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

Suherman Suherman 1*, Baharuddin Hamzah 1, Sri Hastuti V. Pulukadang 1, Siti Rahmawati 1, Muhammad Fakhruhl Hardani 2, Ririen Hardani 1, Andi Saifah 1

1Chemistry Study Program, Mathematics and Sciences Division, Faculty of Teacher Training and Education, Tadulako University, Palu, Indonesia; 2Department of Pharmacy, Faculty of Mathematics and Natural Sciences, Tadulako University, Palu, Indonesia; 3Nursing Study Program, Faculty of Medicine, Tadulako University, Palu, Indonesia

**Abstract**

**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 of 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 block 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 phytochemistry**

| Chemical ingredients | The results | Description |
|----------------------|-------------|-------------|
| Alkaloids            | +           | (+): Contains compounds tested |
| Saponins             | -           | (-): 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 on 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 | 304.33 ± 6.65 | 304.33 ± 6.65 |
| 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 | 116.67 ± 3.65 | 105.17 ± 17.36 |
| Insulin bark extract 125 mg/kg | 1. | 80 | 410 | 325 | 248 |
| | 2. | 74 | 480 | 333 | 303 |
| | 3. | 89 | 356 | 345 | 246 |
| The mean ± SD | 81 ± 5.74 | 415.33 ± 62.17 | 333.67 ± 11.01 | 265.67 ± 32.34 | 265.67 ± 32.34 |
| 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 | 208.67 ± 39.42 |
| Insulin bark extract 500 mg/kg | 1. | 78 | 422 | 350 | 108 |
| | 2. | 104 | 455 | 378 | 150 |
| | 3. | 113 | 498 | 391 | 218 |
| The mean ± SD | 98.33 ± 18.17 | 458.33 ± 38.10 | 373.33 ± 20.95 | 172 ± 57.16 |

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 sulfonfylurea 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.
Antidiabetic activity on insulin stem extract is allegedly due to the chemical content of alkaloids, flavonoids, saponins, and polyphenols. 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. This capability is owned by alkaloids and flavonoids, which work by regenerating damaged pancreatic beta cells and act as an antioxidant flavonoid that protects pancreatic beta cell damage. Extrapancreatic mechanism works by lowering blood glucose levels by increasing insulin sensitivity. 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.

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.

Acknowledgment

The author would like to thank the Faculty of Education for funding research for DIPA 2021, as well as the Chemistry Laboratory assistant who has helped a lot in the completion of this paper.

References

1. Mahendra B. Jenis Tanaman Obat Ampuh. Jakarta: Penebar Swadaya; 2005.
2. Hong L, Guo Z, Huang K, Wei S, Liu B, Meng S, et al. Ethnobotanical study on medicinal plants used by Maonan people in China. J Ethnobiol Ethnomed. 2015;11(1):1-35. https://doi.org/10.1186/s13002-015-0019-1 PMid:25925830
3. Abe R, Ohtani K. An ethnobotanical study of medicinal plants and traditional therapies on Batan Island, the Philippines. J Ethnopharmacol. 2013;145(2):554-65. https://doi.org/10.1016/j.jep.2012.11.029 PMid:23153086
4. Nita Y, Yuda A, Nugraheni G. Pengetahuan pasien tentang diabetes dan obat antidiabetes oral. J Farm Indones. 2012;6(1):38-47.
5. Yuen L, Saeedi P, Riaz M, Karuranga S, Dlkavak H, Levitt N, et al. Projections of the prevalence of hyperglycaemia in pregnancy in 2019 and beyond: Results from the international diabetes federation diabetes Atlas. Diabetes Res Clin Pract. 2019;157:107841. https://doi.org/10.1016/j.diabres.2019.107841 PMid:31518656
6. Saeedi P, Petersohn I, Salpea P, Malanda B, Karuranga S, Unwin N, et al. Global and regional diabetes prevalence estimates for 2019 and projections for 2030 and 2045: Results from the international diabetes federation diabetes Atlas. Diabetes Res Clin Pract. 2019;157:107843. https://doi.org/10.1016/j.diabres.2019.107843 PMid:31518657
7. Otusanya O, Ilior O. Phytochemical screening and the phytotoxic effects of aqueous extracts of Tithonia diversifolia (Hemsl) a gray. Int J Biol. 2012;4(3):97. https://doi.org/10.5539/jib.v4n3p97
8. Thongsom M, Chunglok W, Kuanchuea R, Tangpong J. Antioxidant and hypoglycemic effects of Tithonia diversifolia aqueous leaves extract in alloxan-induced diabetic mice. Adv Environ Biol. 2013;7(9):2116-26.
9. Mwanauta RW, Mtei KA, Ndakidemi PA. Prospective bioactive compounds from Vernonia amygdalina, Lippia javanica, Dysphania ambrosioides and Tithonia diversifolia in controlling legume insect pests. Agric Sci. 2014;5(12):1129. https://doi.org/10.4236/as.2014.512123
10. Ajao AA, Motetee AN. Tithonia diversifolia A. Gray. (Asteraceae: Heliantheae), an invasive plant of significant ethnopharmacological importance: A review. S Afr J Bot. 2017;113:396-403. https://doi.org/10.1016/j.sajb.2017.09.017
11. Tagne AM, Marino F, Cosentino M. Tithonia diversifolia (Hemsl.) A. Gray as a medicinal plant: A comprehensive review of its ethnopharmacology, phytochemistry, pharmacotoxicology and clinical relevance. J Ethnopharmacol. 2018;220:94-116. https://doi.org/10.1016/j.jep.2018.03.025 PMid:28956999
12. Williamson G. Possible effects of dietary polyphenols on sugar absorption and digestion. Mol Nutr Food Res. 2013;57(1):48-57. https://doi.org/10.1002/mnfr.201200511 PMid:23180627
13. Lenzen S. The mechanisms of alloxan-and streptozotocin-induced diabetes. Diabetologia. 2008;51(2):216-26. https://doi.org/10.1007/s00125-007-0886-7 PMid:18087688
14. Ningsih P, Rahmawati S, Hamzah B, Santoso T, Nurbaya N, Hardani MF, et al. Histology of hematoxylin and eosin and immunohistochemical diabetes rat pancreas after giving combination of Moringa leaves (Moringa oleifera) and clove flower (Syzygium aromaticum) extracts. Open Access Maced J Med Sci. 2021;9(A):257-62. https://doi.org/10.3889/oamjms.2021.5928
15. Katzung BG. Farmakologi Dasar dan Klinik. 2nd ed. Jakarta: Salemba Medika; 2002.
16. Lippi G, Valentino M, Cervellin G. Laboratory diagnosis of acute pancreatitis: In search of the Holy Grail. Crit Rev Clin Lab Sci. 2012;49(1):18-31. https://doi.org/10.3109/10408363.2012.658354 PMid:22339380
17. Uyumlu AB. Ratlarda Cerulein Ile Indüklenen Akut Pankreatit Üzerine Verapamil, Dantrolen Ve 2-Aminoetoksidifenil Boratın Etkilerinin İncelenmesi: Dramatik Yapılar; 2011.
18. Román-Ramos R, Flores-Sáenz JL, Partida-Hernández G, Lara-Lemus A, Alarcón-Aguilar F. Experimental study of the hypoglycemic effect of some antidiabetic plants. Arch Invest Med. 1991;22(1):87-93. PMid:1819981