Open Access Research Journal of
Biology and Pharmacy
Journals home page: https://oarjbp.com/
ISSN: 2782-9979 (Online)

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(Research Article)

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The potential of red betel leaf as a Hypoglycemic Agent in Alloxan-Induced Mice

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Open Access Research Journal of Biology and Pharmacy, 2025, 13(02), 001–006

Publication history: Received on 21 January 2025; revised on 25 March 2025; accepted on 28 March 2025

Article DOI: https://doi.org/10.53022/oarjbp.2025.13.2.0023

Abstract

This study aims to determine the effect of red betel leaf extract and its optimal dose in reducing blood glucose levels in alloxan-induced male mice. This experimental study employs a Completely Randomized Design with a quantitative approach, using 6 treatments with 5 replications. The alloxan dose administered is 150 mg/kg body weight (BW), while the red betel leaf extract doses are 50, 100, 150, and 200 mg/kg BW. Glibenclamide is used as a positive control at a dose of 0.65 mg/kg BW, administered via the mouse's vein. Blood samples were collected using a syringe and measured using a glucometer 5 times. Data analysis was conducted using Analysis of Variance (ANOVA), and if a significant effect was observed, it was followed by Duncan's Multiple Range Test at an $\alpha = 5\%$ significance level. Based on the ANOVA analysis of the blood glucose reduction percentage, red betel leaf extract demonstrated an effect in lowering blood glucose levels. Duncan's Multiple Range Test indicated that treatment P3 (150 mg/kg BW) showed a significant difference compared to other treatments, with the highest percentage reduction in blood glucose level of 41.06%. Thus, the optimal dose of red betel leaf extract for lowering blood glucose levels in mice is 150 mg/kg BW. The results of this study are expected to serve as a source of information for developing alternative treatments for diabetes mellitus in humans.

Keywords: The Potentia; Red Betel Leaf; Hypoglycemic Agent; Alloxan-Induced; Mice

1. Introduction

Red betel has long been known as a medicinal plant with a distinct aroma and is often used in traditional ceremonies in Indonesia. Red betel leaves contain various phytochemicals such as essential oils, alkaloids, saponins, and flavonoids. These compounds offer a variety of benefits, including the ability to lower blood glucose levels.

Some phytochemical compounds in red betel leaves, such as alkaloids and flavonoids, are known to have the ability to reduce blood glucose levels. Flavonoids also function as antioxidants that can potentially bind hydroxyl radicals, which can damage the Langerhans cells in the pancreas. For people with Diabetes Mellitus, these antioxidants can help reduce lipid peroxidation. In patients with high blood glucose levels, flavonoids may improve beta cells in the pancreas, thereby increasing insulin secretion [1].

Diabetes Mellitus (DM) is a condition in which blood glucose levels exceed the normal range, due to low production of insulin by the pancreas. Insulin plays a role in facilitating the absorption of glucose into body cells. However, if insulin production is insufficient, glucose cannot enter the cells and remains in the bloodstream. Early symptoms of DM include excessive thirst, frequent urination, unexplained weight loss, slow wound healing, dry skin, and a tingling sensation in the feet [2].

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2. Materials and methods

This study is an experimental study with a Completely Randomized Design and a quantitative approach, using 6 treatments with 5 repetitions. All mice units were induced with alloxan before receiving the treatments. The treatments consisted of: P0 = alloxan and water, P1 = red betel leaf extract 50 mg/kg BW, P2 = red betel leaf extract 100 mg/kg BW, P3 = red betel leaf extract 150 mg/kg BW, P4 = red betel leaf extract 200 mg/kg BW, and P5 = glibenclamide 0.65 mg/kg BW. Blood samples were taken using a syringe and blood glucose levels were measured with a glucometer 5 times.

On the first day, the mice were acclimated for 7 days. On day 8, the mice were fasted. On day 9, blood glucose levels were measured in a fasting state, and on the same day, the mice were induced with alloxan via intravenous injection in the tail. On day 10, the mice were only provided with food and water. On day 11, blood glucose levels were re-measured after alloxan induction, and on the same day, the 6 treatments were administered.

After the treatments were given on day 11, the mice were only given food and water until day 15 (day 4 after extract administration). On day 16 (day 5 after extract administration), blood glucose levels were measured post-treatment. Then, from days 17 to 20, the mice were only provided with food and water, and on day 21, the second blood glucose measurement was taken after treatment (day 10). From days 22 to 25, the mice were only provided with food and water. On day 26, or day 15 after treatment, blood glucose levels were measured.

Data from the measurements were collected to calculate the percentage decrease in blood glucose levels. Data analysis was performed using Analysis of Variance (ANOVA) with a significance level (α) of 0.05. If a significant difference was observed, Duncan's Multiple Range Test at α = 5% was conducted to determine differences between treatments. Analysis was performed on both quantitative and qualitative data.

3. Results and discussion

The results of the study showed that the average blood glucose level of the mice before alloxan induction was below 100 mg/dL. The average data of blood glucose measurements before and after alloxan induction can be seen in Table 1.

Table 1 Average Blood Glucose Levels (mg/dL) of Mice Before and After Alloxan Induction

Treatment	**Average Blood Glucose Levels (mg/dL)**	
	Before Alloxan Induction	After Alloxan Induction
P0	96	140
P1	83.8	134.2
P2	87.2	138.6
P3	99	138.8
P4	82.6	131.4
P5	90.6	152.6

Based on Table 1, the findings indicate that after alloxan induction, the average blood glucose level of the mice increased to 131.4 – 152.6 mg/dL. This shows that the mice were in a diabetic condition and were subsequently given treatments according to the study design. The average blood glucose levels on the fifth, tenth, and fifteenth days can be seen in Table 2 below.

Table 2 Average Blood Glucose Levels of Mice (mg/dL) on Days 5, 10, and 15

Treatment	Average Blood Glucose Levels (mg/dL)		
	Day-5	Day-10	Day-15
P0	143.6	147.8	155
P1	122.8	120.8	118
P2	129	127.4	123.6
P3	122.8	110.2	82
P4	112.2	106.8	98.2
P5	144	126	110

Table 2 shows that the treatments resulted in a decrease in blood glucose levels in mice, except in P0. The measurement data were used to calculate the difference in Blood Glucose Levels, as presented in Table 3.

Table 3 Average Difference in Blood Glucose Levels of Mice on Days 5, 10, and 15

Treatment	Difference in Blood Glucose Levels		
	Day		
	5	**10**	**15**
P0	50.4	54.4	61.6
P1	39	37	34.2
P2	41.8	39.8	36.4
P3	23.8	11.2	-17
P4	29.6	24.2	15.6
P5	53.4	36	19.4

The measurement data in Table 3 was used to calculate the Percentage Decrease in Blood Glucose Levels (Table 4).

Table 4 Average Percentage Decrease in Blood Glucose Levels of Mice on Days 5, 10, and 15

Treatment	Average Percentage Decrease in Blood Glucose Levels		
	Day		
	5	10	15
P0	-2.71	-5.65	-10.91
P1	22.54	26.44	12.18
P2	9.63	12.88	10.90
P3	8.92	19.02	41.06
P4	14.36	18.52	25.06
P5	6.04	16.66	26.83

Table 4 shows the differences in the Percentage Decrease in Blood Glucose Levels in alloxan-induced male mice. The table indicates that P3 has the highest average percentage decrease in blood glucose levels, while P0 has the lowest. To assess the differences in the effects of each treatment on the decrease in blood glucose levels, Duncan's Multiple Range Test (DMRT) was conducted (Table 5).

Table 5 Duncan Multiple Range Test for Percentage Decrease in Blood Glucose Levels of Mice

No	Treatment	Average Blood Glucose Levels (mg/dl)			% Decrease in Blood Glucose Levels	Levels of Blood Glucose Decrease from Highest to Lowest
		Before	Alloxan	After		
1	P0	96	140	155	-10.91[a]	**5**
2	P1	83.8	134.2	118	12.18[bc]	**4**
3	P2	87.2	138.6	123.6	10.90[b]	**5**
4	P3	99	138.8	82	41.06 [e]	**1**
5	P4	82.6	131.4	98.2	25.06 [bc]	**3**
6	P5	90.6	152.6	110	26.83[c d]	**2**

Note: Numbers followed by the same letter in a column indicate no significant difference in the DMRT test at the 5% level.

Table 5 shows the differences in the effects of administering red betel leaf extract on the reduction of blood glucose levels in male mice. Treatment P0 showed a different effect compared to the other treatments. P1 was not significantly different from P2, P4, and P5, but was different from P3. Treatment P3 showed a significantly different effect compared to all other treatments on the blood glucose levels of the mice. P0 experienced the lowest decrease in blood glucose levels at -10.91%, while P5 showed a decrease of 26.83%. On the other hand, P3 demonstrated the highest decrease in blood glucose levels at 41.06%. Based on these results, the optimal dose of red betel leaf extract for lowering blood glucose levels is 150 mg/kg BW (P3). The percentage decrease in blood glucose levels of the mice is displayed in Figure 1.

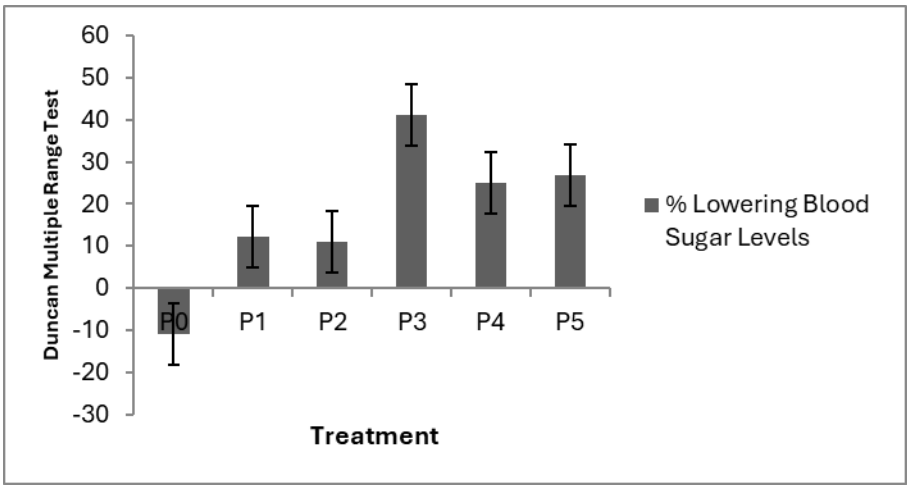

Figure 1 Duncan Multiple Range Test for % Decrease in Blood Glucose Levels of Mice

The results of the Analysis of Variance test indicate that red betel leaf extract affects the reduction of blood glucose levels in mice with varying doses administered. The DMRT post hoc test (Table 5) shows differences in the effects among treatments, marked by different letter notations. Observations were made over 15 days, with blood glucose measurements taken on days 5, 10, and 15. Treatments P1 and P2 showed no significant difference, at 12.18% and 10.90%, respectively. P4 experienced a decrease of 25.06%, and P5 a decrease of 26.83%, which did not show significant differences. This indicates that the doses given to those groups have not yet reached the optimal dose for lowering blood glucose levels in mice. Treatment P3 had a significantly different effect compared to the other treatments, with the highest blood glucose reduction of 41.06%. Based on these results, the optimal dose of red betel leaf extract for lowering blood glucose levels is 150 mg/kg BW (P3).

Treatment P0 did not show a decrease in blood glucose levels because alloxan induction caused damage to the pancreatic beta cells responsible for producing insulin. Without the red betel leaf extract treatment, the mice's bodies could not repair the damage caused by alloxan, resulting in persistently high blood glucose levels [3]; [4]; [5]. According to research conducted by [6], red betel is known for its efficacy in lowering blood glucose levels due to its active compounds such as flavonoids, alkaloids, and tannins, which are effective in reducing blood glucose levels [7]; [8]. Other studies explain that flavonoids work by inhibiting the enzyme α-glucosidase, which reduces carbohydrate hydrolysis into glucose in the intestines [9].

Treatments with various doses of the extract, such as P1, P2, P4, and glibenclamide in P5, did not show significant differences in blood glucose levels. This is likely because the doses of the extract have not yet reached an effective optimal level, so the decrease in blood glucose levels was not significantly different. Other factors that may influence this include the absorption, distribution, and metabolism of the active compounds that have not been optimal. [10] reported that using combinations of herbs in certain proportions can enhance synergistic effects without causing significant toxic effects, as inactive molecules in the mixture will not counteract the effects of other molecules.

Treatment P5 showed an effect in lowering blood glucose levels; however, its effect was not as effective as that of red betel leaf extract. Therefore, the use of red betel leaf extract is preferable to the synthetic drug glibenclamide [11] . Treatment P3 showed the highest decrease in blood glucose levels at 41.06%, which was significantly different from the other treatments. The dose of 150 mg/kg BW of red betel leaf extract is considered the most optimal because this dose provides a significant therapeutic effect without side effects or toxicity. Factors such as effectiveness, safety, and the mechanisms of action of the active compounds support the optimization of this dose. Research by [12] showed that administering ethanol extract of red betel leaves at doses of 50 mg/kg BW and 100 mg/kg BW did not cause toxicity in the kidneys of diabetic mice, indicating the safety of red betel leaf extract as an alternative treatment for lowering blood glucose levels.

Another study by [13] reported that a dose of 100 mg/kg BW provided effects comparable to the positive control at doses of 100, 200, and 400 mg/kg BW. In this study, the researcher added a dose of 150 mg/kg BW, and the results showed that this dose was more effective in lowering blood glucose levels compared to the 100 mg/kg BW dose.

4. Conclusion

Based on the research results, it can be concluded that red betel leaf extract (*Piper crocatum* Ruiz & Pav.) has an effect on reducing blood glucose levels in alloxan-induced male mice (*Mus musculus* L.), with an optimal dose of 150 mg/kg BW

Compliance with ethical standards

Acknowledgments

Gratitude is extended to the head of the Biology Education laboratory at Jambi University for facilitating this research.

Disclosure of conflict of interest

The authors declare no conflict of interest.

Author's contributions

The research involved preparation, implementation, data collection, analysis, paper editing (AJ), field research execution and data collection (VM), data analysis and paper editing (M), paper editing (MN).

Statement of ethical approval

Ethical approval was obtained.

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