The Effect of *Schleichera oleosa* L. on HbA1c Levels in Alloxan-Induced Rats

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Abstract

Diabetes mellitus is a chronic disease that occurs due to the failure of the pancreas to produce sufficient insulin, or the body cannot use the insulin it makes effectively. The HbA1c (glycated hemoglobin) levels show an alteration in blood glucose levels in people with diabetes. As a traditional medicine, the kesambi plant (*Schleichera oleosa* L.) can improve diabetic people's health due to its secondary compounds, such as alkaloids, flavonoids, tannins, steroids, and phenolic compounds, besides several empirically proven as antiulcer, anticancer, and antimicrobial effects. The study aimed to determine the effect of the ethanol extract of Kesambi leaves on HbA1c levels in alloxan-induced rats. This research used a posttest-only design method, using 18 rats divided into six groups of mice and induced by alloxan 150 mg/kg in intraperitoneal with the division of groups: group I without treatment; group II alloxan + Na-CMC; group III alloxan + glibenclamide; groups IV, V, and VI, given alloxan + kesambi leaf extract 200, 400, and 600 mg/kg BW, respectively. All treatments were given orally for 23 days. HbA1c values were measured on day 24 using the Afinion HbA1c. The data obtained were analyzed using the method of comparing the measurement results of each group against the control group. The results showed that the 200, 400, and 600 mg extracts affected HbA1c levels with successive values of 5.47%, 5.37%, and 5.47% compared to the control group, namely 5.10%. This study concluded that giving kesambi leaf ethanol extract can affect HbA1c levels.

Keywords: *Schleichera oleosa* L., alloxan, HbA1c, blood glucose, diabetes mellitus
Background

Diabetes mellitus (DM) is a chronic disease that occurs due to the failure of the pancreas to produce sufficient insulin, or the body cannot use its insulin effectively. The most characteristic endocrine disorder is that carbohydrate and lipid metabolism cannot control through insulin. There are two types of DM; type I diabetes stems from inadequate insulin synthesis by pancreatic cells, while type II diabetes is characterized mainly by insulin resistance (a condition during which peripheral cells usually do not respond to insulin or cell dysfunction) (Wang, 2015). PERKENI data in 2015 showed that the number of people with diabetes in Indonesia reached 9.1 million, and Indonesia was ranked 5th from 7th in the previous ranking. WHO estimates that the number of people with diabetes in Indonesia reached 21.3 million in 2013; this condition is very jumpy compared to the number of diabetic patients, which reached 8.4 million in 2000 (PERKENI, 2015).

The pathophysiology of diabetes based on experimental evidence shows the involvement of free radicals in the pathogenesis of diabetes, resulting in the development of complications of diabetes. Free radicals can damage cellular molecules, DNA, proteins, and lipids, causing changes in cellular function. Some of these studies revealed that antioxidants capable of neutralizing free radicals effectively prevented diabetes due to animal models and reduced the severity of diabetes complications (Dharmojono, 2010; Subronto, 2006; PERKENI, 2006).

HbA1c is an examination used to assess the risk of tissue damage caused by high blood sugar levels, so HbA1c measures how well management has normalized blood glucose in people with diabetes (Richard et al., 2013). According to the WHO, the examination of HbA1c levels can show several categories: normal <5.7%, prediabetes: 5.7-6.4, diabetes: 6.5%. Glycosylated or glycated hemoglobin results from a simple chemical reaction between hemoglobin and sugar after hemoglobin synthesis is complete (Sherwani et al., 2016). The glycosylated hemoglobin assay helps differentiate between diabetes mellitus and stress-induced hyperglycemia (Goldstein et al., 1984).

*Schleichera oleosa* (Lour) or kesambi has several biological effects, such as antiulcer, anticancer, and antimicrobial, and is traditionally used as an antidiabetic (Goswami & Singh, 2019). Several studies reported that kesambi leaves contain secondary metabolites: alkaloids, flavonoids, tannins, steroids, and phenolic compounds (Situmeang et al., 2016). Based on research conducted by Muthukrishnan and Sivakkumar (2017), the ethanolic extract of kesambi leaves can inhibit the activity of α-amylase and β-glucosidase. Therefore, the study aimed to determine the effect of ethanolic extract of kesambi leaves on decreasing HbA1c levels in alloxan-induced rats.

Materials and Methods

This research is experimental research on a laboratory scale. The sample was kesambi (*Schleichera oleosa* L.) leaves taken in the Takalar district, South Sulawesi. The materials in this research were extracting sets, HbA1c measuring instruments (Afinion HbA1c), laboratory glassware, oral cannula, gloves, syringes, Venoject tubes, analytical scales, and animal scales for testing. The materials used include aquadest, alloxan, kesambi, 70% ethanol, EDTA reagent, glacial acetic acid, concentrated sulfuric acid, 70% ethanol, FeCl₃, HCl 2 N, H₂SO₄ 10%, Mg powder, Dragendorff's reagent, Mayer's reagent, and Na-CMC.

Extract preparation

Kesambi leaves simplicia were weighed as much as 100 g and put into a maceration vessel, then moistened with 70% ethanol little by little. After everything was wetted, added all the remaining 70% ethanol in a ratio of 1 : 10. The maceration vessel was tightly closed and stored in a place protected from sunlight for 3 x 24 hours with occasional stirring and then filtered. The residue was then macerated again with the same solvent for 24 hours. The filtrate was then concentrated with
a vacuum rotary evaporator to obtain a thick extract of kesambi leaves. A total of 0.5 g disperses little by little in a warm water mortar while grinding until a colloidal solution is up to 50 mL.

**In vivo test**
The research subjects were 18 rats with healthy criteria. Activity, regular, adult, body weight 150 g, 2-3 months old. The number of experimental animals used was 18 animals into six groups for testing blood glucose levels. Each group consisted of 3 test animals then the animals were acclimatized for one week in order to adapt to their environment.

**Antidiabetic potential test**
The research phase includes an adaptation period of a week with pellet feed and drinking water. Before being given treatment (intraperitoneal induction of alloxan at a dose of 150 mg/kg BW), the rats fasted for 8 hours first; then blood was taken for blood glucose examination as much as 1-2 mL, which was accommodated in a Venoject tube containing EDTA. Test animals should be 18, divided into six groups randomly. Each treatment consisted of 3 rats:

1. Group I (normal control): no treatment
2. Group II (negative control): alloxan + 0.5% Na-CMC induced
3. Group III (positive control): alloxan + glibenclamide induced 5 mg/kg BW
4. Group IV: alloxan induced + kesambi extract 200 mg/Kg BW
5. Group V: alloxan induced + kesambi extract 400 mg/Kg BW
6. Group VI: alloxan induced + kesambi extract 600 mg/Kg BW

**Determination of HbA1c**
In an examination using the Afinion HbA1c measuring instrument, blood was taken through rats’ retroorbital plexus (eye vein). The volume of blood taken was as much as 1-2 mL, which was accommodated in a Venoject tube. The blood previously stored in the tube was taken using the HbA1c test cartridge. The capillary tube in the cartridge was filled and inserted into the Afinion HbA1c tool. Then the HbA1c level was measured in mice. The measurement results would appear on the screen of the Afinion HbA1c tool. The results of the examination will be written in percentages, and the interpretation of the results is: Normal 5.7%; Prediabetes 5.7-6.4%; and Diabetes 6.5%.

**Data analysis**
The data obtained were then analyzed using the post-test-only design method; the analysis was carried out at the end to compare the final results of the control group from each group.

**Result and Discussion**
The sample used in this study was the leaves of the kesambi (*Schleichera oleosa* L.), which were green and fresh. The samples obtained from Bajeng Village, Patallasang District, Takalar Regency, South Sulawesi, were dried after wet sorting, dry sorting, and washing with running water to remove other unwanted parts and impurities that were included in the sample. Drying was carried out for a week at room temperature, supervised, and protected from direct sunlight. The drying aims to reduce the water content and stop the enzymatic reactions so that quality degradation and Simplicia damage can be prevented. The remaining water in the simplicia with a certain level can be a medium for the growth of molds and other microorganisms. The simplicia was then powdered to increase the surface area to facilitate the sample extraction process (Abubakar & Haque, 2020). The extraction method is the maceration method, with immersion for 3 x 24 hours using 70% ethanol as a solvent. The choice of the maceration method was due to the sensitivity of phenolic and polyphenolic compounds to high temperatures. Phenolic and polyphenolic compounds, such as flavonoids in plants, generally bind to sugars to form glycosides, making them more soluble in
polar solvents. In contrast, they are less polar in the form of aglycones (Dias et al., 2021). Ethanol is a solvent with an excellent ability to penetrate cell walls and dissolve almost all organic compounds in the sample, both polar and non-polar. Ethanol also a suitable for the extraction of polyphenols from plants. Therefore, in this study, 70% ethanol was used (Borges et al., 2020). In the maceration process, the liquid filter will penetrate the cell wall and enter the cell cavity containing the active substance. The substance will dissolve, and if there is a difference in concentration between the active substance solution inside and outside of the cell, the active substance (solute) is pulled out. This event repeatedly occurs until a concentration balance occurs between the solution outside and inside the cell (Zhang et al., 2018). From the maceration process of 300 g of simplicia kesambi leaves, 3 L of 70% ethanol were taken, macerated for 3 x 24 hours, and obtained a thick extract of 33.3 g with a percentage yield of 11.1%.

The kesambi leaf was tested for phytochemical screening to determine the metabolite compounds contained. The results of the phytochemical screening of the extract were positive, containing phenolic compounds: alkaloids, flavonoids, saponins, tannins, and terpenoids (Table 1). These metabolite compounds can be used for various benefits, especially for treatment, and then tested on Rattus norvegicus experimental animals because rats have almost the same characteristics as humans. Almost all researchers usually use rats before being tested on humans; therefore, this study used mice as test animals to provide information related to the antidiabetic activity of kesambi leaves to reduce HbA1c levels based on research conducted (Eddouks et al., 2012).

<table>
<thead>
<tr>
<th>Tests</th>
<th>Results</th>
</tr>
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<tbody>
<tr>
<td>Alkaloid</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoid</td>
<td>+</td>
</tr>
<tr>
<td>Saponin</td>
<td>+</td>
</tr>
<tr>
<td>Tannin</td>
<td>+</td>
</tr>
<tr>
<td>Steroid</td>
<td>+</td>
</tr>
<tr>
<td>Terpenoid</td>
<td>-</td>
</tr>
</tbody>
</table>

After induction of rats with alloxan, the rats were hyperglycemic, which was marked by an increase in blood sugar at an average of 500 mg/dL the research conducted (Kumar et al., 2011); then, the treatment was administered, namely the administration of kesambi leaf extract for 23 days. Glucose based on HbA1c measurement parameters on day 24 in mice that had previously been induced by alloxan. The results can be seen in Table 2, which was obtained after measuring HbA1c levels in rats during treatment.

<table>
<thead>
<tr>
<th>No</th>
<th>Groups</th>
<th>HbA1c measurements</th>
<th>Average ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1 % Mmol/L 2 % Mmol/L 3 % Mmol/L</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>I</td>
<td>4 3.8 4 3.8 4 3.8</td>
<td>4 ± 0</td>
</tr>
<tr>
<td>2</td>
<td>II</td>
<td>7.3 9 4.3 4.3 6.2 7.3</td>
<td>5.93 ± 1.51</td>
</tr>
<tr>
<td>3</td>
<td>III</td>
<td>5.8 6.6 5.5 6.2 4 3.8</td>
<td>5.1 ± 0.96</td>
</tr>
<tr>
<td>4</td>
<td>IV</td>
<td>6.7 8 4 3.8 5.7 6.5</td>
<td>5.47 ± 1.36</td>
</tr>
<tr>
<td>5</td>
<td>V</td>
<td>4 3.8 6.5 7.7 5.6 6.3</td>
<td>5.37 ± 1.26</td>
</tr>
<tr>
<td>6</td>
<td>VI</td>
<td>5.7 6.5 4.3 4.2 6.4 7.5</td>
<td>5.47 ± 0.35</td>
</tr>
</tbody>
</table>

Based on Figure 1, it can be seen that the presentation of a decrease in HbA1c levels is close to normal, namely positive control with glibenclamide 2 mg/BW with a concentration of 5.1%, while for the 200, 400, and 600 mg extract treatment groups, the effect of kesambi leaf extract can be
seen on the effect of HbA1c levels successively 5.47%, 5.37%, and 5.47%. This study did not show a significant difference between the negative control group and the treatment group due to the limited time of the study, which was only 23 days, while the cycle of erythrocytes to see the maximum HbA1c level, which is 120 days. According to the WHO, mice with increased levels of HbA1c 7 Mmol/L were considered diabetic based on research conducted by (Shambadiya et al., 2019), who reported that the content of polyphenolic compounds in kesambi leaves was 48.09%, while flavonoids were 25.13%. This shows that kesambi leaf extract can reduce postprandial glucose levels by inhibiting the activity of α-amylase and α-glucosidase, vital enzymes in the breakdown of compound carbohydrates into glucose that can be absorbed in food. However, the effect of glibenclamide is the most remarkable of all.

HbA1c is a parameter that can be used as an illustration of the concentration of sugar bound in the blood during the treatment period because reasonable control of HbA1c levels can reduce chronic complications of diabetes mellitus by 20-30%, while every 1% decrease in hba1c will reduce the risk of complications by 35% (American Diabetes Association, 2010), measurement using the HbA1c parameter, namely the occurrence of posttranslational modification of the HbA molecule to form GHb (glycemic hemoglobin), which is irreversible, which is used as a parameter for blood sugar levels tends to be high or normal GHb measurement of average blood glucose for 120 days. As for the things that limit the measurement of HbA1c levels, namely the occurrence of a hemoglobinopathy, which can cause high or low HbA1c values, which causes a shortened erythrocyte age causing a decrease in HbA1c levels, organ disorders such as kidney disorders, blood cell disorders, bleeding, iron deficiency, liver, and spinal cord disorders.

Figure 1. Blood glucose levels (HbA1c) of rats while testing the antidiabetic activity of ethanol extract of kesambi leaves.

Conclusion

From this study, it can be concluded that all ethanolic extracts of 200, 400, and 600 mg of kesambi leaves showed an effect on HbA1c levels with values of 5.47%, 5.37%, and 5.47%, respectively, when compared to the negative control with 5.93%.

References


