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The effectivity of topical mangosteen pericarp extract cream on wound healing in Swiss albino mice

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Abstract

Wound healing is a complex physiological process consisting of four phases: coagulation, inflammation, proliferation and migration, and remodeling, each with distinct characteristics. Studies have suggested that mangosteen pericarp extract (MPE) may accelerate wound healing. However, the mechanism is not fully understood. This study aims to evaluate the effect of MPE cream in various concentrations in acute wound healing of albino mice, both histologically and macroscopically.

Thirty-two healthy female Swiss albino mice, aged 6-9 weeks, weight 20-30 g, were included in this study. The samples were randomly divided into eight groups each consisting of 4 mice. The

first four groups were treated with MPE cream 5%, 10%, and 20%, and no medication (control group), respectively, and were sacrificed after three days. The other four groups received the same application and were sacrificed after 8 days. Wound bed diameter was measured and biopsy from skin lesion was performed for histopathologic examination. Mann-Whitney test was used to analyze the diameter of the wound bed and histopathological findings of granulation tissue formation, reepithelialization, and inflammation, with $P < 0.05$ considered as significant.

MPE cream significantly improved wound healing by increasing granulation tissue formation, and reepithelialization. In addition, MPE cream application was also shown to decrease the number of inflammatory cells, particularly in 5% and 10% concentrations, both in the 3-day and 8-day groups.

MPE cream application can accelerate wound healing and thus can be used in acute wound treatment.

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Introduction

Cutaneous wound healing is a complex and essential physiological process that depends on the interaction of cytokines, growth factors, chemokines and chemical mediators from cells to perform regulatory events.¹⁻³ Tissue regeneration and repair process begin immediately after an acute injury following any stimuli that interfere with the physical continuity of functional tissue. In general, there are four recognized phases that characterized the cutaneous repair process: coagulation, inflammatory phase, proliferative and migratory phase and remodeling phase. The coagulation and inflammatory phases are sometimes grouped together.^{1,2,4} These phases are distinct in function and histological characteristics. In acute wound, there is an orderly progression from injury to coagulation, inflammation, cell migration, and tissue modelling.^{2,5}

As the inflammation resolves, the involved cells enter into apoptosis and initiate the proliferative phase, which consists of four fundamental stages: reepithelialization, angiogenesis, granulation tissue formation, and collagen deposition.² During reepithelialization, the keratinocytes migrate from wound and multiple regulators such as growth factors and cytokines, integrins, keratins, matrix metalloproteinases (MMPs), chemokines, and extracellular macromolecules stimulate epithelial attachments. Remodeling is marked by the extracellular matrix (ECM) reorganization, cells apoptosis and angiogenesis regression, and type III collagen replacement by type I collagen.²

A variety of active agents has been used to accelerate wound healing process such as antimicrobials and antibiotics, epithelial growth factor, fibroblast growth factor, vitamins and minerals, and wound dressing material from naturally extracts.⁶ Mangosteen (*Garcinia mangostana* Linn.) is a tropical fruit most commonly found in Southeast Asia, including Indonesia. It is also known as the queen of fruits and its pericarp has been used in traditional medicine in Southeast Asia for centuries to treat infection, wound, infection, and diarrhea. Xanthones are among the mangosteen metabolites that have been successfully isolated from the pericarp of mangosteen and they have been attributed to the medicinal properties of the fruit. At least 68 distinct xanthones have been identified in different parts of the plant with 50 being present in a higher concentration in the pericarp than in the edible portion of the fruit. The most abundant xanthones in the pericarp of mangosteen are α - and γ - mangostin.^{7,8} Several studies have revealed that mangosteen pericarp extract (MPE) exhibit antimicrobial, anti-proliferative, antioxidant, anti-inflammatory, and analgesic properties.^{9,10}

A recent study has determined the effect of different concentrations of ethanol extract gel formation of mangosteen pericarp on wound healing in burns.¹¹ However, there is lack of studies evaluating the effectiveness of topical application of MPE cream for wound healing. The present study aims to evaluate the effect of ethanol-based MPE cream in various concentrations in acute wound healing in albino mice, both histologically and macroscopically.

Materials and Methods

This post-test only, true experimental study with control group was conducted in Animal Laboratory and Pathological Anatomy Laboratory of Hasanuddin University, Makassar, Indonesia, from November to December 2018 with the approval from Hasanuddin University Ethics Committee. The samples were 32 Swiss albino mice, with inclusion criteria consisting healthy females aged 6-9 weeks, weight 20-30 g. The mice were excluded if they had died during the study.

MPE cream at 5%, 10%, and 20% concentrations was made in the laboratory of Phytochemical Pharmacognosy, Faculty of Pharmacy, Hasanuddin University. A 5-mm wound was made in each experimental mouse by a 5-mm punch biopsy and treated with twice daily application of MPE cream. The samples were randomly divided into 4 groups each consisting of 4 mice. Each sample in the first four groups was treated with MPE cream 5% (A), 10% (B), 20% (C), and no treatment (E), respectively, before being sacrificed after 3 days. The other four groups received the same treatment, labelled A1, B1, C1, and E1, and were sacrificed after 8 days.

After three days, sample group A, B, C, and E were sacrificed. Wound bed diameter was measured and recorded. Sample of skin tissue was taken from each mouse by excisional biopsy from wound bed and normal adjacent skin for histopathological examination. These specimens were fixed with buffered formalin, embedded in paraffin wax, cut into 3-4 μ m sections and stained with hematoxylin-eosin. The sections were then examined with light microscope (Dual Head Olympus CX41). The same procedure was done with group A1, B1, C1 and E1 after 8 days.

Wound healing was assessed by analyzing granulation tissue formation (GTF), inflammatory cells count (ICC), reepithelialization (RE), and diameter of wound bed in millimeters (D). Granulation tissue formation was graded by score 1-3 (1=0-25% GTF with inflammatory cell domination, 2=50-75% GTF with wide neovascularization and less fibroblast, and 3 \geq 75% GTF with domination of collagen and fibroblast). Reepithelialization was graded by score 1-3 (1=no RE, 2=RE<50%, and 3=RE>50%). Inflammatory cells count was graded by score 1-3 (1=mild macrophage and neutrophil, 2=moderate macrophage and neutrophil, 3=abundant macrophage and neutrophil).

The collected data were analyzed with Statistical Product and Service Solution (SPSS). The analysis was done to compare the score of GTF, ICC, RE and D from each MPE concentration group to unmedicated group. The statistical test used in this study was Mann-Whitney test with $P<0.05$ considered as significant.

Table 1. Granulation tissue formation, reepithelization, inflammatory cell count, and wound bed diameter score for acute wound treated with mangosteen pericarp extract cream and untreated one after 3 and 8 days.

	3 days				8 days			
	N	Group (Concentration)	Mean	Std. Deviation	N	Group (Concentration)	Mean	Std. Deviation
GTF	4	A (5%)	1.75	0.50	4	A1 (5%)	2.75	0.50
	4	B (10%)	1.25	0.50	4	B1 (10%)	2.75	0.50
	4	C (20%)	1.25	0.50	4	C1 (20%)	2.25	0.96
	4	E (unmedicated)	1.25	0.50	4	E1 (unmedicated)	1.75	0.50
RE	4	A (5%)	2.25	0.50	4	A1 (5%)	3.75	0.50
	4	B (10%)	2.75	0.50	4	B1 (10%)	3.75	0.50
	4	C (20%)	2.25	0.50	4	C1 (20%)	3.50	0.58
	4	E (unmedicated)	1.75	0.50	4	E1 (unmedicated)	2.75	0.50
ICC	4	A (5%)	2.50	0.58	4	A1 (5%)	1.25	0.50
	4	B (10%)	2.00	0.00	4	B1 (10%)	1.25	0.50
	4	C (20%)	2.00	0.00	4	C1 (20%)	2.00	0.82
	4	E (unmedicated)	2.50	0.58	4	E1 (unmedicated)	2.50	0.58
D	4	A (5%)	3.98	0.13	4	A1 (5%)	2.00	0.24
	4	B (10%)	4.18	0.24	4	B1 (10%)	1.73	0.33
	4	C (20%)	4.45	0.10	4	C1 (20%)	2.75	0.53
	4	E (unmedicated)	4.55	0.06	4	E1 (unmedicated)	3.20	0.28

GTF, granulation tissue formation; RE, reepithelization; ICC, inflammatory cells count; D, diameter of wound bed.

Results

The result of statistical analysis between the groups are shown in Tables 1-3. Table 1 describes mean score of GTF, ICC, RE and mean wound bed diameter of the 5%, 10 %, and 20% MPE cream, and control group.

A preliminary study was done to measure the level of α -mangostin in MPE; it was revealed that 1 gram MPE contained 2.8 mg α -mangostin (unpublished data).

Three days after mangosteen pericarp extract cream application

As described in Table 2, the wound diameter, RE and GTF were all significantly higher in the MPE cream 5% group compared to those in the control group ($P < 0.05$). In the MPE cream 10% group, compared to the control group, a significantly higher RE (2.75 vs 1.75) and significantly lower wound bed diameter (D) (4.18 vs 4.56) were shown after three days ($P < 0.05$). The results also suggest that ICC was lower compared to the control group. However, there was no significant difference between MPE cream 20% group and control group.

Eight days after mangosteen pericarp extract cream application

As described in Table 3, GTF and RE in the MPE 5% group were significantly higher than the control group (mean 2.75 vs 1.75 and 3.75 vs 2.75, respectively) ($P < 0.05$). In addition, ICC and D (mean=1.25, mean=2.00, respectively) were significantly lower than the control group (mean=2.50 vs 2.50 and 2.00 vs 3.20, respectively) ($P < 0.05$).

In addition, GTF and RE were also found to be significantly higher in MPE cream 10% group than in the control group (mean 2.75 vs 1.75 and 3.75 vs 2.75, respectively) ($P < 0.05$). The ICC and D parameters were significantly lower (mean=1.25, mean=1.73, respectively) than the control group (mean=2.50 and 1.73 vs 3.20, respectively) ($P < 0.05$). However, there was no significant difference between MPE 20% group and the control group.

Discussion

This study showed that topical application of MPE cream significantly influenced wound healing by increasing GTF and RE

Table 2. Comparison of granulation tissue formation, reepithelization, inflammatory cell count, and wound bed diameter score for acute wound treated with mangosteen pericarp extract cream and untreated one after 3 days.

	Group (Concentration)	Mean	Mean of Group E (Control)	P-value
GTF	A (5%)	1.75	1.25	0.17
	B (10%)	1.25		1.00
	C (20%)	1.25		1.00
RE	A (5%)	2.25	1.75	0.17
	B (10%)	2.75		0.04
	C (20%)	2.25		0.17
ICC	A (5%)	2.50	2.50	1.00
	B (10%)	2.00		0.13
	C (20%)	2.00		0.13
D	A (5%)	3.98	4.55	0.02
	B (10%)	4.18		0.04
	C (20%)	4.45		0.10

GTF, granulation tissue formation; RE, reepithelization; ICC, inflammatory cells count; D, diameter of wound bed.

Table 3. Comparison of granulation tissue formation, reepithelization, inflammatory cell count, and wound bed diameter score for acute wound treated with mangosteen pericarp extract cream and untreated one after 8 days.

	Group (Concentration)	Mean	Group E1 mean	P-value
GTF	A1 (5%)	2.75	1.75	0.04
	B1 (10%)	2.75		0.04
	C1 (20%)	2.25		0.36
RE	A1 (5%)	3.75	2.75	0.04
	B1 (10%)	3.75		0.04
	C1 (20%)	3.50		0.10
ICC	A1 (5%)	1.25	2.50	0.03
	B1 (10%)	1.25		0.03
	C1 (20%)	2.00		0.34
D	A1 (5%)	2.00	3.20	0.02
	B1 (10%)	1.73		0.02
	C1 (20%)	2.75		0.19

GTF, granulation tissue formation; RE, reepithelization; ICC, inflammatory cells count; D, diameter of wound bed.

and decreases the number of ICC. These effects were especially observed in 5% and 10% concentrations both 3 days and 8 days after application that were confirmed by histopathology examination. It was also shown that MPE 5% and 10% cream application can accelerate wound bed closure.

Despite its long known anti-inflammatory activity, there is still lack of knowledge about the mechanism of action of xanthenes.^{10,12} This effect mainly mediated by α -mangostin, which is the most abundant xanthone in mangosteen pericarp.⁸

Inflammation begins with coagulation, platelet aggregation, and fibrin clot formation.¹ These processes function to prevent cellular elements loss, to serve as physical barrier to prevent microorganism entry, and to act as provisional matrix, cytokine deposit and growth factors; all of which are fundamental for maintenance of subsequent healing phases.² In the first few hours after injury, neutrophils are recruited and mediate tissue damage through the release of proteases, cytokines, and other factors contained in cytoplasmic granules. These cells generate reactive oxygen species (ROS) and produce antimicrobial proteases (cathepsins, defensins, lactoferrin, and lysozyme) with the function of destroying potentially pathogenic microorganisms.⁴ Neutrophils also produce various types of MMPs, involved in the extracellular matrix degradation. Uncontrolled neutrophil migration generates a cycle of recruitment activation of these cells leading to excessive ROS and proteases production, causing undesired ECM degradation and additional tissue damage, which may progress to chronic inflammation with consequent defective collagen deposition, reduced tissue resistance, and late reepithelialization which limits wound healing.^{4,13}

It has been reported that α -mangostin attenuates inflammation by blocking inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2) expression and by inhibiting prostaglandin E₂ (PGE₂).¹⁰ Gutierrez-Orozco *et al.* have investigated the inhibitory effect of α -mangostin on secretion of pro-inflammatory mediators such as IL-8 or TNF- α by human cells from various tissue origins, and found that α -mangostin stimulated secretion of TNF- α in primary culture of human monocyte-derived macrophage cells.¹² Another study has been done *in silico*, *in vitro*, and *in vivo* and conclude α -mangostin acts via inhibition of NO, IL-1 β , and TNF- α and also established anti-inflammatory effect by NF κ B inhibition and selective COX-2 inhibition together with iNOS blockage.⁸

About four days after a wound onset granulation tissue begins to be made through increasing of fibroblastic proliferation, collagenous and elastic biosynthesis, which forms a three-dimensional extracellular network of connective tissue. The granulation tissue fills the wound and provides covering condition for the newly formed epidermis.⁶ Two or three weeks after, the granulation tissue is gradually remodeled, forming a less cellular and vascular scar tissue.¹

GTF and reepithelialization acceleration were confirmed in this study. However, it is still unclear how α -mangostin induced GTF, especially fibroblast and epithelial migration. Faster wound healing might be due to the potential activity of α -mangostin as anti-inflammatory, antioxidant, and anti-bacterial agent that accelerates healing process.

Conclusions

This study showed that MPE cream application can accelerate wound healing and thus can be used in acute wound for faster wound healing.

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