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HOW CASSIA ALATA L AFFECTS BLOOD GLUCOSE LEVELS IN DIABETES MELLITUS PATIENTS?

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ABSTRACT

Candle bush (*Cassia alata* L.) are plants that are rich in antioxidants. There have been many studies that discuss the efficacy of Candle bush (*Cassia alata* L.) in skin diseases, but there is still less research that discusses the potential of Candle bush leaves as antidiabetics. Therefore, researchers want to test the effectiveness of Candle bush leaves (*Cassia alata* L.) in improving blood glucose levels (antidiabetic) using experimental animals (*Rattus norvegicus* strain wistar) induced with Streptozotocin (STZ). Candle bush leaf extract (*Cassia alata* L.) was made with maceration method using ethanol 70% solution. Experimental animals were divided into 6 groups. Group 1,2, and 3 are the control group, Group 4,5, and 6 are the groups that are given the extract with different doses each. This study proves that extract of Candle bush leaf (*Cassia alata* L.) can improve blood glucose levels of the experimental animals (*Rattus norvegicus* strain wistar).

I. INTRODUCTION

Diabetes Mellitus is a metabolic disorder that occurs due to the body's failure to produce sufficient amounts of insulin or resistance to insulin so that blood glucose levels increase to abnormal levels. High blood glucose levels in diabetes mellitus (DM) sufferers can cause various disease conditions such as stroke, kidney failure, blindness, limb amputation, heart disease, and birth defects (WHO, 2012).

Indonesia is ranked fourth in Diabetes Mellitus cases with a prevalence of 8.6% of the total population, estimated to increase by 8.4 million people in 2000 to around 21.3 million people and in 2030. The results of Basic Health Research (Riskesdas) in 2018 show that the prevalence of Diabetes Mellitus is 2.0%. The prevalence of diabetes mellitus was obtained based on the results of blood sugar examinations in residents aged >15 years (National Riskesdas Report, 2018).

Providing therapy to diabetes mellitus sufferers is divided into 2, namely pharmacological therapy and non-pharmacological therapy. The aim of providing this treatment therapy is to regulate blood sugar levels. In pharmacological therapy for diabetes mellitus, primary health facilities such as community health centers provide antidiabetics such as glibenclamide, glimepirid, glipizide, and metformin. Long-term use of anti-diabetic drugs can cause certain side effects. Some patients stop taking antidiabetic drugs due to the side effects they experience (Renaldi *et al.*, 2021). Each type of drug available will have different side effects depending on the patient's condition. The most common side effects are gastrointestinal disorders such as nausea and diarrhea, followed by an increased risk of hypoglycemia, especially when using sulfonylurea drugs. (Adiputra, 2023).

From the results of data analysis through *Word Clouds*, the majority of patients experience gastrointestinal problems after using antidiabetic drugs and the drugs that have the most potential to cause gastrointestinal side effects such as the problems identified are from the biguanide group (metformin). Using metformin will often cause the side effects of feeling bloated and nauseous, sometimes also causing a feeling of pain in the stomach (Katzung, 2015). Another side effect that can be observed is weight gain, this occurs in 50% of patients. Weight gain usually occurs in the sulfonylurea group such as glimepiride and glibenclamide which have an influence on lipogenesis and increase the risk of heart and blood vessel disease. (Sauriasari *et al.*, 2022).

Indonesia has abundant natural resources that have potential for medical treatment. Research on natural ingredients in plants is important because there are still many compounds in plants whose activities need to be known before being used in traditional and modern medicine. Therefore, there are various alternative herbal ingredients that can be used for treatment, one of which is the treatment of type 2 diabetes mellitus.

One of the plants that is often found in tropical countries, including Indonesia, is the Chinese ketepeng plant (*Cassia alata* L.). This plant is usually used as an

ornamental plant and also as medicine for the community. Nowadays, people usually use Chinese ketepeng leaves (*Cassia alata* L.) as a medicine for fungal skin diseases and is supported by many studies discussing the benefits of Chinese ketepeng (*Cassia alata* L.) for these skin diseases. On the other hand, there is still a lack of research discussing the potential of Chinese ketepeng leaves as an anti-diabetic considering that the incidence of diabetes in Indonesia is also quite high.

Therefore, the use of medicinal plants with antidiabetic properties needs to be developed as an alternative treatment with relatively fewer side effects. One of the plants in Indonesia, especially Southeast Sulawesi that has the potential to be researched is Chinese ketepeng (*Cassia alata* L.).

Considering laboratory research on the efficacy of Chinese ketepeng leaves (*Cassia alata* L.) as an anti-diabetic is still limited, more research is needed to provide strong evidence regarding the efficacy of Chinese ketepeng leaves as an anti-diabetic. Based on the background above, the idea arose to utilize Chinese ketepeng leaves (*Cassia alata* L.) as a raw material to improve blood glucose levels in diabetes mellitus sufferers.

II. METHODS

Research Ethics

This research was carried out with the approval of the Ethics Committee of the Faculty of Medicine, Halu Oleo University with No. 094/UN29.17/PP/2023 and is in accordance with animal welfare principles, namely principles *Replacement, Refinement, And Reduction*.

Time and Place

This research was conducted at the Experimental Animal Laboratory, Faculty of Medicine, Halu Oleo University in June-October 2023.

Tools and materials

The tools used are analytical scales, micropipettes, dropper pipettes, glass jars, stir sticks, test tubes, porcelain cups, pestle mortars, measuring cups, beakers, *vacuum rotary evaporator*, oven, basket *stainless steel*, electromantle, beaker, EDTA tube, eppendorf tube, syringe, surgical board, tissue scissors, scalpel, tweezers, *glucose meter device*, *ELISA reader*, cages and drinking bottles for experimental animals.

The material used for this research was Chinese ketepeng leaves (*Cassia alata* L.), ethanol 70%, distilled water, CMC-Na, dragendorff reagent, FeCl reagent₃, NaOH reagent, HCl reagent, Lieberman Burchard reagent, chloroform, Streptozotocin (STZ), glibenclamide, AD-II and corn feed, and drinking water for experimental animals.

Research Procedures

Samples of Chinese Ketepeng Leaves (*Cassia alata* L.)

Sampling of Chinese ketepeng leaves (*Cassia alata* L.) carried out in Kambu Village, Kambu District, Kendari, Southeast Sulawesi. The samples taken were Chinese ketepeng leaves (*Cassia alata* L.) which is green and the leaves are not too young to obtain good secondary metabolite content. The leaves that have been taken

are dry sorted to separate the leaves and dirt attached to the leaves, as well as separate damaged leaves from those that are still good, then wet sorted to clean the dirt stuck to the leaves in order to obtain simplicia whose quality is guaranteed. The samples were then washed and cut into small pieces with a size of $\pm 1 \times 3$ cm and then dried using an oven at a temperature of 60°C for 4 x 24 hours.

Extraction is carried out through a maceration process by adding 70% ethanol solvent. Maceration is carried out for 3 x 24 hours. After obtaining a clear soak, evaporation is carried out using *rotary vacuum evaporator* so that a thick extract is obtained.

Try Animals

The experimental animals used in this research were white mice (*Rattus norvegicus strain Wistar*), male, 2 months old, average weight 98 grams. First, the experimental animals go through an acclimatization process carried out in the experimental animal laboratory at the Faculty of Medicine, Halu Oleo University for 7 days at a room temperature of $\pm 25^\circ\text{C}$ and are protected from industrial fumes and pollutants. During the acclimatization process, mice will be given standard food and drink ± 20 grams/day ad libitum. The acclimatization process aims to adjust the way of living, eating and conditions of the experimental cage.

The experimental animals were then divided into 6 groups based on the similarity of the body weight of the experimental animals, consisting of 3 control groups and 3 treatment groups.

- 1.K1, as a normal (healthy) control and was given rat food.
- 2.K2, as a negative control (diseased) and induced by STZ.
- 3.K3, as a positive control (sick), was given feed, induced by STZ, and *glibenclamide*.
- 4.K4, as control 1 (sick), was given feed, induced by STZ, and Chinese ketepeng leaf extract (*Cassia alata* L.) (500 mg/KgBW).
- 5.K5, as control 2 (sick), was given feed, induced by STZ, and Chinese ketepeng leaf extract (*Cassia alata* L.) (750 mg/KgBW).
- 6.K6, as control 3 (sick), was given feed, induced by STZ, and Chinese ketepeng leaf extract (*Cassia alata* L.) (1000 mg/KgBW).

After dividing the groups of experimental animals, it was continued with measuring blood glucose levels.

After ± 7 days of treatment, the experimental animals had their blood glucose levels measured again to determine the effect of treatment on blood glucose levels.

Parameters of Blood Glucose and Insulin Levels in Experimental Animals

Parameters used as a reference in measuring blood glucose levels in experimental animals (*Rattus norvegicus strain Wistar*) is 55-135 mg/dL, hyperglycemia is defined when the blood glucose level is >200 mg/dL. (Afriyeni *et al.*, 2023).

Preparation of STZ Induction Solution

To make the STZ induction solution, 0.11 grams of STZ was dissolved in 0.05 M sodium citrate buffer (pH 4.5) until the volume reached 6 ml and then stirred until homogeneous. The volume given to experimental animals was 0.09 ml.

Making Comparative Solutions

The comparison solution made is a solution *glibenclamide* which will be given orally at K3 (positive control). In its preparation, 1 g of NaCMC in 100 ml of distilled water was heated while stirring until homogeneous, then 0.127 g of glibenclamide was added to 20 ml of the NaCMC solution, then stirred until homogeneous and given 3.09 ml orally to the positive control group.

Preparation of Chinese Ketepeng Leaf Extract Test Preparation (*Cassia alata* L.)

The extract given is 0.5 - 1 mg/KgBW according to the experimental animal group. The administration volume is 0.3 ml/oral/head for 7 days.

Data Analysis Techniques

The observation data was then analyzed using the paired T test statistical test (*Paired sample T-test*). The paired T test is a statistical test that compares the average of two data from one sample group to see whether there is a significant difference. Then, statistical tests were carried out *One Way Anova* to see if there are significant differences in each average value between sample groups (Suhron, 2024). Next, a test is carried out *Post-hoc Bonferroni* to carry out pairwise comparisons between group means in analysis of variance to ensure that any findings obtained are based on valid test results. Finally, a correlation test was carried out *Pearson* which is carried out to measure the strength and direction of the linear relationship of two variables.

III. RESULT

The aim of this research is to analyze the active compounds, level of effectiveness, and optimal concentration of Chinese ketepeng leaf extract (*Cassia alata* L.) in improving blood glucose levels in experimental animals (*Rattus norvegicus* strain Wistar).

Table 1. Average Blood Glucose Levels of Experimental Animals

Experimental Animal Group	Initial Glucose Levels (mg/dl)	Glucose Levels after STZ Induction (mg/dl)	Glucose Levels 7th day (mg/dl)
Q1	98,75	-	86,75
K2	97,25	275,25	420,50
K3	109,50	365,50	32,75
K4	83,25	298,50	104,25
K5	95,75	308,00	87,75
K6	89,75	340,00	67,75

Based on the table of average blood glucose measurements, before and after STZ induction, there was an increase in the blood glucose levels of experimental animals; K3 with an initial glucose level of 109.50 mg/dl increased to 365.50 mg/dl, K2

with an initial glucose level of 97.25 mg/dl increased to 275.25 mg/dl, K5 with an initial glucose level of 95.75 mg/dl increased to 308.00 mg/dl, K6 with an initial glucose level of 89.75 mg/dl increased to 340.00 mg/dl, K4 with an initial glucose level of 83.25 mg/dl increased to 298.50 mg/dl, Then, on the 7th day of treatment, there was a decrease in the blood glucose levels of test animals K3, K4, K5, and K6 after being given glibenclamide and Chinese ketepeng (*Cassia alata* L.) leaf extract, while K2 which was only given STZ induction experienced an increase in levels. uncontrolled blood glucose.



Figure 1. Comparison of blood glucose levels of experimental animals before and after treatment

From the results of the Bonferroni post-hoc test, it was found that Chinese ketepeng leaf extract (*Cassia alata* L.) had an effect on reducing blood glucose levels in experimental animals after administering a dose of 500 mg/kgBW, a dose of 750 mg/kgBW, and a dose of 1000 mg/kgBW with the difference in order of reduction is: - 194.2 mg/dl; - 220.5 mg/dl; -272.2 mg/dl. It was also found that the glibenclamide group and the Chinese ketepeng leaf extract group at doses of 750 mg/kgBB and 1000 mg/kgBB had significant differences ($p < 0.05$), which means that Chinese Ketepeng leaf extract (*Cassia alata* L.) and glibenclamide had the same ability. in reducing blood glucose levels in animals, try the DM model.

Table 2. Average Blood Glucose Levels of Experimental Animals

Experimental Animal Group	7th Day Insulin Levels (mIU/mL)
Q1	7,27
K2	1,30
K3	6,97
K4	3,01
K5	4,05
K6	6,34

Table 2 shows that insulin levels on day 7 varied greatly.

IV. DISCUSSION

Administration of Chinese ketepeng extract (*Cassia alata* L.) contains compounds such as flavonoids, alkaloids, tannins, terpenoids and saponins which have the effect of reducing blood glucose levels. The decrease in blood glucose occurs due to the presence of chemical compounds consisting of a mixture of steroid saponins (charantin), insulin-like compounds, namely peptides and alkaloids. Charantin activates *Adenosine Monophosphate-activated Protein Kinase (AMPK)* thereby increasing glycogen formation and increasing glucose uptake in muscle and liver cells. Polypeptide-p is a polypeptide protein that has a mechanism like insulin, stimulating pancreatic β -cells to release insulin. The alkaloid content has the effect of reducing blood glucose by inhibiting glucose absorption in the intestine, increasing glucose transport in the blood, stimulating glycogen formation, and inhibiting glucose formation. (Puspitasari and Choerunisa, 2021). Flavonoids with the action of stimulating insulin release, such as quercetin, will induce hepatic glucokinase and as a result create a hypoglycemic effect so that they can reduce blood glucose. Apart from that, several studies show that flavonoids also work by activating the expression of the PPAR- γ gene thereby improving glucose uptake and insulin resistance, inhibiting the process of hepatic gluconeogenesis and stimulating glucose uptake through GLUT4 regulation and activating the AMPK pathway (Yao *et al.*, 2019). Tannin can also increase glucose uptake through insulin signaling pathway mediators, such as activation of PI3K, P38 MAPK and GLUT-4 translocation (Sumarlin *et al.*, 2020). Saponin can have a hypoglycemic effect because it can regenerate the pancreas which causes an increase in the number of pancreatic β cells and islets of Langerhans so that insulin secretion will increase. This increase in insulin secretion will help reduce levels (Septi Ulandari *et al.*, 2022).

The results of this research are in line with research conducted by Yakubu M. T., Uwazie N. J. and Igunnu A (2016) which states that Chinese ketepeng leaves (*Cassia alata* L.) contains flavonoids, alkaloids, saponins, phenols and tannins which act through various mechanisms as antidiabetics.

V. CONCLUSION

The results of this research provide information that Chinese ketepeng leaf extract (*Cassia alata* L.) has been proven to reduce blood glucose levels in experimental animals (*Rattus norvegicus strain Wistar*). The results in the form of a decrease in blood glucose levels can be used as an antidiabetic indicator from Chinese ketepeng leaf extract (*Cassia alata* L.) (Antique Ketcin). It is hoped that future researchers can develop the use of Chinese ketepeng leaf extract (*Cassia alata* L.) as the main raw material for making antidiabetic drugs.

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