SANGKURIANG CATFISH (CLARIAS GARIEPINUS VAR) SKIN EXTRACT ACTIVITY ON FIBROBLAST AND COLLAGEN SYNTHESIS FOR SKIN BURN HEALING

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Abstract

Background: Burn healing of IIb degree requires a relatively longer time for healing followed by hypertrophic scar formation. Sangkuriang catfish (Clarias gariepinus var) skin extract topical contains amino acids of collagen. Collagen is the main component of skin wound healing. The aim of this research was to determine the influence of Sangkuriang catfish skin extract topical treatment on fibroblast and collagen synthesis for burn healing

Methods: Sangkuriang catfish skin was extracted using HCl 2% for 48 hours and neutralized by NaOH 1 M. Twenty-eight male Rattus novergicus strain Wistar were used for this researchwere, divided into K3, P3, K10, and P10 groups. Each member of group was subjected with IIb degree burn injury on the back, then K3 and K10 groups were treated by using aquades topically twice a day whereas P3 and P10 groups tpicaly treated with Sangkuriang catfish skin extract twice a day.

Results: Sangkuriang catfish skin extract topical treatment increase fibroblast number and significant extensive collagen percentage on 10^{th} day

Conclusion: Topical treatment of Sangkuriang catfish skin extract could accelerate burn healing by increasing number of fibroblast and percentage of extensive collagen.

Keywords: Sangkuriang Catfish, Collagen, Fibroblast, Burn Healing

INTRODUCTION

In Indonesia, burn injury cases were higher than other cases of injury. Burn Injury of IIb degree happens when skin tissue damage reaches almost entire of dermis, however low damage in the rest of epithelial tissue [1]. Based on *"Riset Kesehatan Dasar (Riskesdas)"* the prevalence of burn injury in Indonesia was 70% in 2013[2]. Burn healing requires a relatively longer time and followed by hypertrophic scar formation. Wound healing itself consist of four phases: haemostasis, inflammation, proliferation and remodelling[1].

Collagen is the main component in connective tissue that induce wound healing by generating fibroblast proliferation, stimulate new granulation and epithel tissue formation around burn injury[1][3].Fish skin collagen is better than livestock and poultry collagen because of its superior bioavalability (absorbed up to 1.5 times more efficiently) and because of severe infections (zoonosis), including bovine spongiform encephalopathy, avian and swine inluenza, and tooth-and-mouth disease in bovines, pigs, and bufalo, occur worldwide[4]. Fish skin collagen is also halal to be consumed and it has lower immunoreactivity risk on wound healing[4][5][6]. It has relatively high glycine to keep the moist-state of skin and low hydroxyproline causing fish skin collagen tends to be more elastic than livestock and poultry collagen[6]. The aim of this research is to determine if topical treatment with Sangkuriang catfish skin could accelerate burn healing by increasing synthesis of fibroblast and collagen on wound area.

METHODS

This research used Sangkuriang catfish (*Clarias gariepinus var*) skin extract wich contains collagen amino acid. Sangkuriang catfish skin was taken from home industry waste of catfish nugget and crackers in

Tanggulangin district, Sidoarjo city. Catfish skin extract was extracted using HCl 2% for 48 hours, neutralized by NaOH 1 M until collagen fiber appears[4][5][6][7][8]. Twenty-eight male *Rattus novergicus strain Wistar* were used for this research, divided into 4 groups, control group for 3 days (K3) and 10 days (K10), treatment group for 3 days (P3) and 10 days (P10). Each member of group was subjected to IIb degree burn injury on its back, K3 and K10 groups were treated with aquades topical, whereas P3 and P10 groups were topically treated with Sangkuriang catfish skin extract twice a day.

RESULTS

Topical treatment with Sangkuriang catfish skin extract on groups treatment showed insignificant increase of fibroblast number of fibroblast (p>0,05) on 3 days treatment (K3 and P3), and significant increased (p>0,05) on 10th day treatment (K10 and P10). Results on fibroblast increase was presented on table 1. Table 2 showed p-value of differences mean for each group on table 1.

Table 1. Mean of fibroblast number along burn heling

Group	N	Mean ± Std. Deviation
K3	6	11,056 ± 1,357[a]
P3	6	11,333 ± 1,563[a]
K10	6	21,889 ± 1,425[b]
P10	6	38,111 ± 4,500[c]

*superscript showed difference score statistically significant (p-value < 0,05)

Table 2. p-value of post hoc test on fibroblast number along burn healing

Kelompok Perlakuan	K3	P3	K10	P10
K3		0,854	0,000	0,000
P3			0,000	0,000
K10				0,000
P10				

Sangkuriang catfish skin extract topical treatment on treatments groups show insignificant percentage of extensive collagen increase (p>0,05) on 3 days treatment (K3 and P3), and significant (p>0,05) on 10th day treatment (K10 and P10). The satistic result of extensive collagen mean percentage was presented on table 3. Table 4 showed p-value of post hoc test presenting differences on mean percentage of extensive collagen for each group on table 3. .

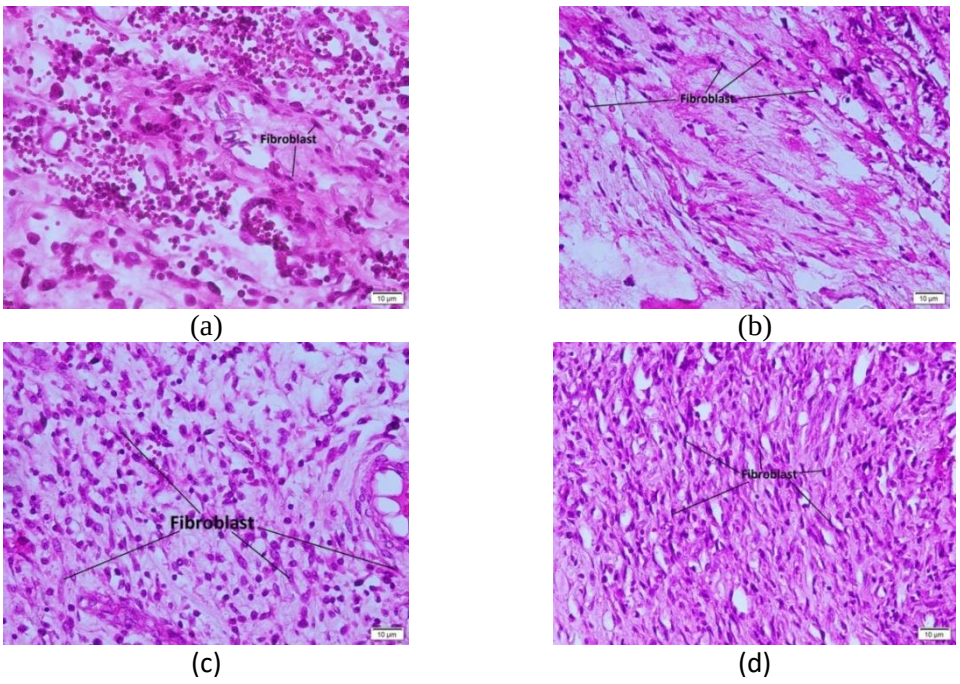
Table 3. Mean of percentage of collagen wide-spaced along burn healing

Group	N	Mean ± Std. Deviation (%)
K3	6	11,222 ± 1,501[a]
P3	6	12,000 ± 1,299[a]
K10	6	22,889 ± 3,673[b]
P10	6	42,611 ± 9,027[c]

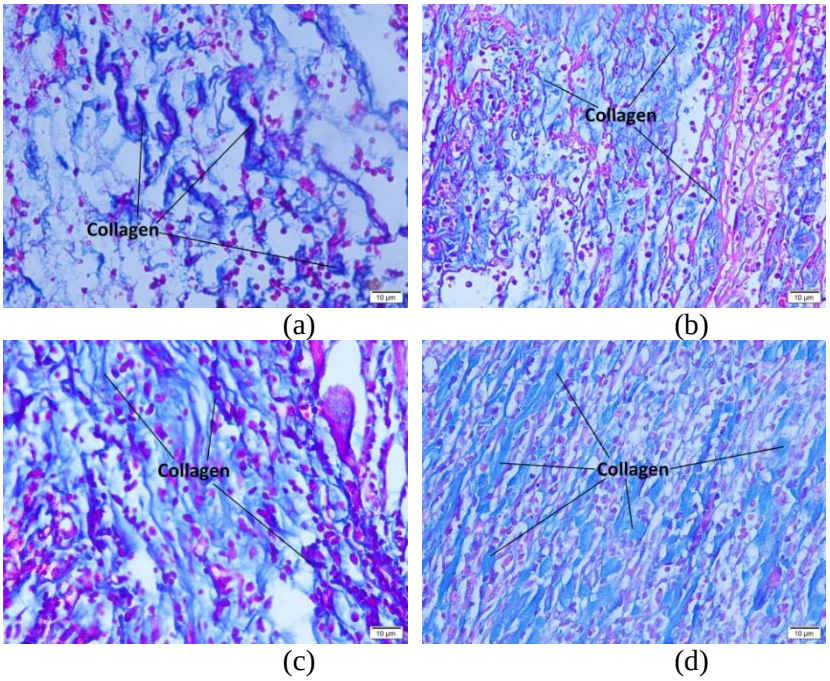
*superscript showed difference score statistically significant (p-value < 0,05)

Table 2. p-value of post hoc test on percentage of extensive collagen on burn injury

Kelompok Perlakuan	K3	P3	K10	P10
K3		0,789	0,001	0,000
P3			0,001	0,000
K10				0,000
P10				



Figures 1. The histopatology of fibroblast number colored with HE on 10 µm scale, magnitude 400x. a) K3; b) P3; c) K10; d) P10. showed fibroblast number of P3 higher than K3 and P10 higher than K10.



Figures 2. The histopatology of collagen fiber colored with MT on 10 µm scale, magnitude 400x. a) K3; b) P3; c) K10; d) P10. showed collagen number of P3 wider than K3 and P10 wide than K10.

DISCUSSIONS

Burn injury induces high morbidity and mortality that could change local and systemic body function, hence patient with burn injury should have special treatment since the early phase until advanced phase of wound care. The burn injury degree is based on wound depth, causative source, agent and duration contact with calor[1]. Normal wound healing is characterized by fibroblast deposition of collagen type I and III[9].

Based on this study, Sangkuriang catfish skin extract topical treatment showed the number of fibroblast was enhanced in treated group, because it contains proline and glycine. Glycine was used as *natural moisturizing factor* on skin and induced collagen synthesi to accelerating burn healing[3]. The results showed group P had number of fibroblast higher than group K ,statistically no significant ($p>0,05$) on 3rd day treatment (K3 and P3), but significant ($p>0,05$) on 10th day treatment (K10 and P10). It was caused 3rd day of wound healing in inflammation phase, but 10th day of wound healing in proliferation phase. Hence, Sangkuriang catfish skin extract could increase the number of fibroblast in extracellular matrix synthesis to accelerate wound healing. Proliferation phase happened along 3-12 days post-injury[1]. Figure 1 showed that P3 has higher fibroblast number than K3 and P10 than K10.

Collagen is biomedically used to promote proliferation and migration of fibroblast cell into wound area[7]. Collagen plays a key role in each phase of w ound healing[9][10][11]. The collagen cleavage products stimulate vascular endothelial cell proliferation. These cells sec rete a variety of GFs (VEGF, βFGF, PDGF), which promote angiogenesis[9]. Granulation is achieved by vascularizing ECM. Collagen cleavage products stimulate also keratinocyte migration and proliferation[10].

Type I collagen from *Clarias batrachus* has significantly enhancing adhesion of fibroblast on culture cell from mice skin[7], it was used as chemoattracttant[7][10]. Sangkuriang catfish skin extract topical treatment on burn injury result showed the percentage of collagen on P3 is wider than K3 but insignificant and P10 was wider than K10 statistically significant. It was caused 3rd day of wound healing during inflammation phase, but 10th day of wound healing during proliferation phase. Glycine and proline in Sangkuriang catfish skin extract was enhancing deposition and increase of extensive collagen percentage in extracellular matrix. Therefore collagen extract from *Clarias batrachus* skin could generate decomposition of collagen and extracellular matrix around wound area, it could be developed as wound dressing or scaffold for tissue engineering, because it could accelerate burn healing by increasing fibroblast number and percentage of extensive collagen.

CONCLUSION

Topical treatment of Sangkuriang catfish skin extract could accelerate burn injury by increasing fibroblast number and percentage of extensive collagen significantly on 10th day of wound healing.

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