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Review

Salicornia herbacea: Botanical, chemical and pharmacological review of halophyte marsh plant

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***Salicornia (S.) herbacea* L. (Chenopodiaceae) is a salt marsh plant and one of the most salt tolerant species on Western coast of Korea. In a long time, *S. herbacea* has been prescribed in traditional medicines for the treatment of intestinal ailments, nephropathy, and hepatitis in Oriental countries. In addition, *S. herbacea* has recently reported to be effective on the atherosclerosis, hyperlipidemia, and diabetes. A variety of pharmacological experiments have revealed that solvent-extracted fractions of *S. herbacea* exhibited anti-oxidative, anti-microbial, anti-proliferative, and anti-inflammatory activities, supporting rationale behind its several traditional uses. Tungtungmadic acid, quercetin 3-O-glucoside, and isorhamnetin 3-O-glucoside have been isolated from *S. herbacea*, and identified as active ingredients of biological and pharmacological activities. Due to the easily collection of the plant and remarkable biological activities, this plant has become the food and medicine in seashore area of Korea. This review presents comprehensively analyzed information on the botanical, chemical, and pharmacological aspects of *S. herbacea*.**

Key words: *Salicornia herbacea*, salt marsh plant, anti-oxidative effect, anti-inflammatory activity, Tungtungmadic acid.

INTRODUCTION

The Salicornioideae are among the most salt-tolerant land plant and frequently occur in saline areas associated with coastlines, tidal floodways, and salt lakes (Ihm and Lee, 1986; Anwar et al., 2002; Shepherd et al., 2005). These halophytes are world-widely distributed and found on every continent with the exception of Antarctica (Shepherd et al., 2005). The Salicornioideae family comprises approximately 15 genera and 80 species (Shepherd et al., 2005). The Salicornia species are:

(i) *Salicornia (S.) herbacea* (syn to *uropeae*).
(ii) *S. indica* (syn. to *arthrocenemum*).
(iii) *S. bigelovii*.
(iv) *S. perenis*.
(v) *S. disarticulate*.

S. herbacea (Figure 1) has been known as 'Tungtungmadi' in Korea and distributed in tidelands on Western coast of Korea (Lee et al., 2004b; Chung et al., 2005). *S. herbacea* grows about 10 ~ 40 cm high and its stem looks deep green and changes into red in the Fall (Jo et al., 2002b). *S. herbacea* has been recently consumed as a food in some recipes and a medicine against obesity, constipation and hepatitis. *S. indica* is a gregarious and wildly distributed on the saline wet soil of Pakistan and India (Anwar et al., 2002). *S. bigelovii* is a leafless annual salt-marsh plant with green and succulent stem, and with most of the seed spikes on the upper one-third of the plant (Anwar et al., 2002). In subtropical regions, it may grow to be a large, upright plant, 50 cm tall. *S. bigelovii* has been considered as a potential seawater oilseed crop from a screening of wild halo-phytes and selected for seawater field trials including determination of seed yield and analysis.

In a long time, *S. herbacea* has been used as a folk medicine for treatment of nephropathy, hepatitis and diarrhea or constipation in Korea. The usage of the plant has been recently extended into the functional food and

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Figure 1. *Salicorna herbacea* L. (courtesy: http://www.nature.go.kr/index.do).

medicinal plant due to the advent of new functional and biological active material. However, review and systemic analysis of botany, chemistry and pharmacology of *Sarconia herbacea* have not been reported yet. This review intended to provide the currently available information on traditional and local knowledge, ethno biological and ethno medicinal issues, identification of pharmacologically important molecules, and pharmacological studies on this useful plant.

BOTANY

S. herbacea is commonly called as Tungtungmadi in Korea. The habitat which plant grows is classified as muddy flat, sand dune and rock face by a gradient of soil properties, and divided into flooding of seawater, salt or seawater in soil and saltern by a gradient of salt influence. The plant is annual succulent shrub growing throughout the Western coast of Korea. The stem is tuberous and succulent without hair or leaf, and is fairly richly branched with distinct articulation. The stem is originally dark green coloured and ultimately flushed pink or red in fall season. The plant flowers during August - September is green, which comprised of paired cymules. Each cymule has 3 flowers. These flowers have 2 - 4 perianth lobes that may be free or fused almost to the apex, 1 - 2 stamens and a single ovary. Central flower is distinctly larger than the two laterals (Davy et al., 2001).

Salicornioideae exhibit considerable phenotypic variation at the population level and taxonomic confusion is exacerbated by the occurrence of species complexes and polyploids (Wilson, 1980; Davy et al., 2001). Although few vegetative and floral features are diagnostic in the Salicornioideae, seed and fruit characters have been recognized as potentially useful parts at both the generic and species levels. The seeds of *S. herbacea* are round to ellipsoid in shape, ranging from 1.3 - 1.7 mm in length. The color of *S. herbacea* seed is green and appears opaque.

CHEMISTRY

One of the active constituents in *S. herbacea* is tungtungmadic acid (3-caffeoyl-4-dihydrocaffeoyl quinic acid), a chlorogenic acid derivative (Chung et al., 2005). Chlorogenic acid, an ester of caffeic acid with quinic acid, is found in many plants and recognized as an antioxidant (Bonita et al., 2007; Bouayed et al., 2007; Medina et al., 2007). Indeed, tungtungmadic acid was found to have higher anti-oxidative activity in 1, 1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging test and in the iron-induced liver microsomal lipid peroxidation assay. In addition, tungtungmadic acid was shown to be effective in protecting the plasmid DNA against strand breakage induced by Fe^{3+}-nitrilotriacetic acid-hydrogen peroxide (Chung et al., 2005). In addition, other active compounds, such as β–sitosterol, stigmasterol, uracil, quercetin 3-O-β-D-glucopyranoside, and isorhamnetin 3-O-β-D-glucopyranoside, were isolated from the methanol extract of *S. herbacea* (Lee et al., 2004b; Park, Kim, 2004; Lee et al., 2005). The structures of these compounds were identified on the basis of chemical and spectroscopic analyses and by comparison with published data (Lee et al., 2004b; Park, Kim, 2004). Lee et al. (2004) reported that methanol

extract of *S. herbacea* contained 4.85 mg/ml of betaine (Lee et al., 2004a), known to diminish the level of homocysteine in blood and thus protecting cardiovascular diseases (Lee et al., 2004a).

Oh et al. (2007) found that ethanol extracts of viscozyme-treated *S. herbacea* exhibited the strongest radical scavenging activity against DPPH, superoxide and hydroxyl radicals. Five phenolic compounds, procatechuic acid, ferulic acid, caffeic acid, quercetin, and isorhamnetin, were isolated and identified by antioxidant assay-guided fractionation and purification (Oh et al., 2007).

Chemical compositions and the contents of amino acids and minerals in *S. herbacea* are shown in Table 1, 2 and 3 respectively (Min et al., 2002a). This plant has been revealed to contain large amounts of salt and minerals, especially calcium, magnesium and iodine

PHARMACOLOGY

Several workers have reported on the different biological activities of *S. herbacea* in various *in vitro* and *in vivo* test models. Different solvent extracts or various compounds of the plant have exhibited immunomodulatory, anti-oxidative, anti-inflammatory, anti-hyperlipidemic, and anti-diabetic activities. These biological activities have been described in details in the following sections.

Anti-oxidative effect

An antioxidant is defined as 'any substance that, when present at low concentrations compared to those of an oxidizable substrate, significantly delays or prevents oxidation of that substrate' (Halliwell et al., 1995; Wiseman et al., 1997; Mates et al., 1999). Antioxidants are of interest to biologists and clinicians because they help to protect the human body against damage induced by reactive free radicals caused in cancer, atherosclerosis, and aging (Halliwell et al., 1995; Mates et al., 1999). There are many reports that natural products and their derivatives have efficient anti-oxidative characteristics, consequently linked to anti-cancer, hypolipidemic, anti-aging, and anti-inflammatory activity (Halliwell et al., 1995; Wiseman et al., 1997; Hogg, 1998; Mates et al., 1999; Aruoma, 2003; Cho et al., 2006).

In order to compare the anti-oxidative capacities of methanol (MeOH), hexane (HEX), chloroform (CHF), ethyl acetate (EA), *n*-butanol (BuOH), and aqueous (AQU) fractions of *S. herbacea*, first, we measured the antioxidant activity of each extract (100 µg/ml) of the plant, by using a xanthine oxidase assay and a DPPH assay. In xanthine oxidase assay, the anti-oxidative activity of EA and BuOH fractions was much higher than those of MeOH, HEX, CHF, and AQU fractions (Figure 2). The IC_{50} value of EA fraction (66.0 µg/ml) calculated from further obtained dose-responsive curve (data not shown) was less than that of the BuOH fraction (82.5

Table 1. Chemical composition and salt contents of *S. herbacea* (Min et al., 2002a).

Items	Leaf (%)	Stem (%)	Root (%)
Moisture	90.9	73.9	66.2
Crude protein	1.7	2.0	2.0
Crude lipid	0.2	0.3	0.3
Crude ash	4.7	6.1	6.2
Salt	3.3	3.9	2.8
Total sugar	2.2	13.4	22.8
Uronic acid	0.3	1.4	1.9

Table 2. Amino acid compositions of *S. herbacea* (Min et al., 2002a).

Amino acid	Leaf (%)	Stem (%)	Root (%)
Taurine	7.6	21.4	37.7
Aspartic acid	137.1	140.2	165.5
Threonine	70.9	69.8	81.2
Serine	67.5	72.7	94.8
Glutamic acid	144.8	160.5	182.3
Glycine	76.9	80.4	122.9
Alanine	76.9	88.7	98.2
Cystine	-#	-	11.1
Valine	72.9	126.1	94.7
Methionine	23.2	52.2	23.3
Isoleucine	110.7	107.5	94.7
Leucine	115.5	98.1	128.4
Tyrosine	10.8	-	-
Phenylalanine	73.2	63.3	67.7
Lysine	79.8	310.2	178.9
Histidine	34.0	79.3	54.4
Arginine	77.0	36.1	57.0
Proline	88.8	18.4	86.8
Total	**1,270**	**1,525**	**1,569**

Table 3. Mineral contents of *S. herbacea* (Min et al., 2002a).

Minerals	Leaf	Stem	Root
Na	1003.4	1218.1	1333.8
Ca	237.5	158.8	22.1
K	650.1	740.1	741.1
Mg	46.5	54.0	52.5
Zn	13.4	29.6	2.4
Fe	31.5	66.2	84.8
Cu	3.1	1.1	2.1
Ni	1.1	0.7	0.4
Mn	7.2	3.9	3.0

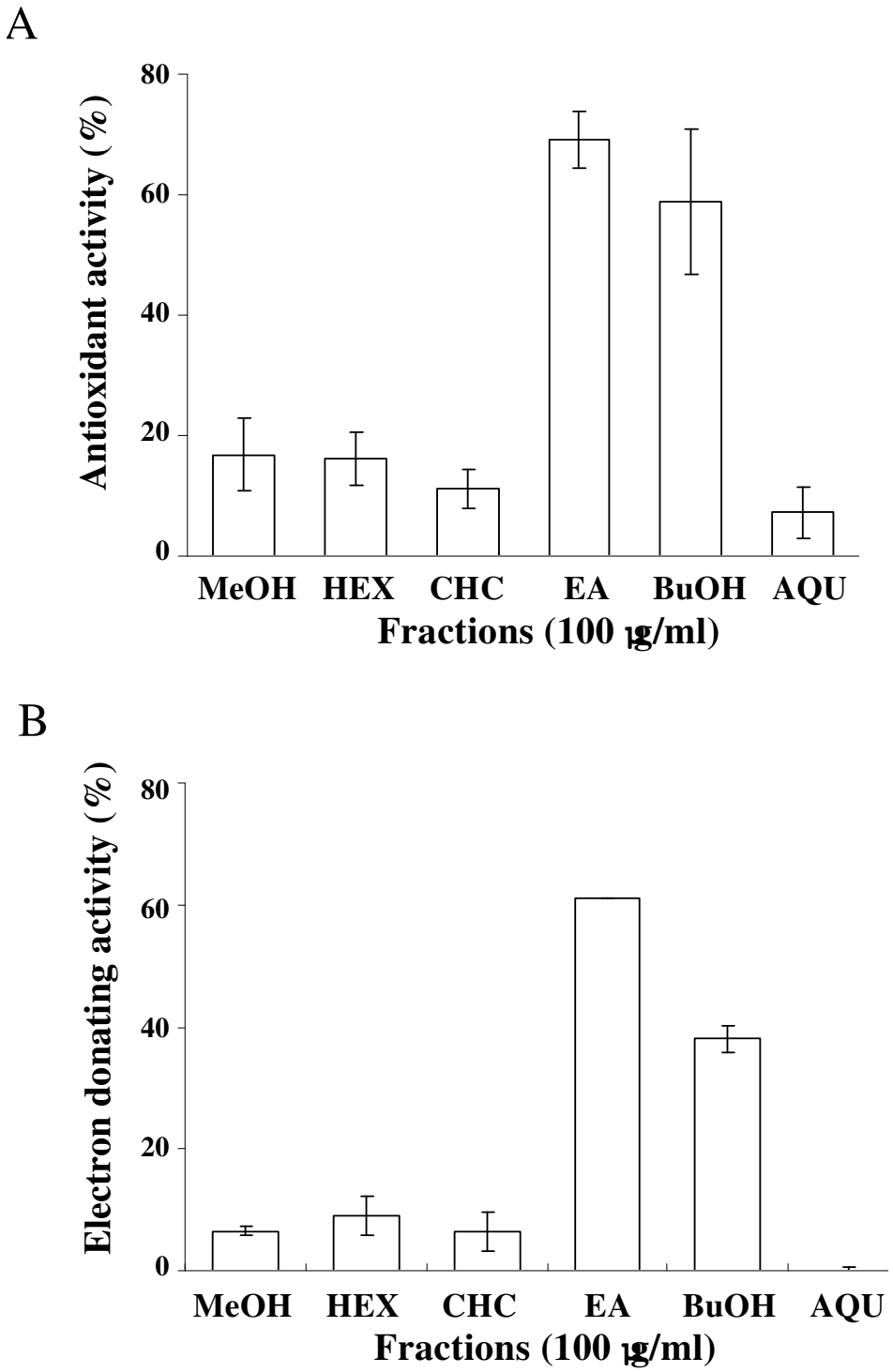

Figure 2. Antioxidant activities of various solvent extracts from *S. herbacea* in xanthine oxidase assay (A) and DPPH assay (B). Either phosphate buffer (0.1 mM, pH 7.4, for xanthine oxidase assay) or acetate buffer (10 mM, pH 5.5, for DPPH assay) and methanol (MeOH), chloroform (CHF), hexane (HEX), ethyl acetate (EA), *n*-butanol (BuOH) or aqueous (AQU) extracts (100 μg/ml) were mixed and assay was carried out. Each value is the mean ± SEM of three determinations, performed in triplicate.

μg/ml). On the other hand, in a DPPH assay, the radical scavenging activity of EA fraction (IC_{50} value = 117.5 μg/ml) was much stronger than those of the others (Figure 2B). The radical scavenging activity (IC_{50} value =

375.0 μg/ml) of the BuOH fraction was less than that of EA fraction.

Ethanol extract of *S. herbacea* leaves and its sub-fractions (EA, CHF, EA, BuOH, and AQU) were also tested for the evaluation of their anti-oxidative activities by using nitrite scavenging, lipid peroxidation, DPPH, and xanthine oxidase assays (Min et al., 2002b). All fractions showed the inhibition of lipid peroxidation with similar anti-oxidative property. The DPPH radical scavenging activity (IC_{50} value = 279.0 μg/ml) of EA fraction was much more effective than other fractions, but less effective than ascorbic acid (IC_{50} = 67 μg/ml). In the nitrite scavenging assay, diethyl ether and EA fractions were found to be more effective than other fractions. Therefore, some ingredients in the diethyl ether and EA fractions of *S. herbacea* leaf seem to play an important role in the anti-oxidative capacity of the salt marsh plant.

Han and Kim (2003) reported on the anti-oxidative effect of *S. herbacea* grown in closed salt paddy. Anti-oxidative activities of the *S. herbacea* were investigated by using Rancimat and TBA method. Oxidative stability of components from the plant stem was shown to exhibit higher than that of root-derived samples.

Two flavonoids, quercetin 3-O-β-D-glucopyranoside and isorhamnetin 3-O-β-D-glucopyranoside, were isolated from the aerial parts of *S. herbacea* by column chromatography. The radical scavenging activity of quercetin 3-O-β-D-glucopyranoside was comparable to that of quercetin. The antioxidative activity of isorhamnetin 3-O-β-D-glucopyranoside, which contains methoxyl group at ring B, was lower than that of 3-O-β-D-glucopyranoside.

Chung et al. (2005) reported the isolation of tungtungmadic acid from *S. herbacea* by chemical and spectral analyses. It has been found that this compound had higher antioxidant activity in the DPPH scavenging assay as well as in the iron-induced liver microsomal lipid peroxidation system with IC_{50} values of 5.1 and 9.3 □M respectively. In addition, plasmid DNA damage (single strand breaks) by H_2O_2 was remarkabley protected by this compound.

The water extract of *S. herbacea* was found to protect against oxidative stress under ovariectomy conditions (Ha et al., 2006). The malondialdehyde (MDA) levels in the liver total homogenate and mitochondrial fractions were markedly increased in the ovariectomized rats and were also decreased by *S. herbacea* up to almost control level. The levels of superoxide dismutase (SOD), catalase, and glutathione peroxidase were also decreased in the ovariectomized rats, which were reversed significantly by the administration of *S. herbacea*. Interestingly, the decreased level of 17 β-estradiol in ovariectomy rats was ecovered by *S. herbacea* treatment. These results imply that estrogen-like mechanism of *S. herbacea* could play a protective role in overiectomic conditions against free radical production.

The anti-oxidative activities of water and ethanol extracts from *S. herbacea* prepared by enzymatic treatments were evaluated by *in vitro* assays against DPPH, superoxide and hydroxyl radicals (Oh et al., 2007). The ethanol extract from viscozyme-treated *S. herbacea* displayed the strongest radical scavenging activity against DPPH, superoxide and hydroxyl radicals. Five phenolic compounds, including procatechuic acid, ferulic acid, caffeic acid, quercetin, and isorhamnetin, were isolated and identified by antioxidant assay-guided fractionation and purification. Most of these phenolic compounds exhibited considerable DPPH, superoxide, and hydroxyl radical scavenging activities. In particular, caffeic acid and ferulic acid more strongly scavenged the reactivity of superoxide and hydroxyl radicals than (+)-catechin, a well-known antioxidant. The levels of five phenolic compounds detected in the ethanol extract of viscozyme-treated *S. herbacea* were highly observed in 1 - 12 mg ranges in one hundred grams of this plant.

Anti-inflammatory and immunomodulatory effects

Macrophages are representative inflammatory cells involved in acute or chronic inflammatory responses by over-production of pro-inflammatory cytokines [eg., tumor necrosis factor (TNF)-α, interleukin (IL)-1β, and granulocyte/macrophage colony-stimulating factor (GMC-SF)] and inflammatory mediators [eg., reactive oxygen species (ROS) and nitric oxide (NO)] (Lundberg, 2003; Walsh, 2003). Indeed, to develop anti-inflammatory drugs, a number of immunopharmacologists have a strategy to suppress the functional activation of macrophages. For this purpose, they employ macrophages to test the *in vitro* efficacy of potential anti-inflammatory agents using artificially activated conditions induced by treatment of inflammatory stimuli such as lipopolysaccharide (LPS) and purified pro-inflammatory cytokines (Gallucci et al., 1998).

Our group has also found that EA fraction (EA-SH) of *S. herbacea* was able to dose-dependently suppress LPS (0.1 μg/ml) induced NO production in macrophage-like RAW264.7 cell (Figure 3A), while BuOH fraction did not (data not shown). In addition, EA fraction diminished the expression of inducible NO synthase (iNOS) in RAW264.7 cells stimulated by 0.1 μg/ml of LPS (Figure 3B). Moreover, EA fraction blocked the mRNA expressions of IL-1β and GM-CSF in LPS-stimulated RAW264.7 cells (data not shown). Therefore, these results indicate that EA of *S. herbacea* may have simultaneously anti-oxidative and anti-inflammatory activities by modulating radical-induced toxicity and various pro-inflammatory responses.

The polysaccharide fraction purified from hot water extract of *S. herbacea* is also another active component to exhibit strong immunomodulatory activity as ones purified from other medicinal plants such as *Aloe vera* and *Panax ginseng* (Im et al., 2003). That is, the fraction

was revealed to stimulate the production of TNF-α and IL-1β, which modulate chemotaxis and activation of inflammatory and antigen-presenting cells and stimulate T cell proliferation. In addition, the polysaccharide fraction activated RAW264.7 cells to increase NO release and the mRNA expression of co-stimulatory molecules such as B7-1 and CD40. Interestingly, weakly adherent RAW264.7 cells were more differentiated into strongly adherent macrophages by this polysaccharide fraction. Im et al. (2007) also reported that the combined treatment of polysaccharide fraction with IFN-γ was synergistically effective in inhibiting the growth of the murine macrophage RAW264.7 cells, and their differentiation into strongly adherent macrophages. The differentiation-inducing activity by the co-treatment accompanied with the increase in the expression of differentiation antigens and adhesion molecules such as CD11b, CD18, and CD24. The combination of polysaccharide fraction and IFN-γ dramatically enhanced the production of cytokines such as TNF-α, IL-1β, and NO, which was tightly correlated with an increased level of their respective transcripts.

Lee et al. (2006) has explored the immnostimulatory mechanisms of the polysaccharide fraction using NO production conditions with mouse peritoneal macro-phages and RAW264.7 cells. They found that the poly-saccharide of this plant strongly induced the production of NO and the mRNA expression of iNOS by mediation of NF-κB/Rel but not Oct transcription factor. Indeed, nuclear translocation and DNA binding activity of NF-κB/Rel as well as NF-κB-dependent reporter gene expression were clearly observed by *S. herbacea* polysaccharide fraction. Therefore, these results strongly suggest that the immunomodulatory activity of *S. herbacea* polysaccharide could be mediated by NF-κB and its relevant pro-inflammatory pathways.

Anti-hyperlipidemic and anti-hyperglycemic effects

Jo et al. (2002) investigated the effect of *S. herbacea* powder on weight gain and the modulation of relevant serum parameters (Jo et al., 2002a). The rats were fed with vehicle, 10 and 20% of the plant powder for 4 weeks. It has been clearly observed that the administration of *S. herbacea* powder was capable of reducing the weight gain. Total and LDL cholesterol contents in serum were significantly down-regulated by the administration of this plant, whereas HDL cholesterol content was significantly up-regulated compared to vehicle group. In addition, total lipid and triglyceride contents were decreased by the administration of *S. herbacea*. These results imply that *S. herbacea* can be applied to ameliorate metabolic and cardiovascular diseases such as diabetes, atherosclerosis and hyperlipidemia, in addition to obesity.

The anti-diabetic effect of *S. herbacea* powder and its underlying mechanism were indeed continuously examined (Bang et al., 2002). The administration of *S. herbacea* powder alleviated hyperglycemia symptom seen in streptozotocin-induced diabetic rats (Bang et al., 2002). The intakes of food and water were significantly decreased in rat group fed with 5% *S. herbacea* powder for 5 weeks but not diabetic group. The level of blood glucose in *S. herbacea* powder-treated group began to be decreased from 3-week administration and lasted until 5-week supplementation. Similar experiment performed by 4-month administration using diabetic rats supported the anti-diabetic activity of this plant, although there was no significant difference of plasma lipid metabolite levels between diabetic and supplemented groups (Kim, 2007).

In a series of investigations to develop potential anti-diabetic or anti-hyperlipidemic agents from Korean indigenous plants, Park et al. (2006) have screened that *S. herbacea* was able to prevent the onset of hyperlipidemia and weight gain induced by high fat diet in mice (Park et al., 2006). The ethanol extract of *S. herbacea* similarly modulated the expression levels of lipogenesis-related genes [e.g., sterol regulatory element binding protein 1 (SREBP1a), fatty acid synthase (FAS), glycerol-3-phosphate acyltransferase (GPAT), steroyl-CoA desaturase-1 (SCD-1)] and gluconeogenesis-related genes [e.g., phosphoenolpyruvate carboxykinase (PEPCK), glucose 6-phosphatase (G6Pase)] in liver (Park et al., 2006).

Conclusion

S. herbacea is a popular medicinal plant useful in various intestinal ailments including diarrhea and constipation; In addition, this plant has been used to treat some inflammatory disorders such as nephropathy and heaptitis. Recent studies also remarkably demonstrated the curative or modulatory roles of *S. herbacea* in diabetes, obesity, and hyperlipidemia. According to various pharmacological studies, the major activity of this plant seems to be its anti-oxidative, anti-inflammatory and immunomodulatory activities. In particular, EA fraction showing higher anti-oxidative property also strongly suppressed LPS-induced NO production and iNOS expression in RAW264.7 cells. Based on that acute or chronic inflammation is now regarded as a major symptom causing various serious diseases such as cancer, autoimmune diseases (eg. rheumatoid arthritis), vascular diseases (eg. atherosclerosis) and metabolic diseases (eg. diabetes), our results could suggest a possibility that this fraction can be further developed as a potential disease-curing remedy. Currently, our group is focusing on addressing this possibility by employing various *in vivo* inflammatory models.

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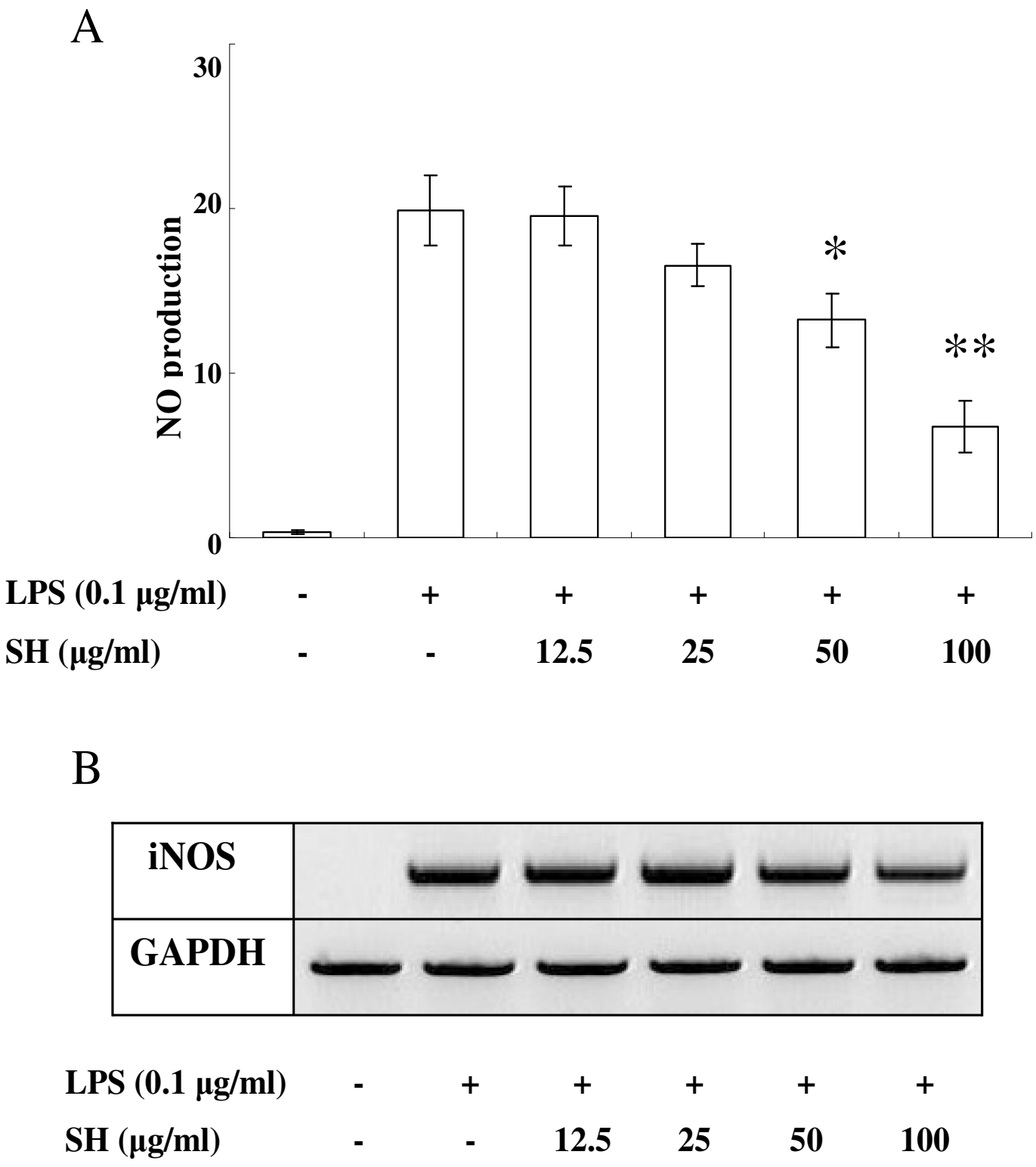

Figure 3. The effects of EA fraction (EA-SF) of *S. herbacea* on the production of NO and on the mRNA expression of iNOS in LPS-activated RAW264.7 cells. RAW 264.7 cells ($1X10^6$ cells/ml) were stimulated by a LPS (0.1 μg/ml) and incubated with ethyl acetate (SH) fraction of *S. herbacea*. (A) Supernatants were collected after 18 h and nitrite formation was determined using Griess' reagent. Means ± SEM was calculated from three independent experiments that were performed in triplicate. (B) The preparation of total RNA and RT-PCR was performed as described. The figures represent the results of three independent experiments. *P < 0.05 and **P < 0.01 compared to control (LPS alone).

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