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Effectiveness of *Cassia alata* L Leaf Extract Decrease Blood Glucose Level on Streptozotocin-induced Male White Rats

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ABSTRACT

Introduction: Diabetes mellitus (DM) is a condition in which the body either fails to produce enough insulin at the right time or fails to use it properly. **Aims**: The goal of this study is to show how *Cassia alata* L. leaf extract affects blood glucose levels in streptozotocin-induced diabetic rats. **Methods**: *Cassia alata* L. leaves therapy is an alternative treatment for DM. In this investigation, laboratory techniques were employed. Streptozotocin was administered intraperitoneally to mice at a dose of 40 mg/kg body weight initially. Except that they produce normal controls. Six groups of 30 rats each received treatment with *Cassia alata* L. leaf extract at doses of 500 mg/kg body weight, 600 mg/kg body weight, and 700 mg/kg body weight in addition to the usual control group. Trial outcome data were first examined using one-way ANOVA to confirm differences between treatments and then put through the DUNCAN trial. **Result**: As a result, it was discovered that secondary metabolites of alkaloids, flavonoids, saponins, and tannins were present in the ethanol extract of *Cassia alata* L. leaves. A dose of 700 mg/kg body weight is an effective blood sugar-lowering dose, with an average reduction of 121 mg/dl. **Conclusion**: Ethanol extract from *Cassia alata* L. leaves has this effect.

KEYWORDS: Blood glucose level, antidiabetes, *Cassia alata* L., streptozotocin, white rats

INTRODUCTION

A disorder known as diabetes mellitus (DM) occurs when the body is unable to produce enough of the hormone insulin, uses insulin ineffectively, or both. Hyperglycemia, or high blood sugar, is one of its defining characteristics. Reactive oxygen species (ROS), also known as free radicals, can be overproduced as a result of hyperglycemia. ROS causes oxidative stress because there are more free radicals than antioxidants in the body. Free radicals can diffuse into the cell membrane by attacking and binding to the electrons of the surrounding molecules which then react with the lipid membrane to produce malondialdehyde (MDA) (Tandi, 2016).

One of the plants that can be used in traditional medicine is *Cassia alata* L. *Cassia alata* L has been traditionally used as a treatment for typhus, diabetes, malaria, asthma, ringworm, scabies, and eczema (Oladeji et al., 2020).

A previous study on *Cassia alata* L. by Herlina (2019) found that *Cassia alata* L ethanolic extract at a dose of 800 mg/kg body weight effectively reduced alloxan-induced blood glucose levels in male white rats at different doses of 200, 400, and 800 mg/kg body weight. Research by Anggraini, 2018 states that the methanol extract of *Cassia alata* L leaves contain steroid compounds, flavonoids, alkaloids, saponins, and tannins. The flavonoid group contained in the *Cassia alata* L leaf plant is kaemferol which has anti-diabetic activity presumably by stimulating glycogen synthesis.

Based on the above explanations, investigators are interested in studying the ethanol extract of *Cassia alata* L leaf on streptozotocin-induced hypoglycemia in male albino rats (*Rattus norvegicus*) to determine the effect of administration of the ethanol extract at different doses. Innovative researchers varied three doses to obtain an effective dose: 500, 600, and 700 mg/kg body weight. This study demonstrates the efficacy of his *Cassia alata* L ethanol extract at various doses in lowering blood glucose levels in male white rats. The purpose of this study is to provide information on effective doses for lowering blood glucose levels as an important antidiabetic parameter.

MATERIAL AND METHODS

Chemicals and Instrument

The tools used are surgical instruments, glassware (pyrex ®), 40 mesh sieve, maceration vessel, blender, rat drinking bottle, glucometer (Accu-Chek ®), test animal cages, mortar and stamper, water bath, dropper, Rotary vacuum evaporator (Heidolph), 3 ml oral sonde (One Med Health Care), 3 ml injection syringe (One Med Health Care), test tube (pyrex ®), rat feeding tray, gram scales, analytical balance (Ohaus), and test animal scales.

The materials used are distilled water (aqua), aqua pro injection (Otsuka), 70% alcohol, concentrated hydrochloric acid (Merck), sulfuric acid (Merck), iron (III) chloride (Merck), citrate-buffer saline (citric acid and sodium citrate), ethanol 96% (Merck), *Cassia alata* L leaf, drag-drop LP, glibenclamide, Hanskun (Sensi), cotton, label paper, filter paper, duct tape, Liebermann-Burchard, mask, 0.5% Na CMC, sodium hydroxide (Merck), sodium chloride (PT. Widatra Bhakti), streptozotocin (Bioworld USA), magnesium P powder (Merck), and tissue.

Preparation Ethanol Extract of *Cassia alata* L Leaf

The maceration process was used to create an ethanol extract from *Cassia alata* L leaves. In other words, 1000 grams of powdered *Cassia alata* L leaf were sieved through a 40-mesh sieve, extracted over the course of three days using 4 liters of a 96% ethanol solvent, divided between two maceration jars with periodic stirring, then filtered through filter paper to obtain a filtrate, and finally evaporat-

Instead, the solution was divided, heated to 60°C in a rotary evaporator to evaporate it, and then concentrated in a water bath to create a thick extract (Dewi et al., 2021).

Phytochemical Screening Test

Flavonoid Test

Extracts weighed up to 0.5 grams, added 10 ml of distilled water and heated over a water bath then filtered then dissolved in 1 ml of ethanol (95%) with the addition of magnesium powder then dissolved in 10 ml of concentrated hydrochloric acid and dripped on a tissue if a red color occurs, purple, yellow, and orange indicate the presence of flavonoids (Widuri, 2018).

Saponin Test

Extracts weighed up to 0.5 grams and put into a test tube. Add 10ml of hot water to cool, then shake vigorously for 10 seconds. If the foam is formed and persists for at least 1 minute up to 10 cm in height, or if the foam does not disappear after adding a drop of 2N hydrochloric acid, this indicates the presence of saponins. (Hasibuan et al, 2021).

Tannin Test

Extracts weighed up to 0.5 grams put into a porcelain cup. Add 20 ml of hot water and 3 drops of 10% NaCl solution, then add FeCl3 solution and drop it on a tissue if a black-blue color forms, indicating the presence of tannin compounds (Tandi et al., 2020).

Alkaloid Test

Extracts weighed up to 0.5 grams and then 5 ml of 2N hydrochloric acid was added. Heat Effectiveness of *Cassia alata* L leaf extract on a water bath for 2 minutes and add 3 drops of Dragendoroff reagent. If the result is a yellow-orange to brick-red precipitate, the sample contains alkaloids (Hasibuan et al, 2021).

Steroids Test

Extracts weighed up to 0.5 grams dissolved with 5 ml of water then added a few drops of chloroform, acetic anhydrous acid, and sulfuric acid through the wall of the test tube and drops on the tissue if a bluish-green color is formed indicating the presence of steroid compounds (Tandi et al., 2020).

Test Materials Manufacturing

The ethanol extract of *Cassia alata* L leaf was weighed to make the test suspension 1 gram (500 mg/kg BW), 1.2 gram (600 mg/kg BW), and 1.4 gram (700 mg/kg BW) respectively. BB) then added 0.5% Na CMC to each extract, made up to 25 ml, then shaken until homogeneous (Dewi et al., 2021).

Preparation of Streptozotocin

Streptozotocin was weighed to 0.32 grams and then dissolved in 100 ml using citrate buffered saline (pH 4.5) and then induced intraperitoneally (IP) in rats. The dose of streptozotocin is 40 mg/kg body weight. (Dewi et al., 2021).

Preparation of Glibenclamide Suspension 0.45 mg/kg BW

The dose of glibenclamide in adults is 5 mg per day. When converted to a rat weighing 200 grams, it is 0.018, giving a rat glibenclamide

dose of 0.45 mg/kg body weight. Weigh an amount equivalent to 3.6 mg of glibenclamide tablet powder, suspended in 25 ml of 0.5% Na-CMC, and shake until homogenous. (Tandi, 2016).

Antidiabetic Effect Testing

Thirty male white rats were divided into six groups: a normal control group (containing standard diet and 0.5% Na-CMC suspension), a positive control group (containing glibenclamide suspension), and a negative control group (containing 0.5% Na-CMC suspension). and treatment groups at doses of 500 mg/kg body weight, 600 mg/kg body weight, and 700 mg/kg body weight. Mice were adapted for 14 days in the laboratory and fed a standard diet. On day 0 after adaptation, rats were fasted for 16 hours, after which initial tail vein blood glucose levels were measured with a glucometer to ensure that all mice had normal blood glucose levels before treatment. On the same day after the initial blood glucose measurement, rats were intraperitoneally administered 40 mg/kg body weight of streptozotocin. After fasting the rats for 16 hours on day 7 after induction, the post-induction rat blood glucose level was measured again. After the rats' fasting blood glucose levels reached hyperglycemia (>200 mg/dl), the rats received oral treatment for 21 days. Record and analyze blood glucose measurement data before and after treatment. (Tandi, 2016).

Determination of Glucose Levels

The blood glucose level of rats was measured from the lateral vein of the tail end using a glucometer. The trick is to slightly pull the tail of the rat from which blood was collected, place it on the wire plunger, wash the tail with 70% alcohol before blood collection, gently massage the tail, and then pierce the tip of the rat's tail with a fine needle. The blood that comes out is dripped into the blood glucose meter within 10 seconds, the blood glucose level is automatically measured, and the result can be read on the blood glucose meter monitor. The working mechanism of this glucometer tool works enzymatically and involves a glucose oxidase reaction that produces the color intensity detected by this tool. This tool is very easy to use, highly sensitive, and specialized for blood glucose measurement. (Tandi et al., 2020).

Data Analysis

The measured data is the blood sugar level and a normality test was performed to ensure that the data were normally distributed. If the data are normally distributed, proceed to statistical analysis of one-way ANOVA at the 95% confidence level and DUNCAN test to detect differences between treatments.

RESULTS AND DISCUSSION

Ethanol extracts of *Cassia alata* L leaves were obtained through an extraction process using the maceration method. The resulting

ethanol extract of *Cassia alata* L. leaves was 394 grams with a yield of 39,4%. Phytochemical studies showed that ethanol extracts from *Cassia alata* L leaf contained secondary metabolites such as alkaloids, flavonoids, saponins, and tannins, as well as unidentified steroids, which can be seen in Table 1.

The study was conducted using test animals in the form of 30 male white rats of the Wistar strain. The test animals used were approved by the Animal Ethics Committee of the Tadulako University Medical Health Research Ethics Committee. To create a diabetes test animal model, test animals were administered 40 mg/kg body weight of streptozotocin. Streptozotocin can produce free radicals in the body that specifically damage the DNA chains in pancreatic β cells resulting in impaired function of pancreatic β cells (Azhari et al., 2016). Intermediate doses of streptozotocin between 40-55 mg/kg BW cause partial insulin disorders such as type 2 DM (Husna et al., 2019) so if the rat's blood glucose level becomes >200 mg/dL, then the rat is considered to have diabetes (Haryoto et al., 2016).

Table 1. Phytochemical test results for *Cassia alata* L leaf extract

Bioactive compound	Result
Alkaloid	+
Flavonoid	+
Flavonoid	+
Saponin	+
Tannin	+
Steroid	-

Note : (+) : Contains tested compound; (-) : Does not contain the compounds tested

Effectiveness of *Cassia alata* L leaf extract

Blood glucose measurements were performed on day 0 before streptozotocin induction to determine the initial blood glucose levels of the test animals and to compare whether streptozotocin intraperitoneal induction was successful in all test groups except normal controls. Streptozotocin enters pancreatic Langerhans β-cells via GLUT 2 and causes alkylation. This was preceded by restriction of mitochondrial adenosine triphosphate formation through the formation of free radicals, an increase in the enzyme xanthine oxidase, and inhibition of the Krebs cycle, causing damage to pancreatic β-cells, resulting in inhibition of insulin production and elevated blood glucose levels. (Saputra et al., 2018).

The results of measuring blood glucose levels on the 28th day (figure 1) showed that the average glucose levels for all groups ranged from 94 mg/dL - 247 mg/dL. The results of the one-way ANOVA statistical test on the 28th day showed a value of P = 0.000 (P <0.05) which indicated that there were significant differences in the test group except for the normal controls, so it was continued with Duncan's test to see significant differences between the test groups.

The results of Duncan's test showed that the group at the dose of 500 mg/kg, dose 600 mg/kg, dose 700 mg/kg was significantly different from the negative control. Affects blood glucose levels in rats. The 500 mg/kg body weight and 600 mg/kg body weight doses were not significantly different from the

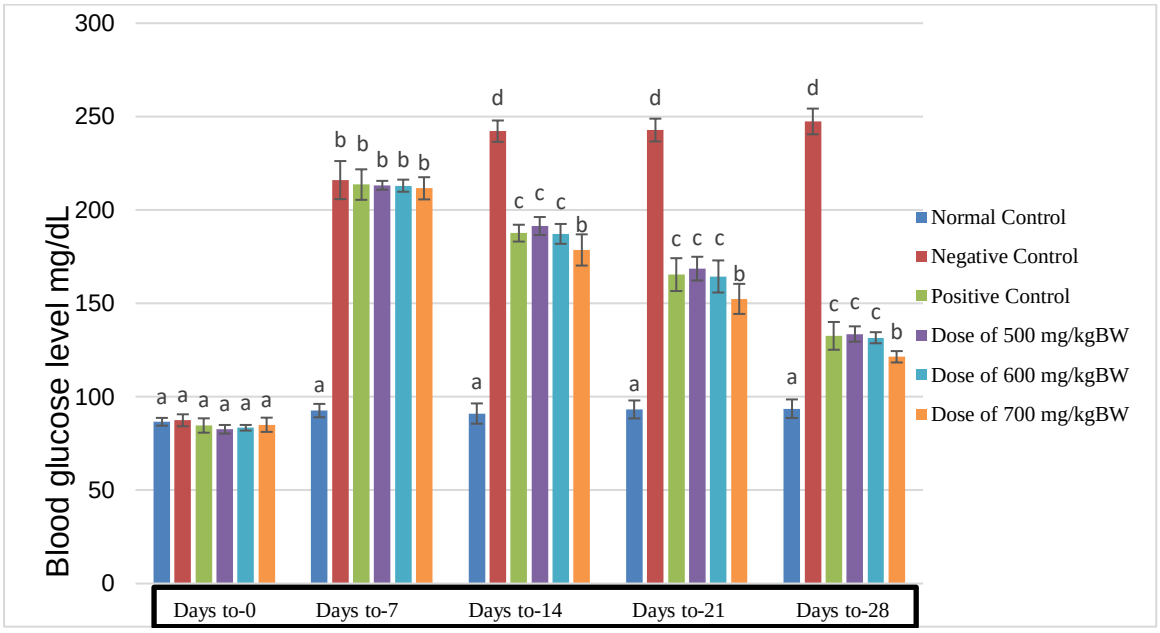

Figure 1. Profile of rat blood glucose levels. Same letters indicate insignificant differences.

positive control. The dose of 700 mg/kg body weight was significantly different from the normal and positive controls and did not reach normoglycemia in the control group, but was already within the normal range for rats, 50-135 mg/dl (Haryoto et al., 2016). This is due to the very high level of damage to pancreatic β-cells and the relatively short time of administration of ethanol extract of *Cassia alata* L leaves, causing the content of secondary metabolites in *Cassia alata* L leaves to not be able to repair pancreatic β cells optimally to produce insulin which can lower blood glucose levels and cause glibenclamide to not work optimally because the test animals have lost their ability to secrete insulin.

The antidiabetic mechanism of flavonoids in *Cassia alata* L is thought to play a key role in reducing oxidative stress and increasing the activity of antioxidant enzymes that can reduce reactive oxygen species (ROS), thus providing protective effects on pancreatic β cells and increasing insulin sensitivity (Chitania et al., 2020). Flavonoids contained in plants can also improve insulin receptor sensitivity and stimulate Ca^{2+} uptake (Anggraini, 2018).

A comparison of studies on *Cassia alata* L leaf ethanol extract induced with a streptozotocin dose of 40 mg/kg body weight showed that a dose of 700 mg/kg body weight averaging 121 mg/dl was superior to previous studies using different inductions, namely the antidiabetic effects of *Cassia alata* L leaf ethanol extract induced by alloxan at doses ranging from 1 to 30 mg/kg body weight. The results indicate that *Cassia alata* L leaf ethanol extract at a dose of 800 mg/kg body weight is an effective dose compared to other therapeutic dose variants due to the lowest average AUC 0-15 value and highest hypoglycemic rate. (Anggraini, 2018).

CONCLUSION

Based on the research results, we can

conclude that doses of 500,600, and 700 mg/kg body weight of *Cassia alata* L leaf ethanol extract have an effect on streptozotocin-induced hypoglycemia in male white rats, with a dose of 700 mg/kg body weight being an effective dose for mean hypoglycemia of 121 mg/dl.

In future studies, quantitative testing of secondary metabolites in *Cassia alata* L leaves and toxicity testing of *Cassia alata* L leaves is possible.

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REFERENCES

Anggraini, P. D. (2018). Efektivitas ekstrak etanol daun ketepeng cina (*Senna alata* L.) sebagai antidiabetes terhadap tikus putih jantan galur wistar yang diinduksi oleh aloksan.

Azhari, D. M., Yuliet, Y., & Khaerati, K. (2016). Uji aktivitas serbuk jamur tiram putih (*Pleurotus ostreatus* (Jacq.) P.kumm) terhadap kadar glukosa darah pada model hewan hiperkolesterolemia-diabetes. *Jurnal Farmasi Galenika (Galenika Journal of Pharmacy) (e-Journal)*, *2*(2), 96–102. https://doi.org/10.22487/j24428744.2016.v2.i2.5971

Chitania, N., Alawiyah, T., Dwi, L. A. M., & Adhany, A. Y. (2020). Potensi ekstrak daun ketepeng cina (*Cassia alata* L.) terhadap penurunan kolesterol darah mencit jantan hiperlipidemia'. *Journal of Pharmaceutical Care and Science*, *1*(1), 36–44.

Dewi, N. P., Kawanga, W. O., & Lakiu, D. R. (2021). Histopathological study of streptozotocin induced liver regeneration of wistar mice after administration of pumpkin leaf ethanol extract. *Ad-Dawaa' Journal of Pharmaceutical Sciences*, *4*(2). https://doi.org/10.24252/djps.v4i2.25115

Haryoto, N. H., Sujono, T. A., & Suhendi, A. M. (2016). Antidiabetes mellitus ekstrak etanol kulit batang tumbuhan sala (*Cynometra ramiflora* L.) terhadap tikus jantan galur wistar yang diinduksi aloksan. *Jurnal Penelitian Farmasi Dan Teknologi*, *147*(March), 11–40.

Hasibuan, A. S., & Edrianto, V. (2021). Sosialiasi Skrining Fitokimia Ekstrak Etanol Umbi Bawang Merah (Allium cepa L.). *Jurnal Pengmas Kestra (Jpk)*, *1*(1), 80–84. https://doi.org/10.35451/jpk.v1i1.732

Herlina, Amriani, A., Solihah, I., Damayanti, P., & Rais, S. W. (2019). Effectiveness of ethanolic extract ketepeng cina leaves (*Senna alata* L.) as antidiabetic activity test in male wistar rats induced by alloxan. *Journal of Physics: Conference Series*, *1282*(1). https://doi.org/10.1088/1742-6596/1282/1/012085

Husna, F., Suyatna, F. D., Arozal, W., & Purwaningsih, E. H. (2019). Model hewan coba pada penelitian diabetes animal model in diabetes research. *Mini Review Article Pharmaceutical Sciences and Research (PSR)*, *6*(3), 131–141.

Oladeji, O. S., Adelowo, F. E., Oluyori, A. P., & Bankole, D. T. (2020). Ethnobotanical description and biological activities of *Senna alata*. *Evidence-Based Complementary and Alternative Medicine*, *2020*. https://doi.org/10.1155/2020/2580259

Saputra, N. T., Suartha, I. N., & Dharmayudha, A. A. G. O. (2018). Agen diabetagonik streptozotocin untuk membuat tikus putih jantan diabetes mellitus. *Buletin Veteriner Udayana*, *10*(2), 116. https://doi.org/10.24843/bulvet.2018.v10.i02.p02

Tandi, J. (2016). Efektivitas ekstrak daun gedi merah terhadap glukosa darah, malondialdehid, 8-Hidroksi-deoksiguanosin, insulin tikus diabetes. *J. Trop. Pharm*, *147*: 11–40.

Tandi, J., Lalu, R., Magfirah, Kenta, Y. S., & Nobertson, R. (2020). Uji potensi nefropati diabetes daun sirih merah

(*Piper croatum* Ruiz & Pav) pada tikus putih jantan (*Rattus norvegicus*). *Kovalen: Jurnal Riset Kimia*, *6*(3), 239–251. https://doi.org/10.22487/kovalen.2020.v6.i3.15323

Widuri, S. A., & Mediawati, I. (2018). Skrining Fitokimia Dan Uji Aktivitas Antioksidan Beberapa Tumbuhan Obat Di Kabupaten Paser, Kalimantan Timur. *Prosiding Seminar Nasional Lingkungan Lahan Basah*, *3*(1), 116–120.