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Inhibitory Effects of Herbal Extracts on CINC-1 Induction in LPS-Stimulated Rat Kidney Epithelioid NRK-52E cells

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Abstract – A rat chemokine, cytokine-induced neutrophil chemoattractant-1 (CINC-1) has chemotactic and activating properties to neutrophils. Rat kidney epithelioid NRK-52E cells contained 4 ng/ml of CINC-1 as a basal level and their CINC-1 production was significantly increased by stimulation with lipopolysaccharide (LPS) of *E. coli*. Maximal induction of CINC-1 was 58 ng/ml when 3 μg/ml of LPS was treated to the NRK-52E cells. Inhibitory effects on CINC-1 induction in LPS-stimulated NRK-52E cells by extracts prepared from herbal medicines and wild plants in Korea were analyzed. At the final concentration of 100 μg/ml, 9 species out of 304 species of herbal extracts exhibited more than 50% of inhibition on the CINC-1 induction. The active extracts prepared from *Artemisia argyi, Lythrum salicaria, Machilus thunbergii, Magnolia sieboldii, Nelumbo nucifera, Prunus persica, Rubus coreanus, Sanguisorba officinalis,* and *Tripterygium regelii* have been sequentially fractionated to obtain methylene chloride, ethyl acetate, butanol, and aqueous layers. Among solvent fractions of the active herbal extracts, methylene chloride fractions of *Artemisia argyi* and *Magnolia sieboldii* exhibited the highest inhibitory effects on CINC-1 induction in LPS-stimulated NRK-52E cells.

Keywords – CINC-1 induction, herbal extracts, *Artemisia argyi, Magnolia sieboldii.*

Introduction

Inflammation is a localized host-defensive response which serves to destroy or dilute both injurious agent and injured tissue. In the inflammatory reactions, chemical mediators increase vascular permeability, augment adherence of circulating leukocytes to vascular endothelium, promote migration of leukocytes into tissues, and stimulate leukocytes to destroy the inciting agent. The accumulation and activation of leukocytes are central events in imflammatory pathogenesis. A variety of mediators known as chemoattractants can migrate leukocytes from the circulation to inflamed site, and activate the locally accumulated leukocytes. As the chemoattractants, leukotriene B_4, platelet-activating factor, formyl-methionyl-leucyl-phenylalanine, and chemokines are well known (Snyderman and Uhling, 1992).

Human chemokines can be classified into CXC-type and CC-type chemokines (Schall, 1994). CXC-type chemokines stimulate neutrophils to be migrated and activated, and CC-type chemokines induce the migration of monocytes but not neutrophils (Baggiolini *et al.*, 1992). CINC-1 is a rat chemokine identified and purified originally in culture supernatants of IL-1β-stimulated rat kidney

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epithelioid NRK-52E cells (Watanabe *et al.*, 1989). Rat chemokines of CINC-2α, CINC-2β, and CINC-3 were later indentified by cDNA analysis of LPS-stimulated rat peritoneal macrophages (Nakagawa *et al.*, 1994). All members of CINC family are structually related to human CXC-type chemokines, and can attract and activate neutrophils in human and rat (Watanabe *et al.*, 1991). In calcium influx and chemotaxis assays, rat neutrophils seem to have two different receptors mediating the biological activities of CINC family, one responding to all memebers of CINC family and the other to CINC-3 only (Shibata *et al.*, 1995).

As a part of our screening study to identify anti-inflammatory agents, inhibitory effects on CINC-1 induction in LPS-stimulated NRK-52E cells by 304 kinds of herbal extracts prepared from herbal medicine and wild plant in Korea have been estimated in this study. Nine species of the herbal extracts exhibited more than 50% of inhibition on the CINC-1 induction at their final concentration of 100 μg/ml. The active extracts were subjected to sequential fractionations with methylene chloride, ethyl acetate, butanol, and aqueous residue. Among solvent fractions of 9 active extracts, methylene chloride fractions of *Artemisia argyi*, and *Magnolia sieboldii* exhibited the highest inhibitory effects on LPS-induced CINC-1 production in NRK-52E cells.

Experimental

Materials – Dulbecco's modified Eagle's medium, bovine serum albumin (BSA), hydrogen peroxide, and Tween-20 were purchased from Sigma Chemical Co., and *o*-phenylenediamine, penicillin G potassium, and streptomycin sulfate from Wako Chemical Ind. Ltd. LPS of *E. coli* 026:B6 was purchased from Difco Lab., fetal bovine serum (FBS) from Gibco Lab., and streptavidin-horseradish peroxidase from PIERCE.

Herbal extracts – Herbal medicines were purchased from a local store, and wild plants were collected at the hills and mountains in Korea. The plants were taxonomically identified with respect to morphology. Each of the plants was sliced, and then extracted twice with 80% methyl alcohol (MeOH) at room temperature. The extract solution was evaporated under reduced pressure at 50℃, and then subjected to lyophilization to be dried. Some of the MeOH extracts were sequentially fractionated to obtain methylene chloride, ethyl acetate, *n*-butanol, and aqueous layers. The solvent fractions were subjected to evaporation under reduced pressure followed by lyophilization. The dried total MeOH extracts and solvent-fractionated extracts were used as samples.

Culture of NRK-52E cells – Rat kidney epithelioid NRK-52E cells were grown in DMEM (13.4 mg/ml Dulbecco's modified Eagle's medium, 24 mM $NaHCO_3$, 10 mM HEPES, 143 units/ml penicillin G potassium, 100 μg/ml streptomycin sulfate, pH 7.1) containing 10% FBS at 37℃ with 5% CO_2. When NRK-52E cells were confluent, the cells were detached from a culture dish (Falcon) with PBS buffer (0.2 M NaCl, 2.7 mM KCl, 10 mM NaH_2PO_4, 1.8 mM K_2HPO_4) containing 0.25% trypsin and 0.02% EDTA, and then centrifuged at 250×g for 10 min after 10-fold dilution with DMEM containing 10% FBS. After washing once with DMEM, NRK-52E cells were resuspended in DMEM containing 10% FBS, and counted their numbers after trypan exclusion. Ten ml of 5×10^4 cells/ml was dispensed to a culture dish with diameter 100 mm for passage of NRK-52E cells.

LPS stimulation and sample treatment – NRK-52E cells were dispensed to 1×10^4 cells per well of a 96-well culture plate (Falcon) and incubated at 37℃ with 5% CO_2 for 48 hr. After washing once with DMEM containing 0.1% BSA, NRK-52E cells were treated with 100 μl of 2 μg/ml of LPS and

100 μl of designated concentration of each sample, and then incubated at 37℃ with 5% CO_2 for 24 hr. Control group was treated with LPS only, and blank group with DMEM containing 0.1% BSA only. LPS was dissolved in DMEM containing 0.1% BSA. Samples were dissolved in 100% dimethyl sulfoxide, and then serially diluted with DMEM containing 0.1% BSA to have less than 0.1% of dimethyl sulfoxide when samples were treated to NRK-52E cells.

CINC-1 quantitation by ELISA – NRK-52E cells were centrifuged at 500×g at 4℃ for 30 min, and their supernatants were subjected to a sandwich ELISA. A 96-well assay plate (Falcon) was coated with 0.4 μg of polyclonal rabbit anti-CINC-1 per well, and incubated at 37℃ for 3 hr. After washing three times with PBS buffer containing 0.05 % Tween-20, the assay plate was added with 200 μl of 1% BSA per well, and incubated at 4℃ for overnight. The assay plate was washed three times with PBS buffer containing 0.05% Tween-20, added with 80 μl of the culture supernatant per well, and incubated at 37℃ for 1 hr. After washing three times with DMEM containing 0.05 % Tween-20, the assay plate was added with 80 μl of 1 mg/ml of biotinylated anti-CINC-1 IgG per well, and incubated at 37℃ for 1 hr. The assay plate was washed three times with PBS buffer containing 0.05% Tween-20, added with 80 μl per well of 10,000 -fold diluted streptavidin-horseradish peroxidase, and incubated at 37℃ for 30 min. After washing extensively with PBS buffer containing 0.05% Tween-20, 100 μl of a substrate solution (1% *o*-phenylenediamine, 0.02% hydrogen peroxide, 50 mM sodium citrate, 100 mM phosphate buffer, pH 5) was added to each well of the assay plate. After incubation at room temperature for 10 to 30 min, 100 μl of 4 N H_2SO_4 was added to each well of the assay plate, and then subjected to measurement of absorbance at wavelength 492 nm (A_{492}) by using a microplate reader.

Statistics – Inhibitory effect by sample was expressed as % of inhibition, [1-(sample A_{492}-blank A_{492})/(control A_{492}-blank A_{492})]×100. Data were represented as mean ±standard error (n=4), and their significances compared with the control were analyzed by Student's t-test.

Results and Discusssion

Chemokines are usually found as basal level in normal state but its production and secretion are amplified and increased in inflamed state (Baggiolini *et al.*, 1992). When inflammatory stimuli are exposed, a variety of cells are known to increase chemokine production. As an inflammatory stimulus, LPS induces chemokine production in phagocytes and endothelial cells but not in tissue cells (Baggiolini *et al.*, 1992). A rat chemokine, CINC-1 is known to be induced in IL-1β, TNF or LPS-stimulated NRK-52E cells, IL-1 or TNF-stimulated rat fibroblast NRK-49F cells, and LPS-stimulated rat peritoneal macrophages (Watanabe *et al.*, 1990; Nakagawa *et al.*, 1993; Lee *et al.*, 1995).

The CINC-1 was originally indentified as a neutrophil chemoattractant in culture supernatants of IL-1β-stimulated NRK-52E cells, and thus designated as cytokine-induced neutrophil chemoattractant (Watanabe *et al.*, 1989). CINC-1 induction in LPS-stimulated NRK-52E cells was analyzed by a sensitive ELISA (Fig. 1). Rat kidney epithelioid NRK-52E cells contained about 4 ng/ml of CINC-1 as a basal level. When LPS with 0.01 μg/ml to 10 μg/ml as the final concentration was treated, NRK-52E cells significantly increased CINC-1 production, and amount of CINC-1 induced was dose-dependent of LPS treated. Maximal CINC-1 induction with 58 ng/ml was obtained when 3 μg/ml of LPS was treated to NRK-52E cells. Compared with NRK-52E cells stimulated with 3 μg/ml of LPS, CINC-1 production seems to be decreased when the cells were treated with 10 μg/ml of LPS. This result would be specul-

ated as a desensitization by excess amount of LPS. This kind of desensitization is also identified in LPS-induced CINC-1 production in rat peritoneal macrophages (Min *et al.*, 1996). Morphology and viability of NRK-52E cells were not changed when LPS was treated.

Steroidal anti-inflammatory drugs including dexamethasone are known to suppress CINC-1 induction in LPS-stimulated rat peritoneal macrophages or in IL-1-stimulated NRK-49F cells (Nakagawa *et al.*, 1993; Lee *et al.*, 1995; Min *et al.*, 1996). CINC-1 induction in LPS-stimulated NRK-52E cells was significantly suppressed by dexamethasone (Fig. 1). Dexamethasone with 1 μM as the final concentration completely inhibited CINC-1 induction in NRK-52E cells stimulated with 0.01 μg/ml to 0.03 μg/ml of LPS. Approximately 50% of CINC-1 production in NRK-52E cells stimulated with 0.1 μg/ml to 10 μg/ml of LPS was inhibited by dexamethasone with 1 μM as the final concentration. Dexamethasone is known to implicate in gene regulation of cytokines and their receptors, inhibiting the production of IL-1, IL-2, IL-3, IL-6, IL-8, TNF or interferon-γ, and enhancing that of IL-1 or IL-6 receptor (Goldstein *et al.*, 1992). Dexamethasone is known to inhibit IL-1-induced IL-8 production in human fibrosarcoma at transcription level (Mukaida *et al.*, 1992). Therefore, inhibitory effect of dexamethasone on LPS-induced CINC-1 production in NRK-52 cells would be attributed to downregulation of CINC-1 gene.

As a part of our screening study of anti-inflammatory agents from natural products, inhibitory effects of herbal extracts on LPS-induced CINC-1 production in NRK-52E cells were estimated (Table 1). Among 304 kinds of herbal extracts, 9 samples with 100 μg/ml as the final concentration exhibited more than 50% of inhibition on the CINC-1 produ-

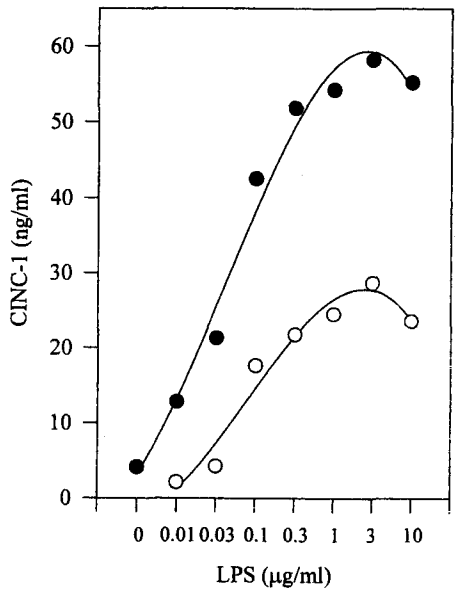

Fig. 1. CINC-1 induction in LPS-stimulated NRK-52E cells and its suppression by dexamethasone. Rat kidney epithelioid NRK-52E cells were stimulated with LPS only (filled circle) or LPS plus 1 μM of dexamethasone (open circle), and CINC-1 amount in the culture supernatants was analyzed by a sensitive ELISA.

Table 1. Inhibitory effects on CINC-1 induction by total MeOH extracts

Plant (part of use)	Inhibition (%)
Acanthopanax sessiliflorus (cortex)	<0
Acanthopanax sessiliflorus (aerial part)	<0
Acer ginnala (aerial part)	<0
Aconitum koreanum (whole plant)	5±1
Acorus gramineus (rhizoma)	<0
Actaea asiatica (aerial part)	<0
Actinidia polygama (whole plant)	<0
Adenophora triphylla var. *tetraphylla* (radix)	12±2
Aeschynomeme indica (whole plant)	<0
Agastache rugosa (herba)	25±2
Agrimonia pilosa (herba)	29±1

Table 1. Continued.

Plant (part of use)	Inhibition (%)
Ainsliaea acerifolia (whole plant)	<0
Albizzia julibrissin (cortex)	<0
Alisma orientale (rhizoma)	12±1
Alpinia oxyphylla (fruit)	<0
Ambrosia artemisiifolia var. *elatior* (aerial part)	12±6
Ambrosia artemisiifolia var. *elatior* (underground part)	23±8
Amomum cardamomum (fruit)	9±1
Amomum tsao-ko (fruit)	<0
Amomum villosum (semen)	<0
Ampelopsis brevipedunculata var. *heterophylla* (aerial part)	<0
Anemarrhena asphodeloides (rhizoma)	34±9
Angelica dahurica (aerial part)	<0
Angelica dahurica (radix)	12±2
Angelica decursiva (whole plant)	<0
Angelica gigas (aerial part)	<0
Angelica gigas (radix)	10±5
Angelica koreana (aerial part)	7±5
Angelica koreana (radix)	11±1
Angelica sieboldii (whole plant)	<0
Aquilaria agallocha (lignum)	<0
Aralia continentalis (radix)	17±7
Aralia elata (aerial part)	5±3
Areca catechu (pericarpium)	7±3
Areca catechu (semen)	21±2
Arisaema consanguineum (rhizoma)	<0
Aristolochia contorta (aerial part)	<0
Artemisia argyi (folium)	65±2
Artemisia capillaris (aerial part)	38±2
Artemisia capillaris (herba)	<0
Artemisia iwayomogi (aerial part)	33±4
Artemisia japonica (aerial part)	28±4
Artemisia montana (aerial part)	37±2
Artemisia princeps (aerial part)	41±3
Artemisia sieversiana (aerial part)	<0
Arundinella hirta (aerial part)	<0
Asiasarum sieboldii (radix)	7±3
Asparagus cochinchinensis (radix)	9±2
Aster scaber (whole plant)	<0
Aster yomena (aerial part)	<0
Astragalus membranaceus (aerial part)	<0
Astragalus membranaceus (radix)	<0
Atractylodes japonica (rhizoma)	7±2
Benincasa hispida (semen)	<0
Betula davurica (aerial part)	<0
Betula schmidtii (aerial part)	<0
Bidens bipinnata (aerial part)	<0
Biota orientalis (semen)	24±1
Boehmeria nivea (aerial part)	<0
Boehmeria sieboldiana (aerial part)	<0
Boehmeria spicata (aerial part)	<0

Table 1. Continued.

Plant (part of use)	Inhibition (%)
Boehmeria tricuspis (underground part)	<0
Boswellia carterii (resin)	6±4
Brassica alba (semen)	<0
Calystegia soldanella (whole plant)	11±3
Cannabis sativa (aerial part)	17±2
Carduus crispus (aerial part)	<0
Carex kobomugi (aerial part)	<0
Carex kobomugi (underground part)	<0
Carex maackii (whole plant)	<0
Carex siderosticta (whole plant)	<0
Carthamus tinctorius (flower)	8±1
Celastrus flagellaris (aerial part)	17±1
Celastrus orbiculatus (aerial part)	6±2
Celastrus stephanotifolius (aerial part)	<0
Chenopodium virgatum (whole plant)	<0
Chenopodium album var. *centrorubrum* (aerial part)	<0
Chenopodium glaucum (aerial part)	<0
Chrysanthemum indicum (flower)	32±3
Cimicifuga heracleifolia (aerial part)	<0
Cimicifuga heracleifolia (rhizoma)	2±5
Cinnamomum cassia (cortex)	<0
Cirsium pendulum (aerial part)	<0
Cistanche salsa (herba)	17±3
Citrus aurantium var. *tachibana* (pericarpium)	<0
Clematis apiifolia (aerial part)	<0
Clematis chinensis (radix)	9±2
Clematis mandshurica (aerial part)	7±2
Clerodendron trichotomum (aerial part)	<0
Clinopodium chinense (aerial part)	<0
Cnidium officinale (rhizoma)	<0
Coix lachryma-jobi var. *ma-yuen* (semen)	<0
Commelina communis (whole plant)	24±3
Commiphora molmol (resin)	<0
Convallaria keiskei (aerial part)	<0
Coptis chinensis (rhizoma)	6±2
Cornus officinalis (fruit)	14±2
Corydalis speciosa (aerial part)	<0
Corydalis yanhusuo (tuber)	<0
Corylus heterophylla var. *thunbergii* (aerial part)	<0
Crataegus pinnatifida (fruit)	25±2
Croton tiglium (semen)	<0
Curcuma zedoaria (rhizoma)	<0
Cuscuta japonica (aerial part)	<0
Cynomorium songaricum (herba)	49±2
Cyperus amuricus (underground part)	<0
Cyperus rotundus (rhizoma)	12±1
Desmodium oldhami (aerial part)	<0
Desmodium oxyphyllum (aerial part)	<0
Deutzia parviflora (aerial part)	<0
Dianthus sinensis (aerial part)	<0
Dioscorea batatas (radix)	16±3

Table 1. Continued.

Plant (part of use)	Inhibition (%)
Dolichos lablab (semen)	28±2
Dryopteris crassirhizoma (aerial part)	<0
Echinochloa crus-galli var. *frumentacea* (aerial part)	<0
Elaeagnus umbellata (aerial part)	<0
Ephedra sinica (herba)	25±2
Epimedium grandiflorum (herba)	10±2
Equisetum arvense (whole plant)	<0
Erigerom annuus (whole plant)	<0
Erigerom canadensis (whole plant)	<0
Eriobotrya japonica (folium)	<0
Eucommia ulmoides (cortex)	<0
Eugenia caryophyllata (flower)	20±4
Euonymus alatus (aerial part)	<0
Euonymus planipes (aerial part)	<0
Eupatorium chinensis var. *simplicifolium* (whole plant)	47±1
Eupatorium lindleyanum (aerial part)	45±4
Euphorbia kansui (radix)	<0
Euphorbia pekinensis (radix)	<0
Euphorbia supina (whole plant)	<0
Euphoria longana (fruit)	21±2
Evodia officinalis (fruit)	19±1
Foeniculum vulgare (fruit)	28±4
Forsythia viridissima (fruit)	11±2
Fraxinus rhynchophylla (aerial part)	<0
Fritillaria verticillata (tuber)	<0
Galium spurium (whole plant)	<0
Galium verum var. *asiaticum* (aerial part)	<0
Gardenia jasminoides (fruit)	7±4
Gastrodia elata (rhizoma)	7±2
Gentiana scabra var. *buergeri* (radix)	15±3
Gentiana uchiyamai (whole plant)	<0
Geum aleppicum (aerial part)	4±2
Ginko biloba (leaf)	<0
Gleditsia sinensis (spina)	16±3
Glycyrrhiza glabra (radix)	<0
Gossipium nanking (semen)	<0
Gyrophora exculenta (lichens)	<0
Hepatica asiatica (whole plant)	39±2
Hibiscus trionum (whole plant)	<0
Hordeum vulgare (semen)	21±5
Humulus japonicus (whole plant)	<0
Hypericum ascyron (whole plant)	23±4
Hypericum erectum (whole plant)	<0
Hypocoeris ciliata (aerial part)	<0
Ilex macropoda (aerial part)	<0
Impatiens textori (aerial part)	<0
Iris ensata var. *spontanea* (aerial part)	2±4
Iris ensata var. *spontanea* (underground part)	<0
Kalopanax pictus (cortex)	19±2
Kummerowia striata (whole plant)	<0
Lactuca indica for. *indivisa* (whole plant)	<0

Table 1. Continued.

Plant (part of use)	Inhibition (%)
Lactuca triangulata (whole plant)	<0
Lathyrus davidii (aerial part)	<0
Lathyrus japonica (whole plant)	<0
Leonurus sibiricus (aerial part)	<0
Lespedeza cyrtobotrya (aerial part)	18±2
Lespedeza tetraloba (aerial part)	<0
Ligusticum tenuissimum (radix)	23±4
Lindera obtusiloba (aerial part)	33±2
Lindera strychnifolia (radix)	10±3
Liriope graminifolia (tuber)	<0
Lonicera japonica (flower)	<0
Lonicera maackii (aerial part)	<0
Loranthus parasiticus (herba)	9±3
Lycium chinense (fruit)	10±1
Lycium chinense (radicis cortex)	16±2
Lyndera erythrocarpa (aerial part)	<0
Lysimachia vulgaris var. *davurica* (whole plant)	45±2
Lythrum salicaria (whole plant)	71±3
Maackia amurensis (aerial part)	<0
Machilus thunbergii (cortex)	55±1
Magnolia kobus (flower)	15±2
Magnolia sieboldii (aerial part)	63±1
Malus baccata (aerial part)	13±2
Melampyrum roseum (aerial part)	<0
Mentha arvensis (herba)	<0
Metaplexis japonica (aerial part)	<0
Microstegium vimineum (whole plant)	<0
Morus alba (cortex)	<0
Nelumbo nucifera (semen)	63±1
Oenothera lamarckiana (aerial part)	9±3
Oplismenus undulatifolius (whole plant)	<0
Orostachys japonicus (herba)	<0
Osmunda japonica (aerial part)	13±3
Osterium sieboldii (aerial part)	<0
Pachyma hoelen (sclerotia)	9±2
Paeonia albiflora (radix)	11±4
Paeonia suffruticosa (cortex)	<0
Panax ginseng (radix)	<0
Parthenocissus tricuspidata (whole plant)	<0
Patrinia scabiosaefolia (whole plant)	<0
Pedicularis resupinata (whole plant)	<0
Perilla frutescens (herba)	15±1
Persicaria hydropiper (whole plant)	19±1
Persicaria senticosa (aerial part)	<0
Persicaria sieboldii (aerial part)	8±3
Persicaria yokussiana (aerial part)	<0
Petasites japonicus (whole plant)	<0
Peucedanum japonicum (radix)	5±3
Peucedanum praeruporum (radix)	<0
Phaseolus nipponensis (aerial part)	<0
Phellodendron amurense (cortex)	16±3

Table 1. Continued.

Plant (part of use)	Inhibition (%)
Phlomis umbrosa (radix)	36±3
Phlox paniculata (aerial part)	<0
Phryma leptostachya var. *asiatica* (aerial part)	<0
Phtheirospermum japonicum (whole plant)	<0
Phyllostachys nigra var. *henonis* (caulis)	<0
Phyteuma japonicum (aerial part)	<0
Phytolacca esculenta (radix)	<0
Picris hieracioides sp. *japonica* (whole plant)	<0
Pilea mongolica (aerial part)	<0
Pinellia ternata (tuber)	3±2
Plantago asiatica (semen)	21±4
Platycarya strobilacea (aerial part)	<0
Platycodon grandiflorum (radix)	<0
Pleuropterus multiflorus (radix)	9±1
Polygala tenuifolia (radix)	<0
Polygonatum sibiricum (rhizoma)	5±1
Polystichum tripteron (whole plant)	<0
Poncirus trifoliata (fruit)	19±6
Potentilla chinensis (aerial part)	20±2
Potentilla chinensis (herba)	42±2
Potentilla freyniana (aerial part)	<0
Potentilla paradoxa (aerial part)	13±5
Prunella vulgaris (herba)	44±1
Prunus armeniaca var. *ansu* (semen)	<0
Prunus persica (semen)	57±1
Pteridium aquilinum var. *latiusculum* (aerial part)	24±1
Pueraria thunbergiana (aerial part)	<0
Pueraria thunbergiana (radix)	44±2
Ranunculus tachiroei (aerial part)	<0
Ranunculus tachiroei (underground part)	<0
Raphanus sativus (semen)	43±3
Rehmannia glutinosa (rhizoma)	<0
Rheum undulatum (rhizoma)	17±3
Rhus chinensis (aerial part)	<0
Rosa laevigata (fruit)	<0
Rubus coreanus (fruit)	78±2
Rubus crataegifolius (whole plant)	<0
Salix floderusii (aerial part)	<0
Salix glandulosa (aerial part)	18±4
Salix hallaisanensis (aerial part)	<0
Salvia chanroenica (aerial part)	11±4
Sanguisorba hakusanensis (whole plant)	<0
Sanguisorba officinalis (radix)	68±3
Sasa borealis (aerial part)	<0
Saxifraga manshuriensis (aerial part)	<0
Schizandra chinensis (fruit)	<0
Schizonepeta tenuifolia (herba)	26±4
Scirpus wichurae (aerial part)	28±4
Scrophularia ningpoensis (radix)	6±1
Scutellaria baicalensis (aerial part)	<0
Scutellaria baicalensis (radix)	<0

Table 1. Continued.

Plant (part of use)	Inhibition (%)
Securinega suffruticosa (aerial part)	<0
Sedum zokuriense (whole plant)	47±2
Sedum erythrostichum (aerial part)	3±7
Sedum sarmentosum (whole plant)	<0
Setaria chodrachne (whole plant)	<0
Setaria glauca (whole plant)	<0
Setaria viridis (whole plant)	11±4
Siegesbeckia glabrescens (aerial part)	27±4
Sium sauve (aerial part)	<0
Solanum nigrum (herba)	3±1
Sophora flavescens (aerial part)	<0
Sparganium stoloniferum (rhizoma)	<0
Spiraea salicifolia (aerial part)	12±5
Stephanandra incisa (aerial part)	9±3
Styrax obassia (aerial part)	<0
Symphytum officinale (aerial part)	<0
Synurus excelsus (aerial part)	<0
Taraxacum mongolicum (herba)	46±1
Teucrium japonicum (aerial part)	<0
Thalictrum filamentosum var. *tenerum* (aerial part)	<0
Thalictrum minus var. hypoleucum (aerial part)	<0
Thuja orientalis (folium)	<0
Trichosanthes kirilowii (radix)	20±3
Trichosanthes kirilowii (semen)	<0
Tripterygium regelii (aerial part)	50±1
Ulmus davidiana (aerial part)	<0
Ulmus parvifolia var. *coreana* (aerial part)	<0
Uncaria rhynchophylla (ramulus)	47±3
Vaccinium koreanum (aerial part)	<0
Vicia bungei (aerial part)	<0
Viola dissecta var. *chaephylloides* (aerial part)	<0
Weigela subsessilis (aerial part)	<0
Xanthium strumarium (fructus)	<0
Youngia chelidonifolia (whole plant)	<0
Zanthoxylum bungeanum (pericarpium)	20±4
Zanthoxylum schinifolium (aerial part)	<0
Zingiber officinale (rhizoma)	5±1
Zizyphus vulgaris var. *inermis* (fruit)	1±2
Zizyphus vulgaris var. *spinosus* (fruit)	25±2

Inhibitory effects on CINC-1 induction are represented as % of inhibition, mean±standard error (n=4), where sample was treated at 100 ug/ml as the final concentration.

ction. The active extracts were prepared from folium of *Artemisia argyi*, whole plant of *Lythrum salicaria*, cortex of *Machilus thunbergii*, aerial part of *Magnolia sieboldii*, semen of *Nelumbo nucifera*, semen of *Prunus persica*, fruit of *Rubus coreanus*, radix of *Sanguisorba officinalis*, and aerial part of *Tripterygium regelii*.

Nine of the active extracts were independently subjected to sequential fractionation with methylene chloride, ethyl acetate, *n*-butanol, and water. Inhibitory effects on CINC-1 induction in LPS-stimulated NRK-52E cells by each of the solvent fractions with 50 μg/

Table 2. Inhibitory effects on CINC-1 induction by solvent-fractionated extracts

Plant (part of use)	Inhibition (%)			
	MC	EtOAC	BuOH	Residue
Artemisia argyi (folium)	88±1*	<0±5	<0±4	3±2
Lythrum salicaria (whole plant)	5±6	15±4	31±3**	45±1*
Machilus thunbergii (cortex)	<0±3	38±1*	58±1*	13±3**
Magnolia sieboldii (aerial part)	83±2*	<0±4	<0±3	<0±2
Nelumbo nucifera (semen)	<0±4	<0±2	13±2**	4±3
Prunus persica (semen)	<0±2	<0±2	<0±1	11±3
Rubus coreanus (fruit)	5±2	<0±8	31±2**	34±2**
Sanguisorba officinalis (radix)	<0±3	<0±1	4±3	37±2*
Tripterygium regelii (aerial part)	60±2*	29±1**	6±1	1±2

Herbal extract was sequentially frationated to obtain methyl chloride (MC), ethyl acetate (EtOAc), *n*-butanol (BuOH), and polar residue layers. Each of the solvent fractions treated was 50 ug/ml as the final concentration. Data are represented as mean±standard error (n=4), and their significances compared with control group are $p<0.001$ (*) and $p<0.01$ (**).

ml as the final concentration were analyzed (Table 2). Methylene chloride fractions of *Artemisia argyi, Magnolia sieboldii*, and *Tripterygium regelii* exhibited the highest inhibitory effects on the LPS-induced CINC-1 production among their four solvent fractions. *Lythrum salicaria*, and *Rubus coreanus* exhibited significant inhibition on the CINC-1 induction in their *n*-butanol and aqueous fractions, but not in their methylene chloride and ethyl acetate fractions. *Machilus thunbergii* exhibited significant inhibitory effects on CINC-1 induction in its ethyl acetate, n-butanol, and aqueous fractions but not in methylene chloride fraction. Nelumbo *nucifera exhibited* significant but weak inhibition on CINC-1 induction in its *n*-butanol fraction only. None of the solvent fractions of *Prunus persica* inhibited the CINC-1 induction significantly. *Sanguisorba officinalis* exhibited significant inhibition on CINC-1 induction in its aqueous fraction only. At the final concentration of 50 μg/ml, methylene chloride fractions of *Artemisia argyi, Magnolia sieboldii*, and *Tripterygium regelii*, and *n*-butanol fraction of *Machilus thunbergii* exhibited more than 50% of inhibition on CINC-1 induction. The highest inhibitory effects on CINC-1 induction in LPS-stimulated NRK-52E cells were identified in methylene chloride fractions of *Artemisia argyi* and *Magnolia sieboldii* among solvent fractions of 9 active extracts in this study. To identify the inhibitors on CINC-1 induction, activity-guided fractionations of herbal extracts prepared from folium of *Artemisia argyi* (Compositae), and aerial part of *Magnolia sieboldii* (Magnoliaceae) are now in progress.

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