Research Article

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Antihyperglycemic activity of *Centella asiatica* (L.) Urb. leaf ethanol extract SNEDDS in zebrafish (*Danio rerio*)

<https://doi.org/10.1515/chem-2021-0200>

received September 17, 2020; accepted November 27, 2020

Abstract: This study aimed to identify the effectiveness of SNEDDS of Pegagan Leaf Ethanol Extract (PLE) to reduce fasting blood glucose (FBG) levels in zebrafish. *Centella asiatica* (L.) Urb. or pegagan is among the medicinal plants widely used to treat diabetes in Indonesia. Maceration was employed with 70% ethanol to obtain a viscous extract for the formulation of SNEDDS with Capryol 90, Tween 80, and PEG 400 (1:6:3). Antihyperglycemic testing was conducted on five groups, consisting of normal, positive control, negative control, P I treatment, and P II treatment. On Day 1, all except the normal group was induced with 300 mg alloxan and soaked in 2% glucose solution for 7 days. On day 8, the treatment consisted of 25 mg/2 L metformin for the positive control, 100 mg/2 L SNEDDS for P I, 200 mg/2 L SNEDDS for P II, and no treatment for the negative control. The SNEDDS characterization obtained 100.6 ± 3.12 nm particle size and -7.93 ± 0.66 mV zeta potential, indicating that the SNEDDS had fulfilled the requirements of good preparation. The anti-diabetic activity test found a 69.90% decline in FBG levels in 100 mg/2 L SNEDDS and 72.20% in 200 mg/2 L SNEDDS.

Keywords: *Centella asiatica*, hyperglycemia, SNEDDS, zebrafish, *Danio rerio*, diabetes

1 Introduction

Diabetes mellitus (DM) is a metabolic disorder characterized by an increase in blood glucose level known as

hyperglycemia due to the inability of the body to produce insulin or because the cells do not respond properly to the insulin produced. The World Health Organization (WHO) estimated an increasing number of people with DM as a global health issue, predictably affecting 629 million people by 2045 [1]. Over the past few decades, the prevalence of DM has risen more rapidly in low- and middle-income countries than in high-income countries. In 2018, the number of patients with DM in Indonesia increased by 2% [2]. Meanwhile, the majority of Indonesians prefers traditional medicine to modern medicine, and one of the plants commonly used to treat DM is Asian pennywort (*Centella asiatica* (L.) Urb.) or pegagan [3]. One of the numerous compounds contained in this herb is flavonoids with their pharmacological activity to decrease blood glucose levels.

Since the ethanol extract of pegagan leaves is nonpolar or poorly water-soluble, the Self Nano-Emulsifying Drug Delivery System (SNEDDS) preparation of this extract is formulated to improve the bioavailability [4]. The antihyperglycemic potential of the SNEDDS preparation of pegagan leaf ethanol extract can be determined by testing it on zebrafish (*Danio rerio*) since the fish have a similar function of blood glucose regulation and can absorb small-sized compounds, including SNEDDS preparation [5]. The purpose of this research is therefore to determine the antidiabetic potential of the Self-Nanoemulsifying Drug Delivery Systems (SNEDDS) of PLE to decrease the fasting blood glucose levels of zebrafish (*Danio rerio*).

2 Materials and methods

2.1 Plant source and extract preparation

The material of this study was pegagan or Asian pennywort (*Centella asiatica* (L.) Urb.) from Kalibawang, Kulonrogo, Yogyakarta. A botanist from the Faculty of Biology,

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Universitas Gadjah Mada Yogyakarta, authenticated the plant taxonomy. Selected pegagan leaves were dried in a cabinet dryer at 35–40°C for 2 days and subsequently processed in a grinder to obtain simplicia powder. Extraction was done by dissolving 1 kg of simplicia powder in 10 L of 96% ethanol at a 1:10 ratio followed by remaceration conducted twice after three days. The extraction result was then filtered using a Buchner funnel, and the supernatant was evaporated in a rotary evaporator (Heidolph L-4000) to obtain a final solvent-free viscous extract [6].

2.2 Formulation of PLE SNEDDS

The SNEDDS composition consisted of Capryol 90 (1 mL) and Tween 20 (6 mL) as the surfactants, PEG 400 (3 mL) as the cosurfactant, and 1,600 mg PLE as the active compound. PLE was weighed and dissolved in Capryol 90 followed by the addition of the surfactant–cosurfactant and 2 min ultrasonication for 4–7 times. After 100-fold dilution, the formation of nanoemulsions was examined, and nanoemulsion separation was visually observed. A spectrophotometer (Shimadzu UV 1800, Japan) was used to measure the transmittance at 630 nm. Only formulations with >90% transmittance would be further investigated for this research [7] (Table 1).

A particle size analyzer was used to identify the particle size and polydispersity index of nanoemulsion droplets. The polydispersity index (PDI) or size distribution represents the standard deviation of mean particle size that determines the uniformity of nanoparticles and reliability of the preparation method.

2.3 Experimental animals

This study involved zebrafish that met the inclusion criteria of 4–6 months old and physically healthy adult fish while dead fish during the study would be excluded. Zebrafish was authenticated at the Research Center for

Biology of the Indonesian Institute of Sciences (LIPI) in Bogor. This study has obtained ethical clearance from the Medical and Health Research Ethics Committee of the Medical Faculty of Universitas Islam Indonesia. Randomly selected male and female zebrafish aged 4–6 months were kept for a minimum of one week before given a treatment. Each group of 10 zebrafish was put in an aquarium with 2 L of water at $28 \pm 2^\circ\text{C}$ and fed on Tetramin Flakes twice a day. A 14–10 light-dark cycle was applied along with observation of water filtration [8].

As much as 300 mg alloxan was mixed with half-normal saline (0.45% NaCl) to soak zebrafish for 60 min at an ambient temperature. In 24 h afterward, the fish were immersed in 2% glucose solution at an ambient temperature for 7 days, and the levels of blood glucose were then measured to identify hyperglycemia [9].

2.4 Experimental design

This study followed the approved protocols for animal research from the Ethics Committee of Universitas Islam Indonesia. Using the posttest-only control group design, the fish were divided into normal, negative control, positive control (100 μM metformin), treatment P I (100 mg SNEDDS), and treatment P II (200 mg SNEDDS) groups each with 10 males and females. On Day 1, 300 mg alloxan was administered to all except the normal group for 1 h followed by administration of 2% glucose for 24 h within 7 consecutive days. Ice-slush anesthetic was given for ± 10 s followed by excision at the back of the head for blood sampling. A glucometer (Autocheck) was then used to check the level of fasting blood glucose.

2.5 Data analysis

Following the examination of blood glucose levels, calculation of the percent reduction against the negative control was performed for the positive control, 200 mg/2 L SNEDDS, and 300 mg/2 L SNEDDS groups.

Percentage of blood glucose reduction

$$\frac{\text{negative control blood glucose level} - \text{normal blood glucose level}}{\text{negative control blood glucose level}} \quad (1)$$

A statistical analysis using independent samples *t*-test was conducted to compare the blood glucose levels

Table 1: SNEDDS formulation of PLE

Material	Function	Quantity (10 mL)
Capryol 90	Oil	1 mL
Tween 20	Surfactant	6 mL
PEG 400	Cosurfactant	3 mL
PLE	Active compound	1,600 mg

of normal and negative control groups. Meanwhile, the Kolmogorov–Smirnov test was used to examine data normality. For normally distributed data, the differences in blood glucose levels were identified through the one-way anova test, while the Kruskal–Wallis test and Mann–Whitney *U* of the *posthoc* test were performed for non-normally distributed data.

Ethical approval: This study followed the approved protocols for animal research from the Ethics Committee of Universitas Islam Indonesia Number 47/KakomEt/70/KE/V/2019.

3 Results and discussion

3.1 PLE SNEDDS formulation

The results of PLE SNEDDS extraction and formulation are shown in Figure 1. The formulation was selected based on literature review and optimization in the laboratory. Capryol 90 has been extensively studied as an oil phase for the development and optimization of nanoemulsions from various water-insoluble drugs both *in vitro* and *in vivo* [10]. Meanwhile, the surfactant and cosurfactant in this study referred to a previous study that used Tween 20 and PEG 400 as the surfactant and cosurfactant, respectively [5].

Tween 20 or polyoxyethylene sorbitan monolaurate is a viscous liquid similar to oil. This liquid has a specific odor of fatty acids and is a type of stable surfactant to be a carrier in nanoemulsions [11]. In addition, PEG 400 with

Table 2: Results of SNEDDS characterization

Type of characterization	Characterization result	Normal range
Particle size (nm)	100.6 ± 3.12	≤ 200
PDI (D)	0.26 ± 0.07	0.20–0.70
Zeta potential (mV)	-7.93 ± 0.66	$\geq +25$ mV or ≤ -25 mV
Percent transmittance (%)	75.42 ± 0.0015	Nearly 100%

such characteristics as a clear, colorless, viscous liquid, specific fatty odor, and mildly hygroscopic property was selected as cosurfactant. In essence, cosurfactants are selected according to their ability to form stable nanoemulsions at minimum concentrations [12].

The smaller the particle size obtained, the greater the surface area produced, thus resulting in higher absorbance. In this study, the obtained particle size (100.6 nm) is in the nanoemulsion range of smaller than 200 nm [4]. Polydispersity Index (PDI) of 0.26 D indicates the uniformity of nanoemulsion particle size (optimum range: 0.20–0.70) [13] (Table 2).

PLE SNEDDS has an average zeta potential of -7.93 mV. A zeta potential greater than $+25$ mV or less than -25 mV indicates a high level of stability [14]. Meanwhile, the percent transmittance is nearly 100%, indicating that SNEDDS produces clear, transparent dispersions.

3.2 Antihyperglycemic effect of PLE SNEDDS

Authentication of zebrafish has identified the name of the species as *Danio rerio* (B-3853/IPH.1/KS.02.03/XI/2017).

Figure 1: (a) PLE and (b) PLE SNEDDS.

Figure 2: Effect of the antihyperglycemic potential of pegagan leaf extract SNEDDS on zebrafish.

Table 3: Percentage of fasting blood glucose reduction of PLE SNEDDS

Group	Glucose level (mg/dL) $\pm$ SD	Blood glucose reduction (%)
Positive control group (metformin)	74.7 $\pm$ 12.4	65.50
Group 1 100 mg/2 L SNEDDS	65.3 $\pm$ 6.9	69.90
Group 2 200 mg/2 L SNEDDS	60.2 $\pm$ 6.16	72.20

The animal experiment strictly followed the protocols from the Research Ethics Committee of Universitas Islam Indonesia Yogyakarta (No. 47/KakomEt/70/KE/V/2019). Zebrafish were selected as the experimental animal since they are normally easy to keep in a laboratory setting, less costly to handle than mammals, and, as a lower vertebrate, more ethical for the screening of drug activity than rats and mice, thus limiting mammals to being involved in a higher phase of preclinical studies [8].

Hyperglycemia was induced by immersing the fish for 60 min. Such process is observable from the difference in the levels of fasting blood glucose ($p < 0.05$) in the negative control group (217 ± 58.9 mg/dL, thus fulfilling the criteria of hyperglycemia) as opposed to that in the normal group (73 ± 9.9 mg/dL, within the normal FBG range of 50–70 mg/dL) [15] (Figure 2).

The results indicate a difference by metformin (74.7 ± 12.4 with $p < 0.05$) between the negative control group and the positive control group. Metformin is an oral anti-diabetic drug of which mechanism of action works by increasing the sensitivity of intrahepatic insulin receptor and accelerating glucose uptake from muscles through facilitating the mobility of glucose transporters, such as GLUT-1 and GLUT-4, to the plasma membrane [8].

In the PI treatment group with 100 mg/2 L SNEDDS, the mean fasting blood glucose level was 65.3 ± 16.9 mg/dL while in P II with 200 mg/2 L SNEDDS for 12 h, the level reached 60.2 ± 6.16 mg/dL. Both results have proved that the administration of PLE SNEDDS at concentrations of 100 and 200 mg/2 L can reduce the fasting blood glucose levels of zebrafish with hyperglycemia which is insignificantly different from that of zebrafish with a normal condition. The results in Table 3 show that the decrease in the fasting blood glucose level of the PLE SNEDDS group is greater than that with metformin. Formulation modification into PLE SNEDDS preparation results in 100.6 ± 3.12 nm particle size which can improve the absorbance and bioavailability of herbal compounds in the body [14].

The SNEDDS of pegagan leaf ethanol extract is an important discovery in the development of traditional

medicine. Of the two concentrations tested, the 200 mg/2 L PLE SNEDDS was more effective to reduce the fasting blood glucose levels of zebrafish than that at 100 mg/2 L.

4 Conclusion

The SNEDDS of pegagan leaf ethanol extract can become an effective antihyperglycemic agent for zebrafish when administered at a concentration of 200 mg/2 L to reduce the levels of fasting blood glucose by 72.20%.

Acknowledgment: The authors thank the Ministry of Research, Technology, and Higher Education for the funding from *Hibah Penelitian Dasar Unggulan Perguruan Tinggi* 2019 (No. 1627.1/LL5/PG/2020). We are also grateful to the Department of Pharmacy of Universitas Islam Indonesia for the facilities, and the Directorate of Research and Community Services of Universitas Islam Indonesia.

Funding: Ministry of Research, Technology, and Higher Education, Hibah Penelitian Dasar Unggulan Perguruan Tinggi 2019 (No. 1627.1/LL5/PG/2020).

Author contributions: F.H. – Conceptualization, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing; L.C. – Conceptualization, Data curation, Formal Analysis, Methodology, Supervision, Validation, Visualization, Writing – review & editing; F.D.S. – Data curation, Formal Analysis, Investigation, Methodology, Validation, Visualization, Writing – original draft; W.S.F. – Data curation, Formal Analysis, Investigation, Methodology, Project administration, Validation, Visualization.

Conflict of interest: The author declares that there are no conflicts of interest regarding the publication of this paper.

Data availability statement: The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

References

- [1] World Health Organization. Global report on diabetes, Chicago; 2016. p. 20–31.
- [2] Kementerian Kesehatan RI. Profil Kesehatan Indonesia 2017, Jakarta 2018.
- [3] Hayati F, Chabib L. Tanaman Obat Indonesia 1. Yogyakarta, Indonesia: Universitas Islam Indonesia; 2017. p. 136.
- [4] Chime SA, Kenechukwu FC, Attama AA. Nanoemulsions: advances in formulation, characterization and applications in drug delivery. In: Sezer AD, ed., Application of nanotechnology in drug delivery. London, UK: InTech; 2014. p. 76–126.
- [5] Hayati F, Chabib L, Dwi Darma D. Antihyperglycemia activity of self-nano emulsifying drug-delivery systems (SNEDDS) of Ipomoea reptans, Poir leaf ethanolic extract in zebrafish (*Danio rerio*). AIP Conference Proceedings 2026. Melville, NY: American Institute of Physics; 2018. p. 02002601–7.
- [6] Hayati F, Chabib L, Tsani SFI, Awaluddin R, Sumayya, Faizah WS, et al. Effects of pegagan (*Centella asiatica* L) ethanolic extract SNEDDS (self-nano emulsifying drug delivery systems) on the development of zebrafish (*Danio rerio*) embryos. J Pharm BioAllied Sci. 2020;12:457–61.
- [7] Syukri Y, Martien R, Lukitaningsih E, Nugroho AE. Novel self-nano emulsifying drug delivery system (SNEDDS) of andrographolide isolated from andrographis paniculata nees: characterization, *in vitro* and *in vivo* assessment. J Drug Delivery Sci Technol. 2018;47:514–20.
- [8] Capiotti KM, Antonioli JR, Kist LW, Bogo MR, Bonan CD, Da Silva RS. Persistent impaired glucose metabolism in a zebrafish hyperglycemia model. Comp Biochem Physiol Behav. 2014;274:319–25.
- [9] Shin E, Hong BN, Kang TH. An optimal establishment of an acute hyperglycemia zebrafish model. Afr J Pharm Pharmacol. 2012;6(42):2922–8.
- [10] Shakeel F, Haq N, Alanazi FK, Alsarra IA. Impact of various nonionic surfactants on self-nanoemulsification efficiency of two grades of Capryol (Capryol-90 and Capryol-PGMC). J Mol Liq. 2013;182:57–63.
- [11] Perugini L, Cinelli G, Cofelice M, Ceglie A, Lopez F, Cuomo F. Effect of the coexistence of sodium caseinate and Tween 20 as stabilizers of food emulsions at acidic pH. Colloids Surf B. 2018;168:163–8.
- [12] Patel V, Kukadiya H. Development of microemulsion for solubility enhancement of clopidogrel. Iran J Pharm Res. 2010;9(4):327–34.
- [13] Mao L, Xu D, Yang J, Yuan F, Gao Y, Zhao J. Effects of small and large molecule emulsifiers on the characteristics of b-carotene nanoemulsions prepared by high pressure homogenization. Food Technol Biotechnol. 2009;47(3):336–42.
- [14] Diba RF, Sedarnawati Y. Nanoemulsifikasi Spontan Ekstrak Jintan Hitam dan Karakteristik Produk Enkapsulasinya. Jurnal Teknologi dan Industri Pangan. 2014;25:134.
- [15] Jorgens K, Hillebrands J-L, Hammes H-P, Kroll J. Zebrafish: a model for understanding diabetic complications. Exp Clin Endocrinol Diabetes. 2012;120:186–7.