



## Effectiveness of Giving Chitosan on Interleukin-6 and Mallondhyaldehyde Levels in Wistar Rats with Chronic Periodontitis

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### Abstrak

Penyakit terbanyak ke-6 yang terjadi di masyarakat Indonesia antara lain karies gigi dan periodontitis yang disebabkan adanya plak gigi yang merupakan tempat berkembang biaknya bakteri seperti *Streptococcus mutans* dan *Phorphyromonas gingivalis*. Tujuan penelitian adalah untuk mengetahui efektivitas kitosan terhadap kadar Interleukin 6 dan Mallondhyaldehyde (MDA), sebagai penanda anti inflamasi menggunakan uji Elisa. Metode penelitian adalah true eksperimental randomized posttest only control group design, dengan 3 kelompok perlakuan yaitu kontrol, kitosan gel 2% dan kitosan 100mg/200g/bb per oral pada 15 ekor tikus Wistar dengan periodontitis yang diinduksi bakteri *Phorphyromonas gingivalis*. Hasil penelitian menunjukkan rerata kadar Interleukin-6 dan Mallondhyaldehyde berbeda nyata ( $p < 0,05$ ) antara kelompok kontrol, perlakuan gel kitosan dan pemberian kitosan oral. Kadar interleukin-6 tertinggi pada kitosan gel ( $8,3091 \pm 1,25570$ ), rata-rata kontrol ( $5,8523 \pm 0,51512$ ), terendah oral ( $3,2563 \pm 1,45952$ ). Demikian pula, kadar Mallondhyaldehyde tertinggi pada kelompok gel ( $2,8850 \pm 0,19353$ ), kelompok kontrol ( $2,2284 \pm 0,00914$ ), dan kelompok oral ( $1,2943 \pm 0,60280$ ). Berdasarkan hasil tersebut, pemberian kitosan oral paling efektif terhadap terapi periodontitis.

**Kata kunci:** Kitosan, Kadar Interleukin, Kadar Mallondhyaldehyde

### Abstract

The 6th highest diseases that occur in Indonesian society include dental caries and periodontitis caused by dental plaque which is a breeding ground for bacteria such as *Streptococcus mutants* and *Phorphyromonas gingivalis*. The purpose of this study was to determine the effectiveness of chitosan on levels of Interleukin-6 and Mallondhyaldehyde (MDA), as an anti-inflammatory marker using the Elisa test. The research method was a true experimental randomized posttest only control group design, with 3 treatment groups namely control, 2% chitosan gel, and 100mg/200g/bb of chitosan orally in 15 Wistar rats with periodontitis induced by *Phorphyromonas gingivalis* bacteria. The results showed that the mean levels of Interleukin-6 and Mallondhyaldehyde were significantly different ( $p < 0.05$ ) between the control group, chitosan gel treatment, and oral chitosan administration. Interleukin-6 levels were the highest in the chitosan gel ( $8.3091 \pm 1.25570$ ), the control average ( $5.8523 \pm 0.51512$ ), the lowest oral average ( $3.2563 \pm 1.45952$ ). Similarly, Mallondhyaldehyde levels were highest in the gel group ( $2.8850 \pm 0.19353$ ), control group ( $2.2284 \pm 0.00914$ ), and oral group ( $1.2943 \pm 0.60280$ ). Based on these results, oral chitosan was the most effective in the treatment of periodontitis.

**Keywords:** Chitosan, Interleukin Levels, Mallondhyaldehyde Levels

## 1. INTRODUCTION

Dental and oral disease is the 6th highest disease that Indonesian people complain about, including dental caries and periodontal disease, namely periodontitis. The main cause of this dental disease is the presence of dental plaque. Dental plaque is a breeding ground for *Streptococcus mutans* bacteria as the main bacteria that cause caries and *Phorphyromonas gingivalis* bacteria as bacteria that cause periodontal disease (Hasibuan et al., 2021; Ikono et al., 2019; Krzyściak et al., 2014). The dental and oral disease that is often experienced by

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Indonesian people is dental caries. Patients with dental caries in Indonesia have a prevalence of 50-70% with the most sufferers being children under five. Periodontal disease in Indonesia ranks second after caries, reaching 96.58%. Based on the Basic Health Research (RISKESDAS) in 2007, dental and oral problems, including periodontal disease, reached 23.5% (Chaparro et al., 2013; Notohartoyo & Suratri, 2016; Trisnawaty et al., 2017). Periodontitis is a destructive inflammation of the supporting tissues of the teeth caused by specific microorganisms, which results in further damage to the periodontal ligament and alveolar bone with pocket formation, gingival recession, or both. The main bacteria found in the subgingival plaque of patients with chronic periodontitis include: *Porphyromonas gingivalis*, *Actinobacillus actinomycetemcomitans*, *Prevotella intermedia*, *Tannerella forsythia*, and *Fusobacterium nucleatum*.

The material from the bacterial cell wall of *Porphyromonas gingivalis* contains Lyphopolysacarida (LPS), this material, directly and indirectly, gives a signal to the host. In the early stages, these bacteria will secrete Lyphopolysacarida, a fatty acid, which will damage the gingival epithelial layer. Lyphopolysacarida will bind to form Lyphopolysacarida Binding Protein and activate CD14 receptors, activated monocytes, and endothelial cells through Toll-Like Receptors (TLRs). This material will stimulate epithelial cells to produce inflammatory mediators such as Interleukin-6, Interleukin-8, Interleukin 1 $\beta$ , prostaglandin E2 (PGE2), matrix metalloproteinases (MMPs) and Tumor Necrosis Factor-alpha (TNF $\alpha$ ) (Fu et al., 2014, 2015; Makama et al., 2016). Examination of the severity of periodontal tissue damage can be done by measuring pocket depth, bleeding on probing, and clinical attachment level (Carvalho et al., 2018; Jepsen & Jepsen, 2016; Mdala et al., 2014).

Periodontitis begins with the formation of dental plaque which is firmly attached to the tooth and gingival surfaces and has a large potential for disease in the hard tissues of the teeth and their supporting tissues. This situation is caused by plaque containing various kinds of bacteria with various metabolic products. *Streptococcus mutans* is the main bacteria that cause periodontitis. The efforts that can be done to prevent and control the formation of dental plaque include: regulating diet and taking chemical action. Chemical control of plaque can be done by suppressing bacterial colonies and suppressing the formation of extracellular polysaccharides. To deal with these conditions, a natural ingredient is needed to reduce the occurrence of resistance and side effects, in this study selected natural ingredients in the form of chitosan. Chitosan is a compound derived from the deacetylation of chitin which is found in many marine animals such as shrimp and crabs and has been proven to be non-toxic, biocompatible, and biodegradable compared to other polymers. Chitosan has been studied to stimulate cell proliferation, increase collagenization and accelerate cell regeneration (reepitelation) in injured skin. Chitosan can stimulate PMN cell migration, activate macrophages and mediate phagocytosis in injured tissues. Chitosan has anti-infective properties, namely antibacterial and antifungal abilities (Campoccia et al., 2013; Rasul et al., 2020). In previous research on the use of chitosan from shrimp shells as an anti-inflammatory against rheumatoid arthritis rats, the results showed a decrease in edema that occurred in the soles of the rats' feet in proportion to the increase in the concentration of chitosan given orally.

This study proves that the anti-inflammatory effect of 100mg/200gr BW of chitosan is equivalent to the effect of diclofenac sodium administration. The anti-inflammatory power of chitosan can be caused because chitosan has a structure similar to glucosamine. The mechanisms of action of glucosamine are to reduce the production of the Cox-2 enzyme so that the expression of Interleukin-1 and Interleukin-6 induced by Cox-2 and Nfkb can be suppressed (Putri & Darmawan, 2022; Qu et al., 2022). Based on this background, the aim of this study was to determine the anti-inflammatory activity of Vanameshrimp chitosan against Wistar periodontitis rats induced by *Porphyromonas gingivalis* bacteria.

## 2. METHODS

The method used in this second phase of research is an experimental study with a randomized posttest-only control group design to determine the effect of chitosan compared to controls. This study was conducted in vivo on 21 white rats of the Wistar Periodontitis strain with three treatments, namely; the control group which only did scaling and root planning, the group that was given oral chitosan 100mg/200gr/bb, the group that was given topical chitosangel.

### Periodontitis rat preparation process

The rats were anesthetized by injection of ketamine HCl i.m in the hamstrings at a dose of 0.2 ml/250 g bw, then a periodontal silk ligature was placed on the anterior mandible in the subgingival area. Then, *Porphyromonas gingivalis* was induced. Induction of *Porphyromonas gingivalis* with the amount of  $3 \times 10^8$  Mac Farland, 0.25 ml once in the buccal area. Installation of the silk ligature for 7 days, after the ligature, is removed, for 3 days no debridement is carried out, with the aim that the bacteria in the plaque will persist until chronic periodontitis occurs on day 11.

### Chitosan Gel preparation process

30 mg chitosan powder was macerated with 500 ml 96% ethanol, for 3x24 hours. Then the maceration results were filtered to obtain the filtrate and concentrated with a rotary evaporator to obtain a thick extract. Prepared as much as 2 grams of carbomer, 5 ml of glycerin, and 100 ml of distilled water. Then mixed and stirred carbomer and distilled water with a mixer until homogeneous. After homogeneous, add glycerin and stir until smooth. Finally, add 3 ml of chitosan extract, stir again until homogeneous, and form a gel-like mass.

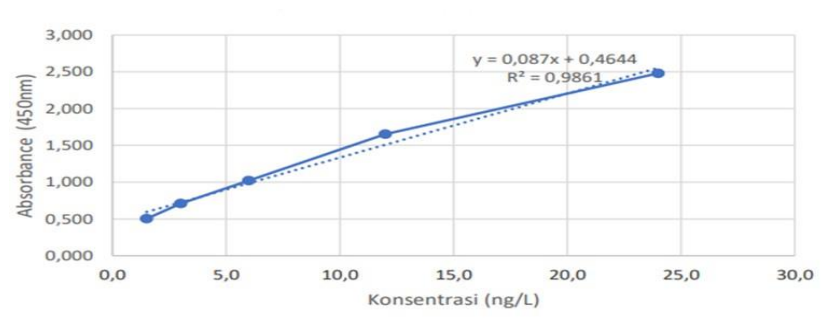
### The process of giving chitosan

The administration of chitosan with oral gavage was given twice a day (at 07.00 and 19.00 WITA). The extract dose, namely: 200 mg/kg/bb given to Wistar rats for 14 days. Topically, chitosan gel was applied directly to the pocket area with the help of a 0.3 ml 1 ml syringe three times a day (7 AM, 1 PM, 7 PM), for 14 days.

## 3. RESULTS AND DISCUSSION

### Result

The results achieved in this study were obtained levels of Interleukin-6 as shown in Figure 1 and Mallondhyaldehyde in Periodontitis Wistar Rats in Figure 2.



**Figure 1.** Standard Curve Graph of Interleukin-6 Level Measurement Results in Wistar Rats with Periodontitis

Figure 1 shows that the lowest levels of Interleukin-6 were found in the chitosan group with oral administration.

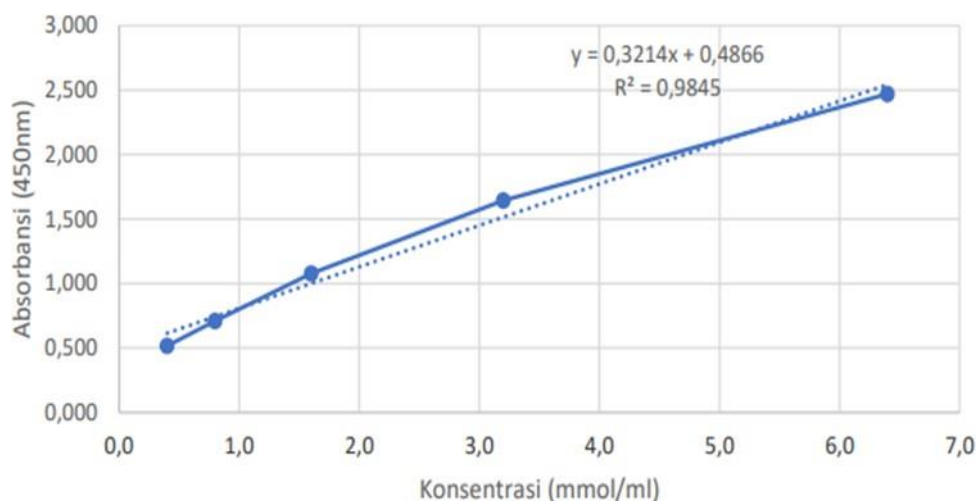


Figure 2. Standard Curve Graph of Mallondhyaldehyde Level Measurement Results in Wistar Rats with Periodontitis

Figure 2 shows that the lowest level of Mallondhyaldehyde is found in the chitosan group with oral administration.

Table 1. Normality Test for Interleukin-6 and Mallondhyaldehyde Levels in Wistar Rats with Periodontitis

Group		Kolmogorov-Smirnov <sup>a</sup>		
		Statistic	df	Sig.
IL_6	Gel	0.156	7	0.200*
	Oral	0.169	7	0.200*
	Control	0.177	7	0.200*
MDA	Gel	0.212	7	0.200*
	Oral	0.261	7	0.152
	Control	0.252	7	0.198

Table 1 shows that the levels of the measurement results of Interleukin-6 and Mallondhyaldehyde in Wistar rats with periodontitis are data that are normally distributed ( $p > 0.05$ ).

Table 2. Test for Homogeneity Levels of Interleukin-6 and Mallondhyaldehyde in Wistar Rats with Periodontitis

	Levene Statistic	df1	df2	Sig
IL_6	2.593	2	18	0.102
MDA	3.419	2	18	0.055

Table 2 shows the results of the homogeneity test of significant data ( $p > 0.05$ ), that the data from the measurement of IL-6 levels and Mallondhyaldehyde levels are homogeneous. So that data testing between groups can be continued with the One-Way ANOVA test.

**Table 3.** Interleukin-6 Level Analysis Test Results in Wistar Rats with Periodontitis Between Treatments Groups

Group	n	Mean ± SD	p
Chitosan Gel	7	8.3091±1.25570	0.000
Chitosan Oral	7	3.2563±1.45952	
Control	7	5.8523±0.51512	

Table 3 shows the levels of Interleukin-6 between the group given topical chitosan gel, oral chitosan administration, and the control group there are significant differences (p<0.05). From the mean shown in the data, the results of the measurement of Interleukin-6 levels showed the smallest average in the oral administration of chitosan.

**Table 4.** Mallondhyaldehyde Levels Analysis Test Results in Wistar Rats with Periodontitis Between Treatments Groups

Group	n	Mean ± SD	p
Chitosan Gel	7	2.8850±0.19353	0.000
Chitosan Oral	7	1.2943±0.60280	
Control	7	2.2284±0.00914	

Table 4 shows the levels of Mallondhyaldehyde between the group given topical chitosan gel, oral chitosan administration, and the control group there are significant differences (p<0.05). From the mean shown in the data, the results of the measurement of Interleukin-6 levels showed the smallest average in the oral administration of chitosan.

**Table 5.** Interleukin-6 Level Analysis Test Results in Wistar Rats with Periodontitis in the Treatment Group

Level	Group	Mean Differences	Sig.
Interleukin-6	Gel - Oral	5.05286	0.000
		2.45686	0.001
Oral - Control	- Gel	-5.05286	0.000
	Control	-2.59600	0.001
Control - Oral	- Gel	-2.45686	0.001
	Oral	2.59600	0.001

Table 5 shows the levels of Interleukin-6 between groups: in chitosan gel with oral there is a significant difference (p<0.05), between the gel and control there is a significant difference (p<0.05), the oral group and the control also show a significant difference. significant difference in interleukin-6 levels (p<0.05).

**Table 6.** Mallondhyaldehyde Levels Analysis Test Results in Wistar Rats with Periodontitis in the Treatment Group

Level	Group	Mean Differences	Sig.
Mallondhyaldehyde	Gel - Oral	1.59071	0.000
		0.65657	0.003
Oral - Control	- Gel	-1.59071	0.000
	Control	-0.93414	0.000
Control - Oral	- Gel	-0.65657	0.003
	Oral	0.93414	0.000

Table 6 shows the levels of Malondialdehyde between groups: in chitosan gel with oral there is a significant difference ( $p < 0.05$ ), between the gel and control there is a significant difference ( $p < 0.05$ ), the oral group and control also show differences in levels. significant interleukin-6 ( $p < 0.05$ ).

## Discussion

Table 1 and Table 2 showed the data on levels of Interleukin-6 and Mallondhyaldehyde from the intervention of chitosan gel, oral administration of chitosan, and control are normally distributed and homogeneous data. Table 3 and Table 5 showed data on Interleukin-6 levels, where there is a significant difference in Interleukin-6 levels between the control group, chitosan gel group, and oral chitosan. Similarly, the data on Interleukin-6 levels were compared between each group, namely: between chitosan gel and oral there was a significant difference ( $p < 0.05$ ), between gel and control there was a significant difference ( $p < 0.05$ ), the oral group with the control also showed a significant difference in interleukin-6 levels ( $p < 0.05$ ). From the mean obtained, it shows that the gel group with the highest mean ( $8.3091 \pm 1.25570$ ), the control average ( $5.8523 \pm 0.51512$ ), the oral average ( $3.2563 \pm 1.45952$ ) on the other hand, had the lowest mean.

The measurement of the levels of Interleukin-6 showed the three data have significant differences and the average difference starts from the smallest average in the oral chitosan group, then the control group, and the largest in the chitosan gel group. Oral administration of chitosan can prove the ability of chitosan to suppress Interleukin-6 which is a marker of inflammation. Inflammation is a biological response to various forms of the disease. One of the markers responsible for the inflammatory process is cytokines, which are proteins that are involved in the initiation and further development process and regulate the duration of the inflammatory response, such as Interleukin-6, tumor necrosis factor (TNF), and nitrate oxygen (NO). Interleukin-6 is a multifunctional cytokine that plays a role in the inflammation of the tooth-supporting tissue which is a characteristic of periodontitis. Several studies have compared Interleukin-6 levels in saliva and gingival crevicular fluid as parameters of chronic periodontitis (Grover et al., 2016; Kurgan et al., 2016; Vemanaradhya et al., 2017).

Oral administration of chitosan proved the ability of chitosan as an anti-inflammatory shown by the levels of interleukin-6 in Wistar periodontitis rats which had significantly different levels from the control, with a much smaller mean. The anti-inflammatory power of chitosan can be caused because chitosan has a structure resembling glucosamine. One of the mechanisms of action of glucosamine is to reduce the production of the Cox-2 enzyme so that the expression of Interleukin-1 and Interleukin-6 induced by Cox-2 and Nfkb can be suppressed (Jones Lipinski et al., 2021; Shao et al., 2013; Sultana & Rasool, 2015). The results of this study are supported by previous research on the use of chitosan from shrimp shells as an anti-inflammatory against rheumatoid arthritis model rats. The results show that there is a decrease in edema that occurs in the soles of the rats' feet in proportion to the increase in the concentration of chitosan given orally. This study proves that the anti-inflammatory effect of 100mg/200gr BW of chitosan is equivalent to the effect of diclofenac sodium administration (Azuma et al., 2015; Chang et al., 2019; Ngo et al., 2015). In addition, chitosan also has the ability as an antibacterial so it can suppress the proliferation of bacteria. Proven from the previous research by measuring the inhibitory power of chitosan against the bacterium *Phorphyromonas gingivitis* which is the bacteria that causes periodontitis (Lee et al., 2018; Ma et al., 2022; Zang et al., 2019).

Table 4 and Table 6 showed data on Mallondhyaldehyde levels, there was a significant difference between the control group, giving chitosan gel and chitosan orally ( $p < 0.05$ ). The mean obtained for the control group ( $2.2284 \pm 0.00914$ ) was still smaller than



the gel group ( $2.8850 \pm 0.19353$ ), while the oral group had the lowest mean ( $1.2943 \pm 0.60280$ ). Similarly, between the levels of Malondialdehyde in each group, namely: between the chitosan gel and oral there was a significant difference ( $p < 0.05$ ), between the gel and the control there was a significant difference ( $p < 0.05$ ) and between the oral group and the control group. also showed a significant difference in malondialdehyde levels ( $p < 0.05$ ).

In periodontitis, oxidative stress also occurs. Oxidative stress is defined as the excess production of free radicals that can cause cell damage. Occurs due to an imbalance between the production of reactive oxygen species (ROS) and antioxidant defenses. Infection in the periodontal tissue causes oxidative stress. Lipopolysaccharide (LPS) and DNA from these bacteria lead to the activation of both pathways, activating protein-1 (AP-1) and nuclear factor kappa- $\beta$  (NF- $\kappa$ B) in fibroblasts, via CD14 and TLR-4 (toll-like receptor) in fibroblasts. gingival and inflammatory cytokine production. The production of IL-1, IL-6, IL-8, TNF- and transforming growth factor beta (TGF- $\beta$ ) cytokines are cytokines and chemokines that respond to inflammation, recruitment and hyperresponsiveness to polymorphonucleate (PMN) activation. Polymorphonucleates produce reactive oxygen species (ROS), through respiratory or oxidative bursts as part of the defense response against infection. Reactive oxygen species are very toxic and can induce lipid peroxidase (LPO) which has an effect on cells. Lipid peroxidation events will result in cell lysis which will result in the release of malondialdehyde (MDA) which in turn will cause damage to all cells. In this study, oral administration of chitosan proved the ability of chitosan to suppress malondialdehyde levels in periodontitis, as evidenced by the malondialdehyde levels of Wistar rats with periodontitis which had lower levels than controls. Chitosan in cancer patients has the ability for biodegradation, biocompatibility, and anti-toxicity which is the role of antioxidants in suppressing the occurrence of ROS (Chen et al., 2022; Mokgehle et al., 2021; Puspitasari et al., 2023). This is supported by previous research on the administration of chitosan for the protection of heart damage in diabetic-treated rats (Abdel-Moneim, A. et al., 2020; Mostafa et al., 2021; Omodanisi et al., 2017). The results showed a decrease in malondialdehyde in rats with chitosan administration and an increase in levels of superoxide dismutase (SOD), and glutathione peroxidase (GPX), an endogenous enzyme that acts as an antioxidant.

The administration of chitosan gel in this study was not in accordance with the hypothesis, where the results showed the opposite, Interleukin and malondialdehyde levels in Wistar rats showed higher results than controls. The weakness of this study is that preliminary research on gel formulation has not been carried out. Gel making refers to three previous studies with a safe addictive ingredient, namely glycerin, with variations in the gel formulation in this study, namely 3 grams of chitosan: 5ml glycerol. The success of this study was supported by the previous research, which stated that a variation of 0.5 grams of chitosan: 4 ml had the lowest and highest swelling test at 0.5 grams: 2 ml of glycerol, as well as data that were not constant for the addition of chitosan, as well as with the addition of glycerol. With the low swelling test results, it is possible that chitosan gel cannot last long in the oral cavity when applied and is immediately dissolved by saliva so the expected effect for periodontitis therapy is not achieved. Therefore, it is necessary to conduct further research for effective chitosan gel as a therapy for periodontitis.

#### 4. CONCLUSION

Based on the results of research that has been carried out on the study of the effectiveness of giving chitosan vename shrimp shells on Interleukin-6 levels and

Mallondhyaldehyde levels, it shows that oral administration of chitosan is effective for the therapy of inflammation in periodontal tissues for the treatment of periodontitis.

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