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Original Article

Protective effects of atung (*Parinarium glaberrimum*) seed extract against streptozotocin-induced pancreatic damage: an in vivo experimental study

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A B S T R A C T

Background: Hyperglycemia, characterized by elevated blood glucose levels, leads to oxidative stress and pancreatic β-cell dysfunction. Streptozotocin (STZ)-induced hyperglycemia in mice is widely used to model pancreatic injury and evaluate antihyperglycemic agents. Atung (*Parinarium glaberrimum*) seed extract contains antioxidant compounds; however, its effects on pancreatic histopathology remain unclear.

Objective: This study aimed to investigate the effect of atung seed extract on pancreatic histopathology in STZ-induced hyperglycemic mice, focusing on islet diameter and histopathological damage.

Methods: Twenty-four male Mus musculus (BALB/c) mice were randomly allocated into six groups: normal control, hyperglycemic control, metformin-treated, and three atung seed extract groups (100%, 75%, and 50%). Hyperglycemia was induced using intraperitoneal streptozotocin (40 mg/kgBW). Treatments were administered orally for 21 days. Blood glucose levels were reassessed on day 22 prior to euthanasia. Pancreatic tissues were collected, fixed in 10% neutral buffered formalin, and stained with hematoxylin–eosin.

Results: Islet diameter differed significantly among groups ($p < 0.05$). The ASE75 group showed a mean diameter of 133.01 µm and a median damage score of 1 (IQR 0), comparable to the metformin group (146.61 µm; 0.5 [IQR 1]), whereas the hyperglycemic control group showed severe atrophy (89.02 µm; 3 [IQR 0]).

Conclusion: Atung seed extract at a 75% concentration effectively preserved pancreatic β-cell structure, as indicated by increased islet diameter and reduced histopathological damage, supporting its potential as a natural antioxidant-based adjuvant therapy for hyperglycemia management.

INTRODUCTION

Hyperglycemia is a metabolic condition characterized by elevated blood glucose levels beyond the normal physiological range and represents a key initiating factor in the development of various metabolic and organ-related complications. Persistent hyperglycemia disrupts cellular homeostasis, leading to oxidative stress, systemic inflammation, and progressive tissue damage. The pancreas, particularly the β-cells of the islets of Langerhans, is highly susceptible to this condition due to its central role in insulin secretion and glucose regulation.[1,2]

Over the past decades, hyperglycemia has become a major contributor to the global burden of metabolic diseases. The World Health Organization reported a substantial increase in diabetes mellitus cases from approximately 200 million in 1990 to more than 830 million in 2022.[3] Similarly, the International Diabetes Federation documented a 63% increase in the number of adults with diabetes in the Western Pacific region, rising from 131.9 million in 2011 to 215 million in 2024.[4] This trend is strongly associated with rapid urbanization, lifestyle changes, and population aging, particularly in low- and middle-income countries.

In Indonesia, diabetes prevalence continues to rise, with the country ranking among the top five globally in total cases. National data from the 2023 Survey Kesehatan Indonesia reported a prevalence of 1.8%, while regional data indicate a consistent increase in case numbers over recent years.

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These findings highlight the growing public health burden of hyperglycemia and its long-term complications.[5,6]

At the cellular level, chronic hyperglycemia induces excessive production of reactive oxygen species (ROS) and impairs endogenous antioxidant defenses, including superoxide dismutase, catalase, and glutathione peroxidase. This imbalance leads to oxidative stress, resulting in β-cell degeneration, structural alterations of the islets, and impaired pancreatic function. These changes can be objectively assessed through histopathological and morphometric analysis.[7]

Natural antioxidant-based therapies have attracted increasing attention as potential strategies to mitigate oxidative damage and preserve pancreatic integrity. One potential source is atung (*Parinarium glaberrimum*), a local plant rich in phenolic and flavonoid compounds with strong antioxidant properties. Previous studies on plant-derived antioxidants, such as Syzygium aqueum, have demonstrated improvements in oxidative stress and partial restoration of pancreatic function in streptozotocin-induced models.[8] However, most studies primarily focus on biochemical parameters and provide limited evaluation of structural pancreatic changes.

To date, no study has specifically evaluated the protective effect of atung seed extract on pancreatic islet integrity using an integrated approach combining histopathological scoring and quantitative morphometric analysis. Therefore, this study aims to investigate the effect of atung seed extract on pancreatic histopathological changes and islet morphology in streptozotocin-induced hyperglycemic mice.

METHOD

Study Design

This study employed a laboratory-based experimental design using a post-test-only control group model.[9]

Study Setting

Animal experiments were conducted at the Biochemistry and Physiology Laboratory, Faculty of Medicine, Universitas Pattimura, Ambon. Histological processing was performed at the Histology Laboratory, Faculty of Medicine, Universitas Pattimura, Ambon.

Materials

The reagents used included streptozotocin (STZ), metformin hydrochloride, atung seed extract, 96% ethanol, citrate buffer (pH 4.5), 10% neutral buffered formalin (NBF), a ketamine–xylazine anesthetic mixture, 10% sucrose, 0.9% NaCl, and distilled water. The equipment included a rotary evaporator, magnetic stirrer, oral gavage (1 mL syringe with a sonde needle), digital balance, microtome, Euromex iScope microscope, and ImageJ software (version 1.53a).

Preparation of Atung Extract

Atung fruits were collected from Soya Village, Sirimau District, Ambon City. The seeds were separated, washed, and sun-dried for 2–3 days, then ground into a fine powder and sieved through a 30-mesh filter. Approximately 800 g of powder was macerated in 96% ethanol for 3 × 24 hours with occasional stirring and protected from light. The extract was filtered and concentrated using a rotary vacuum evaporator to obtain a viscous extract, which was stored at 4°C until use. Three concentrations were prepared: 100% (pure extract), 75% (75% extract + 25% distilled water), and 50% (50% extract + 50% distilled water).

Animals Preparation

This study involved 24 male Mus musculus (BALB/c) mice, aged 8–12 weeks and weighing 20–30 g. Animals were acclimatized for one week under controlled conditions (24 ± 2°C; 12 h light–dark cycle) with free access to standard laboratory feed (AD-II) and water ad libitum. The mice were randomly divided into six groups (n = 4 per group).

In Vivo Procedure

Induction of Hyperglycemia

Hyperglycemia was induced using a modified multiple low-dose STZ protocol adapted from Furman (2021). Mice received intraperitoneal injections of STZ (40 mg/kgBW) for two consecutive days, followed by a 48 h rest period and continued for five additional days. STZ was freshly dissolved in sodium citrate buffer (pH 4.5) and administered within 15 min of preparation.

Experimental Treatment

After confirmation of hyperglycemia, treatments were administered orally for 21 days. The groups consisted of: normal control, hyperglycemic control, metformin-treated, and three groups receiving atung seed extract at concentrations of 100%, 75%, and 50%.

Pancreatic Tissue Collection

On day 22, mice were anesthetized using a ketamine–xylazine mixture (ketamine 100 mg/kgBW and xylazine 10 mg/kgBW diluted in 0.9% NaCl) and euthanized. Pancreatic tissues were dissected, immediately fixed in 10% NBF for 24 h, and processed through dehydration, clearing, and paraffin embedding. Tissue sections were cut using a rotary microtome, mounted on slides coated with Mayer's albumin, and stained with hematoxylin–eosin.

Histopathology Assessment

Morphometric Analysis of Islet Diameter

Islet diameter was measured using ImageJ software (version 1.53a) at 10× magnification by assessing two perpendicular axes (horizontal and vertical). The average value was used for analysis.

Histopathological Scoring

Islet damage was assessed semi-quantitatively based on the proportion of degeneration, vacuolization, and vascular

congestion at 40× magnification. The percentage of affected area was categorized into five levels: normal (no alteration), mild (1/8 affected), moderate (1/4 affected), severe (3/8–1/2 affected), and very severe (>1/2 affected).

Data Analysis

Continuous data were presented as mean ± SD, and ordinal data as median (IQR). Normality and homogeneity were assessed using Shapiro–Wilk and Levene's tests. Parametric data were analyzed using one-way ANOVA followed by post hoc tests, while nonparametric data were analyzed using the Kruskal–Wallis test with post hoc comparisons. A p-value < 0.05 was considered statistically significant.

Ethical Approval

All procedures were conducted in accordance with ethical guidelines for laboratory animal care and were approved by the Ethics Commission of the Faculty of Medicine, Universitas Pattimura, Ambon (No. 176/FK-KOM.ETIK/IX/2025).

RESULTS

Histological evaluation revealed significant differences in Langerhans islet diameter among groups ($p < 0.05$). The hyperglycemic control (HC) group exhibited marked islet atrophy, with a mean diameter of 89.02 µm, compared with the normal control (NC) group (155.83 µm). Treatment with metformin (MET) resulted in substantial restoration of islet size (146.61 µm). Among the atung extract groups, ASE75 demonstrated the most pronounced improvement (133.01 µm), whereas ASE100 (119.17 µm) and ASE50 (106.14 µm) showed partial recovery (Figure 1B).

Histopathological scoring showed significant differences between groups ($p < 0.05$). The HC group exhibited the highest damage score (median 3 [IQR 0]), indicating severe islet degeneration. In contrast, the NC group showed minimal damage (median 0). Treatment with metformin reduced the damage score (0.5 [IQR 1]), while ASE75 demonstrated comparable protective effects (median 1 [IQR 0]). The ASE100 and ASE50 groups showed moderate improvement, with higher damage scores compared with ASE75 (Figure 1C).

Blood glucose levels measured on day 22 showed a similar pattern. The HC group had the highest glucose level (177.2 mg/dL), whereas the NC group remained within normal range (67.4 mg/dL). Treatment with metformin significantly reduced glucose levels (61 mg/dL). Among the extract-treated groups, ASE75 showed the greatest reduction (75.25 mg/dL), followed by ASE100 (117 mg/dL) and ASE50 (128 mg/dL) (Figure 1A).

Microscopic observations supported these findings (Figure 2). The NC group exhibited normal islet architecture, while the HC group showed extensive cellular degeneration, vacuolization, and vascular congestion. In contrast, the MET and ASE75 groups preserved islet structure, with ASE75 showing improved cellular integrity and reduced histopathological alterations.

Overall, the comparative analysis (Figure 3) demonstrated that ASE75 produced the most favorable outcomes among the extract-treated groups, showing improvements in blood glucose levels, islet diameter, and histopathological damage comparable to metformin treatment.

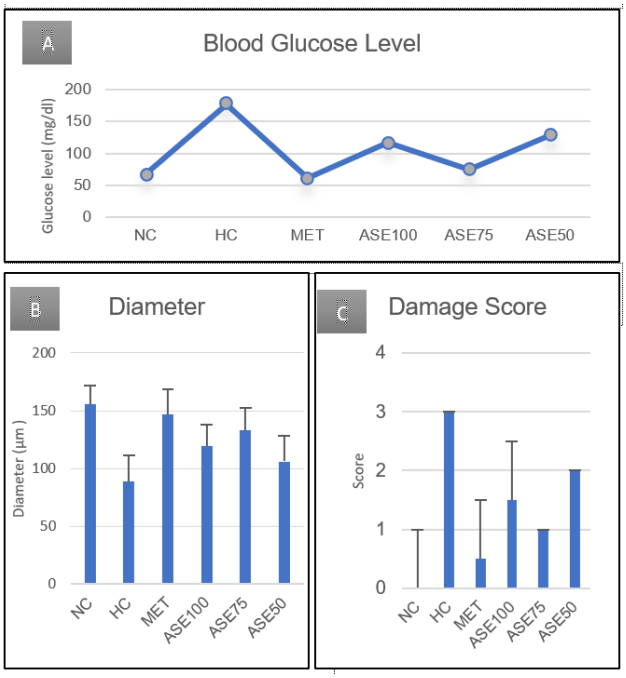

Figure 1. (A) Mean blood glucose levels on day 22 (mg/dL), (B) mean islet diameter (µm), and (C) median histopathological damage scores across experimental groups (n = 4 per group).

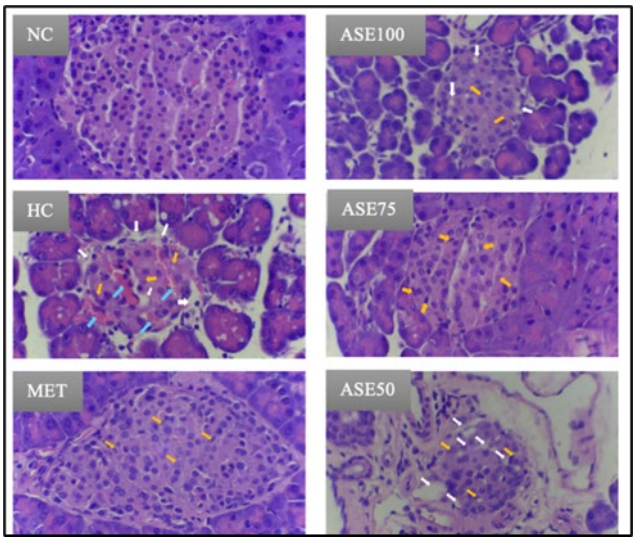

Figure 2. Histopathological Damage Score of Langerhans Islets. [HE, 40x Magnification].

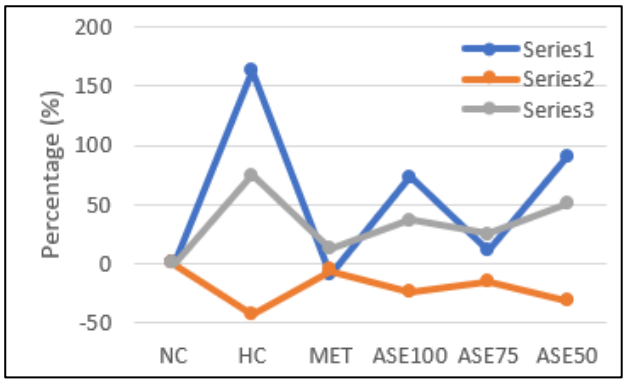

Figure 3. Comparative Analysis of Glucose, Islet Diameter, and Damage Score Across Groups.

DISCUSSION

The main finding of this study is that atung seed extract exerts a protective effect against STZ-induced pancreatic islet injury. Treatment with ASE75 significantly reduced fasting blood glucose levels, increased islet diameter, and decreased histopathological damage compared with the hyperglycemic control group, with effects comparable to metformin, indicating preservation of pancreatic β-cell integrity.[19]

The STZ-induced hyperglycemia model successfully reproduced selective β-cell injury in the islets of Langerhans, as reflected by elevated fasting glucose levels and marked histopathological degeneration in the HC group. This finding is consistent with the established mechanism of STZ toxicity, in which the compound enters β-cells through the GLUT2 transporter and induces DNA fragmentation, oxidative stress, and apoptosis, ultimately impairing insulin secretion. [10-13]

The beneficial effects of ASE75 are likely attributable to the antioxidant and anti-inflammatory properties of atung seed phytochemicals, particularly phenolic and flavonoid compounds such as gallic acid. These compounds reduce oxidative stress and inflammatory signaling, thereby preserving β-cell integrity.[14-16] Similar findings have been reported in studies of plant-derived antioxidants, such as Moringa oleifera, where flavonoids enhance β-cell survival by activating the PI3K/Akt and PPAR-γ pathways, improving insulin sensitivity and glucose regulation. [17,18]

The superior effect of ASE75 compared with ASE100 and ASE50 is consistent with the concept of phytochemical hormesis, in which moderate concentrations of polyphenols exert optimal antioxidant effects, whereas higher concentrations may act as pro-oxidants, leading to redox imbalance.[19,20] Excess phenolic compounds may interact with metal ions to generate reactive intermediates, thereby exacerbating oxidative stress. [21,22] Conversely, the lower efficacy observed in the ASE50 group may be due to insufficient levels of active compounds to effectively neutralize reactive oxygen species and protect β-cell integrity. Thus, ASE75 appears to provide an optimal balance between antioxidant activity and cellular homeostasis. [23,24,25]

Histological analysis further supported these findings, showing that the ASE75 group exhibited preserved islet architecture, minimal vacuolization, and lower degeneration scores than the ASE100 and ASE50 groups. These results indicate that atung seed extract, particularly at a 75% concentration, confers structural protection to pancreatic islets under hyperglycemic conditions. Collectively, these findings suggest that atung seed extract has promising potential as a phytotherapeutic agent for the management of hyperglycemia and warrant further investigation at the molecular and clinical levels.

CONCLUSIONS AND RECOMMENDATION

This study demonstrates that atung seed extract exerts significant protective effects on pancreatic tissue in streptozotocin-induced hyperglycemic mice. The 75% concentration was the most effective in preserving islet integrity, as evidenced by increased islet diameter and reduced histopathological damage. These findings highlight the potential role of atung seed extract as a natural antioxidant-based adjunct therapy for hyperglycemia. Further studies are recommended to investigate the underlying mechanisms using immunohistochemical markers such as insulin or PDX-1, as well as to evaluate its efficacy in clinical settings.

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