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Vacuum drying albumin powder of snakehead (*Channa micropeltes*) potential for wound healing from Central Kalimantan, Indonesia

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Abstract : Giant snakeheads (*Channas micropeltes*) are one of fish species of family *Channidae* that is protein source, has high albumin content, and highly beneficial for wound healing. In Central Kalimantan, the species is highly popular to be consumed by post-partum mothers, since it is believed to be able to accelerate their recovery. The fish are easily obtained and inexpensive. The vacuum drying albumin powder of this fish species was applied in *in vivo* test of wistar rats. Results indicated that the application of 1.5 g of the vaccum drying albumin powder gave the best effect in day-5 and day-10 observstions based on albumin level analysis of the test animals and skin tissue histological analysis.
Keywords : *Channa micropeltes*, Vacuum drying, albumin, wound healing, in vivo.

Introduction

Freshwater fish population in big islands in Indonesia is different. Freshwater fishes are about 394 species in Kalimantan with 149 (38%) endemic, 272 in Sumatera with 30 (11%) endemic, 132 species in Java with 52 (9%) endemic, and 68 species in Sulawesi with 52 (76%) endemic[1]. Kalimantan has the highest number of species among big Indonesian islands, but its endemic level is lower than that in Sulawesi. Kalimantan is also the biggest island in Indonesia with a total area of 736,000 km^2 and squiggly geographic condition that makes Kalimantan possess many river flows with important potential and role as living media for all aquatic biota.

Freshwater potency of Central Kalimantan is 2,290,000 Ha comprising lake of 132,800 Ha or 5.80%, river of 323,500 Ha or 14.13% and swamp of 1,833,700 Ha or 80.07%. river covers an extensive area in central Kalimantan with 11 big rivers of numerous streams. Rivers and streams are associated with lake (as dead river/*oxbow-lake*) and swamp adjacent to the watershed. From 3.6 million Ha of lowland swamp in Kalimantan island, about 1.8 million Ha or about 50% are used for fisheries in central Kalimantan[2]. Fish are one of the altenative food as albumin source highly needed forhuman health, especially wound healing process. The occurrence of family *Channidae* in this area is very dominant and consumed by the local communities. People of other areas know only common snakeheds (*Channa striata*) while the snakeheads possess several species. Channidae has high albumin content, and toman (*C. micropeltes*) is one of the family members selected in this study as the fish usable as albumin source. Until now, the utilization divertification of family Channidae in powder form made through vacuum drying process has not been carried out, but if the fish can be processed to fish powder, it does not smell, the storing period is longer, and could be used anytime and anywhere. The powder can also be easily absorbed, particularly for wound treatment. The potential of vacuum drying powder of toman (*C. micropeltes*) in wound healing neeeds to be quantitatively proved using *in vivo* method on wistar

rats. The albumin –composing amino acids of family Channidae belong to complete proteins made of essential and non-essential amino acids [3,4]. Vacuum drying albumin powder of *C. micropeltes* possesses high tape density of albumin protein and the most complete amino acids compared with other fish albumin powder in one fish family, 8.28% [5].

Materials and Method

This study used an experimental method by applying vacuum drying powder on wistar rats acclimated for 1 week and made a 2 cm-lesion on the posterior part. The material was albumin powder extracted from toman *Channa micropeltes* using vacuum extractor at 35ºC for 12.5 minutes at a pressure of 1 atm. Oral administration of vacuum drying powder was done into the rats with a dose of 0.5g, 0.75g, 1g, 1.25g and 1.5g, respectively.

Vacuum drying powder processing

Fifty percent of fish filtrate from extraction was added with 75% arabian gum and 25% gelatin, 5% lecitin and 10% CMC, then homogenized at 2000 rpm for 15 minutes[6]. The homogenous mixture was poured into a pan and inserted into a vacuum dryer at 49°C for 5 hours until dry and then ground to fine particles and sieved through **60 mesh** [7]. Moreover, the wistar rats treated with vacuum drying powder for 5 and 10 days were taken their blood for albumin level test.

Albumin content test

As much as 2 cc of blood serum of the test animal was taken and tested its albumin level. It was measured using a spectrophotometer, an instrument measuring the transmitance and absorbance of sample as wavelength function**.** Albumin content measurement was done using a spectrophotometer: 2 cc of sample were added with biuret reagent and heated at 37°C for 10 min**.,** then cooled and measured with spectronic-20 and record the absorbance**.** Albumin level was calculated using the following formula:

$$(\%)\ \text{Albumin} = \frac{\text{ppm x 25}}{\text{sample weight x } 10^6} \text{ x } 100\%$$

Data collection and statistical analysis

Data were gathered based on albumin level measurement of the test animal blood serum at day-5 and day-10, and then continued with statistical analysis. Experimental design used Factorial Completely Randomized Design with 3 replications for each treatment.

Results and Discussion

Vacuum drying albumin powder of Toman administered to the test rat is shown in Fig. 1, while the proximate composition value was presented in Table 1.

Figure 1. Vacuum drying powder of toman (*Channa micropeltes*)

Further study was *in vivo* test using 33 individuals of 2–4 month old male wistar rat of ± 150g BW. The use of male rats results from that female rats easily get psychological and hormonal disorders at breeding time. Males are also calmer, easily handled, rarely fight ,parameters use in the study were albumin level and Zn of rat blood serum.

Albumin content of rat blood serum after feeding vacuum drying albumin powder of *C. micropeltes.*

ANOVA showed significant difference ($p \leq 0.05$) and interaction between drying vacuum albumin powder doses of 0.5, 0.75; 1, 1.25 and 1.5 g and 5 days and 10 days of healing time. Therefore, the dose application and healing time gave different outcomes as shown in Fig. 2.

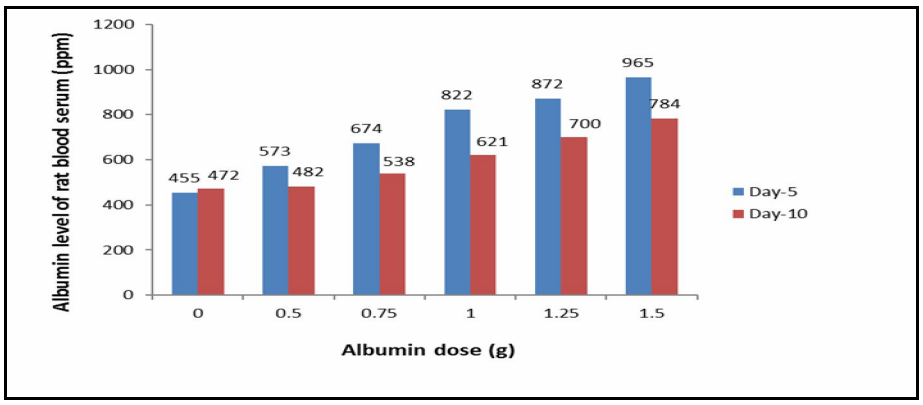

Figure 2. Albumin content of rat blood serum after feeding vacuum drying albumin powder of Toman

The optimum dose of vacuum drying albumin powder of toman is 1.5g**.** Based on Fig. 2. It is apparent that the rat blood serum albumin content response to all doses observed in day-5 be higher than that in day-10. The highest albumin content of the rat blood serum was observed in the dose application of 1.5g, 965.67±24.58 ppm at day-5.

Zn content of rat blood serum after administration of vacuum drying albumin powder of *C. micropeltes.*

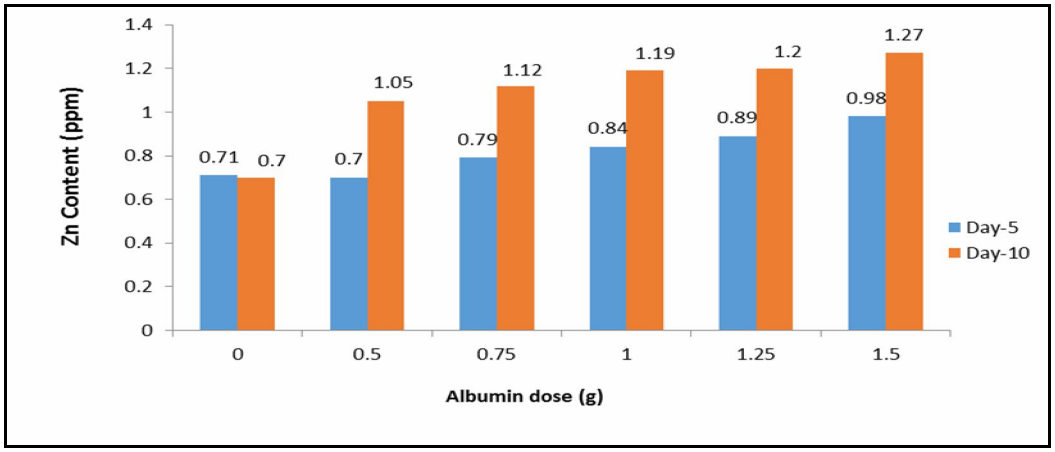

Figure 3. Zn content of rat blood serum after administration of vacuum drying albumin powder of toman.

Zn content of the rat blood serum at day-5 and day-10 is presented in Fig. 3. ANOVA shows that there are significantly different effects on Zn level among the doses of 0.5, 0.75, 1, 1.25 and 1.50g and between wound healing observation time after 5 and 10 days ($p \leq 0.05$), and there is significantly different interaction effect between albumin powder application and wound healing observation time Zn level ($p \leq 0.05$). Thus, the dose of toman albumin powder application and the wound healing observation time have different effects. The use of the LSD test and Duncan Multiple Range Test (DMRT) indicated that the optimum dose of toman albumin powder application was 1.5g.

Table 2. Wound healing of the rat (*Rattus norvegicus*) skin (%)

Vacuum drying Albumin powder dose (g)	Vacuum drying of *C. micropeltes*	
	Day-5	Day-10
Negative control	38.5	51.8
0.5	48.5	63.4
0.75	52.5	71.8
1	63.5	81
1.25	71.8	86.8
1.5	83.5	100

Based on Fig. 3 and Table 2, this study reveals that percent wound healing of the rat's (*Rattus norvegicus*) skin develops with increased vacuum drying albumin powder of toman in the 2 cm long, 4-6 mm wide and 1-3 mm deep wound. In negative control**,** the application of toman vacuum drying albumin powder had percent wound healing of 38.5% at day-5 and 51.8% at day-10. The percent wound healing tended to rise at dose 0.5-1.5 g.

Histological analysis of the rat skin tissue

Skin, an outer body organ covering muscles and protecting the body from various trauma, is bacterial, viral and fungi inhibitor. Skin consists of epiderm (outer layer), derm (inner layer), and hypoderm (tissue under the skin). Epidermis is the outer layer with about 0.1 mm thick on the eyelid and 1 mm thick on the palm and foot. Dermis is the second layer of the skin layer holding collagen and elastin fibers that are important protein to promote skin suppleness and firmness. Hypoderm is inner skin consisting of adipose tissue providing cushion between the skin layer and the muscle and bone[8].

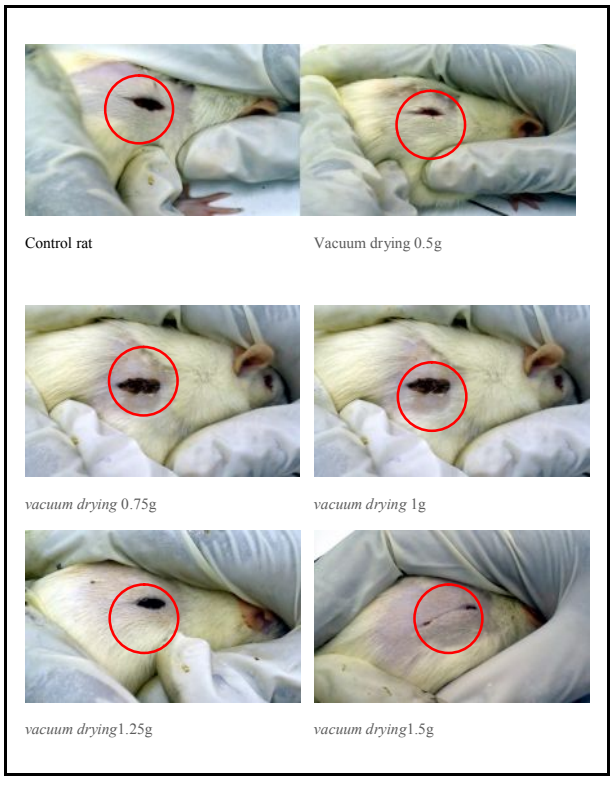

Figure 4. wound conditions of negative control and *vacuum drying* albumin powder at day-5

Cut is one of the acute wounds as traumatic injuries that usually get immediate care and can well recover if there is no complication. This injury category is new lesion, incidental, and its recovery occurs in estimated time.

Wound healing is an establishment to repair tissue damages. Major component in wound healing is collagen beside tha epithelial cells. Fibroblast is cell responsible for collagen sythesis. The wound healing physiology will naturally follow several phases, inflammatory, proliferative or fibroplasia, and maturation or remodelling phases [9]. Fig. 4 shows the injury of control rats and those with wound recovery.

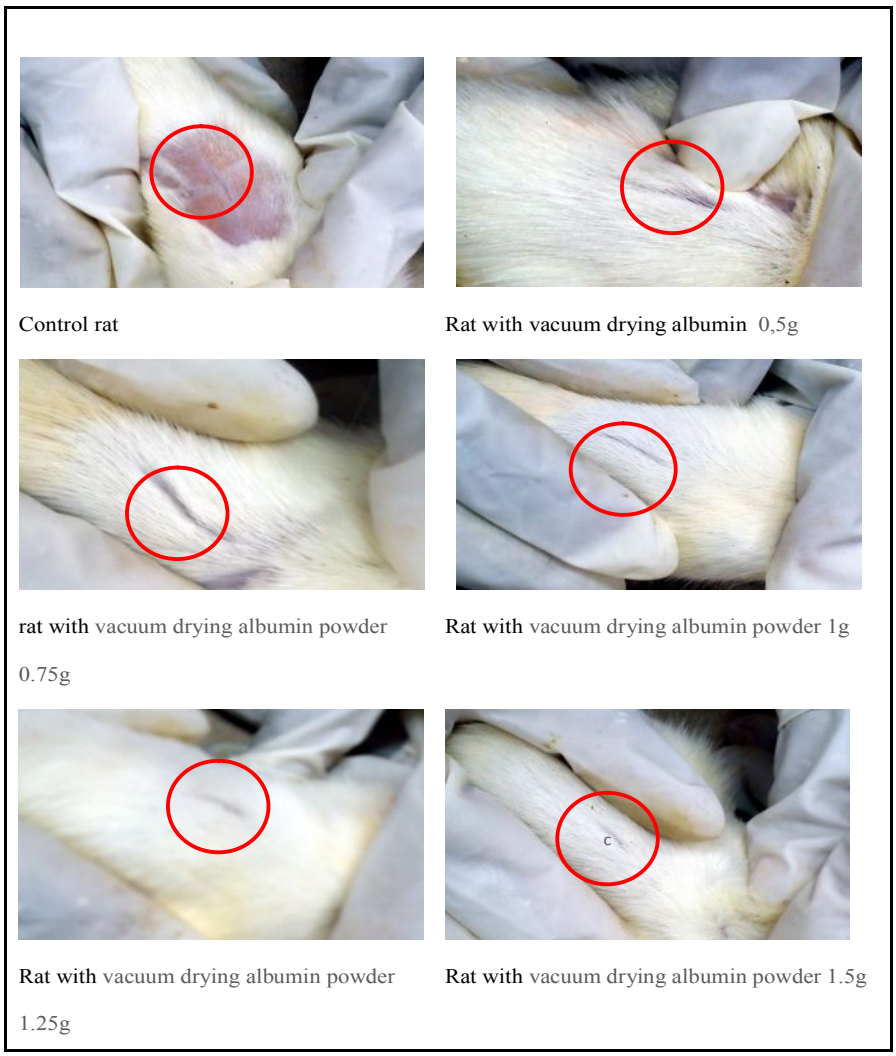

Figure 5. test rats treated with vacuum drying albumin powder at day-10

Skin tissue observations at day-5 and day-10 show that some skin tissues have not reco vered or well healed, while the rats treated with 1.5g of albumin powder exhibit relatively fast wound healing through skin condition approaching to normal. Remodelling phase or maturation phase is the last and the longest phase in wound healing [9,11-13]. In this phase, the dynamic processes, such as collegan remodellng, lesion contraction, and scar maturation, occur. Collegan synthesis and degradation activities are in balance. Remodelling phase could occur from 3 weeks to 2 years. Albumin highly plays a role in accelerating the collegan synthesis and degradation and the distribution to intra- and extra-vascular parts**.** The epidermal layer thickening also declines with increased treament dose. All dose treaments have not still caused vascularization of the tissue with inflammation.

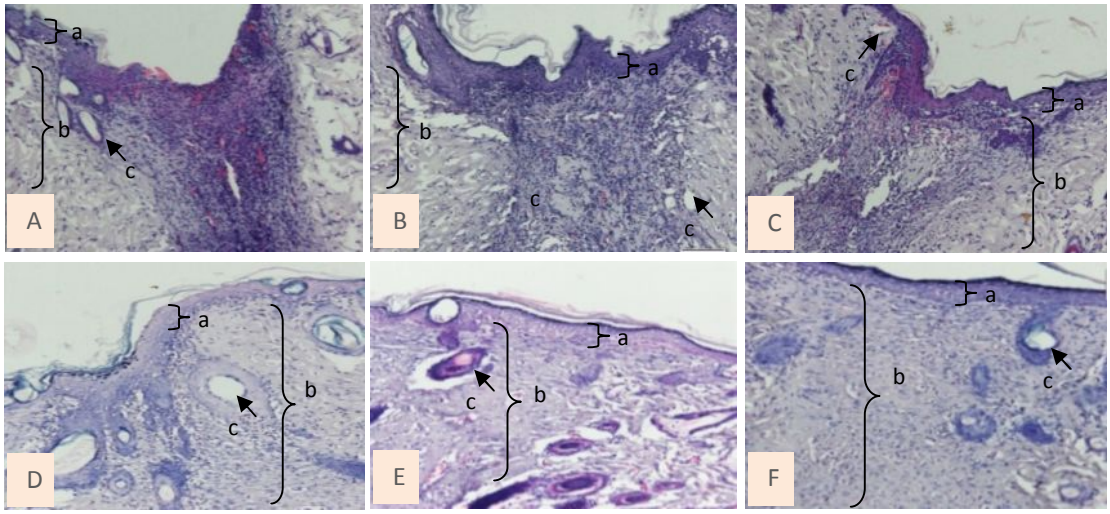

Figure 6.Histology of rat's (*Rattus norvegicus*) skin tissue after treated with vacuum drying albumin powder of toman. Notes: A=control (negative), B=dose of 0.5g, C=dose of 0.75 g, D=dose of 1g, E=dose of 1.25g, and F=dose of 1.5g. 200x enlargement. a= epidermal layer, b =dermal layer, c= dermal papilla.

The application of 1.5 g of toman vacuum drying albumin powder is the most optimum dose based on skin tissue histological analysis. Under this dose, the epidermal layer is thinner than that in other treatments and causes lymphocytic cellular infiltration of the inflammatory tissue.

Fig. 6 demonstrates that negative control rats still have very high lymphocytic cell infiltration in the inflammatory tissues and the epidermal layer has not been properly formed yet. With the dose of 0.5, 0.75, 1, 1.25, and 1.5 g, the epidermal layer was formed and thickened followed with fibroblast cell proliferation. The lymphocytic cell infiltration gradually decreases with increased treatment dose of albumin powder application.

Integument or skin is a tissue covering the body surface, comprising 2 layers:

1. Epithel called epiderms
2. Connective tissue called dermis or corium epidermis originating from ectoderm and dermis coming from mesoderm.

Beneath skin occurs looser connetive tissue called hypodermis which largely contains lipid tissue. The boundary could be found in lips, nose hole, vulva, preputium, and anus. Skin is the body part covering extensive area with a mass about 16% of body weight. It works to cover the body and has some other functions. Therefore, beside epithetial structure and connective tissue, skin is also equipped with additional structure called skin appendix including glandula sudorifera, glandula sebacea, hair follicle, and nail. The surface between epidermis and dermis are not flat due to connective tissue protrusions toward.

The application of toman vacuum drying albumin powder, based on histological analysis, is proved to be able to increase the wound healing of the skin tissue compared with in negative control rats. Fig. 6 shows that the application of toman albumin powder gives wound healing effect due to inflammatory tissue vascularization. Wound healing involves cell types (neutrophyl, macrophage, keratinocyte, endothel and fibroblast), growth factor, kemokin, cytokine, and extracellular matrix working in inflammatory, proliferation, and tissue remodelling phases[10].

Conclusion

Vacuum drying albumin powder of Toman could heal the wound of the wistar rat in 10 days at the feed dose of 1.5g.

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