

Antidiabetic Activity of Diterpene Lactone Fraction of “Sambiloto” (*Andrographis paniculata* Nees.) on Mice (*Mus musculus*) Induced by Alloxan

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Abstract: Objective: The present study was carried out to evaluate the antidiabetic activity of diterpene lactone fraction of “Sambiloto” (*A. paniculata* Nees.) Methods: Antidiabetic was induced experimentally by a single intraperitoneal administration of 3.8 mg/20 g body weight of Alloxan-monohydrate in each male mice. Three days after alloxan administration, blood glucose level was measured using a glucometer. Mice with blood glucose levels above 200 mg/dL were considered diabetic and were used in this study. Then the mice were randomly divided into five experimental groups of six mice each. First group as positive control (Glibenclamide 0.06 mg/20 g body weight). Second group as negative control (CMC-Na 0.5%). Samples were given by oral route at three dosage levels (7.4 mg, 14.9 mg, and 22.3 mg/20 g body weight). After treatment finished, blood sampling was done by sterilizing the tail with alcohol and then nipping the tail at the start of the experiment and this was repeated after 2nd, 4th, 6th, and 24th h. Results: Results were expressed as Mean±SEM of the blood glucose levels per number of animals used in every study point. One-way ANOVA and post-ANOVA (LSD) test were used to compare the means in each group. Conclusion: Diterpene lactone fraction of “Sambiloto” had antidiabetic activity and the most effective dose was at 14.87 mg/20 g body weight of mice.

1 INTRODUCTION

Diabetes mellitus is one of the common metabolic disorders with micro-and macrovascular complications that results in significant morbidity and mortality. It is considered as one of the five leading causes of death in the world (DiPiro *et al.*, 2008). In modern medicine no satisfactory effective therapy is still available to cure diabetes mellitus. There is increasing demand by patients to use natural products with antidiabetic activity due to side effects associated with the use of insulin and oral hypoglycemic agents (Scanlon *et al.*, 2009).

Andrographis paniculata Nees (Acanthaceae) is a traditional medicinal herb, grown as shrub in the moist soil, shady areas of India, China, Indonesia and throughout Southeast Asia. It has been used as antihyperglycemic (Sukardiman *et al.*, 2012; Yulinah *et al.*, 2001).

The past research shown that an extract of “Sambiloto” (*A. paniculata* Nees.) with the main content of diterpene lactone was andrographolide efficacious as antidiabetic drugs (Xuansheng *et al.*,

2013). Therefore further research, antidiabetic activity using diterpene lactone fraction of “Sambiloto” in mice induced by alloxan.

2 MATERIALS AND METHODS

2.1. Plant Material

The diterpene lactone fraction of “Sambiloto” got from Department of Pharmacognosy and Phytochemistry, Faculty of Pharmacy Airlangga University Surabaya Indonesia.

2.2. Preparation Diterpene Lactone Fraction of “Sambiloto”

“Sambiloto” herbs was cutted into small pieces and dried under shade at room temperature, then blended up into powder. Then the powder was extracted by maceration method. It used 2 L of 96% ethanol for 24 h. After extraction, the extract was filtered using a Buchner funnel to obtain filtrate in the form of

liquid extract. Maceration performed four times. The filtrate which four times maceration were collected and evaporated using a rotary evaporator with temperature not exceeding 50 °C and low pressure \pm 2% initial volume. The ethanol extract was measured as much as 100 mL dissolved in water : ethyl acetate (1:1) during three times. Then a solution obtained were separated by a separating funnel to obtain ethyl acetate fraction at the top and ethanol-water at the bottom. Ethanol-water fraction was shaken again with ethyl acetate four times. Ethyl acetate fraction which collected were dried with Avicel and Cab-O-Sil (4:1) (Sukardiman *et al.*, 2012).

2.3. Experimental Design

2.3.1. Antidiabetic of Diterpene Lactone Fraction from “Sambiloto” by Alloxan-Induced Diabetic Model

Experimental animals: The study used male BALB/c mice (8-10 weeks old) that weighed 20-40 g with a mean weight of 30 g. The mice were housed at a temperature of 25°C with 12 h light/12 h darkness photoperiod.

Induction of diabetes: Hyperglycemia was induced experimentally by a single intraperitoneal administration of 186.9 mg/kg body weight of a freshly prepared alloxan-monohydrate (2, 4, 5, 6 tetraoxypyrimidine; 5-6-dioxuracil). Forty-eight h after alloxan administration, blood glucose level was measured using a glucometer. Mice with blood glucose levels above 200 mg/dL were considered diabetic and used in this study. Prior to initiation of

this experiment, the animals were fasted for 8-12 h but allowed free access to water until the end of the experiment (Karau *et al.*, 2012).

2.3.2. Procedure

Experimental design: The mice were randomly divided into six experimental groups of five mice each. These groups included positive control (0.06 mg/20 g body weight glibenclamide), negative control (0.5% CMC-Na) and three tested dose levels (7.4 mg, 14.9 mg, and 22.3 mg/20 g body weight) diterpene lactone fraction of “Sambiloto”. All of groups were given by oral route.

Blood sampling: Blood sampling was done by sterilizing tail with alcohol and nipping tail at the start of the experiment. It was repeated after 2nd, 4th, 6th and 24th h. Every time the blood glucose levels were determined with a glucometer (Karau *et al.*, 2012).

Data management and statistical analysis:

Data was entered in the Microsoft® Excel spreadsheet, cleaned and exported to Statistical Package for Social Sciences (SPSS Version 17.0) software. The results were expressed as Mean \pm Standard Error of Mean (SEM) of the blood glucose levels per number of animals used in every study point. One-way ANOVA and post-ANOVA (LSD) test were used to compare the means in each group. $p \leq 0.05$ was considered statistically significant.

3 RESULTS AND DISCUSSIONS

Table 1. The effect of Diterpene Lactone Fraction of “Sambiloto” on Glucose Level in Mice with Alloxan Induced Diabetes

Mice Group	Treatment	Blood Glucose Levels (mg/dL)				
		0 h	2 h	4 h	6 h	24 h
Diabetic control	0,5 % CMC Na	563 \pm 15.5	559 \pm 14.0	584 \pm 9.99	591 \pm 9.33	582 \pm 6.86
Diabetic control	Glibenclamide (0.06 mg/20 g body weight)	597 \pm 3.33	466 \pm 30.6	326 \pm 19.3	247 \pm 13.1	334 \pm 15.5
Diabetic treated	7.4 mg /20 g body weight	462 \pm 64.7	477 \pm 72.3	439 \pm 79.3	423 \pm 81.7	402 \pm 66.2
	14.9 mg/20 g body weight	409 \pm 52.5	302 \pm 57.8	318 \pm 42.9	301 \pm 55.3	265 \pm 48.8
	22.3 mg/20 g body weight	431 \pm 60.1	356 \pm 65.5	388 \pm 54.9	368 \pm 73.3	321 \pm 55.4

The comparison between groups was done using one way ANOVA, Values were in mean \pm SEM; Number of animals in each group = 6

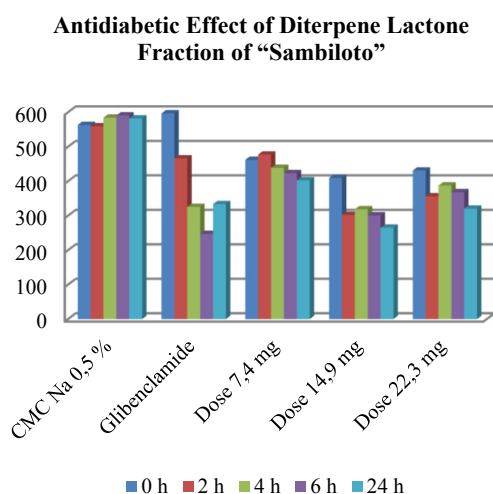


Fig. 1 Effect of Diterpene Lactone Fraction of "Sambiloto" (*A. paniculata* Nees.) on Glucose Level

The result showed that positive control treated with glibenclamide suspension was obtained an average reduction in blood glucose levels of 263 ± 14.3 (44.1%). Negative control, giving a suspension 0.5% CMC Na, didn't give effect in decreasing blood glucose levels of -18.7 ± 13.7 (-3.64%) and it become a baseline of this experiment. Based on SPSS statistical analysis, it showed that positive control had a significant difference to negative control with p calculated value of 0.000 ($p < 0.05$). It indicated that positive control was able to lower blood glucose levels significantly over an 24-hour and antidiabetic test method used in this study was correct.

The first treatment group that containing 7.4 mg/20 g BW diterpene lactone fraction resulted in an average reduction in blood glucose levels of 59.7 ± 18.2 (14.8%). It had p calculated value at 0.013 ($p < 0.05$) when compared to negative control using statistical analysis Anova One Way and Post Hoc LSD. It showed that group 1 was able to lower blood glucose levels significantly.

The second treatment group that containing 4.9 mg/20 g BW diterpene lactone fraction resulted in an average reduction in blood glucose levels of 144 ± 34.6 (35.9%). Based on statistical analysis Anova One Way and Post Hoc LSD result, it had p calculated value at 0.000 ($p < 0.05$) when compared to negative control. It showed that group 2 was able to lower blood glucose levels significantly.

The third treatment group that containing 22.3 mg/20 g BW diterpene lactone fraction resulted in an average reduction in blood glucose levels of 111

± 15.1 (27.6%). Based on statistical analysis Anova One Way and Post Hoc LSD, it had p calculated value at 0.000 ($p < 0.05$) when compared to negative control. It showed that group 3 was able to lower blood glucose levels significantly.

Comparising data between groups 1 and 2 was obtained significantly different with a significance value of 0.008 ($p < 0.05$), whereas between group 1 and 3 and group 2 and 3 were no significant differences with a significance value of 0.095 ($p > 0.05$) and 0.260 ($p < 0.05$), respectively. It can be concluded that group 2 was the most effective dose for lowering blood glucose levels.

Comparising data between positive control and three treatment groups to negative control was obtained no significant difference (p count < 0.05), which means positive control and three treatment groups can lower blood glucose levels in mice significantly for 24 hours. Then three treatment groups compared to positive control showed significant difference (p count < 0.05) and it had greater average value of a decreasing blood glucose levels than positive control. It can be concluded that the fraction of diterpene lactone (group 1, 2 and 3) had antidiabetic activity but their decreasing blood glucose levels were not as big as a positive control.

Thus, the hypothesis in this study accepted that diterpene lactone fraction can decrease blood glucose levels. Compound suspected which has activity lowers blood glucose levels is andrographolide with triggering mechanism secretion of insulin so glucose metabolism increases, it can cause blood glucose levels to decline (Sukardiman *et al.*, 2014).

The results of this study can be used as scientific information and supporting data for further studies in order to develop diterpene lactone fraction as dosage forms of antidiabetic phytopharmaceutical drugs or raw materials. It had been found the effective dose of diterpene lactone fraction that can be used as traditional or complementary drug candidate for antidiabetic drugs is safe. Furthermore, it can be done praformulation study (physical characteristics, physicochemical characteristics, biofarmasetika characteristics, compatibility, and stability) and formulations diterpene lactone fraction from "sambiloto".

4 CONCLUSION

Diterpene lactone fraction of "Sambiloto" at 7.4 mg/20 g body weight, 14.9 mg / 20 g body weight, and 22.3 mg / 20 g body weight had antidiabetic

activity in mice induced by alloxan. The most effective dose was 14.9 mg/20 g body weight.

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