

# Investigation of Astaxanthin effect on the formation of collagen fibers for periodontal tissue repair in bacteria infected periodontitis



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## ABSTRACT

**Introduction:** Periodontitis is an inflammation of periodontal tissue in which the attachment-forming ligament composed of collagen fibers is damaged. Inflammation is a scenario where oxidative stress is abundant which may worsen periodontitis. Antioxidant, such as astaxanthin, is needed to promote collagen fibers formation as a sign of healing.

**Objective:** To explore whether astaxanthin has a desirable effect in the formation of collagen fibers in bacteria-triggered periodontitis in rats.

**Methods:** An animal experimental study were conducted. There were 3 groups, each consisted of 8 rats: negative control, positive control,

and treated group. The treated group received oral preparation of astaxanthin 0,54 mg/kg/day for 7 days. The rats were then sacrificed, and the periodontal tissue were made into paraffin-fixed 5 µm tick slides with hematoxylin eosin staining and trichrome masson to evaluate the collagen expression. The evaluation was carried out by a pathologist.

**Results:** There is a significant difference of collagen expression level between the T and PC groups (p-value<0.05)

**Conclusion:** There is a significant difference between the groups treated with astaxanthin and the group with periodontitis disease.

**Keywords:** Astaxanthin, bacterial periodontitis, collagen-fibers

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## INTRODUCTION

Periodontal disease is one of the most frequently found dental and oral health problems in general population. Individuals aged 35-55 years are at high risk of developing periodontitis. Periodontitis with the risk of tooth loss mostly arises from gingivitis cases.<sup>1</sup> Periodontitis is an inflammation of periodontal tissue in which the attachment-forming ligament composed of collagen fibers is damaged. A chronic asymptomatic periodontitis infection may develop for before clinical manifestations occur, causing delay in treatment.<sup>2</sup> A healing response after the periodontal tissue injury allows wound closure and partial recovery of the tissue structure and function. A combination of epithelial tissue and connective-collagen tissue growth is needed to rebuild the damaged attachment of bones and teeth.<sup>3</sup>

Inflammation is a process in which free radicals are produced.<sup>4</sup> Free radicals imposed oxidative stress to the involved tissues which may worsen the inflammation process but at the same time destroy pathogenic microorganism.<sup>5</sup> Antioxidants are agents that can inhibit oxidation process. Astaxanthin is one of many antioxidants which claim to have a stronger antioxidative effects than carotene group.<sup>6</sup>

We conducted a research to explore whether Astaxanthin has a desirable effect in the formation of collagen fibers in bacteria-triggered periodontitis in rats.

## METHODS

We conducted an animal experimental study. The data were obtained by post-test only. As many as 24 male Wistar rats were divided equally into 3 groups: negative control (NC), positive control (PC), and the treated group (T). The rats in the PC and T groups received a periodontitis inducing procedure recreated by daily injection of *Aggregatibacter actinomycetemcomitans* on the mesial of the lower anterior teeth for 1 week. Only the rats in T group were given oral preparation of astaxanthin 0,54 mg/kg /day for 7 days. On the 7th day the astaxanthin treatment, all animals were sacrificed by decapitation.

The periodontal tissue of each rat was fixated with paraffin, and serially cut into 5 µm thick and made into slides and stained with hematoxylin eosin and trichrome masson to evaluate the expression of collagen fibers. The assessment of the collagen fiber expression was conducted by a pathologist. The slides were rated for the level of the collagen

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fiber expressed as 0 when there was 0% collagen fibers expressed, 1 for under 25%, 2 for 25 to 49%, 3 for 50 to 75%, and 4 for over 75%. The proportion of the rats according to the level of collagen fiber expression were recorded. The data analysis was conducted using SPSS v 23.0 for Windows. P-value < 0.05 is considered significant.

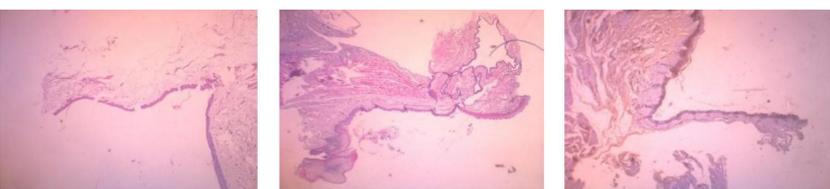
## RESULT

Histopathological pictures of hematoxylin eosin-stained periodontal tissue were taken. The NC group showed healthy periodontal tissue with normal lymphocyte and collagen fibers. The PC group, periodontitis tissue showed a higher density of lymphocytes and collagen fibers. The T group has the highest density of lymphocytes and collagen fibers (See [Figure 1A, B and C](#)).

The Kruskal-Wallis test shows that there is a significant difference of collagen expression level between the NC, PC and T groups with a p-value of less than 0.05. ([Table 1](#)) The post hoc analysis with Mann Whitney test shows that there is a significant difference of collagen expression level between the T and PC groups (p-value<0.05). The collagen expression level comparison between the NC and PC groups and the NC and T groups are significantly different with p-value of less than 0.05.

## DISCUSSION

This the first study known to the researcher that is exploring the effect of astaxanthin to collagen



**Figure 1** Histopathological pictures hematoxylin eosin-stained periodontal tissue; A. NC group: healthy periodontal tissue with normal lymphocyte and collagen fibers; B. PC group: periodontitis tissue showed a higher density of lymphocytes and collagen fibers; C: T group: when compared to PC group, more lymphocytes and collagen fibers were found in T group

**Table 1** The proportion of collagen level between NC, PC and T groups

Groups (n=8)	Collagen Level					p-value
	0	1	2	3	4	
NC	8 (100%)	-	-	-	-	
PC	-	4 (50%)	4 (50%)	-	-	<0.05
T	-	-	4 (50%)	4 (50%)	-	

fiber expression in rats with periodontal disease. Moreover, the histological examination of the tissue for collagen fiber expression can provide information on the disease process and the astaxanthin effects as antioxidants.

Antioxidant is claimed to have an effect of promoting collagen fiber growth favorable for tissue healing.<sup>6</sup> In vivo study shows that antioxidant such as astaxanthin have partial effect on antioxidant alteration in diabetic rats by stimulating glutathione peroxidase activity.<sup>7,8</sup> The regulatory effect on oxidative stress will indirectly stimulate tissue growth. Moreover, other study conclude that the expression of collagen fibers is stimulated with the oral administration of astaxanthin by activating type I collagen.<sup>9,10</sup> Furthermore, the imbalances of matrix metalloproteinases and tissue inhibitors of matrix metalloproteinases in the periodontal disease might contribute to the level of collagen fiber expression.<sup>11,12</sup> The result of our research showed a significant difference of collagen expression between the PC and T groups. This finding suggest that the astaxanthin affects the collagen expression in rats with periodontitis. However, there was no readily available data in which we can compare our result to. Therefore, there is a need for further research in the effects of astaxanthin.

## CONCLUSION

In rats, there is a significant difference of collagen formation expression between the three groups. There is a significant difference between the groups treated with astaxanthin and the group with periodontal disease.

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