Suppressive effect of black tea polyphenol theaflavins in a mouse model of ovalbumin-induced food allergy

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Abstract (204 words)

Food allergy is recognized as a global medical problem with increasing prevalence in recent years. Currently, the treatment of food allergy mainly involves avoidance of allergens and allergen-specific immunotherapy. Barring the spontaneous resolution of food allergy during the growth process, this disease is difficult to treat fundamentally. In recent years, the use of functional food ingredients derived from natural products has been attracting attention for their prophylactic use in food allergy. Theaflavins, i.e. black tea polyphenols, are potent antioxidants that have inhibitory effects on a variety of diseases. However, little is known about the preventive effect of theaflavins on food allergy. In this study, we designed a mouse model of food allergy and examined the effect of theaflavins using the severity of diarrhea, a symptom of food allergy, as an indicator. The administration of a black tea extract rich in theaflavins or theaflavin 1 (subgroup of theaflavins) to mice reduced the severity of diarrhea when compared with a normal diet. A reduction in malondialdehyde levels, a key marker of lipid peroxidation, was also observed. Overall, these data suggest that theaflavins may potentially inhibit food allergy by alleviating oxidative stress in the colon and can be a potential food material for prevention of food allergy.

Keywords:

Black tea extract, diarrhea, food allergy, functional food ingredients, oxidative stress, theaflavins.

Introduction

Food allergy (FA) is globally recognized as an important medical problem. Although there are no clear epidemiological data, FA affects up to 10% of the overall population and has increased in prevalence over the past 20–30 years [1]; the prevalence of FA is about 6% in children and 3–4% in adults [2]. The symptoms of FA include itching, abdominal pain, vomiting, and diarrhea [3]. Allergen avoidance and allergen-specific immunotherapy are common in patients diagnosed with FA [4, 5]. However, avoidance of allergens may result in nutritional deficiencies and impaired growth in children on strictly restricted diets, whereas allergen-specific immunotherapy has been noted for its limited effectiveness, safety, and sustainability. Hence, new and safe functional food ingredients are expected to be utilized to prevent FA or to support the ongoing treatment plan [6].

Black tea, one of the world's most popular beverages, has been shown in epidemiological and laboratory studies to have beneficial properties, including antioxidant [7], and anti-inflammatory activities [8], and reduction of cardiovascular disease risk [9]. Theaflavins (TFs), which are polyphenols abundant in black tea are key to their biological activity [10]. During the fermentation process of black tea, catechins are enzymatically oxidized into two linked forms [11]. TFs have excellent antioxidant activity, and their hydroxyl radical scavenging ability is more effective than that of the leading catechin, epigallocatechin gallate. [12]. TFs have also been reported to exhibit anti-inflammatory [13] and inhibitory effects on digestive enzymes leading to anti-diabetic and anti-obesity properties [14, 15]. However, little is known about the anti-allergic effects of TFs. The subgroups of TFs known include theaflavin 1 (TF1), theaflavin 3-gallate (TF2A), theaflavin 3'-gallate (TF2B), and theaflavin 3, 3'-digallate (TF3). Among these, TF2A and TF3 are effective in preventing oxazolone-induced contact hypersensitivity in male ICR mice by dermal and oral administration [16]. If the suppressive effect of TFs on FA can be demonstrated, it may aid in decreasing the prevalence of FA. In this study, we investigated the preventive effect of black tea extract rich in TFs in a mouse model of FA.

Materials and Methods

Materials

Theaflavin mixture (TFM), TFM-low catechins (TFM-lc) with most of the catechins removed from TFM, and TF1 were provided by Mitsui Norin Co., Ltd. (Tokyo, Japan). Ovalbumin (OVA, grade V) was purchased from Sigma-Aldrich Corp. (St. Louis, MO, USA). Aluminum hydroxide gel (Alum) was purchased from Fujifilm Wako Pure Chemical Corp. (Osaka, Japan).

Animals

The study protocol was approved by the Animal Care and Use Committee of the Graduate School of Pharmaceutical Sciences, Osaka University, Osaka, Japan (protocol number: Douyaku 29-3). All experimental procedures were conducted in accordance with the Guide for the Care and Use of Laboratory Animals [17]. Extra care was taken to minimize animal suffering and the number of animals used. Female BALB/c mice were obtained from Japan SLC Inc. (Shizuoka, Japan) and acclimatized under controlled environmental conditions (22 ± 1°C; 50% ± 10% relative humidity; 12-h light-dark cycle; lights on at 08:00 AM) for some days before the start of the experiment. All animals were fed with normal powdered food (MF, Oriental Yeast Co. Ltd., Tokyo, Japan) and had *ad libitum* access to water [18]. TFM, TFM-lc, and TF1 were each prepared by mixing with powdered food at a concentration of 0.02–0.20% and administration was started 2 weeks prior to OVA sensitization.

OVA-induced FA mouse model

The experimental protocol for the OVA-induced FA mouse model was slightly modified from that of Kunisawa *et al.* [19]. Briefly, eight-week-old mice were sensitized by intraperitoneal injection of 100 μL solution containing 1 mg alum and 1 mg OVA on week 0. Then, from week 1–5, 200 μL of 250 mg/mL OVA was orally administered three times per week. FA symptoms were evaluated using allergic diarrhea as a parameter. Allergic diarrhea was determined 30–60 min after OVA administration by a severity score of 0–3 (0, solid state; 1, semi-solid form; 2, slurry; 3, watery state) for fecal conditions.

Malondialdehyde (MDA) assay

Three days after the last OVA challenge, colon tissue was collected from mice 40 min after oral administration of OVA. Colon samples (approximately 100 mg) were homogenized with phosphate-buffered saline (1:9 w/v). After centrifugation at 10,000 × g for 5 min, the collected supernatant was used for the MDA assay. The MDA levels were assessed using the thiobarbituric acid (TBA) reaction described by Ohkawa et al. [20]. Briefly, 100 μL of colon tissue sample was mixed with 100 μl of SDS lysis solution (50 mM Tris-HCl, 1% SDS, 10 mM EDTA, 1 mM PMSF, and protease inhibitor cocktail) and incubated at room temperature for 5 min. To this mix, 250 μL of 5.2 mg/mL TBA solution was added and incubated at 95°C for 60 min. The samples were cooled to room temperature, and centrifuged at 900 × g for 5 min. Absorbance of the supernatant was measured at 532 nm.

Statistical analysis

Statistical analysis was performed with Dunnett's multiple comparisons test or Dunn's multiple comparisons test using Prism 9 (GraphPad Software, Inc., La Jolla, CA). Data are presented as mean ± standard error.

Results

The maximum (or total) amount of TFs in Assam black tea has been assessed by HPLC and reported to be 2.12% [21]. The extraction of TFs from black tea leaves is difficult owing to the small amount of TFs in black tea [22]. Synthesis of TFs by enzymatic and other methods has been reported previously, but with low yield [22]. As the quantity of TFs available for long-term *in vivo* studies is limited, extracts of black tea are generally used in experiments. In this study, we examined the effect of TFs on FA using TFM, which contains ~42% TFs in the dry matter (Supplemental Table 1). Experimental protocols for induction of FA by OVA and administration of TFs are illustrated in Fig. 1. The oral challenge with OVA was performed 12 times, and the severity of diarrhea was assessed 30–60 min after each challenge. After the 4^{th} challenge, diarrhea scores were lower in the group fed with TFM diet than the group fed with normal diet (ND) (Fig. 2A). Statistical analysis of diarrhea scores at the 12^{th} challenge revealed a significant decrease in diarrhea scores in the 0.2% TFM group compared with the normal diet (Fig. 2B). Overall, these findings indicated that TFM may suppress FA.

Multiple studies have demonstrated that catechins have an inhibitory effect on FA [23, 24]. As TFM contains ~16% catechins in the dry matter, we predicted that the FA suppression effect exhibited by TFM is potentially due to catechins and not TFs. Hence, the effect on FA was further examined using TFM-lc with catechins reduced to ~3% in the dry matter (TFs are ~49%

in the dry matter). To more directly test the suppressive effect of TFs on FA, theaflavin 1 (TF1), a subgroup of TFs, was also tested simultaneously. In this study, the dose of TF1 was set at 0.02% because ~10% in TFM and TFM-lc corresponded to TF1 (Supplemental Table 1). As in Fig. 2, diarrhea scores were lower in the TFM diet group than in the ND group after the fourth challenge (Fig. 3A). The TFM-lc group with low levels of catechins was found to behave similarly to the TFM group. However, the TF1 group behaved the same as the ND group until the 10th challenge, but a decline in diarrhea scores were observed after the 11th challenge. Statistical analysis of diarrhea scores at the 12th challenge showed that a significant decrease in diarrhea scores was observed in all TFM, TFM-lc, and TF1 groups relative to the ND group (Fig. 3B).

Finally, to elucidate the mechanism of the diarrheal symptom suppression effect of TFs, oxidative stress levels in colon tissue were examined. Analysis of the amount of MDA, a marker of lipid peroxidation, showed that it was reduced in all TFM, TFM-lc, and TF1 groups than the ND group (Fig. 4). Taken together, these findings suggested that TFs may suppress FA by alleviating oxidative stress in the colon.

Discussion

Allergic diseases are increasing in prevalence globally, causing significant health and socioeconomic losses in various countries [25]. Lifestyle and dietary changes are known to contribute to the increase and exacerbation of these diseases. For example, dietary changes due to decreased intake of antioxidants such as vitamin E can lead to the development of allergic diseases such as FA, asthma, and rhinitis. Therefore, black tea, which contains a variety of antioxidants such as TFs and catechins and is routinely available, can be a beneficial ingredient as a preventive measure against allergic diseases.

In this study, we found that TFM and TFM-lc had inhibitory effects on FA (Figs. 2, 3). Since administration of TF1 also led to FA inhibition, it was concluded that TF1 is one of the active ingredients responsible for the FA suppression mediated by TFM and TFM-lc (Fig. 3B). On the other hand, no suppressive effect on FA was observed for TF1 prior to the 10^{th} OVA challenge compared to TFM or TFM-lc (Fig. 3A). This may be due to the involvement of TFs other than TF1, ECg, caffeine, and other polyphenols. Oral administration of TF2A and TF3 in mice has been shown to suppress type I allergic symptoms in a dose-dependent manner [16]. ECg also has an inhibitory activity against histamine release from rat basophilic leukemia cell lines [26]. These findings suggest that various components in black tea, including TF1, exert inhibitory effects on FA. OVA-specific antibody production was not altered by administration of TFs during the OVA sensitization or challenge phase (data not shown). Further studies are required to elucidate the details of the suppressive effect of TFs on OVA-induced FA and should focus on mechanisms of action other than antibody production.

Conclusion

In this study, we demonstrated that TFM, TFM-lc, and TF1 suppressed OVA-induced FA. The antioxidant properties of these ingredients were found to contribute to the alleviation of FA. Hence, given the increasing global significance of FA, daily consumption of readily available black tea may aid in the prevention or treatment of FA.

Author Contributions

KI designed the study as the first author, and drafted the manuscript. YK, SO, and SM collected the test data. YA, NH, and MS helped in the interpretation of results. SN directed the research and reviewed the manuscript.

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Declarations

Funding

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Competing interests

Authors Shuichi Otani, Soya Maeda, and Masayuki Suzuki are employed by Mitsui Norin Co. Ltd. The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Availability of data and material

The datasets during and/or analyzed during the current study available from the corresponding author on reasonable request.

Author Contributions

KI designed the study as the first author, and drafted the manuscript. YK, SO, and SM collected the test data. YA, NH, and MS helped in the interpretation of results. SN directed the research and reviewed the manuscript.

Figure Captions

Fig. 1 Schematic diagram representing a mouse model of OVA-induced FA

OVA sensitization, challenge, and TFs administration protocols in this study are shown.

FA, food allergy; i.p., intraperitoneal; OVA, ovalbumin; p.o., peroral. TFs, theaflavins.

Fig. 2 Effect of TFM on diarrhea symptoms in OVA-induced FA mice

Fecal condition was scored by severity when mice were fed normal diet (ND), 0.1% TFM, and 0.2% TFM. (A) Diarrhea symptoms during the study (up to the 12^{th} OVA challenge). (B) Diarrhea symptoms of the 12^{th} OVA challenge. Data are expressed as mean ± standard error (n = 10). $*P < 0.05$ (Dunn's multiple comparisons test).

FA, food allergy; ND, normal diet; OVA, ovalbumin; TFM, theaflavin mixture.

Fig. 3 Effects of TFM, TFM-lc, and TF1 on diarrhea symptoms in OVA-induced FA mice

Fecal condition was scored by severity when mice were fed normal diet (ND), 0.20% TFM, 0.20% TFM-lc, and 0.02% TF1. (A) Diarrhea symptoms up to the 12^{th} OVA challenge. (B) Diarrhea symptoms of the 12^{th} OVA challenge. Data are expressed as mean ± standard error (n = 9 or 10). $*P < 0.05$, $**P < 0.01$ (Dunn's multiple comparisons test).

FA, food allergy; ND, normal diet; OVA, ovalbumin; TFM, theaflavin mixture; TFM-lc, TFM-low catechins; theaflavin 1, TF1.

Fig. 4 MDA levels in colon tissue of OVA-induced FA mice

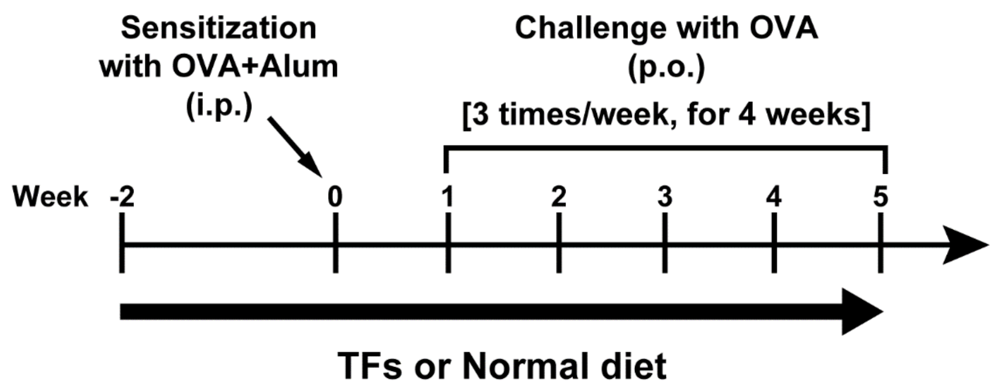
MDA levels in colon tissue were measured when mice were fed normal diet (ND), 0.2% TFM, 0.2% TFM-lc, and 0.02% TF1. Colon tissue was obtained 3 days after the 12th OVA challenge, again from mice that had been orally administered OVA. Data are expressed as mean ± standard error (n = 9 or 10). *$P < 0.05$ (Dunnett's multiple comparisons test).

FA, food allergy; MDA, malondialdehyde; ND, normal diet; OVA, ovalbumin; TFM, theaflavin mixture; TFM-lc, TFM-low catechins; theaflavin 1, TF1.

Supplemental Table 1. The content of TFs and catechins in TFM and TFM-lc as a percentage of dry matter

C, catechin; Cg, catechin gallate; EC, epicatechin; ECg, epicatechin gallate; EGC, epigallocatechin; EGCg, epigallocatechin gallate; GA, gallic acid; GC, gallocatechin; GCg, gallocatechin gallate; TFs, theaflavins; TFM, theaflavin mixture; TFM-lc, TFM-low catechins; TF1, theaflavin 1; TF2A, theaflavin 3-gallate; TF2B, theaflavin 3'-gallate; TF3, theaflavin 3, 3'-digallate.

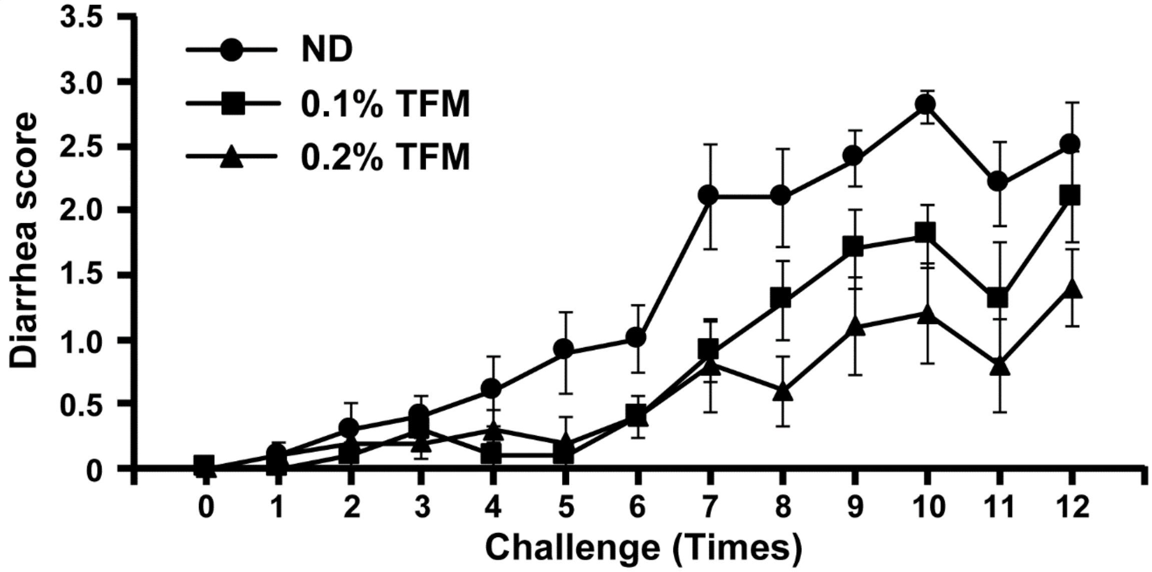
Fig. 1



Sensitization
with OVA+Alum
(i.p.)
Challenge with OVA
(p.o.)
[3 times/week, for 4 weeks]
Week
-2
0
1
2
3
4
5
TFs or Normal diet

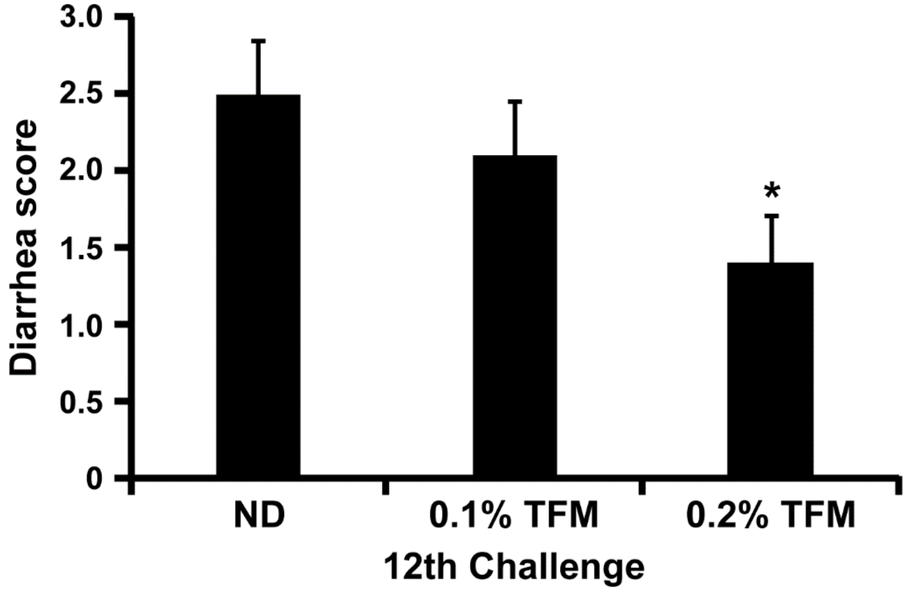
Fig. 2

A



Diarrhea score
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0
ND
0.1% TFM
0.2% TFM
0
1
2
3
4
5
6
7
8
9
10
11
12
Challenge (Times)

B



Diarrhea score
3.0
2.5
2.0
1.5
1.0
0.5
0
*
ND
0.1% TFM
0.2% TFM
12th Challenge

Fig. 3

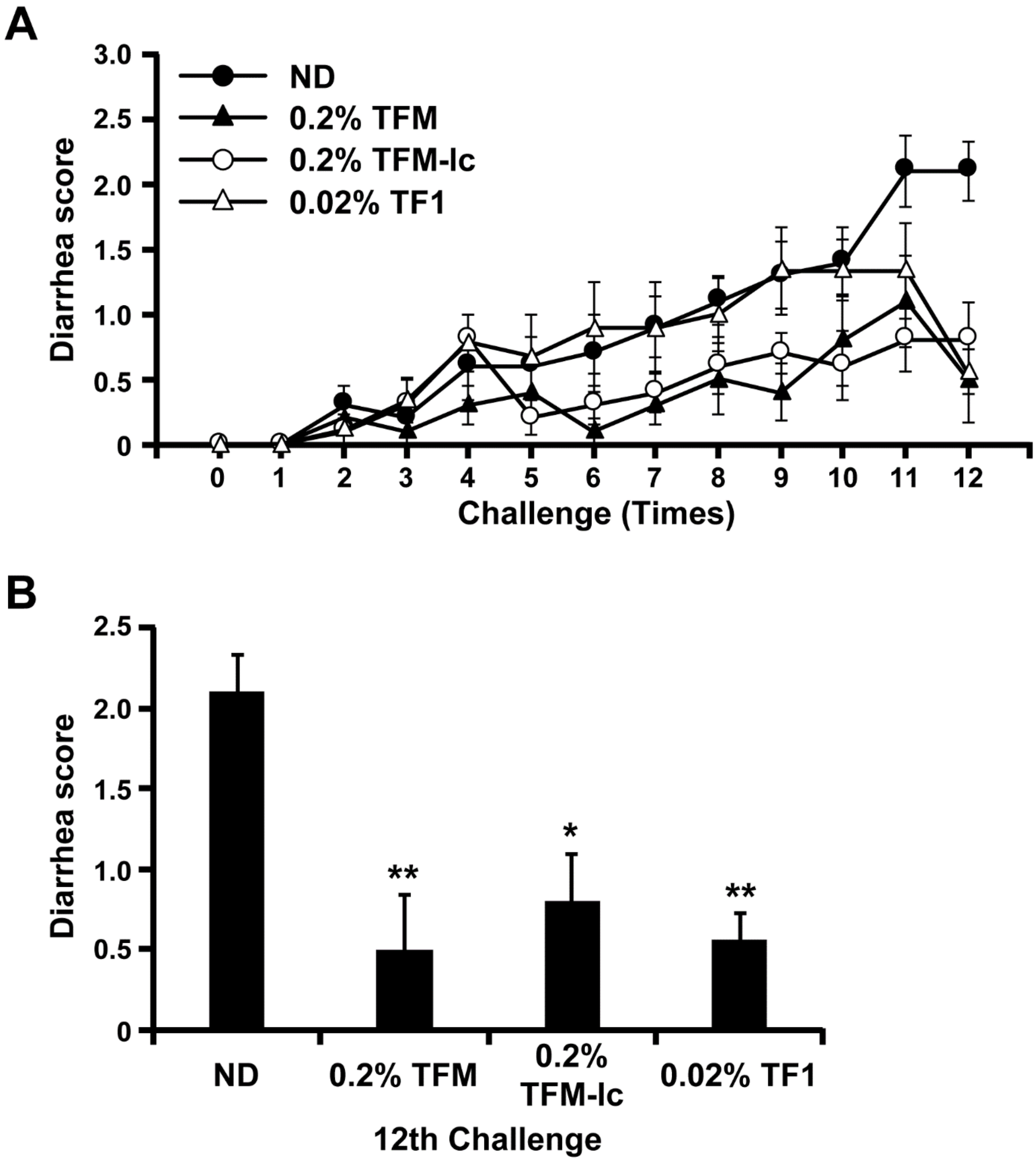
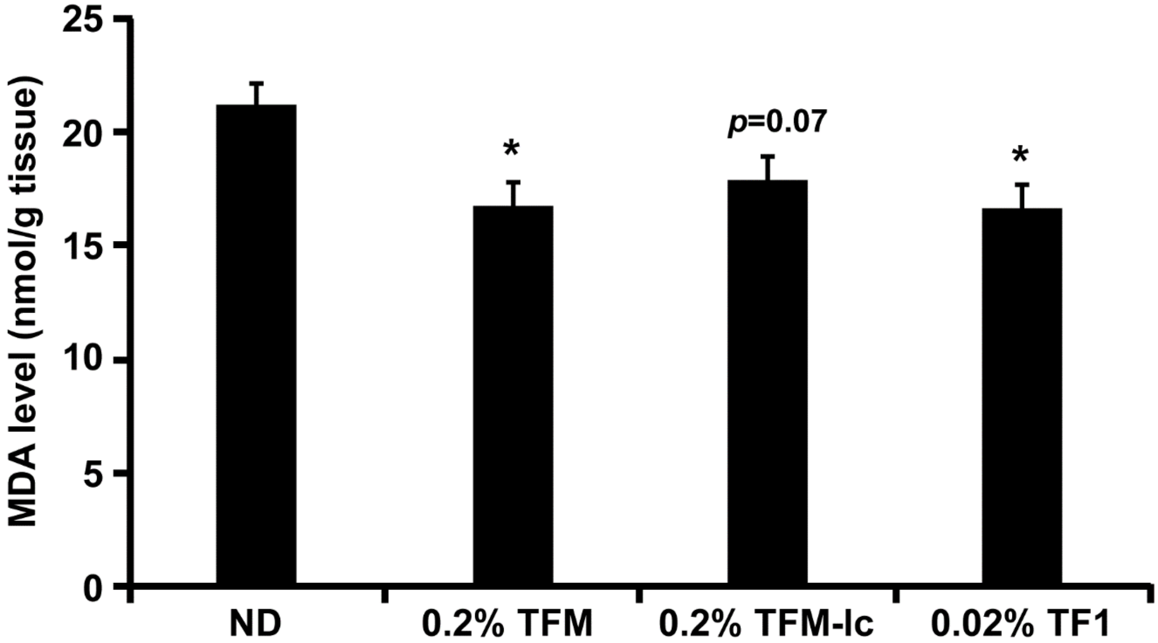

A
ND
0.2% TFM
0.2% TFM-lc
0.02% TF1
Diarrhea score
3.0
2.5
2.0
1.5
1.0
0.5
0
0
1
2
3
4
5
6
7
8
9
10
11
12
Challenge (Times)
B
2.5
2.0
1.5
1.0
0.5
0
Diarrhea score
**
*
**
ND
0.2% TFM
0.2% TFM-lc
0.02% TF1
12th Challenge

Fig. 4



25
20
15
10
5
0
MDA level (nmol/g tissue)
*
p=0.07
*
ND
0.2% TFM
0.2% TFM-lc
0.02% TF1

Supplemental Table 1

Substance	Content (% of dry matter)	
	TFM	TFM-lc
TF1	7.03	8.62
TF2A	12.80	14.30
TF2B	5.80	6.58
TF3	16.39	19.17
GC	0.08	0.00
EGC	0.53	0.00
C	0.25	0.00
EGCg	4.24	0.00
EC	0.74	0.00
GCg	0.46	0.00
ECg	8.94	2.63
Cg	0.55	0.00
Caffeine	3.18	4.30
GA	0.04	0.00
Total theaflavins	42.02	48.67
Total catechins	15.79	2.63