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Characterization of SNEDDS Formulated with Single Bulb Garlic and its Anti-Inflammatory Effect on 3T3-L1

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ABSTRACT

Inflammation is an early symptom of a disease that reduces the level of health. Single-bulb garlic (*Allium sativum L.*) is used medicinally as a plant with a broad pharmacological effect, especially anti-inflammatory activity. Self-nanoemulsifying drug delivery systems (SNEDDS) have offered opportunities to improve drug delivery. The current study aimed to characterize SNEDDS-single bulb garlic extract (SBGE) and determine its potential as an anti-inflammatory agent in 3T3-L1 cells. SNEDDS was formulated from tween-80, glycerol, canola oil, and SBGE. The formula characterization is done using droplet size, polydispersity index, zeta potential, physical stability test, and antioxidant assay. The cytotoxicity test of SNEDDS-SBGE was evaluated using a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. The anti-inflammatory activity was examined using 3T3-L1 cell methylglyoxal (MG) induction, and the expression of cytokines was measured using immunocytochemistry (ICC). The SNEDDS-SBGE had a nanoemulsion size of 42.30±1.39 nm, 0.6±0.03 for the polydispersion index, and -22.63±0.75 mV for the zeta potential. SNEDDS-SBGE was physically stable and had a high antioxidant level (47.579±8.017 %). SNEDDS-SBGE exhibited no toxic effect on 3T3-L1 cells. The administration of 62.5 µg/ml and 125 µg/ml SNEDDS-SBGE could inhibit ($p<0.05$) the expression of IL-1β after methylglyoxal induction. Thus, SNEDDS-SBGE may have potential anti-inflammatory properties.

1. Introduction

Inflammation is a complicated interaction between soluble substances and tissue cells in reaction to traumatic, viral, post-ischemic, toxic, or autoimmune diseases (Ansary *et al*. 2020). The incidence of diseases caused by inflammation is high in Indonesia. Diabetes mellitus 2.1%, asthma 4.5%, dermatitis 6.8%, acute respiratory infections 25.50%, cancer 0.4%, and hepatitis 1.2% are the most common inflammatory disorders in the country (Hadrich 2018; Hanafi *et al*. 2018). Inflammation causes oxidative stress to tissues or cells, disruption of hormonal balance, and chronic inflammatory reactions that trigger pro-inflammatory cytokine production (Brasil *et al*. 2020). Interleukin-1β (IL-1β) are examples of inflammatory mediators

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that can cause an increase in the generation of reactive oxygen species (ROS) (Munteanu 2021). If the gene expression is not balanced, it will cause oxidative stress (OS). ROS can alter cellular proteins, lipids, and Deoxy Nucleic Acid (DNA), which can alter how cells function (Hong *et al*. 2020). A high-carbohydrate diet stimulates adipogenesis and lipogenesis. A crucial mediator of the pro- or anti-lipogenic effects of many hormones and nutrients is Sterol Regulatory Element Binding Protein-1 (SREBP-1) (Kim *et al*. 2020). Another transcription factor related to lipogenesis is peroxisome proliferator-activated receptor-γ (Kim *et al*. 2016). High ROS production and decreased antioxidant capacity cause various diseases (Ghosha *et al*. 2013; Munteanu 2021).

Antioxidant supplementation is necessary to reduce inflammation, which can adversely affect health. 33 different types of sulfur compounds, including diallyl disulfide, ajoene, alliin, allicin, diallyl trisulfide, diallyl sulfide, and S-allyl-cysteine, are found in one bulb garlic (*Allium sativum* L.), a medicinal plant (Borlinghaus *et al*. 2014; Li *et al*. 2021). The pharmacological effect of these active compounds could act as cardioprotective, anti-inflammatory, anticancer, antimicrobial, immunomodulatory, antidiabetic, and antioxidant (Capasso 2013; Sangar *et al*. 2019; Li *et al*. 2021; El Baroroh *et al*. 2024). Antioxidants prevent damage to cells and organs due to oxidation (Shang *et al*. 2019a). The characteristics of volatility, lipophilicity, strong scent, and poor stability in gastrointestinal fluids make the active chemicals in garlic less effective and reduce their bioavailability (Capasso 2013; Lestari *et al*. 2019). Consequently, in order to improve the bioavailability of active ingredients in single-bulb garlic (SBG), a delivery mechanism is required. To increase the low bioavailability of SBG, one drug delivery method is the Self Nanoemulsifying Drug Delivery System (SNEDDS) (Priani *et al*. 2017; Lestari *et al*. 2021; Miasih *et al*. 2022).

The nanoemulsion form is commonly used in SNEDDS formulation because it has an oil content that may transport a single onion extract that is difficult to dissolve in water. In addition to their capacity to spontaneously create nanoemulsions in the digestive system, SNEDDS formulations improve the size of the droplets in nanometer size (Alexander *et al*. 2016; Priani *et al*. 2017; Ujilestari *et al*. 2018). The characteristics of SNEDDS are influenced by its constituent components, such as the oil phase, surfactants, and co-surfactants (Ujilestari *et al*. 2018). Saturated fatty acids (7%), polyunsaturated fatty acids, oleic acid (61%), linoleic acid (21%), and α-linolenic acid (11%) are all present in modest concentrations in canola oil (Lin *et al*. 2013). Canola oil plays a role in lowering blood pressure, reducing inflammation, preventing mutagenesis, and eliminating microbes (Loganes *et al*. 2016). Compared to ionic surfactants, non-ionic surfactants like Tween-80 are safer to swallow and also has good solubility (Pratiwi *et al*. 2017; Ragavan *et al*. 2017). Glycerol was used as a co-surfactant in this study. The surface tension is reduced when glycerol interacts with other surfactants as co-surfactants (Lestari *et al.* 2021).

Single garlic extracts with SNEDDS consisting of Tween-80 surfactant, glycerol co-surfactant, and virgin coconut oil could increase their antioxidant activity by 56.74%, which is higher than single garlic extract without SNEDDS (17.56%) (Lestari *et al*. 2023b). Using Tween-80 surfactant, PEG-400 co-surfactant, and EBT-based canola oil carrier oil, the SNEDDS formulation was found to decrease IL-1β expression in 3T3-L1 cells (Alexander *et al*. 2016). The novelty of the research is the formulation SNEDDS loaded wuith a single garlic extract using tween-80, glycerol, and canola oil. This research will be carried out by optimizing the SNEDDS formula on single bulb garlic extract (SBGE) with characterization, physical stability, antioxidant, and toxicity tests. Thus, the goal of the current work was to characterize SNEDDS-SBGE and assess its potential for using 3T3-L1 cells as an anti-inflammatory drug *in vitro*.

2. Materials and Methods

2.1. Preparation of Single Bulb Garlic Extract (SBGE)

Single bulb garlic (SBG) was obtained from Ngadas, Poncokusumo Malang, East Java. One kilogram of SBG was chopped and air-dried. The maceration process was used to extract SBG (Lestari *et al*. 2023b). The dry weight of SBG was then weighed and mixed with n-Hexane (Merck, Germany) with a ratio of 1:3. After two 24-hour shakes at 37°C, filter paper was used to filter the mixture (Whatman, China). Extraction was then completed with a rotary evaporator (IKA RV 10 digital V-, Germany) to evaporate the solvent to produce the crude extract.

2.2. Preparation of SNEDDS-SBGE

SNEEDS was formulated by mixing surfactants, including tween-80 (Sigma Aldrich, Germany), glycerol (Merck, Germany), canola oil (Mazola, Malaysia), and SBGE, with a ratio of 3.536: 0.500: 0.964: 0.100 (g). The SNEDDS-SBGE formulation was initiated by combining glycerol and tween-80 (mixture 1), followed by 5 minutes of ultraturrax (IKA©-WERKE, Germany) homogenization and 15 minutes of magnetic stirrer (Thermolyne cimarec®2, USA) use. Mixture 2 (canola oil) was gradually blended with 1 mg of SBGE using a stirrer. Following an equal mixing process, the mixture was homogenized for five minutes with an ultratrurrax and fifteen minutes with a magnetic stirrer. Mixtures 1 and 2 were homogenized for five minutes with an ultraturrax, fifteen minutes with a magnetic stirrer, and fifteen minutes with sonication (IWAKI Ultrasonic Cleaner, Japan). To finish the mixing, the SNEDDS system mixture was agitated for one additional hour (Lestari *et al*. 2022).

2.3. Characterization of Droplet Size, Polydispersity Index, and Zeta Potential

The Dynamic Light Scattering (DLS) method was used to characterize the droplet size (Z-Average), polydispersity index (PDI), and zeta potential. To prepare the SNEDDS sample for experimental use, a dilution was performed using double-distilled water at a 1:1000 ratio (v/v) (Ujilestari *et al*. 2018). After one minute of homogenization with a magnetic stirrer, the mixture was examined using a particle size analyzer (Horiba SZ-100z, Japan).

2.4. Physical Stability Test

2.4.1. Hot-Cold Cycle Test

SNEDDS-SBGE was first cooled for 24 hours at 4°C, followed by a 24-hour heating cycle at 40°C. This heating process was repeated up to six times. After completing a 12-day stability test, the cycles concluded with centrifugation at 5,000 rpm for 5 minutes. Observations were conducted on the parameters related to separation, precipitation, creaming, and cracking (Pratiwi *et al*. 2017).

2.4.2. Centrifugation Test

SNEDDS-SBGE underwent centrifugation at 50,000 rpm for 30 minutes. Throughout this process, the key parameters observed included separation, precipitation, creaming, and cracking (Pratiwi *et al*. 2017).

2.4.3. Free Thawing Stability Test

SNEDDS-SBGE samples were frozen at -21°C for 24 hours and then thawed at room temperature (25°C) for another 24 hours, constituting one complete cycle. This procedure was repeated six times, with each cycle concluding with a 5-minute centrifugation at 5,000 rpm. Observations of separation, precipitation, creaming, and cracking were conducted to assess the stability of the formulation (Senapati *et al*. 2016).

2.5. Antioxidant Assay

The DPPH (2,2-diphenyl-1-picrylhydrazyl-hydrate) assay (Sigma Aldrich, Germany) was used to measure the antioxidant content of SNEDDS-SBGE, with methanol serving as a control. Samples and DPPH were both at 2 mg/ml and 50 µM concentrations, respectively. After combining SNEDDS and DPPH in a 1:5 ratio, the mixture was left to sit at room temperature for 30 minutes while it was dark. Using a spectrophotometer (Biochrom Libra S12 UV-Vis, UK) the absorbance was measured at 517 nm and calculated using the formula 1 (Ratananikom *et al*. 2021).

$$\% \text{ DPPH radical inhibition} = [(A-B) / A] \times 100$$

A referred to the absorbance of the DPPH solution without the sample, while B referred to the absorbance of the DPPH solution after the SNEDDS-SBGE had been added.

2.6. Cytotoxicity MTT Assay

The 3T3-L1 pre-adipocyte cell lines were obtained from the Laboratory of Structure, Development, and Physiology of Animals at Universitas Brawijaya. The 3T3-L1 cells were cultured in a complete medium consisting of Dulbecco's Modified Eagle's Medium (DMEM) (Gibco®, USA), 10% fetal bovine serum (Gibco®, USA), and 1% penicillin-streptomycin (Gibco®, USA). These cells were placed in a 25 cm^2 tissue culture flask and incubated at 5% CO_2 and 37°C until they reached approximately 80% confluency, after which they were harvested. About 5 x 10^3 cells were transferred into 100 µL of culture media in each well of a 96-well plate. After washing with PBS 1x, 100 µL of culture media containing SNEDDS-SBGE at varying concentrations (n = 3): 62.5, 125, 250, 500, 1000, 2000, and 4000 µg/ml was added and incubated for 24 hours. Following incubation, the culture media was discarded, and the cells were washed again with PBS. Then, 100 µL of 3-[4,5-dimethylthiazol-2yl]-2,5-

diphenyl-tetrazolium bromide (MTT) (Nacalai Tesque Inc., Japan) solution was added to each well except for the blanks, and the cells were incubated for another 2 to 4 hours until purplish formazan crystals formed. Afterward, 100 µL of DMSO (Merck, Germany) was added to stop the reaction, and the absorbance was measured using a microplate reader at a wavelength of 595 nm and calculated following formula 2. The Acc represents the absorbance of the cell control, while the As corresponds to the absorbance of the sample (Lestari *et al*. 2023a; El Baroroh *et al*. 2024).

$$\% \text{ Cell viability } = \frac{\text{Acc-As}}{\text{Acc}} \times 100$$

2.7. Induction of Inflammation in 3T3-L1 Cell with Methylglyoxal (MG)

The 3T3-L1 cells were induced with methylglyoxal (Sigma Aldrich, Germany) to induce inflammation (Vulesevic *et al*. 2016). A total of 1×10^4 cells were seeded in each 24-well plate with the cover slip inside the well and incubated for 24 h. The addition of 500 µL of culture media containing 5 µg/ml of methylglyoxal to each well, the experiment was incubated for 24 h.

2.8. Immunocytochemistry (ICC)

The expression of IL-1β in 3T3-L1 cells after exposure to SNEDDS-SBGE was evaluated using ICC. After being stimulated with 5 µL/ml methylglyoxal in the culture media, the 3T3-L1 cells were incubated for 24 hours. Following this incubation, the cells were washed with PBS and then treated with varying concentrations of SNEDDS-SBGE, specifically 62.5, 125, and 250 µg/ml, for another 24 hours. The sample fixation at 5 minutes with 100 µL of 4% paraformaldehyde (4% PFA), the cells were rinsed once with PBS. After 30 minutes of adding 100 µL 0.5% Triton-x to the cells, they were incubated in 5% Bovine Serum Albumin (BSA) (BioWest, France) for another 30 minutes. The cells were treated with IL-1β primary antibody (Santa Cruz Biotechnology Inc., USA) overnight. PBS 1X was used to wash the cells, and then a FITC-conjugated secondary antibody (Abcam, USA) was applied for one hour. PBS (100 µL) was used to rewash the cells. The expression of IL-1β in 3T3-L1 cells was seen right away in a dark room at Laboratorium Sentral Ilmu Hayati Universitas Brawijaya (LSIH-UB) using a Confocal Laser Scanning Microscope (CLSM) (Olympus FV1000, Japan). Using Flouview version 17a software (Olympus, Japan), the intensity of IL-1β expression in 3T3-L1 cells was determined (El Baroroh *et al*. 2024).

2.9. Statistical Analysis

All data were presented as mean±SD. According to the results, the characterization, physical stability, antioxidant activity, and cytotoxicity tests of SNEDDS-SBGE were analyzed descriptively. The expression of IL-1β in the 3T3-L1 cell line was evaluated using one-way ANOVA, followed by DMRT ($p<0.05$).

3. Results

3.1. Characterization

The Z-Average value of SNEDDS-SBGE was 42.300±1.389 nm (Table 1). PDI of SNEDDS-SBGE was 0.455±0.034 (Table 1), which indicated that the SNEDDS-SBGE formulation was formed as a mono dispersion due to PDI<0.5. SNEDDS-SBGE has zeta potential value of -22.633±0.751 mV (Table 1).

3.2. Physical Stability Test

The results showed that all physical instability characters of SNEDDS-SBGE indicated negative results in Table 2 and Figure 1. Therefore, it was indicated that SNEDDS-SBGE was physically stable before and after the test. Good stability is exhibited by SNEDDS-SBGE in the absence of separation, precipitation, creaming, or cracking.

3.3. Antioxidant Activity of SNEDDS-SBGE

DPPH can oxidize a radical molecule because it has an electron acceptor or hydrogen ion. The results showed (Figure 2) that the antioxidant activity of SNEDDS-SBGE

Table 1. The characterization of SNEDDS-SBGE

Value	Mean ± SD
Z- Average (nm)	42.300±1.389
Polydispersity Index (PDI)	0.455±0.034
Zeta Potential (mV)	-22.633±0.751

Table 2. Assessment of the physical stability characteristics of SNEDDS-SBGE

Parameter	Freeze thawing test	Centrifugation test	Hot-cold stability test
Separation	-	-	-
Precipitation	-	-	-
Creaming	-	-	-
Cracking	-	-	-

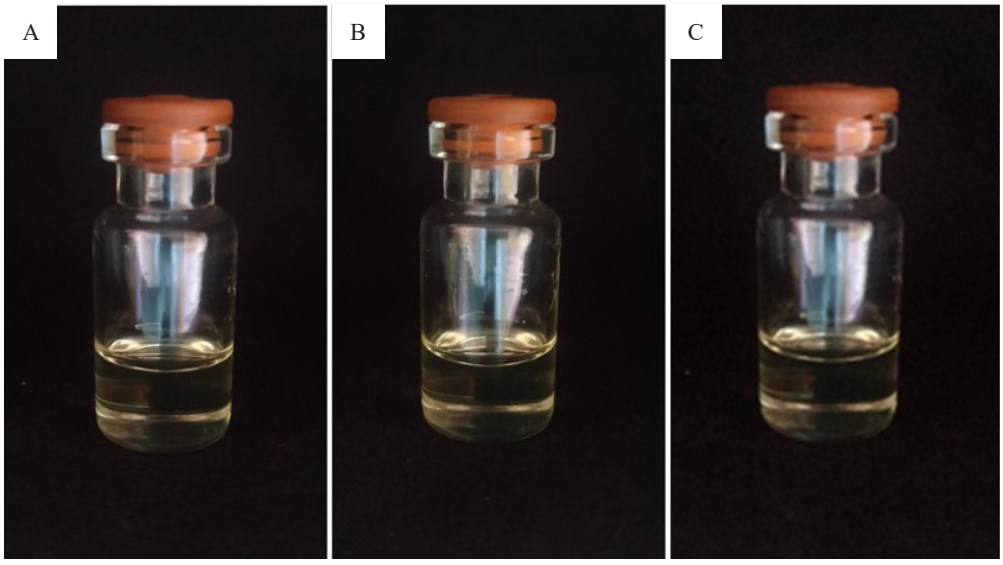

Figure 1. The physical stability test of SNEDDS-SBGE demonstrated excellent performance with no signs of separation, precipitation, creaming, or cracking observe. (A) hot-cold cycle test; (B) freeze-thawing test; (C) centrifugation test

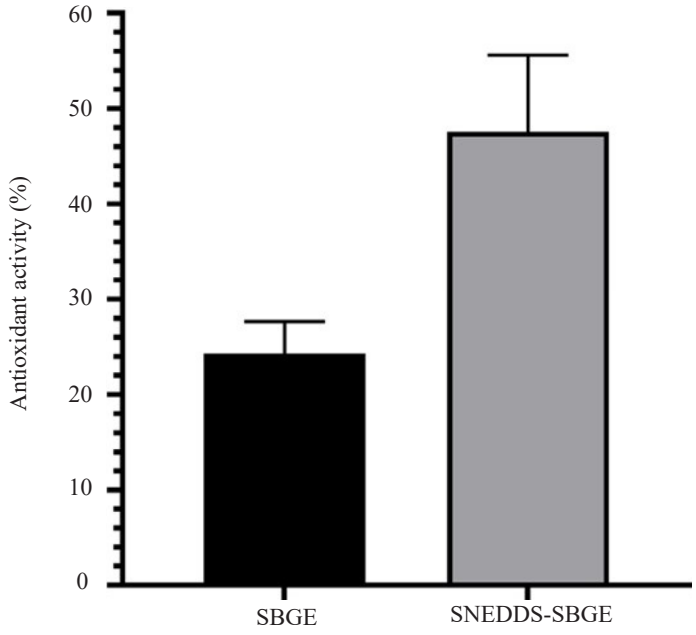

Figure 2. Antioxidant activity of SNEDDS-SBGE and SBGE evaluated using the DPPH assay

(47.579±8.017%) was higher than ($P < 0.05$) SBGE (24.305±3.326%) at the same dose.

3.4. Cytotoxicity Assay of SNEDDS-SBGT on 3T3-L1 Cell

SNEDDS-SBGE exhibited lower toxicity to 3T3-L1 cells than SBGE (Figure 3). Administration of SNEDDS-SBGE and SBGE to 3T3-L1 cells still showed live cell activity (Figure 4). The viability of 3T3-L1 cells after being treated with SNEDDS-SBGE at 62.5-2000 µg/ml was relatively high. However, the viability of 3T3-L1 cells tends to decrease after exposure to SBGE at 125-4000 µg/ml. The optimal drug delivery system intends to maximize the therapeutic effect while reducing toxicity.

3.5. SNEDDS-SBGE as an Anti-Inflammatory Agent

The green fluorescence in 3T3-L1 cells indicates the degree of IL-1β expression (Figure 5). When different concentrations of SNEDDS-SBGE are administered, the intensity of IL-1β is generally higher than SBGE (65.44±3.38 AU). The findings demonstrated that, in

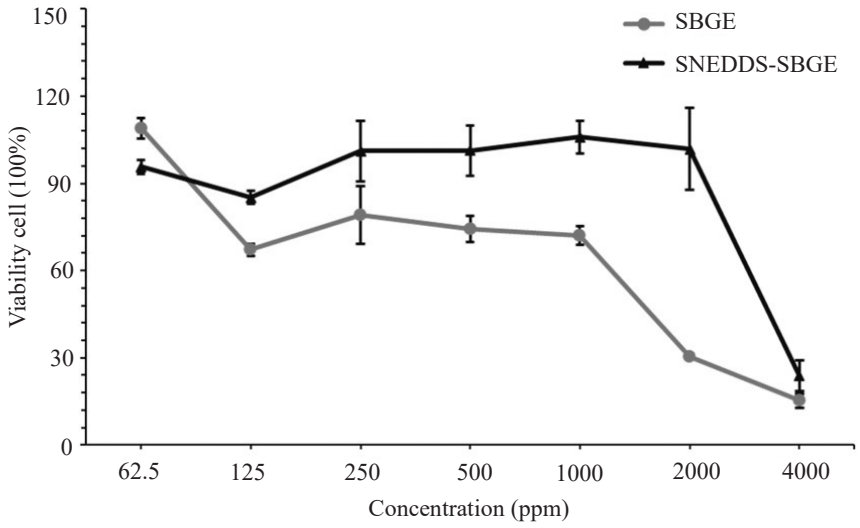

Figure 3. The cytotoxicity of SBGE and SNEDDS-SBGE on 3T3-L1 cells evaluated using the MTT assay

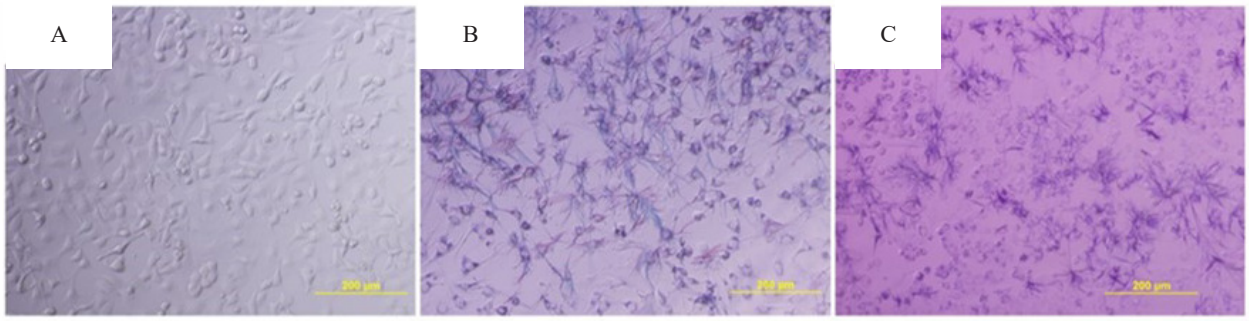

Figure 4. The Formazan crystal formation in 3T3-L1 cells treated with SBGE and SNEDDS-SBGE evaluated using the MTT assay. (A) 3T3-L1 cells before MTT assay. (B) MTT assay results on 3T3-L1 cells after being treated with 1,000 µg/ml SBGE. (C) MTT assay results on 3T3-L1 cells after being treated with 1,000 µg/ml SNEDDS-SBGE

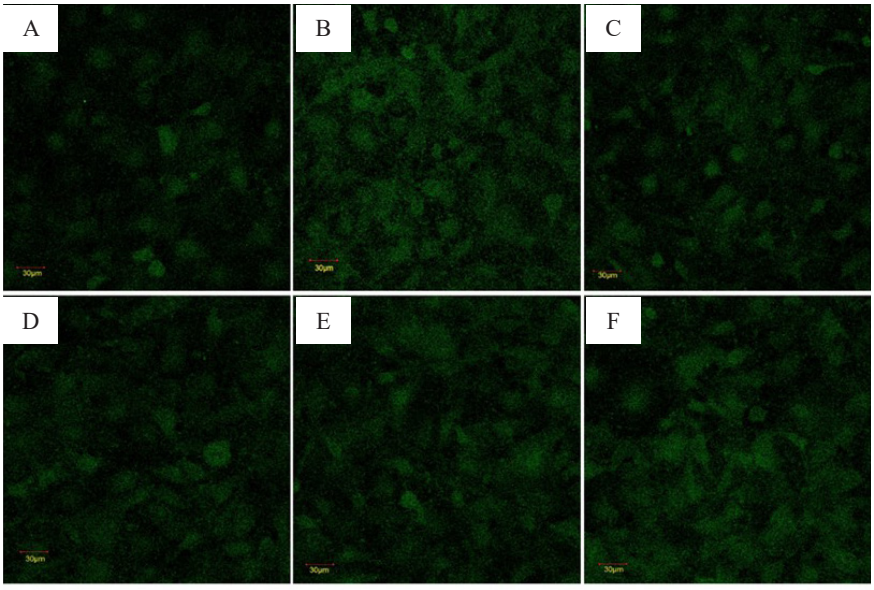

Figure 5. IL-1β expression in 3T3-L1 cells following treatments with methylglyoxal, SBGE, and SNEDDS-SBGE (400x magnification). The following treatments were applied to 3T3-L1 cells: (A) Control; (B) MG; (C) SBGE; (D) SNEDDS-SBGE 62.5 µg/ml SNEDDS-SBGE; (E) SNEDDS-SBGE 125 µg/ml; and (F) SNEDDS-SBGE 250 µg/ml

comparison to control cells, MG-induced 3T3-L1 cells increased the degree of IL-1β expression intensity. The expression of IL-1β cytokines upon MG induction was considerably ($p<0.05$) suppressed by the administration of 62.5 µg/ml (104.44±2.37 AU) and 125 µg/ml (112.63±1.83 AU) SNEDDS-SBGE (Figure 6). It was shown that the treatment of SNEDDS-SBGE, which ranged from 62.5 to 125 µg/ml, could prevent the MG-induced increase in the intensity of IL-1β expression (Figure 5).

4. Discussion

The Z-average value of SNEDDS-SBGE was good because the Z-average was less than 200 nm. PDI analysis was used to determine the degree of non-uniformity of the particle size distribution. Due to the zeta potential of SNEEDS-SBGE > -30 mV, therefore it allowed agglomeration. Smaller particle sizes will influence absorption, resulting in a greater drug surface area (Buya *et al*. 2020; Kazi *et al*. 2020). The nano size of the formed SNEDDS droplets is generally ideal at 20-200 nm (Khan *et al*. 2015; Kazi *et al*. 2019, 2020). PDI value <0.5 indicates homo homogeneously dispersed particles (Ali 2017). A stable mono-dispersion emulsion has a low PDI value, whereas a stable monodispersion emulsion has a high PDI value (Polychniatou 2014). Tween-80 has been reported to have the ability to lower PDI value (Hidajat *et al*. 2020).

Emulsions are stable if their positive or negative zeta potential exceeds 30 mV (Eid *et al*. 2014). Zeta potential reveals the electrostatic repulsion force on the droplet collection formed and will affect the stability and mobility of the surface. The stability of the developed SNEDDS system can be ascertained by measuring the electrostatic strength of the emulsion (Zhang *et al*. 2015). The negative value of the zeta potential of SNEDDS is due to hydroxyl groups in antioxidants between the oil or water surface, which then cause the stability of the nanoparticle formulation (Tripathi *et al*. 2016). To further ensure the robustness of the SNEDDS-SBGE formulation, physical stability studies were performed, as these tests are essential for identifying unstable formulations under various stress conditions

Thermodynamic physical stability studies such as hot-cold tests were conducted to identify unstable formulas (Khan *et al*. 2015). In food and colloidal systems, freezing can alter the physical and chemical

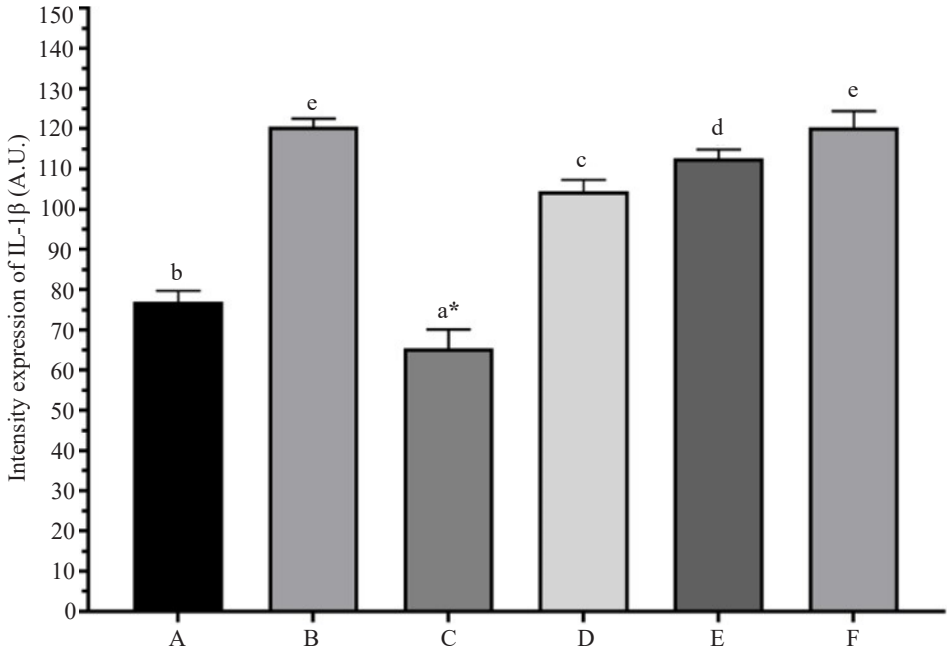

Figure 6. Intensity of IL-1β expression in 3T3-L1 cells following treatments with methylglyoxal, SBGE, and SNEDDS-SBGE cells. The MG treatment (B) significantly increased IL-1β expression compared to the control (A). The data showed with average ± SD, One-way ANOVA followed with duncan's multiple range test (DMRT) (*$p < 0.05$). Conversely, SBGE (C) and SNEDDS-SBGE (D and E) significantly reduced IL-1β expression, with the greatest reduction observed at the SBGE C: (A) Control, (B) MG, (C) SBGE, (D) SNEDDS-SBGE 62.5 µg/ml, (E) SNEDDS-SBGE 125 µg/ml and (F) SNEDDS-SBGE 250 µg/ml

composition. Additionally, during the thawing process, surfactant crystallization damages the nanoemulsion (Galvão *et al*. 2018). The stability of the SNEDDS-SBGE looks quite good during the several cycles, so it can be considered a positive result. Nanoemulsions, which are thermodynamically unstable, can undergo flocculation coalescence, creaming, and settling (Wik *et al*. 2020).

The centrifugation test stresses the SNEDDS system to accelerate the sample deterioration (Wik *et al*. 2020). As a stability test acceleration model, centrifugation will provide pressure to the system. According to reports, centrifugation for 20 minutes at 3500 rpm is equal to delivering gravity for approximately one year. The percentage of stability is determined by system separation. The system will be highly stable if there is a low separation (Restu *et al*. 2015). A test was conducted using the DPPH method to evaluate the success of the SNEDDS formulation process in maintaining the antioxidant activity of SBGE.

The ability of the free radical DPPH to stabilize, as demonstrated by a color shift in the presence of antioxidants, was used to measure the antioxidant activity. Nanoemulsion can increase stability and maintain the antioxidant activity of an active compound (Kalantari *et al*. 2017). Garlic contains more than 20 types of phenolic compounds, with several main phenolic compounds, including β-resorcylic acid, followed by protocatechuic acid, gallic acid, pyrogallol, rutin, and quercetin (Shang *et al*. 2019b). The phenolic compounds in garlic have been reported to have antioxidant properties (Chen *et al*. 2013). We observed an increase in the antioxidant activity of SBGE when it was formulated with SNEDDS. This might be due to the addition of antioxidants from the other SNEDDS constituent components.

The rise in antioxidant activity in SNEDDS-SBGE might be attributed to the carrier oil used. Several studies have shown that canola oil contains antioxidants derived from vitamin E, carotenoids, and phenolic compounds. Canolol, or 2,6-Dymethoxoy-4-vinylphenol, is the most common phenolic compound found in canola oil and is thought to be the most active component of the oil (Loganes *et al*. 2016). Zhao *et al*. (2021) reported that the antioxidant activity of SNEDDS formulated using PEG-40, propylene glycol, and castor oil had higher antioxidant activity by 81.92% than buckwheat flavonoid antioxidants without SNEDDS preparation (37.1 %) Kalantari *et al*. (2017) also revealed that SNEDDS with the composition of Labrasol/Kollipor RH-40, Transcutol HP, and carrier oil Isopropyl Myristate increase the antioxidant activity of Plantago lanceolata extract. Following the antioxidant activity test, the MTT assay was also conducted to ensure that the SNEDDS-SBGE formulation had no cytotoxic effects on 3T3-L1 cells.

MTT assay is used to determine the mitochondrial function of the cells, which is indicated by cell viability. The mitochondrial enzyme dehydrogenase in living cells reduces the yellow tetrazolium MTT salt to purple formazan crystals, which are precipitated on uninjured cells (Wong-a-nan *et al*. 2018; Yu *et al*. 2018). The SNEDDS formula exhibited no cytotoxic activity against 3T3-L1 cells. These results were supported by previous research that the SNEDDS-SBGE had high stability properties and a high antioxidant level (47.579%). Therefore, SNEDDS-SBGE were not toxic to 3T3-L1 cells. Garlic extract has low toxicity to NIH-3T3 fibroblast cells (Borlinghaus *et al*. 2014; Gruhlke *et al*. 2017). Miasih *et al*. (2022) also demonstrated that single-bulb garlic extract was not toxic to the 3T3-L1 cell line. Figure 4 depicts the treated 3T3-L1 cells following the MTT assay. Following treatment with 1,000 µg/ml SBGE (cell viability of 71.96%) and 1,000 µg/ml SNEDDS-SBGE (cell viability of 105.93%), the crystal formazan was seen in the 3T3-L1 cells. Low toxicity can increase drug efficiency, reduce concentrations without losing effectiveness, and minimize side effects (Martínez *et al*. 2020; Miasih *et al*. 2022). Figure 3 showed that the SNEDDS-SBGE at 62.5-2000 µg/ml had no toxic effect on the 3T3-L1 cell with cell viability above 80%, which indicated healthy cells (Chang *et al*. 2021; Lestari *et al*. 2022). In addition to the findings on cell viability, the effects of MG-induced oxidative stress were further examined to understand the underlying mechanisms of inflammation in 3T3-L1 cells.

MG caused cells to produce Reactive Oxygen Species (ROS), which in turn caused inflammation in 3T3-L1 cells. MG will open ATP-sensitive potassium K+ (KATP) channels through the oxidative phosphorylation pathway. Phosphorylation activates the P38 pathway, c-Jun NH2-terminal kinase (JNK), and stress-activated protein kinase (SAPK). Because of P38/SAPK/JNK, cydooxygenase-2 (COX-2) will be released into the cytoplasm. A signal from COX-2 will activate nuclear factor-kappa B (NF-kB), increasing transcription and producing TNF-α and IL-1β. TNF-α and IL-1β, two pro-inflammatory cytokines, will increase inflammation and slow down cell growth (Hadrich 2018; Brasil *et al*. 2020; Miasih *et al*. 2022)

Interestingly, in this study, the lowest dose of SNEDDS-SBGE was able to reduce IL-1β more effectively than the highest dose of SNEDDS-SBGE. The administration of 62.5 µg/ml SNEDDS-SBGE was the optimum concentration for inhibiting IL-1β expression. This may be due to the presence of other components in SNEDDS that can influence IL-1β expression, such as canola oil. As reported, the expression of IL-1β in subcutaneous adipose tissue significantly increased after consuming canola oil for 4 weeks. Data show that IL-1β gene expression rose 1.61-fold 4 hours after consuming the test meal containing canola oil compared to fasting conditions (Kruse *et al*. 2015).

The occurrence of SNEDDS-SBGE effectively prevented the increase in pro-inflammatory cytokine production in 3T3-L1 cells following MG induction compared to the control group. This effect may be attributed to garlic, which has been shown to reduce nitric oxide production through several mechanisms. Specifically, garlic downregulates the activity of inducible nitric oxide synthase (iNOS), inhibits NF-κB signaling, and prevents the nuclear translocation of NF-κB. These combined actions highlight garlic's potential role in modulating inflammatory responses (Altan *et al*. 2020). Numerous investigations have revealed that NF-κB is a crucial modulator of IL-1β gene activation. NF-κB is found in the cytoplasm and is linked to protein inhibitors called IkBN and IkB-α (Lestari 2018; Anggraini *et al*. 2022; Miasih *et al*. 2022). The interaction prevents NF-κB from entering the cell nucleus. In response to cellular stimulation, certain kinases phosphorylate IκB, resulting in the degradation of the NF-κB-IκB complex (Liu *et al*. 2017; Yu *et al*. 2020). The degradation of the NF-κB-IκB complex allows NF-κB to become active and translocate into the nucleus, where it binds to gene promoters to initiate transcription (Gamble *et al*. 2012; Yu *et al*. 2020).

The anti-inflammatory property of garlic was demonstrated by suppressing NF-kB activation, which led to the inhibition of pro-inflammatory cytokine production in human monocytes. In garlic, organosulfur compounds such as alliin and allicin could inhibit TNF-α-induced inflammation by reducing ROS levels (Shang *et al*. 2019b; El Baroroh *et al*. 2020). Furthermore, allicin in single-clove garlic could capture and neutralize ROS, then reduce oxidative stress and prevent cell damage (Le Lay *et al*. 2014). The limitation of this study is that it measures the IL-1β cytokine only, so further study needs to confirm the other cytokines.

The optimal SNEDDS-SBGE met the characterization criteria based on Z-average and PDI but had less optimal zeta potential. The results of the physical stability test showed a stable formula. SNEDDS can increase SBGE antioxidant activity. SNEDDS-SBGE exhibited low toxicity to 3T3-L1 cells, indicated by higher cell viability than SBGE. Therefore, SNEDDS-SBGE can be considered an anti-inflammatory agent on 3T3-L1 cells after being induced with methylglyoxal. Our research suggests testing the other anti-inflammatory markers to know the effectiveness of SNEDDS-SBGE.

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