files (e.g. movies) online.

Author's Response.docx
Anti-obesity potential of glutinous black rice bran extract: anti-adipogenesis and lipolysis induction in 3T3-L1 adipocyte model

Warathorn Chumchoochart\textsuperscript{1,2} and Khaetthareeya Sutthanut\textsuperscript{1,*}

\textsuperscript{1} Department of Pharmaceutical Chemistry, Faculty of Pharmaceutical Sciences, Khon Kaen University, Khon Kaen, Thailand

\textsuperscript{2} Mekong Health Science Research Institute Khon Kaen University, Khon Kaen, Thailand

* Corresponding author, Email address: khaesu@kku.ac.th

Abstract

The ethanolic glutinous black rice bran extract (RBE) was prepared and used in the experiments of determination of phenolics and flavonoids contents, antioxidation using DPPH and TBARS assay, anti-adipogenesis and lipolysis induction in 3T3-L1 pre-adipocyte model to discover its anti-obesity potential. The results showed that RBE was composed of phenolics, flavonoids and anthocyanins with different contents and showed antioxidative activity (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 interruption of adipocyte life cycle; significant anti-adipogenesis effect in reduction of lipid accumulation (50.86 ± 3.26 to 97.06 ± 8.09\% of (-) control) and triglyceride content in 3T3-L1 pre-adipocytes (36.89 ± 2.08 to 90.56 ± 0.67\% of (-) control) and lipolysis induction (relative glycerol content 117.62 ± 1.44 and 163.72 ± 10.18 \% of control) were demonstrated. \textbf{Therefore, to implement into} obesity therapy, \textbf{further research is needed.}

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

Obesity is one of the most common health problems of the globe with associated comorbidities including metabolic syndrome, cardiovascular diseases, muscular-skeleton syndromes, such as diabetes, hypercholesterolemia, hypertension, atherosclerosis, cancer, muscle weakness, heart failure, and mortality. Therefore, obesity prevention and treatment are global community needs and medication is a choice besides behavior modification, exercise, and surgery, yet, with caution of serious central nervous (CNS) and cardiovascular (CVS) side effects. From this, anti-obesity agents from natural sources are interested alternates providing safe, economic, and integrated health promoting in long-term uses as expected advantages for obesity therapy.

Increased numbers of adipocyte can contribute to obesity development. This is regulated by a concert of multiple factors such as genetic, metabolic, nutritional factors (de Ferranti & Mozaffarian, 2008) as well as oxidative stress (Albuali, 2014). Therefore, adipocyte life cycle interruption via the inhibition of adipocyte differentiation (adipogenesis), reduction of lipid accumulation as well as oxidative stress, and induction of lipolysis believed to be the preventive strategies for 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, quercetin and anthocyanins have been reported for anti-obesity via regulation of anti-adipogenesis (Ahn, Lee, Kim, Park, & Ha, 2008). Moreover, they have been extensively known as the health beneficial antioxidants.
Rice bran from glutinous black rice (*Oryza sativa L.*)) consists of a high content 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 which anti-obesity potential is postulated via anti-adipogenesis contributed by phenolics as gallic acid, ferulic acid (Chaiittianan, Chayopas, Rattanathongkom, Tippayawat, & Sutthanut, 2016), 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 anti-obesity of the glutinous black rice bran has 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 (DPPH), 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 (EtOH), formaldehyde were purchased from RCI Labscan Ltd. (Bangkok, Thailand); methanol (MeOH) 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. (Carlshad, CA, USA). potassium chloride (KCl), potassium dihydrogen orthophosphate (KH$_2$PO$_4$), sodium chloride (NaCl), sodium hydroxide (NaOH), di-sodium hydrogen orthophosphate anhydrous (Na$_2$HPO$_4$), sodium carbonate anhydrous (NaCO$_3$), aluminium chloride hydrated (AlCl$_3$), sodium acetate (CH$_3$COONa), ferrous sulphate (FeSO$_4$), acetic acid (CH$_3$COOH), sodium lauryl sulfate (SLS) and hydrochloric acid (HCl) were purchased from Ajax Finechem Pty Ltd. (Albany, Auckland, N.Z.).

2.2 Plant materials

The glutinous black rice bran material was derived from milling process of a variety of Thai glutinous black rice (Oryza sativa L.) cultivated in Baanhad community, Khon Kaen, Thailand. The extraction was performed by maceration technique in 50% ethanol solvent for 24 hours. After filtration, the extract was filtered and concentrated by rotary evaporator and lyophilize by using Freeze-dryer (EYELA FDU-1200, Tokyo, Japan). Finally, the extract (RBE) was obtained with %yield of 10.42 % w/w of rice bran and kept at -20°C temperature until used.

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 μg/mL. Gallic acid (0-100 μg/mL) was used as a reference compound. Briefly, the mixture of 50 μL of
the sample solution, 125 μL of 20% (w/v) Na₂CO₃ and 25 μL of 50% (v/v) of Folin-Ciocalteu reagent was constituted and incubated at 25 ºC for 40 min prior to measurement of absorbance of the mixture at wavelength of 700 nm using a microplate reader (Varioskan™ Flash Multimode Reader, Thermo Scientific®, Waltham, MA, USA). The total phenolics content was expressed in term of mg gallic acid equivalence per gram of extract (mg GA eq/g extract).

2.4 Total flavonoids determination

Total flavonoids content was determined by aluminium chloride colorimetric assay (Chang, Zuo, Harrison, & Chow, 2002). The RBE solution was prepared at various concentrations of 62.5-1000 μg/mL. Quercetin (0-100 μg/mL) was used as a reference compound. Briefly, the mixture of 100 μL of the sample solution and 200 μL of 5% AlCl₃ was constituted and incubated at 25 ºC for 30 min. Then, measured the optical absorbance at wavelength of 437 nm using a microplate reader. The total phenolics content was expressed in term of mg quercetin equivalence per gram of extract (mg Q eq/g extract).

2.5 Total anthocyanins determination

Total anthocyanins content was determined by pH differential method (Shafazila, Lee, & Lee Kong, 2010). The RBE solution was prepared at final concentration of 500 μg/mL. Cyanidin-3-glucoside (200 μg/mL) was used as a reference compound. Briefly, The RBE solution was prepared in KCl solution pH 1.0 and in CH₃COONa pH 4.5 incubated at 25 ºC for 30 min. The optical absorbance was measured at 520 and 700 nm in order to be used in calculation following the below equation. The total anthocyanins content was expressed in term of mg cyanidin-3-glucoside equivalence per gram of extract (mg C3G eq/g extract).
For Review Only

[193x522]For Review Only

[534x746]6

1

2

3

4

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

5

6

TA content = (A \times MW \times \text{dilution factor} \times 100)/\varepsilon

7

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

8

2.6 Antioxidation activity by DPPH assay

Free radical-scavenging activity was determined by 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 ºC for 15 min. Then, measured the optical absorbance at wavelength of 570 nm using a microplate reader. The antioxidative effect was expressed in terms of 50% inhibitory concentration (IC50) and vitamin C equivalence 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 ºC for 1 h prior to 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 at wavelength 532 nm was measured using a microplate reader. The antioxidative effect was expressed in terms of 50% inhibitory concentration (IC50) and BHT equivalence 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 humidified atmosphere of 5% CO₂ in pre-adipocytes medium (PM; the mixture of high-glucose Dulbecco’s modified Eagle medium (DMEM) supplemented with 10% fetal calf serum (FCS), 1% penicillin streptomycin mixture) until 90% confluence was reached. In all experiments, cells were seeded into 24-well plate at a density of 30,000 cells/well and cultured for 2 days until reach 90% confluence. Afterward, cells were induced to differentiate by differentiation media (DM) treatment, DM I (a mixture of DMEM, 10% FCS, 1 µM dexamethasone, 500 µM 3-Isobutyl-1-methylxanthine (IBMX), and 10 µg/mL insulin) and DM II (dexamethasone and IBMX- free DM I), then maintain in adipocyte medium (AM; the mixture of DMEM supplemented with 10% fetal bovine serum (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 96-well plate until 90% confluence. After 24-h preconditioning, 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. 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-buffer saline; PBS) was added into the well followed by 4-h incubation at 37 °C temperature, removed the MTT solution, added with 150 μL DMSO, incubated at ambient temperature for 30 min, and determined the optical absorbance at wavelength of 540 nm using a microplate reader. Compared to the control, the results were demonstrated as pre-adipocyte viability (% of control).

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

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

2.10.1 Oil Red O staining assay

The differentiated adipocytes were washed with phosphate buffer saline (PBS, pH 7.4) and fixed with 4%(v/v) formaldehyde for 30 min, washed with 60%(v/v) isopropanol. Then, stained with freshly prepared 0.5%(w/v) Oil Red O solution at 37 °C in humidified atmosphere of 5% CO2 for 1 h, and photographed the stained cells using an inverted microscope (Axi Vert.A1 FL-LED, ZEISS®, Jena Germany). In addition, lipid accumulation content was determined, the retained dye in adipocytes were extracted with 100% isopropanol, then, the optical absorbance was 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 in 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 Laboeteknik GmbH, Germany). Then, the supernatant was separated by centrifugation at 4 °C temperature, speed 12,000 rpm for 20 min. The triglyceride content in the supernatant of each sample was determined by using triacylglycerol assay kit which the optical absorbance was measured at 540 nm using microplate reader. The results were 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 24-well plate 24,000 cells/well, incubated overnight in DMEM with 0.5% FBS at 37 °C temperature in 5% CO₂ humidified atmosphere, then, treated with phenol red-free DMEM (as negative control) or IBMX (as positive control) or various concentrations of RBE (125, 250, 500, 1000 and 2000 μg/mL), and followed by 24-h incubation. Afterward, released glycerol was quantified using test kit of free glycerol reagent (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 free glycerol reagent at 1:1 (v/v) ratio. Following 15-min incubation, the optical absorbance was determined at 540 nm using the microplate reader. The results were expressed as relative glycerol content (% of control).

2.12 Statistical analysis

Results were expressed as mean ± standard deviation (SD) of values obtained from triplicate determination (n=3) of studies unit. Statistical analysis was performed using using
Oneway ANOVA followed by Bonferroni’s multiple comparison of SPSS version 17.0 (Chicago, IL, USA) which significance was considered at p-value less than 0.05 (P<0.05).

3. Results and Discussion

3.1 Phytochemical contents and antioxidation activity

Phenolics, flavonoids, and anthocyanins contents in RBE were extracted by 50% ethanol, as shown in Table 1. Respect to the like-dissolve-like principle, the extractive solvent used, 50% ethanol, was considered as the factor which resulted of high extracted contents of phenolics and anthocyanins—the polar compounds—in RBE. These compounds: cyanidin-3-glucoside, caffeic acid, p-coumaric acid, ferulic acid, gallic acid, vanillic acid, protocatechuic acid, synaptic acid, syringic acid, peonidin-3-glucoside are phytochemicals in rice bran extract. Although, there is a number of previous reports on wide spectrum of biological activities of phenolics, flavonoids, and anthocyanins including antioxidation and anti-obesity, the differences among them also have 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 obese between antioxidative and anti-obesity effects has been evidenced involving reduction of oxidative stress-induced obesity and complications (Alcalá et al., 2015; Calzadilla et al., 2011), attributed by overproduction of reactive oxygen species (ROS) leads to cell function impairment (Halliwell, Gutteridge, & Cross, 1992) and induction of lipid accumulation (Geon Lee et al., 2016; Y. Kim & Lee, 2017). From this, various antioxidants: vitamin C, green tea, green coffee,
cinnamon, lipoic acid and L-carnitine, have been used for prevention and treatment of obesity (Abdali, Samson, & Grover, 2015) as well as oxidative stress related chronic diseases therapy. Interestingly, RBE expressed antioxidative effects in both the hydrophilic and hydrophobic condition. In hydrophilic condition (DPPH assay), RBE had IC50 value as 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 hydrophobic condition (TBARS assay), RBE showed IC50 2853.10 ± 31.34 μg/mL and 6.06 ± 0.49 mg BHT eq/g extract when compared to BHT (IC50 6.98 ± 0.03 μg/mL) (Table 2). The results suggested the RBE as a potential natural extract to be further researched to evaluate the possibility in applications for health and anti-obesity.

3.2 Cytotoxicity of RBE on pre-adipocyte by MTT assay

The very low cytotoxicity of RBE to pre-adipocytes was demonstrated with high viability of cells, more than 80% cell viability when compared to the control, which ranged between 96.58 ± 5.17 to 104.26 ± 6.86% of control at all tested concentrations (62.5-2000 μg/mL). Therefore, this concentration range was logically safe to be used in further experiments (Figure 1).

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

Adipogenesis or adipocyte differentiation process which pre-adipocyte transforms to mature adipocyte via intercellular adipogenesis, for example, lipid production and accumulation in form of triglyceride. This commonly results in enlargement and coalescence of lipid droplets and morphological change to bigger-size and rounded-shape cells (Niemelä, Miettinen, Sarkanen, & Ashammakhi, 2008). Using Oil Red O staining technique, anti-adipogenesis of RBE at concentration range of 125-2000 μg/mL was demonstrated with a dose dependent trend of
reduction of oil droplets in sizes and numbers when compared to negative control (Figure 2A).

In addition, the lipid contents were significantly reduced at RBE concentrations of 500-2000 μg/mL with lipid content range between 50.86 ± 3.26% to 84.57 ± 1.70% of negative control (Figure 2B). Accordantly, the reduction of lipid accumulation was exhibited by significant diminishing of triglyceride content with dose dependent manner, triglyceride content ranged between 36.89 ± 2.08% to 90.56 ± 0.67% of negative control (Figure 3). This is supported by the reports of anti-obesity potential of other natural extracts which could reduce or inhibit in both adipogenesis and accumulation (Buerger et al., 2017; Niemelä et al., 2008; Walter & Marchesan, 2011).

Remarkably, the result showed slightly increasing of lipid accumulation but reduction of triglyceride content at only low concentration (62.5 μg/mL) of RBE which the attribution of some phytochemicals contained in RBE, which can lead to lipid accumulation, was proposed. As one supporting evidence, cell proliferative induction and total lipid accumulation in adipocytes was found in cyanidin-3-glucoside (Roh & Jung, 2012). From our results, anti-obesity effects of RBE has been manifested. As supporting evidence, the rice bran is pronounced as a rich source of health beneficial agents, such as γ-oryzanol, gallic acid, catechin, chlorogenic acid, caffeic acid, ferulic acid and etc., with owing merits of antioxidation and wide spectrum of biological activities (Furuyashiki et al., 2004; González-Castejón, García-Carrasco, Fernández-Dacosta, Dávalos, & Rodríguez-Casado, 2014; H.-K. 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 is potentiated by a report on its safety in in vivo model (Lee et al., 2013).

However, to achieve the rationale implement for health and clinical applications focusing on anti-obesity purpose, there are several indicative information that need to be defined. These include
evaluation of efficacy in anti-adipogenesis and lipolysis induction in in vivo model and clinical studies, identification of the bioactive compounds, their biomolecular mechanisms and targets.

3.4 Lipolysis inductive effect

Adipocyte lipolysis is a catabolic process leading to the breakdown of triglycerides that are stored in fat cells into fatty acids and glycerols. Lipolysis is hormone-sensitive lipase (HSL) dependent event, in which, HSL expression is regulated by cyclic adenosine monophosphate (cAMP) (Duncan, Ahmadian, Jaworski, Sarkadi-Nagy, & Sul, 2007; Greenberg et al., 2001; Langin et al., 2005). Phosphodiesterases (PDEs) are regulatory feedback enzyme that catalyze the conversion of cAMP to 5'AMP, an important signal-transducing molecule in regulating intracellular concentrations and biological actions of these signal-transducing molecules (Moorthy, Gao, & Anand, 2011). Thus, PDE 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 ± 9.56 % of the control), RBE exhibited the lipolysis induction by increasing of released glycerol contents with dose dependent manner and significant results observed at 1000 and 2000 μg/mL (Figure 4A-B). The 3T3-L1 lipolysis induction effect of RBE may be attributed by the phenolics and flavonoids such as anthocyanidin, proanthocyanidin, quercetin which could increase intracellular cAMP levels in 3T3-L1 adipocytes (Pinent, Bladé, Salvadó, Arola, & Ardévol, 2005; Pinent, Blade, et al., 2005). Based on the result, RBE has been evidenced as a potential natural anti-obesity agent with having adipocytes as a peripheral target of action 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 with dose dependent anti-adipogenesis and lipolysis induction in adipocytes. This finding has suggested RBE as an alternative natural agent of interest for applications in both prevention and treatment of obesity. However, the further studies in evaluation of efficacy of action and identification of biological markers in in vivo models shall be performed.

Acknowledgments

This work was supported by faculty of Pharmaceutical Sciences, Mekong Health Science Research Institute Khon Kaen University and Research and Technology Transfer Affairs (KKU-YN011, fiscal year 2559 and 2560), Khon Kaen University.

References


disease: Where are we now? *The Journal of Laboratory and Clinical Medicine, 119*(6),

anthocyanins inhibit adipocyte differentiation in 3T3-L1 cells. *Nutrition Research, 32*(10), 770-777. doi:10.1016/j.nutres.2012.06.008

during Adipocyte Differentiation in 3T3-L1 Cells. *Preventive Nutrition and Food Science, 22*(2), 118-123. doi:10.3746/pnf.2017.22.2.118

Adipocyte Lipases and Defect of Lipolysis in Human Obesity. *Diabetes, 54*(11), 3190-
3197. doi:10.2337/diabetes.54.11.3190

Hypolipidemic effect of Goami-3 rice (Oryza sativa L. cv. Goami-3) on C57BL/6J mice

(2013). γ-Oryzanol inhibits the adipogenesis of adipose-derived human mesenchymal
doi:10.1096/fasebj.27.1_supplement.lb246


viability and collagen synthesis, and matrix metalloproteinase-2 and -9 inhibition.


Figure 1 The effects on 3T3-L1 pre-adipocyte viability which expressed as relative viability (% of control) of glutinous black rice bran extract (RBE) at concentration range of 62.5-2000 µg/mL which the viability ranged between 96.58 ± 5.17 to 104.26 ± 6.86% of control which were more than 80% of control. This implied the high safe of used concentration range for further experiments, although, the difference was statistically significant found at concentration of 250 µg/mL (96.58 ± 5.17% of control), p-value less than 0.05 when compared to control (*).
Figure 2 The 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) which the reduction in size and number of oil droplets was detected with trend of dose dependent manner, compared to differentiated cells and by (B) relative lipid accumulation (% of (-) control), the significance was found at concentrations of 500-2000 µg/mL with p-value less than 0.05 when compared
to

(-) control (differentiated cells) (*)

Figure 3 The anti-adipogenesis expressed as reduction of adipocyte intracellular triglyceride contents of glutinous black rice bran extract (RBE) at concentration range of 62.5-2000 µg/mL, the significance was found at all tested concentrations with p-value less than 0.05 when compared to (-) control (differentiated cells) (*). Remarkably, RBE at high concentration of 2000 µg/mL was lower than (+) control (Un-differentiated cells)
Figure 4
The 3T3-L1 adipocyte lipolysis induction of the glutinous black rice bran extract (RBE) was expressed as increasing of (A) glyceride concentration (bar graph) and
(B) relative glycerol content (---, % of control) to the differentiated group (-) control) as similar to the 3-isobutyl-1-methylxanthine treated group (+) control, IBMX). The significance was found at RBE concentrations of 1000 and 2000 µg/mL with p-value less than 0.05 when compared to control (a) or IBMX (b).
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, \( r^2 \)) of the correspond standard compound calibration curve: \( y = 0.0052x \) \( (r^2 = 0.9875) \) for gallic acid, \( y = 0.0396x \) \( (r^2 = 0.9888) \) for quercetin.

<table>
<thead>
<tr>
<th>Phytochemical content</th>
<th>180.67 ± 7.88</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenolics (mg GA eq/g extract)</td>
<td></td>
</tr>
<tr>
<td>Flavonoids (mg Q eq/g extract)</td>
<td>0.43 ± 0.34</td>
</tr>
<tr>
<td>Anthocyanins (mg C3G eq/g extract)</td>
<td>2.01 ± 0.03</td>
</tr>
</tbody>
</table>

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, \( r^2 \)) of the correspond standard compound calibration curve: \( y = 3.4883x \) \( (r^2 = 0.9945) \) for vitamin C, \( y = 9.151x - 14.09 \) \( (r^2 = 0.9915) \) for BHT.

<table>
<thead>
<tr>
<th>Antioxidation</th>
<th>IC50 (µg/mL)</th>
<th>Equivalent (eq) amount to reference compounds</th>
</tr>
</thead>
<tbody>
<tr>
<td>in DPPH assay which vitamin C showed IC50 14.46 ± 0.33 µg/mL</td>
<td>1686.70 ± 22.75</td>
<td>8.58 ± 0.14 mg Vit C eq/g extract</td>
</tr>
<tr>
<td>in TBARS assay which BHT showed IC50 6.98 ± 0.03 µg/mL</td>
<td>2853.10 ± 31.34</td>
<td>6.06 ± 0.49 mg BHT eq/g extract</td>
</tr>
</tbody>
</table>