European Journal of Medicinal Plants
16(3): 1-10, 2016, Article no.EJMP.28566
ISSN: 2231-0894, NLM ID: 101583475

SCIENCEDOMAIN *international*
www.sciencedomain.org

Anti-Allergic Action of Aqueous Extract of *Moringa oleifera* Lam. Leaves in Mice

Akinori Hagiwara[1], Muneaki Hidaka[2], Shiro Takeda[3,4], Hiroki Yoshida[5], Hisahiro Kai[6], Chihiro Sugita[5], Wataru Watanabe[7] and Masahiko Kurokawa[5*]

[1]Lien Co. Ltd., Higashimorotuka-gun, Miyazaki, Japan.
[2]Department of Clinical Pharmacy, Graduate School of Clinical Pharmacy, Kyushu University of Health and Welfare, 1714-1 Yoshino, Nobeoka, Miyazaki 882-8508, Japan.
[3]Research Division, Minami Nihon Rakuno Kyodo Co. Ltd., 5282 Takagi, Miyakonojo, Miyazaki 885-0003, Japan.
[4]Department of Animal Science and Biotechnology, School of Veterinary Medicine, Azabu University, 1-17-71 Fuchinobe, Sagamihara, Kanagawa 252-5201, Japan.
[5]Department of Biochemistry, Graduate School of Clinical Pharmacy, Kyushu University of Health and Welfare, 1714-1 Yoshino, Nobeoka, Miyazaki 882-8508, Japan.
[6]Department of Pharmaceutical Health Sciences, Graduate School of Clinical Pharmacy, Kyushu University of Health and Welfare, 1714-1 Yoshino, Nobeoka, Miyazaki 882-8508, Japan.
[7]Department of Microbiology, Graduate School of Clinical Pharmacy, Kyushu University of Health and Welfare, 1714-1 Yoshino, Nobeoka, Miyazaki 882-8508, Japan.

Authors' contributions

This work was carried out in collaboration between all authors. Authors AH and MK designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors MH, ST, HY, HK, CS and WW managed the analyses of the study. Authors HK and CS managed the literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/EJMP/2016/28566
Editor(s):
(1) Karim Hosni, Institut National de Recherche et d'Analyse Physico-chimique, Biotechpole de Sidi Thabet, Tunisia.
(2) Marcello Iriti, Professor of Plant Biology and Pathology, Department of Agricultural and Environmental Sciences, Milan State University, Italy.
Reviewers:
(1) Sameh Mohamed Farouk, Suez Canal University, Ismailia, Egypt.
(2) Maged Refaat, Ain Shams university, Egypt.
Complete Peer review History: http://www.sciencedomain.org/review-history/16171

Original Research Article

Received 26th July 2016
Accepted 6th September 2016
Published 12th September 2016

**Corresponding author: E-mail: b2mk@phoenix.ac.jp;*

ABSTRACT

Aims: The present investigation aimed to evaluate anti-allergic activity of an aqueous extract of *Moringa oleifera* Lam. leaves (AqMOL) in three type I allergy models in mice.
Study Design: Anti-allergic assay of AqMOL was performed using three type I allergy models in mice.
Place and Duration of Study: Department of Biochemistry, Graduate School of Clinical Pharmacy, Kyushu University of Health and Welfare, Nobeoka, Japan, from April 2014 to March 2015.
Methodology: AqMOL (300 mg/kg) was orally administered to mice three times daily from two days before treatments in compound 48/80 (48/80) stimulation and passive cutaneous anaphylaxis (PCA) reaction models, and scratching frequencies and Evans blue levels in the ears, respectively, after treatments were measured. In an ovalbumin (OVA) sensitization model, AqMOL was continuously ingested *ad libitum* by mice from day 0 to day 45, and scratching frequencies of OVA-sensitized mice were measured after an intranasal OVA challenge. Histamine and total and OVA-specific IgE levels in the sera were also measured.
Results: AqMOL significantly reduced scratching frequencies in the 48/80 stimulation and OVA sensitization models. In the PCA reaction model, AqMOL reduced Evans blue levels in the ears of mice after specific IgE injection, although not statistically significantly. In OVA-sensitized mice, AqMOL significantly reduced OVA-specific IgE levels in the serum, but histamine and total IgE levels were not significantly affected by AqMOL administration.
Conclusion: AqMOL was suggested to alleviate allergic symptoms through suppression of mast cell activation and/or improving the Th1/Th2 balance to Th1 dominance in allergic mice. The elucidation of mode of anti-allergy action of AqMOL may provide new insights into the usage of AqMOL as a functional food for the alleviation of type 1 allergy.

Keywords: Type I allergy; Moringa oleifera; compound 48/80; passive cutaneous anaphylaxis reaction; ovalbumin sensitization; IgE.

1. INTRODUCTION

Moringa oleifera Lam. (*M. oleifera*), Moringaceae, is widely distributed in tropical areas. Its leaves, fruits, flowers, roots, seeds, and immature pods are commonly used as a nutritious vegetable, spice, cosmetic oil, and medicine [1,2]. The extract of *M. oleifera* leaves has been shown to exhibit various biological activities, such as antibacterial activity against *Pseudomonas aeruginosa* and *Staphylococcus aureus* [3], reduction of cholesterol level in the serum of high fat diet fed rats [4], regulation of thyroid hormone status in rats [5], and antiviral efficacy against herpes simplex virus type 1 (HSV-1) in mice [6]. It has been also reported to exhibit cytotoxic effects against human multiple myeloma cell lines [7], prevent oxidative damage to major biomolecules by scavenging free radicals [8], and improve lornoxicam-induced liver damage in rats [9]. Recently, *M. oleifera* is cultivated in Japan, and an aqueous extract of *M. oleifera* leaves (AqMOL) is commercially available as a dietary supplement. Previously, we characterized the basis of the biological efficacy of AqMOL in the alleviation of herpetic symptoms in mice and found that oral administration of AqMOL was effective in activating a delayed-type hypersensitivity (DTH) reaction, which is a major host defense immunity against intradermal herpes simplex virus type 1 (HSV-1) infection, and in elevating interferon (IFN)-γ production from splenocytes of HSV-1-infected mice [10]. These results suggested important insights into the mechanism by which oral administration of AqMOL activates cellular immunity through Th1 immunity.

The number of patients diagnosed with type I allergic diseases such as hay fever, food allergy, allergic rhinitis, and atopic dermatitis has been increasing in many countries worldwide. Various kinds of functional foods and dietary supplements are expected to be possible efficacious alternative treatments to alleviate the allergic symptoms prophylactically and therapeutically in order to maintain and increase the quality of life. Type I allergic symptoms are caused by activation of mucosal mast cells and/or basophils [11]. The cross-linking of immunoglobulin E (IgE) intermediated by the binding of multivalent antigens at the surface of mast cells triggers the release of many chemical mediators such as histamine, leukotrienes,

and prostaglandins from these cells, and these chemical mediators cause type I allergic symptoms [11-13]. IgE produced by plasma cells, which have been differentiated from B cells [14], plays a crucial role in type I allergic reactions. The production of IgE is affected by a skewed T helper type 1 (Th1)/T helper type 2 (Th2) cell balance [15-18]. Thus, it is possible that AqMOL activates Th1 immunity and the modulation of the Th1/Th2 balance contributes to the alleviation of type I allergy symptoms.

In this study, to clarify the immunomodulatory activity of AqMOL in the Th1/Th2 balance, its effect on type I allergy was examined using three kinds of type I allergy models in mice, a compound 48/80 stimulation model, a passive cutaneous anaphylaxis (PCA) reaction model, and an ovalbumin (OVA) sensitization model. We found that oral administration of AqMOL was effective in alleviating allergic symptoms in mice sensitized by compound 48/80 and OVA. The mode of anti-type I allergy action was suggested to involve not only adjustment of the Th1/Th2 balance but also suppression of mast cell activation.

2. MATERIALS AND METHODS

2.1 Preparation of AqMOL

Seeds of *M. oleifera* were purchased from Santan International, Tiruchengode, India, and grown in Miyazaki, Japan. The leaves of *M. oleifera* were harvested, dried at 60℃ for 8 h, roasted at 125℃ for 30 min, and cut and powdered. A voucher specimen (No. 130213) was deposited at Lien Co. Ltd., Miyazaki, Japan. The powdered leaves supplied by Lien Co., Ltd. were extracted once with distilled water (1:30, w/v) for 24 h at room temperature, and the filtered extract was lyophilized. Finally, about 30% (w/w) of the powdered leaves were incorporated in AqMOL. For the compound 48/80 stimulation and PCA reaction models, the lyophilized powder was dissolved in distilled water and administered orally in a volume of 0.2 ml/mouse to the mice by gavage. For OVA sensitization model in mice, the powdered leaves (0.12 or 1.2 g) were extracted once with 200 ml of distilled water for 24 h at room temperature. The filtered extract (AqMOL) was freshly prepared every 2 or 3 days and continuously ingested by mice *ad libitum* for 45 days.

2.2 Mice

Specific-pathogen-free female BALB/c mice (5- or 6-week-old) were obtained from Kyudo Animal Laboratory, Kumamoto, Japan. The mice were housed five or seven per cage in specific pathogen-free conditions under a 12 h light/dark cycle at a room temperature of 23±2℃. They were fed a standard pellet diet CE-2 (Clea Japan, Inc., Tokyo, Japan) and water *ad libitum*. The mice were acclimated for 5 days before starting the experiments. The experimental protocols were approved by the animal experiment committee of Kyushu University of Health and Welfare, Japan, and the animal experimentation guidelines of the university were followed in the animal studies.

2.3 Compound 48/80 Stimulation Model in Mice

Systemic allergy was induced by compound 48/80 (Sigma, St. Louis, MO, USA) as previously described by Kim et al. [19,20]. AqMOL at 300 mg/kg was administered orally in a volume of 0.2 ml/mouse to mice by gavage three times daily on days 0 and 1 (Fig. 1A). As a control, mice were given an equal volume of distilled water. In this experiment, we used AqMOL at 300 mg/kg as a biologically active dose for mice without toxicity based on our previous study [10]. On day 2, AqMOL was administered orally 2 h before the injection of compound 48/80 as a mast cell degranulator, and then 100 μL of compound 48/80 dissolved in saline at 1 mg/mL was subcutaneously injected into the back. Immediately after the injection, the number of times each mouse scratched itself was counted for 40 min.

2.4 PCA Reaction Model in Mice

PCA reaction was carried out in mice as described previously [21,22]. AqMOL (300 mg/kg) or distilled water as a control was orally administered by gavage to mice three times daily on day 0 and day 1 as described above (Fig. 1B). On day 2, AqMOL was administered orally once before and twice after an injection of anti-dinitrophenyl (DNP) IgE (Sigma). The anti-DNP IgE dissolved in saline at 2 mg/mL (10 μL) was intradermally injected into an ear under anesthesia by intraperitoneal injection of 0.5 mL saline including 1.0 mg pentobarbital. On day 3, 2 h after the oral administration of AqMOL, mice were intraperitoneally anesthetized with 0.2 mL

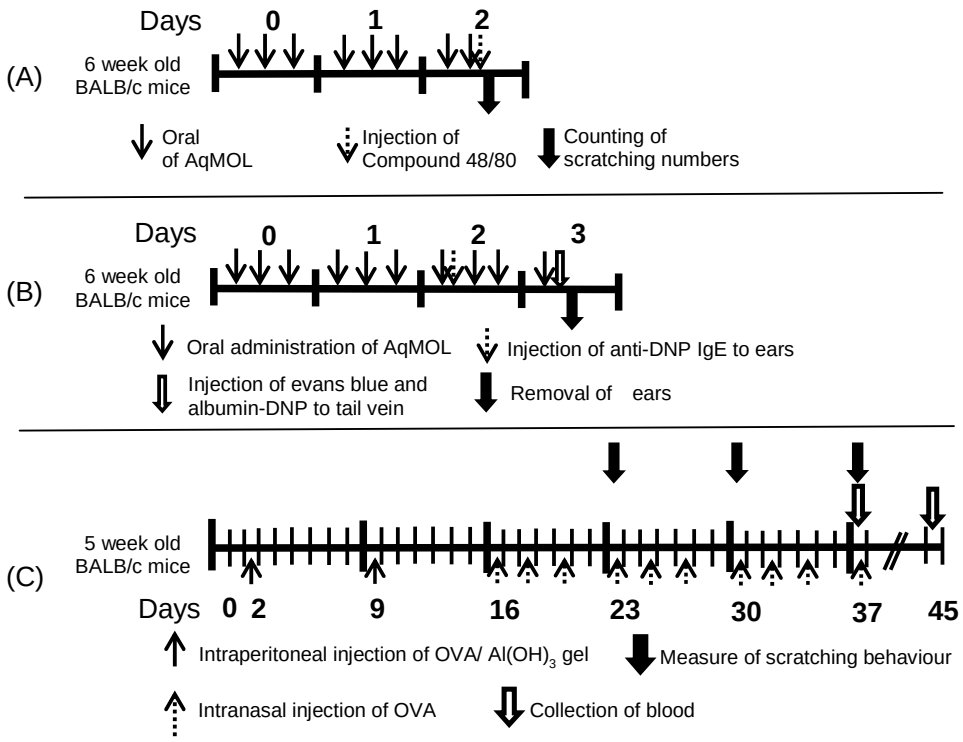

Fig. 1. Schemas of three kinds of type I allergy models in mice. (A) 48/80 stimulation, (B) PCA reaction, (C) OVA sensitization

saline including 1.6 mg pentobarbital, and then 24 h after the anti-DNP IgE injection, 1 mL of saline containing 0.4% Evans blue (Sigma) and 0.1% albumin-DNP was injected into the tail vein of mice. At 30 min after the intravenous injection, the mice were sacrificed, and the ears were removed, weighed, and dissolved in 0.5 mL of 1 mol/L KOH at 37℃ for 24 h. Then, 2 mL of a mixture of acetone and phosphoric acid (5:13, v/v) was added to the KOH solution, and the mixed solution was centrifuged at 3000 rpm for 10 min. The absorbance of the supernatant was measured at 620 nm with a spectrophotometer (UV-1201, Shimadzu, Kyoto, Japan).

2.5 OVA Sensitization Model in Mice

Mice were immunized and boosted with OVA (MP Biomedicals, Santa Ana, CA, USA) in order to induce type I allergic rhinitis according to the method of Tobita et al. [23]. As shown in Fig. 1C, AqMOL at low (0.6 mg/ml) and high (6 mg/ml) doses and distilled water as a control were continuously ingested *ad libitum* by mice (10 mice/each group) as drinking water from day 0 to day 45. On days 2 and 9, the mice were intraperitoneally immunized and boosted, respectively, with 200 µL saline containing 20 µg OVA and 2 mg aluminum hydroxide gel. From day 16 to day 37, the OVA-sensitized mice were intranasally challenged by instillation of 10 µL saline containing 100 µg OVA every 2 or 3 days. On days 23, 30, and 37, the incidence of nose scratching was counted for 20 min before the challenge of OVA instillation, and then immediately after the challenge the incidence of nose scratching was further counted for 20 min. For each mouse, the net scratching number was calculated by subtracting the scratching number before the OVA challenge from the scratching number after the OVA challenge. The body weight of each mouse was measured once daily. AqMOL at low and high doses and water were freshly supplied every 2 or 3 days, and the consumption per mouse in each group was calculated. On day 37, after the OVA challenge, blood was collected from five mice in each group under anesthesia, and the prepared sera were stored at -30℃. From day 38 to day 45, no challenge of OVA instillation was performed, although AqMOL was continuously ingested *ad libitum* by mice as drinking water. On day 45, blood was again collected from five mice in each group under anesthesia, and the prepared sera were stored at -30℃.

2.6 Measurement of IgE and Histamine Levels in Sera of OVA-sensitized Mice

The OVA-specific IgE level was measured using an enzyme-linked immunoassay (ELISA) according to the previously reported method [23]. Briefly, the wells of 96-well microtiter plates were coated with 100 µL OVA (100 mg/mL) in 0.1 mol/L carbonate buffer (pH 9.6) overnight at 4℃ and washed five times with PBS containing 0.05% Tween 20 (PBS-T). The wells were further coated with 300 µL of 0.1 mol/L carbonate buffer (pH 9.6) containing 0.4% bovine serum albumin (BSA) (Sigma) for 2 h at 25℃ and washed five times with PBS-T. The sera were then diluted 100-fold in PBS-T containing 0.4% BSA and added to each well in a volume of 100 µL. The plate was incubated for 2 h at 25℃. After washing the wells as described above, 100 µL horseradish peroxidase-labeled anti-mouse IgE (Bethyl Laboratories, Montgomery, TX, USA) diluted 10000-fold in PBS-T containing 0.4% BSA was added to each well, and the plates were incubated for 1 h at 25℃. The wells were washed with PBS-T, and then 100 µL of 3,3',5,5'-tetramethylbenzidine (Wako Pure Chemical Industries, Osaka, Japan) was added to detect the peroxidase reaction. The plate was then incubated for 30 min at 25℃, and the reaction was stopped by the addition of 100 µL 4 N H_2SO_4. The antigen level was read at 450 nm on a microplate reader (MTP-300, Corona Electric, Ibaraki, Japan). In addition, total IgE levels in sera of OVA-sensitized mice were determined using a mouse IgE ELISA kit (eBioscience, San Diego, CA, USA), and the histamine concentration in sera was determined using a histamine enzyme immunoassay kit (SPI-Bio, Montigny-le-Bretonneux, France) according to the manufacturer's instructions.

2.7 Statistical Analysis

All data are expressed as mean ± standard error (SE). The scratching frequencies of mice in the compound 48/80 stimulation model and Evans blue levels in the PCA reaction model were analyzed statistically by Student's *t*-test. The scratching frequencies in the OVA sensitization model were analyzed statistically by the repeated measures ANOVA to evaluate the continuous differences in repeated measurements with 7-day intervals after the challenges by OVA. A *P* value of less than 0.05 was defined as statistically significant.

3. RESULTS

3.1 Anti-allergic Activity of AqMOL in a Compound 48/80 Stimulation

Compound 48/80 induces an allergic reaction in animals and is known to activate mast cells and the release of histamine and cause pruritus in mice [19,20,24]. AqMOL was examined for its anti-allergic effect on the frequency of scratching induced by compound 48/80 injection in mice. As shown in Table 1, injection of compound 48/80 remarkably increased scratching numbers (180.4 ± 21.5 times in the water-administered mice) as compared with that (44.2 ± 6.4 times) in the water-administered mice without compound 48/80 injection ($P < 0.05$ by Student's *t*-test). The oral administration of AqMOL was significantly effective in reducing scratching frequencies in the compound 48/80-injected mice compared with water administration ($P < 0.05$ by Student's *t*-test). AqMOL reduced the scratching frequencies to less than 50% and was found to possess potent anti-allergic activity.

Table 1. Effects of AqMOL on scratching frequency after injection of compound 48/80 into mice

Treatment	Scratching number (time)	Scratching frequency (%)
Water[a]	44.2 ± 6.4*	24.5
Water[b]	180.4 ± 21.5	100.0
AqMOL	86.3 ± 19.2*	47.9

*AqMOL at 300 mg/kg or distilled water was orally administered three times daily before the injection of compound 48/80 as described in text. The scratching frequencies in mice are shown as mean ± SE (n=7) and are also expressed as percentages of water-administered mice (control group). [a]Water-administered mice without compound 48/80 injection. [b]Water-administered mice with compound 48/80 injection. *P<0.05 versus water-administered mice with compound 48/80 injection by Student's t-test*

3.2 Anti-allergic Activity of AqMOL in a PCA Reaction Model in Mice

The PCA reaction model is a passively sensitized model. In this model, the anti-DNP-specific IgE-dependent mast cell activation followed by the release of chemical mediators including histamine is evaluated, and the mast cell activation also causes an increase of vascular permeability [25,26]. The anti-allergic activity of AqMOL was examined in a murine PCA reaction model. As shown in Table 2, the anti-DNP IgE

injection into the ears remarkably increased the Evans blue levels (1.57 ± 0.06 ng/mg per ear) in the ears of mice as compared with that (0.48 ± 0.02 ng/mg per ear) in the water-administered mice without anti-DNP IgE injection ($P < 0.05$ by Student's *t*-test). AqMOL reduced Evans blue levels in the ears of water-administered mice with anti-DNP IgE injection, although it was not statistically significant ($P = 0.06$ by Student's *t*-test).

3.3 Anti-allergic Activity of AqMOL on Scratching Frequency in OVA-sensitized Mice

AqMOL was further examined for its anti-allergic activity on an OVA-sensitized allergic rhinitis model in mice. In this study, AqMOL at low and high (0.6 and 6 mg/mL, respectively) doses and water were ingested *ad libitum* by mice. The consumptions per mouse in the AqMOL-ingesting group with low and high doses were 4.6 ± 0.1 and 4.8 ± 0.2 mL/day, respectively, and there was no significant difference as compared with the consumption in the water-ingesting (non-AqMOL) group (5.0 ± 0.2 mL/day). The mean body weights of mice in the AqMOL-ingesting group at low and high doses at 37 days were 22.2 ± 0.3 and 21.5 ± 0.4 g, respectively, and also there was not any significance as compared with the body weight of the water-ingesting group (22.4 ± 0.3 g). As shown in Fig. 2, the scratching numbers due to the intranasal ova challenge increased in OVA-sensitized mice administered water in a time-dependent manner for from days 23 to 37 in the OVA-sensitized mice, AqMOL administration at low and high doses significantly reduced scratching number from days 23 to 37 as compared with the water-administered mice ($p < 0.002$ by repeated measures ANOVA). Thus, AqMOL was significantly effective in alleviating allergic symptoms in an ova-sensitized allergic model in mice.

3.4 Effects of AqMOL on Histamine and IgE Levels in Sera of Ova-sensitized Mice

To evaluate the mode of anti-allergic action of AqMOL, histamine, total IgE, and OVA-specific IgE levels in the sera of OVA-sensitized mice on days 37 and 45 were compared between water administration as a control and AqMOL administration at the high dose (6 mg/mL). As shown in Table 3, AqMOL administration reduced histamine levels in the sera of OVA-sensitized mice on days 37 and 45, although there were no statistical significances. The total IgE level in the sera on day 45 was also reduced by AqMOL administration, but the total IgE level on day 37 increased in the sera of OVA-sensitized mice with AqMOL administration. These differences, however, were not statistically significant. On the other hand, AqMOL administration was significantly effective in reducing OVA-specific IgE levels in the sera of OVA-sensitized mice on days 37 and 45 ($P < 0.05$ by Student's *t*-test).

4. DISCUSSION

A functional food is defined as one that satisfactorily demonstrates beneficial effects on one or more target functions in the body, and is expected to improve the physical function and to reduce the risk of specific pathologies by modulating the immune, secretion, nerve, circulating, or digestive system [27]. It is consumed to promote human health and reduce disease risk, and is among the fastest growing sectors of the modern food industry [28]. *Moringa oleifera* Lam. is used as a vegetable, spice, cosmetic oil, and medicine. In recent decades, extracts of the leaves of *M. oleifera* have been shown to exhibit various bioactivities [3-9]. Oral administration of its aqueous extract, AqMOL, has been demonstrated to activate DTH reactions based on cellular immunity in mice intradermally infected with HSV-1 and is suggested to have potential as an immunomodulator [10]. In this study, to verify the immunomodulatory activity of AqMOL, its effects on type I allergy were examined using three kinds of type I allergy models in mice, a compound 48/80 stimulation model, a PCA reaction model, and an OVA sensitization model. The oral administration of AqMOL was found to be significantly effective in alleviating allergic symptoms in mice sensitized by compound 48/80 and OVA (Table 1 and Fig. 2). Even in the PCA reaction model, AqMOL reduced the Evans blue level in the ears of mice after anti-DNP IgE injection, although the reduction was not statistically significant (Table 2). Thus, oral AqMOL was demonstrated to be significantly effective in reducing type 1 allergic symptoms in at least two type I allergy models of the three murine models. The beneficial effect of AqMOL as a dietary supplement might have been demonstrated.

In the compound 48/80 stimulation model, AqMOL was significantly effective in reducing the scratching frequency induced by compound 48/80 injection (Table 1). Compound 48/80

induces an allergic reaction in animals and is known to directly activate mast cells and the release of histamine and cause pruritus in mice [19,20,24]. In an *in vitro* study, it was shown to directly promote mast cell degranulation and histamine release [29]. Thus, it is probable that AqMOL was effective in suppressing the direct activation of mast cells by compound 48/80 and the subsequent histamine release in mice.

Table 2. Effect of AqMOL on PCA reaction in mice

Treatment	DNP-IgE	Evans blue (ng/mg of ear)	Evans blue level (%)
Water (n=5)[a]	-	0.48 ± 0.02	30.6
Water (n=7)[b]	+	1.57 ± 0.06[c]	100.0
AqMOL (n=7)	+	1.49 ± 0.09[d]	94.9

AqMOL at 300 mg/kg or distilled water was orally administered three times daily as described in text. PCA reaction in anti-DNP IgE-injected ears of mice was detected by tail vein injection of albumin-DNP and Evans blue. The levels of Evans blue in ears are shown as mean±standard error (n=5–7) and expressed as percentages of water-administered mice with anti-DNP IgE injection. [a]Water administered mice without anti-DNP IgE injection. [b]Water-administered mice with anti-DNP IgE injection. [c]P<0.05 versus water-administered mice without anti-DNP IgE injection by Student's t-test. [d]P=0.06 versus water-administered mice with anti-DNP IgE injection by Student's t-test

Table 3. Effect of AqMOL on histamine and IgE levels in the sera of OVA-sensitized mice

Treatment	Histamine level (μmol/L)	Total IgE level (μg/mL)	OVA-specific IgE level (A_{450} nm)
Day 37			
Water	3939.2 ± 3130.3	55.4 ± 33.5	0.75 ± 0.05
AqMOL	1348.3 ± 771.8	136.0 ± 78.7	0.66 ± 0.06[a]
Day 45			
Water	3367.9 ± 1360.7	59.4 ± 24.5	0.76 ± 0.04
AqMOL	1915.1 ± 830.6	46.4 ± 29.5	0.65 ± 0.05[a]

AqMOL at high dose (6 mg/ml) or distilled water as a control was continuously ingested ad libitum to mice as drinking water from day 0 to day 45 as described in text. Sera were prepared from five mice per group on days 37 and 45 after the first administration of AqMOL or water. [a]P<0.05 versus water by Student's t-test

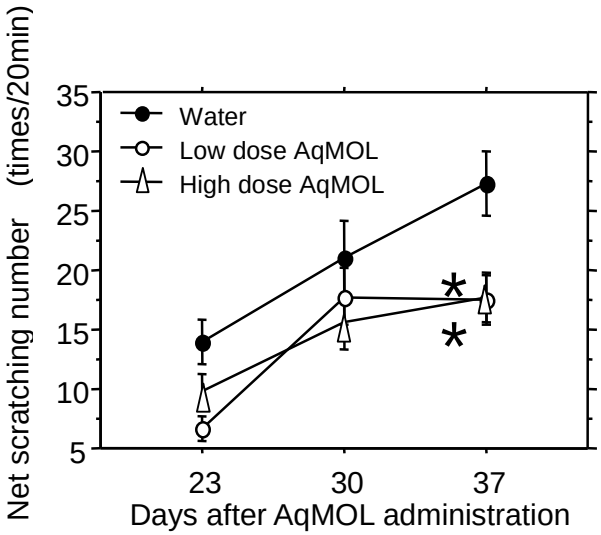

Fig. 2. Effects of AqMOL on the scratching frequencies in OVA-sensitized mice

*The mice (n=10) in each group were administered AqMOL at a low dose (○), AqMOL at a high dose (▲), or distilled water (●), as described in the text. Immediately after every fourth intranasal challenge, on days 23, 30, and 37, scratching behavior was counted for 20 min. Bars indicate standard errors. *P < 0.002, OVA-sensitized mice treated with AqMOL at low or high dose versus OVA-sensitized mice treated with distilled water by two-way repeated measures ANOVA*

In the PCA reaction model, AqMOL reduced Evans blue levels in the ears of anti-DNP IgE injected mice, although the reduction was not statistically significant (Table 2). This is a passively sensitized model, that can be evaluated by the anti-DNP-specific IgE-dependent mast cell activation, followed by the release of chemical mediators including histamine. The mast cell activation also causes an increase of vascular permeability [25,26]. Thus, AqMOL might mildly interfere with the specific IgE-dependent mast cell activation and/or the increase of vascular permeability. It is possible that the mast cell activation through specific IgE was initially reduced by AqMOL.

AqMOL at 300 mg/kg was orally administered by gavage to mice three times daily in the compound 48/80 stimulation and PCA reaction models. Based on our previous study [10], we used 300 mg/kg of AqMOL as a biologically active dose for mice without toxicity. Thus, in these models, the dosage of AqMOL was 900 mg/kg/day. However, in the OVA sensitization model, AqMOL at 0.6 or 6 mg/ml was continuously ingested *ad libitum* by mice. The consumption of AqMOL per mouse was similar to the amount of distilled water (5.0 ml/day). Thus, the daily consumption of AqMOL at 0.6 or 6 mg/ml per 20 g mouse may be deduced to be 150 or 1500 mg/kg/day, respectively, in the OVA sensitization model. The dosages were not very different from that of the 900 mg/kg used in the compound 48/80 stimulation and PCA reaction models and were significantly effective in reducing scratching numbers in the OVA sensitization model (Fig. 2). Therefore, the daily *ad libitum* ingestion of AqMOL at 0.6 or 6 mg/ml might correspond to the range of biologically active dosages.

The OVA sensitization model mimics naturally occurring allergy and involves a process from IgE production to IgE-mediated mast cell activation, followed by increased vascular permeability caused by mast cell mediators. In contrast to the two previous models, the OVA sensitization model involves OVA-specific IgE production *in vivo*. In our OVA sensitization model, oral administration of AqMOL was significantly effective in suppressing scratching numbers (Fig. 2). Thus, AqMOL was suggested to be effective in interfering with not only the process of mast cell activation but also IgE production. OVA-sensitized mice are often used for evaluation of anti-allergic activity, and some oral materials were previously shown to ameliorate the allergic symptoms and suppress total and/or OVA-specific IgE levels in the sera of OVA-sensitized mice [30-32]. In our model, repeated intranasal challenges of the immunized mice with OVA as an antigen significantly suppressed the production of OVA-specific IgE in the sera of OVA-sensitized mice but that of total IgE was not significantly suppressed (Table 3). AqMOL might not affect the overall humoral immunity in the OVA-sensitized mice. The release of histamine was also suppressed by AqMOL, although the suppression was not statistically significant (Table 3). It is possible that the reduction of histamine release resulted mainly from the reduction of OVA-specific IgE levels due to the administration of AqMOL. In the two other allergy models, AqMOL was suggested to be effective in reducing mast cell activation. Thus, AqMOL might be effective in reducing the activation of mast cells by the lower binding of OVA-specific IgE to mast cells. Alleviation of type I allergy symptoms by some bacterial products has been demonstrated to result in reduction of the IgE level in the serum due to a shift in the Th1/Th2 balance from a Th2- to a Th1-dominant state [32,33]. In our OVA sensitization model, AqMOL was significantly effective in reducing OVA-specific IgE production in the sera. AqMOL possibly reduced Th2 immune responses. Thus, AqMOL might be able to shift the Th1/Th2 balance toward Th1 dominance, resulting in a failure to reduce scratching frequency and OVA-specific IgE production. The elucidation of mode of anti-allergy action of AqMOL as a functional food is interesting and may contribute to the improvement of physical function and the alleviation of type 1 allergy.

5. CONCLUSION

Oral administration of AqMOL was shown to alleviate type I allergy symptoms in three kinds of allergy models (compound 48/80 stimulation, PCA reaction, and OVA sensitization models) in mice. AqMOL was suggested to alter the Th1/Th2 balance toward Th1 dominance in these allergy models in mice, resulting in the suppression of mast cell activation, followed by histamine release. AqMOL may be a useful functional food ingredient to alleviate type I allergies and expected to develop anti-allergic compounds included in AqMOL.

CONSENT

It is not applicable.

ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the animal experiment committee of Kyushu University of Health and Welfare, Japan (No. 26-1-11 and 27-1-30).

ACKNOWLEDGEMENTS

We thank Miss Chiemi Watanabe, Mr. Kyohei Michizaki, Mr. Masaharu Iwata, Miss Miu Suzuki, Mr. Tatsuya Yamashita, Miss Yu Imasato, Mr. Kohji Morita, Mr. Takuya Tanimura, Miss Kaori Nishizono, and Miss Yuhki Ichikawa for their great assistance in anti-allergic assays *in vivo* and some parts of ELISA. We thank Ms. Yukiko Shimoda and Ms. Yuko Yanagida for their excellent technical assistance and Ms. Katherine Ono for her editorial assistance. This study was supported by the Industry-Academia-Government Joint R&D Implementation Project of the Miyazaki Prefectural Industrial Support Foundation and partly Grants-in-Aid for Scientific Research (No. 25460183) from the Japan Society for the Promotion of Science.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Anwar F, Latif S, Ashraf M, Gilani AH. *Moringa oleifera*: A food plant with multiple medicinal uses. Phytother Res. 2007; 21(1):17-25.
2. Goyal BR, Agrawal BB, Goyal RK, Mehta AA. Phyto-pharmacology of *Moringa oeifera* Lam.ó. An overview. Nat Prod Rad. 2007;6:347-53.
3. Cáceres A, Cabrera O, Morales O, Mollinedo P, Mendia P. Pharmacological properties of *Morinnga oleifera*. 1: Preliminary screening for antimicrobial activity. J Ethnopharmacol. 1991;33(3): 213-216.
4. Ghasi S, Nwobodo E, Ofili JO. Hypocholesterolemic effects of crude extract of leaf of *Morinnga oleifera* Lam in high-fat diet fed Wistar rats. J Ethnopharmacol. 2000;69(1):21-25.
5. Tahiliani P, Kar A. Role of *Moringa oleifera* leaf extract in the regulation of thyroid hormone status in adult male and female rats. Pharmacol Res. 2000;41(3):319-323.
6. Lipipun V, Kurokawa M, Suttisri R, Taweechotipatr P, Pramyothin P, Hattori M, et al. Efficacy of Thai medicinal plant extracts against herpes simplex virus type 1 infection *in vitro* and *in vivo*. Antiviral Res. 2003;60(3):175-180.
7. Parvathy MVS, Umamaheshwari A. Cytotoxic effects of *Moringa oleifera* leaf extracts on human multiple myeloma cell lines. Trends in Medical Res. 2007;2(1): 44-50.
8. Sreelatha S, Padma PR. Antioxidant activity and total phenolic content of *Morinnga oleifera* leaves in two stages of maturity. Plant Foods Hum Nutr. 2009;64: 303-311.
9. Bahr HI, Farouk SM. Comparative ameliorative effect of basil oil and *Moringa oleifera* on lornoxicam-mediated histological and biochemical alterations in albino rat liver. J Vet Sci Tech. 2016;7(2): 1-11.
10. Kurokawa M, Wadhwani A, Kai H, Hidaka M, Yoshida H, Sugita C, et al. Activation of cellular immunity in Herpes Simplex virus type 1-infected mice by the oral administration of aqueous extract of *Moringa oleifera* Lam. leaves. Phytother Res; 2016.
DOI: 10.1002/ptr.5580
11. Metcalfe DD, Baram D, Mekori YA. Mast cells. Phys Rev. 1997;77(4):1033-79.
12. Hata D, Kawakami Y, Inagaki N, Lantz CS, Kitamura T, Khan WN, et al. Involvement of Bruton's tyrosine kinase in FcepsilonRI-dependent mast cell degranulation and cytokine production. J Exp Med. 1998; 187(8):1235-47.
13. Zhang T, Yang C, Rupa P, Jiang B, Mine Y. Inhibitory effects of *Quillaja saponin* on IgE-mediated degranulation of rat basophilic leukemia RBL-2H3 cells. J Funct Foods. 2012;4:864-71.
14. Takhar P, Smurthwaite L, Coker HA, Fear DJ, Banfield GK, Carr VA, et al. Allergen drives class switching to IgE in the nasal mucosa in allergic rhinitis. J Immunol. 2005;174(8):5024-32.
15. Hopkin JM. The rise of atopy and links to infection. Allergy. 2002;57(s72):5-9.
16. Prescott SL, Macaubas C, Smallacombe T, Holt BJ, Sly PD, Holt PG. Development of allergen-specific T-cell memory in atopic

and normal children. Lancet. 1999; 353(9148):196-200.
17. Shirakawa T, Enomoto T, Shimazu S, Hopkin JM. The inverse association between tuberculin responses and atopic disorder. Science. 1997;275(5296):77-9.
18. Takeda S, Hidaka M, Yoshida H, Takeshita M, Kikuchi Y, Tsend-Ayush C, et al. Anti-allergic activity of probiotics from Mongolian dairy products on type I allergy in mice and mode of anti-allergic action. J Funct Foods. 2014;9:60-9.
19. Kim SH, Choi CH, Kim SY, Eun JS, Shin TY. Anti-allergic effects of *Artemisia iwayomogi* on mast cell-mediated allergy model. Exp Biol Med. 2005;230(1):82-8.
20. Kim SH, Lee S, Kim IK, Kwon TK, Moon JY, Park WH, et al. Suppression of mast cell-mediated allergic reaction by *Amomum xanthiodes.* Food Chem Toxicol. 2007; 45(11):2138-44.
21. Kabu K, Yamasaki S, Kamimura D, Ito Y, Hasegawa A, Sato E, et al. Zinc is required for Fc epsilon RI-mediated mast cell activation. J Immunol. 2006;177(2):1296-305.
22. Lee JH, Kim JW, Ko NY, Mun SH, Her E, Kim BK, et al. Curcumin, a constituent of curry, suppresses IgE-mediated allergic response and mast cell activation at the level of Syk. J Allergy Clin Immunol. 2008;121(5):1225-31.
23. Tobita K, Yanaka H, Otani, H. Anti-allergic effects of *Lactobacillus crispatus* KT-11 strain on ovalbumin-sensitized BALB/c mice. Anim Sci J. 2010;81(6):699-705.
24. Kubes P, Granger DN. Leukocyte-endothelial cell interactions evoked by mast cells. Cardiovasc Res. 1996;32(4): 699-708.
25. Inagaki N, Goto S, Yamasaki M, Nagai H, Koda A. Studies on vascular permeability increasing factors involved in 48-hour homologous PCA in the mouse ear. Int Arch Allergy Appl Immunol. 1986;80(3): 285-90.
26. Inagaki N, Nagai H. Analysis of the mechanism for the development of allergic skin inflammation and the application for its treatment: Mouse models for the development of remedies for human allergic dermatitis. J Pharmacol Sci. 2009; 110(3):251-9.
27. Kumalasari ID, Nishia K, Harmayanib E, Raharjob S, Sugahara T. Effect of bengkoang (*Pachyrhizus erosus*) fiber extract on murine macrophage-like J774.1 cells and mouse peritoneal macrophages. J Funct Foods. 2013;5:582-9.
28. Kapsak WR, Rahavi EB, Childs NM, White C. Functional foods: Consumer attitudes, perceptions, and behaviors in a growing market. J Amer Diet Assoc. 2011;111(6): 804-10.
29. Li GZ, Chai OH, Song CH. Inhibitory effects of epigallocatechin gallate on compound 48/80-induced mast cell activation and passive cutaneous anaphylaxis. Exp Mol Med. 2005;37(4): 290-6.
30. Ishida Y, Bandou I, Kanzato H, Kanzato H, Yamamoto N. Decrease in ovalbumin specific IgE of mice serum after oral uptake of lactic acid bacteria. Biosci Biotechnol Biochem. 2003;67:951-7.
31. Murosaki S, Yamamoto Y, Ito K, Inokuchi T, Kusaka H, Ikeda H, et al. Heat-killed *Lactobacillus plantarum* L-137 suppresses naturally fed antigen-specific IgE production by stimulation of IL-12 production in mice. J Allergy Clin Immunol. 1998;102(1):57-64.
32. Segawa S, Nakakita Y, Takata Y, Wakita Y, Kaneko T, Kaneda H, et al. Effect of oral administration of heat-killed *Lactobacillus brevis* SBC8803 on total and ovalbumin-specific immunoglobulin E production through the improvement of Th1/Th2 balance. Int J Foof Microbiol. 2008;121(1): 1-10.
33. Fujiwara D, Inoue S, Wakabayashi H, Fujii T. The anti-allergic effects of lactic acid bacteria are strain dependent and mediated by effects on both Th1/Th2 cytokine expression and balance. Int Arch Allergy Immunol. 2004;135(3):205-15.

Peer-review history:
The peer review history for this paper can be accessed here:
http://sciencedomain.org/review-history/16171