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RESEARCH ARTICLE

Novel Self-Nanoemulsifying Drug Delivery System (SNEDDS) of Single Garlic Extract: Bio-accessibility, Cytotoxicity, and Anti-Inflammatory Studies

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Abstract Single garlic has many health benefits in which the active compound content five times higher than regular garlic. However, the utilization of the active compound in single garlic was not optimal due to its lipophilic characteristics, volatility, and low stability in gastrointestinal fluids. Self-Nanoemulsifying Drug Delivery System (SNEDDS) has the potential to control the release rate of the active compounds and lead to increase bioavailability in the digestive tract. The study aimed to formulate SNEDDS for single garlic extract (SGE) to increase its bioaccessibility and anti-inflammatory potentials. SNEDDS-SGE was formulated with Tween-80, PEG-400, canola oil, and SGE. SNEDDS-SGE was characterized by response tests including emulsification time, emulsion pH, and transmittance; morphology and droplet size of SNEDDS using TEM; and SGE stability in SNEDDS was evaluated by in vitro gastrointestinal simulation. The cytotoxicity of SNEDDS-SGE was tested using MTT assay, and its anti-inflammatory potential on IL-1ß expression was assessed by the immunocytochemistry (ICC) method on TIG-1 cells induced by methylglyoxal. Response test data and characterization of SNEDDS-SGE were analyzed descriptively, bioaccessibility data were analyzed by T-test, cytotoxicity test by linear regression, IL-1β and C/EBPα expression data by One-way ANOVA. The results showed that SNEDDS-SGE had a fast emulsification time of 41.913 seconds, a stable pH of 7.56, and a high transmittance percentage of 98.027%. The SNEDDS-SGE droplet has a uniform shape with a size of less than 100 nm. SNEDDS-SGE was able to increase the bioaccessibility of SGE by 89.34% for allicin and 89.31% for alliin, also non-toxic at concentrations below 1000 µg/ml. SNEDDS was able to significantly increase the effect of SGE in suppressing the expression of IL-1ß at all concentrations of SNEDDS-SGE 62.5 µg/ml, 125 µg/ml, and 250 µg/ml with the lowest decreased expression of 44.82 ± 4.30 AU. Our study suggested that SNEDDS has the potential to enhance the biological effects of SGE, as well as being a promising anti-inflammatory agent.

Keywords: Single Garlic Extract, SNEDDS, Bioaccesibility, Cytotoxicity, Anti-Inflammatory.

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Introduction

As of early 2021, the COVID-19 disease pandemic had resulted in more than 80 million confirmed positive cases, with a death toll of more than 1.8 million [1]. Although the recommendations for social distancing and the use of masks have shown a decrease in the rate of spread in various countries, the high transmission rate indicates a further wave of transmission that must balance with efforts to identify the pathogenicity of COVID-19 in-depth and specific therapeutic targets [2]. Deaths caused by COVID-19 infection mainly occur through the development of acute respiratory distress syndrome (ARDS), followed by a cytokine storm characterized by hyperinflammatory conditions [2,3]. COVID-19 therapy based on hyperinflammatory treatment is a strategic effort to reduce the increase in mortality due to COVID-19 infection [3].

Utilization of herbal medicines with anti-inflammatory activity can alter immune function through dynamic regulation of molecules such as modulation of cytokine and chemokine secretion; promoter of phagocytosis and activation of macrophages; production of immunoglobulins; and lymphocyte proliferation [4,5]. One of the herbal ingredients that have potential anti-inflammatory activity is single garlic [5–7]. Many studies have shown the remarkable biological functions of garlic, including anti-inflammatory, immunomodulatory, antioxidant, anti-obesity, anticancer, antidiabetic, antibacterial, and cardiovascular protecting [6,8,9]. The main active components of garlic are organosulfur compounds, including diallyl thiosulfonate (allicin), diallyl sulfide (DAS), diallyl disulfide (DADS), diallyl trisulfide (DATS), E/Z-ajoene, S-allyl-cysteine (SAC), and S-allyl-cysteine sulfoxide (alliin) [8,10,11]. Allicin is the main component that has the greatest effect on the biological functions of garlic in the health sector, including anti-inflammatory mechanism [10,11].

Although it has high benefits in the health sector, several studies have shown that garlic's bioactive compounds will undergo transformation and decomposition under certain pH conditions and a certain length of time during the digestion process [12,13]. The mechanism of action of garlic's active compounds is less than optimal due to its low lipophilic characteristics, volatility, and stability in gastrointestinal fluids, which reduces the bioavailability of garlic's active compounds for systemic circulation after oral administration [9,14,15]. The optimal delivery system for garlic's active compounds without reducing its bioavailability is still being developed.

Self-nanoemulsifying drug delivery system (SNEDDS) is a new delivery and dissolving technique that is getting wide attention because of its excellent properties in increasing the solubility and oral absorption rate of active compounds with poor solubility in water [16,17], including the active compounds in single garlic. SNEDDS is an isotropic mixture of drug/active compound, oil, surfactant, and co-surfactant, which forms an oil-in-water nanoemulsion with droplet size <100 nm when introduced into aqueous media, such as gastrointestinal (GI) fluids [18]. The delivery and dissolution system of garlic's active compounds through SNEDDS can control the rate of release of active compounds and lead to increased bioavailability of active compounds in the digestive tract [16,17,19,20].

The results of previous studies showed that the single garlic extract formulation in a mixture of carrier oil, Tween-80, and Glycerol formed a SNEDDS system with good thermodynamic stability and emulsion character [21]. Hence, it can increase the bioavailability and the anti-inflammatory effect of single garlic extract. The study was conducted on the effectiveness of SNEDDS single garlic extract on TIG-1 cells induced by methylglyoxal. SNEDDS single garlic extract (SGE) is expected to be a potential anti-inflammatory candidate in reducing the expression of pro-inflammatory cytokines with low toxicity values.

Materials and Methods

SGE was extracted with ethanol absolute solvent (Merck). SNEDDS was formulated with surfactant Tween-80 (Sigma-Aldrich), co-surfactant Glycerol (Merck), and carrier oil Canola. TIG-1 cell lines obtained from the Laboratory of Structure, Development, and Physiology of Animals, Biology Department, Science and Mathematics Faculty, Brawijaya University. The TIG-1 cell line were grown in Minimum Essential Medium (Gibco®USA), Penicillin-Streptomycin (Gibco®USA), Fetal Bovine Serum USA Origin (Gibco®USA), and induced with methylglyoxal solution (Sigma-Aldrich).

SNEDDS-SGE Preparation

SNEDDS-SGE preparation was carried out by mixing surfactant and co-surfactant (mixture A) using ultra turrax for 5 minutes and stirred for 15 minutes. On the other hand, 100 mg of SGE was added to the carrier oil gradually using a stirrer. The homogenization of the mixture of SGE and carrier oil (mixture B)

was optimized with ultra turrax for 5 minutes and stirred again for 15 minutes. Mixtures A and B were homogenized using ultra turrax for 5 minutes, followed by a stirrer for 15 minutes and a shaker in a water bath at 45°C 500 rpm for 15 minutes. The mixture was stirred again for 15 minutes, sonicated for 15 minutes, and then, the SNEDDS system mixture was rested for 24 hours until it stabilized.

Response Test of SNEDDS-SGE Emulsion

The emulsification time test was carried out by dispersing 50 µl of SNEDDS into 5 ml of aquabidest and homogenized using a vortex, the time was calculated until the system dissolved and formed an emulsion. The pH test was carried out by measuring the pH of the emulsion using a microprocessor pH meter (Hanna Instrument pH 211). The transmittance percentage test was carried out by measuring the transmission of the emulsion using a UV-VIS spectrophotometer (Libra S11/12 Visible & UV Spectrophotometers) with a wavelength of 650 nm with aquabidest as a blank.

Morphological Characterization of SNEDDS-SGE

The morphological characterization of SNEDDS-SGE was carried out by dissolving 50 µl of the SNEDDS system into 5 ml of aquabidest, the morphology of the droplet emulsion was observed using Transmission Electron Microscopy (TEM) Tecnai 200 kV D2360 SuperTwin. TEM observations were carried out at the Integrated Laboratory & Research Center (ILRC), University of Indonesia.

In-Vitro Gastrointestinal Simulation of SNEDDS-SGE

Gastrin phase: Simulation gastrointestinal fluid (SGF) was prepared by dissolving 2 g of NaCl in 7 mL of 37% HCl in aquades until the volume became 1 L and adding 3.2 g of pepsin enzyme. The pH of SGF was adjusted to 1.2 using 1.0 M HCl. 500 µL of SNEDDS-SGE was dissolved in 50 mL of SGF and the pH was adjusted to 2.5 by adding 1.0 M NaOH. The mixture was incubated at 37°C for 2 hours in incubator shaker at 100 rpm.

Intestinal phase: Digesta samples or samples from the gastric phase simulation as much as 30 mL were incubated in an incubator shaker at 37°C for 10 minutes and the pH was adjusted to pH 7 using 1 M NaOH solution. The digesta samples were stirred continuously using a magnetic stirrer, and then, 1 mL $CaCl_2$ 750 mM was added to the mixture, and the pH was maintained at pH 7. About 2.5 mL of pancreatin lipase was added to the mixture, homogenized again with a magnetic stirrer and the pH was maintained at pH 7 using 0.25 M NaOH for 2 hours, until it becomes raw digesta.

Bioaccessibility of Active Compound of SGE

Simulated gastrointestinal or raw digesta samples of 10 mL were centrifuged at 4000 rpm at 25°C for 40 minutes. The results of the centrifuge of raw digesta consist of 3 phases, namely: a cloudy sediment phase at the bottom, a relatively clear micellar phase in the middle, and an oily phase at the top. The micelle phase in the middle is assumed to contain dissolved SGE active compounds. The micelle phase resulted from the centrifugation was aliquoted using a 0.45 µm syringe filter to as much as 5 mL and then, analyzed by HPLC. The bioaccessibility of SGE dissolved in the micellar phase was calculated by equation 1.

$$\% \text{ Bioaccessibility} = \frac{\text{M micelle}}{\text{M emulsion}} x\ 100\ \% \quad \ldots\ldots (1)$$

Description:
M micelles = SGE concentration in micelle phase
M emulsion = SGE concentration on SNEDDS

In-Vitro Cytotoxic Assay of SNEDDS-SGE in TIG-1 Cells

In vitro cytotoxic activity of the SNEDDS-SGE on TIG-1 cell was evaluated by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) reduction assay. The cells were seeded in 96-well plate at density 5×10^3 cells/well in 100 µL medium and incubated overnight at 37ºC, with 5%. Then, SNEDDS-SGE was added to each well with various concentrations of 62.5 ug/ml, 125 ug/ml, 250 ug/ml, 500 ug/ml, 1000 ug/ml, 2000 ug/ml, and 4000 ug/ml [22]. Cells were incubated for 24 hours in an incubator at 37°C, with 5% CO_2. After treatment, the culture media was discarded and the cells were washed using sterile PBS. Then, 100 µl of MTT (0.5 mg/mL) in culture medium was added in each well followed by 4 hours of incubation in an incubator at 37°C, with 5% CO_2. Each well was added with 100 µL of DMSO to dissolve the formazan crystals formed and incubated for 30 minutes at room temperature. The optical density (OD) of each well was determined using ELISA reader at 595 nm. The percentage of cell viability was then calculated by Equation 2.

$$\% \text{ Cell Viability} = \frac{\text{OD of treatment - OD of blank}}{\text{OD of control - OD of blank}} \text{ x } 100\% \quad \ldots\ldots (2)$$

Measurement of IL-1ß Expression in TIG-1 Cells

Expression of IL-1β in TIG-1 cells after SNEDDS-SGE treatment was evaluated by immunocytochemistry (ICC) method. The cells were seeded in 24-well plate with coverslip at density 5×10^4 cells/well in 500 L medium and incubated overnight at 37ºC, with 5%. TIG-1 cells were induced with Methylglyoxal (MG) 5 g/ml and incubated for 24 hours at 37ºC, with 5%. After induction, cells were treated using SNEDDS-SGE with various concentrations and incubated for 24 hours. At the end of the incubation, the culture medium was removed, and the cells were washed with PBS. The cells were fixed with 4% paraformaldehyde (PFA) for 10 minutes and washed with PBS. Cell membrane permeability with 0.5% Triton-X for 30 minutes and blocking buffer with 3% BSA for 30 minutes.

Cells were incubated with IL-1β primary antibodies overnight. Cells were washed with PBS, repeated three times. The cells were incubated with FITC conjugated secondary antibody for 1 hour. After that, the cells were rewashed with PBS for three times. The expression of IL-1β in TIG-1 cells was immediately observed using a confocal laser scanning microscope (Olympus, Japan) at The Central Laboratory of Life Sciences (LSIH), Brawijaya University. The intensity of IL-1β expression in TIG-1 cells was measured using Flouview version 17a software.

Analysis

Response test data and SNEDDS-SGE characterization were analyzed descriptively. Statistical analysis of bioaccessibility data involved T-test, cytotoxicity test was based on linear regression, and IL-1β and C/EBPα expression data was based on One-way ANOVA with $p < 0.05$. All data were presented in the mean ± SD value of three replicates in each treatment.

Results and Discussion

Response and Characterization of SNEDDS-SGE

In the response test, the emulsification time, pH, and percentage of transmittance were tested. Emulsification time is an important parameter to describe the stability of the system and the ease of the process of forming SNEDDS-SGE nanoemulsions in gastric fluid. The average emulsification time of SNEDDS-SGE was 41.913 ± 7.188 seconds which showed that the SNEDDS-SGE had a fast emulsification process because it was below 2 minutes. The short emulsification time is mediated by the action of surfactants and co-surfactants which can quickly form an oil-water interface layer. Co-surfactants will be inserted and form spaces between surfactants with high fluidity which can form nanoemulsions more quickly [20]. A good SNEDDS formula must have the ability to disperse entirely and quickly when in contact with water with mild agitation which can be related to the ease of the process of forming nanoemulsions in a liquid-filled stomach [21,22].

The average pH of SNEDDS EBT was 7.560 ± 0.200. The formulation of SNEDDS at a pH range of 7.0 - 9.0 is stable in acidic media, hence, it is able to withstand hard gastric juices and protect the drug inside. In-vitro drug release kinetic studies showed SNEDDS with optimal pH were able to increase drug release substantially [25]. The average transmittance percentage of SNEDDS-SGE was 98.027 ± 0.730%. The transmittance value of the formulation above 90% indicates the efficiency of self-nano emulsification of the SNEDDS [19].

The morphology of SNEDDS-SGE and the droplet size were observed using TEM microscope as shown in Figure 1. Based on the observation, the nanoemulsion appeared against a light background in which the nanoemulsion droplets were circular with sizes ranging from ±50 nm and almost uniform. Nanoemulsions with droplet dimensions <100 nm have kinetic stability that can minimize the occurrence of aggregation because their small size can reduce the attractive force between droplets [26].

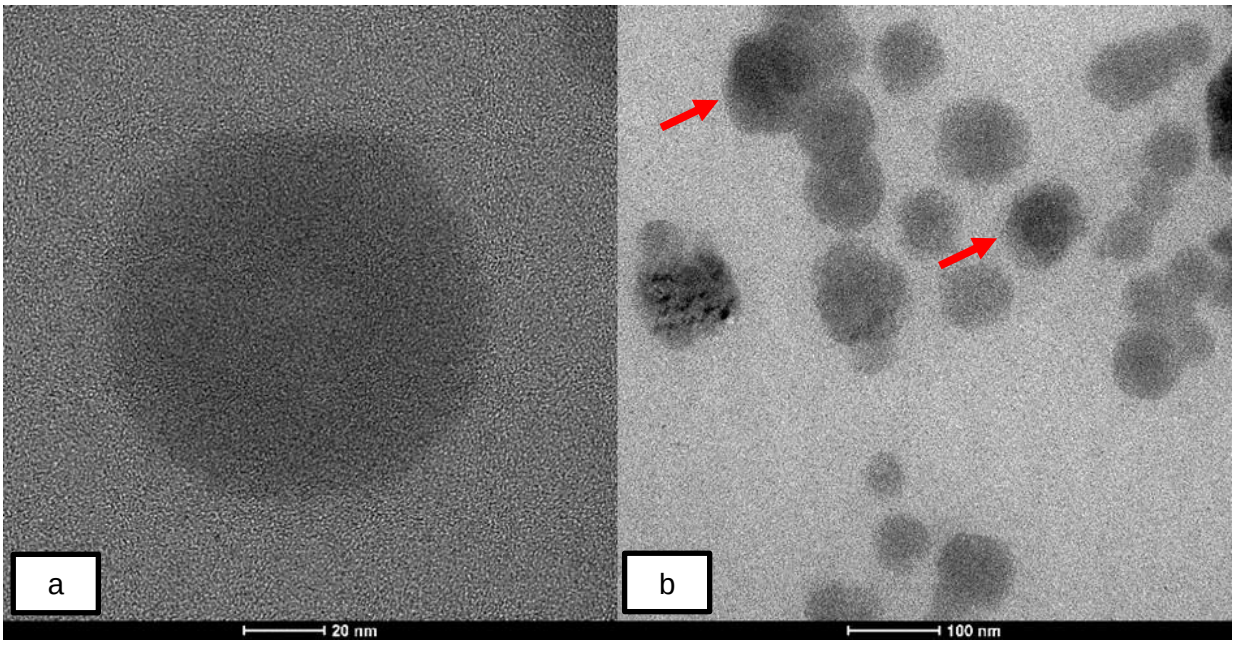

Figure 1. SNEDDS-SGE morphology from TEM observation. (a) The SNEDDS-SGE droplet at 285,000X magnification, (b) The formed SNEDDS-SGE droplet has a spherical structure with a darker center and a lighter surface indicating the SNEDDS-enveloped SGE (red arrow); magnification: 71,000X.

Effect of Gastrointestinal Simulation on Bioaccessibility of Active Compounds on SNEDDS-SGE

In vitro gastrointestinal simulations can be used to predict the effect of dissolution and digestion of lipid-based formulations in digestive fluids. Lipid-based drug delivery formulations must be able to maximize the rate and maintain the availability of active compounds in the gastrointestinal tract until the absorption process [27]. To determine the effect of SNEDDS on maintaining the availability of SGE active compounds, HPLC was performed on allicin and alliin levels in SGE and SNEDDS SGE both before and after the gastrointestinal simulations. The results of allicin and alliin HPLC assays and their bioaccessibility are presented in Table 1.

Table 1. HPLC result and bioaccessibility of Allicin and Aliin on SGE and SNEDDS-SGE

Formulation	Compound Levels Before GI Simulation (µg/ml)		Compound Levels After GI Simulation (µg/ml)		Bioaccessibility (%)	
	Allicin	*Alliin*	*Allicin*	*Alliin*	*Allicin*	*Aliin*
SGE	41959.04 ± 533.84	25031.01 ± 318.76	19345.32 ± 307.11	11527.86 ± 183.38	46.11	46.05
SNEDDS-SGE	18980.44 ± 3204.25	11309.99 ± 1913.32	16956.46 ± 1900.05	10101.43 ± 1134.56	89.34	89.31

The bioaccessibility percentage of allicin in SNEDDS-SGE was 89.34% and alliin was 89.31% higher than the bioaccessibility of allicin and alliin that is not formulated in SNEDDS, allicin was 46.11% and alliin was 46.05%. The results of the SNEDDS study of garlic oil with the formulation of oleic acid as a carrier oil, capryol PGMC (propylene glycol monocaprylate) as a surfactant, and ethanol as a co-surfactant by Sangar, et.al. (2019) showed that after gastrointestinal simulation in vitro 91.80% of the active compounds were able to survive in the gastrointestinal tract [28]. Whereas, in the SNEDDS-SGE study, the content of active compounds after in vitro gastrointestinal simulations that persisted was lower than in previous studies.

The low bioaccessibility of allicin and alliin indicates that the active compound cannot be directly absorbed. Based on the data on bioaccessibility analysis of polymer compounds, 48% can be digested in the small intestine, 42% can be digested in the large intestine, and 10% are not absorbed. [29]. This indicates that the active compound in garlic has a low solubility level and the lipid-based formulation is

able to increase the bioaccessibility of allicin and alliin. This increase in bioaccessibility indicates the availability of the active compound allicin and alliin while in the gastrointestinal tract to the absorption process [30].

Cytotoxicity Effects of SNEDDS-SGE on TIG-1 Cells

Cytotoxicity assay of SNEDDS-SGE on TIG-1 cells was determined by the MTT-assay method, using the mitochondrial dehydrogenase activity of living cells against MTT to form purple formazan crystals. The data obtained was in the form of absorbance of purple formazan crystals measured at a wavelength of 570 nm and then, converted to determine the percentage of living cells presented in Figure 2.

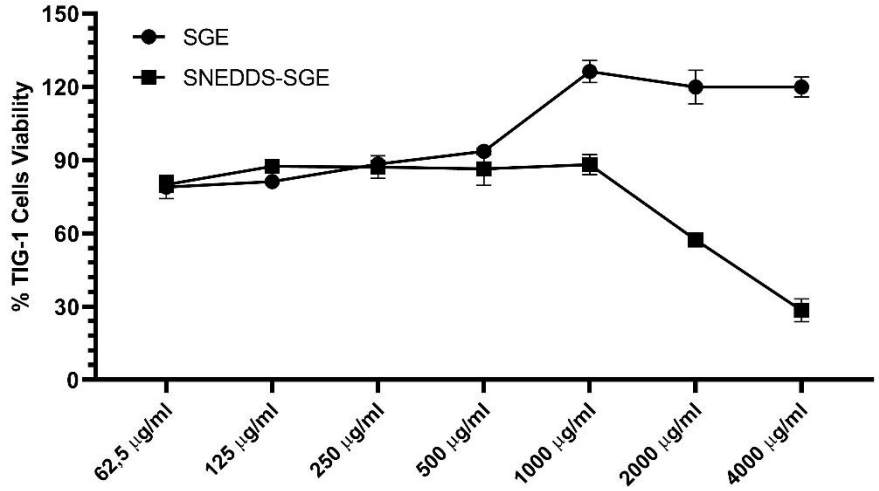

Figure 2. Cytotoxicity profile of SGE and SNEDDS-SGE in TIG-1 Cells

The results of the SGE cytotoxicity test on TIG-1 cells showed that up to the highest concentration of SGE administered at 4000 µg/ml did not show any toxic effects on TIG-1 cells, as evidenced by the cell viability value above 100%. Meanwhile, different results were shown in the SNEDDS-SGE treatment group. At the dose of 2000 µg/ml, the TIG-1 cell viability value of 57.29 ± 0.83% showed moderate toxicity, and non-toxic at concentrations below 1000 µg/ml with a value of viability of 88.14 ± 3.36%.

Determination of the toxicity value of a component based on the ISO-10993-5 standard, which states that the percentage of cell viability above 80% is categorized as non-toxicity, between 80%-60% is categorized as weak toxicity, 60%-40% is in the moderate toxicity category, and below 40% classified as strong toxicity [31]. Garlic extract is known to have a low toxicity value on fibroblast cells [32], but is toxic to cancer cells [33].

The main component in SNEDDS in the form of Tween-80 is a non-ionic surfactant group with HLB > 12, which can spontaneously form SNEDDS with droplet sizes below 100 nm after forming an emulsion in digestive juices in the gastrointestinal tract. In addition, non-ionic surfactants are used in SNEDDS components because of their lower toxicity than the anionic and cationic groups [34].

Effect of SNEDDS-SGE on IL-1β Expression in Methylglyoxal-Induced TIG-1 Cells

The active compound of single garlic has low bioavailability and stability in the digestive tract, so it can decrease its therapeutic effect as an anti-inflammatory. The SNEDDS delivery system is expected to increase the anti-inflammatory potential of SGE. The anti-inflammatory effect of SNEDDS-SGE was evaluated based on its potential to decrease the pro-inflammatory cytokine profile in Methylglyoxal-induced TIG-1 cells using the ICC method shown in Figure 3.

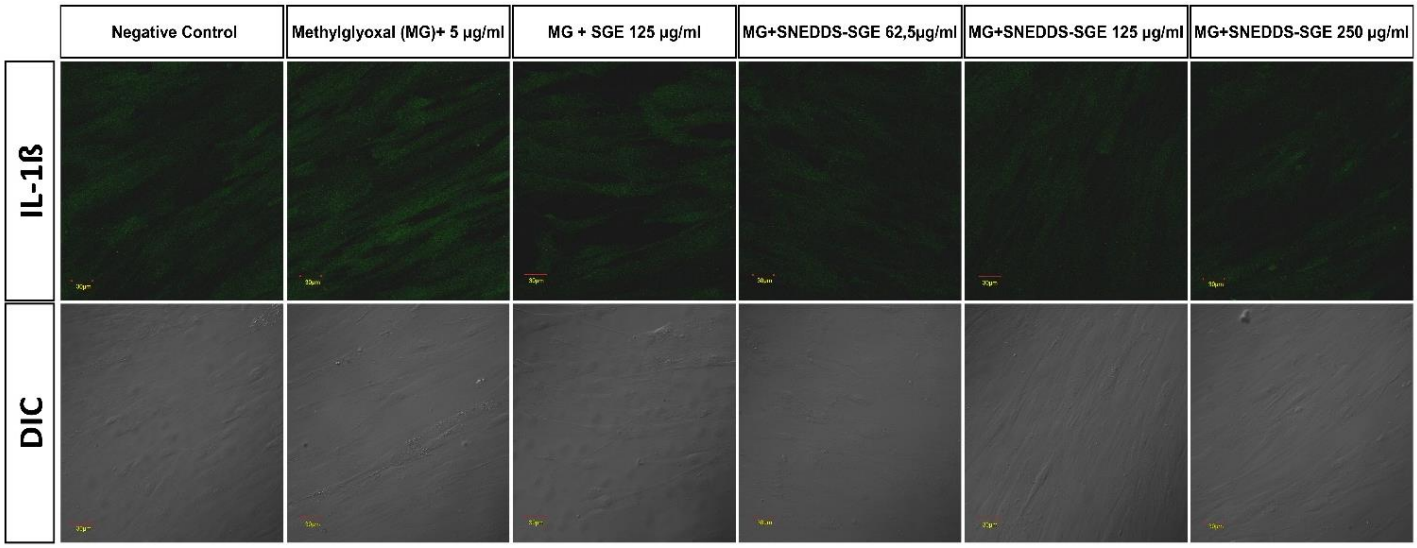

Figure 3. IL-1ß Expression Profile on TIG-1 Cells Induced by Methylglyoxal (MG) with SGE and SNEDDS-SGE Treatment. Magnification: 400x. DIC: Differential interference contrast.

The results of the expression of IL-1ß in TIG-1 cells induced by MG showed that administration of SGE and SNEDDS-EBT was able to reduce ($p < 0.05$) the expression of pro-inflammatory cytokines compared to the MG treatment group (Figure 4). MG increased the expression of the pro-inflammatory cytokine IL-1ß as compared to the control group. The MG plays role in cell inflammation pathways by increasing oxidative stress through the induction of pro-inflammation cytokines of TNF-α, IL-1β, and IL-6, and damage the anti-oxidant ability of cells. In addition, MG could induce inflammation response through the increase in JNK expression and activate the transcription factor of NF-κB [33–35].

Administration of SGE was able to suppress the expression of IL-1ß in TIG-1 cells induced by MG. Extensive studies have shown that garlic with sulfur bioactive compounds involved in regulating the body's immune system, reducing free radicals, and anti-inflammatory [5,10,36]. Garlic and its active components capable of preventing the development of chronic diseases associated with aging, stimulation immune function through macrophage activation, induction of T cell proliferation, reduction of blood glucose levels, radiation protection, protection against microbes, anti-cancer, and anti-hyperlipidemia effects [37-40].

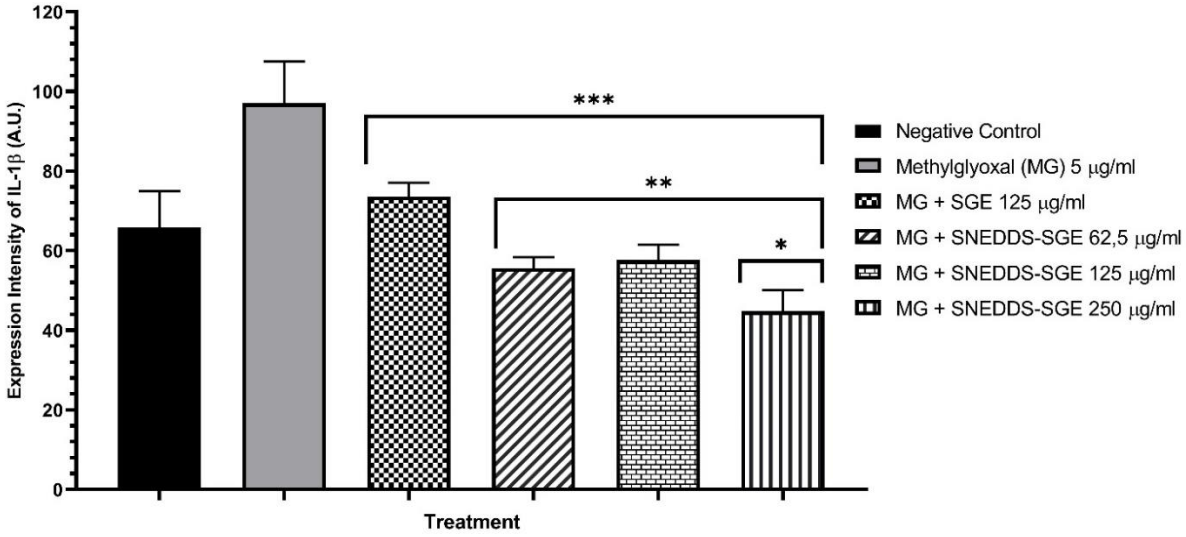

Figure 4. Intensity of IL-1ß Expression on TIG-1 Cells. *Significantly different ($p < 0,05$) from all treatment groups; **Significantly different ($p < 0,05$) from the MG + SGE 125 µg/ml group; ***Significantly different ($p < 0,05$) from the Methylglyoxal (MG) µg/ml group.

On the other hand, the SNEDDS formulation was able to significantly increase the effect of SGE in suppressing the expression of IL-1ß cytokines, with the lowest expression intensity of 44.82 ± 4.30 A.U. at the SNEDDS-SGE concentration of 250 µg/ml. SNEDDS can increase the dissolution and absorption rate of the active compound, reduce the effect of pH variability, and improve the release performance of the active compound [20]. Nanoemulsions help increase the bioavailability of various active compounds through one or a combination of mechanisms in the form of increasing the solubility of the active compound, protection against enzymatic hydrolysis, modification of biochemical functions in the GI tract through the use of surfactants, and inhibition of P-glycoprotein efflux of the active compound [43].

Conclusions

In conclusion, SNEDDS-SGE formulation from this study showed that the nanoemulsion droplet characterization was less than 100 nm, with fast emulsification time, stable pH, and self-nano emulsification efficiency. SNEDDS-SGE can protect and increase the bioaccessibility of allicin and allin compounds almost twice that of SGE. SNEDDS-SGE showed low toxicity at a 2000 µg/ml concentration and non-toxic at below 1000 µg/ml. SNEDDS-SGE treatment on TIG cells induced by methylglyoxal proved that SNEDDS was able to enhance the biologic effect of SGE in suppressing the expression of the pro-inflammatory cytokine IL-1ß. Our research suggests that SNEDDS can protect the active compound SGE and increase its potential as an anti-inflammatory agent.

Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

Acknowledgment

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References

[1] W. H. O. (2021). WHO Coronavirus Disease (COVID-19) Dashboard. *WHO Coronavirus Disease (COVID-19) Dashboard*, https://covid19.who.int (accessed Jan. 04, 2021).
[2] M. A. Torres Acosta and B. D. Singer. (2020). Pathogenesis of COVID-19-induced ARDS: implications for an ageing population. *Eur Respir J.*, *56*(3). Doi: 10.1183/13993003.02049-2020.
[3] P. Mehta, D. F. McAuley, M. Brown, E. Sanchez, R. S. Tattersall, and J. J. Manson. (2020). COVID-19: consider cytokine storm syndromes and immunosuppression. *The Lancet*, *395*(10229), 1033-1034. Doi: 10.1016/S0140-6736(20)30628-0.
[4] Mahima *et al.* (2012). Immunomodulatory and therapeutic potentials of herbal, traditional/indigenous and ethnoveterinary medicines. *Pak. J. Biol. Sci.*, *15*(16), 754-774. Doi: 10.3923/pjbs.2012.754.774.
[5] R. Arreola *et al.* (2015). Immunomodulation and anti-inflammatory effects of garlic compounds. *J Immunol Res*, 2015. Doi: 10.1155/2015/401630.
[6] L. Bayan, P. H. Koulivand, and A. Gorji. (2014). Garlic: a review of potential therapeutic effects. *Avicenna J Phytomed*, *4*(1), 1-14.
[7] J. A. Morales-González *et al.* (2019). Garlic (Allium sativum L.): A Brief Review of Its Antigenotoxic Effects. *Foods*, *8*(8). Doi: 10.3390/foods8080343.
[8] A. Shang *et al.* (2019). Bioactive compounds and biological functions of garlic (Allium sativum L.). *Foods*, *8*(7). Doi: 10.3390/foods8070246.
[9] G. Ragavan, Y. Muralidaran, B. Sridharan, R. Nachiappa Ganesh, and P. Viswanathan. (2017). Evaluation of garlic oil in nano-emulsified form: Optimization and its efficacy in high-fat diet induced dyslipidemia in Wistar rats. *Food Chem. Toxicol.*, *105*, 203-213. Doi: 10.1016/j.fct.2017.04.019.
[10] M. Moutia, N. Habti, and A. Badou. (2018). In vitro and in vivo immunomodulator activities of allium sativum L. *Evidence-Based Complementary and Alternative Medicine*. https://www.hindawi.com/journals/ecam/2018/4984659/ (accessed Dec. 08, 2019).
[11] P. Mikaili, S. Maadirad, M. Moloudizargari, S. Aghajanshakeri, and S. Sarahroodi. (2013). Therapeutic uses and pharmacological properties of garlic, shallot, and their biologically active compounds. *Iran J Basic Med Sci.*, *16*(10), 1031-1048.
[12] T. Park, J.-H. Oh, J. H. Lee, S. C. Park, Y. P. Jang, and Y.-J. Lee. (2017). Oral administration of (S)-Allyl-l-Cysteine and aged garlic extract to rats: determination of metabolites and their pharmacokinetics. *Planta Med.*, *83*(17), 1351-1360. Doi: 10.1055/s-0043-111895.
[13] A. L. Colín-González, R. A. Santana, C. A. Silva-Islas, M. E. Chánez-Cárdenas, A. Santamaría, and P. D.

Maldonado. (2012). The antioxidant mechanisms underlying the aged garlic extract- and S-Allylcysteine-Induced protection. *Oxidative Medicine and Cellular Longevity*, https://www.hindawi.com/journals/omcl/2012/907162/ (accessed Dec. 08, 2019).
[14] H. Wang *et al.* (2015). Influence of pH, concentration and light on stability of allicin in garlic (Allium sativum L.) aqueous extract as measured by UPLC. *J. Sci. Food Agric.*, *95*(9), 1838-1844. Doi: 10.1002/jsfa.6884.
[15] H. Fujisawa, K. Suma, K. Origuchi, H. Kumagai, T. Seki, and T. Ariga. (2008). Biological and chemical stability of garlic-derived allicin. *J. Agric. Food Chem.*, *56*(11), 4229-4235. Doi: 10.1021/jf8000907.
[16] X. Xue, M. Cao, L. Ren, Y. Qian, and G. Chen. (2018). Preparation and optimization of rivaroxaban by self-nanoemulsifying drug delivery system (SNEDDS) for enhanced oral bioavailability and no food effect. *AAPS PharmSciTech.*, *19*(4), 1847-1859. Doi: 10.1208/s12249-018-0991-6.
[17] C. Liu *et al.* (2018). Self-nanoemulsifying drug delivery system of tetrandrine for improved bioavailability: physicochemical characterization and pharmacokinetic study. *BioMed Research International*. https://www.hindawi.com/journals/bmri/2018/6763057/ (accessed Dec. 08, 2019).
[18] M. Kazi *et al.* (2019). Evaluation of self-nanoemulsifying drug delivery systems (SNEDDS) for poorly water-soluble talinolol: preparation, in vitro and in vivo assessment. *Front Pharmacol*. Doi: 10.3389/fphar.2019.00459.
[19] T. Ujilestari, N. D. Dono, B. Ariyadi, R. Martien, and Z. Zuprizal. (2017). Formulation and characterization of self-nano emulsifying drug delivery systems of lemongrass (cymbopogon citratus) essential oil. *Malaysian Journal of Fundamental and Applied Sciences*, *14*(3), 360-363. Doi: 10.11113/mjfas.v14n3.1070.
[20] L. Pratiwi, A. Fudholi, R. Martien, and S. Pramono. (2017). Self-nanoemulsifying drug delivery system (Snedds) for topical delivery of mangosteen peels (Garcinia Mangostana L.,): Formulation design and in vitro studies. *JYP*, *9*(3), 341-346. Doi: 10.5530/jyp.2017.9.68.
[21] S. R. Lestari, A. Gofur, F. Fajaroh, S. Imroatul Maslikah, Y. Annisa, and N. A. N. N. Malek. (2021). Self-nanoemulsifying drug delivery system (SNEEDS) for improved bioavailability of active compound on single clove garlic: Optimization of PEG 400 and glycerol as co-surfactant. *AIP Conference Proceedings 2353*, *Malang, Indonesia*, 030004. Doi: 10.1063/5.0052638.
[22] D. S. Miasih, Y. Annisa, S. R. Lestari, H. Susanto, and S. Sunaryono. (2022). Novel self-nanoemulsifying drug delivery system of single bulb garlic: stability, toxicity, and antiinflammation in 3T3-L1 Cells. *Sci. Technol. Indones*, *7*(4), 417-426. Doi: 10.26554/sti.2022.7.4.417-426.
[23] J. Patel, G. Kevin, A. Patel, M. Raval, and N. Sheth. (2011). Design and development of a self-nanoemulsifying drug delivery system for telmisartan for oral drug delivery. *Int J Pharm Investig*, *1*(2), 112-118. Doi: 10.4103/2230-973X.82431.
[24] E. Wulandari, A. Alverina. (2016). SNEDDS (SELF-NANOEMULSIFYING DRUG DELIVERY SYSTEM) FORMULATION OF β-CAROTENE IN OLIVE OIL (Olea europaea). *IJAR*, *4*(11), 1031-1043. Doi: 10.21474/IJAR01/2179.
[25] T. Zhao, D. Maniglio, J. Chen, B. Chen, and C. Migliaresi. (2016). Development of pH-sensitive self-nanoemulsifying drug delivery systems for acid-labile lipophilic drugs. *Chem Phys Lipids*, 196, 81-88. Doi: 10.1016/j.chemphyslip.2016.02.008.
[26] J. B. Aswathanarayan and R. R. Vittal. (2019). Nanoemulsions and their potential applications in food industry. *Frontiers in Sustainable Food Systems*, *3*. https://www.frontiersin.org/article/10.3389/fsufs.2019.00095.
[27] O. E. Puspita and A. K. Nugroho. (2016). Optimization of self-nanoemulsifying drug delivery system for pterostilbene. *Journal of Food and Pharmaceutical Sciences*, *4*(2), 18-24. Doi: 10.14499/jfps.
[28] P. Sangar, S. A. Bandgar, S. Sardar, P. Pravin, B. Durgacharan, and P. Shitalkumar. (2019). Design, development and evaluation of self nanoemulsifying drug delivery system of garlic oil using capryol PGMC. *Indian Journal of Pharmaceutical Education and Research*, *53*(4), S539-S547. Doi: 10.5530/ijper.53.4s.148.
[29] K. Wojtunik-Kulesza, A. Oniszczuk, T. Oniszczuk, M. Combrzyński, D. Nowakowska, and A. Matwijczuk. (2020). Influence of in vitro digestion on composition, bioaccessibility and antioxidant activity of food polyphenols—a non-systematic review. *Nutrients*, *12*(5). Doi: 10.3390/nu12051401.
[30] F. Shahidi and H. Peng. (2018). Bioaccessibility and bioavailability of phenolic compounds. *Journal of Food Bioactives*, *4*, 11-68. Doi: 10.31665/jfb.2018.4162.
[31] S. H. Chang, J. Y. Liu, M. W. Hsiao, H. L. Yang, G. W. Wang, and J. C. Ye. (2021). Protective effects of ocimum gratissimumaqueous extracts on hacat cells against uvc-induced inhibition of cell viability and migration. *International Journal of Medical Sciences*, *18*(9), 2086-2092. Doi: 10.7150/ijms.54644.
[32] M. C. H. Gruhlke, C. Nicco, F. Batteux, and A. J. Slusarenko. (2017). The effects of allicin, a reactive sulfur species from garlic, on a selection of mammalian cell lines. *Antioxidants*, *6*(1), 1-16. Doi: 10.3390/antiox6010001.
[33] S. K. Vemuri, R. R. Banala, S. GPV, G. R. AV, and M. Thekkumalai. (2018). Apoptotic efficiency of aqueous extracts of turmeric, garlic and their active compounds in combination with Tamoxifen in lung and oral cancers: A comparative study. *Beni-Suef University Journal of Basic and Applied Sciences*, *7*(2), 184-197. Doi: 10.1016/j.bjbas.2017.10.006.
[34] L. Li, C. Hui Zhou, and Z. Ping Xu. (2019). Chapter 14 - Self-Nanoemulsifying Drug-Delivery System and Solidified Self-Nanoemulsifying Drug-Delivery System. In *Nanocarriers for Drug Delivery*, S. S. Mohapatra, S. Ranjan, N. Dasgupta, R. K. Mishra, and S. Thomas, Eds. Elsevier, 421-449. Doi: 10.1016/B978-0-12-814033-8.00014-X.
[35] J. Lu, E. Randell, Y. Han, K. Adeli, J. Krahn, and Q. H. Meng. (2011). Increased plasma methylglyoxal level, inflammation, and vascular endothelial dysfunction in diabetic nephropathy. *Clin Biochem*, *44*(4), 307-311. Doi: 10.1016/j.clinbiochem.2010.11.004.
[36] T. Brenner *et al.* (2014). Methylglyoxal as a new biomarker in patients with septic shock: an observational clinical study. *Crit Care*, *18*(6), 683. Doi: 10.1186/s13054-014-0683-x.
[37] K. M. Desai *et al.* (2010). Oxidative stress and aging: is methylglyoxal the hidden enemy? *Can J Physiol Pharmacol*, *88*(3), 273-284. Doi: 10.1139/Y10-001.
[38] S. R. Lestari, M. F. Atho'illah, Y. I. Christina, and M. Rifa'i. (2020). Single garlic oil modulates T cells activation and proinflammatory cytokine in mice with high fat diet. *Journal of Ayurveda and Integrative Medicine*, *11*(4),

414-420. Doi: 10.1016/j.jaim.2020.06.009.
[39] S. R. Lestari and M. Rifa'i. (2018). Effect of Single Garlic Oil for Homeostasis of CD4$\mathplus$CD25$\mathplus$ Immuno-regulatory T Cells Controlling Hypercholesterolemia. *J. Phys.: Conf. Ser.*, *1093*, 012018. Doi: 10.1088/1742-6596/1093/1/012018.
[40] S. R. Lestari and M. Rifa'. (2019). Regulatory T cells and anti-inflammatory cytokine profile of mice fed a high-fat diet after single-bulb garlic (*Allium sativum* L.) oil treatment. *Trop. J. Pharm Res*, *17*(11), 2157. Doi: 10.4314/tjpr.v17i11.7.
[41] R. R. Ilmawati, A. Gofur, and S. R. Lestari. (2019). Single bulb garlic oil improves interleukin-6 via decreased reactive oxygen species (ROS) in high-fat diet male mice. *Universa Medicina*, *38*(2), 2. Doi: 10.18051/UnivMed.2019.v38.100-107.
[42] S. R. Lestari, Y. I. Christina, M. F. Atho'illah, and M. Rifa'i. (2021). Single-bulb garlic oil regulates toll-like receptors and Nrf2 cross-talk and IL-17 production in mice fed with high-fat diet. *Saudi Journal of Biological Sciences*. Doi: 10.1016/j.sjbs.2021.07.021.
[43] Z. Ke, X. Hou, and X. Jia. (2016). Design and optimization of self-nanoemulsifying drug delivery systems for improved bioavailability of cyclovirobuxine D. *Drug Design, Development and Therapy*, https://www.dovepress.com/design-and-optimization-of-self-nanoemulsifying-drug-delivery-systems--peer-reviewed-fulltext-article-DDDT (accessed Dec. 10, 2019).