



Original article

Breadfruit leaves extract (*Artocarpus altilis*) effect on pancreatic damage in diabetic type II animal model induced by alloxan–nicotinamide[☆]



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ABSTRACT

Objective: Diabetes mellitus is a metabolic disease characterized by chronic hyperglycemia due to abnormal insulin secretion, insulin action, or both. Breadfruit has been used in traditional medicine for diabetes mellitus. This study aims to see the effect of giving ethanol breadfruit leaf extract to pancreatic damage induced by alloxan–nicotinamide.

Methods: Rats were induced with alloxan–nicotinamide and grouped into 4 groups, namely group I (normal) without treatment, group II (positive) given metformin 100 mg/kg body weight (BW), group III (negative) given Na CMC, group IV gave breadfruit leaves extract 100 mg/kg BW, group V given breadfruit leaves extract 400 mg/kg BW. Treatment was given for 14 days and histopathological examination of the pancreas.

Results: The results showed that group I pancreatic histopathology was not damaged, Group II suffered damage of 25–75%, Group III with 25% damage, and group IV showed 50%, and group V showed pancreatic damage from 0 to 25%. Breadfruit leaves extract 400 mg/kg BW has better activity to protect the pancreas and reduced the effect of pancreatic damage in rats.

Conclusion: The administration of breadfruit leaves extract 400 mg/kg BW has a potential effect against pancreatic damage better than metformin. This protection against the pancreas might be caused by the antioxidant activity of breadfruit leaves against ROS. This protection on pancreas against ROS might also affect proper insulin secretion.

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Introduction

Type 2 diabetes mellitus (Non-Insulin Dependent Diabetes Mellitus) is a condition of hyperglycemia caused by inadequate insulin production and the inability of the body to respond fully to insulin, defined as insulin resistance. Type 2 diabetes is the most common type of diabetes, about 90% of all diabetes cases.¹ Preclinical diabetes research can use a combination of alloxan–nicotinamide.² Alloxan is a toxic glucose analog, which selectively destroys insulin-producing pancreatic β cells.³

Breadfruit leaves extract contains polyphenols which can increase antioxidant activity by increasing levels of cellular antioxidant enzymes, such as superoxide dismutase (SOD), catalase and glutathione peroxidase.⁴ Polyphenols are also able to stimulate 16% increase insulin secretion from pancreatic β cells by influencing Peroxisome Proliferator Activated Receptor (PPAR).⁵ This study aimed to determine the effect of ethanol extract from breadfruit leaves on pancreatic damage in rat induced by alloxan–nicotinamide.

Method

Sample collection and preparation

The leaves of breadfruit were collected from the Darul Istiqomah Islamic Boarding School in Timbuseng Village, Patallasang District, Gowa Regency, South Sulawesi. The leaves used are old yellow leaves, picked directly from the tree at 9–10 am.

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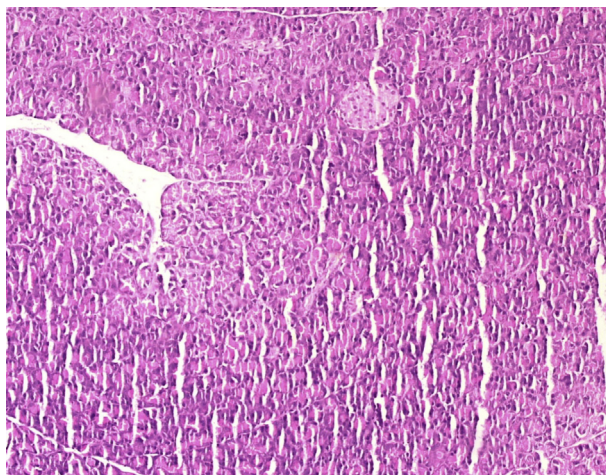


Fig. 1. Normal pancreatic rat tissue in normal group (without induction and without treatment).

Extraction

Two hundred fifty grams of breadfruit leaves washed and cut into small pieces. Extracted with 70% ethanol for three days with stirring. The extract was filtered with filter paper and evaporated in a rotary evaporator.

Experimental animal

Adult male Wistar strain rats (*Rattus norvegicus*) weighing around 180–200 g, were acclimatized for 14 days under standard conditions. Standard pellet diet and water ad libitum were provided. The rat was injected with a single dose of alloxan 170 mg/kg BW by i.p after nicotinamide 50 mg/kg BW injection which have been dissolved in 0.9% NaCl. Significant hyperglycemia will occur three days after induction. Blood glucose levels are calculated using a glucometer. The rat was diagnosed with type 2 DM if blood glucose levels were ≥ 200 mg/dL. Rats were divided into five groups, with three animals in each. Group I is the group without induction and treatment (normal control), group 2 given CMC Na 1%, group 3 given metformin 100 mg/kg BW, group 4 given breadfruit leaves extract 100 mg/kg BW, and group 5 given breadfruit leaves extract 400 mg/kg BW for 14 days, once daily. The Medical Faculty of University Hasanuddin Animal Ethics Committee approved this study.

Histopathological examination

After 14 days, rats from all groups were killed by cervical dislocation and abdominally dissected. The pancreas was removed immediately from each animal and then washed within 0.9% NaCl to remove the blood. Samples were allowed to remain in fixative (10% neutral buffered formalin). Sections were cut at 5 μ thickness and stained with Hematoxyline Eosin (HE). The stained sections were examined and photographed by using a microscope. Histopathological damage analysis was performed by a pathologist anatomy expert using a scoring by Mitchel in Gufron.⁶

Result

The result of this study, normal group without induction and treatment showed normal pancreas without damage (Fig. 1). All rats in normal group were categorized in score 0 are presented in Table 1. Negative control group induced by alloxan–nicotinamide showed that pancreatic damage score

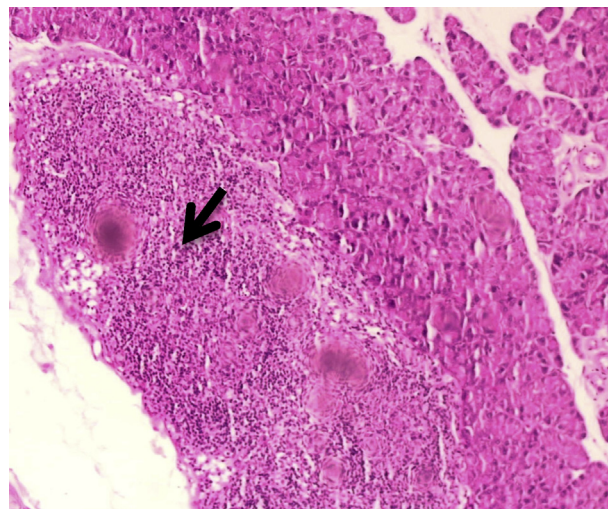


Fig. 2. Rat pancreatic tissue in negative control with inflammation score 2 (induction of alloxan–nicotinamide and administration of Na CMC).

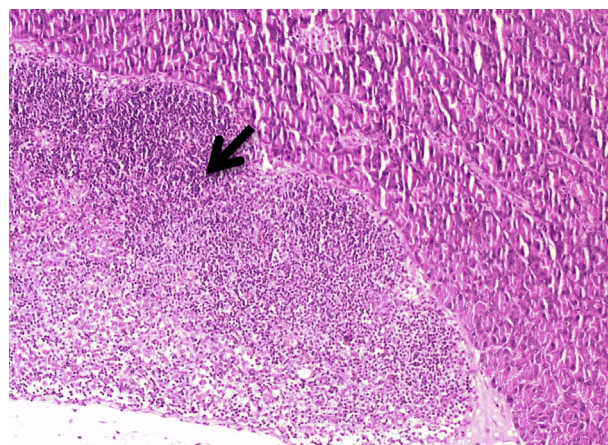


Fig. 3. Rat pancreatic tissue in negative control with inflammation score 3 (induction of alloxan–nicotinamide and administration of Na CMC).

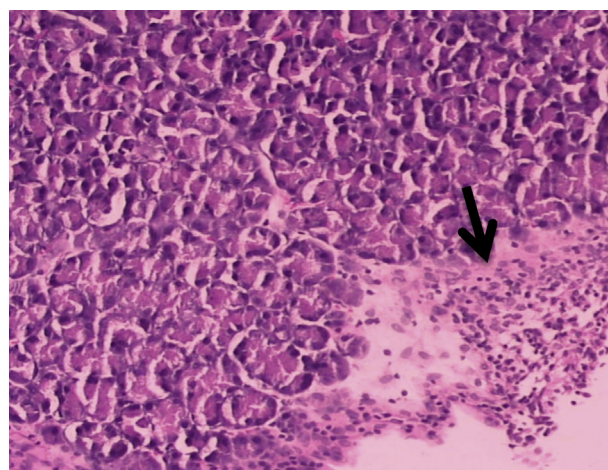
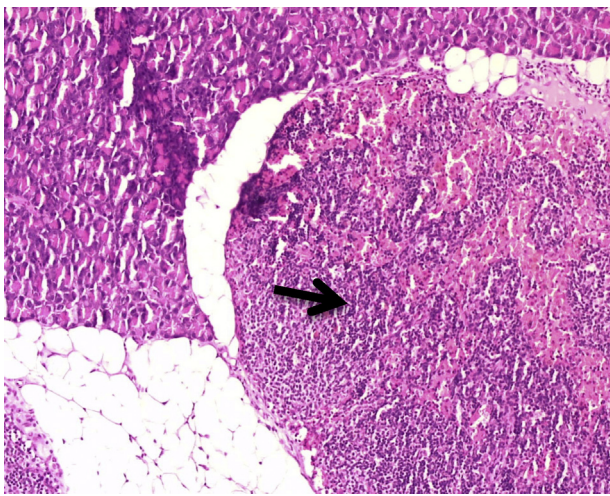
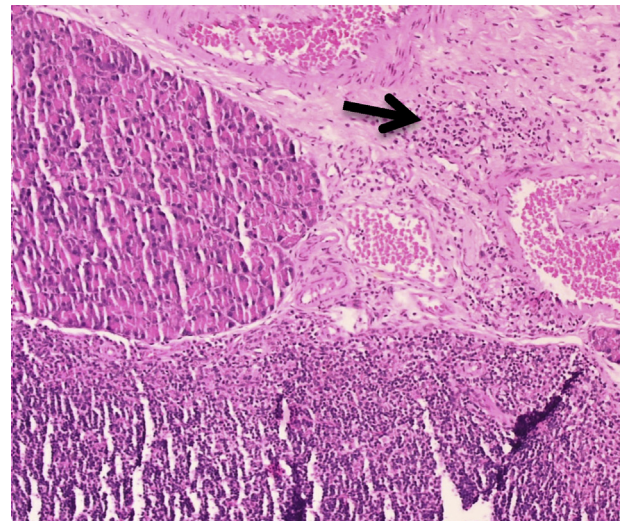


Fig. 4. Rat pancreatic tissue in positive control with inflammation score 1 (induction of alloxan–nicotinamide and administration of metformin 100 mg/kg BW).

of 2 in two rats and score 3 in one rat (Figs. 2 and 3). All rats in the positive control group induced alloxan–nicotinamide showed pancreatic damage score 1 (Fig. 4). Group of breadfruit leaves extracts 100 mg/kg BW showed that pancreatic damage

Table 1
Pancreatic histopathological scoring.

No	Sample	Score	Treatment	Description
1	1EK	0	Normal control	Normal
	1KL	0	Normal control	
	1KKD	0	Normal control	
2	2KKD	2	Negative control	Necrosis, cell inflammation and degeneration up to 50%
	2KKB	3	Negative control	
	2KKID	2	Negative control	
3	3PG	1	Positive control	Necrosis, cell inflammation and degeneration up to 25%
	3KKB	1	Positive control	
	3KKD	1	Positive control	
4	4KKD	2	Extract 100 mg/kg	Necrosis, cell inflammation and degeneration up to 50%
	4KKB	2	Extract 100 mg/kg	
	4 KL	2	Extract 100 mg/kg	
5	5KKD	1	Extract 400 mg/kg	necrosis, cell inflammation and degeneration up to 25%
	5KL	0	Extract 400 mg/kg	
	5KKID	0	Extract 400 mg/kg	

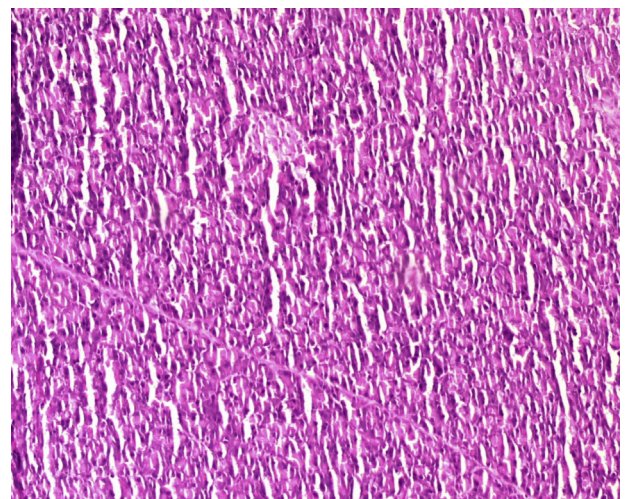
**Fig. 5.** Rat pancreatic tissue in breadfruit leaves extract 100 mg/kg BW with inflammation score 2 (induction of alloxan–nicotinamide, administration of breadfruit leaf extract 100 mg/kg BW).**Fig. 6.** Rat pancreatic tissue in breadfruit leaves extract 400 mg/kg BW with inflammation score 1 (induction of alloxan–nicotinamide, administration of breadfruit leaf extract 400 mg/kg BW).

score 2 in all rats (Fig. 5). Group of breadfruit leaves extracts 400 mg/kg BW showed pancreatic damage in one rat categorized in score 1 and two rats showed no pancreatic damage (score 0) (Figs. 6 and 7).

Discussion

The human pancreas is an organ located in the retroperitoneum. Pancreatic tissue consists of the endocrine and exocrine.⁷ Endocrine cells in the islets of Langerhans is responsible for regulating the body glycemia. Pancreatic β cell damage can be caused by several factors, including genetic factors, infection by bacteria, nutrients, diabetogenic agents and free radicals.⁸

In some groups found abnormalities in pancreatic tissue which are categorized in various scores (0–4). Normal group without induction did not show any abnormalities in rat pancreatic tissue. Negative control group induced alloxan–nicotinamide and treatment with CMC Na showed necrosis and cell inflammation reaching 50–75%. The diabetogenic agent used is alloxan. Alloxan is a toxic glucose analog, which selectively destroys pancreatic β cells through the formation of reactive oxygen species (ROS) such as nitric oxide.³ Oxidative stress plays an important role in the pathogenesis and complications of diabetes mellitus. Oxidative stress causes β cell dysfunction caused by glucose toxicity. β cells are

**Fig. 7.** Rat pancreatic tissue in group breadfruit leaves extract 400 mg/kg BW score 0 (induction of alloxan–nicotinamide, administration of breadfruit leaves extracts 400 mg/kg BW).

very susceptible to oxidative stress and damage because they have low expression and antioxidant enzyme activity.⁹ ROS by alloxan will cause DNA fragmentation in β cell. Nicotinamide is a derivative of vitamin B3 (niacin) is used to protect β cells thereby preventing partial damage to the pancreas.¹⁰

Positive control group given metformin 100 mg/kg BW which also induced by alloxan–nicotinamide compared with negative control (Na CMC) showed the incidence of necrosis and inflammatory cells reaching 25%. Metformin is a biguanide anti-hyperglycemic agent and primarily used for the treatment of type 2 diabetes mellitus. Metformin lowers blood glucose through increased insulin sensitivity from liver and peripheral (muscle).¹¹ Metformin also has antioxidant activity to stop the production of reactive oxygen species (ROS) by the mechanism of directly inhibiting the transfer chain of complex I NADH ubiquitin oxidoreductase (NADH). Inhibition of complex I chain reduces ATP production and increases the ratio of ADP/ATP and AMP/ATP, which are the main stimulants for Adenosine Monophosphate-activated Protein Kinase (AMPK) activation. This complex is involved in encouraging IL-1 β production through ROS. By blocking complex I, metformin inhibits the production of IL-1 β induced by Lipopolysaccharide (LPS)-activated Macrophages and increased IL-10 production.¹²

Breadfruit leaves extract 100 mg/kg BW had no optimal effect on pancreatic damage showed the incidence of necrosis and inflammatory reaching 50% (score 2). Compared to metformin, the effect of breadfruit leaves extracts 100 mg/kg BW is lower. Whereas breadfruit leaves extract 400 mg/kg BW has better potential to protect the pancreas of diabetic rats against stress and oxidative damage. Pancreatic histopathological analysis showed pancreatic damage reaching 25% (score 1) in one rat and score 0 in two rats that did not experience pancreatic damage. When compared with metformin, extract 400 mg/kg has better activity to protect the pancreas.

Chemical analysis of breadfruit leaves extracts identified alkaloids, flavonoids, tannins, phenols and saponins. Breadfruit extract contains polyphenols which can increase antioxidant activity by increasing levels of cellular antioxidant enzymes, such as superoxide dismutase (SOD), catalase and glutathione peroxidase.⁴ Polyphenols as antioxidants are thought to protect pancreatic cells from the toxic effects of free radicals produced under conditions of chronic hyperglycemia.¹³ Polyphenols are also able to stimulate an increase in insulin secretion by 16% from pancreatic β cells by influencing the Peroxisome Proliferator Activated Receptor (PPAR).⁵

Conclusions

This study shows induction of alloxan–nicotinamide causes hyperglycemia condition through the mechanism of ROS. The

administration of breadfruit leaves extracts 400 mg/kg BW has potential effect against pancreatic damage better than metformin. This protection against the pancreas might be caused by the antioxidant activity of breadfruit leaves against ROS. This protection on pancreas against ROS might also affect good insulin secretion.

Conflict of interest

The authors declare no conflict of interest.

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