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Research Paper

# Novel Self-Nanoemulsifying Drug Delivery System of Single Bulb Garlic: Stability, Toxicity, and Antiinflammation in 3T3-L1 Cells

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**Abstract**
Single bulb garlic (SBG) has the potential as an immunomodulator; however, it has low solubility and bioavailability. A lipid-based delivery system, that is, the self-nanoemulsifying drug delivery system (SNEDDS), offers a novel opportunity in drug delivery. SBG can be a suitable candidate for SNEDDS development. This research aims to describe the physical stability, toxicity test of the SNEDDS, and SNEDDS SBG potential as antiinflammation in 3T3-L1 cells. The SNEDDS is made with various ratios of concentrations of carrier oil, surfactants, and cosurfactants, namely, 0.50: 3.45: 0.96, and added with SBG extract (SBGE) of 20 mg/mL. The results of the response test of SNEDDS SBGE formulation indicate an average and standard deviation of emulsification time of 16.38±3.01 (second), pH 7.21±0.08, and transmittance of 98.40±0.23(%). The average nanoemulsion size is 14.333±0.416 nm, polydispersion index of 0.213±0.056, and zeta potential of -14.67±0.72 mV. The results of the physical stability test indicate no segregation, deposition, cracking, or creaming in all nanoemulsion samples and SNEDDS SBG. The MTT test in a dose of 62.5, 125, 250, 500, 100, 2000, and 4000 µg/mL suggests that the highest viability of the 3T3-L1 cells is at a dose of 2000 µg/mL, which is 97.83%±1.55%. Therefore, SNEDDS SBGE can be a potential candidate for oral preparation by increasing bioavailability and reducing toxicity in the 3T3-L1 cells. An antiinflammatory test on the TNF-$\alpha$ and IL-1$\beta$ expressions influences the 3T3-L1 cells. the SNEDDS of SBGE has the potential to reduce the expression of TNF-$\alpha$ and IL-1$\beta$ and increase IL-10 expression in the methylglyoxal-induced 3T3-L1 cells.

**Keywords**
Single Bulb Garlic, SNEDDS, 3T3-L1 Line Cells, TNF-$\alpha$, IL-1$\beta$, IL 10

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## 1. INTRODUCTION

Inflammation is a set of complex interactions between soluble factors and cells that occurs in tissue as a response to traumatic, infectious, postischemic, toxic, or autoimmune injuries. Inflammatory mediators of interleukin-1$\beta$ (IL-1$\beta$) and tumor necrosis factor-alpha (TNF-$\alpha$) could induce the production of reactive oxygen species (ROS). An excess increase in ROS will cause oxidative stress (OS) that triggers metabolic disorders if it occurs for a long time (Fernández-Sánchez et al., 2011; Le Lay et al., 2014).

High production of ROS and a decline in antioxidant capacity lead to metabolic disorders. Supplementation of antioxidants is vital to suppress OS and restore the body to a normal condition. Garlic is one of the plants that can be an antioxidant source and is frequently used by people as medicine. It contains diallyl thiosulfonate (allicin), diallyl disulfide, diallyl trisulfide, dan S-allyl-cysteine sulfoxide (alliin), E/Z-ajoene, S-allyl-cysteine, and diallyl sulfide. Garlic has antibacterial, antiobesity, antiinflammation, immunomodulation, and anticancer properties (Borlinghaus et al., 2014; Papu et al., 2014).

The utilization of active compounds in garlic is less optimal due to its volatility, lipophilicity, strong aroma, and low stability in gastrointestinal (GI) liquids; therefore, it reduces the bioavailability of garlic active compounds (Borlinghaus et al., 2014; Ragavan et al., 2017). In certain conditions, consuming less optimum garlic can result in GI disorders and allergen effects (Abdel-Gawad et al., 2021; Ried and Fakler, 2014). An optimal delivery system of garlic active compounds without reducing its bioavailability is a continuous development at present.

One of the nanosized drug deliveries that are capable of enhancing drug delivery and active compounds' bioavailability is the self-nanoemulsifying drug delivery system (SNEDDS). The SNEDDS is a drug delivery method with an isotropic

mixture of oil, surfactants, cosurfactants, and drug/active compounds that are capable of forming oil nanoemulsion in water with a droplet size of <100 nm when added to watery media, such as GI liquid (Izham et al., 2019; Kazi et al., 2019). The carrier oil used is canola oil since it has low levels of saturated fatty acids (7%), polyunsaturated fatty acids, oleic acid (61%), linoleic acid (2%), and $\alpha$-linoleic acid (11%) (Lin et al., 2013; Mehmood, 2015). Canola oil has antihypertension, antiinflammation, antimutagenic, and antimicrobial properties (Lin et al., 2013; Loganes et al., 2016).

The urgency of the research is based on the decrease of bioavailability and low stability of garlic active compounds in the body. The delivery system and dissolution of garlic active compounds via the SNEDDS have the potential to enhance active compounds' bioavailability in the digestive tract and inhibit proinflammatory cytokine expression. Inflammation seriously impairs quality of life and increases the risk of metabolic syndrome diseases (Ambele et al., 2020; Kazi et al., 2020). 3T3-L1 cells become a model to deeply study the in vitro mechanisms related to inflammation (Ghosh et al., 2013; Yu et al., 2018). The effectiveness test of SNEDDS preparation of canola phase of single bulb garlic extract (SBGE) in the 3T3-L1 cells can be a representative model to explain the effect of the SNEDDS in inhibiting proinflammatory cytokine expression.

This research performs an optimization of the formula, characterization, stability test, and toxicity test with the specific purpose of gaining the formula of the SNEDDS of the SBGE canola phase with high bioavailability and low toxicity levels. The SNEDDS test of the SBGE canola phase in the 3T3-L1 was conducted to evaluate the effectivity of the SNEDDS of the SBGE canola phase as an antiinflammatory agent. It specifically aims to explain the effect of the SNEDDS of the SBGE canola phase on inhibiting proinflammatory cytokine expression of TNF-$\alpha$ and IL-1$\beta$ in vitro.

## 2. EXPERIMENTAL SECTION

### 2.1 Materials

This research used the following materials: single bulb garlic (SBG), 70% ethanol (Merck), canola oil, Tween-80 (Sigma-Aldrich), PEG 400 (Sigma-Aldrich), 3T3-L1 cells were obtained from the Laboratory of Structure, Development, and Physiology of Animals, Biology Department, Science and Mathematics Faculty, Brawijaya University, the liquid medium of Dulbecco's Modified Eagle's Medium (DMEM) Gibco®USA, penicillin–streptomycin Gibco®USA, fetal bovine serum (FBS) Gibco®USA, sodium ethylenediaminetetraacetic acid (Na.EDTA) Nacalai Tesque Inc., Tripcyn Gibco®USA, sodium phosphate anhydrous ($Na_2HPO_4$) Nacalai Tesque Inc., sodium dihydrogen phosphate ($NaH_2PO_4$) Nacalai Tesque Inc., sodium chloride (NaCl) Nacalai Tesque Inc., deionized water Otsuka®, trypan blue Gibco®USA, MTT reagent, demineral water, 4% paraformaldehyde (PFA), dimethyl sulfoxide, Triton-X, pen strep, phosphate-buffered saline (PBS), trypan blue, TNF-$\alpha$ primary antibody, a conjugated secondary antibody of fluorescein isothiocyanate (FITC), IL-1$\beta$ primary antibody, tryptone, methylene blue, Sudan III, aluminum foil, and methylglyoxal (MG).

### 2.2 Methods

#### 2.2.1 Extraction of SBG

The making of SBGE was carried out with maceration using ethanol solvent. One kilogram of SBG (*Allium sativum L.*) was mashed and macerated using ethanol solvent with a ratio of 1:3 SBG to solvent and evaporated using a rotary evaporator. The process produced 100% SBG (*Allium sativum L.*) extract (Fitriana et al., 2019).

#### 2.2.2 SNEDDS SBGE Preparation

The SNEDDS was prepared by weighing the surfactant component of 3.45 g, cosurfactant of 0.96 g, carrier oil of 0.5 g, and SBGE of 50 µL. The components are incorporated in two stages, namely, surfactant and cosurfactants (mixture A) and a carrier oil and SBGE (mixture B). Mixture A and mixture B were prepared using a magnetic stirrer (Thermolyne cimarec®2) for 40 min. The mixture A and B were mixed and homogenized using ultra turrax (IKA©-WERKE) for 5 min and followed by a magnetic stirrer for 40 min. Next, sonication (IWAKI Ultrasonic Cleaner) was carried out for 15 min. SNEDDS SBGE was then stirred using a magnetic stirrer for 60 min and kept for 24 h at room temperature (Ujilestari et al., 2018a).

#### 2.2.3 Response Test of SNEDDS Formula

The emulsification time test was conducted by dissolving 50 $\mu$L of the SNEDDS into 5 mL aquabides (GENERIK) and homogenized using a vortex. The time was calculated until the system dissolved and formed an emulsion. The pH test was done by inserting a probe pH meter into the emulsion solution. %transmittance test was carried out by measuring the emulsion transmission using the spectrophotometer UV–VIS (Libra S11/12 Visible & UV Spectrophotometers) with a wavelength of 650 nm and aquabides as the blank solution (Ujilestari et al., 2018b).

#### 2.2.4 Characterization of SNEDDS Particle Size and Morphology

The measurement of average particle size (Z-average), particle size distribution (PDI), and zeta potential of the SNEDDS of SBGE was carried out using a particle size analyzer (PSA) (HORIBA SZ-100). The morphological characterization of the SNEDDS was identified with observation using a transmission electron microscope (TEM) microscope (Tecnai 200 kV D2360 SuperTwin). Observation using the PSA and TEM took place at ILRC Universitas Indonesia. Samples were diluted three times with aquades at a ratio of 1:1000 (v/v) (Kazi et al., 2019).

#### 2.2.5 Physical Stability Test

Centrifugation test was carried out via the centrifugation of nanoemulsion and SNEDDS at a speed of 5000 rpm for 30

min using a microcentrifuge (IWAKI Micro Centrifuge CFM-1300); segregation, deposition, creaming, and cracking were observed. A hot and cold test was conducted by storing the nanoemulsion and SNEDDS at 4°C and 40°C for 2×24 h (1 cycle) in 6 cycles. Then, centrifugation was conducted at 5000 rpm for 5 min, and observation was carried out to identify segregation, deposition, creaming, and cracking. The freeze-thaw test was conducted by storing the nanoemulsion and SNEDDS at -21°C and 20°C (Izham et al., 2019).

### 2.2.6 3T3-L1 Cell Reproduction

The reagent required must be made and prepared prior to the cell culture. The making of the reagent was conducted in laminar air flow. By mixing approximately 50 mL FBS (10%) and 0.5 mL Penstrep (1%) with 50 mL of liquid medium DMEM, a complete culture medium was prepared. The solution was homogenized until it became homogeneous. Prior to use, the culture media must be warmed up in a water bath at 37°C for 15 min. The stored 3T3-L1 cells must be thawed before use so that the cells can be reactivated after being frozen at -20°C. The 3T3-L1 cells to be used for testing must have a certain number and must be homogeneous between treatment groups; therefore, cells must be calculated to determine the number of cells to be used from the result of cell culture.

### 2.2.7 Toxicity Test of the SNEDDS in 3T3-L1 Cells

The toxicity test of SNEDDS SBGE in the 3T3-L1 cells applied an MTT-assay method. The test materials were removed from each well of the first plate. Then, 50 $\mu$L of MTT reagent (5 mg/mL) was added and incubated for 2 h at 37°C in the $CO_2$ incubator. The MTT solution was then discarded, and 100 $\mu$L of isopropanol was added. The plates were placed on a shaker to solubilize the formations of purple crystal formazan. The absorbance was measured using a microplate reader at a wavelength of 595 nm. The results were used to construct a graph of cell viability percentage against extract concentrations.

### 2.2.8 Inflammation Induction in 3T3-L1 Cells with MG

Before the treatment of SNEDDS SBGE application, inflammatory induction in the 3T3-L1 cell culture was conducted using Methyl Glyoxal (MG). The 3T3-L1 cell culture in the TC flask 25 $cm^2$ with confluent condition ±80% was harvested and the number of total cells was calculated. The well 24 that contained a coverslip was prepared as a cell attachment site. As many as 1×104 cells were transferred into 300 $\mu$L culture media in each 24-well plate with the number of well as needed. The cell condition was observed using a microscope to identify cell distribution. Each well was added with 100 $\mu$L culture media that contained MG of 5 $\mu$g/mL. The treated cells were incubated for 24 h to induce inflammation (Vulesevic et al., 2016; Yamawaki et al., 2008).

### 2.2.9 SNEDDS SBGE Test on the Expression of TNF-$\alpha$, IL-1$\beta$, and IL-10 in 3T3-L1 Cells

TNF-$\alpha$, IL-1$\beta$, and IL-10 expressions of the 3T3-L1 cells after the application of SNEDDS SBGE were evaluated using an immunocytochemistry (ICC) method. The 3T3-L1 cells in the 24-well plate were washed with 100 $\mu$L PBS in each well. Next, PBS was removed using a spuit. The 3T3-L1 cells in each well were given treatments (repeated three times) that consisted of positive control, negative control, cell control, and application of SNEDDS SBGE with an optimum dose. The plates were incubated for 24 h at 37°C with 5% $CO_2$ . At the end of incubation time, the cell media were removed using a spuit. The cells were washed with 100 $\mu$L PBS. The washing was repeated three times. The cells were fixed with 100 $\mu$L 4% PFA for 5 min and then washed with 100 $\mu$L PBS three times. After being washed, the cells were added with 100 $\mu$L 0.5% Triton-x for membrane permeability for 30 min (Vulesevic et al., 2016).

The cells were incubated with primary antibodies for an hour. The cells were washed with the conjugated secondary antibody of FITC for an hour. The cells were once again washed with 100 $\mu$L PBS three times. The coverslip was carefully taken using a tweezer with the help of a needle tip and then placed on a glass object with the cell position at the bottom. The TNF-$\alpha$, IL-1$\beta$, and IL-10 expression in the 3T3-L1 cells were immediately observed using a confocal laser scanning microscope (CLSM) Olympus FV-1000 at Central Laboratory of Life Sciences, Brawijaya University. Prior to the observation, settings were made on the software used. The cell focus filter was set with a magnification of 100–400 times, numerical aperture (NA ¼ 1.4), immersion medium (oil), a working distance (100 mm above the glass coverslip), and type of correction (Plan ApoChromat). The intensity of TNF-$\alpha$, IL-1$\beta$, and IL-10 expression in the 3T3-L1 cells was measured using the Flouview version 4.2a software.

### 2.2.10 Data Analysis

Analysis of the response test, characterization, cytotoxicity test, and expression test of TNF-$\alpha$, IL-1$\beta$, and IL-10 was conducted in SNEDDS nanoemulsion, whereas the physical stability test was carried out in the SNEDDS. Data of the response test and PSA results were input in Microsoft Excel and analyzed according to the average. Data from the physical stability test and TEM results were analyzed descriptively. Data from the toxicity test were analyzed using linear regression. Data from the expression test of TNF-$\alpha$, IL-1$\beta$, and IL-10 were analyzed using a one-way statistical analysis of variance (ANOVA) with provisions of normality test and significant homogeneity. The variance analysis was applied to find out the influence of the preparation of the SNEDDS of the SBGE canola phase on 3T3-L1 cells. If the F-statistic was greater than F-table at a 95% confidence level, the test will be continued with the Duncan's Multiple Range Test (DMRT).

## 3. RESULT AND DISCUSSION

An optimum SNEDDS formulation was reviewed from the response of emulsification time, pH, and percentage of transmittance. The emulsification time plays a crucial role in illustrating

emulsification stability when interacting with water. Table 1 shows the results of the response test.

**Table 1.** Results of Response Test of SNEDDS SBGE Preparation

| Response test | Average ± SD |
|---|---|
| Emulsification time (second) | 16.38 ± 3.01 |
| pH | 7.21 ± 0.08 |
| %Transmittance | 98.4 ± 0.23 |

**Note**: SD, standard deviation

The emulsification time of the SNEDDS SBGE was 16.38± 3.01 s. The result indicates that the emulsification time of the SNEDDS SBGE was within a good category since it was less than 1 min. A good SNEDDS formula must have an ability to perfectly and rapidly disperse when interacting with water with mild agitation. This is related to the easiness of the nanoemulsion formation process (Patel et al., 2011; Wulandari, 2016). Emulsification time is affected by surfactants that play a role in converting oil into smaller particles by reducing the surface tension between the oil and water; thus, forming nanoemulsion faster (Zhao et al., 2016). The smaller the size of the oil phase droplet, the faster the SNEDDS dispersion time (Ujilestari et al., 2018c).

The pH value of the SNEDDS SBGE was 7.21±0.08. The SNEDDS formulation with pH in the range of 7.0–9.0 is stable in an acid medium; hence, it can protect its containing drug from hard gastric juice in the digestive system. pH value determines the chemical stability of the SNEDDS (Zhao et al., 2016). Moreover, a study of in vitro drug release on optimum Ph-SNEDDS indicated a substantial increase in drug release (Izham et al., 2019).

The transmittance percentage of the SNEDDS SBGE was 98.4%±0.23%. The value suggests a self-emulsification process of the SNEDDS SBGE. The nanometer particle size can be seen from the dispersion clarity and transmittance percentage value. A formulation transmittance value above 90% indicates efficient self-nano emulsification of the SNEDDS (Wulandari, 2016). The smaller the size of a particle, the larger its transmittance percentage value is (Priani and Darusman, 2017).

### 3.1 SNEDDS Analysis with PSA

Particle size is an important factor in SNEDDS performance. It determines the level and extent of drug release and absorption. An ideal criterium of the SNEDDS is that it has an average droplet size of under 200 nm to be able to work efficiently. The results of SNEDDS SBGE characterization using PSA are presented in Table 2 and Figures 1 and 2.

The SNEDDS characterization with PSA can be observed from the value of zeta average, polydispersity index (PI), and zeta potential. The PSA results indicate that the size of the nanoemulsion droplet was 14.33±0.4163 nm and the PI value was 0.213±0.058. Nanoemulsion droplet size under 100 nm

**Table 2.** Results of SNEDDS SBGE Characterization with PSA

| Value | Average ± SD |
|---|---|
| Zeta average (nm) | 14.33 ± 0.4163 |
| Polydispersity index | 0.213 ± 0.058 |
| Zeta potential (mV) | -14.667 ± 0.7234 |

**Note**: SD, standard deviation

suggests its efficiency as the SNEDDS (Ambele et al., 2020). Surfactant properties and concentrations determine droplet size. After SNEDDS dilution with water, the microemulsion formed has a very narrow droplet size distribution and it has a crucial role in the effective release of the drug (Baloch et al., 2019). The smaller the particle size, the wider the surface area and, consequently, faster drug absorption and increased SNEDDS bioavailability (Ujilestari et al., 2018b). A PI value that is less than 0.5 has indicated a homogeneous distribution (Baloch et al., 2019). A diagram of the PSA of the SNEEDS SBGE nanoemulsion is illustrated in Figure 1.

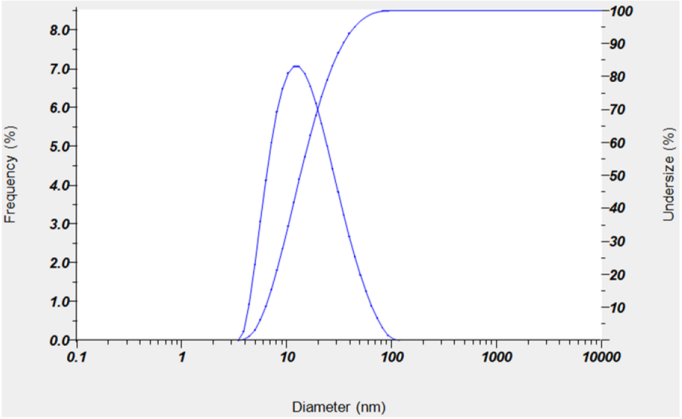


**Figure 1.** Chart of Particle Measurement Results of SNEDDS SBGE using PSA

The zeta potential of the SNEDDS SBGE nanoemulsion is needed to identify SNEDDS nanoemulsion stability to prevent flocculation and aggregation (Betageri, 2019). The zeta potential value of the SNEDDS SBGE was -14.667±0.7234 mV. The zeta potential value that is less than -30 mV indicates a less stable property and allows the formation of aggregate due to attractive force between particles (Pratiwi et al., 2018). Nanoemulsion is stable if its zeta potential value is greater than +30 mV or less than -30 mV (Betageri, 2019). SNEDDS SBGE had a low zeta potential value that triggered a decrease in electrostatic repulsion that will cause phase separation (Priani et al., 2020). A diagram of the zeta potential of the SNEEDS SBGE nanoemulsion is presented in Figure 2.

The physical stability of the SNEDDS formula illustrates the durability of products during storage, utilization, and product shelf life in which the products still have the same charac-

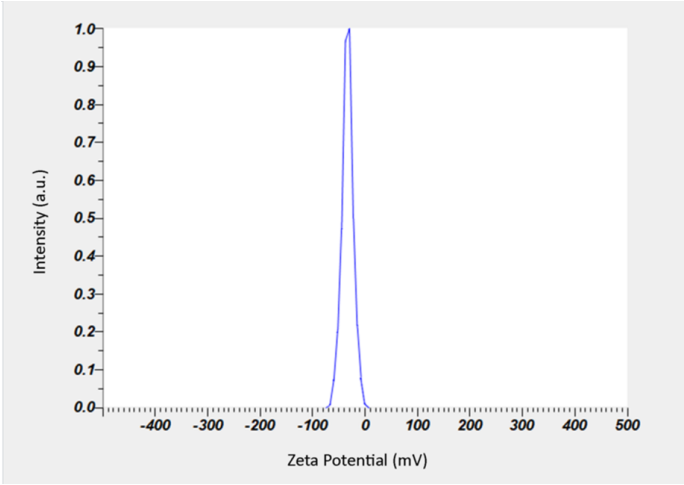


**Figure 2.** Chart of Zeta Potential Measurement Result of SNEDDS SBGE

teristics as when they were produced or experienced changes. The physical stability test of the SNEDDS SBGE consisted of three tests, namely, the hot and cold test, freeze-thawing, and centrifugation. The results of the physical stability test can be seen in Figure 3. In the figure, if the zeta potential is more or less than 30 mv, then the SNEDDS is stable. If the value is between ±30, then the SNEDDS is unstable.


**Figure 3.** Results of Physical Stability Test of the SNEDDS SBGE. (A) Results of the Hot and Cold Test of the SNEDDS SBGE, (B) Results of the Freeze Thawing Test of the SNEDDS SBGE, and (C) Results of SNEDDS SBGE Centrifugation

Figure 3 shows that the results of the physical stability test of the hot and cold test, freeze-thawing, and centrifugation of SNEDDS SBGE did not display segregation, deposition, creaming, and cracking. Figure 3 also indicates that SNEDDS SBGE physically did not experience any changes in shape and color. This suggests that SNEDDS SBGE had thermodynamic stability (Ujilestari et al., 2018c). The small size of SNEDDS particles could minimize Brown movement between nanoemulsion particles that could prevent segregation, deposition, creaming, and cracking. The hot and cold test and freeze-thawing suggest that SNEDDS SBGE could survive temperature changes (Beandrade, 2018). Several factors influence the stability of a drug product, namely, active ingredient stability, the interaction between active ingredients and excipient, manufacturing process, type of preparation, container/closing system for packing, light condition, heat, humidity during delivery, storage, and handling (Zothanpuii et al., 2020).

### 3.2 Toxicity Test with MTT Method

Before performing a toxicity test to ensure whether each component in the SNEDDS SBGE is toxic or not to 3T3-L1 cells, the researcher compared each SNEDDS SBGE concentration with SBGE, cell control, SNEDDS, Tween-80, and PEG 400 in the same concentration. Figure 4 shows the chart of the relationship between each component in SNEDDS SBGE.

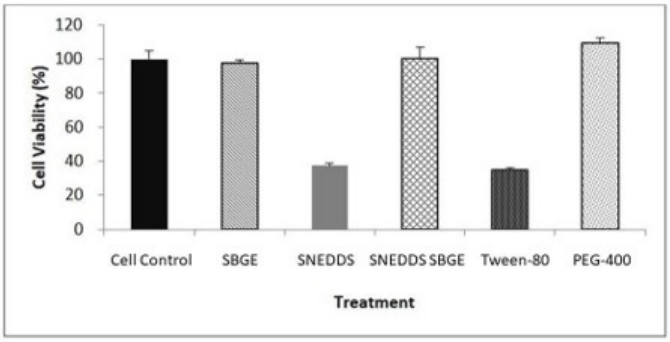


**Figure 4.** Results of Viability Between Treatments of Cell Control, SBGE, SNEDDS, SNEDDS SBGE, Tween 80, and PEG 400 SBGE on 3T3-L1 Cell Viability

Figure 4 shows the cell viability of SNEDDS SBGE was fairly high and almost similar to that of cell control, SBGE, and PEG-400. Treatment of SNEDDS application without SBGE and Tween 80 had low viability. Decreasing or low cell viability below the concentration is due to the decreasing nutrient with the increase in the number of cells and the formation of metabolite results in the cells; therefore, many cells die. Based on the analysis elaborated, a tentative conclusion can be drawn that SNEDDS SBGE is nontoxic to 3T3-L1 cells. The image of 3T3-L1 cells after the application of MTT can be seen in Figure 5.

Figure 5 shows the formation of purple, needle-shaped formazan crystals after the application of MTT. The more the number of formazan crystals formed indicates the more number of living cells, since MTT will only react to living cells to form formazan crystals (Martínez et al., 2020). The values of IC50 from SBGE and SNEDDS SBGE were low. This indicates that SNEDDS SBGE made by comparison between SBG and Tween 80 as a surfactant and PEG-400 as cosurfactants is not toxic to the 3T3-L1 cells in vitro. Therefore, the following test used SNEDDS SBGE with a concentration of 62.5–250 $\mu$g/mL.

Figure 6 shows that groups treated with the application of SBGE and SNEDDS SBGE had a different percentage of living cells. In the SBGE group, the living cell percentage at a concentration of 62.5 $\mu$g/mL continued to increase until

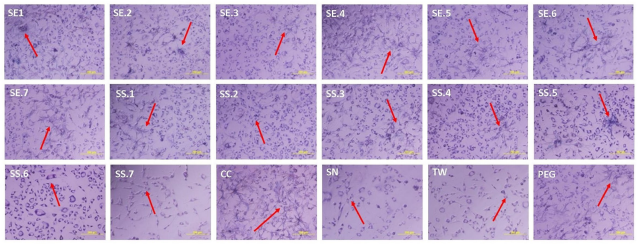


**Figure 5.** Formazan Crystal in the 3T3-L1 Cells After the Application of MTT. SE.1-SE.7 is the Application of SBGE. SS.1-SS.7 is the Application of SNEDDS SBGE. CC is cell control. SN is the SNEDDS only. Tw is Tween-80. PEG is PEG-400. The red arrow (→) indicates the existence of formazan crystal.

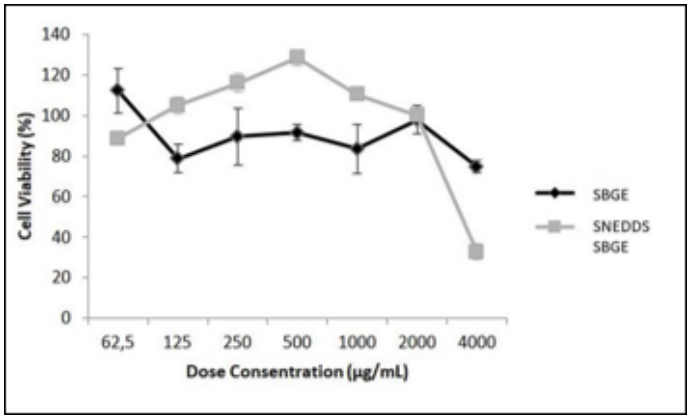


**Figure 6.** Chart of the Relationship Between SBGE and SNEDDS SBGE Concentrations on 3T3-L1 Cell Viability

the concentration of 1000 $\mu$g/mL, and it decreased in the concentration of 2000 $\mu$g/mL and 4000 $\mu$g/mL. The highest living cell percentage was at the concentration of 62.5 $\mu$g/mL for the SBGE group and 500 $\mu$g/mL for the SNEDDS SBGE group.

### 3.3 Data of SNEDDS SBGE Test on the Expression of IL-1$\beta$, TNF-$\alpha$, and IL-10

The research applied an ICC method and Confocal Laser Scanning Microscope (CLSM). The method allows the evaluation of cells in certain samples by producing fluorescence in the desired marker factors, in this case, IL-1$\beta$, TNF-$\alpha$, and IL-10. Based on the measurement results using the ICC method and CLSM, the results of fluorescence intensity are indicated in Table 3 and Figure 7.

The results of the one-way ANOVA of TNF-$\alpha$ indicate a significance level of <0.05 (0.000) suggesting that the application of SNEDDS SBGE of the canola phase had a significant influence on the intensity of TNF-$\alpha$ expression in 3T3-L1 cells. The next step was carrying out a DMRT to find out the differences or similarities in the intensity of TNF-$\alpha$ expression

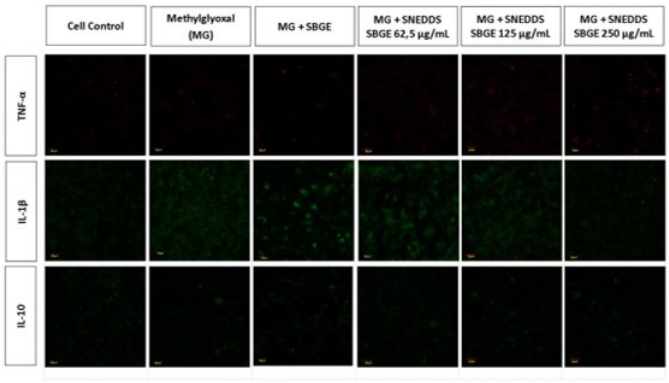


**Figure 7.** Expression of IL-1$\beta$, TNF-$\alpha$, and IL-10 on 3T3-L1 Cells After the Application of Methylglyoxal and Treatments

in 3T3-L1 cells in each treatment group. The chart of the average intensity of TNF-$\alpha$ and the DMRT is illustrated in Figure 8.

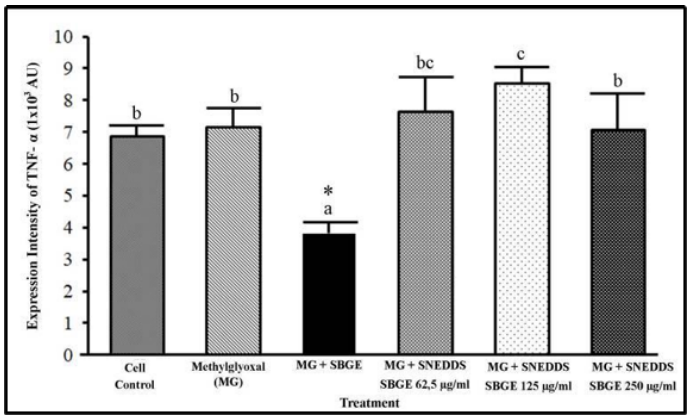


**Figure 8.** TNF-$\alpha$ Expression Toward 3T3-L1 Cells After the Application of Methylglyoxal and Treatments

The results of statistical analysis in the application of SNEDDS SBGE on the intensity of TNF-$\alpha$ expression in the 3T3-L1 cells imply that the application of SNEDDS SBGE at a dose of 62.5 $\mu$g/mL was not significantly different from SNEDDS SBGE at a dose of 125 $\mu$g/mL, SNEDDS SBGE at a dose of 250 $\mu$g/mL, cell control, and MG. SNEDDS SBGE at a dose of 125 $\mu$g/mL, however, was significantly different from SNEDDS SBGE at a dose of 250 $\mu$g/mL and MG+SBGE. The result indicates that the application of SNEDDS SBGE at a dose of 62.5 and 250 $\mu$g/mL was able to inhibit cytokine expression of TNF-$\alpha$ due to MG induction. The application of SNEDDS SBGE at a dose of 62.5 and 250 $\mu$g/mL also caused the TNF-$\alpha$ expression intensity to be not much different from the 3T3-L1 cell control.

In the 3T3-L1 cells applied with SNEDDS SBGE at a dose of 125 $\mu$g/mL, the cytokine expression intensity of TNF-$\alpha$ was not significantly different from MG-induced 3T3-LI cells.

**Table 3.** The Intensity of Cytokine Expression

| Treatment | Average intensity of cytokine expression | | |
|---|---|---|---|
| | TNF-$\alpha$ | IL-1$\beta$ | IL-10 |
| Cell control | 6.893 ± 303 | 84.129 ± 3.851 | 21.425 ± 2.420 |
| Methylglyoxal | 7.169 ± 589 | 116.181 ± 5.350 | 13.044 ± 1.329 |
| MG + SBGE | 3.821 ± 340 | 158.822 ± 6.161 | 12.899 ± 1.110 |
| MG + SNEDDS SBGE 62.5 µg/mL | 7.665 ± 1.055 | 115.815 ± 7.245 | 35.905 ± 3.269 |
| MG + SNEDDS SBGE 125 µg/mL | 8.547 ± 502 | 102.275 ± 2.623 | 15.684 ± 3.693 |
| MG + SNEDDS SBGE 250 µg/mL | 7.085 ± 1.124 | 67.544 ± 4.750 | 23.133 ± 2.942 |

The statistical analysis results indicate that the application of SNEDDS SBGE at a dose of 125 $\mu$g/mL in the MG-induced 3T3-L1 cells caused an increase in the number of TNF-$\alpha$ expression intensity, which was higher than those of 3T3-L1 cells that were only induced by MG. The research results suggest that SNEDDS SBGE at a dose of 125 $\mu$g/mL was unable to inhibit the increase in the TNF-$\alpha$ expression intensity due to the MG induction.

Referring to the analysis of TNF-$\alpha$ expression previously discussed, a tentative conclusion can be drawn that, statistically, the application of SNEDDS SBGE at a dose of 62.5 and 125 $\mu$g/mL was unable to hinder the TNF-$\alpha$ cytokine expression in the 3T3-L1 cells. SNEDDS SBGE at a dose of 250 $\mu$g/mL was capable of inhibiting TNF-$\alpha$ cytokine expression in the 3T3-L1 cells. The application of SNEDDS SBGE at a dose of 250 $\mu$g/mL was the best in inhibiting the TNF-$\alpha$ expression. The result suggests that SNEDDS SBGE affected TNF-$\alpha$ expression in the MG-induced 3T3-L1 cells.

The results of the one-way ANOVA of IL-1$\beta$ indicate a significance level of <0.05 (0.000) suggesting that the application of SNEDDS SBGE of the canola phase had a significant influence on the intensity of IL-1$\beta$ expression in the 3T3-L1 cells. The next step was performing the DMRT to identify any dissimilarities and similarities in the intensity of IL-1$\beta$ expression in the 3T3-L1 cells in each treatment group. The chart of the average intensity of IL-1$\beta$ and DMRTs is illustrated in Figure 9.

The application of SNEDDS SBGE on the intensity of IL-1$\beta$ expression in the 3T3-L1 cells indicates that the application at a dose of 62.5 $\mu$g/mL was significantly different from that of application at a dose of 125 $\mu$g/mL, 250 $\mu$g/mL, cell control, and MG + SBGE. The results imply that the application of SNEDDS SBGE at a dose of 125 and 250 $\mu$g/mL was able to inhibit cytokine expression of IL-1$\beta$ due to MG induction. The application of SNEDDS SBGE at a dose of 125 and 250 $\mu$g/mL also caused the IL-1$\beta$ expression intensity to be not much different from the 3T3-L1 control cells.

In the 3T3-L1 cells applied with SNEDDS SBGE at a dose of 62.5 $\mu$g/mL, the cytokine expression intensity of IL-1$\beta$ was not significantly different from MG-induced 3T3-LI cells. The statistical analysis results indicate that the application of SNEDDS SBGE at a dose of 62.5 $\mu$g/mL in the

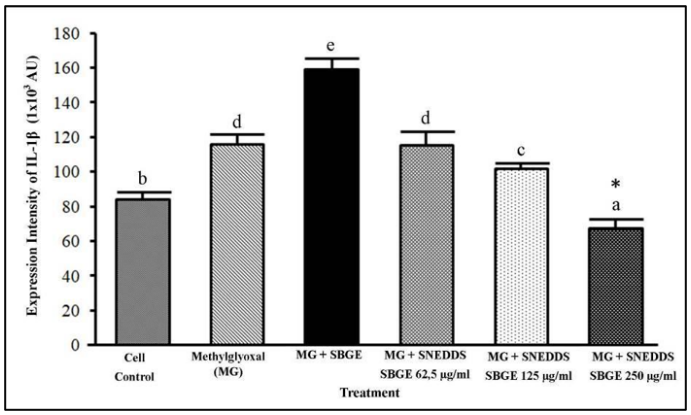


**Figure 9.** IL-1$\beta$ Expression On 3T3-L1 Cells After the Application of Methylglyoxal and Treatments

MG-induced 3T3-L1 cells caused an increase in the IL-1$\beta$ expression intensity, which was higher than those of 3T3-L1 cells that were only induced by MG. The research results indicate that SNEDDS SBGE at a dose of 62.5 $\mu$g/mL was unable to inhibit the increase in the IL-1$\beta$ expression intensity due to the MG induction.

Based on the aforementioned analysis of IL-1$\beta$ expression, a tentative conclusion can be drawn that statistically, the application of SNEDDS SBGE at a dose of 62.5, 125, and 250 $\mu$g/mL was able to inhibit the IL-1$\beta$ cytokine expression in the 3T3-L1 cells compared with the application of SBGE only. According to the analysis results and supporting data, the application of SNEDDS SBGE at a dose of 250 $\mu$g/mL was the best in inhibiting the IL-1$\beta$ expression. The result shows that SNEDDS SBGE affected IL-1$\beta$ expression in the MG-induced 3T3-L1 cells.

The result of the one-way ANOVA of IL-10 indicates a significance level of <0.05 (0.000) signifying that the application of SNEDDS SBGE of the canola phase had a significant influence on the intensity of IL-10 expression in the 3T3-L1 cells. The next step was carrying out a DMRT to identify any dissimilarities and similarities in the intensity of IL-10 expression in the 3T3-L1 cells in each treatment group. The chart of the average intensity of IL-10 and DMRTs is presented in Figure 9.

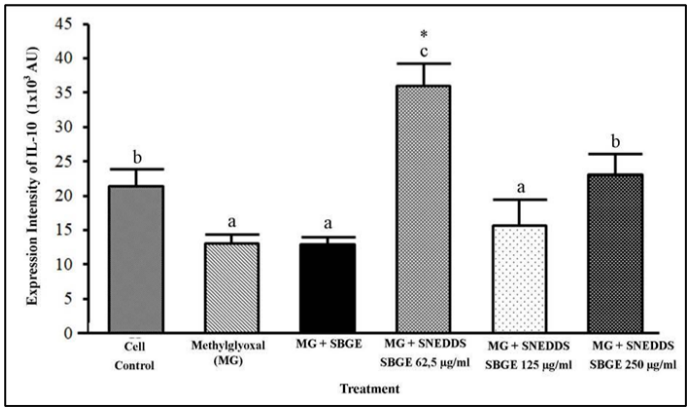


**Figure 10.** IL-10 Expression on the 3T3-L1 Cells After the Application of Methylglyoxal and Treatments

The application of SNEDDS SBGE on the intensity of IL-10 expression in the 3T3-L1 cells points that the application at a dose of 62.5 $\mu$g/mL was significantly different from that of application at a dose of 125 and 250 $\mu$g/mL. The results signify that the application of SNEDDS SBGE at a dose of 125 and 250 $\mu$g/mL was able to increase cytokine expression of IL-10 due to MG induction. The application of SNEDDS SBGE at a dose of 250 $\mu$g/mL also caused the IL-10 expression intensity to be not much different from the 3T3-L1 control cells.

In the 3T3-L1 cells applied with SNEDDS SBGE at a dose of 125 $\mu$g/mL, the cytokine expression intensity of IL-10 was not significantly different from MG and MG + SBGE-induced 3T3-LI cells. The statistical analysis results indicate that the application of SNEDDS SBGE at a dose of 125 $\mu$g/mL in the MG and MG + SBGE-induced 3T3-L1 cells caused a decrease in the number of IL-10 expression intensity. The research results indicate that SNEDDS SBGE at a dose of 125 $\mu$g/mL was unable to increase the IL-10 expression intensity due to the MG induction.

Based on the analysis of IL-10 expression previously elaborated, a tentative conclusion can be drawn that statistically, the application of SNEDDS SBGE at a dose of 62.5, 125, and 250 $\mu$g/mL was able to increase the IL-10 cytokine expression in the 3T3-L1 cells compared to the application of SBGE only. According to the analysis results and supporting data, the application of SNEDDS SBGE at a dose of 62.5$\mu$g/mL was the best in inhibiting the IL-10 expression. The result shows that SNEDDS SBGE affected IL-10 expression in the MG-induced 3T3-L1 cells.

Inflammation in the 3T3-L1 cells was due to MG induction, which is one of the reactive substances that trigger the cells to produce ROS. MG, through the phosphorylation pathway, will open the ATP-sensitive potassium $K^+$ (KATP) channel. The phosphorylation will activate the p.38 pathway, stress-activated protein kinase (SAPK), and c-Jun $NH_2$-terminal kinase (JNK). P 38/SAPK/JNK will induce the secretion of cyclooxygenase-2 (COX-2) in the cytoplasm. COX-2 will give a signal to activate nuclear factor-kappa B (NF-kB) agar; hence, it will enhance transcription to produce IL-1$\beta$ and TNF-$\alpha$. Proinflammatory cytokines of IL-1$\beta$ and TNF-$\alpha$ will hamper proliferation and increase apoptosis and inflammation in cells (Yamawaki et al., 2008; Wang et al., 2019).

Cytokine is a messenger protein that conveys information between cells through molecular receptors on the cell surface. It consists of proinflammatory cytokines that activate macrophage involved in the inflammatory response and antiinflammatory cytokines that relates to derived T cells in the downregulation of the inflammatory response (Park et al., 2010). Cytokines play an essential role in the remodeling and formation of tissue and significantly contribute to homeostatic regulation balance (Song et al., 2020).

Our research explains that the utilization of SNEDDS SBGE and its application along with MG causes the nonoccurrence of an increase in the number of cytokines of TNF-$\alpha$ and IL-1$\beta$ in the 3T3-L1. SNEDDS SBGE was able to prevent the enhancement of proinflammatory cytokine expression of TNF-$\alpha$ and IL-1$\beta$ in the 3T3-L1 cells due to MG induction (Matafome et al., 2013; Vulesevic et al., 2016). SNEDDS SBGE contains bioactive compounds, such as Allicin, which is known as an antioxidant (Birben et al., 2012; Lee and Gao, 2012). Single bulb garlic antioxidant is Allicin and it is required to capture and neutralize ROS to prevent OS and cell damage (Le Lay et al., 2014). Active groups that serve to capture and inhibit free radicals are the -OH group and C=C double bond group (Fernández-Sánchez et al., 2011). The groups can release their one hydrogen molecule so that ROS becomes stable and less reactive new free radical is formed. Flavonoids in the SNEDDS SBGE serve as an antiinflammatory agent (Tran et al., 2014).

Several studies reported that IL-10 can suppress inflammation by inhibiting NF-kB activation (Fernández-Sánchez et al., 2011; Kany et al., 2019). IL-4 and IL-10 are cytokines that generate antiinflammatory effects. IL-10 hampers the production of activated 1L-1$\alpha$, IL-1$\beta$, IL-6, IL-12, and IL-18 (Garlanda et al.; Ghosh et al., 2013; Kany et al., 2019). TNF production and the effect of IL-10 inhibition on IL-1 are crucial in inflammatory activities. These cytokines synergize with inflammatory pathways and processes to increase inflation response by stimulating secondary mediators, such as chemokines and prostaglandins (Park et al., 2010; Vulesevic et al., 2016). IL-10 signaling pathway through JAK/STAT is an immunomodulator pathway with an inhibition effect in the inflammatory mediator that reduces antigen presentation and phagocytosis (Kany et al., 2019).

## 4. CONCLUSION

The research results indicate that SNEDDS SBGE as an optimum SNEDDS had met the criteria of characterization that include droplet size of 14.33±0.42 nm, PI of 0.21, and zeta potential of -14.67±0.72 mV. Moreover, its nanoemulsion morphology did not indicate agglomeration between nanoemulsions. The toxicity test suggests that SNEDDS SBGE was nontoxic to 3T3-L1 line cells. SNEDDS SBGE had higher cell vi-

ability compared with SBGE without being made as SNEDDS. The antiinflammatory analysis of the expression of TNF-$\alpha$, IL-1$\beta$, and IL 10 affected the 3T3-L1 cells. SNEDDS of SBGE had the potential to decrease TNF-$\alpha$ and IL-1$\beta$ expressions, and increase IL-10 expression in the MG-induced 3T3-L1 cells.

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