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RESEARCH ARTICLE OPEN ACCESS

Effect of Anredera cordifolia (Binahong) Extract on C-Peptide Levels in Streptozotocin-Induced Diabetic Sprague–Dawley Rats

Salsabila Hafsah Hasanah[1], Nurlaely Mida Rachmawati[2]*, Luluk Hermawati[3], and Ervina Julien Sitanggang[4]

*Correspondence: nurlaely_pspd@uinjkt.ac.id

[1]Medical Study Program, Faculty of Medicine, Syarif Hidayatullah State Islamic University, Jakarta, Indonesia
[2]Department of Biochemistry, Faculty of Medicine, Syarif Hidayatullah State Islamic University, Jakarta, Indonesia.
[3]Department of Medical Biology, Faculty of Medicine, Sultan Ageng Tirtayasa University, Banten, Indonesia.
[4]Department of Histology, Faculty of Medicine, HKBP Nommensen University, Medan, Indonesia

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ABSTRACT

Background: Diabetes mellitus (DM) is a metabolic disorder characterized by chronic hyperglycemia resulting from impaired insulin secretion, impaired insulin action, or both, and remains a major global health concern. Anredera cordifolia (binahong) is a medicinal plant commonly used in traditional medicine and is known to contain bioactive compounds such as saponins, alkaloids, polyphenols, and flavonoids, which have been suggested to possess antidiabetic and antioxidant properties. **Objective:** This study aimed to evaluate the effect of Anredera cordifolia leaf extract on C-peptide levels as a marker of endogenous insulin secretion in streptozotocin (STZ)-induced diabetic rats. **Results:** A total of 24 male Sprague–Dawley rats were randomly divided into three groups: negative control (without STZ induction or treatment), positive control (STZ-induced), and treatment group (STZ-induced rats receiving Anredera cordifolia leaf extract at a dose of 100 mg/kg body weight/day). Diabetes was induced by a single intraperitoneal injection of STZ at a dose of 50 mg/kg body weight, and rats with random blood glucose levels ≥200 mg/dL were included in the diabetic groups. The extract was administered orally for 14 days. At the end of the intervention period, blood samples were collected, and plasma C-peptide levels were measured using an enzyme-linked immunosorbent assay (ELISA) and analyzed with a microplate reader. Statistical analysis using the Kruskal–Wallis test demonstrated no significant difference in mean C-peptide levels among the three groups ($p = 0.132$). **Conclusion:** These findings indicate that administration of Anredera cordifolia leaf extract at the tested dose and duration did not significantly affect C-peptide levels in STZ-induced diabetic Sprague–Dawley rats, suggesting that its potential antihyperglycemic effects may not be associated with measurable improvement in pancreatic β-cell secretory function under these experimental conditions.

Keywords: Anredera cordifolia, diabetes mellitus, C-peptide, streptozotocin

INTRODUCTION

Diabetes mellitus (DM) is a chronic metabolic disorder characterized by persistent hyperglycemia resulting from impaired insulin secretion, insulin resistance, or a combination of both[1]. As a progressive disease, DM poses a major public health challenge due to its long-term complications and its substantial contribution to morbidity and mortality. Globally, the number of people living with diabetes continues to rise, particularly in low- and middle-income countries, and is projected to increase further in the coming decades [2].

In Indonesia, diabetes remains a growing health concern. Data from the Indonesia Health Survey 2023 indicate an increasing prevalence of diabetes based on blood glucose measurements, highlighting ongoing challenges in prevention, early detection, and disease control[3]. These findings reflect broader issues related to lifestyle changes, limited adherence to treatment, and gaps in routine health monitoring among individuals with diabetes. Consequently, strengthening both preventive and therapeutic strategies is essential to reduce the national burden of DM[2].

From a pathophysiological perspective, particularly in type 2 diabetes, insulin resistance is commonly followed by progressive dysfunction of pancreatic β-cells[1]. Pancreatic β-cells synthesize insulin through the cleavage of proinsulin into insulin and C-peptide, which are released into the circulation in equimolar amounts. Therefore, circulating C-peptide levels are widely used as a marker of endogenous insulin secretion and β-cell function. Unlike insulin, C-peptide is not cleared by the liver and has a longer half-life, making it a more reliable and stable indicator of β-cell secretory capacity. Moreover, C-peptide assessment provides functional information beyond glucose measurements alone, offering direct insight into the degree of β-cell preservation or impairment. A decline in C-peptide levels reflects impaired insulin production and is associated with worsening glycemic control and an increased risk of metabolic complications[4].

Management of diabetes primarily focuses on lifestyle modification, nutritional education, and pharmacological therapy. However, studies have shown that adherence to treatment and sustained lifestyle changes remain challenging for many patients with type 2 diabetes[5]. Improving nutritional knowledge and promoting balanced dietary practices have been shown to play a key role in reducing metabolic risk and improving overall health status[6,7]. In addition, common comorbidities such as hypertension and dyslipidemia frequently coexist with diabetes and further increase the risk of cardiovascular complications[8-10].

Alongside conventional therapy, the use of herbal medicine as a complementary approach has gained increasing attention. Anredera cordifolia (binahong) is a medicinal plant traditionally used in Indonesia and is known to contain bioactive compounds with antihyperglycemic and antioxidant properties. Previous studies have reported that binahong may reduce blood glucose levels and improve metabolic parameters, although its effects on insulin secretion and C-peptide levels remain inconsistent[11,12]. Experimental studies using diabetic animal models have shown that herbal extracts with antidiabetic properties, including those rich in antioxidant compounds, may influence pancreatic histopathological features[13]. Therefore, investigating the effect of binahong leaf extract on C-peptide levels in streptozotocin-induced diabetic rats is important to further clarify its potential role in preserving pancreatic β-cell function.

MATERIALS AND METHODS

This experimental laboratory study used 24 male Sprague–Dawley rats (12 weeks old; 120–160 g) divided into three groups: negative control (no streptozotocin [STZ]), positive control (STZ-induced), and treatment (STZ-induced plus Anredera cordifolia leaf extract, 100 mg/kg body weight/day). Randomization was performed using a simple random allocation method, whereby each rat was assigned a unique number and group allocation was determined using a random number table to ensure equal group sizes (n = 8 per group) and minimize selection bias. Diabetes was induced by a single intraperitoneal injection of STZ (50 mg/kg body weight), and rats with random blood glucose ≥200 mg/dL were included.

The extract was administered orally for 14 days. Blood glucose was measured using a glucometer, while plasma C-peptide levels were quantified by ELISA and read with a microplate reader. Data were analyzed using appropriate parametric or nonparametric tests based on data distribution ($\alpha = 0.05$). Ethical approval was obtained from the Health Research Ethics Committee, Faculty of Medicine, UIN Syarif Hidayatullah Jakarta (Protocol No. 3674022P21113202009220001).

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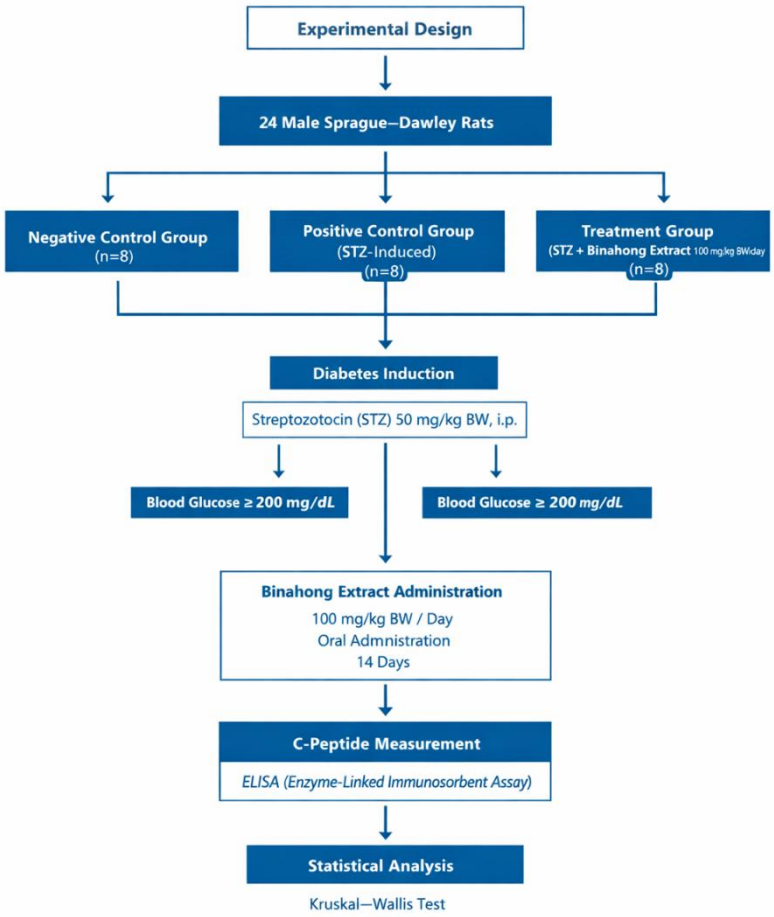

Figure 1. Experimental Workflow of the Study. The diagram illustrates the sequential steps of the study protocol, including animal preparation, diabetes induction via intraperitoneal streptozotocin (STZ) injection (50 mg/kg body weight), blood glucose verification (≥200 mg/dL for inclusion), group allocation, 14-day oral treatment with Anredera cordifolia leaf extract (100 mg/kg body weight/day), blood sample collection during necropsy, and plasma C-peptide quantification by ELISA.

RESULTS

This study evaluated the effect of Anredera cordifolia leaf extract on C-peptide levels in streptozotocin (STZ)-induced diabetic Sprague–Dawley rats. Mean C-peptide concentrations were measured at the end of the intervention period from plasma samples obtained during necropsy. Pre-intervention C-peptide levels were not assessed due to technical limitations related to blood volume collection in live animals.

The mean C-peptide level in the treatment group receiving binahong extract was 1.106 ± 0.231 ng/mL, which was higher than that of the negative control group (0.918 ± 0.696 ng/mL) and the positive diabetic control group (0.585 ± 0.421 ng/mL). The lowest C-peptide concentration was observed in the STZ-induced diabetic control group, indicating impaired β-cell function following STZ administration. In contrast, the treatment group showed a higher mean C-peptide level, suggesting a potential protective effect of binahong extract on pancreatic β-cell activity.

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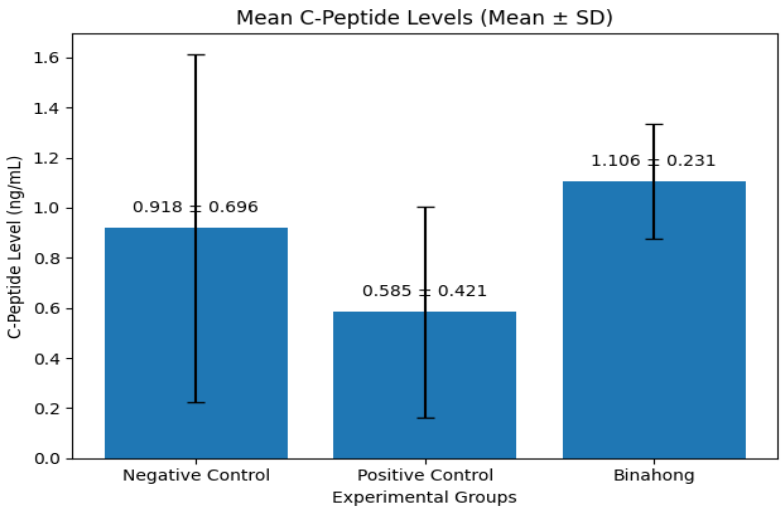

Figure 2. Mean plasma C-peptide levels (mean ± SD, ng/mL) across the three experimental groups at the end of the 14-day intervention period: negative control (no STZ induction, no treatment), positive control (STZ-induced, no treatment), and treatment group (STZ-induced, receiving Anredera cordifolia leaf extract at 100 mg/kg body weight/day). No statistically significant differences were observed among the groups (Kruskal–Wallis test, $p = 0.132$). STZ = streptozotocin; SD = standard deviation.

Normality testing using the Shapiro–Wilk test demonstrated that C-peptide data in all groups were normally distributed ($p > 0.05$). However, Levene's test indicated unequal variances among groups ($p < 0.05$), precluding the use of parametric analysis. Although normality was satisfied, the assumption of homogeneity of variance—required for one-way ANOVA—was violated; therefore, a nonparametric Kruskal–Wallis test was applied as the appropriate alternative, since this test does not assume equal variances across groups. The analysis revealed no statistically significant difference in C-peptide levels among the three groups ($p > 0.05$). Therefore, administration of Anredera cordifolia leaf extract at a dose of 100 mg/kg body weight for 14 days did not result in a significant change in C-peptide levels in STZ-induced diabetic rats.

DISCUSSION

In the present study, administration of Anredera cordifolia leaf extract resulted in a higher mean C-peptide level compared with both the negative and streptozotocin (STZ)-induced diabetic control groups; however, this difference was not statistically significant. These findings indicate that, under the experimental conditions applied, binahong extract did not lead to a measurable improvement in endogenous insulin secretion. Although a numerical increase in C-peptide was observed, this finding should be interpreted cautiously and cannot be considered evidence of meaningful recovery of pancreatic β-cell function.

The lowest C-peptide level was observed in the STZ-induced diabetic control group, which is consistent with the established pathogenesis of STZ-induced diabetes. Streptozotocin induces selective β-cell injury through oxidative stress, DNA damage, and apoptotic pathways, leading to impaired insulin synthesis and secretion[13,14,15]. Because insulin and C-peptide are secreted in equimolar amounts, reduced C-peptide levels reliably reflect β-cell secretory dysfunction, confirming that the animal model used in this study adequately represents experimental diabetes.

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Although the binahong-treated group showed a higher mean C-peptide level, this observation does not necessarily indicate β-cell regeneration or functional restoration. The numerical increase may reflect partial cytoprotection of residual β-cells that potentially mediated by the antioxidant and anti-inflammatory properties of binahong bioactive compounds rather than de novo β-cell proliferation. This interpretation is consistent with the relatively short intervention period and the extent of STZ-induced β-cell damage expected at the administered dose. Previous studies using binahong-based interventions have primarily demonstrated improvements in glycemic control, as reflected by reductions in blood glucose levels, without direct assessment of β-cell secretory markers such as insulin or C-peptide [12,14]. Experimental work using STZ-induced animal models has also reported antihyperglycemic activity of binahong extracts, including ethyl acetate and ethanol fractions, suggesting metabolic modulation rather than definitive restoration of β-cell secretory capacity[16,17].

From a mechanistic perspective, the biological effects of binahong may be related to its flavonoid and polyphenol content, which has been shown to exert antioxidant and cytoprotective effects. Flavonoids are known to reduce oxidative stress, modulate inflammatory signaling, and protect pancreatic β-cells from further damage, thereby helping to preserve residual insulin secretion rather than induce rapid β-cell regeneration [18,19,20]. Specifically, flavonoids such as quercetin and kaempferol which are present in Anredera cordifolia have been shown to attenuate STZ-induced oxidative damage in β-cells by scavenging reactive oxygen species (ROS) and upregulating endogenous antioxidant enzymes such as superoxide dismutase (SOD) and catalase.[21,22] By reducing intracellular oxidative stress, these compounds may protect the remaining functional β-cell mass and preserve their capacity for insulin synthesis and release, which would be reflected in stabilized or elevated C-peptide levels. The modest (non-significant) increase in C-peptide observed in the treatment group in this study may thus represent early cytoprotective effects rather than complete functional restoration. Reviews on herbal medicine in diabetes management also emphasize that β-cell protection and regeneration are gradual processes that typically require prolonged exposure, optimized dosing, or combination therapy to produce measurable functional improvement [23].

The absence of a statistically significant effect in this study may also be influenced by methodological factors, including the use of a single extract dose and a relatively short intervention period. Molecular and pharmacological studies suggest that combining binahong with other herbal agents may enhance insulin sensitivity and metabolic signaling pathways more effectively than single-plant interventions[24,25].

Furthermore, improvements in long-term glycemic markers such as HbA1c are often influenced by behavioral and lifestyle interventions rather than direct β-cell recovery alone, as demonstrated in family-based and supportive interventions for adults with diabetes[26]. Taken together, these findings suggest that while Anredera cordifolia exhibits antihyperglycemic and antioxidant properties, its effect on β-cell secretory function, as reflected by C-peptide levels, remains limited under the conditions tested in this study. Future research should explore varied dosing strategies, longer treatment durations, combination therapies, and complementary outcomes such as pancreatic histopathology to further clarify the role of binahong in supporting β-cell function.

Moreover, the differential behavior observed in the two conditions yielded distinct phenotypes. The variant toxin induced an arborizing cytopathic effect, characterized by thin actin extensions, whereas the other caused classical cell rounding without prominent protrusions. This morphological contrast aligns with previous studies showing that certain TcdB variants that do not glucosylate RhoA produce an arborized phenotype, while those that inactivate RhoA lead to complete rounding [28]. Our assays showed that the strain with intact RhoA generated actin protrusions, whereas the other caused fiber collapse, indicating that RhoA inactivation underlies the rounded morphology. This morphological difference underscores that the toxin's substrate specificity, specifically which GTPase it inactivates, determines the resulting cellular phenotype [29].

The disruption of these adhesion complexes has functional consequences for the cell. Specifically, the loss of FAK–Paxillin signaling and the disorganization of the actin and intermediate filament network accentuate cell rounding. Furthermore, these changes may facilitate processes such as epithelial barrier destabilization or cell motility. The results of this study suggest a decrease in adhesion-associated signals, indicating that adaptor proteins within these complexes are affected [19,30].

The changes observed in paxillin are consistent with a biochemical disassembly of cell–matrix adhesion complexes rather than a direct structural rupture [31,32]. Therefore, in addition to protein disruption, a biochemical disassembly affecting integrin–actin junctions were evident, leaving the cell unable to maintain its normal adherent structure. The selection of NAP1 as the sole variant for the Co-IP assays is justified by the mechanistic redundancy observed among RhoA-inactivating toxins. Since VPI and NAP1 induce the same negative signaling cascade on the paxillin interactome, the Co-IP results obtained with NAP1 provide a valid molecular framework for both. In contrast, the NAP1v variant, by not glucosylating RhoA, preserves the basal signaling of focal adhesions, making its analysis by Co-IP unnecessary for validating the proposed disassembly model, which appears to be a signature exclusive to variants that collapse RhoA activity.

In contrast to the other proteins, plectin exhibited a distinctive quantitative decrease, as evidenced by the quantitative analysis. This protein is indispensable for anchoring intermediate filaments to focal adhesions, thereby playing a pivotal role in cell motility and invasion. Its loss suggests that the toxins undermine cellular integrity in a multidimensional manner, potentially affecting both microfilaments and intermediate filaments[33]. It can be hypothesized that the degradation of plectin is mediated by calpains activated by calcium flux. Calpain-2 is known to regulate FAK proteolysis in a Src phosphorylation–dependent manner [34].

The capacity of NAP1 to disrupt adhesion complexes, in conjunction with its enhanced autoproteolysis, suggests a potential correlation with the clinical severity of the condition. The disintegration of focal adhesions undermines their function in transmitting force or tension to adhesion sites, resulting in diarrhea and subjecting the epithelium to inflammation [35].

Several methodological limitations should be considered when interpreting the findings of this study. First, pre-intervention C-peptide levels were not measured due to technical constraints related to the limited blood volume obtainable from live animals without compromising their welfare; this precluded direct within-animal comparison and precise quantification of changes in β-cell secretory function over the intervention period. Second, only a single dose of Anredera cordifolia extract (100 mg/kg body weight/day) was evaluated, which limits conclusions regarding dose-response relationships and optimal therapeutic dosing. Third, the intervention period of 14 days may have been insufficient to produce measurable restoration of β-cell secretory function, given that cytoprotective and regenerative processes are inherently gradual. Fourth, the relatively small sample size (n = 8 per group) may have reduced statistical power to detect modest differences. Fifth, no histopathological examination of pancreatic tissue was performed, which would have provided complementary structural evidence regarding β-cell integrity. These limitations should be addressed

in future studies through expanded dosing protocols, longer treatment durations, pre- and post-intervention assessments of both C-peptide and insulin levels, and inclusion of pancreatic histopathological endpoints.

CONCLUSION

In conclusion, administration of Anredera cordifolia leaf extract at a dose of 100 mg/kg body weight for 14 days did not significantly affect C-peptide levels in streptozotocin-induced diabetic Sprague–Dawley rats. Although the treatment group showed a higher mean C-peptide level compared with both negative and diabetic control groups, this increase was not statistically significant, indicating that the extract did not produce a measurable improvement in endogenous insulin secretion under the conditions of this study. These findings suggest that, while binahong has been reported to exert antihyperglycemic and antioxidant effects, such properties alone may be insufficient to restore pancreatic β-cell secretory function within a short intervention period and at a single tested dose.

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Authors' contributions

All authors contributed equally to the study design, data collection and analysis, and manuscript preparation. All approved the final version and take full responsibility for the accuracy and integrity of the work.

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Conflict of interest

The authors declare that they have no conflict of interest.

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