

## Inhibitory Effects of *Morus alba* on Compound 48/80-Induced Anaphylactic Reactions and Anti-Chicken Gamma Globulin IgE-Mediated Mast Cell Activation

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We investigated the effects of hot-water extract from the root bark of *Morus alba* (HEMA) on anaphylactic reactions. Using *in vitro* and *in vivo* experiments, we examined whether HEMA could inhibit compound 48/80-induced systemic anaphylactic shock and anti-chicken gamma globulin (CGG) IgE-mediated rat peritoneal mast cell activation. HEMA significantly inhibited systemic anaphylaxis induced by the compound 48/80 in mice. HEMA also significantly inhibited the passive cutaneous anaphylaxis activated by anti-CGG IgE. HEMA had no cytotoxicity on rat peritoneal mast cells (RPMC). Moreover, HEMA dose-dependently inhibited mast cell degranulation, histamine release and calcium uptake into RPMC induced by the compound 48/80 or anti-CGG IgE. When HEMA was added, the level of intracellular cAMP in RPMC showed a transient and significant increase (5-fold) compared with that of control cells. HEMA also inhibited significantly the compound 48/80-induced cAMP reduction in RPMC. These results suggested that HEMA inhibits the compound 48/80- or anti-CGG IgE-induced mast cell activation and its inhibitory effects on mast cell activations were favorably comparable to disodium cromoglycate. And HEMA is a candidate for effective therapeutic tools of allergic diseases.

**Key words** *Morus alba*; anaphylactic reaction; cAMP; calcium; histamine; mast cell degranulation

Mast cells are widely distributed throughout vascularized tissues and certain epithelia.<sup>1)</sup> Mast cells are responsible for a variety of allergic disorders including allergic rhinitis, dermatitis, asthma, and food allergies, as well as catastrophic anaphylactic reactions to insect stings and some drugs. These cells respond to IgE-directed antigens *via* the high-affinity receptor for IgE, Fcε RI, by releasing granules that contain preformed inflammatory mediators and generating inflammatory lipids and cytokines.<sup>2,3)</sup>

Species of the genus *Dorstenia* (Moraceae) are perennial, herbaceous plants that are widely distributed in tropical Africa, Asia, Middle-East, Central and South America.<sup>4,5)</sup> Moraceae comprises a large family of sixty genera and nearly 1400 species, including important groups such as *Artocarpus*, *Morus*, and *Ficus*. The species of *Morus* have been shown to exhibit anti-HIV, anti-oxidative, anti-hypotensive, and cytotoxic activities.<sup>6–8)</sup> Plants of this genus are known to be rich in flavonoids,<sup>9,10)</sup> a group of chemicals shown to have potent antiviral activities against herpes simplex virus, rhinovirus, human immunodeficiency virus, and various respiratory viruses.<sup>11–13)</sup> This herb has been long used as an antiphlogistic, diuretic, and expectorant,<sup>14)</sup> and also applied for control of inflammation, diabetes, and bronchial asthma.<sup>15,16)</sup>

The phenolic constituents and flavonoids of mulberry tree inhibits cAMP phosphodiesterase activity and regulates arachidonate metabolism in rat platelet homogenates.<sup>17)</sup> Although studies have demonstrated that various effects of *M. alba* on numerous disease, the anti-allergic activity of the root bark of *M. alba* is poorly understood. Since calcium and cAMP have been shown to play a pivotal role in the mast cell activations, we have assessed the effect of the root bark of *M. alba* on calcium uptake and change of intracellular cAMP in mast cells. In this paper, we show that the root bark of *M. alba* inhibits the compound 48/80-induced systemic anaphylactic shock and anti-CGG IgE-induced rat peritoneal mast

cell activations and the increased vascular permeability while cAMP level is increased. In addition, its effects are compared with disodium cromoglycate (DSCG), a known cell stabilizer which inhibits the release of pre-formed and newly synthesized chemical mediators from a variety of cells involved in allergic and inflammatory responses.<sup>18)</sup>

### MATERIALS AND METHODS

**Materials** Compound 48/80, bovine serum albumin (BSA), disodium cromoglycate (DSCG) and HEPES, chicken gamma globulin (CGG) were purchased from Sigma Chemical Co. (MO, U.S.A.). DSCG, an antianaphylactic agent, was used as positive control in order to compare with HEMA activity. Percoll solution was purchased from Pharmacia (Uppsala, Sweden). Anti-CGG IgE antibodies were supplied by Hern-Ku Lee works for department of immunology, Chonbuk National University Medical School.

**Experimental Animals** Male ICR mice and Sprague-Dawley rats aged 8–10 weeks were purchased from Dae Han Experimental Animal Center (Daejeon, Korea). They were housed 3–5 per cage in a laminar air-flow cabinet maintained at 22±1 °C and relative humidity of 55±10% throughout the study. Animal Research Committee of Chonbuk National University approved the animal study in accordance with the guidelines of the National Institutes of Health (NIH publication #85–23, 1985).

**Preparation of Hot-Water Extract from the Root Bark of *Morus alba* (HEMA)** The root bark of *Morus alba* used in this study were purchased from Jangso Oriental Pharmacy (Chonbuk, Korea). The air-dried root barks of *M. alba* (100 g) were immersed in 400 ml of distilled water and boiled under reflux for 2 h. This hot-water extraction was conducted twice. The resulting extract was filtered through a 0.45 μm filter, and concentrated to approximately 100 ml

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under reducing pressure. The concentrated extract was finally lyophilized, yielded 12.4 g dried powder and kept at 4 °C until use. A voucher specimen (number 2003-MS203) was deposited at the Chonbuk University Medical School. The dried extract was dissolved in saline or HEPES-Tyrode buffer (136 mM NaCl, 5 mM KCl, 2 mM CaCl<sub>2</sub>, 11 mM NaHCO<sub>3</sub>, 0.6 mM NaH<sub>2</sub>PO<sub>4</sub>, 2.75 mM MgCl<sub>2</sub>, 5.4 mM HEPES, 1.0 mg/ml bovine serum albumin, 1.0 mg/ml glucose, 0.1 mg/ml heparin) before use.

**Compound 48/80-Induced Systemic Anaphylaxis** Systemic anaphylaxis was induced by the mast cell degranulator, compound 48/80.<sup>19,20</sup> Briefly, HEMA (50, 100, 200 mg/kg body weight) administered orally at 24, 12 and 1 h before the injection of compound 48/80. Mice were given an intraperitoneal injection of compound 48/80 (8 mg/kg body weight). Survival rate was monitored for 1 h after the induction of anaphylactic shock.

**Passive Cutaneous Anaphylaxis (PCA)** An IgE-dependent cutaneous reaction was generated by sensitizing the skin with an intradermal injection of anti-chicken gamma globulin (CGG) IgE followed 48 h later with an injection of CGG into the rat's penis vein. The CGG was diluted in saline. The rats were injected intradermally with 500 ng of anti-CGG IgE into each of 4 dorsal skin sites that had been shaved. The sites were outlined with a water-insoluble red marker. Forty-eight hours later each rat received an injection of 20 µg CGG in saline containing 0.5% Evans blue *via* the penis vein. HEMA (50, 100, 200 mg/kg) was administered orally at 24, 12 and 1 h before the challenge. Thirty minutes after the challenge, the rats were sacrificed by ether asphyxiation and the dorsal skin was removed for measurement of the pigment area. The amount of dye was then determined colorimetrically after extraction with 1 ml of formamide, based on the method outlined by Fleming *et al.*<sup>21</sup> The absorbent intensity of the extraction was measured at 620 nm using a spectrophotometer (Spectra MAX PLUS, Molecular Devices, CA, U.S.A.), and the amount of dye was calculated with the Evans blue measuring line.

**Preparation of Rat Peritoneal Mast Cells (RPMC) Suspension and Microscopic Observation** RPMC were isolated as previously described.<sup>22</sup> In brief, rats were anesthetized with ether and injected with 10 ml of calcium-free HEPES-Tyrode buffer into the peritoneal cavity, and the abdomen was gently massaged for about 90 s. The peritoneal cavity was opened, and the fluid was aspirated by a Pasteur pipette, and RPMC were purified by using a Percoll density gradient as described in detail elsewhere.<sup>23</sup> RPMC preparations were at least 95% pure and at least 98% of these cells were viable as assessed by trypan blue exclusion.<sup>24</sup> Purified mast cells (1 × 10<sup>6</sup> cells/ml) were resuspended in HEPES-Tyrode buffer. Mast cells were observed under phase contrast microscope and photographed as described by Cochrane and Douglas.<sup>22</sup>

**Mast Cell Viability Assay** To test the viability of cells, the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) colorimetric assay was performed as described previously.<sup>25</sup> Briefly, RPMC (2 × 10<sup>5</sup> cells/well) were incubated for 2 h after stimulation with or without of HEMA (2.5–250 µg). After addition of MTT (100 µg in 100 µl saline solution), cells were incubated at 37 °C for 2 h. The crystallized MTT was dissolved and the absorbance was

measured at 570 nm in a spectrophotometer.

**Assay of Histamine Release** Mast cell suspensions (2 × 10<sup>5</sup> cells in 200 µl) in HEPES-Tyrode buffer were preincubated with HEMA (2.5–250 µg) or DSCG for 10 min at 37 °C and then incubated with compound 48/80 (125 ng). Mast cells were sensitized with anti-CGG IgE (250 ng) for 2 h and preincubated with HEMA at 37 °C for 10 min prior to the challenge with CGG (500 ng). The reaction was stopped by cooling the tubes in ice. The cells were separated from the released histamine by centrifugation at 150 × g for 10 min at 4 °C. Residual histamine in the cells was released by disrupting the cells with boiling. After centrifugation, histamine content was measured by the radioenzymatic method described by Harvima *et al.*<sup>26</sup> The inhibition percentage histamine release was calculated using the following formula: % inhibition = [(histamine release without HEMA – histamine release with HEMA)/histamine release without HEMA] × 100.

**Measurement of <sup>45</sup>Ca Uptake** The calcium uptake of mast cells was measured according to the method described by Chai *et al.*<sup>27</sup> Purified mast cells were resuspended in HEPES-Tyrode buffer containing <sup>45</sup>Ca (1.5 mCi/ml; 1 Ci = 3.7 × 10<sup>10</sup> becquerels; Du Pont), and incubated for 10 min at 4 °C. Mast cell suspensions were preincubated with HEMA (2.5–250 µg) or DSCG for 10 min at 37 °C and then incubated with compound 48/80 (125 ng). Mast cells were sensitized with anti-CGG IgE (250 ng) for 1 h and preincubated with HEMA or DSCG at 37 °C for 30 min prior to the challenge with CGG (500 ng). The reaction was stopped by the addition of 1 mM lanthanum chloride and centrifuged 3 times at 4 °C for 10 min, then the cells in the pellet were disrupted with 10% Triton X-100 by vigorous shaking. Radioactivity of the solution was measured in a scintillation β-counter (Liquid scintillation Analyzer, A Canberra company, Australia).

**Measurement of cAMP Level** The cyclic adenosine-3',5' monophosphate (cAMP) level was measured according to the method described by Holmegaard.<sup>28</sup> In brief, mast cell suspensions were added to an equivalent volume (200 µl) of prewarmed buffer containing the drug in an Eppendorf tube. The reaction was allowed to proceed for discrete time intervals, terminated by centrifugation, and then added 250 µl of 50 mM sodium acetate buffer (pH 6.2) under vigorous vortexing, followed by snap frozen in liquid nitrogen. The frozen samples were thawed and vortexed, the debris was sedimented by a centrifugation 400 × g at 4 °C for 10 min. The cAMP level in the supernatant was determined by radioimmunoassay using a Rianen assay system (MA, U.S.A.).

**Statistical Analysis** The data were expressed as mean ± S.E.M. of four or five experiments under the same condition. Student's *t*-test and ANOVA with Dunnett's test were used to make a statistical comparison among the groups. Results with *p* < 0.05 were considered statistically significant. Linear regression analysis was used to correlate the linear relationship among the assay values. The correlation coefficients among the assay values were determined using SPSS version 12.0.1 (SPSS Inc., IL, U.S.A.).

## RESULTS

To assess the *in vivo* anti-allergic activity of HEMA, we

first used compound 48/80-induced systemic anaphylactic animal model. After injection of compound 48/80, the mice were monitored for 1 h to determine the mortality rate. Compound 48/80 successfully induced anaphylactic death within 1 h after intraperitoneal treatment with a dose of 8 mg/kg in ICR mice. As shown in Table 1, the compound 48/80-induced anaphylactic reaction was dose-dependently inhibited by HEMA. Anaphylactic death was inhibited by 53% in the group treated 200 mg/kg HEMA.

Another way to test anaphylactic reactions is to induce passive cutaneous anaphylaxis (PCA). Local extravasation was induced by a local injection of anti-CGG IgE followed by an intravenous antigenic challenge. Anti-CGG IgE was injected into the right dorsal skin sites. As a control, the left dorsal skin site of these rats was injected with saline alone. After 24 h, all animals were injected intravenously with CGG injected with Evans blue dye. PCA was best visualized by the extravasation of the dye. As shown in Table 2, anti-CGG IgE induced extravasation of Evans blue. Oral administration of HEMA resulted in inhibition of PCA reactions in a dose-dependent manner. HEMA 200 mg/kg dose showed a marked inhibition on anti-CGG IgE-induced extravasation of Evans blue.

MTT conversion assay was used to determine the viability of RPMC exposed to HEMA. As shown in Fig. 1, the viable cells were almost 100% after exposure to 250  $\mu$ g HEMA for 2 h, and HEMA had no cytotoxicity on RPMC.

To investigate the inhibitory mechanism of HEMA on anaphylactic reactions, we determined inhibitory effects on compound 48/80-induced or anti-CGG IgE-mediated mast cell activation. Figure 2 showed the inhibitory effects of HEMA on compound 48/80-induced mast cell degranulation. Inverted microscopy technique showed that the normal RPMC were generally spherical, or oval, had many fine granules surrounding a prominent nucleus (Fig. 2A). After stimulation with 125 ng compound 48/80 for 5 min, the cell became swollen and exhibited many vacuoles and extruded granules near the cell surface and in the surrounding medium, which is interpreted as mast cell degranulation (Fig. 2B). When RPMC were incubated with HEMA (250  $\mu$ g), RPMC showed similar findings as seen in Fig. 2A (Fig. 2C). After the stimulation of HEMA-pretreated RPMC with compound 48/80 for 5 min, the cell showed swelling with an irregular boundary, but not degranulation (Fig. 2D). HEMA significantly inhibited compound 48/80-induced mast cell degranulation.

Figure 3 shows inhibitory effects of HEMA (Fig. 3A) and DSCG (Fig. 3B) on compound 48/80-induced or anti-CGG IgE-mediated histamine release from RPMC. The histamine release from the RPMC induced by compound 48/80 and anti-CGG IgE amounted to  $88.3 \pm 4.4$  and  $57.2 \pm 5.4\%$ , respectively. However the histamine release from the compound 48/80-treated RPMC after pretreatment of 2.5, 25, or 250  $\mu$ g of HEMA amounted to  $49.6 \pm 3.4$ ,  $11.0 \pm 1.2$  or  $4.2 \pm 1.0\%$ , respectively. And the histamine release from the anti-CGG IgE-treated RPMC after pretreatment of 2.5, 25, or 250  $\mu$ g of HEMA amounted to  $47.1 \pm 2.9$ ,  $27.2 \pm 3.5$  or  $9.4 \pm 0.9\%$ , respectively. HEMA dose-dependently inhibited the compound 48/80-induced or anti-CGG IgE-mediated histamine release at doses of 2.5 to 250  $\mu$ g. These results indicated that HEMA exhibits an ability to inhibit compound 48/80-induced anaphylactic reactions *via* blocking compound

Table 1. Inhibitory Effects of Hot-Water Extract from the Root Bark of *Morus alba* (HEMA) on Compound 48/80-Induced Systemic Anaphylaxis

HEMA (mg/kg BW)	Compound 48/80 (8 mg/kg BW)	Survival rate (%)
0	+	0
50	+	$15.5 \pm 5$
100	+	$35.0 \pm 11$
200	+	$53.6 \pm 13$
200	-	$100 \pm 0$

Groups of mice ( $n=20$ /group) were orally pretreated with 300  $\mu$ l saline or HEMA which was given at 24, 12 and 1 h before the injection of compound 48/80. The compound 48/80 solution was intraperitoneally given to the group of mice. Survival rate (%) within 1 h following the compound 48/80 injections is presented as the numbers of survival mice  $\times 100$ / total numbers of experimental mice.

Table 2. Inhibitory Effects of Hot-Water Extract from the Root Bark of *Morus alba* (HEMA) on the Anti-CGG IgE-Mediated Passive Cutaneous Anaphylaxis in Rats

HEMA (mg/kg BW)	Anti-CGG IgE	Amount of dye ( $\mu$ g/g skin)	Inhibition (%)
0	+	$18.5 \pm 1.5$	0
50	+	$15.3 \pm 1.9$	17.3
100	+	$10.7 \pm 0.6^*$	42.2
200	+	$7.9 \pm 0.4^*$	57.3

The rats were injected intradermally with 500 ng of anti-CGG IgE into each of 4 dorsal skin sites that had been shaved an experiment. The HEMA was administered orally 24, 12 and 1 h prior to the challenge with antigen. Amount of dye is presented as the mean  $\pm$  S.E.M. of four independent experiments. \* $p < 0.01$ ; significantly different from the saline value using a ANOVA with Dunnett's test.

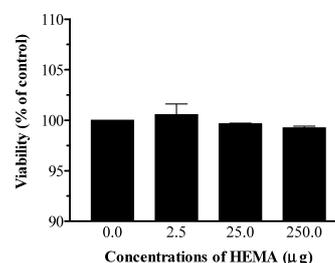


Fig. 1. Effect of Hot-Water Extract of the Root Bark of *Morus alba* (HEMA) on Rat Peritoneal Mast Cells (RPMC) Viability

RPMC were treated with various concentrations of HEMA for 2 h. RPMC viability was determined by MTT assay and its percentage was calculated as a ration of  $A_{570}$  of control cells (treated with HEPES-Tyrodé buffered solution). Each value is the mean  $\pm$  S.E.M. of five independent experiments.

48/80-induced mast cell degranulation and histamine release from RPMC.

The close correlation among concentration of the histamine-releasing stimulus, calcium ion influx, and the amount of released histamine suggests a cause- and effect-relationship between the influx of calcium and the release of histamine. The release of histamine is decreased by an increment in the intracellular cAMP. We also examined the effect of HEMA on calcium uptake and cAMP production. HEMA alone did not affect the calcium uptake into the RPMC, while compound 48/80 induced the calcium uptake into the RPMC. However, HEMA and DSCG inhibited compound 48/80-induced or anti-CGG IgE-mediated calcium uptake into RPMC in a concentration-dependent manner (Fig. 4). The correlation coefficient ( $\gamma$ ) was 0.935, ( $p < 0.01$ ) indicating significant correlation between the histamine release and calcium uptake.

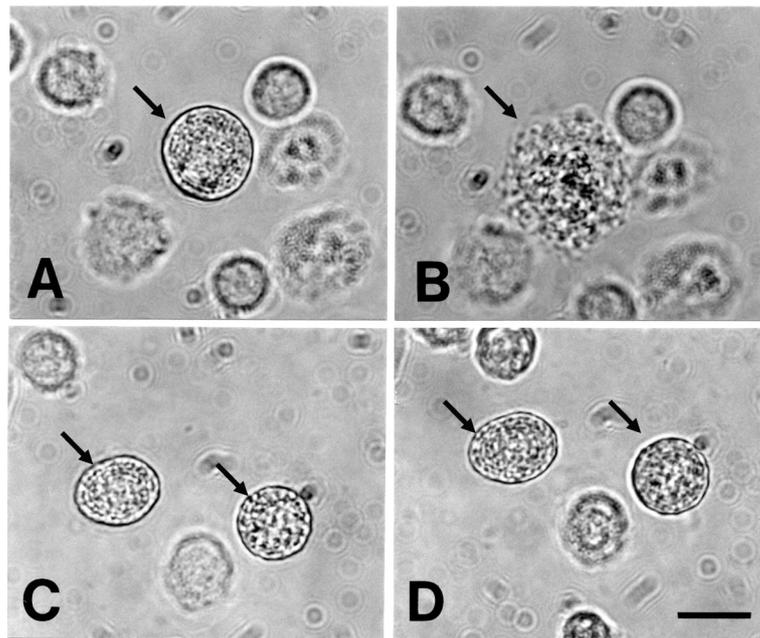


Fig. 2. Light Microphotographs of Rat Peritoneal Mast Cells (RPMC) in HEPES-Tyrode Buffer (A), after Stimulation with 125 ng Compound 48/80 (B), 250 µg Hot-Water Extract of the Root Bark of *Morus alba* (HEMA) (C), and after Being Pretreated with 250 µg HEMA Prior to the Stimulation with 125 ng Compound 48/80 (D)

Normal RPMC are generally characterized by round shape with fine granules and regular surface. Degranulated mast cells become swollen and have many vacuoles, an irregular surface and extruded granules. However, compound 48/80 did not induce degranulation of RPMC pretreated with HEMA. HEMA significantly inhibited compound 48/80-induced mast cell degranulation. Bar=10 µm (arrows: mast cells).

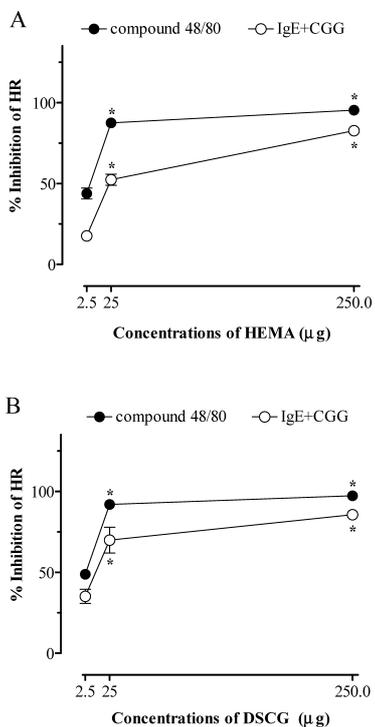


Fig. 3. Inhibitory Effects of Hot-Water Extract of the Root Bark of *Morus alba* (HEMA; A) and Disodium Cromoglycate (DSCG; B) on Compound 48/80 or IgE-Mediated Histamine Release (HR) from Rat Peritoneal Mast Cells (RPMC)

RPMC were preincubated with the drug at 37°C for 10 min prior to the incubation with compound 48/80 or to the challenge with antigen. Each value is the mean±S.E.M. of four independent experiments. \* $p < 0.01$ ; significantly different from the saline value.

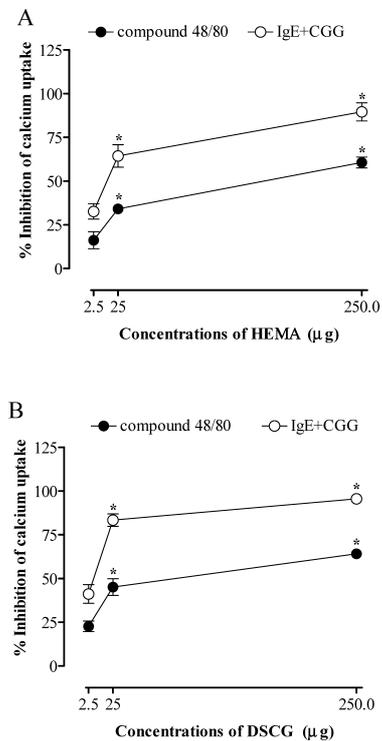


Fig. 4. Inhibitory Effects of Hot-Water Extract of the Root Bark of *Morus alba* (HEMA; A) and Disodium Cromoglycate (DSCG; B) on Compound 48/80 or IgE-Mediated Calcium Uptake in Rat Peritoneal Mast Cells (RPMC)

RPMC were preincubated with the drug at 37°C for 10 min prior to the incubation with compound 48/80 or to the challenge with antigen. Each value is the mean±S.E.M. of six independent experiments. \* $p < 0.01$ ; significantly different from the saline value.

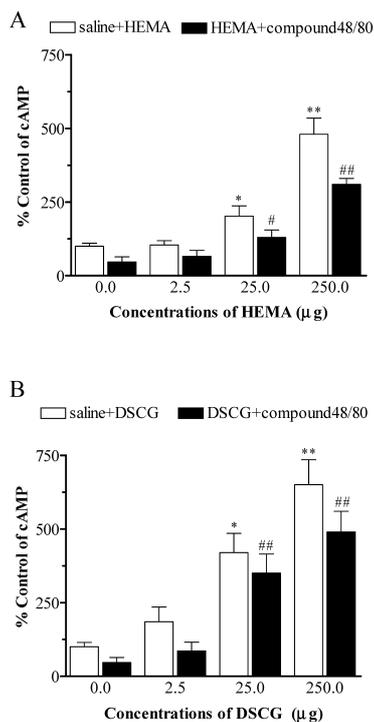


Fig. 5. Inhibitory Effects of Hot-Water Extract of the Root Bark of *Morus alba* (HEMA; A) and Disodium Cromoglycate (DSCG; B) on Compound 48/80-mediated Decrease of cAMP Level in RPMC

Various concentrations of HEMA were added into the rat peritoneal mast cell suspension for 10 min, and cAMP levels were measured. Each value is the mean  $\pm$  S.E.M. of six independent experiments. \* $p < 0.01$  and \*\* $p < 0.001$ ; significantly different from the saline, # $p < 0.01$  and ## $p < 0.001$ ; significantly different from the saline plus compound 48/80.

HEMA itself increased the cAMP level in RPMC by 5-fold as compared to those in control cells. RPMC treated with compound 48/80 caused less than 2-fold decrease in the cAMP level as compared to those treated with buffer only. HEMA and DSCG inhibited the compound 48/80-induced cAMP reduction of RPMC in a dose-dependent manner (Fig. 5). The inhibitory effects of HEMA on the compound 48/80-induced histamine release, calcium uptake and cAMP reduction were less potent than those by DSCG, which may be that HEMA is not purified substance. The correlation coefficient ( $\gamma$ ) was  $-0.577$ , ( $p < 0.01$ ) indicating significant correlation between the histamine release and cAMP production. Also, the correlation coefficient ( $\gamma$ ) between calcium uptake and cAMP production was  $-0.799$ , ( $p < 0.01$ ) indicating significant correlation between the two groups. These results suggest that HEMA contains a bioactive compound which inhibits the compound 48/80- or anti-CGG IgE-induced mast cell activations and vascular permeability *via* blocking of calcium uptake into RPMC, or increasing of cAMP level in RPMC.

## DISCUSSION AND CONCLUSIONS

We have demonstrated that HEMA pretreatment profoundly inhibited compound 48/80-induced systemic allergic reaction and anti-CGG IgE-induced local allergic reaction. HEMA also inhibited the histamine release by compound 48/80 and anti-CGG IgE. These results indicate that mast cell-mediated immediate-type allergic reactions are inhibited

by HEMA. Its inhibitory effects on mast cell activations were favorably comparable to DSCG.

Mast cells are widely distributed, especially in connective tissue and mucosal surfaces, and provide a useful and powerful tool for studying both stimulus-secretion coupling and allergic disorders.<sup>29)</sup> Mast cells are responsible for a variety of allergic disorders including allergic rhinitis, dermatitis, asthma, and food allergies, as well as catastrophic anaphylactic reactions to insect stings and some drugs.<sup>29)</sup> These diseases are becoming more prevalent, and they increase the burden of health-care costs in many countries including Korea. Although there are many approaches to the treatment of these illnesses, such as allergen-specific immunotherapy, DNA vaccination, and antagonists to the receptors of leukotriene and histamine, these therapies still have many problems and side effects.<sup>2,3)</sup> Various forms and models of inflammatory reactions have been observed. For example, there are the inflammatory responses from the airways of asthmatic patients, bone and joints inflammation, microbial infection, anaphylaxis and allergic conditions, and so on. Thus, the adoption of anti-allergic and anti-inflammatory experimental models for the assessment of phytomedicines used in the traditional healthcare system for the management of a variety of diseases such as pain, asthma, arthritis, and rheumatism, are considered desirable and justifiable.

Mulberry trees are widely cultivated in East Asia, and the leaves are used to feed silkworms. The root bark of *M. alba* has been traditionally used in Asian countries for medicinal purposes due to its anti-inflammatory, hypoglycemic, and antibacterial activities.<sup>30)</sup> The root bark of *M. alba*, Cortex mori, has attracted much interest due to its hypotensive, antiviral and anti-inflammatory effects. Topical application of Cortex mori extract has been a common ethnobotanical practice for the treatment of inflammation in Korea. It has long been used as an antiphlogistic, diuretic, and expectorant.<sup>14)</sup> Therefore, this herb has also been applied for the control of inflammation, diabetes, and bronchial asthma.<sup>15,16)</sup> Recently, a few papers have reported the hypotensive<sup>31,32)</sup> and antiviral<sup>33,34)</sup> effects of natural Diels-Alder type adducts or flavonoids or HEMA. Specifically, HEMA inhibits cAMP phosphodiesterase activity,<sup>35)</sup> and flavonoids and related compounds from mulberry tree regulate the arachidonate metabolism in rat platelet homogenates.<sup>17)</sup> HEMA is recently shown to inhibit the spermine-induced histamine release of rat mast cells by blocking calcium uptake,<sup>36)</sup> and Cortex mori extract induces cancer cell apoptosis through inhibition of microtubule assembly.<sup>37)</sup> In this study, we utilized well-established *in vitro* and *in vivo* allergic animal models including *in vitro* degranulation in RPMC by compound 48/80 or CGG antigen and *in vivo* compound 48/80-induced systemic anaphylactic reaction.<sup>19,20)</sup>

Data presented in this paper show that HEMA pretreatment greatly affected compound 48/80-induced mast cell degranulation and histamine release from the RPMC. A close correlation among concentration of the histamine-releasing stimulus, calcium ion influx, and the amount of released histamine suggest a cause- and effect-relationship between the influx of calcium and the release of histamine.<sup>23)</sup> The compound 48/80 causes the release of histamine from mast cells following calcium uptake, but the pretreatment with HEMA inhibits the compound 48/80-induced calcium uptake into the

mast cells. The inhibition of HEMA on histamine release induced by compound 48/80 was more potent than that by anti-CGG IgE, however, in the result of calcium uptake, opposite direction was observed. We think that this phenomenon is because the mechanism of mast cell activation by compound 48/80 was different with that by IgE. Compound 48/80 is a member of the family of polybasic mast cell secretagogues that are known to activate trimeric G proteins directly, primarily those of Gi and Go categories.<sup>38–40</sup> However mast cell activation with IgE and antigen results in mobilization of intracellular calcium and activation of protein kinase C both possibly *via* activation of phospholipase C.<sup>41</sup> According to earlier findings, agents that elevated cAMP levels inhibited mediator release.<sup>19</sup> The possible mechanism of the inhibitory effect on the histamine release from mast cells appears to be related to the increase of adenylate cyclase activity and a subsequent increase in intracellular cAMP.<sup>42</sup> The intracellular cAMP content of the mast cells, when incubated with HEMA, increased more than 5-fold in comparison with that of basal cells. The inhibitory effect of HEMA on mast cell degranulation and histamine release from mast cells may be related to the prevention of an increase of intracellular calcium content owing to elevation of the intracellular cAMP level by inhibition of the cAMP phosphodiesterase.<sup>35</sup> DSCG, an antianaphylactic agent, especially inhibited chemical-mediator release by increasing intracellular cAMP through the inhibition of cAMP phosphodiesterase,<sup>43</sup> and inhibited the release of pre-formed and newly synthesized chemical mediators from a variety of cells involved in allergic and inflammatory responses.<sup>18</sup> Moreover, DSCG has long been used for the prevention of allergy related human ailments but because of its poor gastrointestinal absorption and its beneficial effects have been due to its topical application rather than systemic absorption.<sup>44</sup> On the basis of inhibition of systemic anaphylaxis induced by the compound 48/80 in mice after oral HEMA administration, HEMA may prove superior to DSCG for the prevention of allergy related impediments.

The active components in the root bark of *M. alba* have not been clarified phenolic constituents; however, flavonoids and related compounds isolated from *M. alba* shown to possess anti-inflammatory effects.<sup>17,32,34</sup> Moran A, a glycoprotein known for its hypoglycemic activity has also been isolated.<sup>45,46</sup> This and other studies<sup>47</sup> suggest the possible application of *M. alba* to treat many diseases. In conclusion, our present results provide evidence that HEMA may possess strong antihistamine and antiallergic activity. Studies on the isolation and characterization of the active ingredients are in progress.

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