



Antidiabetic Effect Test of Insulin Stem Extract (*Tithonia diversifolia*) Toward Streptozotocin-Induced Diabetic Rats (*Rattus Norvegicus*)

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Abstract

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BACKGROUND: Insulin plant is a plant that has potency to be used as an antidiabetic drug. This is due to the presence of the chemical content of alkaloids, flavonoids, saponins, tannins, and polyphenols.

AIM: A research on the antidiabetic effects test of insulin stem extract (*Tithonia diversifolia*).

METHODS: Insulin stem was extracted by maceration using 96% ethanol, antidiabetic tests were conducted using test animals – mice, induced with streptozotocin 40 mg/kg intraperitoneally. Tested animals were divided into five groups, call now was given a different treatment. The treatment I was given a suspension of 1% Na CMC as a negative control. Treatments II, III, and IV administered insulin stem extract per call now with a dose of 125 mg/kg, 250 mg/kg, and 500 mg/kg. Treatment of V given glibenclamide suspension as a positive control. Blood glucose levels were measured using glucometer mice. Data were analyzed using ANOVA statistical tests at 95% confidence level.

RESULTS: The analysis results showed that insulin stem extract (*Tithonia diversifolia*) was shown to significantly reduce blood glucose levels in rats after 14 days of treatment.

CONCLUSION: Based on the results of this study, the dose of insulin stem extract was effective in lowering blood glucose levels by 250 mg/kg.

Introduction

The rapid development of the world economy has been implicated in causing the economic crisis the country is no exception for Indonesia. The economic crisis that is part of the world crisis led to the high cost of health care and treatment of a disease. Unfavorable economic conditions are to encourage and urge the public to review and seek alternatives for treatment. Alternative treatment of the most widely used public is by utilizing natural resources. Indonesia is known to be rich in the diversity of medicinal plants. Medicinal plants have been used for thousands of years to maintain health and treat various diseases [1], [2]. Medicinal plants have become a necessity and are widely used by the public in addition to safe treatment because they are relatively inexpensive when compared with medical treatment [2], [3]. Type of disease that often arises in society is diabetes mellitus.

Diabetes mellitus is a group of metabolic diseases with characteristic hyperglycemia (increased

blood sugar levels) that occurs due to abnormal insulin secretion, insulin action, or both. Basic Health Research (RIKESDAS) in 2013 stated diabetes mellitus in Indonesia increased by 2.1% from year to year. The World Health Organization (WHO) predicts diabetes in Indonesia in 2030 reached 21.3 million [4]. International Diabetes Federation (IDF) in 2013 revealed that patients with diabetes mellitus rank 7 in Indonesia with a total of 8.5 million [5], [6].

Plant insulin (*Tithonia diversifolia*) generally grows wild in steep places, for example, in the cliffs, river banks, and ditches. Otusanya and Ilori [7] have phytochemical analysis on *Tithonia diversifolia* indicating bioactive substances in methanol extracts such as alkaloids, saponins, glycosides, flavonoids, tannins, terpenoids, and phenols. Thongsom *et al.* [8] say that the hypoglycemic effect of *Tithonia diversifolia* leaf extract at a dose of 500 mg/kg significantly reduced blood glucose levels in oral glucose tolerance test (OGTT) normal mice. In addition, *Tithonia diversifolia* given in alloxan-induced diabetic rats for 30 days significantly lowered glucose levels. Apart from the leaves, other plant parts of insulin, such as the stems,

are suspected of containing chemicals that can have an antidiabetic effect.

In connection with the above mentioned, the problem in this study is whether there is a difference in dose variation antidiabetic effects stem extract at a dose of insulin and insulin stem extract what is effective in lowering blood glucose levels of mice. Based on these problems, the goal of this study was to determine the antidiabetic effects on stem extract insulin dose variation to decrease blood glucose levels of mice and determining the dose of insulin stem extract is effective in lowering blood glucose levels of mice. The results of this study are expected to provide scientific information to the public about the effectiveness of stem extract *Tithonia diversifolia* in lowering blood glucose levels and help the development of the pharmaceutical field, especially in the use of herbal medicine in Indonesia is increasingly widespread use.

Materials and Methods

Tools and materials

Glucometer (Accu-Check), injection syringes 3 mL and 5 mL oral (Terumo), no mesh sieves 40, Cage test animals, animal scales, and analytical scales (Sartorius) were used. Reagents Lieberman–Burchard, trunk insulin (*Tithonia diversifolia*), streptozotocin, ethanol 96%, magnesium powder, hydrochloric acid (pa), Dragendorff LP, animal testing rat (*Rattus norvegicus*), sodium CMC, and iron (III) chloride were used.

Sample preparation and determination

The sample used is the stem of insulin (*Tithonia diversifolia*) obtained from the village happy, District Palolo, Sigi, Central Sulawesi Province, Palu, and has been determined in the Herbarium UPT, Biological Resources Tadulako Sulawesi.

Intake and processing samples

Tithonia diversifolia plant stems were taken with a knife/machete, collected, and then cleaned and washed with water to clean. Further, perajangan then dried with aerated without direct sunlight until the material dries. After the bulbs crushed and sieved using a mesh sieve No.40.

Preparation of insulin stem extract (*Tithonia diversifolia*)

Making the plant extract is done using the method in which 500 g remaserasi stem powder insulin

(*Tithonia diversifolia*) which has been dried soaked in liquid penyari. Maceration process is done using a glass container. Plant powder was soaked in 96% ethanol penyari fluid for 3 days, then filtered and diremaserasi back to get a plant extract. The results obtained extract are then concentrated by rotary evaporator followed by evaporation of the water to obtain the above tangas condensed extract.

Preparation of solution streptozotocin

Taken streptozotocin 0.26 g then dissolved in about 100 ml API to obtain a solution of 0.26% STZ.

Preparation the colloidal solution of Na-CMC 1% w/v

Colloidal solution of Na-CMC 1% was prepared by dissolving 1 g of Na-CMC little by little into 50 ml of hot distilled water with stirring to form a colloidal solution. Then, distilled water was added until the volume was 100 mL.

Preparation suspension glibenclamide

Taken glibenclamide tablet powder 0.1 g, then crushed in a mortar and added colloidal solution of Na-CMC 1% w/v gradually eroded until homogeneous. Included in the 100 ml flask and then paid back the volume up to 100 ml with a colloidal solution of Na-CMC 1%.

Preparation and selection of test animals

Test animals used were rat (*Rattus norvegicus*) streptozotocin-induced male. This study uses three male rats each group, the number of treatment groups by five groups so that the total number of test animals used in research as much as 15 tails. Criteria for inclusion: Male rats, age 3 months, weight 200 g, and healthy condition (active and not disabled). Exclusion criteria: Rat inappropriately active and the mice die during the study.

Treatment against animal testing

- a. Step I: Research using laboratory animals as much as 15 male rats were divided into five groups, adapted for 1 week in the laboratory and fed standard.
- b. Step II: Initial examination of blood glucose levels of mice that had been fasted for 16 hours in the day 7.
- c. Step III: After the initial examination of blood glucose levels, rats with streptozotocin induced a dose of 40 mg/kg intraperitoneally.
- d. Step IV: After streptozotocin induced, blood

- glucose levels were measured every day to show the fasting blood glucose levels of mice over 126 mg/dL.
- e. Step V: After the rats reached hyperglycemia blood glucose levels, 15 rats were divided into five groups randomly.
 - f. Step VI: Examination of blood glucose levels in mice that had been fasted for 16 h.
 - g. Step VII: After the measurement of blood glucose levels, five groups of mice were treated orally for 14 days.
 - Group I: Suspension Na-CMC
 - Group II: Insulin bark extract (*Tithonia diversifolia*) 125 mg/kg
 - Group III: Stem extract insulin (*Tithonia diversifolia*) 250 mg/kg
 - Group IV: stem extract insulin (*Tithonia diversifolia*) 500 mg/kg
 - Group V: Suspension glibenclamide 0.45 mg/kg
 - h. Step VIII: After treatment for 7 and 14 days, blood glucose levels examined male rats that had been fasted for 16 h.
 - i. Step XI: All data glucose levels before and after treatment were obtained, tabulated, were averaged and analyzed.

Determination of blood glucose levels

Before use, glucometer glucose is turned on and the stick is inserted into the glucometer. Blood is drawn through the tail veins of mice and then dropped into the stick glucometer. Within 10 s of blood glucose levels will be measured automatically and the results can be read on the monitor glucometer.

Data analysis

The research design used is RAK (randomized design). Observed data were analyzed with ANOVA statistical test at 95% confidence level. This test is used to determine whether the dose variation between treatments were significant difference or not significant, followed by Duncan test to determine the effect of a treatment that provides meaningful.

Results and Discussion

The results of research on phytochemical tests are shown in Table 1 and blood glucose levels in rats after treatment with insulin stem extract (*Tithonia diversifolia*) in Table 2.

Table 1: Screening test phytochemistry⁷

Chemical ingredients	The results	Description
Alkaloids	+	(+): Contains compounds tested
Saponin	+	(-): Not containing compounds tested
Steroids	-	
Flavonoids	+	
Tannins	-	
Polyphenols	+	

This study used a sample of insulin stems identified in Unit Biological Resources Sulawesi, intending to ensure the correctness of the plant species used in the study. The results show that the identification of plant samples used was insulin rod (*Tithonia diversifolia*) from Asteraceae tribe. Simplicia powder insulin rod extracted by maceration with 96% ethanol. Extraction results obtained are 500 grams, with a percentage of 5% extract. Then, the extraction results were tested for phytochemical screening. Based on phytochemical screening test results as shown in Table 1, the results showed that insulin rod contains chemical compounds alkaloids, flavonoids, saponins, and polyphenols. This is consistent with the literature mentions that the plant insulin (*Tithonia diversifolia*) contains bioactive substances such as alkaloids, saponins, flavonoids, and phenolic [9], [10], [11].

This study used male rats as test animals. Male rats have drug metabolism that works faster and more stable biological conditions because male rats were not influenced by the hormone estrogen, which can affect blood glucose levels. Mice used in this study first fasted before being treated. The goal is to minimize the dietary factors that can affect blood glucose levels and the rats given the drug absorption [12]. Nevertheless, the biological variation factor of the test animals cannot be removed so that the relative can affect the results because there are differences in the initial blood glucose levels for each test animal. The research design used was a randomized block design in connection with these considerations.

In this study, streptozotocin-induced rats were used as animal models of diabetes. Streptozotocin is a chemical used to induce diabetes in experimental animals. Giving streptozotocin is a quick way to generate experimental diabetic conditions (hyperglycemic) because streptozotocin reacts with damaging essential substance in the beta cells of the pancreas, causing a reduction of insulin in the beta cells of the pancreas [13], [14]. Streptozotocin can be administered intravenously, intramuscularly, intraperitoneally, or subcutaneously in animal experiments. An increase in blood glucose levels cause losses will easily observe after treatment given.

A decrease in the levels of blood glucose is shown in Table 2. The results of the measurement of blood glucose levels after induction of streptozotocin resulted in all test animals induced with diabetes mellitus with blood glucose levels >126 mg/dl. Increased blood glucose levels that occur are from 415.33 to 460.33 mg/dl. The increase in blood glucose levels varies for each rat, nor seen any difference in the average reduction in blood glucose levels that occur after administration of treatment

Table 2: Data of blood glucose levels in mice after treatment of insulin stem extract (*Tithonia diversifolia*)

Treatment group	No.	Initial blood glucose levels (mg/dL)	Induced glucose levels	Glucose levels on day 7	Glucose levels day 14
Negative control (Na-CMC 1%)	1.	71	430	328	312
	2.	77	538	381	300
	3.	55	413	297	301
	The mean ± SD	67.67 ± 11.37	460.33 ± 67.80	335.33 ± 42.47 ^b	304.33 ± 6.65 ^c
Positive control (glibenclamide)	1.	61	485	115	117
	2.	61	490	119	102
	3.	55	338	113	96
	The mean ± SD	59 ± 3.46	437.66 ± 86.34	115.67 ± 3.65 ^A	105 10.81 ± ^a
Insulin bark extract 125 mg/kg	1.	80	410	323	248
	2.	74	480	333	303
	3.	89	356	345	246
	The mean ± SD	81 ± 7.54	415.33 ± 62.17	333.67 ± 11.01 ^b	265.67 32.34 ± ^c
Insulin bark extract 250 mg/kg	1.	79	418	346	202
	2.	90	484	366	173
	3.	110	470	369	251
	The mean ± SD	93 ± 15.71	457.33 ± 34.77	360.33 ± 12.50 ^b	208.67 ± 39.42 ^b
Insulin bark extract 500 mg/kg	1.	78	422	350	108
	2.	104	455	378	190
	3.	113	498	391	218
	The mean ± SD	98.33 ± 18.17	458.33 ± 38.10	373 ± 20.95 ^b	172 ± 57.16 ^b

Description: Alphabetical different shows significant differences, the same alphabet showed no significant difference.

in each group. Each subject responded differently in each group of the same treatment. This is due to the presence of biological variation of the dose given.

The rats that had induced pancreatic glands can still produce insulin because their pancreatic beta cells can still produce. Therefore, glibenclamide used as a positive control/comparison to see the effect of a decrease in blood glucose levels of mice after the administration of insulin stem extract. The use of glibenclamide as a drug for diabetes mellitus sulfonylurea class of compounds has a mechanism of action of drugs that can stimulate the secretion of insulin in the pancreas and is only effective when the beta cells of the pancreas can still produce.

Research results contained in Table 2 show that the average decline in blood glucose levels on day 7, the negative control group, and a dose of 1, 2, and 3 have not been able to lower blood glucose levels of mice, but in the positive control group, decreased blood glucose was significantly. This is because herbal medicine treatment effects are relatively long when compared with treatment using the dosage so that the treatment effect more quickly. On the 14th day of the experiment, five treatments at doses 1, 2, and 3 obtained blood glucose levels of 265.67 mg/dL, 208.67 mg/dL, and 172 mg/dL, respectively. The positive and negative controls were 105 mg/dl and 304.33 mg/dl, respectively. These results indicate that administration of insulin stem extract is proven to lower blood glucose levels of mice. Determining the existence of a significant difference between the doses of insulin stem extract conducted by ANOVA statistical test with a level of 95%. The results of the analysis indicate that there are significant differences between each treatment in lowering blood glucose levels of mice. This shows that there is a dose effect of the extract on rat blood glucose reduction, where the higher the dose the more active substances contained in the stem extract insulin dosage. Determination of the dose of insulin stem extract is effective in lowering blood glucose levels which is done by further analysis using Duncan test.

Based on Duncan's test results, the effect of decreasing blood glucose levels in mice on day 14 on

the use of insulin stem extract at a dose of 250 mg/kg and 500 mg/kg BW was not significantly different. Also found that the use of stem extract insulin at a dose of 125 mg/kg was significantly different from the negative control, proving that the stem extract insulin has antidiabetic effects. Based on the data in Table 2, which is also corroborated by the data obtained by the Duncan test results, the decrease in blood glucose levels of mice on the use of stem extract of insulin at a dose of 250 mg/kg body weight did not differ significantly with a dose of 500 mg/kg, although a decrease in blood glucose levels extracts insulin rod looks lower than the dose of 500 mg/kg (Figure 1). Determination of the dose of 250 mg/kg as the most effective dose is done based on the consideration that the use of stem extract of insulin at a dose of 250 mg/kg can give the effect of a decrease in blood glucose levels of mice which are relatively similar to the use of a dose of 500 mg/kg. This is because the extract at a dose of 250 mg/kg was the best concentration to bind to the receptors so that by adding/increasing dose had no significant effect to effect a given. The intensity of the effect of the drug is directly proportional to the fraction of receptors occupied or tied to the intensity of the effect reaches a maximum when all of receptors occupied by the drug [15]. This condition causes the antidiabetic effects inflicted on the use of a dose of 250 mg/kg and 500 mg/kg which was also relatively common.

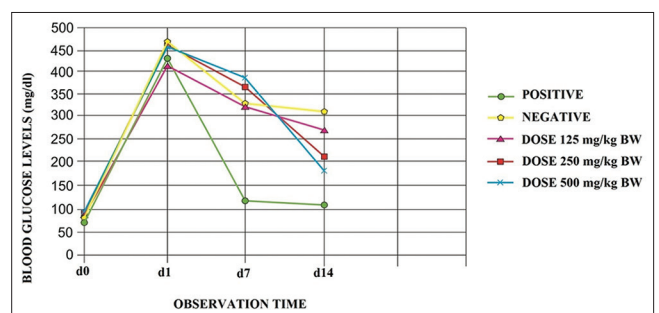


Figure 1: Profile of blood glucose levels. Description: d0 = Initial glucose levels, d1 = Glucose induction, d7 = Glucose levels on day 7, d14 = Glucose 14th day

Antidiabetic activity on insulin stem extract is allegedly due to the chemical content of alkaloids, flavonoids, saponins, and polyphenols [9], [10], [11]. Two mechanisms work to decrease blood glucose levels, which are intrapancreatic and extrapancreatic. The intrapancreatic mechanism works by preventing the damaged pancreatic beta cells, protecting the beta cells, and stimulating insulin release [16]. This capability is owned by alkaloids and flavonoids, alkaloids which work by regenerating damaged pancreatic beta cells and act as an antioxidant flavonoid protects pancreatic beta cell damage. Ekstrapankreatik mechanism works by lowering blood glucose levels by increasing insulin sensitivity [17]. This capability is owned by alkaloid compounds that can inhibit glucose absorption in intestines, stimulates glycogen synthesis, and inhibits synthesis by inhibiting the enzyme glucose 6-phosphatase fructose 1,6-bifosfatase that plays a role in gluconeogenesis [13], [17], [18].

Conclusion

Based on the results of research, discussion, and statistical analysis, we can conclude the following: Stem extract insulin (*Tithonia diversifolia*) is proven to lower blood glucose levels of mice significantly, after treatment for 14 days. The dose of insulin stem extract was effective in lowering blood glucose levels by 250 mg/kg.

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