Therapeutic effects of Eucommia water extract on periodontitis in experimental rats

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Abstract

Herein, we evaluated the potential therapeutic effects of water extracts from Eucommia on periodontitis in experimental rats. We ligated the maxillary second molars of SD rats with 4.0 silk threads and locally smeared *Porphyromonas gingivalis* to induce gingivitis and periodontitis. After successfully establishing the model, we exposed the rats to Eucommia water extracts through topical smearing and intragastric administration and evaluated the therapeutic effect of the extracts on gingivitis (for a 2 week treatment period) and periodontitis (over 4 weeks). We recorded the alveolar bone resorption levels and analyzed histopathological sections of the periodontal tissue, molecules related to periodontal oxidative stress, and periodontal inflammatory factors to understand the feasibility of Eucommia in treating gingivitis and periodontitis. Results showed reduced damage to the periodontal tissue after treatment with extracts, indicating that Eucommia has a positive effect in treating gingivitis and periodontitis in experimental rats. These findings are expected to provide the foothold for future research on secondary metabolites derived from Eucommia and guide the development of novel approaches for preventing and treating periodontal disease.

Introduction

Periodontitis is a chronic infectious disease caused by microorganisms that destroy the periodontal tissue and cause alveolar bone resorption and loss of connective tissue. It is a major oral disease that results in tooth loss in adults and endangers general health [1]. The disease is of significant concern worldwide, owing to its high incidence in both developed and developing countries. An increasing body of evidence suggests that *Porphyromonas gingivalis* (*P. gingivalis*) is one of the main pathogenic bacteria that play an important role in chronic and aggressive periodontitis[2–4].

The commonly used control measures against periodontitis include mechanical approaches such as brushing, flossing and interdental brushing for adjacent tooth cleaning [5], and pharmaceutical interventions such as mouthwashes and fluorides (fluorine-containing coatings, fluorine-containing gels). Despite the current emphasis placed on oral health, not enough of it is directed to preventing periodontal disease (PD). For example, the correct method for brushing teeth has not been popularized, while adjacent surface cleaning measures such as dental floss and interdental brushes have not been accepted by the public [6]. According to the third and fourth National Chinese oral health epidemiological survey results, China's oral health literacy level and health behavior are still very low, warranting an urgent need for improvement.

Although periodontitis is not fatal, the current treatment methods do not lead to the complete recovery of periodontal tissue once the disease has occurred [7]. It is, therefore, necessary to urgently seek a safe, effective and easily acceptable method for prevention and treatment of the disease, thereby improving human health.
Eucommia ulmoides Oliv. is a second-class rare and protected tree species in China with valuable medicinal value and a long history of medicinal use [8]. Over 138 chemical constituents with active ingredients such as lignans, cyclic allene terpenes, phenylpropanoids, flavonoids, polysaccharides, and antifungal proteins, have been identified in this species. An in-depth study of the chemical composition, coupled with assaying for the pharmacological activities and clinical application of these compounds, has revealed the extent of its potential, accounting for its broad application in medicine [9]. Current evidence suggests that the pharmacological effects of Eucommia mainly include antihypertensive activity, enhancement of immunity, regulation of blood lipids, lowering of blood sugar, prevention of osteoporosis, and anti-inflammatory, antibacterial, and anti-tumor activities. Furthermore, compounds from the plant reportedly harbor hepatobiliary and diuretic capabilities, protect nerve cells, regulate bone metabolism, and nourish kidneys. However, the use of Eucommia for the prevention and treatment of periodontal disease remains unknown, warranting further research.

The current study assessed the therapeutic effects of secondary metabolites derived from Eucommia extracts in rat models of periodontal disease. Importantly, our findings can guide future research toward the development of strategies for the prevention and treatment of periodontitis.

1. Material And Methods

1.1 Animals models and plant extracts

A total of 44 male Sprague–Dawley (SD) rats (90g-110g) were obtained from Changsha Tianqin Biotechnology Co., Ltd. (SCXK2019-0013) for this study. Eucommia water extract was obtained from Xi’an Tianfeng Biotechnology Co., Ltd. (Xi’an, China).

1.2 Establishment of gingivitis model and treatment

Twenty SD rats were adaptively fed for 1 week and anesthetized with an intraperitoneal injection of 10% chloral hydrate (0.2 mL/100g, Yulong Seaweed Pharmaceutical Factory, Qingdao, China). The rats’ maxillary second molars were ligated with 4.0 silk threads into the gingival sulcus and locally smeared with P. gingivalis. The gingivitis model was successfully established after 2 weeks.

The 20 modeled rats were randomly assigned into two groups as follows; group 1 was the treatment group subjected to topical smear and intragastric administration of 800 mg/kg Eucommia water extracts once a day for 14 days. Group 2 was the control treated with normal saline for 14 days. All rats were sacrificed after 14 days of treatment, then oxidative stress-related molecules superoxide dismutase (SOD) and catalase (CAT) in the gingival tissues of the second molars were detected. The maxillary bones were taken for HE staining and observation.

1.3 Establishment of Periodontitis and treatment
A total of 24 SD rats, adaptively fed for 1 week, were first anesthetized with an intraperitoneal injection of 10% chloral hydrate (0.2mL/100g), then their maxillary second molars ligated with 4.0 silk threads into their gingival sulcus. They were later locally smeared with \textit{P. gingivalis}, and 4 rats were sacrificed after 4 weeks (based on pre-experiment). Alveolar bone resorption was detected using X-ray and methylene blue staining, while changes in the maxillary second periodontal tissues were detected by HE staining. The remaining 20 rats were randomly assigned into two groups: group 1 (treatment group) rats underwent topical smear and intragastric administration of 800 mg/kg Eucommia water extract once a day for 28 days. Group 2 (control group) mice were treated with normal saline for 28 days. All rats were sacrificed after 28 days of treatment, followed by an HE assay to detect SOD and catalase (CAT) in the gingival tissues of the second molars as well as inflammatory factors IL-1\(\beta\) and IFN-\(\gamma\) in serum,

1.4 Statistical analyses

Data were statistically analyzed using SPSS software version 18.0 and presented as means ± standard errors. An independent student’s \(t\)-test was used to compare two groups, while a one-way analysis of variance (ANOVA) was employed for comparisons among three groups. A 95% confidence level (\(p < 0.05\)) was used to indicate statistically significant values.

2. Results

2.1 Establishment of the gingivitis model in experimental rats

One week after smearing \textit{P. gingivalis}, the rats’ gingival tissues appeared pink, while their gingival margins became red and swollen. No bleeding was detected, and their teeth were not loose. At two weeks, the gingival tissues were swollen with dark red color and bleeding was observed, suggesting successful induction of gingivitis. Subsequently, the rats were sacrificed for sample collection.

2.2 Determination of oxidative stress-related molecules

We quantified the levels of superoxide dismutase (SOD) and catalase (CAT) in the gingival tissues of experimental rats according to the manufacturer’s instructions of the SOD (A001-3-1) and CAT UV (A007-2-1) kits (Nanjing Jiancheng Biotechnology Research Institute). The results showed that the SOD and CAT levels in the treatment group were significantly higher in the controls before modeling (\(p < 0.05\)) (Figure 1).

2.3 Analysis of periodontal tissues of second maxillary molars
The periodontal tissues of the second maxillary molars were analyzed by HE staining. In the treatment group, the position of the combined epithelium was restored to the enamel dentin boundary, the gingival sulcus epithelium showed no epithelial studs, and collagen fibers were formed (Figure 2). In the control group, erosion and epithelial degeneration occurred in the gingival sulcus epithelium. The combined epithelium proliferated and extended to the root of the teeth, while the collagen fibers developed hydropic degeneration (Figure 3).

2.4 Establishment of the periodontitis model in experimental rats

We found that the rats' gum tissues became red and swollen with a dark red color, bleeding spontaneously at four weeks of treatment. In addition, there was a loss of adhesion, and their teeth loosened in the cheek palate direction. This preliminary finding indicated that periodontitis was successfully induced, and 4 rats were sacrificed for further analysis.

X-ray films and methylene blue staining showed alveolar bone resorption in the second maxillary molar in the experimental rats. The alveolar bone of normal rats wrapped around the root, which proved that periodontitis was successfully induced at four weeks of modeling (Figure 4-5).

2.5 Analysis of maxillary periodontal tissues

The periodontal tissues of the second maxillary molars were analyzed following HE staining. The combined epithelium was found at the enamel dentin boundary in normal rats, while the gingival sulcus epithelium had no epithelial studs. In addition, collagen fibers gathered into a bundle and were arranged in a certain direction. In the modeling group, erosion and epithelial degeneration occurred in the gingival sulcus epithelium. The combined epithelium proliferated and extended to the root of teeth. At the same time, the combined epithelium had been separated from the root surface, the periodontal pocket was formed, and significant edema was observed in the collagen fiber (Figure 6).

2.6 Determination of oxidative stress-related molecules

Analysis of SOD and CAT levels in the gingival tissue of experimental rats showed significantly \((p < 0.05)\) higher levels in the treatment group relative to the control group (Figure 7).

2.7 Determination of serum inflammatory factors

We employed the ELISA technique to detect the levels of inflammatory factors IL-1\(\beta\) (AndyGene, AD3023Ra) and IFN-\(\gamma\) (AndyGene, AD3257Ra) in rat serum. Before treatment, there was no difference between the treatment and the control groups. After treatment, the levels of inflammatory factors in the
treatment group decreased, while those in the controls continued to increase. The differences between the two groups were statistically significant at week 8 ($p < 0.05$) (Figure 8).

### 2.8 Analysis of periodontal tissues of the maxillary

Analysis of the periodontal tissues of the second maxillary molars following HE staining indicated that in the treatment group, the position of the combined epithelium was restored to the enamel dentin boundary, the gingival sulcus epithelium had no epithelial studs, and collagen fibers were recovered (Figure 9). In the control group, we observed erosion and epithelial degeneration in the gingival sulcus epithelium. At the same time, the combined epithelium proliferated and extended to the root of teeth and was further separated from the root surface. Furthermore, the periodontal pocket was formed, and the collagen fiber indicated signs of hydropic degeneration (Figure 10).

### 3. Discussion

Oral diseases can occur due to external physical and chemical damage, pathogen invasion, dental and maxillofacial abnormalities and systemic diseases and are increasingly common in China. The most common oral disease globally is periodontitis, which has serious implications on people's health [10–12].

Overwhelming evidence substantiates that periodontal disease is also a risk factor for certain systemic diseases or conditions [13–15]. An association has been found between periodontal disease and poor blood glucose control in diabetic patients [16]. Interestingly, PD has also been implicated in the occurrence and development of cardiovascular diseases [17–18]. It is well-established that periodontal disease results from specific pathogenic bacteria [19], with clinical studies reporting that causative periodontal pathogens are found in atherosclerotic plaques. Besides, PD can cause preterm and low birth weight in infants, therefore, emphasizing the need to develop a safe, effective and easily acceptable method for its prevention and treatment [20].

To date, most research on the medicinal activity of *Eucommia ulmoides* has primarily focused on lowering blood sugar and pressure, lowering blood lipids, its use as an antioxidant, and improvement of osteoporosis and as an antibacterial agent [21]. However, its pharmaceutical value has not been fully exploited [22–23]. In addition, the scope of its current clinical applications is relatively narrow, given that its leaves have not been fully incorporated into clinical applications, with the only use being for adjuvant therapy. Furthermore, few indicators are available for experimental observation, the sample size used in previous studies is relatively small and no scientific theoretical basis has hitherto been established. Indeed, research on the pharmacological mechanism of action and specific targets is not thorough enough, while in-depth, comprehensive and systematic research has not been carried out.

In this study, we assessed the therapeutic effects of Eucommia extracts on gingivitis and periodontitis. We analyzed alveolar bone resorption levels, periodontal tissue pathological sections, and gingival tissue
oxidative stress-related molecules in periodontal tissues in experimental rats. In addition, we determined changes in periodontal tissues, such as inflammatory factors. The results showed an increase in oxidative stress-related molecules SOD and CAT in the gingival tissue of the rats in the treatment group in gingivitis and periodontitis rat models treated with Eucommia extracts by topical smearing and intragastric administration. This phenomenon led to the inhibition of reactive oxygen species, thereby protecting periodontal tissues. We also found a decrease in serum pro-inflammatory factors IL-1β and IFN-γ, indicating that periodontitis inflammation was effectively controlled. The periodontal tissues of the second maxillary molars were analyzed following HE staining and revealed that in the treatment group, the position of the combined epithelium had been restored to the enamel dentin boundary. At the same time, the gingival sulcus epithelium had no epithelial studs, and collagen fibers recovered. In the control group, erosion and epithelial degeneration occurred in the gingival sulcus epithelium. The combined epithelium proliferated, extended to the root of the teeth, and was further separated from the root surface. In addition, a periodontal pocket was formed, and the collagen fiber showed hydropic degeneration. These results suggest that Eucommia extracts played a role in restoring inflammation of the gum tissue.

Taken together, our findings indicate that treatment of gingivitis and periodontitis with Eucommia water extracts reduced the periodontal tissue inflammation and damage, indicating the potential use of this plant in drug development for controlling periodontal disease. It is widely believed that Eucommia can be incorporated into future research to prevent and treat periodontal disease. Eucommia has huge prospects with the application of its secondary metabolites in biomedicine.

Declarations

Ethical approval and consent to participate

Medical ethics committee of the Affiliated Stomatological Hospital of Zunyi Medical University, Approval number: YJSKTLS-2018-2021-018A.

I confirm that all methods are reported in accordance with ARRIVE guidelines for the reporting of animal experiments.

I confirm that all methods were performed in accordance with relevant guidelines and regulations and consult the American Veterinary Medical Association (AVMA) Guidelines for the Euthanasia of Animals (2020).

Consent for publication

The Author confirm that the work described has not been published before and agrees to publication in the Journal by BMC Oral Health.

Availability of data and materials
The data and materials used to support the findings of this study are available from the corresponding authors upon request.

**Competing interests**

The authors declare that there is no conflict of interest.

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**Authors' contributions**

Yueyue Wang: Data curation, Formal analysis, Methodology, Writing – original draft. Fengjiao Zeng: Writing – original draft, Methodology. Xia Liu: Formal analysis; Yuan Tian: Formal analysis. Guohui B: Conceptualization, Funding acquisition, Project administration, Supervision, Writing – review & editing.

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References


**Figures**
Figure 1

SOD and CAT in gingival tissue

*indicates comparison with other groups, the difference has statistically significant \( p < 0.05 \)

Figure 2

Analysis of periodontal tissues of the maxillary second molar in rats in the treatment group following HE staining,

A: Original magnification 20×, Scale bars = 500 \( \mu \)m.

B: Local gums, Original magnification 200×, Scale bars =50 \( \mu \)m.
Figure 3

Analysis of periodontal tissues of the maxillary second molar in rats in the control group by HE staining,

A: Original magnification 20×, Scale bars = 500 μm.

B: Local gums, Original magnification 200×, Scale bars = 50 μm.

Figure 4

Methylene blue staining of periodontal tissues (Original magnification 20×)

A: the normal group B: the periodontitis group
Figure 5

X-ray films of the periodontal tissue
A: the normal group  B: the periodontitis group

Figure 6

Analysis of the periodontal tissue of maxillary second molar following HE staining. Original magnification 20×, Scale bars = 500 μm.
A: the normal group  B: the periodontitis group
Figure 7
SOD and CAT in gingival tissue
*indicates comparison with other groups, the difference has statistically significant ($p < 0.05$)

Figure 8
Levels of IL-1β and IFN-γ in experimental rats.
*indicates the difference has statistically significant ($p < 0.05$)
Figure 9

Analysis of periodontal tissues of maxillary second molar in rats in treatment group following HE staining

A: Original magnification 20×, Scale bars = 500 μm.

B: Local gums, Original magnification 200×, Scale bars =50 μm.

Figure 10

Analysis of periodontal tissues of maxillary second molar in rats in control group by HE staining

A: Original magnification 20×, Scale bars = 500 μm.

B: Local gums, Original magnification 200×, Scale bars =50 μm.

Supplementary Files

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