Effect of black garlic on visceral fat, oxidative stress and insulin resistance in nonalcoholic fatty liver disease rats

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Abstract

Purpose – This paper aims to determine the effect of black garlic (BG) on visceral fat, oxidative stress and insulin resistance (IR) compared with metformin and vitamin E in nonalcoholic fatty liver disease (NAFLD) rats.

Design/methodology/approach – A randomized post-test only design with control group was used in this study. Rats were given high-fat fructose diet enriched with 1.25% cholesterol and 0.5% cholic acid for eight weeks to induce NALFD condition. The administration of BG dose of 450 mg/200 gBW, 900 mg/200 gBW and 1350 mg/200 gBW with a comparative control of 45 mg/200 gBW of metformin and vitamin E of 9 IU/200 gBW were given for four weeks via oral gavage to reduce visceral fat, oxidative stress and improve IR. Statistical analyses were performed to examine differences between groups with one-way analysis of variance and nonparametrics test.

Findings – Rats given with three different doses of BG for four weeks did not reduce body weight from 244 ± 4.4 to 284 ± 4.6 g, 242 ± 2.5 to 272 ± 3.1 g and 240 ± 2.4 to 270 ± 3.6 g, respectively, but significantly reduced visceral fat ($p = 0.001$) on BG groups with 3.7 ± 1.3, 2.7 ± 0.7 and 1.8 ± 0.6 g, respectively. BG improved oxidative stress ($p = 0.001$) with malondialdehyde level 5.1 ± 0.2, 3.0 ± 0.06 and 2.3 ± 0.06 ng/mL, respectively, but did not better than vitamin E group 1 ± 0.03 ng/mL. Significant ($p = 0.001$) improvement on

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insulin resistance with homeostatic model assessment IR in BG groups were 5.3 ± 0.1, 4.4 ± 0.1 and 4 ± 0.1, respectively, but not as good as metformin group 3.7 ± 0.1.

Research limitations/implications – Based on the experiment, there are several limitations including small sample size, performed on animal models in a relatively short time, did not examine organosulfurs compound (OSC) content of BG specifically and OSC affects metabolism in NAFLD remains unclear and will require further investigation.

Practical implications – BG is a functional food made from heated fresh garlic owing to the Maillard reaction and the organosulfur compounds as antioxidants. The higher the dose of BG, the greater the improvement in visceral fat, oxidative stress and IR in model NAFLD rats.

Social implications – NAFLD is a liver disorder caused by excessive fat and energy intake, the treatment strategies among others through diet modification.

Originality/value – In model NAFLD rats, BG administration improved NALFD markers but did not better rather than the metformin and vitamin E result.

Keywords Oxidative stress, Visceral fat, NAFLD, Black garlic, Insulin resistance

Paper type Research paper

Introduction

Nonalcoholic fatty liver disease (NAFLD) is a liver disorder that is histologically similar to alcoholic fatty liver disease but occurs in patients who do not consume alcohol or with consumption limit of >30 g/day in men and >20 g/day in women (Riani *et al.*, 2017; Chalasani *et al.*, 2012). NAFLD is caused by an abnormality in fat metabolism resulting in the accumulation of fat in the liver cells (Riani *et al.*, 2017). Several studies have shown that NAFLD and non-alcoholic steato hepatitis (NASH) prevalence rates have risen from 17% to 33% for NAFLD and 5.7% to 17% for NASH. The amount of fatty liver patients has increased year by year at Dr Kariadi Hospital Semarang (Purnomo *et al.*, 2018). In a subgroup of patients, the inflammatory response of adipose tissue results in the release of several proinflammatory and profibrotic cytokines, which together with steatosis induce oxidative stress and activate fibrogenic hepatic stellate cells to cause NASH, hepatic fibrosis and cirrhosis (Smith and Adams, 2011).

The relation between visceral fat, oxidative stress and resistance to insulin has attracted attention. It was unclear or incomplete to explain this connection, mostly focusing on an individual detail of a specific pathway or pathogenesis (Purnomo *et al.*, 2018). High visceral fat deposition is one of the risk factors for the incidence of severe NAFLD (Riani *et al.*, 2017). In obesity, the deposition of visceral fat will result in the expansion of adipose tissues, which becomes a site for the accumulation of tumor necrosis factor-α stimulates proinflammatory cytokines, causing inflammatory reaction and fibrosis. In addition, Kupffer cells are activated to produce C-reactive proteins and prothrombotic molecules, resulting in the oxidation of fatty acids in the liver cells, which are the source reactive oxygen species (ROS), and then liver tissue damage as described in the second event of the two-hit hypothesis (Zivkovic *et al.*, 2007). NAFLD is strongly associated with insulin resistance (IR) in the liver and adipose tissue and reduced insulin sensitivity throughout the body (Utzschneider and Kahn, 2006). IR is not only involved in hepatic steatosis but also in the development of oxidative stress by reducing mitochondrial beta oxidation leading to the activation of other oxidation pathways, thereby contributing to increased ROS level (Leach *et al.*, 2014).

Current treatment strategies for patients with NAFLD include the identification and management of associated metabolic diseases through weight loss, exercise and pharmacotherapy and administration of hepatoprotective drugs such as antioxidants to protect the liver from oxidative stress (Jurnalis *et al.*, 2014). The pharmacological approach of NAFLD is caused from failure to change lifestyle and eating habits. Therefore, by

assessing the component of NAFLD markers, vitamin E is emerging as a promising therapeutic approach in NAFLD patients (Hadi *et al.*, 2018).

The mechanism of the hepatoprotective of functional foods is related to their antioxidant and anti-inflammatory effects. Garlic (*Allium sativum L.*) is used as a spice, flavoring and herbal medicine (Suleria *et al.*, 2015). The consumption of garlic as a functional food is increasing worldwide owing to its great opportunity for effectiveness, fewer side effects and relatively low cost (Suleria *et al.*, 2015). Naji *et al.* tested the difference in the antioxidant capacity of single clove garlic (SCG) and multi-clove garlic (MCG) and found that the antioxidant capacity at SGC was three times greater than that of MCG (Naji *et al.*, 2017). In addition to the scavenging activity, it has been shown that single clove in SCG has significant ability to donate electrons to free reactive radicals, switching them into less reactive species and blocking free chain reactions more than MCG.

Black garlic (BG) is a functional food made from heating garlic controlled by temperature and humidity for approximately a month (Nur Rochmah, 2017). BG appears wrinkled, light owing to reduced water content and odorless and taste than fresh garlic, so it can be consumed daily without the spicy taste and body odor. The garlic turns into black because of a nonenzymatic browning reaction, known as the Maillard reaction (Nur Rochmah, 2017). BG has stronger antibacterial properties and antioxidant content two times higher than fresh garlic because it contains organosulfurs compound (OSC) (Gasyiya *et al.*, 2018). BG contains water-soluble and fat-soluble OSC. Diallyl sulfide (DAS), dialyl disulfide, dialyl trisulfide (DATS) and dialyl tetrasulfide are fat-soluble compounds, whereas S-allylocysteine (SAC) and S-allylmercaptocysteine are specific water-soluble compounds. This allicin-derived organosulfur compound contributes to antioxidant property (Jeong *et al.*, 2016).

According to Kim *et al.* (2011), BG may protect against liver damage caused by alcohol-induced liver injury in animals experimental (). Chen *et al.* (2014) used methanoic extract black garlic and showed the supplementation could improve the health profile of animal experiment related to metabolic syndrome owing to high fat diet, and Liu *et al.* (2006) reported that garlic and its derivatives (diallyl trisulfide) may have a regulatory effect on glycemic control in diabetic mice. It was reported that rational consumption of garlic had no major side effects (Ghorbani, 2013); thus, BG can be used as an alternative therapy for the treatment of NAFLD. This study aimed to determine the effects of BG on the visceral fat, oxidative stress and IR compared with metformin and vitamin E in NAFLD rats. The findings contribute to a greater understanding of the mechanism of BG as functional food that can be a hepatoprotective agent against NAFLD markers.

Materials and methods

Black garlic preparation

BG was used as a treatment material based on the results of a research in the field of biotechnology by the Agency for the Assessment and Application of Technology, Tangerang, Indonesia. Its commercial name is Natural Black Garlic Lanang which used SCG and produced at a temperature of 70°C and humidity of 90%; BG is made using far infrared technology and gradual heating for 26 days, and then it is placed in an incubator to maintain temperature and humidity stability. Natural Black Garlic Lanang has been tested for acute toxicity and lethal dose at 50% by the Laboratory Toxicity and Safety Materials, School of Pharmacy Bandung Institute of Technology, Indonesia.

Animal experimental design

This study used a randomized post-test only design with control group design. A total of 42 male Sprague Dawley (*Rattus novergicus*) rats (150–200 g) were obtained in the Animal

Laboratory Study Center of Food and Nutrition Laboratory, Gadjah Mada University, Yogyakarta, Indonesia. The rats were housed individually under a 12 h light or dark cycle with temperature (23°C) and humidity (50%). Rats were given a standard diet of 15 g/day, and *ad libitum* water was given until the end of the study. After a week of acclimatization, the rats were randomly defined into seven groups ($n = 6$ rats). The rats in each group were fed as follows: healthy group given standard diet; NAFLD control group; NAFLD with metformin treatment group; NAFLD with vitamin E treatment group; NAFLD with BG 450, 900 and 1350 treatment group, respectively. The study design was approved and reviewed by The Ethics Commission of the Faculty of Medicine, Diponegoro University, and Dr Kariadi Hospital Semarang, Indonesia with certificate ethics no. 11/EC/H/FK-UNDIP/III/2020. This study was part of grand study entitled "Pre-clinical benefits of BG in NAFLD."

Induction and feeding of the rats

The standard diet used was commercial Comfeed AD II (Japfa Comfeed Indonesia Ltd, Indonesia) containing 15% crude protein, 3–7% crude fat, 12% moisture content, 6% crude fiber, 7% ash, 0.5% phosphorus, 0.9–1.1% calcium and vitamins (5.24 kcal/g, 11.1% fat). NAFLD rats were fed a high-fat and high-fructose diet (HFFD) enriched with 1.25% cholesterol and 0.5% cholic acid for eight weeks to six groups. The composition of HFFD was as follows: 177.5 g/kg of pork oil, 172.8 g/kg of fructose, 19.87 kJ/g of energy, 20% protein, 35% carbohydrates and 45% fat. After administering HFFD for eight weeks, which is supposed to induce steatohepatitis, fatty liver disease in rats can be seen. NAFLD rats were classified by using the Lee index (obese index) measure of >300 as classified fatty liver if they were obese.

Metformin dose 45 mg/200g BW and vitamin E dose 9 IU/200g BW as comparative groups and BG dose 450 mg/200g BW, 900 mg/200g BW and 1350 mg/200g BW were weighed and crushed, then dissolved in distilled water and given via oral gavage for four weeks in the morning before feeding the rats for the day. The study lasted for a week of acclimatization, eight weeks of HFFD induction and four weeks of metormin, vitamin E and BG administration. The liver histopathology, dysfunctional liver enzymes, lipid profile and other markers will be published in other studies.

Biochemical analysis, rat termination and adipose tissue retrieval

Rats were weighed weekly during the experiment using an electronic analytical balance (Dor Yang JA-P, Shanghai, China). At the end of the experimental period, the rats were fasted, and blood was drawn through the orbital vein using a capillary tube. Blood samples were centrifuged ($1000 \times g$, 5 min), and the serum was separated and stored at −80°C for later analysis. The level of fasting blood glucose (FBG) was determined spectrophotometrically (Spectroquant Prove 300, Germany) according to the instructions kits (DiaSys, Germany). The levels of fasting insulin and malondialdehyde (MDA) in blood serum was determined using sandwich enzyme-linked immunosorbent assay (ELISA) technique according to the instructions kits (Wuhan Fine Biotech Co., Ltd.) and absorbance was measured with an ELISA reader (BioRad, Japan) at 450 nm. Homeostatic model assessment insulin resistance (HOMA-IR) was measured by [insulin (in μIU/ml) × glucose (in mg/dl)/405].

The rats were terminated by intraperitoneal injection of a mixture of ketamine 75 mg/kgBW and xylazine 5 mg/kgBW, and surgery was performed at the end of the experiment. An incision was made on the stomach and chest of the anesthetized rat. Visceral fat content was measured using the weight of the intra-abdominal fat (white adipose tissue) that was obtained from the sum of the weight of the mesenteric fat, retroperitoneal fat and epididymal

fat, which were weighed using an electronic analytical balance (Dor Yang JA-P, Shanghai, China).

Statistical analysis

Research data are expressed as mean and standard deviation. Statistical analysis was performed using the Kruskal–Wallis test for nonparametric data, followed by the Mann–Whitney comparison test and one-way analysis of variance (ANOVA) between groups for normally distributed data followed by least significant difference (LSD), and Tamhanee multiple comparison test was performed thereafter. The level of significance was set at $p < 0.05$. Statistical analyses were performed using computer software.

Results

Table 1 shows the ratio of BW before and after administration of HFFD and BG for four weeks. After the administration of HFFD, based on the post-hoc LSD test (Table 1), a significant difference was found between the healthy group and all other groups ($p = 0.001$). Six groups fed with HFFD enriched with 1.25% cholesterol and 0.5% cholic acid became obese rats (Lee index > 300, $p = 0.014$) (Table 2). After four weeks of BG treatment, there was an increase in the BW in all groups, and this was related to the growth of rats. Table 3 shows NAFLD markers including visceral fat, oxidative stress and IR. The administration of BG for four weeks affected the weight of the visceral fat in BG groups ($p = 0.001$). MDA levels decreased in the BG groups in comparison with the control group. The NAFLD group had ten times greater MDA levels than the healthy group because of HFFD induction, and administering BG for four weeks improved oxidative stress but did not do better than Vitamin E comparative group. Significant differences show between groups in terms of the

Treatment groups	Body weight (g) Pre	Post	Δ
Control groups			
Healthy	208 ± 3.6	238 ± 4.3[b]	29.3 ± 2.2[c]
NAFLD	242 ± 2.2[a]	305 ± 2.5	62.6 ± 1.6[c]
Comparative groups			
Metformin	240 ± 3.4[a]	271 ± 3.4[b]	31.1 ± 0.9[c]
Vitamin E	242 ± 2.9[a]	273 ± 4[b]	30.6 ± 1.7[c]
BG groups			
BG 450	244 ± 4.4[a]	284 ± 4.6[b]	40.3 ± 1.8[c]
BG 900	242 ± 2.5[a]	272 ± 3.1[b]	30.3 ± 2.7[c]
BG 1350	240 ± 2.4[a]	270 ± 3.6[b]	30.3 ± 1.5[c]
p value	0.001*	0.004*	0.001*

Notes: Values are mean ± SD ($n = 6$); *Statistically significant; [a,b]bearing different superscripts in a same column differ significantly (used Kruskal–Wallis with Mann–Whitney post-hoc test, $p < 0.5$); [c]bearing different superscripts in a same row differ significantly (nonparametric Wilcoxon test); Pre: before intervention, rats fed with high-fat and high-fructose diet (HFFD) enriched with 1.25% cholesterol and 0.5% cholic acid to induce NAFLD for eight weeks; Post: after intervention, rats fed with standard diet and treatments for four weeks, BG: black garlic; Δ: difference between Pre and Post; Control groups: no treatment, Comparative groups: treatment with metformin 45 mg/200 gBW and vitamin E 9 IU/200 gBW, BG groups: treatment with BG 450 mg/200 gBW, 900 mg/200 gBW and 1350 mg/200 gBW, respectively

Table 1. Effect of black garlic treatments in body weight

Treatment groups	Obese index (>300) Pre	Post	Δ
Control groups			
Healthy	286 ± 2.8	287 ± 3.4[b]	1.5 ± 1.5
NAFLD	319 ± 5.7[a]	332 ± 3.2	13.2 ± 6.7[c]
Comparative groups			
Metformin	320 ± 7.8[a]	287 ± 7.1[b]	−32.9 ± 2.6[c]
Vitamin E	319 ± 2.5[a]	287 ± 2.3[b]	−32.8 ± 1.1[c]
BG groups			
BG 450	321 ± 6.7[a]	302 ± 4.9[b]	−18.3 ± 3.6[c]
BG 900	318 ± 4.1[a]	286 ± 3.3[b]	−32 ± 1c
BG 1350	318 ± 8.9[a]	286 ± 3.3[b]	−34.2 ± 4.1[c]
p value	0.014*	0.001*	0.003*

Notes: Values are mean ± SD ($n = 6$); *Statistically significant; [a,b]bearing different superscripts in a same column differ significantly (used Kruskal–Wallis with Mann–Whitney post-hoc test, $p < 0.5$); [c]bearing different superscripts in a same row differ significantly (nonparametric Wilcoxon test); Pre: before intervention, rats fed with high-fat and high-fructose diet (HFFD) enriched with 1.25% cholesterol and 0.5% cholic acid to induce NAFLD for eight weeks; Post: after intervention, rats fed with standard diet and treatments for four weeks, Δ: difference between Pre and Post; BG: black garlic, Control groups: no treatment, Comparative groups: treatment with metformin 45 mg/200 gBW and vitamin E 9 IU/200 gBW, BG groups: treatment with BG 450 mg/200 gBW, 900 mg/200 gBW and 1350 mg/200 gBW, respectively

Table 2.
Effect of black garlic treatments in obese index

Treatment groups	NAFLD markers Visceral fat (g)	MDA (ng/mL)	FBG (mg/dL)	Insulin (pg/mL)	HOMA-IR
Control groups					
Healthy	2.1 ± 0.9	0.9 ± 0.02	77.9 ± 2.3	17.2 ± 0.2	3.3 ± 0.1
NAFLD	8.4 ± 1.6[a]	9.6 ± 0.4	186 ± 2.2	12.2 ± 0.1	5.6 ± 0.1
Comparative groups					
Metformin	1.7 ± 0.6	1.5 ± 0.01	96.9 ± 1.80	15.6 ± 0.4	3.7 ± 0.1[e]
Vitamin E	1.7 ± 0.5	1 ± 0.03[b]	95 ± 1.5	16.5 ± 0.1[d]	3.8 ± 0.1
BG groups					
BG 450	3.7 ± 1.3	5.1 ± 0.2	147 ± 4.5	14.8 ± 0.08	5.3 ± 0.1
BG 900	2.7 ± 0.7	3 ± 0.06	118 ± 2.7[c]	15.3 ± 0.1	4.4 ± 0.1[e]
BG 1350	1.8 ± 0.6[a]	2.3 ± 0.06[b]	101 ± 3.6	16.1 ± 0.1[d]	4 ± 0.1[e]
p value	0.001*	0.001*	0.001*	0.001*	0.001*

Notes: Values are mean ± SD ($n = 6$); *Statistically significant; [a–e]bearing different superscripts in a same column differ significantly (used one-way ANOVA with LSD post-hoc test, $p < 0.5$); BG: black garlic; Control groups: no treatment; Comparative groups: treatment with metformin 45 mg/200 gBW and vitamin E 9 IU/200 gBW, BG groups: treatment with BG 450 mg/200 gBW, 900 mg/200 gBW and 1350 mg/200 gBW, respectively, NAFLD: nonalcoholic fatty liver disease; MDA: malondialdehyde; FBG: fasting blood glucose; HOMA-IR: homeostatic model assessment insulin resistance

Table 3.
Effect of black garlic treatments in NAFLD markers

levels of FBG, fasting insulin and HOMA-IR in all the groups ($p = 0.001$). In HOMA-IR, metformin had the better result rather than other groups.

Discussion

After eight weeks of HFFD enriched with 1.25% cholesterol and 0.5% cholic acid induction, there was an increase in liver-related parameters such as obesity index, visceral fat, oxidative stress status and IR. Similarly, along previous study, hypercholesterolemic diet for 60 days was able to develop NAFLD characteristics as a result of histopathological changes demonstrated by extensive steatosis, presenting liver degeneration with inflammatory cells (Carreira dos Santos *et al.*, 2019).

Fructose increases the deposition of liver fat rapidly and fat acts on the deposition of body fat and fat stores, thereby increasing BW and visceral fat. Increased prevalence of obesity, metabolic syndrome and NAFLD is associated with excess energy intake, with consumption of processed and fast food followed by intake of sugary drinks high in fructose (Cho *et al.*, 2017). The consumption of fructose has now become a lifestyle, increasing the incidence of obesity and associated complications (Fakhoury-Sayegh *et al.*, 2015), but not only fructose, Emamat *et al.*'s (2019) result has shown that galactose intake was significantly associated with higher risk of NAFLD. Diet modification and weight loss are considered important components of NAFLD treatment (Lee *et al.*, 2011).

In this study, the antiobesity activity of BG in HFFD rats was evaluated by measuring the BW, obesity index and visceral fat. The main cause of NAFLD in humans is through the accumulation of lipids in that organ (Gomes *et al.*, 2020). BG administration for four weeks did not reduce BW but reduce obesity index and visceral fat. This is owing to the reduced size and number of adipocyte cells resulting in a decrease in fat tissue mass which is in line with the slowing of the weight gain and the rise of rat length. Similarly, Kang *et al.* (2008) reported that consumption of 3% raw garlic and feed containing BG did not affect the BW of rats fed with 1% cholesterol. When high fat is consumed in excess and chronically, both types of saturated fatty acid and unsaturated fatty acid can produce deleterious effects on the serum lipid profile and abdominal fat accumulation, also increasing the TG/HDL-c ratio (Rocha-Gomes *et al.*, 2020).

The antiobesity effect of garlic may be explained by its thermogenic properties, and this is confirmed by Ince *et al.* (2000), who found that garlic and its derivatives increased oxygen consumption. SAMC can lower serum levels of free fatty acid, which can cause initial pathological changes during NAFLD and significantly decrease lipid accumulation in hepatocytes (Xiao *et al.*, 2013). By inhibiting lipogenesis and regulating lipid metabolism, OSC such as SAC and SAMC are thought to have a certain beneficial effect on obesity (Chang *et al.*, 2017). Another OSC found in BG, such as ajoene and tiacremonone compounds, have shown antiobesity effects through the inhibition of adipocyte differentiation and fatty lipid catabolism of activated protein kinase activation (Lee *et al.*, 2016).

Amor *et al.* (2019) revealed the antioxidant properties of aged black garlic by altering the gene expression of insulin sensitivity and fat metabolism related markers in adipose tissue and by increasing the expression of hypothalamus anorexigenic neuropeptides. Ha *et al.* (2015) showed that the distinctive taste and aroma of BG impacted the rat's appetite, referring to a decline in food consumption and weight loss. These theories are confirmed by adipose hypertrophy owing to the antiobesity effect of BG may be related to the inhibitory effect of OSC on adipocyte differentiation.

Consumption of foods high in fat and energy dense has been shown in experimental models to cause increased oxidative stress (Lasker *et al.*, 2019). The "two-hit hypothesis" theory suggests that fat accumulation and oxidative stress are responsible variables and

major components of NAFLD pathogenesis (Riani *et al.*, 2017). Increased levels of MDA in the NAFLD group suggested a disruption of the tissue antioxidant balance, causing cellular and molecular damage characterized by weakening the liver enzyme antioxidant system and increasing lipid peroxidation products in the liver and plasma (Auberval *et al.*, 2014), also playing an important role in the development of the disease spectrum from NAFLD to NASH (Ji *et al.*, 2014).

In contrast to fresh garlic, improvements in physicochemical properties have been the key factor in increasing the bioactivity of BG (Kimura *et al.*, 2017; Tsai *et al.*, 2019). The high antioxidant capacity, polyphenols, amino acids and organosulfur responsible for the Maillard reaction are not found in fresh garlic (Choi *et al.*, 2014; Sunanta *et al.*, 2020). The antioxidants in OSC found in garlic counteract free radicals by inducing detoxification enzymes with modified redox status (Colín-González and Santamaría, 2017). Simultaneously, SAC contains a thiol group that is responsible for its antioxidant capacity, as this nucleophile can easily donate its protons to electrophilic species to neutralize or make pro-oxidants less reactive (Nasr and Saleh, 2014).

Organosulfur compounds such as DATS are the most effective components of garlic to enhance the detoxification system (Oktari *et al.*, 2020), whereas DAS and its metabolite products can repair drug-induced liver damage (Garlic *et al.*, 2020). The variation between organosulfur compounds in the antioxidant system is related with the relative stability and reactivity of the carbon bonds (Colín-González and Santamaría, 2017). By improving oxidative stress status and suppressing the inflammatory response through CYP2E1 expression, SAMC prevents the production of early stage NAFLD and protects against NASH degree progression. This explains the protective effect of OSC that are found in BG by fat metabolism against oxidative stress (Xiao *et al.*, 2013).

Liver plays important role in glucose and insulin metabolism. HFFD induces an increase in visceral adipocytes, which initiates IR in the liver (Fakhoury-Sayegh *et al.*, 2015) and is one of the main pathophysiologies in the development of NAFLD (Pasarín *et al.*, 2011). Excessive intake without balanced energy expenditure increase mitochondrial NADH (mNADH) and ROS in the citric acid cycle. Excessive development of ROS can reduce the function of pancreatic β-cells and other cells (Amor *et al.*, 2019), whereas hyperglycemia will cause ROS signals to promote glucose-induced insulin secretion (Takahashi *et al.*, 2012).

BG can improve IR in the presence of increased lipogenesis by reducing the activation of enzymes involved in adipose formation in the liver and increasing adiponectin levels (Sangouni *et al.*, 2020), through the antioxidant capacity of OSC contained in BG (Atkin *et al.*, 2016). IR induced by increased pancreatic insulin secretion from β-cells or by indirect production of gastrointestinal hormones is associated with pancreatic insulin secretion, bound insulin release or increased insulin sensitivity. Organosulfur such as allicin can increase serum insulin by fighting insulin-inactivating sulfhydryl compounds, for example, cysteine prevents inactivation of insulin (Capasso, 2013). Liu *et al.* (2006) reported the indirect role of other OSC in improving glycemic control through reduced peripheral concentrations of nonesterified fatty acid (NEFA). The increased concentration of NEFA in the plasma is the main cause of IR and can reduce insulin secretion.

Another possible mechanism is that this study proves that BG has a hypoglycemic effect, which is marked by an improvement in IR in the intervention group owing to the active organosulfur compounds obtained from the heating process that destroys active, volatile and unstable ingredients (Qiu *et al.*, 2020). The total content of phenolic acid in BG increased by around 4.6–7.8-fold compared to fresh garlic (Kim *et al.*, 2013). Nikkhah-Bodaghi *et al.*'s (2019) study has shown that polyphenols are very heterogenic hydrosoluble compounds that could interfere with different ways possibly involved in the pathogenesis of NAFLD.

According to preclinical evidence, polyphenols could protect from hepatic fat accumulation by modulating insulin sensitivity and glucose metabolism, inhibiting lipogenic enzymes and increasing lypolytic enzymes and fatty acid β-oxidation.

The results of this study suggest that consumption of BG can be considered as one of the treatment strategy for NAFLD as a safe and effective functional food. Based on the experiment, there are several limitations including small sample size, performed on animal models in a relatively short time, did not examine OSC content of BG specifically and OSC affects metabolism in NAFLD remains unclear and will require further investigation. This study showed the need for a new perspective on the use of BG in liver pathophysiological management to prevent NAFLD.

Conclusion

BG administration with a dose of 450 mg/200 gBW, 900 mg/200 gBW and 1350 mg/200 gBW for four weeks reduced visceral fat, reduced malondialdehyde level as marker of oxidative stress and improve IR but did not do better than pharmacologically used metformin and vitamin E. BG has an important role in NAFLD parameters through the antioxidant effect of OSC. In humans, long-term consumption is required to obtain similar protection against fatty liver. Further work is needed to investigate the active ingredients in BG and mechanism OSC against NAFLD.

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