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Wound Healing Potential of Virgin Coconut Oil in Combination With Extract of Neem Leaves (Azadirachta Indica L.) in Diabetic Rat Model

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ABSTRAK

Kadar gula darah yang tinggi pada DM dapat menghambat penyembuhan luka dan meningkatkan risiko infeksi. Terapi ulkus diabetik dengan obat antiinflamasi komersial relatif mahal dan memiliki efek samping. Penggunaan VCO dan ekstrak daun intaran memiliki potensi sebagai alternatif terapi dengan efek antiinflamasi, namun perlu penelitian lebih lanjut terkait dosis yang tepat. Tujuan penelitian ini untuk menganalisis pengaruh pemberian VCO dengan suplementasi ekstrak daun intaran (Azadirachta indica L.) terhadap kadar IL-6 pada penyembuhan luka tikus model DM tipe 2. Penelitian ini merupakan penelitian true experimental dengan post-test only control group design. Penelitian ini menggunakan 25 ekor mencit jantan yang dibagi menjadi 5 kelompok, yaitu: kelompok P1 ekstrak daun Intaran 5%, P2 ekstrak daun intaran 10%, P3 ekstrak daun Intaran 15%, kontrol negatif (NaCl 0.9%), dan kontrol positif (Povidone Iodine). Data dianalisis dengan uji statistik Shapiro Wilk, dilanjutkan Uji One-Way Anova, serta uji konfirmasi yaitu Uji LSD. Hasil penelitian menunjukkan bahwa, terdapat perbedaan bermakna kadar IL-6 antara kelompok perlakuan yang dibuktikan melalui hasil uji statistik One Way ANOVA. Didapatkan nilai signifikan pada uji kadar IL-6 hari ke-4 Sig. 0,003 dan hari ke-14 Sig. 0,046. Dapat disimpulkan bahwa kelompok perlakuan pemberian suplementasi VCO dengan ekstrak daun intaran konsentrasi 10% berpengaruh terhadap penyembuhan luka dengan terjadinya penurunan kadar IL-6 hari ke-14. Penelitian ini memberikan kontribusi penting dalam upaya penyembuhan luka DM melalui penggunaan bahan alami yang terjangkau dan berpotensi rendah efek samping.

ABSTRACT

High blood sugar levels in DM can inhibit wound healing and increase the risk of infection. Diabetic ulcer therapy with commercial anti-inflammatory drugs is relatively expensive and has side effects. VCO and diamond leaf extract have the potential as an alternative therapy with anti-inflammatory effects, but further research is needed regarding the right dose. This study aimed to analyze the impact of VCO administration with supplementation of diamond leaf extract (Azadirachta indica L.) on IL-6 levels in wound healing of type 2 DM model rats. This research is a true experimental research with a post-test-only control group design. This study used 25 male mice which were divided into five groups, namely: group P1 5% Intaran leaf extract, P2 10% Intaran leaf extract, P3 15% Intaran leaf extract, negative control (NaCl 0.9%), and positive control (Povidone Iodine). Data were analyzed using the Shapiro-Wilk statistical test, followed by the One-Way Anova test, the confirmation test, and the LSD test. The results showed a significant difference in IL-6 levels between the treatment groups, as evidenced by the One Way ANOVA statistical test results. Considerable value was obtained in the test of IL-6 levels on day 4 Sig. 0.003 and day 14 Sig. 0,046. It can be concluded that the treatment group giving VCO supplementation with intern leaf extract at a concentration of 10% affects wound healing with a decrease in IL-6 levels on day 14. This study provides an important contribution to DM wound healing efforts by using affordable natural ingredients with low potential side effects.

1. INTRODUCTION

Diabetes mellitus (DM) is a chronic metabolic disorder characterized by the body's inability to regulate blood sugar levels effectively due to inadequate insulin production or resistance to insulin. The condition affects the metabolism of carbohydrates, fats, and proteins, impairing various body functions and organs. DM can occur in all age groups, including children, adolescents, and adults, with prevalence increasing globally. One of the serious complications of DM is diabetic ulcers, which are open wounds on the skin that usually appear in the foot area due to damage to blood vessels and nerves. Diabetic ulcers affect about 5-10% of the diabetic population and, if left untreated, can lead to severe infection and amputation. The prevalence of mortality within five years in patients with diabetic neuropathic ulcers, diabetic

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neuroischemic ulcers, and diabetic ischemic ulcers was recorded at 15%, 18%, and 55%, respectively, showing how dangerous these complications are if not treated immediately. The wound healing process involves a complex relationship between cellular, humoral, and connective tissue elements. The phases of wound healing consist of inflammation, proliferation, and maturation, each of which is interrelated. Fibroblasts are the most important cells in remodeling damaged tissue healing. Fibroblasts are the main cellular component of connective tissue and the main synthetic source of protein matrix (Sihotang et al., 2019; Zulkarnain et al., 2023).

The inflammatory phase occurs immediately after trauma and continues until day five post-trauma. In the inflammatory phase, there will be a buildup of neutrophils and increased secretion of proinflammatory cytokines such as Tumor Necrosis Factor-α (TNF-α), Interleukin-1 (IL-6), and Interleukin-6 (IL-6). IL-6 plays a role in regulating inflammatory immune responses and hematopoiesis. Increased levels of IL-6 are associated with inflammation and tissue damage (Salsabila & Sudiono, 2022; Sarihati et al., 2020). The use of commercial anti-inflammatory drugs, such as steroids and nonsteroids, to heal diabetic ulcers is relatively more expensive. Side effects include growth suppression, osteoporosis, aggravating DM disease, susceptibility to infection, and muscle weakness. However, the available topical treatments are still limited. Therefore, other alternative therapies are needed to accelerate the healing of diabetic ulcers from natural ingredients, which are expected to minimize the unwanted side effects of drug use. VCO is known to increase the formation of new blood vessels in the wound-healing process. The anti-inflammatory content of VCO can accelerate the wound-healing process by reducing the wound surface area. VCO also plays a role in improving circulation in diabetic ulcers so that there is an increase in the angiogenesis process and the need for nutrients and oxygen in the wound healing process is well met. The inhibitory power of VCO can be increased by making VCO as hydrolyzed virgin coconut oil or by adding other active ingredients that can be dissolved in VCO (Dafriani et al., 2020; Karta et al., 2022; Suarni & Fitarina, 2019).

The urgency of this study is particularly significant due to the increasing prevalence of diabetic ulcers, which affect approximately 5-10% of individuals with diabetes, leading to high morbidity and mortality rates. The current therapeutic options for diabetic ulcers often have limitations in terms of safety, cost, and effectiveness, necessitating the exploration of alternative therapies that are not only safer but also more affordable. Therefore, this study specifically aims to investigate the impact of Virgin Coconut Oil (VCO) supplementation with neem leaf extract on the regulation of IL-6 levels during the wound healing process in type 2 diabetic rat models. The findings of this research are anticipated to contribute to the development of a novel, natural-based therapy for diabetic wounds that offers enhanced efficacy with minimal side effects. Neem leaves, or *Azadirachta indica*, are known for their wide array of pharmacological properties, including anti-inflammatory, antitumor, diuretic, antifungal, insecticidal, antibacterial, antimalarial, and mosquito larvicidal effects. Phytochemical analysis of neem leaf extract has revealed the presence of several bioactive compounds such as alkaloids, terpenoids, steroids, saponins, tannins, and flavonoids, which have been shown to work synergistically with VCO as a carrier oil to enhance its therapeutic potential (Ibrenna et al., 2023; Jaharuddin, 2020; Jasmine et al., 2022; Karta & Burhannuddin, 2022; Kumari & Singh, 2024). VCO, which is rich in medium-chain fatty acids, serves as an ideal carrier oil for neem extract, allowing for optimal absorption into the skin without causing irritation.

However, it is important to note that neem leaves also contain certain toxic compounds that can have adverse effects, such as hepatotoxicity and nephrotoxicity, if not administered in the correct dosages (Jayalakshmi et al., 2021; Karta et al., 2022; L.R. et al., 2019; Tauheed et al., 2021). This highlights the importance of precise dosing when using neem as a medicinal agent. Given the promising phytochemical profile of neem leaves in promoting wound healing, this study seeks to rigorously assess the combined efficacy of neem leaf extract and VCO in expediting the wound healing process, particularly in diabetic wound models, where impaired healing and chronic inflammation are significant concerns. This research is expected to offer valuable insights into the formulation of a potent, cost-effective, and safe diabetic wound therapy that addresses the limitations of current treatments.

2. METHOD

This experimental study using the Posttest Only Control Group Design which consists of two groups (Husni, 2020; Maryam et al., 2021). This research was conducted in the immunology laboratory of the Poltekkes Kemenkes Denpasar Jl. Sanitation No. 1, Sidakarya, South Denpasar, Denpasar City and the Laboratory of Maintenance and Breeding of Bio Mice and Rat Experimental Animals. Ethical approval for all procedures involving animals was granted by the Ethics Committee of Poltekkes Kemenkes Denpasar (No. DP.04.02/F.XXXII.25/ 0093 /2024). The samples used in this study were utilized white rat population with male sex. Data collection was carried out by measuring immunologically through quantitative analysis of antigen-antibody reactions. The Shapiro-Wilk test was used to determine whether the data were normally

distributed; otherwise, the Kolmogorov Smirnov test was used (Danush K. Wijekularathna & Scariano, 2022; Okoye & Hosseini, 2024). After the data were normally distributed, One Way ANOVA test was conducted to determine the difference in IL-6 levels in mice between the control and treatment groups, followed by Least Significantly Difference (LSD) test to confirm the difference. The materials used in this study include male white rats aged 2-3 months weighing 100-120 gr, standard feed and drinking water, NaCl 0.9%, Streptozocin (STZ), sterile bandages, wound closure plasters, and supplementation materials such as Neem leaves, ethanol, and Virgin Coconut Oil (VCO).

The preparation of Neem leaves simplicial and extracts began with washing the leaves, drying them in the open air, baking for 24 hours, crushing, and extracting with ethanol for seven days. Preparation of VCO supplementation was carried out by making 5%, 10%, and 15% concentrations of pomegranate leaf extract using the formula %b/b to produce a total mass of mixture per concentration of 5 g. Grouping of experimental animals involved 25 rats divided into five groups, that are, negative control (NaCl 0.9%), positive control (povidone-iodine), and three treatment groups with concentrations of 5%, 10%, and 15%. Body weight and STZ administration were performed by weighing the body weight of the rats and injecting STZ 40 mg/kg to induce diabetes. Fasting blood sugar was measured by taking blood from the lateral vein after the rats were fasted for 10 hours. The excision wound was made by shaving, cleaning, and sterilizing the rat's back, then making a circular wound with a diameter of 5 mm. The treatment and wound diameter measurements were carried out daily by measuring the wound diameter vertically, horizontally, and diagonally. Blood sampling was performed on the 4th and 14th days through the retro-orbital sinus, then centrifuged. A Sandwich ELISA test was performed for further analysis.

3. RESULT AND DISCUSSION

Result

Wound Diameter Analysis on VCO Supplementation with Neem Leaves Extract (Azadirachta Indica L.)

The average results of wound diameter measurements that have been carried out in this study are shown in Table 1, and Figure 1.

Table I. Average Wound Diameter in Each Group

Group	n	Wound Diameter Measurement Day (mm)			Asymp. Sig One Way Anova
		Day 1	Day 7	Day 14	
K (-)	4	5.00	4.07	1.58	
K (+)	4	5.00	2.55	0.83	
P1	4	5.00	4.57	1.21	0.008
P2	4	5.00	3.04	0.40	
P3	4	5.00	4.36	1.98	

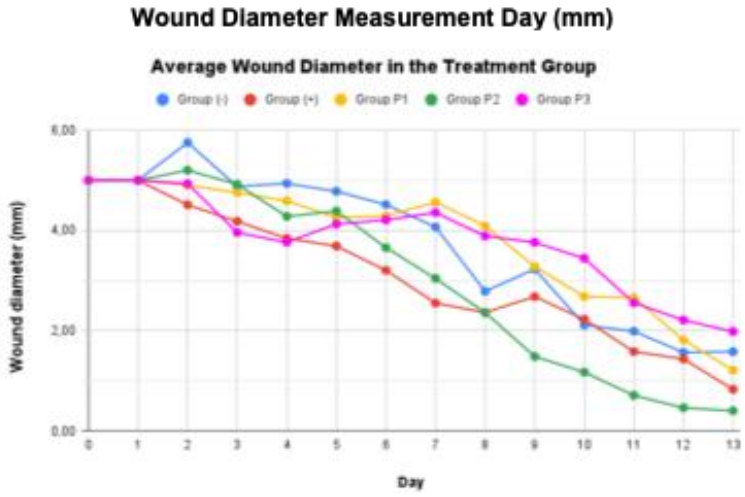

Figure 1. Average Wound Diameter of the Groups

In group P2, the green line with the treatment of VCO supplementation with 10% Neem leaves extract showed the fastest wound closure with an average wound diameter of 0.40 mm. Based on the results

of the study, the diagram above shows that the K (-) and P3 groups have the highest average wound diameter compared to other groups. At P2 group has the average wound diameter smallest, so the P2 group with group P2 with the treatment of VCO supplementation with Neem leaves extract with a concentration of 10% has macroscopically significant activity in healing type 2 diabetes mellitus wounds. VCO supplementation with Neem leaves extract plays a role in shortening the inflammatory phase compared to the K (-) and K (+) groups.

Effect of VCO Supplementation with Neem Leaves Extract (Azadirachta Indica L.) on IL-6 Levels

Table 2. Mean Difference in IL-6 Levels at Day 4 and Day 14.

Group	n	IL-6 Day 4 (mg/dL)	IL-6 Day 4 (mg/dL)	% Decrease in IL-6 Levels
K (-)	4	15.310	10.602	30.75
K (+)	4	13.512	10.509	22.23
P1	4	15.042	9.184	38.94
P2	4	16.087	9.041	43.80
P3	4	17.443	9.812	43.75
Asymp. Sig One Way Anova		**0.003**	**0.046**	

Table 3. Post Hoc LSD (Least Significant Difference) Test Results of the Average Measurement of IL-6 Levels on Day 4 and Day 14

Group Result of IL-6 Levels on Day 4	K (-)	K (+)	P1	P2	P3
K (-)	-	0.036	0.851	0.037	0.099
K (+)	0.036	-	0.025	0.001	0.000
P1	0.851	0.025	-	0.053	0.138
P2	0.037	0.001	0.053	-	0.603
P3	0.099	0.000	0.138	0.603	-
IL-6 Level Result Group 14th Day	**K (-)**	**K (+)**	**P1**	**P2**	**P3**
K (-)	-	0.097	0.010	0. 007	0.108
K (+)	0.097	-	0.251	0. 196	0.953
P1	0.010	0.251	-	0.876	0.229
P2	0.007	0.196	0.876	-	0.178
P3	0.108	0.953	0.229	0.178	-

Based on Table 3, it is known that the difference in mean IL-6 levels on day 4 is most significant in groups K (+) and P3 with a difference in mean IL-6 levels Sig. 0.000. It is known that the average difference in IL-6 levels on day 14 is most significant in group K (-) and P2 with an average difference in IL-6 levels Sig. 0.007.

Discussion

One of the factors in the wound healing process is the immune system that plays a role in recognizing and fighting antigens from the wound, high blood sugar levels can cause nutrients to not be able to enter the cells so that protein and calories decrease, rehydration and wound washing, lack of nutrients, low blood albumin levels, oxygen supply and vascularization, pain that can produce glucocorticoid hormones which play a role in inhibiting the wound healing process, and corticosteroids (Dinparastisaleh & Mirsaeidi, 2021; Krawiec et al., 2023; Kumar et al., 2023; Nasrine et al., 2023; Nurmalasari et al., 2020; Radzikowska-Büchner et al., 2023). Wounds cause inflammation which results in the production of free radicals by phagocytic cells. Increased free radical production can delay the wound healing process, so strategies are needed that can inhibit free radical production with the aim that the therapy can function in action. wound healing. Macrophages are the main cells that factor into wound healing. These phagocytic cells actively remove foreign bodies in the wound area such as bacteria. After that, fibroblasts emerge from the wound tissue and endothelial cells that migrate towards the wound, these fibroblasts play a role in increasing tissue permeability and collagen fiber production (Asrul et al., 2023; Huang & Ogawa, 2020; Spielman et al., 2023).

The presence of alkaloid, terpenoid and steroid compounds, saponins, tannins, and flavonoids in VCO supplementation with Neem leaves extract. The active ingredients in the extract were able to collaborate with VCO. Semi-quantitatively, this mixture contains a lot of tannins and flavonoids that can be used as a supplement to helps the wound healing process of diabetes mellitus rats through antioxidant,

antimicrobial, astringents, and anti-inflammatory activities. The anti-inflammatory content of VCO can improve the wound healing process by reducing the surface area of the wound. This study shows that the P2 group has the fastest average wound healing and is in line with the research of other researchers who state that VCO supplementation with neem leaf extract with a concentration of 10% has the highest inhibition zone diameter with a strong category in inhibiting the growth of P. acnes (Dafriani et al., 2020; Karta et al., 2022; Karta & Burhannuddin, 2022). This study also mentioned that the higher the extract of Neem leaves in VCO does not necessarily have an impact on increasing the strength of inhibition due to the solubility factor and saturation of extracts in the mixture. The higher the concentration, the more saturated the mixture and the absorption is not optimally distributed.

This study showed that the mean ratio of IL-6 levels on day 4, which is the peak of inflammation, was higher in the VCO supplementation group with 15% pomegranate leaf extract at 17.443 mg/dL compared to the mean ratio in the positive control group at 13.512 mg/dL. VCO supplementation with pomegranate leaf extract has an effect on IL-6 levels in type 2 diabetes mellitus model rats. The effect can be seen from the IL-6 levels on day 4 significantly which is addressed by the p value of $p < 0.05$, namely $p < 0.003$. In group K (+) with povidone iodine treatment showed the lowest IL-6 levels due to povidone iodine only having an antimicrobial effect. In line with research by other researchers who state that, povidone iodine is a complex iodine that acts as an antiseptic, which is able to kill microorganisms. The ability of povidone iodine in the inflammatory process is to inhibit IL-1 beta and IL-8. In the P2 group with the treatment of VCO supplementation with 10% Neem leaves extract, the average IL-6 levels decreased faster. The mean IL-6 level in the positive control group was 9.041 mg/dL compared to the 14th day, which was 10.509 mg/dL. The table shows that the P2 group experienced a decrease of 43.80% faster than the K (-) group which experienced a decrease of 22.23%. The role of IL-6 as a pleiotropic cytokine that carries out pro- and anti-inflammatory activities. In the research of other researches mentioned that, IL-6 is considered a pro-inflammatory cytokine and IL-6 also has regenerative and anti-inflammatory activities (Ferdina et al., 2022; Hirano, 2020; Niculet et al., 2021; Spielman et al., 2023).

This study also explains that IL-6 levels will increase at the peak of inflammation. In the inflammatory phase, pro- and anti-inflammatory levels must be balanced because they are important for immune system activity . Neem leaves extract contains flavonoid compounds that have the ability to inhibit nitric oxide (NO) activity, which inhibits IL-6 expression. NO is a free radical with odd electrons that can bind to other molecules such as oxygen, superoxide, and transition metals. As a result, excessive production of NO can lead to increased expression of IL-6 which is associated with various cardiovascular pathogenesis and complications. The presence of IL-6 on day 14 in the wound healing process because on day 7 to day 14 is a proliferation phase that still requires IL-6 as a receptor in stimulating keratinocytes that play a role in the wound healing process. The proliferation phase occurs around day 4 to day 14 after trauma or injury which is characterized by the formation of granulation tissue. IL-6 is needed in the wound healing process in the proliferation phase because IL-6 can stimulate epithelial proliferation. A decrease in IL-6 levels indicates that IL-6 is successful in controlling systemic inflammation and will return to normal because the presence of IL-6 is no longer needed. IL-6 concentration must decrease because the remodeling phase does not require IL-6 and is a sign that there is no infection or chronic inflammation in the wound (Hafizsha et al., 2021; Johnson et al., 2020).

The main obstacle in this study to putting topical therapy into practice is its cost, as it is more expensive than commercial drugs due to the preparation of the extract. More research is required to assess the efficacy and potency of topical interleukin therapy in DM wounds as well as other cost-effective options. Further research is required to study the available treatment modalities for a diabetic wound with respect to possible drug therapies, the cytokine environment of the wound and the side effects of such treatments. Thus, the implications of this study show great potential for developing topical therapies that combine Neem leaf extract and VCO (Virgin Coconut Oil) to accelerate the wound healing process, especially in patients with diabetes mellitus. This study opens up opportunities for the application of natural ingredient-based therapies that are safer and have fewer side effects than commercial drugs such as corticosteroids, which are known to have long-term risks such as osteoporosis and decreased immune function. The results of this study also provide evidence that using a combination of VCO and Neem leaf extract can reduce the production of free radicals and accelerate the reduction of IL-6 levels, which is an indicator of inflammation.

Therefore, with the success of this study, it can be implied that this therapy can be developed as a more affordable alternative for diabetic patients with chronic wounds, especially in areas with limited access to expensive medical care. Furthermore, it could support a more holistic treatment approach by combining natural ingredients' anti-inflammatory, antioxidant, and antimicrobial activities. Further research is needed to explore the mechanism of action of bioactive compounds contained in Neem leaf extract and VCO further, as well as to optimize the appropriate dosage so that the therapy can be applied with better efficacy and safety. In addition, the implication for the clinical world is that this therapy can help

reduce the number of complications associated with diabetic ulcers and improve patients' quality of life. However, the main challenge is the higher cost of producing these natural extract-based therapies than commercial drugs. Therefore, further research is needed to explore more cost-efficient alternatives and strengthen the scientific evidence regarding the potential clinical application of this therapy on a wider scale.

4. CONCLUSION

Based on the findings of this study, it can be conceptually concluded that the combination of Neem leaf extract and Virgin Coconut Oil (VCO) has the potential to enhance the wound healing process in diabetic conditions. The active compounds in Neem leaves, particularly at a 10% concentration, appear to work synergistically with VCO to regulate inflammation, as indicated by their role in modulating immune responses such as IL-6 activity. This suggests that the integration of natural ingredients like Neem leaves and VCO could offer an alternative therapeutic approach for managing diabetic wounds, providing a balance between efficacy and safety.

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