Original Research Article

International Journal of Research and Review
Vol. 10; Issue: 7; July 2023
Website: www.ijrrjournal.com
E-ISSN: 2349-9788; P-ISSN: 2454-2237

Administration of Spirulina Ethanol Extract Gel Made Malondialdehyde Levels Lower and Collagen Density Higher in Wound Healing Wistar Rat Model Diabetes Mellitus

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DOI: https://doi.org/10.52403/ijrr.20230758

ABSTRACT

Background: Wound in diabetes mellitus becomes a major problem. Reactive oxygen species (ROS) in diabetes cause peroxidative phospholipid damage and lead to malondialdehyde (MDA) accumulation. Malondialdehyde levels in diabetes are increasing significantly and cause complications. The MDA reaction affects collagen which interferes with the normal process of wound healing. Flavonoids in Spirulina sp. acts as an antioxidant by increasing antioxidant enzymes to reduce the amount of ROS. Flavonoids increase collagen through stimulating keratinocytes against fibroblasts, preventing oxidative damage to cells thereby accelerating wound healing. This study aims to determine the administration of spirulina ethanol extract gel makes MDA levels lower and collagen density higher in wound healing in Wistar rats' model of diabetes mellitus.
Methods: This research was conducted from November 2022 to January 2023 using an experimental posttest only control group design, using 30 male white rats (Rattus norvegicus) Wistar strain aged 3 months, body weight 180-200 grams, which were divided into five groups, group P1 not induced by streptozotocin (STZ) excised wound and given NaCMC gel, group P2 induced STZ with blood sugar ≥150 mg/dL had wound excised and given 0.1% Gentamicin ointment, group P3 induced STZ had excision wound smeared with 10% Spirulina gel, the STZ-induced P4 group had the wound excised and given 15% Spirulina gel, and the STZ-induced P5 group had the wound excised and given 20% Spirulina gel. Statistical test was used One Way ANOVA.
Results: The results showed that the average amount of MDA in the P5 group (0.470 nmol/mL) was lower than the other groups, where the average amount of MDA in the P4 group (0.592 nmol/mL), the P3 group (0.679 nmol/mL), the P2 group (0.732 nmol/mL) mL) (p=<0.001). The mean number of % pixel collagen density in the comparison of each group was not significantly different, in the P5 group (69.30), the P4 group (64.76), the P3 group (65.97) and the P2 group (49.53) (p= 0.116).
Conclusion: Administration of 20% Spirulina ethanol extract gel was more effective in reducing malondialdehyde levels in the wound healing of Wistar rats' model of diabetes mellitus than control group. Administration of Spirulina ethanol extract gel did not differ in making collagen density higher compared to control group. Spirulina can be used as an alternative drug with the ability as an antioxidant.

Keywords: spirulina ethanol extract gel, malondialdehyde, collagen, wounds of Wistar rats, diabetes mellitus

INTRODUCTION

Aging is a decrease in the condition and function of all organs in living things. Along with the development of science emerged the branch of medicine, Anti-Aging Medicine, which aims to extend life expectancy in a healthy state with a good quality of life. In aging there is a decrease in organ function including the mechanism of skin healing when an injury occurs.

Normal wound healing proceeds through phases of inflammation, proliferation, and remodeling in response to tissue injury. Interference with any of these phases of wound healing can cause the wound to become chronic and usually requires some form of intervention. One of the reasons for the delay in the wound healing process is diabetes and aging (Mathew-Steiner et al., 2021).

Reactive oxygen species in diabetes cause peroxidative phospholipid damage leading to malondialdehyde (MDA) accumulation. Reactive oxygen species (ROS) are important regulators of several phases of wound healing. A low amount of ROS is needed to fight external damage, but a high accumulation of oxidative stress will induce oxidative damage to cell membranes, degradation of the extracellular matrix, prolonged inflammation and cause wounds not to heal, as in diabetes (Deng et al., 2021).

Malondialdehyde levels in diabetes increase significantly and are one of the causes of complications in diabetic patients. Malondialdehyde reactions affect collagen function by making collagen less susceptible to degradative enzymes, changing collagen molecules and influencing collagen cell interactions. This interferes with the normal process of wound healing (Oo et al., 2017).

Delayed and impaired wound healing in diabetic patients is the focus of research. Long-term exposure to a hyperglycemic environment will affect protein and lipid metabolism, induce the production of reactive oxygen species, increase inflammation, increase the risk of ulcerative wounds, and inhibit wound healing (Chen et al., 2020).

Study shows Spirulina sp. extract has potential in wound healing. Spirulina sp. is a blue-green algae known to have anti-inflammatory and antioxidant properties that is used as a food supplement and is known as a "Superfood", the designation given by the World Health Organization (WHO). Spirulina sp. has an effect on cell growth and cell regeneration thereby improving health and quality of life (Elbialy et al., 2021). In addition, Spirulina sp. can be easily obtained, because it can be cultivated in fresh water media, harvested and processed easily (Soni et al., 2017).

The important role of Spirulina sp. in wound healing is through its antioxidant mechanism. The content of flavonoids in Spirulina sp. which act as antioxidants can increase collagen fibers by stimulating keratinocytes and preventing oxidative damage to cells so that they can accelerate wound healing (Liu et al., 2020; Burhan et al., 2021; Mehdinezhad et al., 2021).

Research conducted by Liu et al., showed that MDA levels in wound healing of rats given Spirulina sp. gel. 4% lower than the control group, these results indicate the positive effect of spirulina as an antioxidant in inhibiting MDA during wound healing in rats (Liu et al., 2020).

Spirulina sp. can also accelerate healing by increasing angiogenesis, collagen deposition, reducing histopathological morphological changes and inhibiting scar tissue formation through regulation of basic fibroblast growth factor (BFGF) and angiogenic vascular endothelial growth factor (VEGF) genes and occupying scar tissue-related genes transforming growth factor beta (TGF-β) and smooth muscle actin (-SMA) which provide potential molecular for the treatment of hypertrophic scar tissue as a promising natural biological source in wound healing (Elbialy et al., 2021).

This study used wistar rats (Rattus norvegicus) because their genetic structure, anatomy and physiology are similar to humans, and easy to maintain, and refers to the basic principles of research ethics regarding the use of experimental animals known as 3R, namely Reduction, Replacement, and Refinement (Mutiarahmi et al., 2021).

Wistar rats in this study were induced using streptozotocin (STZ) to achieve hyperglycemia. Researchers use STZ because it has a long half-life, low incidence of ketosis and death (Islam et al., 2017). Referring to the description described above, the researcher is interested in conducting research on the administration of Spirulina sp. ethanol extract gel on decreasing MDA and increasing collagen density in wound healing in Wistar rats model of diabetes mellitus.

LITERATURE REVIEW

Wound

Wound is damage to the integrity of biological tissue which includes skin, mucous membranes, and organ tissue (Herman and Bordoni, 2022). Wounds to the skin will disrupt the barrier between the inside of the body and the outside environment (Liu et al., 2019). Skin wounds occur due to damage to the integrity of the epidermal layer. Any tissue injury with compromised anatomic integrity with functional loss can be described as a wound (Kangal and Regan, 2022).

The skin consists of two layers namely the epidermis and dermis. When there is a disturbance in one or both layers of the skin, the organism will start the wound healing process to regenerate the wound area which involves cellular, molecular and biochemical mechanisms which are divided into three healing phases namely inflammation, proliferation and remodeling (Wilkinson and Hardman, 2020; Gushiken et al., 2021).

Diabetes Mellitus

Diabetes mellitus (DM) is defined as a group of metabolic pathologies characterized by impaired insulin production and/or function, leading to hyperglycemia. Diabetes mellitus greatly affects the quality of life and survival expectations of patients. As a consequence of hyperglycemia, diabetic patients are at increased risk of comorbid conditions affecting multiple organs (Spampinato et al., 2020).

Oxidative stress and inflammation are closely related to aging-related changes. It is known that aging and diabetes have important factors that include oxidative stress and low levels of inflammation. The early stages of diabetes are the same as in aging, there is a persistent accumulation of oxidative damage to biomolecules caused by ROS which are produced in all cells especially by mitochondria. Mitochondria are major producers and also prime targets of ROS because they damage mitochondrial DNA (mtDNA) leading to increased ROS production. An increase in mtDNA mutations accelerates aging (Moura et al., 2019).

Oxidative stress can affect diabetic wound healing through skin injury, neuropathy, ischemic lesions, and topical infections (Gargouri et al., 2016; Deng et al., 2021).

Diabetic wounds have a microenvironment with increased levels of glucose, advanced glycation end products (AGEs), ROS, and inflammatory cytokines. High glucose levels reduced keratinocyte migration and proliferation in vivo and in vitro scratch wound tests. The end products of glycation impair keratinocyte proliferation and migration with effects similar to those induced by high glucose. Reduced migration is associated with high production of MMP9 and decreased expression of the MMP network inhibitor, TIMP. Oxidative stress is a disturbance in the balance of pro-oxidants and antioxidants which is characterized by increased levels of reactive oxygen species. Diabetes increases ROS levels which are harmful to wound healing and inhibits migration and proliferation of keratinocytes. The presence of elevated ROS levels stimulates apoptosis, which may be an additional factor impairing the healing response in diabetes. High ROS levels induce the production of inflammatory cytokines such as TNFα. When TNF is inhibited in diabetic wounds, re-epithelialization is significantly increased. In addition, diabetes causes prolonged expression of chemokines which leads to difficulty in downregulating inflammation, contributing to poor healing outcomes (Wang and Graves, 2020).

Spirulina

Spirulina, a blue-green algae, has been used as a food source since ancient times. Spirulina is commercialized for its abundance of protein, vitamins and minerals. In addition, spirulina has been reported to exhibit pharmaceutical potential due to its anti-inflammatory, antioxidant and anticancer properties (Liu et al., 2020).

The content of flavonoids in spirulina which act as antioxidants can increase collagen fibers by stimulating keratinocytes and increasing DNA synthesis (Mehdinezhad et al., 2021).

Flavonoids are able to increase levels of SOD, CAT, GSH, GST and GPx and reduce the amount of MDA showing antioxidant effects and increasing the wound healing process. Flavonoids that promote the formation of new epithelium are able to increase migration of keratinocytes and attachment of the respective epithelium to the wound. This causes the release of growth factors to promote mitosis and epithelial hyperplasia, ensuring the advancement of the proliferative phase during the healing process (Carvalho et al., 2021).

MATERIALS & METHODS

This research is a laboratory experimental study using a posttest only control group design, using 30 male Wistar rats (Rattus norvegicus) aged 3 months, body weight 180-200 grams, which were divided into five groups, group P1 which was not induced by STZ who were excised and given NACMC gel, group P2 induced by STZ with blood sugar ≥150 mg/dL were excised and given with Gentamycin 0.1% ointment topically, group P3 induced by STZ were excised and given with 10% Spirulina gel topically, group P4 induced by STZ were excised and given with 15% Spirulina gel topically, and group P5 induced by STZ were excised and given with 20% Spirulina gel topically. Thirty (200 ± 20 g and 12 weeks of age) male Wistar rats.

All rats were kept under controlled environmental conditions (24-32°C) in a room with sufficient ventilation, controlled light or dark cycles for 12 hours. Rats were fed standard HPS511 brand feed 20 grams/day. The experimental rats were randomly allocated into 5 groups in 2 experiments (n = 6 in each group), as follows: control group (CG) and Spirulina group (SG) for experiment 1 and control group (CG) and Spirulina group (SG) for experiment 2. After the adaptive period (1 week), the experimental rats (n = 30) were divided into 5 groups (6 rats per group). Four groups of rats were induced by Streptozotocin (45 mg/kg) intraperitoneal. Three days after induction rats 'blood sugar levels were measured with a glucometer, the state of hyperglycemia had not been reached, thus induction of streptozotocin was repeated and observed 3 days later. On the 7th day, all rats reached a state of hyperglycemia. Intraperitoneal injection (IP) of a combination of ketamine (70 mg/kg) and xylazine (7 mg/kg) was used for anesthetizing the rats. The rats back hair was razed and disinfected with ethanol (70%). Full thickness 1.5 × 1.5 cm skin wound excision was done on the back of every rat.

Rats in P1 were excised and the wound was smeared with NA-CMC gel twice a day for 14 days. P2 group was induced by STZ, and excision was performed, the wound was smeared with 0.1% Gentamycin ointment, for 14 days. P3 group was induced by STZ and excision was carried out, the wound was smeared with Spirulina sp. gel. 10% twice a day for 14 days. P4 group 4 was induced by STZ and excision was performed, the wound was smeared with Spirulina sp. gel.

15% twice daily for 14 days. P5 group was induced by STZ and excision was performed, the wound was smeared with Spirulina sp. gel. 20% twice a day for 14 days. After the study on the 14th day all sacrificed rats were euthanized using Ketamine and Xylazine at a dose of 0.5 mL which were injected intramuscularly. A biopsy excision was performed on the scar tissue with a 4 cm square incision with a depth of up to the subcutis.

MDA levels are determined using Colorimetric/Fluorometric test for lipid peroxidation with an ELISA kit which will be calculated in units (nmol/ml) on microplate reader. Collagen density measurement are determined as red fibers in Picrosirius red staining, observed using a CX 41 Optimus microscope with an Optilab camera at 100x magnification at 3 fields of view and input in Adobe Photoshop and Image J, the number of collagens will be calculated in units (% pixel).

STATISTICAL ANALYSIS

The research data were analyzed using SPSS version 26.0 (IBM SPSS Statistics for Windows; IBM Corp., Armonk, NY, USA). The One-Way ANOVA test was used to analyzed the variables for statistical comparison. A P value of less than 0.05 was considered as statistically significant. The best cutoff value was determined using receiver operating characteristics (ROC) analysis.

RESULT

Based on the results of One-Way ANOVA test, the p value of MDA levels <0.001 indicated that there was a significant difference between the 4 matched groups, then a Post Hoc test was performed on LSD to see which group had a significant difference. Malondialdehyde levels between study groups showed significant differences with p values = <0.001 in all group comparisons. The results of One-Way ANOVA analysis on collagen density obtained a p value = 0.116 indicating that there was no significant difference between the groups being compared.

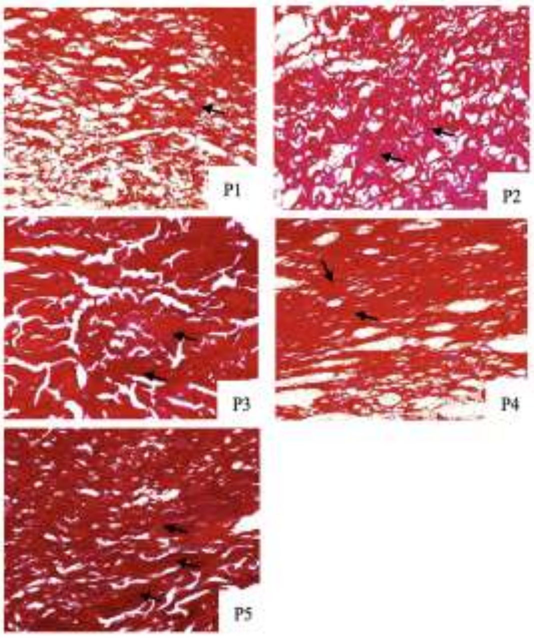
On the variables MDA and collagen density, the descriptive analysis comprises the mean, standard deviation (SD), minimum, and maximum. Effect analysis was tested based on the average MDA and collagen density between groups before and after being given treatment and its increase/decrease. Table 1 displays the findings of the analysis.

Table 1 - Descriptive Analysis Variables MDA and Collagen Density

Variables	Mean	SD	Median	Minimum	Maximum
MDA levels (nmol/ml)					
P1	0,944	0,0064	0,945	0,935	0,952
P2	0,731	0,0027	0,731	0,729	0,736
P3	0,678	0,0065	0,680	0,670	0,686
P4	0,591	0,0022	0,592	0,589	0,594
P5	0,496	0,0059	0,471	0,462	0,476
Collagen density (% pixel)					
P1	31,53	13,27	35,04	13,63	44,76
P2	49,53	19,08	47,75	24,42	73,35
P3	65,97	18,31	67,83	40,12	85,84
P4	64,76	8,12	63,83	56,12	77,50
P5	69,30	7,98	70,59	56,18	78,79

Analysis of the treatment effect was tested based on the MDA and collagen density between groups after being given treatment in the form of excised wound, diabetes induced and Spirulina ethanol extract gel. Malondialdehyde and collagen density data were tested for normality using the Shapiro-Wilk test. The results show that data are normally distributed ($p > 0.05$) and were tested for homogeneity using Levene's test. The findings demonstrate non-homogeneous data for Collagen density ($p < 0.05$) and homogenous data for MDA ($p > 0.05$). Significance analysis using the one-way ANOVA test for MDA and collagen shows that the value of p = <0.001 & p = 0.116. The test for differences in the mean MDA between groups was carried out using the

Post Hoc Least Significant Difference (LSD) test. Based on the difference results on the mean MDA levels between groups, it can be concluded that there were statistically significant differences in MDA levels in all groups because they had a p value < 0.001.



Picture 1. Histological picture of Collagen density in the P5 group was not significantly different compared to the P4, P3, P2 groups (p value = 0.116) (black arrows indicate collagen fibers)

DISCUSSION

Delayed Wound Healing in Diabetes Mellitus

Normal wound healing proceeds through phases of inflammation, proliferation, and remodeling in response to tissue injury. Interference with any of these wound healing phases can cause the wound to become chronic and require some form of intervention. One of the reasons for the delay in the wound healing process is diabetes and aging (Mathew-Steiner et al., 2021).

Diabetes mellitus (DM) is a group of metabolic pathologies characterized by impaired insulin production and/or function, which causes hyperglycemia. Diabetes mellitus greatly affects the quality of life and survival expectations of patients (Spampinato et al., 2020).

Aging and diabetes have important factors including oxidative stress and inflammation. In the early stages of diabetes, there is persistent accumulation of oxidative damage to biomolecules caused by ROS which are produced in all cells especially by mitochondria. Mitochondria are major producers and also major targets of ROS because they damage mitochondrial DNA (mtDNA) leading to increased ROS production. An increase in mtDNA mutations can accelerate aging (Moura et al., 2019).

Diabetic wounds have a microenvironment with increased levels of glucose, advanced glycation end products (AGEs), ROS, and inflammatory cytokines. Oxidative stress is a disturbance in the balance of pro-oxidants and antioxidants which is characterized by increased levels of reactive oxygen species. Diabetes increases ROS levels which are harmful to wound healing and inhibits migration and proliferation of keratinocytes. Elevated ROS levels stimulate apoptosis which impairs the healing response in diabetes. High levels of ROS induce the production of inflammatory cytokines such as TNFα which, when their activity is inhibited, reepithelialization increases significantly. Diabetes also causes prolonged expression of chemokines which causes difficulty in downregulating inflammation, contributing to poor healing outcomes (Wang and Graves, 2020).

The Effect of Spirulina Ethanol Extract Gel on MDA

Malondialdehyde levels in diabetes mellitus increase significantly and cause complications. The reaction of MDA with the lysine and arginine side chains of protein-bound collagen not only affects collagen function by making collagen less susceptible to degradative enzymes but also causes changes in collagen and affects collagen interactions. This MDA reaction interferes with the normal process of wound healing (Oo et al., 2017).

The study by Elbialy showed that Spirulina sp. has potential in wound healing. Spirulina sp. is a blue-green algae that has anti-inflammatory and antioxidant properties that is used as a food supplement and is known as a "Superfood" (Elbialy et al., 2021).

The content of flavonoids in Spirulina sp. able to increase levels of SOD, CAT, GSH, GST and GPx and reduce the amount of MDA showing antioxidant effects and increasing the wound healing process. Flavonoids that promote re-epithelialization are able to increase keratinocyte migration and attachment of the respective epithelium to the wound. This causes the release of growth factors to increase mitosis and epithelial hyperplasia, accelerating the proliferative phase during the healing process (Carvalho et al., 2021).

Flavonoids are able to activate the Ras/Raf/MEK/ERK pathway which promotes proliferation, differentiation and secretion of keratinocytes, and increases the expression of MMP-9 which is an important molecule in reepithelialization and promotes migration through the internal fibroblast (Smad) signaling pathway (Carvalho et al., 2021).

Research conducted by Liu et al (2019) rats with excision wounds that were treated with Spirulina sp. extract. 4% topically showed lower MDA levels compared to the control group. These results indicate that the extract of Spirulina sp. 4% had a positive effect on antioxidants by inhibiting MDA levels during skin wound healing in mice, the MDA value in this group of mice was 0.360 nmol/mg, whereas in the control group the MDA value was 0.980 nmol/mg (Liu et al., 2019).

Gargouri stated in his research, administration of Spirulina to diabetic rats increased the activity of antioxidant enzymes such as CAT and GPx which limited the level of free radicals. Spirulina's antioxidant and protective effects are related to the content of flavonoids, phycocyanins, minerals, vitamins, proteins.

The mean MDA levels in this study were the lowest in the P5 group which received Spirulina sp 20% ethanol extract gel treatment with a yield of 0.470 nmol/mL when compared to the other groups. The highest MDA level was found in the P1 group with a value of 0.944 nmol/ml which received treatment with NaCMC gel smeared on the excision wound, in the macro picture of the excision wound of the P1 group rats there were signs of infection and pus, the wound quality of the P1 group was not good so that the MDA results were in the P1 group tall. The macro picture of the excision wound in group P2 which received Gentamicin ointment, the wound had no signs of infection, no pus, but there was a scar that didn't close properly. The MDA of P2 group rats was 0.731 nmol/mL. The results of the One-Way ANOVA test showed that there was a significant difference with a p value = <0.001, the P5 group had a lower MDA level of 0.262 than the P2 group. Spirulina ethanol extract gel has the ability as an antioxidant to reduce MDA in wound healing in diabetic rat models, while Gentamicin is an aminoglycoside class of antibiotics, does not have antioxidant properties, only has bactericidal properties. These results indicate that Spirulina is an antioxidant and is an antiaging medicine agent by inhibiting the increase in MDA which can fight free radicals.

The Effect of Spirulina Ethanol Extract Gel on Collagen Density

The flavonoid content in spirulina which has anti-inflammatory properties can increase collagen fibers by stimulating keratinocytes and increasing DNA synthesis (Mehdinezhad et al., 2021). C-phycocyanin, purified from spirulina, was reported to increase rat wound healing and fibroblast migration. The effect of spirulina plantesis crude protein (SPCP) on CCD-986sk human fibroblast activity was shown to increase collagen formation in CCD-986sk cells and decrease elastase activity which plays an important positive role in wound repair. Migration and proliferation of CCD-986sk cells have been shown to be driven by SPCP through the phos-phoinositide 3-kinase (PI3K)/protein kinase B (Akt) signaling pathway. The effect of SPCP on human fibroblasts provides a possible application to enhance skin wound repair (Liu et al., 2020).

Research conducted by Liu in 2019, revealed that the expression level of type I collagen was higher in the group of mice with excision wounds treated with 4% Spirulina than in the control group. These results indicate that Spirulina 4% increases the deposition of type I collagen during skin wound healing. Other components of the ECM also play an important role in wound healing, such as fibronectin. Although the ECM plays an important role in wound healing, excessive deposition can lead to fibrosis, scarring, and loss of tissue function. The balance of ECM production is important for perfect wound closure (Liu et al., 2020).

Research by Elbialy on the healing of rat incisions and burns determined by the percentage of the wound surface covered by epidermal regeneration in rats with or without topical Spirulina application showed that wounds treated with Spirulina improved faster. After the 15th day of the incision wound and the 21st day of the burn wound, the wound that was given topical Spirulina did not have a scar, while the control rats had a clear scar. Topical spirulina made a significant contribution to wound healing, compared to the control group.

Histological analysis in Elbialy's study to evaluate the wound healing process on day 15 showed a moderate degree of epithelialization and remodeling of connective tissue with a mild degree of necrosis, granulation tissue formation, and inflammatory cell infiltration in wound healing rats given topical Spirulina. Spirulina can enhance the wound healing process by increasing angiogenesis and collagen deposition (Elbialy et al., 2021).

Wound healing in diabetes is hampered because the ulcerated epidermis causes interference with the extracellular matrix which contributes to the loss of tissue integrity resulting in type I collagen deficiency so that the proliferation and migration of fibroblasts in the wound is hampered.

The results of collagen density in this study obtained a p value = 0.116 indicating that there was no significant difference between the administration of Spirulina ethanol extract gel and the group that was smeared with Gentamicin ointment. The density of collagen in the P4 group that was smeared with 15% Spirulina ethanol extract gel obtained 64.82% pixels was also lower compared to the P3 group that was smeared with 10% Spirulina ethanol extract gel with a yield of 65.97% pixels. Administration of 10% spirulina ethanol extract gel was able to provide an anti-inflammatory effect on wound healing in Wistar rats. Research conducted by Rizqi using Spirulina gel with 96% and 70% ethanol extract on the 3rd day of healing of the gingival wounds of Wistar rats also showed no significant difference.

CONCLUSION

From this study, it is known that administration Spirulina ethanol extract 20% gel was more effective in reducing malondialdehyde levels in wound healing in Wistar rats model diabetes mellitus than the control group. Administration of Spirulina ethanol extract gel did not differ in increasing collagen density compared to control group. Spirulina can be used as an alternative drug with the ability as an antioxidant.

Declaration by Authors

Ethical Approval:
B/219/UN14.2.9/PT.01.04/2022 is the authorization number given by the Animal Ethics Committee of the Faculty of Veterinary Medicine at Udayana University for this study.

Acknowledgement: None

Source of Funding: The author(s) received no financial support for the research, authorship, and/or publication of this article.

Conflict of Interest: The authors declare no conflict of interest.

REFERENCES

1. Blair, M. J. Jones, J D., Woessner, A. E., Quinn, K.P. (2020) 'Skin Structure-Function Relationships and the Wound Healing Response to Intrinsic Aging', *Advances in Wound Care*, 9(3), pp. 127–143. doi: 10.1089/wound.2019.1021.
2. Bolajoko, E. B., Akinosun, O. M. and Khine, A. A. (2020) *Hyperglycemia-induced oxidative stress in the development of diabetic foot ulcers*, *Diabetes*. INC, pp. 35-48. doi: 10.1016/b978-0-12-815776-3.00004-8.
3. Bonifant, H. and Holloway, S. (2019) 'A review of the effects of ageing on skin integrity and wound healing', *British Journal of Community Nursing*, 24(March), pp. S28–S33. doi: 10.12968/bjcn.2019.24.Sup3.S28.
4. Burhan, H. W., Mewo, Y. M. and Assa, Y. A. (2021) 'Efek Antioksidan dari C-Fikosianin pada Spirulina', *Jurnal e-Biomedik*, 9(1), pp. 131–138. doi: 10.35790/ebm.v9i1.31908.
5. Cañedo-Dorantes, L. and Cañedo-Ayala, M. (2019) 'Skin acute wound healing: A comprehensive review', *International Journal of Inflammation*, 2019, pp. 1-15. doi: 10.1155/2019/3706315.
6. Carvalho, M. T. B., Araujo-Filho H., Barreto A. (2021) 'Wound healing properties of flavonoids: A systematic review highlighting the mechanisms of action', *Phytomedicine*, 90(5), pp. 1-15. doi: 10.1016/j.phymed.2021.153636.
7. Chen, L. Y. *et al.* (2020) 'Effects of rutin on wound healing in hyperglycemic rats', *Antioxidants*, 9(11), pp. 1–13. doi: 10.3390/antiox9111122.
8. Davison-Kotler, E., Marshall, W. S. and García-Gareta, E. (2019) 'Sources of collagen for biomaterials in skin wound healing', *Bioengineering*, 6(3), pp. 1–15. doi: 10.3390/bioengineering6030056.
9. Deng, L., Du, C., Song, P. (2021) 'The Role of Oxidative Stress and Antioxidants in Diabetic Wound Healing', *Oxidative Medicine and Cellular Longevity*, 2021(Figure 1), pp. 1-11. doi: 10.1155/2021/8852759.
10. Elbialy, Z. I. Assar, D., Abdelnaby, A. (2021) 'Healing potential of Spirulina platensis for skin wounds by modulating bFGF, VEGF, TGF-ß1 and α-SMA genes expression targeting angiogenesis and scar tissue formation in the rat model', *Biomedicine and Pharmacotherapy*, 137(November 2020), p.111349. doi: 10.1016/j.biopha.2021.111349.
11. Felician, F. F., Yu, R., Li, M. (2019) 'The wound healing potential of collagen peptides derived from the jellyfish Rhopilema esculentum', *Chinese Journal of Traumatology-English Edition*, 22(1), pp.12–20. doi:10.1016/j.cjtee.2018.10.004.
12. Gargouri, M., Magné, C. and El Feki, A. (2016) 'Hyperglycemia, oxidative stress, liver damage and dysfunction in alloxan-induced diabetic rat are prevented by Spirulina supplementation', *Nutrition Research*, 36(11), pp. 1255–1268. doi: 10.1016/j.nutres.2016.09.011.
13. Geahcahan, S., Baharlouei, P. and Rahman, A. (2022) 'Marine Collagen : A promising biomaterial for wound healing ', *Marine Drugs*, 20(61).
14. Gershwin, M. E. and Belay, A. (2008) 'Spirulina in Human Nutrition and Health', *CRC Press*.
15. Gunawan, I. (2013) 'Percobaan Tikus (Rattus novergicus)', *Skripsi*.
16. Gunes, S., Tamburaci, S., Dalay, M. (2017) 'In vitro evaluation of spirulina platensis extract incorporated skin cream with its wound healing and antioxidant activities', *Pharmaceutical Biology*, 55(1), pp. 1824–1832. doi: 10.1080/13880209.2017.1331249.
17. Gushiken, L. F. S., Beserra, F., Bastos, J. (2021) 'Cutaneous wound healing: An update from physiopathology to current therapies', *Life*, 11(7), pp. 1–15. doi: 10.3390/life11070665.
18. Hajati, H. and Zaghari, M. (2019) Spirulina Platensis in Poultry Nutrition, *Cambridge Scholars Publishing Lady*. Cambridge Scholars Publishing Lady.
19. Herman, T. F. and Bordoni, B. (2022) 'Wound Classification', *StatPearls* Finlandia. *Available from* : https://www.ncbi.nlm.nih.gov/books/NBK554456/
20. Islam, M., Rupeshkumar, M. and Reddy, K. B. (2017) 'Streptozotocin Is More Convenient Than Alloxan for The Induction of Type 2 Diabetes', *International Journal of Pharmacological Research*, 7(1), pp. 6–11. Available at: https://dx.doi.org/10.7439/ijpr.

21. Kangal, M. K. O. and Regan, J.-P. (2022) 'Wound Healing', *StatPearls [Internet]*. Finlandia. *Available from :* https://www.ncbi.nlm.nih.gov/books/NBK535406/
22. Kumar, A., Ramamoorthy, D., Verma, D. K., Kumar, A., Kumar, N., Kanak, K. R., Marwein, B. M., Mohan, K. (2022) 'Antioxidant and phytonutrient activities of Spirulina platensis', *Energy Nexus*, 6(April), p. 100070. doi: 10.1016/j.nexus.2022.100070.
23. Labib, A., Rosen, J. and Yosipovitch, G. (2022) 'Skin Manifestations of Diabetes Mellitus', *Endotext [Internet]. South Dartmouth. Available from :* https://www.ncbi.nlm.nih.gov/books/NBK481900/
24. Liu, P., Choi, J., Lee, M., Choi, Y., Nam, T. (2019) 'Wound healing potential of spirulina protein on CCD-986sk cells', *Marine Drugs*, 17(2), pp. 1–14. doi: 10.3390/md17020130.
25. Liu, P. Choi, J., Lee, M., Choi, Y., Nam, T. (2020) 'Spirulina protein promotes skin wound repair in a mouse model of full-thickness dermal excisional wound', *International Journal of Molecular Medicine*, 46(1), pp. 351–359. doi: 10.3892/ijmm.2020.4571.
26. Mathew-Steiner, S. S., Roy, S. and Sen, C. K. (2021) 'Collagen in wound healing', *Bioengineering*, 8(63), pp.1-15. doi: 10.3390/bioengineering8050063.
27. Mehdinezhad, N. Aryaeian, N., Vafa, M., Saeedpour, A., Ebrahimi, A.,Mobaderi, T., Fahimi, R., Hezaveh, Z. (2021) 'Effect of spirulina and chlorella alone and combined on the healing process of diabetic wounds : an experimental model of diabetic rats'. *Journal of Diabetes and Metabolic Disorders,* Springer Nature Switzerland
28. Mendes, A. L., Haddad, V. and Miot, H. A. (2017) 'Diabetes mellitus and the skin', *Anais Brasileiros de Dermatologia*, 92(1), pp. 8–20. doi: 10.1590/abd1806-4841.20175514.
29. Moura, J. *et al.* (2019) 'Immune aging in diabetes and its implications in wound healing', *Clin Immunol*, 200, pp. 43–54.doi: 10.1016/j.clim.2019.02.002.Immune.
30. Mutiarahmi, C. N., Hartady, T. and Lesmana, R. (2021) 'Use of Mice as Experimental Animals in Laboratories that refer to the Principles of Animal Walfare : A Literature Review', *Indonesia Medicus Veterinus*, 9(3), pp. 418-428. doi: 10.19087/imv.2020.10.1.134.
31. Naomi, R. and Fauzi, M. B. (2020) 'Cellulose/collagen dressings for diabetic foot ulcer: A review', *Pharmaceutics*, 12(9), pp. 1–18. doi: 10.3390/pharmaceutics12090881.
32. Okano, J., Kojima, H., Katagi, M., Nakagawa, T., Nakae, Y., Terashima, T., Kurakane, T., Kubota, M., Maegawa, H. (2016) 'Hyperglycemia induces skin barrier dysfunctions with impairment of epidermal integrity in non-wounded skin of type 1 diabetic mice', *PLoS ONE*, 11(11), pp. 1–22. doi: 10.1371/journal.pone.0166215.
33. Oo, A. M., Lwin, M. O., Kanneppady, S. S., Kanneppady, S. K. (2017) 'Oxidative Stress Marker and Fibrinogen Level as Indicators of Severity of Diabetic Foot Ulcer', *Journal of Health and Allied Sciences NU*, 7(4), pp. 31-34. doi: 10.1055/s-0040-1708733.
34. Park, K. H., Kwon, J. B., Park, J. H., Shin, J. C., Han, S. H., Lee, J. W. (2019) 'Collagen dressing in the treatment of diabetic foot ulcer: A prospective, randomized, placebo-controlled, single-center study', *Diabetes Research and Clinical Practice*, 156, p. 107861. doi: 10.1016/j.diabres.2019.107861.
35. Sengupta, P., (2013) 'The laboratory rat: Relating its age with human's', *International Journal of Preventive Medicine*, 6, pp. 624-630.
36. Snyder, D., Sulliva, N., Margolis, D., Schoelles, K. (2020) 'Technology assessment program-Skin substitutes for treating chronic wounds technical brief', *February 2, 2020,* pp. 1–175. Available at: https://www.cms.gov/Medicare/Coverage/DeterminationProcess/downloads/id109TA.pdf.
37. Soni, R. A., Sudhakar, K. and Rana, R. S. (2017) 'Spirulina – From growth to nutritional product: A review', *Trends in Food Science and Technology*, 69, pp. 157–171. doi: 10.1016/j.tifs.2017.09.010.
38. Spampinato, S. F., Caruso, G. I, Pasquale, R. D., Sortino, M. A., Merlo, S. (2020) 'The treatment of impaired wound healing in diabetes: Looking among old drugs', *Pharmaceuticals*, 13(4), pp. 1–17. doi: 10.3390/ph13040060.
39. Tottoli, E. M., Dorati, R., Genta, I., Chiesa, E., Pisani, S., Conti, B. (2020) 'Skin wound

healing process and new emerging technologies for skin wound care and regeneration', *Pharmaceutics*, 12(8), pp. 1–30. doi: 10.3390/pharmaceutics12080735.

40. Tsikas, D. (2017) 'Assessment of lipid peroxidation by measuring malondialdehyde (MDA) and relatives in biological samples: Analytical and biological challenges', *Analytical Biochemistry*, 524, pp. 13–30. doi: 10.1016/j.ab.2016.10.021.
41. Wang, Y. and Graves, D. T. (2020) 'Keratinocyte function in normal and diabetic wounds and modulation by FoxO1', *Journal of Diabetes Research*, 2020. pp. 1-9. doi: 10.1155/2020/3714704.
42. Widawati, D., Santosa, G. W. and Yudiati, E. (2022) 'Pengaruh Pertumbuhan Spirulina platensis terhadap Kandungan Pigmen beda Salinitias', *Journal of Marine Research*, 11(1), pp. 61–70. doi: 10.14710/jmr.v11i1.30096.
43. Wilkinson, H. N. and Hardman, M. J. (2020) 'Wound healing: cellular mechanisms and pathological outcomes: Cellular Mechanisms of Wound Repair', *Open Biology*, 10(9), pp. 1-14. doi: 10.1098/rsob.200223

How to cite this article: Jeane Cynthia Wongso, Agung Wiwiek Indrayani, Ni Made Linawati, Ida Ayu Ika wahyuniari, Susy Purnawati, Putu Ayu Asri Damayanti. Administration of spirulina ethanol extract gel made malondialdehyde levels lower and collagen density higher in wound healing Wistar rat model diabetes mellitus. *International Journal of Research and Review*. 2023; 10(7): 477-487. DOI: *https://doi.org/10.52403/ijrr.20230758*

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