Title: Effects of rice extract of KDML105 cultivar on protein expression associated with adipogenesis and antioxidative status in 3T3-L1 adipocytes.

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Field: Biochemistry.
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Abstract

Obesity is a chronic disease and a risk factor for metabolic disorders including cardiovascular disease, hypertension and type 2 diabetes mellitus. Obesity is characterized by excess lipid contents. Recently, several phytochemicals have been reported to potentially prevent obesity through various mechanisms. The purpose of this study was to determine the effect of germinated brown rice, brown rice, and white rice (polished rice) of Khao Dawk Mali 105 cultivar extract on adipogenesis and antioxidative status in 3T3-L1 adipocytes. The adipocytes were treated with the rice extracts at concentrations of 0.1, 0.5 and 1.0 mg/ml for 9 days from the starting of differentiation. Total proteins were extracted and separated by Western blot technique. The expression of protein involved in adipogenesis (PPARγ) and antioxidant (SOD2 and GPx4) by western blot method and analyzed with Gel documentation system. The results showed that GBR extract significantly decreased expression of PPARγ (p<0.05) in a dose-dependent manner and tended to reduce its expression in cells treated with BR. However, WR did not affect PPARγ protein expression. In addition, GBR and BR were likely to increase the expression of antioxidant proteins, GPx4 and SOD2. This study may be a preliminary study to notify the biological activity of germinated brown rice extract on adipogenesis and oxidative stress and could be a potential functional food for health promotion.

Keywords: KDML105, SOD2, PPAR, Gpx4, 3T3-L1 adipocytes, Oxidative stress

Introduction:

Obesity is a chronic metabolic disorder disease that is a serious risk factor for hypertension, cardiovascular disease, type-s diabetes, and cancer (Mokdad et al., 2001). The obesity is derive from energy balance and energy accumulation with the accumulation of triglycerides.The adipocytes develop form Fibroblast. Adipogenesis is tightly controlled by various transcription factors and adipocyte-specific genes (Gregoire et., 1998) such as peroxisome proliferator-activated receptors (*PPARs*), CCATT-enchancer-binding proteins (*C/EBPs*), and sterol regulatory element binding proteins (*SREBPs*). In addition, the study an increase in PPARγ expression was necessary to adipogenesis in 3T3 -L1 adipocyteing, displays its importance in the differentiation of adipocytes (Rios-Vazquez et al., 2006).

Currently, anti-obesity drug such as orlistat, sibutramine and dinitrophenol are ability used to treat obesity (Filippatos et al., 2008).However, these drugs have been associated with adverse effects human body such as Insomia, mood swings, diarrhea and nervous system. Therefore, the natural medicinal product might be an alternative strategy for the development of effective anti-obesity drugs which include flavonoids, vitamins, capsaicin and resveratrol. Rice extract is have metabolically beneficial effects. Recent studies have shown that rice extracts prevent obesity and type 2 diabetes (Sun, 2012). The rice extract could decrease lipid accumulation in the 3T3-L1 adipocytes (Kayahara, 2013) and have been shown to reduce body weight in obese mice. It also increase enzymatic antioxidant expressions such as superoxide dismutase (SOD2) and glutathione peroxidase (GPx4). The enzymatic antioxidants inhibit or quench free radicals and delay or inhibit cellular damage. Moreover, bioactive compounds found in plants such as flavonoids and anthocyanins (Robert, 2010) have been shown to decrease ROS level of obesity. Previous research has identified the chemical composition of Thai rice cultivars and their antioxidant activities (Norhaizan et al., 2013). The phenolic compounds that found in rice such as GABA and γ-oryzanol (Jannoey et al., 2010; Moongngarm and Saetung, 2010).

Consequently, organizer choose the Germinated brown rice of Kaw dokmali-105 rice (KDML-105) to study effect of rice extract on protein expression SOD2, GPx4 and PPARγ. This study is data for future clinical trials and the rice of KDML-105 might protect or treat obesity. It also add value to money of agricultural products.

Methodology:

1. Bicinchoninic Acid (BCA) method

Prepare stock BSA at 200 µg/ml or 0.2 µg/µl. then prepare a standard graph with a solution of BSA at various concentrations. Prepare protein extract to dilute protein with distilled water in 96 well plate. In addition, prepare BCA solution from BCA protein assay which made of BCA solution A and BCA solution B (50A:1B in ratio), the volume of 200 µl and incubated at 37 ° C for 30 minutes. After that, the absorbance was measured with microplate reader (BioTek, USA). At wavelengths of 562 nm, the values were calculated to determine the amount of protein required for further experiments.

2. SDS page

Prepare a 1 ml polyacrylamide gel electrophoresis by preparing separating gel into a 15mL microcentrifuge tube and bring the vortex to a drop of saturated n-butanol until the gel solidifies and then pour the saturated n-butanol. Stack the gel into a 15 mL microcentrifuge tube, bring the vortex into the glass, and suck into the set glass and put the comb down and wait until the gel is solid. When Sodium dodecyl sulfate (SDS) gel is solid, then separates the protein. Bring gel to install with Electrophoresis Chambers (BIO RAD, USA) filled 1x SDS PAGE buffer and loaded the protein into the hole by loading all proteins and protein ladder volume 5 microlitter, then Install the power supply to the power supply by setting the current at 120V 400mA for 3 hours.

3. Transfer protein

Soaked PVDF membrane which has a pore size of 0.45micrometer (Millipore, USA), which required membrane in 100% methanol (ACILabscan, Thailand) for 5 minutes, then move membrane and filter paper (BIO-RAD, USA) to soak in 1x transfer buffer a place for about 30 minutes, then bring a gel, PVDF membrane and filter paper place. When everything set completely. Take the set into the tank chamber and add a 1X transfer buffer to the chamber, then connect it to the power supply. It will set the current at 50V, 400 mA for 4 hours.

4. Blocking

dye membrane with Ponceau red color (Sigma, USA) about 20-30 minutes to check protein's band on membrane with distilled water and soak in 5% blocking buffer (5% (w / v) and non fat dry milk dissolved in 1X TBST (containing 0.1% Tween 20 and 10X Tris-buffered saline (TBS)) buffer and shake for 1 hour at room temperature.

5. Primary antibody

Immerse membrane in each protein-specific primary antibody and shake it overnight at 4 ° C. Apply membrane to 1X TBST for 3 times, 5 minutes.

6. Secondary antibody and detection

Soaked membrane in the secondary antibody which conjugate with horseradish peroxidase (HRP) conjugate anti-rabbit (MILLIPORE, USA) and shake for 1 hour at room temperature, then wash the membrane with 1X TBST for 3 times 5 minutes Then the molecules of The antigen-antibody complex on the membrane was measured by Luminata ™ Western HRP Chemiluminescence Substrates (BIOLOGIX, USA).

Results

Effect of White rice, brown rice and germinated brown rice extracts on PPARγ, GPx4 and SOD2 expression in 3T3-L1 adipocytes.

Mechanisms of anti-adipogenesis was investigated by measuring protein expression of key protein involved in adipogenesis. Antioxidant, such as SOD_2 and GPx_4 was also observed by western blot.

The GBR treatment at day 9 significantly reduced PPAR protein expression at 0.5 and 1 mg/ml by 20% and 29%, respectively contrast with SOD_2 and GPx_4 protein expression were not statistically significant in WR, BR and GBR treated group.

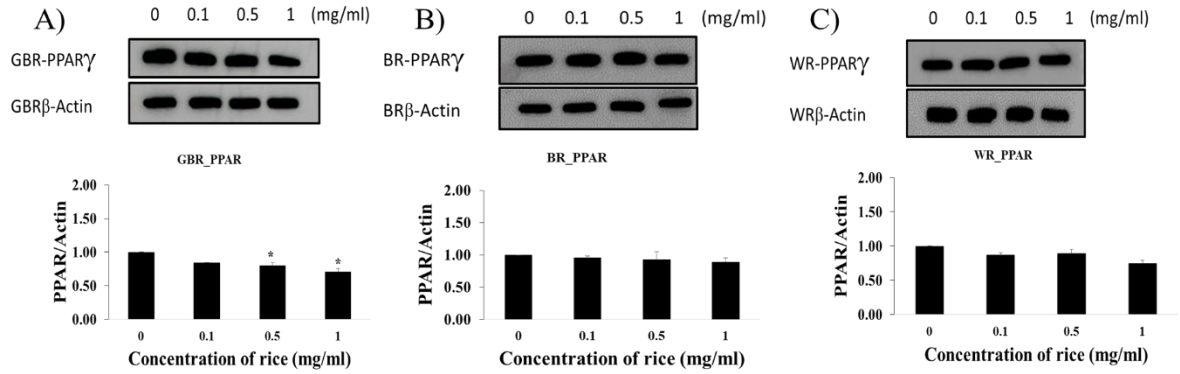

Fig. 1. Effect of GBR (A), BR (B) and WR (C) on protein expression of PPARγ in 3T3-L1 adipocytes. Data are expressed relative to untreated control cells and represent means ± S.D. *p<0.05 compared to the control (0 µg/ml).

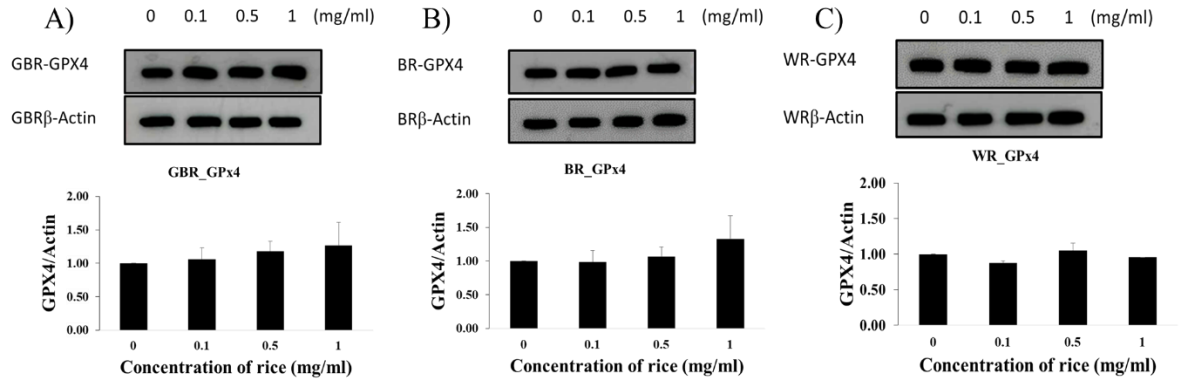

Fig. 2. Effect of GBR (A), BR (B) and WR (C) on protein expression of GPx4 in 3T3-L1 adipocytes. Data are were expressed relative to untreated control cells and represent means ± S.D. *p<0.05 compared to the control (0 µg/ml).

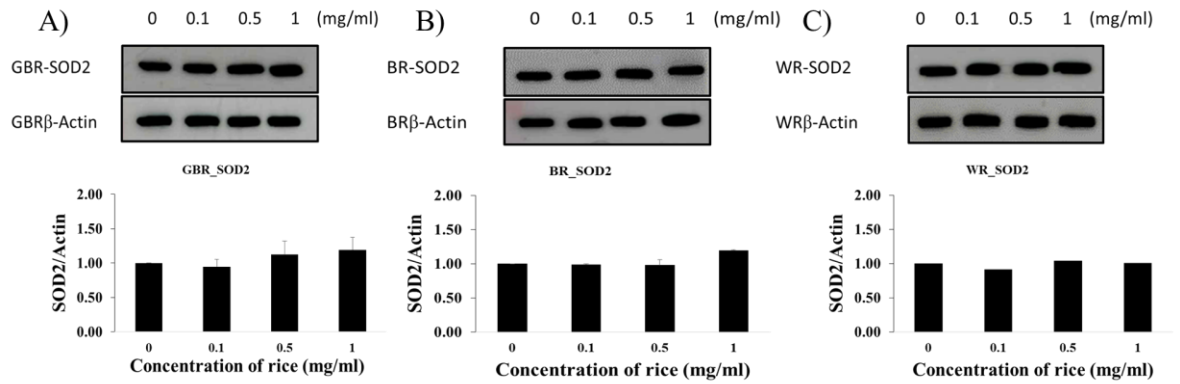

Fig. 3. Effect of GBR (A), BR (B) and WR (C) on protein expression of SOD2 in 3T3-L1 adipocytes. Data are expressed relative to untreated control cells and represent means ± S.D. *p<0.05 compared to the control (0 µg/ml).

Discussion and Conclusions

In this study, we examined the effect of WR, BR and GBR extracts of KDML-105 cultivars on adipocyte differentiation. We also investigated the molecular mechanisms of WR, BR and GBR extracts on adipocyte differentiation through analyzing expressions of protein associated with adipogenesis, and oxidative status by western blot.

BR and GBR contain several antioxidants including γ-oryzanol, phytic acid, ferulic acid, phenolics, and flavonoids that might have effective antioxidant effects (Pastil and Khan 2011; Kaukovirta et al., 2004). Previous study, rice extracts significantly decreased gene expression of adipogenesis such as C/EBPα and PPARγ genes (Jeon T. et al., 2004). Moreover, BR and GBR extracts of Malaysian local rice can increase the activity of antioxidant enzymes (SOD2 and GPx4) in rabbits (Mohd Esa et al., 2013).

The results showed that protein levels of adipogenic transcription factors PPARγ were significantly down-regulated in dose-dependent manner in adipocytes treated with GBR extracts resulting in reduce lipid accumulation.

In conclusion, the present study demonstrated that GBR extracts of KDML-105 Thai rice cultivars disturbed 3T3-L1 adipocyte differentiation by decreasing PPARγ protein expression. However, it did not affect antioxidant protein expressions. Our preliminary results (unpublished data) have shown that GBR extract exhibited radical scavenging activity and reduced intracellular ROS levels. Thus GBR extracts may have health beneficial effects on anti-obesity.

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