

The Efficacy and Safety of Topical Gel Formulation of n-Hexane Fraction of *Curcuma longa* in Wound Healing of Hyperglycemic Mice

(EFEKTIFITAS PENYEMBUHAN LUKA DAN KEAMANAN GEL FRAKSI N-HEKSAN CURCUMA LONGA PADA MENCIT HIPERGLIKEMIK)

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ABSTRACT

Curcuma longa (turmeric) is one of the tropical plant belong to Zingiberaceae family. It has beneficial effects in healing of variety diseases. The aim of this study was to evaluate the efficacy and safety of a topical gel formulation of n-hexane fraction of *Curcuma longa* in the wound healing of hyperglycemic mice. A total of 30 hyperglycemic mice were divided into three groups. They were given topical application, NC as a negative control (gel base), PC as a positive control (Neomycine sulfate 5%), treated groups (HF = n-hexane gel). Histopathological examination of the skin was performed to evaluate the wound healing. The result showed that, there were no changes on the appearance of the gel preparation, physically. Even though upon the pH determination of the gel at six month of storage at 40°C there were significant difference. The effect of n-hexane gel, in terms of decreasing the surface area of wound and histopathological characteristics were significantly ($p < 0.05$) different between the PC group and HF group. Skin irritant test showed neither rashes, swelling, inflammation, redness, irritation, itching nor any other toxicity symptoms following application of the gel. Significant effect of n hexane fraction of *C. longa* gel on wounds without any adverse dermal toxic effects sounds very promising and it may be employed in the management of diabetic wound.

Keywords: turmeric, n-hexane, wound, hyperglycemic

ABSTRAK

Curcuma longa (kunyit) famili Zingiberaceae adalah tanaman yang dipercaya dapat digunakan untuk menyembuhkan berbagai penyakit. Penelitian ini bertujuan untuk menguji stabilitas sediaan gel dari fraksi n heksan kunyit dan aktivitasnya pada proses penyembuhan luka mencit hiperglikemik. Penelitian ini menggunakan tiga puluh ekor mencit hiperglikemik yang dibagi dalam tiga kelompok. Semua kelompok diberikan sediaan gel secara topikal, NC sebagai kontrol negatif diberi basis gel, PC (kontrol positif) diberi gel neomisin sulfat, HF diberi gel n heksan. Preparat histopatologi organ kulit digunakan untuk mengevaluasi efektifitas penyembuhan luka. Hasil penelitian ini menunjukkan tidak terjadi perubahan fisik pada semua sediaan gel yang dibuat, tetapi nilai pH setiap gel pada penyimpanan selama enam bulan (suhu 40°C) mengalami perubahan. Efektifitas penyembuhan luka berdasarkan penurunan luas permukaan luka dan histopatologi menunjukkan hasil yang signifikan pada kelompok PC dan HF. Uji keamanan sediaan menunjukkan tidak terjadi reaksi alergi. Sediaan gel n heksan rimpang kunyit mempunyai aktivitas pada proses penyembuhan luka dan aman digunakan untuk terapi luka diabetik.

Kata-kata kunci: kunyit, n heksan, luka, hiperglikemik

INTRODUCTION

Plants have been one of the important sources of medicines because over 60% of all pharmaceuticals are plant-based. The other name of *Curcuma longa* Linn is *Curcuma domestica* Valetton (Zingiberaceae) or turmeric (locally known as kunyit). Its has been widely used in Indonesia and other parts of Southeast Asia as a spice and a coloring agent in cooking. Turmeric was found to be effective on topical application such as allergy, antibacterial and anti-inflammation (Jain *et al.*, 2007). Turmeric is established as an excellent remedy for wound healing since antiquity. The local application of *Curcuma longa* powder efficiently heals septic wounds in diabetic patient (Pandya *et al.*, 1995). Turmeric contains an essential oils and fatty oils. An isolate from turmeric oil has been reported to have antifungal, antimutagenic, and antibacterial activity. Turmeric were also contains curcuminoids altatone, bisdemethoxycurcumin, dimethoxycurcumin, diaryl heptanoids, and tumerone. Curcumin, a polyphenol compound, is responsible for the yellow color of turmeric and is thought to be the most active pharmacological agent. Turmeric may be standardized to contain approximately 95% curcuminoids per dose. The dried root of turmeric reportedly contains 4-8% curcumin, of which curcumin I is the most abundant, but may not be the most biologically active. The curcumin was known insoluble in water and ether, but soluble in ethanol, dimethylsulfoxide, and other organic solvents. The anti-inflammatory properties of curcumin are thought to be due in part to suppression of prostaglandin synthesis. Prostaglandin synthesis from arachidonic acid is catalyzed by two isoenzymes: COX-1 and COX-2 (Jain *et al.*, 2007).

The foot ulcers in human health were known as an one of the main complications in diabetes mellitus, with a 15% life time risk in all diabetic patients. The problem and features of foot ulcers in human health are infection, ulceration, or gangrene. Neuropathy, poor circulation, and susceptibility to infection are the three major contributors to the development of diabetic foot; which when present, foot deformities or minor trauma can readily lead to ulceration and infection. Not all diabetic foots are preventable, but appropriate preventive measures can dramatically reduce their occurrences. Since all treatments have a partial effect in ulcer improvement and amputation rate; so more

effective treatments are essential (Larijani *et al.*, 2008). By cases problem background as described aboved, these research was designed for explored efficacy and safety of a topical gel formulation of n hexane fraction of *Curcuma longa* in hyperglycemic mice.

RESEARCH METHODS

The rhizome of *Curcuma longa* were collected from the Research Institute of Spices and Medicinal Plants (BALITRO) Bogor and identified by LIPI Research Center determinations in Cibinong Jl. Tentara Pelajar No. 3 Cimanggu Bogor 16111, E-mail : balitro@telkom.net.

Data collection

Ethanol extract and n hexane fraction of *C. longa* was evaluated for presence of various phytoconstituents by performing different qualitative chemical tests reported. It showed the presence of alkaloid, flavonoid, polifenol, saponins and quinon (Khandelwal 2005).

Turmeric powder was macerated with ethanol for 72 h and the extract was concentrated at a temperature not exceeding 60°C, which gave a semisolid extract. The *C. longa* extract was partitioned with n-hexane and gave n hexane fraction (Liu C. *et al.*, 2007).

Gels containing 10% of n hexane fraction of *C. longa* with carbopol, propylene glycol, TEA, methyl paraben, propyl paraben were prepared. The gels were tested for stability and toxicity in human normal skin. The gels were placed in the stability chamber, which was maintained at 25°C and 40°C, 75% relative humidity for 21 days. The gels were then observed for any change in the product performance such as physical appearance, pH, and viscosity (Srikanth *et al.*, 2008)

The gel of n hexane fraction of *C. longa* has been applied on the back hand of ten respondent. Skin irritant monitoring of n hexane fraction of *C. longa* gel, if no showed rashes, swelling, inflammation, redness, irritation, itching nor any other toxicity symptoms, gel safe to used (Moghbel *et al.*, 2007)

Thirty Hyperglycemia mice (*Mus musculus albinus*) as a subject research reffered to hyperglycemia in animal was achieved by intraperitoneal injection of STZ (40 mg kg⁻¹ i.v) (Eshrat and Hussain, 2002; Mazunder *et al.*, 2005) weighing 25-30g were selected. The

animals were housed under normal laboratory conditions. They had free access to food (Standard pellet) and water *ad libitum* and maintained at 24 - 28°C temperature, 60-70% relative humidity. The hair on the back skin were shaved and 2 days later the incision about 1,5 cm were done. The wound bearing animals were divided into three groups of ten animals each. The first group of animals as a negative control (NC) without any treatment, the second group received neomycin sulfate 5% as a positive control (PC) and the last group received n hexane fraction (HF). During 21 days post-operatively, the formulations were applied topically two times daily in wounds mice for 21 days.

On 21th day, the regenerated tissue from the healing wounds was collected for histopathological studies. Sections from regenerated tissue were examined for reepithelization and capillary number. The degree of reepithelization was given a percentage value, 0% being equivalent to no closure and 100% equivalent to complete wound closure. The number of capillary lumens in the granulation tissue was counted in the complete wound cross-section at magnification 40X (Halper *et al.*, 2003; Chen *et al.*, 2005).

Data analysis

Analysis of variance was used to test the statistical significance of difference among groups. Statistical significance in the difference of the mean was determined by Duncan method at significance 0.05%.

RESULTS AND DISCUSSION

Phytochemical investigation of n hexane fraction of *C. longa* showed the presence of alkaloids, saponin, Quinon and polifenol.

Gels of n hexane fraction of *C. longa* were found to be stable in the accelerated stability studies, as there no change in the color, odor and consistency, and there was no phase separation observed during the course of study, but the pH determination in six months storage periods at temperature 40°C showed that each gel had significant differences ($p > 0.05$). Skin irritant test showed neither rashes, swelling, inflammation, redness, irritation, itching or any other toxicity symptoms.

Histological evaluation was carried out for the treated and untreated groups. There was a marked increased blood vessel formation and

enhanced reepithelisation as a result of treatment with n hexane fraction of *C. longa* gel and neomycin sulphate 5%. There was full thickness re-epithelisation, in which epidermis was thin and well organized. The granular layer was well formed and one cell in thickness. Group HF showed comparable results when compared to NC. The wound area has already covered with full thickness epidermal regeneration. Group PC and HF, complete re-epithelisation, vascularisation and hair follicles formation were observed (Fig. 1). Early dermal and epidermal regeneration in group HF also confirmed that the n hexane fraction had a positive effect towards re-epithelisation (Fig.2).

Healing process, a natural body reaction to injury, initiates immediately after wounding and occurs in four stages. The first phase is coagulation which controls excessive blood loss from the damaged vessels. The next stage of the healing process is inflammation and debridement of wound followed by reepithelisation which includes proliferation, migration and differentiation of squamous epithelial cells of the epidermis. In the final stage of the healing process collagen deposition and remodeling occurs within the dermis.

Study on animal models showed enhanced rate of wound healing and drastic reduction in healing time than negative control, which might be due to enhanced epithelisation (Fig.2). The animals treated with n hexane showed significant results when compared to NC groups. The treated wound after seven days itself exhibit marked dryness of wound margins with tissue regeneration. Increased reepithelisation may be due to the metabolic activity of curcuma which might have significantly contributed to healing process. The study reveals that n hexane fraction of *C. longa* treated groups possesses good wound healing properties which may be attributed to the individual or combined action of phytoconstituents like alkaloids, saponins and polifenol present in it (Jain *et al.* 2007). Turmeric is established as an excellent remedy for wound healing since antiquity. The local application of *Curcuma longa* powder efficiently heals septic wounds in diabetic patient, reported by Pandya *et al* (1995). The main causes foot ulceration in diabetic are neuropathy, these case frequently compounded bacterial infection with organisms such as *Staphylococcus aureus* and *Streptococcus pyogenes* (Shi *et al.*, 2007). *Curcuma longa* showed significant antimicrobial activity (Iyengar *et al.*, 1995, Kapoor, 1997,

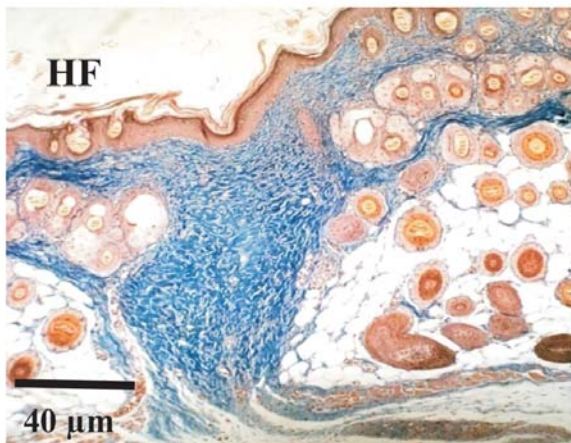
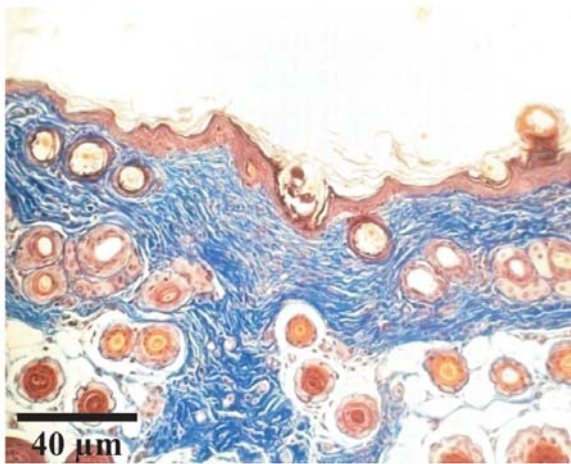
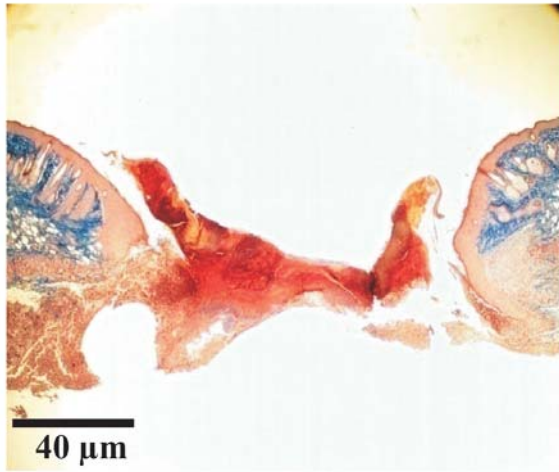


Figure 1. Histopathology of the wound in skin mice at day 21st. Negative Control (NC), Positive Control (PC) and nHexane Fraction (HF). The number of re-epithelization was completely occurred in PC and HF group, but in NC group was not completely. Masson Trichrome staining.

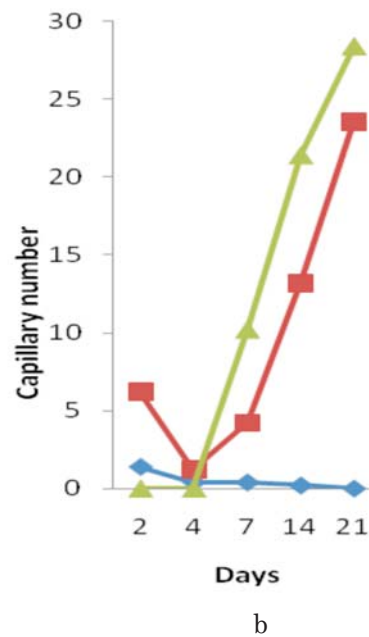
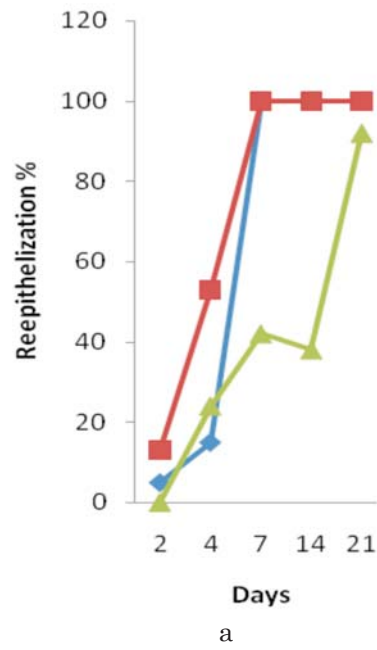


Figure 2. The course of wound healing in reepithelization and capillary number in hyperglycemic mice. a Re-epithelization. b Capillary number. GF (blue circle) and PC (red circle), and the untreated wounds of hyperglycemic mice (NC green circle) are shown in each panel.

Singh *et al.*, 2002). Turmerone and curcumin components of turmeric oil possess excellent antibacterial action against a wide range of microbes, such as *B. cereus*, *B. coagulans*, *B. subtilis*, *S. aureus*, *E. coli*, and *Pseudomonas aeruginosa* (Negi *et al.*, 1999). Further research needed to perform other topical dosage forms and also need to be done by varying doses of n-hexane fraction of turmeric.

CONCLUSION

N hexane fraction of *C. longa* gel increased the wound healing activity in hyperglycemic mice. There was enhanced reepithelization as a result of treatment gel. This study demonstrates the wound healing activity of n hexane fraction and its gel formulation of *C. longa*. *Curcuma longa* found to be effective in the functional recovery of the healing of wounds and also in histopathological alterations.

ACKNOWLEDGMENT

This study was supported by Directorate of Higher Education Competency Grant Number 1/13.24.4/SPK/BG-PD/2009 to Wiwin Winarsih. We acknowledge all participants who have supported this research.

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