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## International Journal of PharmTech Research

ISSN(Online):2455-9563, (ISO 3297, ISSN: 0974-4304)

INDEX	
S.N.( Page Numbers)	Title of Paper and Names of Authors
(436-445)	<u>Synthesis and Antimicrobial of New Substituted 1,3,5-Triazine Derivatives</u> Fatma A, El-Samahy, Mervat Elsedik, Tarek Aysha and Magda A
(446-454)	<u>Bioresponscpe of Microalgae Oscillatoria limnetica to Organophosphorous Pesticide Glyphosate</u> Jasim M. Salman, Esraa Abdul-Adel
(455-460)	<u>Inducing Yield Productivity and Nutrients Content of Peanut Plant Grown on Sandy Soil under Different Rates of Remnants of Freeze Vegetable Factories Compost and P. Fertilization</u> Abd El-Rheem KH.M, Entzar M , Essa and Sahar M
(461-472)	<u>Novel Conductive Textile Fabric Based on Polyaniline and CuONanoparticles</u> S.Sharaf, A. Farouk and M.M Abd El-Hady
(473-482)	<u>Assessment of Some Biochemical Markers in Chronic Kidney Disease Patients in AlNajaf Al-Ashraf Governorate</u> Alaauldeen S.M. Al-Sallami, Rusul Ali Kadhim
(483-491)	<u>A Novel of Cocrystalization to Improve Solubility and Dissolution rate of Simvastatin</u> Iyan Sopyan, Achmad Fudholi, Muchtaridi, Ika Puspitasari
(492-501)	<u>Genotyping of Insulin Plant Costus igneus using trnH-psbA using Intergenic Spacer Gene trnH-psbA (PTIGS) and Biogenic Gold Nanoparticles Syntesis</u> Saranya. R, David. E and Mohanasrinivasan. V
(502-507)	<u>Antioxidant Screening through DPPH-HPLC-PDA Analysis on Phlomis herba-venti L. Subsp Kopetdaghensis</u> Ahmadreza Naghavi Azad, Hamideh Nikoozadeh, Ebrahim Golmakani, Peyman Fezi, Katayoun
(508-513)	<u>The Effect of Penetrant Enhancer Combination towards The Diffusion Rate of Snakehead Fish (Ophiocephalus striatus) Cream in Vitro and Vivo</u> Robert Tungadi, Ani M Hasan
(514-519)	<u>The Behavior of Health Services Finding Effort (A Case Study in Kaili Da'a Tribe Society in The Village of Dombu, West Marawola District, Sigi Regency, Central Sulawesi Province) 2010</u> Esron Sirait, Hari Kusnanto



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INDEX



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Home

PharmTech  
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"References Search":  
Enter any Key words

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Features PharmTech

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  - Novelty level of Research work,
  - Importance of Research work,
  - Quality level of work done,
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  - b) Suitable for publication with Low priority.
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Selected by **EBSCO, USA**

Included in **Scimago**.[Rank].

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Alabama, **RGATE** Database.

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**Research Gate**, **Cite Factor**, **Vikaspsoar**, **e.library.ru**, **SJIF**

**DRJI**-Directory of Research Journals Indexing

and, In positive evaluation by:

Many other International databases.

\*\*\*\*\*



INDEX Page-2.

S.N.(Page Numbers)	Title of Paper and Names of Authors			
(301-306)	Spectrophotometric Determination of Darunavir Ethanolate by Condensation Technique			
	R. Vijayalakshmi, Y. Naga Sri Ramya, A. Dimple Mani and M.D. Dhanaraju			
	<a href="#">Abstract</a>	<a href="#">Full Text PDF</a>	<a href="#">Full Text XML</a>	
(307-315)	Preparation of Spray Nanoemulsion and Cream Containing Vitamin E as Anti-Aging Product Tested in Vitro and in Vivo Method			
	Benni Iskandar, Karsono, Jansen Silalahi			
	<a href="#">Abstract</a>	<a href="#">Full Text PDF</a>	<a href="#">Full Text XML</a>	
(316-324)	Organic food and impact on human health			
	Akram Arianfar, Maryam Sardarodiyani			
	<a href="#">Abstract</a>	<a href="#">Full Text PDF</a>	<a href="#">Full Text XML</a>	
(325-335)	Suppressive effects of medicinal plants and their derivatives on inflammasome complex: a systematic review			
	Mohammad Reza Mahmoudian Sani, Majid Asadi-Samani, Ali Saeedi-Boroujeni, Mehdi Banitalebi-Dehkordi, Mahmoud Bahmani			
	<a href="#">Abstract</a>	<a href="#">Full Text PDF</a>	<a href="#">Full Text XML</a>	
(336-338)	Nutrient Content of Body and Growth as Physiological Responses of Mud Crab Scylla olivacea Reared Male Monosex in Mangrove			
	Muhammad Yusri Karim, Hasni Y. Azis, Muslimin, Akbar Marzuki Tahya			
	<a href="#">Abstract</a>	<a href="#">Full Text PDF</a>	<a href="#">Full Text XML</a>	
(339-342)	Evaluation of linoleic acid oxidation in roots of Astragalus glaucacanthus			
	Somaye Langari, Esmail Ataye Salehi, Ameneh Mohammadi			
	<a href="#">Abstract</a>	<a href="#">Full Text PDF</a>	<a href="#">Full Text XML</a>	
(343-347)	Radical Scavenging Activity of Leaf Extract of Edible Hibiscus (Abelmoschus manihot (L.) Medik) Using 1,1-Diphenyl-2-Picryl Hydrazil (DPPH)			
	Risda Waris, Esti Dewi Pratiwi A.M., Ahmad Najib			
	<a href="#">Abstract</a>	<a href="#">Full Text PDF</a>	<a href="#">Full Text XML</a>	
(348-356)	Cytotoxicity Enhancement of Doxorubicin in Conjugation with PAMAM G4.5 Dendrimer Containing Gold Nanoparticles			

	Erny Sagita, Joshita Djajadisastra and Abdul Mutalib		
	<a href="#">Abstract</a>	<a href="#">Full Text PDF</a>	<a href="#">Full Text XML</a>
(357-365)	Molecular and Cytopathological study on etiological agents responsible of Sexually Transmitted diseases		
	Ali H. Al-Marzoqi, HaiderSh. Obaies, Ali M. Saad		
	<a href="#">Abstract</a>	<a href="#">Full Text PDF</a>	<a href="#">Full Text XML</a>
(366-379)	Antioxidant Properties of Liquid Smoke Production Variation of Pyrolysis Temperature Raw and Different Concentration		
	I Ketut Budaraga, Arnim, Yetti Marlida, Usman Bulanin		
	<a href="#">Abstract</a>	<a href="#">Full Text PDF</a>	<a href="#">Full Text XML</a>
(380-387)	Design and optimization of self-nanoemulsifying drug delivery systems (SNEDDS) of ethyl acetate fraction from mangosteen peel (Garcinia mangostana, L.)		
	Liza Pratiwi, Achmad Fudholi, Ronny Martien, Suwidjiyo Pramono		
	<a href="#">Abstract</a>	<a href="#">Full Text PDF</a>	<a href="#">Full Text XML</a>
(388-394)	In Silico Screening of Hesperetin and Naringenin Ester Derivatives as Anticancer Against Phosphoinositide 3-Kinase		
	Nerdy, Effendy De Lux Putra, Ginda Haro, Urip Harahap		
	<a href="#">Abstract</a>	<a href="#">Full Text PDF</a>	<a href="#">Full Text XML</a>
(395-400)	Correlation of Progesterone Induced Blocking Factor and Neutrophil-Lymphocyte Ratio in Preterm and Term Delivery		
	Udin Sabarudin, Glen M Mose, Budi Handono, Hadi Susiarno, Sofie Rifayani Krisnadi, Beni H Purwara, A. Yogi Pramartirta, Prima Nanda Fauziah, Dwi Davidson Rihibiha, Eisenhower Sitanggang		
	<a href="#">Abstract</a>	<a href="#">Full Text PDF</a>	<a href="#">Full Text XML</a>
(401-408)	Bioactive phytochemicals in Rice Bran: processing and functional properties		
	Maryam Sardarodiyani, Esmail Ataye Salehi		
	<a href="#">Abstract</a>	<a href="#">Full Text PDF</a>	<a href="#">Full Text XML</a>
(409-416)	Essential oils in Food Systems: A systemic review		
	Ramezan Ali Mahian, Ali Mohamadi Sani		
	<a href="#">Abstract</a>	<a href="#">Full Text PDF</a>	<a href="#">Full Text XML</a>
(417-423)	The role of milk protein-derived bioactive peptides in humans		
	Ramezan Ali Mahian, Esmail Ataye Salehi		
	<a href="#">Abstract</a>	<a href="#">Full Text PDF</a>	<a href="#">Full Text XML</a>
(424-435)	Effects of Papua Ant Nests (Myrmecodia pendens) on Level of sFlt-1, PlGF, MDA and NO in Preeclampsia-induced HUVEC Cell Line		
	Jeffry Iman Gunardi, Johannes Mose, Mieke H. Satari, Anita Deborah Anwar, Prima Nanda Fauziah, Triyuli		
	<a href="#">Abstract</a>	<a href="#">Full Text PDF</a>	<a href="#">Full Text XML</a>
(436-445)	Synthesis and antimicrobial activity of new substituted 1,3,5-triazine derivatives		
	Fatma A. El-Samahy, Mervat Elsedik, Tarek Aysha and Magda A. El-Bendary		

	<a href="#">Abstract</a>	<a href="#">Full Text PDF</a>	<a href="#">Full Text XML</a>	
(446-454)	<p><b>Bioresponce of microalgae <i>Oscillatoria limnetica</i> to organophosphorous pesticide Glyphosate</b></p> <p><b>Jasim M. Salman, Esraa Abdul-Adel</b></p>			
	<a href="#">Abstract</a>	<a href="#">Full Text PDF</a>	<a href="#">Full Text XML</a>	
(455-460)	<p><b>Inducing Yield Productivity and Nutrients Content of Peanut Plant Grown on Sandy Soil Under Different Rates of Remnants of Freeze Vegetable Factories Compost and P Fertilization</b></p> <p><b>Abd El-Rheem Kh. M. , Entsar M. Essa and Sahar M. Zaghloul</b></p>			
	<a href="#">Abstract</a>	<a href="#">Full Text PDF</a>	<a href="#">Full Text XML</a>	
(461-472)	<p><b>Novel conductive textile fabric based on polyaniline and CuO nanoparticles</b></p> <p><b>S.Sharaf, A .Farouk, and M. M. Abd El-Hady</b></p>			
	<a href="#">Abstract</a>	<a href="#">Full Text PDF</a>	<a href="#">Full Text XML</a>	
(473-482)	<p><b>Assessment of Some Biochemical Markers in Chronic Kidney Disease Patients in Al-Najaf Al-Ashraf Governorate</b></p> <p><b>Alaauldeen S.M.AL-Sallami, Rusul Ali Kadhim</b></p>			
	<a href="#">Abstract</a>	<a href="#">Full Text PDF</a>	<a href="#">Full Text XML</a>	
(483-491)	<p><b>A Novel of Cocrysalization to Improve Solubility and Dissolution rate of Simvastatin</b></p> <p><b>Iyan Sopyan, Achmad Fudholi, Muchtaridi Muchtaridi, Ika Puspitasari</b></p>			
	<a href="#">Abstract</a>	<a href="#">Full Text PDF</a>	<a href="#">Full Text XML</a>	
(492-501)	<p><b>Genotyping of insulin plant <i>Costus igneus</i> using trnH-psbA using intergenic spacer gene trnH-psbA (PTIGS) and Biogenic gold nanoparticles synthesis</b></p> <p><b>Saranya R, David. E and Mohanasrinivasan. V</b></p>			
	<a href="#">Abstract</a>	<a href="#">Full Text PDF</a>	<a href="#">Full Text XML</a>	
(502-507)	<p><b>Antioxidant screening through DPPH-HPLC-PDA analysis on <i>Phlomis herba-venti</i> L. subsp. <i>Kopetdaghensis</i></b></p> <p><b>Ahmadreza Naghavi Azad, Hamideh Nikoozadeh, Ebrahim Golmakani, Peyman Feizi, Katayoun Roghani</b></p>			
	<a href="#">Abstract</a>	<a href="#">Full Text PDF</a>	<a href="#">Full Text XML</a>	
(508-513)	<p><b>The Effect of Penetrant Enhancer Combination towards the Diffusion Rate of Snakehead Fish (<i>Ophiocephalus striatus</i>) Cream in Vitro and Vivo</b></p> <p><b>Robert Tungadi, Ani M Hasan</b></p>			
	<a href="#">Abstract</a>	<a href="#">Full Text PDF</a>	<a href="#">Full Text XML</a>	
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	<a href="#">Abstract</a>	<a href="#">Full Text PDF</a>	<a href="#">Full Text XML</a>	

Next



[1](#)

[2](#)

[\(Back to Journal section\)](#)



## The Effect of Penetrant Enhancer Combination towards the Diffusion Rate of Snakehead Fish (*Ophiocephalus striatus*) Cream in Vitro and Vivo

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**Abstract** : The aim of this research was to determine amount of albumin in snakehead fish cream which can be transported by stratum corneum using penetrant enhancer combination. The snakehead fish powder, containing albumin, was formulated in cream dosage form which consist of 6 formulas. They consisted of penetration enhancer combination in different concentration such as propylene glycol and oleic acid i.e. (F0) : no penetrant enhancer, (F1) : 2% : 5%, (F2) : 4% : 3%, (F3) : 6% : 1%, (F4) : 0% : 7%, (F5) : 7% : 0%. The all of formulas were tested by diffusion membrane cell in vitro using rat skin. After that, the best formulas which were taken to be tested in vivo using rabbits. It was grouped based on the best formulas and positive control (Madecassol® ointment) which each rabbit was wounded on dorsal by scalpel having wound diameter 2 cm then observed for 12 days by measurement and taking picture of wound diameter compared to positive control. The research result showed that all formulas after testing of diffusion cell showed only 1 formula giving the best result i.e. F5 propylene glycol : oleic acid (7% : 0%). The amount of albumin which can be transported into stratum corneum for F5 was 49.76%. Otherwise, the other formulas were only average 0.5% - 7%. It means that snakehead fish cream containing penetrant enhancer without combination giving the best result to penetrate stratum corneum. It was proven by pre-clinic test using rabbits showing the wound experienced healing process acceleration which marked reduction of wound diameter on the third day.

**Keywords** : snakehead fish, cream, albumin, wound healing.



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### Introduction

Indonesia is an archipelago state which is rich natural products from plants and marine animals. Gorontalo Province is one of provinces in Indonesia having Limboto lake the largest producer of snakehead fish. The snakehead fish (*Ophiocephalus striatus*) is one of freshwater fishes which have biochemical components such as albumin, amino acids (glycine and lysine) and unsaturated fatty acids (arachidonic acid and eicosapentanoic) which is very important to synthesize collagen network for accelerating wound healing

process<sup>1</sup>. Gorontalo people usually utilize snakehead fish traditionally by boiling snakehead fish to gain extract of snakehead fish then was drunk to patients who are malnutrition or wound healing after childbirth.

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, gourami, and so on<sup>2</sup>. 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 discomfortability taken because it smells fishy. Therefore, this is one of appropriate alternative to make snakehead fish water extract in other dosage form<sup>3</sup>.

Topical dosage forms can work effectively unless active compounds in suitable dosage forms which can penetrate into skin layers by trans-epidermal and trans-follicular route. Regarding this, skin has stronger barrier properties compared to other biological membrane. Skin has low permeability relatively so that active compounds of drugs are difficult to penetrate skin<sup>4</sup>. Based on this, it can be utilized modification of permeability properties of stratum corneum by adding penetrant enhancer into topical formulas.

The one of penetrant enhancer materials which was widely used propylene glycol. It was able to increase theophylline permeability<sup>5</sup>. The permeability of fentanyl in penetrating skin layers raised with adding propylene glycol into adhesive patch formula. Adding propylene glycol into topical dosage forms could increase diffusion rate of cream compared to cream without propylene glycol. Besides that, oleic acid also could be utilized as penetrant enhancer on skin which 3% of oleic acid could increase diffusion rate of piroxicam gel into stratum corneum<sup>6</sup>.

Therefore, the aim of this research was to determine amount of albumin in snakehead fish cream which can be transported by stratum corneum using penetrant enhancer combination.

## **Materials and Methods**

### **Preparation of Snakehead fish dry extract**

Snakehead fish were cleaned and cut like dice form. After that, they were entered into atomizer extraction tool to get dry extract of snakehead fish

### **Preparation of snakehead fish cream**

The dry extract of snakehead fish was formulated into cream dosage form (6 formulas). All of formulas contained penetrant enhancer except F0 (Oleic acid : propylene glycol) i.e. F0 : no penetrant enhancer, F1 : 5% : 2%, F2 : 3% : 4%, F3 : 1% : 6%, F4 : 7% : 0% and F5 : 0% : 7%.

### **Penetration Test in Vitro**

All of formulas containing penetrant enhancer including F0 were tested by Franz diffusion cell using rat skin as membrane cell. The sample taking were done at 2'30", 3'00", 3'30", and 4'00". After that, it was measured absorbance by UV-Vis Spectrophotometry.

### **The Effectiveness test of snakehead fish cream in vivo**

The best results of penetration test were continued by the effectiveness test of cream using rabbit skin. The rabbits were divided into 3 groups i.e. group I was applied cream containing the highest albumin levels, group II the second highest albumin levels and group III the control positive of madecassol ointment. Each rabbit was wounded at left dorsal by scalpel to get open wound 2x2 cm. After that, the wounds were applied by snakehead fish cream in accordance with treatment group then each treatment group was done observations for 12 days including measurement of wound diameter and taking picture of wounds each day.

## Result and Discussion

### Diffusion Test in Vitro

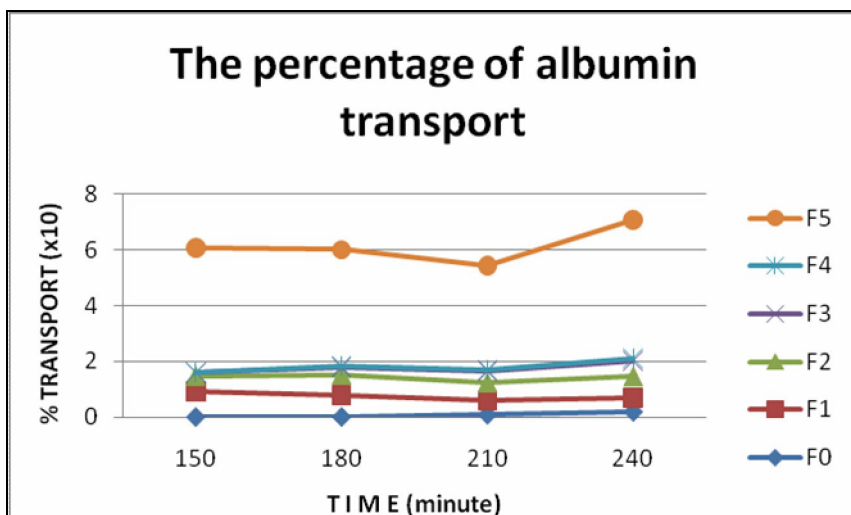
On this research was done diffusion test utilizing rat skin as membrane cell which the sixth formulas were tested and gained the result of spectrophotometry measurement (table 1).

**Table 1. The amount of albumin which can be transported into skin membrane cell**

Formula	Sampling Time	Absorbant
F0	2'30"	0,122
	3'00"	0,111
	3'30"	0,133
	4'00"	0,117
F1	2'30"	0,205
	3'00"	0,183
	3'30"	0,146
	4'00"	0,146
F2	2'30"	0,186
	3'00"	0,214
	3'30"	0,194
	4'00"	0,211
F3	2'30"	0,103
	3'00"	0,138
	3'30"	0,162
	4'00"	0,195
F4	2'30"	0,067
	3'00"	0,081
	3'30"	0,089
	4'00"	0,092
F5	2'30"	0,851
	3'00"	0,774
	3'30"	0,666
	4'00"	0,894

One of important thing to formulate cream dosage form is able to penetrate of active compounds into skin layers. Stratum corneum of epidermis layer is the main protective layer and skin barrier so that drug penetration via stratum corneum is very important to determine diffusion rate<sup>4</sup>. Therefore, snakehead fish dry extract, containing albumin, was formulated into cream dosage form using penetrant enhancer combination (Oleic acid and Propylene glycol) in vitro i.e. F0 : no penetrant enhancer, F1 : 5% : 2%, F2 : 3% : 4%, F3 : 1% : 6%, F4 : 7% : 0%, and F5 : 0% : 7%.





**Figure 1. The percentage of albumin transport**

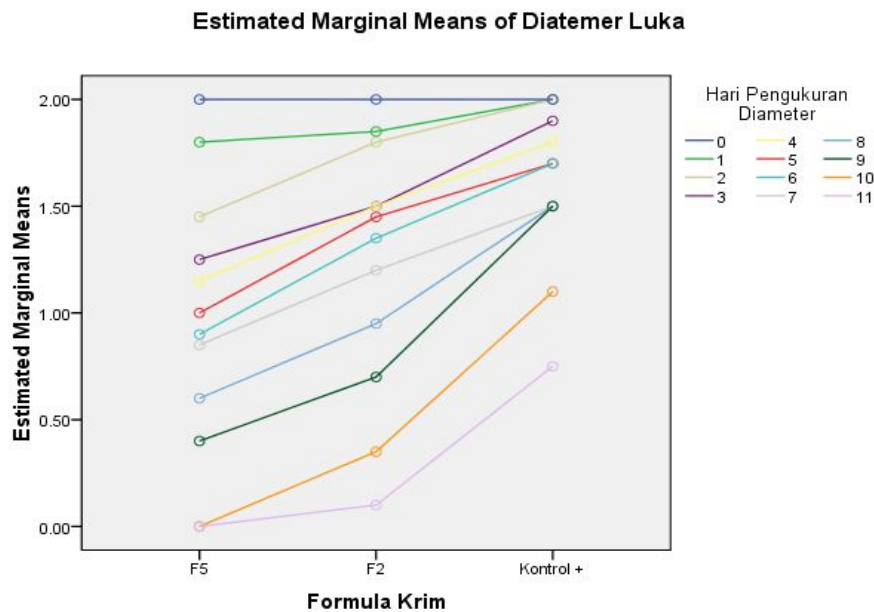
The figure showed that the amount of albumin transport into stratum corneum was 49.8% of Formula 5. It was the highest compared to other formulas then 7.42% of formula 2 respectively. Otherwise, the lowest of albumin transport into stratum corneum was Formula 0, 1, 3, and 4 average around 0.8% - 5.7%.

Formula 5 (F5) contained only propylene glycol 7% which was able to transport albumin penetrating skin layers. It was caused by hydrotropic effect from propylene glycol using destabilization mechanism of lipid components which can affect membrane permeability of stratum corneum<sup>7</sup>. Besides that, propylene glycol had capable as co-solvent of drugs because chemical structure of propylene glycol contained hydrophilic group enabling to gain high concentration in donor compartment. Meanwhile, the amount of albumin transport of Formula 2 (F2), containing oleic acid : propylene glycol 3% : 4%, was 7.42%. It means that cream containing penetrant enhancer combination oleic acid and propylene glycol affected diffusion rate of albumin to penetrate into stratum corneum because oleic acid will inhibit penetration in high concentration. It can be seen that the amount of albumin transport of formula 2 was better than formula 0, 1, 3, 4 because oleic acid and propylene glycol had balance concentration compared to other formula. Besides that, oleic acid was lipophilic group which will affect penetration of albumin into stratum corneum and albumin also had lipophilic group so that the capable of stratum corneum was difficult to experience hydration in stratum corneum.

Based on mechanism of propylene glycol as penetrant enhancer, there are three pathway of main mechanism by disturbing lipid structure on stratum corneum, interacting with intercellular protein, and improving drug partition, co-enhancer or dissolving into stratum corneum<sup>8</sup>.

### Pre-Clinic Test in Vivo

In this research in vivo utilized the best formula from diffusion test i.e. Formula 5 (F5) and formula 2 (F2) which applied on rabbits skin in compliance treatment group. Group I consisted of rabbits which had been wounded 2x2 cm and applied snakehead fish cream containing oleic acid and propylene glycol (0 : 7%). Group II (F2) was treatment group using oleic acid and propylene glycol (3% : 4%). Group III (positive control) using madecassol<sup>®</sup> ointment.



**Figure 2. The estimated marginal means of wound diameter**

From estimated marginal means of wound diameter showed that each formula affected wound healing process on rabbits skin. Group I (F5) described that wound recovery was faster than other groups. As can be seen from the graph that formula 5 (F5), the best formula, gave estimated marginal means of wound diameter about 1.25 cm on third day compared to group II and III approximately 1.5 and 1.9 cm respectively. It proven that the percentage of albumin level which transported into stratum corneum the biggest level will accelerate wound healing process on third day. It was marked by wound area reduction and wound recovery.

Based on pharmacology aspect, snakehead fish cream 2% (F5) was very fast in accelerating wound healing process because the use of propylene glycol as penetrant enhancer which was able to transport albumin and other biochemical components. All of this could increase wound healing process on the skin. Albumin, arachidonic acid and eicosapentanoic acid were strong mediator of pro inflammation causing migration of granulocyte and macrophage. It had important role in forming new granulation tissues which arachidonic acid as precursor from prostaglandin in accelerating wound healing process by increasing prostaglandin synthesis<sup>9</sup>.

In wound healing, epithelialization occurs after healing of granulation tissue causing inflammation. This is formed by improvement process of connective tissue which can be characterized by synthesis and decomposition of fibroblasts result. It can be divided into 3 phase i.e. cell mobilization and proliferation, synthesis and decomposition of collagen product, glykosaminoglikan and extracellular matrix and final arrangements or transformation from scarring<sup>10</sup>.

## Conclusion

Based on results of this research can be concluded that the highest percentage of albumin level into stratum corneum using diffusion test was formula 5 (F5) using oleic acid and propylene glycol (0% : 7%) 49.76%. This can be proven by pre-clinic test in vivo on rabbits skin which formula 5 which can accelerate wound healing on the third day compared to formula 2 (3% : 4%) and positive control (madecassol<sup>®</sup> ointment).

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