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Identification of an active fraction of Kangfuxin in the treatment of periodontitis in a rat model

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ABSTRACT

Introduction. Periodontitis is a serious gum infection that disrupts the soft tissue around teeth. This study aimed to identify the most effective fraction of the Chinese medicine Kangfuxin for periodontitis treatment in a rat model.

Material and methods. Kangfuxin solution was subjected to sequential extraction using chloroform, ethyl acetate, n-butanol, and water. The extracts were evaporated, dissolved in DMSO, diluted in water, and administered to rats *via* gavage (0.5 mL/day) for 2 weeks. The n-butanol extract was further fractionated using macroporous resin chromatography with 10%, 30%, 50%, 70%, and 90% ethanol elution. Levels of inflammatory cytokines IL-6, IL-1β, and TNF-α in periodontitis samples were examined by ELISA. Leukocyte infiltration in the cementum was analysed by haematoxylin and eosin (H&E) staining.

Results. The n-butanol extract showed the best anti-inflammatory effect, reducing IL-6, IL-1β, and TNF-α levels in periodontitis samples and alleviating tissue damage and leukocyte infiltration in the cementum. Further fractionation revealed that the 50% ethanol fraction of the n-butanol extract had the most potent action in attenuating inflammation. This fraction suppressed the activation of the PI3K-AKT-mTOR signalling pathway in periodontitis samples. Application of a PI3K activator counteracted the anti-inflammatory effect of the 50% ethanol fraction.

Conclusions. We identified a potent anti-inflammatory fraction (50% ethanol fraction of the n-butanol extract) of Kangfuxin for periodontitis treatment. This fraction suppressed the activity of the PI3K-AKT-mTOR signalling pathway in periodontitis samples. Further research is needed to isolate and characterise the specific bioactive compounds within this fraction.

Keywords: periodontitis; Kangfuxin; PI3K-AKT-mTOR signalling pathway

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INTRODUCTION

Periodontitis is a chronic inflammatory disease due to severe infection, which is characterised by damage to tooth-supporting tissues, gum swelling, and haemorrhage [1, 2]. It is a prevalent condition, which accounts for the destruction of cementum, and tooth loosening and loss. Globally, approximately 11% of the population suffers from severe periodontitis, 743 million cases were recorded worldwide [3, 4], and oral illnesses such as periodontitis and caries are becoming a serious global public health challenge [5, 6].

Histological features of periodontitis include leukocyte infiltration in the gum tissues, gum recession, radiographic signs of alveolar bone loss, and pathological degeneration of cementum [7–9]. Bacteria such as *Aggregatibacter actinomycetemcomitans* are the common pathogens leading to periodontitis. These microbes produce multiple factors, including peptidoglycans, various catechins, and

lipoteichoic acid, to recruit and activate leukocytes and fibroblasts to produce cytokines, metalloproteinases, transglutaminases, and other proteolytic enzymes to induce tissue inflammation and disrupt tissue integrity [10–12]. Several pro-inflammatory cytokines, such as IL-1β, IL-6, and TNF-α, have been implicated in inflammatory damage to periodontium and alveolar bone degradation [13–15]. Although mild periodontitis can be curbed by antibiotics, severe periodontitis requires orthodontic therapy and surgical intervention [16, 17]. Sufficient daily gum care is critical for preventing periodontitis and its progression [18]. Several kinds of anti-inflammatory medications, including protease inhibitors like doxycycline and NSAIDs, have been proposed for periodontitis treatment, but the clinical efficacy is limited [19, 20].

Recently, various medicinal plant extracts and herbal medications have shown anti-inflammatory and anti-bone resorption properties against periodontal disorders [21]. Kangfuxin solution is a bioactive agent derived from the Chinese medicinal insect *Periplaneta americana*, commonly known as the American cockroach. This medication has been used in traditional Chinese medicine for its potential healing properties in various conditions, including gastric ulcers, oral mucositis, and wound healing [22, 23]. The chemical composition of Kangfuxin is highly complex and not fully characterised. Current research indicates that it comprises multiple active components, including peptides, amino sugars, amino acids, and polyhydric alcohols [22]. Some studies have identified specific compounds such as polysaccharides, fatty acids (*e.g.* oleic acid, linoleic acid), and amino acids (*e.g.* proline, alanine) [24, 25]. However, the exact proportions and complete list of bioactive compounds remain unknown. Kangfuxin has demonstrated anti-inflammatory, mucosal healing, and immune system enhancement activities in various pathological conditions [22–29]. For instance, it has shown beneficial effects in patients with stomach ulcers by reducing gastric juice secretion and preventing gastrointestinal inflammation [24, 25]. These effects are associated with increased cell proliferation of fibrous tissue, capillary hypertrophy, and acceleration of mucosal damage healing [26, 27]. The gastroprotective effects of Kangfuxin against gastric ulcers can also be attributed to the attenuation of oxidative and endoplasmic reticulum stresses [28]. Additionally, Kangfuxin has been shown to mitigate radiotherapy-induced oral mucositis in patients with head and neck squamous cell carcinoma through immunomodulatory effects [29]. Despite these known benefits, the potential therapeutic value of Kangfuxin in periodontitis has not yet been thoroughly explored. Given its anti-inflammatory and tissue-healing properties, we hypothesised that Kangfuxin might have beneficial effects in the treatment of periodontitis. In this study, we aimed to identify the most effective fraction of Kangfuxin for periodontitis treatment in a rat model and investigate the underlying mechanisms.

Materials and methods

Animal model of periodontitis

In this study, male SD rats (6–8 weeks old, weighing 180–210 g) were used to establish an animal model of periodontitis. The Kunming Medical University Animal Research Institute granted approval for the animal protocol. To construct the periodontitis model, rats were fasted for 24 hours and sedated with an intraperitoneal administration of 10% chloral hydrate. The gingival sulcus was stretched with 4.0 silk thread, and the first tooth in the left mandibular was ligated. The gingiva was cut with a 1-mm-deep incision using ophthalmic fine scissors. On the seventh day after the operation, the onset of periodontitis was confirmed by the appearance of redness and swelling in the gingiva. For the treatment group, different extracts of Kangfuxin by organic solvents (chloroform, ethyl acetate, n-butanol) or the ethanol fractions of n-butanol extract were administered for 2 weeks. Rats in the treatment groups were administered with the Kangfuxin extracts or fractions *via* oral gavage once daily for 2 weeks. Each rat received 0.5 mL of the diluted extract or fraction *per* day. The administration began on the seventh day after the periodontitis induction, coinciding with the confirmation of periodontitis onset. Control and model groups received 0.5 mL of water *via* oral gavage following the same schedule to ensure consistent handling across all groups. The following groups were included in the experiment (n = 5 in each group): blank control group (Control, without periodontitis induction); model group (Model: periodontitis induction); treatment groups after periodontitis induction: chloroform group (chloroform group, CG), ethyl acetate group (ethyl acetate group, EAG), n-butanol group (The n-butanol group, TNBG), and water group (water group, WG). At the end of the 2-week treatment period, all rats were euthanised by carbon dioxide inhalation followed by cervical dislocation to ensure complete euthanasia. Immediately after confirmation of death, the periodontal tissues were carefully dissected and collected for further analysis. All euthanasia procedures were performed in accordance with the guidelines approved by the Kunming Medical University Animal Research Institute.

Organic solvent extraction and fraction

To determine an active fraction of Kangfuxin in periodontitis treatment, 200 mL of Kangfuxin solution (Kelun Pharmaeutics, Tianjing, China) was loaded into the Strata C18-E Solid Phase Extraction Column (Phenomenex,

Torrance, CA, USA). After drainage of the solution, different solvents including chloroform, ethyl acetate, n-butanol, and water were applied at 20 mL to elute the fractions retained in the column. The extract was evaporated and then dissolved in 1 mL DMSO as the stock. 0.1 mL of the stock was diluted in 2 mL of water, and then the rats were given 0.5 mL diluted stock *via* gavage *per* day for 2 weeks. The n-butanol extract was subjected to macroporous resin chromatography using OPUS® Pre-packed Column (Repligen, Lansing, IL, USA). Retained materials in the column were eluted with 2 mL of 10%, 30%, 50%, 70%, and 90% ethanol. The eluent was evaporated and then dissolved in 0.5 mL DMSO as the stock. 0.1 mL of the stock was diluted in 2 mL of water, and then the rats were given 0.5 mL diluted stock *via* gavage *per* day for 2 weeks.

IL-6, IL-1β, and TNF-α assay by ELISA

To determine the concentrations of inflammatory markers in the gingival samples, the tissues were ground and homogenised before the lysis. The supernatant of tissue lysate was collected by centrifugation, and 100 μL of the supernatant was used to measure concentrations of interleukin (IL)-1β, IL-6, and tumour necrosis factor (TNF)-α by corresponding ELISA kit (Sigma, St. Louis, MO, USA). Briefly, the micro-assay plates were coated with antibody to rat TNF-α, IL-1 β, or IL-6 at 4°C. The supernatant of tissue lysate and the prepared standards were added to the capture-antibody-coated plate for 4-hour incubation at ambient temperature. After a washing step to remove unbound material, the biotin-labelled detection antibody was added for one-hour incubation, which was followed by incubation with streptavidin-HRP. Next, the chemiluminescent detection reagents were added for signal development, and the optical densities of each sample and the standards were measured at 450 nm using a microplate reader (Infinite 200 PRO; Tecan). The concentration of each cytokine was measured based on the linear regression of the standards and normalised to the tissue weight.

Haematoxylin and eosin (H&E) staining

Due to the presence of bone components, the tissues were preserved in 10% formalin solution for 2 days before being demineralised for 15 days in a solution of 18% disodium ethylenediaminetetraacetate. After decalcification, the tissues were embedded in paraffin and sliced into 5-μm sections in the mesial-distal direction. H&E staining was performed using the H&E Stain Kit (ab245880, Abcam, Cambridge, UK). The deparaffinised/hydrated sections were incubated in haematoxylin Mayer's solution (Lillie's modification) for 5 min. Samples were then rinsed twice with distilled water to remove excess stain before further incubation in Bluing Reagent for 30 sec. After washing with distilled water, the sections were dehydrated in absolute alcohol, followed by staining with Eosin Y Solution for 2 min. After rinsing with pure ethanol, samples were imaged under an inverse light microscope.

Western blotting

RIPA buffer containing 1 mM PMSF and a complete kinase inhibitory cocktail (Roche, Mannheim, Germany) was used to collect proteins from the gingival samples. Ground tissues were lysed in RIPA buffer on ice for 10 min and then centrifuged at 12,000 *g* for 10 min. The protein concentration of the supernatant was quantified by a BCA Protein assay kit (Beyotime Biotechnology P0009; Beijing, China). 20 μg of protein sample was separated by 10% SDS-PAGE gel, and then the separated protein bands were transferred onto a PVDF membrane. After blocking with 5% non-fat milk, the membranes were probed with each primary antibody: TNF-α (1:1000; ABclonal Biotechnology), IL-1β (1:1000; ABclonal Biotechnology), IL-6 (1:1000; ABclonal Biotechnology), PI3K (1:1000 dilution; CST), p-PI3K (1:1000 dilution; CST), AKT (1:1000 dilution; CST), p-AKT (1:1000 dilution; CST), mTOR (1:1500 dilution, Abcam), and p-mTOR (1:1000 dilution, Abcam). After washing the membranes 3 times with TBST buffer, the membranes were further labelled with HRP-conjugated secondary antibody for 1 hour (1:5000 dilution, Bios, Beijing, China). The enhanced chemiluminescent reagent (Pierce, Rockford, IL, USA) was used for signal development, and the protein band density was analysed using the Gel-Pro Analyser (Media Cybernetics, Rockville, MD, USA).

Statistical analysis

The statistical significance of difference was examined using GraphPad Prism Version 8.0 (GraphPad software, Boston, MA, USA). Differences were regarded as statistically significant when $P < 0.05$. The statistical differences between 2 groups were compared using unpaired Student's *t*-test. Comparisons among multiple groups were analysed using one-way analysis of variance (ANOVA), with Tukey's *post-hoc* test for the pairwise comparisons.

Results

N-butanol extract of Kangfuxin reduced the levels of IL-6, IL-1β, and TNF-α in the periodontitis samples

To determine an effective fraction of Kangfuxin in periodontitis treatment, the following experimental groups were established in rats (n = 5 in each group): blank control group (Control, without periodontitis induction); model group (Model: periodontitis induction); treatment groups after periodontitis induction: chloroform group (Chloroform group, CG), ethyl acetate group (Ethyl acetate

group, EAG), n-butanol group (The n-butanol group, TNBG), and water group (Water group, WG). The concentrations of IL-1β, IL-6, and TNF-α in the periodontal tissues were determined by ELISA. The absolute levels of inflammatory cytokines were quantified in pg/mL based on standard curves generated with known concentrations of recombinant cytokines. As expected, the levels of these inflammatory cytokines were significantly elevated in the model group. With the administration of different Kangfuxin extracts for 2 weeks, we found that only the n-butanol extract (TNBG) was able to curtail the production of the inflammatory cytokines after the periodontitis induction (Fig. 1). We also performed Western blot to detect the relative levels of IL-1β, IL-6, and TNF-α in the periodontal tissues. Similarly, the protein levels of these inflammatory factors were significantly increased in the model group compared to the control group. The protein levels of IL-1β and TNF-α were lower in the CG, EAG, and TNBG groups than in the model group. The IL-6 level was reduced significantly in the CG, EAG, TNBG, and WG groups (Fig. 2). For all 3 cytokines, n-butanol extract (TNBG) exhibited the strongest inhibition effect, indicating that n-butanol extract fraction possesses anti-inflammatory properties.

N-butanol extract alleviated leukocyte infiltration and alveolar bone loss in periodontitis samples

To evaluate the beneficial effects of different extracts of Kangfuxin on periodontal tissues, we performed H&E staining in each experimental group. The periodontal histopathology of the model group revealed inflammatory cell infiltration with severe destruction of the cementum and alveolar bone tissues. The Kangfuxin extracts in the CG, EAG, and WG groups did not show remedial effects when compared to the model group. N-butanol extract in the TNBG group showed protective effects against the leukocyte infiltration and the degeneration of cementum (Fig. 3).

50% ethanol eluent of n-butanol extract showed the most potent anti-inflammatory effect and protective effect against periodontitis

N-butanol extract was subjected to macroporous resin chromatography and eluted with a gradient of 10%, 30%, 50%, 70%, and 90% ethanol. Then we also evaluated the effects of different ethanol eluents in the rat model of periodontitis. ELISA analysis in the periodontal tissues revealed that the elevated levels of IL-6, IL-1β, and TNF-α in the model group were strongly suppressed by the 50% ethanol eluent

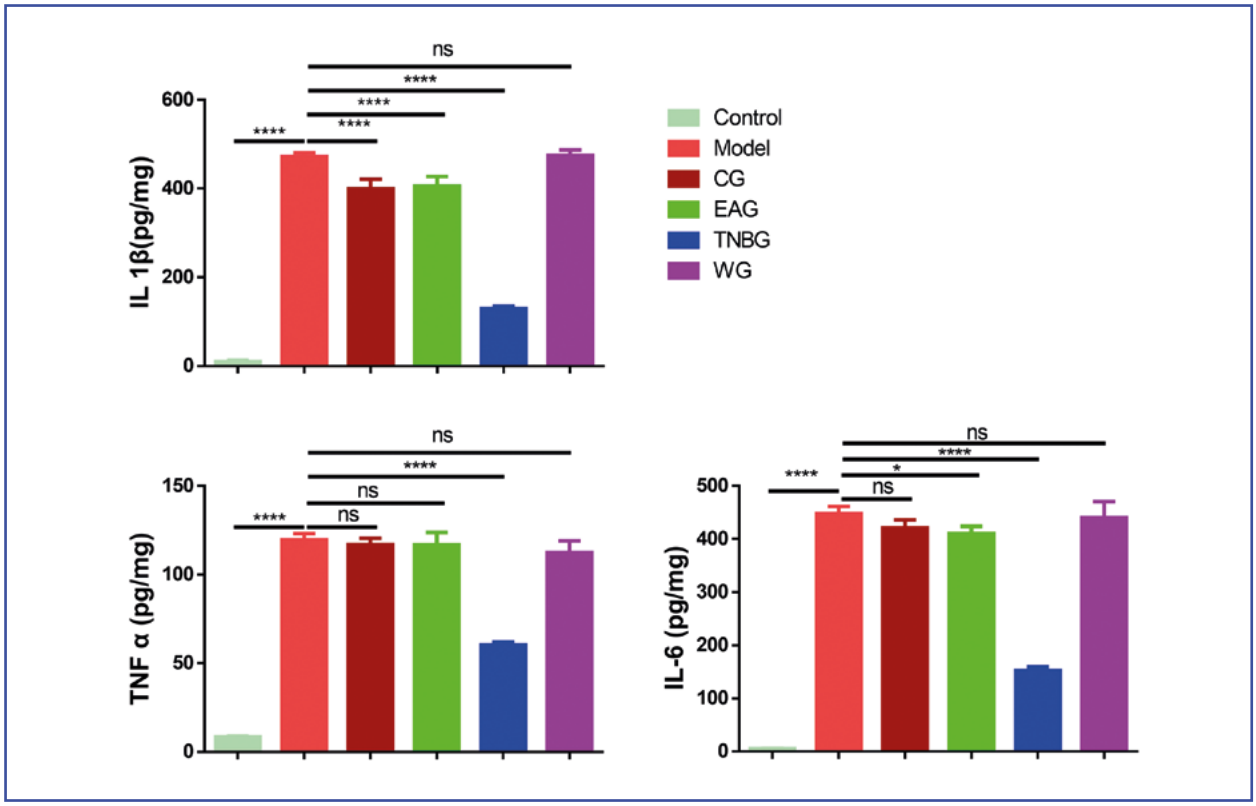

Figure 1. ELISA detection of levels of IL-1β, IL-6, and TNF-α in the periodontal tissues of the control, model, CG, EAG, TNBG, and WG groups (n = 5 in each group). Abbreviations: CG — chloroform group; Control — blank control group; EAG — ethyl acetate group; Model — model group; TNBG — the n-butanol group; WG — water group. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$.

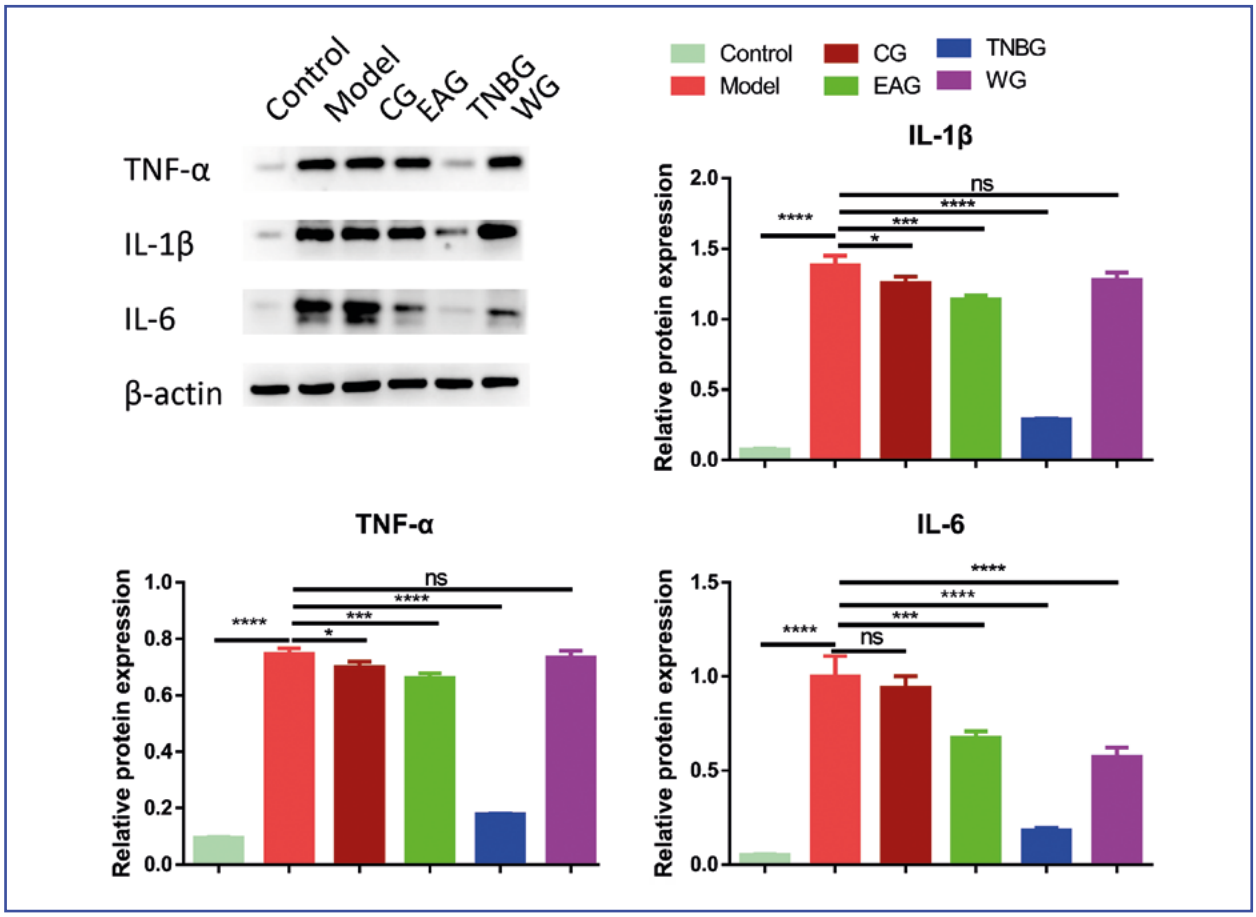

Figure 2. Western blot analysis of the relative protein levels of IL-1β, IL-6, and TNF-α in the periodontal tissues of the control, Model, CG, EAG, TNBG, and WG groups (n = 5 in each group). Abbreviations: CG — chloroform group; Control — blank control group; EAG — ethyl acetate group; Model — model group; TNBG — n-butanol group; WG — water group. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$.

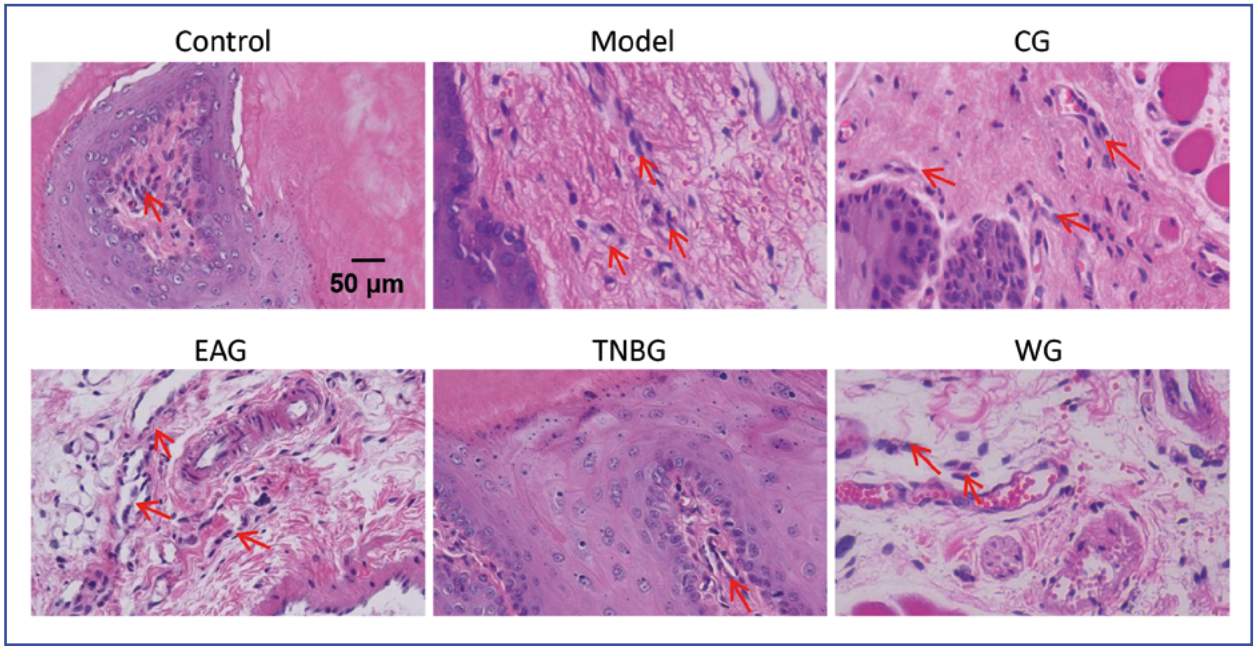

Figure 3. Haematoxylin and eosin staining analysis of the periodontal tissues of the control, Model, CG, EAG, TNBG, and WG groups (n = 5 in each group). Arrows indicate the infiltrated leukocytes. Abbreviations: CG — chloroform group; Control — blank control group; EAG — ethyl acetate group; Model — model group; TNBG — n-butanol group; WG — water group.

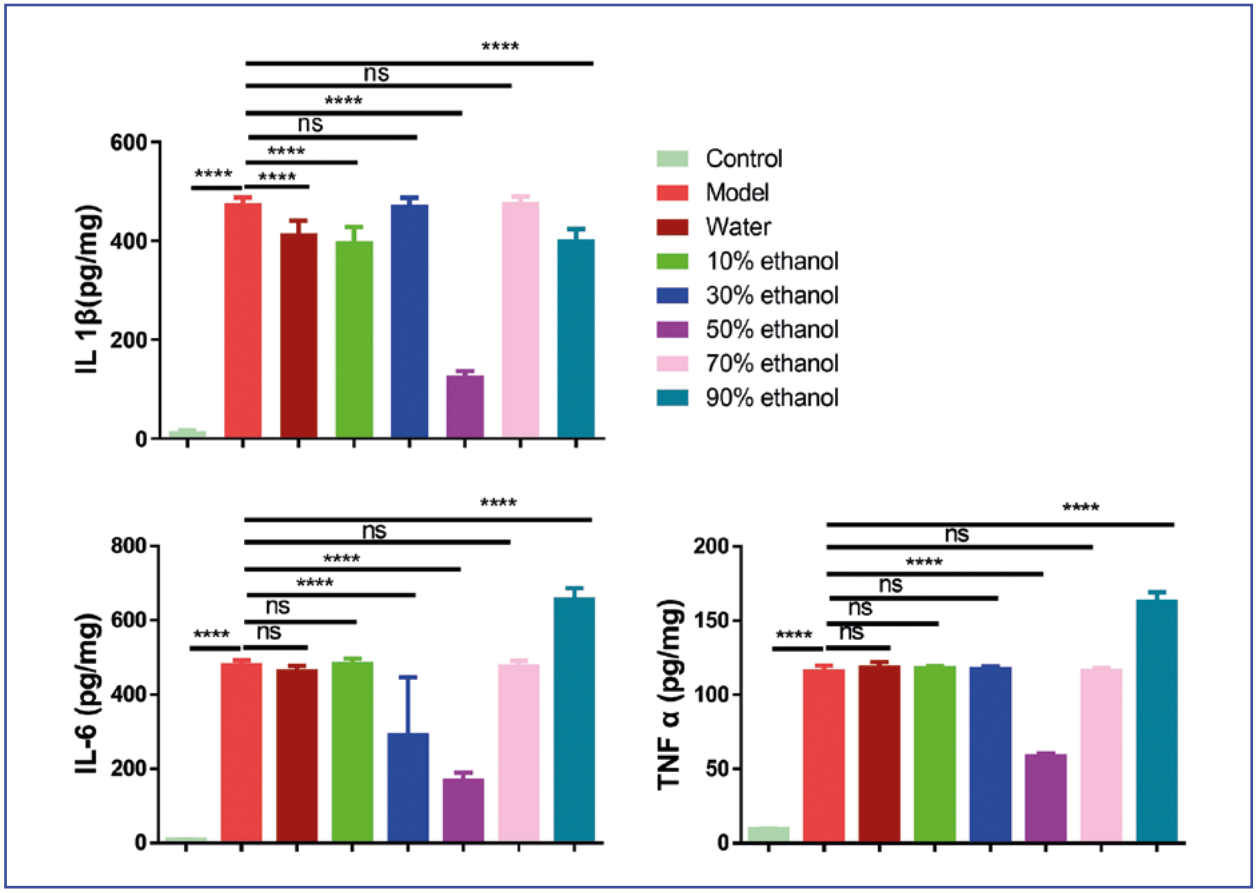

Figure 4. ELISA analysis of the levels of IL-1β, IL-6, and TNF-α in the periodontal tissues of the Control, Model, Water, and 10% to 90% ethanol fraction of n-butanol extract groups (n = 5 in each group). $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$.

(Fig. 4). Similarly, Western blot results showed that the protein levels of IL-1β, IL-6, and TNF-α in the periodontitis samples were heavily reduced by the 50% ethanol eluent, although 10%, 30%, and 70% ethanol eluents also showed inhibitory effects (Fig. 5). The periodontal histopathology analysis revealed that the inflammatory cell infiltration in the model group was significantly suppressed by the 50% ethanol eluent, while other fractions showed little effect (Fig. 6). Together, these findings suggest that the 50% ethanol eluent of n-butanol extract shows promising anti-inflammatory effects.

50% ethanol fraction reduced the PI3K-AKT-mTOR signalling pathway in periodontitis samples

To examine the activity state of the PI3K-AKT-mTOR pathway, Western blot analysis was conducted to measure total AKT, PI3K, and mTOR protein levels, as well as their phosphorylation states (p-PI3K, p-AKT, and p-mTOR). Our results showed that the relative phosphorylation levels of AKT, PI3K, and mTOR were consistently suppressed by the 50% ethanol fraction (Fig. 7), suggesting that the protective effect of this fraction may be due to the inhibition of the PI3K-AKT-mTOR pathway.

PI3K activator abrogated the effect of 50% ethanol fraction on periodontitis

To confirm that PI3K signalling inhibition contributes to the beneficial effect of 50% ethanol eluent of n-butanol extract, the model group was treated with 50% ethanol eluent or together with 740 Y-P (a cell-permeable phosphopeptide activator of PI 3-kinase, 5 mg/kg every 3 days). Periodontal histopathology analysis showed that PI3K activator could reverse the remedial effect of 50% ethanol fraction on tissue inflammation (Fig. 8A). ELISA analysis and Western blot results also showed that the application of PI3K activator significantly promoted the levels of IL-6, IL-1β, and TNF-α in the 50% ethanol fraction treatment group (Fig. 8B, C). These results suggest that the 50% ethanol fraction of n-butanol extract of Kangfuxin exerts remedial effects on periodontitis through PI3K signalling inhibition.

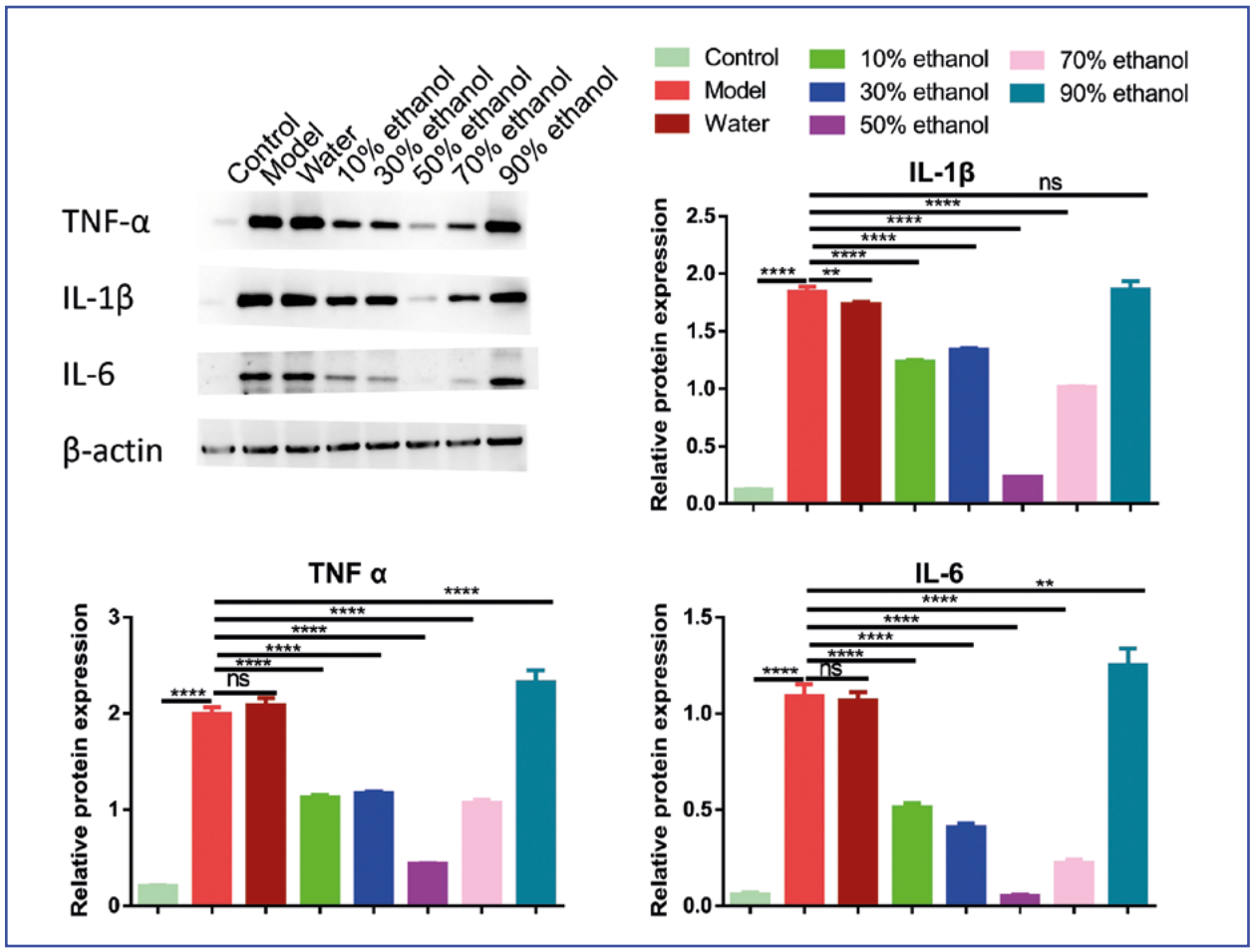

Figure 5. Western blot analysis of the relative protein levels of IL-1β, IL-6, and TNF-α in the periodontal tissues of Control, Model, Water, and 10% to 90% ethanol fraction of n-butanol extract groups (n = 5 in each group). $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$.

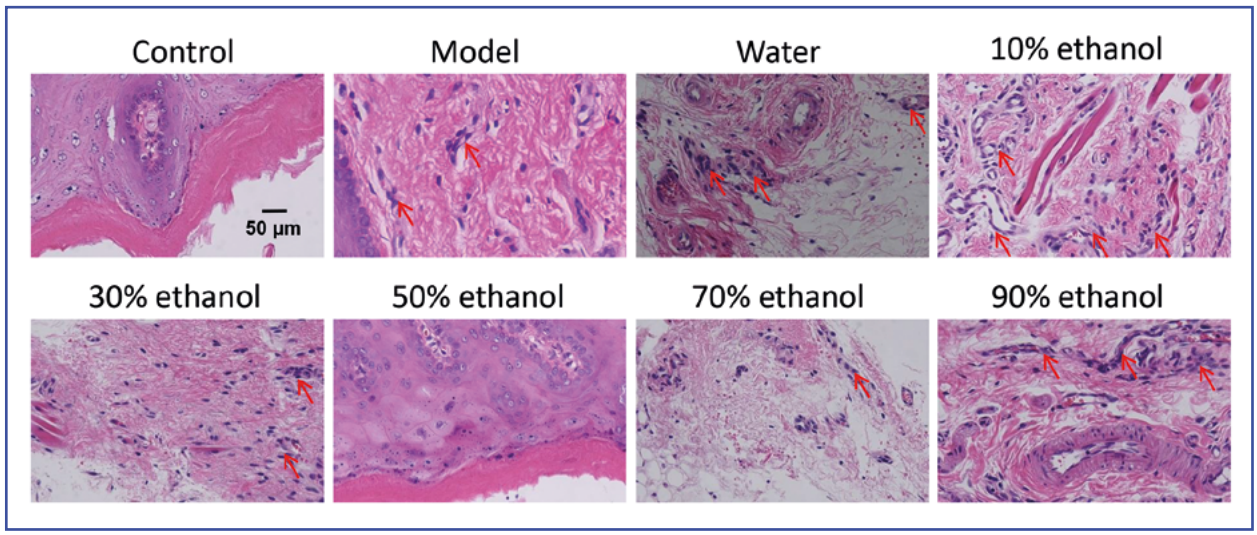

Figure 6. Haematoxylin and eosin staining analysis of the periodontal tissues of the control, Model, Water, and 10% to 90% ethanol fraction of n-butanol extract groups (n = 5 in each group). Arrows indicate the infiltrated leukocytes.

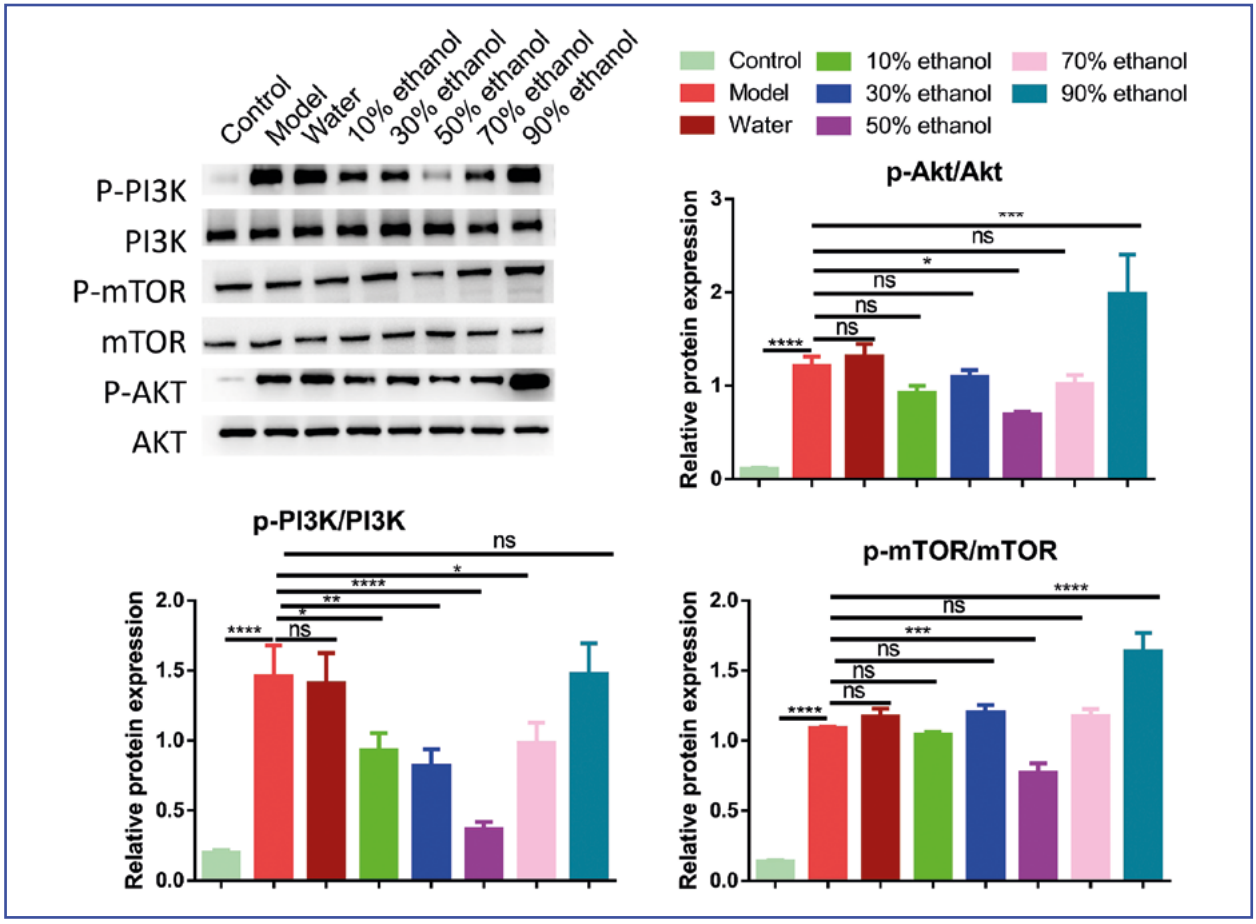

Figure 7. Western bot analysis of the phosphorylation state of PI3K, AKT, and mTor in the periodontal tissues of the Control, Model, Water, and 10% to 90% ethanol fraction of n-butanol extract groups (n = 5 in each group). $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$.

Discussion

Periodontitis is mainly caused by chronic inflammation after severe microbial infection in the mandibular tissues [30]. This condition is accompanied by the development of a series of periodontal diseases [31]. Clinical therapy for severe periodontitis is surgical intervention, which involves the removal of contagious biofilm, flap surgery, dental bone grafting, and gum grafting [32]. Microbe-derived substances such as lipopolysaccharide could induce the activation of macrophages to produce pro-inflammatory cytokines such as TNF-α and IL-1β [33, 34]. The accumulation of inflammatory cytokines not only promotes the tissue infiltration of other immune cells, but also induces bone resorption during the inflammatory process. For example, IL-6 and IL-1β have been shown to promote bone resorption by activating RANK ligand and increasing osteoclast activity [35]. On the other hand, over-activation of the PI3K-AKT-mTOR signalling has been associated with chronic inflammatory conditions [36].

In this study, we showed that n-butanol extraction of Kangfuxin exhibited the best anti-inflammatory effect to reduce IL-6, IL-1β, and TNF-α production in the periodontitis samples. N-butanol extract alleviated tissue damage and reduced leukocyte infiltration in the cementum. Furthermore, the 50% ethanol fraction of N-butanol extract suppressed the activation of the PI3K-AKT-mTOR signalling pathway in periodontitis samples. PI3K activator counteracted the anti-inflammatory effect of the 50% ethanol fraction. Our study reveals a potential workflow to identify an active fraction of Kangfuxin to treat periodontitis, indicating that Kangfuxin active fraction suppresses the activity of the PI3K-AKT-mTOR signalling pathway which is hyperactive in periodontitis samples.

Nowadays, natural plant-based medicines and traditional Chinese medications are becoming viable options for the prevention and treatment of oral disorders [37]. Kangfuxin is an extract from the American cockroach *Periplaneta americana,* which shows tissue healing effects and bacteriostatic properties. Recent studies demonstrated the therapeutic value of Kangfuxin in the treatment of radiotherapy-induced oral mucositis and wound healing [29, 38]. Also, in the mouse model of ulcerative colitis,

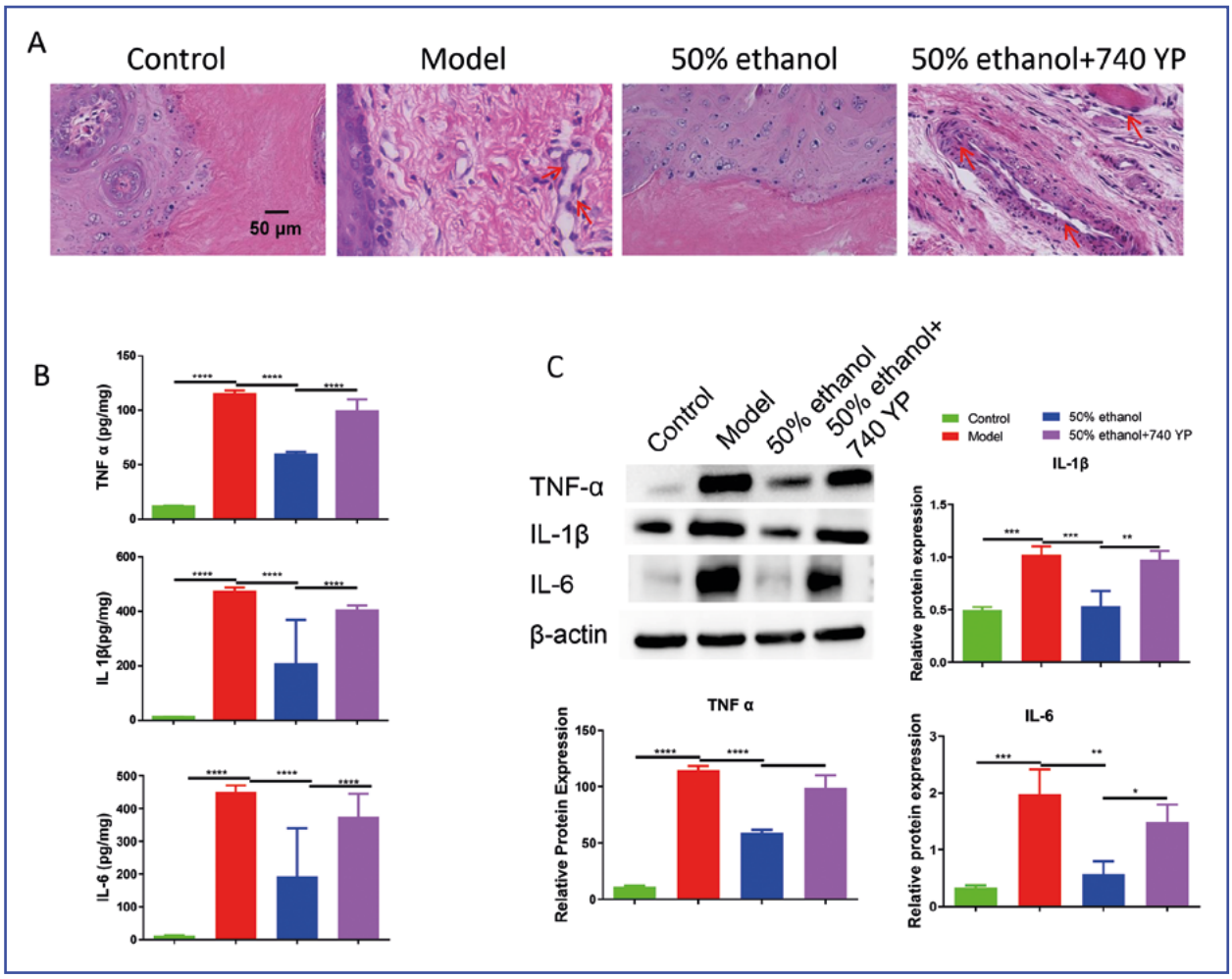

Figure 8. To confirm that PI3K signalling inhibition contributes to the beneficial effect of 50% ethanol eluent of n-butanol extract, the model group was treated with 50% ethanol eluent or together with 740 Y-P (a cell-permeable phosphopeptide activator of PI 3-kinase, 5 mg/kg every three days). **A.** Haematoxylin and eosin staining analysis of the periodontal tissues. Arrow indicates the infiltrated leukocytes. **B.** ELISA analysis of the levels of IL-1β, IL-6, and TNF-α in the periodontal tissues. **C.** Western blot analysis of the relative protein levels of IL-1β, IL-6, and TNF-α in the periodontal tissues. N = 5 in each group. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$.

Kangfuxin administration reduced inflammatory cytokine production, such as IL-6, IL-1β, and TNF-α [39]. Similarly, we showed that n-butanol extraction of Kangfuxin exhibited the best anti-inflammatory effect to reduce IL-6, IL-1β, and TNF-α production in the periodontitis samples, and it can reduce the infiltration of leukocytes. In agreement with our findings, Liu *et al*. demonstrated that Kangfuxin solution can effectively decrease the levels of IL1, IL6, IL17, and TNF in gingival crevicular fluid, improving periodontal conditions [40]. Liang *et al*. also showed that Kangfuxin reduces periodontal inflammation in individuals with gingival oedema and discomfort by suppressing the inflammatory cytokines in gingival crevicular fluid [41]. Importantly, we further showed that the 50% ethanol fraction of n-butanol extract of Kangfuxin has powerful anti-inflammatory effects. A limitation of this study is that we did not isolate or identify specific chemical components within the active 50% ethanol fraction. Future work is needed to characterise the individual bioactive compounds responsible for the observed effects.

Our study further revealed a hyperactivation of the PI3K-AKT-mTOR pathway in periodontitis samples, and the 50% ethanol fraction of n-butanol extract of Kangfuxin was able to dampen the activity of this signalling pathway. The PI3K signalling pathway plays divergent roles in different pathophysiological conditions. Aberrant activation of the PI3K signalling pathway has been widely reported in different cancers, which also facilitates the development of drug resistance [42]. In traumatic spinal cord injury, the PI3K/AKT signalling pathway is required for the recovery of spinal cord function after secondary injury, and activating PI3K/AKT signalling suppresses formation of glial scars in the chronic phase [43]. However, aberrant activation of this pathway is implicated in inflammatory disorders [44].

The PI3K/AKT/mTOR pathway has been recognised as a potential target for anti-SARS-CoV-2 therapy, since over-activation of the mTOR pathway facilitates viral replication. Also, clinical evidence reveals mTOR signalling hyperactivation in the lung tissues after SARS-CoV-2 infection [45]. Different members of PI3K seem to regulate different aspects of the inflammatory response to damage and microbial infection. For instance, PI3Kγ is abundantly expressed in leukocytes and mediates the chemokine-induced recruitment and activation of innate immune cells at sites of inflammation [46]. Furthermore, PI3K signalling inhibition blunts the inflammatory damage in the rat model of osteoarthritis [47]. Thus, our study indicates that targeting the aberrant activation of the PI3K/AKT/mTOR pathway could serve as an intervention to attenuate the inflammatory damage in periodontitis.

It is important to note that the periodontitis model used in this study is a "sterile" model, which does not include infection with bacteria typically associated with periodontitis, such as *Actinobacillus actinomycetemcomitans* [48]. This approach allows us to focus on the inflammatory response and tissue damage aspects of periodontitis. However, we acknowledge that periodontitis in humans is typically associated with bacterial infection, which can significantly influence the disease progression and treatment outcomes. Alternative "non-sterile" models, such as those described by Chipashvili and Bor [49], which combine ligature-induced periodontitis with bacterial infection, may provide additional insights into the efficacy of Kangfuxin in a more clinically relevant setting. The presence of pathogenic bacteria could potentially alter the inflammatory response and the effectiveness of the Kangfuxin fractions. For instance, bacterial factors might interact with or modulate the PI3K-AKT-mTOR signalling pathway differently than in our sterile model. Additionally, whether Kangfuxin fractions could induce bacteriostatic or bactericidal effects could be evaluated in a non-sterile model. Future studies using both sterile and non-sterile models would be valuable to comprehensively evaluate the efficacy of Kangfuxin fractions in periodontitis treatment and to better understand how bacterial presence might influence the observed anti-inflammatory effects.

Conclusions

In summary, our data demonstrated the potent anti-inflammatory effect of 50% ethanol fraction from the n-butanol extract of Kangfuxin on periodontitis. This fraction exerts inhibitory effect on the PI3K-AKT-mTOR signalling pathway. Future work is required to further purify bioactive components in the n-butanol extract of Kangfuxin and characterise the chemical nature of its active compounds.

Article information and declarations

Data availability statement

The data generated in this study are available upon request from the corresponding author.

Ethics statement

This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the Ethics Committee of Kunming Medical University (No. Kmmu20230909).

Author contributions

All authors contributed to the study conception and design. Material preparation, data collection, and analysis were performed byYanli Tang and Jie Pan. The first draft of the manuscript was written by Yanli Tang. Qiyan Li was mainly responsible for the conception of the experimental scheme and the review of the final manuscript, and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Conflict of interest

The authors have no relevant financial or non-financial interests to disclose.

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