Original Article

Artocarpus altilis Leaf Extract Protects Pancreatic Islets and Improves Glycemic Control in Alloxan-induced Diabetic Rats

Abstract

The antioxidant capacity of *Artocarpus altilis* leaf extract may offer protection against stress oxidative-induced damage to pancreatic cells. This study aimed to examine the effect of *Artocarpus* leaf extract on pancreatic islets structure, blood glucose (BG), and insulin serum levels in alloxan-induced diabetic rats. Diabetes mellitus was induced in rats with an intraperitoneal injection of alloxan (155 mg/kg). Rats' BG levels were measured daily. Only rats with BG >250 mg/dL (n = 25) proceeded to receive different treatments: placebo, *Artocarpus* leaf extract at the dose of 100, 200, or 400 mg/kg, or insulin 6 IU/200 g. All treatments were administered daily for 14 days before blood and pancreatic tissue samples were collected. Five healthy rats (n = 5) were included to serve as normal controls. The result shows that alloxan-induced atrophy of pancreatic islets and *Artocarpus* leaf extract administration at all given doses reduced the severity of pancreatic islet's atrophy. However, only at 400 mg/kg dose, *Artocarpus* leaf extract significantly reduced rats' BG level ($P < 0.05$), similar to that of insulin-treated rats. *Artocarpus* leaf extract, especially at 100 and 400 mg/kg doses, also improved insulin serum levels compared with placebo treatment ($P < 0.05$). In conclusion, *Artocarpus* leaf extract protected rats' pancreatic islets against alloxan-induced damage. This protection could improve the BG and insulin serum levels in *Artocarpus*-treated rats.

Keywords: *Alloxan, Artocarpus altilis, blood glucose, insulin, pancreatic islets*

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Introduction

Diabetes mellitus (DM) is a complex metabolic disorder characterized by marked hyperglycemia, polyphagia, polydipsia, and polyuria.[1] The prevalence of DM has been increasing in Asian countries in the past decades, making Asia the continent with the largest diabetic population worldwide.[2] This trend has led to a massive increase in the cost of diabetic treatments because it may require lifelong medication and therapy. Consequently, many studies have been focusing on searching for affordable but effective alternative therapies to control hyperglycemia.[3]

The pathogenesis of DM is mostly related to inadequate or loss of insulin secretion or activity leading to dysfunctional carbohydrate, lipid, and protein metabolisms.[1] The hormone insulin is secreted by β-pancreatic cells located in the islets of Langerhans and is responsible to regulate the blood glucose level. Hyperglycemia manifests when the release of insulin from pancreatic islets is insufficient or the insulin-dependent glucose cellular uptake is impaired.[4]

Several plants have been acclaimed for their antidiabetic properties. Many of their uses are traditionally practiced based on empirical evidence.[5] Among them is Breadfruit plant (*Artocarpus altilis)* [(Parkinson) Fosberg], which belongs to the family of Moraceae. The fruits of *Artocarpus altilis* have been reported to contain triterpenes, flavonoids, stilbenes, and sterols that provide antioxidant, antihyperglycemic, and antimicrobial properties.[6] Meanwhile, the leaves of the plant have been recognized to endow with rich phenolic content, which has been shown to provide potent antioxidant activities.[7] The polyphenols from *Artocarpus altilis* are capable of eliciting insulin-like biological activity, reducing ROS production, and improving cellular antioxidant defense mechanisms. These capacities are beneficial to improve cellular protection against oxidative damage.[8]

The current antidiabetic medications mainly act to enhance insulin secretion, improve insulin sensitivity, prevent liver gluconeogenesis, or reduce glucose absorption. However, it is crucial to uncover an antidiabetic agent that is capable of enhancing β-pancreatic cell regeneration

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and preventing further loss of β-pancreatic cells.[9] The antioxidant capacity of *Artocarpus altilis* leaf compounds may offer protection against stress oxidative-induced damage in pancreatic cells. Therefore, the present study aimed to examine the effect of *A. altilis* leaf extract on pancreatic islets protection, blood glucose, and serum insulin levels in alloxan-induced diabetic rats.

Materials and Methods

Chemicals and drugs

Alloxan monohydrate (Sigma Aldrich®, USA), formaldehyde in 10% PBS, and diethyl ether were purchased from an official chemical distributor in Makassar, Indonesia. Insulin glargine (Lantus®) used in this study was obtained from a regional hospital in Makassar, Indonesia. The level of plasma insulin was measured using rat insulin ELISA kit 96T (BT-Lab®, Gaithersburg).

Artocarpus altilis leaf collection and extraction

Artocarpus altilis leaves (green and yellow) were harvested in the area of Gowa District, South Sulawesi, Indonesia. The samples were identified and authenticated at the Laboratory of Biology, Faculty of Mathematics and Natural Sciences, Makassar State University, Indonesia. Green and yellow leaves were separately extracted using maceration with 70% ethanol (1:10) at room temperature (25°C). The extract was thickened using a rotary evaporator (Heidolp®) at 50°C before stored in a desiccator at room temperature prior to usage. *Artocarpus* leaf extract was freshly prepared in 1% sodium carboxyl methylcellulose (Na CMC) suspension before peroral administration in animals.

Total flavonoid analysis

The total flavonoid contents of *Artocarpus* green and yellow leaf extracts were determined as rutin content using an Ultra-Fast Liquid Chromatography (Shimadzu, Japan). The composition of mobile phase was methanol, acetonitrile, and 5% formic acid at the 25:65:10 ratio and the flow rate was 1 mL/min. The silica-bound octadecyl carbon chain (C18) was used as the stationary phase. The flavonoid compound was measured at a wavelength of 370 nm and calculated as the area under curve (AUC).

Animal procedures

Male Wistar rats age ranging from 2 to 3 months with a bodyweight of 180–250 g were purchased from a rodent breeding facility in Yogyakarta, Indonesia (UD Wistar). The rats were housed and cared for according to the institutional standard for laboratory animal care and use (animal ethical clearance number: 544/UN4.6.4.5.31/PP36/2019). Rats were accustomed to their environment for 14 days prior to initiating treatment protocols. Access to standard pellets and water was provided at all times. Prior to alloxan injection, blood glucose (BG) levels of rats were analyzed to exclude rats that had abnormal baseline levels.

Alloxan monohydrate was prepared in 0.9% of sodium chloride with a pH of 7.[10] DM was induced in rats with a single intraperitoneal injection of alloxan (155 mg/kg).[11] An oral administration of 5% glucose (2 mL) was given after 10 min of alloxan injection to prevent hypoglycemia in rats.[12] Rat BG levels were measured daily using a digital glucometer (Easy Touch®). Rats that exhibited significant elevation of BG levels of more than 250 mg/dL within 3 days post-injection were defined as diabetic and proceeded to receive treatments.

The diabetic rats ($n = 25$) were divided into five treatment groups: 1% Na CMC (PLACEBO); *Artocarpus* leaf extract at 100 mg/kg dose (EXT 100); *Artocarpus* leaf extract at 200 mg/kg dose (EXT 200); *Artocarpus* leaf extract at 400 mg/kg dose; (EXT 400); and insulin at 6 IU/200 g dose (INSULIN). Meanwhile, healthy rats ($n = 5$) that were not subjected to alloxan injection were also included as normal controls. As the mature leaves of *Artocarpus altilis* were traditionally claimed to have a better antidiabetic effect than the young leaves,[13] only the yellow leaf extract was used in the animal study. The doses of *Artocarpus* leaf extract were chosen based on a previous study showing an improvement in pancreatic tissue structure after *Artocarpus* leaf extract administration in alloxan–nicotinamide-treated rats.[14] *Artocarpus* leaf extract in this study was administered peroral and insulin was administered subcutaneously. Insulin dose was chosen based on the most effective insulin dose in treating diabetic rats.[15] All treatments were given daily for 14 days.

Blood glucose test

Following the injection of alloxan and during the 14 days of treatment, the rat BG levels were checked daily using a glucometer (Easy Touch®) to record the day-to-day fluctuation of BG levels. In addition, after the last day of treatment, the BG levels were also analyzed using a Humalyzer 3500 (Human Diagnostic Worldwide, Germany) to confirm the result of the glucometer test.

Insulin level assay

At the end of the experiment, the rats were anesthetized with diethyl ether in a chamber. The blood samples were collected from the tail lateral vein using collecting tubes containing ethylenediamine tetraacetic acid (BD vacutainer®). Blood samples were centrifuged at a rate of 3000 rpm at 25°C for 20 min to obtain rat serum. The level of insulin serum was measured using an enzyme-linked immunosorbent assay (ELISA) reader (Thermo Scientific®) according to the kit's instruction (rat insulin ELISA kit, BT-Lab) at 450 nm.

Histomorphometric analysis of pancreatic islets

Following blood collection, the rats were euthanized with cervical dislocation. The organ pancreas was removed and fixed in 10% formaldehyde for 48 h before undergone automatic tissue processing (Thermo Scientific®). Pancreatic tissues were embedded in paraffin cassettes and cut into 4-µm thickness with a microtome (Thermo Scientific®). The histological slides were stained using hematoxylin and eosin (HE) and observed under a light microscope (Olympus®). Histopathological analysis of

the pancreatic tissues was performed by an observer who was blinded to the treatment groups. The histomorphometric study was carried out according to Noor *et al.* study[16] with some modification. The morphometric data include (1) the number (N) of pancreatic islets observed under ×40 magnification, (2) the diameter of the islets that was calculated as $D = \sqrt{ab}$ [Figure 1]; the mean diameter of the islets per animal that was calculated from the average of five pancreatic islets per section, including the largest and smallest islets observed at ×200 magnification; (3) the area of the islets, which was calculated using the formula $A = \pi(D/2)^2$, assuming that the pancreatic islets are spherical.[17]

Statistical analysis

The numerical data are presented as mean ± SEM. The distribution of data was tested for normality using the Kolmogorov–Smirnov test and the homogeneity of the data was confirmed with Levine's test. If the data were normally distributed, a one-way ANOVA analysis was performed, followed by an LSD post hoc test. A paired *t*-test was also used to analyze the changes in the BG levels in rats following alloxan injection and after 14 days of treatments. A significant difference is defined if the *P*-value is <0.05.

Results

Total flavonoid content of *A. altilis* leaf extract

A comparison of the flavonoid content of the green and yellow leaf extracts of *A. altilis* is shown in Figure 2. It is found that the yellow leaf extract had four times higher concentration of flavonoid compared with the green leaf extract (0.476 vs. 0.144 µg/mg).

The effect of *A. altilis* leaf extract administration on blood glucose level

All rats had normal BG levels at baseline (93 to 128 mg/dL). Following the injections of alloxan, around 50% of the rats had extreme elevations of BG levels. These rats were then randomly assigned into five groups. The mean of BG levels of each group following alloxan injection was ranging from 352 to 422 mg/dL. No statistical difference among groups was found at this stage [Figure 3].

After 14 days of treatments, the PLACEBO, EXT 100, and EXT 200 groups still experienced elevated BG levels above 250 mg/dL. In contrast, rats treated with *Artocarpus* leaf extract at the highest dose (EXT 400) experienced a significant decrease in the BG level (154.5 ± 45.8 mg/dL), which was near their baseline levels. The decrease of BG levels was also significant in rats treated with insulin, from 352.4 ± 33.2 to 191.6 ± 23.1 mg/dL ($P < 0.05$), where 80% of the animals had BG levels lower than 250 mg/dL.

The effect of *A. altilis* leaf extract administration on serum insulin level

In this study, it is shown that insulin serum level in placebo-treated rats was significantly lower (1.79 ± 0.05 mIU/L) compared with that of the normal rats (2.05 ± 0.12 mIU/L) [Figure 4]. Although a reduction in insulin levels was observed in all diabetic groups, the insulin levels of *Artocarpus* leaf

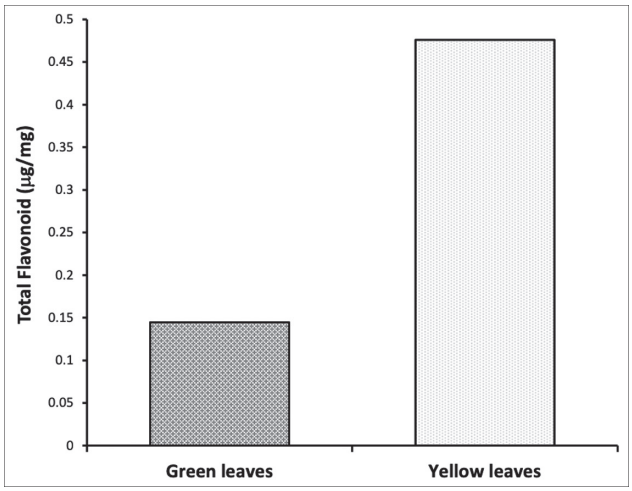

Figure 2: The total flavonoid calculated as rutin content of the green and yellow leaf extracts of *A. altilis*

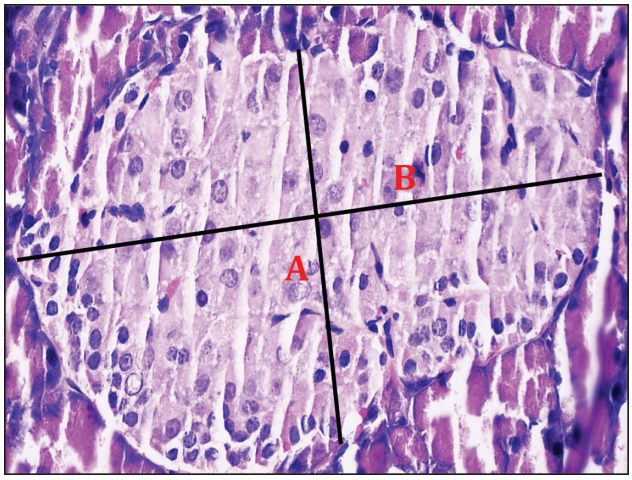

Figure 1: A representative photomicrograph used for the determination of pancreatic islet diameters (A and B)

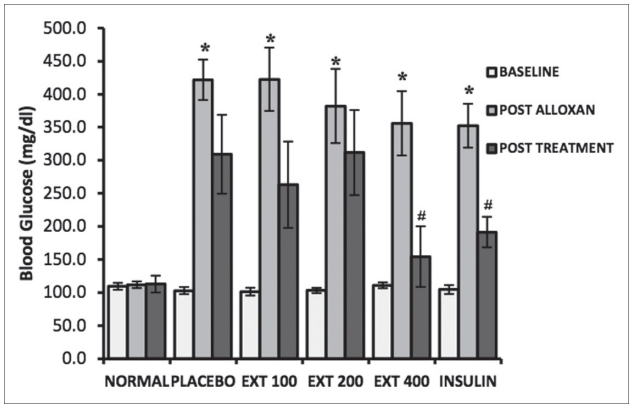

Figure 3: The profiles of blood glucose (BG) levels of normal and diabetic rats treated with placebo, *Artocarpus* extract 100 mg/kg, *Artocarpus* extract 200 mg/kg, *Artocarpus* extract 400 mg/kg and insulin. Baseline: BG prior to alloxan injection; post alloxan: BG post 3-day from alloxan injection; post treatment: 14-day post treatments. **P* < 0.05 using paired *t*-test between post alloxan and baseline values; #*P* < 0.05 using paired *t*-test between post treatment and post alloxan values

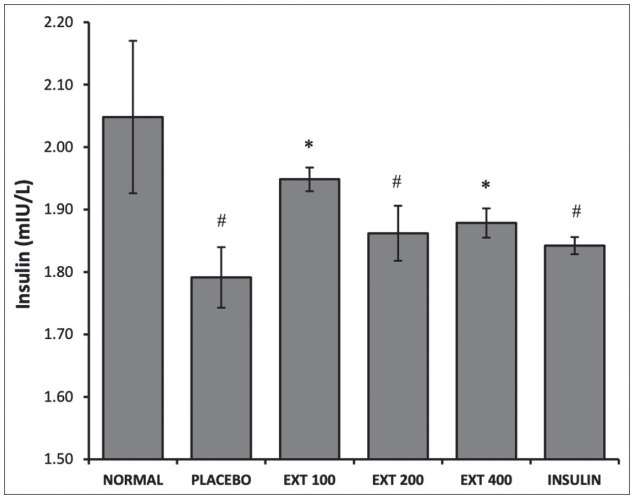

Figure 4: The insulin levels of normal and diabetic rats treated with placebo, *Artocarpus* extract 100 mg/kg, *Artocarpus* extract 200 mg/kg, *Artocarpus* extract 400 mg/kg and insulin. #*P* < 0.05 for placebo, EXT 200 and insulin compared to normal group; **P* < 0.05 for EXT 100 and EXT 400 compared to placebo group

extract groups, EXT 100 and EXT 400, were significantly higher than the placebo group ($P < 0.05$) and were not statistically different from the normal group.

The effect of *A. altilis* leaf extract administration on pancreatic islets

Figure 5 illustrates the photomicrographs of large and small pancreatic islets of normal and diabetic rats treated with different treatments. Normal rats showed a normal appearance of pancreatic islets with smooth-contoured lining containing β and non-β pancreatic cells without the presence of fibrosis or vacuolization. Meanwhile, the injection of alloxan induced degeneration and shrinkage (atrophy) of pancreatic islets, which was evident in the placebo-treated rats. In contrast, rats treated with insulin or *Artocarpus* extract showed minimal histopathological changes in their pancreatic islets, although the size of pancreatic islets was predominantly smaller than that in the normal group.

The morphometric analysis showed that the number, diameter, and area of pancreatic islets in the placebo group were significantly reduced ($P < 0.05$) [Table 1]. A decrease in the number and size of pancreatic islets was also experienced by diabetic rats treated with *Artocarpus* extract or insulin; however, it was not statistically different compared with the normal control. The insulin group had larger pancreatic islets, but the islet number was somewhat lower than the *Artocarpus* extract group; however, these differences were not statistically significant.

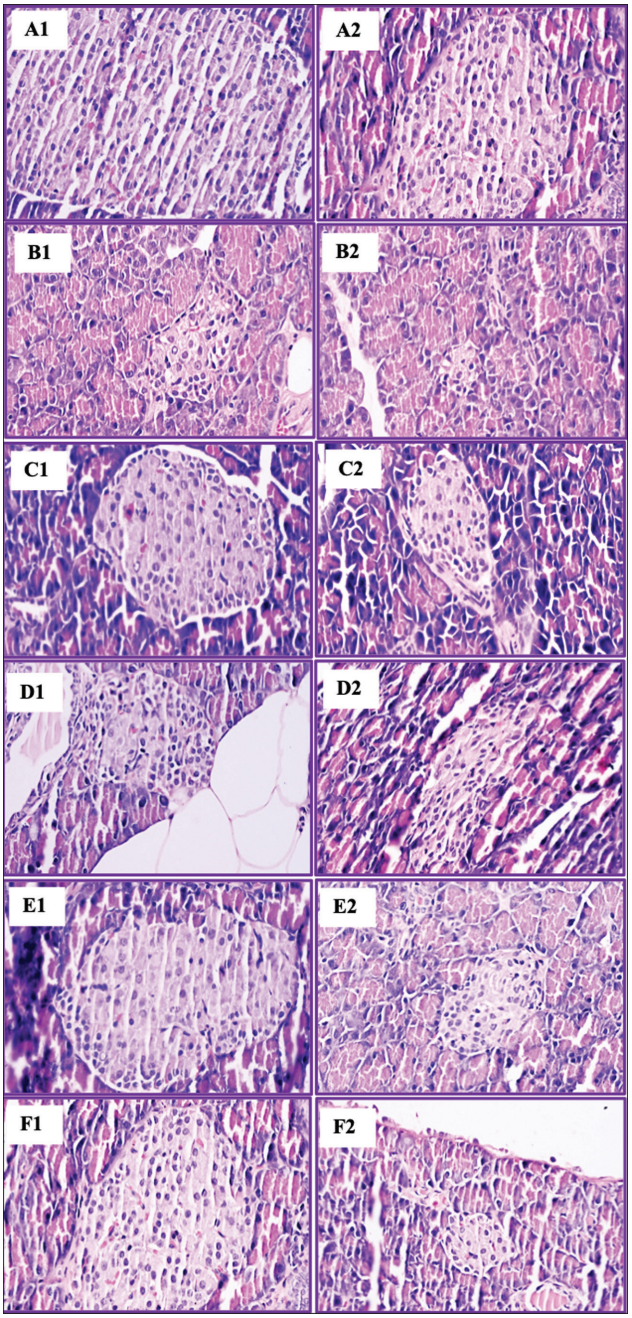

Figure 5: The representative photomicrograph of the pancreatic islets of normal (A1 and A2) and diabetic rats treated with placebo (B1 and B2), *Artocarpus* leaf extract 100 mg/kg (C1 and C2), *Artocarpus* leaf extract 200 mg/kg (D1 and D2), *Artocarpus* leaf extract 400 mg/kg (E1 and E2), or insulin (F1 and F2). The left-hand side pictures represent the islets with the largest size and the right-hand side pictures represent the smallest islet found in the group

Discussion

Artocarpus altilis leaf extract has been presumed to possess antioxidant activities, which are strongly associated with the flavonoid content of the plants. Based on its empirical use, the mature (yellow) leaves of *Artocarpus altilis* are believed to have a more potent antidiabetic effect than the green leaves. The result of this study supports this contention by showing a significantly higher flavonoid content in yellow compared to green *Artocarpus* leaf extract. In addition, a phytochemical study has revealed some dissimilarities in chromatogram profiles of yellow and green *Artocarpus* leaf extract, which might contribute to a more potent alpha-glucosidase inhibition of yellow leaf compared with green leaf extract.[18]

Table 1: The comparison of the number, mean diameter, and mean area of pancreatic islets among groups

Groups	Number of islets	Diameter (μm)	Area (μm²)
Normal	43.0 ± 14.4	79.6 ± 2.7	4980.6 ± 333.8
Placebo	17.0 ± 5.8*	36.0 ± 8.1*	1172.8 ± 523.3*
EXT 100	26.5 ± 11.3	49.3 ± 4.6	1955.0 ± 359.3
EXT 200	29.7 ± 2.0	46.7 ± 6.3	1776.4 ± 485.2
EXT 400	32.3 ± 7.5	44.9 ± 11.6	1900.7 ± 1012.1
Insulin	24.8 ± 11.5	55.9 ± 9.1	2648.5 ± 789.3

The number of pancreatic islets was counted per section with ×40 magnification. The mean diameter and the mean area of islets were measured using ×200 magnification. *$P < 0.05$ compared with normal group

In this study, the antidiabetic effect of *A. altilis* leaf extract was examined against alloxan-induced diabetes in rats. The blood glucose levels of rats were found to fluctuate after alloxan injection, starting with a transient hypoglycemic phase lasting around 10–30 min followed by a rapid elevation of blood glucose levels within hours post-injection. The hypoglycemic phase might be triggered by transient hyperinsulinemia caused by a temporary increase in ATP levels as a consequence of alloxan inhibition on glucokinase.[19] To prevent prolonged hypoglycemic state in rats, 5% glucose was administered in this study approximately 10 min after administering alloxan i.p injection.

Three days after alloxan (155 mg/kg) injection, approximately 50% of total rats experienced a significant elevation of blood glucose levels to more than 250 mg/dL with less than 5% deaths. It has been postulated that animal conditions, including nutritional status and weight, may contribute to their responses to alloxan injection.[20] The mechanisms of alloxan-induced diabetes are associated with two different pathogenesis: (1) a selective inhibition of insulin secretion through the inhibition of glucokinase, i.e., the glucose sensor in β pancreatic cells, and (2) stimulation of ROS formation, leading to a necrotic death of the pancreatic β cells.[20] In this study, only treatment with *A. altilis* extract at a high dose (400 mg/kg) was able to significantly reduce the blood glucose level to less than 200 mg/dL, which was comparable to that seen in rats treated with insulin (154.5 ± 45.8 vs 191 ± 23 mg/dL).

In this study, it is also revealed that the hyperglycemic state in alloxan-induced diabetic rats was concomitantly found with low blood insulin levels. This is not surprising because alloxan injection induces necrosis on insulin-producing β cells in pancreatic tissue.[19] It is found that, with 100 or 400 mg/kg *Artocarpus* leaf extract, both the blood glucose and insulin levels of the rats were significantly improved compared with the placebo group. This improvement may indicate enhanced insulin production and secretion of pancreatic β cells. Unlike *Artocarpus* leaf extract, insulin treatment in alloxan-induced diabetic rats also corrected the hyperglycemia state but it did not significantly improve blood insulin levels.

The improvement of blood insulin level in *Artocarpus* extract groups was in line with improved pancreatic islet histology in the rats. In this study, the pancreatic islets of the alloxan-injected rats treated with placebo were mostly athropic. Alloxan has been shown to induce histopathological changes and severe injury to the pancreatic tissue, including the degeneration of β-cells and vacuolization of the pancreatic exocrine area.[21] In contrast, the pancreatic islets in *Artocarpus* extract groups mostly had a healthy appearance, although the number and the size slightly decreased compared with the normal group. It is found that the treatment with *Artocarpus* extract at 400 mg/kg dose was able to retain 75% of pancreatic islet numbers in diabetic rats, compared with only 40% in the placebo group. Moreover, the diameter and area of pancreatic islets were also improved by 11% and 15%, respectively, compared with the placebo groups. A previous study has uncovered that some plants can stimulate regeneration of pancreatic islets in streptozotocin-induced diabetic rats, which results in antidiabetic capacity of the plants.[16,22,23] It is believed that this is also true for *A. altilis.* This present study indicates that *Artocarpus* leaf extract induces rejuvenation or revitalization of insulin-producing pancreatic β cells. It is assumed that, through its antioxidant capacity, *Artocarpus* leaf extract protects against alloxan-induced damage in pancreatic islets, leading to regeneration or recovery of partially damaged pancreatic cells, enhancing insulin production, and thereby, resulting in better glycemic control in diabetic rats.

Conclusion

The administration of *A. altilis* leaf extract at all given doses appeared to alleviate the atrophy and increase the number and size of pancreatic islets. This pancreatic protection of *A. altilis* leads to the restoration of plasma insulin levels and improvement of blood glucose levels following treatment. Further experiments are required to elucidate the mechanisms of antidiabetic effect of *A. altilis* leaf extract to adequately support the traditional use of *A. altilis* leaf in improving glycemic control in diabetic patients.

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Conflict of interest

The authors declared no conflict of interests.

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