Jurnal Analis Laboratorium Medik
Avalilable Online http://e-journal.sari-mutiara.ac.id/index.php/ALM

THE EFFECTIVENESS OF PRP ON REDUCING BLOOD GLUCOSE LEVELS IN DIABETIC MICE

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ABSTRACT

PRP contains growth factors that have the potential to repair tissue damage, such as pancreatic damage in diabetes mellitus. Pancreas damage in diabetes mellitus is characterized by hyperglycemia. This study aimed to determine the effectiveness of PRP in reducing blood glucose levels in diabetic mice. Male Swiss Webster mice divided into 5 groups (normal group, DM group, PRP1 group, PRP2 group and PRP3 group). The DM, PRP1, PRP2 and PRP3 groups were given streptozotocin 45 mg/kgBW to induce diabetes mellitus. Mice in the PRP1, PRP2, and PRP3 groups that have indicated diabetes mellitus with glucose levels > 200 mg/dL will be given PRP with a dose of 0.1 mL; 0.3 mL; 0.5 mL intraperitoneally for 4 weeks. Data on blood glucose levels were analyzed using one-way ANOVA test and LSD test. The use of PRP in various doses, namely 0.1 mL, 0.3 mL, 0.5 mL, can reduce blood glucose levels in the PRP1 group (195.8 ± 14.04 mg/dl), PRP2 group(176.6 ± 9.37 mg/dl), PRP3 group (121 ± 5.52 mg/dl) compared to the DM group (392.6 ± 18.09 mg/dl). Dose of PRP of 0.5 mL gave a better effect on pancreatic tissue repair than the PRP1 and PRP2 groups, which were characterized by glucose levels that were close to the normal group (106.8 ± 10.61 mg/dl). Based on the results of the study, PRP 0.5 mL is effective in repairing pancreatic tissue which is characterized by a decrease in blood glucose levels in diabetic mice.

Keywords: Diabetes mellitus; PRP; Streptozotocin

INTRODUCTION

Diabetes mellitus cases continue to increase every year. The International Diabetes Federation (IDF) data in 2019, showed that Indonesia is the seventh country with the largest number of people with diabetes mellitus in the world and it will continue to increase until 2045 (International Diabetes Federation, 2021). Diabetes mellitus is a metabolic disease characterized by hyperglycemia (above normal blood glucose levels). Hyperglycemia in diabetes mellitus occurs due to damage to pancreatic cells that are unable to produce insulin in sufficient quantities (Simarmata et al., 2021). The state of hyperglycemia in diabetic patients can cause various complications such as cardiovascular disease, chronic kidney failure, damage to the retina of the eye and many complications of other health problems (Prawitasari, 2019). Blood glucose is the result of carbohydrate metabolism that occurs in the body. Monitoring blood glucose levels is used for the diagnosis of Diabetes Mellitus (Siahaan & Aruan, 2022).

Diabetes mellitus requires serious treatment and must be treated immediately to control blood glucose levels. Currently, there is no drug that can permanently heal diabetes mellitus. The use of synthetic insulin is only to control blood glucose levels and has side effects on the body. Pancreatic cell repair strategies can be used as a long-term treatment to normalize pancreatic function and reduce dependence on using synthetic insulin (Dewi et al., 2016). Currently, using autologous Platelet Rich Plasma (PRP) products is a relatively new approach in

regenerative medicine and has received considerable attention over the last two decades (Pavlovic et al., 2016).

PRP is a platelet concentrate derived from whole blood and rich in growth factors. The number of platelets in PRP is 3-5 times higher than the number of circulating platelets (El Tahawy et al., 2017). PRP applications have been widely used in the healing of skin burns, wound healing in diabetic patients, facilitating bone proliferation in orthopedic surgery, maxillofacial surgery, spine surgery, cardiac surgery, plastic surgery and aesthetic surgery (Cozma et al., 2016). Another study found that using PRP can trigger the repair of endocrine tissue (island of Langerhans) and exocrine tissue (ductal cells and acinar cells) in the pancreas. Administration of PRP can reduce lipid profile levels in diabetic male rats (Simarmata et al., 2021). Growth factors in PRP are biological agents that play a role in the regeneration of pancreatic tissue and have the potential to restore the normal function of the pancreas by producing insulin (Zarin et al., 2019). Currently, there are no studies that have tested the effectiveness of PRP in lowering blood glucose levels in diabetes mellitus. Based on this, it is necessary to conduct research on the effectiveness of PRP in lowering blood glucose levels in diabetic mice.

METHODS

This study was conducted at the Research Laboratory, Hematology Laboratory and Clinical Chemistry Laboratory, John Paul II Health Academy Pekanbaru. This study is an experimental study with a post-test only control group design. The DM group was given PRP at various doses (0.1 mL; 0.3 mL and 0.5 mL) compared with the DM group without PRP and normal group. The animal models used in this study were male Swiss Webster mice.

Thirty male mice aged 3 months with a body weight of 25-30 g were divided into 5 groups. All mice were acclimatized for 7 days and blood glucose measurements were taken. During acclimatization, mice were fed and watered ad libitum 3 times a day. Setting room temperature in the range of 18-19°C, humidity between 30-70% and changing the husks in the cage every 3 days. During the acclimatization process, the test animals were kept away from noise.

Mice were injected intraperitoneally with streptozotocin 45 mg/kgBW to induce diabetes melitus. Streptozotocin was dissolved using 0.1 M citrate buffer with a pH of 4.5 (Dehghani et al., 2019). After 3 days of streptozotocin injection, blood glucose levels were measured. Diabetic mice were characterized by blood glucose levels of > 200 mg/dL.

Whole blood for the PRP preparation procedure used comes from male donors. A total of 5 mL of venous blood was taken using a wing needle connected to an Eppendorf tube which already contained 1 mL of citrate buffer anticoagulant, then centrifuged at 1200 rpm for 10 minutes. The results of the first centrifugation, namely the top layer (plasma) and the middle layer (buffy coat) were transferred to an empty tube without anticoagulant, then centrifuged at 5000 rpm for 10 minutes. After the second centrifugation, the top third of the layer (plasma) is removed and the remaining is a small amount of plasma and pellets) which is called PRP (Rizal et al., 2020).

PRP activation was carried out by adding 0.05 mL of 10% CaCl2 solution to 1 mL of PRP. PRP was injected intraperitoneally in mice for each treatment group (PRP1: 0.1 mL), (PRP2: 0.3 mL), (PRP3: 0.5 mL), once a week for 4 week. Measurement of blood glucose levels of mice was carried out during acclimatization, injection of streptozotocin and after PRP

treatment in week 4. Data from measuring blood glucose levels in week 4 were analyzed using a one-way ANOVA and LSD test.

RESULTS AND DISCUSSION

This study used male mice induced by streptozotocin 45 mg/kg BW to induce diabetes mellitus. The selection of animal models was adapted to the purpose of this study, which only measured blood glucose levels. Mice are animal models that are often used because they have the same metabolism as humans (Mutiarahmi et al., 2021), so mice were chosen as subjects in this study.

Measurement of blood glucose levels of mice was carried out during acclimatization, injection of streptozotocin to induce diabetes mellitus, and after PRP treatment. The results of measuring blood glucose levels in mice in this study can be seen in Figure 1.
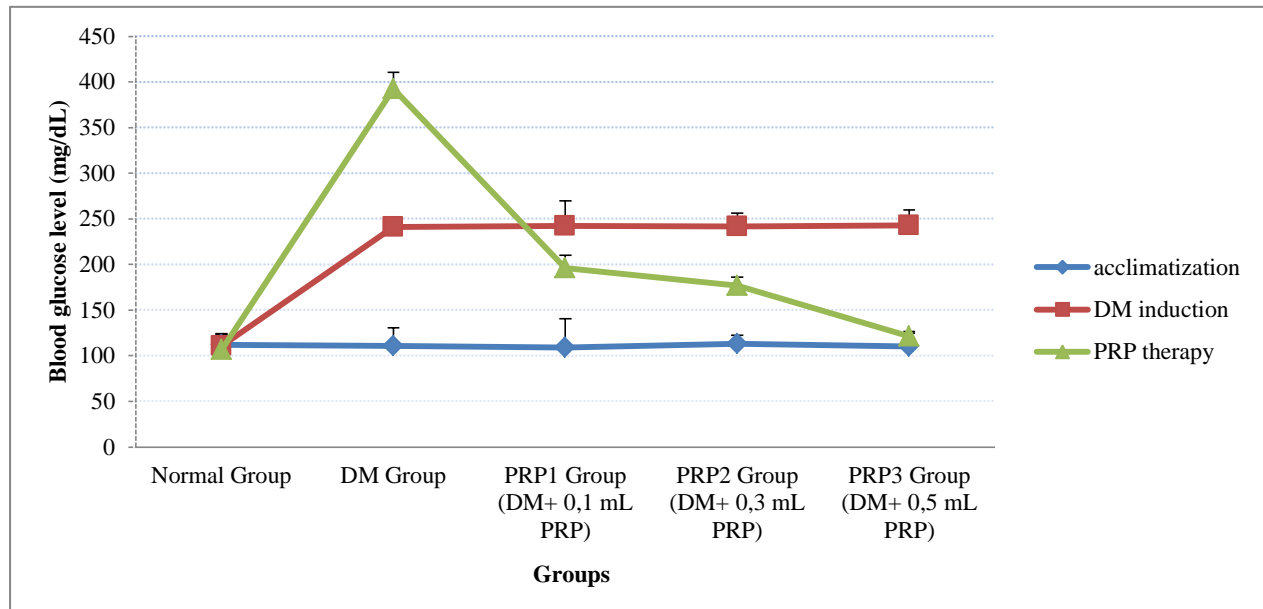

Figure 1. Blood glucose levels of mice during acclimatization, DM induction and PRP therapy

Blood glucose levels in the DM, PRP1, PRP2 and PRP3 groups after 3 days of streptozotocin injection, showed an increase in blood glucose levels above 200 mg/dL when compared to the normal group. The state of moderate hyperglycemia in mice after streptozotocin induction was characterized by blood glucose levels of 200-400 mg/dl (Saputra et al., 2018). The use of a single dose of streptozotocin can increase the blood glucose levels of rats to 383.38 mg/dl (Munjiati et al., 2021). The application of streptozotocin 45 mg/KgBW can have an impact on pancreatic damage, especially beta cells, which are characterized by an increase in blood glucose levels (Aji et al., 2019).

Streptozotocin is an ingredient commonly used for the induction of diabetes mellitus in experimental animals. Streptozotocin works by forming free radicals that can damage pancreatic beta cells, so insulin production can be disrupted (Yuliana, 2021). Streptozotocin has a toxic effect that causes swelling of the pancreas organ, pancreatic beta cell necrosis so that insulin production is disrupted and causes Diabetes Mellitus (Simarmata et al.,2021).

Statistical analysis results of the one-way ANOVA test showed a significant difference between each group ($p<0.05$). The post hoc LSD test showed that the blood glucose levels of mice in the diabetic group

treated with PRP were significantly different when compared to the DM group. The LSD

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post hoc test is presented in table 1.

Table 1. LSD test results of mice blood glucose levels between groups

No	Groups	Mice blood glucose level (mg/dL)					Mean ± SD
		1	**2**	**3**	**4**	**5**	
1	Normal Group	113	94	112	118	97	106.8 ± 10.61[a]
2	DM Group	423	392	381	377	390	392.6 ± 18.09[b]
3	PRP1 Group	201	205	179	183	211	195.8 ± 14.04[c]
4	PRP2 Group	179	188	178	162	176	176.6 ± 9.37[d]
5	PRP3 Group	121	120	130	119	115	121 ± 5.52[a]

Note: different superscripts ([a, b, c, d]) showed significant differences between each group (P<0.05)

Pancreatis beta cell damage will have an impact on increasing blood glucose levels. The use of PRP in various doses, namely 0.1 mL; 0.3 mL; 0.5 mL can reduce blood glucose levels in the PRP1 (195.8 ± 14.04 mg/dL), PRP 2 (176.6 ± 9.37 mg/dL) groups, respectively, PRP3 (121 ± 5.52 mg/dL) compared to the DM group (392.6 ± 18.09 mg/dL). The application of PRP as a treatment has been used to repair tissue damage. PRP can stimulate cell proliferation so that PRP is often applied for therapy in wound healing or tissue repair (Hadi et al., 2019);Ahmed et al., 2017; Irianto et al., 2021). IGF-1 contained in PRP is thought to play an important role in pancreatic regeneration by an autocrine mechanism through stimulation of DNA synthesis (El Tahawy et al., 2017).

The normal group was used as a comparison of healthy mice that did not have pancreatic tissue damage. The decrease in blood glucose levels in the PRP group indicated an improvement in pancreatic tissue damage. PRP stimulates the regeneration of pancreatic exocrine tissues such as acinar cells and ductal cells (El Tahawy et al.,2017). The PRP administration can increase insulin secretion in the pancreas, which is characterized by a decrease in blood glucose levels (Zarin et al.,2019). The decrease in glucose levels in

the PRP1 and PRP2 groups was significantly different from the normal group, but the administration of 0.5 mL of PRP in the PRP3 group was not significantly different when compared to the normal group. PRP at a dose of 0.5 mL gave a faster repair effect on pancreatic tissue than the PRP1 and PRP2 groups, which were characterized by glucose levels that were close to the normal group. The administration of PRP in this study has the potential to improve pancreatic tissue, which is characterized by a decrease in blood glucose levels in the treatment of Diabetes mellitus. Further research is needed to examine more deeply the repair effect of PRP on pancreatic tissue damage so that the application of PRP can be developed to treat diabetes mellitus.

CONCLUSION

The administration of 0.5 mL of PRP in this study was effective in repairing pancreatic tissue which was characterized by a decrease in blood glucose levels in diabetic mice.

ACKNOWLEDGEMENT

Academy who has supported research activities and all parties involved in this

research.

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