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Original Article

Formulating Self-Microemulsifying Drug Delivery Systems from Bay Leaves (Eugenia polyantha Wight) with Virgin Coconut Oil and Its Anti-Diabetic Activity

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Abstract

Insulin resistance is a pathological condition associated with the inability of target tissues to insulin response. Bay leaf (Eugenia polyantha Wight) extract has been used for the treatment of insulin-resistant type-2 diabetes mellitus (IRDM), but it has low solubility and bioavailability. To overcome these problems, chloroform extract of bay leaves was formulated into a self-micro emulsifying drug delivery system (SMEDDS) using virgin coconut oil (VCO) as a carrier oil. This study aims to generate a micro-herbal medicine and to determine the effect of a micro-herbal, derived from bay leaves, as an anti-IRDM agent. Homogeneous formulations were evaluated for extract loading, emulsification time, size, size distribution, and the polydispersity index of the nano-emulsion droplets. In addition, their anti-IRDM activities were investigated on insulin-resistant rats using extracts, SMEDDS, metformin, negative control, and normal groups. Each group consisted of five randomly selected male Wistar rats. Blood cholesterol levels were measured at 0, 80, and 95 days. Data were analyzed by using ANOVA. The results showed that the optimum SMEDDS formula was tween 80: PEG 400: VCO (48%:32%:20%) in a total volume of 5 mL. It has less than 1-minute emulsification time with an average 141.4 µm of droplet size and 0.254 of polydispersity index. Morphological observation revealed that the microemulsion particles were spherical and stable in a variety of pH media. The hypoglycemic effects of single-dose metformin, SMEDDS, and the combination of a half dose of SMEEDS with metformin were 28.3%, 15.6%, and 34.6%, respectively. The combination of a half dose of SMEDDS (91.75 mg/kg BW) and a half dose of metformin (22.5 mg/kg BW) provides the best anti-diabetic activity of bay leaves micro-herbal.

Keywords: ADMRI, antidiabetic, bay leaves, diabetic, SMEDDS, VCO.

1. Introduction

Diabetes mellitus (DM) is a metabolic disease characterized by a high level of glucose in the bloodstream as a result of the beta pancreas' failure to produce insulin (type 1) or a lack of insulin response in the target

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tissue (type 2) [1]. According to the estimation of The International Diabetes Federation, Diabetes Atlas (2013), it was estimated that 382 million people diagnosed with DM in 2013, and eventually this figure will increase to 592 million people in 2035 [2].

Numerous attempts have been made to cure diabetes by a pharmacological approach. One such strategy is to use a natural compound, such as the bay leaf. Studiawan and Santosa (2005), reported that an ethanolic extract of the bay leaf could decrease blood glucose levels in mice after induction by 12.97% alloxan or 2.62 mg/20 g body weight [3]. Another study by Wahjuni *et al*. (2018) also reported a similar effect after alloxan induction [4]. The methanol extract of the bay leaf also exhibits an antihyperglycemic effect to streptozocin-induced mice [5]. Surprisingly, natural products from this extract have low solubility and bioavailability, making them ineffective as therapies [3].

There are numerous techniques to increase solubilities, such as surfactant usage, micronization, salt formation, pH alteration, nano size delivery, solid dispersions, a permeation enhancer, and self-micro emulsifying drug delivery systems (SMEDDS) [6]. Importantly, SMEDDS have been proven to increase the bioavailability of lipophilic drugs administered orally. Technological advances enable SMEDDS to overcome the problem of delivery of drugs with low water solubility [7]. The SMEDSS offer numerous benefit such as physically stable, easy to manufacture, can be applied in soft gelatin capsules that make a drug dispersed easily in the gastrointestinal tract. Enhanced bioavailability and solubility are primary advantages of SMEDSS [6].

The primary components of a SMEDDS formulation are oil, surfactant, and cosurfactant. The surfactant is responsible for decreasing surface tension. The selection of a surfactant in SMEDDS is based on the safety of its application and its hydrophilic-lipophilic balance (HLB). Tween 80 has high HLB and suitable for creating type O/W microemulsions. Moreover, tween 80 is a non-ionic surfactant that is widely used as an emulsifying agent [8]. The cosurfactant determines the emulsification time in the media and the microemulsion size; its small size enables to place itself between surfactant molecules. The cosurfactant usually in the form of an amphiphilic substance like propylene glycol, polyethylene glycol, and glycol ester, which have a high affinity for both water and oil phases [9]. PEG 400 was chosen as the material during the first screening because it increases the solubility of a hydrophilic surfactant or oil-based drug [10]. Oil is also an indispensable part in SMEDDS formulation.

Plant-based oils are the most often used oils in SMEDDS formulation. Plant-based oil is one of the first choices in the formulation. It is

easier to degrade for microorganisms and eco-friendly [11]. In this study, virgin coconut oil (VCO) is used as the oil component of SMEDDS because VCO has medium-chain fatty acids with a high content of lauric acid, which can increase the emulsification time [12].

In a present study, we investigated a micro-herbal formulation from the bay leaf using VCO as carrier. The VCO used to produce a micrometer-sized emulsion. Therefore, it enhances bioavailability of the bay leaf extract through oral route and can be used in low doses for SMEDDS microemulsion. Similar studies have been reportred that the bay leaf anti-diabetic and antihyperblicemic activities, but none of them use SMEDDS system for its administration [3-5]. Besides that, we also evaluated the formulation for its anti-diabetic effectiveness using animal model.

2. Materials and Methods

2.1. Materials

The bay leaf (Eugenia polyantha W.) was obtained from Klaten, Middle Java, Indonesia. It was identified at the Biological Laboratory of Universitas Sebelas Maret (No. 25/UN27.9.6.4/Lab/2016). Distilled water (Ikapharmindo Pharmaceutical Laboratories, Indonesia), chloroform (Merck, Germany), VCO, PEG 400, propylene glycol, tween 20, tween 80, NaCl, H2SO4, MgCl2, CaCl2, NaHCO3, and KCl were used in SMEDDS. The materials from VCO to KCl were obtained from MIPA Terpadu Laboratories, Indonesia. High-fat ransom (food pellet (80%), pig fat (15%), and duck egg yolk (5%), 0.5% Na-CMC, glibenclamide (Generic), Glucose GOD FS reagent Dyasis (GmBH, Germany), metformin (Hexpharm Jaya, Indonesia), and fructose were used to determine the antidiabetic activity.

2.2. SMEDDS Formulation

2.2.1. Bay Leaf Extraction

The bay leaf simplisia powder was extracted by the maceration method. A 500-g quantity of powder was submerged in 4 L chloroform solvent for 5 days. After that, the mixture was evaporated by using a rotary evaporator (RVO 400 SD Boeco, Germany) at 55 °C. After that, the extract was standardized by its presence of alkaloids, carbohydrates, tannins and phenols, glycoside, flavones and flavonoids, steroids, and saponins [13]. The homogeneous extract of the bay leaf was used for further analysis.

The presence of the afromentioned components was performed according to Harbone protocol [14]. Alkaloid was measured by using the Dragendorf, Meyer and Wagner reagent after fractionated with chloroform and H2S04. Molicsh test was used to determine the carbohydrate content. Tannin content was determined with FeCl3 1% after boiling the extract. FeCl3 5% was used to measure the phenol content after 70% ethanol extraction. The flavonoids were determined with magnesium and amil alcohol (37% HCl and 95% ethanol). Glicoside was measured by using methanol extraction and added the glacial acetic acid with 1% FeCl3 and H2SO4. Steroid or triterpenoid was determined by using Lieberman Burchard test. Lastly, the

foaming test was used to determine the saponin content.

2.2.2. Surfactant, Cosurfactant, and Oil Composition

The bay leaf extract was added to 5 mL carrier solution (carrier oil: VCO; surfactant: tween-20 and/or tween-80; and cosurfactant: PEG 400 or propylene glycol) in various ratios. The mixture was homogenized by vortex for 1 minute, sonicated for 10 minutes, and placed at 45 °C in the water bath for 15 minutes. The optimal composition of SMEDDS is obtained when the mixture does not separate after treatment.

2.2.3. SMEDDS Formula Selection

A 100-µL sample of the SMEDDS formula was added up to 5 ml of distilled water [11]. The solution was homogenized by vortex for 30 seconds. The microemulsion procedure was determined visually as an initial confirmation; a clear solution was indicated with homogenized mixture. Subsequently, if the mixture was visually homogen, the transmittance of the SMEDDS sample was measured by using spectrophotometry at a wavelength of 650 nm with distilled water as a blank [9] If the transmittance of the SMEDDS was closely to distilled water (100% T), then the emulsion drop was reached at the micrometer level.

The emulsification time of the SMEDDS was measured in three kinds of media: (1) distilled water; (2) artificial gastric fluid without pepsin (AGF); and (3) artificial intestinal fluid without pancreatin (AIF).

Extract loading optimization was performed by using a weight series of the bay leaf extract: 100, 125, 150, 175, and 200 mg in 5 g of the SMEDDS. The solubility of the extract in the SMEDDS was observed visually. The highest extract concentration produced a clear solution with no sediment. It was considered as the optimum extract of the SMEDDS loading capacity.

2.2.4. Microemulsion Drop Characterization

A particle size analyzer (Horiba SZ-100) was used to measure the size and distribution of the microemulsion particles. The morphology of the emulsion was observed by using a transmission electron microscope (TEM) as well.

2.3. Pharmacological Activity Test

2.3.1. Insulin-Resistant Experimental Animal

Insulin-resistant type-2 DM was induced in rats by administration of 660 mg/200 g of rat body weight (fructose concentration was 300 mg/mL) with fructose diet through oral route and high-fat diet (80% food pellet, 15% pig fat, and 5% duck egg yolk). Both diets were given for 80 days. An insulin resistance test (hypoglycemic and glibenclamide activity) was used to verify the presence of insulin resistance. Rats were considered insulin resistant if the hypoglycemic activity was significantly lower than the normal group [15].

2.3.2. Insulin Resistance Antidiabetic Activity Test

The antidiabetic activity of the bay leaf extract delivered by SMEDDS was evaluated

on 15 days in a completely randomized design. This study had six treatment groups, with five rats per group (n = 5): Group 1, the normal control group, was given distilled water and standard feed; group 2, the negative control, was given a high fructose-fat diet (HFFD) and distilled water; group 3, the positive control, was given the HFFD and 45 mg/kg body weight metformin in 0.5% Na-CMC suspension; group 4 was given the HFFD diet with 183.5 mg/kg body weight in 0.5% Na-CMC suspension of chloroform-fraction bay leaf extract; group 5 was given the HFFD diet with 183.5 mg/kg body weight of SMEDDS-bay leaf extract; and group 6 was given the HFFD diet with a half dose of metformin (22.5 mg/kg body weight) and a half dose of SMEDDS-bay leaf extract (91.75 mg/kg body weight). All treatments (distilled water, metformin, bay leaf extract, and SMEDDS-bay leaf extract) were given through oral route, once per day. The dose formulation was based on [3] and [16] and converted to rat animal model according to the dose of conversion table [17].

Blood sample was obtained from the orbitalis vena in the eye by using a hematocrit (Virtex). The serum was mixed with Glucose GOD FD. The blood glucose level was measured by using a UV/Vis spectrophotometer (Perkin Elmer Lambda 25).

2.3.3. Data Analysis

The blood glucose level on the 80^{th} and 95^{th} days was analyzed with a paired *t*-test to assess the changes of glucose level after treatment. The same data were used to determine the percentage of hypoglycemic activity with the following formula:

$$\text{Hypoglycemic activity (\%)} = \frac{\text{glucose level at 80th day} - \text{glucose level at 95th day}}{\text{glucose level at 80th day}} \times 100$$

The percentage of hypoglycemic activity for each group was represented graphically. The acquired data were further analyzed by using ANOVA with a 95% confidence level for between-group comparisons [18].

3. Results and Discussion

3.1. Extract Standardization

The bay leaf extract was standardized before utilizing the formulation. The rendement result from raw materials to extract was 2.51% with these following phytochemical content (Table 1).

Table 1. The phytochemical substances of the vay leaf extract.

Substances	Result
Alkaloids	+
Carbohydrates	-
Tannins and Phenols	+
Glycosides	+
Flavones and Flavonoids	+
Steroids	+
Saponins	-

+: positive test
- : negative test

According to Patel *et al*. (2012), the antidiabetic activity of herbal plants was indicated by the presence of polyphenols, flavonoids, terpenoids, and coumarins. The bay leaf extract had numerous of phytochemical substances that potential as anti-diabetic activity. Therefore, it was used for further analysis.

Table 2. Surfactant, cosurfactant, and oil optimization.

Surfactant	Surfactant: cosurfactant composition	VCO:surfactant-cosurfactant (1:4)	
		PG	PEG
Tween 80 (T80)	1 : 1	x	v*(F1)
	2 : 1	x	x
	3 : 1	x	x
	3 : 2	x	v*(F2)
	2 : 3	x	x
	1 : 3	x	x
	1 : 2	x	x
Tween 20 (T20)	1 : 1	v*(F3)	x
	2 : 1	x	x
	3 : 1	v*(F4)	x
	3 : 2	x	x
	2 : 3	v*(F5)	x
	1 : 3	x	x
	1 : 2	v*(F6)	x
T80 : T20	**Composition of surfactant**	**T80/T20 : PG (3 : 1)**	**T80/T20 : PEG (3 : 1)**
	1 : 1	x	x
	2 : 1	x	x
	3 : 1	x	x
	3 : 2	x	x
	2 : 3	x	x
	1 : 3	x	x
	1 : 2	x	x

x: not homogenous; v: homogenous

3.2. Surfactant, Cosurfactant, and Oil Composition

As presented in table 2, the optimal composition that produced a homogenous mixture was the mixture of tween 80 and PEG in a 1:1 or 3:2 ratio and mixed with VCO in a 4:1 ratio (first and second formula). In addition, tween 20 and PG (1:1, 3:1, 2:3, and 1:2) mixed with VCO in a 4:1 ratio (formulas 3, 4, 5, and 6) also produced a homogenous mixtures.

3.3. SMEDDS Formula Selection

This process was performed to determine the optimum surfactant-cosurfactant-oil formula, which was formula 1, 2, 3, 4, 5, and 6. Observation of the transmittance (%) showed that formulas 2 and 3 had >80% transmittance at a wavelength of 650 nm (Table 3). A transmittance level was closely with distilled water. It indicates that the emulsion drops are small and around 50-500 nm in size [19]. Both formulas 2 and 3 were measured to determine their emulsification time.

As a shown in table 4, formula 2 produced faster emulsion in media with a pH value as similar as artificial gastric fluid. In addition, formula 3 also produced faster emulsion in media with a pH value as similar as artificial intestinal fluid and distilled water.

Table 3. Transmittance (%) test of each optimal composition formula.

Formula	Surfactant	Cosurfactant	Ratio Surfactant: Cosurfactant	% Transmit (x±sd)
1	$\frac{Tween}{80}$	PEG 400	1:1	71.47667±0.371
2			3:2	83.8633±0.021*
3			1:1	80.32333±0.55*
4	$\frac{Tween}{20}$	PG	3:1	44.59±0.907
5			2:3	58.83±2.49
6			1:2	73.37±0.21

*=selected formula

Table 4. Emulsification time.

F	**Distilled water (seconds)**	**AGF (seconds)**	**AIF (seconds)**
2	19.62 ± 0.44	19.87 ± 0.52	13.67 ± 0.68
3	10.45 ± 0.36	24.59 ± 1.463	12.31 ± 0.52

3.4. Microemulsion Drop Characterization

The microemulsion drop was conducted to elucidate the microemulsion drop size. The required of microemulsion drop size for SMEDDS is around 50-500 nm [19]. The size and PDI index showed that the microemulsion drop size was in the range of 50-500 nm, more specifically, 118.74-193.48 nm. The microemulsion size of formula 2 was considered sufficient for the subsequent analysis. This result corresponded with the transmittance result, which obtains the first insight into the microemulsion size (Table 3). The polydispersity index (PDI) of the microemulsion was less than 1, indicating a homogenous distribution of drops. This result showed that formula 2 and the production method of SMEDDS were reliable.

Extract loading optimization was conducted to elucidate the maximum capacity of the drug in the SMEDDS system in order to have the optimal therapeutic effect and avoid an overdose. The inclusion of the drug into the system is a critical point due to its effects to emulsification process and eventually changes the optimal ratio of oil, surfactant, and cosurfactant [11]. The extract loading test showed that the highest concentration of the bay leaf extract was 150 mg/mL in the SMEDDS system.

The formula 2 of SMEDDS microemulsion showed the spherical morphology of microemulsion drops, with a small portion of non-spherical drops (Figure 1). The morphology is esssential, as spherical drops will ease the particle contact and leads to aggregation [20].

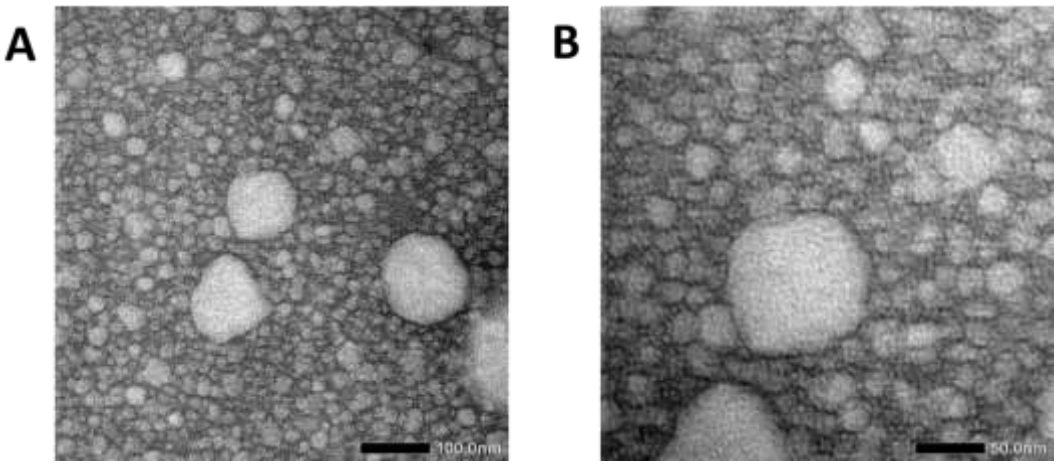

Figure 1. The morphology of formula 2 in SMEDDS system using TEM: (A) 10^5 times magnification; (B) $2x10^5$ times magnification.

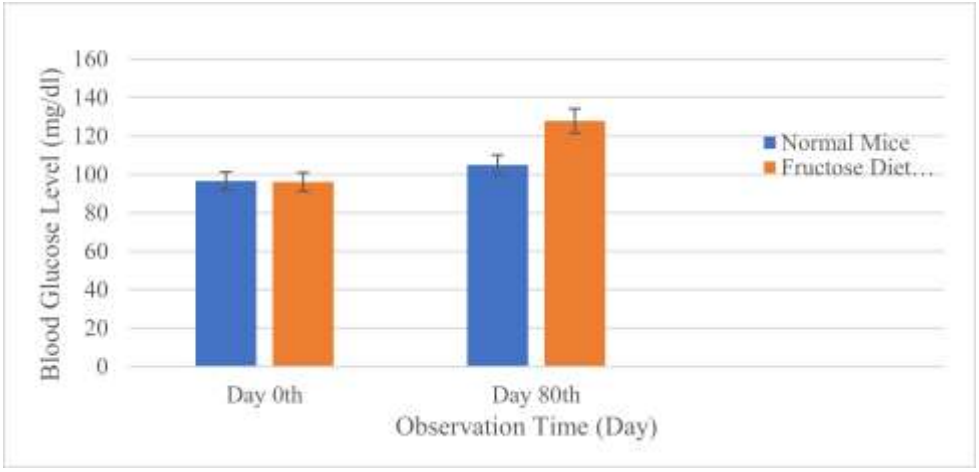

Figure 2. Blood glucose levels on days 0 and 80. The data are represented as mean + standard error of mean (SEM).

3.5. Antidiabetic Activity to DM Type 2

The adaptation period for insulin resistant type 2 DM was 80 days. The blood glucose levels before and after the adaptation period for the normal diet and HFFD were presented (Figure 2).

Statistical analysis using an independent t-test showed no significant difference ($p > 0.05$) between rats fed normal and HFFDs on day 0 (baseline).

Rats fed a HFFD showed an increase in blood glucose levels after the adaptation period. Meanwhile, rats fed a normal diet did not show a significant increase (Figure 2), which was also supported by the statistical test ($p > 0.05$). The statistical test revealed a significant increase ($p < 0.05$) in blood glucose levels on day 0 and day 80 in HFFD-fed rats. On the 80th day, an independent-sample t-test showed a significant difference ($p < 0.05$) in the blood glucose level between the normal-

and HFFD-fed rats. This indicates that 80 days of HFFD feeding increases the blood glucose level of Wistar rats and the animals were ready for the next treatment.

Fructose was used in this study due to it is preferentially metabolized into fat rather than glycogen in the liver. Meanwhile, a high-fat diet is intended to induce insulin resistance as a response to high cholesterol, triglycerides, and fatty acid [21]. A study of experimental animals showed that the excess saturated fat disrupted the insulin resistance [22].

This study used 10 mg/kg body weight glibenclamide administration to determine the insulin resistance. Findings of this study revealed that the fructose-diet mice had lower hypoglucemic potential than the normal ones ($p < 0.05$). It indicates that the fructose-diet mice have insulin resistance.

Blood glucose levels were measured to verify insulin resistant on the 80th day: before and after glibenclamide treatment. Glibenclamide was used due to its ability to induce insulin secretion from beta pancreatic cells.5 Glibenclamide administration had a hypoglycemic effect on rats fed in a normal diet but not on those fed an HFFD diet. The statistical test showed a significant difference in the rate of glucose disposal between normal- and HFFD-fed rats ($p < 0.05$), indicating that the HFFD-fed rats had developed insulin resistance. This condition is not caused by a deficit of insulin but by the decreased insulin sensitivity of the tissues [23].

Statistical analysis using a paired t-test showed a significant difference ($p < 0.05$) between the 80th and 95th days in groups 3 and 6. Meanwhile, groups 1, 2, 4, and 5 did not show any significant difference ($p > 0.05$) between the 80th and 95th days. However, a decrease in the mean blood glucose level was observed in groups 1, 2, 4, and 5 (Table 5).

Table 5. Mean blood glucose level decrease in each treatment group

Group of Treatment	Average of blood glucose level (ml/dl) ±SD			Decreased of blood glucose levels (ml/dl) ±SD
	0 Day	80th Day	95th Day	95th Day
I	96.5±6.2	104.9±11.4	90.0±5.1	14.9
II	87.8±12.6	117.5±20.5	113.9±11.9	3.6
III	104.1±16.3	127.8±7.0	91.6±2.1	36.3*
IV	96.3±2.4	139.9±20.0	110.7±2.9	29.2
V	103.5±15.9	139.7±1.7	109.7±9.9	30.0
VI	96.2±8.2	127.2±12.5	87.3±6.7	39.9 *

* significant reduction in blood glucose levels

Hypoglycemic activity was measured to determine the effectiveness of each treatment in decreasing blood glucose levels. The change in hypoglycemic activity in the various treatments over 15 days is presented in figure 3.

The significant difference analysis showed insignificant differences ($p > 0.05$) between groups 3, 4, 5, and 6 at day 95. This result

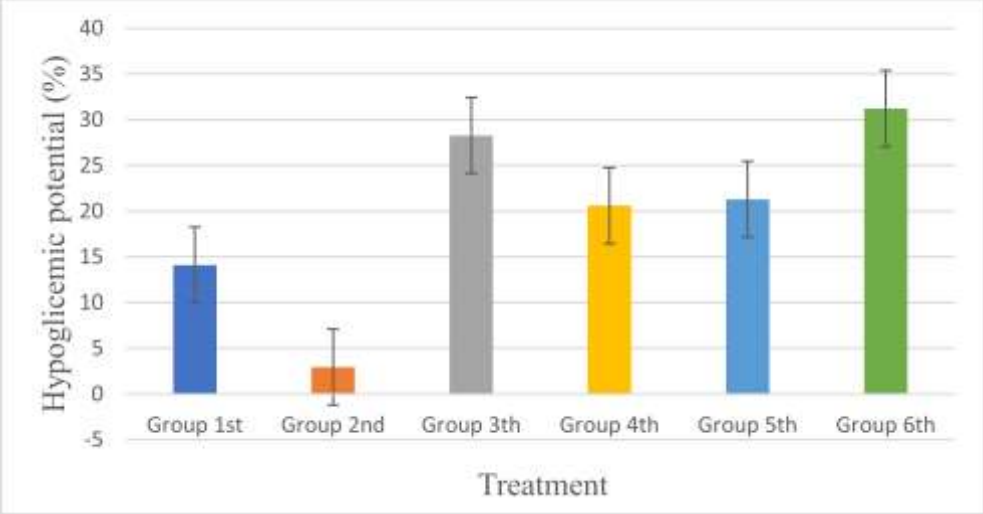

Figure 3. Hypoglycemic activity after treatment for 15 days. The data are represented as mean ± SEM. Group 1: Normal; Group 2: HFFD + distilled water; Group 3: HFFD + 45 mg/kg body weight metformin; Group 4: HFFD + 183.5 mg/kg body weight bay leaf extract; Group 5: HFFD + group 5 was given the HFFD diet with 183.5 mg/kg body weight of SMEDDS-bay leaf extract; Group 6: HFFD + half metformin and half SMEDDS-bay leaf.

indicates that the bay leaf chloroform extract, SMEDDS with bay leaf extract and metformin, and SMEDDS with bay leaf extract only are equivalent to single metformin treatment in decreasing blood glucose levels. Interestingly, the hypoglycemic activity in groups 4 and 5 was lower than group 3. The hypoglycemic activity on group 5 was slightly higher than group 4. It indicates the effect of the delivery system in its anti-diabetic activity. Meanwhile, group 6 had less 50% of metformin than group 3, had higher hypoglycemic activity (31.198%) than group 3 (28.324%) (Figure 3). These results imply that it is safe to use the bay leaf extract in the SMEDDS with metformin. They work synergistically to decrease the blood glucose levels in experimental insulin resistant type 2 DM animals. Moreover, the less amount of metformin seems to a slightly better result in decreasing of metformin's toxicity and side effect to the subject.

In this study, the hypoglycemic activity of the bay leaf extract was observed. This activity is likely due to the secondary metabolite content that was extracted from the fractionation process with the chloroform solvent. According to Sudarsono *et al*., the secondary metabolites in the bay leaf are saponins, triterpenoids, flavonoids, polyphenols, alkaloids, tannins, and essential oils, sesquiterpenes, lactones, and phenols [24]. Flavonoids can decrease blood glucose and increase insulin secretion [25]. Moreover, Bnouham *et al*. (2006) reported that alkaloids have antidiabetic activity and decrease the

blood glucose level in diabetic mice [26]. Interestingly, these findings are similar to the report by Prihapsara *et al*., which used the similar delivery system. In addition, it has been reported that a half extract and a half metformin had a better anti-diabetic activity than a full single dose of metformin [27].

4. Conclusion

Optimization of a SMEDDS and the bay leaf extract system produced an optimal microemulsion concerning to emulsion drop size, polydispersity index (PDI), and particle morphology. A SMEDDS with chloroform-fractionated bay leaf extract and VCO as a carrier decrease the blood glucose levels in rats. A half dose of metformin combined with a half dose of the SMEDDS-bay leaf combination is equal with the single dose of metformin in its ability to decrease blood glucose levels in insulin resistant type 2 DM rats.

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