Effect of Mangkokan (Polyscias scutellaria) Leaf Extract on Blood Sugar Levels in Alloxan-Induced Male White Rats

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Abstract

Type 2 diabetes is a metabolic disorder caused by insulin resistance and is associated with oxidative stress. In Indonesia, the mean prevalence of diabetes ranges from 1.4% to 1.6%; however, some areas have a much higher diabetes prevalence such as Pekajangan (2.3%) and in Manado (6%). The 2013 Indonesian Basic Health Research (IBHR) stated that the number of people with DM in Indonesia has reached an alarming rate. This study aimed to determine the antidiabetic effect of Polyscias scutellaria on alloxan-induced male Wistar. This was an experimental study conducted in July 2020 in the Faculty of Medicine, Prima Indonesia University. This study used 25 rats that were grouped into 5 treatment groups: control group (Na-CMC), standard (Metformin), and 3 extract groups with different doses (125 mg/kg BW, 250 mg/kgBW, and 500 mg/kgBW). Mangkokan leaf extract was obtained through the maceration method. All rats were induced intraperitoneally using alloxan monohydrate 10% at a dose of 175 mg/kgBW. The parameters used in this study were fasting blood glucose level before induction, after induction, and after treatment and body weight before treatment. It was observed that there was a significant change in blood glucose level between the extract groups. The blood sugar level in the 125 mg/kgBW group was 495.00 mg/dL while in the 250 mg/kgBW and 500 mg/kgBW, the blood glucose levels were 317.00 mg/dL and 126.00 mg/dL, respectively, with the 500 mg/kgBW dose as the most effective dose (P-value=0.001). Thus, mangkokan leaves have the potential to reduce blood glucose level but are not as good as the standard group.

Keywords: Blood glucose level, mangkokan leaves, metformin, pancreas

Pengaruh Ekstrak Daun Mangkokan (Polyscias scutellaria) terhadap Kadar Gula Darah Tikus Putih Jantan yang Diinduksi Aloxan

Abstrak

Diabetes Mellitus Tipe 2 merupakan gangguan metabolisme disebabkan oleh resistensi insulin dan berkaitan dengan stress oksidatif. Di Indonesia, rata-rata diabetes berkisar antara 1,4–1,6%, namun, beberapa daerah memiliki prevalensi diabetes yang jauh lebih tinggi seperti Pekajangan (2,3%) dan di Manado (6%). Berdasar atas laporan Riskesdas 2013, menyebutkan bahwa jumlah penderita DM di Indonesia sudah mencapai angka yang mengkhawatirkan. Penelitian ini bertujuan mengetahui efek antidiabetik dari daun mangkokan pada tikus wistar jantan yang diinduksi dengan aloksan. Penelitian ini merupakan penelitian eksperimental yang dilakukan pada bulan Juli 2020 di Fakultas Kedokteran Universitas Prima Indonesia. Penelitian ini menggunakan 25 ekor tikus yang dikelompokkan dalam 5 kelompok perlakuan, yaitu kelompok kontrol (Na-CMC), standar (Metformin), dan 3 kelompok ekstrak dengan dosis berbeda (125 mg/kgBB, 250 mg/kgBB, dan 500 mg/kgBB). Ekstrak daun mangkokan diperoleh melalui metode maserasi. Seluruh tikus yang digunakan diinduksi terlebih dahulu dengan menggunakan aloksan monohydrate 10% dengan dosis 175 mg/kgBB) secara intraperitoneal. Parameter penelitian yang digunakan dalam penelitian ini adalah kadar gula darah (KGD) puasa sebelum induksi, sesudah induksi, dan setelah perlakuan, serta berat badan sebelum perlakuan. Diantara yang berat ini yang menunjukkan dalam kadar glukosa darah antara kelompok ekstrak. Kadar gula darah pada kelompok 125 mg/kgBB adalah 495,00 mg/dL sedangkan pada kelompok 250 mg/kgBB dan 500 mg/kgBB berturut-turut adalah 317,00 mg/dL dan 126,00 mg/dL dengan 500 mg/kgBB sebagai dosis paling efektif (P=0,001). Simpulan, daun mangkokan berpotensi menurunkan KGD namun tidak sebagus kelompok standar.

Kata kunci: Daun mangkokan, etanol, kadar gula darah, metformin, pankreas
Introduction

Diabetes mellitus (DM) is a group of metabolic diseases characterized by hyperglycemia that occurs due to abnormalities in insulin secretion, insulin action or both. Meanwhile, the World Health Organization (WHO) has previously formulated that DM is something that cannot be expressed in one clear and concise answer, but in general, it can be said to be a collection of anatomic and chemical problems resulting from a number of factors such as absolute insulin deficiency or relative and insulin function disorders.¹

Type 1 diabetes is characterized by absolute insulin deficiency caused by lesions or necrosis of beta cells of Langerhans, loss of beta cell function may be due to viral invasion, action of chemical toxins or the action of autoimmune antibodies against beta cells. As a result of the destruction of pancreatic beta cells, they fail to respond to glucose input.²

Based on the estimation results from the International Diabetes Federation (IDF) in 2013, it was stated that the increase in prevalence was in line with global trends, where in 2000, the IDF estimated the prevalence of diabetes was 3.2%, which continued to increase to 6.5% in 2013. This increase will continue happens which will reach 10.1% by 2035.³

Meanwhile, in developing countries it is estimated that there will be an increase in prevalence which is also quite significant, especially in countries that adopt a westernized culture. There are 10 countries with the highest prevalence of type 2 DM, namely Tokelau (37.5%), Federated States of Micronesia (35%), Marshall Islands (34.9%), Kiribati (28.8%), Cook Islands (25.7%), Vanuatu (24%), Saudi Arabia (24%), Nauru (23.3%), Kuwait (23.1%), and Qatar (22.9%). ¹ In Indonesia, the prevalence of diabetes ranges from 1.4% to 1.6%, except in some places, namely in Pekajangan 2.3% and in Manado 6%. Based on the 2013 Riskesdas report, it shows that the number of people with DM in Indonesia is very large. With the possibility of an increase in the number of people with DM in the future it will be a very heavy burden to be handled by specialists/subspecialists or even by all existing health workers.⁴ This can be seen from the increase in the prevalence of diabetes mellitus based on doctor’s diagnosis from 1.3 in 2013 to 2.0% in 2018. The five provinces with the largest prevalence of diabetes mellitus in Indonesia are DKI Jakarta, East Kalimantan, DI Yogyakarta, North Sulawesi, and East Java. With the prevalence of diabetes mellitus incidence more commonly found in women (1.8%) than men (1.2%), with the peak prevalence of diabetes mellitus at the age of 55-64 years of 6.3%.²⁵⁶

The increasing prevalence of obesity in childhood seems to affect the onset of type 2 diabetes, especially in children and young adults, especially in risk ethnic groups.⁷ There are still many other risk factors that affect the prevalence of diabetes, such as physical activity, exposure to smoke, blood pressure, etc.⁸

This inflammation is supposed to restore inflamed tissue, but recent research has shown that this chronic inflammation can lead to the release of several chemicals known as Reactive Oxygen Species (ROS) which further classify the pre-inflammatory cascade and pose a risk of developing a hyperglycemic state.⁹

Patients with type 1 diabetes mellitus often require exogenous insulin to treat hyperglycemia, if not treated, it is associated with the occurrence of ketosis which often occurs in adolescents and sometimes adults.⁵

Based on the background description above, it is important to find effective drugs at affordable prices with local resources with relatively safe side effects, one of which is mangkokan leaf which offers various phytochemicals such as flavonoids and saponins, where these compounds have antioxidant activity and can improve oxidative stress in the body. Several previous studies have been conducted to explore other pharmacological effects of this plant such as hair growth and antibacterial effects.¹⁰¹¹ However, no previous studies have explored the effects of mangkokan leaves on fasting blood sugar levels. So the researchers are interested in exploring the effects of mangkokan leaf extract on fasting blood sugar levels as a diabetes control in male Wistar rats as experimental animals induced with alloxan.

Methods

This study is an experimental study with a post-test only control group design research design that aims to assess differences in blood sugar levels in each group of rats. The protocols and ethical clearance were approved by the Health Research Ethics Committee of Faculty of Medicine, University of Prima Indonesia (Ethical Clearance No: 041/KEPK/UNPRI/V/2020).

This research was conducted at the White Rat
Laboratory (Riandi Animal House) in Medan City from July-August 2020. In this study there were 5 treatment groups, so the number of experimental animals needed were:

\[(5-1) \geq 15 \]
\[(4) (N - 1) \geq 15 \]
\[(N-1) \geq 15/4 \]
\[N-1 \geq 3.75 \]
\[N \geq 4.75 \approx 5 \]

Based on this formula, in this study the experimental animals used were 5 white rats in each research group, so that the total number of experimental animals used was 25 white rats.

The mangkokan leaf samples used in this study were collected from several places around the Medan Petisah sub-district, which were then identified in the Medanese Herbarium at FMIPA, University of North Sumatra.

The identified mangkokan leaf samples were then cleaned and then aerated for 7 days until they became dry simplicia, which were then crushed into dry simplicia powder. The dry simplicia powder was macerated using ethyl acetate as a solvent in a ratio of 1:15 (g/mL) for 5 days, the mixture was stirred regularly and constantly every day. After 5 days, the mixture was filtered with Whatmann filter paper no. 1, the residue is re-macerated in the same way but the solvent used is half of the previous maceration volume. The maceration process is carried out 3 times, then the filtrate of each maceration and re-maceration evaporates with a rotary evaporator at a temperature of 70°C and then followed by drying using an oven at 40°C to become a thick extract.

In this research, the phytochemical test used a modification of the Fansworth method consisting of identification of phenols, steroids/triterpenoids, terpenoids, saponins, flavonoids, tannins and alkaloids.

All male wistar rats were housed in several standard polypropylene cages under an environment suitable for conditions with maintained light-dark cycles. The White Rats were adapted for one week to a normal pellet diet and fed ad libitum. After one week, male wistar rats were fasted overnight before alloxan was injected intraperitoneally. The induction process of male wistar rats was carried out using Alloxan Monohydrate 10%. A total of 0.35 mL (175 mg/kg body weight of rats) Alloxan monohydrate 10% was injected intraperitoneally. To ensure the induction was successful, fasting blood sugar levels after 72 hours, the mice were said to be diabetic if their blood sugar levels were more than 200 mg/L (11.1 mmol/L).

Anti-diabetic activity testing was carried out on 25 rats which were grouped into 5 treatment groups and all mice were induced with alloxan, as follows:

Blood sugar levels measured in this study were fasting plasma glucose (FPG) levels. FPG was measured in mice that had been fasted for 10-12 hours before measuring blood sugar levels. Blood samples from mice were taken from the veins of the rats 72 hours after induction (FPG 0) and on day 28 (FPG 28) after the rats were given mangkokan leaf extract and metformin as standard.

Data analysis was performed using IBM SPSS 25. Data in the form of phytochemical screening results, FPG 0, FGP 28. Then the data for FPG 0, and FPG 28 were analyzed for data normality using the Shapiro-Wilk test. If the data is normally distributed, then the analysis is continued with testing.

One-way ANOVA and Post Hoc Test. However, if the data is not normally distributed, the data analysis is continued with a non-parametric test in the form of the Kruskal-wallis test.

| Treatment Group | Treatment |
|-----------------|-----------|
| Control         | Rats in this group received 1 ml of 0.5% Na-CMC suspension. Food and drink are given ad libitum. |
| Standard        | Rats in this group received 1 ml of metformin oral suspension 250 mg/kgBW. Food and drink are given ad libitum. |
| Extract Dosage 125mg/kgBW | Rats in this group received 1 ml of the extract oral suspension at dose of 125 mg/kgBW. Food and drink are given ad libitum. |
| Extract Dosage 250 mg/kgBW | Rats in this group received 1 ml of the extract oral suspension at dose of 250 mg/kgBW. Food and drink are given ad libitum. |
| Extract Dosage 500 mg/kgBW | Rats in this group received 1 ml of the extract oral suspension at dose of 500 mg/kgBW. Food and drink are given ad libitum. |

12,13 Data analysis was performed using IBM SPSS 25. Data in the form of phytochemical screening results, FPG 0, FGP 28. Then the data for FPG 0, and FPG 28 were analyzed for data normality using the Shapiro-Wilk test. If the data is normally distributed, then the analysis is continued with testing.

One-way ANOVA and Post Hoc Test. However, if the data is not normally distributed, the data analysis is continued with a non-parametric test in the form of the Kruskal-wallis test.
Results

As a first step in this research, samples of mangkokan leaves that had been obtained were identified in the Herbarium Medanese, University of North Sumatra. The results of the identification of the leaf samples are as follows:

**Kingdom**: Plantae  
**Division**: Spermatophyta  
**Class**: Dicotyledoneae  
**Order**: Apiales  
**Family**: Araliaceae  
**Genus**: Polycias  
**Species**: *Polycias scutellaria* (Burm. F) Fosberg  
**Local Name**: Mangkokan Leaf

Prior to the antidiabetic activity test of the ethanol extract of mangkokan leaves, the ethanol extract of the mangkokan leaves was screened for phytochemicals on the extract. The results of phytochemical screening on the mangkokan leaf extract can be seen in the table below.

Table 2, it can be seen that the ethanol extract of mangkokan leaves contains several phytochemicals, namely: alkaloids, triterpenoids and steroids, saponins, flavonoids, and tannins.

Before testing the hypothesis on each parameter, first the data normality analysis was carried out using the Shapiro-Wilk test. The results of the data normality analysis can be seen in the following table.

Table 3 Results of Data Normality Analysis on Each Parameter

| Parameter                          | Treatment Group                        | P Value |
|-----------------------------------|----------------------------------------|---------|
| **Blood Sugar Levels Before Induction** | Extract Dosage 125 mg/kgBW             | 0.532   |
|                                   | Extract Dosage 250 mg/kgBW             | 0.115   |
|                                   | Extract Dosage 500mg/kgBW              | 0.024   |
| Control                           |                                       | 0.792   |
| Standard                          |                                       | 0.627   |
| **Blood Sugar Levels After Induction** | Extract Dosage 125 mg/kgBW             | 0.255   |
|                                   | Extract Dosage 250 mg/kgBW             | 0.995   |
|                                   | Extract Dosage 500mg/kgBW              | 0.015   |
| Control                           |                                       | 0.042   |
| Standard                          |                                       | 0.029   |
| **Blood Sugar Levels After Treatment** | Extract Dosage 125 mg/kgBW             | 0.159   |
|                                   | Extract Dosage 250 mg/kgBW             | 0.837   |
|                                   | Extract Dosage 500mg/kgBW              | 0.220   |
From the Table 3, it can be seen that for each parameter evaluated in this study, only body weight is normally distributed because the P value is >0.05. Meanwhile, blood sugar levels were not normally distributed, because the P value was <0.05. Based on the results of the data normality analysis, the data were analyzed using the One Way Anova test, while the blood sugar data were analyzed using the Kruskall-Wallis test.

In analyzing the antidiabetic activity of ethanol extract of mangkokan leaves, several parameters were assessed, namely: blood sugar levels before induction, blood sugar levels after induction, and blood sugar levels after treatment. From the Table 4, it can be seen that there is no significant difference in blood sugar levels before and after induction between each treatment group, this can be seen from the P value >0.05. This shows that blood sugar levels before and after induction between each group were quite uniform.

**Discussion**

The result of this study has fulfilled the purpose of this study to explore the effect of the mangkokan leaf extract on fasting blood glucose. The mangkokan leaf extract not only significantly decreased fasting blood glucose. On the other hand, this study also showed that the ethanol extract of mangkokan leaves (Polycias scutellaria) contains several phytochemical compounds such as alkaloids, triterpenoids and steroids, saponins, flavonoids, and tannins.

The results of this study are in line with the results of research conducted by Nasution et al. and Revina et al. who reported that the ethanol extract from mangkokan leaves contains phytochemical compounds in the form of alkaloids, saponins, flavonoids, and tannins. The phytochemical content of this ethanol extract provides various benefits such as antioxidants, hair growth, wound healing, and antibacterial, as well as anti-diabetic. 10–12,17,18 From the various benefits of mangkokan leaves, in this study it can be seen that the ethanol extract of mangkokan leaves has anti-hyperglycemic effects. The anti-hyperglycemic effect of mangkokan leaves is due to the saponin content in mangkokan leaves. Saponins contained in mangkokan leaves are in the form of olenolic acid (Olenolic acid) which inhibits the action of the α-glucosidase and α-amylase enzymes, so that they can reduce glucose absorption in the digestive tract and reduce post-prandial blood sugar levels.

Apart from interfering with glucose absorption in the digestive tract, the results of this study are in line with the results of research conducted by Ighoadaro et al. who reported ethanol extract of mangkokan leaves also provides an antioxidant effect to reduce blood sugar levels in mice. This is related to the mechanism of pancreatic damage caused by alloxan. Alloxan will be reduced by GSH to form dialuric acid, where this diuric acid is unstable and can undergo antioxidant to form alloxan radicals.

These alloxan radicals will damage pancreatic beta cells through damage to the DNA structure of pancreatic beta cells and inhibition of the glucokinase enzyme thiol group. Damage to the DNA structure of pancreatic beta cells will cause death in beta cells, while the observation of the thiol group in the glucokinase enzyme will interfere with the formation of ATP in pancreatic beta cells, thereby causing a decrease in insulin secretion. 20 Based on the mechanism of action of the alloxan. So the ethanol extract of mangkokan leaves containing saponins and flavonoids is able to provide an antioxidant effect by donating electrons to the alloxan radicals formed so that they are able to form more stable alloxan compounds and reduce the danger of these alloxans to the pancreatic tissue. 12 This can be seen from the results of this study which show

**Table 4 Comparison of Blood Sugar Levels in Each Treatment Group**

| Treatment                  | Before Induction | After Induction | After Treatment |
|----------------------------|------------------|-----------------|-----------------|
| Control                    | 105.50 (24.00)   | 425.00 (14.00)  | 583.50 (35.00)  |
| Standard                   | 116.50 (48.00)   | 416.00 (33.00)  | 114.00 (16.00)  |
| Extract Dosage 125 mg/kgBW | 106.50 (40.00)   | 405.50 (27.00)  | 495.00 (59.00)  |
| Extract Dosage 250 mg/kgBW | 103.50 (9.00)    | 402.00 (17.00)  | 317.00 (198.00) |
| Extract Dosage 500 mg/kgBW | 104.50 (7.00)    | 400.50 (31.00)  | 126.00 (21.00)  |
| P value                    | 0.608            | 0.198           | 0.001           |
that increasing the dose of mangkokan leaf extract shows an improvement in the structure of the pancreatic tissue. Overall, it can be concluded that the ethanol extract of mangkokan leaves has an antidiabetic effect on alloxan-induced rats because of the saponins and flavonoids that inhibit the action of the glucosidase enzyme in the gastrointestinal tract and improve oxidative stress status.

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