Antihyperglycemia activity of self-nano emulsifying drug-delivery systems (SNEDDS) of Ipomoea reptans, Poir leaf ethanolic extract in zebrafish (Danio rerio)

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Antihyperglycemia Activity of Self-Nano Emulsifying Drug-Delivery Systems (SNEDDS) of *Ipomoea reptans,* Poir Leaf Ethanolic Extract in Zebrafish (*Danio rerio*)

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Abstract. *Kangkung* (*Ipomoea reptans*, Poir) is an Indonesian plant possessing antioxidant and antidiabetic activities. The ethanolic extract of *Ipomoea reptans*, Poir leaves is a water-insoluble constituent because of its non-polar characteristic and therefore is formulated into SNEDDS (self-nano emulsifying drug-delivery systems) preparation to improve drug absorption. In this research, the antidiabetic activity of SNEDDS of *Ipomoea reptans*, Poir leaf ethanolic extract was tested to identify its effectiveness in decreasing the fasting blood glucose level in zebrafish. *Ipomoea reptans*, Poir leaves were extracted through maceration using 96% ethanol, and the viscous extract was made into SNEDDS preparation with Capryol, Tween 20, and PEG 400 formulations (1:7:2). The SNEDDS characterization resulted in 94.3 nm ± 2.2 particle sizes and -24.6 mV ± 0.2 zeta potential, proving that it has fulfilled the criteria of good preparation. The SNEDDS of *Ipomoea reptans*, Poir leaf ethanolic extract proved to decrease the fasting blood glucose level of zebrafish induced by 300 mg/100ml alloxan on the first day and 2% glucose for 7 days. The SNEDDS of *Ipomoea reptans*, Poir leaf ethanolic extract in a dose of 200 mg/2L could reduce fasting blood glucose level to 69.03%, while a dose of 300 mg/2L equaled to 67.07% reduction.

INTRODUCTION

Diabetes mellitus (DM) is one of the chronic diseases commonly found in any countries with ever increasing number and significance. The International Diabetic Federation (IDF) reported that the number of DM patients in the world in 2015 within the age range of 20-79 years reached 415 million people with the death toll rising to 5 million people. Indonesia ranked 7th among countries with the largest number of DM patients in 2015 with 10 million people affected [1].

Kangkung (Ipomoea reptans, Poir) is a plant widely used as a foodstuff and has empirically been used as an alternative therapy for DM treatment. It contains carbohydrates, fiber, vitamins, and secondary metabolites, such as β carotene that has an antioxidant activity [2]. The ethanolic extract of *Ipomoea reptans*, Poir leaves has an antihyperglycemic effect on 50 mg/kgBW STZ-induced male rats. From previous research, it is reported that the dose of 200 mg/kgBW *Ipomoea reptans*, Poir leaf ethanolic extract (IPE) shows 64.77% antihyperglycemic activity, while at the dose of 400 mg/kgBW has 24.38% activity [3]. The toxicity test show the safety of repeated administration of *Ipomoea reptans*, Poir leaf ethanolic extract to mice consecutively for 14 days [4].

Ipomoea reptans, Poir *(Kangkung)* leaf extract is a water-insoluble constituent due to its non-polar property and formulated into a self-nano emulsifying drug-delivery systems (SNEDDS) preparation. The SNEDDS is a thermodynamically stable, isotropic mixture of oils, surfactants, co-surfactants, and two drugs that form spontaneous nanoemulsion of oil in water [5]. The SNEDDS preparation can improve the absorption and penetration properties of Ipomoea reptans, Poir leaf extract. Based on the previous study, The SNEDDS of IPE is produced by mixing

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myritol as an oil phase, and tween 20 as surfactant, and its stability is tested physically through the centrifugation test, heating-cooling cycle, as well as freeze-thaw cycle [6].

Meanwhile, in recent decades, zebrafish have been used as a model organism in biomedical studies. Several research projects have used zebrafish as a model for measuring the effects of antidiabetics [7-9]. A total of 101 studies related to glucose homeostasis screening have been conducted through observations of zebrafish blood glucose [10]. Zebrafish have genetic and physiological similarities with human and have the ability to regulate water content and total solutes in the body, making them easily absorb molecules from water [11-12]. The high reproductive capability makes zebrafish easy to breed, and their treatment is relatively less costly than rats or mice [12]. In this study, the antihyperglycemic effect of IPE SNEDDS was measured in zebrafish. This study was aimed to determine the effectiveness of SNEDDS kangkung darat leaf extract on reducing fasting blood glucose level in zebra fish (*Danio rerio*).

MATERIALS AND METHODS

Plant source and extract preparation

The leaves of Ipomoea reptans, Poir aged 21-25 days were obtained from the area of Gantiwarno in Klaten. The taxonomical authentication was performed by a botanist of the Pharmaceutical Biology Laboratory of Universitas Islam Indonesia Yogyakarta. The leaves were chopped and dried at 40^{o}C in an oven. Using the maceration method, two kilograms of Ipomoea reptans, Poir powder was extracted with ethanol 96%. Re-maceration was conducted twice for 6 days to obtain the extract, which was then evaporated for about 2 hours in a rotary evaporator (Heidolph-L4000) at 60ºC and 60 rpm speed [3,13].

Formulation of IPE SNEDDS

The SNEDDS formula of IPE was obtained from a previous study and presented in Table 1.

TABLE 1. IPE SNEDDS Formula

Material	Function	Quantity
IPE	Active Ingredient	500 mg
Capryol-90	Oil phase	0.5 mL
Tween 20	Surfactant	3.5 mL
PEG 400	Co-surfactant	1 mL

After being carefully weighed, the IPE was dissolved completely in the oil phase (Capryol-90). The surfactant and co-surfactant were then added gradually to the solution, and ultrasonication was conducted 4-7 times for 2 minutes.

Experimental Animals

The subject of the study was zebrafish with the following inclusion criteria: a) adult aged 4-6 months and b) physically healthy, and the exclusion criteria was the death of fish during the experiment. Identification of zebrafish was conducted at the Biology Research Center, Indonesian Institute of Sciences (LIPI), Bogor. Ethical clearance was submitted to the ethics committee of the Medical and Health Research of the Faculty of Medicine, Universitas Islam Indonesia. The zebrafish used were randomly selected male and female adults aged 4-6 months and kept at least one week prior to the experiment. The fish were placed in an aquarium with a temperature of 28±2°C in 2L water, and each group had 10 fish. Water filtration was observed and maintained under the photoperiod cycle (14 hours of daylight and 10 hours of dark) [14]. Fish were fed twice daily on Tetramin Flakes fish food.

Determination of alloxan dose referred to the study of Shin et al., in which zebrafish were soaked in 100 ml half-normal saline solution (0.45% NaCl) with 300mg alloxan for 1 hour at a room temperature. They were then transferred to 2% glucose solution within 24 hours at a room temperature. Measurement of blood glucose levels of

the fish was performed to determine whether they had been in a hyperglycemia condition. Each group consisted of 10 adult zebrafish [11].

Experimental Design

All the animal experiments were performed under the protocols approved by the Research Ethics Committee of Islamic University of Indonesia, Yogyakarta. The research design was the post-test only control group design. The treatment group consisted of normal group, negative control, positive control (administered with 100 μM metformin), treatment I (200 mg SNEDDS), and treatment II (300 mg SNEDDS). Each group had 10 male and female fish. All groups except the normal group were given 300 mg alloxan on the first day for 1 hour and 24-hour 2% glucose for 7 days. Prior to blood sampling, the fish were anesthetized with iced water for ± 10 seconds then the blood was taken by excision at the back of the fish head followed by examination of fasting blood glucose level using a glucometer (Autocheck®).

Data Analysis

After the Blood Glucose level in each experimental animal was examined, the percentage of FBG reduction was calculated for the positive control group, 200 mg/2L SNEDDS group, and 300 mg/2L SNEDDS group against that of the negative control group using the following formula:

$$\text{percentage of Blood Glucose reduction} = \frac{negative\ control\ Blood\ Glucose\ Level - normal\ Blood\ Glucose\ Level}{negative\ control\ Blood\ Glucose\ Level}$$

The data obtained was then statistically analyzed using the Independent Sample t-test to compare the differences between Blood Glucose level of the normal group and that of the negative group. Normality test was performed using the Kolmogorov-Smirnov method. If the data were normally distributed, the One-way Anova test would be proceeded to identify the difference in Blood Glucose level levels of the zebrafish. If data were not normally distributed, the Kruskal-Wallis test would be done followed by the Post-hoc test using the Mann-Whitney U Test.

RESULTS AND DISCUSSION

Evaluation of IPE SNEDDS formulation

The SNEDDS formulation comprises oils, surfactants, cosurfactants, and drugs that form a clear and transparent preparation in the aqueous phase; therefore, it is important to identify the solubility of a drug in a preparation to produce a stable SNEDDS formulation [6]. The solubility test of an extract in oil component is carried out to determine the appropriate formula for the IPE SNEDDS. The oil used in this test is capryol, virgin coconut oil, and olive oil. *Kangkung* leaf extract can dissolve in capryol and virgin coconut oil but not in oleic acid. Capryol 90 has a medium-chain triglyceride structure with 8 carbon atoms and COOH groups, while virgin coconut oil has 12 carbon atoms. Olive oil is a highly unsaturated fatty acid with a long-chain triglyceride structure. The 25 long-chain triglycerides are more difficult to form nanoemulsion than medium- and short-chain triglycerides [15].

(a) (b)

FIGURE 1. (a) IPE (b) IPE SNEDDS

The *Ipomoea reptans*, Poir leaf extract was weighed and then dissolved in Capryol 90. The gradual addition of surfactant and co-surfactant to the solution was followed by ultrasonication using the Model 300 VT of Biologics, Inc. for 2 minutes and 4-7 times.

TABLE 2. Data of IPE SNEDDS Characterization

Response	Characterization Results
Particle Size	94.3 nm ± 2.2
Zeta Potential	-24.6 mV ± 0.2

Small particle sizes (below 200 nm) will produce clear and transparent SNEDDS preparations and cause Brownian motion in SNEDDS which can prevent sedimentation or creaming that often occurs in emulsions, thereby increasing the stability of SNEDDS [15]. The zeta potential illustrates the difference in the potential of a strongly binding layer in the preparation associated with the stability of the colloidal dispersion. A stable zeta potential value ranges from ±30 mV and an acceptable minimum value of ± 20 mV [15]. The SNEDDS of IPE has good stability proven by the absence of phase separation in such tests as centrifugation, heating-cooling cycle, as well as the freeze-thaw cycle [6].

Antihyperglycemic Effect on Zebrafish

The results of zebrafish identification showed the species name: *Danio rerio* (B-3853/IPH.1/KS.02.03/XI/2017). The experiment involving animals was conducted based on the protocols issued by the Research Ethics Committee of Universitas Islam Indonesia, Yogyakarta (No.39/KakomEt/70/KE/III/2018). The use of zebrafish (*Danio rerio*) as an experimental animal was based on several considerations, including its ease of maintenance in laboratory conditions with relatively less handling costs than mammals' as well as the use of fish, a lower vertebrate, for drug activity screening which is more ethical than using rats or mice since it may limit the use of mammals only in a more serious pre-clinical stage of research [14].

In this study, alloxan with a concentration of 300 mg/100ml 0.45% NaCl for 1 hour is selected as it is able to increase the blood glucose levels of fish from normal to hyperglycemia and is not toxic. The alloxan is specifically accumulated and selectively toxic to pancreatic beta cells, and the body will improve by increasing insulin secretion [16]. The induction of hyperglycemia for 7 days can maintain a stable diabetes condition and avoid the toxic effects of long-standing glucose induction. Replacement of induced water is done to reduce turbidity and performed every other day to avoid frequent water replacement, which can cause stress on fish. On the 7th day, blood sampling is done to determine the blood glucose levels in each group.

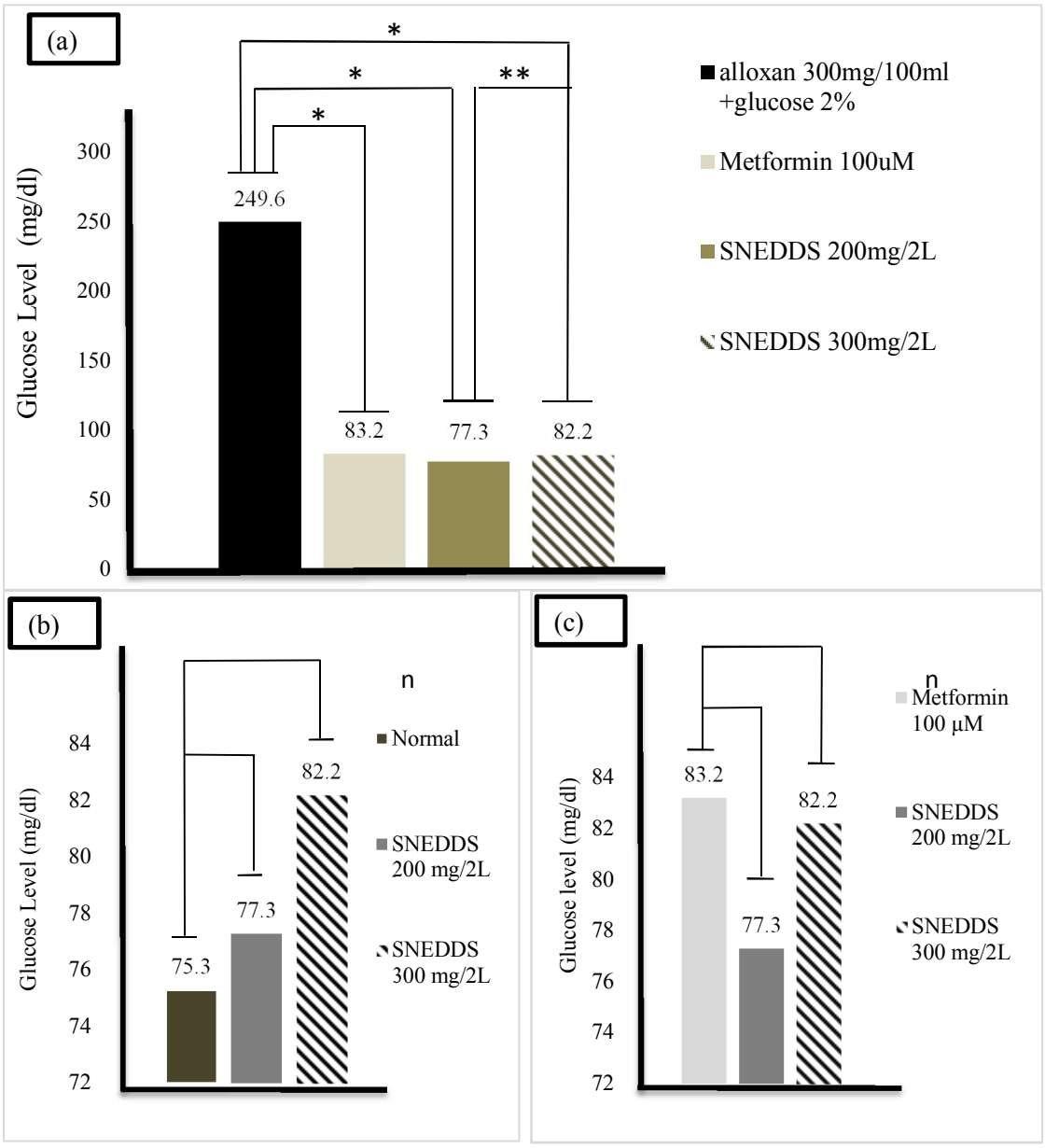

FIGURE 2. Effect of anti hyperglycemic of IPE SNEDDS in zebrafish. Effect of alloxan 300 mg/100ml + 2% glucose (a), normal group (b), and 100 µM metformin (c). Data was analyzed using the Mann-Whitney U Test $^{*}p<0.05$

Metformin affects the regulation of glucose uptake, gluconeogenesis, glycolysis, and glycogen synthesis, making it often recommended as the first-line treatment for patients with type 2 diabetes [17]. Metformin increases the activity of insulin receptors and glucose uptake by increasing the translocation of glucose transporters, such as GLUT-1 and GLUT-4, to plasma membranes [14].

The treatment group I with a dose of 200mg/2L SNEDDS for 12 hours has a mean Blood Glucose level of 77.3 ± 23.4 mg/dl, while the treatment group II administered with 300mg/2L SNEDDS for 12 hours has 82.2 ± 24.8 mg/dl Blood Glucose level. In Fig. 2 (a), both of the SNEDDS treatment group I and group II have significant differences compared to the negative group ($p=0.000 <0.05$). This has proved that the administration of SNEDDS of Ipomoea reptans, Poir leaf extract in a dose of 200 mg/2L and 300 mg/2L can lower the blood glucose levels in hyperglycemic fish. In Fig. 2 (b), there is no significant difference between the normal group and the groups administered with 200 mg/2L and 300 mg/2L of SNEDDS ($p>0.05$). Therefore, it is identified that the decreased FBG levels in zebrafish with SNEDDS can approach the normal condition.

The effectiveness of the IPE SNEDDS as an antidiabetic is compared with metformin, which is a conventional medicine, in figure 2(c). The results show no significant differences ($p>0.05$) between metformin and SNEDDS in a dose of 200 mg/2L or 300 mg/2L, marking the effectiveness of IPE SNEDDS. There is no significant difference ($p>0.05$) between the effectiveness of SNEDDS in a dose of 200 mg/2L and that of 300 mg/2L although the 200 mg/2L SNEDDS has a greater Blood Glucose Level reduction compared with the 300 mg/2L SNEDDS (Figure 2(a)).

TABLE 3. Percentage of Blood Glucose Level reduction (mg/dl) in zebrafish compared to the negative control group

Group	Glucose Level (mg/dl)±SD	% Glucose Level Reduction
100 µM Metformin	83.2 ± 26.36	66.67%
200 mg/2L SNEDDS	77.3 ± 23.4	69.03%
300 mg/2L SNEDDS	82.2 ± 24.8	67.07%

Based on this study, the ethanolic extract of Ipomoea reptans, Poir leaves formed into a SNEDDS preparation has a small particle size (94.3 nm ± 2.2), resulting in a greater surface area of molecules and an increased absorption of the molecules of extract. The presence of oils, surfactants, and cosurfactants that form an emulsion system leads to an increase in the bioavailability of extract molecules in the fish body. When given the ethanolic extract of *Ipomoea reptans*, Poir leaves in a dose of 200 mg/KgBW, streptozotocin-induced rats experienced a decreased Blood Glucose Level by 43.3% and 42.1% in a 300 mg/KgBW dose (Hayati, 2017). The effectiveness of Ipomoea reptans, Poir leaf ethanolic extract has proved to be better after being formulated into SNEDDS preparation. To sum up, the administration of the SNEDDS of *Ipomoea reptans*, Poir leaf ethanolic extract has been able to control the level of fasting blood glucose in fish and is effective as an antidiabetic.

CONCLUSION

The SNEDDS of *Ipomoea reptans*, Poir leaf ethanolic extract proved to be able to decrease the fasting blood glucose level in zebrafish induced with alloxan of 300mg/100ml on the first day and 2% glucose for 7 days. The IPE SNEDDS in a dose of 200 mg/2L can decrease the blood glucose level up to 69.03% and to 67.07% in a dose of 300 mg/2.

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REFERENCES

1. International Diabetes Federation (IDF), Diabetes Atlas, 5th edition. *Russels, Belgium*, 2015, pp. 17-63.
2. K. N. Prasad, G. R. Shivamurthy, and S. M. Aradhya, Int. J. Bot. **4**, **1**, 123-129 (2008).
3. F. Hayati, S. Widyarini, L. Lanova, and M. Wijayanti, The effect of *Ipomoea reptans*, Poir ethanolic extract on the histopathological parameters of pancreas in streptozotocin-induced diabetic rats, AIP Conference Proceedings (American Institute of Physics, Melville, NY, 2017) pp. 1-8.
4. Hayati, F., Murwanti, R. and Zabrina, G. Jurnal Ilmiah Farmasi, **10**, **1**, 1-6 (2013).
5. R. N. Dash, H. Mohammed, T. Humaira and D. Ramesh, Saudi Pharm. J. **23**, **5**, 528-540 (2015).
6. L. Chabib, W. K. Muhtadi, Z. Ikawati, R. Martien and H. Ismail, Int. J. Innovation Res. **3**, **2**, 590-594 (2017).
7. M. P. Sarras, A. A. Leontovich, R. V. Intine, *Comp. Biochem. Physiol*. **178**, 3–7 (2015).
8. K. Marques, D. Almeida, D. Moraes, F. Peres, L. Wilges, M. Reis, et al. Behav Brain Res. **274**, 319–25 (2014).
9. K. Jörgens , J. L. Hillebrands, H. P. Hammes, J. Kroll, Exp Clin Endocrinol Diabetes. **4**, 186-7 (2012).
10. C. A. MacRae, and R. T. Peterson, Nat Rev Drug Discov, **14**, **10**, 721 (2015).

11. E. Shin, B. N. Hong and T. H. Kang, Afr J Pharm Pharmacol. **6**, **42**, 2922-2928 (2012).
12. N. Tabassum, H. Tai, D. W. Jung and D. R. Williams, Evidence-based Complement Altern Med. **23**, 201(2015).
13. F. Hayati, A. Wibowo, P. Jumaryatno, A. Nugroho, D. Amalia, Jurnal Ilmu Kefarmasian Indonesia. **23**, **2**, 151-7 (2015).
14. K. M. Capiotti, R. A. Junior, C. Denise, R. Souza, D. Silva, Comp Biochem Physiol Part B Sci, **171**, 58–65 (2014).
15. B. S. Makhmalzadeh, S. Torabi, A. Azarpanah, Iran J Pharm Res. **11**, **1**, 47 (2012).
16. T. Gohil, N. Pathak, N. Jivani, V. Devmurari, J. Patel, *African J Pharm Pharmacol*. **4**, **5**, 270–5 (2010).
17. American Diabetes Association. Diagnosis and Classification of Diabetes Mellitus. *Diabetes care*. 1;37(Supplement 1) S81-90 (2014).