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Clinically Considerations About Induced Experimental Periodontitis in Rats Treated by Photodynamic Therapy

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RESEARCH ARTICLE

Abstract

Periodontitis is a chronic inflammatory condition that affects the soft and hard tissue supporting the tooth, favoring the adhesion of bacteria that can generate dental laxity and bone resorption. This study aimed to test an experimental protocol for inducing periodontitis which allows a close clinical assessment and to evaluate the effectiveness of a photodynamic therapy (PDT) protocol to reverse the associated clinical signs. Fifteen Wistar male rats were used for this study, divided into three equal groups. Tooth scaling, rooting and planning of the first superior left molar were performed in all rats, causing the displacement of the gingiva in order to create an accumulation of plaque. A 5-0 cotton ligature was placed, provoking an inflammatory response removed after ten days. One group received one session of PDT; another group received 3 PDT; the last one represented the control group, with no treatment. Clinical evaluation was represented by assessing the body weight, mobility index and bleeding. A rat grimace scale was used to determine the pain. The results showed clinical aspects of periodontitis and healing tissue proved microscopically. The tested procedure can provide all the key biological factors present in periodontal disease and an option for reversing the clinical aspects.

Keywords: alveolar bone loss; chronic inflammation; periodontium, photodynamic therapy.

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INTRODUCTION

Periodontal disease is a complex, chronic, inflammatory condition that can occur both in humans and animals due to a local bacterial infection that generates a change in the host's response to bacterial aggression. This condition can have unwanted effects, such as tooth loss by destroying the tooth socket's supporting tissue and the alveolar bone that supports the dental root. Certain environmental factors but also individual predisposition can influence the development of this pathology (Hajishengallis, 2015) Clinically this condition begins with gingivitis manifested by bleeding gums due to their friability and also with the accumulation of bacterial plaque on the dental surface, especially under the free extremity of the gums, thus creating local inflammation (AlAhmari et al., 2019; Goyal et al., 2014) Periodontitis in pets is a disease almost identical to the pathology found in humans in terms of evolution and clinical presentation (Oz and Puleo, 2011). The accelerated rate of disease progression reported in pets compared to humans may be caused by poor hygiene and lack of routine dental care (García-Salinas et al., 2018). Thus, this disease is diagnosed in animals most often clinically, observing the food preferences of the animal that favors the consumption of soft, moist food to the detriment of dry and hard or solid food. In more advanced cases, the disease

is diagnosed incidentally when the animal shows evident dental laxity that causes tooth loss which could sometimes be late to save the tooth.

Conventional mechanical debridement (scaling and root planning) can temporarily decrease the subgingival levels of pathogens. However, mechanical therapy alone cannot remove organisms from most periodontal pockets. In addition, various systemic and local chemical antimicrobial agents have been introduced to treat periodontitis, suppressing periodontopathogens more effectively than mechanical techniques and improving the results of conventional mechanical therapeutic techniques. Some disadvantages of antimicrobial agents use (such as antibiotics) include an increase in the counts of bacteria resistant to these agents, the need for the use of different antibiotics due to the diversity of periodontopathogens, an increase in the number of immunosuppressed patients and the incidence of unfavorable reactions (Dascalu et al., 2022; Alves et al., 2019). Antimicrobial photodynamic therapy (PTD) is a new and promising method that aims to reduce or even eliminate pathogenic microorganisms. New approaches are currently being researched that may be bactericidal but also have advantages over traditional antibiotic therapy. PDT is a non-thermal photochemical reaction that requires the co-participation of three factors: visible light at an appropriate wavelength, oxygen and a photosensitizer (Dascalu et al., 2020). There are particular protocols for the use of photodynamic therapy for different types of tissues and pathologies.

By implementing these therapies with laser and also taking into account the fact of combining photodynamic therapy (PDT) with some biomaterials with an antibacterial effect, we want to create a periodontal treatment more efficient and to find ways to extrapolate these therapies to pets in order to avoid the side effects due to analgesic medication expressed by gastrointestinal disorders and the emergence of antibiotic resistance when using antibacterial medication which could be a good start, because all of these represent ways of conventional methods of treating periodontitis (AlAhmari et al., 2019)

Therefore, this experimental framework was implemented to test the 3R principle (refinement) for laboratory animals by clinical and pain evaluation in induced periodontitis. Also, another main aim of the study was to adjust the photodynamic therapy (PDT) that could potentially reverse the associated clinical and pathological symptoms.

MATERIALS AND METHODS

Animals

Fifteen medium-weight male, Wistar rats, represented the study material; all were used to induce experimental periodontitis. Ten days later, after the induction was done, the ligature was removed, and the animals were divided into 3 equal groups, as follows: The Control group without treatment, the second group treated with one session of photodynamic therapy (PDT) and the third one treated with three sessions of PDT. In the beginning, the appetite test was performed to evaluate the general condition, and then all the rats were weighed to determine precisely the body loss that rats may suffer after the ligation. Therefore, the body weight fluctuation was monitored daily after the ligature placement and after performing every root and scaling procedure for four consecutive days. This monitoring was realized to establish whether there is necessary the implementation of a supportive therapy or to adjust this therapy. Furthermore, an evaluation of the bleeding and the laxity of the molar after one or three sessions of photodynamic therapy (PDT) was performed.

The precise and careful clinical evaluations in each step were performed in 5 episodes for all the rats as follows: on the first day before placing the ligature, on the tenth day after removing the ligature, on the 17th day after the first session of treatment with photodynamic therapy, on the 24th day after two sessions of therapy and at the end, on the 31st day of the study, after three sessions of photodynamic therapy.

Accommodation

Good accommodation conditions were ensured throughout the study, food in the form of standard granulated feed for rodents and also a high-calorie diet with a softer consistency for the first days after the surgery was provided (from the Cantacusino Institute, Bucharest, Romania) plus fresh water ad libitum, in order to help the rats in the post-surgical stage.

Clinical evaluation - Body Mass, Index of Mobility and Bleeding and Rat Grimace Scale Evaluation

A correct and careful clinical evaluation of this pathology for this species was realized. The evaluation, which was performed through several methods, was done to evaluate and assess the applicability of these techniques and their effect from a clinical point of view. In addition, the induced pathology was treated with different protocols of photodynamic therapy.

In the first phase, all the rats were weighed to have an adequate body condition scoring as a clinical tool for a good evaluation and animal welfare. The body mass is considered a clinical endpoint, especially in periodontal experimental protocols where the prehension capacity of the animal is intensely affected. Other clinical endpoints for laboratory animals are their behavior, reluctance to move, dehydration and pain (Hickman and Swan, 2010). Therefore, on the first day of the study, all the rats were weighed and clinically evaluated by following their

dehydration status and behavior and evaluating the pain scale by determining the grimace scale; the evaluation was realized after the method of Sotocinal (Sotocinal et al., 2011).

After the induction of the periodontitis, dental laxity and gingival bleeding score were evaluated every seven days starting with the tenth day. So the mobility of each first superior left molar was classified according to the following scale: 0 = physiological mobility; 1 = slight mobility. (buccal-palatal); 2 = moderate mobility (buccal and mesial-distal); 3 = severe mobility (tooth moves in and out of the socket), evaluation being adapted to Xie's method (Xie et al., 2011) and Dhingra's method (Dhingra and Vandana, 2011).

The gingival bleeding index was used to classify the level of inflammation by probing the molars in the region of the periodontal pocket for ten seconds with movements of lateralization on each side, so the levels were scored based on: 0, normal gingival; 1, mild inflammation, a slight change in color, slight edema, no bleeding on probing; 2, moderate inflammation, redness, edema and glazing, bleeding on probing; 3, severe inflammation, marked redness and edema, ulceration, a tendency to spontaneous bleeding (Xie et al., 2011; Deng et al., 2018; Löe and Silness, 1963).

This technique represents an essential part of the clinical evaluation protocol. The same operator must perform these surgical procedures for all the rats.

Surgical procedure

The surgical procedures were performed only under the effect of general injectable anesthesia. The study involved the experimental induction of periodontitis in Wistar rats by ligating their first left superior molar under the effect of anesthesia and analgesia, this was an injectable type and was performed by administering the following anesthetic substances: Xylazine, an injectable solution of 7 mg/kg IM; Ketamine, injectable solution 70 mg/kg IM. To extract the ligature, the following were administered: Xylazine, an injectable solution of 7 mg/kg IM and Ketamine, an injectable solution of 70 mg/kg IM. The animals were euthanized 31 days' post-induction. If the side effects have significantly weakened the animals, according to the bioethical protocol, they were euthanized before the end of the study period, based on previously established criteria.

After a careful clinical examination, the anesthesia was performed, and tooth scaling and root planning of the first superior left molar was performed, causing the displacement of the gingival tissue in order to create an accumulation of plaque, then a 5-0 cotton ligature was placed (Figure 1), all this provoking a local inflammatory response. After ten days, the ligatures were removed, and a yellowish discoloration could be observed, also the retraction of the gum with an accumulation of food debris. Then the molar teeth were subjected to tooth scaling, root planning and soft movements to create an accumulation of plaque, flattening and the displacement of the gum, with the help of mini-curettes by distal-medial traction movements in the oral and lingual planes, repeated ten times.

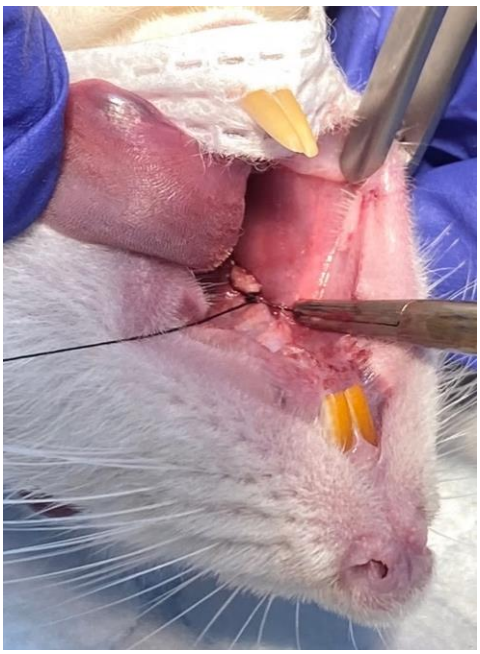

Figure 1. The aspect of the molar, right after ligature placement, after creating the displacement of the gingival tissue, also inflammation, redness and reacted mucosa can be seen. Source: Original

Laser treatment

In this experiment, a standard protocol was applied using the Diode laser, to which a preset program was set for the therapy of periodontal diseases. Pathogenic bacteria involved in periodontal disease are known to be sensitive to PDT using a light-emitting diode (LED). The penetrability of the LED light set at a wavelength of 650 nm through the gingival mucosa and the activation of the antibacterial and anti-biofilm effect can be considered critical aspects for the treatment of periodontitis by PTD. The frequency was set to 200 mV, with a dose of 2.00J/cm and a surface area of 0.40〖cm〗^2. One of the purposes of the study was to compare the therapeutic effects of laser therapy through one exposure, respectively three exposures within three weeks, so the implemented protocol of PDT was performed in only one session for 5 rats and another 5 rats, three sessions of PDT were performed, every 7 days. The last five rats without therapy represented the control group. All the treatments were applied locally, after removal of the ligature, following the procedure used in standard human treatment (Lin et al., 2010): hydrogen peroxide irrigation for 1 minute and photodynamic therapy (40 seconds), although the specifications were modified according to the species being studied. Low-intensity laser therapy was applied perpendicularly, in contact with the gum of the first molar of the left maxilla in four equidistant points on each side of the tooth, buccal and lingual (Figure 2). Right after that, the region exposed was rinsed with 1 ml of Kaqun® oxygen enriched water (Harghita, Romania) using a graded syringe.

Figure 2. Example of application of photodynamic therapy on the first superior left molar in the rat. Source: original.

After every treatment session, the same protocols for clinical evaluation were performed for all the rats to compare the results between the groups. In addition, a pre-determined amount of special food in a softer consistent high-calorie diet was administered to check their post-surgery appetite. Finally, after 31 days' post-induction, the rats were euthanatized, after which the left mandible was sampled for histopathological analysis to confirm the onset of periodontitis.

Microscopical analysis

For euthanasia, the deep narcosis technique with Isoflurane was used for cervical or axoatloid de-spinalization. Right after that, the necropsy was performed. Grossly, yellow discoloration of the molars, mobility within the alveolus and gums reactivity with fibrous tissue have been noticed for the control group (Figure 3). Therefore, gum, molar and alveolus bone sampled from the injured region have been submitted for histological examination for having the standard gold method to confirm the onset of periodontitis and observe the efficacy of the implemented protocol of PDT. Therefore, after the fixation in 10% buffered neutral formalin, the maxillary samples were decalcified using a mix of means 1:1 (formic acid and clorhydric acid) for 24 hours and embedded in paraffin. Five-micron thickness sections were stained by the hematoxylin-eosin method (HE). The slides were examined under a BX51 Olympus microscope, and images were taken with an Olympus UC 30 digital camera and processed using Olympus basic stream software. Sections were examined by an independent observer blinded to the experimental protocol.

Figure 3. The aspect of the molar from a rat from a control group during the necropsy, there is observed a noticeable yellow discoloration, gingival bleeding, gum retraction, suppurative processes and food debris

RESULTS AND DISCUSSIONS

Clinical evaluation - Score of Mobility and Bleeding Index

Gingivitis was observed in nine subjects, more moderate, in six others more acute, so dental laxity was recorded in all of the rats, suggesting the success of the induction of periodontitis, confirmed later by histological examination.

The dental laxity and the level of bleeding and inflammation were scored in all 15 rats as described in the following tables. For all the rats, the mobility and bleeding score on the first day before placing the ligature was 0 and all the rats had healthy gums, no visible plaque on teeth, and no sign of gingivitis and inflammation (Table 1).

Table 1. Results of evaluation of the control group. M=mobility score; B=bleeding score

Control group	Day 1	Day 10	Day 17	Day 24	Day 31
Rat 1	M0B0	M3B3	M3B3	M3B3	M3B3
Rat 2	M0B0	M2B3	M2B3	M2B2	M2B2
Rat 3	M0B0	M3B2	M3B2	M2B3	M2B3
Rat 4	M0B0	M2B3	M2B3	M2B3	M2B3
Rat 5	M0B0	M2B3	M2B3	M2B3	M2B3

For the control group but also for the other two groups at ten days after placing the ligature, visible signs of inflammation were present; also, the color of the ligatured molars was changed, having a yellowish shade, and the plaque became visible, especially on the buccal side with the presence of food debris around the molar.

The tooth mobility was recorded in grade 2 for 8 rats, having moderate mobility (grade 2), and seven rats presented severe mobility (grade 3) on the molar, having an impressive laxity into the socket (Figure 4).

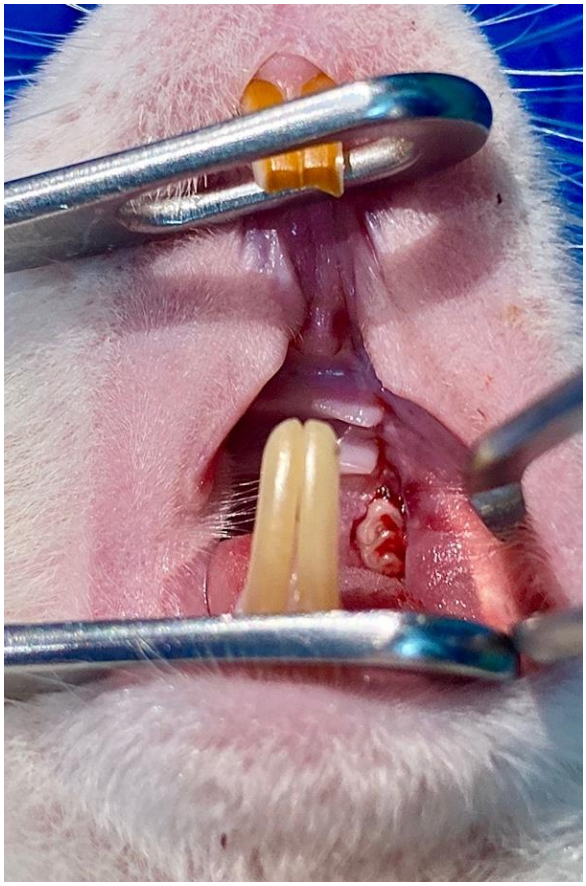

Figure 4. The aspect of the molar with induced periodontitis after the extraction of the ligature, severe bleeding with laxity into the socket was noticed

Following the evolution of the rats with a single session of PDT, on the 17th day of the experiment, one week after the single dose of PDT (Table 2), an improvement was seen in all the rats. More precisely, 5 rats presented slight mobility (grade 1) of the treated molar, and 5 presented moderate laxity (grade 2) of the molar.

Table 2. Results of evaluation of the group treated with one session of photodynamic therapy. PDT=photodynamic therapy, M=mobility score, B=bleeding score

1x PDT group	Day 1	Day 10	Day 17	Day 24	Day 31
Rat 6	M0B0	M3B3	M2B2	M2B2	M2B2
Rat 7	M0B0	M2B2	M2B2	M1B0	M1B0
Rat 8	M0B0	M3B2	M2B1	M2B1	M2B1
Rat 9	M0B0	M2B3	M1B2	M1B2	M1B2
Rat 10	M0B0	M3B3	M1B2	M1B1	M1B1

Also, the level of the inflammation was improved; therefore, 3 rats presented mild inflammation, a slight change in color, slight edema and no bleeding in the moment of probing. For the other 6 rats treated with one PDT session as well, a moderate inflammation was observed, with the presence of redness and edema with moderate bleeding on probing; for only one rat, the inflammation was severe, scored at 3, with marked redness and edema, ulceration and tendency to spontaneous bleeding.

A remarkable better evolution was noted in the group with three PDT sessions (Table 3), where the mobility score was reduced for 4 rats after two and three sessions of PDT (on the 24th and the 31st day of evaluation), initially, 4 rats presented grade 1 of mobility after two sessions of PDT, and only one rat had grade 2 of mobility, as later on the last day of evaluation, the mobility became minimal, 4 rats presented physiological mobility (grade 0), and only one rat presented a slight laxity on the treated molar.

Table 3. Results of evaluation of the group treated with three sessions of photodynamic therapy every 7 days. PDT=photodynamic therapy, M=mobility score, B=bleeding score

3x PDT group	Day 1	Day 10	Day 17	Day 24	Day 31
Rat 11	M0B0	M2B3	M1B3	M1B1	M0B1
Rat 12	M0B0	M2B2	M1B2	M1B1	M0B0
Rat 13	M0B0	M3B2	M2B1	M2B1	M1B1
Rat 14	M0B0	M2B3	M1B2	M1B2	M0B1
Rat 15	M0B0	M3B3	M2B2	M1B1	M0B0

Regarding the level of inflammation in this group, on the 24th day, for 4 rats, a mild inflammation was observed on their gingival tissue all around the molar, but with no bleeding on the probe (Figure 5) and for only one rat, the inflammation was moderate with a bit of redness noticed and bleeding on probing. In the end, on the last clinical evaluation, after 3 sessions of PDT, normal gingival tissue was observed in 3 rats and minimal inflammation for 2 rats.

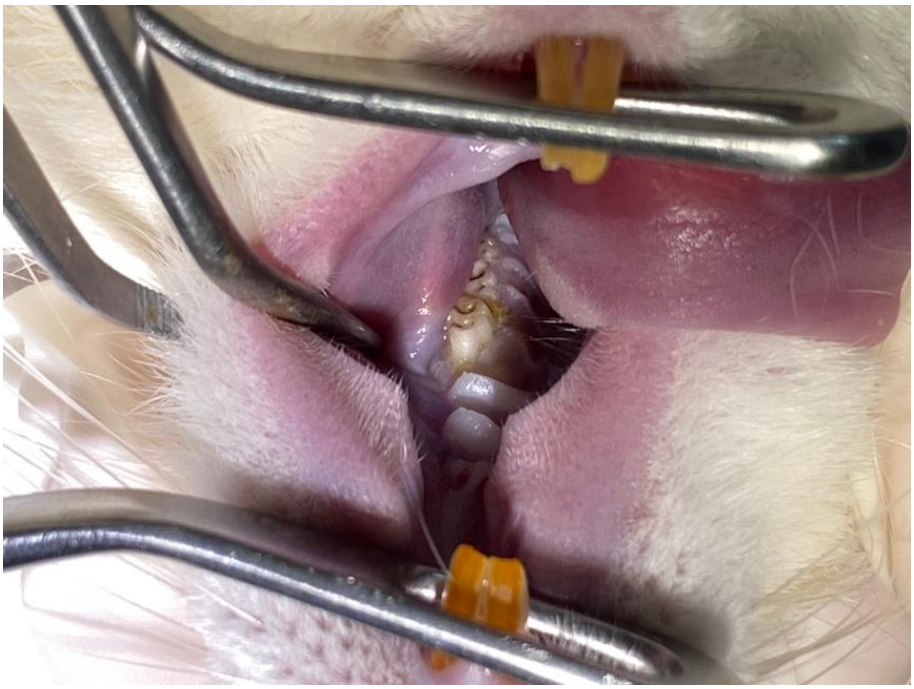

Figure 5. The aspect of the molar with induced periodontitis from a rat from 3 x PDT group, after 14 days of extracting the ligature, no bleeding on probe, minimal inflammation and a slight discoloration

Meanwhile, the other groups were clinically evaluated as well on the 24th day, and 31st day of the experiment, and no significant changes were registered for none of the groups.

Rat Grimace Scale Evaluation

Another clinical parameter reported in this study was the pain score, which was evaluated by determining the rats' grimace scale. Pain in laboratory animals can be effectively alleviated by recognizing and assessing its severity. Traditional pain assessment methods based on monitoring behavior and clinical signs are time-consuming and may be limited. Research has shown that changes in facial expression provide a reliable and rapid means of assessing pain in mice and rats. "Grimace scores" have been developed for these species based on changes in facial expressions. They should only be used with the animal awake (Sotocinal et al., 2011). There are 4 elements tracked in determining the pain score in rats: orbital constriction, flattening of the nose and cheeks, changes in the ears (rounded shape, tilting outwards), and change in whisker position (tendency to the crowd).

Depending on the intensity of these changes, the degree of pain is noted with numbers from 0-2; thus, "0" represents the absence of pain, "1" represents moderate pain and "2" refers to a prominent, intense pain. During the study, different pain levels were noticed at every session of clinical evaluation; the results are presented in Table 4.

Table 4. Results of clinical evaluation of the pain for each group during the evaluation days; PDT=photodynamic therapy

Groups	Change in whisker position	Flattening of the nose and cheeks	Changes in the ears	Orbital constriction (frowning)
DAY →	0, 10, 17, 24, 31	0, 10, 17, 24, 31	0, 10, 17, 24, 31	0, 10, 17, 24, 31
Control group	0, 2, 2, 1, 2	0, 2, 2, ,2 ,2	0, 2, 1, 2 , 2	0, 1, 2, 1, 1
1x PDT group	0, 2, 1, 0, 1	0, 1, 0, 1, 1	0, 2, 1, 1, 1	0, 2, 1, 0, 1
3x PDT group	0, 2, 1, 0, 0	0, 1, 1, 0, 0	0, 1, 1, 1, 0	0, 1, 0, 0, 0

As the results presented in Table 4 indicate the presence of various pain levels, it can be observed that the pain is reduced as the PDT is applied every 7 days, and the change is considerable; the best evolution is seen in the group with 3 sessions of PDT.

Body Mass Exam

The body weight fluctuation was monitored daily after the ligature placement for 4 consecutive days and another 4 consecutive days after performing the root and scaling procedure. This represents an essential parameter of this research because appetite and body weight directly affect oral diseases. This monitoring was realized in order to establish whether there is necessary the implementation of a supportive therapy or to adjust this therapy. Figure 6 is represented the fluctuation of the body weight for the control group, showing the average body weight for each clinical evaluation session (composed of 4 weight checks in 4 consecutive days).

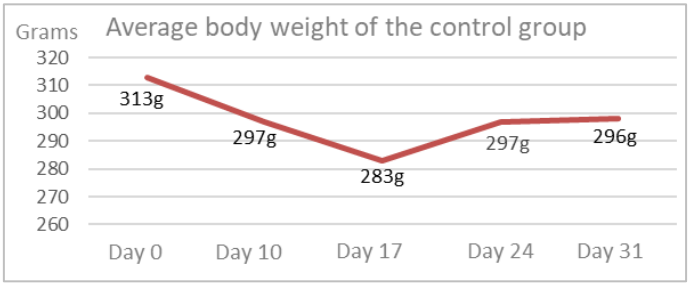

Figure 6. Body weight fluctuation was expressed in grams for the control group during the evaluation session; the value expressed in the graphic represents the average body weight made from the checks realized in the 4 consecutive days within an evaluation session.

Compared with the results of the other two groups, the body weight in the control group decreased as periodontitis lesions settled. However, once these injuries persisted during the experiment, the rats could no longer gain weight, so supportive therapy and a hyper caloric diet were implemented.

For the two groups treated with one and three sessions of PDT, the body weight decreased, and the oral lesions were settled, but an increase of body weight is observed as the therapy sessions were applied. In Figure 7, there is a noticeable change in the body weight after 10 days after the first PDT applied

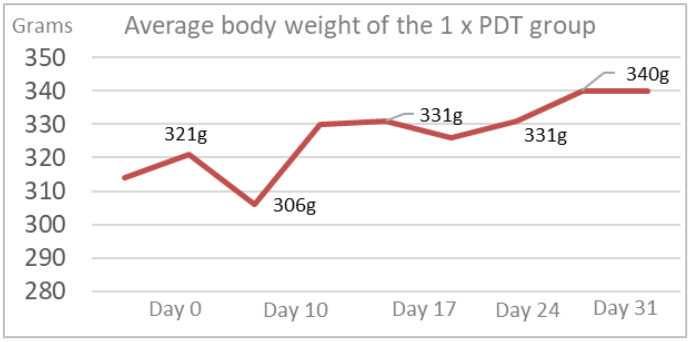

Figure 7. Body weight fluctuation expressed in grams for the 1 x PDT group during the evaluation session; the value expressed in the graphic represents the average body weight made from the checks realized in the 4 consecutive days within an evaluation session.

After 3 sessions of PDT applied, the body weight has a remarkable increase, especially after the second application of PDT in the 17th day (Figure 8), the body weight at the end of the therapy is higher than the initial weight before starting the study which means that PDT had a positive effect for the oral lesions because the prehension capacity of the animal is intensely affected and this therapy regenerates the tissue and stimulates the appetite, having a benefic response.

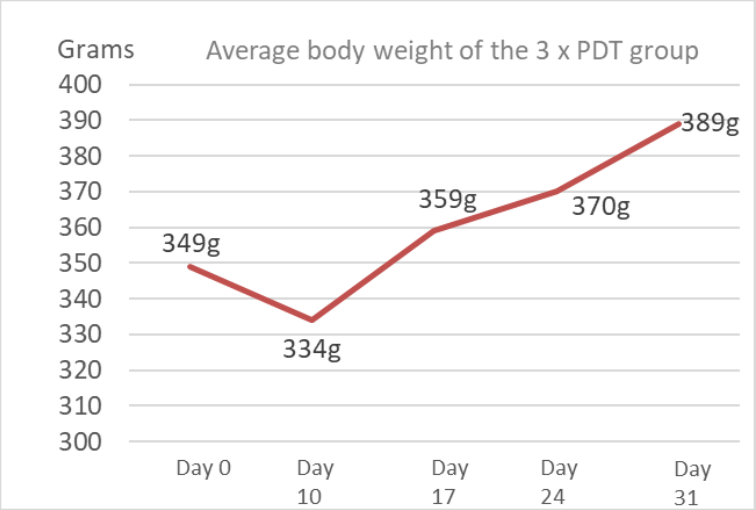

Figure 8. Body weight fluctuation expressed in grams for the 3 x PDT group during the evaluation session; the value expressed in the graphic represents the average body weight made from the checks realized in the 4 consecutive days within an evaluation session

Microscopical analysis

At the end of this study, the rats were euthanatized in order to realize the necropsy of the oral cavity, respectively the left maxilla from each rat was taken for the histopathological analysis in order to confirm the establishment of periodontitis because this is the standard gold method (for the control group) and also to check the histopathological aspects for the groups treated with PDT in order to inspect the efficacity of the implemented protocols. Grossly, yellow discoloration of the molar, mobility within the alveolus, gum reactivity, and food debris have been observed for the control group. However, for the other two groups treated with implemented protocols, the macroscopic aspect was different, no food debris was noticed, the color of the molar was whiter, the laxity presented differences between the groups but was improved, slight fibrosis of the gum was observed.

Regarding the histological analysis, chronic and superficial focal gingivitis was noticed in the control group (Figure 9, A.).

Inflammation was present in the interdental space, also gum retraction was observed. The dental ligament (A) is expanded by granulation tissue (A), and the gingival is focally ulcerated (C) and infiltrated by many mononuclear leukocytes. Lesions such as bone remodeling consisting of osteogenesis and osteoclastic resorption (B), thinning, and demineralization could be seen in the maxilla of the control group. On the inflammation site, a superficial layer of bacteria, food debris and suppuration processes were observed, provoking the segmental osteoclastic resorption (B). In the subgingival region, a layer characterized by granulation tissue was present (B), delimiting the infectious process. A progressive mononuclear cell infiltrative process (C) and the presence of osteoclasts (B) demonstrated the bone rarefaction and demineralization, confirming that periodontitis was well installed.

For the group treated with only one session of PDT, the dental ligament is expanded by granulation tissue (Figure 10 - A, B), the gingiva is focally ulcerated and infiltrated by a few mononuclear leukocytes, with the presence of hyperplasia and hyperkeratosis.

Thus, a mild suppurative process with degenerated neutrophils, reactive fibroblasts and slight segmental osteoclastic resorption of the hard tissue was noticed. However, in comparison with 3 x PDT administrations group, for this group, the gingival ulceration is absent, and the subgingival inflammation is minimal; no suppurative process was observed, only fibroblasts and partially oriented fibro-vascular connective tissue.

Because fluctuations of body weight were noted, decreasing more than usual and the pain levels were increased, supplementary injectable therapy was assured to manage and control the pain for the rats in need. Analgesia was provided by administering Tramadol in a dose of 10 mg/kg body weight subcutaneously. Also, to implement a supportive therapy, administration of 1 ml NaCl 0.9% combined with 1 ml Glucose 10%, was administered subcutaneously. These procedures were performed to maintain the welfare status of the rats at a high level throughout the experiment. Another way of managing the loss of appetite and loss of body mass involved

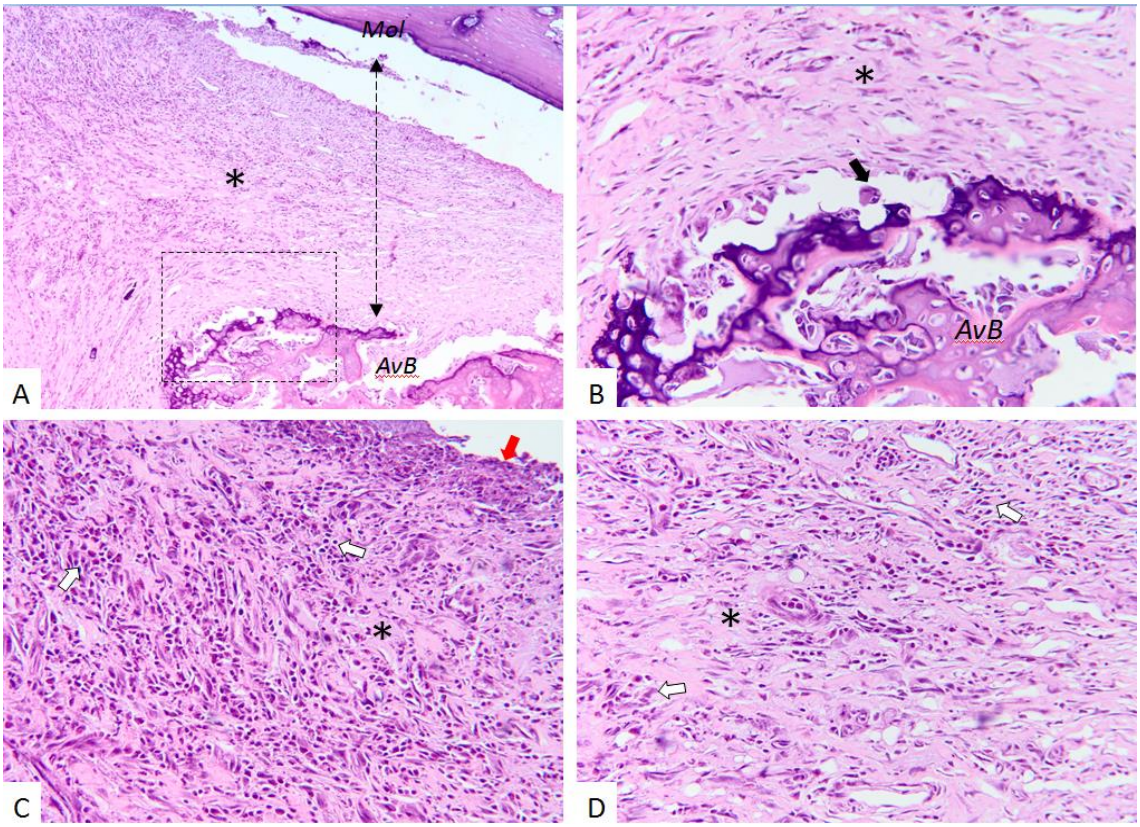

Figure 9. Sham Group. The dental ligament (marked by the double-edged arrow) is expanded by granulation tissue (*), and the gingival is focally ulcerated (red arrow) and infiltrated by many mononuclear leukocytes (marked by the white arrows). In addition, the alveolar bone (AvB) shows major remodeling, consisting of osteogenesis and osteoclastic resorption (black arrow). HE, x 10 (image A) and ob x40 (images B, C, and D). Mol=molars, AvB=Alveolar bone.

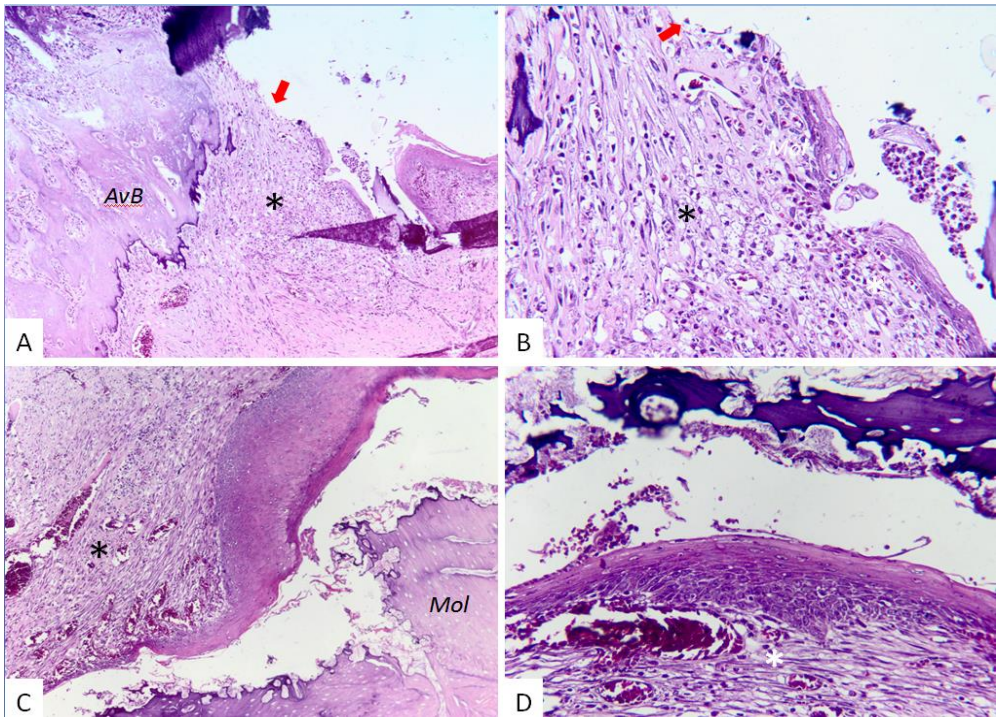

Figure 10. 1 x PDT administration group (images A and B). The dental ligament is expanded by granulation tissue (marked by an asterisk), and the gingiva is focally ulcerated (red arrow) and infiltrated by a few mononuclear leukocytes. 3 x PDT administrations group (images C and D). The gingival ulceration is absent, and the subgingival inflammation is minimal (asterisk). HE, of x 10 (images A and C) and ob x40 (images B, and D). Mol=molars, AvB=Alveolar bone.

the administration of a special diet consisting of pellets with a soft, crumbly texture, which was palatable, hypercaloric and hyperlipidemic, thus ensuring optimal nutritional intake. Of course, this food was not provided discreetly not to influence the results of the clinical evaluations. However, it was administered only to the rats that required assistance, their prehension being affected, and unable to consume the usual food, especially after the painful operations.

CONCLUSIONS

In the present study, we successfully demonstrate the experimental induction of periodontitis in 15 rats by correctly placing the cotton ligature on the molar for ten days, which was not removed during the induction, creating local inflammation and specific lesions of periodontitis. Furthermore, we also demonstrated that experimental periodontal disease has general systemic biological implications, poor body conditions (respiratory disorders, stress diarrhea, etc.), decreased body weight - secondary to loss of prehension and increased oral pain, also investigating a correlation between periodontal disease and general health, for this reason, we closely followed the discomfort created by inspecting the grimace of the rats.

This experimental protocol followed exactly the surgical steps by performing ligatures and scaling and root planning demonstrated macroscopic and microscopic lesions. This was created to clearly explain all the steps necessary to achieve successful experimental periodontitis and to emphasize the importance of the number of applications in terms of photodynamic therapy. The differences in the protocols between the two photodynamic therapies used consisted of the number of exposures and the device settings. Even though compensation was attempted by changing the settings of the first protocol that involved a single photodynamic exposure, it was clearly shown that at least 3 exposures are required to reverse the effects of our long-term induced periodontal process. At the clinical examination, in the groups treated with photodynamic therapy, the clinical signs of periodontitis were reduced during the treatment period, in some cases even reaching complete healing.

The histopathological examination demonstrated the usefulness of photodynamic therapy by preventing bacterial superinfection, respectively the appearance of a suppurative process at the local site, highlighting its antioxidant, anti-inflammatory and antibacterial properties in the studied pathology.

According to all the results obtained in this study, it is confirmed. Therefore, it can be recommended as a useful method of treatment of periodontitis, especially the use of photodynamic therapy and definitely more effectively, according to the literature, is the addition of biomaterials in the therapeutic protocol.

The tested protocol can provide all the key biological factors present in periodontal disease, while this study presents the appropriate characteristics for the field of biomaterials testing. Another main objective of this study is to demonstrate the efficacy of regenerative therapy with photodynamic therapy, reversing all the effects of periodontitis induced by the initially placed ligature.

Author Contributions: A.D., S.M., C.R., and R.D. conceived and designed the analysis; S.M., C.D., and R.D. collected the data; S.M., A.D. and C.R. were involved in the design of the study and the implementation of the study at USAMV. A.R.P., B.S. and S.P. contributed with analysis tools, and statistical analysis; F.T., R.P. and S.M. performed the necropsy and the histological examination; S.M. and A.D. wrote the paper. L.O. was involved in the supervision of the Ph.D. study.

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