Supplementary effects of black rice (Oryza sativa L.) aleurone layer extract on body fat, serum lipid, and serum hormone levels in ovariectomized rats

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  * black rice extract (BRE), post-menopausal model
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Introduction

- Rice (Oryza sativa L.) is widely consumed throughout the world and rice consumers have been paying attention to various values of rice.

- With growing concerns regarding health, black, purple, and red rice with unique flavor, nutritional and biological properties have been developed.
Introduction

- The supplementation of black rice and its anthocyanin pigment reduced atherosclerotic lesions in hypercholesterol-emic animal models.
- Dietary black rice supplementation alleviated hepatic steatosis by increasing fatty acid oxidation in the mice fed a high fat diet.
- Black rice is a kind of **brown rice** and is not well digested. So most of functional materials existing in aleurone layer of black rice are hard to be observed.
- Bio-functional effects of black rice have been tested with it’s extracts and major compounds were known as polyphenols and flavonoids.
* Thus numerous *in vitro* and *in vivo* studies have shown us that **black rice** may be preventive against chronic diseases by reducing fat weight or its ratio in the body.
The average life span has been increasing.
Introduction

- Obesity % is higher in women than in men for over 60 years old peoples.

Comparison of obesity percentage by age and gender
Introduction

- For post-menopausal women, body fat tends to shift to the abdomen and body composition changes with higher ratio of fat in the body.

- The women experience increased risk of metabolic syndrome and heart disease.
Introduction

For post menopausal women, keeping good health is so important because their health condition continues to/decides the health condition of their old age.

Exogenous estrogen has been shown to be protective against metabolic abnormalities. But long-term usage of hormone replacement therapy may increase the risk of breast cancer and cardiovascular diseases in postmenopausal women.
Introduction

- People have been interested in natural foods such as black rice to prevent and control obesity or body fat accumulation.

Hypothesis

1. Extracts from aleurone layer of black rice (BRA) may effectively prevent obesity and reduce body fat accumulation in the menopausal women.
2. BRA is 8% of black rice. If we use BRA rather than whole black rice as brown rice for functional material extraction, we can save ethanol as extraction solvent and reduce its amount to 1/13.
Objective

- This study was conducted to evaluate suppressive effects of black rice aleurone layer extract (BRE) on body fat, serum lipid, and hormone levels in ovariectomized model.
Materials and Methods – *in vitro* study

**Preparation of extracts**

1. **Oryza sativa** L.
2. Preparation of extracts
3. **8.0%** aleurone layer (AL)
4. **92.0%** milling rice yields (white rice, WR)
5. + 50, 70 %%(v/v) EtOH
6. 40°C, 48 h

Brown rice (BR)
Results - in vitro

Fig. Comparison of anthocyanin contents in black rice and Black rice aleurone layer

- Cyanidin 3-glucoside
- Peonidin 3-glucoside
- Total anthocyanin

- Black rice
- Black rice aleurone layer
Experimental design - *in vitro*

- Comparing
  - part of rice: BR, AL, WR
  - ethanol concentration: 50%, 70%

- Cells: 3T3L1
- Factors: cell differentiation, gene expression, lipid concentration
Results - *in vitro*

Fig. Comparison of BR and AL effect on adipocyte differentiation in 3T3L1 cells

Fig. Effects of BRE-AL on adipocyte differentiation in 3T3L1 cells
Fig. Effects of BRE-AL on adipocyte differentiation-related gene expression in 3T3L1 cells
Results - *in vitro*

Cell differentiation – Oil Red O

Fat oxidation and synthesis

**Fig. Effects of BRE-AL on adipocyte differentiation, fatty acid oxidation, and its synthesis in 3T3L1 cells**
Fig. Effects of BRE-AL on lipid accumulation-related gene expression in 3T3L1 cells at 9th day
Chosen material for *in vivo and human studies*

1. *Oryza sativa* L.

2. Preparation of BRE

   - 8.0% aleurone layer (BRE, BRE-AL)

3. 92.0% milling rice yields

   + 50% (v/v) EtOH
   
   40°C, 48 h

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Brown rice

Black rice extract (BRE)
### Experimental design - *in vivo*

- Metabolic changes induced by estrogen depletion from **ovariectomy** share many similar characteristics with changes such as weight gain, increased adiposity, and the development of fatty liver in menopausal women.

<table>
<thead>
<tr>
<th>0 week</th>
<th>12 week</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Adaptation period)</td>
<td></td>
</tr>
</tbody>
</table>

- **5-week-old SD rats**
- **Operation**
  - Sham or Ovariectomy

<table>
<thead>
<tr>
<th>Groups</th>
<th>12 week</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham</td>
<td></td>
</tr>
<tr>
<td>OVX</td>
<td>Control</td>
</tr>
<tr>
<td></td>
<td>I.F 10mg/kg B.W</td>
</tr>
<tr>
<td></td>
<td>BRE 30 or 90mg/kg B.W</td>
</tr>
</tbody>
</table>
**Measuring factors - *in vivo***

- Body weight gain, fat pad weight
- Adipocyte size (adipocyte index)
- Serum triglyceride, total-, LDL-, HDL-cholesterol
- Serum leptin, adiponectin concentration
- Hepatic and fecal total lipids, triglyceride, total cholesterol

**Statistical analysis**

- Windows SAS package program (ver. 9.2)
- One-way ANOVA ($\alpha=0.05$)
- Duncan’s multiple range test
Results - *in vivo*

Fig. Treatment effects of BRE-AL on body weight gain of ovariectomized rats
Results - *in vivo*

Fig. Treatment effects of BRE-AL on body fat weight of ovariectomized rats
Results - in vivo

Fig. Treatment effects of BRE-AL on adiposity index of ovariectomized rats, *fat weight/body weight.
Results - in vivo

Fig. Treatment effects of BRE-AL on serum lipid profiles of ovariectomized rats
Results - *in vivo*

Fig. Treatment effects of BRE-AL on serum adiponectin and leptin levels of ovariectomized rats
Fig. Treatment effects of BRE-AL on hepatic lipid profiles of ovariectomized rats
Experimental design - human study

Measure Food and nutrient intake

Body weight and fat content

<table>
<thead>
<tr>
<th>factor</th>
<th>BRE-AL</th>
<th>Placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>53</td>
<td>52</td>
</tr>
<tr>
<td>Age (46 ~69)</td>
<td>56.9</td>
<td>57.3</td>
</tr>
<tr>
<td>Body weight</td>
<td>68.7</td>
<td>68.3</td>
</tr>
</tbody>
</table>
Experimental design - human study

- Treat with BRE-AL:
  - 500 mg/capsule, 2 capsules/day, 12 weeks

- Factors: fat weight, ratio
  - Bioelectrical impedance analysis (BIA),
  - Dual-energy x-ray absorptiometry (DEXA),
  - Computed tomography (CT)

- Statistical analysis: SASV(version 9.4)
Results – Human study

BIA – Body fat ratio

Fig. Treatment effects of BRE-AL on fat ratio of post menopausal women
Results – Human study

DEXA – Body fat weight

Fig. Treatment effects of BRE-AL on abdominal fat weight of post menopausal women
Results – Human study

DEXA – total fat weight

Fig. Treatment effects of BRE-AL on total fat weight of postmenopausal women
CT – visceral fat

Fig. Treatment effects of BRE-AL on visceral fat area of postmenopausal women
Summary

< in vitro>
- BRE-AL treatment significantly decrease adipocyte differentiation and lipid accumulation.

< in vivo>
- Body weight gain, body fat weight, and adiposity index increased in the OVX group, but they significantly decreased in the groups supplemented with IF or BRE-AL.
- Serum triacylglyceride and leptin levels decreased in BRE-AL groups while serum adiponectin level significantly increased compared to that of the OVX group.
- The reduced fat weight may be explained by the changed gene expression or hormone levels.

< human study>
- Body fat in the BRE treated group was significantly lower than placebo group.
Discussion

- BRE-AL effectively reduced body fat weight in post menopausal model at 30 or 90 mg/kg BW though whole black rice extract showed possibility of fat weight reducing at 200 mg/kg BW (Jang et al., 2015).

- BRE-AL can be used in people who have been interested in natural foods to control obesity or body fat accumulation.

- Using BRE-AL can reduce extracting solvent volume, prevent environment, and make white rice as foods.

- The team has been working to register the extract(BRE-AL) as a healthy food.
The results suggest that black rice aleurone layer may be a useful food source to decrease obesity and its related diseases by modulating lipid metabolism in estrogen-deficiency model.
References

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- Jang HH, Park MY, Kim HW, Lee YM, Hwang KA, Park JH, Park DS, Kwon O. Black rice (Oryza sativa L.) extract attenuates hepatic steatosis in C57BL/6J mice fed a high-fat diet via fatty acid oxidation. Nutrition & Metabolism 2012;9:27-38
Thank you for your attention!