THE EFFECT OF LEAF EXTRACT MANGKOKAN (POLYSCIAS SCUTELLARIA) ON BLOOD SUGAR LEVELS AND MALONDIALDEHYDE (MDA) LEVELS IN MALE WHITE RATS INDUCED BY ALOXAN

Sri Lestari Ramadhani Nasution¹, Ali Napiah Nasution², Sri Wahyuni Nasution³, Chrismis Novalinda Ginting⁴, Inyoman Ehrich Lister⁵

¹,²,³,⁴,⁵Faculty of Medicine Universitas Prima Indonesia, Medan Indonesia
¹Email: srilestariramadhaninasution@unprimdn.ac.id

ABSTRACT

Type 2 diabetes is a metabolic disorder caused by insulin resistance and is associated with oxidative stress. 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. This study aims to determine the antidiabetic effect of mangkokan leaves on male wistar rats induced with alloxan. This was an experimental study conducted in July 2020 in the Faculty of Medicine University Prima of Indonesia. This study used 25 rats that were grouped into 5 treatment groups, namely the control group (Na-CMC), standard (Metformin) and 3 extract groups with different doses (125,250,500 mg / kgBW). Mangkokan leaf extract was obtained through the maceration method. All rats used were induced intraperitoneally using alloxan monohydrate 10% at a dose of 175 mg / kgBW. The research parameters used in this study were fasting blood glucose level before induction, after induction, after treatment, and MDA levels in each treatment group. From the results of this study it was found that all treatment groups in this study showed a significant change in blood glucose level (P value = 0.001) after treatment blood glucose level groups given ethanol extract at the test dose showed significant differences with the control and standard groups. And administration of extract of mangkokan leaves could significantly reduce MDA levels compared to the control group, however, it did not decrease serum MDA levels closer to MDA levels in the standard group (P value = 0.002). So it can be concluded that mangkokan leaves have the potential to reduce blood glucose level and MDA levels but not as good as the standard group.

Keywords: Blood Glucose Level, Mangkokan leaves, Malondialdehyde level, Pancreas.

I. 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 where absolute insulin deficiency or relative and insulin function disorders [1].

The prevalence of type 2 diabetes mellitus in whites ranges from 3% - 6% of the adult population. In Singapore, the frequency of diabetes has increased rapidly in the last 10 years. In the United States, diabetes sufferers increased from 6,536,163 in 1990 to 20,676,427 in 2010 [2].

The number of diabetes cases progressively increased worldwide between 1980 and 2008 by more than double in adults. Approximately 90% of this increase was cases of type 2 diabetes, which is in line with the increase in the incidence of obesity and in the elderly population. 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%) [3]. 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% [2] [5] [6].

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, pressure blood, etc [8].

Diabetes mellitus type 2 is a multifactorial disorder that causes metabolic disorders of fat, protein, and carbohydrates that lead to insulin resistance. Several studies currently report that type 2 diabetes is associated with oxidative stress. Type 2 diabetes mellitus can cause inflammation at a low enough level (low-grade inflammation) by the innate immune system (innate immune system), causing chronic inflammation.

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 ROS (Reactive Oxygen Species) which further classify the pre-inflammatory cascade and pose a risk of developing a hyperglycemic state [9].

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 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 [10] [11]. However, no previous studies have explored the effects of mangkokan leaves on fasting blood sugar levels, MDA, and catalase enzymes, as well as the histopathological features of pancreatic tissue. So the researchers are interested in exploring the effects of mangkokan leaf extract on fasting blood sugar levels as a diabetes control as well as MDA and catalase enzymes as parameters of oxidative stress as well as a histological picture of the pancreas in male Wistar rats as experimental animals induced with alloxan.

II. 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 Ethical Committee of the Faculty of Medicine, University of Prima Indonesia (Ethical Clearance No: 041/KEPK/UNPRI/V/2020).

This research was conducted at the White Rat Laboratory (Riwandi 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:

\[(5 - 1) (N - 1) \geq 15\]

\[(4) (N - 1) \geq 15\]

\[(N - 1) \geq 15/4\]

\[N - 1 \geq 3.75\]

\[N \geq 4.75 \approx 5\]
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 (gr / 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 is evaporated 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 [12] [13].

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 [14].

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) [15].

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:

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Rats in this group received 1 ml of 0.5% Na-CMC suspension. Food and drink are given ad libitum.</td>
</tr>
<tr>
<td>Standard</td>
<td>Rats in this group received 1 ml of metformin oral suspension 250 mg/kgBW. Food and drink are given ad libitum.</td>
</tr>
<tr>
<td>Extract Dosage 125mg/ kgBB</td>
<td>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.</td>
</tr>
<tr>
<td>Extract Dosage 250 mg/ kgBB</td>
<td>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.</td>
</tr>
<tr>
<td>Extract Dosage 500 mg/ kgBB</td>
<td>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.</td>
</tr>
</tbody>
</table>

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 [16].

www.turkjphysiotherrehabil.org 10976
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.

III. 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 : Polyciasscutellaria (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 themangkokan leaf extract can be seen in the table below:

<table>
<thead>
<tr>
<th>Phytochemicals</th>
<th>Method</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>Bouchardart</td>
<td>+</td>
</tr>
</tbody>
</table>
Maeyer - Wagner + Dragendorff +
Salkowsky + Lieberman-Burchad -
Saponins + Aquadest + Alkohol 96% +
Flavonoids + FeCl3 5% + Mg(s) + HCl(p) - H2SO4(p) -
Tannins + FeCl3 1%

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

In analyzing the antidiabetic activity of ethanol extract of mangkokan leaves, several parameters were assessed, namely: body weight, blood sugar levels before induction, blood sugar levels after induction, and blood sugar levels after treatment, as well as a histopathological picture of pancreatic tissue. 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

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Treatment Group</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood Sugar Levels Before Induction</td>
<td>Control</td>
<td>0.780</td>
</tr>
<tr>
<td></td>
<td>Standard</td>
<td>0.627</td>
</tr>
<tr>
<td></td>
<td>Extract Dosage 125 mg/kgBB</td>
<td>0.532</td>
</tr>
<tr>
<td></td>
<td>Extract Dosage 250 mg/kgBB</td>
<td>0.115</td>
</tr>
<tr>
<td></td>
<td>Extract Dosage 500mg/kgBB</td>
<td>0.024</td>
</tr>
<tr>
<td>Blood Sugar Levels After Induction</td>
<td>Control</td>
<td>0.792</td>
</tr>
<tr>
<td></td>
<td>Standard</td>
<td>0.266</td>
</tr>
<tr>
<td></td>
<td>Extract Dosage 125 mg/kgBB</td>
<td>0.255</td>
</tr>
<tr>
<td></td>
<td>Extract Dosage 250 mg/kgBB</td>
<td>0.995</td>
</tr>
<tr>
<td></td>
<td>Extract Dosage 500mg/kgBB</td>
<td>0.015</td>
</tr>
<tr>
<td>Blood Sugar Levels After Treatment</td>
<td>Control</td>
<td>0.042</td>
</tr>
<tr>
<td></td>
<td>Standard</td>
<td>0.029</td>
</tr>
<tr>
<td></td>
<td>Extract Dosage 125 mg/kgBB</td>
<td>0.159</td>
</tr>
<tr>
<td></td>
<td>Extract Dosage 250 mg/kgBB</td>
<td>0.837</td>
</tr>
<tr>
<td></td>
<td>Extract Dosage 500mg/kgBB</td>
<td>0.220</td>
</tr>
</tbody>
</table>

From the table data above, 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 rats' weight data were analyzed using the One Way Anova test, while the blood sugar data were analyzed using the Kruskall-Wallis test.

Table 4. Results of Normality Analysis of MDA Levels Data

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>P Value</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard</td>
<td>0.001</td>
<td>Data distributed abnormally</td>
</tr>
<tr>
<td>Control</td>
<td>0.677</td>
<td>Data is normally distributed</td>
</tr>
<tr>
<td>Mangkokan Leaf Extract 125 mg / kgBW</td>
<td>0.130</td>
<td>Data is normally distributed</td>
</tr>
<tr>
<td>Mangkokan Leaf Extract 250 mg / kgBW</td>
<td>0.071</td>
<td>Data is normally distributed</td>
</tr>
<tr>
<td>Mangkokan Leaf Extract 500 mg / kgBW</td>
<td>0.267</td>
<td>Data is normally distributed</td>
</tr>
</tbody>
</table>

From the table data above, it can be seen that the data on MDA levels in each group is normally distributed, except for MDA levels in the standard group. So, based on the results of the normal test, the data is assumed to be distributed abnormally and the data analysis is continued with non-parametric testing, namely Kruskall-wallis and Mann-Whitney. The results of the analysis can be seen in the following table.

Table 5. Comparison of MDA Levels for Each Treatment Group

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>MDA Levels (mmol/L)</th>
<th>P Value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median</td>
<td>Min-Max</td>
<td></td>
</tr>
</tbody>
</table>
From the table data above, it can be seen that there is a significant difference between each treatment group, this can be seen at the value of P = 0.002. In addition, all groups given the leaf extract showed significant differences between the standards and the controls. Thus, it can be said that the leaf extract was able to reduce the MDA level much better than the control group but it was unable to reduce the MDA level closer to the value in the standard group.

### IV. 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, oxidative stress MDA levels. The mangkokan leaf extract not only significantly decreased fasting blood glucose, but also it can be seen that the lowest MDA level is found in the standard group and the highest is in the control group. Whereas in the group that received the extract, MDA levels were inversely proportional to the extract dosage where an increase in the dose of leaf extract would reduce serum MDA levels. On the other hand, this study also showed that the ethanol extract of mangkokan leaves (*Polyciaasscutellaria*) 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, 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 diuoric acid is unstable and can undergo autocidation to form alloxan radicals [19].

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 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.
Mangkokan leaves have the potential to reduce blood glucose level and MDA levels but not as good as the standard group. The Mangkokan leaf extract not only significantly decreased fasting blood glucose, but also it can be seen that the lowest MDA level is found in the standard group and the highest is in the control group. Whereas in the group that received the extract, MDA levels were inversely proportional to the extract dosage where an increase in the dose of leaf extract would reduce serum MDA levels.

REFERENCES