



Short communication

Mangosteen xanthones, α - and γ -mangostins, inhibit allergic mediators in bone marrow-derived mast cellHee-Sung Chae^a, Sei-Ryang Oh^a, Hyeong-Kyu Lee^a, Sang Hoon Joo^b, Young-Won Chin^{c,*}^a Immune Modulator Research Center, Bio-Therapeutics Research Institute, Korea Research Institute of Bioscience & Biotechnology, 685-1 Yangcheon-ri, Ochang-eup, Cheongwon-gun, Chungbuk 363-883, Republic of Korea^b College of Pharmacy, Catholic University of Daegu, Gyeongbuk 712-702, Republic of Korea^c College of Pharmacy, Dongguk University-Seoul, 26, Pil-dong-3-ga, Jung-gu, Seoul 100-715, Republic of Korea

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ABSTRACT

Mangosteen (*Garcinia mangostana* L.), one of top-selling botanical dietary supplements in the USA, has been used as food and folk medicines in southeastern Asia. Herein, α - and γ -mangostins, major constituents of mangosteen, were investigated for their effect on the bone marrow-derived mast cell (BMMC) mediated allergy mechanism induced by phorbol 12-myristate 13-acetate (PMA) plus A23187. α - and γ -mangostins demonstrated to inhibit Interleukin (IL)-6, prostaglandin D₂ (PGD₂) and leukotriene C₄ (LTC₄) production as well as degranulation in PMA plus A23187 induced BMMC. Furthermore, α - and γ -mangostins were found to repress cyclooxygenase (COX)-2 expression in the present study. Thus, these results suggest that α - and γ -mangostins may be useful in alleviating mast cell-mediated allergic inflammatory responses.

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1. Introduction

Garcinia mangostana L. (Clusiaceae), called as mangosteen or “mangkut”, is referred to in Thailand as the “queen of fruits” (Farnsworth & Bunyapraphatsara, 1992; Moongkarndi et al., 2004). Mangosteen is being used widely as one of top-selling botanical dietary supplements in the United States, mainly in the form of a fruit juice. The perceived benefits of mangosteen have been well documented by several reviews (Chin & Kinghorn, 2008; Obolskiy, Pischel, Sirifatanametanon, & Heinrich, 2008; Pedraza-Chaverri, Cardenas-Rodriguez, Orozco-Ibarra, & Perez-Rojas, 2008) and thereafter publications, disclosing antioxidant (Valko et al., 2007), antibacterial activity (Sakagami, Iinuma, Piya-sena, & Dharmaratne, 2005), anticancer (Chao, Hsu, Liu, & Kuo, 2011; Han et al., 2009) and anti-inflammatory (Chen, Yang, & Wang, 2008; Yamakuni et al., 2006) activity of mangosteen extracts or individual ingredients. However, its anti-allergic effect on the bone marrow-derived mast cell (BMMC) remains unknown.

Mast cells are known to participate in allergic and anaphylactic reactions related to immune responses. Upon activation by various stimuli, mast cells release a number of biologically active molecules, prestored mediators including histamine and serotonin, and *de novo* synthesized mediators such as Interleukin-6 (IL-6), leukotriene C₄ (LTC₄), and prostaglandin D₂ (PGD₂), leading to allergic and inflammatory diseases, especially skin inflammation and allergic asthma (Metcalfe, Baram, & Mekori, 1997; Wasserman, 1989).

In the present study, α - and γ -mangostins (Fig. 1), main constituents of mangosteen, were evaluated for their inhibitory effect on the IL-6, PGD₂, and LTC₄ production, COX-2 expression, and degranulation in PMA plus A23187-induced BMMC.

2. Materials and methods

2.1. Animals

Female BALB/c mice, 6–8 weeks of age (Daehan Biolink Co. Ltd., Seoul, Korea), were used under protocols approved by the Institutional Animal Care and Use Committee of the Korea Research Institute of Bioscience and Biotechnology.

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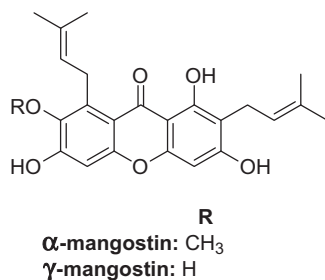


Fig. 1. Structures of α - and γ -mangostins.

2.2. Preparation and activation of bone marrow-derived mast cell (BMMC)

Bone marrow cells from male Balb/c mice were cultured for up to 10 weeks in 50% enriched medium (RPMI 1640 containing 2 mM L-glutamine, 0.1 mM nonessential amino acids, antibiotics, and 10% fetal calf serum) and 50% WEHI-3 cell conditioned medium as a source of IL-3. After 3 weeks >98% of the cells were bone marrow-derived mast cell (BMMC) when checked by the previously described procedure (Murakami, Austen, & Arm, 1995).

2.3. Drugs and chemicals

RPMI 1640, penicillin, and streptomycin were obtained from Hyclone (Logan, UT, USA). Bovine serum albumin, phorbol 12-myristate 13-acetate (PMA) and calcimycin A23187 hemicalcium (A23187) were purchased from Sigma (St. Louis, MO, USA). α - and γ -Mangostins were purified from the pericarp of *G. mangostana* (700 g) according to the literature (Jung, Su, Keller, Mehta, & Kinghorn, 2006) and identified by comparing ^1H -NMR and ^{13}C -NMR with the published values (Chen et al., 2008). Anti-mouse IL-6 antibody and biotinylated anti-mouse IL-6 antibody were purchased from BD Biosciences (BD Pharmingen, San Diego, CA). An RNA extraction kit was purchased from iNtRON Biotech (Daejeon, Republic of Korea). COX-2 and IL-6 oligonucleotide primers were purchased from Bioneer Corp. (Daejeon, Republic of Korea).

2.4. Cell viability

The cell viability was examined by 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Briefly, BMMCs were seeded at a density of $5 \times 10^4/\text{ml}$ in 96 well plates (Nunc, Denmark). Each batch of cells included a non-treated group as control. Sample was then added to each well, after which the plates were incubated for 24 h at 37°C under 5% CO_2 . Next, MTT solutions (5 mg/ml) were added to each well, and the samples were then cultured for an additional 4 h. The supernatant was then discarded and 100 μl of dimethyl sulfoxide (DMSO) was added to each well. Next, the optical density was read at 590 nm. The cytotoxicity was then calculated by subtracting the ratio of the mean absorbance value for treated cells over the mean absorbance value for untreated cells from one.

2.5. Enzyme-linked immunosorbent assay (ELISA)

Cells were seeded at $1 \times 10^6/\text{ml}$ per well in 24 well tissue culture plates and pretreated with various concentration of α - and γ -mangostins (3, 6 and 12 μM) for 30 min before PMA (50 nM) plus A23187 (1 μM) stimulation. The supernatant was decanted into a new microcentrifuge tube, and the amount of IL-6 determined using ELISA kit according to the procedure described by the

Manufacturer (BD Bioscience, USA). All subsequent steps took place at room temperature, and all standards and samples were assayed in duplicates.

2.6. Determination of prostaglandin D_2 (PGD_2) and leukotriene C_4 (LTC_4) levels

The BMMCs were activated with PMA (50 nM) plus A23187 (1 μM) at 37°C for 1 h in the presence or absence of mangostins dissolved in PBS. The supernatants were isolated for further analysis by enzyme immunoassay. PGD_2 and LTC_4 were determined using an EIA kit. (Cayman Chemical, Ann Arbor, MI, USA) Data are reported as the arithmetic mean of triplicate determinations.

2.7. Assay of β -hexosaminidase release

β -Hexosaminidase (β -HEX), a marker of mast cell degranulation was quantified by spectrophotometric analysis of the hydrolysis of p-nitrophenyl-2-acetamido-2-deoxy- β -D-glucopyranoside hydrolysis (Sigma Chemical Co.). Briefly, after harvesting supernatant, cells were lysed in the same volume of medium by freeze and thaw three times. Ten milliliters of the BMMCs lysate or supernatant samples were mixed with 50 ml of β -HEX substrate solution (1.3 mg/ml p-nitrophenyl-2-acetamido-2-deoxy- β -D-glucopyranoside in 100 mM sodium citrate, pH 4.5) in each well of 96-well plates and then incubated at 37°C for 60 min. The reaction was stopped by adding 150 μl of 0.2 M Glycine-NaOH (pH 10.7). The absorbance at 410 nm was measured in a micro plate reader. The percentage of β -HEX released into the supernatant was calculated by the following formula: $[S/(S-P)] \times 100$, where S and P are the β -HEX contents of supernatant and cell pellet.

2.8. RNA extraction and reverse transcription-polymerase chain reaction (RT-PCR)

Total cellular RNA was isolated using an easy-BLUETM RNA extraction kit according to the manufacturer's instructions. Briefly, total RNA (2 μg) was converted to cDNA by treatment with 200 units of reverse transcriptase and 500 ng of oligo-dT primer in 50 mM Tris-HCl (pH 8.3), 75 mM KCl, 3 mM MgCl_2 , 10 mM DTT, and 1 mM dNTPs at 42°C for 1 h. The reaction was then stopped by incubating the solution at 70°C for 15 min, after which 3 μl of the cDNA mixture was used for enzymatic amplification. PCR was then performed using a reaction mixture comprised of 50 mM KCl, 10 mM Tris-HCl (pH 8.3), 1.5 mM MgCl_2 , 0.2 mM dNTPs, 2.5 units of Taq DNA polymerase, and 0.1 μM each of primers specific for COX-2, and β -actin. The amplification conditions were as follows: denaturation at 94°C for 3 min for the first cycle and then 35 cycles of 94°C for 45 s, annealing of COX-2 at 53°C for 45 s annealing of IL-6 at 57°C for 45 s with a final extension at 72°C for 7 min. The PCR products were then electrophoresed on a 1.5% agarose gel and stained with ethidium bromide. The primers used were 5'-GCCCAACAATACAAATGACCCTA-3' (sense) and 5'-TCCTGTTGTTTCTATTTCTTTGT-3' (antisense) for IL-6, 5'-ACTCAGTTTGTTGATCATTC-3' (sense) and 5'-ATTAGTACTGTAGGGTTAATG-3' (antisense) for COX-2, 5'-TGAAGATCCTGACCGAGCGT-3' (sense) and 5'-ACGCAGCTCAGTAACAGTCCG-3' (antisense) for β -actin.

2.9. Statistical analysis

The data from the experiments are presented as the mean $\pm$ SEM. The level of statistical significance was determined by analysis of variance (ANOVA) followed by Dunnett's t-test for multiple comparisons. P values less than 0.05 were considered to be significant.

3. Results and discussion

The cell viability test with α - and γ -mangostins in bone marrow-derived mast cell (BMMC) showed that no toxicity was observed at up to 12 μ M (Data not shown). Interleukin-6 (IL-6) acts as a pro-inflammatory cytokine (Muraguchi et al., 1981), and is secreted by T cells and mast cells to evoke immune response to trauma, burns or other tissue damage leading to allergic reaction and inflammation (Naka, Nishimoto, & Kishimoto, 2002). α - and γ -Mangostins suppressed IL-6 level in BMMC induced by PMA plus A23187 in a dose-dependent manner with IC₅₀ values 5.11 and 7.06 μ M, respectively (Fig. 2). In addition, it was observed that IL-6 mRNA expression was diminished by the treatment of these compounds (Fig. 4). As lipid mediators, PGD₂ and LTC₄ released from mast cells have been reported to be implicated in the pathogenesis of allergic inflammation (Bochenek, Nizankowska, Gielicz, Swierczyńska, & Szczeklik, 2004; Dahlén et al., 1981). Thus, the effect of α - and γ -mangostins on PGD₂ and LTC₄ production in this BMMC model was evaluated and found to lower PGD₂ and LTC₄ level compared to the group induced by PMA plus A23187 (Fig. 3). COX-2, responsible for diverse allergy and inflammatory process, is known to participate in synthesis of prostaglandins including PGD₂. Pretreatment of α - and γ -mangostins onto BMMC, prior to adding PMA plus A23187, attenuated COX-2 mRNA expression in RT-PCR analysis (Fig. 4) when compared with the group without pretreatment of α - and γ -mangostins. Thus, it seems that COX-2 expression is closely associated with PGD₂ production in PMA plus A23187-induced BMMC. Based on the above results, α - and γ -mangostins may exert the inhibitory activity against PGD₂ production via COX-2 suppression. Prestored inflammatory or allergic mediators including biogenic amines (histamine) are released in acute allergic reactions when an allergen interacts with mast cells (Dannhardt & Kiefer, 2001). Similarly, calcium ionophore (A23187) is able to induce degranulation as well (Kitamura, 2005). The extent of histamine release is indirectly quantified by measuring β -hexosaminidase (β -HEX), a marker of mast cell degranulation (Han et al., 2010). In the present study, α - and γ -mangostins were found to inhibit the degranulation of β -hexosaminidase in PMA plus A23187-induced BMMC, indicating block of histamine release and thereby subsequent inhibition of acute allergic reaction (Fig. 5).

In conclusion, from PMA plus A23187-stimulated BMMC assay system, it was found that α - and γ -mangostins present in mango-steen dietary supplements may modulate the production of IL-6, PGD₂ and LTC₄ as well as degranulation of histamine pertinent to

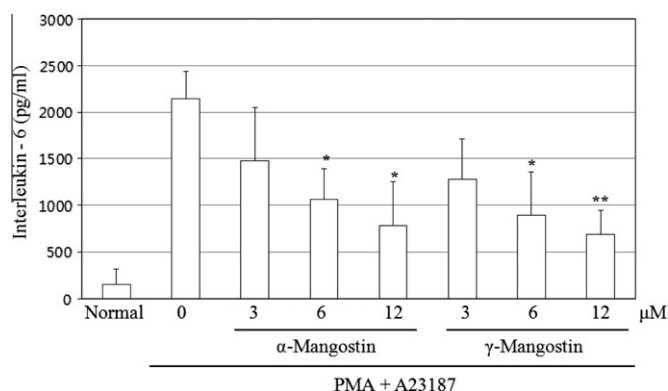


Fig. 2. Effects of α - and γ -mangostins on the secretion of IL-6 in PMA plus A23187-stimulated BMMCs. The cells (1×10^6 cells/ml) were pretreated with α - and γ -mangostin (3–12 μ M) 30 min prior to stimulation with PMA (50 nM) plus A23187 (1 μ M). 2 h after PMA plus A23187 stimulation, the IL-6 level of the supernatants was measured by ELISA. Statistical significance: * $P < 0.05$ and ** $P < 0.005$, when compared to the PMA plus A23187 treated group. Significant differences between treated groups were determined using the Dunnett's *t*-test. Values shown are the mean $\pm$ SE of duplicate determinations from three separate experiments.

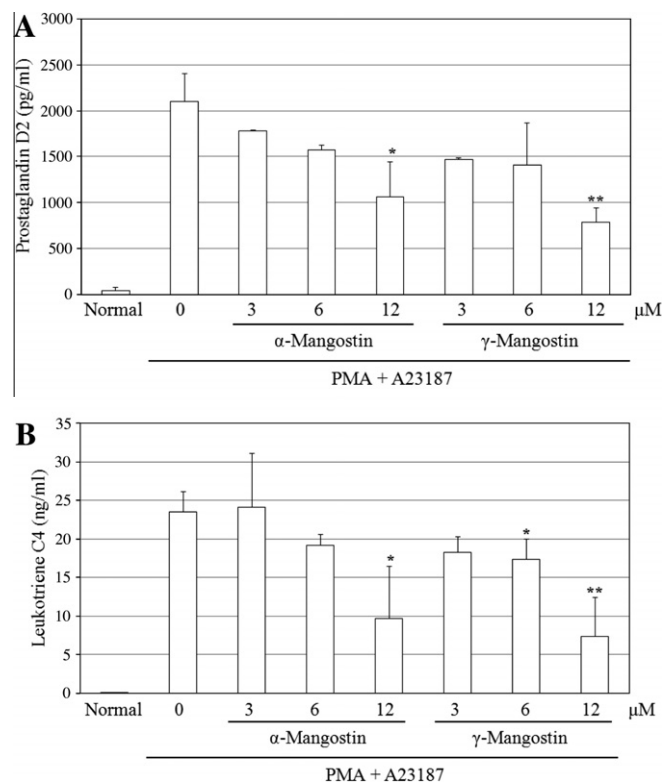


Fig. 3. Effects of α - and γ -mangostins on the production of PGD₂ and LTC₄ in PMA plus A23187-stimulated BMMCs. The cells (1×10^6 cells/ml) were pretreated with α - and γ -mangostin (3–12 μ M) 30 min prior to stimulation with PMA (50 nM) plus A23187 (1 μ M). 2 h after PMA plus A23187 stimulation, the PGD₂ levels of the supernatants were measured by ELISA. Statistical significance: * $P < 0.05$ and ** $P < 0.005$, when compared to the PMA plus A23187 treated group. The cells (1×10^6 cells/ml) were pretreated with α - and γ -mangostin (3–12 μ M) 30 min prior to stimulation with PMA (50 nM) plus A23187 (1 μ M). At 1 h after PMA plus A23187 stimulation, the LTC₄ levels of the supernatants were measured by ELISA. Statistical significance: * $P < 0.05$ and ** $P < 0.005$, as compared to the PMA plus A23187 treated group. Significant differences between treated groups were determined using the Dunnett's *t*-test. Values shown are the mean $\pm$ SE of duplicate determinations from three separate experiments.

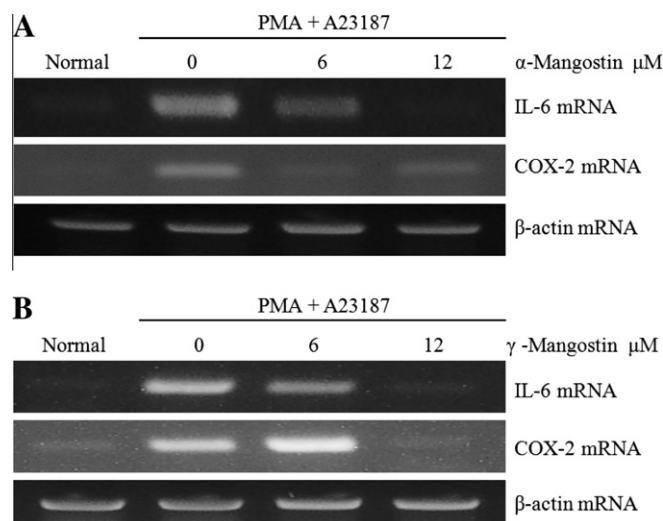


Fig. 4. Effects of α - and γ -mangostin on PMA plus A23187-induced COX-2 protein expressions in BMMCs. BMMCs were pretreated with the indicated concentrations of alpha and gamma mangostin for 30 min prior to being incubated with PMA (50 nM) plus A23187 (1 μ M) for 2 h. Equal amounts of mRNA (2 μ g) were then separated by agarose gel. The β -actin mRNA was carried out in parallel to confirm equivalency of cDNA preparation.

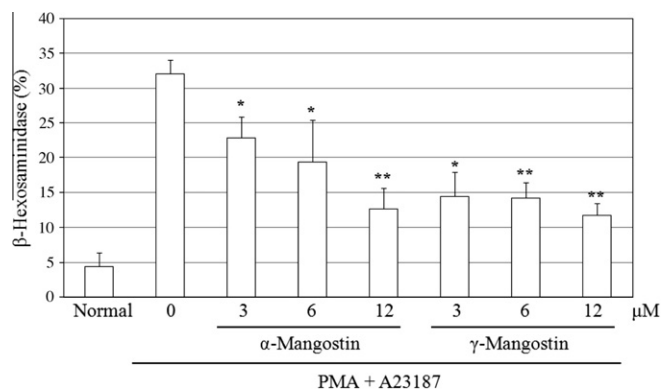


Fig. 5. Effect of α - and γ -mangostin on the release of β -hexosaminidase in PMA plus A23187-stimulated BMMCs. BMMCs (1×10^5 cells/ml) were pretreated with α - and γ -mangostin 30 min prior to stimulation with PMA (50 nM) plus A23187 (1 μ M). At 90 min after PMA plus A23187 stimulation, the β -HEX released into the supernatant cell lysate was measured by ELISA. The procedure is described in detail in the Materials and methods section. All data are the arithmetic mean of triplicate determinations. Statistical significance: * $P < 0.05$ and ** $P < 0.005$, as compared to the PMA plus A23187 treated group. Significant differences between treated groups were determined using the Dunnett's t -test. Values shown are the mean $\pm$ SE of duplicate determinations from three separate experiments.

allergic reactions. Furthermore, it was found that down regulation of COX-2 mRNA expression was involved in this anti-allergic process by the treatment of α - and γ -mangostins in BMMC. Both α - and γ -mangostins showed similar activity in stimulated primary cultured mast cell even though there is structurally small difference. Thus, aforementioned results suggest potentials of α - and γ -mangostins as anti-allergic molecules in preventing or treating various allergic diseases.

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