Effect of Catechin Diet on Gingivitis in Cats

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ABSTRACT

We examined the effect of catechin diet on gingivitis in cats. Gingival inflammation, oral malodor, and percentage of *Porphyromonas* were decreased after feeding of diet with catechin compounds. We suggest that feline gingivitis can be controlled by phytomedical activities of catechin in pet food. Twenty nine cats affected with gingivitis were fed either a catechin diet of 0.8 mg/g or 0.4 mg/g of catechin or a control diet and assessed for gingival inflammation, oral malodor, and percentage of the genus *Porphyromonas* present. The catechin diet showed an anti-inflammatory effect, a deodorant effect, and a decrease in *Porphyromonas* compared with the control diet. This suggests that the catechin in diet has the potential to reduce gingivitis resulting from the bacterial growth and virulence factors in cats.

INTRODUCTION

It has been reported that gingivitis is frequently seen in cats.[1,2] Asaccharolytic pigmented *Porphyromonas* species are predominant isolates from the plaque of cats with the disease.[3,4] Norris et al[4] reported that their study has established *Porphyromonas* as anumerically significant and a highly prevalent genus in feline gingivitis. The genus *Porphyromonas* was the major periodontopathic bacteria in man and animals.[5-7] There is a direct relationship between the development of gingivitis and accumulation of dental plaque with the increase of genus *Porphyromonas*. Therefore, prevention and treatment of the disease are necessary to target to the bacteria. Genus *Porphyromonas* are sensitive to Japanese green tea extract (catechin).[8] We showed that catechin was effective in the inhibition of canine gingivitis.[9]

Phytochemical medicine has been noticed because the public is becoming increasingly aware of problems with excessive use and misuse of antibiotics. It has been interested in useful antimicrobial phytochemical substances, which can be divided into several categories.[10] Catechin is a polyphenolic derivative, mainly extracted from leaves of *Camellia sinensis*. The purpose of the study reported here was to evaluate the reduction in gingival inflammation, oral malodour, and genus *Porphyromonas* using diet with catechin.

MATERIALS AND METHODS

Subjects

A total of 29 (mongrel, male 15, female 14) cats were studied. Their mean age was 9.1 ± 3.4 years (range, 2-17 years). These cats were affected with gingivitis.

Catechin and Preparation of Diet

Catechin compounds were extracted from the leaf of *C sinensis* with 95% hot ethanol, previously described.[8,9] After filtration and charcoal treatment, samples were analyzed for composition by high-performance liquid chromatography and gas chromatography. The final product contained 41.6% catechin. In the extract, 5 major compounds was detected, (-)-epigallocatechin gallate (17.8%), (-)-epigallocatechin (11.8%), (-)-epicatechin gallate (4.2%), (-)-epicatechin (2.8%), and D-(+)-catechin (0.4%). The special diet with catechin (0.4 and 0.8 mg/g) was prepared.

Experimental Protocol

All cats were fed the commercial control diet (INABA Foods Co., Japan) for 14 days, prior to being put on the experimental diet containing catechin for 45 days. Diet with catechin (0.4 mg/g and 0.8 mg/g) was prepared for cats. Animals were given the feeds twice daily during the test period. Gingival index (GI), oral malodor, and percentage of the genus *Porphyromonas* in the subgingival microbiota were examined.

Parameter of Gingivitis: Gingival Index (GI)

The degree of gingival inflammation was estimated according to the criteria of the GI system[11] by Löe and Silness with some modifications.[9] Gingival index was examined on the only buccal aspect of all teeth although the original method has indicated to be scored on 4 gingival sites of each tooth region.

Measurement of Oral Malodour

Measurement of oral malodor was determined using a volatile sulphur compound sensor as methylmercaptan standard (Tokuyama Soda, Co., Tokyo, Japan) to assess oral malodor directly from the oral cavity, as previously described.[9] Production of volatile sulphur compounds from isolated *Porphyromonas* was also detected.

Examination of Genus *Porphyromonas*

Gingival plaque was taken from the maxillary premolars of cats with a scaler or a paper point. Each specimen was immediately inoculated to Brain Heart Infusion agar (BHI agar, Nissui Co., Tokyo, Japan) with 7% horse blood. They were placed in a gas pack system (BBL GasPak Pouch anaerobic system, Becton Dickinson, Maryland, USA). BHI agar is the non-selective one for total counts. A part of the bacteria produced black-pigmented colonies, which were considered as genus *Porphyromonas* (major population) or genus *Prevotella* (minor population). The count of black-pigmented colonies was calculated as genus *Porphyromonas* and the percentage of the bacteria in the plaque was determined. Identification of representative colonies was carried out using an API-ZYM system (API system S. A., Montaliu, France), previously described.[9]

Statistical Analysis

Wilcoxon (matched-pairs) signed-ranks test was used for comparison between control and catechin diet.

RESULTS

A catechin diet showed an anti-inflammatory effect in cats with periodontitis. All cats (control diet) manifested mild to severe gingivitis before being fed the diet with catechin, especially in premolars and molars. The mean GI (0.38 ± 0.46) in cats fed a catechin diet (0.8 mg/g) was significantly lower than that of the GI (0.93 ± 0.52) in cats fed the control diet ($P < 0.01$, Table 1). Similarly, the mean GI (0.82 ± 0.63) in cats fed a catechin diet (0.4 mg/g) was significantly lower than that of the GI (1.51 ± 0.52) in cats fed the control diet ($P < 0.01$, Table 1). Gingival inflammation was reduced to half level after feeding the catechin diet. Figure 1 shows decreased pattern from control diet to catechin diet (0.8 mg/g). The anti-inflammatory effect of catechin was recognized in 12 of 13 cats with only 1 exception.

Table 1. Anti-inflammatory effect of catechin diet in cats with periodontal disease

Catechin Concentration in Diet	GI*		
	Catechin Diet (After)	Control Diet (Before)	*P* Value†
0.8 mg/g	0.38 ± 0.46	0.93 ± 0.52	<0.01
0.4 mg/g	0.82 ± 0.63	1.51 ± 0.52	<0.01

*Mean ± SD.

†Wilcoxon (matched-pairs) signed-ranks test.

Figure 1. Effect of catechin (0.8 mg/g) on gingival inflammation in individual cat with periodontal disease.

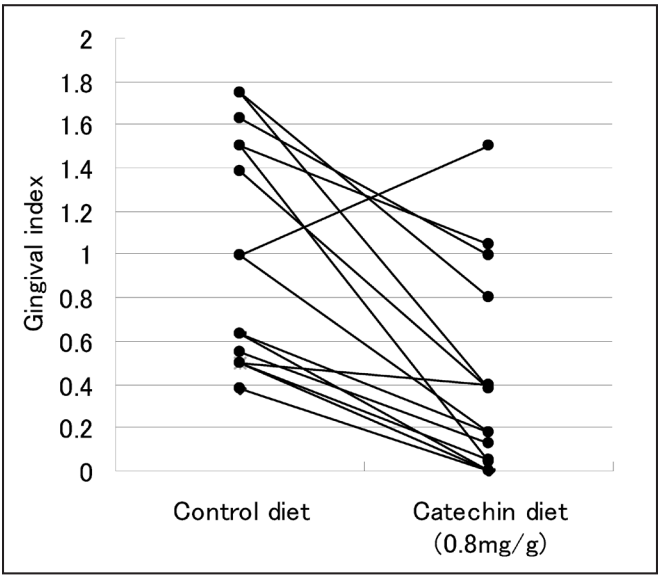


Table 2. Deodorant effect of catechin diet in cats with periodontal disease.

Catechin Concentration in Diet	Oral Maladour (ppm)*		
	Catechin Diet (After)	Control Diet (Before)	*P* Value†
0.8 mg/g	0.21 ± 0.42	0.80 ± 1.13	<0.05
0.4 mg/g	0.17 ± 0.51	0.58 ± 1.67	NS

NS = not significant.

*Mean ± SD; †Wilcoxon (matched-pairs) signed-ranks test.

The catechin diet showed a deodorant effect in cats with periodontitis. The mean concentration of oral malodor (0.21 ± 0.42 ppm) in cats fed a catechin diet (0.8 mg/g) was significantly lower than that of oral malodor (0.80 ± 1.13 ppm) in cats fed the control diet ($P < 0.05$, Table 2). The mean concentration of oral malodor (0.58 ± 1.67 ppm) in cats fed a catechin diet (0.4 mg/g) was lower than that of oral malodor (1.51 ± 0.52 ppm) in cats fed the control diet, although there was no significant difference (Table 2). Thus, oral malodor was reduced to 1/4 level with the 0.8 mg/g catechin diet and 1/3 level with the 0.4 mg/g catechin diet. Figure 2 shows a decreased pattern from control diet to catechin diet (0.8 mg/g). Oral malodor was detected in 11 of 13 cats before being fed a diet with catechin. After being fed the catechin diet, 9 of 11 cats showed a decrease of oral malodor.

Before being fed a catechin diet, black-pigmented *Porphyromonas* was isolated from the plaque of 25/29 cats and recognized as a major bacteria in the microbiota. They were indole-positive and asaccharolytic gram-negative rods. *Porphyromonas gingivalis* (catalase-positive and -negative types), *P salivosa*, *P circumdentaria*, and *Porphyromonas* spp were identified by the API ZYM system. The percentage of these *Porphyromonas* decreased after feeding of the catechin diet. The mean percentage of the bacteria (6.2 ± 10.9) in cats fed a catechin diet (0.8 mg/g) was significantly lower than the percentage (33.9 ± 30.9) of cats fed a control diet ($P < 0.01$, Table 3). The mean percentage of genus *Porphyromonas* (12.4 ± 14.6) in cats fed a catechin diet (0.4 mg/g) was lower than the percentage (34.6 ± 21.2) of cats fed the control diet ($P < 0.05$, Table 3). Figure 3 shows a decreased pattern of black-pigmented *Porphyromonas* in plaque of cats from control diet to catechin diet (0.8 mg/g). Genus *Porphyromonas* was detected in 12 of 16 cats before being fed a diet with 0.8 mg/g catechin. After feeding of the 0.8 mg/g

Figure 2. Deodorant effect of catechin diet (0.8 mg/g).

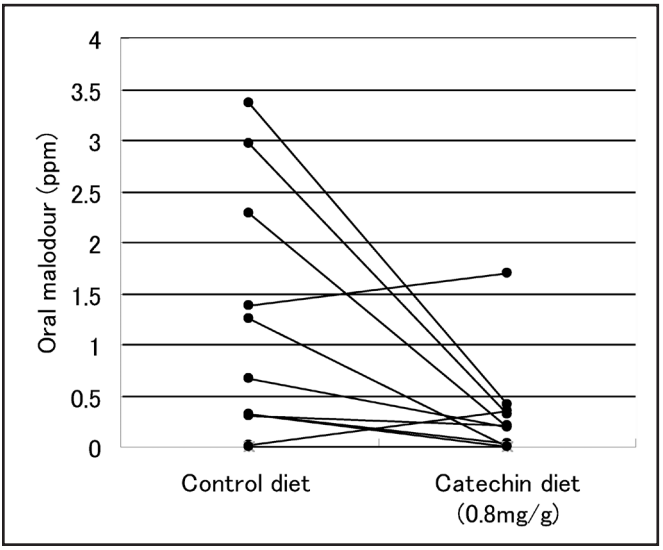


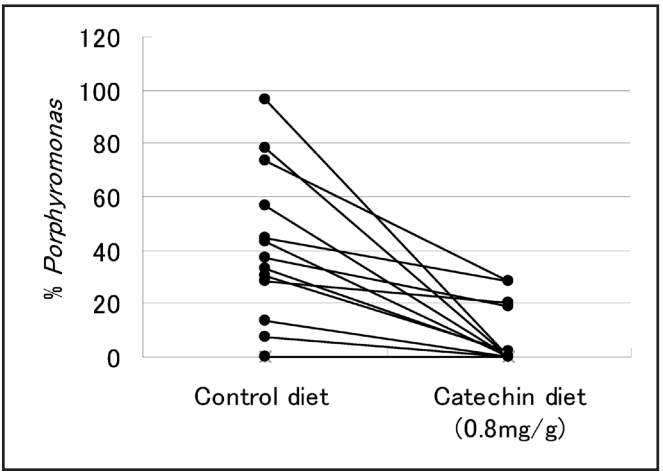
Table 3. Efffect of catechin diet on percentage *Porphyromonas*.

Catechin Concentration in Diet	% *Porphyromonas**		
	Catechin Diet (After)	Control Diet (Before)	*P* Value†
0.8 mg/g	6.2 ± 10.9	33.9 ± 30.9	<0.01
0.4 mg/g	12.9 ± 14.6	34.6 ± 21.2	<0.05

*Mean ± SD.

†Wilcoxon (matched-pairs) signed-ranks test.

Figure 3. Effect of catechin (0.8 mg/g) on reduction of *Porphyromonas* in the plaque of cats with periodontitis.



catechin diet, 12 of 12 showed decrease of the percentage of *Porphyromonas* in plaque (Figure 3). Genus *Porphyromonas* was detected in 13 of 13 cats before being fed a diet with 0.4 mg/g catechin. After feeding of the 0.4 mg/g catechin diet, all showed decrease of the percentage of *Porphyromonas* in plaque (Figure 3).

DISCUSSION

In our study, all of the cats showed gingivitis, especially in premolars and molars. Inflammatory gingivitis occurred with a high frequency in domestic cats. Radiographic evidence of alveolar bone loss was observed in ~77.3% of all premolars and molars in 15 cats averaging 6.8 years old.[12] Lommer et al[2] reported 72% of cats had some degree of periodontitis. Thus, gingivitis is common in cats. It is well-known that plaque forms on teeth and gingival sulcus when no oral hygiene is performed. Therefore, an effective method such as catechin diet can improve oral health in cats.

A diet with catechin was effective in the inhibition of gingivitis in cats. Catechin can control oral health as an anti-periodontitis agent via anti-bacterial effects in cats, as similar to dogs.[9] These trials are based on plaque control by specific diet. In the first step of the dental treatment, growth inhibition of periodontopathic bacteria is very important.

Gingivitis in animals appears to have a rather high prevalence; the etiology and pathogenesis are not understood; and no successful form of therapy exists.[13] A catechin diet is highly effective for not only prevention but also treatment of gingivitis. The endpoints were 1) decrease of periodontopathic bacteria, genus *Porphyromonas*, 2) decrease of gingival inflammation, and 3) inhibition of oral malodor. The success of this treatment may

be owed to inhibition of inflammation in the gingiva. Yang et al[14] reported that green tea polyphenols regulate TNF-α gene expression by modulating nuclear factor-κB activation through their antioxidant properties. It is well known that TNF-α stimulates immune cells in the process of inflammation. Catechins can act to prevent the inflammation via an anti–TNF-α effect. Recent study suggests that major compound (-)-epigallocatechin gallate may prevent the alveolar bone resorption that occurs in gingivitis by inhibiting the expression of matrix metalloproteinase-9 in osteoblasts and the formation of osteoclasts.[15]

Genus *Porphyromonas* has been shown to have pathogenic potential and produce virulence factors.[16] Cystein proteases are regarded as important virulence determinants, as demonstrated in vitro and in vivo. It has been reported that there is an inhibitory effect of green tea catechin on cystein proteases in *P gingivalis*.[17] Sakanaka S et al[18] reported the inhibitory effects of green tea catechin on the production of a virulence factor of the gingivitis-causing anaerobic bacterium *P gingivalis*.[18] We showed that viable *Porphyromonas* species are sensitive to green tea catechin and the growth can be inhibited.[8] We suggest that the catechin in diet has the potential to reduce gingivitis resulting from the bacterial growth and virulence factors in cats.

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