RESEARCH ARTICLE

Black Rice Extract Reduces Body Weight, Waist Circumference, Body Mass Index and Lipopolysaccharide in Obese Subjects: A Preliminary Study

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Abstract

BACKGROUND: The prevalence of obesity, or an excessive fat accumulation, is keep increasing. In obesity, inflammation can be induced by leaky gut due to the intestinal tight junction barrier dysfunction. Zonula occludens-1 (ZO-1) plays a role in developing intestinal tight junction barrier dysfunction and gut microbiota imbalance, thus promote the translocation of bacterial endotoxin characterized by lipopolysaccharide (LPS) into circulation. Black rice extract (BRE) has been known to have anti-inflammatory property. This study was conducted to investigate the effect of BRE on body weight (BW), waist circumference (WC), body mass index (BMI), ZO-1 and LPS of obese patients.

METHODS: Twenty-three male subjects were divided into non-obese group (NOG), obese group (COG) and BRE-obese group (BOG). Subjects in BOG received a daily dose of 5.6 g/day BRE for 4 weeks. BW, WC and BMI, serum ZO-1 and LPS were measured before and after treatment.

RESULTS: BRE was prepared successfully and free from microbial contamination. Treatment of BRE for 4 weeks reduce BW (95.40±5.78 *vs*. 94.59±6.00 kg, p=0.043), WC (109.25±3.55 *vs*. 107.50±3.46 cm, p=0.000) BMI (32.65±1.86 *vs*. 32.18±1.80, p=0.000) and LPS (222.27±38.63 *vs*. 131.63±9.70 ng/mL, p=0.020) of obese subjects. The pre-post ZO-1 levels in all groups were not significantly different (p>0.05).

CONCLUSION: Treatment of 5.6 gr BRE daily for four weeks can reduce BW, WC, BMI and serum LPS, but not serum ZO-1 in obese patients. Therefore, BRE may reduce inflammation in obesity.

KEYWORDS: black rice, obesity, BW, WC, BMI, LPS, ZO-1

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Introduction

Prevalence of obesity keep increasing rapidly, it was estimated that more than one billion people in the world are now living with obesity, nearly 880 million adults and 159 million children and adolescents aged 5-19 years, and about four million people die every year due to obesity and its comorbidities.(1-4) Obesity is an abnormal or excessive fat accumulation that may impair health due to an energy imbalance between calories consumed and calories expended.(5,6) Obesity is characterized by the increase of pro-inflammatory cytokines released from adipose tissue and the infiltration of leukocytes, especially macrophages, leading to chronic low-grade inflammation.(7,8)

Obesity has been associated with gut microbiota composition changes. One of the changes is an increase in Firmicutes and a decrease in Bacteroidetes, which will

contribute to the development of pro-inflammatory status in obesity through alteration in the intestinal barrier.(9) Zonula Occludens-1 (ZO-1) has been known to link tight junction proteins with the cytoskeleton and to provide integrity of the paracellular barrier, hence ZO-1 has been used as a biomarker of intestinal barrier integrity.(10,11) When the intestinal barrier was dysfunction, an endotoxin called lipopolysaccharide (LPS) could be transported into circulation. LPS has been reported to increased pro-inflammatory cytokines (12), therefore, the circulatory-transported LPS will cause metabolic endotoxemia and the production of pro-inflammatory cytokines leading to the development of chronic low-grade inflammation.(9)

Black rice is one variant of rice which has black pigment containing anthocyanins.(13) Compared with white rice, black rice has an abundance of phenolic compounds, which are associated with antioxidant activity. Black rice extract (BRE) was reported to have an anti-inflammatory effect on the splenocytes of a diabetes mellitus mouse model. (14) Another study also indicated that supplementation of BRE for 12 weeks had an effectiveness in reducing fat accumulation in postmenopausal women aged between 45 and 69 years.(15) Although the effects of BRE on oxidative stress and inflammation (16,17), hyperlipidaemia and hyperglycemia (18,19), body weight gain (20), lipid accumulation (21), and gut microbiota (22) have been elucidated, to our knowledge, the effect of BRE on intestinal barrier dysfunction and metabolic endotoxemia in subjects with obesity has not been clearly understood. Therefore, present study was conducted to investigate the effectiveness of BRE on ZO-1 and LPS in subjects with obesity.

Methods

Production of BRE Solution

From Toraja, South Sulawesi, 20 kg of Black rice (*Oryza sativa* L.) was obtained. The rice was milled into powder, macerated with 32 L of 70% ethanol, sonicated for 30 min, and left overnight. The next day, the solution was filtered, evaporated at 40°C, and dried at 60°C. Resulted paste was weighted, solubilized in sodium carboxymethylcellulose (Na-CMC), added with 0.5% citric acid to reach pH=3, and finally added with sorbitol to sweeten the solution.

Microbial Contamination Test

BRE solution was tested for possible contamination of microorganism with Total Plate Count (TPC) Analysis. Briefly, BRE was serial-diluted, poured and spread evenly on Plate Count Agar (PCA), then incubated in an incubator at 37°C for 24 hours. After incubation, the formed colonies were counted.

For *Staphylococcus aureus* and *Salmonella* sp. tests, BRE solution was serial-diluted, spread evenly on Baird-Parker Agar (BPA) for *S. aureus* while Xylose Lysine Deoxycholate (XLD) Agar for *Salmonella* sp. Then the agar was incubated in an incubator at 37°C for 24 hours. After incubation, the formed colonies were counted.

Subject Recruitment and Criteria

Male subjects with age of 18-35 years old were recruited during the period of April-March 2021 at Hasanuddin University Medical Research Center (HUMRC) and at Ibnu Sina Hospital. Subjects with history of smoking, strict diet; chronic metabolic disorders (diabetes mellitus, hypertension, systemic lupus erythematosus, and rheumatoid arthritis) were excluded. Prior to the enrolment, all subject was informed and asked for their willingness to participate by signing a written informed consent form. This research protocol was approved by the Ethics Committee of the Faculty of Medicine, Hasanuddin University, Makassar (No. 300/UN4.6.4.5.31/PP36/2020). This study has been registered at clinicaltrials.gov under the registration number NCT04827628.

Anthropometric Measurement

Body weight (BW) was measured in kilogram (kg), body height was measured in centimetre (cm), waist circumference (WC) was measured in the halfway between subjects' lowest rib and the top of the hipbone, Body Mass Index (BMI) was calculated as weight (kg) divided by height squared (m^2). BMI score was used to differentiate between normal weight (18.5–22.9), overweight (23–24.9), or obesity (≥25).

Subject Intervention and Sample Collection

Subjects were divided into 3 groups: non-obese group (NOG), obese group (OG), and BRE-treated obese group (BOG) for 4 weeks. Serum ZO-1 and LPS was conducted before and after treatment with BRE. After overnight fasting, 5 mL venous blood was drawn, left at room temperature for 15 minutes, then centrifuged at 3000 rpm for 15 min. Afterward, the serum was collected, aliquoted and stored at −80°C for Enzyme-linked Immunosorbent Assay (ELISA) quantifications.

ELISA for ZO-1 and LPS

Collected serum was used to determine ZO-1 and LPS levels using Human Tight Junction Protein 1 (ZO-1) ELISA Kit

(Cat No. MBS2605490; MyBioSource, San Diego, CA, USA) and Human Lipopolysaccharides (LPS) ELISA Kit (Cat No. MBS266722; MyBioSource). Both kits utilized the double antibody sandwich ELISA technique. Anti-Human ZO-1 monoclonal antibody or anti-Human LPS monoclonal antibody was the precoated antibody, while a biotinylated polyclonal antibody was used as the detection antibody. The TMB that was used as the substrate, was reacted to form a blue product and finally turns to yellow after addition of the stop solution. For obtaining optical density (OD), microplate reader was set at 450nm. ZO-1 ELISA kit could detect at the range of 1.56-100 ng/mL with sensitivity of 0.5 ng/mL, while LPS ELISA kit could detect at the range of 15.6-1,000 ng/mL with sensitivity of 5 ng/mL.

Results

The BRE solution in concentration of 93.33 mg/mL and total volume of 28 L was prepared successfully. For the microbial test results, TPC for BRE was 4.6 x 10^3 CFU/g, while *S. aureus* and *Salmonella* sp. counts were both negative per 0.1 g of sample.

Forty male subjects were included in the study. Based on the BMI, 15 subjects were non-obese (included in NOG) and 25 subjects were obese. The obese subjects were then divided randomly into 2 groups: 12 subjects in OG and 13 subjects in BOG. Subjects in BOG consumed 60 mL BRE solution containing 5.6 g BRE daily for 4 weeks.

However, not all subjects could complete the study, 8 subjects in NOG, 4 subjects in OG and 5 subjects in BOG were dropped out due to their health conditions during the Coronavirus Disease 2019 (COVID-19) pandemics. Therefore, in the end of the of the study there were 7 subjects in NOG, 8 subjects in OG, and 8 subjects in BOG that completed the study and assessments. All subjects of all groups had similar age (p=0.382, Kruskal Wallis), for NOG 21.60±0.61 years old, for OG 20.13±0.91 years old and for BOG 22.33±0.49 years old.

BRE reduced BW, WC and BMI

In the pre-treatment stage, subjects in OG and BOG had similar BW, WC and BMI, but higher than NOG. Treatment of BRE for 4 weeks could reduce significantly the BW, WC and BMI of obese subjects, as shown in the BOG (Table 1). The BW, WC and BMI of all groups were analysed further by calculating the pre-post differences (Δ) of each group (Figure 1). All ΔBW, ΔWC and ΔBMI showed significant differences between OG and BOG, suggesting that BRE could certainly reduce BW, WC and BMI of obese subjects.

BRE reduced LPS, but did not affect ZO-1

Similar to BW, WC and BMI, in the pre-treatment stage, subjects in OG and BOG had similar LPS level, but higher than NOG. Treatment of BRE for 4 weeks could also reduce significantly the LPS level of obese subjects, as shown in the BOG (Table 2). The ZO-1 level in NOG was higher than the one in OG and BOG. The pre-post ZO-1 levels in all groups were not significantly different.

Discussion

The current study showed that four weeks of BRE consumption can significantly reduce BW, WC, BMI and LPS, but not ZO-1 level. It has been widely reported that obesity is related with chronic inflammation, which is marked by LPS in the present study. The LPS are cellular wall components of gram-negative bacteria that contain a pathogen-associated molecular pattern, Lipid A, able to interact with the toll-like Receptor 4 via the myeloid differentiation primary response 88 protein. This interaction results in the activation of the pathway downstream and nuclear factor (NF)-κB translocation, thus increasing the gene transcription of cytokines such as tumor necrosis factor (TNF)-α, interleukin (IL)-1, and IL-6.(23) Normally, LPS concentrations are highest in the gut lumen and low or undetectable in the circulating plasma because LPS in the gut lumen do not penetrate the healthy intestinal epithelium.(24) BRE was known to contain high level

Table 1. Pre-post BW, WC and BMI of NOG, OG and BOG groups.

Parameter	NOG (n=7)			OG (n=8)			BOG (n=8)		
	Pre	Post	*p*-value	Pre	Post	*p*-value	Pre	Post	*p*-value
BW (kg)	60.39±3.04	60.33±3.03	0.103‡	99.83±5.47	99.99±5.42	0.135‡	95.40±5.78	94.59±6.00	0.043‡*
WC (cm)	77.71±2.83	77.76±2.85	0.180#	112.75±4.06	112.85±4.08	0.291#	109.25±3.55	107.50±3.46	0.000‡*
BMI	21.84±0.75	21.86±0.78	0.736‡	34.08±1.58	33.96±1.65	0.831#	32.65±1.86	32.18±1.80	0.000‡*

Data are presented in mean±SEM. ‡Paired-Samples T Test; #Wilcoxon Signed Rank Test; *significant with p<0.05.

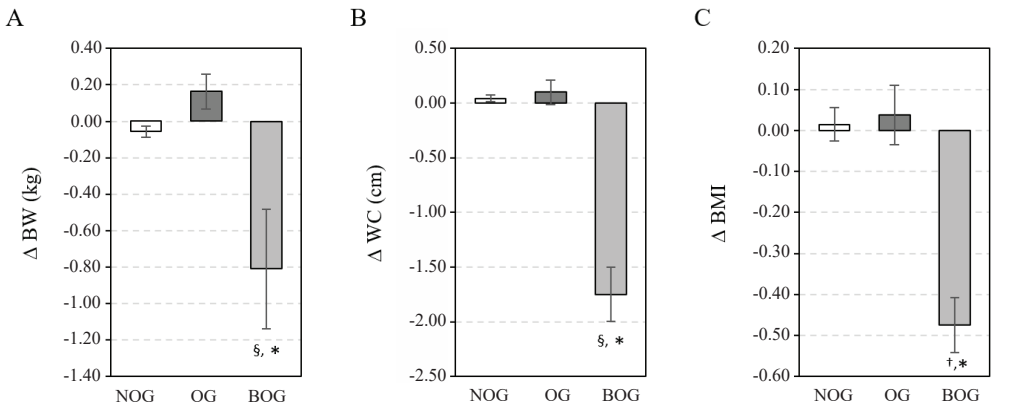

Figure 1. The Pre-post differences of BM, WC and BMI. Data of Table 1 was used to calculate the differences of pre-post of NOG, OG and BOG (mean±SEM). Δ: Pre-post difference. §Independent Samples T Test (compared with NOG); †Mann-Whitney Test (compared with NOG); *significant with $p<0.05$.

of anthocyanin. The anthocyanin in BRE, which has an anti-inflammatory effect, can modulate I-kappa-B-alpha (IκB-α) phosphorylation leading to lower expression of pro-inflammatory cytokines such as TNF-α, interferon (IFN)-γ, and ILs.(13)

It has been reported that pro-inflammatory cytokines regulated the tight junction protein ZO-1 expression.(10) Previous study reported also that high-fat diet feeding in mice could reduce the expression of ZO-1 in the jejunum. (25) In this study, the LPS level was reduced by BRE, however the ZO-1 level was not affected. Therefore, based on our present data, we suggested that the ZO-1 levels might not be detected well in the circulation. However, further larger cohort research is needed to clarify this issue.

Conclusion

Consumption of 5.6 gr BRE daily for four weeks can reduce BW, WC, BMI and serum LPS, but not serum ZO-1 in obese patients. Therefore, BRE may reduce inflammation in obesity.

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Authors Contribution

AM and AB were involved in the conceptualization of the study, preparation of methodology, and the investigation. AM prepared the study resources and drafted the original manuscript. NAT and A gave critical suggestions. AM and FS performed the data analysis, prepared the visualization of the data, as well as revised and edited the manuscript,

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Table 2. Pre-post ZO-1 and LPS levels of NOG, OG and BOG groups.

Parameter	NOG (n=7)			OG (n=8)			BOG (n=8)		
	Pre	Post	*p*- value	Pre	Post	*p*- value	Pre	Post	*p*- value
ZO-1 (ng/mL)	17.26±2.09	17.55±2.97	0.932‡	14.27±2.06	14.63±1.95	0.901‡	14.62±2.77	14.41±1.25	0.954‡
LPS (ng/mL)	149.00±20.83	139.82±14.35	0.778‡	214.26±41.48	206.04±25.58	1.000#	222.27±38.63	131.63±9.70	0.020‡,*

Data are presented in mean±SEM. ‡Paired-Samples T Test; #Wilcoxon Signed Rank Test; *significant with $p<0.05$.

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