OPTIMAL DOSE OF GELL PEPTIDE FOR DIABETIC WOUND HEALING

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Abstract

Various studies show that diabetes cases are increasing. Epidemiological data also reported 10.7 million diabetes cases in Indonesia in 2019. Diabetic foot syndrome is a common complication in individuals with DM. Its treatment is costly and prolonged. Thus, we conducted an in vivo study to assess the optimal peptide dose and its effectiveness in accelerating diabetic wound healing. This experimental laboratory study with a randomized pre-post test control group design was conducted in Wistar rats with diabetic wounds receiving different doses of gel peptides**.** One Way Anova test was applied to compare the effectiveness of the three doses of gell peptide (0.05 gr, 0.1 gr, 0.15 gr). The significance level was determined with $P<0.05$. Significant improvement in all groups was started on day 7 ($p=0.000$). The best outcome was obtained in group B, in which the wound had complete closure ($p=0.000$) The optimal dose of 0.1 gr gell peptide is effective for accelerating diabetic wound healing and closure.

Keywords: Peptide, Wound Healing, Diabetes Mellitus.

Introduction

Diabetes mellitus is a metabolic disorder caused by a deficiency of insulin secretion, damage to pancreatic cells, or insulin resistance. Diabetes mellitus is one of the global problems in the world of health, and it is estimated that there are around 400 million people with diabetes worldwide. Current epidemiological data revealed 10.7 million people in Indonesia suffered from DM in 2019. Diabetes mellitus has various complications, such as nephropathy, neuropathy, retinopathy, blood vessel damage, chronic wounds, and other complications. (Padhi et al., 2020)ꞏ(Aschner et al., 2021)ꞏ(Saeedi et al., 2019)

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Diabetic wounds are chronic or open wounds that are complications of diabetes mellitus (DM) commonly seen on the feet and hands. A diabetic wound can lead to infection, severe pain, and deep tissue damage. Diabetic foot syndrome is one of the most common complications of DM, and this complication is a chronic wound condition on the skin that has been around for a long time or is difficult to heal in diabetic patients. This syndrome is one of the most common causes of non-traumatic amputations. (Ouyang et al., 2020)'(Noor et al., 2015)'(Lathifah, 2017)'(Beckmann et al., 2014)

Wound healing involves various molecular and cellular activities to repair the damage. It comprises four phases: hemostasis, inflammation, proliferation, and remodeling. Diabetic wound healing is difficult because of the imbalance in the healing phases, such as the hemostasis, inflammation, proliferation, and remodeling phases. In diabetic wounds, the phase lengthening occurs, making it difficult for wound healing caused by hyperglycemia from diabetes mellitus. (Gonzalez et al., 2016)'(Patel et al., 2019)

Wound healing in humans and mice has similarities in complex molecular and cellular stages. Rats have panniculus carnosus, a thin layer of muscle tissue that provides a potential skin contraction that can help close the wound, and humans do not possess this. (Gonzalez et al., 2016)'(Rittié, 2016)'(Zomer & Trentin, 2018)

Wound treatment using peptides has been studied and proven in several studies. Research reports that a peptide containing 11 amino acids can modulate cell proliferation with the name Tiger 17, keratinocyte cell migration, and trigger epithelial rearrangement in the skin in mice. Various therapeutic options for chronic wounds are being developed, one of which is amino acid therapies. The amino acids arginine and glutamine are essential in metabolism and wound healing. Hence, we conducted an in vivo study assessing the optimal dose of peptide gel and its effectiveness in accelerating diabetic wound healing in Wistar strain rats.(Tang et al., 2014)'(Molnar et al., 2016)

Research Methods

We performed an experimental laboratory study with a randomized pre-post control group design in male Wistar rats weighing 150-200 g with diabetic wounds. Based on the Federer formula, the research subjects used in each group were six male Wistar rats. The number of treatment groups is three groups, where group A is the group to which 0,05 g of peptide gell will be applied, group B is the group to which 0.1 g of peptide gel will be applied, and group C is the group to which 0.15 g of peptide gel will be applied. The addition of samples was carried out to anticipate dropouts so that each group would be added 1 sample of experimental animals. Thus, the study's total number of animal samples was 21 Wistar rats with diabetic wounds.

Mice are often used in experimental research because they are cheaper and can be intervened by genetic changes so that they can expand the scope of research. The inclusion criteria of the research sample were male Wistar rats, aged 8-12 weeks, with rats body weight of 150-200 grams, and during the study were kept in special cages and

fed BW 1. The study sample would be excluded if they were sick or died at the time of the study. (Grada et al., 2018)

Wistar rats were measured for blood sugar, weighed, and injected with Alloxan with a dose of 150 mg/kg BW intraperitoneally. After three days of alloxan injection, the Non fasting blood glucose level was measured again. If the sugar value is higher than 200 µg, it means diabetes. The process of making DM in rats is confirmed to be successful by checking GDS on day seven after alloxan induction. This indicates stable glucose levels above normal levels so that the rats are in a stable diabetic condition. (Sekiou et al., 2020)

Wounds were made with the help of laboratory assistants. Rats were given general anesthesia with ketamine at a dose of 10 mg/kg body weight intramuscularly. After being anesthetized, the rats were placed on a surgical board in a prone position. The back area to be wound was shaved and cleaned with gauze moistened with 70% alcohol and 10% povidone-iodine, rinsed with gauze moistened with 0.9% NaCl then a total thickness wound to the subcutaneous tissue was made using a 10 mm biopsy tube. Bleeding is controlled by applying sterile gauze to the lesion. After the wound is made, stitches will be done to minimize contractions in healing the rat's wound.

Each research animal will be given daily therapy as planned for each group with a different dose. Wounds from research animals will be observed on days 0, 7, and 14. Wounds will be photographed at the same distance and position, measured using millimeter blocks, and analyzed using the J image application. Will the research data be analyzed to compare each research group's healing? All study samples will be terminated on day 14 after wound measurement.

Before conducting the analysis, the data will be tested for normality to see whether the data is normally distributed or not using the Shapiro-Wilk test because the research sample is less than 50 samples. If the data distribution is normal ($p > 0.05$) and the study consists of 3 groups, then the One Way ANOVA analysis is used to assess the acceleration of wound healing during the study, then to assess the significant differences in each group based on the results of the study, the Post Hoc Bonferroni test is used. Suppose the data distribution is not normal ($p < 0.05$), the Kruskal-Wallis test analysis was used to assess the acceleration of ulcer healing during the study and continued with the Mann-Whitney test to determine the significant differences in each group based on the results of the study. The group difference is significant if $p > 0.05$. Wound observations were carried out three times during the study on days 0, 7, and 14. Wound measurements were carried out using millimeter blocks and processed using Image J software as research data.

Results and Discussion

The study was conducted for 14 days, and documentation of wound size was carried out on days 0, 7, and 14 of the study. From group B, it seems that there has been complete wound closure. The table below is the wound size documentation in each group's research animals.

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Table 1
Documentation on Wound Size from Each Group

Treatment day	A	B	C
Day 0			
Day 7			
Day 14			

The study animals were measured for wounds on days 0, 7, and 14. The table below shows the average data on wound size in each group of research.

Table 2
Average Wound Size from Each Group

Treatment day	Group	Average Wound Size
Day 0	A	1.19
	B	1.19
	C	1.19
Day 7	A	0.84
	B	0.59
	C	0.77
Day 14	A	0.41
	B	0.11
	C	0.37

Description: The average value of wound size between the three treatment groups on days 0, 7 and 14.

Before the data was analyzed, the Shapiro-Wilk normality test was carried out to assess the distribution of the data. The table below shows the results of the normality test of the research data.

Table 3
Result of Shapiro Wilk Test Wound Size

Treatment day	Group	*Shapiro Wilk*		
		Statistic	df	p score
Day 0	A	0.914	10	0.312

	B	0.933	10	0.479
	C	0.907	10	0.263
Day 7	A	0.941	10	0.565
	B	0.908	10	0.268
	C	0.961	10	0.799
Day 14	A	0.951	10	0.684
	B	0.905	10	0.250
	C	0.962	10	0.805

Researchers compared wound closure in each group using the one-way ANOVA test. Comparisons were made on wound closure days 0, 7, and 14. The table below shows the result of this study's one-way ANOVA test.

Table 4
Result of One Way Anova Test Wound Size

Treatment day	Group	Sig ANOVA
Day 0	A	0.998*
	B	
	C	
Day 7	A	0.000**
	B	
	C	
Day 14	A	0.000**
	B	
	C	

Description:*not significant,**significant 1%

Researchers compared the significance of differences in wound closure in each group using the Post Hoc Befferoni test, the comparison of significance was carried out on wound closure days 7 and 14. The table below shows the result of this study's Post Hoc Befferoni test.

Table 5
Result of Post Hoc Befferoni Test

Treatment day	Group	*Post Hoc*		
		Group	Mean	p score
Day 7	A	B	0.25	0.000**
		C	0.78	0.064*
	B	C	-1.74	0.000**
Day 14	A	B	0.290	0.000**
		C	0.034	0.850*

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	B	C	-2.62	0.001**

Description:*not significant,**significant 1%.

Discussion

In this study, observation of diabetic wound closure was carried out by observing three study groups with different doses of peptides gel. Group A with a dose of 0.05 g of peptide gell, group B with 0.1 g of peptide gell, and group C with 0.15 g of peptide gell. Quantitative observations were made on days 0, 7, and 14 using image J software to calculate the wound size.

Table 1 shows an overview of the documentation of wound size in research animals for each group. In all groups, it was seen that the diabetic wounds had indeed healed with a reduction in the size of the wound, and it was seen that in group B the wound had been completely closed. Table 2 shows the average wound size for each study group. On day 7, it was found that group B had the smallest average wound size of 0.59 cm, while in group A the average wound size was 0.84 cm, and in group C was 0.77 cm. On day 14, it was found that group B also had the smallest average wound size of 0.11 cm, while group A was 0.41 cm and group C was 0.37 cm. This shows that peptide gell can help heal diabetic wounds in all research groups, especially in group B with a dose of 0,1 g of peptide gell.

This study aims to find the optimal peptide gel dose by comparing the progress of diabetic wound closure in Wistar rats with various doses of peptide gel, normality test to determine the distribution of data is needed before carrying out other analytical tests. Table 3 shows the results of the Shapiro-Wilk normality test and obtained in each group the p value> 0.05, which means that all the research data are typically distributed.

The One Way Anova test was carried out to compare the wound closures in groups A, B, and C on days 0, 7, and 14. Table 4 shows the One Way Anova test results on day 0. The p-value was 0.998, on day seven was 0.000, and on the 14th day of 0.000. In the One Way Anova test, it can be said to be statistically significant if the p-value < 0.05. So on day 0, there was no significant difference between groups because the wound had just been made with the exact mechanism and condition. Meanwhile, on the 7th and 14th days, a p-value <0.05 was obtained, which means a significant difference between groups on that day.

Based on the results of the One Way ANOVA test, the Post Hoc Befferoni test was carried out on days 7 and 14 to assess the significance of the difference between diabetic wound closures between the three study groups. . In table 5, it was found that on day seven there were significant differences between group A and group B (p-value = 0.000) and in group B and group C (p-value = 0.000). However, in groups A and C there was no difference. significant (p-value = 0.064). On day 14, there was a significant difference between group A and group B (p-value = 0.000) and group B and group C (p-value = 0.001), while in group A and C, there was no significant difference (p-value = 0.850). From the results of this study, it can be concluded that the treatment in group B was most effective in accelerating the healing of diabetic wounds in Wistar

rats.

In this study, researchers used a peptide gell containing various peptides, including L-Arginine, L-Histidine, L-lycine, L-Phenylalanine, L-Isoleucine, L-Leucine, L-Tyrosine, L-Methionine, L-Valine. , L-Glutamic acid, L-Aspartic acid, L-Cysteine, L-Threonine, L-Serine, L-Glycine, and L-Tryptophan. Wound healing is a process that is influenced by many things and is not linear, and this process depends on extrinsic and intrinsic factors such as growth hormones and cytokines. This process can also experience setbacks and progress at the stages of wound healing. (Arribas-lópez et al., 2021)

Recent studies have shown that protein deficiency resulting in amino acid deficiency can interfere with the body's immune system. The role of amino acids in improving the work of the immune system in the body is one of the keys to improving health and treating infectious diseases. Arginine is a non-essential amino acid in humans. This amino acid functions as a regulator of nitric oxide, a mediator for the regulator of the immune system against tumor cells and some microorganisms. In animals, it can increase the proliferation of lymphocytes, NK cells, and macrophages, increase resistance to bacteria and help wound healing.(Dukes, 2015)

Amino acids play a significant role in wound healing, one of which is in the inflammatory phase, played by arginine and glutamine. In the inflammatory phase, glutamine is also involved in wound healing by regulating leukocyte apoptosis, superoxide production, phagocytosis, and triggering the secretion of anti-inflammatory cytokines such as IL-4 and IL-10. Arginine can inhibit the secretion of pro-inflammatory cytokines such as IL-6 and TNF-α.(Barchitta et al., 2019),19.

Angiogenesis is the process of forming new blood vessels from existing blood vessels, and this process requires adequate levels of oxygen and nutrients in order to produce good neovascularization. Arginine is also a precursor of nitric oxide (NO), which is a molecule that can stimulate the secretion of angiogenesis factors and trigger vasodilation in blood vessels.(Aleem et al., 2019)

Theoretically, and by conducting a study to compare the optimal dose of topical peptide for diabetic wound therapy, it was found that the optimal dose of peptide gel is 0.1 g. This study also proves that topical peptides can accelerate diabetic wound healing in Wistar rats and is in line with previous research studies. The limitations of this study are the small number of research animals, and the wound closure is only studied from 1 assessment so that future research can be carried out with more samples and more wound closure assessment factors.

Conclusion

The researcher concluded that from this study, it was found that, in general, peptides gel accelerated wound healing and obtained the optimum dose for diabetic wound closure in Wistar rats of 0.1 g. Complete wound closure occurred on the 14th day of the study.

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Irene Ardiani Pramudya (2023)