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Original Article

EFFICACY OF CHITOSAN NANOPARTICLES LOADED 0.7% TETRACYCLINE ON CLINICAL PARAMETERS AND PLATELET-DERIVED GROWTH FACTOR-BB IN RAT MODELS

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ABSTRACT

Objective: Tetracycline is a variety of antibiotic therapy for periodontal disease that has been chemically changed to serve as an antibacterial, anti-collagenase, and anti-inflammatory pharmaceutical, decreasing clinical indicators while increasing PDGF-BB healing factor. Chitosan nanoparticles can be used for drug delivery and have qualities such as delayed, sustainable, more absorption, and resilience drug reveal. The aim of the study is to observe the sub-gingival impact of chitosan nanoparticles loaded with 0.7% tetracycline on clinical parameters and PDGF-BB expression by immunohistochemistry observation.

Methods: *In vivo* experimental research with a before-after test control group design. 33 rats were enrolled initially, but only 31 completed the study, two samples perished when the observation was performed. The control group received 0.7% tetracycline once daily for 3 and 8 days, as well as placebo gel once daily for the same period. Clinical parameters and PDGF-BB expression were investigated on baseline, 3rd, and 8th day post-intervention with different trials.

Results: The research found that daily tetracycline delivery for 8 d improved clinical measures and raised PDGF-BB expression on 3rd day post-therapy, with significantly analyzed findings ($p<0.05$). In this study, PDGF-BB expression between all groups after d 3 and 8 wasn't significantly different ($p>0.05$). This might be because of the mild severity of periodontitis and shallow pockets in all rat periodontitis model samples.

Conclusion: Scaling by applying chitosan nanoparticles loaded with 0.7% tetracycline every day for 8 d improved clinical measures and enhanced PDGF-BB expression on 3rd day. However, PDGF-BB levels on day 8 did not reach statistical significance.

Keywords: Periodontal disease, tetracycline, chitosan nanoparticles, Platelet-derived growth factor

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INTRODUCTION

Periodontitis is a degenerative and severe problem that impacts tooth-bearing components such as the gingiva, periodontal ligament, cementum, and alveolar bone [1]. The clinical assessment in patients with periodontitis is characterized by the presence of bacterial plaque, supragingival and subgingival calculus, gingival swelling and inflammation and loss of gingival stippling, gingival margin changes, periodontal pocket formation, bleeding on probing, loss of attachment level, alveolar bone loss, root furcation involvement, increased tooth mobility, tooth drifting to edentulism [2]. The pathogenic of this disease is the interactivity of host defense mechanisms, dental bacterial biofilms, lifestyle, psychosocial factors, genetics, and proinflammatory mediators [3, 4].

Mechanical periodontal treatment attempts to eliminate subgingival plaque and calculus, minimizing pathogen growth and supragingival development of biofilm. Debridement is often performed with mechanical devices such as curettes and scalers. This standard technique, known as scaling and root planing (SRP), has been demonstrated to be the primary goal of periodontal treatment for most severe periodontitis [5]. SRP has several limitations, including difficulty accessing deep regions, convoluted pockets, furcation involvement, and struggle to eradicate pathogens from dentinal tubules, root furcations, and soft tissues. As a result, when combined with mechanical treatment, an antibiotic regimen can be quite effective [6].

The administration of antibiotics has a more beneficial effect because it can penetrate deep pockets with minimal doses, interact directly with infections, and reduce undesirable effects [7]. Subgingival administration of antibiotics might support SRP therapy. Despite no described norms, the primary indication is probing depth ≥ 5 mm. Tetracycline is a broad-spectrum bacteriostatic antibiotic that can stimulate the anti-collagenase effect to inhibit tissue damage and help regeneration [6, 8, 9].

In periodontal therapy, the delivery system used for tetracycline administration plays a critical role in maximizing efficacy. Traditional delivery methods, such as systemic oral antibiotics, may not effectively reach deeper periodontal pockets or achieve sufficient local concentrations at the site of infection [6]. Local drug delivery systems, such as hydrogels, microspheres, and nanoparticles, offer targeted and sustained release, allowing for more effective treatment [7]. Hydrogels, for example, can provide a moist environment conducive to healing, but they may suffer from issues like rapid degradation and limited control over drug release. Microspheres, while effective at controlling drug release, may not be as flexible in adapting to the irregular surfaces of periodontal pockets.

Chitosan has become a matrix that is beneficial to the spreading and constant delivery of drugs. Chitosan, as a gel-like base, is shown as a suitable system to promote local drug release through periodontal pockets [10]. Research by Susanto *et al.* regarding the antibacterial effect of chitosan-based tetracycline gel shows that 0.7% of tetracycline gel-based chitosan has strong antibacterial activity [11]. Andrew *et al.*'s research showed the nontoxic properties of 0.7% tetracycline in 4% chitosan gel against 3T3 fibroblast cells [12]. The same thing happened in Ervina *et al.*'s research, which showed improved clinical parameters and increased fibroblast tissue density in the sub-gingival appeal group of 0.7% chitosan hydrogel-based tetracycline [13]. El-Alfy *et al.* discovered a nanoparticle version of chitosan with more antibacterial capabilities than chitosan. Chitosan nanoparticles offer multiple benefits, including delayed and precise drug absorption qualities, improved drug absorption and rigidity, and less toxicity [14].

Chitosan nanoparticles, however, offer distinct advantages over these other systems. Due to their small size, high surface area, and biocompatibility, nanoparticles can achieve more precise and

controlled drug delivery to the targeted periodontal tissues. Additionally, chitosan nanoparticles have been shown to enhance drug absorption, extend release time, and improve stability, all while minimizing toxicity. Studies such as those by El-Alfy *et al.* (2015) have demonstrated that chitosan nanoparticles loaded with tetracycline have superior antibacterial properties compared to bulk chitosan, making them an ideal candidate for local periodontal therapy [14].

The 0.7% tetracycline concentration is particularly effective, as demonstrated by studies such as Susanto *et al.* (2017), which found that a 0.7% tetracycline-chitosan gel exhibited strong antibacterial activity against periodontal pathogens while also being biocompatible [10, 11]. This concentration is optimal for ensuring effective tissue penetration and sustained therapeutic levels without causing undue side effects.

Wound healing is a tissue repair process that involves the tissue's response to injury. This biological response begins as hemostasis, which involves inflammatory, proliferative, and remodeling phase responses. Biologically active molecules known as growth factors control and regulate each stage. Platelet-derived Growth Factor (PDGF) is chemotactic for osteoblasts and fibroblasts, thereby increasing coronal migration from periodontal ligament cells [15]. PDGF-BB is a subtype of PDGF that has a primary role in early wound recovery by attracting macrophage, mesenchymal, fibroblast, and osteoblast cells to approach the wound area [16]. According to research by Mumford *et al.*, PDGF has proven to be an essential regulator in periodontal wound repair and an activator of cell expansion and motility. The PDGF's effect on cellular activity in the periodontal ligament demonstrates a more intense proliferation reaction to PDGF-BB [17].

MATERIALS AND METHODS

This research is an *in vivo* experiment with a before-after test and a control group design for clinical and immunohistochemistry parameters. The Health Research Ethics Committee, Faculty of Mathematics and Natural Sciences, Universitas Sumatera Utara (No.0249/KEPH-FMIPA/2021) provided ethical approval for animal behavior studies, as did the Health Research Ethics Committee of the Faculty of Medicine, Universitas Sumatera Utara (No: 402/KEP/USU/2021) for PDGF-BB expression assessment. All procedures conformed to ARRIVE guidelines and humane endpoints; analgesia was provided via intraperitoneal injection of ketamine (20 mg/kg body weight (BW)) and xylazine (5 mg/kg BW) to minimize discomfort and ensure humane treatment during all surgical and experimental procedures.

This research began with steps to develop a rat periodontitis model that included 33 healthy wistar rats between 2-3 mo, male with weight 200-250 g. The rats were first adapted for 10 d and kept in cages placed in a room with sufficient air and light circulation. During the adaptation period, the animals were monitored daily including food and drink intake, urination, weight gain or loss as well as general animal behavior to evaluate the stress level of the animals. The cages were cleaned daily from feces and food residue to keep them dry. Animals were fed and watered three times a day during the trial period.

The technique starts with analgesia via intraperitoneal injection of ketamine 20 mg/kg Body Weight (BW) and xylazine 5 mg/kg BW to achieve drowsiness. Sulcular incisions were performed in the vestibular regions of both left and right mandibular incisors. A full-thickness flap reveals the alveolar bone in the vestibular area. Applying a carbide bur creates an alveolar bone defect in the vestibular region by lowering the height of the alveolar bone by 1.5 mm and its breadth by 3 mm. Then, the flap is fixed to its normal place, and the ligature wire is attached around the cervical section of the teeth with interrupted suture method. The samples were subsequently fed a high-carbohydrate diet. The *Porphyromonas gingivalis* ATCC® 33277™ is induced in the gingival sulcus. On the 3rdday of initiation, a swab was performed from the gingival sulcus region for biological cultivation, revealing samples were infected with *Porphyromonas gingivalis* ATCC® 33277™. Periodontitis, represented by redness, gingival swelling, periodontal pockets, and gingival attachment loss, emerged on the sixth day. The high carbohydrate diet was discontinued after the samples were proven to be infected with *Porphyromonas gingivalis* ATCC® 33277™ and could receive the intervention. Samples induced by *Porphyromonas gingivalis* ATCC® 33277™ were then divided into seven groups: four treatment groups, two positive control groups, and one negative control group.

Table 1: Treatment and sample size study group

Group	Treatment	Sample size
KA1	Scaling accompanied by applying subgingival nanoparticles of chitosan loaded tetracycline 0.7% (every day) then the parameters are checked clinical and PDGF-BB on 3rd day (rats have been executed).	5
KA2	Scaling accompanied by applying subgingival nanoparticles of chitosan loaded tetracycline 0.7% (every day) then the parameters are checked clinical and PDGF-BB on 8th day (rats have been executed).	5
KB1	Scaling accompanied by applying subgingival nanoparticles of chitosan loaded tetracycline 0.7% (once only), and then the examination was carried out parameter clinical and PDGF-BB on the 3rd d (rats have been executed)	5
KB2	Scaling accompanied by applying subgingival nanoparticles of chitosan loaded tetracycline 0.7% (once only), and then the examination was carried out parameter clinical and PDGF-BB on the 8th d (rats have been executed)	5
K+1	Scaling and utilization of placebo gel once a day, then clinical indicators and PDGF-BB were examined on 3rd d (rats have been executed)	4
K+2	Scaling and utilization of placebo gel once a day, then clinical indicators and PDGF-BB were examined on 8th d	4
K-	No treatment, PDGF-BB parameters were observed at baseline (rats have been executed)	4

PDGF= Platelet-derived Growth Factor

Chitosan nanoparticles have been produced by combining chitosan and sodium tripolyphosphate (0.005 mg) with ratio 4:1 in an ionic gelation at ambient temperature. Chitosan (2 g) with molecular weight 250 kDa and 70% degree of deacetylation was added in 1% acetic acid (50 ml) and agitated for one day on a magnetic stirrer at 4 Mot speed at room temperature. The pH was corrected to 5.5 by adding 0.01 N NaOH (15 ml) to the chitosan mixture. 0.005 mg Tripolyphosphate was dispersed independently in deionized water (0.5 ml) and then introduced by drops to the chitosan solution while agitating at room temperature. For 1 min, the combination was honed with a homogenizer set at 1000 rpm. The particle size analyzer (PSA) test was performed to determine the dimension variation of the gel molecules, resulted 0.02132 µm in particle diameter [18]. The chitosan nanoparticle gel was treated with 0.7% tetracycline dissolved in water 0.35 g (based on the composition of 0.7% tetracycline gel based on chitosan hydrogel). Mixing for another 20 min yielded the last combination of chitosan nanoparticles loaded with 0.7% tetracycline [19].

All specimens underwent supragingival and subgingival scaling using an electric scaler. A 1 cc syringe was used to inject chitosan nanoparticles with 0.7% tetracycline and placebo gel. The intervention teeth were outlying first, and the bevel of the syringe needle was placed into the prepared pocket without force to minimize tissue harm. The gel is pumped into the pocket until surplus gel emerges and pools around the tooth's neck (fig 1A and B). The rat is then monitored and given no food or water for an hour. [20]. Clinical parameters were examined using a Kohler probe (fig 1C).

Samples prep begins with elimination by the neck detachment procedure. The mandible of the rat was then severed and put in a tube with 10% formalin. The tissue samples were decalcified in 10% ethylenediaminetetraacetic acid (EDTA) at normal temperature for 3-14 d, histologically examined, and placed into paraffin blocks. Each paraffin block was recut once for PDGF-BB spotting. Paraffin block samples sliced lightly (4 μm) are adhered to the glass slide. Place thin strips in warm water to separate and flatten them. Once it has enlarged, move it to the object glass. The glass is then heated on a hotplate at 50-60^{0}C. Counterstain with Mayer-Hematoxylin for 1 min. Wash in running water, dip in distilled water, drop intellan, and cover with a cover slip. Immunohistochemistry observations of PDGF-BB expression were carried out under a binocular microscope with a magnification of 400 times connected to the software.

The data obtained include BoP clinical indicators pre-post intervention in each group, PD clinical indicators pre-post intervention in each group, and PDGF-BB expression pre-post intervention in each group. Qualitative data analysis was tested using the Fisher's Exact test. Normality and homogeneity tests were performed to see that the data was normal and homogeneous distributed using the Shapiro-Wilk Then, the Wilcoxon test was used to observe the difference in means pre-post treatment. The mean PDGF-BB was analyzed using the Kruskal-Wallis and Mann-Whitney tests were conducted because the data were not normally distributed and homogeneous.

Fig. 1: (A). The application of chitosan nanoparticle gel loaded 0.7% tetracycline. (B). Application of placebo gel. (C). Examination of clinical parameters

RESULTS

Two samples perished when the observation was performed. Until the end of the investigation, the remaining samples totaled 31 mice.

Clinical parameter values post subgingival application of chitosan nanoparticles loaded 0.7% tetracycline

Data differences in BoP test and PD measurement before and after the subgingival addition of chitosan nanoparticles loaded 0.7% tetracycline in periodontitis samples on the 3rd and 8th d are presented in table 2-5.

Table 2 shows a lower percentage of BoP value after 3 d of treatment in all groups. The percentage decrease in BoP test was higher in the group adding chitosan nanoparticles containing 0.7% tetracycline every day; statistical tests showed no significance in each group ($p>0.05$).

According to table 4, it can be seen that there was a decrease in PD after 3 d of treatment in all groups and was statistically significant ($p<0.05$).

According to table 5, PD was reduced after each group's 8th d of treatment. The lowering in PD was significant statistically only in the given scaling treatment group with daily application of 0.7% tetracycline ($p<0.05$).

Table 2: Percentage differences of bleeding on probing (BoP) test pre-post 3 d of treatment

Bleeding on probing (BoP) test			
Group	**Pre-treatment (%)**	**Post-treatment (%)**	**p-value**
KA1: Scaling and chitosan nanoparticles 0.7% tetracycline daily (n = 5)	100	60	0.444
KB1: Scaling and chitosan nanoparticles 0.7% tetracycline once only (n = 5)	100	80	1
K+1: Scaling and placebo (n = 4)	100	80	1,000
* Fisher's Exact test, significance p<0.05			
BoP= Bleeding on Probing			

Table 3 shows a decrease in the BoP percentage after 8 d of treatment in every group. The decrease in BoP was statistically significant only in given scaling treatment with daily application of 0.7% tetracycline chitosan nanoparticles group ($p<0.05$).

Table 3: Percentage differences of bleeding on probing (BoP) test pre-post 8 d of treatment

Bleeding on probing (BoP) test			
Group	**Pre-treatment (%)**	**Post-treatment (%)**	**p-value**
KA2: Scaling and chitosan nanoparticles 0.7% tetracycline daily (n = 5)	100	0	0.008 *
KB2: Scaling and chitosan nanoparticles 0.7% tetracycline once only (n = 5)	100	25	0.143
K+2: Scaling and placebo (n = 4)	100	50	0.429
*Fisher's Exact test, significance p<0.05, BoP= Bleeding on Probing			

Table 4: Difference in average pocket depth (PD) measurement pre-post 3 d of treatment

Pocket depth (PD) measurement (mm)			
Treatment group	**Mean PD (mm±SD)**		
	Pre-treatment	**Post-treatment**	**p-value**
KA1: Scaling and chitosan nanoparticles 0.7% tetracycline daily (n = 5)	1.60±0.89	0.60±0.89	0.025*
KB1: Scaling and chitosan nanoparticles 0.7% tetracycline once only (n = 5)	1.40±0.55	0.60±0.55	0.046*
K+1: Scaling and placebo (n = 4)	1.60±0.89	0.80±0.84	0.046*
*Wilcoxon test, significance p<0.05, PD= Pocket Depth; SD= Standart Deviation			

Table 5: Difference in mean pocket depth (PD) measurement pre-post 8 d of treatment

Pocket depth (PD) measurement (mm)			
Treatment group	**Mean PD (mm±SD)**		
	Pre-treatment	**Post-treatment**	**p-value**
KA2: Scaling and chitosan nanoparticles 0.7% tetracycline daily (n = 5)	1.60 ±0.89	0.20 ±0.45	0.038*
KB2: Scaling and chitosan nanoparticles 0.7% tetracycline once only (n = 5)	1.75 ±0.96	0.25 ±0.50	0.063
K+2: Scaling and placebo (n = 4)	1.50 ±0.57	0.25 ±0.50	0.059
*Wilcoxon test, significance p<0.05, PD= Pocket Depth; SD= Standart Deviation			

Differences in PDGF-BB expression after 3 and 8 D treatment

The difference in mean PDGF-BB expression pre-post intervention of chitosan nanoparticles loaded 0.7% tetracycline in the periodontium of the rat periodontitis samples after the 3rd 8th d is presented in table 6-8.

From fig. 2, it can be seen that there was an increase in PDGF-BB expression on the 3rd d for all groups and a reduction in PDGF-BB expression on the 8th d for all groups. A higher increase in PDGF-BB expression was shown in the group with the intervention of 0.75% tetracycline chitosan nanoparticles every day on d 3, and the decrease in PDGF-BB expression was seen to be lower in the intervention of chitosan nanoparticles loaded 0.7% tetracycline every day and only once on 8th d.

Table 6 shows that the difference in mean PDGF-BB expression is statistically significant between all groups on d 3 after treatment (p<0.05).

According to table 7, it was seen that the comparison of PDGF-BB expression was not significant between groups (p>0.05). The results of the Mann-Wintney test stated that the lowest p-value between the group that applied 0.7% tetracycline chitosan nanoparticles every day and the group that applied chitosan nanoparticles loaded 0.7% tetracycline only once and the placebo application group (p = 0.053).

According to table 8, the difference in mean PDGF-BB expression across groups on d 8th of treatment was not statistically significant (p>0.05). The lowest mean PDGF-BB expression was seen in the both chitosan nanoparticle application group, which applied 0.7% tetracycline daily and once only.

Table 6: Differences in PDGF-BB expression on 3 d of treatment

PDGF-BB expression measurement		
Treatment group	**PDGF BB expression ($\bar{x}$ ±SD)**	**p-value**
KA1: Scaling and chitosan nanoparticles 0.7% tetracycline daily (n = 5)	5.00 ±1.00	0.031 *
KB1: Scaling and chitosan nanoparticles 0.7% tetracycline once only (n = 5)	4.00 ±0.00	
K+1: Scaling and placebo (n = 4)	4.00 ±0.00	
*Kruskal-Wallis test, significance p<0.05, PDGF= Platelet-derived Growth Factor; SD= Standart Deviation		

Table 7: Comparison of PDGF-BB expression between groups on 3 d of treatment

Comparison of PDGF-BB expression result		
Treatment group		**p-value**
KA1: Scaling and chitosan nanoparticles 0.7% tetracycline daily (n = 5)	*Scaling*+0.7% tetracycline chitosan nanoparticles once only (n = 5)	0.053
	Scaling+placebo (n = 5)	0.053
KB1: Scaling and chitosan nanoparticles 0.7% tetracycline once only (n = 5)	*Scaling*+placebo (n =5)	.000
*Post Hoc Mann-Whitney test, significance p<0.05, PDGF= Platelet-derived Growth Factor		

Table 8: Differences in PDGF-BB expression on 8 d of treatment

PDGF-BB expression measurement		
Treatment group	**PDGF-BB expression ($\bar{x}$ ±SD)**	**p-value**
KA2: Scaling and chitosan nanoparticles 0.7% tetracycline daily (n = 5)	2.00 ±0.00	0.325
KB2: Scaling and chitosan nanoparticles 0.7% tetracycline once only (n = 5)	2.00 ±0.00	
K+2: Scaling and placebo (n = 4)	2.25 ±0.50	
*Kruskal-Wallis test, significance p<0.05, PDGF= Platelet-derived Growth Factor; SD= Standart Deviation		

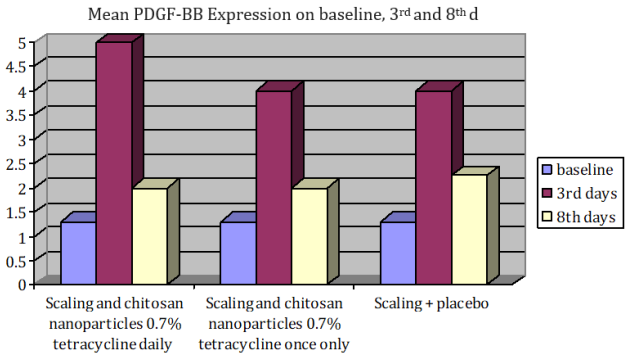

Fig. 2: Mean PDGF-BB expression in each group on baseline, 3rd, and 8th d

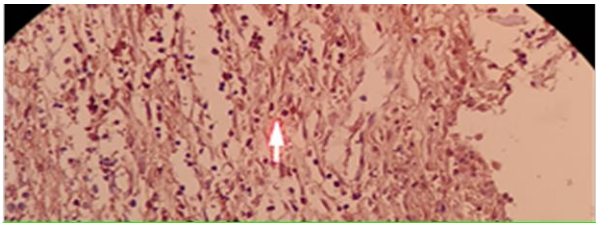

Fig. 3: PDGF-BB expression is stronger in images when chitosan nanoparticles are added to the daily load of 0.7% tetracycline. The arrow shows PDGF-BB cells where brown indicates the cytoplasm and purple indicates the nucleus

DISCUSSION

Tetracycline is an antibiotic that is effective against periodontal pathogenic bacteria. This antibiotic is absorbed by the tooth root's surface and released slowly in active form. Tetracycline produces potential actions to promote periodontal healing and tissue regeneration [8]. Chitosan-loaded tetracycline's effectiveness against periodontal pathogenic bacteria has been widely studied. The current development of nanotechnology, making chitosan nano-sized, aims to make chitosan's absorption more effective by expanding the surface of the chitosan [12]. *A. actinomycete mcomitans* and *P. gingival is* may penetrate and damage sub epithelial connective tissue. Therefore, mechanical debridement cannot totally eradicate them [21]. Subsequently, delivering localized antibiotic treatment is advantageous since it can reach the deeper site of the pocket.

Effect of sub-gingival application of chitosan nanoparticles loaded 0.7% tetracycline on clinical parameters

This research showed that *scaling* accompanied by adding chitosan nanoparticles loaded with 0.7% tetracycline each day for 8 d showed a statistically significant reduced percentage of bleeding on probing and pocket depth ($p<0.05$). The group was given chitosan nanoparticle gel loaded with 0.7% tetracycline only once, and the control group did not show statistically significant differences on d 8th. Sinha *et al.* examined the impact of tetracycline HCL on the therapy of severe periodontitis with a probing depth ≥ 5 mm. Also, they showed a statistically significant lowering in pocket depth and an increase in clinical attachment in the SRP group with tetracycline over the control group [7]. Babrawala *et al.*, in a study, used 1% natural chitosan gel as a transport agent as an intervention to SRP in unsurgical treatment. Chitosan restricts the proliferation of bacteria, has anti-inflammatory effects, and accelerates wound recovery. The examination of 10 patients showed that chitosan mixed with local antibiotics could significantly decrease pocket depth, gingival index, and bleeding index [22].

Clinical findings showed a decrease in BoP on d 3, but it wasn't significantly different ($p>0.05$). This may be because the scaling and tetracycline effects have not worked optimally so that on the 8th d, we can see a significant decrease in BoP. Giving tetracycline only once does not show any significant decrease in BoP, and this may be because tetracycline does not remain long in the periodontal pocket. Natalina *et al.* observed the impact of a single irrigation of 10% tetracycline HCL solution after SRP on changes in clinical parameters of chronic periodontitis with pockets of 4-6 mm where the BoP between the experimental side and the control side was also not significantly different [23]. This may be because the tetracycline irrigation is immediately removed from the pocket by gingival crevicular fluid.

The goal of initial phase therapy is to stop the progression of the disease. SRP aims to remove bacteria and biofilm microorganisms from the tooth surface. Subgingival antimicrobial administration effectively reduces pocket depth and attachment loss over SRP alone [6]. As Goodson reported, tetracycline is the most widely used local antimicrobial in periodontal treatment [24, 25]. The giving of chitosan nanoparticles loaded with 0.7% tetracycline after SRP is expected to be a treatment option for periodontitis sufferers with deep pockets and difficult accessibility.

Effect of subgingival application of chitosan nanoparticles loaded 0.7% tetracycline on PDGF-BB expression indicators

An improvement in PDGF-BB expression was seen on 3rd d post-intervention in all groups. Still, it was seen to be higher in the scaling group with daily application of 0.7% tetracycline and a significant difference ($p<0.05$) between every group. PDGF-BB expression decreased on the 8th d in all groups but wasn't significant between all groups ($p>0.05$). The samples used on d 0, 3, and 8 were from different mice. This happens because on the 3rd d after treatment (9th d after periodontitis induction), the peak period of the proliferative phase of wound healing enters so that by administering 0.7% tetracycline chitosan every day, there is an increase in PDGF-BB expression because it is needed for wound healing, then decreased the following day on the 8th d after treatment (14th d after periodontitis induction) because the proliferation phase here had begun to end so that PDGF-BB no longer played a role at the end of this phase. The subsequent decline in PDGF-BB expression observed on d 8 (14 d after periodontitis induction) may be attributed to the transition from the proliferative phase to the maturation or remodeling phase of healing. During this stage, cellular activity decreases, and the need for growth factors like PDGF-BB diminishes as tissue repair stabilizes and collagen remodeling begins. Histological evidence from previous studies supports this phase-specific expression pattern. For instance, Alzahrani (2017) reported that PDGF-BB and VEGF levels in gingival crevicular fluid were significantly increased during the early healing stages (d 1 to 14) following periodontal flap surgery but began to decline thereafter as healing progressed into the remodeling phase [25]. Research by Joshi *et al.* also showed that PDGF-BB levels in gingival crevicular fluid on the 3rd and 7th d after surgery increased between the groups treated with PRF membrane and collagen, although not significant, the PDGF-BB decreased significantly on the 2nd, 14th and 30th d between both groups [26].

The platelet-derived growth factor is an influential mitogenic and chemical part of mesenchymal cells, especially periodontal ligament cells and osteoblasts. PDGF-BB was discovered to be much more efficient than other isoforms, such as PDGF-AA and PDGF-AB, in increasing periodontal cell proliferation. The PDGF-BB phenotype was beneficial in boosting fibroblast proliferation and adhesion [25, 26]. This proliferation phase takes place from d 2-21 after injury. In this phase, macrophages will stimulate fibroblasts to produce growth factors. Fibroblasts comprise vital units in wound repair, such as eliminating fibrin clots, generating new extracellular matrix, and producing collagen structures to support other cells involved in wound healing and closure. The fibroblasts involved explicitly in wound contraction are myofibroblasts. Various factors influence the differentiation of fibroblasts into myofibroblasts, the two factors that play the most roles are TGF-β1 and PDGF [27]. In the healing phase diagram, the peak increase in PDGF-BB can be seen on the 8th d and decreases on the following day.

Tetracycline may aid tissue repair or regeneration by increasing fibroblast function and root surface conditioning by blocking collagenase action. Among the antibiotics used for localized use, tetracycline stands out due to its superior resorption, capability to attach to proteins, dispersion into tissue structures, and prolonged implementation length [28]. Pradnyani's research stated that the administration of 0.7% tetracycline gel could increase the number of fibroblast cells in the periodontal ligament of rats experiencing periodontitis [8].

Several studies have shown the efficacy of chitosan against bacteria. The effect of chitosan was investigated *in vitro*, and an antimicrobial reaction was observed at lower concentrations. In addition, chitosan can selectively penetrate pathogenic bacteria into biofilms. Chitosan

is vital in the early stages of wound healing because it promotes polymorphonuclear cell (PMN) infiltration, which leads to fibroblast collagen synthesis. Fibroblast stimulation promotes the production of IL-8, which is critical for chemotaxis and angiogenesis. Chitosan also functions in macrophage activation, cytokine generation, giant cell migration, and stimulation of collagen formation. Silva *et al.* concluded that chitosan is tolerable by gingival fibroblasts and that a synergistic response with several platelet-derived gowth factors.

(PDGF is one among numerous growth factors that supervise the expansion and division of cells and serve a substantial part in angiogenesis) can stimulate cell proliferation in gingival fibroblasts [29].

In this study, PDGF-BB expression between every group after d 3 and 8 wasn't significantly different ($p>0.05$). This may be because of the severity of periodontitis and shallow pockets in all rat periodontitis model samples. The discussion shows that daily use of chitosan nanoparticles loaded with 0.7% tetracycline shows greater effectiveness than a single application and placebo. Periodontal dressings may be used to retain tetracycline longer in the periodontal pocket if tetracycline 0.7% is to be administered only once.

Although the findings are promising, this study has several limitations that need to be acknowledged to provide context for the results. The statistical power to identify more nuanced differences between groups may have been constrained by the relatively small sample size, especially on the 8th d when variations in PDGF-BB expression did not reach statistical significance. Future research could use a larger sample to validate and reinforce these findings. Moreover, employing various mice for each time point brings about possible variability. The severity of periodontal lesions may vary based on individual reactions to ligature placement. Although ligature-induced periodontitis is commonly utilized in preclinical models, it can lead to variations in lesion depth and inflammatory response, potentially affecting the levels of biomarker expression. It is crucial to recognize these factors for a careful interpretation of the data and to guide the design of more controlled longitudinal studies moving forward.

CONCLUSION

Based on research results, giving chitosan nanoparticles loaded with 0.7% tetracycline every day as a support for initial therapy can reduce the percentage of BoP, which is significantly different ($p<0.05$), and once it isn't significantly different ($p>0.05$). Giving chitosan nanoparticles loaded with 0.7% tetracycline every day and only once as a support for initial therapy can significantly reduce the average PD ($p<0.05$)**.** Giving chitosan nanoparticles loaded with 0.7% tetracycline daily and once as an addition for initial therapy can increase PDGF-BB expression on the 3rd d and decrease on the 8thd. However, PDGF-BB levels on d 8 did not reach statistical significance.

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AUTHORS CONTRIBUTIONS

Irma Ervina-Conception, writing original draft preparation and revision manuscript, data design and analysis, performed the experiments; Martina Amalia-Supervision and visualization, revision of manuscript; Erdi Effendi Nasution-Revision manuscript, data design and performed the experiments; Harry Agusnar-Supervision and visualization, revision of manuscript; Denny Satria-Visualization and revision of manuscript.

CONFLICT OF INTERESTS

The authors state no conflicts of interest in publishing this research.

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