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## EVALUATION OF PERIODONTAL DISEASE IN RATS SUBMITTED TO THE ADMINISTRATION OF CYTHOCBACTERIA ARTHROSPIRA SP. ("SPIRULINA SP.") AS A FUNCTIONAL SUPPLEMENT

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### ABSTRACT

**Objective:** To evaluate the therapeutic effect of cyanobacteria *Arthrospira* sp. in the evolution of periodontal disease. **Methods:** Eight rats, aged 90 days weighing  $280 \pm 300$ g, were used from the Animal Experimentation Center Animal Hospital of the Department of Pharmaceutical Sciences of the Federal University of Pernambuco. After an adaptation period of 10 days, the animals were randomly divided into three groups, namely: Group I - Control Rats, without administration of cyanobacteria; Group II - Rats with periodontal disease without administration of cyanobacteria; Group III - Rats with periodontal disease with cyanobacteria administration. All animals were induced to periodontitis, which was made by ligature with cotton thread, and this wire was placed around the gingival sulcus of the left upper first molar, with this application there was accumulation of residues, causing the formation of dental biofilm. **Results:** After the morphological analysis of the experimental groups, we observed that there was a significant effect among the studied groups, where the periodontal ligament was preserved (treated group) or showed absence of tissue integrity (untreated group). **Conclusion:** The administration of *Arthrospira* sp. in rats with periodontal disease, suggests to be an important ally in the fight against the progression of periodontitis, thus acting in the control of this disease.

**Keywords:** periodontal disease, rats, spirulina, functional supplement.

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## INTRODUCTION

Periodontal disease is characterized as a public health problem mainly because of its high prevalence, affecting about 50 to 90% of the world population. This one presents a multifactorial origin, having as main factor for its origin in many cases the bacterial plaque or biofilm, being the oral hygiene of fundamental importance for its prevention. (KLOKKEVOLD, 2004)<sup>1</sup>.

Studies have demonstrated the negative association of periodontal disease in relation to systemic diseases such as cardiovascular diseases, diabetes, tuberculosis and premature labor (DOMINGUES et al., 2010)<sup>2</sup>. Taking into account the fact that periodontal diseases may be a risk factor for some systemic diseases, the control of periodontal diseases become essential in the treatment of these diseases.

There are three mechanisms that explain the association of periodontal diseases in systemic diseases. The first is the translocation of the microorganism, where in periodontal disease there is an area in which there is connective tissue, exposed blood vessels that may allow the passage of bacteria from the oral cavity (specifically from the periodontal pocket into the bloodstream). The second mechanism is the passage of the products of the microorganisms into the bloodstream. Bacteria produce toxins, glycoposaccharides, various virulence factors that can enter the blood and be understood as a foreign body and the body will handle a reaction against that antigen. The third mechanism is that every time you have a bacterium, even if it is not transmitted or translocated into the blood, but if it is in the periodontal pocket there will be a production of immune response of the body. This response is a greater production of proteins and defense cells (chemical mediators of the inflammatory system) that promotes an area of inflammation. (CARRANZA et al., 2004)<sup>3</sup>.

Periodontal disease has as one of its most common forms chronic periodontitis, which has as its main characteristic the slow development

of an inflammatory process that reaches the periodontium of support, all of this occurring due to the response of the organism to the presence of the subgingival biofilm. It is known that the immune response of the host is part of the multifactorial etiology of the disease, and that this immune response against the antigens of the microorganisms can often be responsible for the destruction of the periodontium. Therefore, studies have been carried out to find alternatives to the routine treatment of periodontitis, many of these alternative treatments seek a way to modulate the immune response of the host, because apparently tissue and bone destruction is more linked to the immune response of the host than the action of pathogenic bacteria (RÊGO et al., 2010, p.69)<sup>4</sup>.

The inflammatory reaction in periodontal disease is an important process and is the means by which the organism protects its organic structures, such as tissues, for example. In the case of periodontal disease, the inflammatory process and the immune system act in the gingival tissue, in order to prevent pathogenic microorganisms from entering the tissues of the oral cavity. However, when the body's defense response is exacerbated it can promote harmful effects. In the case of periodontal disease, a good part of this response may affect deeper tissues such as the alveolar bone (ROXO, GABRIELLI, 2004, 196)<sup>5</sup>.

It is well known in the scientific literature that a balanced diet is a great ally in the fight against diseases, acting in the proper functioning of the body and immune system. Foods with antioxidant potential may, for example, decrease inflammatory responses, and thus speed up the healing process of a disease. In the case of periodontal disease, the oxygen free radicals produced during inflammation activate the metalloproteinases, degrading the collagen of the periodontal ligament, compromising the fixation of the tooth in the alveolar process, increasing the chance of loss of the dental element. A diet rich in polyunsaturated fatty acids, vitamin C and E, which has proven

antioxidant action can act in the inflammatory process, aiding the body in the fight against chronic inflammation (ALVEZ et al., 2007, p 1054)<sup>6</sup>.

The use of cyanobacteria as food is not new, and records have been found since prehistoric times, some authors claim that this practice intensified with the Chinese more than 200 years old, who used it in times of food shortages. There are cyanobacteria in their biomass a number of nutrients necessary for the proper functioning of the body. Research has proven this high nutritional potential of these microorganisms. Moreira et al. (2013)<sup>7</sup>, report in their research that the intake of *Arthrospira* sp. (*Spirulina*) allowed the recovery of Wistar rats at nutritional level, acting to improve their hematopoietic, biochemical and physiological system. Review studies show that *Arthrospira* sp. ("*Spirulina*") has a number of compounds essential to the body such as proteins, essential amino acids, minerals, polyunsaturated fatty acids and vitamins. Some studies also report that this cyanobacterium produces antitumor effects, increasing immunity, and acting as antioxidants since it has high content of phenolic compounds and phycocyanin. Also acting in the fight against malnutrition and hypercholesterolemia, as well as, in the diabetic, also having antifungal and anti-inflammatory action due to the presence of phycocyanin in its biomass. Thus, the recent worldwide research has focused on the great pharmaceutical and therapeutic potential of this cyanobacterium (AMBROSI et al., 2008)<sup>8</sup>. Taking into account the effects on the immune system and the anti-inflammatory and antibacterial action of the cyanobacterium *Arthrospira* sp. and may lead to a possible control of periodontal disease, thus being an ally in the fight against the progression of this disease. This study aimed to evaluate the therapeutic effect of the cyanobacterium *Arthrospira* sp. in the evolution of periodontal disease.

## OBJECTIVE

To evaluate the therapeutic effect of cyanobacteria *Arthrospira* sp. in the evolution of periodontal disease, analyzing more specifically the integrity of the periodontal ligament.

## METHODOLOGY

This work was submitted to the Ethics Committee on the Use of Animals - CEUA of UFPE, through the protocol no. 23076018148 / 2013-45 and approved.

Eight rats (*Rattus norvegicus albinus*), of the Wistar line, weighing  $280 \pm 300$  g, from the Animal Experimentation Center of the Department of Pharmaceutical Sciences of the Federal University of Pernambuco, were used. After an adaptation period of 10 days, the animals were randomly divided into three groups, namely: Group I - Control Rats, without administration of cyanobacteria; Group II - Rats with periodontal disease without administration of cyanobacteria; Group III - Rats with periodontal disease with cyanobacteria administration.

The model of experimental periodontal disease in rats will be according to the methodology of (BEZERRA, et al., 2009, p.66)<sup>9</sup>. All animals were induced to periodontitis (Fig. 1), which was made by ligation with cotton thread, and this wire was placed in the interproximal space at the level of the gingival sulcus around the upper left first molar, this application of the wire caused a build up of residues, leading to the formation of the dental biofilm.

The administration of *Arthrospira* sp. was orally (gavage) using an endoscopic probe at a dose of 20mg / kg daily for 15 days. The cyanobacterium was acquired as commercial dry biomass and diluted in aqueous solution. After ligature placement, the animals were submitted to dissociative anesthesia based on 2% xylazine hydrochloride and 10% ketamine hydrochloride in the 5 and 15 day periods. After being anesthetized, their jaws were collected (Fig. 2) and fixed in 10% formaldehyde for 48h, and then they underwent a 10% nitric acid decalcification process, and then they were

processed by light microscopy, where the fragments were dehydrated in ethyl alcohol in increasing concentrations, diaphanized by xylol, impregnated by liquid paraffin in an oven regulated at 59 ° C and included in paraffin. Then, the blocks were cut into a microtome of the Minot type, adjusted to 5 micrometers (mm). The obtained sections were placed on slides previously anointed with MAYER albumin, and

kept in a regulated oven at 37 ° C for 24 hours for drying and glazing, stained with hematoxylin and eosin according to the methodology of Junqueira & Junqueira (1983)<sup>10</sup>. The slides were analyzed in Light Microscope and the cuts were photographed in Nikon 50E Trinocular Biological Microscope with VT 480 videomicroscopy and IMAGELAB image analyzer.



Figure 1. Induction of periodontal disease. Insertion of the wire around the upper left first molar (arrow).



Figure 2. Presence of the thread after the animal's sacrifice (arrow).

## RESULTS

After the morphological analysis of the experimental groups, we observed that there

was a significant effect among the studied groups, where the periodontal ligament was preserved (treated group) or showed absence of tissue integrity (Figures 3, 4, 5 and 6).

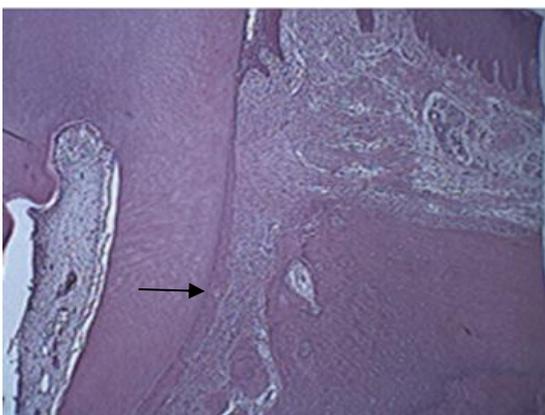


Fig.3. Photomicrography of the maxilla (left side, 1st molar) of rats with periodontitis treated with *Arthrospira* sp. (10x). Observe the integrity of the periodontal ligament (arrow). HE coloration.



Fig.4. Photomicrography of the maxilla (left side, 1st molar) of rats with periodontitis treated with *Arthrospira* sp. (10x). Observe the integrity of the periodontal ligament (arrow). HE coloration.

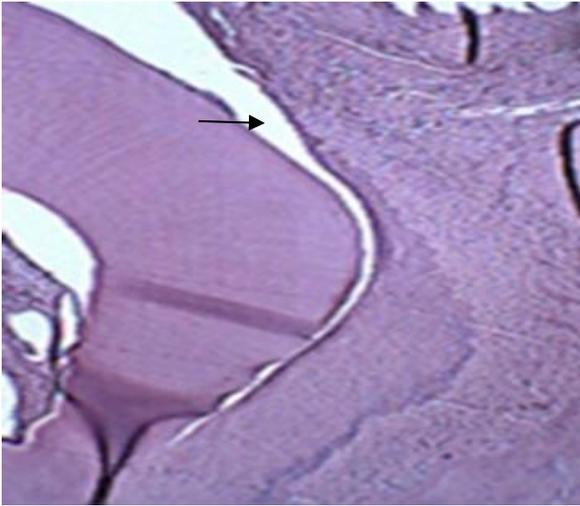


Fig.5. Photomicrography of the maxilla (left molar side) of rats with periodontitis, not treated with *Arthrospira* sp. (10x). Observe the absence of the periodontal ligament (arrow). HE coloration.

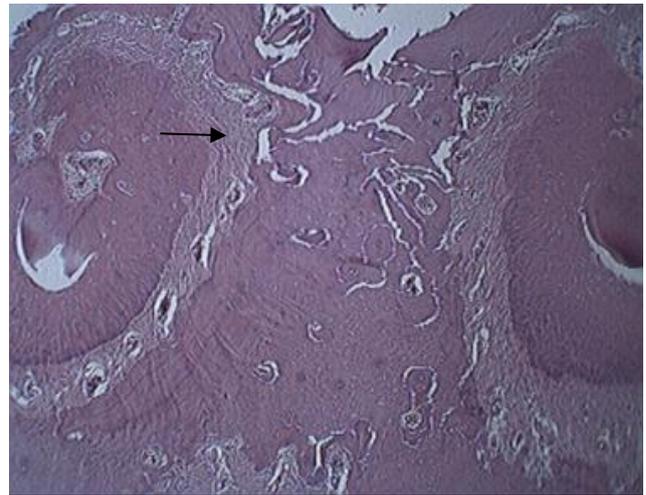


Fig.6. Photomicrography of the maxilla (left side, 1st molar) of control rats (10x). Observe the integrity of the periodontal ligament (arrow). HE coloration.

According to the histological analyzes, it can be seen that the rats that were induced with periodontal disease and were not treated with the cyanobacterium had a very marked destruction of the periodontal ligaments, whereas those that were induced with the disease and were treated had their preserved ligaments identical to the control group. Taking into account the effects on the immune system and the anti-inflammatory and antibacterial action of the cyanobacterium *Arthrospira* sp. (Moreira et al., 2013)<sup>7</sup>, it is possible to see that it acted effectively in the control of periodontal disease, showing itself as a possible ally in the fight against this disease. According to Parisi, et al., 2009<sup>11</sup>, *S. aureus* was satisfactorily inhibited when administered solution of *Arthrospira* sp. *S. aureus* is a gram positive bacteria normally found in diseased sites in periodontitis. This may lead one to assume that *Arthrospira* sp. may have acted to combat this bacterium by controlling the worsening of periodontitis. Taking into account that the microorganisms present in periodontitis interact with each other and that the higher the level of interaction the greater its pathogenesis, it can be inferred that with the inhibition of *S. aureus*, there was also a change

in the interaction between the other microorganisms present in the disease. These interactions often cause more pathogenic bacteria to only act on the disease if other bacteria are already present. So when *S. aureus* bacteria was inhibited probably other more pathogenic bacteria were also indirectly.

It is worth noting also the anti-inflammatory action of *Arthrospira* sp., This anti-inflammatory action results from the presence of numerous compounds such as polyunsaturated fatty acids omega-3 and omega-6. According to Sete et al. (2013)<sup>12</sup>, omega 3 can help in the resolution of inflammation, because when omega-3 is used as a coadjuvant in traditional periodontal therapy, which consists of scaling and smoothing, the treatment has given better results. Thus, it can be assumed that *Arthrospira* sp. may have played a role in the resolution of inflammation by altering the host's response to established infection.

It is not by chance that the pharmaceutical industry has given much attention to the work focused on the therapeutic effects of this cyanobacterium, which has shown promise in the pharmacological field, because its biomass as we have a series of compounds essential to

the organism. And again this work will prove the satisfactory effects of *Arthrospira* sp. now when directed to the control of periodontitis. (OLIVEIRA et al., 2013)<sup>13</sup> It is clear that further studies are needed to understand the mechanism of action of *Arthrospira* sp. in periodontitis, that is, how it acted on the integrity of the periodontium, because as we have seen this may have acted in several ways as bactericidal or even modulating the immune response of the host.

## CONCLUSION

The administration of *Arthrospira* sp. in rats with periodontal disease, suggests to be an important ally in the fight against the progression of periodontitis, thus acting in the control of this disease. Further research should be conducted to gain further insight into the effect of cyanobacteria on periodontitis.

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