

The effect of cyperus rotundus root ethanol extract on the epithelization in the healing process on skin excision wound

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ABSTRACT

Ch. Tri Nuryana, E. Suryadi & Harijadi - *The Influence of Ethanol Extract of Purple Nutsedge (Cyperus rotundus) on Epithelization of Skin Wound Healing Process*

Background: If skin is wounded, epithelial cells will migrate to the wound area in order to keep homeostatic. Epithelization consists of mitotic and proliferation of epithelial cells. Rhizome of purple nutsedge (*Cyperus rotundus*) contains *cyperene*, *flavonoid*, *2-sitosterol* dan *ascorbic acid* that can accelerate epithelization in wound healing process and has been used in traditional medicine.

Objective: To investigate the influence of ethanol extract of Purple Nutsedge (*Cyperus rotundus*) in epithelization of wound healing process.

Methods: Fourty five Balb/c male mice 8-10 weeks old, 25-35 g weight, were excised punch biopsy on back 0.5 cm right and left of columna vertebralis, 2.5 cm in cutis from the ear area. The mice were divided into 5 groups. Negative control group was treated with vehicle of extract, positive control group was treated by gel containing placenta extract 1% & neomycin sulfate 0.5% and the others were treated with *C. rotundus* 1%, 2%, and 4% in concentration. Each of the groups was divided into 3 subgroups. Each of the subgroups composed of 3 mice based on the period of termination, i.e.: 3rd, 7th and 12th day after wounded. Histological evaluation was done to investigate the thickness of epithelial layer.

Results: The data were analyzed by Two-way Anova and the results showed that there was a significant difference ($p < 0.05$) in every decapitation period, groups, and interaction between decapitation period and groups. The results of LSD test showed that there was a significant difference ($p < 0.05$) in the thickness of epithelial layer between negative control and *C. rotundus* groups.

Conclusion: *Cyperus rotundus* extract accelerates epithelization process of wound healing in mice.

Key words: Wound healing - *Cyperus rotundus* - epithelization - mice

ABSTRAK

Ch. Tri Nuryana, E. Suryadi & Harijadi - *Pengaruh ekstrak etanol umbi teki (Cyperus rotundus) terhadap epitelisasi pada proses penyembuhan luka eksisi kulit*

Latar belakang: Jika kulit terluka, sel epitel akan bermigrasi ke daerah luka untuk mempertahankan homeostasis. Epitelisasi terdiri atas mitosis dan proliferasi sel epitel. Umbi teki (*Cyperus rotundus*) mengandung *cyperene*, *flavonoid*, *2-sitosterol* dan *ascorbic acid* yang dapat meningkatkan proses epitelisasi luka dan telah digunakan dalam pengobatan tradisional.

Tujuan: Untuk mengetahui pengaruh ekstrak etanol *Cyperus rotundus* terhadap proses epitelisasi pada penyembuhan luka.

Bahan dan cara : Empat puluh lima tikus Balb/c umur 8-10 minggu, berat 25-35 g, dibiopsi tusuk pada punggung 0.5 cm kanan dan kiri columna vertebralis, di kulit 2.5 cm dari daerah telinga. Tikus dibagi menjadi 5 kelompok yaitu : kontrol negatif diberi perlakuan dengan bahan pelarut ekstrak, kontrol positif diberi perlakuan dengan gel yang mengandung ekstrak plasenta 1% & neomisin sulfat 0,5%, sedangkan yang lain diberi perlakuan dengan *C. rotundus* kadar 1%, 2%, and 4%. Masig-masing kelompok dibagi selanjutnya menjadi 3 subkelompok berdasarkan terminasi percobaan, yaitu hari III, VII, dan XII setelah perlakuan. Evaluasi histologis dilakukan untuk menilai ketebalan lapisan epitel yang timbul.

Hasil: Dengan analisis Anova-dua jalan ditemukan ada perbedaan bermakna ($p < 0.05$) pada tiap periode dekapitasi, dalam kelompok, dan interaksi antara periode dekapitasi dan kelompok Hasil uji LSD menunjukkan bahwa terdapat perbedaan bermakna ($p < 0.05$) dalam ketebalan lapisan epitel antara kontrol negatif dan kelompok perlakuan dengan *C. rotundus* groups.

Simpulan : Ekstrak *Cyperus rotundus* mempercepat proses epitelisasi dalam penyembuhan luka pada tikus.

INTRODUCTION

All of the body surface is lined with epithelial cells whose integrity is needed to maintain homeostasis. If the surface of the body is injured, the epithelial cells will migrate towards the wound to cover the injured skin¹. The epithelial cells of the skin are of keratinized stratified squamous type. Keratinocytes will move towards the area of the wound in 24-48 hours after the injury, and will stop when they meet the keratinocytes from the other side of the wound. Suprabasal keratinocytes are the first cells that migrate to the wound. Epithelization is started by the increase in epithelial cell mitosis activity towards the margin of the wound, followed by their proliferation. Epithelial cells slide from the margin of the wound are in amoeboid movement. They use fibrin bands and extracellular matrix (ECM) components such as fibronectin as their guide. Epithelial cells from the remains of the epithelial structure separate their hemidesmosome bonds and reattach on the basal membrane and move rapidly over the wound. The proliferation of epithelial cells is affected by several growth factors and an enzyme that responsible for the migration of keratinocytes and epithelization, i.e. matrix metalloproteinase-1 (MMP-1)^{2,3}.

The high cost of treatment has caused the community to choose traditional alternative treatment. One of traditional drugs used to cure wounds is purple nutsedge (*Cyperus rotundus*) root. *C. rotundus* is a plant that grows in tropical and subtropical areas, especially in open and shaded areas, and is an ineradicable weed. Several studies have been done to find out the contents and the effects of *C. rotundus*⁴.

C. rotundus contains essential oils, terpenes, flavonoids, b-sitosterol, and ascorbic acid. The main

terpenes in *C. rotundus* are cyperenes, which include sesquiterpene hydrocarbones^{5,6,7}. Cyperene has a role in protein synthesis and an inducer of the growth factors needed in epithelization^{8,9}. Flavonoids can restrict the quantity of free radicals and maintain the stability of collagen fibers^{6,9}. b-sitosterol is a natural steroid with estrogenic effect and able to maintain the humidity of the wound area to enable the cell growth¹⁰. Ascorbic acid has a role in stimulating the synthesis of collagen, fibroplasia (?) and epithelization^{11,12}.

Previous studies on the activity of *C. rotundus* on the wound healing showed that 2% w/w cream of *C. rotundus* root alcohol extract had higher activity in wound healing compared to nitrofurazone ointment⁸.

The aim of this study was to find out the effect of *C. rotundus* root ethanol extract given topically on the epithelization in wound healing process.

MATERIALS AND METHODS

It was an experimental study with posttest-only with control group design, done in the Department of Anatomy, Embryology and Anthropology and the Department of Pathology Anatomy, Faculty of Medicine, Gadjah Mada University, in 2007 The subjects were 45 Balb/c male mice of 8-10 weeks old, obtained from Unit Pengembangbiakan Hewan Percobaan (UPHP). Two 4-mm diameter punch biopsies were performed on the back skin of the mice, each was 2.5 cm from the ear and 0.5 cm from vertebral column. The subjects were divided into 5 groups : negative control group was treated with the solvent, positive control group with gel contained 1% placental extract and 0.5% neomycin

sulphate, and three treatment groups with topical *C. rotundus* root ethanol extract in the concentration of 1%, 2%, and 4%, respectively. Each group was put into individual cages. Each group was divided further into 3 subgroups based on the decapitation period, that is, day 3, 7, and 12 after the wound. Histological slides were made with HE staining for evaluation of the epithelial layer growing over the wound..

C. rotundus root were obtained from Yogyakarta Province, ±1000 m from sealevel, in January 2007. *C. rotundus* root ethanol extract in this study was 96% ethanol extract dissolved in simple cream base. The extraction was conducted in the Department of

Pharmacy, Faculty of Medicine, Gadjah Mada University.

RESULT AND DISCUSSION

Epithelization process was assessed by measuring the thickness of epithelial layers. TABLE 1 shows that on day 3, the negative control group had no epithelization, while the other groups showed some epithelization process. The layer of epithelial cells in negative control group was the thinnest, followed by 1%, 4%, and 2% *C. rotundus* groups.

TABLE 1. Mean and standard deviation of the thickness of epithelial layers (micrometers) in the skin excision wound healing process after the application of *C. rotundus* root ethanol extract (400x magnification, 10 visual fields).

Day	n	The thickness of epithelial layer (mean ± standard deviation)				
		(-) Control	(+) Control	1% <i>C. rotundus</i>	2% <i>C. rotundus</i>	4% <i>C. rotundus</i>
3	6	0.0	14±1.20	33±0.10	56±0.40	35±0.30
7	6	53±2.50	110±0.800	68±0.50	67.5±0,50	85±0.40
12	6	44±0.60	33±0.70	32±0.70	19±0.20	32.5±0.40

Note: n: sample size

On day 7, positive control, 4% and 1% *C. rotundus* groups had the thickest epithelial layers, the 2% *C. rotundus* group started showing a thinning of epithelial layer, while negative control group still showed hyperplasia or a thickening of epithelial layer. On day 12, 2% *C. rotundus* group had the thinnest epithelial layer, almost similar to

the thickness of normal epithelial layer (10 micrometer), while negative control group still showed hyperplasia. A graph illustrated the thickness of *C. rotundus* groups and control groups epithelial layers in each decapitation periods showed in FIGURE 1.

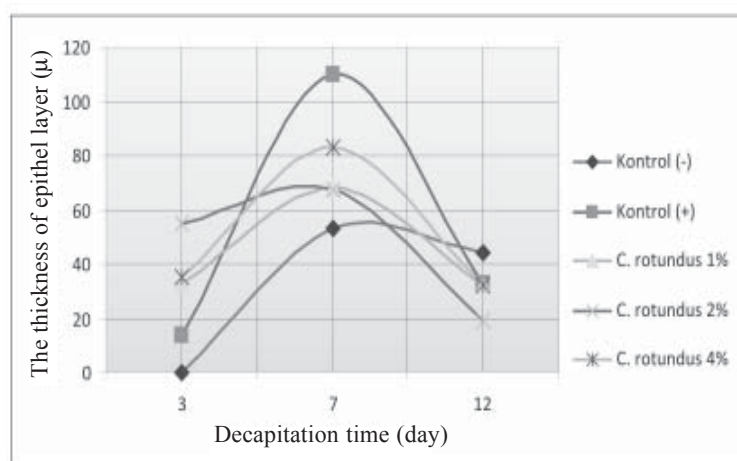


FIGURE 1. The graph of the thickness of *C. rotundus* groups and control group epithelial layers in excision wound healing process on Balb/c mice back skin in each decapitation periods

FIGURE 1 showed that each group had a specific graphic pattern. The epithelial layers of *C. rotundus* groups were thicker than positive and negative control groups on day 3. On day 7, positive control, 4%, and 1% *C. rotundus* groups had the thickest epithelial layers, and then they were thinning back to the normal epithelial layer thickness on day

12. On day 7, 2% *C. rotundus* group started showing a thinning in epithelial layer, while negative control group still showed hyperplasia. Two-way Anova analysis was used to find out the difference between decapitation periods and groups (TABLE 2).

TABLE 2. Two-way Anova analysis on the thickness of epithelial layers (micrometer) between decapitation periods and groups in excision wound healing process on the back skin of Balb/c mice

	F _{hit}	Sig.
Decapitation periods	730.529	0.0001*
Groups	37.225	0.0001*
Decapitation periods x Groups	80.154	0.0001*

Note: * : significant (p<0.05)

TABLE 2 showed a significant difference (p<0.05) in decapitation periods (F=730.529, p=0.0001), in groups (F=37.225, p=0.0001), and between decapitation periods and groups (F=80.154, p=0.0001).

The difference between groups was analyzed with LSD to find out the dominant effect between groups. The analysis showed that there was a significant difference (p<0.05) between negative control, positive control, and 1% *C. rotundus* groups. The difference between positive control and 4% *C. rotundus* groups was not significant (p>0.05). The 1% *C. rotundus* group was not significantly different (p>0.05) from 2% *C. rotundus* group, but significantly different (p<0.05) from 4% *C. rotundus* group. The 2% *C. rotundus* group was significantly different (p<0.05) from positive control group, but not significantly different (p>0.05) from 4% *C. rotundus* group.

On day 3, the epithelial layer in 2% *C. rotundus* group was the thickest and significantly different from positive control, negative control, 1%, and 4% *C. rotundus* groups; followed by 4%, 1% *C. rotundus*, positive control and negative control

groups. It showed that the epithelization process in *C. rotundus* groups occurred earlier than in control groups. This was caused by cyperene, the active compound in *C. rotundus*, which induced the growth factors, such as EGF and FGF. Positive control group showed thinner epithelial layer compared to *C. rotundus* groups, probably caused by the negative effect of growth factor TGF- β 1^{8,9,13}.

Based on histological observations (FIGURE 2), on day 7, the thickness of epithelial layer in 2% *C. rotundus* group had decreased, with higher granulation tissues. The peak of hyperplasia of epithelial layer in 2% *C. rotundus* group was assumed to be occurred on day 5, and after that, it was thinning back to its normal thickness. Positive control group still showed process towards hyperplasia, similar with the process in 1% *C. rotundus* group. It was assumed that keratinocytes in 2% *C. rotundus* group migrated and had mitosis more optimal than other groups. The epithelization in this group is facilitated by ascorbic acid^{12,14}. On day 12, all groups showed epithelial layer thickness almost similar with normal tissue.

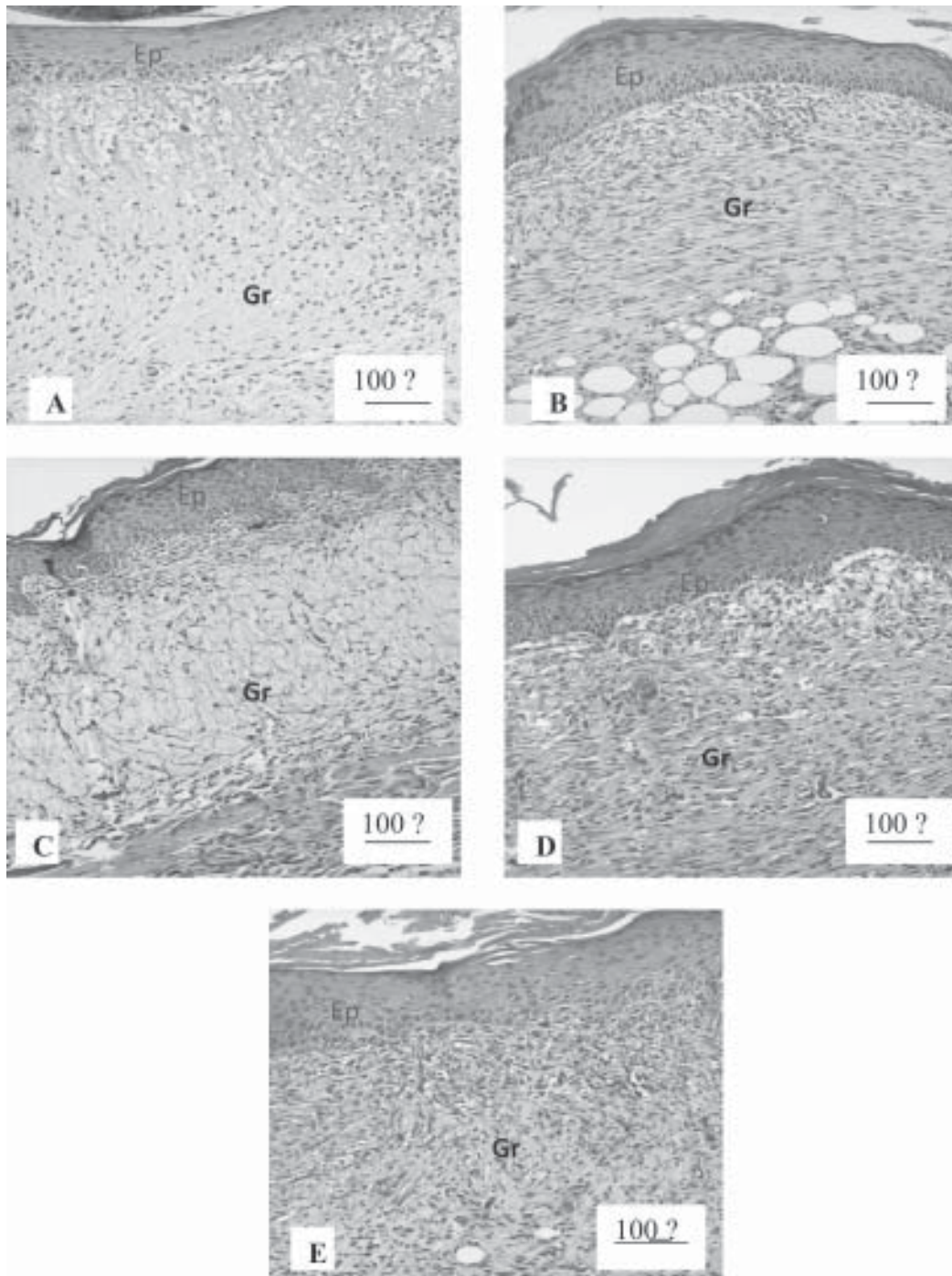


FIGURE 2. The microscopic pictures on day 7, with HE staining (100x magnification), of *C. rotundus* and control groups. (A) Negative control, (B) Positive control, (C) 1% *C. rotundus*, (D) 2% *C. rotundus*, (E) 4% *C. rotundus* groups. (Ep) epithelial cells, (Gr) granulation tissues.

Previous studies used Wistar male rats as subjects, and the study showed that 2% w/w *C. rotundus* root alcohol extract cream had a higher activity wound healing (100% wound closure on day 18) compared to nitrofurazone ointment. The study used three kinds of wounds, i.e. excision to observe the wound closure and contraction, incision to observe the inflammatory cells, and dead space to observe the granulation⁸. The result of that study was consistent with the result of this study, which concluded that *C. rotundus* could accelerate the wound healing.

Meanwhile, Zhu *et al.*¹⁵ reported that *C. rotundus* had cytoprotective effect on gastric ulcer. This study used rats as subjects, who were given *C. rotundus* root ethanol extract with the dose of 0.25 gr/kgBW orally, after induced with alcohol per oral.

C. rotundus contains cyperene, an inducer of the growth factors such as EGF that stimulates the epithelization. EGF acts as a mitogen on epithelial cells, keratinocytes, and fibroblasts, and stimulates the migration of keratinocytes and the formation of granulation tissues^{14,16}. In the wound healing process, EGF is produced by keratinocytes, macrophages, and other inflammatory cells migrating to the wound area. FGF has functions in angiogenesis, fibroblast proliferation, and epithelial migration to compose new epidermis. TGF- β in placental extract given in positive control is an inhibitor in epithelial cell growth^{13,17}.

Epithelization is also influenced by matrix metalloproteinase-1 (MMP-1) produced by epithelial cells, fibroblasts, myofibroblasts, chondrocytes, osteoclasts, endothelial cells, and leukocytes. Overproduction of MMP causes pathological conditions, so that an inhibitor is needed, that is, tissue inhibitor of metallo-proteinase (TIMP) to control the activity of MMP^{18,19,20}.

CONCLUSION

Topical *C. rotundus* root ethanol extract could accelerate the epithelization in wound healing process in mice.

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