Effect of Pakistani medicinal plants on IgE/antigen- and ionophore-induced mucosal mast cells degranulation

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Abstract: Cumulative evidence has now demonstrated the stimulation of mucosal mast cells by both allergic and non-allergic triggers and their inhibition as a potential therapeutic target in many diseases like food allergy and ulcerative colitis. Hence, we screened medicinal plants from Pakistan against antigen- and ionophore-induced degranulation of mucosal mast cells. Aqueous ethanol extracts were screened. IgE/antigen- and A23187-induced degranulation of mucosal-type murine bone marrow derived mast cells (mBMMCs) were screening assays and β-hexosaminidase released from degranulated mBMMCs was measured. Real time-polymerase chain reaction was employed to examine the expression of TNF-α and IL-4 mRNA. Acetoxychavicol acetate, was examined by degranulation assays and real time-PCR. Among the ten plants screened against IgE/antigen stimulated degranulation, five plants; *Alpinia galangal*, *Mentha arvensis*, *Myrtus communis*, *Polygonum bistorta* and *Syzygium aromaticum* demonstrated significant ($p<0.01$) suppression of the degranulation at 100 μg/ml. Of them, *Alpinia galangal* showed significant ($p<0.01$) inhibition at 32 μg/ml. In A23187-induced degranulation, all plants showed significant ($p<0.01$) inhibition at 100 μg/ml except *Tamarix dioica*. Again *Alpinia galangal* exhibited significant ($p<0.01$) suppression at 32 μg/ml. In a concentration dependent assay, *Alpinia galangal* revealed significant suppression at 10 μg/ml against A23187-stimulated degranulation. Acetoxychavicol acetate demonstrated significant ($p<0.01$) inhibition at 3.2 μM in IgE/antigen-treated cells and at 10 μM in A23187-treated cells. Furthermore, both *Alpinia galangal* and acetoxychavicol acetate suppressed the IgE/antigen- and A23187-enhanced mRNA expression of inflammatory cytokines, TNF-α and IL-4, in mBMMCs. Our findings revealed the suppressive effect of *Alpinia galangal* and acetoxychavicol acetate on degranulation of mBMMCs by allergic and non-allergic stimuli, which can be utilized for future drug development against food allergy or ulcerative colitis.

Keywords: *Alpinia galangal*, acetoxychavicol acetate, Pakistani medicinal plants, mucosal mast cells.

INTRODUCTION

Mast cells are one of the foremost munitions of human innate immunity. They not only play a crucial role in allergic disorders like asthma, dermatitis and food allergy (FA) but also act as a vital component in other inflammatory/functional diseases like ulcerative colitis (UC) and irritable bowel syndrome (IBS) (Mekori and Metcalfe, 2000; Theoharides *et al.,* 2012). Upon stimulation, mast cells are degranulated resulting in a release of inflammatory mediators, such as proteases, inflammatory cytokines and lipid mediators, which are extensively involved in the pathogenesis of above mentioned diseases (Rivera and Gilfillan, 2006). Although specific medicines against mast cells such as mast cell stabilizers (tranilast, ketotifen and cromolyn) are developed for some allergic diseases, like asthma, but they are not as effective in FA (Kageyama-Yahara *et al.,* 2010). This might be due to the occurrence of two distinct populations of mast cells namely connective tissue mast cells (CTMCs) and mucosal mast cells (MMCs). CTMCs are located in connective tissues such as skin whereas MMCs are matured in mucosa of intestinal and bronchial tissues. Recent findings have vividly demonstrated that MMCs are morphologically, biochemically and functionally distinct from connective tissue mast cells (Bienenstock *et al.,* 1982). Furthermore, MMCs demonstrate a more contributing role in gastrointestinal (GI) anaphylaxis and protective responses against parasitic infections than CTMCs (Yu and Perdue, 2001). This ignites the search for novel agents to develop innovative therapies against MMCs.

Herbal medicines have always provided a lead to develop new therapeutic candidates. Varieties of herbal medicines are used as traditional remedies for treating numerous diseases, including allergic diseases particularly in Asian countries. Chinese investigators have reported herbal formula, food allergy herbal formula-1, ameliorates peanut-induced anaphylaxis in a murine FA model (Li *et al.,* 2001). A study from Korea demonstrated preventive effects of skullcap (*Scutellaria baicalensis*) extract in a

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mouse model of food allergy (Shin *et al.,* 2014). We have also documented that Kakkonto, a traditional medicine frequently used in Japan and China, suppressed mucosal-type murine bone marrow derived mast cells (mBMMCs) degranulation and alleviated allergic symptoms induced by food antigens (Kageyama-Yahara*et al.,* 2010; Yamamoto *et al.,* 2009). To further identify potential candidates, we screened 80 medicinal herbs frequently used in Japan using rat basophilic leukemia (RBL)-2H3 mast-like cells and found water extracts of *Arecae* Semen, *Cinnamoni* Cortex, *Curcumae* Rhizoma, *Rhei* Rhizoma and *Zanthoxyli* Fructus (ZF) significantly inhibited the antigen-induced degranulation. Among them, ZF suppressed mBMMCs activities by novel mechanism of sphingosine kinase 1-dependent mechanism (Wang *et al.,* 2012). As herbal medicines are extensively employed in other parts of the world, we thought to explore medicinal plants from different countries to identify potential candidates. In the present study, we explore the effect of selected medicinal plants from Pakistan which are used for the treatment of various GI disorders (Zaidi *et al.,* 2009a; Zaidi and Sugiyama, 2013). Among these plants, *Alpinia galangal* Willd. exhibited most potent inhibitory activity on the degranulation of mBMMCs.

Alpinia galangal (AG) is a member of ginger family and is widely cultivated in China, India, and Southeast Asian countries such as Thailand, Indonesia, and Philippines. AG rhizomes have been traditionally used to treat bronchial ailments, as carminative and for the treatment of stomachalgia, dyspepsia, obesity, rheumatoid arthritis, and diabetes (Warrier *et al.,* 1993-1995; Usmanghani *et al.,* 1997). Several pharmacological studies of AG and its constituents demonstrated various biological activities but none of the research so far examined the ability of AG to inhibit mBMMCs degranulation. Hence, we investigated the effect of AG and its major constituent, acetoxychavicol acetate (ACA), on both antigen- and calcium ionophore-induced degranulation of mBMMCs. Furthermore, inflammatory cytokines like tumor necrosis factor-alpha (TNF-α) and interleukin-4 (IL-4) were also investigated to examine the anti-inflammatory potential.

MATERIALS AND METHODS

Animals

Male BALB/c mice (5 week old) were purchased from Japan SLC Inc. (Shizuoka, Japan). All mice were housed with free access to food and water in the experimental animal facility at University of Toyama. All animal care and experiments were approved by the Animal Experiment Committee in University of Toyama (Authorization No. S-2009 INM-9).

Reagents

Recombinant murine SCF, recombinant murine IL-3, recombinant murine IL-9 and TGF-β1 were purchased from Peprotech (London, UK). RPMI-1640 medium was purchased fromWako (Osaka, Japan). A23187 was purchased from Sigma (St. Louis, MO, USA). Anti-dinitrophenyl (DNP: antigen) IgE was purchased from Yamasa (Tokyo, Japan). Acetoxychavicol acetate was purchased from LKT Laboratories, Inc. (St. Paul, MN, USA). DNP-bovine serum albumin (BSA) was purchased from EMD Millipore (Billerica, MA). RNeasy Plus Micro was purchased from Qiagen (Hilden, Germany).

Preparation of extracts

Total ten medicinal plants were employed in this study which is given in table 1 with families, abbreviations, and traditional uses. These medicinal plants were randomly selected on the basis of their use in GI disorders. All the plants were purchased from a local market of Karachi, Pakistan, authenticated by Dr Iqbal Ahzar, Department of Pharmacognosy, University of Karachi, Karachi, Pakistan and authentic voucher specimens have been deposited in the Museum of Materia Medica, Analytical Research Center for Ethnomedicines, Institute of Natural Medicine, University of Toyama, Toyama, Japan (Zaidi *et al.,* 2009a).

As described previously, the powdered plant material (5-50 g) was soaked twice with 50-100ml of aqueous ethanol (30:70) for 48 h at room temperature (Zaidi *et al.,* 2012). Solvent was evaporated under reduced pressure and each extract was dissolved at 100mg/ml with dimethyl sulfoxide (DMSO) and the final concentration of DMSO was <0.1% in the cell culture medium which has no effect on any of the experiment performed in this study.

Cell culture

Mucosal-type murine bone marrow-derived mast cells (mBMMCs) were prepared form the femurs of BALB/c mice as described previously (Kageyama-Yahara*et al.,* 2008). Briefly, bone marrow cells were cultured in RPMI-1640 medium (Sigma, St. Louis, MO) supplemented with 10% heat-inactivated fetal calf serum (FCS) (JRH Biosciences, Lenexa, KS), 10μM 2-mercaptoethanol (Wako, Osaka, Japan), 20 mM Hepes buffer (Sigma), 1 mM sodium pyruvate (Sigma), 100 μM MEM non-essential amino acids (Sigma), 2 μg/ml gentamicin solution (Sigma), 20μl/ml penicillin-streptomycin solution stabilized (Sigma), 20 ng/ml recombinant murine interleukin-3 (IL-3; Peprotech, London, UK), 40ng/ml recombinant murine SCF (Peprotech), 5ng/ml recombinant murine IL-9 (R&D, Minneapolis, MN) and 1 ng/ml TGF-β1(Sigma) at 37°C in a humidified 5% CO_2 atmosphere.

Degranulation assay

The degree of degranulation was assessed by measuringβ-hexosaminidase secretion as previously described (Kageyama-Yahara *et al.,* 2008). Briefly, cells were sensitized with anti-DNP IgE and incubated with test

drugs. Then, the cells were stimulated with DNP-BSA. Alternatively, cells were activated with calcium ionophore A23187.

Real-time PCR

Total RNA was extracted from mBMMCs using RNeasy Plus Micro kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. Quantitative real-time PCR was performed previously as described (Zaidi *et al.,* 2009b). The following primer pairs were used: IL-4, forward: 5'-GGTCTCAACCCCCAGCTAGT-3'; reverse: 5'-GCCGATGATCTCTCTCAAGTGAT-3'; TNF-α, forward: 5'-AAGCCTGTAGCCCACGTCGTA-3'; reverse: 5'-GGCACCACTAGTTGGTTGTCTTTG-3'; GAPDH, forward: 5'- TGACCACAGTCCAT-GCCATC -3'; reverse: 5'- GACGGACACATTGGG-GGTAG -3'. Target mRNA was normalized to GAPDH mRNA as an internal control in each sample.

STATISTICAL ANALYSIS

Data are presented as the mean ±SE. Statistical comparisons were performed using Student's t-test or repeated measures one-way ANOVA followed by post-hoc Dunnett's test. Values of $p<0.05$ or $p<0.01$ were considered significant.

RESULTS

Pre-treatment with plant extracts inhibited antigen-stimulated degranulation in mBMMCs

Ten medicinal herb extracts were screened for their inhibitory effect on IgE/DNP-stimulated degranulation in mBMMCs, and five plants namely AG, MA, MC, PB, and SA demonstrated significant ($p<0.01$) suppression of degranulation at 100μg/ml (fig.1). Of them, AG showed strong inhibition (p value <0.01) even at the concentration of 32μg/ml.

A23187-induced degranulation in mBMMCs was inhibited by plant extracts

Next we examined the effect of extracts on calcium ionophore-induced degranulation to identify the role of these extracts in non-allergic GI diseases. Of the ten plants, only one plant, TD, didn't show any inhibition at both the concentrations employed (fig 2). To our surprise, all of the other nine extracts demonstrated significant ($p<0.01$) suppression at 100μg/ml. Among them, eight extracts namely AG, CT, MA, MC, OR, PB, RD and SA further exhibited significant ($p<0.01$) inhibition at the concentration of 32 μg/ml. Only extract of TC showed less significant ($p<0.05$) suppression at 32 μg/ml. Again AG demonstrated the strongest inhibition at both concentrations among all extracts evaluated.

AG inhibited both antigen- and ionophore-stimulated degranulation in mBMMCs in a concentration dependent manner

As shown above, AG exhibited the strongest suppression of degranulation in both IgE/DNP- and A23187-induced degranulation. This prompted us to further evaluate AG against both allergic and non-allergic stimuli in a concentration dependent assay. As shown in fig. 3, AG suppressed degranulation by both stimuli in a concentration dependent manner. IgE/DNP-stimulated degranulation was significantly ($p<0.01$) inhibited at even 10 μg/ml while A23187-induced degranulation was significantly ($p<0.01$) inhibited by AG at 32 μg/ml (fig 3A and B respectively). This implies the therapeutic potential of AG in both allergic and non-allergic origin of GI diseases.

Pre-treatment with ACA suppressed antigen-and ionophore-induced degranulation in mBMMCs

After the revelation of strong inhibition by AG extract in IgE/DNP- and A23187-stimulated degranulation, we next aimed for the active principal in AG. Several constituents have been isolated from AG so far but ACA, pungent constituent of AG, has been extensively evaluated in several biological activities (Chudiwal *et al.,* 2010). Hence, we hypothesized that this inhibitory activity of AG might be due to the presence of ACA in it. The results revealed suppression of both IgE/DNP- and A23187-induced degranulation by ACA in a concentration dependent manner (fig 3C and D). In case of IgE/DNP stimulation, ACA significantly ($p<0.01$) inhibited degranulation at as low as 3.2 μM of concentration while in A23187-stimulated cells, ACA significantly ($p<0.01$) suppressed at 10μM. Interestingly, these results slightly contradict with the inhibitory spectrum of AG extract which showed stronger inhibition in A23187-treated cells compared to IgE/DNP-stimulated cells. This might be due to the presence of other active constituents in AG or synergistic activity of multiple chemical entities in AG against both stimuli. This creates a dire need to further investigate various principals found in AG against both allergic and non-allergic stimuli.

AG and ACA reduced mRNA expression of inflammatory cytokines in antigen-and ionophore-stimulated mBMMCs

Mast cells also generate and release pro-inflammatory and Th2-related cytokines including TNF-α, IL-4 and IL-13 by various stimuli, which further aggravate the pathogenesis of disease (Mekori and Metcalfe, 2000). Hence, we further evaluated the effect of AG and ACA against IgE/DNP and A23187-induced expression of TNF-α and IL-4. As shown in fig. 4A and B, AG significantly ($p<0.05$) suppressed IgE/DNP-induced expression of TNF-α and IL-4 at the concentration of 100 μg/ml. In case of A23187-stimulated cells, although no significant inhibition was found in case of TNF-α, the expression was markedly reduced compared to untreated cells (fig 4C). However, AG significantly ($p<0.01$)

reduced the expression of IL-4 at both 32 and 100 µg/ml (fig 4D). Similarly, ACA also significantly ($p<0.05$) inhibited the expression of IL-4 at the concentration of both 10 and 32 µM while there was suppressive effect of ACA on the expression of TNF-α but not significant in IgE/DNP-treated cells (figs. 5A and B). In A23187-stimulated cells, again IL-4 expression was significantly ($p<0.05$ and $p<0.01$) reduced at 10 and 32 µM respectively (fig. 5D). TNF-α suppression by ACA was again prominent but not significant in A23187-induced expression (fig 5C). This demonstrates the potential of AG and ACA in not only suppressing the degranulation but also expression of inflammatory cytokines at the site of MMCs activation.

DISCUSSION

Development of intestinal immune diseases such as colitis and food allergy is the consequence of severance in gut immune homeostasis. This physiological balance is achieved with the help of intimate coordination between innate and acquired immune systems. Several host immune cells enjoys a central role in maintaining normal environment and healthy state. Among these players of immune system, mast cells are indispensable especially when it comes to mucosal immunity of gut. However, aberrant MMCs stimulation leads to inflammation or food allergies (Kurashima and Kiyono, 2014). Abnormal activation of MMCs either by allergens or non-allergens leads to degranulation, which ultimately ends up in disruption of both intestinal immune and physical barriers (Rivera and Gilfillan, 2006). Therefore, suppression of MMCs activation provides a novel tool to prevent or treat related diseases.

Here in, we demonstrated inhibition of degranulation by selected Pakistani medicinal plants while employing mBMMCs as homologous to mouse MMCs. Although five plants suppressed either IgE/DNP- or A23187-induced degranulation but AG surpass all with significant inhibition at low concentration of 10 µg/ml. AG has been used for the treatment of various diseases in different systems of traditional medicine. In Unani and Ayurvedic systems, it is used to improve appetite, voice, sore throat, taste, rheumatic pains, and bronchitis. Interestingly in Thai system of medicine, along with other uses, AG is also used for itching (Kirtikar and Basu, 1996). As mast cells play a key role in itching and dermatitis, AG effectiveness in itching might be due to suppression of mast cell activation. Evidence based pharmacological evaluation of AG and its chemical constituents revealed several biological activities including anti-inflammatory, hypoglycaemic, anti-microbial, gastroprotective, anticancer, and anti-allergic (Min *et al.,* 2009; Akhtar *et al.,* 2002; Oonmetta-aree *et al.,* 2006; Al-Yahya *et al.,* 1990; Lee and Houghton, 2005; Matsuda *et al.,* 2003). Later study was the first to show antiallergic principles from AG by demonstrating the inhibition of *β*-hexosaminidase release from IgE-treated RBL-2H3 cells and the suppression of ear passive cutaneous anaphylaxis reactions in mice. Recently, a study from Korea revealed promising antiasthmatic potential of ACA in ovalbumin-induced asthma in mice and pose it as an antiasthmatic drug candidate (Seo*et al.,* 2013). The authors supported their conclusion by demonstrating the reduction in infiltration of eosinophils and IgE levels in lungs as well as suppression of Th2 (IL-4 and IL-13) and Th1 (IL-12 and interferon-γ) cytokines. The above mentioned studies justified the use of AG in asthmatic or anaphylactic conditions but none of the study so far demonstrated the potential use of AG in GI diseases like colitis or food allergy by evaluating its effect on MMCs degranulation. As discussed earlier, inflammatory cytokines also play an important role in the pathogenesis of diseases associated with MMCs degranulation. In this study, we not only demonstrated the suppressive effect of AG and ACA on degranulation of mBMMCs but also exhibited the inhibition of inflammatory cytokines expression in both IgE/DNP- and A23187-treated cells. This might be beneficial in justifying AG use in both allergic and non-allergic diseases like food allergy and colitis respectively.

Beside AG, four plants (MA, MC, PB, and SA) markedly reduced degranulation in both IgE/DNP- and A23187-stimulated cells. Among them, inhibition of degranulation by PB is of quite significance with respect to its frequent ethnomedicinal use in diarrhea. Several reports have shown anti-inflammatory activity of PB including one from our group in *H. pylori*-infected cells (Zaidi *et al.,* 2012). However, to the best of our knowledge, none of the study documented PB activity against degranulation of mast cells. As mast cell degranulation leads to diarrhea in food allergies and IBD/IBS, this might be helpful in justifying PB use in these diseases. Further extensive studies are required to gainfully utilize this plausible use of PB in such disorders. Similarly, MC is also frequently employed for the treatment of diarrhea in Unani system of traditional medicine (Zaidi *et al.,* 2009a). Clinical and experimental studies on MC suggest it possesses a broader spectrum of pharmacological and therapeutic effects such as anti-oxidative, anticancer, anti-diabetic, antiviral, antibacterial, anti-fungal, hepatoprotective and neuroprotective activity (Alipour *et al.,* 2014). Only one study so far postulated MC use in allergic diseases by demonstrating suppressive effect of myrtucommulone and semimyrtucommulone, chemical constituents of MC, on the biosynthesis of eicosanoids along with formation of reactive oxygen species and the release of elastase (Feisst *et al.,* 2005). However, no study so far scientifically justifies the use of MC in diarrhea. The results from our study might give a novel insight into the use of MC against diarrhea in disorders associated with increased MMCs degranulation like food allergy.

Table 1: Details of selected Pakistani medicinal plants employed against degranulation assay

Plant Name	Abbreviation	Family	Therapeutic applications*
Alpinia galangal Willd.	AG	Zingiberaceae	Carminative, expectorant, anti-asthmatic
Cinnamomumtamala(Ham.) Nees	CT	Lauraceae	Indigestion, stomach ache, carminative
Menthaarvensis L.	MA	Lamiaceae	Stomach ache, antispasmodic, topical antipruritic
*Myrtuscommunis*L.	MC	Myrtaceae	Diarrhea, indigestion, stomach tonic
Oligochaetaramosa (Roxb.) Wagenitz	OR	Asteraceae	Diarrhea, cough, fevers
Polygonumbistorta L.	PB	Polygonaceae	Dysentery, diarrhea, stomach and intestine tonic
Rosa damascene Miller	RD	Rosaceae	Stomach and intestine tonic, inflammation, utricaria
Syzygiumaromaticum L.	SA	Myrtaceae	Carminative, antiseptic, inflammation
*Tamarixdioica*Roxb.	TD	Tamaricaceae	Diarrhea, dysentery, carminative
Terminaliachebula Retz.	TC	Combretaceae	Stomach and brain tonic, diarrhea, carminative

*Therapeutic indications were taken from Usmanghani *et al.* (1997). Indusyunic Medicine, Research Institute of Indusyunic Medicine, Pakistan.

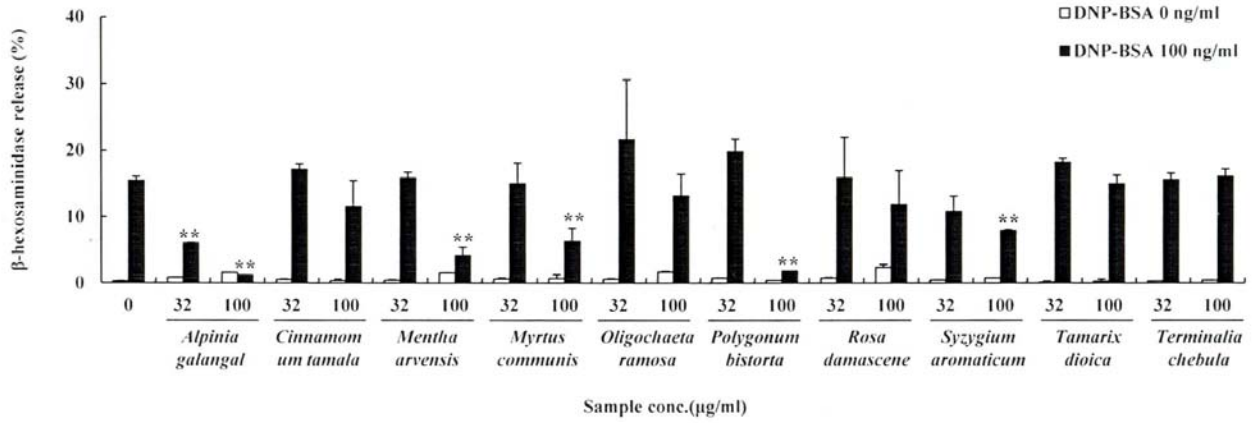

Fig. 1: Effect of medicinal plants (32 and 100 μg/ml) on the degranulation induced by IgE-DNP in mBMMCs. Anti-DNP/IgE sensitized (24 h) mBMMCs were incubated with plant extracts or vehicle for 30 min, then stimulated with 100 ng/ml DNP-BSA (filled) or without (open) for 1h and then β-hexosaminidase release was determined. Data are expressed as mean ± SD of duplicate of two independent experiments. **p<0.01 compared with vehicle.

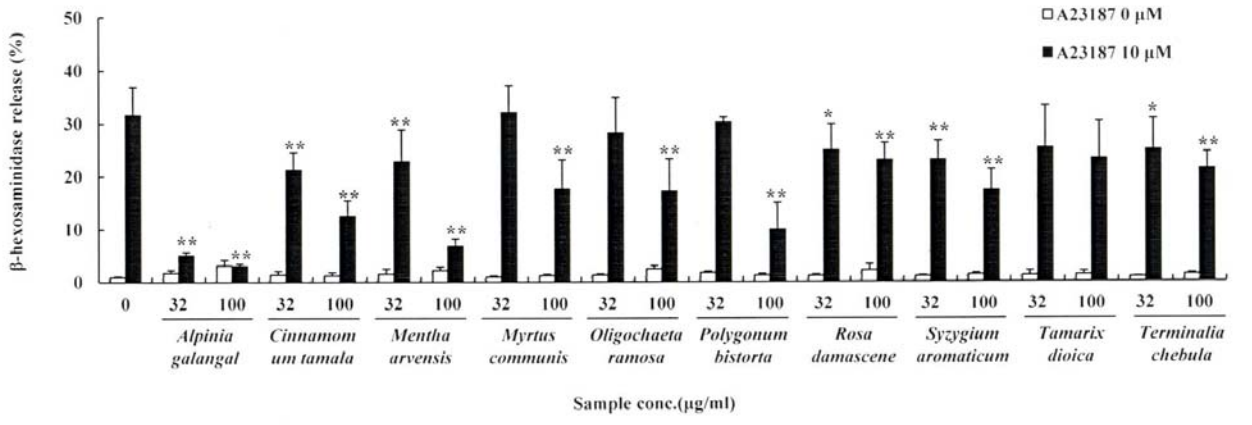

Fig. 2: Effect of medicinal plants (32 and 100 μg/ml) on the degranulation induced by A23187 in mBMMCs. mBMMCs were incubated with plant extracts or vehicle for 30 min, then stimulated with 10 μM calcium ionophore A23187(filled) or without (open) for 30 min. The release of β-hexosaminidase release was determined. Data are expressed as mean ± SD of duplicate of two independent experiments.*p<0.05 and **p<0.01 compared with vehicle.

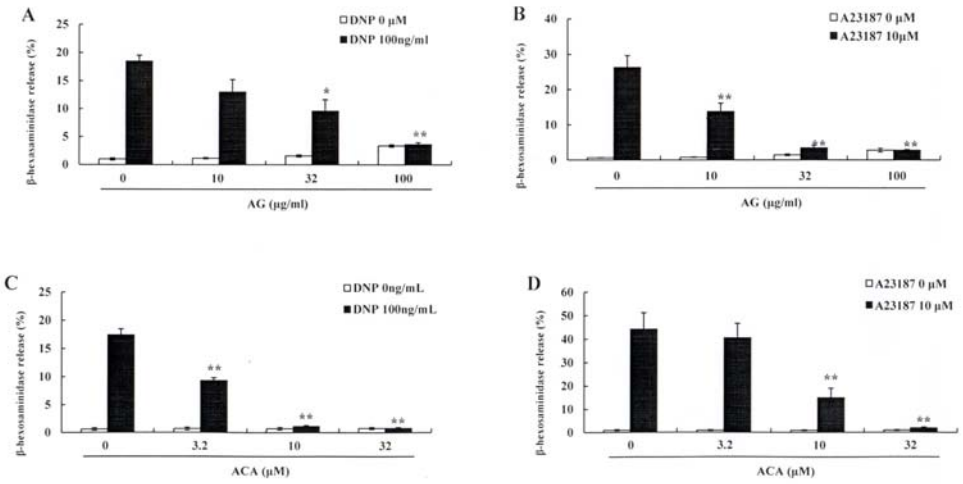

Fig. 3: Inhibitory activity of *Alpinia galangal* (AG) and acetoxychavicol acetate (ACA) on the degranulation induced by allergic and non-allergic stimulation. (A) Anti-DNP/IgE sensitized mBMMCs (24 h) were incubated with AG (10, 32, and 100 μg/ml) or vehicle for 30 min, then stimulated with 100 ng/ml DNP-BSA (filled) or without (open) for 1 h, and then β-hexosaminidase release was determined. Data are expressed as mean ± SD of triplicate of three independent experiments. *p<0.05 and **p<0.01 compared with vehicle. (B) mBMMCs were incubated with AG (10, 32, and 100 μg/ml) or vehicle for 30 min, then stimulated with 10 μM calcium ionophore A23187(filled) or without (open) for 30 min. The release of β-hexosaminidase release was determined. Data are expressed as mean ± SD of duplicate of two independent experiments. **p<0.01 compared with vehicle. (C) Anti-DNP IgE sensitized mBMMCs (24 h) were incubated with ACA (3.2, 10, and 32 μM) or vehicle for 30 min, then stimulated with 100 ng/ml DNP-BSA (filled) or without (open) for 1 h, and then β-hexosaminidase release was determined. Data are expressed as mean ± SD of triplicate of three independent experiments.**p<0.01 compared with vehicle.(D) mBMMCs were incubated with ACA (3.2, 10, and 32 μM) or vehicle for 30 min, then stimulated with 10 μM calcium ionophore A23187(filled) or without (open) for 30 min. The release of β-hexosaminidase release was determined. Data are expressed as mean ± SD of duplicate of two independent experiments.**p<0.01 compared with vehicle.

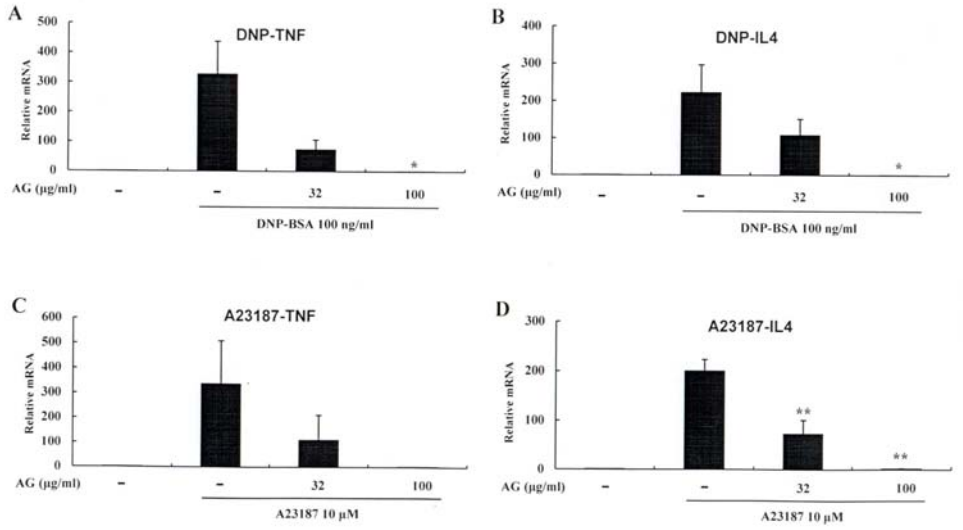

Fig. 4. Suppressive effect of AG (32 and 100 μg/ml) on the mRNA expression level of TNF-α and IL-4. (A, B) mBMMCs were incubated with anti-DNP IgE or vehicle for 30 min and stimulated with or without DNP-BSA and mRNA levels of TNF-α (A) and IL-4 (B) were analyzed by real-time PCR. Data are expressed as mean ± SD. #p<0.05 compared with normal, *p<0.05 compared with vehicle (n=3). (C, D) mBMMCs were incubated with AG (32 and 100 μg/ml) or vehicle for 30 min, then stimulated with 10 μM calcium ionophore A23187 (filled) or without (open) for 30 min. mRNA levels of TNF-α and IL-4 were analyzed by real-time PCR. Data are expressed as mean ± SD. #p<0.05 compared with normal, *p<0.05 compared with vehicle (n=3).

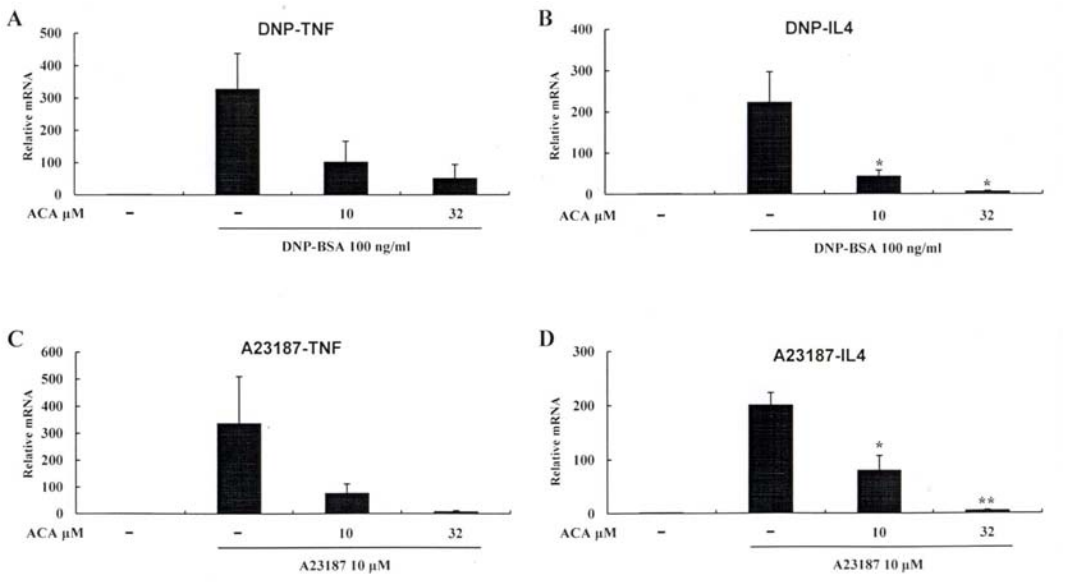

Fig. 5: Reduction of the mRNA expression level of TNF-α and IL-4 by ACA (10 and 32 μM) in stimulated cells. (A, B) mBMMCs were incubated with anti-DNP IgE or vehicle for 30 min and stimulated with or without DNP-BSA and mRNA levels of TNF-α and IL-4 were analyzed by real-time PCR. Data are expressed as mean ± SD.# $p<0.05$ compared with normal, *$p<0.05$ compared with vehicle (n=3). (C, D) mBMMCs were incubated with ACA (10 and 32 μM) or vehicle for 30 min, then stimulated with 10μM calcium ionophore A23187 (filled) or without (open) for 30 min. mRNA levels of TNF-α and IL-4 were analyzed by real-time PCR. Data are expressed as mean ± SD.# $p<0.05$ compared with normal, *$p<0.05$ compared with vehicle (n=3).

The remaining MA and SA are documented to possess anti-allergic properties. Shin TY reported the inhibition of immunologic and nonimmunologic stimulation-mediated anaphylactic reactions by the aqueous extract of *Mentha arvensis* (Shin, 2003). A study on SA showed suppression of immediate hypersensitivity by inhibition of histamine release from mast cells in vivo and in vitro (Kim *et al.,* 1998). However, the later study employed rat peritoneal mast cells not MMCs. Hence our study is the first report on the inhibitory effect of MA and SA on degranulation of mBMMCs, which might open new directions for their use in allergic or inflammatory diseases of gut.

CONCLUSION

Taken together, this study is the first screening report on medicinal plants against allergic and non-allergic-induced degranulation of mBMMCs. Furthermore, the present findings suggest that AG and ACA may provide a lead candidate in the treatment of diseases which have abnormal activation of MMCs. Additionally, our results might provide scientific justification for the use of these agents in GI complains like diarrhea associated with allergic or inflammatory/functional diseases.

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