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The effect of sappan wood extracts in treating diabetes induced in mice

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Abstract

Background: Since 2015, an estimated 415 million people had diabetes worldwide. Although, there is no scientific research related to the effectiveness of chemical substance (brazilin) in Caesalpinia sappan L. that can decrease blood glucose level in humans, many people in Sulawesi consume this wood for diabetes treatment. This study aimed to prove the effect of sappan wood extract on decreasing blood glucose levels in mice and to identify the most effective dose.

Methods: Experimental research (pretest and posttest randomized controlled group design) was conducted on 20 male albino mice [body weight (bw): 20–30 g] used as alloxan-induced diabetic models and were divided into four treatment groups according to alloxan dose: control and 0.25, 0.50, and 0.75 g/kg bw (n = 5 for all groups) groups. Results: Significant effects of sappan wood extract on decreasing blood glucose levels in mice were noted in the pretest and posttest (p values are 0.754 and 0.901 respectively). Conclusions: Sappan wood extract could reduce blood glucose levels in mice with diabetes induced by alloxan at 0.25, 0.50, and 0.75 g/kg bw. The extract with 0.50 g/kg bw was the most effective in decreasing glucose levels in hyperglycemic mice.

Keywords: antihyperglycemic, diabetes mellitus, heartwood

Introduction

Diabetes is one of the most common metabolic disorders worldwide and has been increasing in the last decades.¹ The International Diabetes Federation has been reporting estimates of diabetes prevalence since 2000.² Diabetes is a chronic disease that occurs either when the pancreas does not produce enough insulin or when the body cannot use the insulin it produces effectively.³ Moreover, the treatment of obesity has been less than adequate. The serious danger that can be caused by diabetes is long-term complications, such as heart attacks, strokes, glaucoma, blindness, kidney diseases, and wounds that cannot heal and therefore require amputation of the infected site. In extreme conditions, diabetes can also result in death. Efforts to prevent and deal with complications caused by diabetes must be made. The effects of diabetes mellitus include long-term damage, dysfunction, and failure of various organs. Diabetes doubles the risk of cardiovascular diseases,⁴ and approximately 75% of deaths in patients with diabetes are because of coronary artery diseases.⁵ Other macrovascular diseases include stroke and peripheral artery diseases.

The incidence of diabetes has been increasing over the last few decades. Approximately 7 million people develop diabetes annually, and the most drastic increases in type 2 diabetes have been fueled by rapid urbanization, nutrition transition, and increasingly sedentary lifestyles.⁶ Both diabetes types 1 and 2 are chronic diseases that affect the way our body regulates blood sugar or glucose. Individuals with type 1 diabetes do not produce insulin, whereas those with type 2 diabetes do not respond to insulin as well as they should, and later in the disease, they often do not produce enough insulin. The main difference between type 1 and type 2 diabetes is that type 2 diabetes is not an autoimmune condition. In this study, we used alloxan, an oxidation product of uric acid, to induce diabetes in mice by destroying the insulin-secreting islet cells of the pancreas. Because it selectively kills the insulin-producing beta-cells found in the pancreas, alloxan was used to induce diabetes in laboratory animals.⁷

Recently, some medicinal plants have been reported to be useful in diabetes treatment worldwide and have been empirically used in antidiabetic and antihyperlipidemic remedies.⁸ The essential value of some plants have long been published, however, a large number of these plants remain unexplored to date. Therefore, there is a necessity to explore their uses and to ascertain their therapeutic properties. These plants mainly include Allium cepa, Anacardium occidentale, Andrographis paniculata, Brassica oleracea, Cinnamomum tamala, and Withania somnifera with etiological factors implicated in the development of diabetes and its complications.⁹ Caesalpinia sappan L. is a plant of Leguminosae family, commonly known as Brazil or sappan wood. This wood
is distributed in Southeast Asia, and its dried heartwood has been used as a traditional ingredient in food or beverages.\textsuperscript{11}  

\textit{Caesalpinia sappan} L. is a plant that has shown to exhibit anti-inflammatory, antioxidative, antimicrobial, antiviral, antitumor, antiatherosclerosis, hypoglycemic, and spasmylytic activities that promote blood flow.\textsuperscript{12} The important part of \textit{Caesalpinia sappan} L. is the heartwood that is pale red, hard, and heavy with even and fine structure.\textsuperscript{13} Brazilin is an antioxidant compound that has catechol in its chemical structure and was found in sappan wood. Brazilin is closely related to the blue–black dye precursor hematoxylin, having one less hydroxyl group. Brazilein, the active dye agent, is an oxidized form of brazilin.\textsuperscript{14} The chemical structure of brazilin (C\textsubscript{16}H\textsubscript{16}O\textsubscript{5}) shown in (Figure 1) has a molar mass of 286.283 g mol\textsuperscript{-1} and is a red pigment obtained from the wood of \textit{Caesalpinia sappan} L. Brazilin has been used since at least Middle Ages to dye fabric and make paints and inks. The specific color produced by the pigment depends on its matter of preparation: in an acidic solution brazilin will appear yellow, but in an alkaline preparation solutions it will appear red.\textsuperscript{15}

Indonesian individuals, especially those in South Sulawesi, have been consuming this heartwood as medicine for diabetes treatment. Although, to the best of our knowledge, there is no previous research that has proved the effect of wood extract of \textit{Caesalpinia sappan} L. on diabetes, it is believed that this wood can be consumed as an antihyperglycemic drug. Therefore, we aimed to conduct a research to prove the effectiveness of sappan wood extract. In this case, firstly we intended to identify whether the sappan wood extract can lower the blood glucose level, and secondly to find out the most effective dose in decreasing blood glucose level in hyperglycemic mice. Furthermore, this study result can be beneficial for societies especially those people in South Sulawesi who had been consuming this wood plant for diabetes treatment in long time but they do not have any scientific knowledge in the previous. Finally, we hope through this study, people can have a reference in science on consuming \textit{Caesalpinia sappan} L. for diabetes treatment.

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{chemical_structure.png}
\caption{Chemical structure of brazilin}
\end{figure}

\textbf{Methods}

\textbf{Research design.} This research was an experimental research with pre and posttest randomized controlled group design conducted from June 2015 to September 2015 in a biological and chemical laboratory, Mathematics and Science Faculty, Makassar State University, Indonesia.

\textbf{Ethical statement.} The research complied with all legal requirements for research in Indonesia. The research permit was issued by the head of Biology department of Makassar State University. Permission to collect plant samples was obtained from LIPI (Indonesian Institute of Science) and the Forestry organization of Enrekang Regency. All experimental procedures were conducted in accordance with the law of Indonesian animal protection on the use of experimental animals. The study design, including the number of animals used and possibility of animal death without euthanasia due to experimental intervention, was approved by the Ethical Committee Biology Department of Makassar State University. The administration of dose volume to experimental animals was based on the guidelines for drug and chemical administration of Institutional Animal Care and Use Committee WSU IACUC policy number 35.

\textbf{Experimental animals.} A total of 20 2–3 month-old male adult albino mice (\textit{Mus musculus}) weighting 20–30 g were used in this study. This animal species is the most commonly used mammalian research model for research in genetics, physiology, medicine, and other scientific disciplines.

\textbf{Plant extract preparation.} The wood of \textit{Caesalpinia sappan} L. was obtained from Enrekang Regency, South Sulawesi, Indonesia. Firstly, the bark of this wood was not removed because it also comprises flavonoid substances. The wood was aerated and powdered in a mechanical grinder (Mortar Grinder Pulverisette 2 made in China). The powdered material was then macerated by 96\% ethanol at 30 °C for 24 hours.\textsuperscript{[9]} After 24 hours, the filtrate was concentrated under vacuum rotary evaporator (Cole-parmer rotary evaporator). This process was repeated four times until a colorless solvent was obtained, and the extracts were then dried using a freeze drier to obtain the dry wood extract.

\textbf{Sample size and animal allocation to experimental groups.} The sample was obtained using simple random sampling. The mice were divided into four experimental groups (n = 5 for all groups). These animals were maintained under standard laboratory conditions and were provided diet and water for 1 week. After fasted for 8 hours, these animals were then injected with alloxan (110 mg/kg) in a volume of 1 ml. Blood samples were collected from the tail vein, and fasting glucose levels were estimated using a glucometer (Pharma supply). Furthermore, to determine the effect of sappan wood...
extract on reducing blood glucose levels in these alloxan-induced diabetic mice, glucose levels were measured using the following steps: (1) Measurement of the initial glucose level, i.e., before being induced by alloxan; (2) Pretest: Measurement of blood glucose levels after being induced by alloxan, where the blood glucose levels in mice reach hyperglycemic levels, and the mice are then considered as diabetic; (3) Posttest: Measurement of the blood glucose levels in mice after being treated with sappan wood extracts for 14 days.

**Experimental design.** The experimental design is described in Figure 2.

**Estimation of blood glucose levels.** Blood samples were collected from the tip of the tail vein, and blood glucose levels were estimated using glucose–oxidase–peroxidase reactive strips (pharma supply) and a glucometer.17

**Hypothesis.**  
H₀: there is no significant effect of sappan wood extract on decreasing blood glucose in mice.  
Ha: there is a significant effect of sappan wood extract on decreasing blood glucose in mice.

**Statistical analysis.** Values of blood glucose level in mice were expressed as mean ± SD (Standard Deviation) (Table 1). The normality and paired statistical tests were carried out to determine the p value of each treatment group in the comparison between the pretest and posttest. We applied Kolmogorov-Smirnov and Shapiro-Wilk as normality test (Table 2) to measure the sappan wood extract levels normally distributed. Then, they were analyzed by paired t-test (Table 3).

The criteria we applied in this normality test are as follows:  
H₀ is accepted if -t table <t count <t table  
Hₐ is rejected if -t calculates < -t table or t count > t table

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**Figure 2.** Flowchart of research design
Results

Table 1 shows the average of blood glucose levels in mice in all treatment groups and control group (pretest and posttest). Treatment of mice with ethanol extract of Caesalpinia sappan L. significantly lowered the blood glucose level in mice by 0.754 and 0.901 respectively as compared to pretest (Table 2). The result of paired t-test shows the value of t is 16.289, whereas the value of t table is 4.302, which was obtained from table t. Meanwhile, significance 2-tailed value is 0.001 (p < 0.05). Hence, in this case, Ho is rejected and Ha is accepted, indicating that there was a significant effect of sappan wood extract on decreasing blood glucose levels in mice.

Discussion

The result of this study showed that the sappan wood extract with doses of 0.25, 0.5, and 0.75 g/kg bw that were injected in alloxan-induced diabetic mice could significantly reduce the blood glucose levels in mice.

Table 1. Statistical measurement of blood glucose levels in mice (n = 5)

<table>
<thead>
<tr>
<th>Treatment group number</th>
<th>Pretest (mg/dl) mean ± SD</th>
<th>Posttest (mg/dl) mean ± SD</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>167.0 ± 25.21</td>
<td>149.5 ± 26.29</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>136.2 ± 12.24</td>
<td>114.3 ± 8.23</td>
<td>0.001</td>
</tr>
<tr>
<td>3</td>
<td>158.6 ± 15.19</td>
<td>135.0 ± 24.01</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>143.8 ± 10.96</td>
<td>123.0 ± 11.22</td>
<td></td>
</tr>
</tbody>
</table>

1: control group, 2: sappan wood extract 0.25g/kg body weight of mice, 3: sappan wood extract 0.5 g/kg body weight of mice, 4: sappan wood extract 0.75 g/kg body weight of mice; SD: standard deviation, SE: standard error, t: t value, df: degrees of freedom, Sig: significance

The treatment groups of mice in which sappan wood extract was administered showed a significant difference in decreasing glucose levels compared with the group of mice in which only distilled water, and not sappan wood extract, was administered. Although all the groups administered with sappan wood extract experienced a significant change in blood glucose levels, dosing which showed a graph of the highest decrease in glucose levels was at the 0.5 g/kg bw dose. This was because the dose of 0.5 g/kg bw is more in line with the average body weight of the test animal. Compounds that have an important role in reducing glucose levels in mice are brazilin and flavonoids. Type II diabetes is characterized by an increase in hepatic glucose production and insulin resistance. Increased gluconeogenesis is the main cause of elevated hepatic glucose levels in patients with diabetes. Meanwhile, flavonoids are also found in sappan wood and act as inhibitors of important enzymes that play a role in the breakdown of carbohydrates to monosaccharides, which can be absorbed by the intestine, namely the alpha amylase and alpha glucosidase enzymes. Inhibition of these two enzymes results in disruption of the process of breaking down carbohydrates into monosaccharides so the intestine cannot absorb them. Thus, blood glucose levels do not increase after consuming foods containing glucose.18

Previously, to the best of our knowledge, there have been no studies related to the effect of sappan wood extract on reducing glucose levels in alloxan-induced diabetic mice; therefore, the effectiveness of sappan wood extract cannot be compared. In this study, we found that due to the sappan wood administration into mice’s body, the pancreas has functioned, as it should. The sappan wood extract administration could stabilize the pancreas function after the alloxan solution induced to mice giving a diabetogenic effect that is capable to pancreatectomy effects and selectively damaging cells from the islets of Langerhans in the pancreas, which secrete the insulin hormone. Therefore, it increased the blood glucose level. Moreover, insulin hormone is the only hormone that can reduce blood glucose levels.19-21 Based on this, it is increasingly supportive that the compounds contained in sappan wood extract can reduce blood glucose levels in mice even though blood glucose levels are very high due to the production of the insulin hormone was not produced.

In general, the intake of sappan wood extracts with various formulations and doses (0.25, 0.50, and 0.75 g/kg bw) resulted in a decrease in blood glucose levels in mice. However, the administration of formulation of brazilin and flavonoid compounds in sappan wood at a dose 0.50 g/kg bw had the highest effect on decreasing blood glucose levels in mice. This due to the brazilin and flavonoid substances in the dose of 0.50 g/kg bw that exhibited a significant effect on insulin production. In this study, we did not measure the exact proportion of brazilin and flavonoid compounds in sappan wood extract. Moreover, we did not observe the physiological processes in mice body cells or analyze the reaction of pancreatic cells toward the treatment further. Therefore, further studies investigating the effects of this wood extract in terms of considering the exact proportion of each chemical substance, which can decline blood glucose levels effectively in alloxan-induced diabetic mice models, are warranted in the future.

Conclusions

In conclusion, sappan wood extracts at any dose could reduce blood glucose levels in alloxan-induced diabetic mice. The dose of 0.5 g/kg bw is considered as the most effective dose because it could reduce blood glucose levels in mice better than aquadest (control) could.

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**Conflict of Interest Statement**

There are no conflicts of interest to declare.

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**References**