PROCEEDING OF
MAKASSAR INTERNATIONAL SYMPOSIUM
ON PHARMACEUTICAL SCIENCE (MIPS)
Makassar - South Sulawesi
Indonesia
March 19-20, 2009

Faculty Of Pharmacy
Hasanuddin University

DAAD
Deutscher Akademischer Austausch Dienst
German Academic Exchange Service
CONTENTS

1. Transformation of Cocrystalline Phase in Binary Mixture of Trimethoprim and Sulfamethoxazole by Slurry Technique
   Erzal Zaini, Yevri C. Simbirepasu, Suntari N. Suwandi and Aizel Halim

2. Evaluation of Anti-emetic use in pediatrics with Retinoblastoma at Dharmais Cancer Hospital (National Cancer Center)
   Azrifivia, Edi S. Tehutera and Dinyah Siti Rahmah

3. Quality of Life Research on Geriatric Patients with Multipathology in Sardjito Hospital Yogyakarta
   Dyah Aryani Perwima, Indri Oktasari and I Dewa Putu Pramukta

4. The Influence of Morinda citrifolia, L. Fruit Extract as Adjuvant on IgY Production Raised in Laying Chickens against Avian Influenza Vaccine
   Ediati Sasmito and Sri Herawati

5. Chloroform Fraction of Areca (Areca catechu L.) Induces Apoptosis and Decreases Bel-2 Expression on MCF-7 Cells
   Eddy Miejanto, Sri Handayani and Rama Asmoh Susidarti

6. The Influence of Niosome System (SPAN 20/60-Cholesterol) on The Preparation Characteristics and Released of Dichlorphenac Sodium from HPC: HEC Gel Based
   E silia Hendradi, Tuiek Purwanti, Desy Dwi Listyam and Ika Rosadiati Pribadi

7. The Delivery of Crushed Tablets Using Food and Beverages: Is There a Problem? A Case Study Using Amlodipine Tablet
   Zakky Cholisok, Lisa Nisenn and Kathryn Strandman

8. Formulation Of Chewable Tablet Containing Temu Patuh (Cucumis Zedoaria (Berg) Roscoe : Combination Of Mannitol-Lactose And Sorbitol-Lactose As Filler
   Setyo Nurwadi, Mufadil, Eka Yuliana Dian Prastesti and Wulan Ratna Ningtyas

9. Formulation Of Lozenges Containing Extract Of Kemangi (Ocimum Sanctum L.) Using Sodium Carboxy Methyl Cellulose And Gelatin As Binders
   Erindyah R Wikantoyaning, Setya Nurwadi, Raditya Taufan and Asepia Yusiandri

10. Antipyretic Effect Of Centella Asiatica L. Infusion On Male Swiss Mice
    Ratna Yuliawati, EM Surisno and Windi Irawati

11. Isolation of Antidermatophyte Active Compound from Eleuthereine americana L. Merr.
    Enik Rosanah, Irawan Wijaya Kusuma and Titin Asmunah

12. Determination of Mercury (Hg) on Perna Viridis in Semarang Bay Using Atomic Absorption Spectrophotometry
    Broto Santoso, Sabikis and Adhi Prayitno
13. The Physicomechanical Characteristics Of Corn Starch (Zea Mays, L.) As Exipients In Solid Dosage Form Formulation
Yandi Sinkri, Rania Dewi and Feris Firdaus

14. Acute Renal Failure Of Aeci Used In The Treatment Of Congestive Heart Failure Cross Sectional Study
Vitarani Dwi Aminda Ningrum, Lutfi Chabib and Saeudin

15. Formulation of Gel Containing Robusta Coffee Extract (Coffea canephora L.) for Skin Wound Healing
Yudi Padmadisastra, Anang Subghana and Nurdil Lesari

16. Drug Utilization 90% (DU90%) Profile of Antibiotic Use During 2002-2007 at a Private Hospital In Central of Java
Saeudin, Vitarani D.A. Ningrum, Iwan S. Pradita and Meny Suanini

17. Antioxidant Activity Of Ethyl Acetate Fraction of Kenikir (Cosmos caudatus H. B. K.) Herb Extract and TLC Profile
Suparmi, Kepunahan Kurniasih and C.J Soegihardjo

18. Evaluation of Gel Containing Essential Oil of Citrus aurantium Leaf and In Vitro Antibacterial Activity
Anito Sukawati, Mufidz, Ratna Yuliani and Perti Gailuh Y Rossyad

19. Style Of Nuclear Magnetic Resonance (Nmr) Spectrum Of 2,5-Dibenzylidene Cyclopentanone Series Of Curcumin Analog
Sarifinam

20. Study Of Antiemetic To Cancer In Patients With Cytostatic Therapy In Ds Hospital Madiun, East Java, Indonesia
Enok Muzayen, Reino Yastrawati and R.A. Octori

21. The effect of Snakehead (Ophiocephalus stratus) Fish Water Extract Cream on the Recovery of Rabbit’s (Oryctolagus cuniculus) Skin Wound Histopathologically
Robert Tungadi, Faisal Attamimi, Eva Firmita Sabu and Rama P. Hiola

22. The Influence of Sambung Nyawa [Gynura Procumbens (Lour.) Merr] Leaf Extract on Lymphocyte Culture of Breast Cancer Mice (C3H) Cells
Shirly Kurnadi, Kartina and Kusmardi

23. Radiolabeling of Carcinomembryonic Antigen (CEA) Monoclonal Antibody with Generator-Produced Rhenium-188 for Radioimmunotherapy
Muhammad Yanis Masdia

24. Antifungal Activity From Snail Mucus (Achatina fulica Bowdich) Against Fungi Trichophyton mentagrophytes and Trichophyton rubrum
Nurmeilis, Megga R. Pikoli and Rahmah Garmila

25. Antiangiogenic Activity Of Etanolic Extract From Rambutan Pericarp (Nephelium Lappaceum L.)
Hadi Anshory, Asti Wibowo, Saeudin and Nita Pujianti
26. Inventory, Relocation, Antimicrobial and Phytochemical Screenings of Some Traditional Medicinal Plants of South East Sulawesi (Zingiberaceae)
   Sahidin I, Ruslin and Archangye

27. Antibacterial Properties Of Some Traditional Medicinal Plants Of South East Sulawesi (Indonesia)
   Ruslin, Archangye and Sahidin I

28. Histamin Content In Some Fishery Products Distributed In The Market In Makassar
   Merta Mahendradatta

29. Formulation of Cacao Bean (Theobroma cacao Linn) In Scrub Cream
   Pakki E, Syukur R, Nuriah, Sumantri and Jusnri, D

30. Ethnopharmacy and Chemical Component Identification Study of Permot Herb (Passiflora foetida L.) at 3 Ethnic of South Sulawesi
   Aksar Roskamah Ahmad, Asni Anin and Iskandar Zulkarnain

31. Formulation of Ethanol Extracts Betel Nut (Areca cathechu) as Mouthwash
   Mudzakkar Rawa, Erminia Pakki, Nuriah Hasrin, Faridah and Mufiharia

32. The Dissolution Test of Formulation Phenylbutazone Tablets Using Polysorbate-80
   Amran Ilyas Tandjung

33. Design of Oral Multiple Dose Simulator Program with One Compartment Model
   Yusradi

34. A Computer Application for Simulation of Drug Plasma Concentration of Amoxicillin, Ciprofloxacine, and Tetracycline after Oral Multiple Dose Administration
   Yusradi

35. Novel CYP3A4 and CYP2D6 Inhibitors from Indonesian Medicinal Plants
   Subetan, Shigetomi Kato and Yasuhiro Tazuka

36. The Effect Of Nk-1 Antagonist Administration On Dopaminergic Cell Death In Parkinson’s Disease Animal Model
   Yulia Yusrinia Djahir

37. Zidovudin Analysis Method Validation in Plasma in vitro Using HPLC
   Yahidiana Harhany, Rika Andalusia and Tania Surya

38. Effect Of Bitter Melon (Momordica Charantia) Leaves Juice On Immunoglobulin M (Igm) And Immunoglobulin G (Igg) Activity Of Male Mice (Mus Musculus)
   Ari Aritandi K., Hajar Astuti, Randga Medianto A. and Mufidah

39. Test Effect Extract Etanol Leaves Murbet (Morus alba L.) as Antimalaria to Male Mice (Mus musculus) Use in vivo Method
   Dewi Yuliana, Rusli and Asni Amin
40. Isolation EndoFit Microbial of Turbinaria Murayana Seaweed
Rushli, Muzakkir Baiz and Amindalah

41. Antimicrobial Activity of Cassia alata L n-Butanol Extract from Wajo, South Sulawesi Toward a Range of Microorganisms by Bioautography-TLC.
Amindalah, Rushli, Habibie and Zainal Abidin

42. Analysis of Neem Leaves (Azadirachta Indica A. Juss) Ethanolic Extract Effect on Immunoglobuline M (IgM) Activity of Male Mice (Mus musculus)
Habibie, Kus Haryono, Marianti A. Mangga, Mufidah and Amindalah

43. The Antimicrobial Activity of Turbinaria sp Ethyl Acetate Extract Toward a Few Microorganism by Bioautography-TLC.
Muzakkir Baiz, Rushli, Zaraswati and Zainal Abidin

44. Anti Platelet Aggregation and Free Radical Scavenging Activities of Mezzetia parviflora Becc. Wood bark Ethanolic Extract
Mufidah, Marianti A. Mangga, Hasyini Baruan and Genini Alam

45. In Vitro and in Vivo Study: Antimicrobial Properties and TLC Bioautography Profiling on Vibrio colera, and Antidiarrhoea Activity of Peromt Herb (Passiflora foetida Linn.) Extract from South Sulawesi to Mice (mus musculus)
Assi Amin, Mirawati, Abd Mulik, Vira Haridyani, Ibertina and Lukman Labassy

46. The Influence of Polyvinylpyrrolidone K-30 to the Dissolution Rate of Phenylbutazone Prepared in Solid Dispersion
Latifah Rahma, Aliyah Patroan and Irawan Setiawan

47. Single Drop Microextraction in Pharmaceuticals Analysis
Ganien Supriyanto

48. Soil Actinomycetes of Podor Coastal in Larantuka, East Flores: Isolation and Primary Screening for Antimicrobial Activities
Sarini M. Natsir Djide, Usman, and Septia Buha Odel

49. Gastrointestinal Absorption Of Grisecofulvin From Liquid Organic Acids And Esters In Rats
Syahrudin Kadir, Firzan Nainu, Teruo Murakami and Noboru Yata

50. Possible Factors Behind the Enhanced Gastrointestinal Absorption of Grisecofulvin from Liquid Organic Acid Ester Solutions in Rats
Syahrudin Kadir, Samarheni, Yulio Yusrini Djidir, Noboru Yata.

51. Effect of Humidity Aging on Disintegration, Dissolution and Cumulative Urinary Excretion of calcium p-aminosalicylate formulation
Syahrudin Kadir, Sartini and Samarheni

52. Anti-atherosclerosis Effect of Physalis angulata Herb in Hypercholesterolemic Mice
Marianti A. Mangga, Lukman Muslimin, Genini Alam, Syahrudin Kasim, Habibie
The effect of Snakehead (*Ophiocephalus streatus*) Fish Water Extract Cream on the Recovery of Rabbit's (*Oryctolagus cuniculus*) Skin Wound Histopathologically

Robert Tungadi, Faisal Attamimi, Eva Firmina Sabu, and Rama P. Hiola
Gorontalo State University, Faculty of Health and Sport Sciences, Gorontalo City

**Abstract**

The aims of the study were to discover the effect of cream with several concentrations, duration of wound recovery, effective level needed and skin histological difference of snakehead fish water extract cream and control on wound recovery. The research design used was repeated measure design. The samples of the study were nine rabbits divided into three groups of treatment: group I (0.3%), group II (1%), and group III (2%). Each rabbit in each group was wounded at the left and right dorsal parts as wide as 4 cm² with scalpel to make open wound. Wound at the left dorsal was applied with snakehead fish cream and the right dorsal with basic cream as control according to concentration of each group. The observation was done on day 3, day 6 and day 12 by measuring the size of wound and taking photo at the wounded area. Then on day 12, skin incision was done on each group to observe histopathology. The data were analyzed statistically by applying SPSS 15 program of repeated measures test. The results of the study indicated that group III (2%) had significant effect on recovery of wound by narrowing the size of wound each day (p<0.05) and this is proven by observing the skin histopathology showing 2% concentration improves the granulation tissue very quickly on day 12 compared to 0.3% and 1% concentration. From this study, it can be concluded that the application of snakehead fish cream 2% concentration for 12 days can accelerate the wound recovery.

**Keywords:** Cream, histopathology, snakehead fish, wound recovery

---

**Introduction**

All provinces in eastern part of Indonesia are rich in natural products from sea plants and animals. Gorontalo province is one of them. The snakehead fish is many found in Gorontalo Province especially in marsh and lakes. The snakehead fish is fresh water fish that has many function in improving public health and reported can increase skin wound healing. The snakehead fish have biochemistry component such as amino acids and fatty acids are very important to synthesis of collagen fiber during wound healing process.1 One problem faced by people and surroundings is less information about the benefit of the snakehead fish. The snakehead fish is other alternative of albumin protein sources. Albumin is one of the greatest number of proteins in plasma liquid that achieves 60% for new tissue cell formation. In medicine, this albumin is used to accelerate restoration of human body cell tissue broken by surgery.2

At the moment, particularly Indonesia, albumin serum often used by patients is hard to come by. For once surgery, by using this serum can reach three times of 100 ml. From the result of Suprayitno’s researcher (2003), on some fish in Indonesia, the large number of albumin and amino acids were found on snakehead fish compared to those in other fish such as catfish, goldfish, gurami, etc.3 Giving albumin therapy with snakehead water extract orally can assist wound healing process faster. Making snakehead fish water extract as wound healing at post-operation is not only cheaper than albumin serum but the wound also recover faster within three days than albumin serum. It takes three bottles of albumin serum that are very expensive. Besides that, the snakehead fish has discomfort taken because it smells fishy. Therefore, this is one of appropriate alternative to make snakehead fish water extract in other dosage forms.4

Cream is semi solid dosage form like thick emulsion containing no less than 60% water and intended to external application.5 Cream o/w is good cream for delivering active compound to target tissues, gratifying appearance and feeling comfortable after using it. This cream is not greasy and
easy to be cleaned. There are many doctors and patients prefer using cream to using ointment. In this case, cream is a lot easier spread on skin layer than ointment especially cream o/w.7

Damage to the skin frequently provokes wounds or extensive loss of tissues and to reestablish functional integrity a complex process of wound healing begins. This sequence involves the migration of cells to the wound, proliferation of different cells types, and changes in the synthetic and secretory cellular activities. Arachidonic acid is one of the most prominent polyunsaturated fatty acids in the skin, and its functional role depends largely on the generation of biologically potent oxidative metabolites. Eicosanoid, arachidonic acid derivates, act as inflammatory mediators. They are chemotactic agents for leukocyte, stimulating vessel dilation and induce platelet aggregation. The eicosanoid family consists of prostaglandins, thromboxones, leukotrienes, and other many physiological and pathologic processes and are potent regulators of cell function.10

The present work evaluates the clinical and histopathological aspects of cutaneous application of snakehead fish cream with three concentrations of cream (0.5%, 1%, 2%) on the healing of surgically created open wounds on rabbits.

Materials and Methods

The study protocol and procedures were reviewed and approved by the health research ethics committee of faculty of Medicine, Hasanuddin University.

Sample extraction

The snakehead fish were bought from traditional market of equal body weight standard. The samples were cleaned by water and cut head and tail then the body of fish cut and taken bones. The sample 5 kg that have been processed and cleaned till cut small box. The samples were put into caldron by using 1 liter of water, after that steamed for 15 minutes and settled 60°C, afterwards gained snakehead fish water extract. It was put into funnel then extracted with hexan (1:1), shook and rested till formed two layers. The water extract layer was taken and the hexan liquid layer was thrown. The snakehead fish water extract was steamed till dry with using vacuum evaporator and got dry extract.

Snakehead Fish Cream Preparation

The snakehead fish dry extract was weighed and formulated into cream o/w dosage form in the following way: Oil phases were made by melting some materials that dissolve in oil phase. The temperature was kept at 69°C. Water phases were made by dissolving some materials that dissolve in water phase including the snakehead dry extract. The temperature was kept at 60°C. Emulsion was made by adding oil phase into water phase while mixed with electric mixer for 3 minutes then rested for 20 seconds while back mixed till formed homogenous emulsion. It was put into 10 g cream tube.

Histopathology Examination

Nine clinically healthy young male rabbits 2 - 2.5 kg of weights were used in the experiment. Three rabbits per group were maintained in a box (0.64 m²) with some vegetables and carrots.

Local subcutaneous anesthesia with lidocaine 2%, using the inverted-L technique, was administered on all rabbits. The samples of the study were nine rabbits divided into three groups of treatment: group I (0.5%), group II (1%), and group III (2%). Each rabbit in each group was wounded at the left and right dorsal parts as wide as 4 cm² with scalpel to make open wound. Wound at the left dorsal was applied with snakehead fish cream (1 g) and the right dorsal with basic cream as control according to concentration of each group. A bandage was placed around wound of rabbits dorsal perimeter, with the same width as the gauze that covered the wounds. After the surgical procedures, the animals continued their normal diet, and did not receive any antibiotic or anti-inflammatory treatment.

The wounds were rinsed every day observation (day 3, day 6 and day 12) with physiologic saline solution and new rayon gauze containing snakehead fish cream. The basic cream was placed on the control wounds. This procedure was repeated for 3, 6, 12 days for the first, second and third groups respectively.

The observation was done on day 3, day 6 and day 12 by measuring the size of wound and taking photo at the wounded area. Skin biopsies were taken on day 12 postoperatively in order to take tissue adjacent to the wound, fragments were extended 1 cm from each margin and deepened to the muscular plane. The fragments were immersed in formalin buffer solution. For histological examination, the fixed specimens were processed routinely and embedded in paraffin. Sections with 3 micron thickness were stained with hematoxylin and eosin.11-12,13,14

The data were analyzed statistically by applying SPSS 15 program of repeated measures test.

Results

Effect of snakehead fish cream of wound areas

On the 3rd, 6th, 12th day of each group, the experimental wounds treated with snakehead fish cream showed a statistically significant reduction (p<0.05) of measured area (Tables 1, 2, 3, 4). This showed that there was difference of giving influence snakehead fish water extract cream in several concentration and control on wounds area healing process.

On the other hand, the observation days (on the 3rd, 6th, 12th days) affected wounds area healing. They could be showed by significant value almost approximate 0.009 (table 5). Furthermore, it was also showed that not only the interaction between duration of observation days and cream concentration that used it but also wound area healings on significant value (p<0.05). Besides that, very good clinical progress of treated wounds on the 12th day especially cream of 2% concentration had the fastest healing effect in accordance with duration of observation days. Meanwhile control wounds were in the final stage of the
healing process (Table 3) and exhibited small central areas of granulation tissue. However, these control wounds still needed some more time to complete healing. In Figure 1, it described average plot of wound area marginal based on observation result. This showed that cream of 0.3%, 1%, 2% concentration had same pattern in wounds healing on rabbit skin and good design especially cream 2% compared to the other concentrations. Because the result of observation showed the smallest wound area compared to the other concentrations on all of observation days.

<table>
<thead>
<tr>
<th>Table 1. Area (cm²) of Wounds on the 3rd day</th>
<th>Experimental groups</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rabbits</td>
<td>Control</td>
</tr>
<tr>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>3</td>
<td>4</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Table 2. Area (cm²) of wounds on the 6th day</th>
<th>Experimental groups</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rabbits</td>
<td>Control</td>
</tr>
<tr>
<td>1</td>
<td>3.6</td>
</tr>
<tr>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>3</td>
<td>3.6</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Table 3. Area (cm²) of wounds on the 12th day</th>
<th>Experimental groups</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rabbits</td>
<td>Control</td>
</tr>
<tr>
<td>1</td>
<td>2.7</td>
</tr>
<tr>
<td>2</td>
<td>3.1</td>
</tr>
<tr>
<td>3</td>
<td>1.7</td>
</tr>
</tbody>
</table>

| Table 4. The influence of giving concentration of snakehead fish cream and control on wounds area |
|--------------------------------|----------|-------------|--------|-------|-------|-------|
| Source                      | Type III Sum of Squares | df | Mean Square | F    | Sig. | Partial Eta Squared |
| Interceptor                 | 396.365  | 1           | 396.365 | 1947.561 | 0.994 |
| Konsent                     | 18.963   | 5           | 3.793  | 18.635 | 0.886 |
| Error                       | 2.442    | 12          | 0.204  |        |      |

| Table 5. The influence of day on wound healing that was spread snakehead fish water extract cream compared to control |
|--------------------------------|----------|-------------|--------|-------|-------|-------|
| Effect | Value | F | Hypothesis df | Error df | Sig. | Partial Eta Squared |
| Days   | Pillai’s Trace | 0.931 | 73.657 | 2.000 | 11.000 | 0.000 | 0.931 |
|        | Wilk’s Lambda   | 0.069 | 73.657 | 2.000 | 11.000 | 0.000 | 0.931 |
|        | Hotelling’s trace | 13.392 | 73.657 | 2.000 | 11.000 | 0.000 | 0.931 |
|        | Roy’s Largest Root | 13.392 | 73.657 | 2.000 | 11.000 | 0.000 | 0.931 |
| Day + Konsent | Pillai’s Trace | 1.051 | 2.056 | 10.000 | 24.000 | 0.024 | 0.525 |
|        | Wilk’s Lambda   | 0.171 | 3.125 | 10.000 | 22.000 | 0.012 | 0.587 |
|        | Hotelling’s trace | 3.562 | 3.562 | 10.000 | 20.000 | 0.008 | 0.640 |
|        | Roy’s Largest Root | 3.151 | 7.362 | 3.000 | 12.000 | 0.002 | 0.759 |
On the 12th day, the control wounds were still visible hyperemia, crust and edema on the wounds especially control wound of cream 0.5% and 1% (Figure 2C, 4C). Whereas control wound of cream 2% had small areas of central granulation tissue, though it needed more time for total healing resolution (Figure 6C). At the same time, the treated wounds showed complete epithelial resurfacing with hair follicles lining the borders of wounds characterizing a centripetal evolution of contraction and presented better clinical evolution than the control wounds (Figures 1D, 3C, 5C and Table 3).

Treated wounds (Cream 0.5%)

Figure 1A (the 1st day)  
Figure 1B (the 3rd day)

1C (the 6th day)  
1D (the 12th day)

Control Wounds (Cream 0.5%)

Figure 2A (the 3rd day)  
2B (the 6th day)

2C (the 12th day)
Histological evaluation

On the 12th day after excision, the control wounds had a great presence of crust, highly infiltrated granulation tissue with inflammatory cells, and many active spindle-shaped cells, which were fibroblasts (Figure 7B). It showed a mild process of epithelialization beneath an area of crust and highly infiltrated connective tissue with polymorphonuclear leukocytes (Figure 7B). This process needed more time to conclude epithelial resurfacing due to the evident area of epithelium discontinuity. This also could be compared to normal skin (Figure 7A), and the other treated wounds (cream of 0.5%, 1%, 2% concentrations) histopathologically (Figures 7C, 7D, 7E).

On the 12th day after excision, the treated wound (Cream 0.5%) showed that the presence of epidermal crusts between the transitional area of hyperplastic epithelium and healing tissue. Granulation tissue was more developed with minor inflammatory infiltration, the presence of plasma cells, active fibroblasts around new formed capillaries, and abundant extracellular matrix (Figure 7C).

On the 12th day after excision, the treated wound (Cream 1%) presented a strong epidermic hyperplasia, though no epidermic crusts moving towards the subjacent connective tissue were detected. Furthermore, fibroblasts exhibited a high activity of synthesis and a mild inflammatory infiltration in the subepidermic tissue next to the transitional area between the skin and cicatricial tissue. In treated wounds the epithelium still presented a hyperplastic appearance, and fibroblasts with characteristic arrangement of miofibroblasts were observed. Also, the treated wound (Cream 1%) presented a reduction of inflammatory cells in ground substance and in the newly formed vascular bed (Figure 7D).

On the 12th day after excision, the treated wound (Cream 2%) described a better clinical progress and microscopic healing process, than those of control wounds and treated wounds (Cream 0.5% and 1%) at 12 days. Wound treated with cream 2% showed total epithelial resurfacing.
and subjacent connective tissue very active fibroblastic cells in the synthesis of extracellular matrix, especially collagen fibers. In addition, the amorphous ground substance had a mild quantity of inflammatory cells (Figure 7E).

Figure 7A. The Normal Skin consists of epidermis layer (arrow), dermis layer (star), and sub-cutan tissue (arrowhead).

Figure 7B. The Control Wound (12th day), observe extensive area of crust (large arrow) over the granulation tissue. The granulation tissue presenting several active fibroblasts (arrow) and inflammatory cells (arrowheads) surrounding capillaries (star).

Figure 7C. The Treated Wound (Cream 0.5%), note the hyperplastic epithelium near the transitional region of the wound bed and the presence of epidermal crusts (large narrow). The granulation tissue exhibiting intense production of extracellular matrix (arrow). Deep areas of granulation tissue with capillaries (star) surrounded by plasma cells.

Figure 7D. The Treated wound (Cream 1%), healing transitional area showing epidermal crest of hyperplastic epithelium migrating to the connective tissue. The hyperplastic epithelium showing intense inflammatory infiltration (large arrow). The connective tissue presenting mielofibroblastic cells organized in the traction area (arrows), under the hyperplastic epithelium (large arrow). Deep areas of granulation tissue with capillary (star) surrounded by fibroblasts (arrow).

Figure 7E. The Treated Wound (Cream 2%), the epithelialization region (large arrow) and subjacent connective tissue in remodeling process (star). Detail of hyperplastic epithelium without crests (large arrow) and ground substance of connective tissue. Detail of connective tissue showing active fibroblast and few polymorphonuclear cells (arrows).

Discussion

Essential fatty acids (EFA) effectiveness on problems related to skin lesions has been studied since 1929, when the first observation of skin lesions provoked by a shortage of EFA levels in foods were made.16 Linn and shepered17 described the cure of those alterations by topical application of EFAs. Essential fatty acid, linoleic acid and arachidonic acid are polyunsaturated vegetable lipids that can not be synthesized by the animal organisms, necessitating renewal by diet.18

According to Prettey19 linoleic and arachidonic acids are important in the maintenance of a cutaneous barrier to water loss and as precursor of prostaglandins, which are thought to be involved in both the regulation of cell division and differentiation of epidermis and consequently in
the control of skin scaliness, Elias and Brown showed that EFA are precursors of pharmacologically active substances such as thromboxanes, prostacyclins, prostaglandins and others that are involved in cellular division regulation and epidermal differentiation.

The snakehead fish is fresh water fish that contain many nutrients such as albumin and amino acids i.e. lysin, methionin, isoleusin, tripotan and fisin. Besides that, they also have unsaturated fatty acids such as arachidonic acid, eicosapentaenoic acid and minerals such as vitamin A, Ca, Mg, Cu, Fe, Mn, Ni, Co and Zn. All of biochemistry component from the snakehead fish can increase wound healing process on the skin.1,2,3,4

The arachidonic acid and eicosapentaenoic acid are a powerful pro-inflammatory mediator that causes a migration of granulocytes and macrophages as well as important changes in granulation tissue.1,3 The arachidonic acid is a precursor from prostaglandin that important act in wound healing with increasing of prostaglandin synthesis.

In open wounds, epithelialization occurs after the development of a granulation bed, which is also formed when the repair process of connective tissue is necessary. By definition, this is an inflammatory growth of connective tissue characterized by synthesis and deposition of products of fibroblastic cells that can be divided in three phases: (a) cell mobilization and proliferation; (b) synthesis and deposition of collagen products, glycosaminoglycans and extracellular matrix; and (c) a final organization or remodeling of the wound scar.1,2

This paper evaluated the evolution of the wound healing process in adult rabbits utilizing cutaneous application of snakehead fish cream with designing of snakehead fish water extract in cream dosage form with three concentrations i.e. cream 0.5%, 1% and 2%, describing a simple methodology for wound management and the progression of wound healing.

By the twelfth day after excision, the treated wound of cream 0.5% was more evolved macroscopically than the control wounds due to a great development of granulation tissue, contraction in the outer edge of borders and absence of secretion. On the other hand, the control wounds had extensive areas of crust, hyperemia and edema. Histopathological data in treated wound of cream 0.5% described a more advanced repair process than control wounds, due to fewer inflammatory infiltration and presence of active fibroblasts around newly formed vessels.

While evaluating wounds on the twelfth day of cream 1% had well arranged fibroblastic cells in the contraction area while in the control wounds these cellular types were in high synthesis activity of matrix components showing little organization in wound borders.

By the twelfth day after excision, the treated wound of cream 2% showed effective concentration in accelerating of wound healing process statistically. In this case, the acceleration of the inflammatory process can be explained by biological and biochemical features of the arachidonic acids and amino acids. This polyunsaturated fatty acid is changed by desaturation and elongation of its molecule to arachidonic acid which is metabolized via the 5-lipoxygenase and cyclooxygenase pathway in leukotrienes (LTB4, LTC4, and LTD4), prostaglandins (PGIE, PGI2, PGF2, and PGI2) and thromboxane A2 by polymorphonuclear cells. These substances produced by arachidonic acids have pro-inflammatory properties that can stimulate new vascularization locally, cell migration, proliferation and fibroblastic differentiation as well as extracellular matrix synthesis.1,3,4,5

On the area of wound contraction, mioblast cells had very important role in closing wound from outer margin to wound center. In this case, it can be showed by cream 2% that indicated the importance of cytokines and growth factors in the wound healing process. Many of these factors, such as the epidermal growth factor (EGF), fibroblast basic growth factor (bFGF); platelet-derived growth factor (PDGF), transforming growth factor alpha (TGF-α) and interleukin-1 (IL-1) are cytokines that stimulate keratinocyte proliferation. The topical use of bFGF, PDGF and TGF-β in wounds not only facilitates the migration of monocytes, neutrophils, macrophages and fibroblasts, but also stimulates the proliferation of the granulation tissue. Histological analysis of the treated tissue showed that the fish oil acted as a local immunogen causing the migration of both polymorphonuclear leukocytes and macrophages, and resulting in proliferative activity during the appearance of granulation tissue.

In addition, in the snakehead fish contain many fish oils such as arachidonic acid, eicosapentaenoic and high protein such as albumin and amino acids especially lysin, fisin and minerals especially Zn, Fe. All of the above can accelerate wound healing process.

The present work showed by morphometrical, clinical and histological assays that snakehead fish cream improved the granulation tissue formation and epithelial resurfacing of wounds. Therefore, the snakehead fish cream containing high concentration of unsaturated fatty acids and amino acids, can be indicated as a therapeutic alternative for wound healing process.

Conclusion

1. The giving of snakehead fish water extract cream with 0.5%, 1%, 2% concentrations and control gave significant influence of wound areas healing.
2. The duration of giving snakehead fish water extract cream on all of cream concentration effected wound areas healing significantly and occurred interaction between observation days and concentrations of cream on wound areas healing on significant value α = 5%.
3. The effective level of snakehead fish water extract cream that could accelerate wound healing process was 2% concentration.
4. The histological pictures of rabbit skin on the 12th day gave significant difference on 0.5%, 1%, 2% concentrations and control concerning wound healing process.
References


