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Periodontitis causes abnormalities in the liver of rats

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Abstract

Background: Periodontitis not only causes injury to the periodontium, but also damages other tissues such as: articulate, renal, cardiac and hepatic. The objective of this study was to investigate periodontitis induced alterations in liver function and structure using an experimental model.

Methods: Twenty female rats (*Rattus norvegicus*) were allocated into two groups: control and periodontitis. Gingival bleeding index and oxidative stress parameters and specific circulating biomarkers were measured. Immunohistochemistry was carried out using alkaline phosphatase (AlkP) staining of the liver. Hepatic tissues, cytokines and lipid contents were measured. Histopathological evaluation of the liver was carried out using light and electron microscopy.

Results: Liver histopathological and immunohistochemistry assessment showed increase in steatosis score, and presence of binucleate hepatocytes and positive cells for AlkP in periodontitis vs. control group. Ultrastructural evaluation showed significant increase in size and number

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Authors' contributions

ACCGV, DFPV contributed with the conception, design, data acquisition, analysis, and interpretation; they also drafted and critically revised the manuscript. FRPS, LFCF, EHPA, DL and LSP contributed to the part of the experiments, data acquisition, and interpretation. PDN, FSM, ALRB, FSMR, JVRM contributed with part of the experiments. AM and APO contributed critically by revising the manuscript. All authors gave final approval and agreed to be accounted for all aspects of the work.

Conflict of interest

The authors declare there is no conflict of interest.

of lipid droplets (LD), distance between the cisterns of rough endoplasmic reticulum (RER), mitochondria size, foamy cytoplasm and glycogen accumulation in the liver of the periodontitis group compared with the control group. In addition, plasma levels of AlkP, high-density lipoprotein (HDL), triglycerides and total cholesterol were also changed.

Conclusion: Experimental periodontitis caused immunohistochemistry, histopathological, ultrastructural, oxidative and biochemical changes in the liver of rats.

SUMMARY SENTENCE:

A relationship between periodontitis and non-alcoholic fatty liver disease (NAFLD) has been suggested, but not yet established. Our findings are of great significance for dentists and periodontists, since this is a two-way relationship and abnormal liver function may be associated with periodontitis despite normal liver function tests.

Keywords

periodontal diseases; liver; oral medicine; cytokines; inflammation

Introduction

Periodontitis is an infectious and inflammatory disease, affecting 30–45% of the adults and is an important problem in the world associated to the oral diseases.^{1,2} Tissue damage caused by periodontitis results from imbalances in the host response caused by bacteria, periodontal pathogens and other end-metabolites that stimulate the over-production of inflammatory mediators. Reactive oxygen and nitrogen species (ROS/RNS) released by defense cells in response to bacteria, periodontal pathogens, end-metabolites and lipopolysaccharides (LPS) contribute to periodontal damage.^{3–5}

Periodontitis not only causes injury to the periodontal tissues but also damages other tissues such as: articulate,⁶ renal,⁷ cardiac⁸ and hepatic.^{9–12}

The non-alcoholic fatty liver disease (NAFLD) is characterized by fatty liver accumulation in more than 5% of the hepatocytes in the absence of excessive ethanol ingestion.¹³ NAFLD is estimated to affect 30% of the general population around the world.¹⁴ Abnormalities in the metabolism of carbohydrates and lipids are hallmarks of NAFLD.¹⁵ Oxidative stress is one of the key elements associated to the pathophysiological mechanism of inflammatory conditions that happen in NAFLD.¹⁶ Furthermore, cytokines such as interleukin-1 β (IL-1 β) and tumor necrosis factor- α (TNF- α) can accelerate the accumulation of fat in the liver, augmenting the inflammatory process and promoting the evolution to fibrosis or liver cirrhosis.^{16,17}

In addition, gut microbiota have been implicated as an emerging cause of NAFLD.^{18,19} In this regard, some studies have demonstrated that bacteria associated with periodontitis such as *Porphyromonas gingivalis*¹⁰ and *Aggregatibacter actinomycetemcomitans*²⁰ may influence the development/progression of NAFLD. These and other bacteria associated with periodontitis present LPS in their cell wall, which can be catalyzed by alkaline phosphatase (AlkP), a superfamily of metalloenzymes, removing the lipid A phosphates of LPS.²¹

This catalytic process reduces the affinity for Toll-like receptor 4.²² In addition, AlkP can regulate the uptake of lipoproteins and act as a marker for liver diseases.^{23,24}

Several tools for the clinical diagnosis of NAFLD have been developed, including biomarkers and imaging techniques, but the gold standard for diagnosis is still histopathological assessment.²⁵ Besides fatty liver accumulation into the hepatocyte, hepatocytes of the fatty liver may present one or more nuclei, a feature that can be associated with cell division. It has been shown that oxidative stress may cause cytokinesis failure, leading to incomplete cell division upon and binucleation.²⁶ In addition, oxidative stress is associated with ultrastructural changes in the hepatocytes of liver diseases.^{27,28}

We have demonstrated previously that the ligature-induced periodontitis caused microvesicular steatosis and this process can be reverted after the removal of the ligature from the teeth.¹¹ Another study from our research team demonstrated that the pericytes were associated with changes in the hepatic tissues.¹² Although this association between periodontitis and NAFLD has been proposed,^{9–12,29,30} several points still remain unclear.

No study has evaluated immunohistochemistry to cells positive for alkaline phosphatase (AlkP), cytokines (TNF- α and IL-1 β) and the evaluation for histopathological (binucleate hepatocytes) and ultrastructural components in liver. Additionally, we assessed liver oxidative stress, lipid levels, and blood biomarkers. In order to clarify these points, the model of periodontitis induced with ligature was used in this study.

Material and Methods

Animals

This study was approved by the Ethics Committee of the Federal University of Piauí (384/17). Twenty female Wistar rats (222.4 $\pm$ 4.8g) were kept in an air-conditioned room at 23–25°C, relative humidity of 50–70%, and 12h dark/light cycle. The rats had free access to food and water and were maintained for one week for acclimation before the experiment.

Experimental Design

Rats were randomly divided into two groups (10 animals per group, female): control (no ligature) and periodontitis (rats with ligature). Experimental periodontitis was performed with intramuscular solution of 2% xylazine hydrochloride (15 mg/Kg)[#] and ketamine (40 mg/Kg)^{**}. A nylon ligature 3–0,^{††} was inserted around the first lower molar of each animals bilaterally.^{12,31} After 20 days,^{11,12} rats were euthanized and blood was collected for biochemical tests. Following, the liver and body weights were measured in total (g) to calculate liver index (% , liver weight $\times$ 100/total body weight).

[#]Rompum-Bayer®, São Paulo, SP, Brazil.

^{**}Francotar-Virbac®, Roseira, SP, Brazil.

^{††}Shalon®, Goiania, GO, Brazil.

Gingival Bleeding Index (GBI) and assay for Myeloperoxidase (MPO)

The score was evaluated according to Liu et al.¹⁴ in score 0, 1, 2, 3, 4, and 5 for the 1st lower molars bilaterally. For MPO, the assay was performed following that described by Chaves et al.³²

Mobility (M) of 1st lower molars assessment and Probing Pocket Depth (PPD)

The mobility of the first mandibular molars was measured with a periodontal probe in the labiolingual direction and scored as: 0, physiological mobility; 1, slight mobility; 2, moderate mobility; 3, severe mobility.³³ Values of PPD were evaluated with round-ended probe, tip 0.4 mm in diameter.¹⁴

Alveolar Bone Loss (ABL)

The assessment of ABL followed Vasconcelos et al.¹² and images captured were evaluated with an image analysis system.^{‡‡}

Histopathological evaluation of the liver

The livers of the rats were collected from the left lobe and routinely processed according to Vasconcelos et al.¹²

Steatosis score was categorized, according to the percentage of fatty hepatocytes distributed in the liver, as: 1, 0<25%; 2, 26<50%; 3, 51<75%; and 4, >76%. Inflammation and necrosis score were graded as: one focus per field, 1 and two or more focus per field, 2.^{31,34}

Regarding the number of binucleate hepatocytes, we counted an area of 45,000 μm^2 of hepatic tissue with light microscopy.^{§§} For the assessment of mast cell density (DMCS), the total number of positive cells on toluidine blue stained was counted according to Franceschini et al.³⁵

Immunohistochemistry for alkaline phosphatase (AlkP)

Hepatic tissues were stained for AlkP and the intensity was measured for an area of 45,000 μm^2 . After routine histological preparation, paraffin was removed and endogenous peroxidase was blocked with 5% H_2O_2 in water. After blocking non-specific reactivity with bovine serum albumin (3%) in phosphate-buffered saline, goat polyclonal primary antibody (1% bovine serum albumin/ phosphate-buffered saline): anti-rat AlkP^{|||} 20 $\mu\text{g}/\text{ml}$ was applied to the sections, overnight at 4 °C. After that, the sections were washed in phosphate-buffered saline and incubated with biotinylated secondary antibody,^{¶¶} followed by streptavidin–peroxidase complex^{¶¶} for 40 min at 37.5 °C. Diaminobenzidine solution (3,3 diaminobenzidine) was used for brown staining in the sections and hematoxylin was used for counterstaining.

‡‡ImageJ v.1.48 Software.

§§NOVA®, Piracicaba, SP, Brazil.

|||Sigma, St. Louis, MO, USA.

¶¶LSAB2 system, DAKO, Glostrup, Denmark.

Transmission Electron Microscope (TEM) assessment of hepatocytes

Liver pieces with 1 mm³ of medial and lateral lobes were processed according to López-Navarro et al.³⁶ Sections were viewed and photographed using a transmission electron microscope^{##} at 80 kV. Digital images were captured using a digital camera system.^{***} In order to evaluate Lipid Droplets (LD), we counted and measured images from the livers of both groups on the 6,000x magnification of each photomicrograph containing at least one hepatocyte nucleus, according to Watanabe et al.³⁷ In addition, the distance between the cisterna of rough endoplasmic reticulum (RER) and the size of mitochondria were measured on the 15,000x magnification. The foamy cytoplasm was performed using a 100-point mesh in an area of 1,170 µm² on the 8,000x magnification. The glycogen accumulation was scored in: 0, physiological storage; 1, slightly accumulated storage; 2, moderately accumulated storage; and 3, severely accumulated storage. All ultrastructures examined were based on Ghadially.³⁸ All images were analyzed using an image analysis system.^{††}

Quantitative enzyme-linked immunosorbent assay (ELISA)

For the evaluation of NO₃/NO₂ concentration, the liver homogenate was incubated in a microplate with nitrate reductase for 12 hours to convert nitrate (NO₃) into nitrite (NO₂). Nitric oxide production was determined by measuring nitrite concentrations in an ELISA plate reader at 540 nm.³⁹

Regarding the cytokine measurements for IL-1β and TNF-α, samples of hepatic tissue were collected and homogenized in sterile saline. Subsequently, the IL-1β and TNF-α levels were performed using ELISA kits according to the manufacturer's recommendations. The homogenates were centrifuged at 0.8g at 4°C for 11min, and supernatants were stored at -70°C until further analysis.

For blood biomarkers, levels of AlkP, aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma glutamyltranspeptidase (GGT), glucose, total cholesterol, high-density lipoprotein (HDL), and triglycerides were measured. For the liver evaluation, concentration of total cholesterol and triglycerides were measured according to Folch Less and Sloane Stanley,⁴⁰ and both tests for blood and liver used commercial ELISA kits.^{†††}

Glutathione (GSH), malondialdehyde (MDA) and glucose levels

In order to investigate the systemic effect of periodontitis on hepatic oxidative stress, we used GSH levels, which were measured according to Sedlak et al.⁴¹

The MDA levels were used as an indicator of lipid peroxidation. The MDA concentration for the liver was measured according to Mihara et al.⁴²

In order to evaluate the random glucose levels in the blood, we used a glucometer,^{†††} previously to the euthanasia of the animals.

^{##}JEM 1400, JEOL, Japan.

^{***}Gatan, Pleasanton, CA, United States

^{†††}Labtest®, Belo Horizonte, MG, Brazil.

^{†††}Accu-Chek Active, São Paulo, SP, Brazil.

Statistical Analysis

The results were expressed as mean or mode ($\pm$ S.E.M. and/or minimum-maximum). Kolmogorov-smirnov test was used to check the distribution of the data. Differences between the groups were examined with the Mann-Whitney U test, for data with non-normal distribution and with the unpaired T-test for normally distributed data. * $p < 0.05$.

Results

GBI, mobility of 1st molar, MDA and MPO levels

The animals with experimental periodontitis (periodontitis group) presented altered color, severe edema and bleeding of gingival papilla compared to the control group (Figure 1 A and 1 B, control and periodontitis groups respectively). GBI (Figure 1 C), mobility of 1st molar (Figure 1 D), MDA (Figure 1 E) and MPO levels (Figure 1 F) were significantly higher in the animals with experimental periodontitis than in the animals without experimental periodontitis, as seen in figure 1.

Evaluation of ABL and PPD

The periodontitis group presented significantly increased values for ABL (Figure 1 G) and PPD (Figure 1 H).

GBI, M, MDA, MPO, sum of ABL and PPD values showed robust evidence of the presence of periodontitis, supporting subsequent systemic assessments.

Body, liver weights, blood and liver biomarkers

The body and liver weights did not present difference ($p > 0.05$) between the groups (Table 1).

Biomarkers, AlkP, HDL, triglycerides and total cholesterol were significantly changed in the periodontitis group in comparison with the control group (Table 1). Plasma GGT, AST, ALT and glucose presented higher levels in the periodontitis group than in the control group ($p > 0.05$). In addition, hepatic and plasma levels of total cholesterol and triglycerides were significantly higher in the periodontitis group compared to the control group (Table 1).

NO₃/NO₂, MDA, GSH and MPO content in liver

In the hepatic tissue, NO₃/NO₂, MDA and MPO concentrations were shown to be significantly increased in the periodontitis group, compared with the control group (Table 1). Hepatic oxidative stress, assayed by GSH levels, was significantly decreased in the periodontitis vs. control group (Table 1).

Cytokine measurements for IL-1 β and TNF- α

As shown in the table 1, IL-1 β and TNF- α levels in the liver were not significantly different between the groups.

Histopathological and Immunohistochemistry hepatic tissue assessment with light microscopy

Control group presented normal histological structure for the hepatic tissue evaluation. (Figure 2 A and C). On the other hand, liver tissues from the periodontitis group demonstrated marked histopathological changes, such as: hepatocytes with microvesicular steatosis, loss of cordon organization near the central vein and congestion in sinusoid blood vessels (Figure 2 B and D). AlkP positive cells were significantly increased in the periodontitis group compared to the control group (Figure 2 E, F and I). In addition, the periodontitis group had higher liver steatosis score (Figure 2 G) and binucleated hepatocytes (Figure 2 H), compared to control group. There were no significant differences in inflammation degree, necrosis and DMCS count between the groups (Inflammation, 1 (1–2) for control group, 1 (1–2) for periodontitis group; necrosis, 1 (1–1) for control group, 1 (1–1) for periodontitis group; DMCS count, 1.7 ± 0.5 for control group, 1.8 ± 0.5 for periodontitis group; $p > 0.05$).

Transmission Electron Microscope (TEM) assessment of hepatocytes

The periodontitis group caused a significant increase in size and number of the LDs per hepatocytes, compared to the control group (Figure 3).

As for the assessment of the RER, figure 4 A represents normal RER in the control group. Changed RER is shown in the figure 4 B, illustrating the dilation of cistern of the RER for the periodontitis group, 75.8% higher (Figure 4 C).

The mitochondria showed normal structures for the control group (Figure 4 A). In contrast, mitochondria appear significantly larger in the periodontitis group (Figure 4 D). In addition, the mitochondria of the periodontitis group were presented as less electron-dense with less distinct cristae (Figure 4 B).

In relation to the ultrastructural evaluation of smooth endoplasmic reticulum (SER) into hepatocyte, normal SER from the control group was observed (Figure 5 A and B). On the other hand, in the cytoplasm of the hepatocyte from the periodontitis group, there was the appearance of abundant foamy cytoplasm (Figure 5 C and D). Foamy cytoplasm stem from the proliferation of SER (Figure 5 D and E) and glycogen accumulation (Figure 3 D), and a significant difference was observed between the groups (Figure 5 E for foamy cytoplasm and figure 5 F for glycogen).

Discussion

Our study was designed to provide a better understanding for the association between periodontitis and liver abnormalities. Herein, we showed with robust data relevant changes in the liver of animals with ligature-induced periodontitis. This is the first study in the literature, showing the development of significant alterations in the histopathology of the liver, including increase in number of binucleate and AlkP positive hepatocytes in experimental periodontitis compared to control group. In addition, our results demonstrated significant increase in size and number of LDs, distance between the cisterns of RER, mitochondria size, foamy cytoplasm and glycogen accumulation as well as for NO_3/NO_2

levels in the liver of periodontitis group vs. control group. Besides, serum AlkP levels were significantly higher in the periodontitis group.

Our research team has previously demonstrated microvesicular steatosis in rats with periodontitis,^{11,12} but the increase in the number of binucleate hepatocytes in animals with periodontitis had not been previously reported in the literature by then. Nonetheless, binucleate hepatocytes stand out as an attempt by damaged hepatocytes to regenerate.⁴³ A strategy of our study was to use female rats to obtain higher number of binucleate hepatocytes per gram of liver caused by sexual dimorphism.⁴⁴ This approach possibly increased the success of our investigation. Testing the reproducibility of our data using male rats might be interesting.

Regarding measures of steatosis ($p<0.05$), the mean values for inflammation and necrosis scores were higher for rats with periodontitis than for rats without periodontitis, ($p>0.05$), which is consistent with previous studies^{11,12}, again confirmed here by our research team. We did not find macrovesicular steatosis or hepatic fibrosis in the livers. MPO data together with the cytokines levels for IL-1 β and TNF- α demonstrated no significant increase between the groups, indicating that the process of steatosis caused by periodontitis was light in comparison with steatohepatitis, in which the inflammatory process is evident, with significant elevated levels of proinflammatory cytokines.^{17,45}

We have previously demonstrated that the microvesicular steatosis is associated with higher levels of GSH and MDA caused by periodontitis.^{11,12} In this study, for the first time, in NO₃/NO₂ levels, we demonstrated that this marker was higher in the periodontitis group than in the control group. These elevated levels brought together several findings from our study. A study showed that hepatocytes do not complete cell division upon oxidative stress, leading to cytokinesis failure and binucleation,²⁶ as observed in our study. We believe that an eventual increase in binucleate hepatocytes points out to compensatory proliferation associated with liver injury caused by periodontitis.

This study is the first in the literature to describe ultrastructural changes in the liver associated with periodontitis. It was possible to observe the main characteristic of steatosis, which is the fatty accumulation in the hepatocytes. The increase in oxidative and NO₃/NO₂ levels caused the surplus release of lipid peroxidation products,⁴⁶ as also demonstrated by our data with elevated MDA hepatic levels in rats with ligatures. In addition, the data of total cholesterol and triglycerides in the blood as well as in the liver (Table 1) supported this finding. In order to evaluate the ultrastructure of LDs in hepatocytes, we used TEM, which enabled us to count the number and size of LDs. The periodontitis group presented LD size 92.3% larger than the control groups (Figure 3); this finding is similar to the other study that evaluated fatty accumulation in liver of rats treated with ethanol.⁴⁷ Another alteration observed by us is that the number of LDs was also higher (53.1%) in the periodontitis group (Figure 3). LDs occupy the cytoplasmic space of hepatocytes, diminishing cellular functions.⁴⁸ LDs are not just inclusions, but dynamic organelles that perform several biological activities and are in balance with blood lipids,¹⁵ according to our results for increased levels of total cholesterol and triglycerides both in blood and in the hepatic tissue.

We demonstrated previously¹¹ that the blood lipid profile of rats with periodontitis was similar to these data (Table 1). An emerging hypothesis is linked with the lipid metabolism and bacteria involved with periodontitis increasing the risk of the development/progression of NAFLD.^{10,20} Further studies could elucidate the association between the ligature-induced periodontitis and the mechanisms of the development of NAFLD. In addition, the ligature-induced periodontitis model used currently is the way of simulating the periodontitis-related bacteria in humans and their systemic effects.

In relation to the ultrastructural evaluation, we also observed that the mitochondria of hepatocytes from the periodontitis group were larger than those from the control group. Besides that, the mitochondria from rats with periodontitis were appeared as less electron-dense and less distinct cristae (Figure 4), indicative of degraded membrane constituents, as shown in rats with alloxan-induced diabetes.²⁸ Mitochondria play a key role in ATP production and the increase in oxidative stress is associated with the fall in ATP production, causing mitochondrial damage.⁴⁹ These findings are consistent with increased GSH, MDA and NO₃/NO₂ levels, as well as with ultrastructural alteration observed in the size of mitochondria.

We speculate that the high levels of AlkP in the rat liver is associated with LPS from bacteria involved in periodontitis,^{10,20} because AlkP mediates host-bacterial interactions through their capacity to dephosphorylate lipid A of the bacterial cell wall lipopolysaccharide.⁵⁰ In addition, AlkP activity is also related to lipid metabolism.²³ Our data regarding the AlkP are supported by previous findings and brought an association with the induced periodontitis still not reported in the literature.

The experimental periodontitis caused changes in the LDs, mitochondria and endoplasmic reticulum, both smooth and rough observed by TEM. The hepatocytes from rats with periodontitis showed significant dilation in the RER in comparison with rats without periodontitis, as observed in the figure 4. Similarly, SER also presented significant changes (Figure 5) that are associated with possible damages caused by imbalance of oxidative stress associated with periodontitis. The SER from rats with periodontitis demonstrated foamy cytoplasm (Figure 5 C, D and E), resulted from the proliferation and glycogen accumulation (Figure 3 D and 5 F). These novel findings of the ultrastructural changes in rat hepatocytes are similar to those found in human with NAFLD,²⁷ although these findings must be viewed with caution, because in the periodontitis model with ligature the inflammation is rapid in onset and severe.

Conclusion

In conclusion, we demonstrate here major changes in the liver structure and function of animals with periodontitis induced by ligature. This is the first study in the literature demonstrating a significant increase in the number of binucleate and AlkP positive hepatocytes, increase in size, number of LDs, the distance between the cisterns of RER, mitochondria size, foamy cytoplasm and glycogen accumulation in the liver of rats with periodontitis.

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Figure 1.

A) represents clinical aspect from control group, tongue is represented in T and the first molar by arrow. B) Clinical aspect of the periodontitis group, presenting gingival papilla with alteration in color, severe edema and bleeding after slight probe. C) Gingival Bleeding Index (GBI) scores. D) Mobility (M) of the 1st molar. E) MDA level. F) Myeloperoxidase (MPO) levels. G) Sum of alveolar bone loss (ABL). H) Probing Pocket Depth (PPD). GBI scores, M, MDA and GSH levels, ABL and PPD presented significantly higher values in periodontitis group than control group. * $p < 0.05$.

Figure 2.

Liver in A) and C) demonstrating hepatocytes with normal organization surrounding the central vein (CV). Fatty liver (arrows) is illustrated in B) and D) for the periodontitis group, congestion in sinusoid blood vessels (circle) and binucleate hepatocytes (double arrow) are also observed. The periodontitis group presented higher values for G) steatosis score, H) number of binucleate hepatocytes and I) positive cell for AlkP compared with control group. E) illustrate positive cells for AlkP in the control group and F) in the periodontitis group. A and B) toluidine blue, C and D) hematoxylin and eosin, E and F) immunohistochemistry for AlkP, all photomicrographs are at 600x original magnification. * $p < 0.05$.

Figure 3.

A) Hepatocyte with normal ultrastructures from control group, white rectangle shows B) area at higher magnification, demonstrating normal aspect of mitochondria (M), rough endoplasmic reticulum (RER) and nucleus (N). On the other hand, C) the periodontitis group shows hepatocyte with increase in size and number of lipid droplets (LD), D) area at higher magnification, representing detail from LD and glycogen accumulation (arrows). Higher values for E) size and F) number of LD were observed in the periodontitis group rather than in the control group. E, erythrocyte; M, mitochondria; N, nucleus. Images A) and C) of TEM are at $\times 10,000$ original magnification, and B) and D), at $\times 15,000$ original magnification. * $p < 0.05$.

Figure 4.

A) Hepatocytes with normal mitochondria (M) and RER are observed in the control group. B) illustrates hepatocyte with dilatation of RER and mitochondria larger in the periodontitis group, when compared with control group; in addition, the mitochondria of periodontitis group were presented as less electron-dense with less distinct cristae. Periodontitis group presented C) dilation of cistern of the RER and D) larger mitochondria in comparison with the control group. LD, lipid droplets; N, nucleus; RER, rough endoplasmic reticulum. Images of TEM are at $\times 15,000$ original magnification. * $p < 0.05$.

Figure 5.

A) Two normal hepatocytes are observed in the center of the image of the control group, B) white rectangle shows the area at higher magnification, demonstrating normal RER, rough endoplasmic reticulum (RER), N, nucleus. C) shows hepatocytes with abundant foamy cytoplasm (FC) from the periodontitis group. D) presents the area from the white rectangle with higher magnification, demonstrating details of FC in the hepatocyte from rats with periodontitis. Periodontitis caused increase in the E) FC and F) in the glycogen storage into hepatocytes compared with hepatocytes from the control group. N, nucleus. Images A) and C) of TEM are at $\times 2,500$ original magnification, and B) and D), at $\times 8,000$ original magnification. * $p < 0.05$.

Table 1.

Weight parameters, blood and liver biomarkers.

Groups	Control	Periodontitis
Weight parameters		
Body (g)	218.4 ± 5.0	226.5 ± 4.6
Liver		
Relative (%)	3.2 ± 0.1	3.0 ± 0.1
Absolute (g)	7.1 ± 0.3	7.0 ± 0.4
Blood biomarkers		
AlkP (U/dL)	46.1 ± 6.9	67.9 ± 5.7 *
AST (U/dL)	67.2 ± 2.3	87.6 ± 6.1
ALT (U/dL)	28.5 ± 2.5	34.9 ± 1.7
GGT (U/dL)	10.1 ± 2.5	15.4 ± 3.8
Glucose [#] (mg/dL)	268.1 ± 14.5	259.6 ± 18.6
Total cholesterol (mg/dL)	62.4 ± 2.2	80.3 ± 4.9 *
HDL (mg/dL)	77.4 ± 3.0	38.2 ± 1.4 *
Triglycerides (mg/dL)	58.1 ± 3.8	84.7 ± 3.6 *
Liver biomarkers		
Total cholesterol (mg/dL)	2.4 ± 0.4	5.2 ± 1.0 *
Triglycerides	97.2 ± 5.0	134.3 ± 11.8 *
IL-1β (pg/mg)	307.5 ± 7.6	302.7 ± 13.1
TNF-α (pg/mg)	239.4 ± 4.1	247.4 ± 3.7
NO ₃ /NO ₂ (mM)	0.2 ± 0.0	0.3 ± 0.0 *
MDA (mmol/g of the tissue)	84.0 ± 3.7	122.0 ± 9.7 *
GSH (μmol/g of the tissue)	461.7 ± 37.9	150.0 ± 45.3 *
MPO (mmol/g of the tissue)	3.2 ± 0.2	3.7 ± 0.3

* $p < 0.05$ comparing to periodontitis versus control groups (unpaired T-test).

Results were expressed as mean ± S.E.M.

[#] Random Blood Glucose.