ORIGINAL ARTICLE

Stimulation of Dental Socket Healing by *Pangasius Djambal* Gelatin: Evaluation of Growth Factor Expression

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ABSTRACT

Introduction: Growth factors play a lot of roles in the process of wound healing following tooth extraction. The effect of *Pangasius djambal* gelatin on expression and pattern of distribution of these growth factors have not been investigated in early phase of wound healing after tooth extraction. This study aimed to observe the pattern of distribution of growth factors in dental socket healing of albino rats (*Rattus norvegicus*) following tooth extraction. **Methods:** 24 albino rats were used as an experimental method with randomized posttest-only control group design. Treatment groups were treated with *P. djambal* gelatin after tooth extraction, while control groups were left untreated after tooth extraction. The present immunohistochemical study used polyclonal antibodies specific for Platelet-Derived Growth Factor (PDGF), Vascular Endothelial Growth Factor (VEGF), Fibroblast Growth Factor-2 (FGF-2), and Epidermal Growth Factor (EGF). **Results:** There was a statistically significant increase of PDGF, VEGF, FGF-2, and EGF expression in the treatment groups compared with the control groups. **Conclusion:** The application of *P. djambal* gelatin to dental socket post tooth extraction could increase the level of growth factors expression during dental socket healing process.

Keywords: Growth factor; *Pangasius djambal*; Gelatin; Tooth extraction; Wound healing

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INTRODUCTION

The healing process after tooth extraction is similar to healing in other parts of the body. This process involves complex interactions between cells and multiple growth factors. It is a dynamic process of tissue inflammation, proliferation, and remodeling (1). Some key factors influencing the healing process are growth factors, which, in addition to accelerating healing maturation, stimulate gene transcription and activate cell proliferation. The most important of the growth factors are platelet-derived growth factor (PDGF), vascular endothelial growth factor (VEGF), fibroblast growth factor-2 (FGF-2), and epidermal growth factor (EGF), which contribute to wound healing. Regulates the healing process (4-7).

Many studies tried to find materials which may increase these growth factors. Novel application of extracted gelatin is potential in the biomedical field (8). Gelatin is a modified form of collagen that is water soluble and commonly used to improve the functional characteristics of foods by increasing elasticity, consistency, and stability. In the fields of biotechnology and biomedicine, gelatin has a variety of uses (9,10). Currently, the primary sources of gelatin are derived from pig/ porcine skin, and cow/bovine hide and bones. However, pork-based gelatin might pose religious issues in some countries. In addition, fish skins from many fish species are another source that has been extensively studied as a source of gelatin production. (11). Therefore, there is growing interest in the production of fish gelatin as an alternative to mammalian gelatin.

Pangasius djambal is one of the fourteen pangasius species now recorded in Indonesia. This catfish species may be found in the main rivers of Java,

Sumatra, and Kalimantan islands. Furthermore, its whitish flesh is preferred over other fish, not only in Indonesia but also in other markets, in Asia, Europea, and North America markets which constitute potential export destinations (12). Protein content in gelatin derived from catfish is higher than that of in commercial gelatin. The high protein content in catfish gelatin is also in correlation with the large amount of amino acids in it (13). The catfish skin is considered environmentally unfriendly waste. Fish skin is solid waste produced by fish processing industries with no use or added value. In the previous study, the effect of P. djambal gelatin on the number of macrophages, fibroblast, epithelialization, and collagen deposition was examined. The present study aimed to evaluate the level of growth factor expression on dental socket healing following use of gelatin extracted from P. djambal.

P. djambal is one of 14 Pangasius species currently registered in Indonesia. This species of catfish is found in the main rivers of the islands of Java, Sumatra and Kalimantan. Moreover, its pale flesh is preferred over other fish not only in Indonesia, but also in other potential export markets in Asia, Europe and North America (12). The protein content of catfish gelatin is higher than commercial gelatin. The high protein content of catfish gelatin is also correlated with high amounts of amino acids (13). The catfish skin is considered environmentally unfriendly waste. Fish skin is solid waste produced by fish processing industries with no use or added value. The skin of the fish is a solid waste that is generated by the fish processing industry that has no use or added value. A previous study examined the effect of P.djambal gelatin on the number of macrophages, fibroblasts, epithelialization and collagen deposition. The purpose of this study was to evaluate the expression levels of growth factors on dental socket healing following use of gelatin extracted from P. djambal.

MATERIALS AND METHODS

This study used an experimental method with a randomized posttest-only control group design. The animal model involved in this study was Wistar strain (Rattus norvegicus) male albino rats. A total of 24 rats, each divided into 6 groups. Sample size was determined based on the resource equation approach. All experiments complied with the Research Ethics Committee approval (No.198/EC/KEPK-s1-FKG/08/2018).

Preparation of Pangasius djambal gelatin

The skin of P. djambal was cleaned from the remaining flesh and fat (degreasing), and stored at -20°C for 12 hours. After thawing at room temperature, thawed skins were first cut into small pieces (about 1 cm^2). The skins were further washed with lemon water to remove other foreign body from the skins, rinsed and soaked in a citric acid solution for 12 hours to break down the collagen fiber structure. Subsequently, the skin was repeated with water repeatedly until the skin reached the neutral pH (6 to 7), and then extracted with distilled water at 60°C. during the swinging bath. The extracted gelatin solution was filtered with Whatman #1 filter, and cooled to room temperature until the gelatin gel was formed.

Extraction wound model

Prior to extraction of the left lower incisor, all animal models were anesthetized with 0.18-0.2 ml of 1000 mg/10 ml ketamine intraperitoneally according to the weight of each animal. After tooth extraction, the sockets were cleaned with normal saline and dried. A total of 1 ml of P. djambal gelatin was applied to the extracted sockets of the rats in treatment groups, while the extracted sockets in control groups were left untreated. Evaluation as carried out on third, fifth, seventh days post extraction, according to their respective groups.

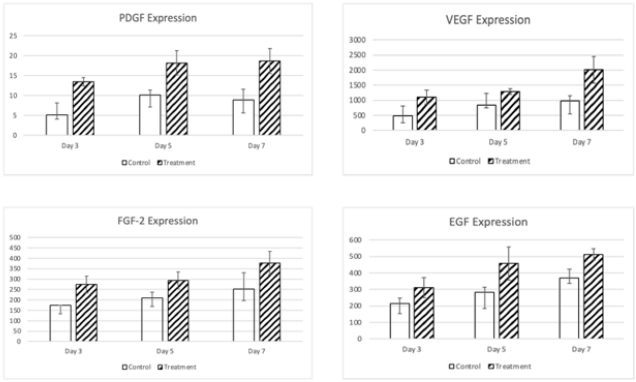
Sample preparation and immunohistochemistry (IHC) analysis

After three, five and seven days, euthanization of the rats using anesthetic overdose of ketamine injection (three times the anesthetic dose) was performed according to their respective groups. Breathing, heartbeat and blinking response were checked to confirm death. Subsequently, the rats were decapitated and the samples of mandibles containing the extraction site were immersed in 10% formalin for 18-24 hours and labeled prior to immunohistochemical staining. The stained specimens were analyzed by two observers under 400x magnification. The specimens were analyzed for (1) PDGF, VEGF, FGF-2 and EGF expression, and (2) cytoplasm and nuclear reactions in the mesenchymal components.

Statistical analysis

All data were analyzed using the personal computer version of SPSS software for Windows (SPSS/PC, Inc. version 22.0, Chicago, IL, USA). Normality was checked using the Shapiro-Wilk test (n=50) and homogeneity of variances was checked using the Levene's test, followed by one way ANOVA test to determine whether there are any statistically differences of growth factors expression between control and treatment group. $P < 0.001$ was considered statistically significant.

RESULTS



Growth Factors	Groups	Day 3	Day 5	Day 7	P Values
PDGF	Control	5.17 ± 2.93	10.17 ± 1.17	8.83 ± 2.79	0.001
	Treatment	13.5 ± 1.05	18.17 ± 3.06	18.67 ± 3.14	
VEGF	Control	486.5 ± 326.75	839.5 ± 389.50	985.75 ± 166.40	0.001
	Treatment	1104.5 ± 236.41	1289 ± 96.58	2008.25 ± 441.80	
FGF-2	Control	174.5 ± 53.88	209.75 ± 28.09	253 ± 77.50	0.001
	Treatment	274.25 ± 40.59	293.25 ± 41.41	377.25 ± 55.97	
EGF	Control	214.5 ± 31.47	283.25 ± 30.09	370.45 ± 52.16	0.001
	Treatment	310.35 ± 61.83	457.7 ± 100.40	512.4 ± 35.44	

Fig. 1 : Mean values of PDGF, VEGF, FGF-2 and EGF expression on days 3,5,7 in control and treatment groups.

Platelet-derived growth factor (PDGF) expression

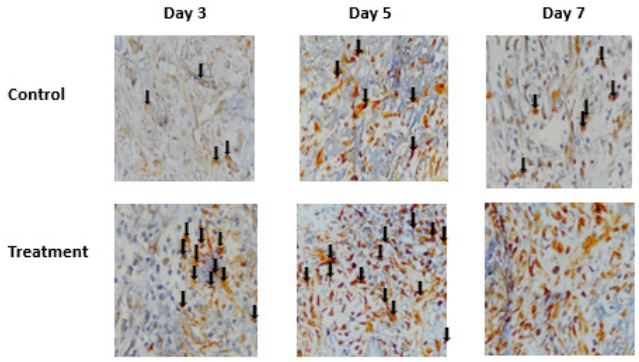


Fig. 2 : The results of histopathological examination of PDGF expressions on third, fifth, seventh days in control and treatment groups.

The highest level of PDGF expression occurred at seventh days in treatment groups, while the lowest occurred at third days in control groups (Fig.1). The expression of PDGF was higher in treatment groups than that of control groups (Fig.2). Compared to the control group, PDGF immunoexpression has been significantly increased statistically in the P. jambal gelatin treatment group ($p < 0.001$).

Vascular endothelial growth factor (VEGF)

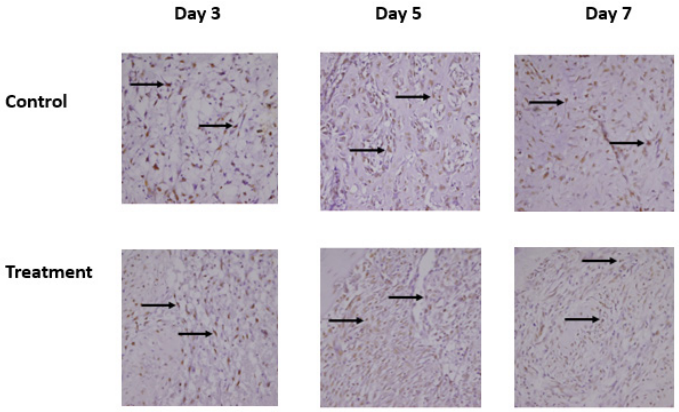


Fig. 3 : The results of histopathological examination of VEGF expressions on third, fifth, seventh days in control and treatment groups.

(Fig.1) shows that the mean expression of VEGF in treatment groups was higher than that of in control groups. The highest expression of VEGF occurred at seventh days in treatment groups. Data shown in (Fig.3) were in accordance with the result of microscopic immunohistochemical examination of VEGF expression in each group on on third, fifth, seventh days. There was a statistically significant increase in VEGF immunoexpression in the treatment group compared to the control group ($p<0.001$).

Fibroblast growth factor 2 (FGF-2) expression

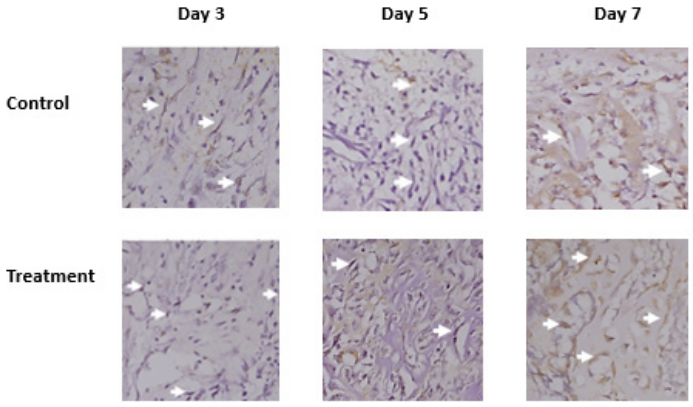


Fig. 4 : The results of histopathological examination of FGF-2 expressions on third, fifth, seventh days in control and treatment groups.

Regarding quantitative assessment of FGF-2 expression, the treatment groups showed higher expression than control groups (Fig.1). There was a statistically significant increase of FGF-2

immunoexpression in the treatment groups compared to the control group (p<0.001). (Fig. 5) shows that the highest expression of VEGF occurred at seventh days in treatment groups.

Epidermal growth factor (EGF) expression

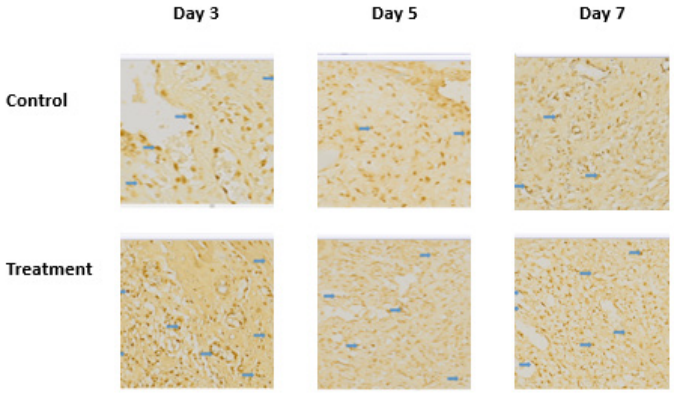


Fig. 5 : The results of histopathological examination of EGF expressions on on third, fifth, seventh days in control and treatment groups.

(Fig.1) shows that the mean values of EGF expressions on on third, fifth, seventh days was higher in the treatment group than in the control group. The highest expression of EGF occurred at seventh days in groups treated with P. djambal gelatin. The statistical test showed a significant difference in total EGF expression between control groups and the treatment groups (p<0.001). (Fig.5) shows that the results of histopathological examination of EGF expression on on third, fifth, seventh days in the groups treated with P. djambal gelatin were higher than that of the control groups. The highest expression of EGF occurred at seventh days in the treatment groups.

DISCUSSION

Wound healing processes after tooth extraction require growth factors to repair the damaged tissue. Growth factor is an endogenous signal transmission molecule that regulates cell response such as migration, proliferation, and differentiation, all of which are essential for wound healing (1). At the inflammatory stage, PDGF and VEGF are released by macrophage, which are necessary for the triggering and propagation of new tissue in the lesioned area, and also during the proliferative stage (14). Meanwhile, at the proliferative and remodeling stage, FGF-2 plays a role to induce proliferation of fibroblast in wound sites which then release collagen in collaboration with EGF. EGF stimulates fibroblast and keratinocyte proliferation and migration (4,6). All these growth factors act in concert during all stages of wound healing (14). In the present study, we evaluated the effect of P. djambal gelatin applied to the dental socket post extraction on the expression of several growth factors (PDGF, VEGF, FGF-2, and EGF) that play important roles in wound healing. P. djambal gelatin contains various amino acids. P. djambal derived gelatin affected collagen expression during the healing process of dental socket after tooth extraction. The group supplemented with P. djambal gelatin had higher quantitative collagen expression compared to the control group (15). Collagen can be completely degraded and absorbed by wounds, contributing to the formation of fibroblasts and collagen fibers (16).

PDGF promotes the differentiation of fibroblasts to myofibroblasts. During the proliferative phase, myofibroblasts are involved in collagen matrix synthesis and wound contraction (14). In this study, PDGF expression was significantly increased in the P. djambal gelatin-treated group compared with the control group (Fig. 1). Increased PDGF expression may be related to the binding of his PDGF receptor to amino acids contained in P. djambal gelatin. PDGF contains several amino acids such as Asn-115, Arg-154 and Ile-158, which are likely part of the PDGF active site (17, 18).

VEGF is produced by a variety of cells including fibroblasts, endothelial cells, platelets, neutrophils, macrophage and smooth muscle cells. Its role is very dominant in the formation of new blood vessels called angiogenesis (19). In our study, there was a statistically significant increase in VEGF expression in the treatment group compared with the control group (Fig. 1). This is probably due to the glycine content of P. djambal gelatin. Glycine is an amino acid involved in VEGF signaling. Increased VEGF expression within the present study is in line with another study that showed increased VEGF expression between third and seventh days after mandibular tooth extraction in a diabetic rat model. A significant increase occurred between 7 and 21 days. It indicates that VEGF plays a role in improving bone repair in the socket (20). Angiogenesis provides the necessary inflammatory cells, growth factors and progenitor cells for the inflammatory and proliferative stages of the wound healing process after tooth extraction. Alveolar bone regeneration and wound healing are directly dependent on the angiogenic process (21). In previous studies, P.djambal gelatin increased angiogenesis after tooth extraction in rats (22).

Cell proliferation in the wound site is essential for wound healing. FGF-2 can promote cell proliferation, reduce local inflammation, improve capillary angiogenesis and achieve great re-epithelialization (6,23). The present study showed a statistically

significant increase of FGF-2 expression in the P. djambal gelatin treated group compared to the control group (Fig.1). Increased FGF-2 expression may be related to arginine and glutamine content in P. djambal gelatin. Arginine has a role that is in line with FGF-2. Arginine plays a role in reducing inflammation and regulating cytokines at the wound site. Arginine can induce fibroblast cell proliferation and plays a role in regulating immune cell apoptosis during acute inflammatory stage (24,25). In addition, glutamine may also play a role in FGF-2 expression, which promotes wound healing by acting on various stages of wound healing such as collagen synthesis, wound contraction and epithelialization (26). In addition, glutamine may also play a role in FGF-2 expression in promoting wound healing by acting on different stages of wound healing such as wound contraction, collagen synthesis and epithelialization (26).

Glutamine plays a role in the wound healing process because its metabolic pathway can regulate epidermal growth factor receptor (EGFR) signaling (27). EGF affects keratinocyte migration, fibroblast function, and granulation tissue formation (4). Increased EGF expression in our study was observed on days 3, 5 and 7 in both control and treatment groups (Fig. 1), although there was a difference and statistically significant increase in EGF expression between control and treatment groups. EGF expression usually increased by day 8 and then decreased (28). The increased EGF expression in the treatment group is probably due to the glycine content of P.djambal gelatin. In addition, glycine receptors are expressed in gingival tissue, suggesting an immunomodulatory role for glycine in response to inflammatory conditions (29).

The role of P. djambal gelatin in wound healing had been demonstrated in our previous study. In comparison to the control groups, supplementation of P. djambal gelatin in treatment groups showed a beneficial effect on wound healing. The number of macrophages, fibroblasts, epithelialization, and collagen deposition were all shown to be significantly higher on histological examination (22). The content of various amino acids in P.djambal gelatin is considered to be strongly correlated with the expression of these growth factors. A limitation of this study is that different concentrations of his P.djambal gelatin were not tested. Further research is needed to establish a correlation between the values found for P.djambal gelatin and each of these growth factors in the wound healing process. In the medical and pharmaceutical industries, gelatin is used in hydrogels, nanomicrosphere containers, nanofibers, pharmaceutical excipients, and cell transplantation carriers (11,30).

Future studies need to investigate the application of P. djambal gelatin combined with other drugs or materials as a new approach in wound healing management, especially in oral and maxillofacial surgery.

CONCLUSION

In conclusion, the application of Pangasius djambal gelatin to dental sockets in white rats (Rattus norvegicus) following tooth extraction significantly increased the expression of PDGF, VEGF, FGF-2, and EGF .

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