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Effectiveness Test of Tome-tome Fruit Ethanol Extract (*Flacourtia inermis*,Roxb) against Antidiabetes in White Rats (*Rattus norvegicus*) Induced by Alloxan

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Abstract

Diabetes mellitus is a chronic metabolic syndrome disorder of carbohydrates, lipids, and fats caused by insulin deficiency in the body causing blood glucose above the normal threshold (hyperglycemia). Tome-tome (*Flacourtia inermis*, Roxb) contains anthocyanins which have the ability to lower blood sugar levels. This study aimed to determine the effectiveness of the ethanolic extract of tome-tome (*Flacourtia inermis*, Roxb) and to see what dose and day treatment was most effective against diabetes in white rats (*Rattus norvegicus*) induced by alloxan. The test animals used were 25 white rats (*Rattus norvegicus*) divided into 5 treatment groups, namely normal control, negative control, positive control (glibenclamide) and ethanol extract of tome-tome fruit with doses of 150 mg/kgBW and 300 mg/kgBW. Blood glucose measurements were carried out on day 1, day 7, and day 14. The results showed that the effectiveness of tome-tome fruit ethanol extract against alloxan-induced diabetic rats decreased blood glucose levels. A dose of 150 mg/kgBW ethanol extract of tome-tome fruit was more effective in reducing blood glucose levels compared to a dose of 300 mg/kgBW rats.

Key words: *Flacourtia inermis*, antidiabetic, *Rattus norvegicus*, dose, blood glucose.

1. INTRODUCTION

Diabetes mellitus or known as high blood sugar or diabetes is a chronic metabolic syndrome disease of carbohydrates, lipids and fats caused by a lack of insulin in the body, causing an increase in blood glucose exceeding the normal threshold (*hyperglycemia*) (Ozougwu et al 2013). Lack of insulin occurs because the production of insulin from the pancreas gland cells is reduced and due to damage to pancreatic gland cells due to tumors, viruses, or autoimmune diseases.

Indonesia is one of several countries with the most diabetes sufferers at the age of 20-79 years, which is ranked 7th with a number of sufferers of 8.5 million people (IDF, 2013). The International Diabetes Federation (IDF) in 2012 revealed that in Indonesia diabetes mellitus was approximately 4.8% and

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more than half (58.8%). IDF also revealed that million people in the world had diabetes mellitus with undiagnosed diabetes mellitus, namely 46%, it is estimated that diabetes mellitus will continue to increase to reach 592 million people in 2035 (Artanti *et al*, 2015).

Diabetes mellitus requires long-term treatment and is expensive. Treatment performed by people with diabetes is insulin injections and oral antidiabetic drugs which have side effects such as allergic skin reactions, hypoglycemia, cholestasis, aplastic anemia, and hemolytic anemia. In line with the advancement of existing modern medicine, it is also considered necessary to further develop traditional medicine in order to achieve a healthy balance to maintain and improve people's quality of life.

Indonesia is a country with abundant bioavailability of plants that are rich in various substances that have a positive effect on health. Various tropical fruits and vegetables contain high anthocyanins which are a group of flavonoids that have not received much attention in relation to their effects on health.

Tome-tome (*Flacourtia inermis*,Roxb) contains anthocyanins due to the purplish red color of the fruit. Anthocyanins have pharmacological activities, one of which is anti-inflammatory related to their chemical structure and can also capture free radicals that play a role in preventing aging, cancer, and degenerative diseases (Reis *et al*, 2016). Apart from that, anthocyanins are also able to prevent liver function disorders, are anticarcinogenic, antihypertensive, and antidiabetic (Husna *et al*, 2013).

2. RESEARCH METHODS

2.1 Research Time And Place

This research has been carried out at the Pharmacy Laboratory of Trinita University, Manado from April to June 2021.

2.2 Tools And Materials

Beaker, measuring cup, test tube, stirring rod, pipette, micropipette, blender, maceration vessel, filter paper, rotary evaporator (Buchi), analytical balance, injection apparatus, oral sonde, 1 and 2 ml filter, mortar, pestle, weighing scale rat, glucometer (Accu-Check), blood glucose test stick, scissors. Tome-tome fruit (*Flacourtia inermis*, Roxb), white rat (*Rattus norvegicus*), alloxan monohydrate (Merck), glibenclamide (PT. Indofarma), 70% ethanol, aquades, physiological NaCl.

2.3 Sample Processing

The tome-tome (*F. inermis*) fruit samples that had been collected were then sorted, weighed,

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then washed with running water and then drained. Furthermore, it is sliced into several parts, separated from the seeds from the fruit flesh and skin and then dried at room temperature protected from direct sunlight for approximately 14 days to obtain dry weight.

2.4 Simplician Extraction

Simplicia tome-tome (*F. inermis*) fruit as much as 800 grams was put in two maceration containers, each 400 grams added with 70% ethanol as much as 4000 ml in each maceration container. Simplicia is macerated for 3 x 24 hours while stirring occasionally. The obtained maserate was then filtered using a filter to obtain the filtrate and then evaporated by evaporator until the extract was obtained, namely the ethanolic extract of tome-tome fruit.

2.5 Animal Preparation

The test animals used in this study were white rats (*R. norvegicus*) aged 2-3 months with a body weight of 150-250 grams, as many as 25 rats were separated into 5 treatment groups. Before the treatment the rats were acclimatized for 7 days by being fed and drinking ad libitum.

2.6 Alloxan Induction

The acclimatized rats were then weighed. Four treatment groups (Groups II-V) were given injections of alloxan 90 mg/kgBW (Maliangkay and Rumondor, 2018). Which was dissolved in 0.2 ml of physiological NaCl and induced intraperitoneally or through the stomach of rats (Syed *et al*, 2005).

2.7 Blood Glucose Measurement

Three days after the rats were induced by alloxan, the blood sugar was checked by taking blood from the tip of the rat's tail, massaged by the rat's tail until the blood came out and measured with an Accu-Chek (Roche) glucometer using a blood sugar strip (Maliangkay & Rumondor, 2018). Measurement of blood sugar after alloxan was induced from the 1st day or before giving the extract. Furthermore, the measurement of blood sugar levels was carried out again on the 7th and 14th days after the treatment of tome-tome fruit extract and glibenclamide.

2.8 Animals Treatment

After the test animals were induced by alloxan 90 mg/kgBW and on the third day blood sugar levels were measured, the rats with an increase in blood sugar levels of more than 147 mg/dL (Syed et al, 2005) were then treated with glibenclamide (positive group) and Tome-tome fruit ethanol extract at a dose of 150 mg/kgBW and 300 mg/kgBW (Groups 4 and 5) was administered by means of an oral probe to each test group according to the dose of the rat. The test material was given every day for 14 days with

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the method of giving once a day.

2.9 Data Analysis

Data analysis of rat blood glucose levels was statistically using ANOVA (Analysis Of Variance) and continued with the LSD (Least Significant Different) test using the IBM SPSS 20 Software program.

3. RESULTS AND DISCUSSION

3.1 Results

The results of the measurement of blood sugar levels of rats after alloxan induced and after treatment with glibenclamide and ethanol extract of tome-tome fruit is presented in the table below :

Table 1. Analysis of blood glucose levels in white rats during the 14-day treatment

Treatment Group	Blood Glucose (mg/dl)		
	Day 1	Day 7	Day 14
Normal	90,6±5,68	85,2±3,27	89±2,92
Negative	394±168,31	402,8±154,21	428,8±153,85
Positive	430,6±140,62	288±150,41	98,8±19,16
Dosage 150 mg/kg BW	481,2±64,23	309±53,37	97,8±12,87
Dosage 300 mg/kg BW	527,6±61,99	305,6±88,78	103,8±9,6

*expressed in mean ± standard deviation

In the negative group with alloxan treatment on day 1, blood glucose levels were 394 ± 168.31, then increased on day 7 to 402.8 ± 154.21 and increased again on day 14, namely 428.8 ± 153.85. In the positive group that was given alloxan treatment and glibenclamide administration, it was seen that it was able to reduce blood glucose levels from 430.6±140.62 on day 1, then decreased on day 7 which was 288±150.41 and decreased again on day 14 which was 98.8 ±19.16.

Table 2. Percentage of decrease in blood glucose levels in white rats during 14 days of treatment

Treatment	**Percentage**	
	Hari ke 7	Hari ke 14
Normal Group	5.96%	4.46%
Positive Group	33.12%	65.69%
Dosage 150 mg/kgBW	35.79%	68.35%
Dosage 300 mg/kg BW	42.08%	66.03%

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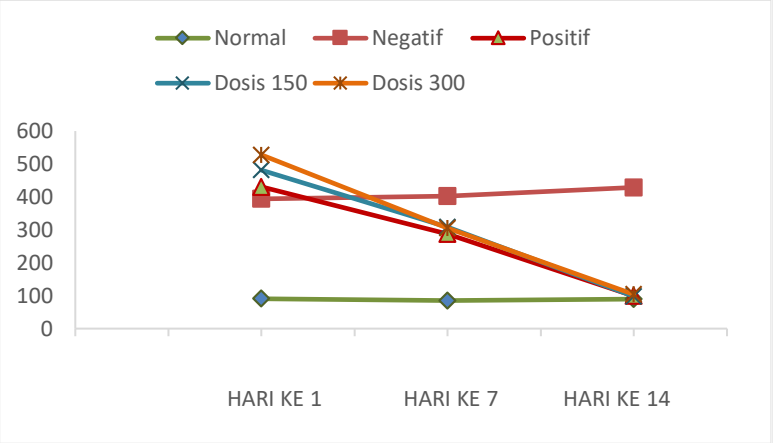
From table 2, the percentage decrease in blood glucose levels shows that on the 7th day there was a decrease of 35.79% and on the 14th day it was 68.35%, the percentage decrease in blood glucose levels shows that on the 7th day there was a decrease of 42.08% and on the 14th day it was 66.03%. The graph below illustrates the average decrease in blood glucose levels of white rats in each group during the 14-day treatment.

Figure 1. Graph of the average decrease in blood glucose levels in white rats during the treatment of tome-tome fruit extract and glibenclamide.



The average blood glucose levels in the treatment group can be seen from the graph above (Figure 1). There was a decrease in blood glucose levels of rats after treatment in the glibenclamide group, at a dose of 150 mg/kgBW and a dose of 300 mg/kgBW ethanol extract of tome-tome fruit.

3.2 Discussion

Data in tables shows that in the normal group there was no significant difference in blood glucose levels from day 1 (3 days after alloxan injection) to the end of the experiment (day 14). Meanwhile, the negative control group (alloxan 90 mg/kgBW) showed an increase in blood glucose levels from the first day to the end of the experiment. This situation is caused because alloxan is one of the diabetogenic parts that is toxic to pancreatic cells (Prameswari and Widjanarko, 2014). In the 2016 Maliangkay study, rats induced by alloxan 90 mg/kgBW intraperitoneally could cause hyperglycemia, with an increase in blood glucose levels >147 mg/dL and an increase of 50%. Also supported by histopathological observations where the pancreatic islets of Langerhans begin to shrink and this is a symptom that the pancreatic cells are damaged and fail to secrete insulin which eventually causes an increase in blood glucose levels (Maliangkay, 2016).

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Glibenclamide is a drug that has the therapeutic effect of lowering blood glucose levels. The way glibenclamide works to lower blood sugar levels is by increasing the release of insulin from the pancreas. In humans, the most effective dose for glibenclamide is 5 mg/kg body weight. For test animals, namely white rats, this dose was converted to a rat dose.

In the treatment group, the ethanolic extract of tome-tome fruit showed a decrease in blood glucose levels on day 7 to day 14 although it was not completely close to normal. However, the blood glucose levels of the 150 mg/kgBW and 300 mg/kgBW dose groups showed an effect that was close to the same as the positive control.

The decrease in blood glucose levels in the treatment of tome-tome fruit ethanol extract at a dose of 150 mg/kgBW is considered more effective than a dose of 300 mg/kgBW because it has a greater percentage reduction in blood glucose levels. The effect of reducing blood glucose levels in rats is due to the chemical compounds contained in tome-tome fruit, namely alkaloids, flavonoids, phenols, triterpenoids, saponins, and tannins. The results of the phytochemical analysis, namely the secondary metabolite content of the methanol extract of the tome-tome fruit, showed that the fruit had a fairly good content of secondary metabolic compounds and high antioxidant activity (Bahruddin. 2018).

Based on the results of the one way ANOVA test between the treatment groups, there was a significant difference in the reduction of blood glucose levels in alloxan-induced rats. Because there were significant differences between groups, it was continued with the LSD (Least Significant Different) test to see which treatment groups showed different effects.

4. CONCLUSION

Treatment of ethanolic extract of tome-tome (*F. inermis*) fruit at a dose of 150 mg/kgBW and 300 mg/kgBW was effective in reducing blood glucose levels in alloxan-induced diabetic white rats (*R. novergicus*) with a percentage decrease on day 14, namely 68.35% and 66.03%. The ethanol extract of tome-tome (*F. inermis*) fruit at a dose of 150 mg/kgBW was the most effective in reducing blood glucose levels compared to a dose of 300 mg/kgBW.

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