Pharmacy Reports

https://doi.org/10.51511/pr.54

RESEARCH ARTICLE

Open Access

Effect of pamelo orange (*Citrus maxima*) peel ethanol extract on burn healing in white rats

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Abstract: Burns are injuries resulting from tissue damage caused by contact with a heat source. The ethanol extract of pamelo orange peel (Citrus maxima) contains alkaloids, tannins, flavonoids, and saponins, which have been shown to enhance wound healing by accelerating epithelialization. This study aimed to evaluate the effect and determine the most effective concentration of ethanol extract of pamelo orange peel on burn wound healing in white rats. Sixteen rats were divided into four groups: a negative control group treated with 1% CMC-Na, two treatment groups receiving 3% and 6% ethanol extract of pamelo orange peel, and a positive control group treated with Lanakeloid-E®. Burns were induced on the rats' backs using a hot plate, followed by topical treatment for 21 days. Wound area measurements were taken on days 1, 4, 8, 12, 16, and 21 using a caliper. The results indicated that the ethanol extract of pamelo orange peel significantly promoted wound healing in the treated rats. Both the 3% and 6% concentrations reduced wound diameter, with the 3% concentration proving to be the most effective, achieving an 86.86% wound healing rate. The effectiveness of the extract is likely due to the presence of alkaloids, tannins, flavonoids, and saponins.

Keywords: burns, citrus maxima, healing, pamelo orange peel, wound diameter

Introduction

Burns are injuries to the skin caused by various forms of heat trauma, including fire, hot liquids, electricity, chemicals, and radiation. If not properly treated, burns can be fatal. According to the World Health Organization, approximately 11 million people worldwide suffer burns each year, with 180,000 fatalities resulting from these injuries [1]. Effective burn management, particularly through local therapy, aims to promote rapid healing. However, many commonly used treatments are associated with significant side effects, including argyria, leukopenia, liver and kidney toxicity, and allergic reactions. Additionally, the use of antibiotic ointments in burn care raises concerns about the potential for antibiotic resistance [2].

Pamelo orange (*Citrus maxima*), commonly known as grapefruit, is a fruit larger than typical oranges, with significant production in Eastern Indonesia, particularly in Pangkajene and the Archipelago Regencies. It is a leading commodity in Pangkep District, especially in Ma'rang District [3]. The peel of the pamelo orange is often discarded as waste, despite research indicating that its ethanol extract contains alkaloids, tannins, flavonoids, and saponins. Flavonoids, for instance, are known to accelerate wound healing by inducing the production of Transforming Growth Factor (TGF)-beta, which speeds up epithelialization. One study demonstrated that the addition of *Citrus sinensis* peel extract to biocellulose reduced the diameter of burns in rats, with a 3% concentration of the extract showing the greatest reduction in burn size [4].

Tannins contribute to wound healing by promoting fibroplasia, the formation of dermal tissue in the wound area. Saponins enhance wound healing by increasing macrophage migration to the wound site, thereby boosting growth factor production, stimulating new blood vessel formation, and promoting the migration and proliferation of fibroblasts within the wound bed [5]. Alkaloids, meanwhile, exhibit antimicrobial activity by disrupting the synthesis of peptidoglycan in bacterial cell walls, leading to cell death [6]. Based on these findings, this study was conducted to evaluate the efficacy of pamelo orange peel ethanol extract in healing burns in white rats (*Rattus norvegicus*).

Methods

Sample preparation

Pamelo orange peel was separated from the fruit, and the green outer layer (flavedo) was carefully removed from the white inner layer (albedo). The flavedo was

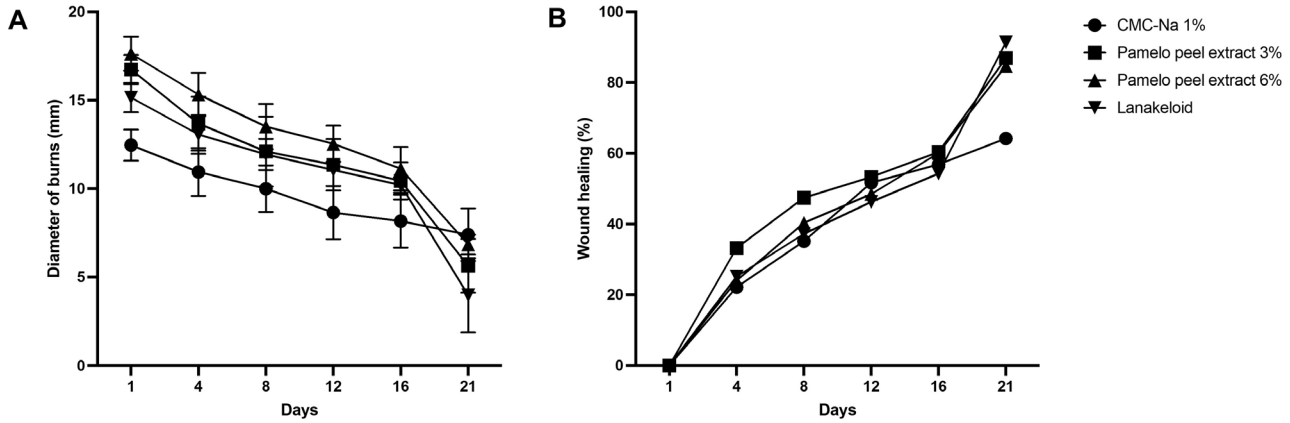

Figure 1. Wound healing activity of pamelo orange peel extract. (A) Changes in burn diameter. (B) Percentage of burn healing in rats over 21 days of treatment. Data A is presented as mean ± SD

then washed, drained, and cut into small pieces. These pieces were dried at room temperature and subsequently ground into a fine powder using a blender, followed by sieving to obtain a uniform powder.

Preparation of pamelo orange peel ethanol extract

The powdered pamelo orange peel was weighed using an analytical scale and then subjected to maceration in 96% ethanol. The macerate was concentrated using a rotary evaporator to obtain a thick ethanol extract of the pamelo orange peel.

Pamelo orange peel ethanol extract suspension

The test extract was prepared by suspending the pamelo orange peel ethanol extract in CMC-Na (carboxymethyl cellulose sodium). To prepare the suspension, 1 gram of CMC-Na was gradually added to 50 mL of hot distilled water while stirring until a homogeneous solution was formed. The solution was then diluted with distilled water to a final volume of 100 mL. The ethanol extract was weighed at 0.3 g and 0.6 g to prepare concentrations of 3% and 6%, respectively, and each was suspended in 10 mL of 1% CMC-Na until a homogeneous suspension was achieved.

Animals

Sixteen adult Wistar strain white rats, aged 2-3 months and weighing 150-200 grams, were used in the study.

Treatment of test animals

All test animals were acclimatized for one week prior to the experiment. On the day before treatment, the hair on the back of each rat was shaved over a 2x2 cm area, cleaned with alcohol, and the animals were anesthetized with 0.2 mL of intraperitoneal ketamine. A burn model was created by applying a hot plate to the shaved area for approximately 5 seconds, inducing a second-degree burn characterized by redness and edema formation. The animals were then divided into four groups: a negative control group (treated with 1% CMC-Na), two treatment groups receiving 3% and 6% concentrations of the pamelo orange peel ethanol extract, and a positive control group treated with Lanakeloid-E®. The treatments were applied topically once daily for 21 days. The diameter of the burns was measured on days 1, 4, 8, 12, 14, 16, and 21 using a digital vernier caliper.

Data analysis

The burn diameter and the percentage of burn healing were calculated using the formula: (wound diameter)^2 / (healing diameter)^2 * 100%.

Results

The effect of the ethanol extract of pamelo orange peel on burn healing was assessed by measuring the wound surface area using a caliper. Burns were induced on the back skin of the rats using a hot plate, resulting in second-degree burns. The key parameters observed in this study were the reduction in burn diameter and the percentage of burn healing in the treated rats [7]. The burn diameters were monitored over a 21-day period for each treatment group, with the results presented in Figure 1A, and the corresponding percentage of burn healing shown in Figure 1B.

Discussion

The test animals were divided into four groups: a negative control group treated with 1% CMC-Na, two treatment groups receiving 3% and 6% ethanol extract of pamelo orange peel, and a positive control group treated with Lanakeloid-E®. All treatments were applied topically to ensure a localized effect, which is known to expedite the wound healing process. Treatments were administered once daily, consistent with the usage guidelines for the positive control, and continued for 21 days, corresponding to the typical burn healing period of 3 to 9 weeks [8].

The group treated with 1% CMC-Na showed the smallest reduction in wound diameter and the lowest percentage of wound healing among all groups. This outcome can be attributed to the fact that CMC-Na, as a solvent, does not influence wound healing [9]. CMC-Na is neutral, has a strong affinity for active substances, and is both non-toxic and non-irritating [10]. In the negative control group, the slower healing observed is due to the lack of active wound-healing agents in CMC-Na, meaning the wounds healed primarily through the rats' natural defense mechanisms [11].

The group treated with Lanakeloid-E® exhibited a significant reduction in wound diameter, with a wound healing percentage of 91.35%. This efficacy is likely due to the presence of *Centella asiatica* (gotu kola) extract and vitamin E in Lanakeloid-E®, which contain triterpenoid bioactive components, such as asiaticoside, asiatic acid, madecassoside, and madecassic acid, known for their wound-healing and anti-inflammatory properties [12].

The results indicate that the 3% and 6% concentrations of the ethanol extract of pamelo orange peel were effective in reducing wound diameter. The 3% concentration was the most effective, with a wound healing percentage of 86.86%. This effectiveness can be attributed to the presence of alkaloids, tannins, flavonoids, and saponins in the extract.

Flavonoids function as antioxidants, preventing or delaying cell necrosis, enhancing blood vessel formation, and exerting anti-inflammatory effects. They can also form complexes that inhibit bacterial metabolism by denaturing proteins and damaging bacterial DNA, leading to bacterial cell death by inhibiting the topoisomerase enzyme [4,13,14]. Saponins contribute to wound healing by stimulating collagen formation. The combination of flavonoids and saponins, both present in the pamelo orange peel, synergistically accelerates wound healing and may also reduce blood sugar levels. Tannins, with their astringent properties, help shrink skin pores and stop bleeding in wounds [14]. Alkaloids exhibit antibacterial activity by disrupting peptidoglycan synthesis in bacterial cell walls, preventing the formation of an intact cell wall and resulting in cell death [6].

Conclusion

The ethanol extract of pamelo orange peel effectively promotes burn healing in white rats, with the 3% concentration proving to be the most effective, achieving a wound healing percentage of 86.86%.

Acknowledgment

None.

Declaration of interest

The authors declare no competing interests.

Author contributions

SW and AMK conceptualized the study design, AMK and NSAE conducted the experiment, SW was the project coordinator and looked for the funding, SW, and AMK investigated the data, SW, AMK, and NSAE wrote original draft, SW, AMK, and NSAE reviewed and edited final version.

Received: May 31, 2023
Revised: August 27, 2024
Accepted: August 28. 2024
Published online: August 29, 2024

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