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Original Article

POTENTIAL FOR DIABETIC WOUND HEALING WITH HYDROGEL PATCH OF NEEM LEAF EXTRACT (*AZADIRACHTA INDICA*)

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ABSTRACT

Objective: Disruptions in the wound healing process occur because of two main factors in diabetes: high blood sugar levels and chronic inflammation. This study evaluated the effectiveness of hydrogel patches containing neem leaf extract in the healing of diabetic wounds.

Methods: The experimental method used a post-test control group design that included extract preparation, phytochemical screening, hydrogel patch preparation, stability testing, and testing of hyperglycemic rats by observing wound size, histopathology, and TNF-α and TGF-β cytokine levels on days 7, 14, and 21.

Results: The neem leaf extract contains secondary metabolites that support anti-inflammatory and wound-healing activities. Stability tests showed that the hydrogel patch met the standards for organoleptic properties, weight uniformity, and moisture absorption. The group given NLEHP (Neem Leaf Extract Hydrogel Patch) showed a decrease in wound area (0.5±0.1 cm^2) not significantly different from the non-diabetic group with a reduction (0.5±0.1 cm^2) ($p>0.05$) and significantly different from the negative group with a decrease (1.1±0 cm^2) ($p<0.05$). Histopathological analysis of skin thickness of the NLEHP group (19.2±1.6 µm) was not significantly different from the non-diabetic group (18.2±0.8 µm) ($p>0.05$) and significantly higher than the negative control group (10.7±2.4 µm) ($p<0.05$). The TNF-α levels showed a significant increase ($p<0.05$) in the negative control group, whereas the non-diabetic and NLEHP groups showed a non-significant decrease ($p>0.05$). The levels of TGF-β progressively increased in the non-diabetic and NLEHP groups, unlike the decline observed in the negative control.

Conclusion: These results indicate that the neem leaf extract hydrogel patch is an effective alternative for diabetic wound healing therapy.

Keywords: *Azadirachta indica*, Anti-inflammatory, Hydrogel patch, Diabetic wound healing

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INTRODUCTION

Diabetic wounds are a complication of diabetes mellitus caused by several factors, including nerve damage (neuropathy) and peripheral arterial disease caused by uncontrolled high blood sugar levels. These wounds are difficult to heal and can lead to serious infections and tissue death (gangrene) [1, 2]. Hyperglycemia can inhibit healing through cell dysfunction, impaired production of growth factors, and increased oxidative stress in the impaired diabetic wound healing process, macrophages produce excessive pro-inflammatory cytokines and have difficulty transitioning into anti-inflammatory cytokines. TNF-α is a proinflammatory cytokine produced by macrophages and other immune cells. TNF-α levels are often elevated in diabetic wounds, which can prolong the inflammatory phase and hinder healing. Elevated levels of TNF-α contribute to insulin resistance and increased oxidative stress, which worsen wound conditions [3, 4]. In contrast, TGF-β functions as an anti-inflammatory mediator and plays a role in the proliferative phase of wound healing by stimulating the synthesis of collagen and other extracellular matrix components. TGF-β levels are often impaired in diabetic wounds, which can inhibit tissue regeneration. Increased TGF-β levels can help improve the healing process by reducing inflammation and promoting new tissue formation [5]. In diabetic wounds, there is an imbalance between TNF-α and TGF-β levels. TNF-α levels tend to be high, which can prolong the inflammatory phase and inhibit wound healing. The production of TGF-β, which is often disrupted, can effectively reduce the effect of wound healing.

Diabetic wound healing requires comprehensive therapeutic interventions to address various cellular and molecular abnormalities that occur. Neem leaf extract *(Azadirachta indica)* is a phytotherapeutic agent that can be investigated for its potential use in diabetic wound management. Neem is an herbal plant that is rich in benefits and contains active components such as nimbidin, nimbin, and nimbidol, which have antibacterial, anti-inflammatory, antiviral, and antifungal properties that play an important role in optimizing wound healing. The high mineral, vitamin, and amino acid content in this plant also supports the formation of new pigments during the proliferation phase [6, 7].

To increase the effectiveness of the topical use of leaf extracts, hydrogel patch preparations can be prepared. Hydrogels with three-dimensional tissue structures are soft materials that can be physically and chemically customized for biomedical applications. Hydrogels have characteristics that resemble the natural extracellular matrix, making them an optimal choice for diabetic wound management. Hydrogel patch-based delivery systems offer several advantages, including ease of application, reduced frequency of drug administration, maintenance of bioavailability, and prevention of first-pass metabolism effects. These characteristics result in a significant reduction in the amount of drug entering the systemic circulation, increasing the overall therapeutic efficacy [7-10]. In contrast to other topical preparations, hydrogels possess a high water content that facilitates the formation of an optimal moist environment for wound healing, controlled drug release through passive diffusion and polymer matrix degradation, and viscoelastic properties that facilitate good skin adhesion and enhance transdermal penetration [11].

MATERIALS AND METHODS

Material

The materials used in this study are simplicia of neem leaves (*Azadirachta indica*) obtained from Tanggamus District, Lampung, 96% ethanol (analytical grade), aquadest, HPMC, PVA, Polyethylene Glycol (PEG 400) (pharmaceutical grade), concentrated HCl, NaOH, Mg powder, Mayer's reagent, Wagner's reagent, Dragendorff's reagent, $FeCl_3$, 2 N HCl, chloroform, and sulfuric acid (analytical grade), (hematoxylin and eosin (HE) (histological grade), TNF-α (Elikine™), TGF-β (Elikine™), Protein Extraction (ExKine™), GOD-PAP reagent (®Dyasis), and alloxan monohydrate (Sigma-Aldrich®).

Methods

This research is based on Laboratory Experimental with posttest control group design.

Preparation of extracts and identification of metabolite compounds of neem leaf extracts

The process of preparing neem leaf extract and identifying metabolite compounds was carried out by smoothing the dried leaves, macerating them using 96% ethanol, and thickening the filtrate with a rotary evaporator. The thick extract obtained was tested by phytochemical screening for flavonoids, alkaloids, tannins, saponins, steroids, and terpenoids. A clearer procedure can be seen in fig. 1 [11-15]:

Hydrogel patch preparation

The procedure for making the hydrogel patch from neem leaf extract begins with dissolving 5 g of PVA in distilled water at 80 °C using a magnetic stirrer. Separately, 1 g of HPMC was slowly added with stirring at a speed of 50 rpm for 50 min. Five (5)g of neem leaf extract and Aquadest were added to a volume of 50 ml while stirring at a temperature of 40 °C, followed by the addition of 2 ml of PEG 400 and 1 ml of glycerin. The preparation was then cast using a primary hydrogel patch with a specific thickness and dried at 40 °C for 24 h. Stability tests include organoleptic observations (color, odor, shape), weight uniformity, and moisture absorption by storing the patch in a desiccator for 24 h at 40 °C in a climate chamber. For more details, please refer to fig. 2 [12, 13].

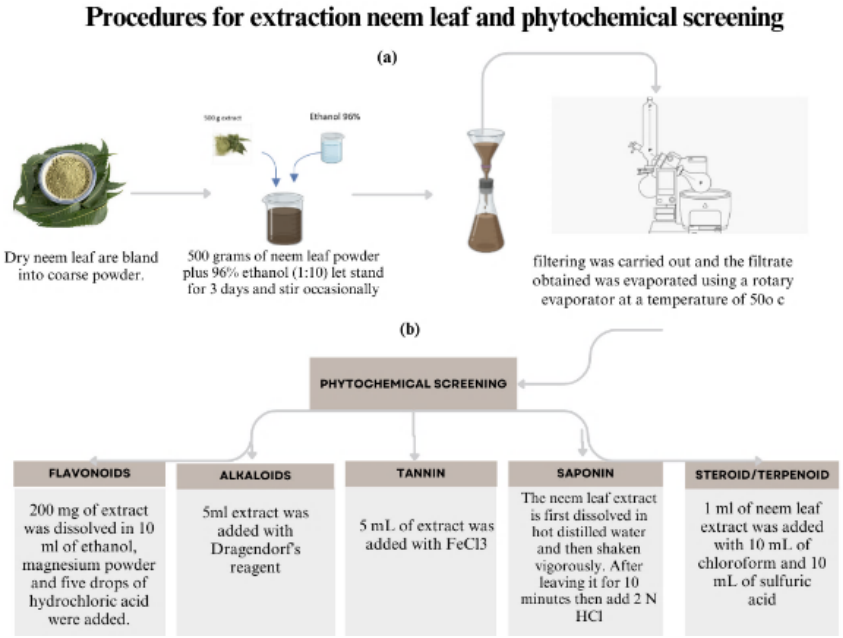

Fig. 1: (a) Extract preparation procedure and identification of metabolite compounds of neem leaf extract, (b) Phytochemical screening, which includes flavonoid, alkaloid, tannin, saponin, steroid and terpenoid tests

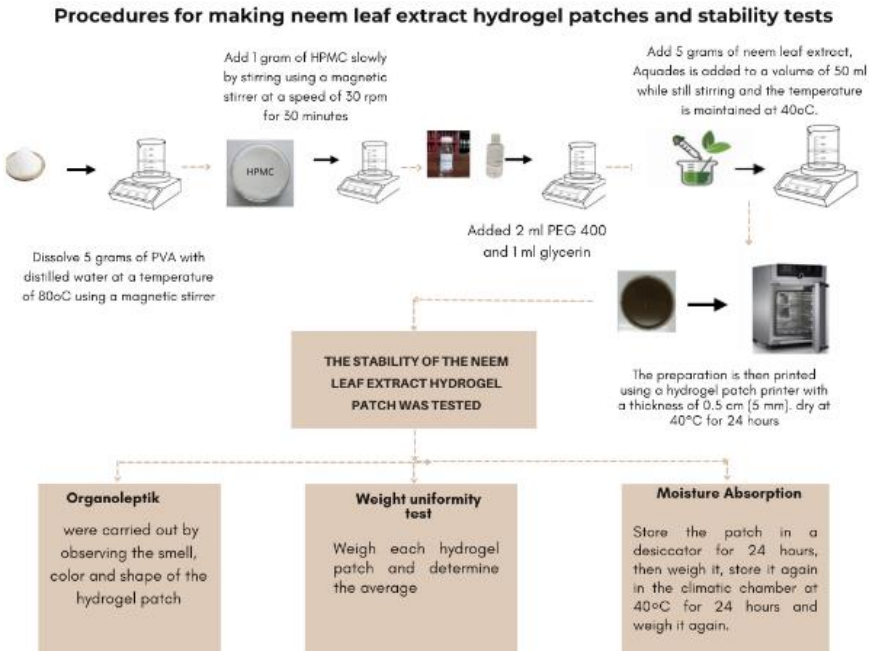

Fig. 2: Procedure for making hydrogel patch from neem leaf extract and stability test

Animal modelling

The research has received ethical approval (No.5262/B.1/KEPK-FKUMS/V11/2024). Using male Wistar white rats obtained from the UMS Pharmacology Laboratory, aged 2-3 mo (200-300g), maintained at a temperature of 25±1 °C with ad libitum access to food and water [18].

Preparation of test animal models

The manufacture of test animal models and treatment of test animals is carried out by making hyperglycemia in test animals using alloxan according to the dose, after the test animals are said to be hyperglycemia, then proceed with the manufacture of excision wound models. For clear procedures can be seen in fig. 3 [14-21]:

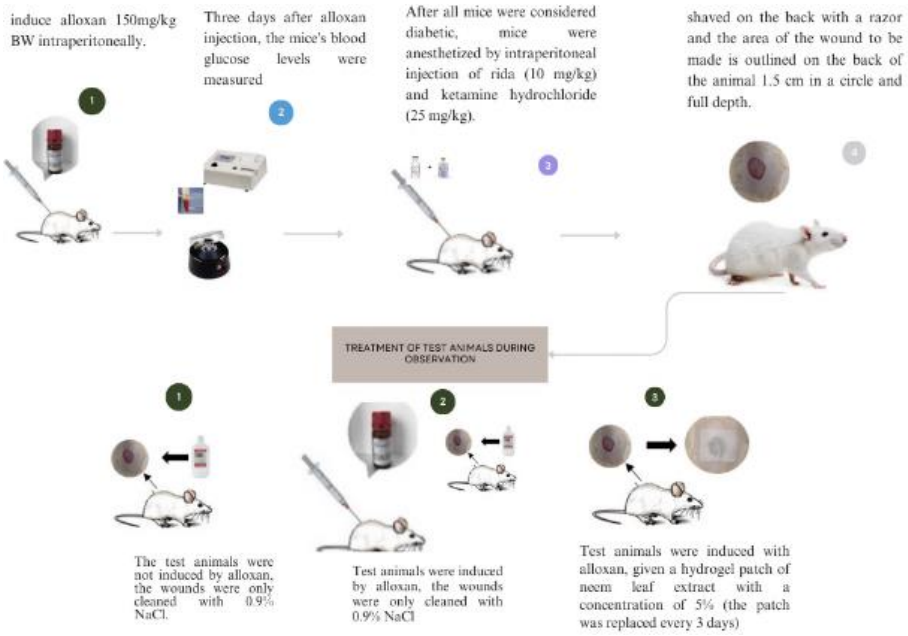

Fig. 3: Test animal model and treatment of test animals for 21

Wound healing measurement

Wound healing was assessed by measuring the wound area at fixed time interval [19]. The wound area was calculated at regular intervals on days 0, 7, 14, and 21, and histopathology assays using formalin-fixed wound tissue samples were cut into 4 µm sections and stained with hematoxylin and eosin (H and E) for histopathological examination. Tissues were graded based on epidermal thickness [17-23], cytokine assay by taking wound tissue biopsies, transferring them to tubes containing protease inhibitor buffer for ELISA, centrifuging, and using tissue homogenates or cell culture supernatants for TNF-α (Tumor Necrosis Factor-alpha) and TGF-β1 expression assays [24].

Statistical analysis

Phytochemical screening data of neem leaves and evaluation of hydrogel patches were analyzed descriptively because they are qualitative data that describe the characteristics of the samples. For wound healing data, the analysis began with the Shapiro-Wilk normality test to determine the distribution of the data normally distributed data, followed by one-way ANOVA test to compare differences between groups, followed by the Tukey test to identify significantly different groups.

RESULTS AND DISCUSSION

Phytochemical screening results

The results of phytochemical screening are shown in (table 1).

Hydrogel patch evaluation

Organoleptic test

The organoleptic test for the neem leaf extract hydrogel patch assessed the product characteristics using the five senses. The following is an image of the appearance of the neem leaf extract hydrogel patches cut to a size of 2 cm.

Table 1: Phytochemical screening

Metabolite compounds	Reagent	Observation results	Information
Flavonoid	Concentrated Magnesium and Hydrogen Chloride Powder	There is a red color	+
Alkaloid	Hydrogen Chloride and Dragendr of reagent	Orange precipitate formed	+
Saponin	Hydrogen chloride 2N	Foam-shaped 1 cm	+
Tannin	FeCl3(Ferric Chloride)	Blue or blackish-green hue	+
Terpenoid	Chloroform and sulfuric acid	Reddish-brown color	+
Steroid		The top layer is red and the bottom layer is greenish	+

Notes:+= Positive,-= Negative

The Organoleptic evaluation of the neem leaf extract hydrogel patch, with a combination of PVA and HPMC polymers, shown in fig. 4, shows that the product has a distinctive odor, blackish-green color, and chewy texture. This indicates that the hydrogel patch produced was of good physical quality.

Weight uniformity and moisture absorbency

The results of the weight uniformity test and moisture absorption of the neem leaf extract hydrogel patches are presented in (table 2).

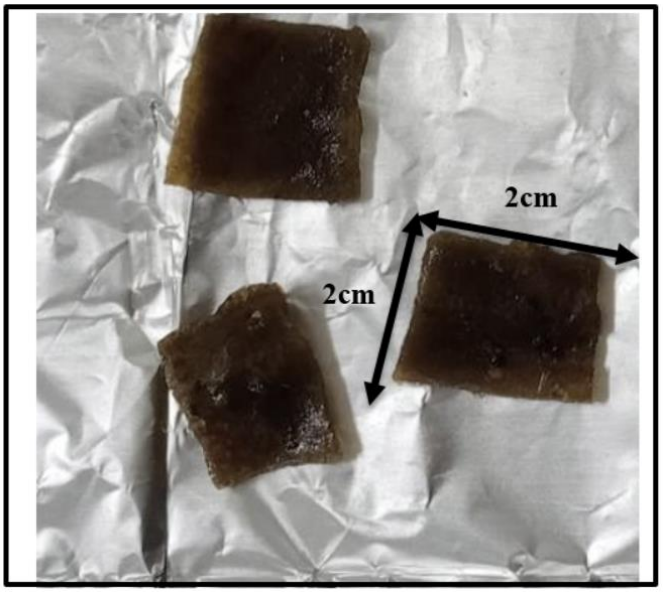

Fig. 4: Neem leaf hydrogel patch cut with 2x2 cm size

Table 2: Weight uniformity of neem leaf extract hydrogel patches (NLEHP)

Name	Average weigh (mean±SD)		CV value
	Before	After	
Weight Uniformity	-	0.3±0.1	3.0
Moisture uptake	23.7%	8.3 %	-

Notes: Data in the table is described in mean±SD. Number of samples (n=5), As shown in (table 2), the CV value of the weight uniformity of neem leaf extract hydrogel patches with a combination of PVA and HPMC polymers was 3.0, and the average moisture absorption values were 23.7% and 8.3 %, respectively.

Weight monitoring of test animals

The results of body weight monitoring before and after treatment can be seen in (table 3):

Based on (table 3) the average body weight of the test animals showed changes in body weight of the test animals in the three treatment groups for 21 days after alloxan induction. The non-diabetic group maintained a relatively stable body weight. The Negative group experienced a drastic weight loss from 243.9 g to 115.2 g. The NLEHP group showed a moderate decrease from 263.5 grams to 150.8 g.

Monitoring blood sugar levels of rats

Monitoring of blood sugar levels in the test animals is shown in table 4.

Table 3: Average body weight of test animals (grams) pre and post-aloxan induction

Group	Weight of test animal (g) (Mean±SD)				
	Baseline	Post induction aloxan	H7	H14	H21
Negative	243.9±30.3	213.8±34.1*	187.9±41.9*	143±46.1*	115.2±11.3*
Non-Diabetic	254.9±41.8	230±37.4#	234.6±35.9#	225.9±40#	254±46.8#
NLEHP	263.5±33	216.7±36.4*	194.2±17.9*	172.2±16.5*	150.8±12.6*

Notes:*= There was a significant difference in body weight in the negative group and the Neem Leaf Extract Hydrogel Patch group with the non-diabetic group #= There was no significant difference in body weight in the negative and Leaf Extract Hydrogel Patch groups. Data in the table is described in mean±SD (n=5)

Table 4: Blood sugar level (mg/dl) before and during observation (21 D)

Group	Blood sugar levels (mg/dl) before and during observation (Mean±SD)				
	Before treatment		During observation		
	Baseline	Post induction aloxan	H7	H14	H21
Negative	92.3±13.5	359.9±134.7*	347.5±89.4*	382.1±84.5*	414.8±60.1*
Non-Diabetic	98.4±9.0	92.8±8.1#	89.8±5.4#	87.7±9.1#	91±5.8#
NLEHP	86.5±12.7	363.2±69.3*	326.1±46.6*	365.3±49.2*	358.2±32.9*

Notes: *= There is a significant difference (<0.05) in blood sugar levels in the negative group Neem Leaf Extract Hydrogel Patch group vs the non-diabetic group. #= There is no significant difference (>0.05) between negative and Neem Leaf Extract Hydrogel Patch group. Data in the table is described in mean±SD. (n=5).

Based on the data (table 4), Variations in blood sugar levels between test groups are shown in table 4. The non-diabetic group maintained stable blood sugar levels (98.4 mg/dl baseline until day 21 of 91±5.8 mg/dl). The negative group increased from 92.3 mg/dl (baseline) to 359.9 mg/dl (post-alloxan induction), continuing to 414.8 mg/dl on day 21. The NLEHP group showed an initial increase (363.2 mg/dl) but experienced a slight decline. To 358.2 mg/dl on day 21, although it was still above the normal threshold (>200 mg/dl).

Wound area monitoring

Wound area monitoring was conducted for 21 D by comparing three different treatment groups, where visual documentation was taken on days 0, 7, 14, and 21 to evaluate the wound healing process in each group, as shown in (fig. 5 and table 5).

The results of wound area monitoring for 21 d based on (table 5) obtained the average value of wound area at the beginning of the study (day 0), and all groups had the same wound area of 1.5±0 cm^2. Over time, there were differences in the speed of wound healing between groups. The NLEHP-treated group showed the most significant decrease in wound area, reaching 0.5±0.1 cm^2on day 21. The Nondiabetic group also showed improvement, with a final wound area of 0.5±0.1 cm^2. Meanwhile, the Negative group showed the slowest healing, with a wound area of 1.1±0.1 cm^2 on day 21.

Histopathology test of skin thickness

The histopathology test results and average skin thickness are shown in (fig. 6):

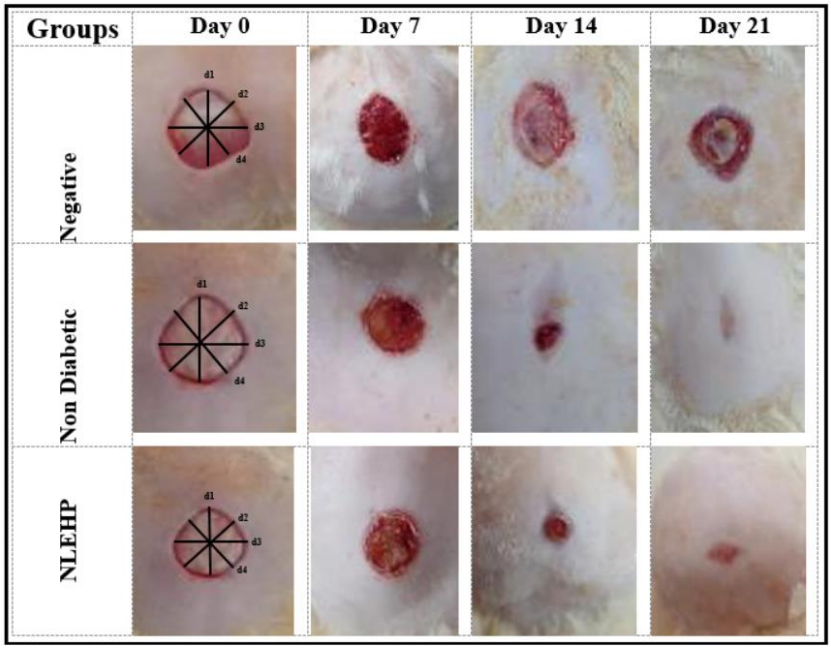

Fig. 5: Wound area reduction on days 0, 7, 14 and 21
Notes: The wound area is calculated using the following formula: $dx = \frac{d1+d2+d3+d4}{4}$

Table 5: Average wound area on days 0, 7, 14 and 21

Groups	Mean±SD wound area (cm^2) (mean±SD)				CI (95%)
	H0	H7	H14	H21	
Negative	1.5±0	1.5±0	1.4±0	1.1±0.1 #	1.3
Non-Diabetic	1.5±0	1.4±0	1.1±0	0.5±0.1 *	0.4
NLEHP	1.5±0	1.3±0.1	1.0±0.1	0.5±0.1 *	0.4

Notes: *= There was significant difference (<0.05) between negative vs. Neem Leaf Extract Hydrogel Patch and Nondiabetic group; #= No significant difference (>0.05) between the non-diabetic group and Neem Leaf Extract Hydrogel Patch group. Data in the table is described in mean±SD. (n=5).

As shown in fig. 6A, the histopathology test results of the skin condition on days 7, 14, and 21 in the negative control experienced tissue damage that appeared to worsen over time, as evidenced by the uneven skin structure and increasing gap between the layers. In contrast, the non-diabetic group showed a more regular skin structure with clear dermis and epidermis layers. In contrast, the NLEHP group showed progressive improvement in skin structure, as evidenced by the increased regularity of the layers and fewer gaps. Furthermore, fig. 6B shows the average skin thickness on days 7, 14, and 21; the negative group showed the lowest or insignificant thickness at all time points, while the non-diabetic and NLEHP groups showed a more significant increase at all time points.

Cytokine assay by elisa method

The cytokine assay with the elisa can be seen in (fig. 7):

As shown in fig. 7A, the average TNF-α levels in the negative control group were relatively high at all time points measured. The non-diabetic and NLEHP groups showed a decrease in TNF-α levels at each time point, with no significant difference (>0.05) between the two groups. Fig. 7B shows the average TGF-β level. In the negative group, TGF-β levels were relatively low and decreased at each time measured at all times measured. In the non-diabetic and NLEHP groups, TGF-β levels increased at each time measured, and there was no significant difference (>0.05) between the two groups.

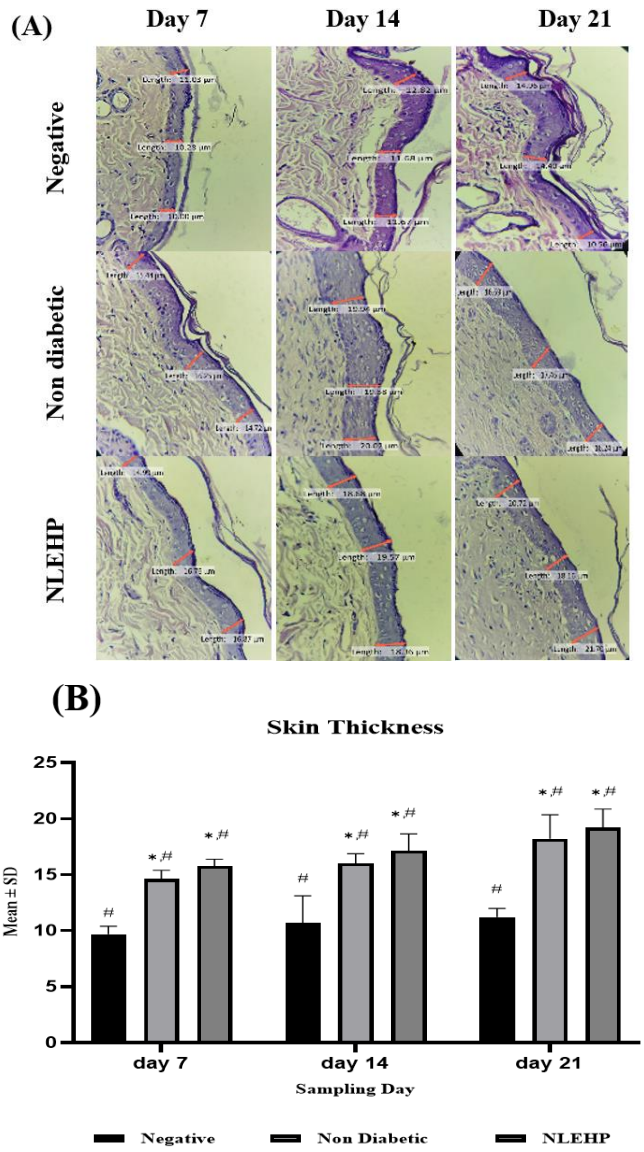

Fig. 6: (A) Histopathological examination of wound skin thickness was performed on days 7, 14, and 21 using 400x magnification. (B) The average skin tissue thickness was measured on days 0, 7, 14, and 21
Notes: *= There was a significant difference (<0.05) between the negative group vs Neem Leaf Extract Hydrogel Patch and Nondiabetic. #= There was no significant difference (>0.05) between Nondiabetic and Neem Leaf Extract Hydrogel Patch Data in the fig. is described in mean±SD. (n=5)

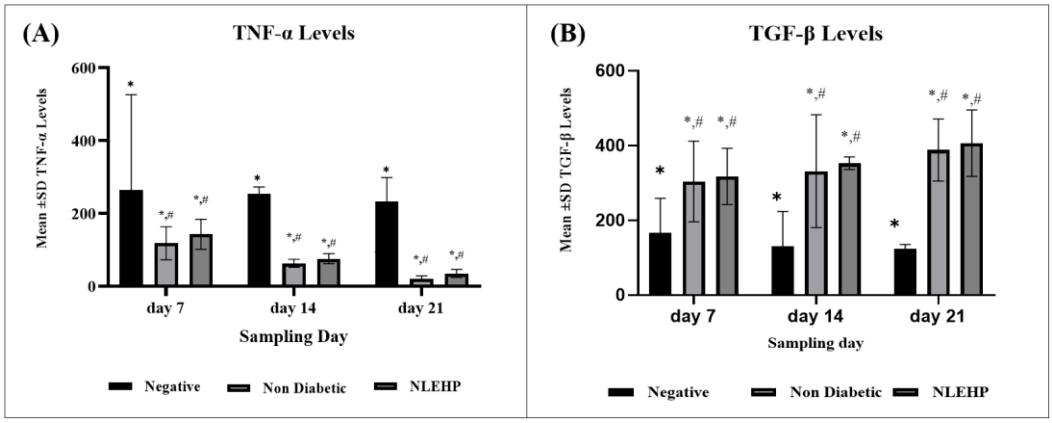

Fig. 7: (A) TNF-α and (B) TGF-β levels
Notes: *= There is a significant difference (<0.05) between the negative group (vsthe Neem Leaf Extract Hydrogel Patch and Nondiabetic groups #= There is no significant difference (>0.05) between the non-diabetic and Neem Leaf Extract Hydrogel Patch groups.
Data in the fig. is described in mean±SD. (n=5)

DISCUSSION

Diabetic wound healing is a significant clinical challenge caused by impaired healing processes resulting from hyperglycemia and chronic inflammation. The neem leaf extract (*Azadirachta indica*) contains secondary metabolites that have potential as therapeutic agents for diabetic wounds (table 1). Flavonoids act as antioxidants and anti-inflammatory compounds by reducing oxidative stress and inhibiting the production of ROS, which inhibits tissue regeneration [21-23]. Alkaloids have various biological activities, including analgesic and anti-inflammatory effects. Alkaloids can inhibit the NF-κB signaling pathway, which is a key regulator of inflammatory responses [25, 26]. Although saponins play a role in stimulating the activity of fibroblasts, they are responsible for the synthesis of collagen and the extracellular matrix. By increasing collagen production, saponins help repair damaged tissue structure [25-27]. Tannins have astringent properties that help accelerate the process of hemostasis or blood clotting. By binding to proteins, tannins can form complexes that inhibit blood flow to the wound area, reduce blood loss, and facilitate early healing [33]. Terpenoids and steroids in neem leaves have also been reported to accelerate wound healing through their antioxidant activities and increased collagen production [6]. In addition, the main components of neem leaves such as nimbin and azadirachtin, have mechanisms to inhibit NF-κB activity to reduce the production of pro-inflammatory cytokines TNF-α and can also modulate the activity of macrophages and neutrophils, enhance phagocytosis, and stimulate the production of growth factors (VEGF, TGF-β) for angiogenesis and extracellular matrix deposition. These two compounds also exhibit antioxidant effects through the activation of the Nrf2/ARE pathway, which induces the expression of antioxidant enzymes (SOD, catalase), and increase the expression of ECM proteins (type I and III collagen) and MMP activity for tissue remodeling [30, 31]. The use of neem leaf extract shows advantages compared to the isolation or fractionation of single compounds because secondary metabolites work synergistically through multiple targets and complementary molecular pathways, providing a more comprehensive therapeutic effect. Additionally, the total extract approach has practical advantages in terms of cost and accessibility, as its production process is simpler compared to isolation, which requires more expensive technology and resources. The complexity of phytochemicals in total extracts also has the potential to reduce the risk of microbial resistance compared to the use of single antimicrobial compounds [32, 33].

Dosage evaluation was conducted on the hydrogel patches containing neem leaf extract combined with PVA and HPMC polymers. Initial testing included organoleptic analysis, as illustrated in (fig. 4). Testing continued with the evaluation of weight uniformity as well as water absorption capacity, with the results listed in (table 2). In patch formulation, weight uniformity is a key indicator of quality, with a standard CV value ≤ 5% to ensure dose uniformity in each patch [9-27]. The moisture absorption test was designed to ascertain the water absorption capacity of the transdermal patch after conditioning at 40 °C for 24 h. The water absorption level of the patch during use was demonstrated by the moisture absorption of the patch on the epidermis. The quality of a patch is influenced by its resistance to moisture, which can result in a decrease in elasticity and an increased susceptibility to tearing [9]. Previous studies have shown that hydrogels have extracellular matrix-like properties that support wound healing by increasing water retention and providing an environment conducive to cell proliferation [28, 29].

Body weight in test animals was observed before and after treatment and used as an indicator of treatment, as shown in (table 3) which shows that body weight in the negative group and hydrogel patch after alloxan induction until observation day 21 decreased body weight. A decrease in the body weight of the test animals can also be a sign of hyperglycemic disease. This is due to an increase in gluconeogenesis and glycogenolysis, which increase the storage of glycogen and fat in muscle tissue and adipose cells [41]. The non-diabetic group did not experience a decrease because it did not undergo alloxan induction. In addition to observations of body weight, blood sugar levels were also measured to support the success of observing diabetic wounds.

Based on (table 4) displays the results of observations of blood sugar levels in three treatment groups for 21 d. The negative control and NLEHP groups showed increased blood sugar levels. This is because alloxan damages the pancreatic β cells, disrupts insulin production, and causes hyperglycemia [42]. Compared to the non-diabetic group, the blood sugar levels on day 21 were relatively stable because the non-diabetic group was not induced by alloxan, so the blood sugar levels were still within the normal range of fasting blood glucose [43].

The wound healing parameters were observed in the wound area and cytokine assays (table 5) shows the development of the wound area in the three treatment groups over 21 d. All groups started with the same initial wound area (1.5 cm), but showed different healing patterns. The negative control group had the slowest healing compared to the non-diabetic group, and the NLEHP group showed the fastest healing, indicating that neem leaf extract has bioactive components that have the potential to significantly affect the wound healing process. These metabolite compounds in neem leaves play a role in regulating the synthesis and secretion of crucial growth factors, especially Vascular Endothelial Growth Factor (VEGF) and Transforming Growth Factor-beta (TGF-β). This modulation can optimize the wound microenvironment, promote angiogenesis, and accelerate granulation tissue formation. The potential effectiveness of neem leaf extract in accelerating wound healing may be due to the anti-inflammatory and antimicrobial properties of bioactive compounds, such as nimbin and azadirachtin contained in neem leaves [34], the non-diabetic group showed good healing (0.5±0.1 cm at day 21). This occurred because normal physiological conditions with stable blood glucose levels allow for an orderly wound-healing process to occur through the phases of inflammation, proliferation, and tissue remodeling [44]. Normal glucose levels also support adequate function of inflammatory cells, collagen production, and angiogenesis for tissue repair [45]. This difference emphasizes the importance of intervention in the wound healing process. The observed pattern of wound area reduction is in line with the normal stages of wound healing, including the inflammatory, proliferative, and remodeling phases [46].

Comprehensive analysis of histopathology test data of skin thickness measured at three points showed the effectiveness of neem leaf extract hydrogel patches in enhancing skin tissue regeneration. This can be seen in fig. 6A and B. In the negative group, on days 7–21, the skin thickness tended to remain thin, indicating significant tissue damage and obstacles in the healing process. Hyperglycemia causes oxidative stress and inflammation, which inhibit skin regeneration [47]. The group treated with the neem leaf extract hydrogel patch showed a significant increase in skin thickness, especially on days 14 and 21. Neem leaf extract in hydrogel patches seems to have a good healing effect, accelerating the regeneration process of skin tissue, even under hyperglycemic conditions. The anti-inflammatory and antioxidant properties of the neem leaf extract may play a role in accelerating the healing process by reducing inflammation and increasing the proliferation of skin cells [45]. This increase in cell proliferation is related to the upregulation of gene expression that supports the synthesis of collagen and growth factors that are essential for tissue regeneration [48]. In the non-diabetic group, which was allowed to heal without any treatment, skin thickness gradually increased from day 7 to day 21. This indicated that natural healing occurred over time without significant impediments, reflecting the normal process of efficient skin regeneration [39, 40].

Based on the data shown in fig. 7A, TNF-α levels in the group administered NLEHP showed a significant anti-inflammatory effect, indicating that this hydrogel patch is effective in reducing inflammation faster than the negative control group and close to the non-diabetic group. This is in line with other studies showing that neem leaf extract exerts anti-inflammatory effects by inhibiting the production of pro-inflammatory cytokines, such as TNF-α [51]. Meanwhile, TGF-β levels fig. 7B showed that TGF-β levels in the NLEHP group increased significantly compared to the non-diabetic group and inversely proportional to the negative control group, which decreased. The consistent pattern of increasing TGF-β levels in the neem leaf extract hydrogel patch group indicated the potential of this formulation to accelerate wound healing. This is supported by other

studies that have found that topical application of plant extracts can increase TGF-β levels and accelerate wound closure [52].

CONCLUSION

The formulation of hydrogel patches from neem leaf extract (*Azadirachta indica*) is effective in healing diabetic wounds through specific mechanisms. The compound content in neem leaf extract has been proven to reduce the expression of the pro-inflammatory cytokine TNF-α in the wound area. The developed hydrogel patch also demonstrated the ability to maintain optimal moisture and facilitate the continuous release of bioactive neem compounds into the wound tissue, resulting in enhanced granulation tissue formation and faster re-epithelialization than the control. These results indicate the potential of neem leaf extract hydrogel patches as a promising wound dressing alternative, particularly in managing diabetic wounds that require optimal inflammatory control and healing environments. The development of NLEHP for diabetic wound healing necessitates additional research on a variety of diabetes models (type 1 and 2), as well as the assessment of vascular complications.

Additionally, formulation optimization is necessary to improve bioavailability and stability before clinical trials. Clinical trials are conducted in stages, beginning with phase I (safety evaluation), followed by phase II (dose optimization), and phase III (efficacy validation). The parameters of these trials include patient quality of life, tissue quality, and healing rate. The development of NLEHP into a safe and effective therapeutic product necessitates collaboration among researchers, clinicians, and the pharmaceutical industry.

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Nil

AUTHORS CONTRIBUTIONS

NWL: As the first author, I was responsible for data collection, data analysis, interpretation of research results, and preparation of the initial manuscript draft. DWW: provided substantive input related to the research methodology, validated the data analysis, and contributed to revising the manuscript content. ASW: as the corresponding author and supervisor.

CONFLICTS INTERESTS

The authors declare no conflict of interest in the publication of this manuscript.

REFERENCES

1. Yang L, Rong GC, WU QN. Diabetic foot ulcer: challenges and future. World J Diabetes. 2022;13(12):1014-34. doi: 10.4239/wjd.v13.i12.1014, PMID 36578870.
2. Saputra MK, Masdarwati M, Lala NN, Tondok SB, Pannyiwi R. Analysis of the occurrence of diabetic wounds in people with diabetes mellitus. fulltext PDF. 2023;12(1):143-9. doi: 10.35816/jiskh.v12i1.915.
3. Liu ZW, Zhang YM, Zhang LY, Zhou T, LI YY, Zhou GC. Duality of interactions between TGF-B and TNF-A during tumor formation. Front Immunol. 2021;12:810286. doi: 10.3389/fimmu.2021.810286, PMID 35069596.
4. Jang DI, Lee AH, Shin HY, Song HR, Park JH, Kang TB. The role of tumor necrosis factor-alpha (TNF-A) in autoimmune disease and current TNF-A inhibitors in therapeutics. Int J Mol Sci. 2021;22(5):2719. doi: 10.3390/ijms22052719, PMID 33800290.
5. Wang HL, Wang L, Zhao CY, Lan HY. Role of TGF-beta signaling in beta cell proliferation and function in diabetes. Biomolecules. 2022;12(3):373. doi: 10.3390/biom12030373, PMID 35327565.
6. Nasrine A, Narayana S, Gulzar Ahmed M, Sultana R, Noushida N, Raunak Salian T. Neem (*Azadirachta indica*) and silk fibroin associated hydrogel: boon for wound healing treatment regimen. Saudi Pharm J. 2023;31(10):101749. doi: 10.1016/j.jsps.2023.101749, PMID 37663591.
7. Batra N, Kumar VE, Nambiar R, De Souza C, Yuen A, LE U. Exploring the therapeutic potential of neem (*Azadirachta indica*) for the treatment of prostate cancer: a literature review. Ann Transl Med. 2022;10(13):754. doi: 10.21037/atm-22-94, PMID 35957716.
8. Zhang S, GE G, Qin Y, LI W, Dong J, Mei J. Recent advances in responsive hydrogels for diabetic wound healing. Mater Today Bio. 2022 Dec 1;18:100508. doi: 10.1016/j.mtbio.2022.100508, PMID 36504542.
9. Wardani VK, Saryanti D. Papaya seed ethanol extract transdermal patch formulation (*Carica papaya* L.) with hydroxypropyl methylcellulose base (HPMC). Med J. 2021;4:38.v4i1.43613.
10. Sharma P, Aggarwal A, Tandon S. Review article enhancing transdermal delivery of poorly water-soluble NSAIDs: effective strategies. Int J Curr Pharm Sci. 2024;16(6):1-5. doi: 10.22159/ijcpr.2024v16i6.5070.
11. Jacob S, Nair AB, Shah J, Sreeharsha N, Gupta S, Shinu P. Emerging role of hydrogels in drug delivery systems tissue engineering and wound management. Pharmaceutics. 2021;13(3):357. doi: 10.3390/pharmaceutics13030357, PMID 33800402.
12. Novriyanti R, Putri NE, Rijai L. Phytochemical screening and antioxidant activity test of lime peel ethanol extract (*Citrus aurantifolia*) using the DPPH method. Proceeding Mulawarman Pharm Conf. 2022;15(1):165-70. doi: 10.25026/mpc.v15i1.637%0A1.
13. Dubale S, Kebebe D, Zeynudin A, Abdissa N, Suleman S. Phytochemical screening and antimicrobial activity evaluation of selected medicinal plants in Ethiopia. J Exp Pharmacol. 2023 Feb 8;15:51-62. doi: 10.2147/JEP.S379805, PMID 36789235.
14. Muhtadi M, Haryoto H, Azizah T, Suhendi A, Yen KH. Antidiabetic and antihypercholesterolemic activities of citrus sinensis peel: *in vivo* study. Natl J Physiol Pharm Pharmacol. 2015;5(5):382-5. doi: 10.5455/njppp.2015.5.2807201561.
15. Rusnedy R, Febrina M, Sari CP. Uji aktivitas wound healing ekstrak etanol buah Averrhoa bilimbiL. (Belimbing Wuluh) pada mencit putih jantan (Mus musculus). Pharmacon. 2023;20(1):50-60. doi: 10.23917/pharmacon.v20i1.22677.
16. Hanistya R, Samlan K. Formulation and physical characteristics of hydrogel plaster preparations of Ciplukan leaf extract (*Physalis angulata* L.) and cinnamon bars (*Cinnamomum burmannii*). J Muhammadiyah Med Lab Technol. 2021;4:201.v4i2.11151.
17. Bin JW, Muthia R, Kartini K, Setiawan F, Juhrah S, Yulida N. Formulation and evaluation of transdermal patches from Eleutherine bulbosa Urb. bulb extract with plasticizer variations. Int J Appl Pharm. 2024;16(1):94-7. doi. 10.22159/ijap.2024v16i1.46421.
18. Wahyuni AS, Munawaroh R, Dai M. Antidiabetic mechanism of ethanol extract of black rice bran on diabetic rats. Natl J Physiol Pharm Pharmacol. 2016;6(2):106-10. doi: 10.5455/njppp.2015.5.1111201590.
19. Rahati S, Kamalinezhad M, Ebrahimi A, Eshraghian M, Pishva H. Accelerated wound healing induced by spinach extract in experimental model diabetic rats with streptozotocin. Sci Rep. 2023;13(1):14933. doi: 10.1038/s41598-023-42033-0, PMID 37696865.
20. Maan P, Yadav KS, Yadav NP. Wound healing activity of *Azadirachta indica* a. juss stem bark in mice. Pharmacogn Mag. 2017;13 Suppl 2:S316-20. doi: 10.4103/0973-1296.210163, PMID 28808399.
21. Muhtadi M, Haryoto H, Azizah T, Suhendi A, Yen K. Antidiabetic and antihypercholesterolemic activities of Citrus sinensis peel: *in vivo* study. Natl J Physiol Pharm Pharmacol. 2015;5(5):382-5. doi: 10.5455/njppp.2015.5.2807201561.
22. Salrian AA, Behzadi A, Oloumi MM, Farajli Abbasi M, Delshad S, Moghadaszadeh M. Amplification of wound healing by propolis and honey ointment in healthy and diabetic rat models; histopathological and morphometric findings. Arch Razi Inst. 2022;77(5):1673-81. doi: 10.22092/ARI.2022.357191.1991, PMID 37123150.
23. Gunawan SA, Berata IK, Wirata IW. Histopathology of skin on incision wound healing of white rats after administration of

extracellular matrix (ECM) derived from pig urinary bladder. Indones Med Veterinus. 2019;8:313-24. doi: 10.19087/imv.2019.8.3.313.

24. Masson Meyers DS, Andrade TA, Caetano GF, Guimaraes FR, Leite MN, Leite SN. Experimental models and methods for cutaneous wound healing assessment. Int J Exp Pathol. 2020;101(1-2):21-37. doi: 10.1111/iep.12346, PMID 32227524.
25. Chagas MD, Behrens MD, Moragas Tellis CJ, Penedo GX, Silva AR, Goncalves DE Albuquerque CF. Flavonols and flavones as potential anti-inflammatory, antioxidant and antibacterial compounds. Oxid Med Cell Longev. 2022;2022(1):9966750. doi: 10.1155/2022/9966750, PMID 36111166.
26. Panche AN, Diwan AD, Chandra SR. Flavonoids: an overview. J Nutr Sci. 2016;5:e47. doi: 10.1017/jns.2016.41, PMID 28620474.
27. Al Ishaq RK, Abotaleb M, Kubatka P, Kajo K, Busselberg D. Flavonoids and their anti-diabetic effects: cellular mechanisms and effects to improve blood sugar levels. Biomolecules. 2019;9(9):430. doi: 10.3390/biom9090430, PMID 31480505.
28. Wati DP, Ilyas S. Renoprotective potential of flavonoids rich against doxorubicin-induced in animal models: a review. Int J App Pharm. 2024;16(6):28-37. doi: 10.22159/ijap.2024v16i6.51741.
29. Souza CR, Bezerra WP, Souto JT. Marine alkaloids with anti-inflammatory activity: current knowledge and future perspectives. Mar Drugs. 2020;18(3):147. doi: 10.3390/md18030147, PMID 32121638.
30. Yadav JP, Verma A, Pathak P, Dwivedi AR, Singh AK, Kumar P. Phytoconstituents as modulators of NF-κB signalling: investigating the therapeutic potential for diabetic wound healing. Biomed Pharmacother. 2024;177:117058. doi: 10.1016/j.biopha.2024.117058, PMID 38968797.
31. Berry CE, Brenac C, Gonzalez CE, Kendig CB, LE T, An N. Natural compounds and biomimetic engineering to influence fibroblast behavior in wound healing. Int J Mol Sci. 2024;25(6):3274. doi: 10.3390/ijms25063274, PMID 38542247.
32. Shita AD, Dharmayanti AW, Meilawaty Z, Lestari M, Mazaya IM. Increasing fibroblasts and gingival collagen density in periodontitis rats by using cassava leaf extract. J Taibah Univ Med Sci. 2023;18(6):1321-8. doi: 10.1016/j.jtumed.2023.05.006, PMID 37293131.
33. Kalalinia F, Amiri N, Mehrvarzian N, Fazly Bazzaz BS, Iranshahi M, Shahroodi A. Topical green tea formulation with anti-hemorrhagic and antibacterial effects. Iran J Basic Med Sci. 2020;23(8):1085-90. doi: 10.22038/ijbms.2020.41397.9782, PMID 32952956.
34. Alzohairy MA. Therapeutics role of *Azadirachta indica* (neem) and their active constituents in diseases prevention and treatment. Evid Based Complement Alternat Med. 2016;2016:7382506. doi: 10.1155/2016/7382506, PMID 27034694.
35. Nagini S. Neem limonoids as anticancer agents: modulation of cancer hallmarks and oncogenic signaling. Enzymes. 2014;36:131-47. doi: 10.1016/B978-0-12-802215-3.00007-0, PMID 27102702.
36. Jayalakshmi M. Effect of neem leaves extract irrigation on the wound healing outcome in nurse managed diabetic foot ulcers. Natl J Physiol Pharm Pharmacol. 2020;11(1):1. doi: 10.5455/njppp.2021.10.09238202008092020.
37. Treichel TL, DO Prado TD, DO Amaral AS, DE Sousa Gomes Y, DA Rocha Pinto L, Martins GT. Use of ointment or aqueous extract of neem (*Azadirachta indica*) for the repair of experimental skin lesions in sheep. Am J Plant Sci. 2020;11(5):693-706. doi: 10.4236/ajps.2020.115051.
38. Patel DP, Setty CM, Mistry GN, Patel SL, Patel TJ, Mistry PC. Development and evaluation of ethyl cellulose-based transdermal films of furosemide for improved *in vitro* skin permeation. AAPS Pharm Sci Tech. 2009;10(2):437-42. doi: 10.1208/s12249-009-9224-3, PMID 19381831.
39. Gounden V, Singh M. Hydrogels and wound healing: current and future prospects. Gels. 2024;10(1):43. doi: 10.3390/gels10010043, PMID 38247766.
40. Deng X, Gould M, Ali MA. A review of current advancements for wound healing: biomaterial applications and medical devices. J Biomed Mater Res B Appl Biomater. 2022;110(11):2542-73. doi: 10.1002/jbm.b.35086, PMID 35579269.
41. Hatting M, Tavares CD, Sharabi K, Rines AK, Puigserver P. Insulin regulation of gluconeogenesis. Ann N Y Acad Sci. 2018;1411(1):21-35. doi: 10.1111/nyas.13435, PMID 28868790.
42. Lenzen S. The mechanisms of alloxan and streptozotocin-induced diabetes. Diabetologia. 2008;51(2):216-26. doi: 10.1007/s00125-007-0886-7, PMID 18087688.
43. American Diabetes Association. Classification and diagnosis of diabetes: standards of medical care in diabetes-2021. Diabetes Care. 2021;44 Suppl 1:S15-33. doi: 10.2337/dc21-S002, PMID 33298413.
44. Rodrigues M, Kosaric N, Bonham CA, Gurtner GC. Wound healing: a cellular perspective. Physiol Rev. 2019;99(1):665-706. doi: 10.1152/physrev.00067.2017, PMID 30475656.
45. Wulandari D, Noviyanti NP, Wardani L, Yowani SC. Efektivitas nanoemulgel kombinasi ekstrak daun mimba dan lidah buaya untuk terapi skabies. JMPI. 2023;9(2):461-74. doi: 10.35311/jmpi.v9i2.403.
46. Gurtner GC, Werner S, Barrandon Y, Longaker MT. Wound repair and regeneration. Nature. 2008;453(7193):314-21. doi: 10.1038/nature07039, PMID 18480812.
47. Berlanga Acosta JA, Guillen Nieto GE, Rodriguez Rodriguez N, Mendoza Mari Y, Bringas Vega ML, Berlanga Saez JO. Cellular senescence as the pathogenic hub of diabetes-related wound chronicity. Front Endocrinol (Lausanne). 2020;11:573032. doi: 10.3389/fendo.2020.573032, PMID 33042026.
48. Panichakul T, Ponnikorn S, Tupchiangmai W, Haritakun W, Srisanga K. Skin anti-aging potential of *Ipomoea pes-caprae* ethanolic extracts on promoting cell proliferation and collagen production in human fibroblasts (CCD-986sk cells). Pharmaceuticals (Basel). 2022;15(8):969. doi: 10.3390/ph15080969, PMID 36015117.
49. Reinke JM, Sorg H. Wound repair and regeneration. Eur Surg Res. 2012;49(1):35-43. doi: 10.1159/000339613, PMID 22797712.
50. Xue M, Jackson CJ. Extracellular matrix reorganization during wound healing and its impact on abnormal scarring. Adv Wound Care. 2015;4(3):119-36. doi: 10.1089/wound.2013.0485, PMID 25785236.
51. Ruslie RH, Darmadi D. Administration of neem (Azadirachta indica A. Juss) leaf extract decreases TNF-α and IL-6 expressions in dextran sodium sulphate induced colitis in rats. J Adv Vet Anim Res. 2020;7(4):744-9. doi: 10.5455/javar.2020.g476, PMID 33409321.
52. Rajendran P, Renu K, Abdallah BM, Ali EM, Veeraraghavan VP, Sivalingam K. Nimbolide: promising agent for prevention and treatment of chronic diseases (recent update). Food Nutr Res. 2024;68:1-14. doi: 10.29219/fnr.v68.9650.