

Melatonin protects liver and periodontal tissues from inflammation exacerbated by metabolic syndrome–periodontitis association.

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Abstract

Metabolic syndrome (MetS) and periodontal disease (PD) are prevalent inflammatory conditions with global health implications. Their bidirectional interaction amplifies systemic and metabolic inflammation, worsening periodontal destruction while PD exacerbates metabolic disturbances. This study evaluates the combined impact of MetS and PD on periodontal and hepatic health and examines melatonin (MEL) as a therapeutic agent. Male Wistar rats were assigned to Control, MetS, PD, MetS + PD, and MetS + PD+MEL groups. MetS was induced with 10% fructose intake for 35 days and PD by ligature placement for 4 days. MEL (10 mg/kg) was administered to assess its anti-inflammatory effects. Body weight, glycemia, lipid profile, and liver enzymes were measured. Radiographic and histomorphometric analyses were performed on the jaw, and NLRP3 and IL-10 expression assessed by immunohistochemistry in jaw and liver. MetS + PD animals showed aggravated hyperglycemia, dyslipidemia, hepatic injury, and severe periodontal destruction, with increased NLRP3 and reduced IL-10 levels. MEL attenuated these alterations, improving metabolic outcomes, preserving periodontal bone, restoring hepatic histology and enzyme levels, and modulating inflammation by decreasing NLRP3 and increasing IL-10. These findings indicate that MetS and PD synergistically intensify inflammatory and metabolic disturbances, and that MEL counteracts this exacerbated inflammatory burden emerging as a promising adjunct therapeutic candidate for systemic–oral inflammatory conditions.

Key message

- Metabolic syndrome and periodontitis synergistically exacerbate liver and periodontal damage.

Melatonin mitigates these combined metabolic and inflammatory alterations.

Melatonin protective effect is associated with reduced NLRP3 activation and restored IL-10 expression.

INTRODUCTION

Metabolic syndrome (MetS) encompasses a cluster of interrelated conditions, including abdominal obesity, dyslipidemia, hypertension, and insulin resistance, all driven by chronic low-grade systemic inflammation [1, 2]. This inflammatory state, marked by elevated proinflammatory cytokines like tumor necrosis factor- α (TNF- α), fuels insulin resistance and metabolic dysfunction. Among the major complications associated with MetS is non-alcoholic fatty liver disease (NAFLD), characterized by excessive hepatic fat accumulation [3]. NAFLD is a growing global health concern, closely linked to obesity and type 2 diabetes, and is now recognized as a leading cause of liver-related morbidity [4, 5]. Despite its high prevalence, approved pharmacological options for preventing or reversing NAFLD remain limited.

Periodontitis (PD), a chronic inflammatory disease affecting the supporting structures of the teeth, is a significant comorbidity, affecting nearly 50% of adults worldwide [6]. A robust bidirectional relationship exists: MetS's systemic inflammation and metabolic dysregulation exacerbate periodontal tissue

destruction, while PD's chronic local inflammation and bacterial products (e.g., lipopolysaccharides, LPS) enter the circulation, worsening metabolic parameters and accelerating disease progression [7–9]. This interplay fosters a self-perpetuating cycle that complicates their management.

To investigate this complex interaction, we utilize established experimental models: a high-fructose diet to induce MetS features, mimicking Western diets by promoting hepatic lipogenesis, insulin resistance, and systemic inflammation [10–12]; and ligature-induced periodontitis, which simulates bacterial biofilm accumulation and leads to alveolar bone loss [13].

The core mechanism linking MetS, PD, and NAFLD is chronic systemic inflammation and immune dysregulation [14]. A critical player is the activation of the NLRP3 inflammasome (NOD-like receptor family pyrin domain containing 3), which processes and triggers the release of the highly inflammatory cytokines interleukin-1 β (IL-1 β) and interleukin-18 (IL-18) [15–17]. NLRP3 activation is triggered by pathological signals from both MetS (high glucose, FFAs) and PD (LPS) and is implicated in NAFLD progression and PD severity [18, 19], highlighting its role as a key therapeutic target.

Melatonin (MEL), an indoleamine known for regulating circadian rhythms [20], has garnered significant interest due to its powerful antioxidant, anti-inflammatory, and immunomodulatory properties [21–23]. MEL's protective effects stem from neutralizing reactive oxygen species (ROS) and enhancing antioxidant enzymes (e.g., superoxide dismutase (SOD) and glutathione peroxidase (GPx)) [25]. Crucially, MEL regulates the NLRP3 inflammasome by inhibiting NF- κ B and MAPK signaling cascades, thereby reducing systemic inflammation [26, 27]. In models of steatosis, MEL limits NLRP3 activation, reduces liver inflammation, and maintains mitochondrial stability [28, 29], making it a promising candidate for NAFLD. Regarding PD, MEL not only counteracts inflammation but also enhances the osteogenic differentiation of stem cells and promotes bone mineralization [22].

It also plays a role in balancing pro- and anti-inflammatory cytokines, including IL-10 [30], supporting its potential as an adjunct therapy for chronic inflammatory diseases and potentially improving systemic conditions linked to PD, such as type 2 diabetes [31].

To our knowledge, no previous study has simultaneously evaluated hepatic and periodontal inflammatory responses in a combined MetS (10% fructose-supplemented diet) and PD (ligature placement) model while examining NLRP3 inflammasome modulation by MEL. Most investigations have addressed either isolated metabolic or periodontal models. The present study introduces an integrated dual-hit approach, allowing the characterization of systemic inflammatory crosstalk between oral and hepatic tissues and assessing whether MEL can interrupt this amplification loop. This integrative perspective provides novel mechanistic insight into the oral–liver axis under metabolic stress conditions.

MATERIALS AND METHODS

Animals and Ethical Approval

Ten-week-old male Wistar rats (250–300 g) were housed under controlled environmental conditions with a 12 h light/dark cycle and ad libitum access to chow and water. All procedures followed national and international regulations and were approved by the Institutional Committee for the Care and Use of Laboratory Animals (CICUAL), of the Schools of Dentistry and Medicine of the National University of Córdoba (UNC). Procedures requiring anesthesia were performed under isoflurane inhalation to ensure adequate analgesia and minimal distress, and animals were euthanized on day 35 with isoflurane overdose. Experiments were carried out at least in duplicate to ensure reliability and reproducibility.

Experimental Design

Rats were randomly assigned to five experimental groups, as shown in Fig. 1 (n = 6 per group). All determinations were performed in triplicate.

1. Control: Standard chow and potable water for 35 days.
2. MetS: 10% fructose in drinking water for 35 days [10].
3. PD: Ligature-induced PD on day 31 for four days, using a 3.0 cotton thread around the left lower first molar [32]; the contralateral molar was used as an anatomical reference for histomorphometric comparisons, as previously described in experimental models combining systemic metabolic alterations with ligature-induced periodontitis [12, 32]. Although systemic inflammatory changes may affect both sides, the ligature model induces a localized mechanical-biofilm challenge that results in asymmetric bone destruction, allowing intra-animal structural comparison.
4. MetS + PD: Combined induction of MetS and PD as described above.
5. MetS + PD+MEL: Animals received melatonin (10 mg/kg/day, infragingival) dissolved in 0.5% ethanol/saline from day 31 to day 35.

The study evaluated the inflammatory exacerbation induced by the coexistence of MetS and PD and melatonin's capacity to modulate this effect. A melatonin-only group was initially assessed but excluded, as it produced no measurable changes in healthy rats. Given the well-documented protective effects of melatonin in isolated models [28, 29, 31, 42], additional MEL-only or single-condition groups were omitted to avoid redundancy and unnecessary animal use, in accordance with the Reduction principle of the 3Rs. Our objective was not to reconfirm melatonin's isolated effects, but rather to determine whether it could attenuate the pathological amplification observed in a combined inflammatory–metabolic context.

Anthropometric and Biochemical Analyses

Body weight and abdominal perimeter were recorded. Visceral adiposity was quantified by weighing mesenteric and perirenal fat pads. Blood collected by cardiac puncture was analyzed for glucose, triglycerides, total cholesterol, HDL-cholesterol, GPT/ALT, and GOT/AST using enzymatic assays (Architect i1000, Abbott).

Radiological Analysis

Alveolar bone loss was assessed on dissected jaws using a digital radiovisiography system (Carestream RVG 5200). Radiopacity intensity was measured in the apical-root and root-furcation areas using Image Pro-Plus software.

Histological and Histomorphometric Analysis

Mandibular samples were fixed in 10% formalin (PBS), decalcified in 10% EDTA at 4°C for 30 days, and paraffin-embedded. Mesiodistal sections (7 µm) at the level of the first lower molar were obtained with a sliding microtome (Jung, Germany) and stained with H&E for histomorphometric evaluation. Interradicular images were captured using an Olympus BX-50 microscope (Sony CCP-370 camera) and analyzed for bone and periodontal thickness in four sectors using the furcation as a reference. All images were digitized with a Scanjet HP G-3110 scanner (2400 dpi) for quantitative analysis.

Immunohistochemical (IHC) Analysis

Mandibular and liver sections were deparaffinized, rehydrated, blocked with 0.5% hydrogen peroxide, and incubated with primary antibodies against NLRP3 (PA5-79740, Invitrogen) and IL-10 (ARC0102, Invitrogen) at 1:1000 (2 h at 37°C or overnight at 4°C). Detection employed a biotinylated secondary antibody and a streptavidin–peroxidase system (Mouse/Rabbit ImmunoDetector DAB HRP Brown, BioSB Inc.) with DAB visualization. After counterstaining and mounting in Permount, sections were examined using a Zeiss photomicroscope with a Canon Powershot 640 camera, and images were saved in CZI format. Positive cells were quantified in ten non-overlapping fields/sample using ZEN 3.6 Blue Edition, and results were expressed as the percentage of positive cells relative to the total field count, based on two independent blinded observers.

Statistical Analysis

All experiments were performed in triplicate. Statistical analyses and graphing were conducted in GraphPad Prism 9.5.0. Normality was assessed prior to analysis. Group differences were evaluated using one-way or two-way ANOVA with Tukey's post hoc test. Data are expressed as mean ± SEM, and $p < 0.05$ was considered statistically significant.

RESULTS

Characterization of the Association Between MetS and PD and the effect of MEL

To characterize the experimental model and evaluate the effects of MEL treatment, weight, abdominal perimeter, and biochemical parameters of glycemia and lipid profile were measured. Only the relevant

experimental groups are presented in the figures. The preliminary data from the melatonin-treated group are available in the supplementary material for reference.

Body Weight and Abdominal Perimeter

As shown in Fig. 2, the MetS group exhibited a significant increase in body weight and abdominal perimeter with increased white adipose tissue accumulation compared to the control group. These parameters were further exacerbated in the MetS + PD group, highlighting the compounded impact of both conditions. Notably, MEL treatment significantly reversed these alterations in the MetS + PD+MEL group, restoring values to levels comparable to the control group.

Glycemic Profile

Fructose administration induced marked hyperglycemia in the MetS group, as depicted in Fig. 3A. An increase in fluid intake (polydipsia) and urinary excretion (polyuria) was also qualitatively observed. These effects were significantly amplified in the MetS + PD group. However, treatment with MEL effectively reversed hyperglycemia in the MetS + PD+MEL group, indicating its therapeutic potential.

Lipid Profile

Figures 3B-D illustrate significant dyslipidemia in the MetS group, characterized by increased serum triglycerides and cholesterol levels, alongside decreased HDL cholesterol, compared to the control group. These disturbances were more pronounced in the MetS + PD group. Importantly, MEL treatment normalized these lipid parameters, restoring them to control levels.

These findings confirm that the experimental model of 10% fructose administration successfully induced metabolic syndrome, with further exacerbation observed when combined with experimental periodontitis. MEL treatment reversed these metabolic disturbances, suggesting its protective role against the synergistic effects of MetS and PD.

Bone Alterations in the Jaw

Radiographic Analysis

Radiographic images (Fig. 4A) revealed radiolucent areas and a widening of the periodontal space in the PD group, corresponding to mild marginal crest loss. In the MetS + PD group, more severe bone destruction was evident. Treatment with MEL mitigated these effects, as evidenced by the preservation of marginal bone crest integrity.

Histological and Histomorphometric Findings

Histological analysis (Fig. 4B) demonstrated inflammatory infiltrates, areas of bone resorption, and collagen matrix destruction in the MetS and MetS + PD groups. In contrast, these pathological features

were absent in the MetS + PD+MEL group, indicating a protective effect of MEL. Histomorphometric analysis corroborated these findings, showing that MEL preserved bone volume in the jaws (Fig. 4C).

Inflammatory and Anti-inflammatory Markers in the Jaw

Immunohistochemical analysis (Fig. 5A) revealed cytoplasmic expression of inflammatory marker NLRP3 in the MetS and MetS + PD groups. MEL treatment significantly reduced the percentage of NLRP3-positive cells to levels comparable to the control group.

Figure 5. **Inflammatory and anti-inflammatory markers in the jaw. A) Immunohistochemical detection of NLRP3** in rat mandibular tissue. **B) Immunohistochemical detection of IL-10** in rat mandibular tissue. Representative sections of the interradicular region of the first lower molar showing NLRP3 and IL-10 immunoreactivity in the different experimental groups. Positive staining (brown DAB) is indicated by a red arrow. Sections were counterstained with hematoxylin, visualized using a Zeiss photomicroscope, and analyzed with ZEN software. Barr graphs represent the percentage of positive cells in relation of total cell count in ten fields in CONTROL, Metabolic Syndrome (MetS), Metabolic Syndrome+Periodontal Disease (MetS + PD) and Metabolic Syndrome+Periodontal Disease+Melatonin Treatment (MetS + PD+MEL) groups. Values are means $\pm$ SEM (n = 6). **p < 0.01. One-way ANOVA with Tukey's multiple comparison.

Liver Alterations

Biochemical Parameters

Elevated levels of liver enzymes GPT/ALT and GOT/AST were observed in the MetS group, with further increases in the MetS + PD group (Fig. 6A). MEL treatment significantly reduced these enzymes to baseline levels.

Histological Analysis

Liver histology (Fig. 6B) revealed mild epithelial hyperplasia, polymorphonuclear infiltration, vacuolization and hepatocyte ballooning in the MetS group. The MetS + PD group exhibited severe epithelial hyperplasia, collagen matrix resorption, Mallory body formation, and fibrosis, indicative of advanced liver damage. In the MetS + PD+MEL group, tissue restoration and normal histological architecture were evident, demonstrating MEL's protective effects.

Inflammatory and Anti-inflammatory Markers in the Liver

Similar to the findings in the jaw, NLRP3 expression was elevated in the MetS and MetS + PD groups but significantly reduced in the MetS + PD+MEL group (Fig. 7A). Conversely, IL-10 expression was notably increased in the MEL-treated group, indicating a protective and resolving effect of MEL on liver inflammation (Fig. 7B).

DISCUSSION

This study investigated the effects of fructose-induced metabolic syndrome (MetS) in a ligature-induced periodontitis (PD) rat model, focusing on the potential therapeutic benefits of melatonin (MEL). The high-fructose dietary model (10% for 35 days) was chosen for its ability to replicate the high-carbohydrate intake of Western diets, which triggers key metabolic alterations such as lipogenesis and adipose tissue expansion, leading to the systemic inflammation characteristic of MetS [10]. Fructose consumption led to metabolic alterations consistent with MetS, including increased body weight, abdominal fat accumulation, hyperglycemia, and dyslipidemia [33]. These results highlight that even moderate fructose intake can trigger significant metabolic disturbances, reinforcing the relevance of our approach for capturing early metabolic dysfunction before severe organ damage occurs [34, 35].

Although insulin resistance is a hallmark of MetS, the 10% fructose model is well established as an early-stage MetS model, inducing hyperglycemia, dyslipidemia, visceral adiposity, and hepatic alterations within 4–6 weeks, even without overt hyperinsulinemia. Prior studies under similar conditions have reported impaired insulin signaling [10, 11, 35]. Thus, the metabolic profile observed here is consistent with experimental MetS.

Clinical and experimental evidence confirms the association between MetS and PD [7, 36, 37]. It is crucial to investigate the shared pathological mechanisms that reciprocally exacerbate both conditions

The microbial dysbiosis and inflammatory burden of PD accelerate MetS progression. In turn, MetS metabolic disturbances exacerbate periodontal inflammation, establishing a vicious cycle [8, 9, 38]. Our results validate this pathological synergy: PD potentiated MetS metabolic dysfunction, significantly deteriorating the glycemic and lipid profiles in the MetS + PD group. This statistically significant difference contrasts with previous trends [12] and aligns with clinical findings associated with PD severity in MetS patients with dyslipidemia and insulin resistance [40].

Regarding the impact of MetS on periodontal health, our study demonstrated that MetS exacerbates periodontal bone loss, evidenced by more severe alveolar destruction in MetS + PD animals. These results concur with the higher prevalence of severe periodontitis reported in individuals with MetS [41, 37, 40].

Mechanistically, the exacerbated phenotype observed in the MetS + PD group may be explained by convergence of metabolic and microbial danger signals at the level of innate immune activation. Fructose-induced lipotoxicity increases mitochondrial ROS production and promotes NF- κ B activation, priming NLRP3 expression in hepatic and peripheral tissues. Concurrently, periodontal dysbiosis provides pathogen-associated molecular patterns such as LPS, acting as a secondary activation signal for inflammasome assembly. This dual signaling promotes IL-1 β and IL-18 maturation, amplifying systemic inflammation and contributing to hepatocellular injury and alveolar bone resorption.

Melatonin likely interferes with this cascade at multiple levels: (i) reduction of mitochondrial ROS generation, (ii) inhibition of NF- κ B nuclear translocation, (iii) suppression of NLRP3 inflammasome assembly, and (iv) upregulation of IL-10–mediated resolution pathways. This multimodal regulatory capacity may explain its effectiveness in disrupting the pathological synergy between metabolic and periodontal inflammation.

Despite this severe combined pathology, MEL treatment effectively reversed these effects. MEL not only counteracted systemic metabolic deterioration (restoring glycemic and lipid profiles) but also mitigated periodontal destruction, preserving marginal bone crest integrity and bone volume. This dual protective effect distinguishes MEL from other interventions, such as omega-3 supplementation, which failed to protect against PD-associated bone loss [12]. Our findings are consistent with Balci Yuce et al. [42], who showed that systemic MEL reduced alveolar bone loss and osteoclastic activity in diabetic rats with PD.

MEL's protective mechanism is linked to the modulation of key inflammatory pathways. NLRP3 inflammasome activation (a crucial factor in MetS, PD, and NAFLD pathogenesis [18, 19]) was markedly elevated in the jaw and liver tissues of the MetS + PD group. MEL treatment exerted a potent immunomodulatory effect by significantly reducing NLRP3 activation while increasing the expression of the anti-inflammatory cytokine IL-10 in both tissues. This dual action (pro-inflammatory suppression and resolution promotion) is well-documented [30, 26, 27] and aligns with MEL reducing TNF- α improving oxidative stress markers, and enhancing insulin sensitivity [43, 44]. By counteracting NLRP3-driven inflammation, MEL prevents metabolic deterioration associated with PD in MetS conditions [45].

A finding of great clinical relevance is that MetS and PD synergistically exacerbate liver damage. Elevated enzyme levels (GPT/ALT, GOT/AST) and advanced histological signs (fibrosis, Mallory bodies) in the MetS + PD group indicate accelerated progression toward NASH-like injury. This reinforces the bidirectional link between periodontal inflammation and systemic metabolic disturbances [14, 46]. MEL significantly mitigated this hepatic inflammation and fibrosis, restoring architecture and reducing NLRP3 activation. This hepatoprotective effect is attributed to MEL's capacity to modulate oxidative stress and inflammation [47], helping to break the cycle of NAFLD progression (fructose-induced) via NLRP3 protection [48].

In summary, MetS and PD coexistence establishes a destructive pathological synergy that accelerates alveolar bone loss and liver injury. Melatonin (locally administered) emerges as a promising adjuvant therapy. By improving metabolic parameters, preserving bone integrity, and protecting the liver via NLRP3/IL-10 axis modulation (Fig. 8), MEL offers an integrated strategy against comorbidities driven by chronic inflammation and oxidative stress. Future research must focus on the underlying molecular mechanisms and its clinical potential for integrated management of MetS-related periodontal and hepatic complications.

CONCLUSIONS

This study demonstrates that the co-occurrence of metabolic syndrome (MetS) and periodontitis (PD) leads to a synergistic exacerbation of systemic inflammation, metabolic dysfunction, and tissue damage, particularly in the liver and periodontium. The experimental model effectively replicated key features of both diseases, showing significant impairments in glycemic control, lipid metabolism, alveolar bone integrity, and hepatic structure and function.

MEL treatment proved to be a potent therapeutic intervention. By reducing NLRP3 inflammasome activation and enhancing IL-10 expression, MEL alleviated systemic and local inflammation, restored metabolic parameters, preserved bone volume, and reversed hepatic damage. These findings highlight the potential of MEL as a multifunctional agent capable of disrupting the pathogenic crosstalk between MetS and PD.

By targeting the inflammatory crosstalk between metabolic and periodontal tissues, melatonin may represent a candidate molecule for integrated management strategies in patients presenting overlapping metabolic and chronic inflammatory disorders.

Overall, MEL emerges as a promising adjunct therapy for mitigating inflammation-driven comorbidities associated with MetS and PD. Future studies should further explore the molecular mechanisms underlying its protective effects and assess its clinical applicability in managing complex metabolic-inflammatory disorders.

Abbreviations

ALT

Alanine aminotransferase (also known as GPT)

AST

Aspartate aminotransferase (also known as GOT)

DAB

3,3'-Diaminobenzidine

EDTA

Ethylenediaminetetraacetic acid

GPT

Glutamic-pyruvic transaminase (ALT)

GOT

Glutamic-oxaloacetic transaminase (AST)

HDL

High-density lipoprotein

H&E

Hematoxylin and eosin

IL

10-Interleukin-10

IL
1 β –Interleukin–1 beta
IL
18–Interleukin–18
MAPK
Mitogen–activated protein kinase
MetS
Metabolic syndrome
MEL
Melatonin
NAFLD
Non–alcoholic fatty liver disease
NF
 κ B–Nuclear factor kappa–light–chain–enhancer of activated B cells
NLRP3
NOD–like receptor family pyrin domain containing 3
PBS
Phosphate–buffered saline
PD
Periodontal disease
ROS
Reactive oxygen species
SOD
Superoxide dismutase
TNF
 α –tumor necrosis factor–alpha

Declarations

COMPETING INTERESTS

The authors declare no competing interests.

ETHIC APPROVAL AND CONSENT TO PARTICIPATE

All experimental procedures complied with national and international ethical guidelines for the care and use of laboratory animals and were approved by the Institutional Committee for the Care and Use of Laboratory Animals (CICUAL), Faculty of Medical Sciences, UNC, 2018.

Clinical trial number: not applicable.

CONSENT FOR PUBLICATION

Not applicable.

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AUTHOR CONTRIBUTIONS

M.E.P.L. and A.R.C. contributed to the conceptualization of the study. A.P.V.M., G.V.C., and F.M.M. contributed to the methodology. Formal analysis was performed by A.P.V.M., M.E.P.L., and A.R.C. Investigation was carried out by A.P.V.M., M.E.P.L., and A.R.C. Resources were provided by E.M.M., M.E.P.L., and A.R.C. Data curation was performed by E.M.M., M.E.P.L., and A.R.C. F.M.M., M.E.P.L., and A.R.C. wrote the original draft. F.M.M. prepared all figures. M.E.P.L. and A.R.C. reviewed and edited the manuscript. Supervision was provided by E.M.M., M.E.P.L., and A.R.C. Project administration was the responsibility of A.R.C. and L. Funding acquisition was secured by E.M.M. and A.R.C. All authors reviewed the manuscript.

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DATA AVAILABILITY

Data can be made available upon reasonable request.

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49. STATEMENTS & DECLARATIONS

Figures

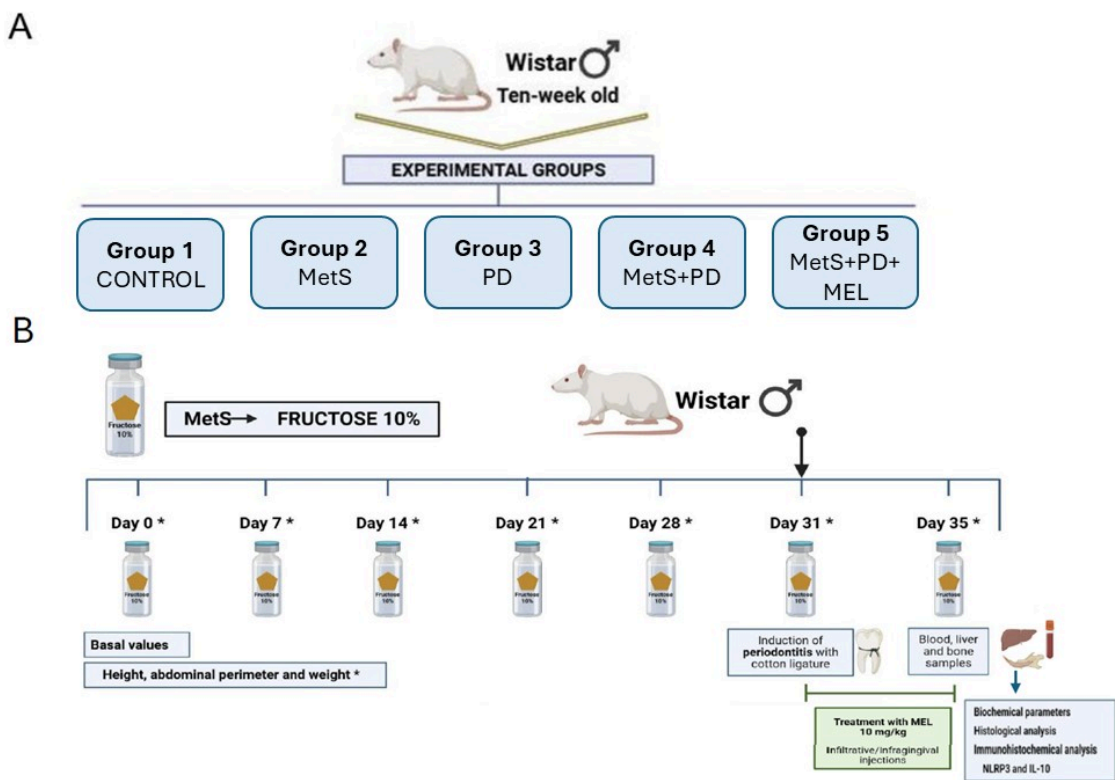


Figure 1

Experimental design. A) General study design and group distribution. Animals were distributed into six groups: GROUP 1-CONTROL, GROUP 2-MetS (Metabolic Syndrome), GROUP 3-PD (Periodontal Disease), GROUP4-MetS+PD, GROUP 5-MEL (Melatonin Treatment) and GROUP 6-MetS+PD+MEL. **B) Graphical representation of GROUP6:** Rats which received fructose 10% to develop MetS and at day 31 were subjected to PD by ligature and received MEL from day 31 to day 35, when euthanasia was performed. All experiments were carried out in triplicate to ensure reliability and reproducibility.

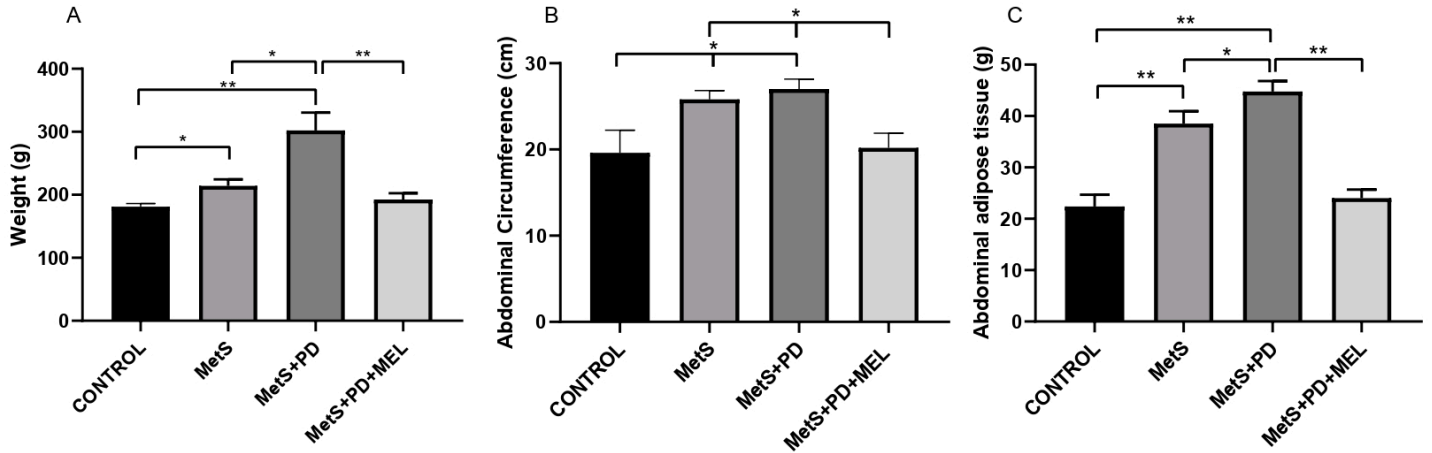


Figure 2

Anthropometric parameters: A) Body weight (g), **B)** Abdominal circumference (cm) and **C)** Abdominal adipose tissue (g) at the end of the study in CONTROL, Metabolic Syndrome (MetS), Metabolic Syndrome+Periodontal Disease (MetS+PD) and Metabolic Syndrome+Periodontal Disease+Melatonin Treatment (MetS+PD+MEL) groups. Values are means $\pm$ SEM (n=6). *p<0.05; **p<0.01. One-way ANOVA with Tukey's multiple comparison.

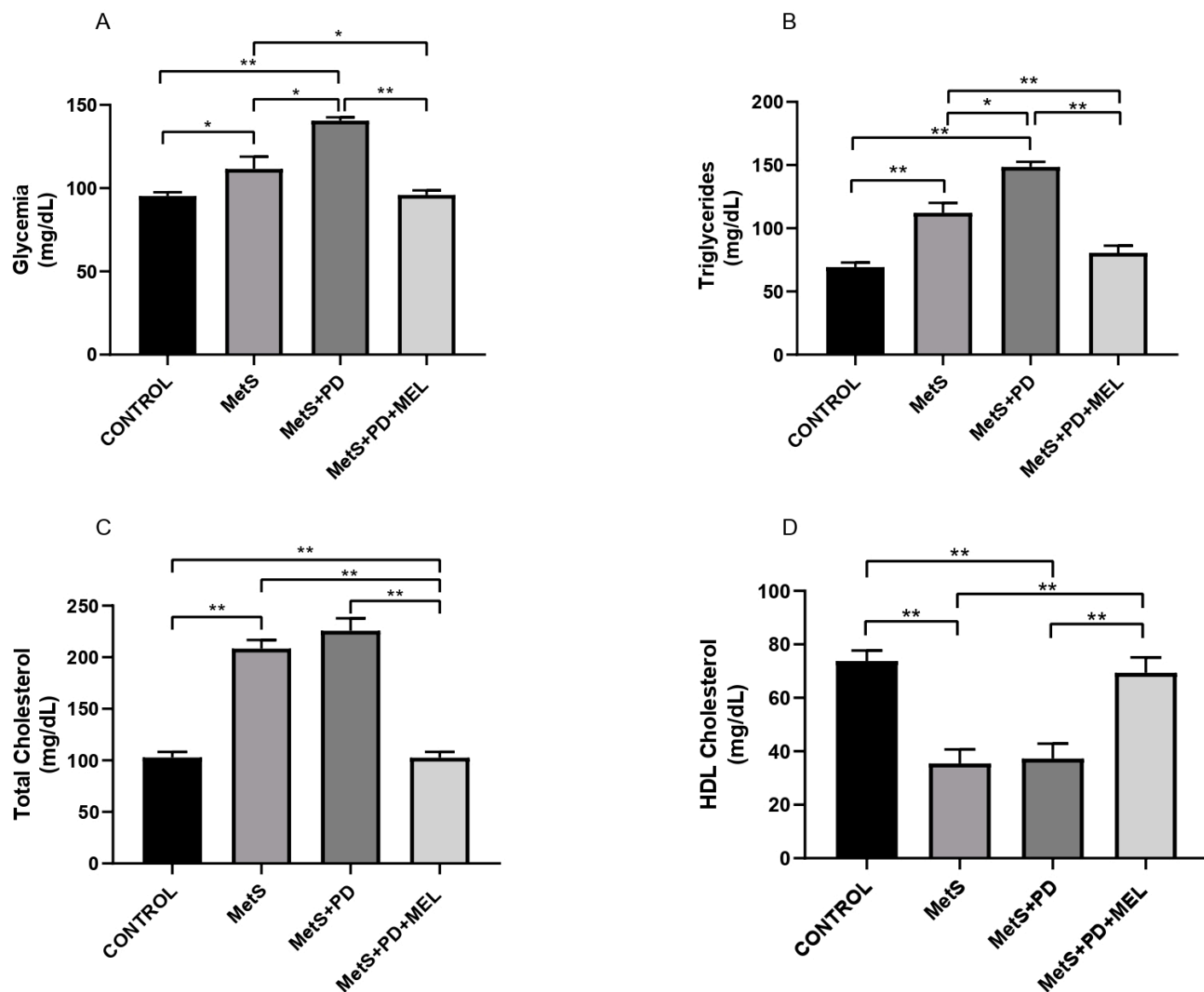


Figure 3

Biochemical metabolic parameters in serum: A) Glycemia (mg/dL), Lipid Profile: **B)** Triglycerides (mg/dL), **C)** Total cholesterol (mg/dL) and **D)** HDL Cholesterol (mg/dL) in CONTROL, Metabolic Syndrome (MetS), Metabolic Syndrome+Periodontal Disease (MetS+PD) and Metabolic Syndrome+Periodontal Disease+Melatonin Treatment (MetS+PD+MEL) groups. Values are means $\pm$ SEM (n=6). *p<0.05; **p<0.01. One-way ANOVA with Tukey's multiple comparison.

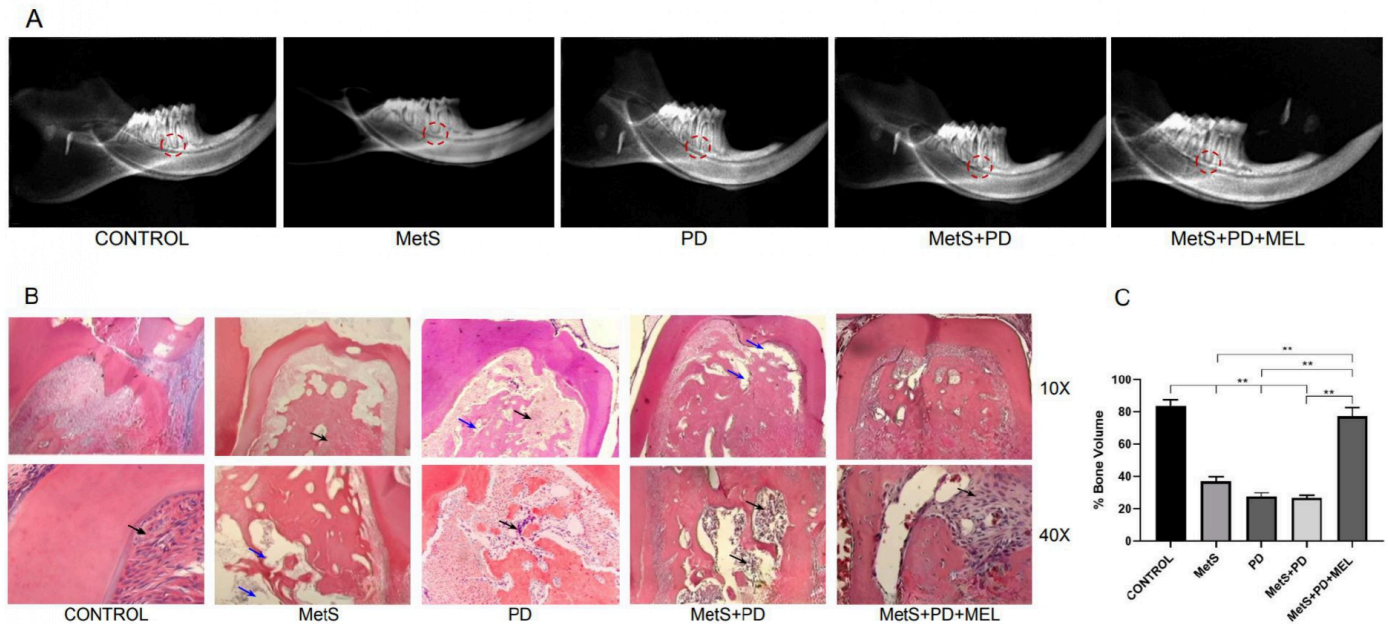


Figure 4

Bone alterations in the jaw. A) Radiovisiographic images of the jaw: representative images of the jaws of rats belonging to different experimental groups, used to assess alveolar bone loss. The radiographs show the region of the first molar, focusing on the apical and furcation areas. The region of interest is highlighted by a red dashed circle. **B) Histological analysis of rat jaws.** Representative H&E-stained sections of the interradicular area of the first lower molar from different experimental groups. Images show inflammatory cell infiltration (black arrow) and alveolar bone structure loss (blue arrow). **C) Histomorphometric measurements.** Comparison of percentage of bone volume of different groups calculated by histomorphometric analysis. MetS (Metabolic Syndrome), PD (Periodontal Disease) and MEL (Melatonin Treatment). Values are means $\pm$ SEM (n=6). **p<0.01. One-way ANOVA with Tukey's multiple comparison.

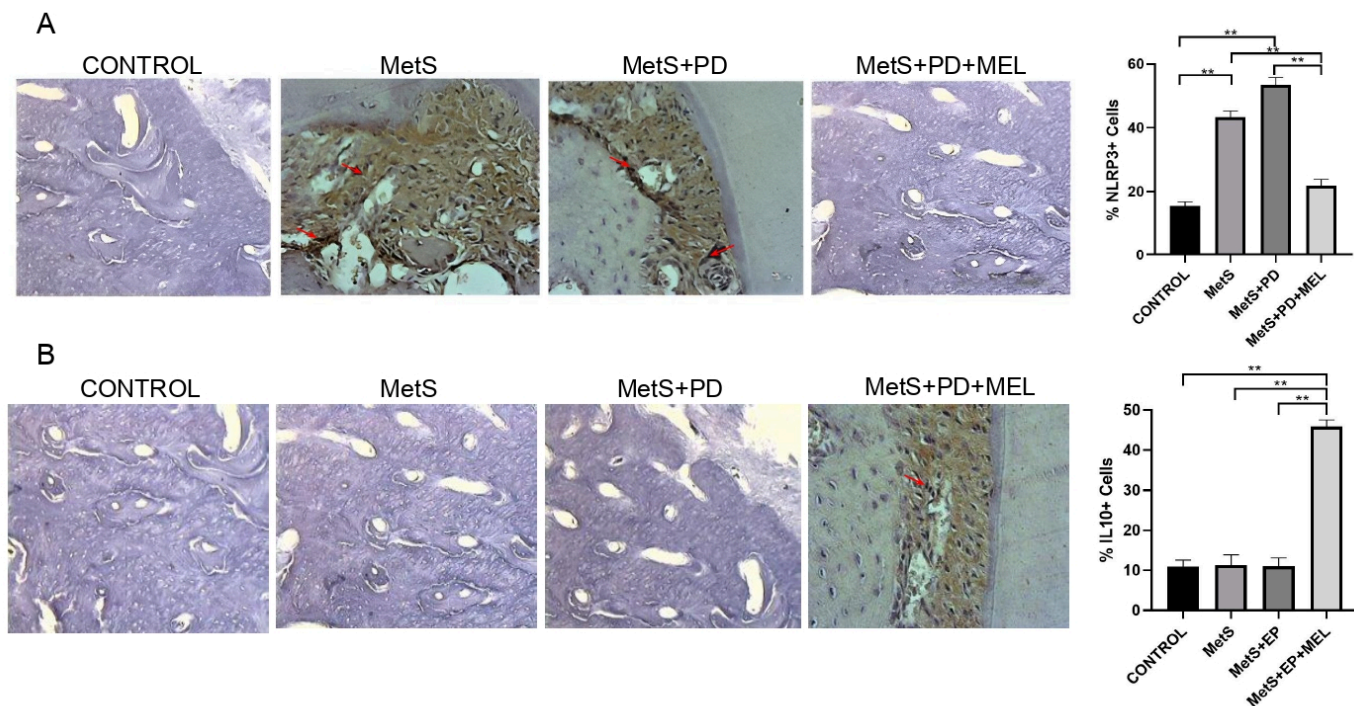


Figure 5

Inflammatory and anti-inflammatory markers in the jaw. A) Immunohistochemical detection of NLRP3 in rat mandibular tissue. **B) Immunohistochemical detection of IL-10** in rat mandibular tissue.

Representative sections of the interradicular region of the first lower molar showing NLRP3 and IL-10 immunoreactivity in the different experimental groups. Positive staining (brown DAB) is indicated by a red arrow. Sections were counterstained with hematoxylin, visualized using a Zeiss photomicroscope, and analyzed with ZEN software. Barr graphs represent the percentage of positive cells in relation of total cell count in ten fields in CONTROL, Metabolic Syndrome (MetS), Metabolic Syndrome+Periodontal Disease (MetS+PD) and Metabolic Syndrome+Periodontal Disease+Melatonin Treatment (MetS+PD+MEL) groups. Values are means $\pm$ SEM (n=6). **p<0.01. One-way ANOVA with Tukey's multiple comparison.

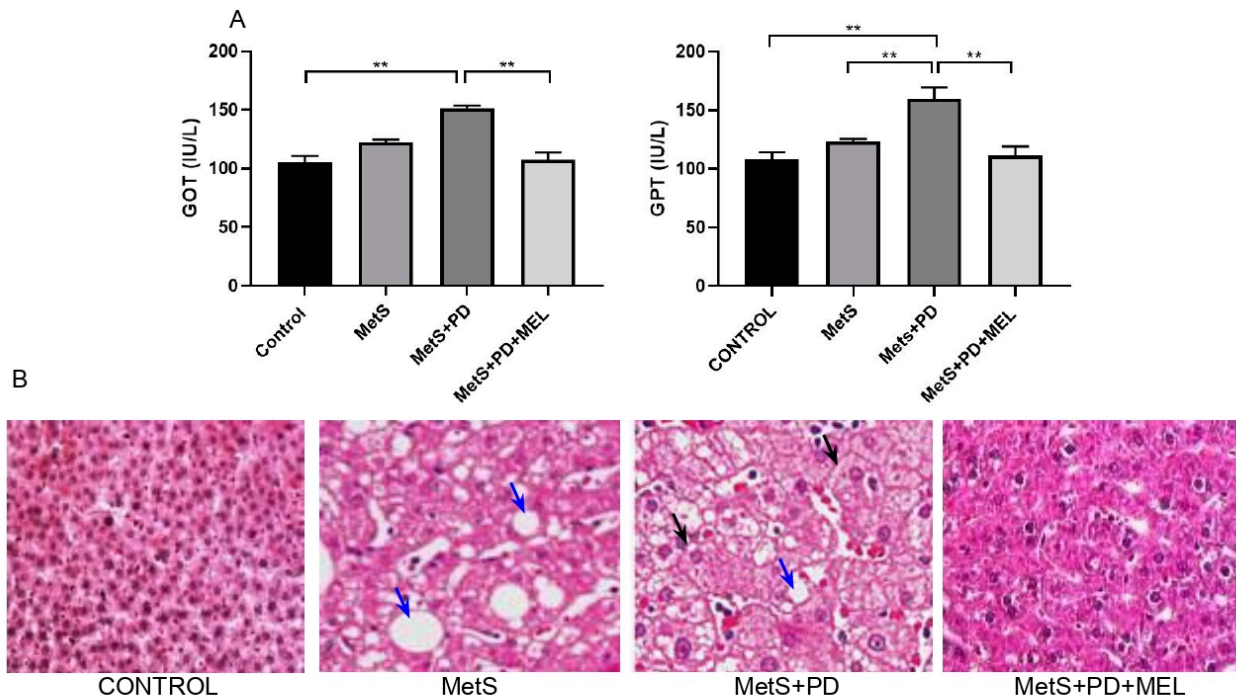


Figure 6

Liver alterations. A) Biochemical parameters: Comparison of plasma transaminases GOT and GPT in different experimental groups. **B) Histological analysis** of H&E-stained sections of the liver of rats belonging to different experimental groups. Blue arrow indicates vacuoles; black arrow indicates cellular ballooning. Analyzed groups: CONTROL, Metabolic Syndrome (MetS), Metabolic Syndrome+Periodontal Disease (MetS+PD) and Metabolic Syndrome+Periodontal Disease+Melatonin Treatment (MetS+PD+MEL). Values are means $\pm$ SEM (n=6). **p<0.01.

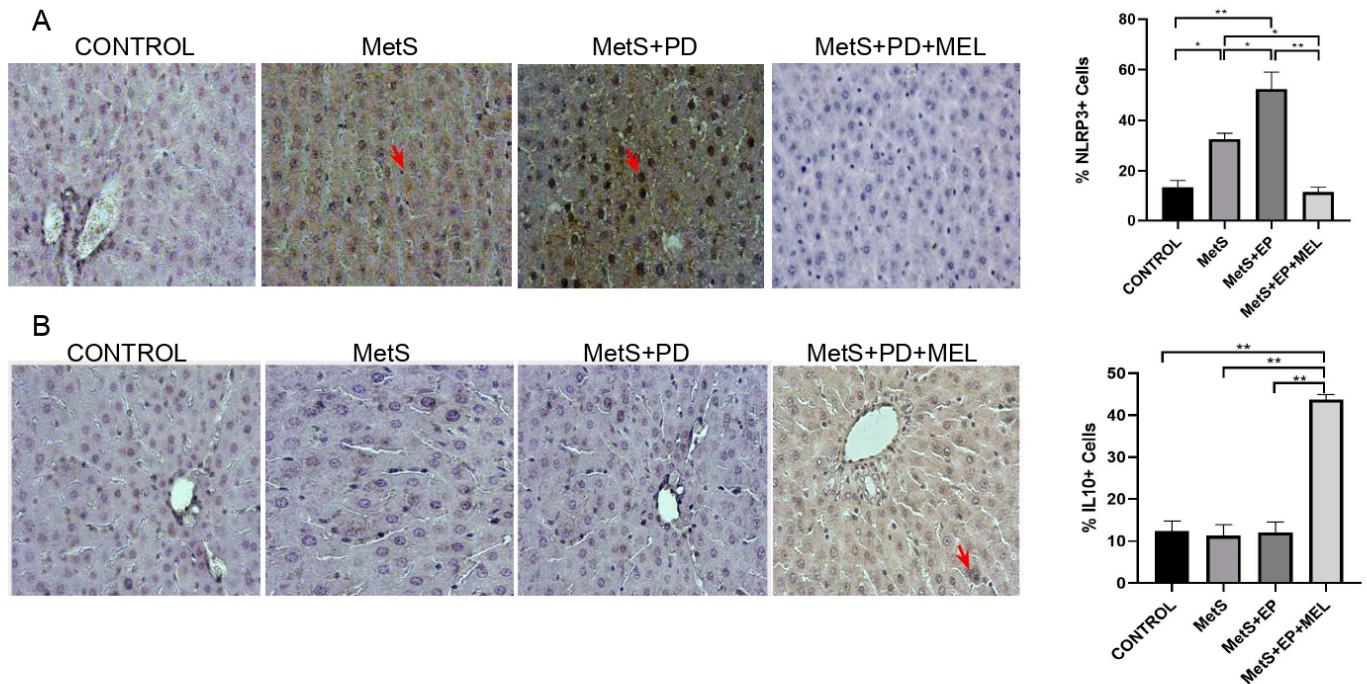


Figure 7

Inflammatory and anti-inflammatory markers in the liver. A) Immunohistochemical detection of NLRP3 in rat hepatic tissue. B) Immunohistochemical detection of IL-10 in rat hepatic tissue.

Representative sections of the liver showing NLRP3 and IL-10 immunoreactivity in the different experimental groups. Positive staining (brown DAB) is indicated by a red arrow. Sections were counterstained with hematoxylin, visualized using a Zeiss photomicroscope, and analyzed with ZEN software. Barr graphs represent the percentage of positive cells in relation of total cell count in ten fields in CONTROL, Metabolic Syndrome (MetS), Metabolic Syndrome+Periodontal Disease (MetS+PD) and Metabolic Syndrome+Periodontal Disease+Melatonin Treatment (MetS+PD+MEL) groups. Values are means $\pm$ SEM (n=6). *p<0.05; **p<0.01. One-way ANOVA with Tukey's multiple comparison.

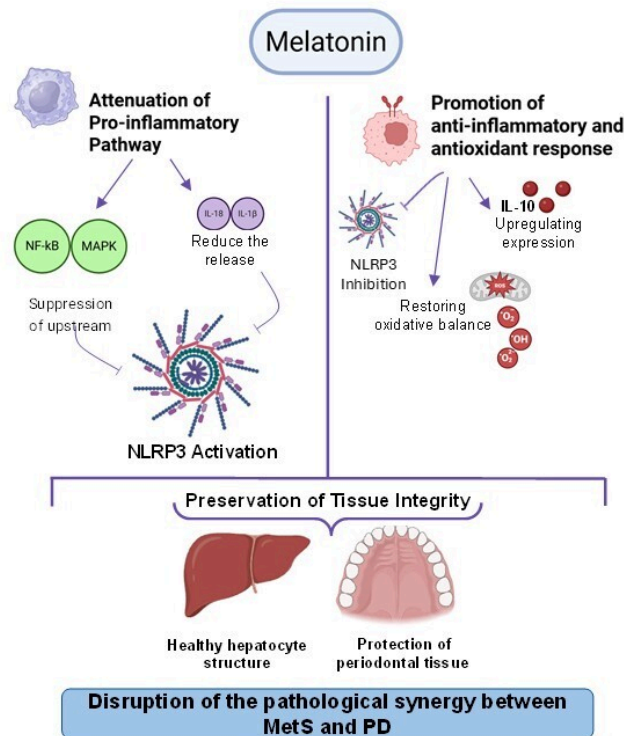


Figure 8

Potential molecular mechanisms of MEL to reduce the synergistic effects of MetS and PD. MEL mitigates the exacerbated inflammatory damage induced by the combined presence of metabolic MetS and PD by targeting key pro-inflammatory pathways and enhancing resolution mechanisms. Specifically, melatonin inhibits NLRP3 inflammasome activation through suppression of upstream NF-κB and MAPK signaling, thereby reducing the release of IL-1β and IL-18 in both hepatic and periodontal tissues. Concurrently, melatonin promotes an anti-inflammatory environment by upregulating IL-10 expression and restoring oxidative balance through the enhancement of antioxidant enzyme activities. This dual action (attenuation of pro-inflammatory cascades and stimulation of anti-inflammatory mediators) contributes to the preservation of hepatocyte structure and function, and the protection of periodontal bone and connective tissue, ultimately disrupting the pathological synergy between MetS and PD.