Anti-Diabetic Effect of Snake Fruit Skin Extract in Alloxan-Induced Wistar Rat

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Abstract.
Basic Health Research Report shows that an average prevalence of diabetes mellitus (DM) in urban areas is 5.7% in the population aged over 15 years. Many studies have been conducted to explore the pharmacological effects of snake fruit skin as different preparations against DM. In this study, the effect of snake fruit skin extract (SFSE) on body weight and blood glucose levels induced by alloxan was tested on 25 male Wistar rats. All rats were initially induced with 5% alloxan (150 mg/kg BW) then after 48 hours were grouped into: control (Na-CMC 0.5%), standard (Metformin), SFSE-I (60 mg/200 gBW), SFSE -II (120 mg/200 gBW), and SFSE-III (240 mg/200 gBW). The treatment was given for 14 days. The results showed that the tendency for the highest blood glucose levels of about 333 mg/dl (after 28 days of treatment) was found in the control group, followed by SFSE-I (222 mg/dl), -II (205 mg/dl), -III (138 mg/dl), and the lowest in the standard group was about 129 mg/dl. Hence, it can be concluded that SFSE which has the best antihyperglycemic effect was obtained with the highest dose of 240 mg/200 gBW.

Keywords: *Snake fruit, skin, alloxan and ethanol.*

I. INTRODUCTION

The prevalence of type 2 diabetes in the white race ranged from 3%-6% in the adult population. In Singapore, the frequency of diabetes has rapidly increased in the last 10 years. Meanwhile people with diabetes increased in the United States from 6,536,163 people in 1990 to 20,676,427 people in 2010. Furthermore, the prevalence of diabetes in Indonesia ranged from 1.4%-1.6%, except in some places viz. Pekajangan (2, 3%) and Manado (6%) [1].The Basic Health Research (*Riset Kesehatan Dasar*/ Riskesdas) Report by the Ministry of Health showed that the average prevalence of diabetes mellitus (DM) in urban areas is 5.7% among the people aged more than 15 years old, meanwhile the lowest prevalence is found in Papua about 1.7%, and the highest is found in Maluku Utara and Kalimantan Timur about 11.1%. On the other hand, the prevalence of impaired glucose tolerance (TGT) ranged from 4.0% in Jambi to 21.8% in Papua Barat with an average of 10.2% [2].The prevalence of the DM in Indonesia that diagnosed by a doctor is 1.5 %, meanwhile the prevalence of Diabetes Mellitus based either by a doctor diagnosed or symptoms is 2.1%. The highest prevalence of diabetes diagnosed by doctors are obtained in DI Yogyakarta (2.6%), DKI Jakarta (2.5%), Sulawesi Utara (2.4%) and Kalimantan Timur (2.3%). The highest prevalence of diabetes either by a doctor diagnosed or symptoms are obtained in Sulawesi Tengah (3.7%), Sulawesi Utara (3.6%), Sulawesi Selatan (3.4%) and Nusa Tenggara Timur (3.3%). The prevalence of DM was found to be higher in women than men. Furthermore, the prevalence rate of diabetes diagnosed by a doctor is 1.8 percent, and the prevalence is diagnosed by a doctor or symptom by 2.3 percent[2].

Recently, it was found that herb is a drug with high demand. According to data resolution Promoting the Role of Traditional Medicine in Health System: Strategy for the African Region, about 80% of people in WHO member countries in Africa use traditional medicine for health purposes. Meanwhile, the WHO Regional Office for the Americas (AMOR/PAHO) also reported that 71% of Chile's population and 40% of Colombia's population used herbal medicines [3].Indonesia, which has a tropical climate, is the second largest country in the world after Brazil, which is rich in biodiversity. About 30,000 species of plants can be found in Indonesia and around 2,500 species of these plants are herb [4]. One of these herbs is snake fruit which has a lot of pharmacological effects including immunostimulator, antioxidant, antidiabetic, and improve profile lipid. This pharmacological effect is supported by various phytochemicals in the snake fruit skin, including quercetin, cholinergic acid, gallic acid, caffeic acid, ferulic acid, and rosmarinic acid [5, 6].Many studies have been performed to explore the pharmacological effects of snake fruit skin, one of which is the anti-diabetic effect both in vitro [7]–[9] and in-vivo [10]–[13]. Some of these studies have even

explored the anti-diabetic effects of preparations such as *kambucha* and snake fruit vinegar. However, in vitro and in vivo study on the anti-diabetic effect from the snake fruit skin is still limited. An In vitro study on snake fruit skin extract reported that the highest anti-diabetic activity in the form of inhibition of the enzyme glucosidase is found in the snake fruit skin from Manonjaya *salak* (IC_{50}: 17.9 µl/dl) [7]. Meanwhile, in vivo study on anti-diabetic effects conducted by Kanon et al. (2012) showed that the ethanol extract from the skin of the snake fruit had an anti-diabetic effect at a dose of 150 mg/kgBW with a rat model loaded by oral sucrose [14].

Several previous studies have explored the anti-hyperglycemic effect of snake fruit skin extract. Saleh et al. reported that in vitro the ethanolic extract of Snake Fruit fruit peel has an anti-hyperglycemic effect through inhibition of the -glucosidase enzyme with IC_{50} value: 11.62 ± 0.67 g/ml. Another study reported that ethanolic extract of salka peel showed a significant effect of lowering blood glucose levels at a dose of 840 mg/kgBW in mice induced with alloxan. Meanwhile, in this study, the ethanolic extract of snake fruit skin showed a significant effect at the highest dose of 240 mg/200 gBW. The difference in the results of this study is thought to be caused by the extraction technique used, namely ethanol which is 96% more concentrated than 70% (previously). This is reflected in the extraction yield value of only 1.46% against 3% (previously). The increase in yield value is inversely proportional to the quality of the extract, this causes the quality of the extract in this study to be more optimal than in previous studies. Based on those information, this study aims to determine the effect of bark extract on body weight and blood glucose levels in alloxan-induced rats.

II. METHODS

A. Study Design

This study is an experimental study with aapproach *Pre-test and Post-test group only control design* that uses male wistar rats as experimental animals. This research was conducted for about 6 (six) weeks, with the first week being the process of making ethanol extract of Snake Fruit skin (*Salacca zalacca*), the next 1 week being the acclimatization process of rats, and 4 weeks being the treatment of male wisatr rats. All of this research was conducted at USU's Herbarium Medanese FMIPA, USU's Pharmacognosy Laboratory, Faculty of Pharmacy, and USU's Pharmacology Laboratory.

B. Research Sample

The experimental animal samples in this study were male wistar rats that had been provided at Riwandi Animal House. The sample size in this study was calculated using the Federer formula ((r-1) (t-1) ≥ 15)/ Thus, it can be concluded that at least 5 male wistar rats (*Rattus norvegicus*) were required in each treatment group.

C. Research Procedure

1. Production of Snake Fruit Skin Extract *(*SFSE*)*

Extracts were made by collecting samples of Snake Fruit skin from one of the traditional markets in Medan City, which were then identified at the Medanese Herbarium at the Faculty of Mathematics and Natural Sciences, University of North Sumatra.A total of 500 grams of fresh simplicia snake fruit skin dried with aerated for 7 days to become dry simplicia, which is then mashed to become dry simplicia powder. The dry simplicia powder was extracted by the remaceration method. A number of dry simplicia powder was macerated using 96 % ethanol as a solvent in a ratio of 1:6 for 5 days, the mixture was stirred regularly every day. After 5 days, the mixture was filtered with filter paper, then the residue was re-remacerated with 600 ml of 70% ethanol for 4 days. Then the remaceration results were filtered and the filtrate from the maceration and remaceration results is evaporated with a *rotary evaporator* at a temperature of 70ºC and then continued with drying using an oven at a temperature of 40ºC to become a thick extract [14].

2. Phytochemical Screening

In the phytochemical test study using a modified Fansworth method consisting of identification of phenols, steroids/triterpenoids, terpenoids, saponins, flavonoids, tannins and alkaloids [15]–[19].

3. Preparation of SFSE and Glibenclamide as Standard
4. Preparation of 0.5% Na-CMC Suspension

As much as 0.5 grams of Na-CMC was mixed with about 30 ml of hot distilled water in a mortar, this mixture was allowed to stand for 15 minutes to form a transparent mixture. Then the mixture was ground until homogeneous and diluted with distilled water using a 100 ml volumetric flask to the mark [14].

5. Preparation of Oral Suspension SFSE

Preparation of ethanolic SFSE oral suspension was carried out by mixing 300 mg, 600 mg, and 1200 mg of the oral suspension with doses of 60 mg/200 gBW, 120 mg/200 gBW, and 240 mg/200 gBW, respectively, with Na suspension. -CMC 0.5% using a 5 ml volumetric flask to the limit mark [14].

6. Manufacture of Metformin Oral Suspension as Standard

The dose of metformin in humans is 500-2000 mg/day [2]. Then the dose is converted by multiplying the dose in humans (mg/kgBW) by a conversion factor of 6.2 to get the dose in rats in mg/kgBW [18]. Then the dose of metformin in rats was 10.33 mg/200 gBW to 41.33 mg/200 gBW. Metformin oral suspension was prepared by mixing 100 mg (20 mg/200 g of body weight rats) tablets of mashed metformin with 0.5% Na-CMC suspension using a 5 ml volumetric flask to the mark.

7. Induction Process of Male Wistar Rat (Rattus norvegicus)

The induction process for male wistar rats was carried out using *Alloxan Monohydrate* 10%. A total of 0.35 ml (175 mg/kg BW rats) Alloxan monohydrate 10% was injected intraperitoneally. To ensure the induction was successful, fasting blood glucose levels after 72 hours, rats were said to be diabetic if their blood glucose levels were more than 200 mg/L (< 11.1 mmol/L) [20]–[22].

8. Anti-diabetic Activity Test of SFSE

The activity of anti-diabetic test were performed on 25 rats which divided into 5 groups and all mice induced by alloxan, as in Table I.

Table 1. Group Treatment Mice

Group Treatment	Treatment
Control	Rats in this group receiving 1 ml suspension of Na-CMC 0.5%. Food and drink were administered ad libitum.
Standard	Rats in this group received 1 ml of metformin oral suspension. Eating and drinking was administered ad libitum.
SFSE-I (60 mg/200 gBW)	Rats in this group received 1 ml of oral suspension of Snake Fruit Skin extract at a dose of 60 mg/200 gBW. Eating and drinking was administered ad libitum.
SFSE-II (120 mg/200 gBW.)	Rats in this group received 1 ml of oral suspension of Snake Fruit Skin extract at a dose of 120 mg/200 gBW. Eating and drinking was administered ad libitum.
SFSE-III (240 mg/200 gBW).	Rats in this group received 1 ml oral suspension of Snake Fruit Skin extract at a dose of 240 mg/200 gBW. Food and drink is provided ad libitum

9. Measurement of Blood Glucose Levels of Male Wistar Rats (Rattus norvegicus)

In this study, the measured blood glucose level was Fasting Plasma Glucose (FPG). The FPG was measured in rats that had been fasted for 10-12 hours prior to measurement. Rat blood samples were taken from the vein in the tail of rats 72 hours after induction (FPG 0) and on day 28 (FPG 28) (after rats were given bark extract and glibenclamide as standard). [21-26].

D. Data analysis

Data analysis was performed using IBM SPSS 25. Data in the form of extract yield, phytochemical screening results, rat weight, FPG 0, and FGP 28 were analyzed with descriptive statistics. Then the data of rat BB, FPG 0, and FGP 28 were analyzed for normality of the data using the Shapiro-Wilk test. If the data is normally distributed, then the analysis is continued with One Way ANOVA and Post Hoc Test. However, if the data is not normally distributed, then a data transformation is carried out to normalize the data distribution and the results of the transformation are analyzed with One Way ANOVA and Post Hoc Tests. If after being transformed the data is still not normally distributed, then the data analysis is continued with a non-parametric test in the form of a test. Kruskal-wallis.

III. RESULTS AND DISCUSSIONS

E. Plant Determination

As the first step of this research, samples of salayang fruit peel were first identified at the Medanese Herbarium, University of North Sumatra. The results of the identification of the leaf samples are as follows:
Kingdom: Plantae
Division: Magnolinophyta
Class : Liliopsida
Order: Arecales
Family: *arecales*
Genus: Salacca
Species: *Salacca edulis*
Local Name: Snake Fruit Fruit

F. Extract Characteristics

After extraction with the maceration method on the samples of snake fruit skin used, the characteristics of the extract were obtained as given in Table II.

Table 2. Characteristics of the SFSE

Characteristics	P-Value
Fresh Simplicia Weight (gr)	3,000 grams
Dry Simplicia Powder Weight (gr)	1,000 grams
Solvent Volume (ml)	8000 ml
Extract Weight (gr)	14.57 grams
Yield (%)	1.46 %

From the data in the Table II, it can be seen that from 3,000 grams of Snake Fruit fruit peel samples, 1,000 grams of extract were found. Thus, the obtained yield from the snake fruit skin extract was 1.46%.

G. Phytochemical Screening Results

Before analyzing the effect of the ethanolic extract of the snake fruit skin extract on blood glucose levels and body weight, the ethanolic extract of the SFSE was screened for phytochemicals on the extract. The results of phytochemical screening on the SFSE can be seen in the Table III.

Table 3. Screening Phytochemicals Results of the SFSE

Phytochemicals	Reagent	Results
Alkaloids	Bouhardart	+
	Maeyer	+
	Dragendroff	+
	Wagner	-
Stroida and Triterpenoid	Salkowsky	+
	Lieberman-Burchad	+
Saponin	Aquadest + Alcohol 96%	-
flavonoida	FeCl3 5%	-
	Mg + HCL (p)	-
	10% NaOH	-
	H2SO4(p)	-
Tannins	1% FeCl3	+
Glycosides	Mollish	-

As shown in the Table III, it was found that the snake fruit skin contains phytochemicals in the form of alkaloids, steroids/triterpenoids, flavonoids, and tannins.

H. Body Weight

As one of the parameters assessed in this study, the body weight of rats before induction and after treatment needs to be analyzed for data distribution using the Shapiro-Wilk normality test. The results of the normality analysis of the data with Shapiro-wilk on body weight of rats can be seen in the Table IV.

Table 4. Normality Analysis Of Rat Body Weight In All Treatment Groups With Shapiro-Wilk

Parameters	Group	P Value	Distribution
Body Weight	Control	0.581	Normal
	Standard	0.737	Normal

Before Induction	Snake Fruit Skin Extract-I	0.876	Normal
	Snake Fruit Skin Extract-II	0.592	Normal
	Snake Fruit Skin Extract-III	0.527	Normal
Body Weight After Induction	Control	0.558	Normal
	Standard	0.824	Normal
	Snake Fruit Skin Extract-I	0.915	Normal
	Snake Fruit Skin Extract-II	0.876	Normal
	Snake Fruit Skin Extract-III	0.998	Normal

Table IV show that the weight data before induction and after treatment indicated in the normal data distribution form. Therefore, the comparison of body weight of mice before induction and after treatment was analyzed by One-Way Anova. The results of the one-way ANOVA analysis can be seen in the Table V.

Table 5. Comparison Of Rat Body Weight Before Induction And After Treatment In All Treatment

Groups	Body Weight (grams)	
	Before Induction	AfterTreatment
Control	196.20±14.74	188.40±22.59
Standard	194.80±13.39	180.80±14.53
SFSE-I	192.80±13.22	179.20±15.22
SFSE-II	191.40±13.41	177.20±15.58
SFSE-III	190.00±13.23	175.60±17.81
P-Value	0.952	0.804

Data are presented as mean ± SD and P values obtained from the analysis of One-Way ANOVA

Table V shows that there was no significant difference in body weight of rats before induction and after treatment. This results was supported by their P-value before induction and after treatment which are 0.952 to 0.804, respectively.

I. Blood Glucose Level

In addition to body weight, another parameter that was also assessed in this study was the blood glucose levels of rats measured before induction, after induction, and after treatment. Before comparing the blood glucose levels of rats, data on blood glucose levels were analyzed for normality with Shapiro-wilk. The results of the normality analysis of the data can be seen in the Table VI.

Table 6. Normality Analysis of Rat Blood Glucose Levels in All Treatment Groups with Shapiro-Wilk

Parameter	Group	P-Value	Distribution
Blood Glucose Levels Before Induction	Control	0.213	Normal
	Standard	0.897	Normal
	SFSE-I	0.973	Normal
	SFSE -II	0.623	Normal
	SFSE -III	0.608	Normal
Blood GlucoseAfter Induction	Control	0.023	Not Normal
	Standard	0.922	Normal
	SFSE -I	0.299	Normal
	SFSE -II	0.812	Normal
	SFSE -III	0.180	Normal
Blood Glucose Level AfterTreatment	Control	0.138	Normal
	Standard	0.337	Normal
	SFSE -I	0.025	Not Normal
	SFSE -II	0.304	Normal
	SFSE -III	0.145	Normal

From the Table VI, it can be seen that the distribution of blood glucose level data before induction was normal, while the distribution of blood glucose level data after induction and after treatment was not normal. Therefore, the analysis was continued with the Kruskall-Wallis test on the blood glucose levels of rats before induction, while the data on blood glucose levels of rats after induction and after treatment were analyzed by one-way Anova. Comparison of blood glucose levels before induction, after induction, and after the treatment can be seen in the Table VII.

Table 7. Comparison of Blood Glucose Levels of Rats in All Treatment

Groups	Blood Glucose Levels		
	Before Induction*	After Induction**	After Treatment**
Control	102.00±6.04	215 (200-548)	333 (198-415)[a]

Standard	99.00±12.77	317 (218-600)	129 (104-150)[b]
SFSE -I	88.20±12.28	339 (228-600)	222 (185-415)[a]
SFSE -II	101.60±9.66	405 (228-600)	205 (174- 218)[a]
SFSE -III	99.00±9.14	389 (219-600)	138 (109-200)[b]
P value	0.238	0.354	0.004

From the table VII, it can be seen that there is no significant difference in the blood glucose levels of rats before and after induction, this can be seen from the P value > 0.05 (ie., before: 0.238 and after: 0.354). This indicates that blood glucose levels before and after induction in all treatment groups were uniform. The average blood glucose level before induction in rats was at a normal value, which was below 200 mg/dl. However, after induction, all rats uniformly experienced an increase in blood glucose levels > 200 mg/dl. Furthermore, after being given treatment for 28 days, there was a significant change in blood glucose levels in all treatment groups, this was seen from the P value < 0.05 (P value = 0.004). The control group showed the highest tendency for blood glucose levels after 28 days of treatment, namely 333 mg/dl, followed by the SFSE-I (222 mg/dl), -II (205 mg/dl), -III (138 mg/dl), and the lowest in the standard group was 129 mg/dl. Both SFSE-I and -II did not significantly decrease the blood glucose level compared to the control group. However, either SFSE-III or standard significantly decreased the blood glucose level, lower than the control group. This can be seen from the superscript in the Table VII.

According to the results of this study it can be seen that the ethanolic extract of snake fruit skin contains several phytochemical compounds such as alkaloids, steroids/triterpenoids, flavonoids, and tannins. The results of this study are in line with the results of research conducted by others contain phenols, flavonoids, tannins, and monoterpenoids. Another study also reported that snake fruit skin extract contains flavonoids, saponins, phenols, tannins, steroids/triterpenoids, and alkaloids. The phytochemical content of the ethanolic extract of snake fruit skin provides various benefits such as antioxidants or skin lightening.In addition to the quality of the extract, the antihyperglycemic effect of the SFSE was also related to the phytochemical content of the extract. Saponins and flavonoids have antioxidant and inhibitory effects on glucosidase enzymes. The inhibitory effect of the -glucosidase enzyme is able to absorb glucose in the digestive tract, thereby reducing postprandial blood glucose levels.In addition, the antioxidant effect of the SFSE ethanol also contributes to the antidiabetic effect. This is related to the mechanism of pancreatic damage caused by alloxan. Alloxan will be reduced by GSH which forms unstable dialuric acid and can undergo autoxidation to form alloxan radicals.

These alloxan radicals have the potential to damage pancreatic beta cells through damage to the DNA structure of beta cells and inhibition of the thiol group of the glucokinase enzyme. Damage to the DNA structure of pancreatic beta cells causes beta cell death while inhibition of thiol groups on the glucokinase enzyme will interfere with the formation of ATP in pancreatic beta cells, causing a decrease in insulin secretion.Based on the mechanism of action of alloxan, the SFSE containing saponins and flavonoids is able to provide an antioxidant effect by donating electrons to the formed alloxan radicals so that they can form more stable alloxan compounds and reduce the danger of alloxan in pancreatic tissue. This can be seen from the results of the study which showed that with increasing doses of mangrove leaf extract, there was an improvement in the structure of pancreatic tissue.

IV. CONCLUSION

Experiment result indicated that snake fruit ethanol extract has anti-hyperglycemic effect at the lowest dosage (60 mg/200 gBW). Higher dosage of extract does not significantly reduced the blood glucose level. The result of this study showed that control group showed the highest tendency for blood glucose levels after 28 days of treatment, namely 333 mg/dl, followed by the SFSE-I (222 mg/dl), -II (205 mg/dl), -III (138 mg/dl), and the lowest in the standard group was 222 mg/dl.

V. ACKNOWLEDGMENT

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