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Intravenous Wharton's Jelly stem cell increased the number of β cells pancreas and reduced the fasting blood glucose level in diabetes mellitus Wistar rat male (*Rattus norvegicus*)

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ABSTRACT

Introduction: Diabetes mellitus is a metabolic disease characterized by hyperglycemic and disorders of carbohydrate, protein and fat metabolism due to abnormalities in insulin secretion, insulin sensitivity or both. Based on previous research, Wharton's Jelly's mesenchymal stem cells have anti-inflammatory, immunoregulatory properties that can improve metabolic control and have the ability to differentiate into pancreatic lineage cells that function as insulin-producing cells in vitro culture. The purpose of this study was to prove that administration of mesenchymal stem cells Wharton's Jelly can increase the number of pancreatic beta cells and reduce fasting blood glucose levels in male rats (*Rattus norvegicus*) Wistar strain of diabetes mellitus.

Methods: The research design used was pure experimental with post-test only control group design using 36 white male rats. All samples were induced with Streptozotocin and Nicotinamide. The selected samples were divided into two groups: the control group given glibenclamide + 0.9% NaCl, and the treatment group was given glibenclamide + mesenchymal stem cells from Wharton's Jelly. Calculation of the number of pancreatic beta cells and measurement of blood glucose levels were carried out after 14 days of treatment.

Result: The results showed that the treatment group had a higher number of pancreatic beta cells than the control group (105.17 ± 16.379 cells/field of view vs. 54.00 ± 11.366 cells/field of view) ($p < 0.001$). In addition, the treatment group had lower fasting blood glucose levels than the control group (109.06 ± 16.71 mg/dl vs 122.78 ± 10.14 mg / dl) ($p < 0.05$).

Conclusion: It was concluded that intravenous administration of Wharton's Jelly mesenchymal stem cells increased the number of pancreatic beta cells and decreased fasting blood glucose levels in male rats (*Rattus norvegicus*) Wistar strain of diabetes mellitus.

Keywords: Wharton's Jelly's mesenchymal stem cells, pancreatic beta cells, fasting blood glucose levels, diabetes mellitus.
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INTRODUCTION

Aging is a natural process that occurs in living things, including humans. The decline in various organ functions characterizes this cycle. This progressive decline will make humans lose resistance to infection, and there will be a buildup of structural metabolic distortions that can cause degenerative diseases (such as hypertension, diabetes mellitus, and cancer).[1] One form of decreased organ function due to the aging process is the emergence of metabolic diseases such as diabetes mellitus, obesity and hypertension. Diabetes mellitus (DM) is often considered a biological model of the premature aging process. Diabetes mellitus is a group of metabolic diseases characterized by hyperglycemia that occurs due to abnormalities in insulin secretion, insulin action or both.[2] Diabetes management aims to control blood glucose levels within normal limits and help prevent early complications. The long-term complications of DM can ultimately be life-threatening. Various epidemiological studies have shown an increasing trend in the incidence and prevalence of type 2 DM in various parts of the world. WHO predicts an increase in the number of people with diabetes in Indonesia from 8.4 million in 2000 to around 21.3 million in 2030. The report shows an increase in the number of people with DM by 2-3 times in 2030.[2,3]

Various types of antidiabetic medications have been used for centuries to treat diabetes. However, in some DM sufferers, it is very difficult to get glucose control within normal limits, and most of these patients will need insulin therapy.[4] Pancreatic transplantation is one promising therapy to prevent diabetic retinopathy and various other

complications. However, this pancreas transplant carries the risk of rejection by the body's immune system. In recent years, research on mesenchymal stem cells (MSC) has been growing. In this respect, MSCs emerge as attractive candidates for various therapeutic applications. Several animal studies and clinical trials have shown that mesenchymal stem cell transplantation can improve glycemic control and pancreatic beta cell function through improved inflammation and immunoregulation.[5]

In recent years, Wharton's Jelly (Wharton's Jelly Mesenchymal Stem Cell/WJ-MSC) mesenchymal stem cells from the umbilical cord have received great attention because cells can be easily isolated (non-invasive procedure), are not subject to ethical problems and come from tissue obtained after birth so that there is no decrease in the characteristics of MSC.[6] Wharton's Jelly mesenchymal stem cells can differentiate into Insulin-Producing Cells. Insulin-Producing Cells can release insulin and C-peptide. Implantation of differentiated MSCs into diabetic mice reduced glucose levels to normal levels. Therefore, IPCs derived from MSC induction are seen as an ideal source of β cells for cell replacement therapy in diabetes.[7] Therefore, the researchers wanted to conduct a study on the benefits and safety of WJ-MSC transplantation in type 2 DM patients, showing that WJ-MSC can lower glucose levels and improve C-peptide levels and beta cells function, and reduce systemic inflammatory markers and the number of T lymphocytes. With the increasing number of scientific research, it is hoped that WJ-MSC will become a new therapeutic agent for the management of DM.

METHODS

Research Design

This study was an experimental study carried out with a post-test only control group design, using 36 white male rats (*Rattus norvegicus*) from the Wistar strain with diabetes mellitus conditions, with fasting blood glucose level ≥126 mg/dL, with a bodyweight of 190-200 grams, and an age of 2.5–3 months as samples.

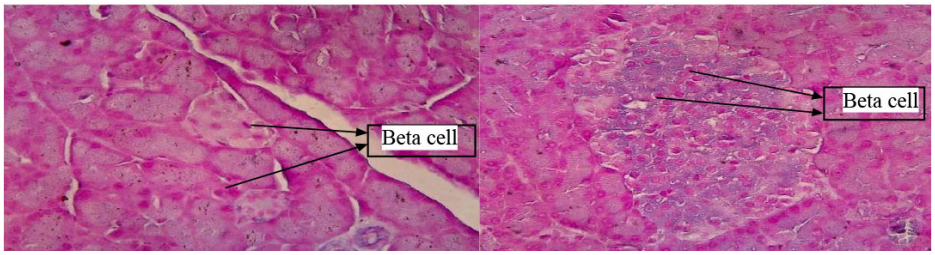

Figure 1. Immunohistochemistry of group P0 rat pancreas (left) and group P1 rat pancreas (right) with Gomori Aldehyde Fuchsin Stain and 400 x Magnification.

Table 1. The results of the descriptive analysis of the data on the number of pancreatic beta cells

Group	n	Mean (mg/dl)	SD (mg/dl)	Minimum – Maximum (mg/dl)
P0 *post-test* (23th day)	18	54,00	11,366	28 - 73
P1 *post-test* (23th day)	18	105,17	16,379	72 - 133

n = sample number; SD = standard deviation

Treatment of Samples

Male white rats (*Rattus norvegicus*) from the Wistar strain with diabetes mellitus were divided into 2 (two) groups, namely the control group (P0) was given 0.9% intravenous NaCl injection and given 5 mg/kg of glibenclamide, and the treatment group (P1) was given an injection. WJ-MSC intravenously and given 5 mg/kg of glibenclamide.

Observation of the Number of Pancreatic β Cells

Observation of pancreatic β cells was carried out by examination using the mouse pancreatic tissue samples immunohistochemistry. In the immunohistochemical examination of mouse pancreatic tissue, Gomori Aldehyde Fuchsin Stain was used to differentiate α and β cells of the pancreas. The count of pancreatic β cells was carried out with an Olympus CX41 (Japan) microscope at 400x magnification.

Data analysis

The results of this study were analyzed using descriptive data analysis, data normality test, homogeneity test, and treatment effect test. Descriptive data analysis was used to present data on the post-test results for each treatment group. The data normality test was performed using the Shapiro-Wilk test with $p>0.05$ was considered normal distribution. The homogeneity test was carried out using the Levene's test with $p<0.05$ was considered homogeneous. Treatment effect analysis was tested using an independent T-test and Mann Whitney U-test.

RESULTS

The Number of Pancreatic β Cells

The results of the analysis of the number of pancreatic β cells after treatment (post-test data) in each group P1 had a greater mean of pancreatic beta cells (105.17 mg/dl) compared to the P0 group (54.00 mg/dl) (Table 1). Pancreatic histology between groups can be seen in Figure 1.

Fasting Blood Glucose Level

After diabetes induction on day 23 after treatment in each group, fasting blood glucose levels were observed (Table 2). Group P1 had a lower mean fasting glucose level (109.06 mg/dl) than group P0 (122.78 mg/dl).

Data Homogeneity Test between Groups

The homogeneity test used Levene's test. Post-test pancreatic β cells showed that

Table 2. **The results of the descriptive analysis of data on fasting blood glucose levels**

Group	n	Mean (mg/dl)	SD (mg/dl)	Minimum - Maximum (mg/dl)
P0 *post-test* (23th day)	18	122,78	10,138	104 - 135
P1 *post-test* (23th day)	18	109,06	16,710	78 - 128

n = sample number; SD = standard deviation

Table 3. **Homogeneity test results data on the number of pancreatic β cells and fasting blood glucose levels between groups**

Variables	n	p	Note
Number of pancreatic β cells	18	0,191	Homogeneous
Fasting blood sugar level	18	0,003	Not homogenous

n = sample number; p = p-value

Table 4. **The average number of pancreatic beta cells between groups after treatment**

Groups	n	R	SD (mg/dl)	t	p
P0	18	54,00	11,366	10,89	0,000
P1	18	105,17	16,379		

n = sample number; R = mean; SD: standard deviation

Table 5. **The average fasting blood glucose levels between groups after treatment**

Groups	n	Mean	SD (mg/dl)	t	p
P0	18	122,78	10,14	87,00	0,017
P1	18	109,06	16,71		

t = count-t; p = p-value; SD: standard deviation

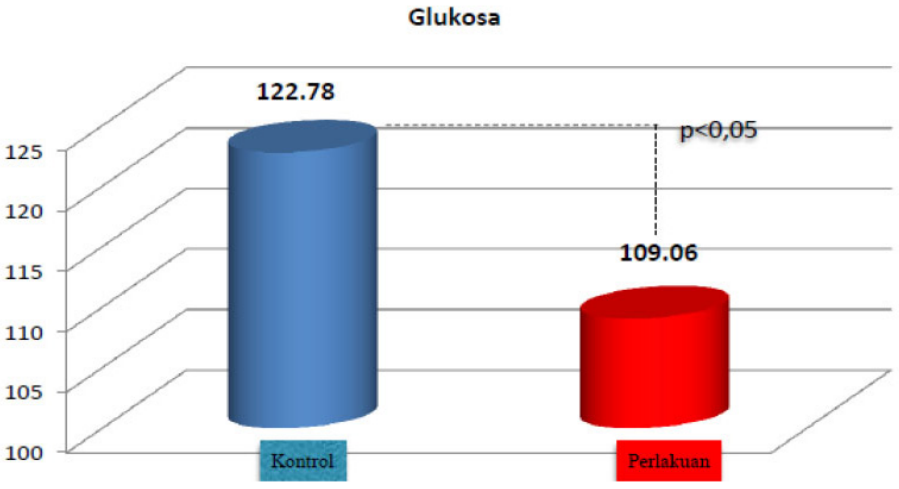

Figure 2. Graph of comparison of fasting blood glucose levels after treatment between groups

the data variant of the post-test results was homogeneous (p<0.05). However, fasting blood glucose levels showed that the data was not homogeneous (p<0.05) (Table 3).

Analysis of the Effects of Treatment of Mesenchymal Stem Cells Wharton's Jelly on Pancreatic Beta Cells

The t-test analysis showed there was a difference significantly in the number of pancreatic beta cells in the P0 group of 54.00 ± 11.366 cells/field of view and in the number of pancreatic beta cells in the P1 groups of 105.17 ± 16.379 cells/field of view (p-value = 0.000) (Table 4).

Analysis of the Effects of Treatment of Mesenchymal Stem Cells Wharton's Jelly on Fasting Blood Glucose Levels

Mann-Whitney U-test showed significantly (p=0.017) the mean fasting blood glucose level in the P0 (122.78 ± 10.14) and P1 (109.06 ± 16.71) (Table 5 & Figure 2).

DISCUSSION

The results showed that the administration of glibenclamide accompanied by intravenous administration of Wharton's Jelly stem cells at a dose of 2 x 106 cells/kg BW reduced fasting blood glucose levels and reduced fasting blood glucose levels significantly (p <0.05). These results show that the administration of glibenclamide and WJ-MSC can reduce fasting blood glucose levels in diabetic Wistar rats. The decrease in blood glucose levels in the treatment group with glibenclamide and WJ-MSC was greater than the decrease in fasting blood glucose levels in the treatment group using glibenclamide and NaCl 0.9%. The increase in the number of pancreatic beta cells in the glibenclamide treatment group accompanied by WJ-MSC was also higher than the increase in the number of pancreatic beta cells in the treatment group glibenclamide and 0.9% NaCl.

Wharton's Jelly's mesenchymal stem cells can differentiate into insulin-producing cells. Induced Insulin-Producing Cells can release insulin and C-peptide. Implantation of differentiated MSCs into diabetic mice can reduce glucose levels to normal levels.[7] Wharton's Jelly stem cells induced with nicotinamide

and β-mercaptoethanol or neurogenic differentiation one gene (NeuroD1) gradually change fibroblasts' morphology into a spherical cell. Immunohistochemical examination revealed positive expression of insulin and glucagon from these cells. RT-PCR examination showed that huMSC expresses insulin and the PDX-1 gene after induction.[5] Research on diabetes mellitus has shown that intravenous administration of intrapancreatic and endovascular intrapancreatic stem cells from mesenchymal stem cells can reduce fasting blood glucose levels with a maximum decrease for the first six months and then stabilize fasting glucose levels for 12 months without administering antidiabetic drugs.[4]

Wharton's Jelly's mesenchymal stem cells can differentiate into IPC. Chao et al. Differentiated WJ-MSC to IPC through several stages of culture using a neuro-conditioned medium. Wu et al. conducted a comparative study comparing the ability of WJ-MSC and BM-MSC to differentiate into IPC phenotypes. Both cell types can form islet-like clusters on the first day of culture with a culture medium containing nicotinamide, activin, HGF, exendin-4 and pentagastrin. The researchers found higher Pdx-1 expression in the differentiated WJ-MSC compared to the differentiated BM-MSC.[8]

With the production of growth factors and cytokines, stem cells systemically transplanted into the body can induce other stem cells in various organs of the patient's body to proliferate and move towards damaged tissues/organs.[9] Bone marrow mesenchymal stem cells (BM-MSCs) express C-peptide at differentiation days 14 and 18, with the greatest expression on day 14. WJ-MSC shows C-peptide expression at differentiation days 5 and 10.[10]

Further research is needed with a longer study time, multiple sample sizes, and different doses. With the increasing number of scientific research, it is hoped that the WJ-MSC research can be advanced to the clinical trial stage. As a reference and basis for future research, it is necessary to carry out further research with a larger number of samples to determine the optimal concentration of WJ-MSC administration in increasing the number of pancreatic beta cells and reducing fasting blood glucose levels.

CONCLUSION

Intravenous administration of WJ-MSC can increase the number of pancreatic beta cells and reduce fasting blood glucose levels in DM rats. Also, it is necessary to carry out further research on DM patients in the form of clinical trials to obtain data on the effectiveness of WJ-MSC in increasing the number of pancreatic beta cells and reducing fasting blood glucose levels.

DISCLOSURES

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The authors declared no grant or third-party supported this research.

Conflict of Interest

The authors declared no conflict of interest that influenced objectivity during the study, interpreting the result, and writing the manuscript.

Author Contribution

WP and AAGB involved in concepting, designing and supervising the manuscript. NP conduced the study and analyses the data. All authors prepare the manuscript and agree that this final version of the manuscript will be submitted to this journal.

Ethical Statement

This research has been approved by the Ethical Commission of Faculty of Medicine, Universitas Udayana, with letter number 03/UN.14.2/Litbang/2016.

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