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Original Article

COMBINED CHIP APPLICATION'S EFFECT OF CLINICAL PARAMETERS AND *TNF-α* EXPRESSIONS

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ABSTRACT

Objective: Scaling root planing is the main mechanical treatment to remove local deposits. Supportive local and systemic antimicrobial therapy is used to eliminate or reduce the number of pathogenic bacteria. The chip application of the combination of snakehead fish extract and betel leaf can change the clinical parameters for the better and increase the expression of *TNF-α* as a pro-inflammatory. To see the effect of the application of a chip combination of snakehead fish extract and betel leaf in male Wistar rats induced by periodontitis as a support for initial therapy on clinical parameters and expression of *TNF-α.*

Methods: Laboratory experimental study with a posttest control group design in which 24 male Wistar rats induced periodontitis were in four treatment groups (2.5%, 5%, 10% snakehead fish extract and placebo). Examination of clinical parameters and expression of *TNF-α* was carried out on day 0, day 3 and day 7. Data were analyzed by the Mann-Whitney test.

Results: The results showed that the clinical parameters; gingival index, pocket depth and attachment level looked better on the 7th day after treatment with statistically significant results ($p \leq 0.05$) and the highest anti-inflammatory *TNF-α* expression occurred at a concentration of 5%.

Conclusion: The application of a chip combination of snakehead fish extract and betel leaf as a support for initial therapy is effective in reducing the gingival index pocket depth and increasing the level of attachment, which is statistically significant, but there is no significant difference in *TNF-α* expression.

Keywords: Periodontitis, Periodontal chip, Clinical parameters, *TNF-α*

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INTRODUCTION

Periodontitis is a chronic inflammatory condition of the periodontium involving interactions between bacterial products, a number of cell populations and inflammatory mediators. Periodontitis affects approximately 50% of the adult population in the United States over the age of 30. The national prevalence of people with dental and oral problems in Indonesia reached 57.6%, according to Basic Health Research (RISKESDAS) in 2018. The definition of periodontitis is based on a number of clinical criteria, including inflammatory response, bleeding on probing, periodontal pocket depth and clinical attachment loss [1-3].

Bacteria that play a role in chronic periodontitis are gram-negative anaerobic bacteria (*Porphyromonas gingivalis, Tannerella forsythia, Treponema denticola, Aggregatibacter actinomycetemcomitans, Campylobacter rectus, Eikenella corrodens, Prevotella intermedia,* and *Prevotella nigrescens*) which are present in the subgingival plaque. *Porphyromonas gingivalis* influences the severity of periodontal disease and induces a periodontal inflammatory response. Mahalakshmi *et al.* examined 128 people with chronic periodontitis; they found that the most dominant bacteria in chronic periodontitis was *Porphyromonas gingivalis* with a prevalence of around 80.5% [4].

Cytokines are polypeptides produced in response to microbes and other antigens that modulate immune and inflammatory reactions. *Tumor Necrosis Factor* (TNF) is a major cytokine in the acute inflammatory response to gram-negative bacteria and other microbes. Severe infection can trigger the production of large amounts of TNF that cause systemic reactions. *TNF-α* as a proinflammatory cytokine and modulator of the immune system, has a major role in the pathogenesis of inflammation. *TNF-α* also functions in the immune response to bacterial, viral, fungal, and parasitic invasions [5, 6].

Non-surgical periodontal treatment through initial therapy includes plaque control, patient education, scaling and root planing, caries excavation and restoration, antimicrobial therapy (local and systemic), occlusal therapy, orthodontic movement and splinting. Clinically, after scaling and root planing, it has been shown to reduce pocket depth and increase attachment levels. In cases of tissue invasion by periodontal pathogens such as *P. gingivalis* or *A. actinomycetemcomitans*, mechanical therapy is sometimes not enough to remove bacteria from the pocket. Therefore, in addition to adequate mechanical treatment, antimicrobial adjunctive therapy is required because of the rapid progression of attachment loss and alveolar bone resorption [7].

Although mechanical elimination significantly reduces the level of microorganisms in the subgingival area, it does not completely eradicate the pathogen due to the complexity of the root anatomy. The limitations of conventional therapy in periodontal treatment can be overcome by using antibiotics and antiseptics as supporting therapy. Conventional therapy can control the development of periodontal disease more effectively. Local antimicrobials directly in the pocket can achieve 100 times the concentration of the same drug given orally. One of the products is a periochip containing chlorhexidine gluconate, which is inserted into the periodontal pocket. Chlorhexidine gluconate is an antimicrobial agent in which the in-situ drug concentration was found to remain above the minimum inhibitory concentration of more than 99% against periodontal pocket bacteria for 9 d [8-10].

Tissue damage due to injury will be followed by a healing process, which is a pathophysiological factor with the aim of restoring damaged tissue. Wound healing occurs through three phases, namely the inflammatory phase, the proliferative phase, and the remodeling phase. If the immune response and inflammatory reaction are unable to fight off the bacteria, the chronic inflammatory response develops into periodontal inflammation

(redness, swelling and bleeding) and periodontal breakdown (loss of clinical attachment) [1, 4, 11].

This study aims to see the effect of applying a combination of snakehead fish and betel leaf extract chip to wistar rats with induced periodontitis as a support for initial therapy of clinical parameter and *TNF-α* expression.

MATERIALS AND METHODS

The type of research used is laboratory experimental. The research sample used male Wistar rats obtained from the Biology Laboratory of the Faculty of Mathematics and Natural Sciences, Universitas Sumatera Utara (No. 0057/KEPH-FMIPA/2022). Any 24 rats sample age 2-3 mo with a weight of 200-300 grams with a healthy condition. The sample size used in the study was divided into four large groups, with eight treatments with 24 rats. Mice were adapted for 7 d before treatment. The rat model was injected with ketamine 20 mg/kg body weight, then incised in the anterior part of the lower jaw to make a bone defect using a fine carbide bur, then continued with the installation of a ligature to trigger plaque retention and inflammation. 7 d after applying the ligature, the rat model was induced with *P. gingivalis* bacteria with the aim of accelerating periodontitis. Periodontitis occurs characterized by the presence of redness, swelling of the gingiva, the presence of periodontal pockets and loss of attachment.

There are 4 major groups in this study, consist of: 1] Control placebo group; 2] Group 1: the group that was given a chip combination of 2.5% snakehead fish extract and 2% betel leaf extract; 3] Group 2: the group that was given a chip combination of 5% snakehead fish extract and 2% betel leaf extract; 4] Group 3: the group that was given a chip combination of 10% snakehead fish extract and 2% betel leaf extract.

Before chip application, scaling is done first on all mouse models. Examination of clinical parameters was performed after scaling. Dry the area around the pocket using a cotton roll, then apply the chip into the periodontal pocket using tweezers. Cover the pocket area with a periodontal pack to prevent the chip from escaping.

Each group was divided into 2 observation groups with different days, namely the 3rd and 7th days. After being treated, it was continued with segment retrieval after the rat model was killed by the neck dislocation technique. The mandibular segment was placed in a pot containing 10% formalin for approximately 24 h, then decalcified using 10% EDTA solution at 4 °C for 14-21 d. The filtered tissue is put into an automatic tissue processor. After the process of clearing and infiltrating liquid paraffin at a temperature of 57 °C-59 °C to form a paraffin block. Each paraffin block was sliced 3-4 µm thick using a microtom. After the mounting procedure is done so that the preparations become durable and clear. Tissue staining by IHC method. Then read the preparations using a microscope.

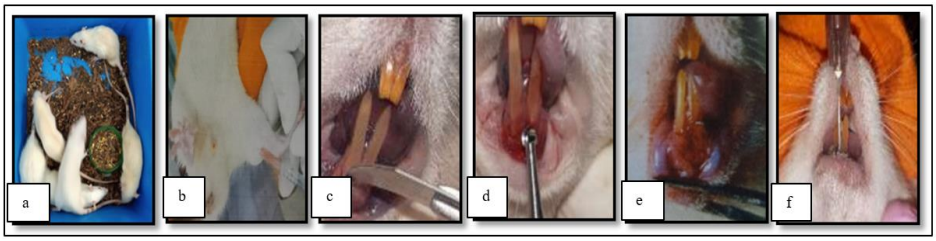

Fig. 1: Process of developing periodontitis, a. Adapted mouse, b. Injected with ketamine, c. Mandibular anterior incision, d. Bone defects, e. Installation of ligatures, f. Induced with *P. gingivalis*

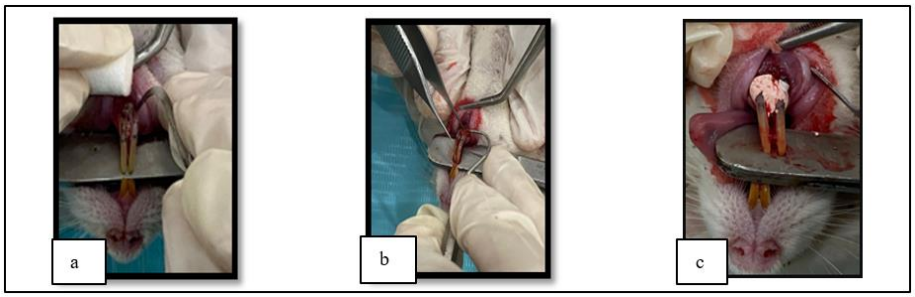

Fig. 2: Chip application procedure, a. Scaling sub and supra gingiva, b. Chip combination application at the base of the sulcus, c. Pack installation after combination chip application

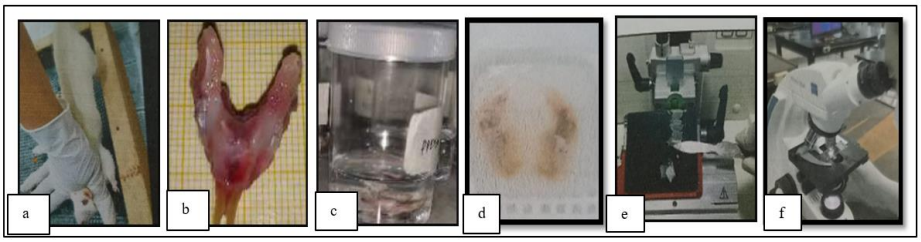

Fig. 3: a. Mouse euthanasia, b. Mandibular specimen, c. Immersion of the specimen in 10 % formalin, d. Paraffin blocks, e. Paraffin block sectioning using a microtom, f. Tissue reading using a microscope

This study was conducted to evaluate clinical parameters and expression of *TNF-α* after application of a chip combination of snakehead fish and

betel leaf extract with different concentrations (2.5%, 5%, 10%) in Wistar rats induced periodontitis as a support for initial therapy. Evaluation was carried out on the 3rd and 7th day. The samples used in this study were 24 male Wistar rats which had been induced by *P. gingivalis* bacteria. During the study, there were two rats that died, so that the remaining samples at the end of the study amounted to 22 rats. Evaluation of clinical parameters of differences in gingival index application of subgingival chip combination of snakehead fish and betel leaf extract in wistar rats induced periodontitis on days-0 and 3 is presented in full in table 1.

Table 1: Differences in gingival index on days 0 and 3

Group	Gingival index		*p-value*
	Day 0	Day 3	
Concentration combination chip 2.5%	1.0±0.00	0.8±0.45	0.013*
Concentration combination chip 5%	1.0±0.00	0.3±0.52	
Concentration combination chip 10%	0.83±0.41	0.3±0.52	
Placebo	0.4±0.55	0.4±0.55	

Data is given as mean±SD, *Significant (p≤0.05)

Table 1 shows that there was a statistically significant difference in the gingival index after subgingival chip application of the combination of snakehead fish and betel leaf extract in Wistar rats induced periodontitis as a support for initial therapy on day 0 and day 3 (p=0.013).

Table 2: Differences in gingival index on days 0 and 7

Group	Gingival index		p-value
	Day 0	Day 7	
Concentration combination chip 2.5%	1.0±0.00	0.3±0.58	0.002*
Concentration combination chip 5%	1.0±0.00	0.0±0.00	
Concentration combination chip 10%	0.83±0.41	0.0±0.00	
Placebo	0.4±0.55	1.0±0.00	

Data is given as mean±SD, *Significant (p≤0.05)

Table 2 shows that there was a significant difference in gingival index after subgingival chip application of a combination of snakehead fish and betel leaf extract in Wistar rats induced periodontitis as a support for initial therapy on days 0 and 7. (p=0.002).

Table 3: Differences in gingival index on days 3 and 7

Group	Gingival index		*p-value*
	Day 3	Day 7	
Concentration combination chip 2.5%	0.8±0.45	0.3±0.58	0.321
Concentration combination chip 5%	0.3±0.52	0.0±0.00	
Concentration combination chip 10%	0.3±0.52	0.0±0.00	
Placebo	0.4±0.55	1.0±0.00	

Data is given as mean±SD.

Table 3 shows that there was no statistically significant difference in the gingival index after subgingival chip application of a combination of snakehead fish and betel leaf extract in Wistar rats induced periodontitis as a support for initial therapy on days 3 and 7. (p≤0.05)

Differences in gingival index after subgingival chip application of a combination of snakehead fish extract and betel leaf in Wistar rats induced by periodontitis as a support for initial therapy on days 0.3 and 7 at each concentration can be seen in fig. 4.

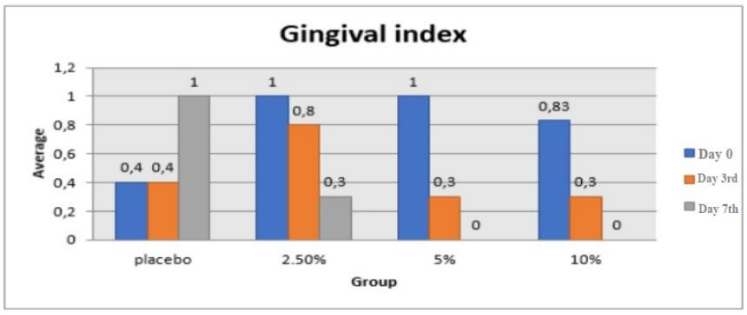

Fig. 4: Differences in gingival index on days 0, 3, and 7

Fig. 4 shows the gingival index after application of the subgingival chip combination of snakehead fish and betel leaf extract in Wistar rats induced by periodontitis as a support for initial therapy was seen to decrease on days 3 and 7 at concentrations of 5% and 10%, whereas in placebo it increased on day 7.

Evaluation of clinical parameters of differences in pocket depth after subgingival chip application of a combination of snakehead fish and betel leaf extract in wistar rats induced periodontitis on days-0, 3 and 7 is presented in the table 4, 5 and 6.

Table 4: Differences in pocket depth on day 0 and 3

Group	Pocket depth		p-value
	Day 0	Day 3	
Concentration combination chip 2.5%	2.9±0.31	2.9±0.13	0.398
Concentration combination chip 5%	2.6±0.52	2.4±0.35	
Concentration combination chip 10%	2.7±0.37	2.5±0.35	
Placebo	2.4±0.22	2.4±0.26	

Data is given as mean±SD.

Table 4 shows that there was no statistically significant difference in pocket depth after subgingival chip application of a combination of snakehead fish and betel leaf extract in Wistar rats induced by periodontitis as a support for initial therapy on day-0 and day-7. (p≤0.05).

Table 5: Differences in pocket depth on days 0 and 7

Group	Pocket depth		p-value
	Day 0	Day 7	
Concentration combination chip 2.5%	2.9±0.31	2.6±0.23	0.039*
Concentration combination chip 5%	2.6±0.52	1.9±0.52	
Concentration combination chip 10%	2.7±0.37	2.2±0.6	
Placebo	2.4±0.22	2.6±0.14	

Data is given as mean±SD, *Significant (p≤0.05)

Table 5 shows that there is a statistically significant difference in pocket depth after subgingival chip application of a combination of snakehead fish and betel leaf extract in Wistar rats induced periodontitis as a support for initial therapy on day-0 and day-7. (p=0.039).

Table 6: Differences in pocket depth on days 3 and 7

Group	Pocket depth		p-value
	Day 3	Day 7	
Concentration combination chip 2.5%	2.9±0.13	2.6±0.23	0.083
Concentration combination chip 5%	2.4±0.35	1.9±0.52	
Concentration combination chip 10%	2.5±0.35	2.2±0.6	
Placebo	2.4±0.26	2.6±0.14	

Data is given as mean±SD.

Table 6 shows that there was no significant difference in pocket depth after subgingival chip application of a combination of snakehead fish and betel leaf extract in Wistar rats induced periodontitis as a support for initial therapy on day-3 and day-7. (p≤0.05)

Differences in pocket depth after subgingival chip application of a combination of snakehead fish extract and betel leaves in Wistar rats induced by periodontitis as support for initial therapy on days 0, 3 and 7 at each concentration can be seen in fig. 5.

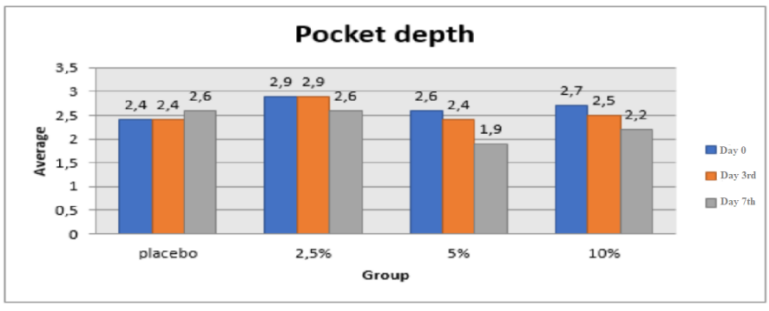

Fig. 5: Differences in pocket depth on days 0, 3 and 7

Fig. 5 shows the pocket depth after application of the subgingival chip combination of snakehead fish and betel leaf extract in Wistar rats induced by periodontitis as a support for initial therapy was seen to decrease on day 3 and 7 at concentrations of 5% and 10%, whereas in placebo it increased on day 7.

Evaluation of clinical parameters of differences in attachment levels after subgingival chip application of a combination of snakehead fish and betel leaf extract in Wistar rats induced periodontitis on days 0, 3 and 7 is presented in the following table 7, 8 and 9:

Table 7: Differences in attachment levels on days 0 and 3

Group	Attachment level		p-value
	Day 0	Day 3	
Concentration combination chip 2.5%	2.92±0.18	3.88±0.42	0.001*
Concentration combination chip 5%	2.98±0.33	3.52±0.41	
Concentration combination chip 10%	3.08±0.18	3.55±0.42	
Placebo	2.44±0.20	2.84±0.54	

Data is given as mean±SD, *Significant (p≤0.05)

Table 7 shows that there is a statistically significant difference in the level of attachment after subgingival chip application of a combination of snakehead fish and betel leaf extract in wistar rats induced by periodontitis as a support for initial therapy on day-0 and day-3. (p=0.001)

Table 8 shows that there was no statistically significant difference in the level of attachment after subgingival chip application of a combination of snakehead fish and betel leaf extract in Wistar rats induced by periodontitis as a support for initial therapy on day-0 and day-7. (p≤0.05).

Table 8: Differences in attachment levels on days 0 and 7

Group	Attachment level		*p-value*
	Day 0	Day 7	
Concentration combination chip 2.5%	2.92±0.18	3.73±0.06	0.512
Concentration combination chip 5%	2.98±0.33	3.00±0.30	
Concentration combination chip 10%	3.08±0.18	3.40±0.17	
Placebo	2.44±0.20	3.60±0.14	

Data is given as mean±SD.

Table 9: Differences in attachment levels on days 3 and 7

Group	Average attachment level		*p-value*
	Day 3	Day 7	
Concentration combination chip 2.5%	3.88±0.42	3.73±0.06	0.001*
Concentration combination chip 5%	3.52±0.41	3.00±0.30	
Concentration combination chip 10%	3.55±0.42	3.40±0.17	
Placebo	2.84±0.54	3.60±0.14	

Data is given as mean±SD, *Significant (p≤0.05)

Table 9 shows that there is a statistically significant difference in the level of attachment after subgingival chip application of a combination of snakehead fish and betel leaf extract in Wistar rats induced by periodontitis as a support for initial therapy on day-3 and day-7. (p=0.001)

Differences in the level of attachment after application of the subgingival chip combination of snakehead fish extract and betel leaf in wistar rats induced by periodontitis as a support for initial therapy on days 0.3 and 7 at each concentration can be seen in fig. 6.

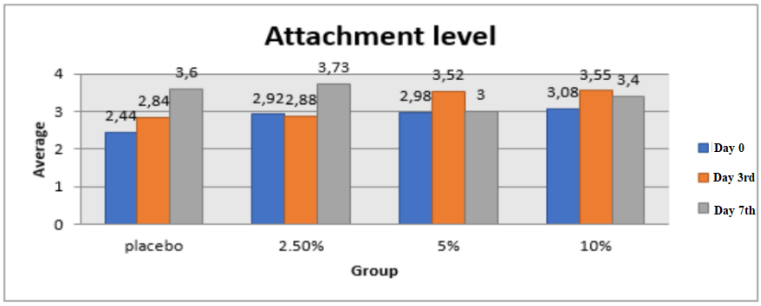

Fig. 6: Differences in attachment levels on days 0, 3 and 7

Fig. 6 shows the level of attachment after the application of the subgingival chip combination of snakehead fish and betel leaf extract in Wistar rats induced by periodontitis as a support for initial therapy was seen to increase on days 3 and 7 with a concentration of 5%, whereas in placebo it increased on day 7.

Differences in *TNF-α* expression levels after application of the subgingival chip combination of snakehead fish and betel leaf extract in Wistar rats induced periodontitis on days 3 and 7 are presented in the table 10.

Based on table 10, the results showed that there was no statistically significant difference in *TNF-α* expression after subgingival chip application of a combination of snakehead fish and betel leaf extract in Wistar rats induced periodontitis as a support for initial therapy in all treatment groups on day 3 and day 7. (p≤0.05)

*TNF-α e*xpression after subgingival chip application of a combination of snakehead fish extract and betel leaf as a support for initial therapy on days 3 and 7 can be seen in fig. 7.

Table 10: Differences in *TNF-α* expression on day 3 and day 7

Group	TNF-α		p-value
	Day 3	Day 7	
Concentration combination chip 2.5%	2.00±0.000	2.00±0.000	0.142
Concentration combination chip 5%	3.00±0.000	3.00±0.000	
Concentration combination chip 10%	2.67±0.577	2.67±0.577	
Placebo	2.00±1.000	2.50±0.707	

Data is given as mean±SD.

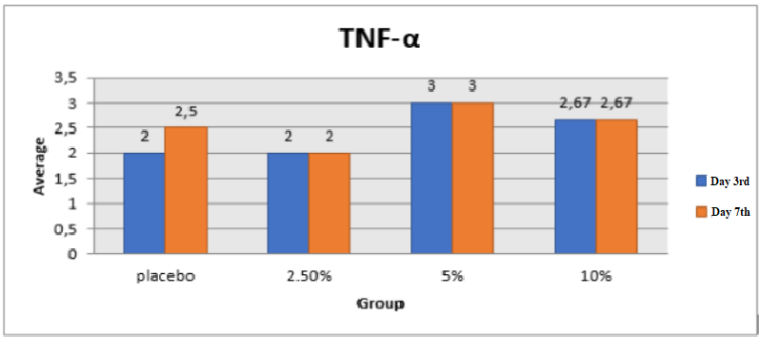

Fig. 7: *TNF-α* expression on day 3 and 7

The results of the study in the fig. 7 show that the expression of *TNF-α* after the application of the chip combination of snakehead fish and betel leaf extract at a concentration of 5% is the highest compared to other concentrations. This shows that the anti-inflammatory power of the chip combination of snakehead fish and betel leaf extract increases at a concentration of 5%.

Immunohistochemical examination taken on the chip combination of snakehead fish and betel leaf extract at a concentration of 10% on D-3, D-7 and placebo.

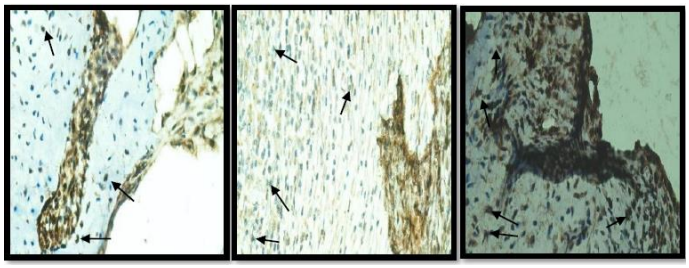

Fig. 8: Immunohistichemical fig. **of *TNF-α* expression in mandibular specimens after subgingival chip application of a combination of snakehead fish and betel leaf extract at a concentration of 10% on D-3, D-7 and placebo (Magnification 400x)**

Fig. 8 shows a change in *TNF-α* expression after the application of the chip combination of snakehead fish and betel leaf extract at a concentration of 10% on D-3, D-7 and placebo.

DISCUSSION

Cleaning of periodontal pathogens and their products with SRP is not sometimes optimal because there are parts that cannot be accessed by SRP tools, so systemic or local administration of antimicrobials is recommended to improve the results of SRP therapy.

The first clinical parameter examined in this study was the gingival index. The gingival index is an index of gingival health which was proposed in 1963 as a method to assess the severity and quantity of gingival inflammation in patients [12]. Based on the results of the study it was shown that there was a decrease in index gingival inflammation after application of the chip combination of snakehead fish and betel leaf extract in Wistar rats induced periodontitis on days 3 and 7, while it increased in the placebo group. In a study conducted by Tyaqi *et al.* stated that a chip containing pomegranate extract could reduce the gingival index on the 21st day [13].

The results showed that there was an increase in pocket depth in the placebo group, whereas in the Wistar rat group which applied the chip combination of snakehead fish and betel leaf extract to the subgingival there was a decrease in pocket depth on days 3 and 7. From the results of this study, it can be stated that the subgingival application of a combination of snakehead fish and betel leaf extract was effective in reducing periodontal pocket depth. In a study conducted by Tyaqi *et al.* (2021) stated that there was a decrease in pocket depth after being given pomegranate chips for 21 d compared to baseline [13].

Based on the results of the study, the level of attachment on days 3 and 7 after subgingival chip application of a combination of snakehead fish and betel leaf extract increased in Wistar rats induced by periodontitis and decreased in the placebo group. From the results of this study, it can be stated that the chip combination of snakehead fish and betel leaf extract can reduce the level of attachment. In a previous study conducted by Tyaqi *et al.* stated that after giving pomegranate chips it caused a decrease in the number of attachment levels on day 21 [13]. Öhm and Sanz suggested that subgingival cleaning, combined with proper control of supragingival plaque, is an effective treatment technique in reducing pocket depth and improving clinical adhesions [14]. The main objective of treating periodontal disease is to regenerate periodontal tissue lost due to the infection process, including alveolar bone, periodontal ligament and cementum [15]. Thus, the results of this study show that there

were improvements in all clinical parameters of the study, gingival index, pocket depth and attachment level after the application of the subgingival chip combined with snakehead fish and betel leaf extract. This is possibly due to the healing process that occurred after all samples received periodontal treatment.

The chip combination of snakehead fish and betel leaf extract can be an antimicrobial agent, so it can help SRP act to reduce inflammation caused by periodontitis [16]. The results of Achmad *et al.*'s research stated that snakehead fish extract (*Channa striata*) has an antibacterial effect on peri-pathogenic bacteria. Glycoproteins, peptides, albumin, minerals, zinc (Zn), and the amino acid arginine contained in snakehead fish extracts can inhibit the growth of pathogenic bacteria [17].

TNF-α is a pro-inflammatory cytokine that regulates the body's immune response and bone metabolism and is one of the dominant cytokines associated with periodontitis [18]. *TNF-α* levels will increase in patients with chronic periodontitis [19]. Based on the research of Gokul *et al.* and Noh *et al.,* which stated that as disease severity increases, *TNF-α* levels will also increase [6].

Based on the results of this study, it was shown that the expression of *TNF-α* after the application of the chip combination of snakehead fish and betel leaf extract at a concentration of 5% was higher than other concentrations. This shows that the anti-inflammatory power of the chip combination of snakehead fish and betel leaf extract increases at a concentration of 5%. From the results of this study, it was also seen that the application of the subgingival chip combination of snakehead fish and betel leaf extract concentrations of 5% and 10% reduced inflammation due to periodontitis faster than placebo, although there was no statistically significant difference ($p \leq 0.05$).

Local administration of antibacterial agents can be a useful adjunct to conventional mechanical therapy. In periodontitis cases, antibacterial agents applied locally into the subgingiva will be very beneficial for pocket depths of 5 mm or more, bleeding on probing, and unresponsive to primary therapy, including SRP. The ability of this material is probably caused by the active compounds contained therein. Snakehead fish extract contains albumin, Zn, Cu and Fe, which accelerate the healing process of inflammation and tissue regeneration. This can be seen from the results of a study conducted by Adam *et al.,* which stated that the application of snakehead fish extract significantly reduced the expression of *TNF-α* [20]. The results of the study by Isa *et al.* concluded that snakehead fish is an effective topical anti-inflammatory agent in a mouse model with chronic inflammation [21]. It is still largely unknown which bioactive compounds from snakehead fish are involved in the anti-inflammatory mechanism. Snakehead fish also has a high content of *docosahexaenoic acid* (DHA), which can contribute to anti-inflammatory action. DHA has been shown to suppress the production of several inflammatory mediators, including *TNF-α*, *IL-1β*, and *COX-2* [20].

Betel leaf is also efficacious as an anti-inflammatory. The anti-inflammatory effectiveness of betel leaves can be seen from the results of Seo *et al.*'s research that methanol extract from betel leaves inhibits *lipopolysaccharide* (LPS)-stimulating the production of nitric oxide and prostaglandin E2 by reducing the expression of inducible nitric oxide synthase and cyclooxygenase-2 in macrophages without affecting cell survival. Furthermore, the inhibitory effect of betel leaf on pro-inflammatory cytokines such as tumor necrosis *factor-α, interleukin-1β,* and *interleukin-6* is caused by nuclear inhibition. Hydroxychavicol, a major constituent of betel leaf, suppresses LPS-induced NF-κB p65 translocation from the cytoplasm to the nucleus [10].

The results of this study also found that the subgingival chip application of a combination of snakehead fish and betel leaf extract at 5% concentration was the most effective concentration than the 10% concentration as a support for initial therapy. This situation may be caused by arachidonic acid contained in snakehead fish. The presence of arachidonic acid in snakehead fish in high doses appears to contradict its anti-inflammatory properties. Arachidonic acid will produce prostanoids which in general, have an important function in inflammation. This reason is the reason why the chip combination of snakehead fish extract and 5% betel leaf is better at reducing inflammation.

CONCLUSION

The application of a chip combination of snakehead fish extract and betel leaf as a support for initial therapy is effective in reducing the gingival index, pocket depth and increasing the level of attachment, which is statistically significant, but there is no significant difference in *TNF-α e*xpression.

FUNDING

Nil

AUTHORS CONTRIBUTIONS

Jasmaniar-Conception, writing; Rini Oktavia Nasution-Revision of manuscript; Syafruddin Ilyas-Original draft preparation, data design, and performed the experiments; Armia Syahputra-Writing-original draft preparation, data design, and performed the experiments; Indra Nasution-Revision of Manuscript.

CONFLICT OF INTERESTS

Declared none

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