Effect of Single Clove Black Garlic Extract on Lipid Accumulation During Adipocyte Differentiation Using 3T3-L1 Cell Line

Efri Mardawati, Erin Nur Fitriani, Desi Ayu Sundari, Tenny Putri Wikayani, Nurul Qomarilla, Nova Rachmadona, Eko Fuji Ariyanto, Roni Kastaman, Totok Pujianto, and Devi Maulida Rahmah

Abstract Obesity, defined as an excessive adipose tissue mass, is a major factor in increasing the risk of serious diseases such as heart disease, hypertension, and diabetes. Obesity is associated with the expansion of adipose tissue by excessive dietary fat intake, which results in adipocyte hyperplasia and hypertrophy. Thus, inhibiting adipocyte differentiation and accumulation of lipids are important targets for preventing obesity. As the mechanism of single clove black garlic (SCBG) extract affecting lipid metabolism in adipocytes remains unclear, this study aimed to evaluate the effect of SCBG extract on lipid metabolism in mature 3T3-L1 adipocytes. The analysis revealed that SCBG extract contained 23.15 mg/g of polyphenol and 9.75 mg/g of flavonoid compounds. SCBG extract had stronger capacities to scavenge α,α-diphenyl-β-picrylhydrazyl (DPPH) than fresh single clove garlic (FSCG) with half-maximal inhibitory concentration (IC50) values of 0.602 mg/mL. The treatment of SCBG extracts at a concentration of 2.5–7.5 mg/mL had a cytotoxic effect that reduced cell viability. However, there was no significant difference between the

E. Mardawati (✉) · E. N. Fitriani · D. A. Sundari · R. Kastaman · T. Pujianto · D. M. Rahmah
Department of Agroindustrial Technology, Faculty of Agroindustrial Technology, Universitas Padjadjaran, Jatinangor 45363, Indonesia
e-mail: efri.mardawati@unpad.ac.id

E. Mardawati · N. Rachmadona · T. Pujianto · D. M. Rahmah
Research Collaboration Center for Biomass and Biorefinery Between BRIN and Universitas Padjadjaran, Jatinangor 45363, Indonesia

T. P. Wikayani · N. Qomarilla
Cell Culture Laboratory, Faculty of Medicine, Universitas Padjadjaran, Jl. Eckyman No. 38, Bandung 40161, Indonesia

N. Rachmadona
Department of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Padjadjaran, Jatinangor 45363, Indonesia

E. F. Ariyanto
Division of Biochemistry and Molecular Biology, Department of Biomedical Sciences, Faculty of Medicine, Universitas Padjadjaran, Jatinangor 45363, Indonesia

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concentration of extract to the cell viability of adipocytes ($p < 0.05$). Furthermore, SCBG extract at 2.5 and 5 significantly reduced lipid accumulation ($p < 0.05$), and 7.5 mg/mL significantly reduced lipid accumulation ($p < 0.01$) compared to cell control indicating potential in anti-obesity effect.

Keywords Black garlic · Phenolic · Flavonoid · Obesity · Lipid accumulation · 3T3-L1 C

1 Introduction

Obesity is a chronic, systemic disease defined as a pathologically increased fat mass associated with an increased health risk (Bischoff and Schweinlin 2020). About 35 percent of the adult population worldwide is overweight and obese, according to a World Health Organization (WHO) scientific survey (WHO 2018). Obesity might increase the risk of developing chronic diseases such as diabetes, cardiovascular, musculoskeletal disorders, and behavioral disorders (Chooi et al. 2019). Obesity is an abnormal or excessive fat accumulation in adipose tissue (Torres-Villarreal et al. 2019). Fat accumulation in adipose tissue involves two metabolic processes: an increase in lipid size in adipocytes (hypertrophy) and an increase in the number of adipose cells (hyperplasia). The process of differentiation of preadipocytes into mature adipocytes is also called the adipogenesis process (Bahmad et al. 2020). 3T3-L1 cells are a cell line used widely as a cell model in learning to control molecular adipogenesis and are associated with obesity (Romao and Guan 2015).

The 3T3-L1 cell line is beneficial in identifying molecular markers, transcription factors (TFs), and various interactions that occur in adipogenesis (Zebisch et al. 2012). The amount of differentiation on the 3T3-L1 cell can be seen from the accumulation of lipids after cells are given several pro-differentiating agents. These agents include insulin, dexamethasone (DEX), and 1-methyl-3-isobutyl xanthine (IBMX), which can increase intracellular cAMP levels through the presence of fetal bovine serum (FBS) (Morrison and McGee 2015). 3T3-L1 cells have been used extensively to evaluate the effects of compounds or nutrients on adipogenesis, to establish the molecular mechanisms underlying adipogenesis, and to evaluate the potential applications of various compounds and nutrients in the treatment of obesity (Kang et al. 2016). In particular, compounds such as quercetin (Eseberri et al. 2015) and resveratrol (Chang et al. 2016) are known to inhibit adipogenesis in 3T3-L1 adipocytes.

Garlic *(Allium Sativum Linn)* is a plant considered to have a pharmacological effect that can be useful in various treatments, including obesity (Batiha et al. 2020). The organosulfur compounds contained in garlic have been shown to have various biological activities. The four most critical organosulfur compounds, which are considered to be the main biological agents, are diallyl sulfide (DAS), diallyl disulfide (DADS), diallyl trisulfide (DATS), and allyl-methyl trisulfide (Yang et al. 2018). A study by Li et al. (2017) evaluated the effects of a garlic compound (DATS) on pre-adipocytes

3T3-L1 and the mechanisms involved. The test results indicated that the administration of (20–75 μM) DATS could inhibit CCAAT or enhancer-binding protein (C/EBP α and β). Proliferator-activated receptor (PPAR) c mRNA that causes decreased expression of fatty acid synthase (FAS) and lipid accumulation in 3T3-L1 cells suggests that DATS compounds may inhibit the differentiation of preadipocyte cells 3T3-L1 becomes an adipocyte. Garlic is therefore considered to be useful for the prevention of obesity.

Black garlic is a derivative of garlic products obtained from the fermentation process for a while (1–2 months) at a controlled high temperature (60–90 °C) with high humidity (80–90%) (Angeles et al. 2016). Compared to fresh garlic, black garlic does not have a distinctive taste and a sharp scent that customers are less interested in due to the reduced content of allicin compounds that are unstable at high temperatures. Allicin is converted during fermentation into more stable compounds such as S-allyl cysteine (SAC), bioactive alkaloids, polyphenols, and flavonoids known to be antioxidants (Kimura et al. 2017).

Currently, research into black garlic products is still based on the form of multi-clove garlic, and research into the potential of single-clove garlic is still limited. In research by Chen et al. (2019), a comparison was made with the bioactive content of 4 varieties of garlic, including multi-clove and single-clove garlic, and the results of this study showed that the content of bioactive compounds in single-clove garlic was higher compared to multi-clove garlic. Considering the potential of single-clove garlic varieties, currently evaluating the potential of black garlic with single-clove garlic as raw materials against 3T3-L1 anti-obesity is still very limited. In this study, we examine the effect of single clove black garlic (SCBG) extract on lipid accumulation in the adipocyte differentiation process using 3T3-L1 cell lines as a reference for the potential use of SCBG in the treatment of obesity.

2 Materials and Method

2.1 Preparation of Single Clove Black Garlic (SCBG) Extract

Single-clove garlic was manufactured for 21 days. SCBG at 1 kg was homogenized and extracted with 70% methanol (MeOH; 5L) for 24 h. The mixture was then filtered with Whatman No. 3 filter paper. The total filtrate was concentrated by evaporation, and the residue of SCBG was dissolved in deionized water.

2.2 Determination of Bioactive Compound

The method described previously by Kim et al. (2013) with slight modification. Briefly, 3 g of each sample was weighed in an extraction tube, and 10 mL of 70%

methanol was added. The extract was mixed for 20 min. The extracts were centrifuged at 6000 rpm for 10 min. The supernatant was decanted in a graduated conical tube. The extraction step was repeated third times. Both extracts were pooled, and the volume was adjusted to 10 mL with 70% methanol. One milliliter of the extract was diluted with water to 50 mL.

2.2.1 Total Phenolic Compound

The total polyphenol compound was determined spectrophotometrically, using gallic acid as a standard, according to the method described by the International Organization for Standardization (ISO) 14502-1. Briefly, 1.0 mL of the diluted sample extract was transferred in triplicate to separate tubes containing 5.0 mL of a 1/10 dilution of Folin Ciocalteu's reagent in water. Then, 4.0 mL of a sodium carbonate solution (7.5%, w/v) was added. After 1 h at room temperature, the absorbance was read at (λ) = 756 nm. All values were expressed as mg gallic acid equivalents (GAE)/mg dry matter of the garlic sample.

2.2.2 Total Flavonoid Compound (TFC)

The total flavonoid compound was determined using a colorimetric method described previously by Kim et al. (2013). A portion of 0.5 mL extract was taken, and 0.5 mL of 2% ethanolic solution of $AlCl_3$ was added. After 1 h at room temperature, the absorbance was read at (λ) = 420 nm. All values were expressed as mg quercetin equivalents (QE)/mg dry matter of garlic.

2.3 Determination of Antibacterial Activity

Antibacterial activity analysis refers to clinical and international laboratory committee standards (Riyanti et al. 2020). There are several test procedures, including the affiliation of tools and materials, the provision of test bacteria, preparation and standardization of bacterial suspense, and analysis of antibacterial activity.

Petri dishes containing the media are to be prepared at room temperature for 10–15 min. Vortex bacterial suspense until homogeneous, then insert the swab into the suspension and apply it to the media evenly. Let stand for 15 min. Then, prepare a paper disk that has been saturated by the sample and store it on the top layer of the agar media. Then, incubate at 37 °C for 24 h. After that, observations were made using the calipers (measuring the diameter/radius of the zone of inhibition).

2.4 Determination of Antioxidant Activity

The free radical scavenging activity of SCBG was determined based on the scavenging activity of the stable DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical using the method described by Choi et al. (2014) with slight modification. A sample of SCBG 5 mg dissolved in 5 mL methanol solution as a stock solution, stock solutions diluted on a range of 0.2, 0.4, 0.6, and 0.8 (mg/mL) and 0.2 mL DPPH was added dissolved in methanol solution (1 mL). After incubating the solution at room temperature in the dark for 30 min, the absorbance was measured at (λ) = 517 nm.

2.5 Cell Culture and Cell Viability Assay

3T3-L1 preadipocyte cells were cultured using 12-well plates in DMEM medium (Stigma, USA) containing 10% FBS (Stigma, USA) in a humidified atmosphere of 5% CO_2 at 37 °C. To induce adipogenesis, the cells were cultured for 2 days until confluency. This induction was in the presence of the differentiation mixture containing 0.5 mM methyl-isobutyl-xanthine (Sigma, USA), 1 μM dexamethasone (Sigma, USA), and 10 μg/ml insulin (Sigma, USA).

Cell viability assay was performed according to the method described by Torres-Villarreal et al. (2019). Four-well plates were used in this experiment. Each well was added with different concentrations of C. longa extracts (0, 2.5, 5, and 7.5 mg/mL), and cells were then incubated for 48 h.

The cells were incubated for 48 h. After 48 h, on day 3, the medium was replaced with DMEM containing 10 μg/m insulin to optimize glucose uptake into the cells and lipogenesis during the differentiation process. On day 5, the medium was then replaced with DMEM. On day 7, the culture medium was replaced again with DMEM. On day 12, the optimal adipocyte differentiation was obtained in concentration control (0 mg/mL).

2.6 Determination of Lipid Accumulation by Oil Red O Staining

Oil Red O staining was performed using Kim's protocol (Nam et al. 2018) with minor modifications. The morphology of the cells was examined under an inverted microscope, and the images were captured. The colored oil droplets were dissolved in 100% isopropanol, and the relative lipid accumulation content was measured by reading the absorbance at a wavelength (λ) = 520 nm with an ELISA reader.

2.7 Statistical Analysis

All experiments were carried out in triplicate, and data were expressed as mean ± standard deviation (SD) using SPSS 25.0 version. One-Way analysis of variance (ANOVA) and Duncan's multiple comparison tests were used to determine the significance of the difference among samples with a significance level of ($p < 0.05$).

3 Result and Discussion

3.1 Bioactive Compound of Black Garlic

3.1.1 Total Phenolic Compound

Hydroxycinnamic acid derivatives are garlic's major phenolic acid compounds (Kim et al. 2013). There is an increase in hydroxycinnamic acid and phenolic acid derivatives during the development phase of black garlic products (Kimura et al. 2017). In making black garlic, the content of phenolic compounds may be caused by the process of creating complex compounds bound by esterification and glycolysis reactions, increasing free phenolic forms (Lu et al. 2017).

The total phenolic compound in SCBG was (23.15 ± 0.15 mg GAE/g dry basis), which was 2.5 times higher compared to TPC content in FSCG (9.26 ± 0.20 mg GAE/g dry basis). These results are consistent with those obtained by Choi et al. (2014), who reported that, compared to fresh garlic (13.91 ± 1.62 mg GAE/g dry basis), the concentration of polyphenol compounds in black garlic heated for 21 days was significantly higher (58.33 ± 1.90 mg GAE/g dry basis). In another study, Jang et al. (2018) showed. an increase in total polyphenol content in samples of black garlic containing (43.01 ± 2.85 mg GAE/g dry basis), compared to fresh garlic (2.86 ± 0.14 mg GAE/g dry basis). The difference between the value of phenolic compounds may be related to the different sources of garlic varieties. The increase of TPCs in SCBG compared to fresh single-clove garlic (FSCG) is shown in Fig. 1.

The antioxidant activity possessed by phenolic compounds was further established for treating various diseases. In this case, phenolic compounds are also considered essential in stabilizing lipids against peroxidation and inhibiting different forms of oxidizing enzymes to better treat obesity (Anyanwu et al. 2020). The mitochondrial-targeted antioxidant action of phenolic compounds can be a potential mechanism to treat obese inflammation. An array of phenolic compounds has displayed AMPK-activating properties in adipocyte models (Zhang and de Mejia 2020). According to Wang et al. (2014), intake of polyphenol compounds can help prevent obesity by reducing lipogenesis, increasing lipolysis, stimulating fatty acid oxidation (FA), inhibiting adipocyte differentiation and growth, weakening the inflammatory response, and suppressing the occurrence of oxidative stress.

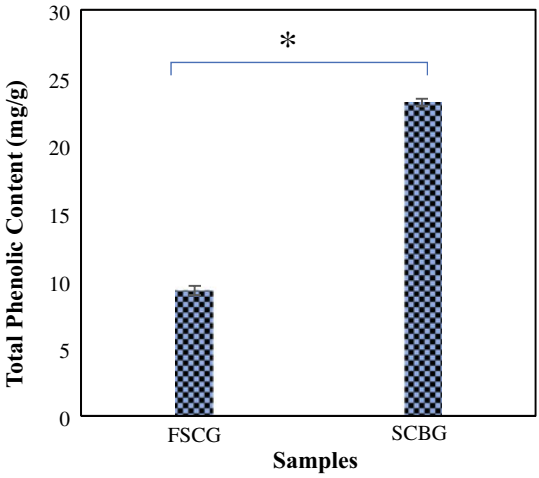

Fig. 1 A comparison of total phenolic compound (mg GAE/g dry basis) of FSCG and SCBG extract. Data represent mean ± SD, $n = 3$; *$P < 0.05$ significant difference between the two groups

3.1.2 Total Flavonoid Compound

Several in vitro studies have shown that flavonoid class compounds affect adipocyte cells, where flavonoids can reduce cell viability and proliferation, inhibit triglyceride aggregation, promote lipolysis, and reduce inflammation. (Herranz-López et al. 2017). The anti-adipogenic effect of flavonoids is mainly via their effect on the regulation of several pathways, such as induction of apoptosis, suppression of key adipogenic transcription factors, activation of AMPK and Wnt pathways, inhibition of clonal expansion, and cell-cycle arrest (Khalilpourfarshbafi et al. 2019). Quercetin and structurally similar luteolin are ubiquitous dietary flavones found in a wide range of herbs, and their anti-obesity effects are well established; this is believed to be mediated by increasing the expression of AMPK, which subsequently reduces the differentiation and proliferation of human preadipocytes 3T3-L1 and induces apoptosis (Woon and Toh 2014).

Flavonoid compound (TFCs) levels have been expressed as Quercetin equivalents. The increase of TFCs in SCBG compared to FSCG is shown in Fig. 2. From the results, the total flavonoids in the SCBG sample were (9.75 ± 0.27 mg QE/g dry basis). This level increased by 6 times when compared to the total flavonoid content FSCG (1.35 ± 0.11 mg QE/g dry basis).

Quercetin has been shown to minimize intracellular ROS in the hypertrophic adipocyte model (Leiherer et al. 2016). Anthocyanins can suppress lipid accumulation in adipocytes due to widespread inhibition of transcription factors that control lipogenesis, such as active peroxisome receptors and binding proteins to the CCAAT conjugator (Lee et al. 2014). In studies with animal models, flavonoid compounds have also shown positive results in preventing and treating obesity. Based on research from You et al., flavonoids can increase energy expenditure or inhibit food intake through various processes that suppress oxidative stress and release gastrointestinal

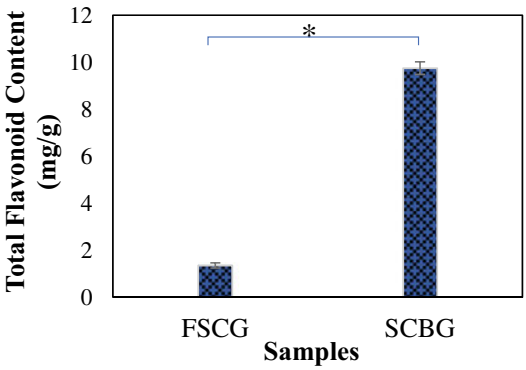

Fig. 2 A comparison of total flavonoid compound (mg QE/g dry basis) of FSCG and SCBG extract. Data represent mean ±SD, $n = 3$; *$P < 0.05$ significant difference between the two groups

Table 1 Comparison of total phenolic and total flavonoid compound

Samples	Total phenolic compound (mg/g)	Total flavonoid compound (mg/g)
FSCG	9.26 ± 0.20^{a}	1.35 ± 0.11^{a}
SCBG	23.15 ± 0.15^{b}	9.75 ± 0.27^{b}

Data represent mean ±SD, $n = 3$; values by different letters were indicated significantly different ($p < 0.05$), according to Duncan's test
Different symbols indicate significant differences in each aspect/column (consisting of: total phenolics, total flavonoids, and antioxidant activity) based on the post-hoc test (DMRT, $p < 0.05$)

hormones. The recapitulation of total phenolic and total flavonoid compounds of FSCG and SCBG is shown in Table 1.

In addition, black garlic's total polyphenol and flavonoid contents significantly increased during the aging period. It shows the potential use of black garlic in various diseases, including obesity.

3.2 Antibacterial Activity

The antibacterial activity of SCBG can be seen based on the size of the precise zone diameter (mm) formed around the paper disk against the bacteria tested, namely *Escherichia coli and Staphylococcus aureus.* A clear zone is an area not overgrown with bacteria and looks more apparent than the surrounding area. The greater the inhibitory zone formed, the greater the ability of antibacterial activity. Based on the analysis made on samples against the growth of *E. coli and S. aureus,* a zone of inhibition formed around the paper disk. The inhibition zone decreased for the sample with more longer heating duration.

The antibacterial activity of *S. aureus* looks better when compared to *E.coli* due to differences in the cell wall structure in the test bacteria. *E.coli* is a gram-negative

Table 2 Comparison on minimum inhibition of antibacterial activities

Samples	Inhibition Zone (mm)	
	E. coli	S. aureus
FSCG	4.5	6.58
SCBG	2	0.65

Data represent the measurement results of the clear zone average diameter

bacterium that has a cell wall, higher lipid content, and a multilayer cell wall structure consisting of lipoprotein, phospholipid outer membrane, and lipopolysaccharide, which causes gram-negative bacterial cell walls to be difficult to penetrate by antibacterial substances while gram-positive bacteria have a layer of peptidoglycan on the outside and have less role in effective defense permeability. The measurement results of the apparent zone average of FSCG and SCBG diameter are shown in Table 2.

The results showed that FSCG in this study showed higher antibacterial activity compared to black garlic. The compound that acts as an antibacterial is an organosulfur compound, which is allicin (Lawson and Hunsaker 2018). However, allicin compounds are unstable against high temperatures (Zhang et al. 2015). The heating carried out in producing black garlic with high temperatures causes the loss of and damage to the allicin compound. Compared to fresh garlic, black garlic does not cause a strong taste because of reduced allicin compounds converted into antioxidant compounds such as S-ally-cysteine (SAC), bioactive alkaloids, polyphenols, and flavonoid compounds (Botas et al. 2019).

3.3 Antioxidant Activity

The antioxidant activity of SCBG extracts was investigated using the DPPH scavenging assay. The DPPH assay is widely used due to the relatively short time required for the analysis. The method is based on the scavenging of DPPH by antioxidant compounds, which includes a reduction reaction that decolorizes the DPPH methanol solution (Lu et al. 2017). The DPPH radical scavenging activity indicates the ability of an antioxidant compound to donate electrons or hydrogen, thereby converting DPPH into a more stable molecule with a reduced absorbance (Wu et al. 2020). The DPPH radical scavenging activity of black garlic samples is shown in Fig. 3.

Based on the analysis of the percent inhibition value of the sample against DPPH radicals, it was found that there was an increase in the percent inhibition of SCBG compared to FSCG. There was an increase in the percent inhibition in the sample where the FSCG extract had a percent inhibition range (of 9.07–60.6%) while the SCBG extract was (41.10–95.78%). The DPPH free radical scavenging activity of SCBG within 21 days of the heating process was significantly higher by approximately 3-fold from FSCG ($p < 0.05$). The increase in the percent inhibition of SCBG extract may be due to an increase in antioxidant compounds in black garlic, such as

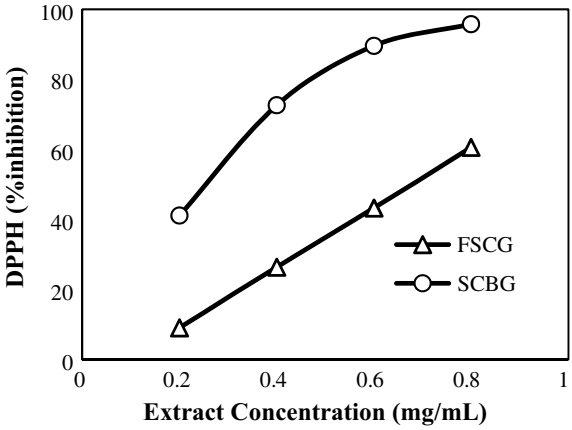

Fig. 3 Radical scavenging activity determined using DPPH method. The logistic model in origin was used to calculate half-inhibition (IC50). "X" represents the different FSCG and SCBG extract concentrations, and "Y" represents the radical scavenging activity rate

total phenolic and flavonoid compounds, where there is an increase in the content of these compounds, which are also known to have antioxidant activity (Bae et al. 2012).

The concept of the half maximal inhibitory concentration (IC_{50}) is extensively used in the pharmaceutical world to measure its effectiveness in inhibiting biological or biochemical functions. The IC_{50} value indicates the inhibitor concentration required to inhibit a given biological or biochemical function by half. In other words, large IC_{50} values denote inhibitors that interact less effectively with an enzyme than those with small IC_{50} values (Caldwell et al. 2012). The IC_{50} value is inversely related to the percentage value of inhibition or the ability of the compound to inhibit free radical activity, which is related to the concentration of an extract.

The IC_{50} value of FSCG and SCBG is shown in Table III. Based on the result, SCBG has a lower $IC_{50,}$ indicating a more significant free radical scavenging activity (Table 3).

The abilities to scavenge DPPH radical (%) of SCBG extract (4 different concentrations) at the same time were used to calculate the IC_{50}. According to Duncan's test, values by different letters were indicated significantly differently ($p < 0.05$).

Bae et al. (2012), Choi et al. (2014), and Jang et al. (2018) have described similar results for DPPH scavenging activity and reducing power using samples of fresh garlic purchased in local Korean markets and subjected to heat treatment by the authors.

Table 3 Comparison of IC_{50} value of single fresh clove black garlic (FSCG) and single clove black garlic (SCBG)

Samples	IC_{50} (mg/mL)
FSCG	0.602 ± 0.91^{a}
SCBG	0.320 ± 0.36^{b}

Different symbols indicate significant differences in each aspect/column (consisting of: total phenolics, total flavonoids, and antioxidant activity) based on the post-hoc test (DMRT, $p < 0.05$)

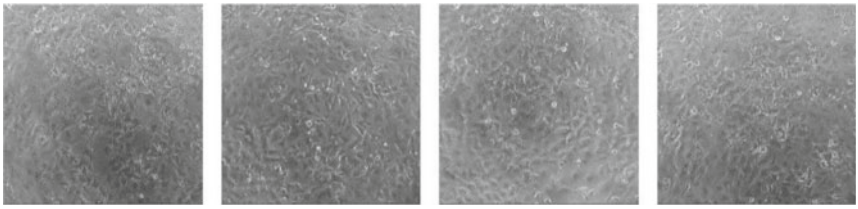

Fig. 4 Representative photographs under a 200x magnification microscope for the cell viability effect of SCBG extract to the 3T3-L1 cells line

3.4 Cell Viability

When a new drug, either derived from natural or synthesized material, is being developed, it is necessary to check its safety for the host cell or its cytotoxic effect on cells. This test is known as the cell viability test. Among the viability tests that depend on converting substrates to chromogenic products by living cells, the MTT test is one of the most widely used tests (Kumar et al. 2018).

To investigate the effect of SCBG extract on viability in mature 3T3-L1 adipocytes, the cell was treated with various concentrations of SCBG extract for 72 h. When viewed from the condition of the photogenic observation cells under the microscope, there was no significant damage to the 3T3-L1 cells affected by the SCBG extract. Representative photographs under a 200x magnification microscope for the cell viability effect of SCBG extract are shown in Fig. 4.

Based on cell viability measurement, in preadipocytes, SCBG extract at 2.5, 5, and 7.5 mg/mL significantly decreased cell viability, resulting in cell viability values of 84.70 ± 0.80, 79.12 ± 2.92 and 79.37 ± 4.30%. A diagram of the percent cell viability can be seen in Fig. 5.

There was a decrease in the viability of cells given SCBG extract at 2.5, 5, and 7.5 mg/ mL compared to the control. However, the concentration of the extract did not significantly decrease cell viability or the number of dead cells. To ensure that SCBG extract can inhibit lipid accumulation, several tests, such as inhibiting CCAAT or enhancer-binding protein (C/EBP α and β) and proliferator-activated receptor (PPAR) c mRNA, should be performed.

3.5 Effects of Single Clove Black Garlic on Lipid Accumulation in 3T3-L1 Preadipocytes Differentiation

The inhibitory effect of SCBG extract on lipid accumulation was evaluated by Oil Red O (ORO) staining. ORO staining is widely used to detect lipids in cells and tissues. The increase in adipocytes is known to be closely related to the accumulation of lipid

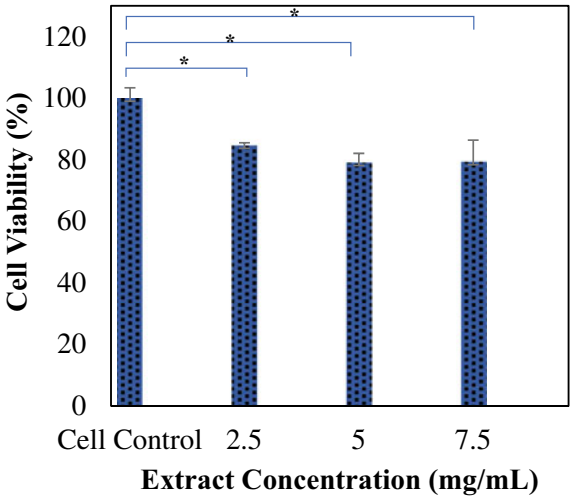

Fig. 5 Cell viability. Cell viability was determined by CCK-8 assay. 3T3-L1 preadipocytes were treated with 0, 2.5, 5, and 7.5 mg/mL SCBG extract for 72 h. Data represent mean ±SD, $n = 9$; $^*P < 0.05$ significant difference between the two groups

content (Kang et al. 2021). Therefore, ORO-stained cells indicate the degree of lipid accumulation in 3T3-L1 adipocytes.

The differentiation of 3T3-L1 preadipocytes was initiated with an inducer after two days of contact inhibition (when preadipocytes exited from the growth cycle). 3T3-L1 preadipocytes differentiated with the treatment of SCBG extract at the indicated concentration for 18 days. After the differentiation of preadipocytes along with the treatment of SCBG for 18 days, ORO staining and subsequent quantification were performed to examine intracellular lipid accumulation.

As seen in Fig. 6, microscopic observations show that the treatment of black garlic extract can reduce lipid data in the 3T3-L1 cell line. The lipid droplets in differentiation media-treated cells became larger with a deeper red color; however, the treatment decreased these phenomena, which is indicated by the reduction of red cells. It could be related to the synthesis of triglycerides in mature adipocytes and the increased hydrolysis of intracellular triglycerides, which in turn prevents the accumulation of lipids in adipocytes, thereby inhibiting fat cell hypertrophy.

After Oil-Red O staining, the stained oil droplets were dissolved in 100% isopropanol, and the relative triglyceride content was measured by reading the absorbance at the wavelength (λ) = 520 nm with an ELISA reader. Based on the results of the calculation of the percentage of accumulated lipids, black garlic extract at concentrations of 2.5, 5, and 7.5 mg/mL resulted in lipid accumulation values of 76.15 ± 6.50, 71.93 ± 8.49 and 57.84 ± 4.10%, diagram of the accumulation of lipid content can be seen in Fig. 7.

The measurement results of cell lipid accumulation added with black garlic, and the value was smaller when compared to control cells ($p < 0.05$), ($p < 0.01$), the value of lipid accumulation in the sample significantly decreased with the higher concentration of the extract added.

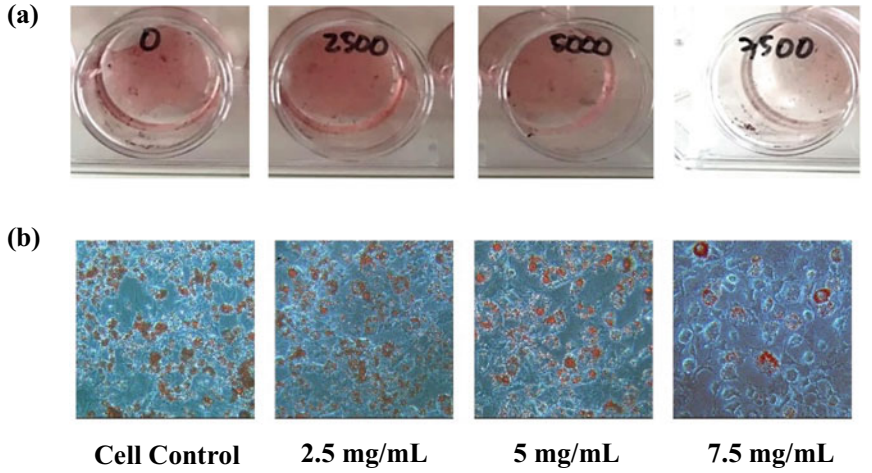

Fig. 6 Lipid accumulation. 3T3-L1 preadipocytes were treated with SCBG extract with 0, 2.5, 5, and 7.5 mg/mL. Mature 3T3-L1 adipocytes were stained with oil red O after 18 days. **a** Lipid accumulation was observed in cell culture media. **b** Microscopic pictures were taken at 200x magnification

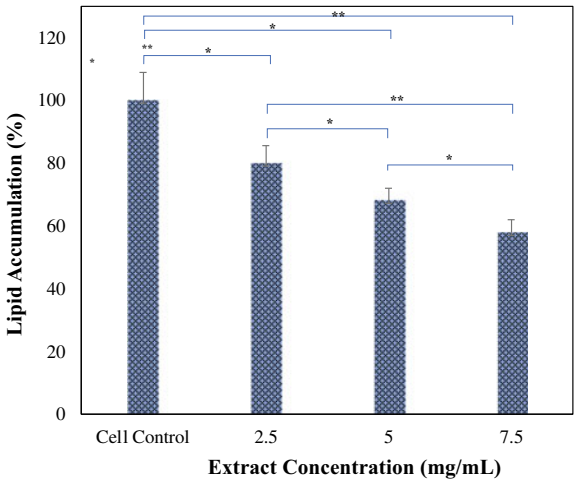

Fig. 7 Lipid accumulation. Values were measured from isopropanol elution of the oil red O stained cells. Data represent mean ±SD, $n = 9$; $^{*}P < 0.05$ significant difference between the two groups. $^{**}P < 0.01$ significant difference between the two groups

Wu et al. specifically tested melanoidin compounds in black garlic to determine their anti-obesity effect, based on the results of research on the effect of melanoidin on C57BL/6 J mice with obesity induced by a high-fat diet (HFD) that the administration of melanoidin orally, has a significant effect in reducing weight gain and white adipose tissue weight and reversing glucose tolerance, especially at high doses. Nam et al. reported that aged black garlic (ABG) extract at 2.5 and 5 mg/mL significantly reduced protein expression of proliferator-activated receptor c (PPARc) and perilipin

in mature 3T3-L1 adipocytes. The hormone-sensitive lipase (HSL) and Ser563-pHSL levels were significantly reduced by treatment with 5 mg/mL of ABG extract.

4 Conclusion

Single-clove black garlic (SCBG), produced by aging single-clove garlic for 21 days, has shown higher antioxidant properties when compared to fresh single-clove garlic (FSCG). Measurement results of lipid accumulation added with SCBG. The value was lower compared to control cells ($p < 0{,}05$), ($p < 0.01$), and the value of lipid accumulation in the sample decreased significantly with a higher concentration of the added extract. These results suggest that SCBG extract may have anti-lipogenic effects on adult 3T3-L1 adipocytes that could be associated with potential. These results suggest that black garlic extract may have anti-lipogenic effects on mature 3T3-L1 adipocytes, which could be associated with the potential for black garlic in treating obesity.

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