Jambura Medical and Health Science Journal, Vol.3 No.2 (August 2024)
p-ISSN 2830-0580 | e-ISSN 2830-4608

ORIGINAL ARTICLE **Open Access**

Utilization of Nanotechnology in Metformin Delivery: The Morphometric Study of Pancreatic Islets of Diabetic Rat Model

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ABSTRACT

Introduction: In diabetic conditions, damage of β cells and changes the structure of pancreatic islets was exhibited. Metformin can improve this condition. The nanoparticle form of metformin can improve bioavailability and accelerate cell regeneration, and pancreatic islets can be repaired. The aim of this study to know the effect of nanoparticles metformin on fasting blood glucose levels and pancreatic islet morphometry in diabetic rat models.
Method: An experimental research with posttest-only controlled group design was conducted on 16 white male Wistar rats. The streptozotocin (STZ) 40 mg/kgBB were injected i.p. Rats were divided into four groups: K1: normal control; K2: negative control (diabetes model); K3: diabetes model treated with metformin 100mg/kgBB; K4: diabetes model treated with nanoparticle metformin 100mg/kgBB. The body weight and fasting blood glucose levels were measured periodically. The histology of pancreatic islets was performed with hematoxylin-eosin staining and quantified using ImageJ software. The data were analyzed with GraphPad Prism 8.0.0 using nonparametric Kruskal-Wallis test.
Results: Metformin therapy decreased the fasting blood glucose levels in K3 starting on day 21 and K4 starting on day 7, but there was no statistical difference (p=0.0597). Pancreatic islet morphometry showed the pancreatic islet area was found to be statistically different (p=0.026), and the perimeter was not statistically different (p=0.115).
Conclusion: Metformin nanoparticle form decreased the fasting blood glucose levels and effectively improved the area and perimeter of pancreatic islets of the diabetic rats model, but the perimeter of the pancreatic islets is not statistically significant.

Keywords: Diabetes, metformin, nanoparticles, pancreatic islets, morphometry.

JMHSJ Jambura Medical and Health Science Journal

Published by:
Universitas Negeri Gorontalo

Address:
Jl. Jend. Sudirman No.6, Gorontalo City, Gorontalo, Indonesia

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+62852 3321 5280

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Article History:
Received 18 April 2024
Accepted 05 September 2024
Published 07 September 2024
DOI:
https://doi.org/10.37905/jmhsj.v3i2.24937

Introduction

Diabetes mellitus (DM) is a chronic disease caused by abnormalities in insulin secretion, action, or both, which is characterized by increased glucose levels.[1-3] This increase in insulin levels is caused by the impairment of pancreatic cells, the dominant cells in the pancreatic insula. The hyperglycan usecemic condition due to the destruction of cells triggers the inflammation, stimulating the formation of connective tissue or fibrosis.[4-5] This can be followed by changes in the overall structure of the pancreatic islets. The use of animal model such as rat has been widely used in diabetes research. To obtain hyperglycemia conditions in rat, streptozotocin (STZ) the most prominent diabetogenic chemical that is widely used.

Metformin is an oral antidiabetic drug recommended as first-line treatment in type 2 diabetes mellitus.[6] Metformin induces glucagon-like peptide-1 (GLP-1)[7] and enhances insulin actions.[8] The bioavailability of metformin in the gastrointestinal system is only about 50-60% with conventional preparations. This leads to unfavorable effects in glycemic level control, resulting in low improvement of insulin resistance and secretion in pancreatic cells.[9] Reducing the particle size in its delivery system improves the bioavailability of a drug. Reducing the particle size of drug can leads to increase in surface area, this condition can increases in rate of dissolution and rate of diffusion (absorption). In many studies the use of nanotechnology to make the metformin preparations has been carried out with good results. Metformin with nano size has a higher bioavailability, leading to more effective pancreatic cell repairment.[10] Improvement or regeneration in pancreatic islets cells will be able to improve their structure. The aim of the study was to determine the effect of metformin nanoparticles on changes in fasting blood glucose levels and pancreatic islets morphometry in diabetic rats.

Methods

Animal Samples

This experimental study was designed with posttest only controlled group. This study using resource equation' approach the sample size, that is included a total of 16 male Wistar rats, 8 weeks of age, with body weight of 250 -350 grams. Intraperitoneal injection are the way to administer the streptozotocin (STZ) at a dose of 40 mg/kg/BW. The rats were assigned into 4 treatment groups; K1: normal control; K2: negative control (diabetic model); K3: diabetes model + 100mg/kg/BW metformin single dose daily; K4: diabetes model + 100mg/kg/BW metformin in nanoparticles single dose daily. Formulation of the chitosan metformin nanoparticles preparation using the ionic gelation method. The evaluation of the particle size by scanning electrone microscopy (SEM) using particle size analyzer and

measured with ImageJ software. The body weight and fasting glucose levels (the rat were fasted overnight) were assessed with a glucometer on a regular basis every week until the 28th day. This study has obtained approval from the Medical and Health Research Ethics Commission, Faculty of Medicine, Universitas Tadulako (No.6507/UN 28.1.30/KL/2020).

Histology Analysis

The rats were terminated on day 28 and their pancreatic tissue was necropsied. The tissues were embedded in paraffin blocks and cut to a thickness of 5 μm. The tissues were stained with hematoxylin eosin (HE). Five randomly selected fields of view were observed with 400× magnification and 1.25 numerical aperture (NA) Olympus CX23 light microscope and optilab software. The images were quantified using ImageJ software to measured the area and perimeter of the Langerhans islets.

Statistical Analysis

Data were analyzed by GrapPhad Prism 8.0.0 using Kruskal-Wallis non-parametric test.

Result

The Wistar rats were assigned into four groups and treated until the 28th day post-hyperglycemia. The body weights are illustrated in Figure 1. The mean of the body weight of rats at the beginning of the study (before STZ induction) was, ranging from 294 ± 43,64 to 331.5 ± 37,72 gram, in the K1 group body weight of the rats tended to be stable from the beginning to the end of the study (H+28). The K2, K3 and K4 group that induced by STZ showed a decrease in weight at the H0 or the day of diabetes diagnosed. The mean body weight decreases in K2 to 281.25 ± 16,01 grams, K3 decrease to 264.67 ± 30,08 grams, and K4 decrease to 276 ± 16,25 grams. The body weight in K2 group continued to decrease until the end of the study to 272.25 ± 56,01 grams. The K3 and K4 groups showed stable body weight until the end of the study with mean body weight of 305 ± 15,55 grams and 283 ± 7,54 grams, respectively. One-way Anova test for the mean weight showed a statistically significant mean weight differences for each group at each measurement time ($p = 0.0001$).

The fasting blood glucose levels of the Wistar rats are describe in Figure 2. At the beginning of the study (before STZ induction), the mean fasting blood glucose levels ranged from 82.75 ± 6,7 to 90.5 ± 12,79 mg/dL. The STZ-induced K2, K3 and K4 group showed an increase in fasting blood glucose levels above normal level (reference value ≤126 mg/dL) at day 7 after STZ induction. The mean fasting blood glucose levels of K2, K3 and K4 were 105.5 ± 29,56, 202.33 ± 48,19, and 239 ± 190,6 mg/dL, respectively. The K1 group showed stable, normal level at each measurement time with fasting blood glucose level of 116.25 ± 25,61 mg/dL at day 28. The K2 group showed continuously high, above the reference level,

up to 184.75 ± 76,02 mg/dL at day 28. The K3 and K4 group showed a decrease in the mean blood glucose level began on day 7. The decline in fasting blood glucose levels in the K3 group were gradual and reached the normal at day 21 and day 28 that is 122 ± 11,31. The K4 group started to show the decrease at day 7 and were stable at day 28 with a mean fasting blood glucose of 79 ± 15,39 mg/dL. Kruskal-Wallis statistical test was performed on the mean level of fasting blood glucose, yielding no statistically significant differences in each group at each measurement time (p = 0.0597).

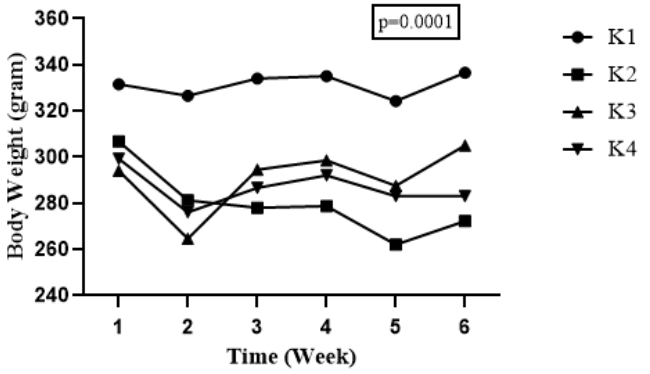

Figure 1. The mean body weight of the Wistar rats. Note: K1: Normal rat control group; K2: Group of diabetic rats; K3: Group of diabetic rats treated with metformin; K4: Group of diabetic rats with metformin in nano preparations. 1: STZ Pre Induction; 2: the establishment of diabetes diagnosis; 3: 7 days post diabetes; 4: 14 days post diabetes; 5: 21 days post diabetes; 6: 28 days post diabetes.

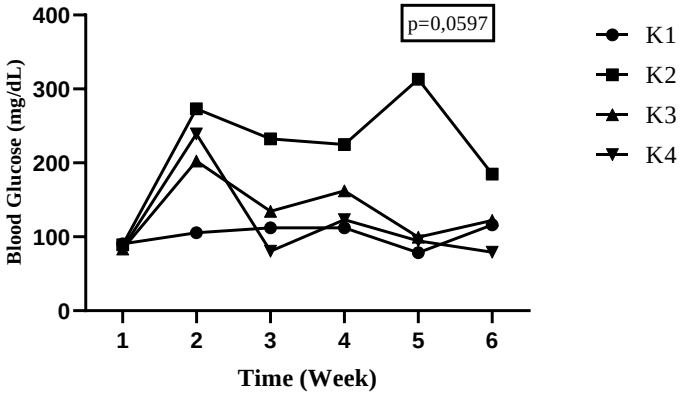

Figure 2. The mean fasting blood glucose levels of the Wistar rats. Normal rat control group; K2: Group of diabetic rats; K3: Group of diabetic rats treated with metformin; K4: Group of diabetic rats with metformin in nano preparations. 1: STZ Pre Induction; 2: the establishment of diabetes diagnosis; 3: 7 days post diabetes; 4: 14 days post diabetes; 5: 21 days post diabetes; 6: 28 days post diabetes.

Pancreatic Islets Morphometry

This research was carried out by measuring the area and perimeter of the pancreatic islets of a diabetic rat model treated with nanoparticle metformin. The pancreas was preparations using HE staining. The results of the examination showed differences in area and perimeter in each treatment group. area The histopathological appearance of the pancreatic insula in all groups can be seen in Figure 3.

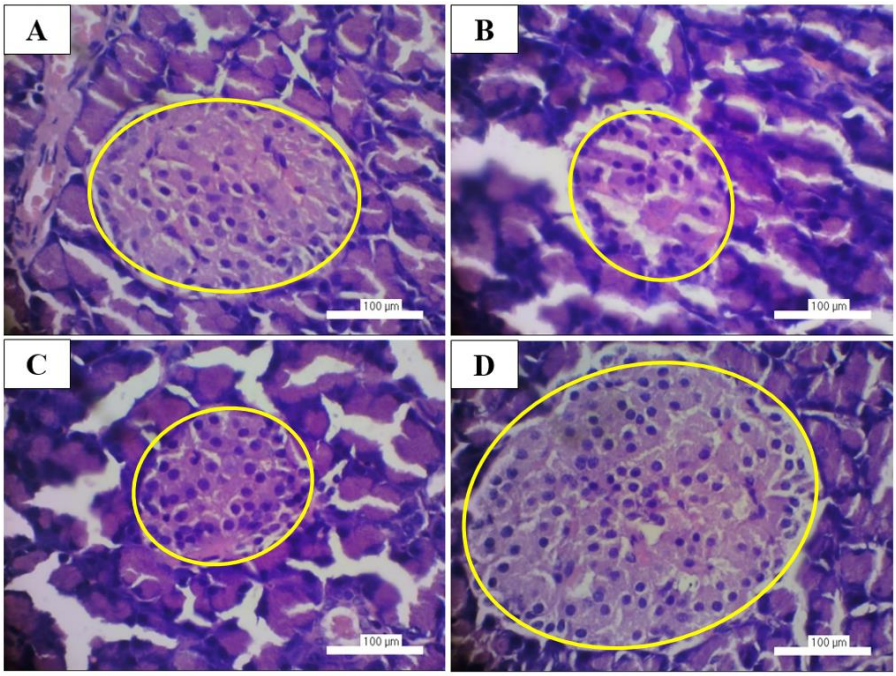

Figure 3. Histopathological of the pancreatic islets of a diabetic rat model at D+28 with HE staining. Magnification 400 times. Description: (A) Group K1, (B) Group K2, (C) Group K3, (D) Group K4. The perimeter is measured along the entire yellow line on the pancreatic islets, while the area is a calculation of the entire shape within the yellow line.

Pancreatic Islets Area

The data on the mean area of the pancreatic islets can be seen in Figure 4. The mean pancreatic islets area of K1 53,13 ±10,78µm^2, K2 has the smallest area that is 23,09±8,29 µm^2 respectively. The K4 group showed comparable pancreatic islets area with K1 57,79±21,39µm^2, while the K3 showed comparable pancreatic islets area with K2 group 24,37 ±3,29 µm^2. Kruskal-Wallis non-parametric test was performed on the pancreatic islets area, yielding a statistically significant difference (p=0.026). a Mann Whitney test was further performed, resulting in a statistically significant difference between K1 against K2, and K2 against K4

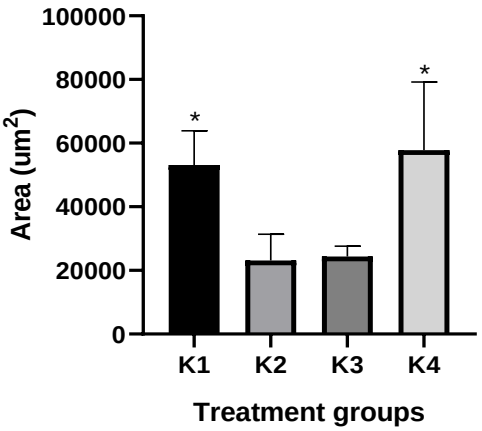

Figure 4. The mean area of the pancreatic islets. Kruskal Wallis test p=0.026. Mann Whitney post hoc test: K1 with K2 (p=0.02), K1 with K3 (p=0.06), K1 with K4 (p=0.724), K2 with K3 (p=0.355), K2 with K4 (p=0.034), K3 with K4 (p=0.085). *=p<0.05 vs K2.

Pancreatic Islets Perimeter

Figure 5 shows the perimeter measurement of the pancreatic islets. The K1, K2, K3, and K4 pancreatic islets perimeter were 957,01±65,39, 622,06±305,15, 708,89±127,12 and 960,00±185,20 µm, respectively. The K2 perimeter is the smallest than the other, K1 pancreatic islets perimeter was comparable to K4, while the K2 pancreatic islets perimeter was comparable to K3. The Kruskal Wallis test on the pancreatic islets perimeter showed no statistically significant differences (p = 0.115).

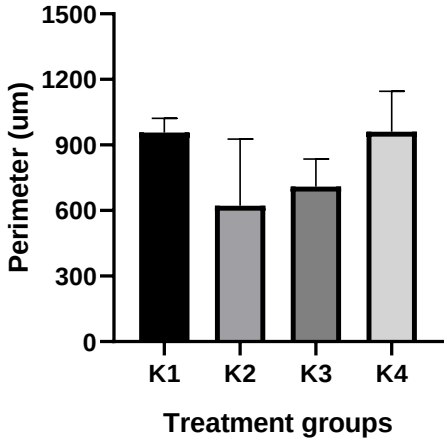

Figure 5. The mean perimeter of the pancreatic insula. Kruskal Wallis test p=0.115.

Discussion

This study was conducted by inducing STZ intraperitoneally in Wistar rats assigned to K2, K3, and K4 group. Streptozotocin is a diabetogenic substance that damage the

pancreatic cells directly on the nitrosourea group and induce an increase in reactive oxygen species (ROS).[11] The STZ is widely used in animal test because it increases blood glucose level by triggering the excess free radical production.

The mean fasting blood glucose level in K1 showed normal results in all measurement times. The K2 showed high, above normal level fasting blood glucose levels started from day 0 to day 28. This is because STZ injures the pancreatic islets cells that causes an increase in blood glucose level.[12] Treatment with 100 mg/kg/BW (K3) metformin and 100 mg/kg/BW (K4) nano-metformin were able to affect the fasting blood glucose levels in diabetic rat models. The diabetic rats treated with 100 mg/kg/BW metformin showed a decrease in fasting blood glucose levels started at day 21, while diabetic rats treated with 100 mg/kg/BW nano-metformin showed a decrease in fasting blood glucose levels started at day 7.

In addition to conventional metformin administration, the animal tests were given nano-metformin to induce hypoglycemic effect. This effect improves pancreatic function by regenerating pancreatic cells. Treatment with nanoparticles has shown its effectiveness in diabetes mellitus. The STZ-induced diabetes causes degeneration in the islets of Langerhans causing injuries. Metformin increases the glucose uptake via insulin and is widely used because it can reduce oxidative stress that increase hyperglycemia.[13]

The area of the pancreatic insula from each group was different based on the treatment. The K1 group as the control group had round or oval pancreatic islets and well-appearance of cells. The K2 group showed smaller, irregular shape pancreatic islets area. The K3 group that received 100mg/kg/BW metformin showed wider area of pancreatic islets area than the K2 group. The K4 group that received 100mg/kg/BW nano-metformin showed wider, uniform shape, comparable to the K1 group. Statistical analysis showed statistically significantly differences in the pancreatic islets area. Mann Whitney's post hoc test showed that there were statistically significant differences between K1 and K2 and K2 and K4. This indicates that nano-metformin given to K4 improved the pancreatic islets area in diabetic rats.

The K1 showed comparable pancreatic islets perimeter to K4. In contrast, the K2 as the untreated diabetic rat group showed a small perimeter. The perimeter size of K3 is not much different from that of K2. This indicates that metformin preparations did not rapidly improve the size of the pancreatic islets perimeter in diabetic conditions. The state of hyperglycemia can be caused by damage to pancreatic β cells leading to inability to produce insulin optimally. Changes in the structure of the pancreatic islets were correlated with

pancreatic function and the amount of insulin produced. Injuries to pancreatic islets cells affect the changes in morphometry in terms of the area and perimeter.[14]

Nanoparticles increases bioavailability with minimal side effects due to their various natural, synthetic, and semi-synthetic polymers that are useful in the formulation delivery. This study showed that nano-metformin reduces the fasting blood glucose levels. Thus, it improves the pancreatic islets structure. Metformin has lower bioavailability leading to poorer glycemic control and subsequent lower improvement in insulin resistance and pancreatic cell secretion impairment.[15] Nano-metformin has a small particle size, triggering an increase in bioavailability and rapid improvement in pancreatic islets.[16]

Conclusion

The nano-metformin preparation reduced the fasting blood glucose levels in diabetic Wistar rat model although the difference was not statistically significant. The nano-metformin preparation improved the area and the perimeter of the pancreatic islets, but the perimeter of the pancreatic islets is not statistically significant.

Conflicts of Interest

There is no conflict of interest in this research

Funding sources

We acknowledge the contribution of the Medical Faculty of Universitas Tadulako for funding this study.

Acknowledgments

The authors wished to thank Anisah S.Si for excellent technical assistance.

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