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Anti-*Streptococcus mutans* and anti-inflammatory effects of ginsenoside Compound K and enzyme-treated red ginseng extract (BTEX-K)

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ABSTRACT

Objectives: Dental caries, or tooth decay, is an oral health issue worldwide. Oral healthcare researchers are considering how to develop safe and effective preventive measures and treatments for dental caries. This study evaluated the potential applications of Compound K and BTEX-K, a Compound K–rich red ginseng extract, for the prevention and treatment of dental caries. Moreover, this study briefly confirmed its inhibitory effect on inflammation, an important factor in dental health.

Methods: The amount of organic acids produced by bacteria in biofilm was determined using in vitro and in vivo assays. The ability of these extracts to promote tooth remineralization and microhardness was evaluated using an in vivo mouse assay. We evaluated their anti-inflammatory potential by inhibiting proinflammatory cytokine expression and lipopolysaccharide-induced nitrous oxide production in cell lines.

Results: Compound K (10–20 μg/mL) and BTEX-K (50–100 μg/mL) effectively inhibited the growth of *Streptococcus mutans* bacteria, demonstrating significant antibacterial properties. They can potentially prevent biofilm formation by reducing lactic acid production in the teeth. These compounds showed a strong ability to promote tooth remineralization and improve the microhardness of acid-producing bacteria. They also possess potent anti-inflammatory properties that downregulate proinflammatory cytokine (interleukin-6, interleukin-1β, inducible nitric oxide synthase) expression, suppress nuclear factor-kappa B transcription factor activation (~1.6 times), and reduce nitrous oxide production in lipopolysaccharide-induced RAW264.7 cells.

Conclusions: Compounds K and BTEX-K may provide a novel approach to dental caries prevention as well as inflammation prevention and treatment.

1. Introduction

Dental caries, commonly known as tooth decay, is a global oral health concern affecting people of all ages [1,2]. It involves the interplay of several factors, including oral microbiota, dietary habits, oral hygiene practices, and host susceptibility [1,2]. *Streptococcus mutans* (*S. mutans*), a key bacterium in the oral microbiome, plays a crucial role in the initiation and progression of dental caries by forming biofilms and producing acids that erode tooth enamel [1–3]. Current strategies for preventing and managing dental caries usually involve mechanical removal of the decayed tooth structure and the use of fluoride-based interventions. However, there is growing interest in exploring natural compounds with potential therapeutic applications for combating dental

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Abbreviations

ANOVA	analysis of variance
COX-2	cyclooxygenase 2
ELISA	enzyme-linked immunosorbent assay
HPLC	high-performance liquid chromatography
IL-1β	interleukin 1β
IL-6	interleukin 6
iNOS	inducible nitric oxide synthase
LPS	lipopolysaccharide
MAPK	mitogen-activated protein kinase
NF-|B	nuclear factor kappa B
NO	nitric oxide
Nrf2	nuclear factor erythroid 2-related factor 2
NSCLC	non-small cell lung cancer
PPAR γ	peroxisome proliferator-activated receptor gamma
SD	Sprague-Dawley
TNF-α	tumor necrosis factor alpha

caries.

Ginsenosides, which are the active components of ginseng, have gained attention for their bioactive properties and potential health benefits [4,5]. Ginsenoside Compound K (20-*O*-*β*-(D-glucopyranosyl)-20 (S)-protopanaxadiol) is a metabolite produced during the processing of ginseng root [5–7]. It is derived from the protopanaxadiol-type ginsenosides, primarily ginsenoside Rb1, Rb2, and Rc, via enzymatic conversion during the steaming and fermentation [6,8]. Extensive research has demonstrated the diverse pharmacological effects of Compound K, including anti-inflammatory, antioxidant, anticancer, and antimicrobial activities [4,5,9]. The antioxidant effects of Compound K have been found to alleviate sodium valproate-induced hepatotoxicity in rats [10]. Compound K was also shown to suppress the tumor growth induced by colitis-associated colorectal cancer at a 60 mg/kg dosage without exogenous interference of gut microbiota [11]. Furthermore, Compound K dose-dependently inhibited the cell viability of non-small cell lung cancer (NSCLC) via HIF-1 Î± mediated metabolic alteration, contributing to novel anticancer therapy by targeting glucose metabolism [12]. Regarding anti-inflammation activity, Compound-K improved insulin resistance by reducing inflammation through the PPAR γ/NF-κ B signaling pathway [13]. In addition, Compound K, at dosages of 40 and 160 mg/kg, reduced the spleen index and marginal zone in the spleen and alleviated hyperplasia of lymph nodes, resulting in downregulation of memory B cells in adjuvant-induced arthritis rats [14]. Especially, Compound K and BTEX-K have demonstrated the ability to inhibit the growth of *S. mutans* from our pretest results [15]. This helps in preventing the initiation and progression of dental caries.

BTEX-K, a Compound K-enriched red ginseng extract through enzymatic treatment, has been found to contain a rich source of bioactive compounds. The use of enzymatic treatment increases the bioavailability and efficacy of ginsenosides, including converting important ginsenosides into more active forms like Compound K [16]. A study has reported that another Compound K enriched extract has potential antioxidant and anti-inflammatory properties by scavenging reactive oxygen species and reducing pro-inflammatory cytokines in RAW264.7 cells [15,16].

These potential pharmacological properties of Compound K and BTEX-K have prompted investigations into their possible application in preventing and treating dental caries. In this study, we explored the inhibitory effects of Compound K and BTEX-K on *S. mutans*, their potential to modulate biofilm formation, and their influence on the expression of critical enzymes and cytokines involved in inflammatory pathways. Additionally, we examined the mechanisms through which Compound K and BTEX-K may affect dental caries. By investigating these effects, we hope to elucidate the potential of these compounds as natural therapeutic agents for managing dental caries and associated inflammatory conditions.

2. Materials and methods

2.1. Chemicals and reagents

2.1.1. Ginsenoside (compound K)

Standard ginsenoside Compound K was purchased from Sigma-Aldrich (St. Louis, MO, USA).

2.1.2. Preparation method for enzyme-treated red ginseng extract powder (BTEX-K)

Dried Korean red ginseng (Panax Ginseng C.A. meyer) root was purchased from a local market (Geumsan, South Korea). The Korean red ginseng (1 kg) was extracted three times with 10 vol of 50% ethanol (10 L) at 60 °C for 12 h (three times), and the extract was concentrated in vacuum conditions until less than 60 brix (P0). The concentrate was extracted again by passing it through a DIAION HP20 resin-packed column (Mitsubishi Chemical Industries, Tokyo, Japan) and powdered in vacuum conditions (P1). The P1 was dissolved in water and enzyme hydrolysis was performed by adding alpha-galactosidase from *Aspergillus niger* to maximize ginsenoside Compound K content (raw ginsenoside was hydrolyzed to Compound K) in mild acidic (pH 4–5) and thermophilic (50–60 °C) conditions. Among the reactants, only the precipitate was collected; it was dissolved in ethanol, filtered, concentrated in vacuum conditions, and powdered using a spray dryer (P2). BTEX-K, the final product, was prepared by mixing P2 and red ginseng extract powder, which was prepared by drying P0 in a 50%:50% ratio. The Compound K content in BTEX-K was 100.08 mg/g (Table 1).

2.1.3. Cell lines and bacterial species

In vitro assays were performed on RAW264.7 cells and *S. mutans* KCTC 3065 (ATCC 25175). The cells and bacteria were cultured according to the protocol provided in **Supplementary Information 1.1. (SI 1.1)**.

2.2. Reduction of biofilm

Five milliliters of a BHI liquid medium containing 1% sucrose was added to a 50 mL glass tube: 50 μL of Compound K (0.1, 1, and 2 mg/mL) and BTEX-K (0.5, 5, and 10 mg/mL) prepared for each concentration were added. The final concentration was 1, 10, and 2 μg/mL for Compound K and 5, 50, and 100 μg/mL for BTEX-K. *S. mutans* was inoculated at a concentration of 1×10^5 CFU/mL. The glass tube was tilted 30° and incubated at 37 °C for 24 h. After culturing, the cells attached to the wall were suspended in 5 mL of 0.5 M NaOH solution. The reduction in dental plaque was determined by measuring the absorbance of the suspension (1 mL) at OD_{550nm}.

2.3. Inhibition of bacterial growth causing dental caries in oral cavity

The inhibition of bacterial growth of Compound K (0.1, 1, and 2 mg/mL) and BTEX-K (0.5, 5, and 10 mg/mL) were assessed through the paper disc and optical density methods (**SI 1.2**). The final concentrations in bacterial media were 1, 10, and 20 μg/mL for Compound K and 5, 50, and 100 μg/mL for BTEX-K.

2.4. Evaluation of the anti-inflammatory activity of red ginseng extracts

The biocompatibilities of Compound K (final concentration: 1, 10, 20 μg/mL) and BTEX-K (final concentration: 5, 10, 20, 30, 40, 50 μg/mL) were performed by MTT assay RAW264.7 cells (**SI 1.3**) [17]. Then, the NO production inhibitory properties of these extracts were also assessed (**SI 1.4**).

Table 1
Ginsenoside content of BTEX-K.

Ginsenoside	Rg1	Re	Rh1	Rg2	Rb1	Rb2	Rc	Rd	Rg3(S + R)	F2	Compound K
Content (mg/g)	2.21	4.18	8.12	12.52	11.51	3.61	2.01	2.91	25.53	11.52	100.08

The ELISA kit was used to determine the inhibition of inflammatory cytokine expressions (interleukins (IL-1β and IL-6) and tumor necrosis factor-α (TNF-α)) upon treatment with Compound K (final concentration: 1, 5, 10 μg/mL) and BTEX-K (final concentration: 5, 10, 20 μg/mL). Western blotting assay and immunofluorescence analysis were designed to detect the expression of inflammatory response proteins (COX-2, iNOS, and NF- κB) in RAW 264.7 cells [18]. The cells were treated with different concentrations of Compound K (final concentration: 1, 5, 10 μg/mL) and BTEX-K (final concentration: 5, 10, 20 μg/mL) for 24 h before being exposed to 100 ng/mL LPS (*Escherichia coli* 0111; B4). The detailed protocols were fully described in **SI 1.5** [19].

2.5. Induction of dental caries

Sprague-Dawley (SD) rats (male, 3–4 weeks old) were fed ad libitum for 6–8 weeks (evaluation at two-week intervals, with a two-week introduction period). Dental caries was induced by feeding the experimental rats a high-concentration sugar-added feed and 20% sugar water for eight weeks. *S. mutans* and *Streptococcus sobrinus* were smeared on the teeth of the experimental rats two days per week (three times per day). One day before smearing cavity-causing bacteria, 10 μL of red ginseng extract (Compound K (40 μg/mL), BTEX-K (40 μg/mL)) was smeared on the teeth of the experimental rats two days per week (three times per day). The rats were divided into five groups, including negative control (normal diet), positive control (feed containing 40% sugar, water containing 20% sugar and bacteria causing dental caries), ethanol group (feed containing 40% sugar, water containing 20% sugar, bacteria causing dental caries and 1% EtOH), Compound K group (feed containing 40% sugar, water containing 20% sugar, bacteria causing dental caries and Compound K (40 μg/mL)), and BTEX-K group (feed containing 40% sugar, water containing 20% sugar, bacteria causing dental caries and BTEX-K (40 μg/mL)).

The jaws of SD rats were aseptically removed and the teeth surfaces were rinsed with 30 μL of glucose solution (275 mM) for 5 min. The carboxylic acids in the biofilm were then isolated using HPLC analysis (**SI 1.6).**

The Kruskal-Wallis test was used to compare the average microhardness values for each experimental group based on the measurement location. Moreover, the microhardness was also measured again after the demineralization process. Microhardness was measured three times during the experiment: before exposure to the dental caries solution, after exposure to the dental caries solution, and after remineralization. The protocols were provided in **SI 1.7 and SI 1.8**.

2.6. Statistical analysis

Independent experimental results are presented as the mean ± standard deviation (SD). Comparisons of three or more groups of normally distributed data were accomplished using one-way ANOVA, followed by multiple comparison tests to determine the least significant difference. Statistical analyses were performed using GraphPad Prism software (ver. 7.0; San Diego, CA, USA). All experiments were conducted in triplicate; the results were considered statistically significant differences when the *p*-value was less than 0.05.

3. Results

3.1. Reduction of biofilm

Absorbance measurement of the suspended solution at 550 nm revealed that significant biofilm reduction occurred with 50 μg/mL BTEX-K ($p < 0.05$) and 10 μg/mL Compound K ($p < 0.0001$) (Fig. 1). Compared to the control group, biofilm showed a sudden significant decrease at a BTEX-K concentration of 100 μg/mL and a Compound K concentration of 20 μg/mL ($p < 0.0001$). The same statistical significance was confirmed in the statistical treatment results compared to 1% EtOH group.

3.2. Inhibition of bacterial growth causing dental caries in the oral cavity

According to the results obtained using the paper disc method (Fig. 2a), BTEX-K showed significant inhibitory effects at concentrations of 50 μg/mL and 100 μg/mL ($p < 0.0001$). However, there was no significant difference in the magnitude of inhibition at 50 μg/mL and 100 μg/mL. Compound K exhibited significant inhibitory activity at concentrations of 10–20 μg/mL ($p < 0.0001$).

The absorbance measurement results using the optical density method were similar to those using the paper disc method (Fig. 2b). When the comparison group was set as the control group or the 1% EtOH group respectively, the same statistical significance was confirmed in the statistical processing results.

3.3. Cell viability assay

As the solvent in which the sample dissolved was ethanol, the cell viability of RAW264.7 cells was investigated in an ethanol solvent. The results showed that cell viability was reduced by over 40% at an ethanol concentration of 2% or more (**Figure SI 2.3a**). Thus, the ethanol concentration of the sample solvent was fixed at 1% or less.

The viability of RAW264.7 cells decreased after treatment with Compound K at a concentration of 20 μg/mL (**Figure SI 2.3a**); thus,

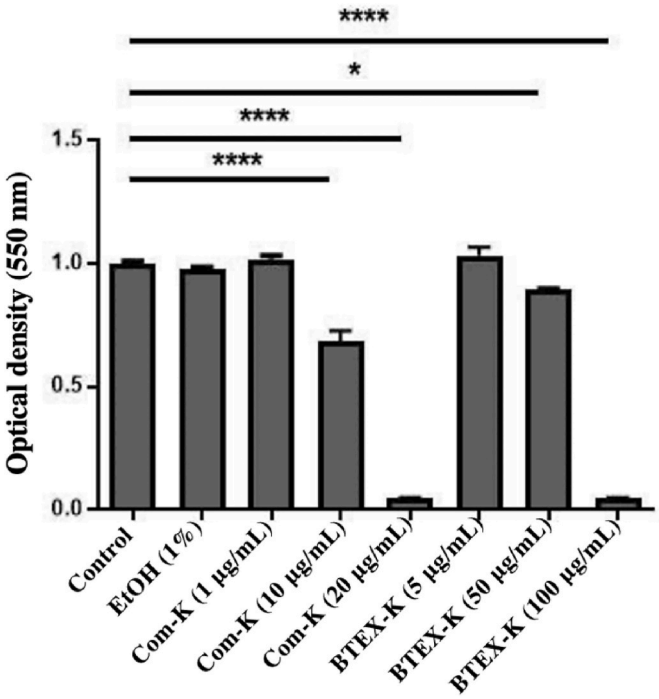

Fig. 1. Biofilm reduction results (*$p < 0.05$, ****$p < 0.0001$ compared with control group).

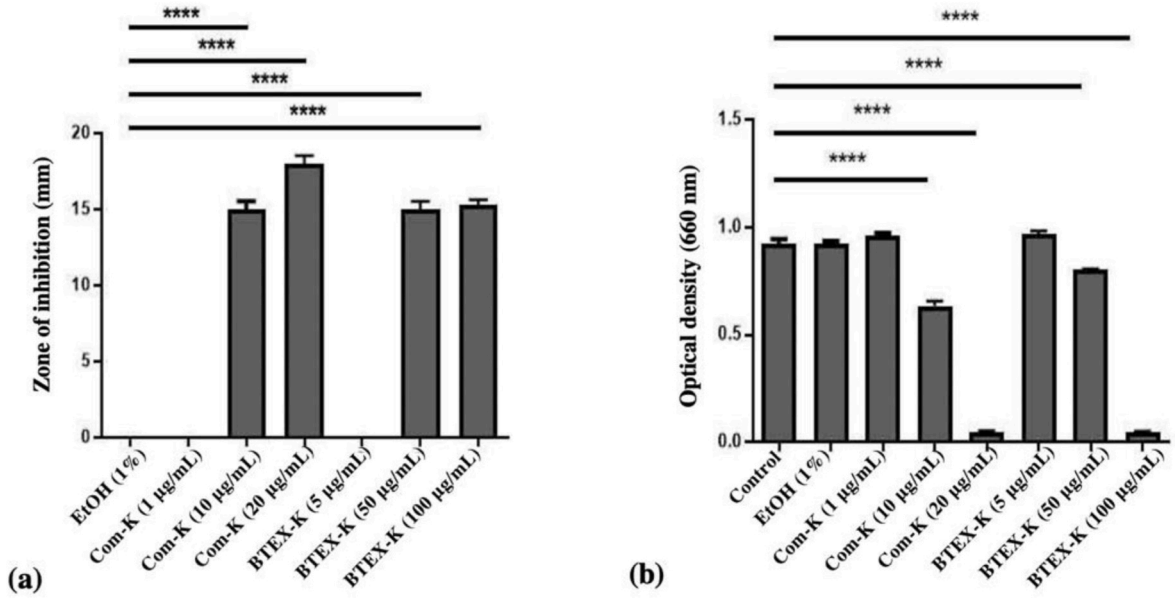

Fig. 2. Statistical processing of experimental results for inhibition of bacterial growth causing dental caries: (a) paper disc method; (b) optical density method (*****$p < 0.0001$ compared with ethanol).

subsequent experiments were performed using sample Compound K at 1, 5, and 10 μg/mL. The viability of RAW264.7 cells decreased after treatment with 30 μg/mL BTEX-K (**Figure SI 2.3a**), suggesting the sample concentrations for subsequent experiments in the range of 5, 10, and 20 μg/mL.

The viability and NO production of RAW264.7 cells after treatment with LPS, an inflammation-inducing reagent, were investigated at each concentration (**Figure SI 2.3b**). Cell viability increased at LPS concentrations of 100 ng/mL and 200 ng/mL; NO production increased from an LPS concentration of 100 ng/mL. Thus, subsequent experiments were performed using a 100 ng/mL LPS.

3.4. Inhibition of NO production

The RAW264.7 cells were pre-treated with Compound K and BTEX-K at different concentrations for 24 h and then treated with 100 ng/mL LPS. After 48 h, the NO production showed a significant decrease only at BTEX-K concentration of 20 μg/mL ($p < 0.01$) compared to LPS (Fig. 3). Statistical processing compared to EtOH with the LPS group also confirmed the same statistical significance ($p < 0.01$).

3.5. Inhibition of inflammatory cytokine and inflammatory response

The RAW264.7 cells were pre-treated with BTEX-K and Compound K at different concentrations for 24 h and then treated with 100 ng/mL LPS. After 48 h, the production of pro-inflammatory cytokine was evaluated (Fig. 4a). Treatment with Compound K did not result in the reduction of IL-6, IL-1β, and TNF-α concentrations. Only BTEX-K (20 μg/mL) significantly reduced the expression of IL-6 and IL-1β compared to LPS and LPS-EtOH groups.

For Compound K, the higher the treatment concentration, the lower was the expression of iNOS and COX-2 (Fig. 4b). With a 10 μg/mL Compound K concentration, iNOS was significantly reduced (1.9 times) compared to LPS ($p < 0.05$). The expression of COX-2 decreased as the concentration of Compound K increased; however, statistical significance was not confirmed at all concentrations. For pre-treatment with BTEX-K before inducing inflammation with LPS, the expression of iNOS and COX-2 decreased with exposure to BTEX-K at 10 μg/mL. At BTEX-K concentrations of 10 μg/mL and 20 μg/mL, iNOS expression was reduced by approximately 1.4–1.6 times compared to LPS; however, statistical significance was not confirmed. For COX-2, expression was reduced to a similar level with both low and high concentrations of BTEX-K; however, significance was not confirmed at all concentrations (Fig. 4c).

In addition, Compound K inhibited expression of inflammatory marker (NF-κ B) expression. The results indicated that the higher the concentration of Compound K, the lower the fluorescence intensity of NF-κ B compared to LPS. At Compound K concentrations of 5 μg/mL and

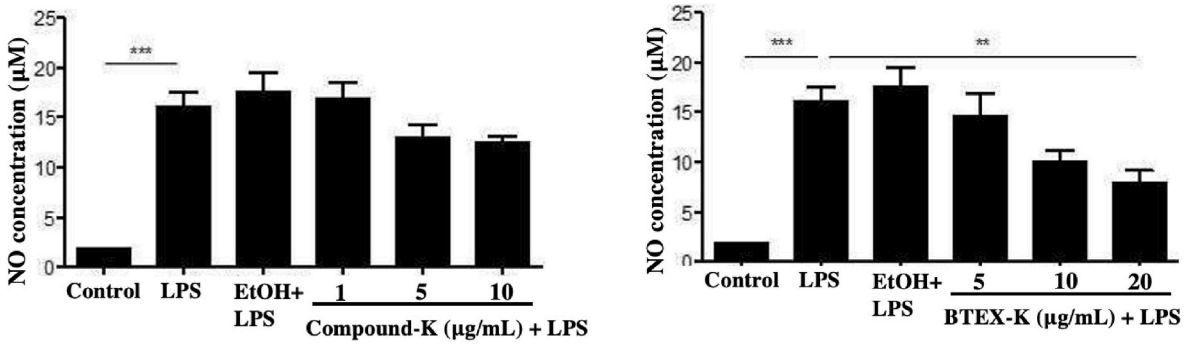

Fig. 3. NO production after 48 h of LPS-induced inflammation after treatment with Compound K (1, 5, and 10 μg/mL) and BTEX-K (5, 10, and 20 μg/mL) (**$p < 0.01$ and ***$p < 0.001$ compared with LPS).

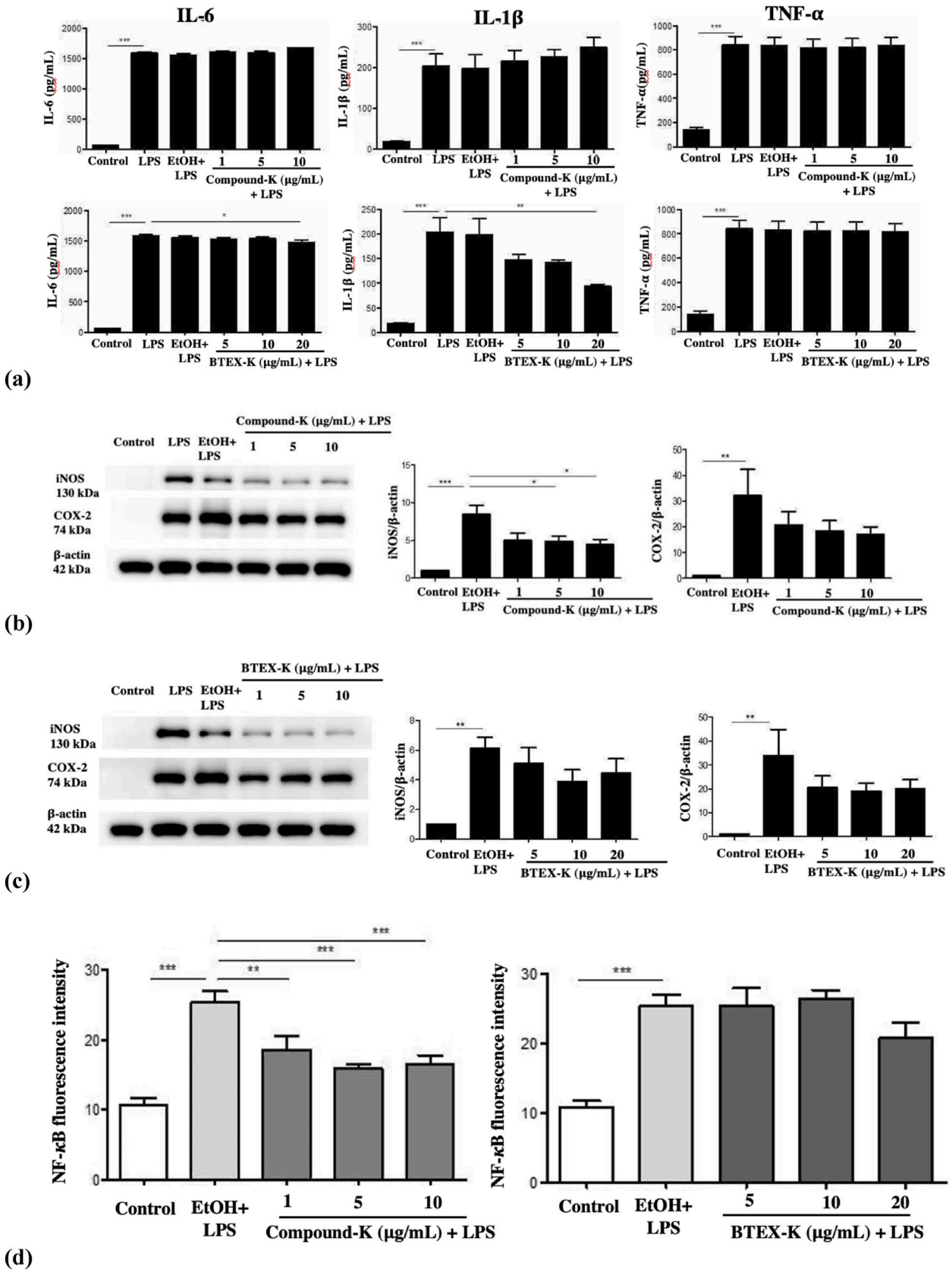

Fig. 4. (a) Cytokine production results after 48 h of LPS-induced inflammation after treatment with Compound K and BTEX-K samples (*$p < 0.05$, ***$p < 0.001$ compared with LPS); (b) Western blot analysis of inflammatory response proteins after treatment with Compound K (*$p < 0.05$,***$p < 0.001$ compared with EtOH with LPS); (c) Western blot analysis of inflammatory response proteins after treatment with BTEX-K (*$p < 0.05$,***$p < 0.001$ compared with EtOH with LPS); (d) Nuclear fluorescence intensity of RAW264.7 cells after treatment with LPS-EtOH, Compound K, and BTEX-K.

10 μg/mL, the fluorescence intensity of NF-κ B was 1.6 and 1.5 times lower than that of LPS, respectively; statistical significance was confirmed ($p < 0.001$) (Fig. 4d). The fluorescence intensity of NF-kB was found to be lower than that of LPS at a concentration of 20 μg/mL than at other BTEX-K concentrations; however, statistical significance was not confirmed (Fig. 4d).

3.6. Induction of dental caries

The tooth condition of each experimental group was evaluated after 9 days after dental caries induction. The color of the teeth in the positive control group was generally yellow compared to that of the negative control group (**SI 2.1a**). In addition, several residues similar to those in the feed were observed in the teeth of the experimental group, in which the teeth were smeared with Compound K and BTEX-K red ginseng extracts (**SI 2.1a**).

After four weeks, the overall tooth color was yellow in the positive control group, compared to the negative control group. In the Compound K and BTEX-K treatment groups, the color of the teeth was generally more yellow than that in the negative control group, although remnants of feed were not observed in the teeth (**Figure SI 2.1b**).

After eight weeks, the overall tooth color changed to yellow in both the control and positive control groups. In addition, what appeared to be food debris was observed on the teeth; the color of the teeth was generally yellow in the Compound K and BTEX-K treatment groups too (**Figure SI 2.1c**).

Although the overall tooth color changed to yellow in all of the test groups including the control and positive control groups, no items corresponding to Keyes score [20], which evaluates caries lesions in typical rats, were observed.

3.7. Amount of organic acid produced in the biofilm

For in vitro test, HPLC analysis showed that the lactic acid amount at 100 μ g/mL for BTEX-K and at 20 μg/mL for Compound K, was reduced compared with that in the control group (Fig. 5a). Lactic acid was detected at the same retention time as in the organic acid standard sample. After preparing a calibration curve for the standard lactic acid sample, statistical processing was performed by analyzing the difference in lactic acid content between the control group and the experimental groups. Compared to the control group, Compound K was reduced by approximately two-fold ($p < 0.0001$) and BTEX-K was reduced by approximately 3.2-fold ($p < 0.0001$) (Fig. 5a).

In addition, the lactic acid on the teeth was analyzed using an in vivo assay. Eight weeks after inducing dental caries, the lactic acid content was significantly decreased in the experimental group treated with red ginseng extract (Compound K and BTEX-K) (Fig. 5b). Compared to positive control, lactic acid production was reduced by approximately 2.2 and 1.5 times in the BTEX-K and Compound K treatment groups, respectively ($p < 0.01$, $p < 0.05$). Statistical processing compared to the EtOH group also confirmed the same statistical significance.

3.8. Microhardness measurement

Microhardness was measured by indentation, twice in the enamel and three times in the dentin, with each measurement location spaced 30 μm apart parallel to the deepest part of the fissure. In the enamel, the measurements were labeled as enamel 1 and enamel 2, while in the dentin, the measurements were labeled as dentin 3, dentin 4, and dentin 5.

The average microhardness values according to the measurement location for each experimental group were compared using the Kruskal-Wallis test. For Enamel 1 and Enamel 2 measured at the enamel depth, the microhardness tended to be the lowest in the positive control group (76.37 ± 37.86 and 110.66 ± 22.58, respectively). This suggests that dental caries progressed in the positive control group, compared with other experimental groups, although the difference was not statistically significant. Moreover, there were no significant differences in the microhardness of Dentin 3, 4, and 5 in each experimental group. Thus, it can be concluded that the dental caries did not progress to the dentin depth (Fig. 6a).

As a result of the first microhardness analysis, a trend was confirmed, but significance was not confirmed, so caries was induced again in new SD rats and a microhardness analysis experiment was performed using the same method. BTEX-K group was compared with the positive control and 1% EtOH groups. Treatment with BTEX-K resulted in significantly increased microhardness in Enamel 1, Enamel 2, and Dentin 3 (1.27, 1.24, and 1.34 times, respectively) compared to the positive control

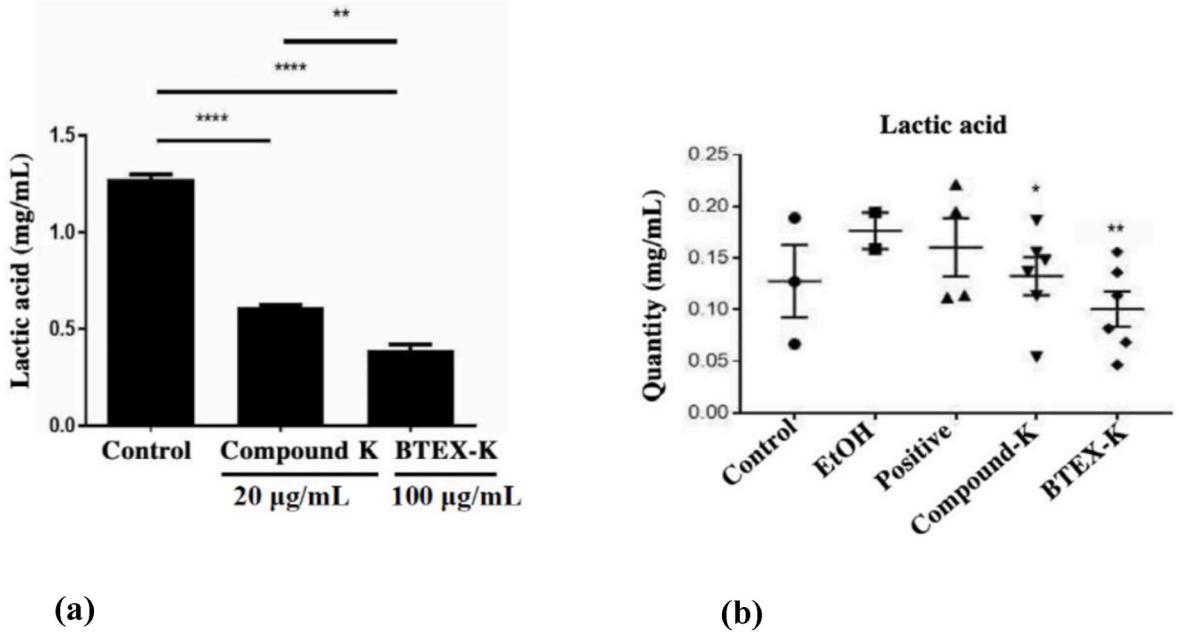

Fig. 5. Quantitative statistical processing of HPLC analysis results for organic acid production (a) the result of in vitro test analysis (b) the result of in vivo test analysis (****$p < 0.0001$ compared with control; **$p < 0.01$ compared with Compound K and BTEX-K).

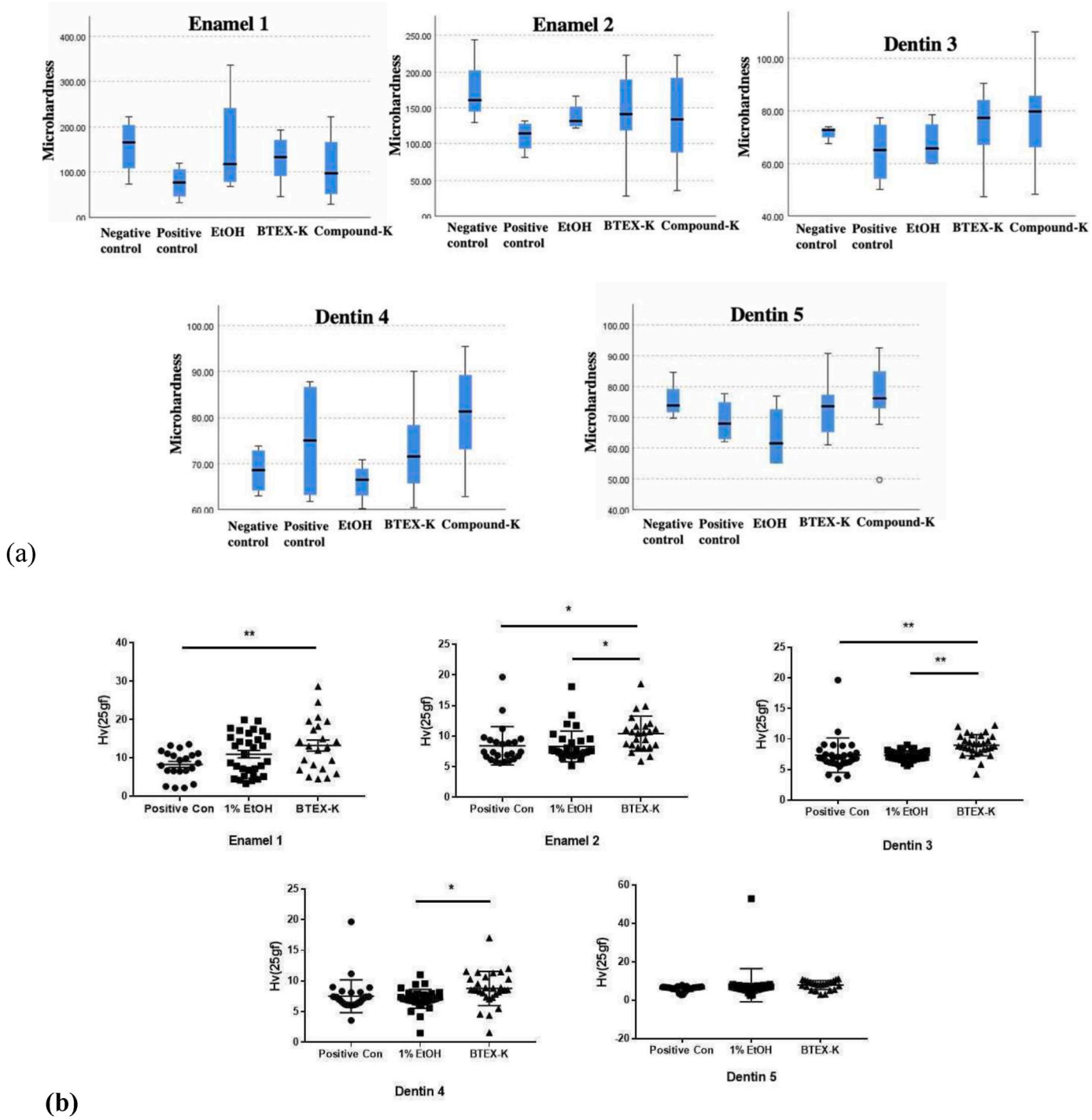

Fig. 6. (a) 1st Microhardness results for Enamel 1, Enamel 2, Dentin 3, Dentin 4, and Dentin 5 in different groups, including negative control, positive control, EtOH, BTEX-K, and Compound K groups; (b) 2nd Microhardness results for Enamel 1, Enamel 2, Dentin 3, Dentin 4, and Dentin 5 in different groups, including positive control, EtOH, and BTEX-K groups (**$p < 0.01$, *$p < 0.05$ compared with positive control or 1% ethanol group).

group (each $p < 0.01$, $p < 0.05$, and $p < 0.01$). Comparing the effectiveness of BTEX-K and 1% EtOH, the microhardness increased significantly in Enamel 2 and Dentin 3, and Dentin 4 by approximately 1.25, 1.21 and 1.23 times, respectively ($p < 0.05$, $p < 0.01$, and $p < 0.05$). The microhardness in Dentin 5 showed no significant difference in all groups (Fig. 6b). These results confirm the ability of BTEX-K red ginseng extract to significantly inhibit dental caries induction.

3.9. Recrystallization measurement

The recrystallization measurement test was performed as indicated in SI. 1.8. After demineralization, microhardness decreased in all three groups (control, Compound K, and BTEX-K groups) (Table 2). In contrast, microhardness increased after remineralization in all three groups. The largest increase in microhardness was observed in the Compound K treatment group (283.77 ± 23.19), compared to control and BTEX-K groups (246.20 ± 22.71 and 242.91 ± 21.24, respectively) (Table 2). The averages of the microhardness measurements after

Table 2
Average and standard deviation of microhardness test for recrystallization according to measurement location.

	Control	Compound K group	BTEX-K group
Before demineralization	311.50 ± 9.31	310.26 ± 8.32	313.28 ± 14.30
After demineralization	203.04 ± 17.03	224.48 ± 13.31	213.70 ± 21.11
After remineralization	246.20 ± 22.71	283.77 ± 23.19[a]	242.91 ± 21.24

[a] Statistically significant results were confirmed in the compound K treatment group compared to the control group ($p < 0.05$).

remineralization were compared using the Kruskal-Wallis test. There was a significant difference in the microhardness of recrystallization only between the Compound K treatment and control groups ($p < 0.05$); on the other hand, no statistically significant differences were observed between the BTEX-K treatment group and control groups (Table 2).

4. Discussion

Ginsenosides are saponins, which are the major pharmacologically active components of ginseng root. The ginseng root contains 2–3% ginsenosides. More than 40 ginsenoside compounds have recently been identified in red ginseng extracts, including Rb1, Rb2, Rc, Rd, Re, Rg1, and Rg3 [21]. Compound K and BTEX-K are derived from the metabolization of these ginsenosides through the action of gut bacteria [7,22,23]. They are considered to be the most potent and pharmacologically active metabolites of ginseng, with anti-inflammatory, antioxidant, anti-cancer, anti-diabetic, anti-obesity, and neuroprotective properties [7,22,23]. In this study, we found that Compound K and BTEX-K could be considered as potential oral health protectant owing to their anti-inflammatory and antibacterial activities.

S. mutans is a type of bacteria known to contribute to dental caries (tooth decay) by producing acid that damages tooth enamel [3,24]. Thus, inhibition of *S. mutans* activity by Compound K and BTEX-K was investigated. The results confirmed that Compound K (>10 μg/mL) and BTEX-K (50–100 μg/mL) significantly inhibited *S. mutans* activity. They may interfere with the bacterial cell membrane integrity, disrupt essential metabolic processes, and inhibit bacterial growth and proliferation [3,24]. *S. mutans* is also known for its ability to form biofilms, which are communities of bacteria plaque embedded in a protective matrix [3,24]. Biofilms play a critical role in the development of biofilm and tooth decay. In this study, both Compound K and BTEX-K showed an ability to inhibit the formation of biofilm caused by *S. mutans* at concentrations of 20 μg/mL and 100 μg/mL, respectively, reducing the ability of *S. mutans* to adhere to tooth surfaces and contribute to dental caries.

In addition, the anti-inflammatory properties of Compound K and BTEX-K compound play important roles in their anti-caries abilities. Inflammatory cytokines are signaling molecules that play a crucial role in initiation and regulation of immune response during inflammation. Excessive or uncontrolled production of inflammatory cytokines contributes to chronic inflammation and inflammatory disease. Several studies have indicated that Compound K and BTEX-K inhibit the expression of certain inflammation cytokines. Jang et al. (2013), suggested that pre-administration of Korean red ginseng extract inhibited microglial activation, iNOS expression, and proinflammatory cytokine production (IL-1 β, IL-6, and TNF-α) [25]. Compound K and BTEX-K also inhibit the release of proinflammatory cytokines such as IL-6, IL-1 β, and TNF- α in LPS-activated microphages through the reduction of the NF-κ B signaling pathway [26]. Park et al. (2012) suggested that Compound K suppressed inflammatory molecules by modulating the production of ROS, MAPK, NF-κ B, and OH-1 signaling pathways in LPS-stimulated microglia [27]. Moreover, Compound K and BTEX-K have been investigated for their potential to inhibit the expression of the enzymes COX-2 and iNOS involved in inflammatory processes [28]. These compounds may regulate the activity of transcription factors such as NF-κ B and AP-1 (activator protein-1) involved in the activation of COX-2 and iNOs gene expression [8,28]. By interfering with these transcription factors, they can downregulate the expression of COX-2 and iNOS. They have also been found to activate anti-inflammatory signaling pathways, such as the MAPK (mitogen-activated protein kinase) and Nrf2 (nuclear factor erythroid 2-related factor 2) pathways [8,28]. Activation of these pathways can suppress the expression of COX-2 and iNOS, reducing inflammation. In this study, Compound K did not significantly reduce cytokine expression, whereas BTEX-K treatment effectively inhibited the production of IL-1 β. The results showed that Compound K (5–10 μg/mL) and BTEX-K (10–20 μg/mL) inhibited the expression of COX-2 and iNOS by approximately 1.5 and 1.4–1.9 times, respectively. It was confirmed that Compound K and BTEX-K effectively suppressed LPS-induced NO production, and mRNA and protein expression of COX-2 and iNOS. In addition, this study indicated that Compound K (5–10 μg/mL) effectively suppressed the activation of NF-κ B transcription factor by approximately 1.6 times in LPS-induced RAW264.7 cells, compared to the control group. The results suggest that both Compound K and BTEX-K are potential candidates for oral health through anti-caries and anti-inflammation application.

It is known that *S. mutans* is the main factor in producing lactic acid, playing a role in tooth decay and biofilm formation [3,24]. Thus, we investigated the ability of Compound K and BTEX-K to reduce lactic acid production and improve oral health. In terms of inhibition of bacterial growth, Compound K and BTEX-K effectively inhibited the growth of *S. mutans*, leading to the suppression of lactic acid production in teeth. According to the in vitro and in vivo results, Compound K and BTEX-K effectively reduced the lactic acid production by approximately 2–2.2 times and 1.5–3.2 times, respectively.

A primary goal in dental caries management is promotion of tooth remineralization, which involves restoration of minerals to tooth structures demineralized by acid-producing bacteria [29,30]. This study indicates that both Compound K and BTEX-K enhanced the remineralization by promoting the deposition of minerals such as calcium and phosphate onto tooth surfaces, offering a novel approach for strengthening tooth surfaces and potentially reversing early-stage caries lesions. These preliminary findings on Compound K and BTEX-K for dental caries management are promising; standardization of dosage formulations is necessary for consistent results and clinical applications.

5. Conclusions

Compound K and BTEX-K are promising natural compounds with potential applications in the management of dental caries. Their antibacterial, anti-inflammatory, and remineralization properties make them an attractive candidate for prevention and treatment of dental caries. However, further research and clinical trials are needed to optimize delivery systems and establish their safety and efficacy in clinical settings. With continued investigation and advancements in Compound K and BTEX-K research, these compounds may become useful tools for oral health professionals in the fight against dental caries. On the other hand, although we have confirmed the potential for development as an anti-inflammatory agent at an early stage, further research using periodontists cell lines (such as Ca9-22) and animal models is necessary.

Author contribution

Jin-Hwan Oh, The conception and design of the study, SangJoon Mo, The conception and design of the study, Le Thi Nhu Ngoc, Drafting the article and revising it critically for important intellectual content, Jonghyuk Lee, The conception and analysis and interpretation of data, Moon-Young Kim, The conception and analysis and interpretation of data, Hae-Seo Park, The conception and analysis and interpretation of

data, Jin-Hee Kim, The conception and analysis and interpretation of data, Yu-Jin Ha, The conception and analysis and interpretation of data, Lee-Sung, The conception and analysis and interpretation of data, Young-Chul Lee, Final approval of the version to be submitted, Youl-Hour, Final approval of the version to be submitted

Ethical statement

All experiments were performed in accordance with the guidelines of the Intramural Animal Use and Care Committee of Dankook University.

Declaration of competing interest

There is no conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.job.2024.08.001.

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