

Exploring the Potent Anti-Hyperglycemic Properties of Red Betel Leaf Ethanol Extract Fraction on Male Wistar Rats

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ABSTRACT

Hyperglycemia can be addressed through traditional treatment with Red Betel Leaf. Red betel leaf contains phytochemical compounds, including flavonoids. Empirically, the flavonoid content in red betel leaves has been shown to lower blood glucose levels and cure diabetes mellitus. An effectiveness test of the anti-hyperglycemic properties of the ethanol extract fraction of red betel leaf was conducted. This research aimed to determine the effectiveness of the ethanol extract fraction of red betel leaf on male white rats. The method employed in this study was laboratory experimentation. Fifteen rats were divided into five treatment groups: Group I, healthy control without treatment; Group II, negative control with Na.CMC administration; Group III, positive control with glibenclamide administration; Group IV, the polar group with polar extract suspension of red betel leaf administration; Group V, the nonpolar group with nonpolar extract suspension of red betel leaf administration. In conclusion, administering the polar fraction was more effective in reducing blood glucose levels than the nonpolar fraction.

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Introduction

A sedentary lifestyle, irregular eating patterns, and dietary choices that do not align with the body's needs can trigger obesity (excessive body weight) and diabetes mellitus. According to the World Health Organization, diabetes mellitus is a chronic hyperglycemic disease that occurs when the pancreas fails to produce sufficient insulin, or the body cannot efficiently utilize its insulin. Insulin is a hormone responsible for regulating blood sugar levels [1]. Diabetes mellitus is a syndrome characterized by chronic hyperglycemia, which can gradually lead to various complications such as retinopathy, neuropathy, and macroangiopathy, including atherosclerosis, leading to coronary heart disease and stroke [2].

According to the International Diabetes Federation (IDF) data for 2021, an estimated 537 million adults (aged 20-79 years), or 1 in 10 individuals globally, live with diabetes. In Indonesia, with a population of 179.72 million, the prevalence of diabetes stands at 10.6%. IDF reports that 81% of people with diabetes reside in low- and middle-income countries [3]. Various epidemiological studies have reported a diabetes mellitus prevalence of 5.8% in Indonesia, with 9 million cases in 2018. Diabetes mellitus cases among individuals aged 20-79 reached 9,116 [4].

In individuals with diabetes mellitus, the pancreas, which produces insulin, does not produce enough insulin. Insulin is crucial in the metabolic process, converting glucose into energy and synthesizing fats. Low insulin levels in the body result in elevated blood sugar, known as hyperglycemia. It leads to imperfect carbohydrate utilization, increased urination, thirst, weight loss, and fatigue [5]. The treatment of diabetes mellitus primarily involves oral medications from the Sulfonylurea and Biguanide drug classes. However, they have side effects such as gastrointestinal disturbances and central nervous system issues. Gastrointestinal disturbances include nausea, diarrhoea, headaches, and abdominal pain. Central nervous system disturbances may manifest as vertigo, confusion, and ataxia. Sulfonylureas (e.g., glibenclamide) can cause higher rates of hypoglycemia than glipizide, and hypoglycemia in elderly patients increases the risk of kidney function impairment [6]. Biguanides (e.g., metformin) are hypoglycemic drugs suitable for children, adolescents with type 2 diabetes mellitus, and patients who are overweight. Their use in elderly patients can lead to acidosis [7]. Some people still rely on traditional remedies, whether in simple forms sourced directly from nature or packaged forms produced by herbal medicine companies or industries [8].

Indonesia boasts a rich diversity of medicinal plants. One such plant known for its potential to lower blood sugar levels is the red betel leaf (*Piper crocatum*). Red betel leaves thrive well without excessive exposure to sunlight [9]. Red betel leaves (*Piper crocatum*) contain phytochemical compounds, including flavonoids. Flavonoids in red betel leaves

possess antioxidant properties. These antioxidants can neutralize hydroxyl radicals that damage the beta cells in the Langerhans islets of the pancreas, optimizing insulin production.

Empirically, the flavonoid content in red betel leaves has been shown to reduce blood sugar levels and treat diabetes mellitus [9]. Relevant to this research, a study by Ref. [10] reported that male mice given red betel leaf extract (*Piper crocatum*) at a dose of 2.8 g/kg body weight for seven days exhibited a blood glucose level of 51.75 mg/dl after receiving the extract. Ref. [5] also stated that a dose of 20g/kg body weight of red betel leaf decoction is considered safe for consumption.

The difference between this study and the research conducted by Ref. [10] and Ref. [5] is that this study uses male White Rats (*Rattus norvegicus*) as test subjects, while others used male mice (*Mus musculus L.*). Given this background, this research aims to evaluate the effectiveness of the anti-hyperglycemic fraction of ethanol extract from red betel leaves on male Wistar White Rats.

Material And Methods

The sample collection was performed purposively, without comparing its growth with plants from other regions. Fresh red betel leaves (*Piper crocatum*) were collected from the Pampang Raya Road, Panakkukang Subdistrict, Makassar, South Sulawesi. Fresh red betel leaves were cleaned, washed thoroughly with water, cut into small pieces, drained, and dried under sunlight while covered with black cloth. Once dried, the samples were ground into powder. Five hundred grams of the powdered sample was macerated for 24 hours using 96% ethanol (250 mL) as a solvent. The extract was then filtered, with the filtrate collected and the residue subjected to another round of maceration using 96% ethanol (2x250 mL). Maceration was continued until a colour change was observed. The resulting maceration extract was filtered using cotton and filter paper. The filtrate obtained from maceration was combined, and the remaining solvent was evaporated using a rotary evaporator at 50°C, resulting in a concentrated extract. A small amount of 70% ethanol was added to the ethanol extract, stirred until dissolved, and then added distilled water and n-hexane. The mixture was shaken and left to stand separately. The n-hexane fraction was separated and repeatedly washed with n-hexane until the apparent n-hexane fraction no longer reacted positively with the Liebermann-Burchard reagent. Ethyl acetate was added to the residue (correct procedure for n-hexane fraction), yielding an apparent ethyl acetate fraction (no positive reaction with FeCl₃ reagent), while the remaining fraction was water. The n-hexane, ethyl acetate, and water fractions were combined and evaporated using a rotary evaporator, followed by freeze-drying at -40°C.

Phytochemical analysis was conducted using Willstatter and 10% NaOH reagents. The presence of flavonoids was examined by reacting several millilitres of the sample with a few drops of concentrated HCl and two pieces of Mg metal. A qualitative examination for flavonoid

content was conducted using a 10% NaOH reagent. Two millilitres of the sample were mixed with 2-4 drops of 10% NaOH. A positive reaction with Willstatter and 10% NaOH reagents resulted in a colour change.

Male Wistar White Rats, aged 2-3 months and weighing 150-250 grams, were acclimated for one week. They were housed in cages with dimensions of 120 cm length, 70 cm width, and 60 cm height, ensuring cage cleanliness by changing the bedding every three days. The rats were given AD 2 feed at a rate of 10% of their body weight, equivalent to approximately 10-15 grams per rat per day. Feeding occurred twice daily, in the morning at 08:00 and the evening at 16:00. Water was provided ad libitum, with water changes daily. The acclimatization process allowed the rats to adapt to their new environment over seven days.

Diabetes induction was performed by injecting rats with alloxan monohydrate at 150 mg/kg body weight. The intraperitoneal injection was administered at 2-3 times the intravenous dose of 65 mg/kg body weight—the choice of 150 mg/kg dose aimed to preserve beta cells' ability to produce insulin. The development of hyperglycemia was assessed daily. A 2% w/v suspension of red betel leaf extract was prepared by adding red betel leaf extract (2 g) dropwise to distilled water. The resulting suspension was then placed in a 100 mL measuring flask, with the 2% concentration chosen to facilitate administration in the experimental animals.

The human adult dose of glibenclamide is 5 mg. When converted to rats weighing 200g, this equates to 0.09 mg/kg body weight. The glibenclamide tablet does not dissolve in water, so it was administered as a suspension in 0.5% Na.CMC. The dose of glibenclamide was then dissolved in 0.5% Na.CMC to achieve a concentration of 100 mL based on body weight. The experimental animals, 15 male Wistar White Rats, were divided into five groups. Each group consisted of 3 rats. The groups were as follows:

- Group I: Healthy control, no alloxan induction or treatment (Kn).
- Group II: Negative control, induced with alloxan but given 5% Na.CMC suspension (K⁻).
- Group III: Positive control, induced with alloxan and given glibenclamide suspension as a reference (K⁺).
- Group IV: Induced with alloxan and given polar ethanol extract suspension of red betel leaf (K1).
- Group V: Induced with alloxan and given nonpolar ethanol extract suspension of red betel leaf (K2).

Blood samples were collected, and blood glucose levels were measured. After obtaining baseline glucose levels, all experimental animals, except the healthy control group, were induced with alloxan at a volume of 5 mL. Glucose levels were measured 30 minutes post-induction, aiming for a target 200 mg/dL glucose concentration. If the target was not achieved on the first day, measurements were repeated on day two and, if necessary, on day 3. Once a 200 mg/dL of blood glucose concentration was achieved, glibenclamide suspension (5 mL) was administered on day 4 to the positive control group.

In contrast, the K1 group received the polar ethanol extract suspension of red betel leaf, and the K2 group received the nonpolar ethanol extract suspension. This treatment was continued daily. On day 7, blood glucose levels were recorded for all experimental animals 30 minutes after treatment. Further measurements were taken on day 14, and all data were collected. Blood glucose data were analyzed using ANOVA, and Duncan's post hoc test was employed to identify significant differences among treatments. Statistical analysis was conducted using SPSS 16.0 for Windows.

Results

This research was conducted to determine the anti-hyperglycemic effectiveness of ethanol extract fractions from red betel leaves (*Piper crocatum*) on male white rats (*Rattus norvegicus*). This study used 15 male white rats due to their more stable hormonal system compared to females. However, the biological factors of the experimental animals could not be eliminated, potentially influencing the research results. Therefore, there were variations in initial blood concentrations for each test subject. The experimental animals were divided into five groups: Group I, the healthy control without treatment; Group II, the negative control receiving Na. CMC; Group III, the positive control receiving glibenclamide; Group IV, the polar group receiving a suspension of polar ethanol extract from red betel leaves. Group V, the nonpolar group, received a suspension of nonpolar ethanol extract from red betel leaves. The results of phytochemical screening for polar and nonpolar samples are presented in Table 1.

Table 1. Phytochemical screening for polar and nonpolar samples

Chemical content	Reagent	Observations		Bibliography results		Remark	
		Polar	Non-polar	Polar	Non-polar	Polar	Non-polar
Flavonoid	HCL	Formed yellow color	No yellow color formed	Formed yellow color	No yellow color formed	+	-
	NaOH 10%	Formed orange color	No orange color formed	Formed orange color	No orange color formed	+	-

Alloxan is administered to male albino rats 24 hours before the treatment to elevate blood glucose levels in male albino rats—Alloxan functions by damaging pancreatic beta cells, thereby causing cellular damage. The mechanism of hyperglycemia induced by alloxan occurs

when aloksan in the bloodstream binds to GLUT-2 (glucose transporter), facilitating its entry into the cytoplasm of pancreatic beta cells. Inside the pancreatic beta cells, alloxan induces excessive depolarization of mitochondria due to the influx of ions and excessive energy consumption. The mechanism of alloxan-induced diabetes mellitus also involves an increase in Reactive Oxygen Species (ROS) through a reaction cycle that ultimately results in dialuric acid. Dialuric acid undergoes redox cycling, forming superoxide radicals. Subsequently, these radicals are dismutated into hydrogen peroxide and catalyzed by iron in the final stage to form hydroxyl radicals. It is these hydroxyl radicals that cause damage to pancreatic beta cells, leading to the development of diabetes mellitus.

Table 2. Average decrease in rat blood sugar levels on days 7 and 14

Treatment	Rat	Blood Sugar Level (mg/dL)				
		Beginning	After induction	H-7	H-14	Decrease %
Na.CMC	1	101	188	195	184	2.12
	2	94	205	207	152	25
	3	88	337	360	297	11.86
	Average	94.3	243.3	254	211	13.27
Glibenklamid	1	120	338	93	78	76.92
	2	113	195	100	63	67.69
	3	93	293	116	71	75.76
	Average	108.6	275.5	103	70.6	74.37
Polar	1	90	369	93	82	77.28
	2	91	308	115	85	72.40
	3	98	174	102	93	46.55
	Average	93	281	103.3	86.6	69.18
Nonpolar	1	84	356	206	161	54.77
	2	89	210	186	156	25.71
	3	98	186	159	135	27.41
	Average	93	250.6	183.3	150.6	39.90

Medications for hyperglycemia can be derived from natural sources, as demonstrated in this study using red betel leaves (*Piper crocatum*), which contain phytochemical compounds, including flavonoids. Flavonoid compounds in red betel leaves have antioxidant properties. These antioxidants can bind to hydroxyl radicals, which damage the β -cells of the pancreas, allowing insulin production to reach its maximum potential. Based on the study's results, blood glucose levels decreased after administering the test preparations in the negative control, positive control, polar, and nonpolar groups. Blood glucose measurements were taken on the 14th day after the rats were induced with alloxan.

In the negative control group, the first rat had a 101 mg/dl blood glucose level before aloksan induction. After alloxan induction, the rat's blood glucose level increased to 205 mg/dl. Following treatment (administration of Na. CMC) for 14 days, the blood glucose level decreased to 184 mg/dl. The second rat had a blood glucose level of 94 mg/dl before induction,

which increased to 188 mg/dl after alloxan induction. After 14 days of treatment (Na. CMC administration), the blood glucose level decreased to 152 mg/dl. The third rat had a blood glucose level of 88 mg/dl before induction, which increased to 337 mg/dl after alloxan induction. After 14 days of treatment (Na. CMC administration), the blood glucose level decreased to 297 mg/dl. The negative control group showed no significant reduction in blood glucose levels (as seen in the table), and the rats still experienced hyperglycemia. It occurred because Na. CMC only served as a comparator and did not have pharmacological capabilities to lower blood glucose levels. The study's reduction in blood glucose levels is suspected to be due to environmental factors causing stress in the rats.

In the positive control group, the first rat had a 120 mg/dl blood glucose level before aloksan induction. After alloxan induction, the rat's blood glucose level increased to 338 mg/dl. After 14 days of treatment (administration of glibenclamide), the blood glucose level decreased to 78 mg/dl. The second rat had a blood glucose level of 113 mg/dl before induction, which increased to 195 mg/dl after alloxan induction. After 14 days of treatment (glibenclamide administration), the blood glucose level decreased to 63 mg/dl. The third rat had a blood glucose level of 93 mg/dl before induction, which increased to 293 mg/dl after alloxan induction. After 14 days of treatment (glibenclamide administration), the blood glucose level decreased to 71 mg/dl. Glibenclamide was used as a reference sample because it has pharmacological effects in lowering blood glucose levels by increasing insulin secretion. This group experienced a significant reduction in blood glucose levels, considering that glibenclamide is a synthetic chemical drug that can quickly lower blood glucose levels.

In the polar group, the first rat had a blood glucose level of 90 mg/dl before aloksan induction. After alloxan induction, the rat's blood glucose level increased to 361 mg/dl. After 14 days of treatment (administration of polar red betel leaf extract suspension), the blood glucose level decreased to 82 mg/dl. The second rat had a blood glucose level of 91 mg/dl before induction, which increased to 308 mg/dl after alloxan induction. After 14 days of treatment (administration of polar red betel leaf extract suspension), the blood glucose level decreased to 85 mg/dl. The third rat had a blood glucose level of 98 mg/dl before induction, which increased to 174 mg/dl after alloxan induction. After 14 days of treatment (administration of polar red betel leaf extract suspension), the blood glucose level decreased to 93 mg/dl.

In the nonpolar group, the first rat had 84 mg/dl of blood glucose before aloksan induction. After alloxan induction, the rat's blood glucose level increased to 356 mg/dl. After 14 days of treatment (administration of nonpolar red betel leaf extract suspension), the blood glucose level decreased to 161 mg/dl. The second rat had a blood glucose level of 89 mg/dl

before induction, which increased to 210 mg/dl after alloxan induction. After 14 days of treatment (administration of nonpolar red betel leaf extract suspension), the blood glucose level decreased to 156 mg/dl. The third rat had a blood glucose level of 80 mg/dl before induction, which increased to 186 mg/dl after alloxan induction. After 14 days of treatment (administration of nonpolar red betel leaf extract suspension), the blood glucose level decreased to 135 mg/dl.

This study aimed to determine the effectiveness of the anti-hyperglycemic fraction of ethanol extract from red betel leaves (*Piper crocatum*) in reducing blood glucose levels in male albino rats. Based on the obtained results (as shown in the table), it can be concluded that the polar fraction is more effective in reducing blood glucose levels. It aligns with previous research [11] that states fractionation methods are used to obtain flavonoid and tannin compounds with antioxidant properties from ethanol extracts using a polar solvent (ethyl acetate). Most flavonoids are empirically known to possess antioxidant activity, which can counteract diseases caused by hydroxyl radicals that damage the β -cells of the pancreas, allowing maximum insulin production [12]-[14].

The variations in average blood glucose levels among the rat groups can be attributed to differences in the doses of alloxan administered intraperitoneally, which were adjusted based on each rat's body weight [15]. Additionally, varying physiological responses among the rats contributed to the differences in blood glucose levels [16].

Indeed, this study is not without its limitations. These limitations encompass a relatively small sample size, the method of oral administration for the preparations, variability in the ingestion of the substances by the rats, variations in the time at which the rats met hyperglycemia criteria, and independent measurements of blood glucose levels, which may introduce the possibility of measurement errors.

Nevertheless, the statistical analysis reveals that the polar group exhibits a p-value of less than 0.05, signifying a statistically significant difference when compared to the negative control group. This statistical significance suggests that the polar fraction of red betel leaf extract may have a notable effect in lowering blood glucose levels. Despite these limitations, the study provides valuable insights into the potential anti-hyperglycemic properties of the polar fraction and underscores the importance of further research with larger sample sizes and more controlled conditions to validate and refine these findings.

Conclusion

In conclusion, this research aimed to evaluate the anti-hyperglycemic effectiveness of the polar and nonpolar fractions of red betel leaf ethanol extract (*Piper crocatum*) in reducing blood glucose levels in male albino rats. The results of this study indicated that both polar and nonpolar fractions of the red betel leaf extract exhibited anti-hyperglycemic effects. However, the polar fraction demonstrated a more significant reduction in blood glucose levels compared to the nonpolar fraction. The findings align with previous research emphasizing the potential of natural compounds, such as flavonoids found in red betel leaves, in managing hyperglycemia. Flavonoids, known for their antioxidant properties, can counteract the detrimental effects of hydroxyl radicals on pancreatic β -cells, thereby optimizing insulin production. Despite the observed reductions in blood glucose levels in both the polar and nonpolar groups, it's important to note that this study had several limitations. These limitations included a limited number of subjects, potential variations in the administration of the preparations, differences in physiological responses among the rats, and independent blood glucose measurements, which may introduce measurement errors. Nevertheless, the results from this research indicate that the polar fraction of red betel leaf extract shows promise as an anti-hyperglycemic agent. Further investigations with larger sample sizes and rigorous controls are warranted to validate these findings and explore the full potential of red betel leaves in managing hyperglycemia. This research contributes to the growing body of knowledge regarding natural remedies for diabetes management and underscores the importance of exploring phytochemical compounds in the quest for effective treatments for hyperglycemia.

Conflict of Interest

The authors declare that there is no conflict of interest.

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