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RESEARCH ARTICLE
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Activity of Manau Rattan (*Calamus manan*) Seeds Extract on Reducing Blood Glucose Levels of Male *Swiss Webster* Mice

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Abstract:

Diabetes mellitus is a degenerative disease characterized by high blood sugar levels caused by disturbances in insulin secretion in the body. One alternative in dealing with diabetes mellitus is traditional medicine using natural ingredients. One of the plants that has the potential to have anti-diabetic activity is the Manau Rattan Seed (Calamus manan). This study aims to determine the decrease in blood glucose levels of Swiss Webster male white mice using the experimental method with the glucose tolerance method. The test animals were divided into seven groups, namely group I being a negative control given glucose 2 g/Kg BW, groups II and III being positive controls each given metformin at a dose of 1.3 mg/Kg BW, and glibenclamide at a dose of 0 .65 mg/Kg BW, and groups IV, V, VI and VII became the test material from manau rattan seed extract with respective levels of 100 mg/Kg BW, 200 mg/Kg BW, 300 mg/Kg BW, and 400 mg/Kg BW. Based on the results of the data, the percentage of decreased blood glucose levels (%) showed that the best reduction in glucose levels was in group III (glibenclamide) with an average yield of 41.95%. Parametric analysis used One Way ANOVA with a significance value of >0.05, namely 0.431, so there was no significant difference.

Keywords: Diabetes Mellitus; Manau Rattan Seeds; Glucose Tolerance Method.

Introduction

Diabetes Mellitus (DM) is a type of metabolic disease characterized by hyperglycemia which occurs due to abnormalities of insulin secretion, insulin action or both. Diabetes mellitus is a metabolic disorder that is genetically and clinically heterogeneous with manifestation in the form of loss of tolerance to carbohydrates.[1] Diabetes mellitus is caused by metabolic disorder that occur in the pancreas organ which is characterized by increased blood sugar or often referred to as hyperglycemia which is caused by a decrease in the amount of insulin from the pancreas.[2]

The increasing number of Diabetes Mellitus sufferers every year, expensive medical cost and continuous use of medication, encourage people to used traditional medicine as an alternative treatment.[3] So the testing is needed for its anti-diabetic potential, one of that is Manau rattan seeds.[4]

Decrease of blood glucose is an activity provided by certain compounds that can treat diabetes. Testing for decrease of blood glucose or antidiabetic activity is usually carried out on herbal plants.[5] Based on the results of research conducted by Salusu at 2021, it shows that the skin and seeds of Manau rattan fruit contain secondary metabolites such as flavonoid, alkaloid, tannin and triterpenoid. Flavonoid can increase insulin secretion through the regeneration of pancreatic β cells.[6]

Based on the background above, the purpose of this research is to analysis the potential of Manau Rattan Seed (Calamus manan) extract to reduce blood glucose levels and as an antidiabetic in male Swiss Webster strain mice using the glucose tolerance method.

Research Method

Sample preparation

The preparation stage involved the setup of tools and materials required for the study, including the process of secondary metabolite testing of Manau Rattan seed extract (*Calamus manan*). Preparation also included the selection and grouping of test animals. A total of 30 male mice were randomly allocated into seven treatment groups. Group K1 received Sodium CMC as the negative control, while groups K2 and K3 were administered Metformin and Glibenclamide as positive controls, respectively. Groups K4, K5, K6, and K7 were given ethanol extracts of Manau Rattan seeds at dosages of 100 mg/kg BW, 200 mg/kg BW, 300 mg/kg BW, and 400 mg/kg BW, respectively.

Before induction, blood glucose testing was carried out by first adapting the mice for seven days with controlled food and water intake. Prior to measurement, the mice were fasted for 24 hours but still allowed access to water. After the fasting period, their baseline blood glucose levels were measured. Following this, blood glucose testing after induction was performed. At time zero (t-0), fasting blood samples were collected from all mice. Each mouse was then orally induced with glucose at a dose of 0.65 mg/kg BW. Five minutes after induction, blood glucose was measured again (t-5) to determine post-induction glucose levels. The assigned treatments were then administered orally to all groups. Thirty minutes after treatment (t-30), blood samples were collected by cutting the tips of the mice's tails, and the blood was collected into an Eppendorf tube. From this tube, one drop of blood was drawn using a capillary tube and measured with a glucose testing device.[7]

Blood glucose levels were measured using a glucose testing device. The decrease in blood glucose after treatment was calculated using the following formula: **%** Decrease in glucose = (t5 − t30) / t5 × 100. This calculation represents the percentage reduction in blood glucose levels from the post-induction value at t-5 to the value measured at t-30.

Tools and Materials

The tools used in the research is oral probes, syringes, glucose tests, test strips, and capillary tubes. The materials used in the research is Manau Rattan Seed Extract (Calamus mannan), glucose, Sodium CMC, metformin, glibenclamide, and male white Swiss Webster mice.

Data Analysis

The data obtained were analyzed using the Analysis of Variance (ANOVA) test

Results

The phytochemical compounds contained in these plants also provide various potential benefits for the health of the human body. The phytochemical screening results in Table 1 show that C. manan seed extract contains flavonoids, alkoloids, tannins and triterpenoids.[8]

Secondary metabolite testing of Manau Rattan Seed (*Calamus manan*) extract was carried out by testing flavonoids, saponins and tannins which have the potential to reduce blood sugar levels.

Table 1 Phytochemical Screening of Manau Rattan Seeds (*Calamus manan*)

No	Flavonoid	Alkaloid	Tanin	Saponin	Triterpenoid
1.	+	+	+	-	+

Tests on flavonoid compounds showed positive results, indicating that the ethanol extract of Rattan Manau seeds contains flavonoid compounds. The principle of the flavonoid test reaction is an oxidation-reduction reaction, where the flavonoid compound will be reduced by hydrogen gas resulting from the reaction between the Mg and HCl bands. Furthermore, the reduced compound will form a complex compound with Mg2+ which is a red compound.

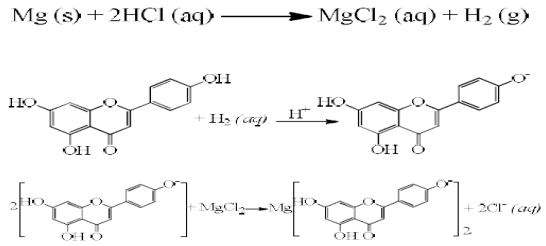

Figure 1. Flavonoid Phytochemical Test Reactions (Andersen *et al.,* 2006)

The positive test for the phenolic compound group was because the ethanol extract of ramania Manau Rattan seeds formed a complex with the Fe3+ ion from FeCl3. Phenolic compounds are compounds consisting of an aromatic ring and one or more hydroxy groups (-OH).

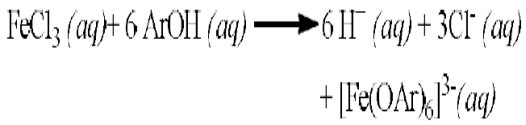

Figure 2. Phenolic Phytochemical Test Reactions (Nafisah dkk, 2014)

The newest source of therapy can be found from Manau rattan seeds which contain secondary metabolites such as flavonoids, saponins and tannins.[9]

Testing of Manau Rattan Seed extract (*Calamus manan*) was carried out to

determine its antidiabetic activity. This test is carried out using the glucose tolerance method. The oral glucose tolerance test (OGTT) is one of the examination methods for diagnosing diabetes which functions to measure the the body's ability to absorb sugar (glucose). The test animals used in this study were male mice of the *Swiss Webster* strain because male mice do not have an estrus cycle which involves the hormone estrogen which plays a role in glucose metabolism, which plays a role in regulating insulin biosynthesis, insulin secretion and pancreatic β cell survival.[10]

All groups had been induced with glucose. Group I was given Sodium CMC solution which was used to see the characteristics of blood glucose levels in test animals. In group II they were given the drug metformin and in group III they were given the drug glibenclamide. Group II and group III were used to see the decrease in glucose levels caused by the comparison drug. Groups IV, V, VI, and VII were given Manau Rattan Seed (*Calamus manan*) extract with different dosage variations. This group was used to see the decrease in glucose levels caused by administering the extract.[7]

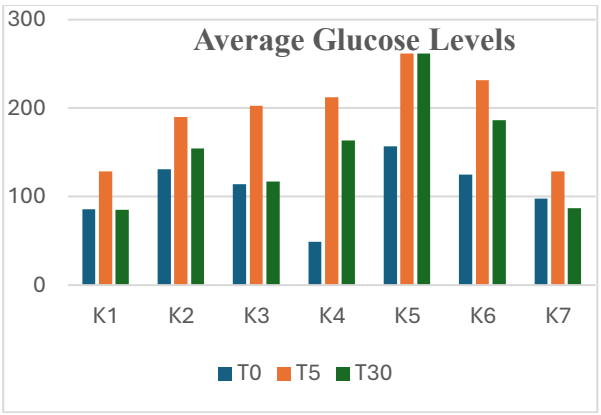

Figure 3. Average Blood Glucose Levels of Mice Before Induction (T0), After Induction (T5), and After Therapy (T30).

Testing blood glucose levels in mice before induction must be adapted for seven days. After that, the mice were fasted for 24 hours without eating but still given water. This is done to avoid the influence of food that can increase glucose levels.[11] Next, blood glucose levels are measured before being given glucose or treatment which is expressed in time 0 (t-0). Testing of blood glucose levels in mice was induced by 0.65 mg/kg bw of glucose orally in all mice. After 5 minutes, the second time (t-5) was measured as blood sugar after glucose induction. Next, treatment was carried out orally for all groups. Blood sampling after 30 minutes (t-30) as glucose levels after therapy.

Table 2 Phytochemical Test Results of Manau Rattan Seeds (*Calamus manan*)

No	Test	Reagents	Description	Results	Documentation
1.	Flavonoid	NaOH	The color of the solution is intense yellow	+	
2.	Tanin	Gelatin	white precipitate formed	+	
3.	Saponin	Aquades	No foam formed	-	

Figure 1 shows that the average fasting blood glucose level in each group of male mice before induction was 108.57 mg/dl, still within the normal range (60-130 mg/dl). Induce glucose at 0.65 mg/Kg BW orally in all mice to create hyperglycemia conditions.[12] Next, blood was drawn after 30 minutes (t-30). Blood was taken from the tails of mice by cutting the mice's tails. Blood is used as a sample because glucose will accumulate in the blood. The results obtained can be seen in Figure 1. Next, the reduction in blood glucose levels is calculated. The average decrease in blood glucose levels in group I was 30.84%, group II was 18.65%, group III was 41.95%, group IV was 23.48%, group V was 29.15%, group VI is 23.58% and group VII is 31.11%. Based on the data from table 1. (%) the percentage of reduction in blood glucose levels shows that the best reduction in glucose levels was in group III (glibenclamide) with an average result of 41.95%. Glibenclamide is a sulfonylurea class of drugs whose receptors are found in the pancreas.[13,14]

Table 3. Percentage Decrease in Blood Glucose Levels'

Treatment Group	Mean (%) Glucose Decrease
Sodium CMC (G1)	30,84%
Metformin (G2)	18,65%
Glibenclamide (G3)	41,95%
Extract 100 mg/Kg (G4)	23,48%
Extract 200 mg/Kg (G5)	29,15%,
Extract 300 mg/Kg (G6)	23,58%
Extract 400 mg/Kg (G7)	31,11%.

The mechanism of action of this drug is by binding to its receptor in the pancreas which causes the potassium channels to close and then depolarization occurs which causes the calcium channels to open, calcium ions entering the pancreatic β cells will stimulate the insulin granules to release insulin so that it can reduce blood glucose levels.[7]

Table 4. Test of Homogeneity of Variances

Levene Statistic	df1	df2	Sig.
0,145	6	28	0,989

In all groups, the results of the Shapiro-Wilk normality test obtained a significance value of >0.05 so that it was normally

distributed, then continued with the homogeneity of variances test, a significance value of >0.05 was obtained, so that the resulting data was homogeneously distributed, then continued with parametric analysis using One Way ANOVA. with a significance value of >0.05, namely 0.431, so there is no significant difference, so there is no influence from group variations.

Table 5. One Way ANOVA

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1699,233	6	283,205	1,023	0,431
Within Groups	7751,445	28	276,837		
Total	9450,678	34			

Conclusions

Based on the data results, the percentage of reduction in blood glucose levels (%) shows that the best reduction in glucose levels was in group III (glibenclamide) with an average result of 41.95%. Parametric analysis uses One Way ANOVA with a significance value of >0.05, namely 0.431 so there is no significant difference.

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