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Research Article
Effectiveness of Gel from Andaliman Fruit (*Zanthoxylum acanthopodium* DC.) Extract on Wound Inflammation

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Abstract

Background and Objective: Pain is caused by damaged tissue on the outside or inside of an organ and it is regulated by chemicals. Synthetic drugs are used to ease pain because they are analgesics in the field of medicine. Traditional medicine is known to help people all over the world, in both rich and developing nations. The Andaliman fruit, or *Zanthoxylum acanthopodium* DC., comes from a spice plant that grows naturally in Toba Regency, North Sumatra, Indonesia. This study aims to determine the formulation of the nanoherbal analgesic spray gel preparation of Andaliman fruit (*Zanthoxylum acanthopodium* DC.) in terms of its effectiveness as a pain reliever. **Materials and Methods:** There were three amounts of spray gel made, namely 5, 10 and 15% and tests were done to see how well they worked. *Staphylococcus aureus* bacteria were used in the bacterial blocking test. To test how well painkillers worked, five groups of mice were used. Using a hot iron, tests for anti-inflammatory activity and wound healing were done. The tissue was then watched for 14 days and analysed using Hematoxylin and Eosin (H&E) stains. **Results:** The 15% concentration reduces pain and the time it takes for the body to respond to it. The clear zone size is the same as (K^+) and it can lower the number of inflammatory cells and help wounds heal by adding fibroblast and collagen cells. These findings are supported by significant data results ($p<0.05$, $p = 0.018$). **Conclusion:** Finally, analgesic gel spray made from the Andaliman fruit at a 15% concentration can help with pain and also be antibacterial, reduce inflammation and help wounds heal.

Key words: Analgesic, collagen, Andaliman fruit, fibroblast, wound healing, *Zanthoxylum*

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Pain is characterised as a multifaceted phenomenon encompassing both emotional and sensory components, capable of eliciting sensations of pain[1]. This experience arises from the presence of injured tissue, either externally or internally, within an organ and is mediated by chemical agents[2]. Both pharmaceutical and non-pharmacological interventions can be employed to proactively address pain[2]. Within the realm of pharmacology, synthetic medicines are employed to alleviate pain by virtue of their analgesic qualities. Analgesics refer to chemical compounds or pharmaceutical substances that possess the ability to alleviate pain without inducing adverse consequences such as loss of consciousness[2]. Traditional medicine is well recognised for its numerous advantages around the globe, encompassing both developed and developing nations[3]. The utilisation of traditional medicine remains prevalent among a significant proportion, up to 65%, of the population residing in affluent nations, hence indicating its enduring popularity[3]. This assertion is substantiated by the "back to nature" perspective, contending that the risk of adverse effects associated with herbal medications is comparatively reduced when compared to synthetic chemical medicines[3].

The Andaliman fruit, scientifically known as *Zanthoxylum acanthopodium* DC., is a spice plant that is native to the Toba Samosir and North Tapanuli Regencies in North Sumatra[4]. The Andaliman fruit is commonly employed as a spice and traditional medicinal remedy, with one of its notable applications being its potential analgesic properties[4]. The pharmacological effects of the essential oils present in Andaliman fruit encompass a range of properties, including larvicidal, anti-inflammatory, analgesic, antioxidant, antibiotic, hepatoprotective, antiplasmodial, cytotoxic, antiproliferative, antiviral, anticonvulsant and antifungal activities[4,5]. Indonesian individuals opt for herbal remedies over conventional medications because of their affordability and limited adverse effects[5]. The fruit plant known as Andaliman (*Zanthoxylum acanthopodium*) exhibits the capacity to effectively sequester free radicals, hence mitigating the occurrence of oxidative damage within the Indonesian Region. The utilisation of fruit from this particular plant is employed as a means of enhancing the taste and aroma of customary Batak culinary preparations[5,6]. In addition to the aforementioned, studies have indicated that the Andaliman fruit exhibits potential therapeutic properties in the treatment of cervical cancer[6-10], placental cells[11,12], preeclampsia[13] and diabetic wounds[14].

The potential of nano herbal derived from Andaliman fruit as a molecular herbal medicinal preparation is believed to be significant[12]. This nanoherbal exhibits surface features and particle size that facilitate its rapid and efficient penetration into specific cells and tissues[14]. Nanoherbals possess remarkable efficacy, safety and potential to administer substantial doses while mitigating adverse effects, owing to their diminutive size and heightened capacity[14]. Hence, it is imperative to develop a nanoherbal formulation of Andaliman fruit in the form of a spray gel to enhance its usability. The spray gel formulation is deemed to be more effective and convenient for portability purposes. The utilisation of gel spray has several benefits, including its invigorating properties, ability to hydrate the skin, user-friendly application and rapid absorption into the dermal layers. The gel formulation exhibits a significantly elevated water content. This particular preparation is favoured due to its ability to mitigate the likelihood of skin inflammation[15]. Additionally, it possesses the desirable qualities of transparency, elasticity, efficient and effective medicine release and absence of an oily residue upon application to the skin[15]. The objective of this study was is to investigate the efficacy of the nanoherbal analgesic spray gel formulation derived from Andaliman fruit (*Zanthoxylum acanthopodium* DC.) as a pain reliever. Additionally, this research aims to explore the potential anti-inflammatory, antibacterial and wound healing properties exhibited by the fruit of this plant.

MATERIALS AND METHODS

Extract preparation: The study was carried out from June, 2023 to December, 2023. The cultivation of Andaliman fruit is primarily observed in the Toba Samosir and North Tapanuli Districts of North Sumatra. Subsequently, the item undergoes a cleansing process involving washing and subsequent air drying. Following the drying process, the sample underwent blending and sieving. Subsequently, the specimens were transported to the Integrated Research Laboratory at the University of Sumatera Utara (USU) to undergo ball milling at a rotational speed of 1500 revolutions per minute over a duration of 60 min, resulting in the transformation of the samples into nanoherbals. Following this, a particle size analyzer (PSA) examination was conducted to determine the dimensions of the particles. Subsequently, the SEM (Scanning Electron Microscopy) examination should be conducted in order to ascertain the morphological characteristics and elemental composition of the specimen. The preparation of a spray gel formulation involves the addition of carbopol to a 100 mL glass beaker, followed by stirring with hot water until complete dissolution is achieved[16]. Following the process of homogenization, proceed to agitate the triethanolamine in order to achieve the formation of a visually clear gel mass,

referred to as mixture 1. Transfer all of the Hydroxypropyl Methylcellulose (HPMC) into the heated water contained in a 100 mL glass beaker (referred to as mixture 2)[16]. Combine mixes 1 and 2, followed by the addition of propylene glycol and DMDM hydantoin. Stir the resulting mixture until achieving homogeneity[17]. Furthermore, the incorporation of Andaliman fruit nanoherbal is recommended. Subsequently, incorporate 100 mL of distilled water in a progressive manner, ensuring continuous stirring until homogeneity is achieved. Subsequently, transfer the resulting liquid into a suitable container, such as a spray bottle[17]. The assessment of spray gel formulations: (a) In order to meet the required standards, compositions for spray gel must successfully undergo organoleptic testing, which encompasses a visual examination to assess attributes such as colour, scent, clarity and texture, (b) The utilisation of a homogeneity test is necessary in order to detect particles or substances that have not undergone complete combination prior to homogenization. The experiment was conducted using a spray gel formulation that was applied onto a transparent glass substrate. The pH test is conducted to ascertain the pH level by employing a universal pH indicator that encompasses a pH range suitable for the experiment's preparation, specifically ranging from 4.5 to 7. The spray pattern test involves the application of the substance onto a plastic sheet of known weight, which has been specifically designated at intervals of 3, 5, 10 and 15 cm. Once the preparation has been applied via spraying, the plastic sheet is subjected to weighing in order to determine the weight of the preparation adhered to it. This weight is then utilised to compute the quantity of preparation released per spray (measured in grammes). Additionally, the spray formation pattern and the width of the spray gel spray pattern are noted. The sticky spreadability test involves applying the preparation onto the upper arm at a distance of 3 cm, followed by an assessment of its spreadability. The evaluation of the adherence of the preparation to the skin surface occurs 10 sec after the gel has been sprayed. The goal of the spray condition test was is to assess the functionality of the applicator in dispensing the preparation, specifically determining whether it is capable of effectively spraying the substance. Each preparation was applied by spraying onto a vacant mica plastic surface from a distance of 3 cm. Subsequently, the spray circumstances were are observed in accordance with the obtained spray findings[17].

Bacterial inhibition test: The bacterial inhibition test is a laboratory technique used to assess the ability of certain substances to inhibit the growth of bacteria. A bacterial inhibition assay was conducted to evaluate the inhibitory potential of a nano herbal spray gel derived from Andaliman fruit against *Staphylococcus aureus*. The experiment was performed using Mueller Hinton Agar (MHA) as the growth medium. *Staphylococcus aureus* was reconstituted in sterile distilled water and subsequently standardised to McFarland turbidity (OD600 = 0.1). The Petri plates were labelled with the quantity of Andaliman fruit nanoherbal spray gel sprays and they were filled with MHA medium. A sterile cotton swab is aseptically obtained and subsequently immersed in a suspension containing pathogenic bacteria. The swab is then gently scraped across the surface of the MHA (Mueller-Hinton Agar) media. Next, a micropipette was used to inoculate a sterile blank disc with 10 µL of the endophytic bacterial suspension, which was then placed in the designated location. Subsequently, the specimen was subjected to incubation for 24 hrs at a temperature of 37°C. The measurement of the clean zone's diameter was conducted using a calliper[18].

Experimental design: The research was conducted at the University of Sumatera Utara's Biology Laboratory, the Pathology and Anatomy Laboratory of the Faculty of Medicine. A cohort of male white rats, aged between 8 and 11 weeks and weighing between 180 and 250 g, was divided into five groups. Each group consisted of five rats. Group 1 served as the negative control and did not receive any treatment. Group 2 served as the positive control and was administered oparin gel. Group 3 served as an additional negative control and received Andaliman fruit nanoherbal gel spray at a concentration of 5%. Group 4 received Andaliman fruit nanoherbal gel spray at a concentration of 10%, while group 5 received Andaliman fruit nanoherbal gel spray at a concentration of 15%. The rats were provided with unrestricted access to food and water[14].

An investigation into the efficacy of spray gel as an analgesic: Prior to administering the test treatment, the tails of individual rats were subjected to immersion in a water bath maintained at a temperature of 40°C for 10 sec. The duration of time taken by each rat to withdraw its tail from the water bath was thereafter measured and recorded. The determination of a typical rat reaction to painful stimuli involves the computation of an average based on previously recorded time response data. The data collection process involved conducting three repetitions of each series of observations, with a 2 min interval between each repetition. The rats in this study were divided into five groups for the purpose of administering different test preparations. Group 1, serving as the negative control, did not receive any treatment. Group 2, the positive control, was given oparin gel.

Group 3 was treated with a 5% concentration of Andaliman fruit nanoherbal spray gel. Group 4 received a spray gel with a concentration of 10%, while group 5 received a spray gel with a concentration of 15%. Following the administration of the therapy, the reaction time data in response to painful stimuli were reevaluated after 10 min. If the rat does not exhibit tail wagging within a 10 sec timeframe upon exposure to hot water at a temperature of 40°C, it can be inferred that the therapy has the potential to alleviate the pain caused by the stimulus, indicating a lack of awareness of the painful sensation. Following the administration of the test treatment, subsequent measurements of reaction times to painful stimuli were conducted at intervals of 20, 30, 60 and 90 sec[19].

Anti-inflammation and wound healing test: A series of experiments were conducted to evaluate the anti-inflammatory and wound healing properties of a 15% spray gel. The experiments were performed on a total of 24 male rats, divided into six groups with six rats in each group. The groups included a control group (H^+), a group in which rats underwent surgery on day 3 (H3), a group in which rats underwent surgery on day 5 (H5), a group in which rats were dissected on day 7 (H7) and a group in which rats were dissected on day 14 (H14). Subsequently, the rats were administered ketamine in order to induce anaesthesia. Subsequently, the rats were subjected to thermal injuries by means of a heated iron with dimensions of 2×2 cm, applied to the dorsal region. Additionally, the rats were administered a 15% spray gel, in accordance with the assigned treatment group. The process of wound healing was documented through the utilisation of descriptive accounts. The inflammatory cells were analysed by doing photo observations and performing surgery on the 14th day, followed by the preparation of skin tissue samples with hematoxylin and eosin staining[14].

Statistical analysis: Data analysis used the one-way ANOVA (Analysis of Variance) test. If the p-value was less than 0.05, there was a significant difference between groups and if the p-value was higher than 0.05, there was no difference between groups and the Kruskal Wallis test (for non-parametric data) was used to analyze the data in the SPSS 22 program.

Ethical consideration: This study utilises laboratory animals that have received approval for handling from the University of Sumatera Utara (USU) Health Research Ethics Approval Team, with the assigned number 450/KEPH-FMIPA/2023.

RESULTS

SEM and PSA analysis of Andaliman fruit: The nanoherbal derived from the Andaliman fruit possesses inherent analgesic properties, which effectively alleviate pain. The Scanning Electron Microscopy (SEM) analysis reveals the presence of agglomerations in the nanoherbal derived from Andaliman fruit, as depicted in Fig. 1. At a magnification of 100X in Fig. 1a, the objects appear to be crystals, but they are not clearly visible from a distance. However, when the magnification is increased to 2500X (Fig. 1b) and 5000X (Fig. 1c), the structure becomes clearly evident, like a cluster of interconnected crystals. The dimensions of the Andaliman fruit nanoherbal, as determined by the PSA (Particle Size Analyzer) test conducted on the ball mill output, measure 117 nm (Fig. 2).

Bacterial inhibition test: The findings from the bacterial inhibition test demonstrated that the application of a nanoherbal spray gel containing Andaliman fruit at a concentration of 15% resulted in the formation of a distinct zone of inhibition, comparable in size to the positive control (Table 1). This observation suggests that the 15% concentration effectively inhibits the growth of *Staphylococcus aureus* bacteria. Furthermore, the statistical analysis revealed a significant result ($p<0.05$, $p = 0.018$).

Comparative analysis of the efficacy of Andaliman fruit gel as an analgesic: A pain alleviation experiment was conducted on individual rats, wherein each rat was subjected to immersion in a water bath maintained at a temperature of 40°C for 10 sec. The experimental group that received a spray gel concentration of 15% exhibited a more prolonged pain response compared to the other treatment groups. A higher proportion of 15% of the groups exhibited a stronger concentration of nanoherbal content derived from Andaliman fruit compared to the remaining groups. This finding demonstrates that the 15% concentration exhibits a significant level of analgesic action, as indicated in Table 2.

Anti-inflammatory and wound healing test results: The results of the tests conducted on anti-inflammatory and wound healing properties are presented in Table 3. Additionally, Fig. 3 and 4 illustrate the progress of wound healing. The experimental findings indicate that the use of a 15% concentration of Andaliman spray gel has anti-inflammatory properties and promotes wound healing by the 14th day. These results are further substantiated by statistically

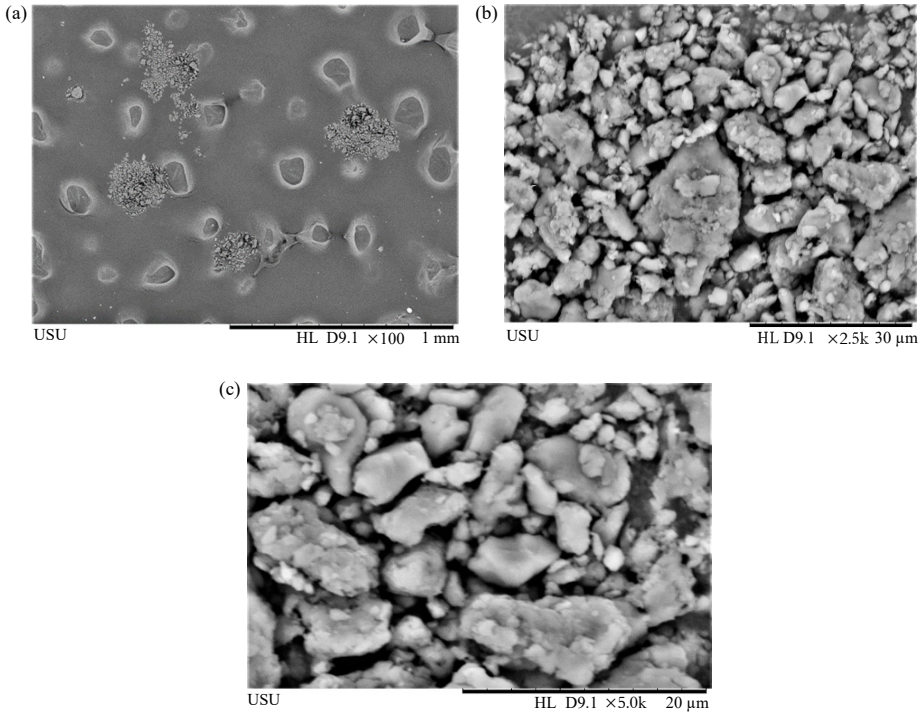

Fig. 1(a-c): Results of scanning electron microscope Andaliman fruit (*Zanthoxylum acanthopodium*), (a) 100×, (b) 2500× and (c) 5000×

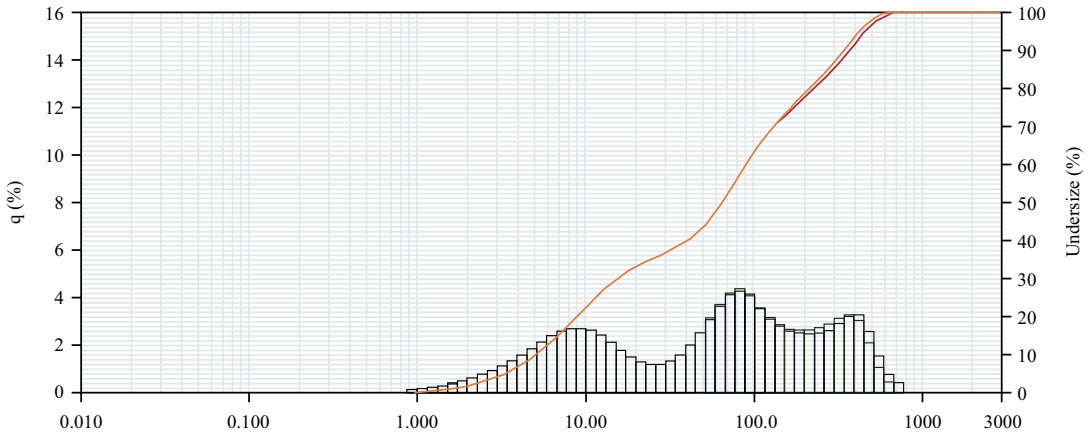

Fig. 2: Analysis graph from the particle size analyzer (PSA) of Andaliman fruit (*Zanthoxylum acanthopodium*)

significant evidence. In the event of an injury, there is a consequential impairment to the integrity of the skin cells. An inflammatory reaction combined with an exudation process causes damage to all layers of the epidermis. Healing happened spontaneously within two weeks after the injury to the majority of the dermis layer. Skin without wounds looks to have a healthy morphological structure (Fig. 3a). After the wound has formed, the patient will go through the first phase (Fig. 3b), which will last 3-4 days. In the inflammatory phase, there are two processes: Hemostasis and phagocytosis. Hemostasis refers to the cessation of bleeding in the wound region. During the hemostasis process, a scab forms on the wound surface (tissue that is slightly dark red and quite hard) to keep microbes out (Fig. 3c). This inflammatory reaction is critical to the healing process because it triggers blood clotting, which prevents blood loss and evident sloppy or untidy epithelial arrangements (Fig. 4a). If there is no infection, this period will end quickly. The inflammatory phase begins in 4. Following 5 days of damage, collagen and basic components known as proteoglycans are synthesised (Fig. 4b).

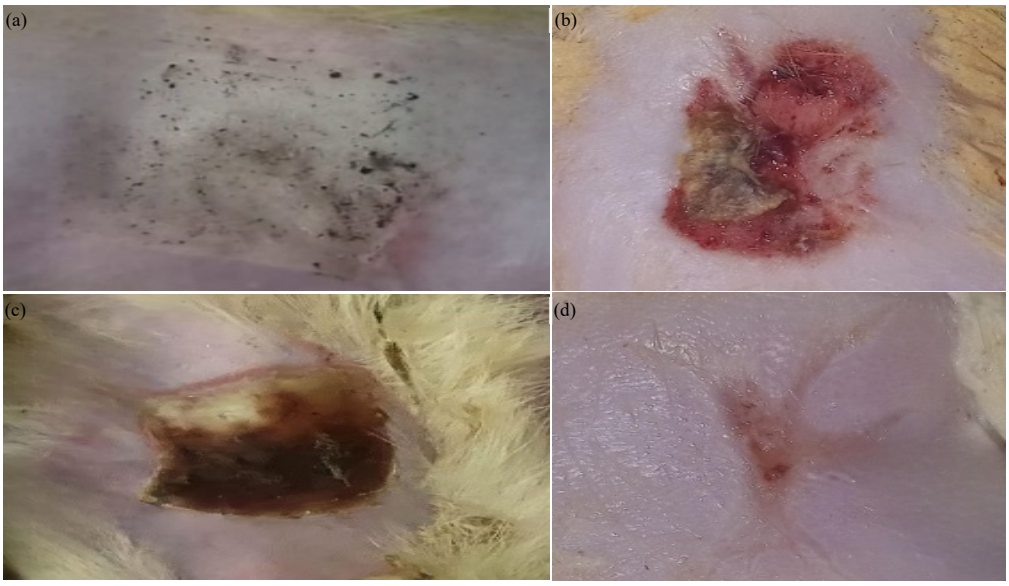

Fig. 3(a-d): Andaliman in healing burns, (a) Normal, (b) 5th day of wounds, (c) 7th day of wounds and (d) 14th day of wounds

Table 1: Bacterial inhibition test results with Andaliman fruit nanoherbal

	Clear zone diameter (mm)			
Concentration	U1	U2	U3	Average
K^+	22.8	19.4	19.1	20.43±3.22
5%	13.2	12.4	14.5	13.36±4.32*
10%	17.8	15.9	16.7	16.80±5.03*
15%	20.5	19.7	19.3	19.83±5.12^{Ns}

U1: Replication 1, U2: Replication 2, U3: Replication, K^+: Chloramphenicol, 5%: 5% concentration of Andaliman fruit nanoherbal, 10%: 10% concentration of Andaliman fruit nanoherbal, 15%: 15% concentration of Andaliman fruit nanoherbal, *p<0.05 compared to K^+ and ^{Ns}p>0.05 compared to K^+

Table 2: Response time to pain

	Response time to pain (sec)						
	Before treatment	After treatment					
Test group	Average	10	20	30	60	90	Average
Control (-)	1.37±0.19	1.39	1.34	1.39	1.35	1.26	1.34±0.02
Control (+)	1.33±0.16	2.46	2.98	3.66	4.63	4.08	3.56±2.21*
Spray gel 5%	1.49±0.11	2.36	2.80	3.65	4.46	4.08	3.47±1.97*
Spray gel 10%	1.39±0.06	2.93	3.62	4.60	5.32	5.11	4.31±2.91*
Spray gel 15%	1.35±0.20	3.18	4.42	5.99	7.47	7.15	5.64±4.28**

*p<0.05 compared to control (-), **p<0.01 compared to control (-) and ^{Ns}p>0.05 compared to control (-)

Table 3: Data on anti-inflammatory test results

Treatment	Number of fibroblast cells	Collagen	Inflammatory cells
Days-0	7.22±0.21	8.44±2.01	15.11±3.78
Day-5	9.23±0.12	10.23±2.45	14.23±3.34
Day-7	14.22±1.24*	17.32±3.22*	9.23±2.34*
Day-14	17.89±2.23**	18.09±3.89*	6.11±2.01**

*Significant at p<0.05 and **Significant at p<0.01

Collagen, a protein found in the human body, can raise the surface tension of wounds. The greater the amount of collagen, the stronger the wound surface, making it less prone to open. Epithelial tissue forms across the wound (epithelialization), increasing blood flow and supplying oxygen and nutrients required for wound healing (Fig. 4c). Finally, when fibroblasts continue to synthesise collagen, the scar shrinks, lose flexibility and leave a white line. The creation

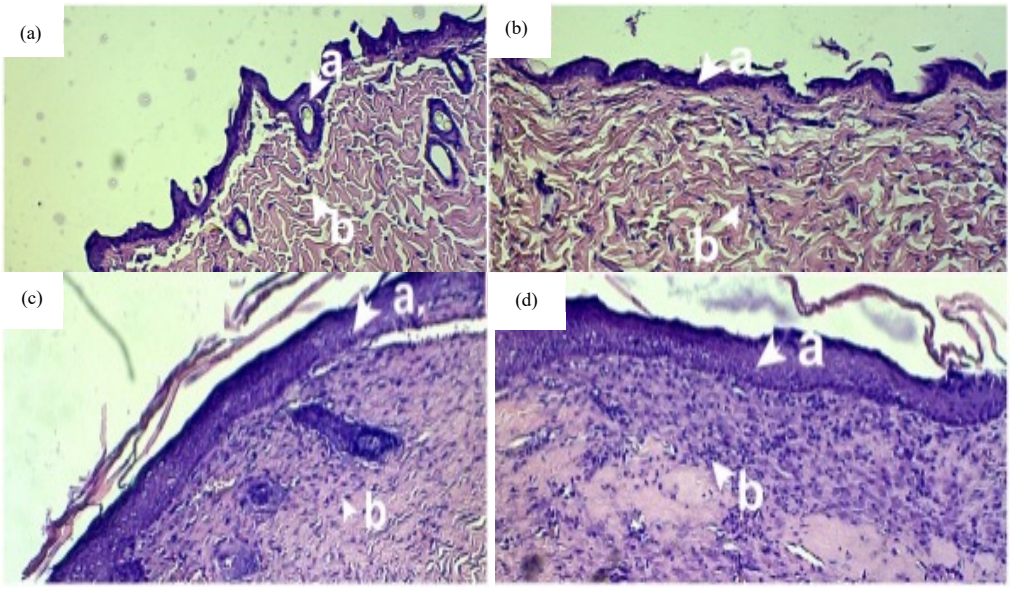

Fig. 4(a-d): Anti-inflammatory test results, (a) Day-0, (b) Day-5, (c) Day-7 and (d) Day-14
Arrow white (a) Epidermis and (b) Dermis

of new collagen alters the contour of the wound while increasing tissue strength. Scar tissue forms that are nearly as strong as the original tissue. The restored tissue's cellular activity and vascularization gradually decreased (Fig. 4d).

DISCUSSION

The inflammatory response results in damage to the entire epidermis layer, while recovery typically happens naturally within two weeks after harm to the majority of the dermis layer, particularly when exposed to the highest dosage of Andaliman spray gel. Pain manifests as a result of physical trauma, encompassing both external and interior injuries[20]. These wounds can serve as a habitat for dangerous bacteria, including *Staphylococcus aureus*, which is responsible for the formation of pus[21]. The Andaliman fruit exhibits antibacterial properties, capable of inhibiting harmful germs when applied at appropriate concentrations. Numerous researchers have documented the presence of a group of distinct components derived from the *Zanthoxylum* family, including lupeol, β-sitosterol, (+)-sesamin, trans-dimethyl matairesinol, hesperidin, (-)-cis-N-methylcanadine, sucrose, liriodenine, sesamin, lichexanthone, piperitol, savinin, liriodenine and decarine, which possess antibacterial properties[4,5].

A pain alleviation experiment was conducted on individual rats, wherein each rat was subjected to immersion in a water bath maintained at a temperature of 40°C for a duration of 10 sec. The experimental group that received a spray gel concentration of 15% exhibited a more prolonged pain response compared to the other treatment groups. A higher proportion of 15% of the groups exhibited a stronger concentration of nanoherbal content derived from Andaliman fruit compared to the remaining groups. This finding demonstrates that the 15% concentration exhibits a significant level of analgesic action, as indicated in Table 2. It is frequently used to treat wounds, rheumatism, arthralgia, stomach aches and other painful conditions since members of the *Zanthoxylum* family have anti-inflammatory qualities and eliminate potential pain[22]. The analgesic actions of the *Zanthoxylum* family are caused by signalling pathways that are connected with inflammation-induced hyperalgesia. These pathways can be found in both the peripheral and central nervous systems[22,23].

Pain is characterised as a distressing amalgamation of sensory and emotional components that is linked to the presence or possibility of harm to bodily tissues. Within the presynaptic nerve terminal, the activation of Voltage-Gated Calcium Channels (VGCCs) is triggered by an action potential, leading to the subsequent release of diverse neurotransmitters. These neurotransmitters then traverse the synaptic cleft and bind to their respective receptors located on the postsynaptic membrane[24]. The interaction between morphine and the μ-opioid receptor results in the suppression of pain-transmitting neurons[25]. The reduction of pain is achieved by diminishing the release of pain-inducing neurotransmitters, such as substance P, from presynaptic

terminals located in the spinal cord[25]. Experimental findings indicate that essential oils derived from the *Zanthoxylum* family exhibit the ability to decrease the input of extracellular calcium and release of intracellular calcium. These oils are believed to possess a synergistic analgesic effect[24,26].

These results are further substantiated by statistically significant evidence. In the event of an injury, there is a consequential impairment to the integrity of the skin cells. The wound healing process consists of three distinct phases, the first of which is referred to the inflammatory phase[14]. The time frame ranges from 0 to 5 days. During this initial stage, the blood vessels undergo constriction to enclose the wound and initiate phagocytosis for the purpose of eliminating germs. This is followed by the subsequent phase known as the proliferation phase, which spans a duration of 6 to 14 days[26]. During this particular phase, a notable decline is observed in the quantity of cells undergoing inflammation. Concurrently, the process of blood vessel creation, epithelialization and fibroblast cell generation commences. The fibroblast cells proliferate until their population surpasses that of the inflamed cells, marking the transition to the subsequent phase known as maturation/remodeling[14,26]. The final stage of wound healing, known as the 14-2 year phase, during this particular stage, there will be a notable augmentation in the quantity of collagen and fibroblasts, resulting in the gradual fading and potential restoration of the scar to its original appearance (Table 3). A solution with a concentration of 15% has the potential to alleviate pain and exhibit antibacterial, anti-inflammatory and wound healing properties. The *Zanthoxylum* genus exhibited significant efficacy in promoting wound healing on the dermal tissue of laboratory mice subjected to scalding injuries caused by exposure to high-temperature water[27]. The potential correlation between the use of substances derived from the *Zanthoxylum* family and enhanced wound healing, characterised by early re-epithelialization and accelerated wound closure, may be attributed to several mechanisms[27]. These mechanisms include heightened antioxidant activity, as indicated by elevated levels of superoxide dismutase (SOD) and reduced levels of malondialdehyde (MDA)[28]. Additionally, the anti-inflammatory effects of these substances may be mediated through the NF-κB signalling pathway[28]. Furthermore, the observed acceleration of collagen synthesis may be facilitated by the downregulation of Matrix Metalloproteinase-2 (MMP-2) and Matrix Metalloproteinase-9 (MMP-9) expression[29]. The enhanced capacity for wound healing can be ascribed to the phytoconstituents present in the substance, which may be attributable to their individual efficacy or their combined synergistic impact[29,30].

The implications of this research that localised natural content can be used as a form of pain treatment. The utilisation of a nanoherbal analgesic spray gel incorporating Andaliman fruit at a concentration of 15% has promising potential in diminishing pain, displaying antibacterial activity, possessing anti-inflammatory qualities and promoting wound healing. Consequently, it holds promise for future development as a pain treatment.

CONCLUSION

This study discovered that utilising a nanoherbal analgesic spray gel comprising Andaliman fruit at a 15% concentration holds promise in diminishing pain, exhibiting antibacterial activity, possessing anti-inflammatory effects and promoting wound healing. A novel hypothesis has surfaced regarding the efficacy of Andaliman nanoherbal fruit as a feasible analgesic. This theory is supported by the ongoing synthesis of collagen by fibroblasts, the reduction in size of scars along with their diminished flexibility, the formation of white lines and the development of epithelial tissue throughout the wound (epithelialization). Additionally, this fruit is believed to enhance blood circulation and provide the necessary oxygen and nutrients for the healing of wounds.

SIGNIFICANCE STATEMENT

This research found that Andaliman fruit nanoherbal analgesic spray gel with a concentration of 15% can be used as a herbal medicine to reduce pain, antibacterial, anti-inflammatory and wound healing. This research will help researchers to reveal the role of *Zanthoxylum acanthopodium* in the treatment of pain. Thus a new theory emerges about the role of nanoherbal Andaliman fruit as a pain reliever that can be achieved.

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