Does Zn-mediated regulation of the kynurenine pathway provide the link between periodontal disease and diabetes?

Ebru Afşar[1]*, Kadirhan Doğan[2], Erdem Arslan[3], Işıl Eranil[4], Neşe Oral[5], Bahadır Kadir Kuzzu[6],Tayfun Ceylan[7], Mehmet Öz[8]

[1]*:Department of Biochemistry, Faculty of Dentistry, Cappadocia University, 50400, Nevşehir, Turkey

[2]: Department of Anatomy, Faculty of Dentistry, Cappadocia University, 50400, Nevşehir/Turkey

[3]: Department of Pharmacology, Faculty of Medicine, Aksaray University, 68100, Aksaray / Turkey

[4]: Department of Medical Services and Techniques, Cappadocia Vocational School, Cappadocia University, 50400, Nevşehir, Turkey

5: Department of Radiology, Faculty of Dentistry, Cappadocia University, 50400, Nevşehir/ Turkey

6: Department of Otolaryngology Head and Neck Surgery, Antalya Ataturk State Hospital, 07040, Muratpaşa/Antalya.

[7]: Department of Histology and Embryology, Faculty of Dentistry, Cappadocia University, 50400, Nevşehir/ Turkey

[8]:Department of Physiology, Faculty of Medicine, Aksaray University, Bahçesaray, 68100 Aksaray / Turkey

*__Correspondence author:__

Ebru Afşar, Department of Biochemistry, Faculty of Dentistry, Cappadocia University, 50400, Nevşehir/Turkey
Telephone: + 90384 353 5009-1439 E-mail address: ebru.afsar@kapadokya.edu.tr

It has long been known that there is a relationship between periodontal diseases and diabetes. The present study aimed to assess the effect of pancreatic zinc (Zn) levels on Kynurenin pathways (KP) and glucose homeostasis and the impact of Thymoquinone (TQ) in the periodontal disease animal model. **Methods:** 10 µl *Porphyromonas gingivalis*-Lipopolysaccharide (*P. gingivalis*-Lps) (1mg/ml) was injected at 48-hour intervals into the palatal gingiva of rats, and TQ was given by oral gavage (10 mg/kg per day) for 2 weeks. Glucose homeostasis was assessed using the Homeostatic Model Assessment (HOMA-IR), and β-cell function (HOMA-β Levels). Kynurenine (KYN), Tryptophan (TRP), kynurenic acid (KYNA), quinolinic acid (QA), KYN 3-monooxygenase (KMO), kynureninase, interferon-γ (IFN-γ), insulin, ZIP10, and caspase-3 levels measured by the enzyme-linked immunosorbent assay (ELISA). Zinc levels in the pancreas tissue and plasma samples were measured using a colorimetric method. Morphological changes in the pancreas were identified by hematoxylin and eosin staining, and X-ray radiography determined bone resorption in the maxillary bone. **Results:** In the LPS group, pancreas ZIP10 and Zn levels increased, the KP pathway was altered to favor KYNA, and impaired glucose homeostasis was observed. TQ administration decreased pancreatic Zn levels, changed KP to favor QA, and improved morphological changes in the pancreas. **Conclusion:** During the progression of periodontal diseases, KP may be altered by Zn levels through ZIP10 in the pancreas, thereby impairing pancreatic function. Regulation of Zn levels may be key to shared pathways between periodontal diseases and diabetes.

Key Words: Kynurenine, *Porphyromonas gingivalis*, Diabetes, Thymoquinone, Zinc

1. Introduction

Microbes highly colonize the gingival tissues in the oral cavity surrounding each tooth. Correlations have been found between oral health and cardiovascular disease (1), pregnancy outcomes (2), rheumatoid arthritis (3), and type II diabetes mellitus (T2DM) (4). *Porphyromonas gingivalis* (*P. gingivalis*) is a gram-negative anaerobic bacterium found in the mouth. This species plays a crucial role as a keystone pathogen in the progression of periodontal disease (PD) and contributes to various systemic diseases (5). *P. gingivalis* or virulence factors such as lipopolysaccharide (LPS) can spread systemically through active periodontal lesions or by invading cells directly (6-8). Therefore, inflammatory conditions caused by LPS of *P. gingivalis* may be the cause of the molecular link between periodontal health and diabetes (6). Previous studies have shown a relationship between *P. gingivalis* and diabetes (5, 9). However, many unknown points exist about the molecular connections between oral health impaired by *P. gingivalis* and the pathogenesis of T2DM.

Zinc (Zn^{2+}) dysregulation has been implicated in the development of insulin resistance and diabetic complications (10). Moreover, recent studies suggest a potential causal link between Zn^{2+} imbalance and the pathogenesis of periodontitis (11). Thus, Zn^{2+} disruption may represent a molecular link connecting PD and T2DM.

Previous studies have shown that PD and T2DM mellitus share many common pathological pathways, including the kynurenine pathway (KP) (12-15). Tryptophan (Trp) is an essential amino acid metabolized by the serotonin or the KP to various bioactive molecules. Pro-inflammatory cytokines, such as interferon-γ (IFN-γ), upregulate the enzyme indoleamine 2,3-dioxygenase (IDO), causing decreases in L-Trp and increasing the metabolite kynurenine (KYN) levels (15). Then, KYN is converted to kynurenic acid (KYNA) by kynurenine aminotransferase (KAT) or quinolinic acid (QA) by two different pathways: 1) KYN can be converted to anthranilic acid by kynureninase and then converted to QA, or 2) KYN can be converted to 3-hydroxyKYN (3-HK) by KYN 3-monooxygenase (KMO) and then converted to QA by kynureninase-mediated pathway. It is evident from a previous study that increasing concentrations of Zn^{2+} inhibit kynureninase, whereas they activate KAT (16). However, it is unknown how the change in Zn^{2+} levels caused by *P. gingivalis* alters the KP pathway and whether it contributes to the link between PD and diabetes.

Thymoquinone (TQ) is one of the main active components of the essential oil obtained from black cumin (Nigella sativa) seeds. Many studies have demonstrated TQ's antibacterial, hypoglycemic, anti-inflammatory, and anti-oxidative activities (17, 18). Thus, this study investigated the effect of Zn^{2+} on KP metabolism in the pancreas on glucose regulation in impaired oral health rat models created by *P. gingivalis*-LPS injection and whether TQ affects this change.

Our study is the first to investigate the molecular connection between *P. gingivalis*-induced deterioration of periodontal health and diabetes in pancreatic tissue. It may contribute to understanding the pathogen-specific molecular link between periodontal diseases and diabetes.

2. Methods

2.1 Animals

All experimental procedures involving male rats were conducted in accordance with the guidelines approved by the Institutional Animal Care and Use Committee of Aksaray University (Approval No: 2024/9-56). Male albino Wistar rats (3 months old, weighing 200–300 g) were obtained from the Aksaray University Animal Care Unit. Throughout the study, rats were housed in stainless steel cages under standard laboratory conditions (23 ± 1 °C temperature, 50 ± 5% relative humidity, and a 12:12 h light-dark cycle). All animals were fed a standard commercial rat chow obtained from Nukleon (Ankara, Turkey), and tap water was provided *ad libitum* throughout the experimental period. The relative humidity and temperature of the animal room were continuously monitored and automatically regulated using a central HVAC system. Environmental parameters were recorded daily to ensure consistency throughout the experiment.

2.2 Establishment of animal model and treatment

P. gingivalis is one of the pathogens that mediates PD; for this reason, we injected *P. gingivalis*-LPS into the palatal gingiva to create a model of impaired periodontal health according to previous studies (19-22). TQ was supplied by Sigma Aldrich (St. Louis, MO, USA), with a purity of> 98%. TQ administration was performed using the dose previously reported in the literature. The water solubility of TQ is reported to be > 0.5 mg/mL, which is enough to exert pharmacologic effects (23). Animals were randomly divided into four groups (n=8 for each group). **The Control group** received saline injections and tap water; **the LPS group (LPS)** received *P. gingivalis*-LPS injections and tap water; **the TQ group (TQ)** received saline injections and TQ; and the **LPS+TQ group**

(LPS+TQ) received *P. gingivalis*-LPS injections and TQ. As shown in Figure 1, oral injections were given 7 times at 48-hour intervals (on days 1, 3, 5, 7, 9, 11, and 13) into the palatal gingiva between the first and second upper molars on the right and left sides, using 10 µl of *P. gingivalis*-LPS or saline each time. Tap water or TQ solution (10 mg/kg per day) was administered daily by oral gavage for 14 days, starting on Day 1—the same day as the first LPS injection. At the end of the experiment, the rats were examined orally, and their clinical signs of periodontitis, such as swelling, redness, and gingival bleeding, were assessed [2]. The experimental design is shown in Figure 1. After being anesthetized intraperitoneally with a mixture of ketamine (80 mg/kg) and xylazine hydrochloride (5 mg/kg), the rats were sacrificed.

2.3 Tissue Collection and Preparation

The maxillary jawbone was separated for X-ray analysis, and the other was separated for biochemical analysis. Some pancreatic tissues were stored at -80 °C for biochemical analysis, and some were separated for hematoxylin and eosin staining. The excised pancreas were first rinsed with ice-cold saline and weighed. Each sample was then homogenized in 10 volumes of phosphate-buffered saline (PBS, pH 7.4) using a glass-Teflon homogenizer on ice. The homogenates were centrifuged at 10,000 × g for 5 minutes at 4°C, and the supernatants were collected for analysis. The supernatant obtained from tissue homogenates was used in the ELISA kit, and zinc levels were analyzed in pancreatic tissue. Plasma samples were obtained by centrifugation of whole blood at 1,500 × g for 15 minutes (24). It was used in all biochemical analyses performed on plasma samples.

2.4 Biochemical Analysis

KYN, TRP, KYNA, QA, IFN-γ, caspase-3 levels, KMO, and ZIP10 levels were measured in the pancreas tissue, and insulin levels were also measured in plasma samples using the enzyme-linked immunosorbent assay technique, employing commercial kits from Bioassay Technology Laboratory (BT Lab) according to the manufacturer's instructions. Zn levels in the pancreatic tissue and plasma samples were determined using a colorimetric method (Fully Automatic Clinical Biochemistry Analyzer, Mindray BS400), following the same procedure previously employed in similar studies (25, 26).

Fasting Blood Glucose Measurement: At the end of the 14-day experimental period, rats were subjected to an overnight fast for 12 hours with free access to water. The following morning, blood samples were obtained from the tail vein using a sterile lancet, while the rats were gently restrained without anesthesia. Fasting blood glucose levels were measured immediately using a calibrated portable glucometer (plusMED Blood GlucoseMeter, Accuro, pM1-300, Bionime Corporation, Taiwan) and compatible glucose test strips. All measurements were performed between 8:00 and 10:00 a.m. to minimize circadian variation.

Homeostatic model (HOMA) assessment: Insulin resistance was assessed using the Homeostatic Model Assessment (HOMA-IR) (insulin (mIU/L) multiplied by fasting blood glucose (mM) divided by 22.5) (27). β cell function was assessed using the analysis of HOMA-β levels, calculated by the equation (360 x fasting plasma insulin)/(fasting plasma glucose -63) (%) (28).

Determination of Kyn/Trp ratio (KTR): The change in the Kyn/Trp ratio reflects the degradation rate of Trp, and therefore, the Trp/Kynurenine ratio is used to determine IDO activity (29).

Protein measurements: Protein concentrations of tissue samples were determined at 595 nm using a modified Bradford assay with Coomassie Plus reagent and bovine serum albumin as a standard (Pierce Chemical Company, Rockford, IL) (30).

2.5 Histopathologic examinations of pancreatic tissues

The pancreas tissues were fixed in a 10% paraformaldehyde/phosphate-buffered saline solution and then subjected to a standard paraffin-embedding process, obtaining 5-6 μm sections. Finally, the sections were stained with Hematoxylin-eosin (H&E) and evaluated under light microscopy (Leica DFC450, Almanya) (31, 32).

2.6 X-ray films of the periodontal tissue

The dissected maxillary bone was examined with a periapical X-ray device (KaVo Focus (KaVo Dental, Bieberich, Germany) at 10x magnification to determine the alveolar bone level. The distance between the cemento-enamel junction (CEJ) and the alveolar bone crest (ABC) between the alveolar bone was measured medially and distally. The CEJ was connected with a line drawn in silico and indicated on the tooth's mesial and distal surface projections. Then, perpendicular lines were drawn to the alveolar bone crest along the CEJ, and the distance was automatically measured by the computer-aided system (CLINIVIEW™ software, Instrumentarium Dental, Tuusula, Finland) (33, 34).

2.7 Statistical analysis

The statistical analyses of the obtained data were performed using SPSS 23.0 (SPSS, Chicago, IL, USA) software for Windows. Biochemical parameters were analyzed by a one-way analysis of variance (ANOVA) followed by Tukey's Post Hoc Test for normally distributed variables, and Kruskal-Wallis followed by the Mann-Whitney U test for non-normally distributed variables. Results are expressed as the mean ± SEM. A p-value <0.05 was considered significant.

3. Results

3.1 Oral examination and radiography results of the experimental groups

We observed that the rats' gum tissues became red and swollen, displaying a dark red color and experiencing spontaneous bleeding after two weeks of LPS injection. These preliminary findings indicated the successful induction of impaired oral health. Oral findings of rats in the TQ group were similar to those of the healthy groups. In addition, the gingival tissues of the LPS+TQ group appeared pink, while their gingival margins became red and swollen. No bleeding was detected. X-ray films revealed slightly alveolar bone resorption in the second maxillary molar in the rats injected with LPS, in contrast to the control and TQ-administered rats. Also, alveolar bone resorption was alleviated in the LPS+TQ group (Figure 2).

3.2 Analysis of Zn-related parameters

As shown in Table 1, ZIP10 levels in the pancreatic tissue of the control groups were significantly higher compared to the TQ group and the LPS+TQ group ($p = 0.001$, $p = 0.042$). ZIP10 levels in the pancreatic tissue of the LPS group were significantly higher compared to the control, TQ, and LPS+TQ groups ($p=0.000$, $p=0.000$, $p=0.000$). The plasma Zn levels of the control group were significantly higher compared to the TQ and LPS groups ($p=0.017$, $p=0.000$) and significantly lower compared to the LPS+TQ group ($p=0.001$). Also, the plasma Zn levels of the

LPS+TQ group were significantly increased compared to the control, TQ, and LPS groups (p=0.001, p=0.000, p=0.000). Pancreas Zn levels of the control group were significantly higher compared to the TQ and LPS+TQ groups (p=0.000, p=0.000), and significantly lower compared to the LPS group (p=0.003). Additionally, pancreas Zn levels in the LPS+TQ group were significantly decreased compared to the LPS group (p = 0.000).

3.3 Analysis of Cytokine and Caspase-3 Levels in Pancreas Tissue

Pro-inflammatory cytokine levels in various groups are shown in Table 1. IFN-γ levels in pancreas samples of the control group were lower compared to the TQ, LPS, and LPS+TQ groups (p=0.055, p=0.025, p=0.037). Also, IFN-γ levels in pancreas samples of the LPS+TQ group were higher compared to the LPS group. However, it is not significant. Caspase-3 levels in pancreas samples of the LPS group significantly decreased compared to the control group and markedly reduced compared to the LPS+TQ group (p=0.026, p=0.055).

3.4 Analysis Of Glucose Homeostasis

Firstly, we investigated the fasting glucose and insulin levels in various groups. As seen in Table 2, the fasting glucose levels of the control group were significantly lower compared to the LPS and the LPS+TQ groups (p=0.000, p=0.000). Also, the fasting glucose levels of the TQ group were significantly lower compared to the LPS and the LPS+TQ groups (p=0.000, p=0.000). Plasma insülin levels of the TQ group were significantly lower compared to the control, LPS, and LPS+TQ groups (p=0.030, p=0.000, p=0.000). Also, TQ administration markedly increased plasma insülin levels of the LPS+TQ group compared to LPS; however, this trend did not reach significant levels.

Then, we determined HOMA-IR and HOMA-β levels. The HOMA-IR levels of the control groups were lower compared to the LPS and LPS+TQ groups (p=0.000, p=0.000). The HOMA-IR levels of the TQ groups were lower compared to the LPS and LPS+TQ groups (p=0.000, p=0.000). Also, the HOMA-IR levels of the TQ groups were slightly lower compared to the control group, and the HOMA-IR levels of the LPS+TQ groups slightly higher compared to the LPS group; however, these trends did not reach significant levels. Additionally, HOMA-β levels were higher in the control groups compared to TQ, LPS, and LPS+TQ groups (p=0.002, p=0.001, p=0.006). HOMA-β Levels of LPS+TQ groups markedly increased compared to the LPS group. However, this increasing trend did not reach significant levels.

3.5 Analysis of Change in KYN Pathways

3.5.1 Analysis of Trp, KYN, KYNA, and QA levels in the pancreas tissue and plasma samples

We investigated differences in the various groups' Trp and KYN levels of the pancreas tissue. There was no difference in the Trp and KYN levels of pancreas tissue (p =0.567, p = 0.071). TRP levels determined for the control group 99.727 ± 47.40 ng/g protein, the LPS group 43.495 ± 8.16 ng/g protein, the LPS+TQ group 9.369 ± 31.75 ng/g protein, and KYN levels determined for the control group 0.510 ± 0.132 ng/mg protein, the LPS group 0.871 ± 0.151 ng/mg protein, TQ group 2.156 ± 0.770 ng/mg protein, LPS+TQ group 2.737 ± 0.868 ng/mg protein (Figure 3. A and Figure 3C).

Pancreatic KYNA levels of the LPS group (27.328 ± 3.553 ng/mg protein) were higher than those of the control group (18.601 ± 3.319 ng/mg protein); however, with this increasing trend, there was no significant difference (p=0.078). KYNA levels of the LPS+TQ group (5.679 ± 0.643 ng/mg protein) were decreased compared to the

control group, the TQ group (38.023 ± 9.697 ng/mg protein), and the LPS group (p=0.016, p=0.004, p=0.004) (Figure 3B). QA levels of the LPS group (1.029 ± 0.207 ng/mg protein) were lower than those of the control group (2.585±0.317 ng/mg protein) and LPS+TQ group (11.109 ± 2.400 ng/mg protein) (p=0.004, p=0.004) (Figure 3. D).

3.5.2. Analysis of Kyn/Trp ratio, KMO, and Kynureninase levels in the pancreas tissue

Significant differences in the Kyn/Trp ratio of various groups in the pancreas tissue. The pancreas Kyn/Trp ratio is lower in the control group (0.0110 ± 0.0013 µg/ng) compared to the LPS group (0.0213 ± 0.0024 µg/ng) and the LPS+TQ group (0.0301 ± 0.0026 µg/ng) (respectively, p=0.010, p=0.004). TQ administration increased the Kyn/Trp ratio in the pancreas of LPS+TQ group rats compared to the LPS group. However, this increasing trend does not reach statistically significant levels (p = 0.078) (Figure 4A).

The pancreatic KMO levels of the control group (144.022 ± 25.075 ng/g protein) were higher than those of the LPS group (54.046 ± 11.874 ng/g protein) and the LPS+TQ group (48.342 ± 8.306 ng/g protein) (p=0.048, p=0.033). Also, KMO levels of the TQ group (156.276 ± 34.649 ng/g protein) were higher than those of the LPS group (p=0.021, p=0.015) (Figure 4B).

It was observed that the pancreatic kynureninase levels of the LPS group (1.399 ± 0.059 ng/mg protein) decreased compared to the control group (3.136 ± 0.712 ng/mg protein) and compared to the LPS+TQ group (6.401 ± 2,848 ng/mg protein) (p=0.025, p=0.004). TQ administration increased kynureninase levels of the TQ group (7.328 ± 3.171 ng/mg protein). However, with this increasing trend, there was no significant difference (Figure 4C).

3.6 Histological analysis.

The results of HE of the pancreatic tissues were evaluated regarding edema, inflammatory cell infiltration, acinar cell degeneration, and hemorrhage parameters (Figure 5). The severity of each criterion was graded from 0 to 3: 0: absent or rare, 1: mild, 2: moderate, 3: severe (31, 32). As seen in Table 3, the damage scores of the LPS group were statistically higher than those of the control group (p=0.05). The TQ group scores were similar to those of the control group. The damage score of the LPS+TQ group decreased statistically significantly compared to the LPS group, but increased significantly compared to the control and TQ groups (p = 0.05).

4. Discussion

Periodontal health and diabetes have long been recognized to have reciprocal relationships (35); however, the mechanisms by which these connections are established remain unclear. In this study, we investigated the effect of oral pathogen *P. gingivalis*-LPS on glucose homeostasis by changes in Zn-mediated KYN metabolism and whether TQ affects these conditions.

Our findings suggest that *P. gingivalis*-LPS injection may contribute to disturbances in glucose homeostasis, potentially through peripheral insulin resistance and impaired β-cell function. Insulin resistance and defects in pancreatic beta cells are two significant pathophysiologic abnormalities that underlie T2DM (35), resulting in hyperglycemia (36). Additionally, reduced insulin signaling and/or the presence of insulin resistance, accompanied by impaired glucose transport, leads to a compensatory increase in pancreatic insulin secretion, resulting in hyperinsulinemia. Notably, elevated insulin levels may precede the clinical onset of T2DM, serving as an early indicator of individuals at risk for developing the disease (37). Therefore, the increased insulin synthesis observed

in the present study may represent an adaptive response to LPS treatment. However, despite elevated plasma insulin levels, HOMA-β may remain low due to persistent hyperglycemia, insulin resistance, inadequate β-cell responsiveness, and β-cell exhaustion (38). Similarly, Abdelmageed et al. demonstrated that T2DM rats exhibited significantly elevated fasting glucose and insulin levels, increased HOMA-IR, and reduced HOMA-β compared to controls (39).

These results appear to be consistent with previous studies reporting an association between PD and T2DM (40), and may contribute to a better understanding of this relationship. Additionally, Zn^{2+} levels decreased in the plasma of the LPS group, which is consistent with reduced plasma Zn^{2+} levels in the T2DM and (38) periodontitis patients (10). This reduction may be explained by the inflammatory response triggered by LPS, which induces redistribution of Zn^{2+} from the plasma to peripheral tissues (41, 42). Inflammatory cytokines upregulate specific zinc transporters, particularly ZIP10, promoting Zn^{2+} influx into tissues (43, 44). Accordingly, ZIP10 and Zn^{2+} levels were increased in the pancreas tissue of the LPS group. This local accumulation of Zn^{2+} may represent a compensatory mechanism against *P. gingivalis*-LPS and reflects similar alterations observed in the pathophysiology of T2DM (45).

Zn^{2+} is an essential component for the normal function of the pancreas, and deficiency and overload of Zn^{2+} are linked to diverse disorders, including diabetes and obesity (46). The physiological and cellular Zn^{2+} concentrations are regulated by Zn^{2+} transporters (ZnTs), and Zn importers (ZIPs, Zrt- and Irt-like proteins) (47). ZIP10 is part of the ZIP (SLC39) class of transporters that enhance Zn^{2+} concentration in the cytoplasm by facilitating its influx from the extracellular space or promoting its efflux from intracellular vesicles. Research has shown that ZIP10 transcripts are expressed in pancreatic alpha (48) and β-cells (49), suggesting that ZIP10 may regulate glucose homeostasis (50). This view supports the idea that in isolated breast cancer cells treated with glucose concentrations equivalent to those found in humans with hyperglycemia, the expression of ZIP10 is reported to be upregulated, accompanied by a concomitant increase in cellular Zn^{2+} concentrations (51). Moreover, Zn^{2+} is crucial in insulin synthesis, crystallization, storage, secretion, and signaling in the pancreatic β-cells (46). Our results suggest that increased ZIP10 may lead to the transport of Zn^{2+} into β-cells and increased insulin synthesis.

Furthermore, Zn^{2+} deficiencies or imbalances in Zn^{2+} levels have been associated with increased susceptibility to periodontal disease, as Zn^{2+} is essential for the activity of various enzymes and transcription factors regulating inflammatory responses (10, 11). Also, the KP is implicated in immune modulation and inflammation within periodontal tissues. Dysregulation of KP metabolites such as KYN, KYNA, and QA can influence local immune responses, oxidative stress, and apoptosis, thereby contributing to tissue destruction and disease progression (52, 53). Emerging evidence suggests that Zn^{2+} may modulate KP activity, for instance, by influencing enzyme functions such as kynureninase and KAT, thereby affecting the balance of KP metabolites (54). Therefore, the interplay between Zn^{2+} homeostasis and KP activity may represent a molecular link between periodontal disease and systemic conditions such as diabetes, highlighting potential therapeutic targets for intervention. Supporting this notion, a previous study demonstrated that Zn^{2+} promotes Trp degradation via IDO and enhances KYN production in dendritic cells (DCs), while concurrently suppressing pro-inflammatory responses triggered by Toll-like receptor (TLR) ligands (55). Our study observed elevated levels of the pro-inflammatory cytokine IFN-γ and increased IDO activity in the pancreas of the LPS group, along with a notable rise in pancreatic Zn^{2+} concentrations.

Notably, it has been shown that Zn^{2+} inhibits kynureninase—the enzyme responsible for converting KYN to QA—while potentially activating KAT, which facilitates the conversion of KYN to KYNA (16). Consistent with these findings in our study, in the *P. gingivalis* LPS group, QA levels decreased while KYNA levels increased, accompanied by a reduction in kynureninase levels. These findings suggest that elevated pancreatic Zn^{2+} levels observed under *P. gingivalis* LPS exposure may have contributed to increased KAT levels or activity, thereby shifting the kynurenine pathway toward KYNA production. Collectively, these findings suggest that *P. gingivalis* LPS may be involved in metabolic alterations, potentially through Zn^{2+}-mediated modulation of KP enzymes, pointing to a possible mechanism by which periodontal inflammation could influence systemic metabolic processes. However, further studies are needed to directly assess KAT expression and enzymatic activity in order to confirm this hypothesis and elucidate the precise role of Zn^{2+} in modulating KP dynamics.

Also, our results show that caspase-3 levels decreased in the pancreas of the LPS group, suggesting increased apoptosis. Caspase-3 is a key zymogen in the process of cell apoptosis. It is not activated until it is cleaved by initiator caspases during apoptotic flux (56). Therefore, decreasing zymogen form caspase-3 levels may increase cleaved caspase-3, active caspase form. Previous in vivo and in vitro studies have demonstrated that QA exhibits apoptotic properties (37), whereas KYNA displays anti-apoptotic properties (57). However, Arya et al. showed that the combination dose of quercetin (QE) and QA (50 mg/kg) exhibited maximum inhibition of the pro-apoptotic protein Bax expression and enhanced the anti-apoptotic protein Bcl-2 expression, suggesting a protective role in the kidneys of diabetic rats (58). Therefore, our results suggest that the decreased QA levels in the pancreas of the LPS group may be associated with the decrease in HOMA β levels and alterations in pancreatic β-cell structure. Importantly, increased ZIP10-mediated Zn^{2+} levels may be responsible for these changes. Supporting this view, TQ administration decreased ZIP10 and Zn^{2+} levels in the pancreas tissue of the LPS-TQ group. Also, IDO activity increased more in the LPS+TQ group than in the LPS group, while QA levels increased and KYNA levels decreased, contrary to the LPS group. Decreased Zn^{2+} levels and increased IDO activity may be related to Zn's dose-dependent effects. Moreover, our results suggest that QA levels in the pancreas tissue of the LPS+TQ group, due to the suppressive effect on kynureninase, may be eliminated by decreasing the Zn^{2+} level, as mentioned earlier (16). Also, results showed that insulin levels in the plasma and zymogen form of caspase-3 levels in the pancreas were increased, and pancreatic morphology was improved in the LPS+TQ group.

QA is an agonist of neuronal N-methyl-D-aspartate receptors (NMDARs) [45], and KYNA is a competitive NMDAR antagonist (59). Lockridge et al. showed that D-serine can have acute antidiabetic effects in mice and potentiates insulin secretion through excitatory β-cell NMDAR co-agonism (60). Thus, our results suggest that changes in QA and KYNA levels may be responsible for decreasing β-cell number and function in the LPS group's pancreas. TQ administration may improve this by regulating Zn^{2+} levels through ZIP10. Our findings were consistent with previous studies, which showed that TQ protects against STZ-induced diabetes by repressing apoptosis of β-cells, ameliorating β-cell ultrastructure, and leading to insulin secretion (61, 62); KYNA, which has potentiated hyperglycemic effects by inhibiting proinsulin synthesis and insulin secretion in rat pancreatic islet cells (13) and an apoptotic effect on cancer cells [55, 56].

Additionally, TQ administration decreased plasma insulin levels may be related to suppressed β-cell function and insulin secretion due to the effect of TQ on insulin sensitivity (63). This may be due to the increased KYNA levels in the pancreas of rats administered only TQ because KYNA and QA continue to act oppositely in inflammation and other functions (64).

Notably, our results suggested a rise in inflammatory cytokine levels in the pancreas following TQ administration in the LPS+TQ group. Cytokines are central mediators of immune responses, and T helper (Th) cells are professional cytokine-producing cells. Once activated, CD4+ T helper cells further differentiate into Th1 cells, which specialize in producing IL-2, IL-6, IL-12, and IFNγ. Therefore, agents that can influence the Th cells' differentiation have the potential to alter the adaptive immune response in various diseases and medical conditions (65). Th1 cells are crucial for host defense against intracellular pathogens, including viruses, protozoa, and bacteria. One of their primary functions is to activate macrophages by producing IFN-γ (66). Additionally, our findings were consistent with the observation that purified protein extracts of N. sativa seeds significantly enhanced the production of TNF-α from unstimulated and PWM-activated lymphocytes [52] and serum IFN-γ levels in CMV-infected mice (67). Also, our results are consistent with the study's findings, which show that TQ reduces bone resorption in an experimental periodontitis model (68). Therefore, our results suggest that activation of the immune response by TQ has a beneficial effect on impaired glucose regulation and bone resorption induced by *P. gingivalis*-LPS.

When the results obtained from our research are evaluated together, it can be inferred that the increase in pancreatic Zn^{2+} concentration via ZIP10, induced by LPS treatment, triggers the KP pathway and enhances insulin synthesis as an adaptive mechanism against the disturbance of pancreatic function. TQ treatment reversed the effect of LPS on KP pathways, improved the pancreas morphological structure, and increased insulin synthesis.

This study has certain limitations, primarily due to the use of an animal model that may not fully reflect the complexity of human pathophysiology. Additionally, further research is required to elucidate the precise molecular mechanisms involved. Future investigations using ZIP10 knockdown or knockout models, as well as exploring other zinc transporters such as ZnT1, ZnT8, and ZIP14, will be essential to understand better the role of zinc regulation in the pathophysiological link between periodontitis and type 2 diabetes.

5. Conclusıons

In conclusion, our results suggest that oral application of *P. gingivalis*-LPS causes an increase in pancreatic Zn levels by ZIP10, and causes a change in KP metabolites, favoring KYNA production and resulting in apoptosis of pancreatic cells. TQ administration reverses these changes induced by *P. gingivalis*, alleviating the impairment of β-cell function.

Our study examined the relationship between periodontal diseases and diabetes in the early period when oral health deterioration and bone resorption begin through the Zn-mediated changes in the KP pathway, specifically for *P. gingivalis*. According to the results of our study, the *P. gingivalis* pathogen may mediate the formation of diabetogenic conditions via the KP pathway in the deterioration of oral health. The results obtained from our research are expected to contribute to understanding this link.

LEGENDS OF FIGURES

FIGURE 1: Experimental design.

Abbreviations: LPS, *Porphyromonas gingivalis*-lipopolysaccharide (*P. gingivalis*-LPS); **TQ,** Thymoquinone

FIGURE 2: X-Ray Films of the Periodontal Tissue.

Abbreviations: LPS, *Porphyromonas gingivalis*-lipopolysaccharide (P. gingivalis-LPS) injected group; **TQ,** Thymoquinone administered group; **LPS+TQ,** *P. gingivalis*-LPS injected and TQ administered group.

FIGURE 3: Analysis of Trp, KYN, KYNA, and QA levels

A: The Trp levels of pancreas tissue; **B:** KYNA levels of pancreas tissue; **C:** The KYN levels of pancreas tissue; **D:** The QA levels of pancreas tissue.

Statistical analyses were done using Kruskal-Wallis's One Way Analysis of Variance on Ranks. All pairwise multiple comparison procedures were done using the Mann-Whitney U test. All values are mean ± SEM and n=6 for each group. *, p<0,05 vs control; **, p<0,01 vs control; ***, p<0,001 vs control; $^{\#}$, p<0,05 vs TQ; $^{\#\#}$, p<0,01 vs TQ; $^{\#\#\#}$, p<0,001 vs TQ; $^{\varepsilon}$, p<0,05 $^{\varepsilon\varepsilon}$, p<0,01 vs LPS $^{\varepsilon\varepsilon\varepsilon}$, p<0,001 vs LPS.

Abbreviations: Trp, Tryptophan; **KYN,** Kynurenine; **KYNA,** Kynurenic acid; **QA,** quinolinic acid

FIGURE 4:Analysis of Kyn/Trp ratio, **KMO, and Kynureninase levels**

A: Kyn/Trp levels of pancreas tissue; **B:** KMO levels of pancreas tissue; **C:** Kynureninase levels. Statistical analysis of KMO levels of pancreas tissue was done using a one-way analysis of variance (ANOVA) followed by Tukey's Post Hoc. Statistical analysis of **Kyn/Trp** and Kynureninase levels in pancreas tissue was done using Kruskal-Wallis's One Way Analysis of Variance on Ranks. All pairwise multiple comparison procedures were done using the Mann-Whitney U test. All values are mean ± SEM and n=6 for each group.*, p<0,05 vs control; **, p<0,01 vs control; ***, p<0,001 vs control; $^{\#}$, p<0,05 vs TQ; $^{\#\#}$, p<0,01 vs TQ; $^{\#\#\#}$, p<0,001 vs TQ; $^{\varepsilon}$, p<0,05 $^{\varepsilon\varepsilon}$, p<0,01 vs LPS $^{\varepsilon\varepsilon\varepsilon}$, p<0,001 vs LPS.

Abbreviations: Trp, Tryptophan; **KYN,** Kynurenine; **KMO,** Kynurenine 3-monooxygenase.

FIGURE 5: Histological changes in the pancreas with hematoxylin and eosin stain (magnification × 40). **Control:** Photomicrograph of the pancreas from control rats showing normal parenchyma cells with collagen fibrils. **TQ:** Photomicrograph of the pancreas from TQ group rats showing typical tissue architecture. **LPS:** Photomicrograph of the pancreas from LPS groups showing abnormal acinar cells with mononuclear cell infiltration, inflammation, and hemorrhage. **LPS+TQ:** Photomicrograph of the pancreas from rats from the LPS+ TQ groups showing many areas of typical tissue architecture with mild inflammatory changes.

Graphical Abstract

A: The effect of *P.gingivalis*-LPS injection on pancreatic KP and glucose homeostasis

B: The effect of TQ administration on changes of *P.gingivalis*-LPS-Induced pancreatic KP and glucose homeostasis

Abbreviations: ***P.gingivalis*-LPS,** *Porphyromonas gingivalis*-lipopolysaccharide**; Trp,** Tryptophan; **KYN,** Kynurenine. **KP,** Kynurenine pathway; **KYNA,** kynurenic acid; **QA,** quinolinic acid; **KMO,** Kynurenine 3-monooxygenase, **IDO**, indoleamine 2,3-dioxygenase **TDO**, tryptophan-2,3-dioxygenase.

References

1. Al-Qutub MN, Braham PH, Karimi-Naser LM, Liu X, Genco CA, Darveau RP. Hemin-dependent modulation of the lipid A structure of Porphyromonas gingivalis lipopolysaccharide. Infect Immun. 2006;74(8):4474-85.
2. Corbella S, Taschieri S, Francetti L, De Siena F, Del Fabbro M. Periodontal disease as a risk factor for adverse pregnancy outcomes: a systematic review and meta-analysis of case-control studies. Odontology. 2012;100(2):232-40.
3. Renvert S, Berglund JS, Persson GR, Söderlin MK. The association between rheumatoid arthritis and periodontal disease in a population-based cross-sectional case-control study. Bmc Rheumatol. 2020;4(1).
4. Genco RJ, Graziani F, Hasturk H. Effects of periodontal disease on glycemic control, complications, and incidence of diabetes mellitus. Periodontology 2000. 2020;83(1):59-65.
5. Blasco-Baque V, Garidou L, Pomie C, Escoula Q, Loubieres P, Le Gall-David S, et al. Periodontitis induced by Porphyromonas gingivalis drives periodontal microbiota dysbiosis and insulin resistance via an impaired adaptive immune response. Gut. 2017;66(5):872-85.
6. Zenobia C, Darveau RP. Does Oral Endotoxin Contribute to Systemic Inflammation? Front Oral Health. 2022;3.
7. Verma A, Azhar G, Zhang XM, Patyal P, Kc G, Sharma S, et al. -LPS Induces Mitochondrial Dysfunction Mediated by Neuroinflammation through Oxidative Stress. International Journal of Molecular Sciences. 2023;24(2).
8. Fine N, Chadwick JW, Sun C, Parbhakar KK, Khoury N, Barbour A, et al. Periodontal Inflammation Primes the Systemic Innate Immune Response. Journal of Dental Research. 2021;100(3):318-25.
9. Radhakrishnan P, Anbalagan R, Barani R, Mani M, Seshadri KG, Srikanth P. Sequencing of Porphyromonas gingivalis from saliva in patients with periodontitis and type 2 diabetes mellitus. Indian J Med Microbi. 2019;37(1):54-9.
10. Pushparani DS. Zinc and type 2 diabetes mellitus with periodontitis - a systematic review. Curr Diabetes Rev. 2014;10(6):397-401.
11. Liu Y, Li X, Liu S, Du J, Xu J, Liu Y, et al. The changes and potential effects of zinc homeostasis in periodontitis microenvironment. Oral Dis. 2023;29(8):3063-77.
12. Koenig P, Nagl C, Neurauter G, Schennach H, Brandacher G, Fuchs D. Enhanced degradation of tryptophan in patients on hemodialysis. Clin Nephrol. 2010;74(6):465-70.
13. Kurgan S, Önder C, Balci N, Akdogan N, Altingöz SM, Serdar MA, et al. Influence of periodontal inflammation on tryptophan-kynurenine metabolism: a cross-sectional study. Clin Oral Invest. 2022;26(9):5721-32.
14. Oxenkrug G. Insulin resistance and dysregulation of tryptophan-kynurenine and kynurenine-nicotinamide adenine dinucleotide metabolic pathways. Mol Neurobiol. 2013;48(2):294-301.
15. Sudar-Milovanovic E, Gluvic Z, Obradovic M, Zaric B, Isenovic ER. Tryptophan Metabolism in Atherosclerosis and Diabetes. Curr Med Chem. 2022;29(1):99-113.
16. el-Sewedy SM, Abdel-Tawab GA, el-Zoghby SM, Zeitoun R, Mostafa MH, Shalaby SM. Studies with tryptophan metabolites in vitro. Effect of zinc, manganese, copper and cobalt ions on kynurenine hydrolase and kynurenine aminotransferase in normal mouse liver. Biochem Pharmacol. 1974;23(18):2557-65.
17. Aboubakr M, Elshafae SM, Abdelhiee EY, Fadl SE, Soliman A, Abdelkader A, et al. Antioxidant and Anti-Inflammatory Potential of Thymoquinone and Lycopene Mitigate the Chlorpyrifos-Induced Toxic Neuropathy. Pharmaceuticals (Basel). 2021;14(9).
18. Isaev NK, Chetverikov NS, Stelmashook EV, Genrikhs EE, Khaspekov LG, Illarioshkin SN. Thymoquinone as a Potential Neuroprotector in Acute and Chronic Forms of Cerebral Pathology. Biochemistry-Moscow+. 2020;85(2):167-76.
19. Khuda F, Baharin B, Anuar NNM, Satimin BSF, Nasruddin NS. Effective Modalities of Periodontitis Induction in Rat Model. J Vet Dent. 2023.
20. Dumitrescu AL, Abd El-Aleem S, Morales-Aza B, Donaldson LF. A model of periodontitis in the rat: effect of lipopolysaccharide on bone resorption, osteoclast activity, and local peptidergic innervation. J Clin Periodontol. 2004;31(8):596-603.
21. Khuda F, Baharin B, Anuar NNM, Satimin BSF, Nasruddin NS. Effective Modalities of Periodontitis Induction in Rat Model. J Vet Dent. 2024;41(1):49-57.
22. Dumitrescu AL, Abd-El-Aleem S, Morales-Aza B, Donaldson LF. A model of periodontitis in the rat: effect of lipopolysaccharide on bone resorption, osteoclast activity, and local peptidergic innervation. J Clin Periodontol. 2004;31(8):596-603.
23. Zhou XJ, Wang FF, Wu HS, Chen XW, Zhang Y, Lin JT, et al. Thymoquinone Suppresses the Proliferation, Migration and Invasiveness through Regulating ROS, Autophagic Flux and miR-877-5p in Human Bladder Carcinoma Cells. Int J Biol Sci. 2021;17(13):3456-75.
24. Afsar E, Kantar D. How does zinc chelation affect liver sphingolipid metabolism in an Alzheimer's-like model? J Trace Elem Med Biol. 2025;87:127589.

25. Zentrichova V, Pechova A, Kovarikova S. Zinc Concentration in Blood Serum of Healthy Dogs. Biol Trace Elem Res. 2023;201(7):3356-66.
26. Lu JC. [Automatic detection and clinical application of semen biochemical markers]. Zhonghua Nan Ke Xue. 2018;24(4):291-6.
27. Yang S, Chen Z, Cao M, Li R, Wang Z, Zhang M. Pioglitazone ameliorates Abeta42 deposition in rats with diet-induced insulin resistance associated with AKT/GSK3beta activation. Mol Med Rep. 2017;15(5):2588-94.
28. Beamish CA, Gaber AO, Fraga DW, Hamilton DJ, Sabek OM. Pretransplant HOMA-β Is Predictive of Insulin Independence in 7 Patients With Chronic Pancreatitis Undergoing Islet Autotransplantation. Transplant Direct. 2022;8(10).
29. Strasser B, Berger K, Fuchs D. Effects of a caloric restriction weight loss diet on tryptophan metabolism and inflammatory biomarkers in overweight adults. Eur J Nutr. 2015;54(1):101-7.
30. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem. 1976;72:248-54.
31. Periyanayagam S, Arumugam G, Ravikumar A, Ganesan VS. Thymoquinone ameliorates NLRP3-mediated inflammation in the pancreas of albino Wistar rats fed ethanol and high-fat diet. J Basic Clin Physiol Pharmacol. 2015;26(6):623-32.
32. Dur A, Kose H, Kocyigit A, Kocaman O, Ismayilova M, Sonmez FC. The anti-inflammatory and antioxidant effects of thymoquinone on ceruleine induced acute pancreatitis in rats. Bratisl Lek Listy. 2016;117(10):614-8.
33. Wang Y, Fan Q, Xu Y, Zeng F, Liu X, Zhao D, et al. Effect of Eucommia water extract on gingivitis and periodontitis in experimental rats. BMC Oral Health. 2022;22(1):326.
34. Parvaneh M, Witting PK, Ku J, Moradi T, Eroglu E, Freedman B, et al. Periodontitis induces endothelial dysfunction in mice. Sci Rep. 2021;11(1):14993.
35. Hu H, Nakagawa T, Honda T, Yamamoto S, Mizoue T. Should insulin resistance (HOMA-IR), insulin secretion (HOMA-β), and visceral fat area be considered for improving the performance of diabetes risk prediction models. Bmj Open Diab Res Ca. 2024;12(1).
36. Tripathi BK, Srivastava AK. Diabetes mellitus: complications and therapeutics. Med Sci Monit. 2006;12(7):RA130-47.
37. Zhang AMY, Wellberg EA, Kopp JL, Johnson JD. Hyperinsulinemia in Obesity, Inflammation, and Cancer. Diabetes Metab J. 2021;45(4):622.
38. Saisho Y. beta-cell dysfunction: Its critical role in prevention and management of type 2 diabetes. World J Diabetes. 2015;6(1):109-24.
39. Abdelmageed ME, Shehatou GSG, Suddek GM, Salem HA. Protocatechuic acid improves hepatic insulin resistance and restores vascular oxidative status in type-2 diabetic rats. Environ Toxicol Pharmacol. 2021;83:103577.
40. Lee CY, Kuan YH, Tsai YF, Tai CJ, Tsai TH, Huang KH. Correlation between diabetes mellitus and periodontitis in Taiwan: A nationwide cohort study. Diabetes Res Clin Pr. 2019;150:245-52.
41. Prasad AS. Zinc in human health: effect of zinc on immune cells. Mol Med. 2008;14(5-6):353-7.
42. Liuzzi JP, Cousins RJ. Mammalian zinc transporters. Annu Rev Nutr. 2004;24:151-72.
43. Kambe T. [Overview of and update on the physiological functions of mammalian zinc transporters]. Nihon Eiseigaku Zasshi. 2013;68(2):92-102.
44. Foster M, Petocz P, Samman S. Inflammation markers predict zinc transporter gene expression in women with type 2 diabetes mellitus. J Nutr Biochem. 2013;24(9):1655-61.
45. Li YV. Zinc and insulin in pancreatic beta-cells. Endocrine. 2014;45(2):178-89.
46. Zhao T, Huang Q, Su Y, Sun W, Huang Q, Wei W. Zinc and its regulators in pancreas. Inflammopharmacology. 2019;27(3):453-64.
47. Sun W, Yang J, Wang W, Hou J, Cheng Y, Fu Y, et al. The beneficial effects of Zn on Akt-mediated insulin and cell survival signaling pathways in diabetes. J Trace Elem Med Biol. 2018;46:117-27.
48. Gyulkhandanyan AV, Lu H, Lee SC, Bhattacharjee A, Wijesekara N, Fox JE, et al. Investigation of transport mechanisms and regulation of intracellular Zn2+ in pancreatic alpha-cells. J Biol Chem. 2008;283(15):10184-97.
49. Gyulkhandanyan AV, Lee SC, Bikopoulos G, Dai F, Wheeler MB. The Zn2+-transporting pathways in pancreatic beta-cells: a role for the L-type voltage-gated Ca2+ channel. J Biol Chem. 2006;281(14):9361-72.
50. Foster M, Chu A, Petocz P, Samman S. Zinc transporter gene expression and glycemic control in post-menopausal women with Type 2 diabetes mellitus. J Trace Elem Med Biol. 2014;28(4):448-52.
51. Takatani-Nakase T, Matsui C, Maeda S, Kawahara S, Takahashi K. High Glucose Level Promotes Migration Behavior of Breast Cancer Cells through Zinc and Its Transporters. Plos One. 2014;9(2).
52. Kurgan S, Onder C, Balci N, Akdogan N, Altingoz SM, Serdar MA, et al. Influence of periodontal inflammation on tryptophan-kynurenine metabolism: a cross-sectional study. Clin Oral Investig. 2022;26(9):5721-32.
53. Yang J, Hao T, Liu Y, Huang J, Wu W, Wu J, et al. Th17/Treg balance and indoleamine 2,3 dioxygenase activity in periodontitis-associated atherosclerotic patients. J Int Med Res. 2022;50(2):3000605221080877.
54. Garay E, Schuth N, Barbanente A, Tejeda-Guzman C, Vitone D, Osorio B, et al. Tryptophan regulates Drosophila zinc stores. Proc Natl Acad Sci U S A. 2022;119(16):e2117807119.
55. George MM, Subramanian Vignesh K, Landero Figueroa JA, Caruso JA, Deepe GS, Jr. Zinc Induces Dendritic Cell Tolerogenic Phenotype and Skews Regulatory T Cell-Th17 Balance. J Immunol. 2016;197(5):1864-76.
56. Asadi M, Taghizadeh S, Kaviani E, Vakili O, Taheri-Anganeh M, Tahamtan M, et al. Caspase-3: Structure, function, and biotechnological aspects. Biotechnol Appl Biochem. 2022;69(4):1633-45.

57. Gáspár R, Nógrádi-Halmi D, Demján V, Diószegi P, Igaz N, Vincze A, et al. Kynurenic acid protects against ischemia/reperfusion injury by modulating apoptosis in cardiomyocytes. Apoptosis. 2024;29(9-10):1483-98.
58. Arya A, Al-Obaidi MM, Shahid N, Bin Noordin MI, Looi CY, Wong WF, et al. Synergistic effect of quercetin and quinic acid by alleviating structural degeneration in the liver, kidney and pancreas tissues of STZ-induced diabetic rats: a mechanistic study. Food Chem Toxicol. 2014;71:183-96.
59. Hogan-Cann AD, Anderson CM. Physiological Roles of Non-Neuronal NMDA Receptors. Trends Pharmacol Sci. 2016;37(9):750-67.
60. Lockridge A, Gustafson E, Wong A, Miller RF, Alejandro EU. Acute D-Serine Co-Agonism of beta-Cell NMDA Receptors Potentiates Glucose-Stimulated Insulin Secretion and Excitatory beta-Cell Membrane Activity. Cells-Basel. 2021;10(1).
61. El-Shemi AG, Kensara OA, Alsaegh A, Mukhtar MH. Pharmacotherapy with Thymoquinone Improved Pancreatic β-Cell Integrity and Functional Activity, Enhanced Islets Revascularization, and Alleviated Metabolic and Hepato-Renal Disturbances in Streptozotocin-Induced Diabetes in Rats. Pharmacology. 2018;101(1-2):9-21.
62. Abdelmeguid NE, Fakhoury R, Kamal SM, Al Wafai RJ. Effects of Nigella sativa and thymoquinone on biochemical and subcellular changes in pancreatic beta-cells of streptozotocin-induced diabetic rats. J Diabetes. 2010;2(4):256-66.
63. Shaukat A, Zaidi A, Anwar H, Kizilbash N. Mechanism of the antidiabetic action of Nigella sativa and Thymoquinone: a review. Front Nutr. 2023;10:1126272.
64. Badawy AA. Hypothesis kynurenic and quinolinic acids: The main players of the kynurenine pathway and opponents in inflammatory disease. Med Hypotheses. 2018;118:129-38.
65. Majdalawieh AF, Fayyad MW. Immunomodulatory and anti-inflammatory action of Nigella sativa and thymoquinone: A comprehensive review. Int Immunopharmacol. 2015;28(1):295-304.
66. Zhu J. T Helper Cell Differentiation, Heterogeneity, and Plasticity. Cold Spring Harb Perspect Biol. 2018;10(10).
67. Salem ML, Hossain MS. Protective effect of black seed oil from

against murine cytomegalovirus infection. Int J Immunopharmaco. 2000;22(9):729-40.
68. Ozdemir H, Kara MI, Erciyas K, Ozer H, Ay S. Preventive effects of thymoquinone in a rat periodontitis model: a morphometric and histopathological study. J Periodontal Res. 2012;47(1):74-80.

Statements & Declarations

Funding

This work was supported by *KÜN.2024-BAGP-012* Grant numbers and Author E.A. has received research support from the Cappodoccia University Research Foundation, Turkey

Competing Interests

The authors declare no conflict of interest.

Author contribution statement

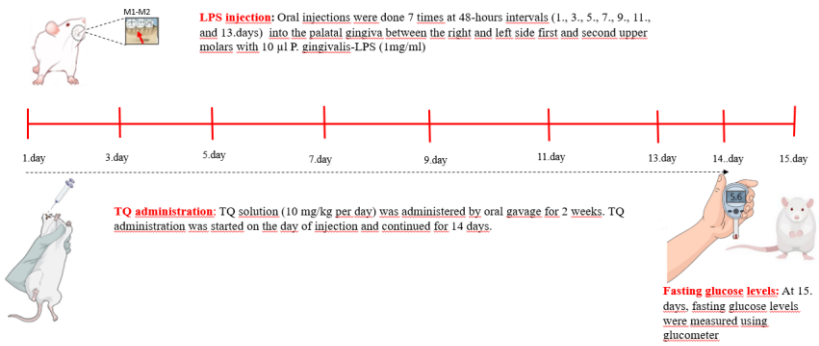
EA (Ebru Afşar), EA (Erdem Arslan), and MO did a *P.gingivalis* injection and oral gavage application. EA (Erdem Arslan) and MO also monitored laboratory animals' living conditions and nutrition. KD and TC sacrificed experimental animals and collected samples. They were also responsible for transferring samples from the experimental animal unit to Cappadocia University, where the analyses would be conducted. NO performed X-ray analyses and evaluated these analyses. IE performed hematoxylin and eosin staining analyses and evaluation of these analyses. SS performed oral examinations of experimental animals before and after the injection procedure. EA (Ebru Afşar) and SS conducted the literature review and designed the study. EA (Ebru Afşar) designed the study, performed biochemical and statistical analyses, and interpreted the analysis results.

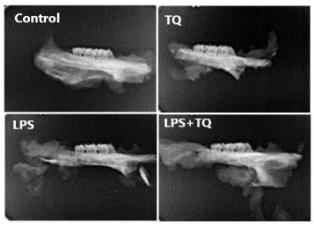
Data Availability Statement

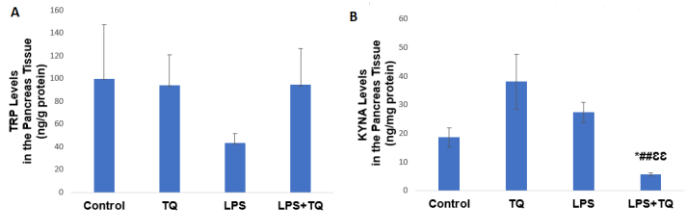
The data supporting this study's findings are available from the corresponding author upon reasonable request.

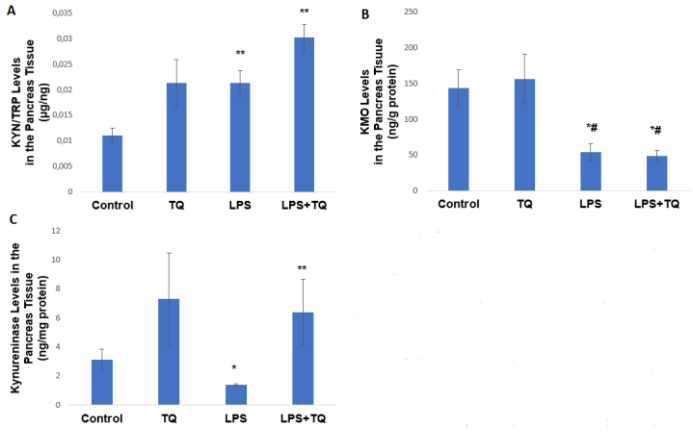
Ethics approval

Ethics approval provided by the Institutional Animal Care and Use Committee at Aksaray University (2024/9-56).

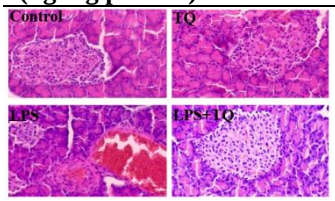








	Control	TQ	LPS	LPS+TQ
Plasma Zn Levels (µg/dL)	81.715±1.095	66.175±0.817*	53.514±3.937***	102.475±5.188$^{**\#\#\#ƐƐƐ}$
Pancreas Zn Levels (µg/mg protein)	5.690±0.117	3.655±0.154***	7.155±0.415**	3.063±0.223^{***ƐƐƐ}
Pancreas ZIP-10 Levels (ng/g protein)	83.069±3.548	57.300±3.656*	120.780±4.027$^{***\#\#\#}$	66.234±5.106^{*ƐƐƐ}
Pancreas IFN-γ levels (ng/g protein)	5.367±0.576	13.635±3.431	9.275±1.969*	16.347±4.710*
Pancreas Caspase- 3 Levels (ng/mg protein)	0.874 ± 0.327	0.841 ± 0.348	0.185 ± 0.021*	0.673 ± 0.2260



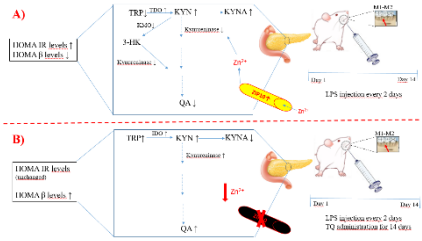


TABLE 1

Evaluation of cytokine, caspase-3, and Zn related paratameters between groups

Statistical analysis was done using Kruskal Wallis's One Way Analysis of Variance on Ranks. All pairwise multiple comparison procedures were done using the Mann-Whitney U test for analysis IFN-γ, and caspase 3 levels. Statistical analysis was performed using a one-way analysis of variance (ANOVA) followed by the Tukey post hoc test for analaysis of Zn and ZIP10 levels in the pancreas tissue, and Zn levels in tle plasma samples. All values are mean ± SEM. n=6 for each group . *, p<0.05 vs control; **, p<0.01 vs control; ***, p<0.001 vs control; #, p<0.05 vs. TQ; ##, p<0.001 vs. TQ; ###, p<0.000 vs. TQ, Ɛ, p<0.05 vs LPS; ƐƐ, p<0.01 vs LPS; ƐƐƐ, p<0.001 vs LPS.

Abbreviations: IFN-γ, interferon-γ; **Zn,** zinc

TABLE 2

Evaluation of glucose homeostasis between groups

Parameters	Control	TQ	LPS	LPS+TQ
	Median (25-75)	Median (25-75)	Median (25-75)	Median (25-75)
Total score	0 (0-0)	3 (2-3)	0 (0-1)*	2 (2-2)$^{*\#\text{Ɛ}}$
Levels (IU/L)	30.364±1.762	21.494±0.881*	37.470±3.542$^{\#\#\#}$	43.648±0.791$^{***\#\#\#}$
HOMA-IR Levels	6.266±0.392	4.982±0.288	10.734±0.868$^{***\#\#\#}$	12.362±0.422$^{***\#\#\#}$
HOMA-β Levels (%)	589 ±87.744	270±40.051**	257±35.838**	310±19.468**

Statistical analysis was done using a one-way analysis of variance (ANOVA) followed by Tukey's Post Hoc Test. All values are mean ± SEM. n=6 for each group *, p<0.05 vs control; **, p<0.01 vs control; ***, p<0.001 vs control; #, p<0.05 vs. TQ; ##, p<0.001 vs. TQ; ###, p<0.000 vs. TQ, Ɛ, p<0.05 vs LPS; ƐƐ, p<0.01 vs LPS; ƐƐƐ, p<0.001 vs LPS.

TABLE 2

Histopathological scores of pancreatic injury

Statistical analysis was done by Kruskal Wallis's One-Way Analysis of Variance on Ranks. All pairwise multiple comparison procedures were done using the Mann-Whitney U test. All values are mean ± SEM and n=6 for each group.*, p<0.05 vs control; #, p<0.05 vs TQ; Ɛ, p<0.05 vs LPS.