

Combination treatment of metformin and red fruit (*Pandanus conoideus* L.) extract increased pancreatic β cell density but had no effect toward fasting blood glucose and glycated albumin levels in diabetic male wistar rats (*Rattus norvegicus*)

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ABSTRACT

Background: Diabetes mellitus (DM) is a degenerative disease associated with premature aging characterized by hyperglycemia. Hyperglycemia in people with DM causes oxidative stress and increases Glycated Albumin level, which is the initial precursor to the formation of AGEs. In turn, AGEs will lead to pancreatic β cell damage and apoptosis. Red fruit (*Pandanus conoideus* L) contains phytochemicals with antioxidant that has the potential to reduce diabetic complications. Therefore, this study aimed to evaluate the effect of metformin and red fruit extract combination toward blood glucose, glycated albumin and pancreatic β cells density in diabetic rats (*Rattus norvegicus*).

Methods: A post-test only control group study was conducted using 36 male Wistar rats as subject. All subjects were induced for type-2 DM with Streptozotocin and Nicotinamide. The diabetic rats were then divided into 2 groups: the positive

control group treated with metformin + placebo and the treatment group treated with metformin + red fruit extract. Pancreatic β cell, fasting blood glucose and glycated albumin assessments were performed after 21 days of treatment.

Results: The results showed the mean number of pancreatic β cells in the treatment group was higher than the control group (116.11 ± 33.14 vs 83.20 ± 23.94 cells/visual field; $p=0.002$). But the mean fasting blood glucose in the treatment group was not significantly different compared to the control group (107.67 ± 14.93 vs 113.67 ± 11.19 mg/dl; $p=0.181$). The same result also found in the glycated albumin level (Treatment vs control: 6.97 ± 3.63 vs 6.42 ± 4.01 ng/ml; $p=0.666$).

Conclusion: It can be concluded that the administration of red fruit extract increased the density of pancreatic β cell but did not reduce fasting blood glucose and glycated albumin levels in diabetic Wistar rats.

Keywords: red fruit, pancreatic β cells, fasting blood sugar, glycated albumin, diabetes

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INTRODUCTION

Diabetes mellitus (DM) is an endocrine disorder that characterized by high glucose condition (hyperglycemia) that lead to chronic progressive multi-organ damage.¹ Hyperglycemia also leads to oxidative stress and free radical formation that potentially harms and damages pancreatic β cells. High glucose exposure will cause a progressive decrease in pancreatic β cell function, insulin resistance and an increase in pancreatic β cell apoptosis.²

Advanced glycated End-Products (AGEs) is a product of protein modification resulted from reduction reaction which ligate glucose molecule to amino acids residues. Glycated albumin is the initial precursor to the formation of AGEs which triggers an increase oxidative stress and will eventually activate the apoptosis pathway in pancreatic beta

cell.³ Albumin is the main protein component in serum with a half-life of 20-25 days, which can be used as an option for monitoring glycemic status more quickly and accurately to better anticipate the development of DM complications.⁴

Metformin is an oral anti-diabetes which belongs to the biguanid group and is a first-line drug commonly used in the treatment of type 2 DM.⁵ It is a hypoglycemic drug that has the main effect of reducing liver glucose production (gluconeogenesis) and improving glucose uptake in peripheral tissues.⁶

Several studies have shown that antioxidants can reduce the effects of oxidative stress and is useful to reduce the severity and complications of diabetes. One of the medicinal plants rich in antioxidants is red fruit (*Pandanus conoideus* L). Traditionally, red

fruit has been consumed by Papuans for generations due to its multiple benefits for treating various diseases, such as eye disorder, worms infection, skin and improving stamina.⁷ In addition, Papuans believe that red fruit could cure degenerative diseases such as cancer, arteriosclerosis, rheumatoid arthritis and stroke.⁸ Red fruit contains high natural antioxidant compounds, including carotene, beta-carotene and α -tocopherol. It also contains other compounds such as omega-3, omega-9 and oleic acid which are active compounds that counteract the formation of free radicals in the body.⁸

Winarto (2007) proved that the administration of red fruit oil and glibenclamide in mice could reduce blood sugar levels and improved pancreatic langerhans islets.⁹ In contrast, another study reported that red fruit was failed to significantly reduce blood glucose levels in alloxan-induced diabetic mice but was able to reduce MDA levels.¹⁰

Based on the background stated above, this study was conducted to prove that the administration of red fruit (*Pandanus conoideus* L) extract could increase the number of pancreatic β cells, reduce fasting blood glucose levels and glycated albumin in diabetic Wistar rats.

METHODS

An experimental post-test only control group study was conducted in Faculty of Medicine Udayana University. The subjects of this study were 36 Wistar rats (*Rattus Norvegicus*), aged 2-3 months and weighing 180-200 grams. Diabetic condition was induced by using streptozotocin and nicotinamide and the blood glucose level was assessed after the induction. Only rats with fasting blood glucose levels ≥ 135 mg/dL were included as samples. The rats were then divided into 2 groups with 18 rats/group. The control group (P0) was a group of diabetic rats treated with 10 mg/200gr BW metformin and 2cc/200gr BW aquadest. Treatment group (P1) was a group of diabetic rats treated with 10 mg/200gr BW metformin and 0.87 ml/200gr BW red fruit extract per day. After 21 days, blood serum from all rats were drawn for fasting blood glucose and glycated albumin levels examination. Then, all rats were euthanized using 10% ketamine HCL with an injection dose of 0.2 ml/200 gr BW. Necropsy was performed to obtain pancreatic tissue samples to assess pancreatic β cells density. Pancreatic tissue examination was conducted using routine HE staining and observed using standard microscopy under 400x magnification. The data obtained were analyzed by using Spss for windows.

RESULTS

The results showed the mean number of pancreatic β cells in the treatment group was higher than the control group (116.11 \pm 33.14 vs 83.20 \pm 23.94 cells/visual field; $p=0.002$). However, the mean fasting blood glucose in the treatment group was not significantly different compared to the control group (107.67 \pm 14.93 vs. 113.67 \pm 11.19 mg/dl; $p=0.181$). In addition, the glycated albumin level was also similar between the two group (Treatment vs control: 6.97 \pm 3.63 vs 6.42 \pm 4.01 ng/ml; $p=0.666$). This comparative analysis indicated that after 21 days of treatment, red fruit extract was able to enhance pancreatic β cells density but had no effect toward blood glucose and glycated albumin level (Table 1).

DISCUSSION

Effects of Red Fruit Extract on the Number of Pancreatic Beta Cells

Inflammation and oxidative stress due to streptozotocin injection could activate NO pathway and NF κ B, resulted in increased production of toxic molecules such as ROS, which lead to DNA damage and pancreatic β cell death.^{11,12} The results of this study indicate that the average number of pancreatic β cells in the treatment group with metformin and red fruit extracts was higher than the control group. This finding is in line with previous research which stated that the combination of red fruit oil and glibenclamide could reduce blood sugar level and improved pancreatic langerhans in diabetic mice.⁹

Uncontrolled hyperglycemia often leads to oxidative stress and free radical formation in diabetic individuals. Free radicals damage pancreatic beta cells and initiate pancreatic beta cell apoptosis which play a major role in the development of diabetic complications. Sequentially, it leads to progressive decrease in pancreatic β cell function, insulin resistance and increased pancreatic β cell apoptosis.² Considering anti-oxidant phytochemical compounds in red fruit extract, the first working mechanism of red fruit extract is to prevent progressive damage of pancreatic β cells due to hyperglycemia. Red fruit extract contains active compounds of 11267.14 mg/100 gr tannin, 105.50 mg/100gr flavonoids, 427.36 mg/100 gr vitamin C, 26.92 mg/100 gr phenol, 313.08 μ g/gr α -tocopherol and 54931.14 μ g/gr β -carotene, which are able to improve the pancreatic endocrine function and improve insulin secretion by increasing beta cells density.¹³

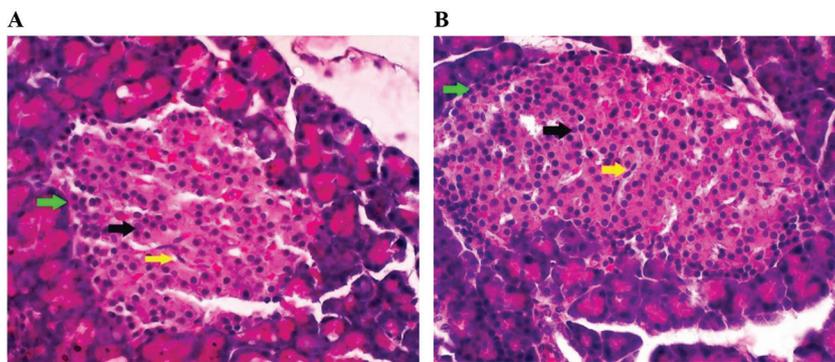


Figure 1. The histopathologic images of pancreatic islet from control group with metformin treatment (A) and pancreatic islet from treatment group with metformin and red fruit extract (B) with 400x magnification with routine Hematoxylin Eosin (HE) staining.

Table 1. Comparative Analysis

Variable	Groups		t	P
	Control (Mean ± SD)	Treatment (Mean ± SD)		
Pancreatic β Cells (cells/field of view)	83.20 ± 23.94	116.11 ± 33.14	-3.415	0.002
Fasting Blood Glucose (mg/dl)	113.67 ± 11.19	107.67 ± 14.93	1.364	0.182
Glycated Albumin (ng/ml)	6.42 ± 4.01	6.97 ± 3.63	-0.436	0.666

Theoretically, β -carotene could protect pancreatic tissues from free radicals by inactivating free radicals so that the pancreas can work optimally to produce insulin. β carotene has also been reported to protect pancreatic β cells by reducing oxidative stress in streptozotocin-induced mice.¹³

Red fruit also contains flavonoids which are protective to pancreatic β cell and improved insulin sensitivity.¹⁴ Flavonoids can also prevent the inflammatory process by inhibiting NF κ B which highlighted its potential as an alternative treatment for diabetes.¹⁴ More recent studies also showed that flavonoids in plants could induce pancreatic β cells regeneration through the cAMP-PKA-CREBP-CBP pathway. Due to the presence of flavonoids in red fruit extract, there is possibility that it can also increased pancreatic β cells density through this pathway.¹⁵

The effect of red fruit extract on fasting blood sugar

In contrast, administration of red fruit extract and metformin did not appear to produce any significant effect on fasting blood sugar compared

to control group. This phenomenon is contradict from previous studies which stated that red fruit significantly reduced blood glucose levels.^{13,14,15}

In addition, it is possible that increased density of pancreatic beta cells in the red fruit extract group and metformin only occurred in the third week (day 21), thus, it might take a longer time for glucose lowering effect to appear. A study suggested that a significant increase in beta cell mass on the 20th day of examination did not follow by an increase in insulin levels and the insulin levels only began to increase on the 28th day of inspection.¹⁶ This at least provides evidence that improvement in insulin level tends to take more time after initial increase in pancreatic β cells density.

Previously, preliminary research showed that 0.87 ml/200gr BW red fruit extract in rats was an optimum dose that can be applied in main part of the study. However, it appears that the time of experiment need to be prolonged in order to get higher insulin and lower fasting blood glucose levels.

Effect of Red Fruit Extract on Glycated albumin

Similar with blood glucose level, glycated albumin assessment also resulted in non-significant finding. Metformin and red fruit extract administration failed to produce any significant decrease in blood glucose level in treatment group which had roughly similar blood glucose level with control after 21 days of treatment. As stated above, it seemed that the research timing was too short to be able to produce any significant change in blood glucose or glycated albumin.

Studies showed that glycated albumin is a product of albumin and glucose cross-linkage and long term blood glucose levels can be represented accurately by it. However, there are studies that showed that bioactive compounds such as flavonoids had anti-glycation activity that prevents the formation of glycated albumin despite high blood glucose levels.¹⁸ On the other hand, in this study, it seemed that the flavonoid content in red fruit extract was not high enough (105.50 mg/100gr QE), so it was not effective enough to inhibit glycation process. Nevertheless, previous studies have shown that lavender extract with 38740.84 mg/100g QE of flavonoids could effectively reduce glycated albumin levels.¹⁸

Regardless of all of our findings, this study has a weakness that is it used a post test only method so basal conditions could not be compared between

the two groups. It was possible that the glycated albumin level was significantly different between the two group even before treatment.

CONCLUSION

The results of this study suggest that the administration of red fruit (*Pandanus conoideus*L.) extract significantly increased the density of pancreatic β cell but failed to reduce fasting blood glucose and glycated albumin levels in diabetic Wistar rats (*Rattus norvegicus*). Further research is needed with a spectrum of dose of red fruit and longer research time to determine the optimal dosage as well as to be able to observe significant changes between treatment and control group.

CONFLICT OF INTEREST

All authors declared that there is no conflict of interest regarding this publication

AUTHOR CONTRIBUTION

All authors contributed equally in the writing of this article

FUNDING

This study was self-funded without any contribution from third party.

ETHIC APPROVAL

This study had been ethically approved by ethical commission of Faculty of Medicine Udayana University with approval letter number 393/KE-PH-Lit-2/VII/2018.

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