Original Article

Effect of Apigenin Nanoemulgel on Inf14lammatory Phase Acceleration in Burn Wound Healing: In Silico and In Vivo Studies

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ABSTRACT

Background: Burns are destructive wounds that trigger inflammation, neuropathic pain, and the risk of infection. The nuclear factor kappa B (NF-κB) signalling pathway plays an important role in regulating inflammation and tissue regeneration. Apigenin, the main flavonoid in celery (*Apium graveolens)*, has anti-inflammatory, antioxidant, and angiogenic effects, but its bioavailability is low due to its lipophilic nature.

Objectives: We analyzed the effects of apigenin-loaded nanoemulgel (NA) administration in modulating inflammation and accelerating burn wound healing in silico and in vivo.

Methods: In silico analysis was conducted using molecular docking against NF-κB, MMP-1, and COX-2. In vivo testing involved 12 rats, each with three burns (±100 °C, 5 seconds), divided into three treatment groups: Apigenin-loaded NA, positive control (bioplacenton®), and negative control (placebo). Macroscopic and histopathological evaluations were conducted on days 3, 7, 14, and 21. Histopathological parameters included re-epithelialization, neutrophil count, lymphocyte count, and collagen density.

Results: In vivo administration of apigenin-loaded NA accelerated the closure of macroscopic wounds, reduced the number of neutrophils, and significantly increased collagen density ($P<0.05$). There were no significant differences in lymphocyte counts and increased re-epithelialization, as determined by statistical testing. In silico validation of apigenin showed stronger binding affinity to MMP-1 and COX-2 than to native ligands, while a lower affinity was observed for NF-κB.

Conclusion: Apigenin-loaded NA is an effective topical agent in accelerating the initial inflammatory phase and promoting wound healing in burn injuries.

Keywords: Apigenin-loaded nanoemulgel (NA), Burn, NF-κB, COX-2, MMP-1

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Introduction

Burns are one of the most destructive types of wounds, often accompanied by an inflammatory response, neuropathic pain, tissue damage, infection, disability, and an increased risk of death (Guo et al., 2017). One of the main pathways involved in inflammation and regeneration in burns is the nuclear factor kappa B (NF-κB) molecular pathway in immune response and inflammation (George et al., 2020). The wound healing process aims to restore tissue homeostasis after injury. Wound healing has four phases, including hemostasis, the inflammatory phase, the proliferation phase, and the remodeling phase, which are interrelated (Rodrigues et al., 2018). The significant impact that burns have on quality of life makes it important to explore effective treatments that utilize natural resources (Utoyo et al., 2025).

Celery (*Apium graveolens)* is one of the plants that is often used in traditional medicine in Indonesian society. It contains many metabolite compounds, including the main flavonoid, apigenin. Apigenin is believed to have benefits in the regulation of oxidative stress, which can activate the inflammatory process (Syukur et al., 2023). Apigenin exerts anti-inflammatory effects and improves vascular regeneration after ischemia by increasing the expression of vascular endothelial growth factor (VEGF) (Ma et al., 2021). Compared to other flavonoids, apigenin has low toxicity and mutagenicity (Xu et al., 2017).

The bioavailability of active ingredients derived from natural materials limits their effectiveness in therapeutic applications, necessitating higher doses (Prasetyo et al., 2018) an inclusion complex was formed using beta-cyclodextrin (BCD). The limitation of apigenin's low solubility properties can be addressed by implementing an efficient drug delivery strategy currently found in processed products called nano (Sholiha et al., 2023). Nanotechnology produces materials as small as nanometers (10-9 m). The advantage of nanoparticle technology is its ability to be combined with other technologies, thereby opening up opportunities to create more effective drug delivery systems. Nanoparticles are materials that consist of particles with a size of less than 100 nm (Khan et al., 2019).

Apigenin in celery has the potential to be developed as a burn wound healing drug and shows excellent prospects for formulation into pharmaceutical preparations in the form of nanoemulgels (NAs) to increase bioavailability and support the commercialization of apigenin as a wound medicine. The activity of the NA, for skin regeneration in this study was tested using a burn wound healing model in rats.

Materials and Methods

Place and time

The research was conducted from November 2024 to January 2025 at the Pharmacy Laboratory, Pathology Laboratory, and Veterinary Teaching Hospital of the School of Veterinary Medicine and Biomedical Sciences at IPB University.

Materials and equipment

The materials used in this study consisted of wire and plastic boxes for mouse cages, surgical instruments (scalpel and anatomical scissors for surgery), rulers, and equipment for preparing histopathological specimens, such as object glasses and a microtome. A microscope was used for histopathological observation. The experimental animals used were white rats (*Rattus Norvegicus*) obtained from the Laboratory Animal Management Unit (UPHL) of the Faculty of Veterinary Medicine at IPB University. The rats were randomized and placed in cages with partitions. Each cage contained two rats. The rats were acclimatized for 7 days, and on the 8th day, burn injuries were inflicted. The rats were fed a standard diet and provided with water ad libitum.

The materials used in this study included apigenin-loaded NA preparations obtained from the Pharmacy Laboratory at the School of Veterinary Medicine and Biomedical Sciences, IPB University, commercial gel (bioplasenton®) preparations, placebo gel preparations, apigenin, ketamine and xylazine for anaesthesia, 10% neutral buffered formalin (NBF) solution for fixation, cotton, and materials for histopathological preparation, including Mayer's hematoxylin solution, eosin solution, xylene, alcohol with varying concentrations (70%, 80%, 90%, 95%, and 100%), lithium carbonate solution, distilled water, 1% acetic acid, Schiff's reagent, sulfuric acid, mordant solution, Carrazzi's hematoxylin solution, 0.75% orange G solution, Ponceau xylidine fuchsin solution, 2.5% phosphotungstic acid solution, aniline blue, and paraffin.

Research methodology

This study is an experimental study conducted using in silico and in vivo tests in the wound healing process of burns to determine the effectiveness of apigenin-loaded NA.

Use of experimental animals

Twelve rats were used in this study, divided into three treatment groups. Each rat had three burns created with different treatments: Apigenin-loaded NA, positive control, and negative control. Thus, each rat effectively represented three biological replicates or three independent units of measurement. Repeated measurements were taken on days 3, 7, 14, and 21 based on the skin sampling schedule and histopathological observations. This approach allows for more data without increasing the number of experimental animals, while still adhering to the ethical principles of laboratory animal use.

Burn treatment

Before treatment, all rats were adapted to the prepared cages. Rats grouped into the positive control group were given a commercial gel, the negative control group was given a placebo gel, and the treatment group received apigenin-loaded NA at 0.1% obtained from the pharmaceutical laboratory, School of Veterinary Medicine and Biomedicine, IPB University. Burn wound preparation followed the method described by Caliari-Oliveira et al. (2016). Burns were created using a metal plate (2 cm diameter) heated to approximately 100 °C, which was then placed on the skin of the back of the rat for 5 seconds. Prior to creating the wound, rats were anesthetized using a combination of ketamine (50 mg/kg) and xylazine (4 mg/kg). The hair around the wound area was shaved and then cleaned with alcohol. After the burn wound was created, the analgesic ibuprofen was administered at a dose of 15 mg/kg body weight. The administration of NA was done topically by applying it to the wound on the rat every day, from day 1 to day 21 after the wound was made, twice a day.

Macroscopic and histopathological analysis

On the 3rd, 7th, 14th, and 21st days, prior to the euthanasia of the rats, animal model euthanasia was performed using EUTHASOL® (pentobarbital sodium and phenytoin sodium) at a dose of 0.2 mL/kg body weight. Macroscopic observations were made regarding the decrease in wound area. The skin samples obtained were then fixed with a 10% NBF solution and left at room temperature for approximately 48 hours to prepare for histopathological examination.

Histopathological observation involved counting the number of cells observed. The parameters used were docking the epidermal layer (re-epithelialization) and the number of inflammatory cells (lymphocytes and neutrophils). Histopathological observations were conducted using a microscope. Furthermore, statistical analysis using a one-way ANOVA was performed to compile the wound morphopathology data, including wound area, neutrophil count, lymphocyte count, re-epithelialization, and collagen deposition in the wound area. These data were tested statistically using the Shapiro-Wilk test and other appropriate statistical tests. A statistically significant difference was defined as $P<0.05$.

In silico study

This procedure began with the preparation of protein structures, specifically the NF-κB p65 receptor, COX-2, MMP-1, and the test ligand apigenin. The three-dimensional (3D) structures of COX-2, MMP-1, and NF-KB were obtained from the Protein Data Bank (PDB) (RCSB, 2025) (PDB: 1PXX, PDB: 966C, and PDB: 4KIK). The test ligand structure for apigenin (PubChem CID 5280443) (Pubchem, 2025) was obtained from PubChem website. The ligand structure was transformed into 3D using Discovery Studio software and saved in PDB format. All ligands used were optimized using AutoDock Tools 1.5.7 by adding hydrogen atoms and were saved in PDBQT format. Molecular docking was performed using the AutoDock Tools 1.5.7. 2D and 3D visualizations of the docking were performed at the best docking sites. The 2D visualization was performed using Discovery Studio Visualizer software. The parameters analyzed included the type of ligand-protein bonds, hydrogen bonds, inhibition constant (Ki), and interacting amino acid residues. The 3D visualization was performed using Discovery Studio Visualizer software to view the ligand binding site in the protein surface structure. The analysis was performed by comparing the energy affinities, visualizing the binding sites of the test ligands on the receptor, and comparing them with the reference ligands.

Results

Macroscopic analysis

The results of the morphopathology of the burn area, as seen in Figure 1, show the development of the burn wound healing process in the three treatment groups observed until day 21.

Data in Table 1 and Figure 1 show that the apigenin-loaded NA treatment group experienced a more significant reduction in wound area than the negative control group on days 3 and 7 ($P<0.05$).

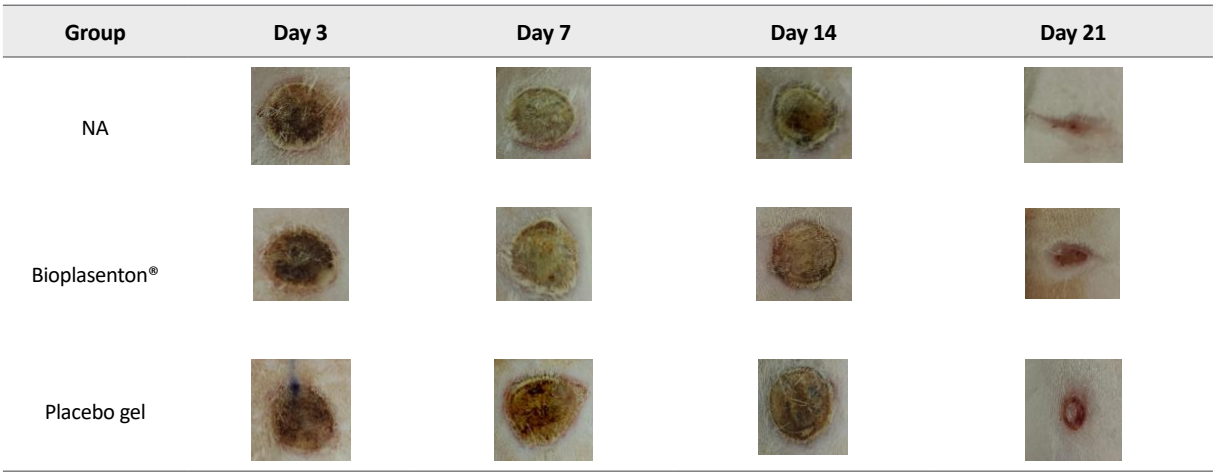

Figure 1. Macroscopic evaluation related to the time course of burn wound healing after 21 days of topical application of apigenin-loaded NA, bioplasenton®, and placebo gel

Neutrophil count

The results of neutrophil counts in Table 2 and Figure 2 show that the average pattern of neutrophil counts in the three experimental groups was relatively similar, with high numbers on the initial day followed by decreasing numbers on subsequent days. The study recorded the total number of neutrophils on days 3, 7, 14, and 21 after burn treatment.. The apigenin-loaded NA -treated group had consistently lower neutrophil counts than the negative control group, especially on days 3, 7, and 14 ($P<0.05$).

On day 14, the apigenin group showed the lowest neutrophil count among all groups, indicating that inflammation had resolved sooner. On day 21, there was no significant difference between groups ($P>0.05$), indicating that all groups had entered the final healing phase.

Lymphocyte count

Although statistically no significant differences were found between the treatment and control groups on each observation day ($P>0.05$; Table 3 and Figure 2), the distribution pattern of lymphocyte counts demonstrated relevant biological trends. Descriptive analysis showed that on day 7, the negative control group had the highest lymphocyte count at 24±11.13 cells/µL, indicating stronger immune activation, possibly due to uncontrolled inflammation. In contrast, the apigenin-loaded NA -administered group showed a lower lymphocyte count of 15.6±8.08 cells/µL.

On day 14, the apigenin group exhibited a descriptively significant decrease in lymphocyte count at 8.67±7.93 cells/µL, while the control groups remained high, with the positive control at 18.2±2.6 and the negative control at 18.6±6.02 cells/µL.

Table 1. Surface area (cm) of burn wound healing

Day	Mean±SEM		
	Group		
	Apigenin-loaded NA	Positive Control	Negative Control
3	1.23±0.12^{a}	1.37±0.12^{ab}	1.63±0.15^{b}
7	1.2±0^{a}	1.3±0.1^{ab}	1.47±0.06^{b}
14	1.17±0.12^{a}	1.23±0.15^{a}	1.37±0.06^{a}
21	0.27±0.15^{a}	0.37±0.15^{a}	0.7±0.17^{a}

Notes: Different letters (superscripts) in the same row indicate significant differences ($P<0.05$).

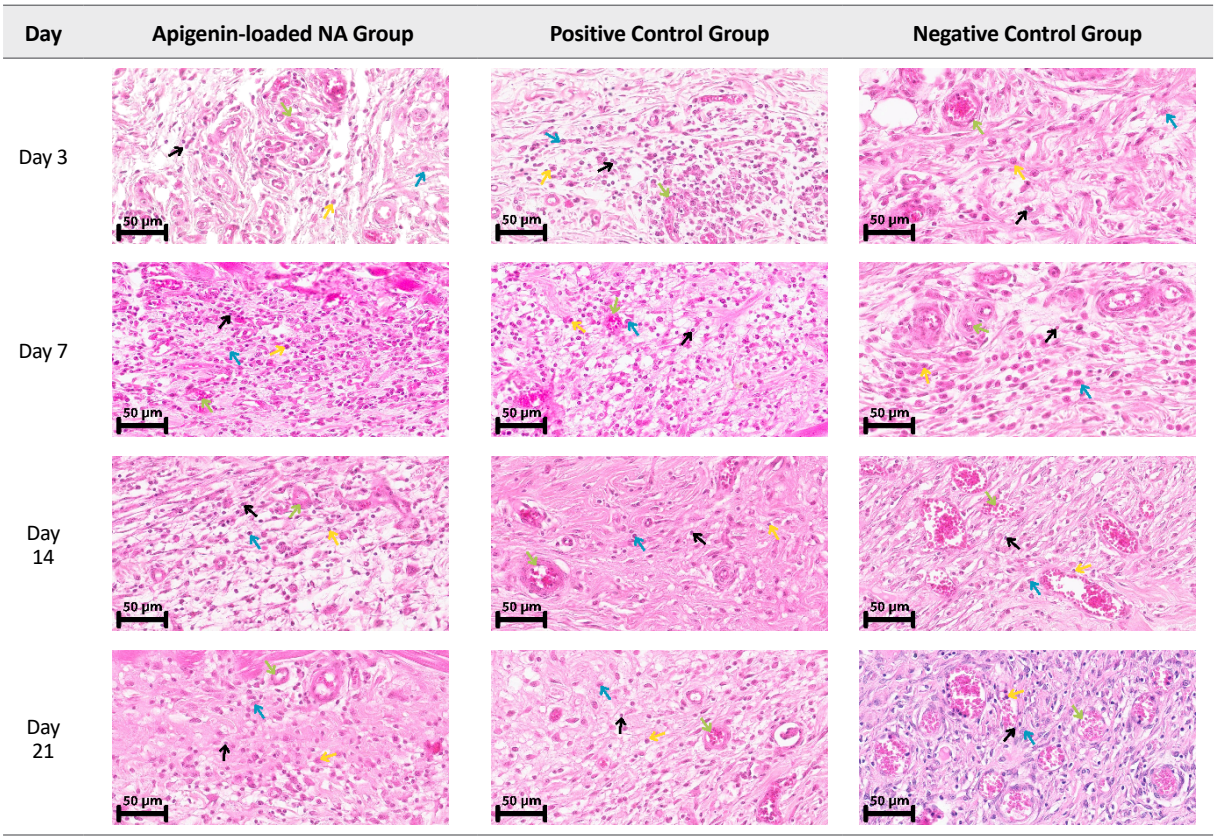

Figure 2. Microscopic analysis of inflammatory cells using hematoxylin-eosin staining on days 3, 7, 14, and 21 in the apigenin-loaded NA, positive control, and negative control groups

Note: Neutrophils (black arrows), lymphocytes (yellow arrows), macrophages (blue arrows), and angiogenesis (green arrows) are shown with a 50 µm scale.

Re-epithelialization

The results of re-epithelialization in the group treated with apigenin-loaded NA, the positive control group, and the negative control group on days 3, 7, 14, and 21 are shown in Table 4 and Figure 3. On day 3, the group treated with apigenin-loaded NA showed a re-epithelialization value of 23.63±6.24%, which was higher than that of the positive control group (14.55±1.64%) and the negative control group (21.1±1.42%). The increase continued on day 7, where the apigenin-loaded NA group showed a rate of 39.93±6.13%, while the positive control group displayed a rate of 34.15±10.55%, and the negative control group showed a rate of 24.12±9.24%.

On day 14, the apigenin-loaded NA group had a re-epithelialization rate of 69.32±10.26%, which was higher than that of the positive control group at 49.65±3.82% and the negative control group at 48±10.72%. On the 21st day, the apigenin-loaded NA group reached a peak re-epithelialization rate of 91.67±6.69%, while the positive control group only reached 86.02±8.73% and the negative control group reached 63.77±8.05%. However, the increase in re-epithelialization was not accompanied by statistically significant results (P>0.05) on each observed day.

Collagen density

On day 3, the apigenin-loaded NA group showed a collagen density of 61.71±5.03 mg/mL, which increased to 69.62±5 mg/mL on day 7, 77.91±4.15 mg/mL on day 14, and reached 81.74±2.77 mg/mL on day 21. The positive control group exhibited a slower increase, rising from 58.47±4.62 mg/mL to 74.57±1.22 mg/mL, while the negative control group had the lowest values, increasing from 41.12±3.9 mg/mL to only 68.04±3.41 mg/mL on day 21. These results showed statistical significance (P<0.05) between apigenin-loaded NA and the negative control groups.

All these differences were statistically significant (P<0.05). The consistent and substantial increase in collagen density, as shown in Table 5 and Figure 4, in the apigenin-loaded NA group confirms the synergistic role of apigenin and the NA delivery system in accelerating dermal tissue remodeling after burns.

Table 2. Total number of neutrophils (cells/µm) involved in burn wound healing

Day	Mean±SEM		
	Group		
	Apigenin-loaded NA	Positive Control	Negative Control
3	24.47±10.87[a]	28.13±9.71[ab]	46.13±4.61[b]
7	28.2±6.13[a]	42.4±10.55[ab]	52.73±9.24[b]
14	19.67±10.26[a]	38.73±3.82[ab]	41.87±10.72[b]
21	26.6±6.69[a]	30.13±8.73[a]	41.8±8.05[a]

Note: Different letters (superscripts) in the same row indicate significant differences (P<0.05).

In silico molecular docking analysis

The interaction between apigenin and COX-2 and MMP-1 receptors resulted in an affinity energy close to that of the native ligand on the NF-κB receptor. In contrast, for COX-2 and MMP-1 receptors, the affinity energy of apigenin was lower than that of the native ligand, indicating a higher binding affinity. Molecular docking results showed that the affinity energy and Ki for the native ligand interaction with COX-2 receptor were -7.85 kcal/mol and 1.76 µM, respectively. Meanwhile, apigenin exhibited a lower affinity energy of -10.21 kcal/mol, with an Ki of 32.76 nM (Tables 6 and 7 and Figure 5).

Molecular docking showed that apigenin interacts with NF-κB receptor with an affinity energy of -10.58 kcal/mol, which is slightly higher than that of the native ligand, which has an affinity energy of -12.19 kcal/mol. The Ki of apigenin was 17.59 nM, greater than that of the native ligand, which was 1.16 nM; however, it still demonstrates potential in inhibiting NF-κB activity. This interaction involved hydrogen bonding with amino acid residues such as ASP 166, GLU 97, and CYS 99 (Tables 6 and 7 and Figure 5).

Based on the molecular docking results between the MMP-1 receptor and the test ligand, apigenin also showed a higher affinity toward the MMP-1 receptor than the native ligand. The affinity energy of the interaction between apigenin and MMP-1 was recorded at -12.26 kcal/mol, which is lower than that of the native ligand, which only reached -11.23 kcal/mol. The Ki for apigenin was 1.04 nM, significantly smaller than that of the native ligand, which was 5.84 nM. This indicates that apigenin has a stronger inhibitory ability toward the MMP-1 receptor. This interaction involves the formation of hydrogen bonds with amino acid residues, such as ALA 182, ALA 234, LEU 181, THR 241, and TYR 237.

Discussion

This study used rats with second-degree burns, which were observed on days 3, 7, 14, and 21. Second-degree burns can affect the papillary layer (upper dermis) or reticular layer (deeper dermis), presenting with white or yellow coloration, blistered skin, and a moist appearance (Mofazzal Jahromi et al., 2018). Apigenin-loaded NA administration reduced the wound area faster than both the positive and negative controls, and it was statistically significantly faster than the negative control (P<0.05).

Table 3. Total number of lymphocytes (cells/µL) involved in burn wound healing

Day	Mean±SEM		
	Group		
	Apigenin-loaded NA	Positive Control	Negative Control
3	9.8±6.24[a]	9±1.64[a]	12.07±1.42[a]
7	15.6±2.78[a]	15.27±8.08[a]	24±11.13[a]
14	8.67±7.93[a]	18.2±2.6[a]	18.6±6.02[a]
21	12.87±4.13[a]	14.53±3.61[a]	21.93±6.99[a]

Note: Different letters (superscripts) in the same row indicate significant differences (P<0.05).

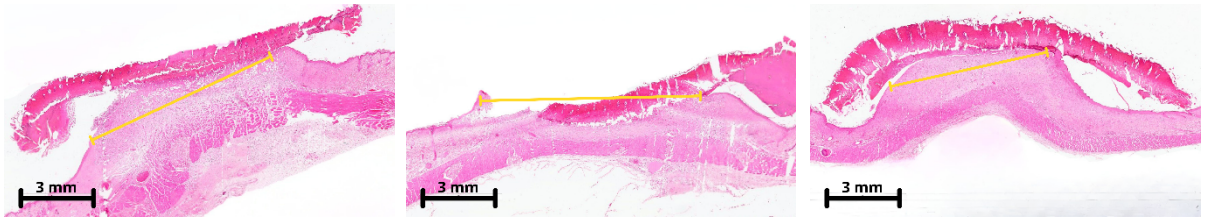

Figure 3. Wound re-epithelialization image on day 14

a) Apigenin-loaded NA group, b) Positive control group (bioplasenton®), c) Negative control group

Note: Areas that have not been re-epithelialized are indicated (yellow line) with a 3 mm scale.

Histopathological analysis showed a statistically significant decrease in the number of neutrophils in the apigenin-loaded NA group compared to the negative control group ($P<0.05$), indicating that the inflammatory phase can resolve more quickly. Neutrophils are the primary immune cells involved in the inflammatory phase, as they phagocytize pathogens and clear tissue debris (Guo & DiPietro, 2010). The inflammatory response is an important component of the wound healing process, as it plays a crucial role in fighting infection and preventing tissue injury and septicemia (Ghasemi et al., 2024). Neutrophils contribute to the innate immune response in burns, but their uncontrolled activation can lead to prolonged inflammation and delayed healing (Neely et al., 2014). The presence of pro-inflammatory cytokines in the wound environment can trigger excessive neutrophil infiltration, potentially inhibiting healing (Dinh et al., 2023).

Lymphocytes play a role in wound healing by secreting lymphokines that activate fibroblasts for collagen synthesis and tissue regeneration. As anti-inflammatory agents, lymphocytes help balance pro-inflammatory and anti-inflammatory cytokines, preventing excessive inflammation (Prakoso & Kurniasih, 2018). However, excessive or prolonged increases in lymphocytes can trigger chronic inflammation that inhibits optimal wound healing (Holzer-Geissler et al., 2022). Although lymphocyte counts between groups were not statistically significantly different ($P>0.05$), the fluctuations in lymphocyte counts still provide important information. The high lymphocyte counts in the negative control group on day 7 indicated persistent inflammation. In contrast, the NA group showed better control of lymphocyte activity, with apigenin stabilizing the adaptive immune response during the wound healing process.

The decrease in inflammatory cells after day 7 shows that apigenin-loaded NA can accelerate the inflammatory process by reducing the number of inflammatory cells after day 7 compared to the other groups. This anti-inflammatory effect accelerates the transition from the inflammatory phase to the proliferative phase, which is characterized by reduced neutrophil infiltration and increased activity of fibroblast cells and keratinocytes for tissue regeneration (Eming et al., 2007). Li et al. (2022) showed that apigenin has immunomodulatory activity through the inhibition of the NF-κB and STAT1 path-

Table 4. Re-epithelialization in burn wound healing process

Day	Mean±SEM		
	Treatment Group		
	Apigenin-loaded NA	Positive Control	Negative Control
3	23.63±1.99[a]	14.55±1.71[a]	21.1±0.8[a]
7	39.93±2.12[a]	34.15±0.39[a]	24.12±3.29[a]
14	69.32±2.64[a]	49.65±2.23[a]	48±4.5[a]
21	91.67±2.89	86.02±2.48[a]	63.77±2.35[a]

Note: Different letters (superscripts) in the same row indicate significant differences ($P<0.05$).

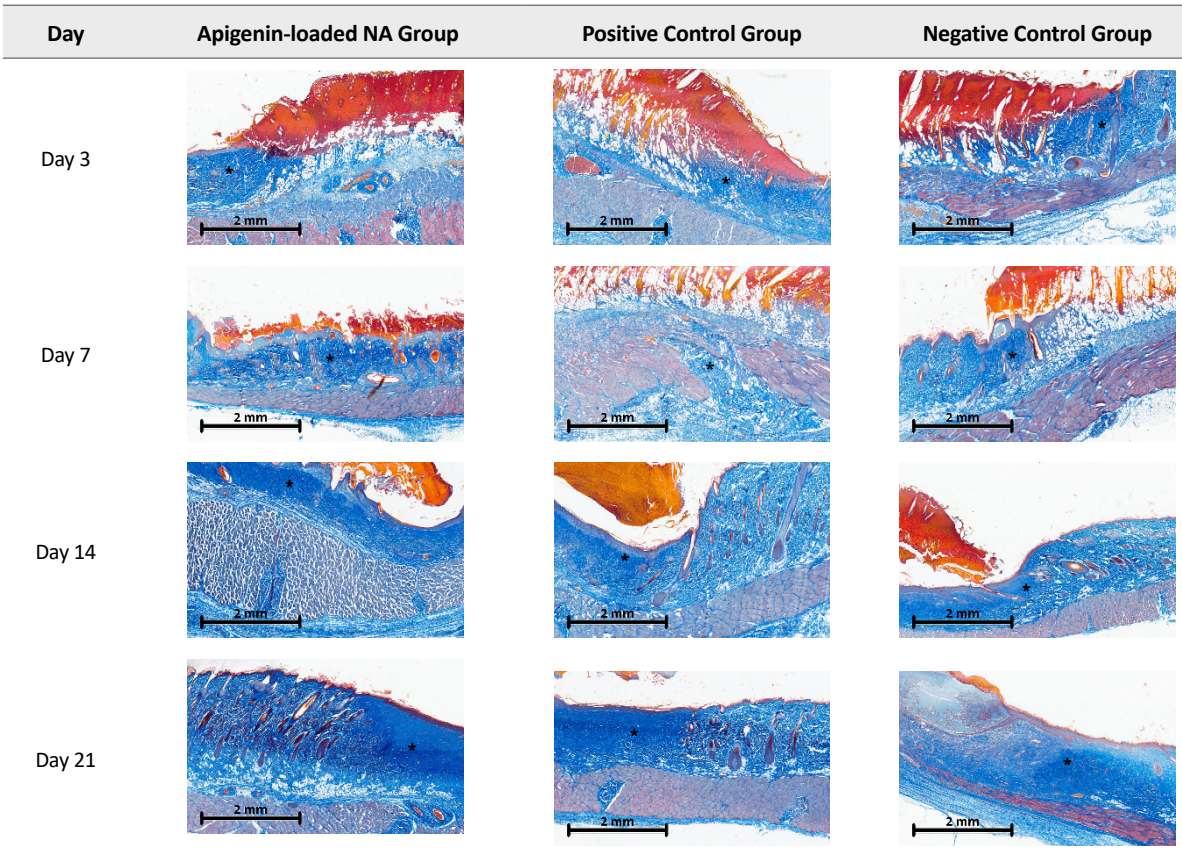

Figure 4. Microscopic analysis of collagen density using Masson's trichrome staining on days 3, 7, 14, and 21 of burn wound healing in the apigenin nnoemulgel (NA), positive control, and negative control groups

Note: Collagen density is indicated by an asterisk (*) and shown with a 2 mm scale.

ways, which play a role in suppressing T cell activation and the production of proinflammatory cytokines, such as IL-2 and IFN-γ. In addition, apigenin increases the expression of IL-10, an anti-inflammatory cytokine that plays a role in ending the immune response and accelerating the healing process (Zhang et al., 2014).

Re-epithelialization is a process in wound healing characterized by the speed of epidermal regeneration and wound closure, thereby accelerating wound healing (Gupta & Kumar, 2015). The involvement of keratinocytes is significant during re-epithelialization because these cells function to close the wound by renewing the damaged epidermal layer (Agung et al., 2016). Slow re-

Table 5. Collagen density in burn wound healing process

Day	Mean±SEM		
	Treatment Group		
	Apigenin-loaded NA	Positive Control	Negative Control
3	61.71±5.03[a]	58.47±4.63[a]	41.12±3.9[b]
7	69.62±5[a]	62.85±1.41[a]	48.76±5.97[b]
14	77.91±4.15[a]	73.85±2.47[a]	61.81±0.27[b]
21	81.74±2.77[a]	74.57±1.22[a]	68.04±3.41[b]

Notes: Different letters (superscripts) in the same row indicate significant differences (P<0.05).

Table 6. Docking analysis of interaction between ligands and COX-2, MMP-1, and NF-κB receptors

Receptor	Ligands	Affinity Energy (kcal/mol)	Ki (μM)
COX-2	Apigenin	-10.21	32.76
	Diclofenac	-7.85	1.76
MMP-1	Apigenin	-12.26	1.04
	RS2	-11.23	5.84
NF-κB	Apigenin	-10.58	17.59
	Staurosporine	-12.19	1.16

epithelialization is often associated with the risk of infection, excess scar tissue formation, and poor recovery of skin function. Apigenin accelerates epidermal regeneration by inhibiting the NF-κB inflammatory pathway, promoting keratinocyte proliferation and migration, and protecting cells from oxidative stress. These effects support re-epithelialization and enhance wound closure (Ma et al., 2021; Zhang et al., 2014).

Collagen is a significant component in the extracellular matrix that supports the strength and stability of newly formed tissues. Collagen comes into play during the proliferation phase when fibroblasts begin producing

Table 7. Interaction between receptor and test ligand

Receptor	Ligand	Amino Acid Residues	Hydrogen Bond Distance
COX-2	Apigenin	MET 522 SER 530 TYR 385	2.02 Å 2.22 Å 2.25 Å
MMP-1	Apigenin	ALA 182 ALA 234 LUE 181 THR 241 TYR 237	1.80 Å 1.94 Å 2.80 Å 2.12 Å 3.09 Å
NF-κB	Apigenin	ASP 166 GLU 97 CYS 99	1.89 Å 2.09 Å 1.67 Å

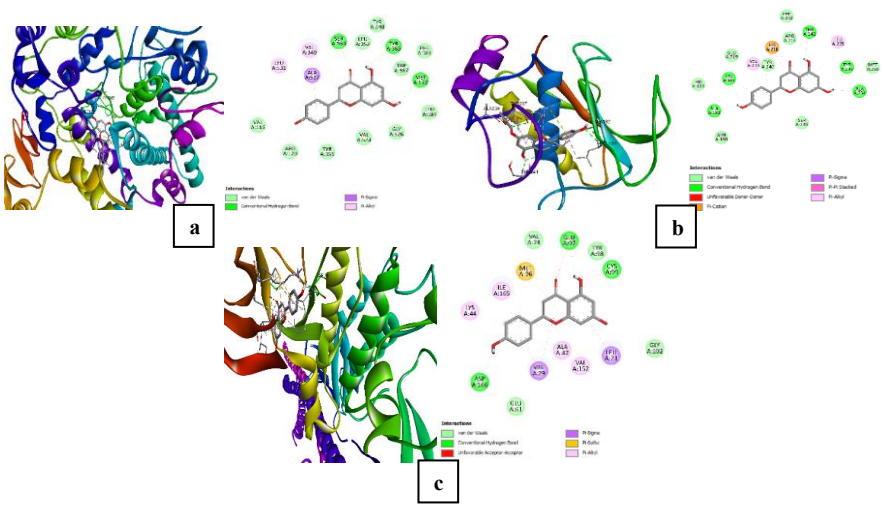

Figure 5. 2D and 3D in silico visualization

a) Apigenin interaction with COX-2; b) Apigenin interaction with MMP-1; c) Apigenin interaction with NF-KB

collagen to repair damaged tissue (Al Marwah Asrul et al., 2023). A progressively increasing collagen density reflects the success of the wound healing process, as the new tissue formed will be stronger and more stable (Nanda et al., 2017). The higher collagen density in the apigenin nanomelugel group shows that apigenin-loaded NA not only accelerates the formation of new tissue but also improves the quality of the formed tissue. Apigenin has protective effects against oxidative stress indicators (Ibrahem et al., 2020). It also increases the expression of TIMPs, which function as MMP inhibitors, leading to reduced collagen degradation and ultimately increased tissue repair (Ji et al., 2021).

In silico analysis also supports the in vivo data by demonstrating that apigenin has a strong binding affinity to key targets in the wound healing process, such as MMP-1, COX-2, and NF-κB. The molecular interaction between apigenin and active residues on these enzymes indicates a potential inhibition of tissue-damaging pro-inflammatory and proteolytic activities. This explains the synergistic effect between apigenin's biological activity and efficient delivery via the NA system (Adel et al., 2022).

Apigenin's ability to inhibit MMP-1 is essential, considering that this enzyme plays a role in collagen degradation during the remodeling phase. Inhibition of MMP-1 helps maintain the integrity of the extracellular matrix and accelerates the restoration of dermal structure (Han et al., 2018). According to, inhibition of MMP-9 can also accelerate collagen deposition, thereby accelerating wound healing. In addition, the binding of apigenin with COX-2 may explain the anti-inflammatory effects observed through decreased neutrophil counts and stabilization of the immune response.

The synergistic mechanism between anti-inflammatory activity, enhanced epidermal regeneration, and stimulation of collagen synthesis makes apigenin-loaded NA a promising candidate for the topical therapy of burn wounds. The effectiveness of apigenin-loaded NA in accelerating wound healing suggests that combining natural bioactive compounds, such as apigenin, with nanotechnology can improve the clinical efficacy of herbal-based products.

Conclusion

The results of the study indicate that administering apigenin-loaded NA can reduce the initial inflammatory phase, thereby accelerating wound healing. In silico analysis supports the idea that apigenin can control inflammatory modulation receptors, such as MMP-1, COX-2, and NF-κB, indicating that apigenin-loaded NA has the potential to be developed into a wound-healing drug in the future.

Ethical Considerations

Compliance with ethical guidelines

All animal procedures were performed following the standards outlined in the guidelines of the Animal Welfare, Ethics, and Experimentation Committee of the Animal Ethics Committee, School of Veterinary Medicine and Biomedical Sciences, IPB University, Bogor, Indonesia (Code: 196/KEH/SKE/IV/2024).

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Authors' contributions

Conceptualization and supervision: Bayu Febram Prasetyo, and Vetnizah Juniantito; Methodology, project administration and formal analysis: Bayu Febram Prasetyo, Vetnizah Juniantito, Rini Madyastuti Purwon, and Baharun Rasyid; Software, data curation and data analysis: Vetnizah Juniantito and Baharun Rasyid; Data collection and investigation: Baharun Rasyid; Validation, visualization, and writing: All Authors.

Conflict of interest

The authors declared no conflict of interest.

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