Investigation of the changes in hepatic 5-hydroxytryptamine 2A receptor levels caused by lipopolysaccharide of Porphyromonas gingivalis and the effect of Thymoquinone on these changes

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Abstract
The present study aimed to assess the effect of Porphyromonas gingivalis-Lipopolysaccharide (P.gingivalis-LPS) on hepatic disorder mediated by 5-hydroxytryptamine receptor 2A (HT2AR) and whether there is any impact of Thymoquinone (TQ) administration. 10 µl P. gingivalis-LPS (1mg/ml) injected 6 times at 48-hour intervals into the palatal gingiva of rats. TQ was given by oral gavage (10 mg/kg per day) for two weeks. HT2AR and pro-caspase-3 levels were measured by the enzyme-linked immunosorbent assay (ELISA). Morphological changes in the liver were identified by hematoxylin and eosin staining. In the P.gingivalis-LPS injected group, the hepatic HT2AR levels increased while pro-caspase-3 levels decreased, disturbing the liver morphological structure. TQ administration increased pro-caspase-3 levels and improved morphological structure despite increased HT2AR levels. Our results suggest that oral application of P. gingivalis-LPS decreased pro-caspase 3, an inactive form of caspase-3 by HT2AR, probably due to increased cleaved caspase-3 levels, and TQ administration increased pro-caspase-3 levels, leading to improved liver morphological structure. Therephore, our study indicated that oral pathogen P. gingivalis might impact the liver HT2AR-mediated, and TQ may have a beneficial effect.

Keywords: *5-Hydroxytryptamine 2A receptors, porphyromonas gingivalis, thymoquinone*

INTRODUCTION

Oral pathogens cause many periodontal diseases, including periodontitis. However, it is accepted that the inflammatory state that develops in periodontal diseases increases the susceptibility to many diseases, including diabetes, pneumonia, arthritis, cardiovascular diseases, and chronic liver diseases (Lontchi-Yimagou et al., 2013; Vernerova et al., 2022), and contributes to the clinical worsening of these diseases (Albuquerque-Souza & Sahingur, 2022). Additionally, periodontal pathogens can enter the bloodstream and trigger the release of various pathogenic factors, such as endotoxins and exotoxins, thus contributing to the inflammation of different tissues and finally leading to systemic diseases (Bui et al., 2019). Porphyromonas gingivalis (P. gingivalis), which is a critical oral pathogen that can activate inflammatory and immune responses in the periodontium (Hao et al., 2023), P. gingivalis or virulence factors such as lipopolysaccharide (LPS) can spread systemically through active periodontal lesions or by directly invading cells (Zenobia & Darveau, 2022). Understanding the molecular changes caused by oral pathogen P. gingivalis in liver tissue may contribute to the understanding of the connection between oral diseases and liver diseases. However, the molecular pathways contributing to liver diseases in impaired oral health are unclear.

5-hydroxytryptamine (5-HT), or serotonin, is derived from the amino acid tryptophan. Peripheral 5-HT is mainly expressed in the gut's enterochromaffin (EC) cells (Nonogaki, 2022). Peripheral serotonin has been shown to have complex effects as an endocrine regulator for lipid metabolism. It may lead to induced hepatic steatosis and oxidative stress in the liver through interaction with 5-HT receptor 2A (HT2AR) and 2B (HT2BR) (Li et al., 2023). Additionally, ıt has been shown that the upregulation of 5-HT2AR and 5-HT2BR levels is accompanied by increased lipid synthesis in liver tissue, leading to steatosis. Also, 5HT2AR blockade has been shown to reduce hepatic fibrosis and steatosis (Pagire et al., 2024). Therefore, these findings suggest that oral pathogens may affect hepatic 5HT2AR and mediate hepatic damage; furthermore, modulation of this receptor may contribute to preventing liver damage. Understanding the molecular mechanisms underlying P. gingivalis-mediated hepatic injury may provide new targets for the development of new therapeutic strategies.

Thymoquinone (TQ) is the main active component that is obtained from the essential oil of black cumin (Nigella sativa) seeds, and it is known to have antibacterial, anti-inflammatory, and antioxidant properties (Ali et al., 2021). In addition, previous studies have shown that TQ treatment alleviates periodontal disease pathogenesis (Ozdemir et al., 2012). Therefore, we investigated how P. gingivalis affects hepatic 5HT2AR-mediated hepatic damage, and whether TQ has a beneficial effect. Our study examined the hepatic 5HT2AR-mediated effect of P. gingivalis for the first time.

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MATERIALS AND METHODS

Animals and Experimental Design

Our study involved 24 male Wistar rats, each three months old, weighing 250 and 300 grams. During the experiment, standard rat food, and tap water were given ad libitum, and the animals were housed in stainless steel cages at standard conditions (23 ± 1 °C and humidity 50 ± 5%) with 12:12 h light-dark cycles at all times (16). All procedures carried out with experimental animals were carried out according to the standards determined by the Aksaray University Institutional Animal Care and Use Committee (2025/2-9).

Our study injected LPS of P. gingivalis (P. gingivalis-LPS) into the palatal gingiva to create a model of impaired periodontal health (Leira et al., 2019). P. gingivalis-LPS was provided in liquid form (1 mg/ml) dissolved in saline (InvivoGen, San Diego, CA, USA). TQ was supplied (Sigma Aldrich, Saint Louis, MO, USA) with >98% purity. Administration was performed using the dose previously reported in the literature (TQ's water solubility is reported to exceed 0.5 mg/mL, sufficient to demonstrate pharmacological effects) (Ali et al., 2021). TQ administration was applied by using oral gavage.

Figure 1 illustrates the experimental design. Animals were randomly divided into four groups, with n=6 in each group. **1. Control group**, received 10 µL salin injections into the palatal gingiva between the first and second maxillary molars on the right side, 6 times at 48-hour intervals and were given tap water by oral gavage for 14 days starting from the day of injections. **2.TQ group,** received 10 µL salin injections into the palatal gingiva between the first and second maxillary molars on the right side, 6 times at 48-hour intervals and were given TQ solution (10 mg/kg per day) by oral gavage for 14 days starting from the day of injections. **3.PGL group,** received 10 µL LPS injections into the palatal gingiva between the first and second maxillary molars on the right side, 6 times at 48-hour intervals and were given tap water by oral gavage for 14 days starting from the day of injections. **4. PGL-TQ group** received 10 µl P.gingivalis-LPS and were given TQ solution (10 mg/kg per day); animals in the control group received saline injections and tap water. All LPS injections were performed under anesthesia (Bhattarai et al., 2016). Finally, rats were anesthetized with a mixture of ketamine and xylazine hydrochloride (respectively, 80 mg/kg and 5 mg/kg) intraperitoneally and sacrificed, and liver tissues were taken. Tissues taken for biochemical analyses were suddenly frozen by liquid nitrogen and stored at -80° until analyzed, while tissue samples taken for histological analyses were stored in a solution containing 10% formalin (Lu et al., 2021).

Biochemical Analysis

The liver tissues were homogenized with cold phosphate buffer saline (PBS) and centrifuged at 10,000×g for 5 min. Finally, the supernatant was separated for biochemical analysis.

Caspase-3 levels: Caspase-3 levels were measured using the enzyme-linked immunosorbent assay technique, employing commercial kits from Bioassay Technology Laboratory (BT Lab) according to the manufacturer's instructions. The amount of protein in all samples was analyzed and expressed as caspase-3 levels ng/mg protein.

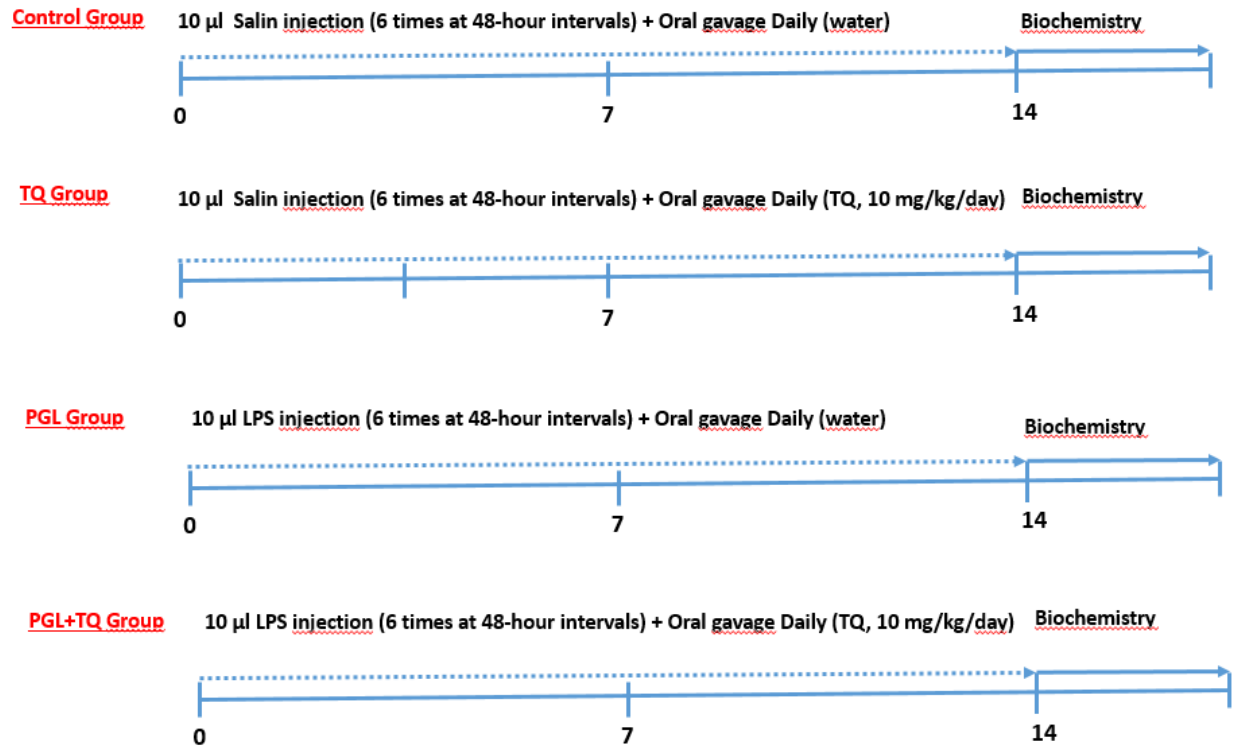

Figure 1. *Experimental design. TQ group, Thymoquinone administrated group; PGL group, P. gingivalis-lipopolysaccharide (P. gingivalis-LPS) injected group; PGL+TQ group, P.gingivalis-LPS injected and TQ administrated group.*

5-HT2AR levels: 5-HT2AR levels were measured using the enzyme-linked immunosorbent assay technique, employing commercial kits from Bioassay Technology Laboratory (BT Lab) according to the manufacturer's instructions (Afsar & Kantar, 2025). The amount of protein in all samples was analyzed and expressed as 5HT2A ng/mg protein.

Protein Measurements: The total protein concentration of all samples was determined using a modified Bradford assay (Bradford, 1976) on a microplate reader (Relassay Diagnostics).

Histopathological Examinations of Liver Tissues

The liver tissues were fixed using a 10% paraformaldehyde/PBS solution and then subjected to a standard paraffin-embedding process, obtaining 5-6 µm sections. Finally, the sections were assessed under light microscopy after being stained with Hematoxylin-eosin (H&E) (Leica DFC450, Almanya) (Lu et al., 2021). Scoring was applied to all experimental groups regarding histopathological changes in liver tissue sections. During histopathological scoring, hemorrhage, necrotic hepatocytes, and mononuclear cell infiltration were evaluated according to the criteria. Scoring was applied as: 0 = none, 1 = 0–25%, 2 = 26–45%, 3 = 46–75%, and 4 = 76–100% (Atteya et al., 2019).

Statistical Analysis

The statistical analyses of all data were conducted using SPSS 23.0 (SPSS, Chicago, IL, USA) software for Windows. Firstly, all data were evaluated to see whether they were usually distributed. After that, whether there is a difference between the groups was assessed by the Mann-Whitney U test after the Kruskal-Wallis test for non-normally distributed variables and Tukey's Post Hoc Test after one-way analysis of variance (ANOVA) for normally distributed variables.

RESULTS

Caspase-3 levels in liver tissue

As seen in Figure 2A, there were significant differences in the pro-caspase-3 levels between the various groups. The levels of pro-caspase-3 levels in the TQ group (0.230 ± 0.010 ng/mg protein) were significantly reduced compared to the control group (0.3233 ± 0.055 ng/mg protein), while the levels of pro-caspase-3 levels in the PGL group (0.196 ± 0.037 ng/mg protein) markedly reduced compared to the control group (respectively p=0.026 and p=0.150). However, this decreasing trend does not reach significant levels. Also, the pro-caspase-3 levels of the PGL-TQ group (0.432 ± 0.038ng/mg protein) significantly increased compared to the PGL group and TQ group (p=0.004 and p=0.004).

5-HT2AR levels in liver tissue

As seen in Figure 2 B, the 5-HT2AR levels of the various groups were significantly different.

The 5-HT2AR levels in the TQ group (229.309 ± 28 595 ng/g protein) and PGL group (268.956 ± 29.215 ng/g protein) were increased compared to the control group (170.444 ± 7.675 ng/g protein). However, this increasing trend does not reach significant levels. Also, the 5-HT2AR levels of the PGL-TQ group (417.102 ± 51.769 ng/g protein) increased in comparison with the control group, TQ group, and PGL group (respectively, p=0.000, p=0.004, p=0.024).

Histopathological analysis

The liver tissues' H&E results were evaluated, as seen in Figure 3. The liver tissues of the control group had a typical morphological structure, and the TQ group section had the same appearance as the control group. However, some areas showed signs of bleeding. Necrotic hepatocytes, congestion, and mononuclear inflammatory cellular infiltration were observed in the PGL group. The morphological appearance of hepatocytes in the PGL+TQ group was similar to that of the control and TQ groups. Also, the scoring results of the histopathological examinations of liver tissues are given in Table 1. When the PGL group's damage scores were examined, it was determined that they were statistically higher than the control group. The TQ group scores decreased statistically significantly compared to the PGL and PGL+TQ groups. The PGL+TQ group damage score significantly reduced compared to the PGL group but increased compared to the control and TQ groups (p<0.5).

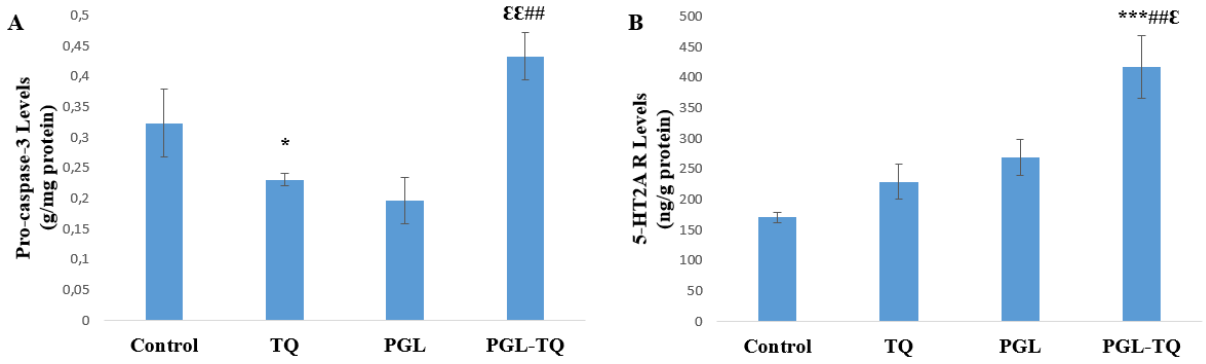

Figure 2. *The result of biochemical analysises. A: The pro-caspase-3 levels of liver tissue. B: The HT2AR levels of liver tissue. Statistical analysis of the pro-caspase-3 levels of liver tissue was done using Kruskal Wallis's One Way Analysis of Variance on Ranks. All pairwise multiple comparison procedures were done using the Mann-Whitney U test. Statistical analysis of the HT2AR levels of liver tissue was done using a one-way analysis of variance (ANOVA) followed by Tukey's Post Hoc. 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; Ɛ, p<0,05 vs PGL, ƐƐ, p<0,01 vs PGL ƐƐƐ, p<0,001 vs PGL.*

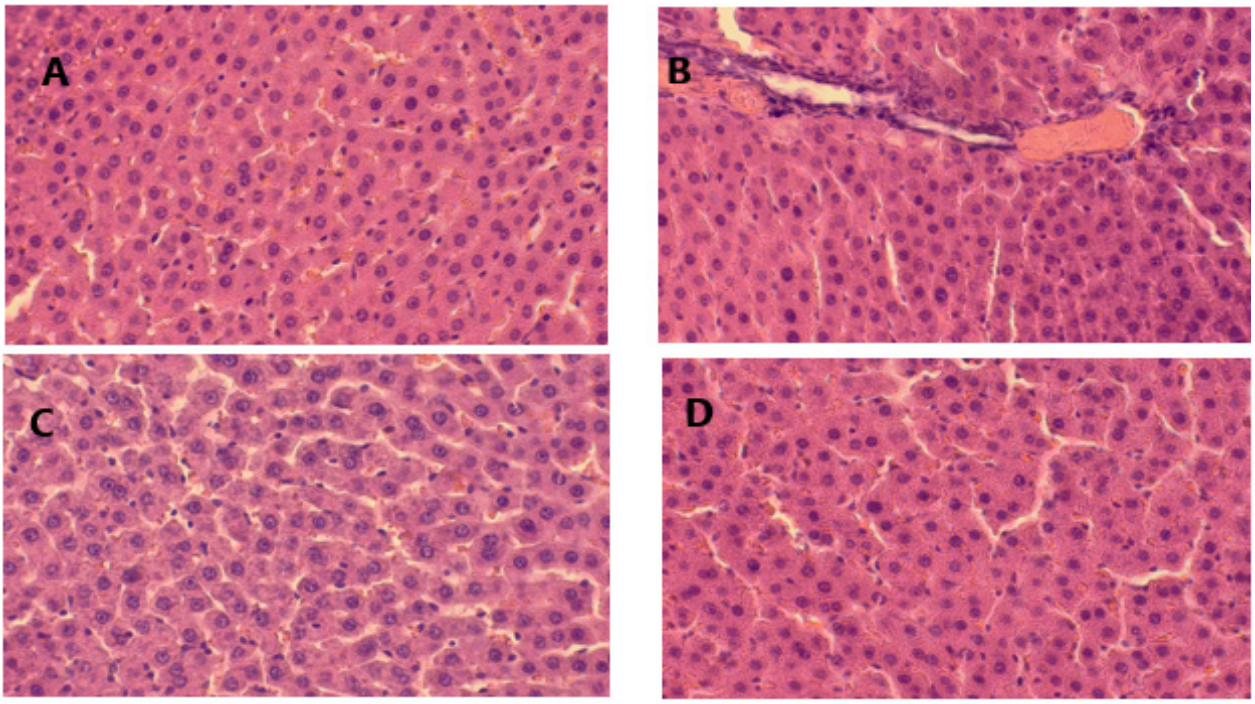

Figure 3. *Histological changes in the liver with H&E stain (magnification × 40). A: The control group displayed a typical hepatic structure; B: The PGL group displayed necrotic hepatocytes, congestion, and mononuclear cell infiltration; C: The TQ group had a histological appearance similar to the control group, bleeding areas were observed in some places; D: PGL+TQ group displayed the morphological appearance of hepatocytes was similar to the control group and the TQ group.*

Table 1. *Histopathological scores of liver injury*

Parameters	Control	TQ	LPS	LPS+TQ		
	Median (25-75)	Median (25-75)	Median (25-75)	Median (25-75)	Test İst.	p
Total score	0 (0-1)^{A}	3 (2-3)^{B}	1 (0-1)^{A}	1 (1-1,75)^{C}	99.3174	<0.001

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, A<C<D<B, p<0,05.

DISCUSSION

Periodontal diseases are chronic, inflammatory, and low-progressing. They can induce systemic inflammation and inflammatory response and may lead to cross-talk between periodontal disease and systemic disease, including liver disease (Albuquerque-Souza & Sahingur, 2022). Some researchers suggest impaired oral microbiota may lead to systemic inflammation and contribute to pathogenesis in cirrhosis individuals (Bajaj et al., 2015). Furthermore, recent studies indicate that oral pathogens such as P. gingivalis or virulence factors such as LPS (Zenobia & Darveau, 2022) can be easily transferred from the oral cavity to the intestines and lead to disruption of the intestinal microbiota composition, which may be a causal link between periodontal disease and liver disease (Rincic et al., 2021). Our study injected P. gingivalis-LPS into the palatal gingiva to create a model of impaired periodontal health (Leira et al., 2019). Thus, we investigated the impact of impaired oral health on hepatic 5-HT2AR-mediated hepatic morphological changes and the impact of TQ on these changes. Our result showed that oral P. gingivalis-LPS injection leads to impaired hepatic morphology. These results are compatible with previous studies that showed relationships between periodontal diseases and liver diseases (Albuquerque-Souza & Sahingur, 2022; Lu et al., 2023). However, many unknowns exist about how oral pathogens affect liver health or which molecular pathways mediate these relationships.

Many studies have focused on 5-HT's effect on the liver. Beaudry et al. found that the levels of 5-HT in the blood of individuals with cirrhosis increased, particularly in the early stages, more than in healthy individuals (Beaudry et al., 1994). Additionally, growing studies revealed that 5-HT has various impacts on liver diseases (Lesurtel et al., 2012). The roles of 5-HT depend on its different receptors in the tissues. 5-HT2AR and 5-HT2BR are mainly found in the liver (Liang et al., 2013). However, there is conflicting data on the role of 5-HT2R in the liver. Ebrahimkhani et al. showed that the activity of 5-HT2BR encourages hepatocyte regeneration and prevents ischemia/reperfusion injury after partial liver transplantation (Ebrahimkhani et al., 2011). At the same time, it worsens inflammation due to elevated levels of oxidative stress in the liver (Breard & Grillon, 2009). On the other hand, inhibiting the 5-HT2 receptor (5-HT2R) reduces prolife-

ration and increases apoptosis in the hematopoietic stem cells (HSCs) (Ruddell et al., 2006).

Our data showed that 5HT2AR levels increased while pro-caspase-3 levels were reduced in the liver of the PGL group. Caspases, apoptosis markers, are produced in an inactive form (pro-caspase), and apoptotic stimuli can induce cleavage of caspase-3 to cleaved-caspase-3 (Eskandari & Eaves, 2022). Therefore, our results suggested that 5HT2AR decreases caspase-3 levels due to an increase in cleaved-caspase-3 levels. By contrast with our data, Kim et al. showed that inhibiting 5-HT2AR suppresses cell viability and increases apoptosis in human hepatic stellate cells (Kim et al., 2013). However, previous studies consistent with our results showed that increasing 5-HT2A and 5-HT2B expression impaired hepatocyte lipid metabolism (Fu et al., 2016). Thus, our study showed that impaired oral health might affect hepatic health by increasing 5HT2AR levels for the first time.

Interestingly, TQ administration increased the 5HT2AR levels and decreased pro-caspase levels, similarly to the PGL group. which also didn't affect morphological structure; however, ıt seen that some areas showed signs of bleeding in the liver of the TQ group. The liver is an important detoxification organ, and various agents will inevitably affect the liver (Afsar & Kantar, 2025). Therefore, even if therapeutic efficacy has been demonstrated (Ali et al., 2021), it should not be overlooked that the agents used may also have adverse effects on the liver. Thus, our results indicate that caution should be exercised when using therapeutic agents.

Furthermore, when TQ was administered to rats injected with P. gingivalis-LPS, it was observed that there was an improvement in liver morphological changes, and both 5HT2AR and pro-caspase-3 levels increased. It is clearly shown that TQ has an increasing effect on hepatic 5HT2AR levels in TQ and PGL+TQ group rats. We suggest that these results are due to TQ's decreasing effect on pro-caspase-3 expression (Ates et al., 2022) and cleaving of caspase-3 (Dera et al., 2020) in the PGL+TQ group. Similarly, previous studies showed that TQ administration could decrease hepatic injury by caspase-3 expression (Ates et al., 2022; Atteya et al., 2019) and cleaving caspase-3 (Dera et al., 2020).

The obtained data are evaluated together; our results suggest that P. gingivalis-LPS increased 5HT2AR and active form caspase-3 levels and caused changes in hepatic morphology. Additionally, in PGL+TQ group rats, TQ administration improved hepatic changes, although the causes of increased pro-caspase-3 levels were probably due to decreasing cleavage of pro-caspase-3, as in previous studies (Dera et al., 2020).

CONCLUSION

In conclusion, our study suggests that 5-HT2AR is involved in P. gingivalis-LPS-mediated hepatic injury, and TQ may improve hepatic injury by decreasing caspase-3 activity. Our study showed that first-time impaired oral health affects liver health by hepatic 5-HT2AR.

Ethics Committee Approval

The Institutional Animal Care and Use Committee at Aksaray University granted ethical approval for this work (ethics approval date and number: 30.01.2025-2025/2-9).

Data Sharing Statement

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

Conflict of Interest

The authors report no conflicts of interest in this work

Authorship Contributions

EA (Ebru Afşar) conducted the literature review and designed the study. EA (Erdem Arslan), and MO did a P.gingivalis injection and oral gavage application. EÖ monitored laboratory animals' living conditions and nutrition. KD and EÖ performed the sacrifice of experimental animals and sample collection. IE performed hematoxylin and eosin staining analyses and evaluated these analyses. EA (Ebru Afşar) performed biochemical and statistical analyses and interpreted the study results.

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Availability of data and materials

All data can be used with permission.

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