Original Research Article

Korean J. Plant Res. 24(3) : 324～329 (2011)

Inhibitory Effects of the Extracts from *Eleutherococcus senticosus* Maxim. on Histamine-release from Rat's Mast Cell

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Abstract - *Eleutherococcus senticosus* Maxim. has been successfully used as an oriental medicine for various diseases including allergic disorders. Histamine is a major factor on various allergic responses and it is reported that histamine was released from mast cells by sensitization of allergens. In this study, ethanol extracts from *E. senticosus* Maxim. were prepared and the composition was analyzed by high performance liquid chromatography. The eleutheroside B as a primary effective component of *E. senticosus* was contained approximately 225 mg/kg in root bark extracts. The extracts were found to significantly inhibit compound 48/80-induced histamine release form mast cells in dose dependent manner. However the extracts had low cytotoxicity on the mast cells with MTT assay. These results showed that *E. senticosus* Maxim. extracts may be the effective materials on inflammatory disorders.

Key words - *Eleutherococcus senticosus*, Histamine, Mast cell, Eleutheroside, Root bark

Introduction

Eleutherococcus senticosus Maxim. (Araliaceae; also called Siberian Ginseng) has been used as a rough drug to treatment of various physiological disorders (Nishibe *et al.*, 1990; Fujikawa *et al.*, 2005). The leaves or stems of *E. senticosus* have also been used clinically for treatment of various allergic diseases in Asia. But, it is still unclear the action mechanism, and a few studies documented the anti-inflammatory effects of *E. senticosus* extract using *in vitro* (Jung *et al.*, 2003; Tokiwa *et al.*, 2006). On the other hand, the eleutherosides were well identified as a primary contents of *E. senticosus* Maxim, and these have been shown to be responsible for the adaptogenic properties of this plant (Hikino *et al.*, 1986). Syringin and its alycone (eleutherosides B) are widely regarded as two important substances of the eleutheroside group (Niu *et al.*, 2008).

Atopic dermatitis (AD) is a chronic inflammatory skin disease with a wide range of severity, and is usually the first phenomenons of atopic disease. Expression of AD results from a complex interaction of environmental factors, defects of skin barrier, and immune disorders (Dubrac *et al.*, 2010). It is one of the most common skin disorders in developed countries, affecting approximately 20% of children and 1-3% of adults (Simpson, 2010). AD is generally reported to be a model of typical type 2 helper T cell (Th2)-mediated disorders and has been consistently shown that skin-infiltrating Th2 chemokines producing cells (Nakazato *et al.*, 2008).

Mast cells have been reported major role in the development of many physiologic changes during allergic responses including of atopic dermatitis (Kim and Lee, 1999). Mast cells activations were induces degranulation and the external release of granule-associated stored mediators such as histamine, neutral proteases, acid hydrolyses, proteoglycans, chemotactic factors, cytokines, etc (Metcalfe *et al.*, 1981). Especially, histamine is the well-identified and most potent vasoactive mediator implicated in the acute phase of immediate hypesensitivity (Petersen *et al.*, 1996), therefore regulation of histamine release from mast cells were given a methods for amelioration of allergic disorders. Compound 48/80 has been used as a direct and convenient

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reagent to study the mechanism of mast cell activation-mediated allergic disorder like anaphylaxis (Kim *et al.*, 1998a). In the present study, we examined the effects of the extracts of *E. senticosus* Maxim. on mast cell activation-mediated histamine releases.

Materials and Methods

Reagents

Compound 48/80, Hank's bufferd salt solution (HBSS), Dulbecco's modified eagle's medium (DMEM), MTT (3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide) and other all reagents were obtained from Sigma-aldrich (St Louis, USA).

Extraction of plant materials

Dried powders of root barks, stem barks and leaves (10 g) were extracted with 70% ethanol (500 mL) in 80℃, refluxed for 1 h, twice, and filtered through filter paper (Advantec, Toyo, Japan). The ethyl alcohol extracts were evaporated to dryness and dissolved in 50 ml of HPLC grade water. The water fraction was washed two times with the same volume of ether. The water fraction was evaporated to dryness and dissolved in 5 ml of 70% ethanol and filtered through 0.45 μm PTEF filter (Gelman, USA).

Animals

Male Sprague-Dawley 6-week-old rats were purchased from Orient Bio (Sungnam, Korea), were acclimated to the facility for 5 d and fed and ad libitum with regular chow Animals were maintained in an environmentally controlled rearing system and used for experiments.

Isolation of peritoneal mast cells

Rat peritoneal mast cells were isolated from adult female Sprague-Dawley rats, 170-200 g, by the modified method of Kim *et al.* (1998b). Briefly, after intraperitoneal injection of 10 ml of cold DMEM with 5 unit/ml of heparin, the abdomen was gently massaged for 2 min. Mast cell suspension was carefully aspirated and centrifuged at 400 ×g for 10 min. Mast cells were purified by Percoll density gradient according to the method of Hachisuka *et al.* (1988). Purified mast cells were washed twice with HBSS containing 1 mg/ml bovine serum albumin.

Measurement of Cytotoxicity

Cytotoxicity was assayed by MTT assay (Mosmann, 1983). Mast cells (100 μl as 2×10^4 cells/ml) were incubated in 96 well plate (SPL Laboratory Co, Pocheon, Korea) for 1 h and treated extracts with various doses for 1 h in CO_2 incubator. After the cells were incubated in PBS containing 0.5 mg/ml MTT for 3 h at 37℃, they were centrifuged at 1,500 × g for 10 min to recover of cells. Cells were dissolved with dimethylsulfoxide (DMSO) for 20 min and the absorbances were read at 570 nm with multiwell plated reader (Molecular devices, USA).

Histamine release by compound 48/80 and histamine assay

The mast cell suspension was divided into three parts, negative control, compound 48/80 group, and compound 48/80 + extracts, each containing 0.1 ml of cell suspension and incubated in CO_2 incubator for 1 h. Then, 0.1 ml of compound 48/80 (12 μg/ml) was added to all the samples except negative control and the suspensions were further incubated for 15 min at 37℃. After centrifugation at 15,000 × g for 10 min, the supernatants were collected and cold-dried (Speed-Vac: Servant, USA), and re-suspended with 100 μl of PBS for histamine assay. Enzyme linked immunosorbent assay kit for histamine was obtained from ALPCO diagnostics (Salem, NH, USA) and used according to the manufacturer's instruction.

HPLC Analysis

The eleutherosides fraction was analyzed by using HPLC system (Waters 2690 separation module; Waters 996 photodiode array detector, Waters millenium 2010 chromatography manager, Milipore Inc, Milford, MA, USA), on a NovaPak C18 column (3.9 X 150 mm, 4 μm), with water and acetonitrile. The rate of water and acetonitrile for initial, 10, 30, 40, 45, 46, and 50 min, were 95:5, 90:10, 60:40, 50:50, 45:55, 95:5, and 95:5, respectively. Flow rate of the mobile phase was 0.8 ml/min and monitoring of eleutherosdies was 220 nm. The authentic eleutheroside B and E were purchased from Chromadex Inc. (CA, USA).

Statistical Analysis

Data are expressed as mean S.E.M. Statistical comparisons were performed using oneway ANOVA. Significant differences between groups were determined using the unpaired Student's *t* test. Statistical significance was set at $p<0.05$.

Results and Discussion

Analysis of active substance from *Eleutherococcus senticosus* extracts

Analysis of active substance from *E. senticosus* extracts was carried out by HPLC. Table 1 shows the contents of eleutheroside B and E, primary effective substances of *E. senticosus* in extracts from various plant parts (root bark, stem bark, and leaf). The amounts of eleutheroside B in extracts of root bark, stem bark, and leaf were 225 ± 12.4, 85.8 ± 8.2, and 45.6 ± 5.6 mg/kg raw materials, respectively. The amounts of eleutheroside E, the other effective substance of *E. senticosus*, were 378 ± 28.6, 388 ± 35.3, and 406 ± 38.6 mg/kg raw materials from root bark, stem bark, and leaf, respectively (Table 1). Especially, extracts from root bark of *E. senticosus* contained significantly high amount of eleutheroside B, compared to stem bark, and leaf extracts. However, no differences in eleutheroside E contents between extracts of both compartments was observed. Therefore, we chose root bark extracts and used them for further experiments.

Cytotoxicity of *E. senticosus* extracts on mast cells

The cytotoxicity of *E. senticosus* extracts on rat peritoneal mast cells was evaluated by MTT assay (Fig. 1). When cells were treated with 0.06 to 2 mg/ml of extracts which is relatively high concentration, 1 mg/ml of extract was induced decrease of viability to 79.2 ± 2.9%. Indeed, when treated with 2 mg/ml of extract, the viability was decreased to 61.5 ± 1.3%. However, no significant difference in their cytotoxicity was observed when cells were treated with 0.5 mg/ml extract and lower concentration. Therefore we decided safe dosage to 0.125 mg/ml of extract, which is maintained >90% viability, for further experiments.

Table 1. Eleutheroside contents of root barks, stem barks and leaves in *E. senticosus*.

Source	Eleutheroside (mg/kg)	
	B	E
Root bark	225 ± 12.4†	378 ± 28.6
Stem bark	85.8 ± 8.2	388 ± 35.3
Leaf	45.6 ± 5.6	406 ± 38.6

†Data represent the mean values ± S.E.M of three independent experiments.

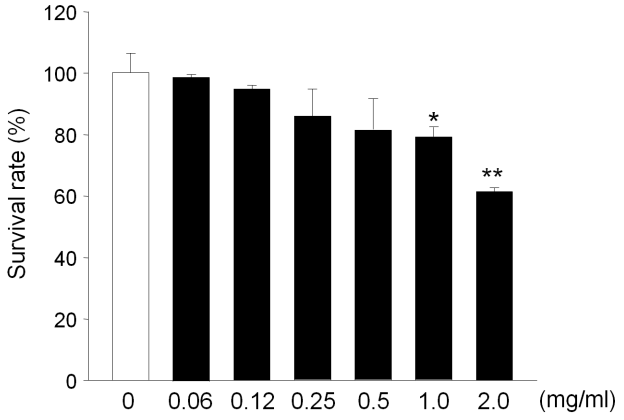

Fig. 1. Cytotoxicity of *E. senticosus* extracts. Mast cells (100 μl as 2×10^4 cells/ml) were incubated in 96 well plate for 1 h and treated extracts with various doses for 1 h in CO_2 incubator. After the cells were incubated in PBS containing 0.5 mg/ml MTT for 3 h at 37℃, they were centrifuged at 1,500 × g for 10 min to recover of cells. Cells were dissolved with DMSO for 20 min and the absorbances were read at 570 nm with multiwell plated reader. Data are expressed as a mean ± S.E.M from three independet experiments. *$p<0.05$ and *$p<0.01$ versus non-treated group.

Inhibition of compound 48/80-induced histamine release *Eleutherococcus senticosus* extracts

We next evaluated the effect of *E. senticosus* extracts on histamine release from mast cells (Fig. 2). Mast cells were pretreated with extracts (0.125 mg/ml) or HBSS for 15 min and stimulated with compound 48/80 (final concentration: 6 μg/ml). When mast cells were stimulated with compound 48/80, histamine releases were increased 27.4 ± 1.5% then negative control group. However pretreatment of *E. senticosus* extracts to mast cell completely reduced compound 48/80-induced histamine release from mast cells. Indeed, *E. senticosus*

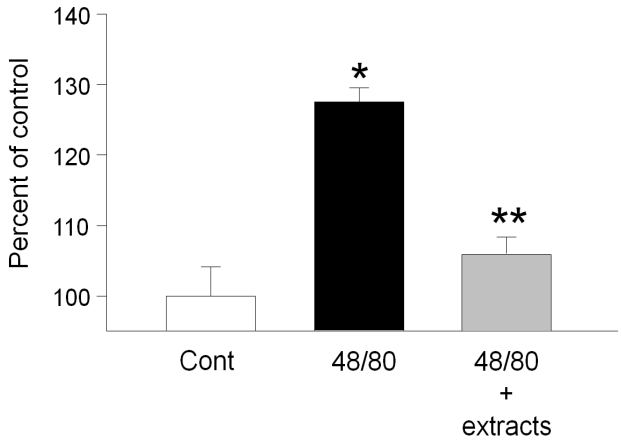

Fig. 2. *E. senticosus* extracts were reduced compound 48/80-induced histamine release from mast cells. The mast cell suspension was divided (100 μl as 2×10^4 cells/ml) and incubated for 1 h, After treatment with presence or absence of extracts (0.125 mg/ml) for 15 min in CO_2 incubator, 0.1 ml of compound 48/80 (12 μg/ml) was added to all the samples except negative control and the suspensions were further incubated for 15 min at 37℃. After centrifugation at 15,000 × g for 10 min, the supernatants were collected and cold-dried. Re-suspended samples were measured histamine contents. Data are expressed as a mean ± S.E.M from three independet experiments. *$p<0.05$ and *$p<0.01$ versus non-treated group.

extracts did not induce histamine releases (data not shown). These results suggest that *E. senticosus* extracts have a preventive effects of mast cells activation.

Discussion

E. senticosus is known to contain several substances such as acanthosides, eleutherosides, chiisanoside, senticoside, triterpenic saponin, syringin, flavone, vitamins and minerals, and they are responsible for many biological activities (Davydov and Krikorian, 2000). Indeed, some researchers examined ameliorative effects of whole extracts from root, stem, and leaf of *E. senticosus* (Yi *et al.*, 2001, 2002) or cultured plants (Jeong *et al.*, 2001) against allergic responses or allergic diseases, but did not observe protective effect against histamine release from mast cells.

Cytotoxicity tests with *in vitro* condition are important for the evaluation of biosafety of biometerials or medicines (Mosmann, 1983). In this study, we pre-treated the extracts with 0.125 mg/ml to mast cells because it maintained higher cell viability (Fig. 1), and these results were similar to other reports with various models (Jang *et al.*, 2003; Lin *et al.*, 2007, 2008).

Eleutheroside B and E as primary substances of *E. senticosus* were analyzed in present study. The contents of these biological substances in extracts were dependent on species of e*leutherococcus,* part of plants, and extraction methods (Kim *et al.*, 1996; Kim *et al.*, 2000; Lee *et al.*, 2005). In present study, eleutheroside B, but not eleutheroside E, showed different content between plant parts of the *E. senticosus* (Table 1). Eleutheroside B was highly present in the root bark extract but not stem bark and leaf extracts. Therefore we used root bark extracts for present study because contained high eleutheroside B from root bark. Indeed, many previous reports were also used root part for their experiments (Hikino *et al.*, 1986; Niu *et al.*, 2008; Davydov and Krikorian, 2000), and suggested that eleutheroside B in root bark has crucial role in various physiological disorder. Eleutheroside B is major member of effective substances from *E.* senticosus (Niu *et al.*, 2008) but has not reported the effects to the allergic disorders such as atopic dermatitis, asthma. Therefore, further studies are required to fully understand the recovery effects of eleutheroside B on *in vivo* and *in vitro* with allergic disorder model such as activated mast cells or atopic dermatitis mice.

E. senticosus extracts contained many substances including eleutheroside B and E, and showed prevention effects of compound 48/80-induced rat mast cells activation. These extracts were non-toxic in specific dosage on mast cells (Fig 1), and completely reduced compound 48/80-induced histamine releases from mast cells (Fig 2). These results suggested that *E. senticosus* extracts are useful materials for managing of immune disorder.

Acknowledgement

This study was financially supported by Jeonnam Provincial University, 2011.

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(Received 10 May 2011 ; Accepted 19 June 2011)