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Effect of hesperidin on deltamethrin toxicity in rat periodontal membrane

Fikret İpek[1], **Engin Deveci**[2], **Polat İpek**[3]

[1] Dicle University Faculty of Dentistry, Department of Periodontology, Diyarbakır, Türkiye
[2] Dicle University, Faculty of Medicine, Department of Histology and Embryology, Diyarbakır, Türkiye
[3] Dicle University, Faculty of Veterinary Medicine, Department of Physiology, Diyarbakır, Türkiye

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Correspondence:

Dr. Fikret İPEK
Dicle University Faculty of Dentistry, Department of Periodontology, Diyarbakır, Türkiye
E-mail: melihsemih21@hotmail.com

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Abstract

Aim: Deltamethrin (DM), a hydrophobic and lipophilic substance used for insect control, easily accumulates in the body, increasing ROS production and causing mucosal inflammation and damage. The aim of this study was to explore the prophylactic effects of hesperidin on DM toxicity in periodontal membrane.

Methods: The study was conducted for 14 days on 21 male Wistar albino rats. The experimental animals were divided into three groups (n=7): The Sham group received 0.9% NaCl (1 mL). The deltamethrin group received 0.5 mg/kg DM (1 mL, dissolved in dimethyl sulfoxide). The deltamethrin + hesperidin group received DM (0.5 mg/kg) + hesperidin (100 mg/kg) (both drugs dissolved in 1 mL dimethyl sulfoxide). All treatments were administered daily via the orogastric route for 14 days. At the end of the experiment, the rats were euthanized by cervical dislocation, and biochemical and histological analyses were performed.

Results: The biochemical tests showed significantly higher malondialdehyde (MDA) levels in the deltamethrin group than in the Sham group. Combined application of hesperidin and deltamethrin resulted in significantly lower MDA levels in the deltamethrin + hesperidin group than in the deltamethrin group. Histopathological examination of the periodontal tissues of the deltamethrin group revealed cell degeneration at the alveolar bone-tooth junction, including atypical cells, irregularity in collagen fibers, dilatation and congestion of the blood vessels, and inflammatory cell infiltrates distributed in aggregate and solitary forms. The deltamethrin + hesperidin group showed substantially less cell degeneration.

Conclusion: Hesperidin application helped alleviate deltamethrin toxicity by preventing oxidative stress and reducing cell degeneration.

Keywords: Hesperidin, deltamethrin, periodontal membrane, rat

Introduction

Deltamethrin (DM), a hydrophobic and lipophilic substance used in insect control, easily accumulates in the body. It is a widely available insecticide and is typically used for extended periods; consequently, DM can show toxicity effects on the environment, animals, and even humans [1]. DM exposure increases reactive oxygen species (ROS) production, which causes damage and inflammation to many mucosal tissues, including the periodontal membrane.

Increases in oxidative stress are noted in periodontal disease as changes in local and systemic biomarkers for oxidative stress and periodontitis. DM-mediated ROS production can increase lipid peroxidation, tissue injury, nucleic acid damage, protein misfolding, and oxidation of vital cellular enzymes, and DM exposure activates signaling molecules and mediators associated with inflammation [2, 3]. Treatments to alleviate periodontal inflammation due to DM toxicity should therefore be aimed at reducing oxidative stress.

Previous work has identified hesperidin (3,5,7-trihydroxy-4′-methoxyflavanone) as a natural compound that has a healing effect on alveolar bone loss and lessens the intensity of inflammatory processes [4, 5]. Hesperidin belongs to the flavanone class of flavonoids and is a low molecular weight molecule ($C_{28}H_{34}O_{15}$). It is usually found as a ß-glycoside form, hesperidin-7-O-rutinoside, and is common in citrus fruits, especially oranges [6].

Hesperidin can prevent inflammation and oxidation and shows anti-metastatic properties and beneficial effects against neurological, cardiovascular, and other diseases. In the present study, hesperidin was investigated for its prophylactic effects against periodontal membrane deltamethrin toxicity.

Materials and Methods

For this research, ethics committee approval was received from Dicle University Sabahattin Payzın Health Sciences Research and Application Center Experimental Animals Local Ethics Committee (DUHADEK) (Decision no: 2022-1). The research was conducted at Dicle University Sabahattin Payzın Health Sciences Research and Application Center (DUSAM), and its budget was covered by Dicle University Scientific Research Projects Coordinatiorship (DUBAP) with decision number DİŞ.22.011.

Experimental Animals

The study continued for 14 days, and 21 male Wistar albino rats were used.

The rats were divided into three groups (n = 7). All animals had unlimited access to water and food and were housed in a controlled environment at 23 ± 2 °C and 12 h light/12 h darkness (8:00 a.m.–8 p.m.). The agents were administered at the same time of day. The animals' general condition was checked every day.

1. **Control Group:** During the experiment, 1 mL of 0.9% NaCl was given daily via the orogastric route.
2. **Deltamethrin (DM) group:** During the experiment, 1 mL DM (0.5 mg/kg) dissolved in DMSO was given daily via the orogastric route.
3. **Deltamethrin + Hesperidin group:** During the experiment, 1 mL DM (0.5 mg/kg) + hesperidin (100 mg/kg) dissolved in DMSO was given daily via the orogastric route.

At the end of the experiment, the rats were euthanized by cervical dislocation.

Histopathologic Analysis

Dental tissues were removed by separating the anterior incisors under ketamine hydroxide anesthesia and softened by applying ethylenediaminetetraacetic acid solution. Dental tissue was fixed in 10% formaldehyde for 24 h at room temperature, followed by dehydration in a graded ethanol series, embedding in paraffin, and sectioning with a microtome. The sections were stained with eosin and hematoxylin, and the histopathology of the periodontal tissues was evaluated by light microscopy (Carl Zeiss, Germany).

Biochemical Analyses

Dental samples were processed for standard biochemical protocols. Dental tissues were centrifuged for 5 min at 1550 × g. The supernatants were discarded, and the pellets were transferred to plastic tubes. All samples were stored at −80°C for further analysis of the malondialdehyde (MDA) content.

MDA was analyzed following the protocol described by Draper and Hadley [1]. MDA values were expressed as nmol/g of wet tissue.

Cardiac blood was also collected for biochemical analysis according to Ermiş's protocol [2]. The blood was centrifuged for 5 min to separate the serum, which was then used in spectrophotometric assays to measure the total antioxidant status (TAS) and total oxidant status (TOS) with a biochemical autoanalyzer (AU5800; Beckman Coulter, Inc., La Brea, CA, USA). The TAS values were recorded as µmol H_2O_2 eq/L, and TOS values were recorded as µmol Trolox eq/L.

Statistical analysis

The data were analyzed using IBM SPSS 25.0 software (IBM, Armonk, New York, USA).

The Shapiro–Wilk test was used for data distribution analysis. The Kruskal–Wallis test with a post hoc Bonferroni test was used for multiple comparisons. A value of $p < .05$ was considered statistically significant.

Results

Hematoxylin and eosin staining of incisive tooth section control group

In the sections from the control group, irregular collagen fibers and fusiform fibroblasts were observed in the periodontal membrane. Cells were mainly seen individually within the extracellular matrix. Macrophage cells were sporadically visible. Fibroblasts and other connective tissue cells were prominent in collagenized areas. Although inflammatory cells were observed in some areas, the periodontal membrane, in general, appeared normal (Fig. 1a).

Hematoxylin and eosin staining of the periodontal membrane section in the deltamethrin group

In the sections from the deltamethrin group, collagen deterioration was observed in the periodontal membrane, especially in the region near the tooth and alveolar bone junction. Inflammatory cells were distributed both in clusters and individually. Degeneration and thinning were detected in some collagen fibers, and blood vessel dilation was also observed. In the sections from this group, the hypertrophic and hyperplasic appearance of the cells, especially in the parts close to the tooth, was notable. Atypical cells were visible. Overall, the periodontal membrane structure, which is crucial for adhesion, was disrupted by deltamethrin, resulting in an increase in inflammation (Fig. 1b).

Incisive tooth section – hematoxylin and eosin staining in the deltamethrin + hesperidin group

In the sections from this group, the collagen fibers in the parts of the periodontal membrane close to the tooth were parallel to each other, and the fibroblast cells between them had a shuttle-like shape. Solitary inflammatory cell infiltrates were observed, especially on the alveolar bone side. Although dilation was observed in blood vessels, there was evidence of protection when compared to the deltamethrin group. Hesperidin appeared to be generally protective for connective tissue, and it may help to prevent collagen fiber degeneration and protect the vascular structures. However, the presence of inflammatory cell infiltration indicated that it did not significantly reduce inflammation (Fig. 1c).

Biochemical examination revealed a significant increase in MDA in the group that received deltamethrin compared to the sham group. It was observed that hesperidin application after deltamethrin resulted in a significant decrease in MDA compared to the deltamethrin group.

In the sham group, the TAS value decreased with deltamethrin application, while the TOS value was higher in the deltamethrin group than in the sham group. With deltamethrin + hesperidin application, the TAS value was close to that of the sham group, while the TOS value was higher than in the sham group and lower than in the deltamethrin group (Table 1)

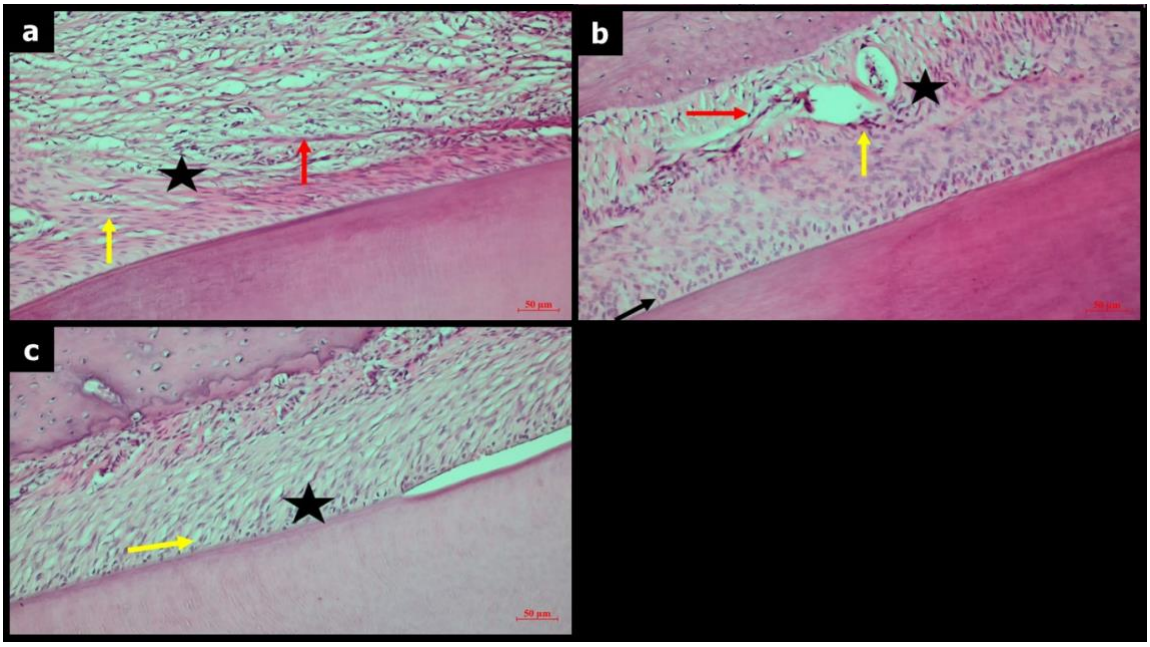

Figure 1. (a) Hematoxylin-eosin staining of the periodontal membrane section control group: Collagen fibers are irregular (red arrow), fibroblasts are fusiform (yellow arrow) and cells in the extracellular matrix are solitary (star); **(b)** Deltamethrin group hematoxylin-eosin staining: Deformations in collagen in the periodontal membrane (star) and inflammatory cell infiltration (yellow arrow), degeneration and thinning in some collagen fibers (red arrow) and cells in the parts close to the tooth are hypertrophic and hyperplasic (green arrow); **(c)** Periodontal membrane section - deltamethrin + hesperidin group hematoxylin and eosin staining: Collagen fibers running parallel to each other (yellow arrow) and solitary inflammatory cell infiltrates (asterisk).

Table 1. Differences between groups.

Parameter	Groups	n	Mean+SD	P value
MDA	Sham	10	5.35±2.14	p < 0.001*
	Deltamethrin	10	24.34±3.54	p < 0.001*
	Deltamethrin+Hesperidin	10	9.23±1.21	
TAS	Sham	10	1.92±0.34	p < 0.001*
	Deltamethrin	10	0.83±0.71	P < 0.001**
	Deltamethrin+Hesperidin	10	1.38±0.42	
TOS	Sham	10	13.55±3.69	p < 0.001*
	Deltamethrin	10	34.71±7.05	p < 0.001**
	Deltamethrin+Hesperidin	10	21.19±4.62	

* Group 1 vs Group 2; ** Group 2 vs Group 3

Discussion

The use of deltamethrin has different effects on different organs. It is associated with neurotoxicity symptoms, including ataxia, loss of coordination, hyperexcitation, tremors, convulsions, and paralysis [3]. Deltamethrin is a type II synthetic pyrethroid that is used as an insecticide against pests and parasites worldwide [1, 2]. However, pyrethroid contamination has become a concern in many agriculture-based countries, as oral ingestion of deltamethrin-contaminated water and food is harmful to humans [3]. Deltamethrin metabolites are known to cause toxicity due to oxidative stress. Deltamethrin (10 µM) has been shown to significantly increase ROS production in PC12 cells [7]. According to Kumar et al. [8], the administration of 25 and 50 µM of deltamethrin for 1 h increased ROS levels, which is a marker of apoptosis in thymocytes. In another study [7], deltamethrin (1 and 5 µM) induced cell death and ROS production in rat primary hepatocytes. These studies demonstrate that deltamethrin could directly cause oxidative stress damage through ROS production [9]. Moreover, Issam et al. [10] investigated the toxicity of deltamethrin when given to male Wistar rats for 30 days, 45 days, and 60 days (2, 20 and 200 mg/kg b.w., subcutaneous injection), reporting increased MDA levels.

In our study, deltamethrin application led to an increase in MDA, indicating increased lipid peroxidation and subsequent cell degeneration due to oxidative stress. The TAS and TOS values supported this increase in oxidative stress. In the group treated with hesperidin, the MDA value approached that in the sham group, and the TAS and TOS values were significantly balanced (Table 1).

Hesperidin, a known flavanone, consists of C28H34O15 and has a light molecular weight. It has many antioxidant structures, including flavanones and flavonoids. Hesperidin has beta glycoside bonds with bioflavonoid activity and is found in many fruits, such as oranges. For centuries, hesperidin has been used as an alternative medication to treat disease. It has radical scavenging activities and cleans oxidant enzymes. Indeed, numerous studies investigating the biological activities of hesperidin have shown that it can be used as an oxidant remover. Hesperidin treatment removes free radicals, which are dangerous to cells, including superoxide molecules. It also regulates vascular permeability, prevents inflammation signaling, reduces the production of microbes, and eliminates fungal and viral particles. Many studies have shown that hesperidin prevents metastatic activity and exhibits anticancer properties. In studies on skin disease, hesperidin treatment has also been shown to have anti-allergic properties [11-13].

In our study, histopathological examination revealed cell degeneration in the periodontal membrane at the alveolar bone–tooth junction, atypical cells, irregular collagen fibers, dilatation and congestion in blood vessels, and inflammatory cell infiltrates with both aggregate and solitary distributions in the deltamethrin group. Further, a decrease in cell degeneration was observed in the group receiving hesperidin, as well as a tightening of collagen fibers, a decrease in blood vessel dilatation, and a solitary distribution of inflammatory cells.

Conclusion

Based on the results, hesperidin application against deltamethrin toxicity is effective in preventing oxidative stress and reducing cell degeneration, and it may be an important antioxidant for regulating the angiogenetic mechanism. However, it should be supported by another treatment in cases of inflammation.

Disclosures

Acknowledgments: This study was carried out at Dicle University Sabahattin Payzın Health Sciences Research and Application Center (DUSAM).

Ethics Committee Approval: Ethics committee approval was received for this study from Dicle University Sabahattin Payzın Health Sciences Research and Application Center Experimental Animals Local Ethics Committee, with the approval number: 2022-1.

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Conflict of Interest: There is no any conflict of interest.

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