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Research article

Inhibitory effect of polysaccharides extracted from Changbai Mountain *Ganoderma lucidum* on periodontal inflammation

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ABSTRACT

As the main bioactive substance of *Ganoderma lucidum*, *Ganoderma lucidum* polysaccharide (GLP) has anti-inflammatory, antibacterial, and other biological activities. Studies have shown that GLP can regulate the expression of multiple inflammatory cytokines in different inflammatory models and diseases as part of the anti-infection immune response. We extracted crude Changbai Mountain *Ganoderma lucidum* polysaccharides (CGLPs), analyzed their physical and chemical properties, and then applied them to the periodontitis model to verify whether they have an inhibitory effect on mouse periodontitis. CGLP was determined to be a heteropolysaccharide with dextran as the main component. Its molecular weight was 17.40 kDa. In vivo experiments in mice showed that CGLP can inhibit the alveolar bone loss and reduced inflammation caused of periodontitis by regulating the expression of the inflammatory factors IL-1β, TNF-α, and IL-10 in a concentration-dependent manner.

1. Introduction

Periodontitis is a type of chronic progressive inflammation related to microorganisms that is mediated by host immunity and resulting in periodontal attachment loss and tooth loss. It is the main cause of adult tooth loss (M. S [1]. and the sixth-most prevalent disease globally [2]. According to the fourth National Oral Health Survey (2015–2016) of China, the prevalence of periodontitis in three age groups (35–44, 55–64, and 65–74 years of age) was 52.8%, 69.3%, and 64.6%, respectively. The prevalence of severe periodontitis was 10.6%, 37.3%, and 43.5%, respectively [3]. It is estimated that the annual global productivity loss caused by severe periodontitis alone is as high as $54 billion [4]. In addition, studies have confirmed that periodontitis is closely associated with various systemic diseases, including cardiovascular diseases, rheumatoid arthritis, adverse pregnancy outcomes (premature infants,

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low-birth-weight infants), and cancer [5–8]. Causative therapy and mechanical removal of dental plaque and calculus has been the mainstay of periodontitis treatment. However, for some patients with severe periodontitis, the discomfort and high cost of surgical treatment are unbearable [9]. In addition, the effectiveness of causative therapy is limited by the skill level of the clinician and the patient compliance and it remains ineffective specifically in cases of severe periodontitis due to the limited visibility and accessibility to root, furcation, and underlying bone defects [10]. In recent years, some scholars have advocated the use of laser therapy, photodynamic therapy, or air polishing therapy to solve this problem [11]. But, for some periodontitis patients, such as patients with systemic diseases, and other susceptibility factors, it is necessary to use systemic or local anti-inflammatory or antibacterial drugs [12]. A kind of commercially available matrix-based minocycline HCL (2%) has been widely used. Many studies on periodontitis suggested that matrix-based minocycline HCL could improve the CAL of patients [13]. But the increase of drug resistance is always inevitable after recurrent applications of antibiotics [14]. In order to avoid this situation, anti-inflammatory substances from natural products such as polysaccharides may play a positive role in treating periodontitis.

Ganoderma lucidum, a common woody basidiomycetes mushroom in Asia, has a medicinal history of more than 2000 years and is believed to strengthen the body and prolong life [15]. There are more than 100 kinds of *Ganoderma lucidum*, of which about 70 are distributed in China, Japan, and the Korean Peninsula [16]. Studies have shown that *Ganoderma lucidum* contains about 400 different bioactive compounds [17], including polysaccharides, steroids, triterpenoid alkaloids, glycoproteins, fatty acids, inorganic elements, and lignin. In addition, it has anti-tumor, anti-inflammatory, anti-microbial, anti-atherosclerotic, hypolipidemic, anti-diabetes, and anti-aging effects. It has been used to treat and prevent various immune diseases such as gastritis, hepatitis, and lupus erythematosus [18].

Ganoderma lucidum polysaccharide (GLP) is considered the main pharmacologically active substance of *Ganoderma lucidum*. The quality of *Ganoderma lucidum* was evaluated by polysaccharide content in Chinese Pharmacopoeia [19]. At present, 220 polysaccharides have been isolated from *Ganoderma lucidum*, with molecular weights of 2–800 kDa [20]. GLP is a compound polymerized by various monosaccharides, including glucose, D-mannose, D-galactose, fucose, xylose, and other β-glucans. GLP has various biological activities such as anti-tumor, anti-oxidative, anti-inflammatory, and antibacterial effects [21–23]. Studies have shown that in different inflammatory models and inflammatory diseases, it participates in the anti-infection immune response by regulating the expression of multiple inflammatory cytokines such as IL-1, TNF-α, IL-6, IL-10, INF-γ, and i-NOS [24,25]; L [26,27]. Presently, sufficient evidence has confirmed that these inflammatory cytokines are closely related to the occurrence and development of periodontal inflammation [28–30]. Therefore, we speculate that GLP can control periodontal inflammation and inhibit the further development of periodontitis.

Changbai Mountain in Jilin province is one of the main areas of *Ganoderma lucidum* production in China. It has also been demonstrated that Changbai Mountain *Ganoderma lucidum* has a high polysaccharide content [31]. Therefore, in this study, we extracted Changbai Mountain *Ganoderma lucidum* polysaccharide (CGLP), analyzed its physical and chemical properties, and then applied it to a mouse model of periodontitis to verify whether it has an inhibitory effect. Our findings provide an experimental basis for the application of CGLP in the treatment of periodontitis.

2. Materials and methods

2.1. Materials

Ganoderma lucidum product were collected from Jilin Province (China). The SPF C57BL/6 mouse were provided by the Experimental Animal Welfare Ethics Committee of Jilin University. Ethylenediamine tetraacetic acid disodium salt (EDTA) was purchased from Beijing Reagent (China), and 4% paraformaldehyde buffer were purchased from Solarbio (China). PCR primers were synthesized and provided by Sangon Biotech (China). All the PCR related kits were purchased from Yeason (China). All chemicals were used without further purification. The water used in all experiments was purified by distillation for three times.

2.2. Extraction of crude CGLP

The crude polysaccharides of Ganoderma lucidum were extracted from dried Ganoderma lucidum product (Changbai Mountain, China). The powdered Ganoderma lucidum (500 g) was extracted it twice in a sterile water bath at 100 °C with a liquid-to-material ratio of 40:1 for 2 h each time. Then, the filtrate was mixed and concentrated to 2 L at 80 °C, and the threefold volume of 95% alcohol was added. The mixed solution was precipitated for 24 h at 4 °C temperature, resulting in the formation of a precipitate. Then, the precipitate was collected by centrifugation (6500 rpm, 5min). Finally, the obtained crude polysaccharides were dried stored at room temperature.

2.3. Characterization of crude CGLP

2.3.1. Analysis of molecular weight

The molecular weight of GLP was analyzed by a Multi-Angle laser light scattering gel chromatography system. The differential detector was Optilab T-rEX (Wyatt Technology, CA, USA), and the laser light scattering detector was DAWN HELEOS II (Wyatt Technology, CA, USA). A 10 mg polysaccharide sample was dissolved in a 1 mL mobile phase (0.1 M $NaNO_3$) and centrifuged at 14,000 rpm for 10 min, passed through a 0.22 μm filter. Then 100 μL filtrate were injected into a Ohpak SB-805 HQ column (8 × 300 mm) maintained at 45 °C and eluted by the mobile phase (0.1 M $NaNO_3$) at 0.45 mL/min.

2.3.2. Analysis of monosaccharide composition

The monosaccharide composition of polysaccharides was determined by high-performance anion-exchange chromatography (HPAEC). Preparation of standard product: a 15 mL centrifuge tube was taken, and 8 mL sterile water was added. Then, 100 mg of fucose, arabinose, galactose, glucose, xylose, rhamnose, mannose, fructose, ribose, galacturonic acid, and glucuronic acid was successively added. After the monosaccharides dissolved, the volume was fixed at 10 mL in a volumetric flask, and stock solution of 10 mg/mL was prepared. The above mixed stock solution was diluted 100 times to prepare 100 μg/mL working liquor. The machine recorded the standard chromatogram. Hydrolysis of polysaccharide sample: CGLP (5 mg) was hydrolyzed with trifluoroacetic acid (TFA) (5 mL, 2 M), heated at 121 °C for 2 h, and then blow-dried with nitrogen. The reaction mixture was evaporated repeatedly to dryness with addition of methanol to remove the excess TFA. Sterile water was added to dissolve the sample, which was then transferred into the chromatographic flask for testing. The Thermo ICS5000+ ion chromatography system (ICS5000+, Thermo Fisher Scientific, USA) and DionexTM CarboPacTM PA10 (250*4.0 mm, 10 μm) liquid chromatography column were used, with a sample volume of 20 mL. Mobile phase A was sterile water and mobile phase B was 100 mM NaOH. The column temperature was 30 °C. The monosaccharide components were analyzed by an electrochemical detector. The gradient and flow rate for HPAEC method are shown in Table 1.

2.3.3. Fourier transform infrared spectroscopy (FTIR)

About 1–2 mg of CGLP powder was mixed with dried potassium bromide (KBr) powder, and thoroughly ground. The mixture was then pressed, and an IR spectrum between 400 cm^{-1} and 4000 cm^{-1} was obtained using an IRPrestige 21 FTIR spectrometer (Shimadzu, Tokyo, Japan).

2.4. Effects of CGLP on periodontitis inflammation in vivo

2.4.1. Establishment of periodontitis model in mice

All animal experiments were reviewed and approved by the Experimental Animal Welfare Ethics Committee of Jilin University (Permit Number: 20,200,429). Forty-eight SPF female C57BL/6 mouse (8–11 weeks of age) were selected and fed for one week under standard conditions. The mice were randomly divided into four groups (n = 12): healthy mice without periodontitis (Control), periodontitis without treatment (Periodontitis), periodontitis treated with low dose CGLP (50 mg/kg) (low dose CGLP), and periodontitis treated with high dose CGLP (100 mg/kg) (high dose CGLP). The C57BL/6 mice were anesthetized using pentobarbital sodium (6 μL/g body weight). Then, a double-segment 5-0 suture (approximately 2.5 mm in length) was placed between the first and second right maxillary molars. The half of each group were sacrificed after 1 week gavage administration and the other half were sacrificed after 2 weeks gavage administration, starting from day 1 after periodontitis modeling. CGLP was dissolved in normal saline, and the gavage dose was 15 mL/kg body weight. The Control and Periodontitis groups were given the same amount of normal saline.

2.4.2. Micro-CT analysis and histological analysis

The isolated right maxillary molars were fixed overnight in 4% paraformaldehyde buffer. Micro-CT (Scanco, Switzerland) analysis was performed on fixed specimens where each sample was exposed to an X-ray with 70 kV voltage, 200 mA node current, 220 threshold value, and 300 ms exposure time. Three-dimensional tissue reconstruction was performed, and the distance between the cementoenamel junction and alveolar bone crest (CEJ-ABC) of the right maxillary first molar was accurately measured. After scanning for micro-CT, the fixed samples were decalcified in 10% EDTA solution (pH 7.0) for 2 weeks, with the EDTA solution refreshed once every 2 days. Then, the samples were dehydrated in a graded series of ethanol, embedded in paraffin, and cut into 3 μm sections parallel to the mesial and distal directions of the maxillary molars. Sections were stained with hematoxylin and eosin (H&E) for morphological analysis. The inflammatory response and the histology of the periodontium were observed by microscope.

2.4.3. qRT-PCR

Liquid nitrogen was used to freeze the gingival tissue sample, which was then crushed. Total RNA was isolated using a HiPure Total RNA kit (Yeason, China) according to the manufacturer's instructions. The mRNAs were was subjected to reverse transcription using Hifair II 1st Strand cDNA Synthesis SuperMix for qPCR (Yeason, China) and qPCR using Hifair qPCR SYBR GREEN Master Mix (Yeason, China). The PCR products were evaluated with a MxPro Mx3005 P real-time PCR detection system (Agilent Technologies, Santa Clara, CA, USA). The internal control for mRNAs was β-actin. The cycling conditions of mRNAs were as follows: 95 °C for 5 min, followed by 40 cycles of 95 °C for 10 s, 60 °C for 30 s, and 72 °C for 1 min. The $2^{-\Delta\Delta Ct}$ method was used to calculate the relative expression levels,

Table 1
The gradient and flow rate for HPAEC method.

Time (min)	Flow rate (ml/min)	phase A (%)	phase B (%)
0.0	0.5	97.5	2.5
30.0	0.5	80	20
30.1	0.5	60	40
45	0.5	60	40
45.1	0.5	97.5	2.5
60	0.5	97.5	2.5

and the obtained values were averaged from triplicate measurements. The primers were as follows:

TNF-α(F):5′-ACCCTCACACTCACAAACCAC3’/
(R):5′-ACAAGGTACAACCCATCGGC-3’;
IL-10(F):5′-GCATGGCCCAGAAATCAAGG-3’/
(R):5′-ACACCTTGGTCTTGGAGCTTATTA-3’;
IL-1(F):5′-GCCACCTTTTGACAGTGATGAG-3’/
(R): R:5′-AGCTTCTCCACAGCCACAAT-3’.

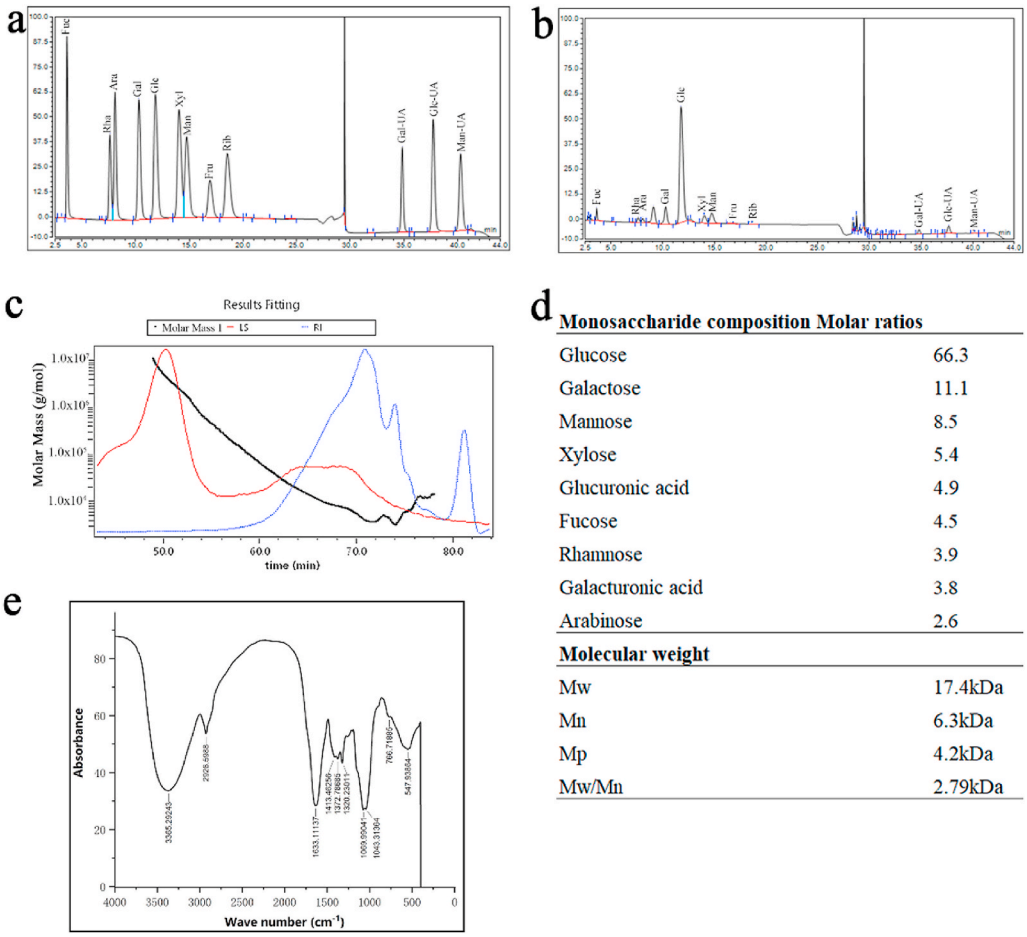
2.4.4. Toxicity assay of CGLP in vivo

The thymus, liver, spleen, and kidney of the mice were harvested and weighed. The organs were fixed in 4% paraformaldehyde for an additional 24 h. Tissues were dehydrated in a graded series of ethanol and embedded in paraffin, and 5 μm sections were prepared for H&E staining to evaluate if the CGLP caused any toxicity. The organ index was calculated according to the following formula:

$$\text{organ index} = \frac{\text{organ weight(g)}}{\text{body weight(g)}} \times 100$$

2.5. Statistical analysis

SPSS 20.0 software was used for statistical analysis of the experimental data, Mimics 19.0 software was used for 3D reconstruction of micro-CT, 3-Matic Research 11.0 software was used to measure the distance of CEJ-ABC accurately, and Origin 2019 software was used to draw the infrared spectrum. The experimental results were expressed as the mean ± standard deviation, and one-way ANOVA was used to compare groups. Differences with a value of $P < 0.05$ were considered statistically significant.



Monosaccharide composition Molar ratios	
Glucose	66.3
Galactose	11.1
Mannose	8.5
Xylose	5.4
Glucuronic acid	4.9
Fucose	4.5
Rhamnose	3.9
Galacturonic acid	3.8
Arabinose	2.6
Molecular weight	
Mw	17.4kDa
Mn	6.3kDa
Mp	4.2kDa
Mw/Mn	2.79kDa

Fig. 1. Characterization of crude CGLP. a-b, Chromatograms of momosaccharide composition. c, Chromatogram of the molecular weight distribution. d, Monosaccharide composition Molar ratio and Molecular weight. e, FTIR spectrum of crude CGLP.

3. Results and discussion

3.1. Physicochemical features of crude CGLP

As shown in Fig. 1A and B, crude CGLP was mainly composed of glucose, galactose, mannose, xylose, glucuronic acid, fucose,

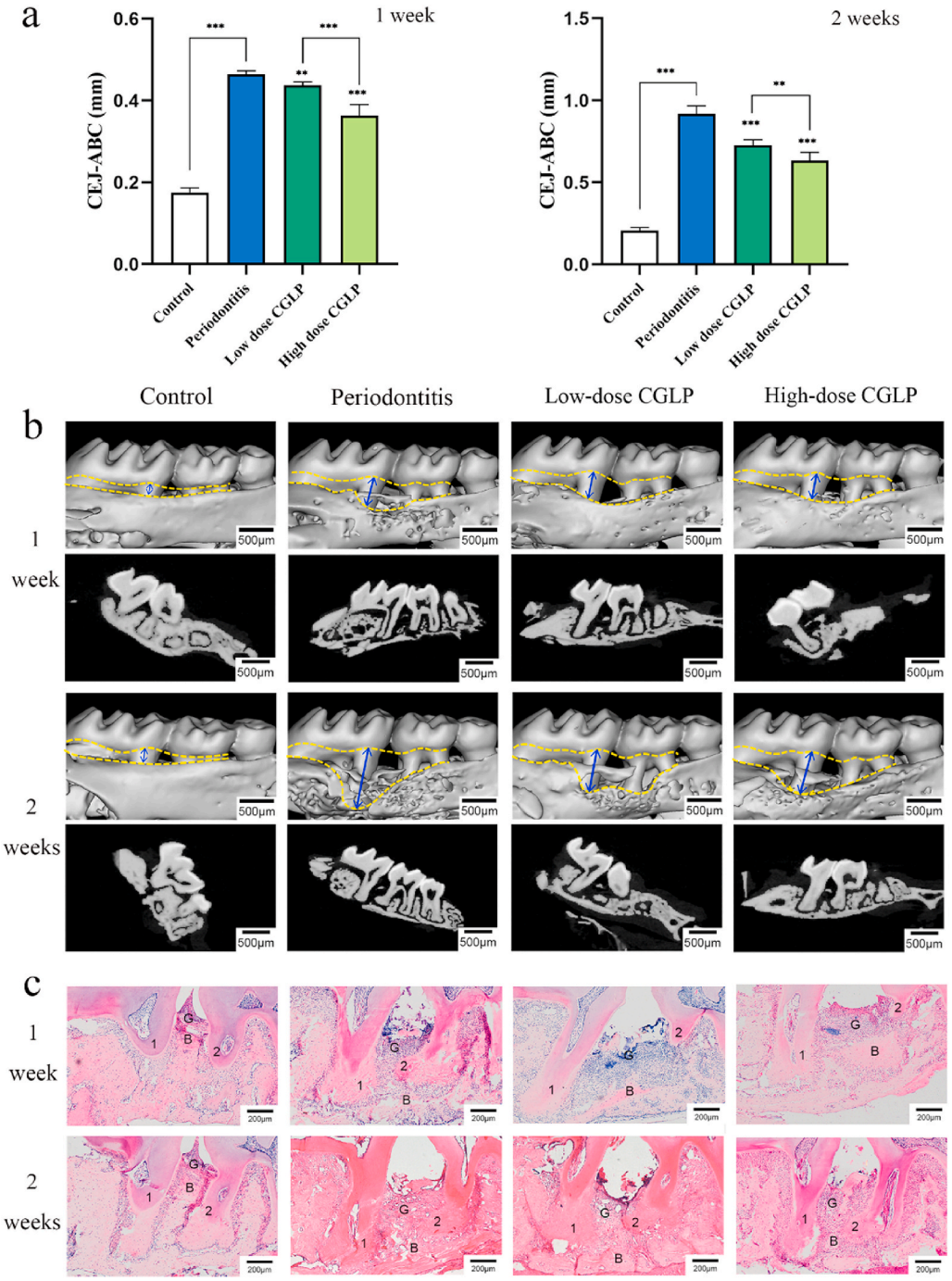


Fig. 2. CGLP can inhibit alveolar bone resorption in mice. a, The distance of CEJ-ABC in the buccal distal root of the right maxillary first molar. b, Micro-CT images of maxillary alveolar bone surrounding the maxillar first molars and maxillary second molars the treatment. c, H&E staining images of the periodontium after the treatment: 1, the root of the first molar; 2, the root of the second molar; B, alveolar bone; G, gingivel epithelium. *P < 0.05, **P < 0.01, ***P < 0.001.

rhamnose, galacturonic acid and arabinose in a molar ratio of 66.3:11.1:8.5:5.4:4.9:4.5:3.9:3.8:2.6 (Fig. 1d). Therefore, the weight-average molecular weight (Mw), number-average molecular weight (Mn), peak molecular weight (Mp), and polydispersity index (Mw/Mn) of crude CGLP were calculated as 17.4 kDa, 6.3 kDa, 4.2 kDa, and 2.79, respectively (Fig. 1c and e).

FTIR is an effective tool for identifying organic groups in polysaccharides [32]. Fig. 1d illustrates the FTIR spectrum in the range of 400–4000 cm^{-1} for identifying characteristic organic groups of crude CGLP. The broad peak at 3365 cm^{-1} was considered the stretching vibration of *O*–H in polysaccharide molecules. The absorption peak at 2927 cm^{-1} was related to the stretching vibration of C–H. The band at 1633 cm^{-1} indicated the existence of C═O groups. The characteristic absorption peaks of the C–H bond were observed in the range of 1400–1200 cm^{-1}, and the absorption peaks in the range of 1200–1000 cm^{-1} indicated the presence of C–*O*–C and C–*O*–H groups. Characteristic peaks at 900–500 cm^{-1} were indicative of the skeletal pattern of the pyranose ring (Y [33–35].

The above results indicated that CGLP was successfully extracted with moderate molecular weight, which may have good biological activity (S. Y [36]. Compared with the GLPs extracted by Zhang Yiwen [37] and Zhou Dong [38], CGLP has obvious differences in monosaccharide composition and polysaccharide molecular weight, indicating that the composition of GLPs differ according to the *Ganoderma lucidum* species, place of origin, and extraction method [39,40]. The CGLP extracted in this experiment may contain new polysaccharide components and structures.

3.2. CGLP-mediated inhibition of bone resorption caused by periodontitis

The occurrence and degree of alveolar bone absorption are the main basis for diagnosing and classifying periodontitis [41]. Animal models of periodontitis need to be established to verify the effect of CGLP on periodontal inflammation. Commonly used animals include dogs, rats, mice, miniature pigs, and non-human primates, and there are many construction methods such as ligation, bacterial inoculation, and dental calculus implantation [42,43]. The ligation model in mice has many advantages, such as low cost and ease of feeding [44]. However, due to the small mouth and tooth size, there are technical difficulties in placing the ligation line around the mouse teeth [45]. We established a simple ligation-induced periodontitis model by consulting the literature to reduce the technical challenges of ligation line placement in mouse gingival tissue. In this ligation model, two-knot sutures with a length of 2.5 mm were placed between the 2 M of the mice to allow the accumulation of endogenous microbiota, resulting in gingival tissue inflammation and alveolar bone absorption [46]. From the first day after establishing the periodontitis model, CGLP was administered daily by

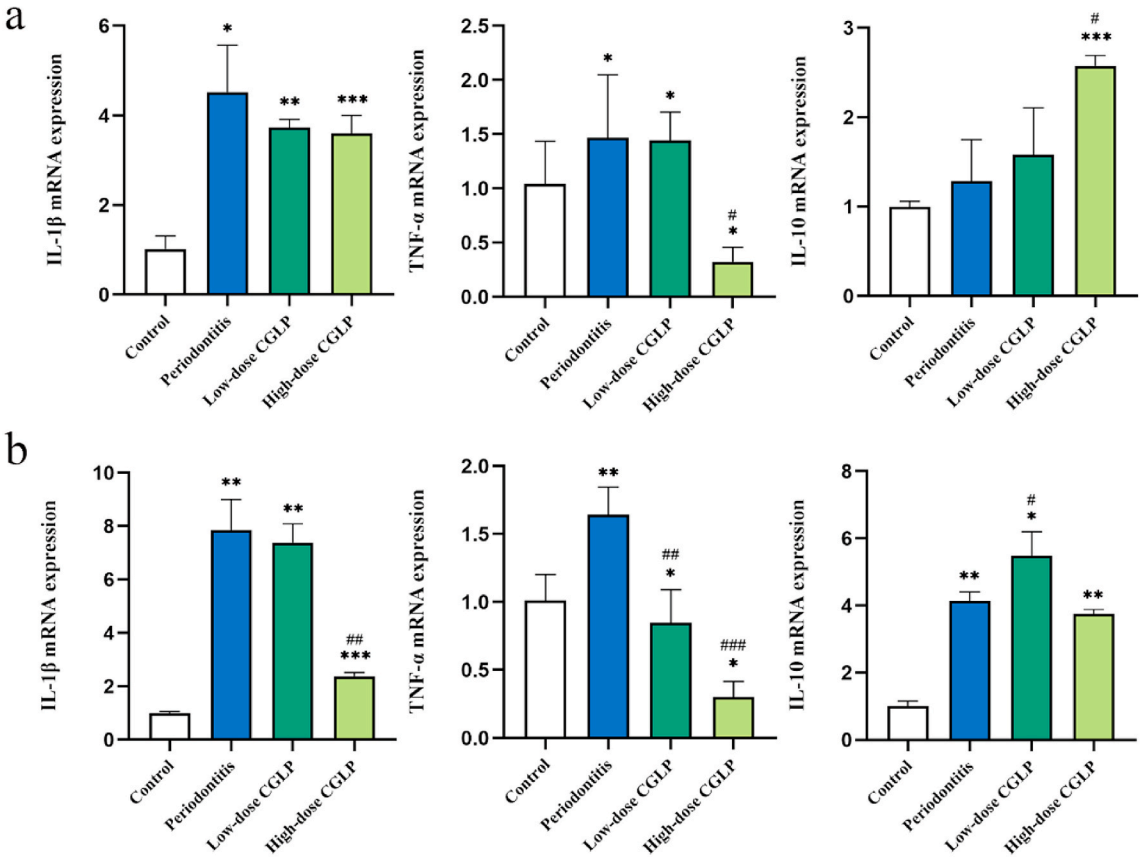


Fig. 3. Effects of CGLP on ecpression of IL-1β, TNF-α and IL-10 in mice. a, the result of week 1. b, the result of week 2. (*means compared with control group,*P < 0.05, **P < 0.05, ***P < 0.001; # means compared with periodontitos group,#P < 0.05, ##P < 0.01, ###P < 0.001).

intragastric administration. The literature confirmed that the mouse periodontitis model exhibits obvious bone resorption 1 week after the operation and the bone resorption is stable 15 days later. Therefore, we selected two time points at 1 and 2 weeks after administration to detect the inflammatory state of the periodontal tissues.

The main features of periodontitis are the loss of attachment and resorption of alveolar bone [47]. Alveolar bone loss is the most direct indicator of the severity of periodontitis. Under this condition, the vertical distance between the cemento-enamel junction (CEJ) and the apex of alveolar bone crest (ABC) represents the degree of alveolar bone loss and the severity of periodontitis (X [48]. Mimics 19.0 software was used for 3D reconstruction of the micro-CT data, and the value of CEJ-ABC in the distal buccal root of the right maxillary first molar was measured accurately. As shown in Fig. 2a, compared with that in the control, the value of CEJ-ABC in the periodontitis group was significantly increased ($P < 0.001$), indicating that the mouse periodontitis model was successfully established. Compared with the periodontitis group, the low-dose and high-dose CGLP groups exhibited significant inhibition of alveolar bone absorption ($P < 0.01$). The inhibitory effect became more significant with the increase of polysaccharide concentration ($P < 0.001$). In addition, when the reconstructed 3D model was compared with the sagittal screenshot, except for the difference in CEJ-ABC value, the thickness, fullness, and integrity of alveolar bone tissue in the experimental group were significantly stronger than those in the periodontitis group, and the effect became increasingly significant with the increase in GLP concentration (Fig. 2b).

To further confirm the above results, H&E staining was performed to evaluate the inflammatory status and periodontium regeneration. The periodontal tissue in the control group was normal, and there was obvious inflammation in the periodontitis, low-dose CGLP, and high-dose CGLP groups, which indicated different degrees of gingival erosion and alveolar bone resorption. Compared with the periodontitis group, the low-dose and high-dose CGLP groups retained more alveolar bone tissue between the first and second molars, and the retention of alveolar bone tissue increased with the increase in CGLP concentration (Fig. 2c). This indicated that CGLP could effectively inhibit the bone resorption caused by periodontitis and the effect was concentration dependent. All of these results show CGLP has a good inhibitory effect on periodontal inflammation.

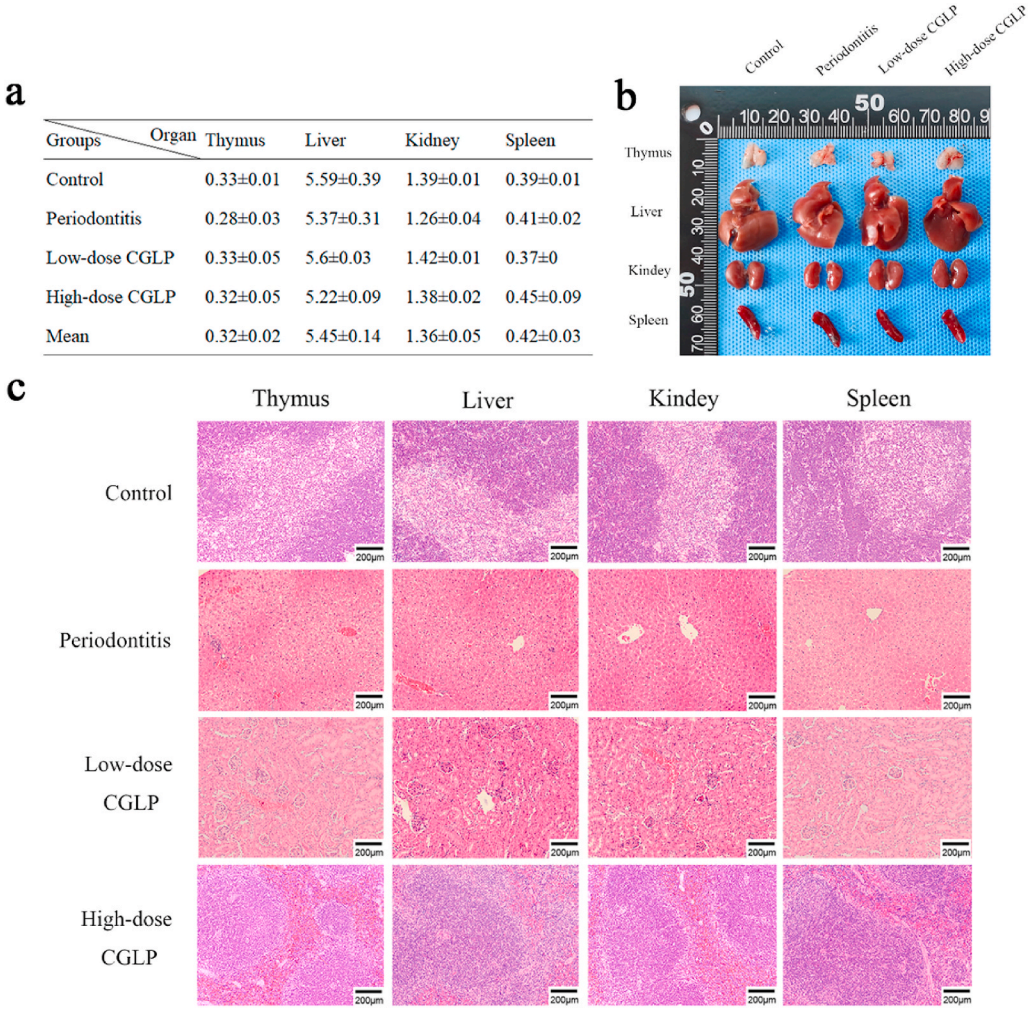


Fig. 4. Biocompatibility of CGLP in vivo. a, Organ index of the mice in each group after 2 weeks of gavage ($P > 0.05$). b-c, General observation and histological analyses of ograns in mice.

3.3. Effect of CGLP on the expression of related inflammatory factors

Studies have shown that in different inflammatory models and inflammatory diseases, CGLP can inhibit the expression of pro-inflammatory cytokines, such as IL-1, TNF-α, and IL-6, and promote the expression of anti-inflammatory cytokines such as IL-10, thereby participating in the anti-infection immune response [25,27,49]. Meanwhile, substantial evidence shows that IL-1 β, TNF-α, and IL-10 are closely related to periodontitis [28–30]. Therefore, we used qRT-PCR to detect the expression of the pro-inflammatory cytokines IL-1β and TNF-α and the anti-inflammatory cytokine IL-10 in periodontal tissues. The expression of inflammatory factors in the right maxillary gingiva of mice in each group was detected by qPCR after intragastric administration for 1 week and 2 weeks (Fig. 3a and b).

Compared with the control group, the expression of IL-1β, TNF-α, and IL-10 in the periodontitis, low-dose CGLP, and high-dose CGLP groups increased to varying degrees ($P < 0.05$). In the first week, compared with the periodontitis group, the high-dose CGLP group exhibited significantly lower TNF-α gene expression ($P < 0.05$) and significantly higher IL-10 gene expression ($P < 0.05$). At week 2, compared with the periodontitis group, the high-dose CGLP group exhibited lower expression of the IL1-β gene ($P < 0.01$). The expression of TNF-α was inhibited both in the low-dose ($P < 0.01$) and high-dose CGLP groups ($P < 0.001$). Low dose CGLP significantly promoted the expression of the IL-10 gene ($P < 0.05$). The results indicated that CGLP could regulate the expression of IL-1β, TNF-α, and IL-10 in periodontal tissue to reduce the inflammatory response to periodontitis in mice. The expression of IL-1β changed in high-dose CGLP group at week 2, and TNF-α changed only in high-dose CGLP group at week 1, but changed both in low-dose and high-dose CGLP groups at week 2. These results again suggest that CGLP inhibits periodontal inflammation in a concentration-dependent manner. After intragastric administration, low-dose of CGLP could not immediately cause changes in local periodontal tissue inflammatory response, but gradually played a role with the accumulation of CGLP in vivo. Unexpectedly, the expression of IL-10 actually decreased in the high-dose CGLP group in the second week compared to the first. Polysaccharides have usually been regarded as a healthy ingredient. However, they can also show toxicity when administered in high doses (H [50]. This may be the reason for the decline of IL-10. Another possible factor is that the CGLP is crude polysaccharide, and may contain some impurities. When there is a certain amount of CGLP in mice, the impurity may have an impact on the anti-inflammatory effects of CGLP. It will be explored in further experiments.

3.4. Biocompatibility of CGLP in vivo

Although *Ganoderma lucidum* and its active components have various pharmacological effects and wide applications, it is difficult to effectively detect and supervise the quality and safety of *Ganoderma lucidum* and its related products [51]. The pollution of heavy metals and other toxic substances in the growth process of *Ganoderma lucidum* is an urgent problem [52]. Therefore, we conducted a preliminary evaluation of the biosafety of CGLP. The organ index of the mice in each group after 2 weeks of gavage is shown in Fig. 4A. Compared with those in the control group, the thymus, liver, kidney, and spleen indexes in the periodontitis, low-dose CGLP, and high-dose CGLP groups did not change significantly ($P > 0.05$). Finally, general observation and histological analyses demonstrated no significant abnormalities in the thymus, liver, kidney, or spleen samples of the four groups (Fig. 4B and C). These results indicate that CGLP, as a natural bioactive substance, may have good biosafety. However, this needs further experimental confirmation.

4. Conclusion

CGLP, a heteropolysaccharide with dextran as the main component, was extracted in this study. Its molecular weight was 17.40 kDa. In vivo experiments showed that CGLP can inhibit the absorption of alveolar bone and reduce the inflammation of periodontitis in mice by regulating the expression of inflammatory factors IL-1β, TNF-α, and IL-10 in a concentration-dependent manner. Thus, the inhibitory effect of CGLP on periodontal inflammation was preliminarily verified. However, for drug development, it is necessary to improve the purity of CGLP and separate it into single components. The therapeutic effects of different polysaccharide components and the optimal concentration of CGLP for periodontitis treatment still need further exploration.

Author contribution statement

Zhen Chen: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper. Wenguang Qin; Hongbing Lin; Yang Liu; Xuetao Zhao: Performed the experiments; Contributed reagents, materials, analysis tools or data. Yue Tian; Tong Ding: Performed the experiments. Yawei Wang; Tianjiao Mao: Analyzed and interpreted the data. Jiang Li: Conceived and designed the experiments. Yuqin Shen: Conceived and designed the experiments; Wrote the paper.

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Data availability statement

Data will be made available on request.

Additional information

No additional information is available for this paper.

Declaration of competing interest

The authors declare no competing interests.

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