Chapter 10

Enormous Potential for Development *Liriope platyphylla Wang et Tang* as a Therapeutic Drug on the Human Chronic Disease

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Additional information is available at the end of the chapter

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1. Introduction

Liriope platyphylla Wang et Tang (*L. platyphylla*) is named *Liriope muscari* with Binomial name and called big blue lilyturf, lilyturf, border grass and monkey grass with common name [1]. It is widely used as one of the 50 fundamental herbs in traditional Oriental medicine; the species is one member of low, herbaceous flowing plats, which grows commonly in the shady forests of East Asia including China, Korea and Japan at elevation of 100 to 1400 m. Also, it typically grows 30-45 cm tall and have grass-like evergreen foliage and lilac-purple flowers which produce single seeded berries on spike in the fall [1,2]. Their roots are long fibrous with terminal tubers. Its flower is showy form which an erect spikes with tiered whorls of dense, white to violet-purple flowers rising above the leaves as like grape hyacinth. This flower differents into blackish berries which can maintain their status into winter season (Fig. 1A). Furthermore, *L. platyphylla* bear a strong likeness to *L. spicata* (creeping lilyturf) which is the most common species in this genus. Although the prominent flower spike extending above the leaves is alike, the leaves of *L. platyphylla* were wider and longer than those of *L. spicata* [3,4].

L. platyphylla is easily well grown in most condition of soil including average, medium, well-drained type in full sun shines to partial shade although the ideal condition for it growth are fertile and moist soils with partial shade. Furthermore, it has a wide range of tolerance for light, heat, humidity, drought and soil condition. Because of these advantages, they widely used as one of the most popular border plant and groundcover in southeastern USA [1].

Of several parts in *L. platyphylla*, only roots have generally been used for a variety of purposes, including as a therapeutic drug and in teas (Fig. 1B and C) [1]. However, there are a few studies

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which purified and elucidated constituents in *L. platyphylla*. Total 13 chemical constituents were isolated from the chloroform fraction and *n*-BuOH fraction from EtOH extract of *L. platyphylla*. These constituents included beta-sitosterol-3-*O*-beta-D-glucopyranosile, palmic acid, ruscogenin, LP-C, LP-D, 25 (S) -ruscogenin 1-*O*-beta-D-xylopyranoside-3-*O*-alpha-L-rhamnopyranoside, lupenone, lupeol, ursolic acid, beta-sitosterol, diosgenin, LP-A and LP-B [5,6].

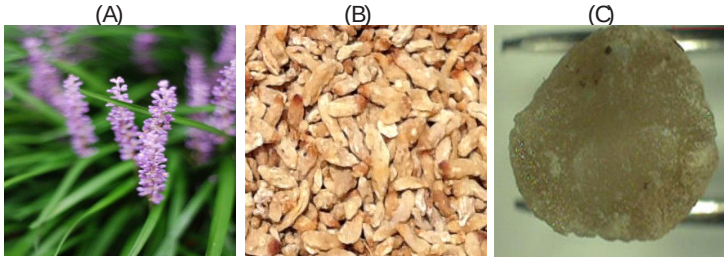

Figure 1. Flowers and roots of *L. platyphylla*. (A) The violet-purple flowers of *L. platyphylla* rise above the leaves as like grape hyacinth. (B) Its dried roots generally used to therapeutic effects. (C) A cross section of *L. platyphylla* root.

A variety of previous pharmacological studies have suggested that *L. platyphylla* may exert beneficial biological effects on inflammation, diabetes, neurodigenerative disorder, obesity and atopic dermatitis (Table 1). However, there are no reviews to publish the report for the therapeutic effects of *L. platyphylla* on the human chronic disease. Therefore, this chapter describes the important results of an experiment using *L. platyphylla* which may prove valuable in the development of a therapeutic drug for the treatment of human chronic disease.

Target Disease	Functional effects	References
Inflammation	-Inhibition of bacteria activity -Inhibition of airway inflammation and hyperresponsiveness	[7,8]
Diabetes	-Worked as insulin sensitizer -Stimulation of insulin secretion -Relief of diabetes symptom	[9-14]
Neurodigenerative disorder	-Stimulation of NGF expression and secretion -Induction of neurite outgrowth -Improvement of learning and memory ability -Induction of neuronal cell survival and neuritic outgrowth	[15-18]
Obesity	-Prevention of obesity and hypertriglyceridemia	[19]
Atopic dermatitis	-Relief of atopic dermatitis	[20]

Table 1. Summary of the pharmacological activities of *L. platyphylla*.

2. Therapeutic effects of *L. platyphylla* on human chronic disease

This main section described experimental results regarding the biological effects of *L. platyphylla* on five chronic disease including inflammation, diabetes, neurodigenerative disorder, obesity and atopic dermatitis.

2.1. Effects of *L. platyphylla* on inflammation

L. platyphylla has been long-used for the treatment against asthma and bronchial and lung inflammation. Firstly, the inhibitory activity of recombinant sortase was evaluated in 80 medicinal plants. In order to test these effects, a total 240 medicinal plant fractions were sequentially purified from 80 plants with *n*-hexane, ethyl acetate and water. As show Table 2, the high inhibition activity was detected in the ethyl acetate fractions of *Cocculus trilobus*, *Fritillaria vertillata*, *L. platyphylla* and *Rhus verniciflua.* Especially, the greatest activity was observed in the ethyl acetate fractions of *Cocculus trilobus* [7].

Medicinal plants(part of use)	IC_{50}(μg/ml)
p-Hydroxymercuribenzoic acid	40.55
Achyranthes bidentata (root)	15.48[a]
Benincasa cerifera (seed)	21.57[a]
Cibotium barometz (rhizome)	39.40[a]
Cimicifuga heracleifolia (rhizome)	26.58[a]
Cocculus trilobus (rhizome)	1.52[a]
Coptis chinensis (rhizome)	16.73[a]
Cuscuta austrailia (fruit)	21.12[a]
Ecodia offcinalis (fruit)	13.51[a]
Fritillaria verticillata (tuber)	8.41[a]
Gleditsia japonica (fruit)	27.74[a]
Liriope platyphylla (tuber)	7.96[a]
Rhus verniciflua (bark)	3.22[a]
Zanthoxylum bungeanum (fruit)	27.29[a]

Table 2. Inhibition effects of medicinal plat extracts on recombinant sortase [7] a Ethyl acetate fraction, b Water fraction

Furthermore, anti-asthmatic effects of *L. platyphylla* were investigated in ovalbumin (OVA)-induced airway inflammation and asthma murine model. OVA treatment induced significant accumulation of eosinophils into the airway, but co-administration if *L. platyphylla* induced the decrease of eosinophils and total lung leukocytes (Fig. 2). Also, the level of several important cytokines such as IL-5, IL-13, IL-4 and IgE concentration in Broncho Alveolar

Lavage Fluid (BALF) and serum dramatically decreased in *L. platyphylla* treated group compare to control group (Fig. 3). Therefore, these experiments suggested that *L. platyphylla* has anti-inflammation and anti-asthmatic activity through regulating the correlation between Th1/Th2 cytokine imbalance [8].

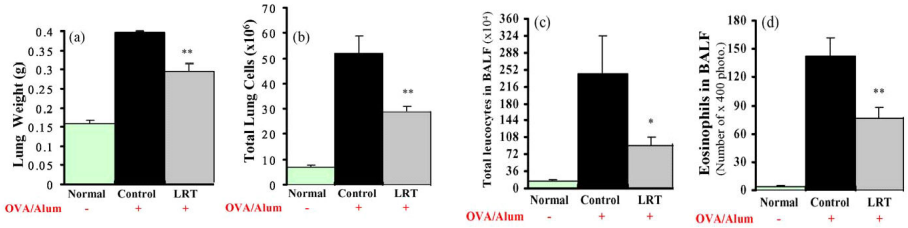

Figure 2. Effect of *L. platyphylla* on total leucocytes number and lung cells in OVA-induced murine model of asthma. *L. platyphylla* was treated into C57BL/6 mice injected with OVA for 8 weeks. Normal: normal C57BL/6 mice; OVA-control: ovalbumin inhalation (control); OVA+ *L. platyphylla* (150 mg/kg) [8].

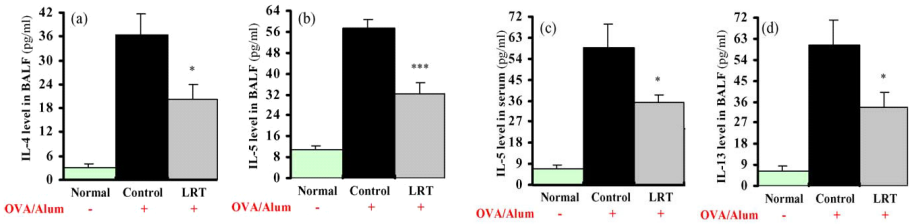

Figure 3. Effect of *L. platyphylla* cytokine level in BALF and serum in OVA-induced murine model of asthma. After final treatment, the BALF and serum were collected from animals of three groups and cytokine concentration were analyzed by ELISA kit. Normal: normal C57BL/6 mice; OVA-control: ovalbumin inhalation (control); OVA+ *L. platyphylla* (150 mg/kg) [8].

2.2. Effects of *L. platyphylla* on diabetes

2.2.1. Role as insulin sensitizer

The therapeutic effects of *L. platyphylla* on diabetes have been well studied for short period. Early study on insulin action was firstly reported by Choi et al. [9]. In this study, the extract of *L. platyphylla* was extracted with 70% methanol for 12 hours. And then, this extracts was further separated by passage through a Diaion HP-20 and silica gel column chromatography a stepwise elution by the gradients of chloroform and methanol (9:1, 6:1, 3:1, 2:1 and 1:1). Of these fractions, the 9:1 fraction induced the increase of glucose uptake 1 ng/mL up to glucose uptake 50 ng/mL insulin in 3T3-L1 adipocytes (Fig. 4). Also, this fraction contained several active compounds including homoisoflavones, methylophiopogonone A, ophiopogonone A,

methylophiopogonanone A and ophiopogonanone A. Furthermore, this study showed that the insulin stimulated glucose uptake has been regulated by the increase of glucose transporter (Glut 4) contents in plasma membrane and insulin receptor substrate 1 (IRS1)-PI3 kinase-Akt signalling mechanism (Fig. 5). Particularly, Gluts are a group of membrane protein that facilitate the transport of glucose molecule across a cell membrane. Isoform of Glut was classified by a specific role in glucose metabolism including the expression pattern in tissue, substrate specificity, transport kinetics and expression in different physiological conditions [21]. Until now, 13 members of Gluts have been identified on the basis of sequence similarities [22]. Of these, four Gluts (1-4) were well characterized. Glut-1 highly expressed in erythrocytes and endothelial cells of barrier tissues, and was responsible for the low-level of basal glucose uptake. Glut-2 was widely distributed in renal tubular cells, small intestinal epithelial cells, liver cells and pancreatic beta cells. Especially, in the liver cells, it was required to uptake glucose for glycolysis and release the glucose produced from gluconeogenesis. Most of Glut-3 was expressed in neurons and placenta and Glut-4 was founded in adipose tissue and striated muscle [23]. Therefore, this study suggested possibility that homoisoflavone-enriched fraction of *L. platyphylla* may have the potential role as an insulin sensitizer [9].

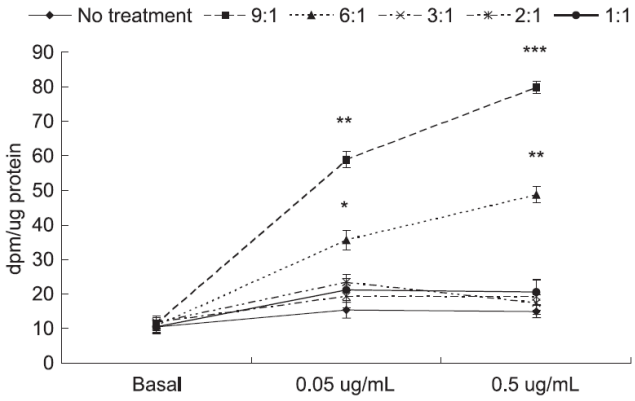

Figure 4. Glucose uptake pattern with 1 ng/mL insulin and fractionated extracts of *L. platyphylla* in 3T3-L1 adipocytes. The basal state was not treated both of insulin and extracts. *Significantly different from no treatment group in 0.05 ug/mL or 0.5 ug/mL treatment group at a=0.01. ** at a=0.001.

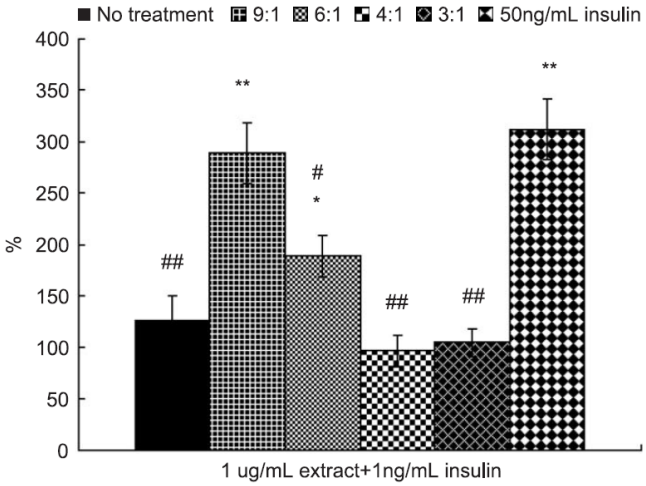

Figure 5. Glut-4 contents in plasma membrane of 3T3-L1 adipocytes. **Significantly different from no treatment group at a=0.001. #Significantly different from 50 ng/mL insulin treated group at a=0.01. ## at a=0.001.

2.2.2. Role as insulin stimulator in vitro

Recently, a role as insulin stimulator of *L. platyphylla* is being extensively researched with our group. Ten novel extracts including LP-H, LP-E, LP-M, LP-M80, LP-M50, LP-H20, LP9M80-H, LP9M80-C, LP9M80-B, LP9M80 were newly extracted from *L. platyphylla* with MeOH, EtOH, BuOH and hexan. The insulin secretion ability of these extracts was measured through the detection of insulin concentration in supernatant of HIT-T15 cells (hamster pancreatic beta cells). Of then extacts, the highest concentration of insulin was detected in LP9M80-H treated group, followed by the LP-H, LP-M, LP-E and LP9M80-C treated group. Furthermore, the optimal concentration of LP9M80-H was determined at approximately 100-125 μg/ml using cell viability test and insulin ELISA in HIT-T15 cells (Fig. 6)[12]. This study suggested that novel extracts, LP9M80-H could be considered as potential candidate for enhancement of insulin secretion.

In addition, the new approach such as steaming had applicated to *L. platyphylla* in order to to increase the level or efficacy of their functional components and to induce chemical transformation of specific components. In our previous study, Red *L. platyphylla* (RLP) has been manufactured with steaming technique under different steaming time and frequencies. Among these, maximum insulin secretion was induced by RLP steamed for 3 hours with two repeated steps (3 hours steaming and 24 hours air-dried) carried out 9 times (Fig. 7). Also, the expression and phosphorylation levels of most components in insulin receptor signalling pathway were significantly enhanced in INS cells (rat pancreatic beta cells) treated with RLP. Furthermore, a significant alteration of glucose transporter expression was detected in same group. Meanwhile, in the study using streptozotocine-induced diabetic model

(Type I), the treatment of RLP for 14 days induced the down-regulation of glucose concentration and upregulation of insulin concentration (Fig. 8)[13]. These data showed that steaming processed *L. platyphylla* may be regared as a potential candidate for a relief and treatment of diabetes.

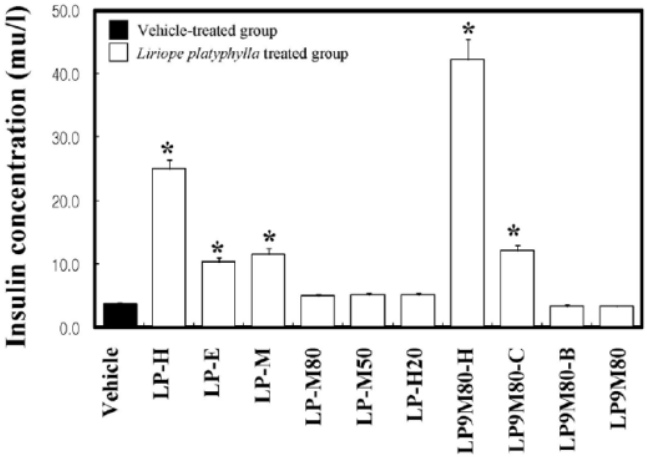

Figure 6. An insulin secretion ability of ten extracts in HIT-T15 cells. Cells were cultured with one of the ten extracts in DMSO at 500 μg/ml concentrations for 24 hr. An insulin concentration in the supernatants was measured using anti-insulin ELISA kit. The values of data represented mean±SD of three experiments. *p<0.05 is the significance level compared to the vehicle treated group.

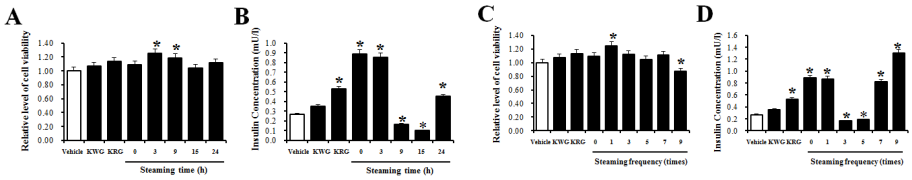

Figure 7. Effects of the different steaming time (A and B) and frequency (C and D) of *L. platyphylla* on toxicity and insulin secretion in INS cells. INS cells were cultured with one of the 5 different extracts manufactured under the different concentrations for 24 hrs. INS cell viability was measured via MTT assays (A and C). Insulin concentration in the supernatant was measured using an anti-insulin ELISA kit (B and D). The values of data represented the means±SD of three experiments. *P<0.05 is the significance level relative to the vehicle-treated group.

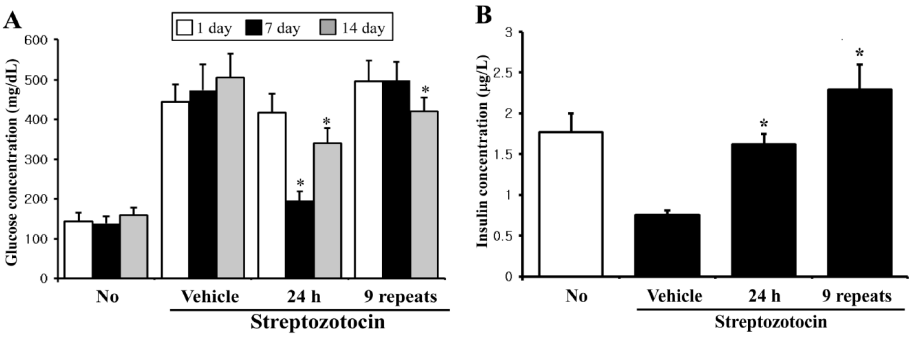

Figure 8. Effects of RLP on glucose and insulin regulation of streptozotocin-induced diabetic mice. During the treatment of RLP for 14 days, the glucose concentration was measured at three different times using the sensitive strip of the Blood Glucose Monitoring System (A). The insulin concentration was detected in the serum of streptozotocin-induced diabetic mice on the final day (B). The values of data were expressed as the means±SD of three experiments. *P<0.05 is the significance level compared to the vehicle-treated group.

2.2.3. Role as insulin stimulator in vivo

Early work was performed with aqueous extract of *L. platyphylla* (AELP). In this study, AELP was administrated into nonobese diabetic (NOD) mice showing type I diabetes with 200 mg/kg body weight for 14 weeks. Glucose concentration was significantly suppressed in NOD mice treated with AELP, while this level was increased in vehicle-treated NOD mice (Fig. 9). Also, AELP treated NOD mice showed higher insulin concentration than control NOD mice, although IL-4 and IFN-γ level was decreased in AELP treated NOD mice (Table 3)[13]. Therefore, these results indicated that AELP have a components down-regulating glucose concentration via enhancement insulin concentration.

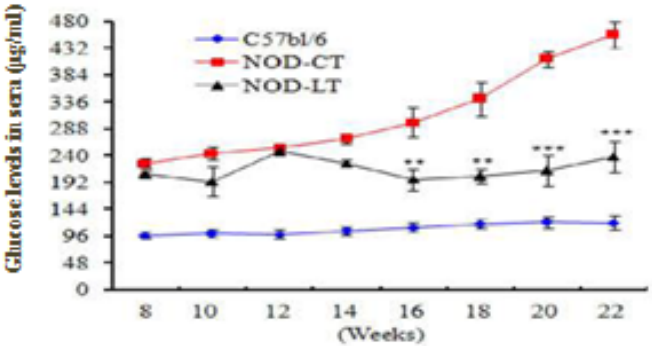

Figure 9. Alteration of glucose concentration in serum of NOD mice after AELP treatment. Glucose concentration was measured in whole blood collected from tail vein of NOD. CT indicated control group, LT indicated AELP treated group.

	C57	NOD(Control)	LT
IL-4(pg/ml)	9.1±0.41	13.4±1.1	11.0±0.7
IFN-γ(pg/ml)	4.2±0.20	45.2±2.5	12.5±3.1
Insulin(pg/ml)	9.8±0.4	3.8±0.8	25.3±0.6

Table 3. Concentration of insulin and cytokines in serum of NOD mice after AELP treatment. C57 indicated 22-week-old C57BL/6 mice, control indicated vehicle-treated group, LT indicated AELP treated group.

In addition, the therapeutic effects of LP9M80-H which had insulin secretion ability in HIT-T15 cells [12] had been investigated in normal animals and diabetic model by our group. Firstly, LP9M80-H was administrated into ICR mice for 4 days to investigate the correlation between Glut biosynthesis and the insulin signalling pathway activated by LP9M80-H. The ICR mice treated with LP9M80-H showed lower glucose concentration and higher insulin concentration than vehicle-treated group, although their body weight was remained constant over 5 days (Fig. 10). Also, the expression of Glut-3 was significantly down-regulated through p38 MAP kinase signalling pathway in liver, while the expression of Glut-1 was up-regulated by Akt and PI3-K pathway in liver and brain of LP9M80-H treated mice (Fig. 11) [10]. Thus, these study showed the first evidences that LP9M80-H could regulate Glut-1 and Glut-3 biosynthesis through the Akt and p38 MAPK signalling pathway in ICR mice.

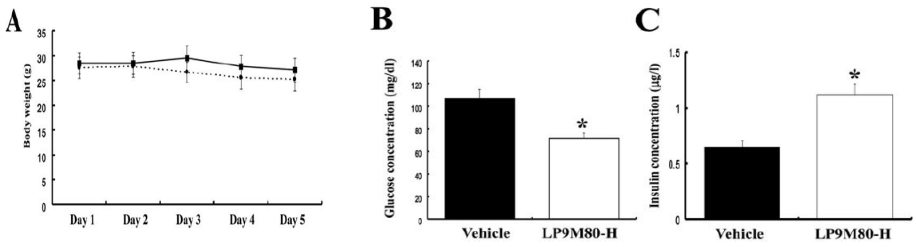

Figure 10. Effects of LP9M80-H on body weight (A), glucose concentration (B) and insulin concentration (C) of ICR mice. After oral gavage with LP9M80-H (10 μg/g body weight/day) for 5 days, blood glucose and insulin concentration were determined with Blood glucose monitoring system and Insulin ELISA kit. Values are means ± SD. *p<0.05 is the significance level compared to the vehicle-treated group.

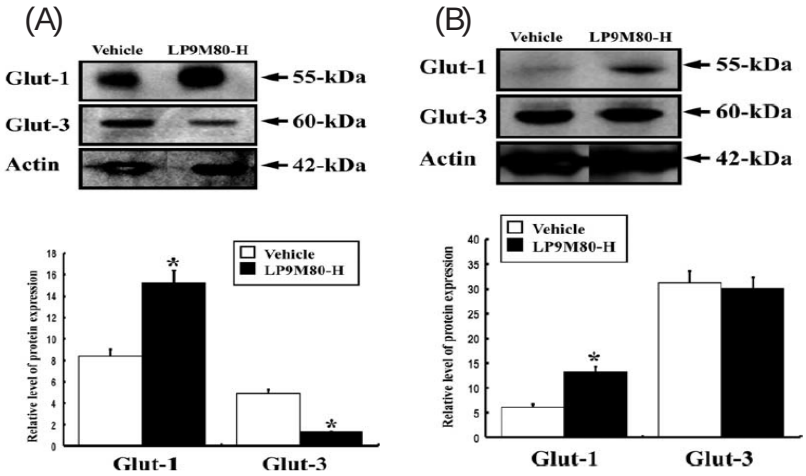

Figure 11. Effects of LP9M80-H on Glut expression in liver (A) and brain of ICR mice. The Glut-1 and Glut-3 protein expression in the liver and brain was detected with anti-Glut-1, anti-Glut-3 primary antibodies, and horseradish peroxidase-conjugated goat anti-rabbit IgG. The intensity of the Glut protein was calculated using an imazing densitometer. The values are the mean ± SD. *$p<0.05$ is the significance level compared to the vehicle-treated group.

Furthermore, the effects of LP9M80-H were also investigated in OLEFT rats showing type II diabetes to determine whether or not the therapeutic effects on the pathology of diabetes and obesity. After the oral administration for 2 week, the abdominal fat mass were significantly lower in LP9M80-H treated group than vehicle treated group, although there are no difference in body weight between two group. Also, a significant alteration on glucose and insulin concentration was detected in LP9M80-H treated OLETF rats compare with vehicle treated rats (Fig. 12). Furthermore, LP9M80-H treated OLETF rats showed the decrease of lipid and adiponectin concentration as well as the enhanced expression of insulin receptor and insulin receptor substrate. Especially, the Glut-2 and Glut-3 expression was decreased whereas Glut-4 expression increased by LP9M80-H in liver tissue of OLETF rats (Fig. 13) [14]. Therefore, this paper showed that LP9M80-H may also relief the symptoms of diabetes and obesity in Type II model.

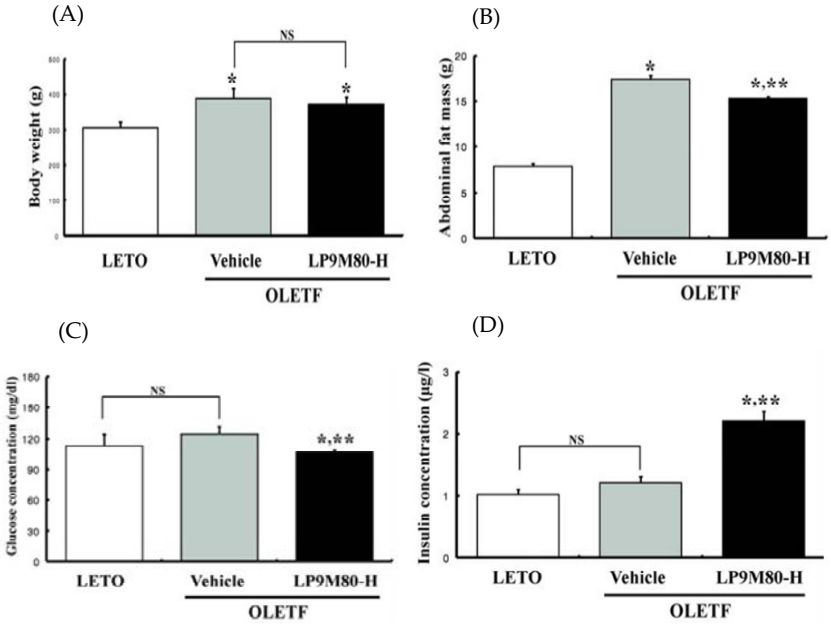

Figure 12. Effects of LP9M80-H on body weight (A), abdominal fat (B), blood glucose (C) and insulin concentration (D) of OLETF rats. At 24 hr after final LP9M80-H treatment, the body weight and abdominal fat mass of OLETF rats were measured with an electronic balance. Glucose level and insulin was measured using blood collected from the abdominal veins of rats. The data represents the mean±SEM from three replicates. *p<0.05 is the significance level compared to LETO group. **p<0.05 is the significance level compared to the vehicle-treated group.

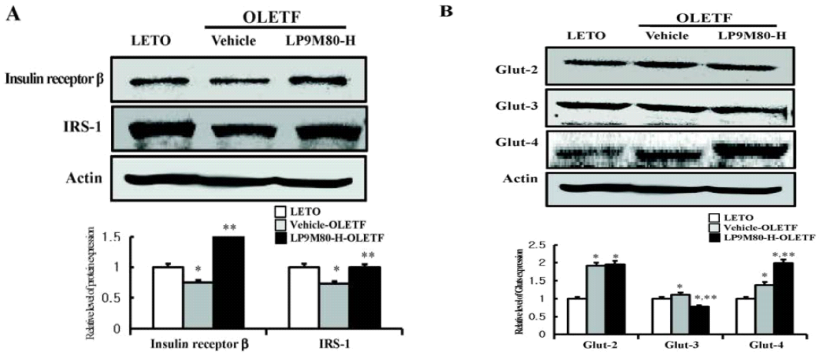

Figure 13. Effects of LP9M80H on insulin receptor-related factors and Gluts expression in liver of rats. Insulin receptor, IRS-1 and Gluts protein expression was detected with each primary antibody and horseradise peroxidase-conjugated goat anti-rabbit IgG. The intensity of each protein was calculated using an imaging densitometer. The data represents the mean±SEM from three replicates. *p<0.05 is the significance level compare to the LETO rats. **p<0.05 is the significance level compare to the vehicle-treated OLETF rats.

2.3. Effects of *L. platyphylla* on neurodegenerative disorder

2.3.1. Induction effects of Nerve Growth Factor (NGF) secretion in vitro

NGF was one of neurotrophic factors that regulated the neuronal development and maintenance within central nervous system (CNS) and peripheral nervous system (PNS)[16]. Many perivous studies showed that NGF could reduce the cholinergic neuronal damage induced from surgical injury [16] and prove the cognitive ability of aged rodents [24]. Therefore, NGF was considered as a therapeutic drug for the treatment of neurodegenerative disorder such as Alzheimer's disease and cerebrovascular dementia [25,26].

Firstly, the effects of butanol extract isolated from *L. platyphylla* (BELP) were investigated in undifferentiated PC12 cells (pheochromocytoma of the rat adrenal medulla) using conditional medium of C6 and primary astrocytes [15]. In order to collect conditional medium, C6 and primary astrocytes were incubated with BELP during 24 hr and then media harvested from these cells. In these results, the neuritic outgrowths of PC12 cells were significantly induced with dose-dependent manner. The maximum length of neurite-bearing cells was observed at a concentration of 10 μg/ml of BELP conditional medium (Fig. 14). Furthermore, the expression and secretion of NGF was determined in C6 cells and primary astrocytes. The NGF concentration was higher in the culture media of BELP-treated C6 and primary astrocytes than these of control. The RT-PCR results showed that BELP treatment induced the increase of the expression level of NGF mRNA (Fig. 15). Therefore, these results suggested that BELP may induce the enhancement of the expression and secretion of NGF in astrocytes.

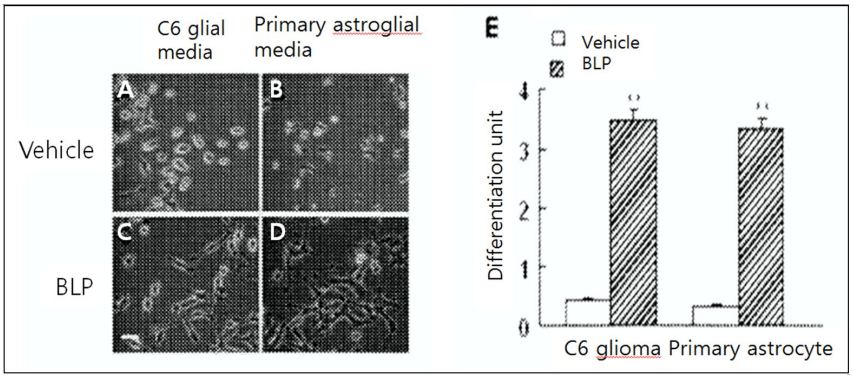

Figure 14. Effect of BELP-conditioned media on the neurite outgrowth of PC12 Cells. After 48 h incubation with four different conditioned media including (A) C6 glial media conditioned by vehicle (DMSO, 0.01%), (B) Primary astroglial media conditioned by vehicle (DMSO, 0.01%), (C) C6 glial media conditioned by BLP (10μ g/ml), (D) Primary astroglial media conditioned by BLP (10μ g/ml), the morphology of PC12 cells were detected using a camera attached to a microscope. (E) The differentiation of PC12 cells was scored as follows: cells without neurite outgrowth; 0, cells bearing neurite as long as one cell diameter; 1, cells bearing neurite two times longer length than their diameter; 2, and cells which had a synapse-like neurite. The results are expressed as the mean± SEM. The asterisks indicate a significant difference from the treatment with media conditioned by vehicle (** $p < 0.01$).

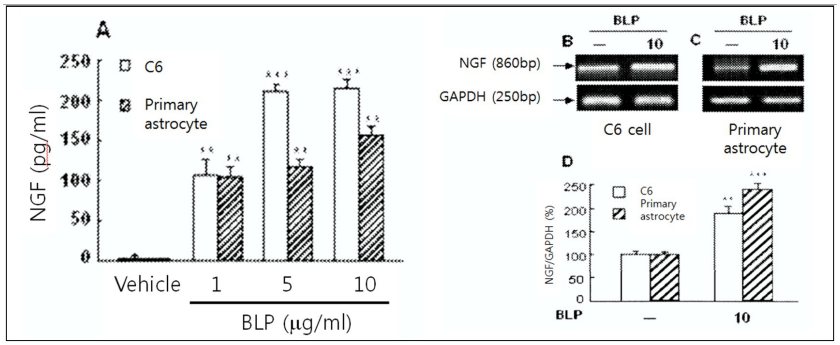

Figure 15. Effect of BELP on NGF synthesis and secretion of astrocytes. After BELP treatment for 6 h, NGF secretion and mRNA expression was measured using an ELISA kit (A) and RT-PCR analysis (B-D). The results are expressed as the mean± SEM. The asterisks indicate a significant difference from the treatment with media conditioned by vehicle (** $p < 0.01$).

Meanwhile, Hur et al [16] studied the effects of spicatoside A on NGF secretion and NGF receptor signalling pathway. Spicatoside A used in this study was isolated from dried tubers of *L. platyphylla* using bioactivityguided isolation techniques and their structure was determined with 1H NMR and 13C NMR analysis (Varian U1500, 500 MHz, CD3OD)(Fig. 16). Then, their effects on neurite outgrowth in undifferentiated PC12 cells were investigated. The neuritic outgrowth was significantly increased in PC12 cells treated with spicatoside A and their effects observed at 10 μg/ml were very similar to that of NGF at 50 ng/ml (Fig. 17). In most of animal cells, Trk, a high affinity NGF receptor regulated the cell survival and neuritic outgrowth via ERK1/2 and PI3-kinase signaling pathway [27]. Therefore, the effects of spicatoside A on NGF receptor and their downstream signaling pathway in undifferentiated PC12 cells were examined using western blot analysis to investigate NGF ability. The high level of TrkA phosphorylation was detected in the spicatoside A-treated PC12 cells (Fig. 18). Also, spicatoside A-treated PC12 cells showed the increase level of ERK1/2 and Akt phosphorylation level compare with control group (Fig. 18). In conclusion, spicatoside A may induce the neuritic outgrowth of PC12 cells through TrkA signaling pathway including ERK1/2 and PI3-kinase pathway.

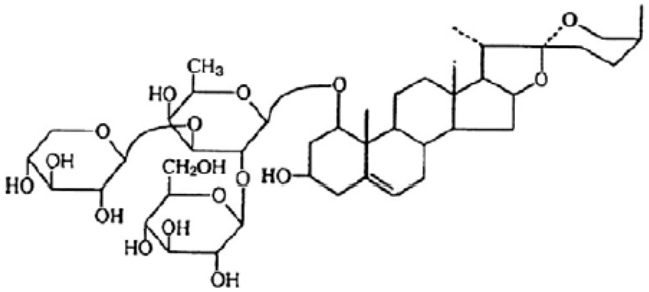

Figure 16. Chemical structure of spicatoside A isolated from *L. platyphylla*.

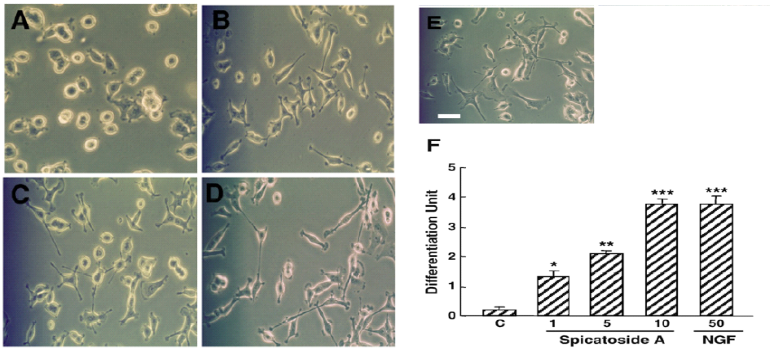

Figure 17. Effects of spicatoside A on the neurite outgrowth of PC12 cells. The morphology of PC12 cells were were observed using a camera attached to a microscope (×100) under the different treatment condition. (A) Vehicle, (B) Spicatoside A (1 μg/mL), (C) Spicatoside A (5 μg/mL), (D) Spicatoside A (10 μg/mL), (E) NGF (50 ng/mL). (F) indicates the length of the PC12 cell neurite outgrowth. The results are expressed as the mean±SEM. The asterisks indicate a significant difference from the control (*P<0.01, **P<0.005, and ***P<0.001).

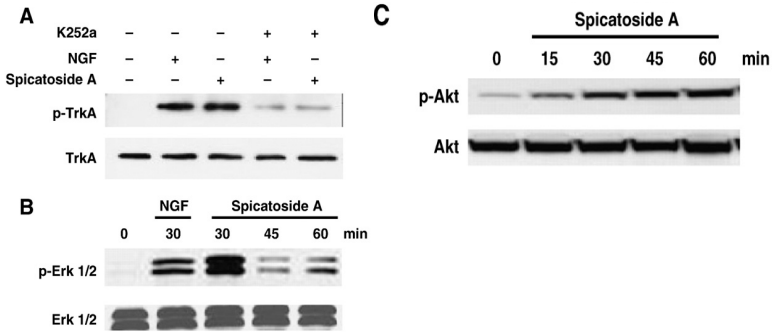

Figure 18. Effects of spicatoside A on NGF receptor signaling pathway. (A) The phosphorylation level of the TrkA receptor was detected in undifferentiated PC12 cells stimulated for 30 min with either spicatoside A (10 μg/mL) or NGF (50 ng/mL) with or without K252a (potent inhibitor of various protein kinases)(100 nM). (B) The intracellular ERK1/2 phosphorylation was measured in the undifferentiated PC12 cells stimulated with spicatoside A (10 μg/mL) for 30, 45, and 60 min or with NGF (50 ng/mL) for 30 min. (C) After the PC12 cells were stimulated with spicatoside A (10 μg/mL) for 30, 45, 60, and 120 min, the proteins containing phosphotyrosine were immunoprecipitated using anti-phosphotyrosine antibody agarose beads. The Akt phosphorylation was detected in the PC12 cells stimulated with spicatoside A (10 μg/mL) for 15, 30, 45, and 60 min.

2.3.2. Induction effects of NGF secretion in vivo

The NGF stimulation effects of *L. platyphylla* observed in several cell lines was further investigated base on behavioural and physiological features in mice. Firstly, the effects of ethanol (70%) extract of roots of *L. platyphylla* (EELP) on learning and memory was measured in ICR mice using the passive avoidance task. The sub-chronic treatment of four different concentrations of EELP induced a significant group effect on the step-through latency in retention

trial. Especially, these latencies were significantly loger in EELP-treated group than those in vehicle-treated group (Fig. 19). In addition, EELP effect on the BDNF and NGF expression was detected in brain of ICR mice using immunohistochemistry. BDNF immunoreative cells were dramatically increased in CA1 region of hippocampus and dentate gyrus region in a dose dependent manner. Furthermore, NGF immunostaining cells were increased by treatment of EELP in dentate gyrus region of ICR mice, while those cells did not detected in CA1 region of hippocampus (Fig. 20). Above results showed that EELP administration could improve the learning and memory of mice through the increase of BDNF and NGF expression.

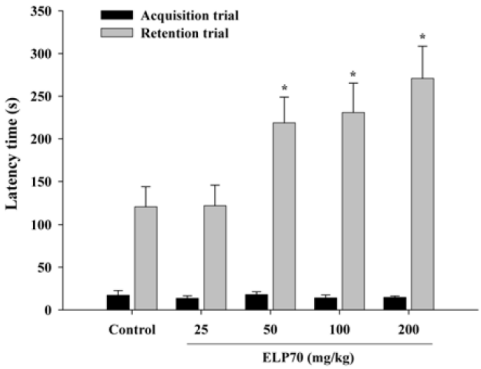

Figure 19. Effects of EELP administration on learning and memory of ICR mice. After final administration of four different concentrations EELP, the retention trials were carried out 24 hr after acquisition trials. The results data are expressed as the mean±SEM. *P<0.005, compare with the vehicle control group.

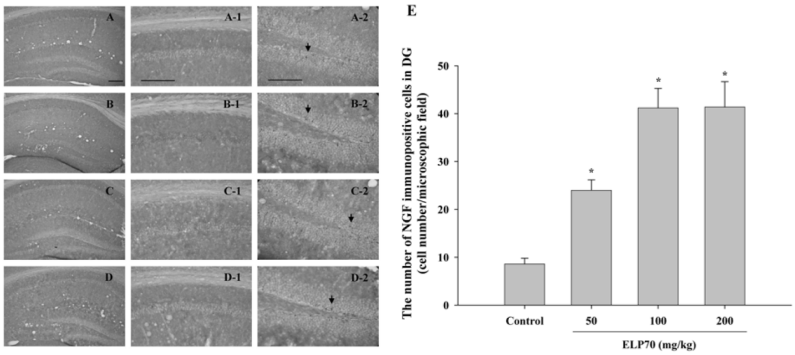

Figure 20. Effects of EELP administration on NGF expression. The NGF positive cells were detected in the hippocampa (A-D), dentate gyrus (A-1, B-1, C-1, D-1), CA1 region (A-2, B-2, C-2, D-2) of brain after administration of vehicle (A, A-1, A-2), EELP 50 mg/kg (B, B-1, B-2), EELP 100 mg/kg (C, C-1, C-2) or EELP 200 mg/kg (D, D-1, D-2). (E) The number of NGF positive cells in the dentate gyrus was calculated in mice treated with three different concentration (50, 100, 200 mg/kg). The results data are expressed as the mean±SEM. *P<0.005, compare with the vehicle control group.

Meanwhile, Nam et al. [18] reported the 100% methanol extracts isolated from *L. platyphylla* (MELP) on NGF metabolism. Firstly, they collected a total 13 novel extract from the roots of *L. platyphylla* using various solvent such as ethylacetate, methanol, hexan, butanol and chloroform. Of these extracts, only four extracts (LP-E, LP-M, LP-M50 and LP2E17P) induced the NGF secretion and mRNA expression in neuroblastoma cells, although the NGF-induced neuritic outgrowth from PC12 cells was only induced by LP-E, LP-M and LP-M50 (Fig. 21). Furthermore, *in vivo* effect of LP-M was investigated in C57BL/6 mice treated with 50 mg/kg of LP-M for 2 weeks. The expression level of NGF mRNA was significantly increased in LP-M treated mice compare with vehicle treated goup. The signaling pathway of TrkA NGF receptor was dramatically activated in hippocampus of mice via LP-M treatment, while the signaling pathway of $p75^{NTR}$ was inhibited in the cortex by LP-M treatment (Fig. 22). Then, these results suggested the possibility that four novel extracts of *L. platyphylla* was considered to be a good candidate for a neurodegenerative disease-therapeutic drug.

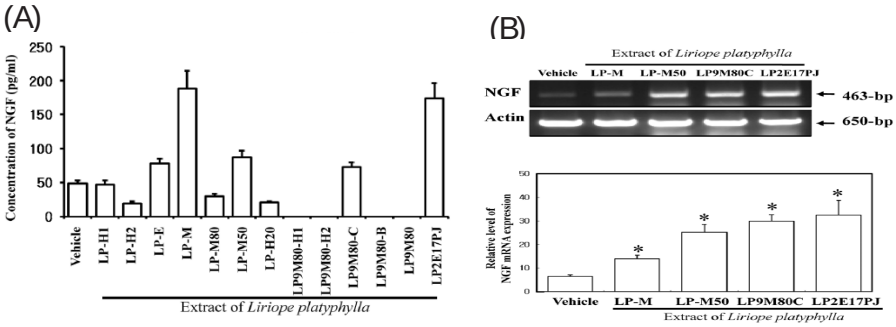

Figure 21. Novel extracts of *Liriope platyphylla* on the NGF secretion and mRNA expression. (A) After the 13 extracts treatment into B35 cells for 24 hr, An NGF concentration in the supernatant was measured using anti-NGF ELISA kit. (B) The density of NGF mRNA was quantified using a Kodak Electrophoresis Documentation and Analysis System 120. The β-actin signal was used as the endogenous control, and the transcript (650-bp) indicates the RNA loading. The values of data represented mean±SD of three experiments. *p<0.05 is the significance level compared to the vehicle treated group.

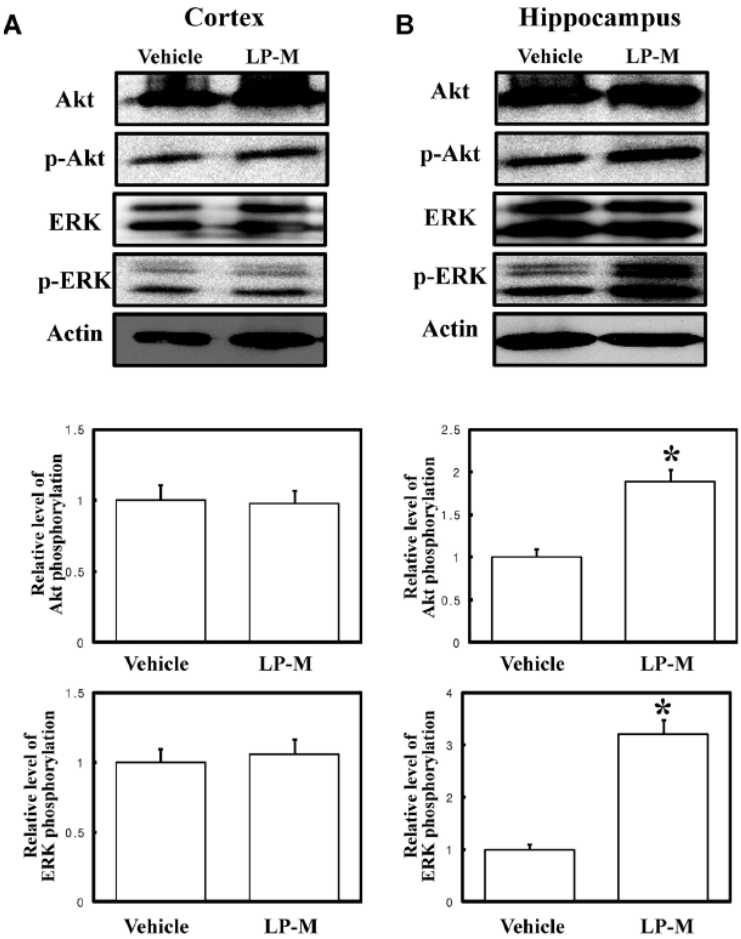

Figure 22. Effect of LP-M on down-stream signaling pathway of NGF high affinity receptor in cortex (A) and hippocampus (B) of C57Bl/6 mice. The values are the mean±SD. *p<0.05 is the significance level compared to the vehicle treated group.

2.4. Therapeutic effects on other diseases

2.4.1. Prevent effects on obesity

Obesity was caused by an energy imbalance induced by an increase ration of caloric intake to energy expenditure. Recently, a development of novel drug for obesity has received attention as important topics. In an effort of develop drug for the treatment of obesity, Hur et al. [16] investigated the therapeutic effects of Gyeongshingangjeehwan which composed of four medicinal plants, *L. platyphylla*, *P. grandiflorum*, *S chinensis*, and *E. sinica* using OLEFT rats. Firstly,

abdominal fat area was significantly decreased by 12.1% in GGEx (X5) treated OLETF rats and 42.8% in GGEx (X10) group (Fig. 23A and B). Compare with the LETO group, the leptin level that reflects changes in body weight and adipose tissue mass [28] were 172% higher in the vehicle treated OLETF rats. But, GGEx treatment decreased leptin levels by 23.5% in vehicle treated OLETF rats with similar level to those in sibutramine (oral anorexiant)-treated obese rats (Fig. 23C). Also, the beneficial effects of GGEx on circulating lipid profile including triglyceride and total cholesterol were examined. GGEx treated OLETF rats showed a reduction of plasma triglycerides by 28.7% although total cholesterol level were unaffected by GGEx treatment (Fig. 24). Furthermore, the hepatic lipid accumulation were markedly lower in GGEx treated OLETF rats than those in vehicle treated OLETF rats (Fig. 25), while the mRNA level of PPARα target enzymes were upregulated by GGEx administration [19]. These results suggest that GGEx including *L. platyphylla* may effectively prevent obesity and hypertriglyceridemia through the inhibition of feeding and the activation of hepatic PPAR.

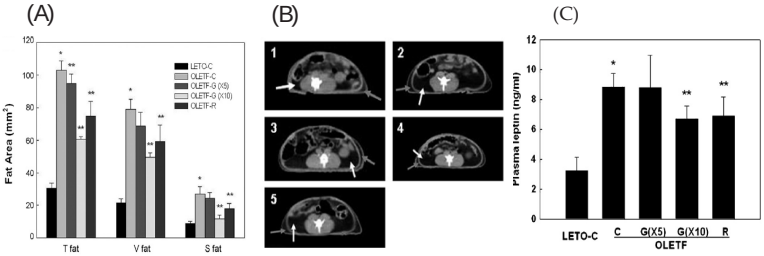

Figure 23. Effects of GGEx on abdominal fat mass and leptin concentration in genetically obese OLETF rats. Adult male LETO and OLETF rats were treated with water, GGEx, or sibutramine for 8 weeks. (A) Rats underwent CT to measure cross-sectional abdominal subcutaneous and visceral fat areas. (B) Representative CT images are shown. Gray and white arrows indicate subcutaneous and visceral fat. (C) The leptin plasma levels were measured by ELISA kit. All values are expressed as the mean±S.D. for n=4 rats. *$p < 0.05$ is the significance level compared to the LETO control rats. **$p < 0.05$ is the significance level compared to the vehicle treated OLETF rats.

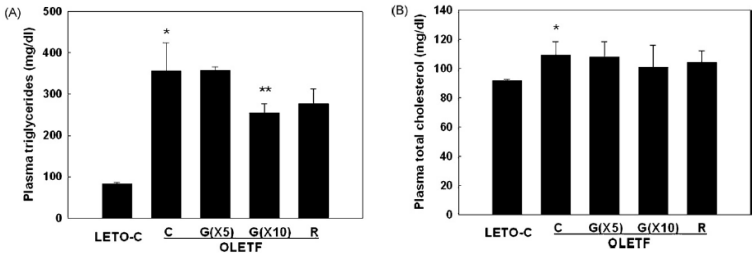

Figure 24. Effects of GGEx on circulating triglycerides and total cholesterol in genetically obese OLETF rats. After water, GGEx, or sibutramine for 8 weeks, Plasma concentrations of triglycerides (A) and total cholesterol (B) were measured and all values are expressed as the mean±S.D. for n=4 rats. *$p < 0.05$ is the significance level compared to the LETO control rats. **$p < 0.05$ is the significance level compared to the vehicle treated OLETF rats.

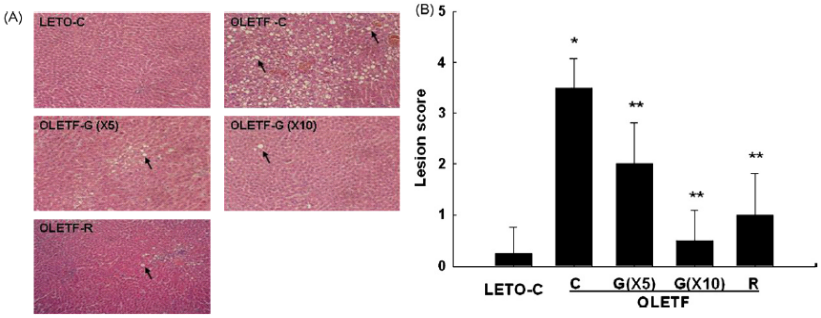

Figure 25. Effects of GGEx on inhibition of hepatic lipid accumulation in genetically obese OLETF rats. (A) Representative hematoxylin- and eosin-stained sections of livers are shown (original magnification 200×). (B) Histological analyses of hepatic lipid accumulation. Pathological scores of hepatic lipid accumulation are as follows: 0, no lesion; 1, mild; 2, moderate; 3, severe; 4, very severe. All values are expressed as the mean±S.D. for n=4 rats. *$p < 0.05$ is the significance level compared to the LETO control rats. **$p < 0.05$ is the significance level compared to the vehicle treated OLETF rats.

2.4.2. Therapeutic effects on atopic dermatitis

Atopic dermatitis was a typical skin disorder showing inflammatory, chronically relapsing, noncontagious and pruritic symptoms. Also, they were induced by several factors such as epidermal barrier dysfunction, allergy, microwave radiation, food allergy, histamine intolerance and other biological factors. Recently, Kim et al. [20] has investigated the effects of aqueous extracts of *L. platyphylla* (AELP) on atopic dermatitis of NC/Nga mice after phthalic anhydride (PA) treatment. In this animal model, 10% AELP treated mice showed the significant decline of the pathological phenotypes of atopic dermatitis such as erythema, ear thickness, edema, scab and discharge compare to control group (Fig. 26). Also, the weight of immune related organs including lymph node and thymus were gradually decreased in AELP treated groups, while the weight of spleen was slightly increased in same group (Fig. 27). The significant histological changes including inflammation, edema, epidermal hyperplasia were observed in 5% and 10% AELP treated group. Furthermore, toluidine blue staining analysis, a method used to specifically identify the mast cell, showed that the decrease of master cell infiltration into the dermis were statistically observed in 5% AELP and 10% AELP treated groups (Fig. 28). The IgE concentration was lower in only 10% AELP treated group than that in control group although this level was not affected in 5% AELP treated group. Therefore, these results indicated that the aqueous extracts of *L. platyphylla* may contribute the relieve of atopic dermatitis symptoms.

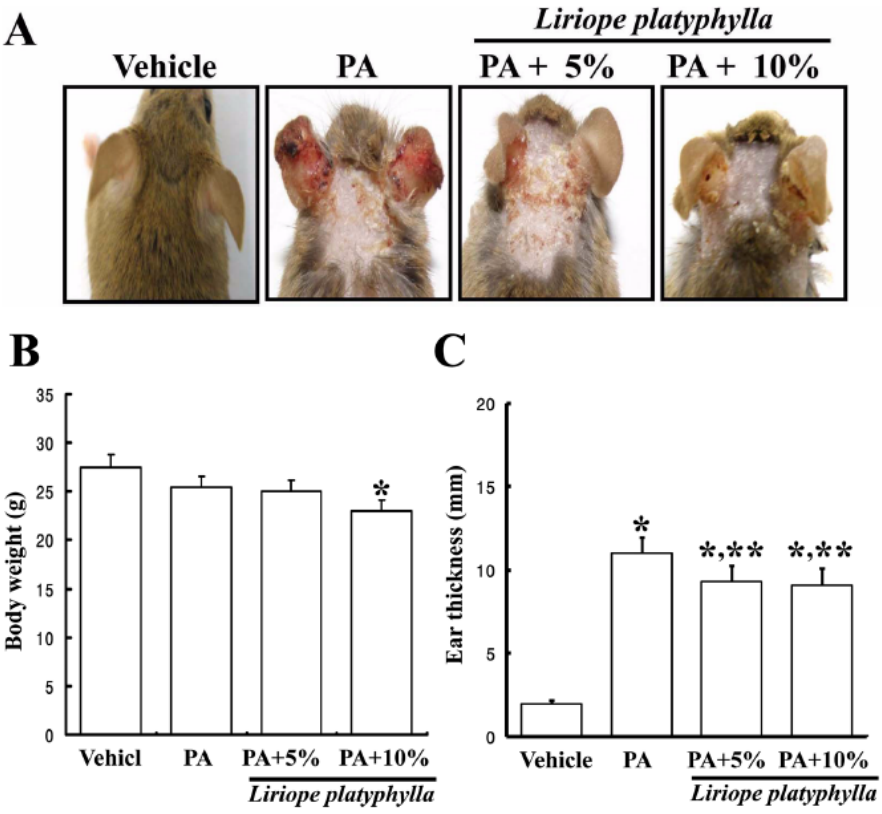

Figure 26. Effects of AELP on the ear pathological phenotypes, the body weight and the ear thickness. PA solution was repeatedly applied to the dorsum of the ear and back skin of NC/Nga mice. After 2 weeks, the change of ear pathological phenotype (A), the body weight (B) and the ear thickness (C) were detected in the mice. Data shown are the means±SD (n=5). *P<0.05 is the significance level compared to the vehicle treated group. **P<0.05 is the significance level compared to the PA treated group.

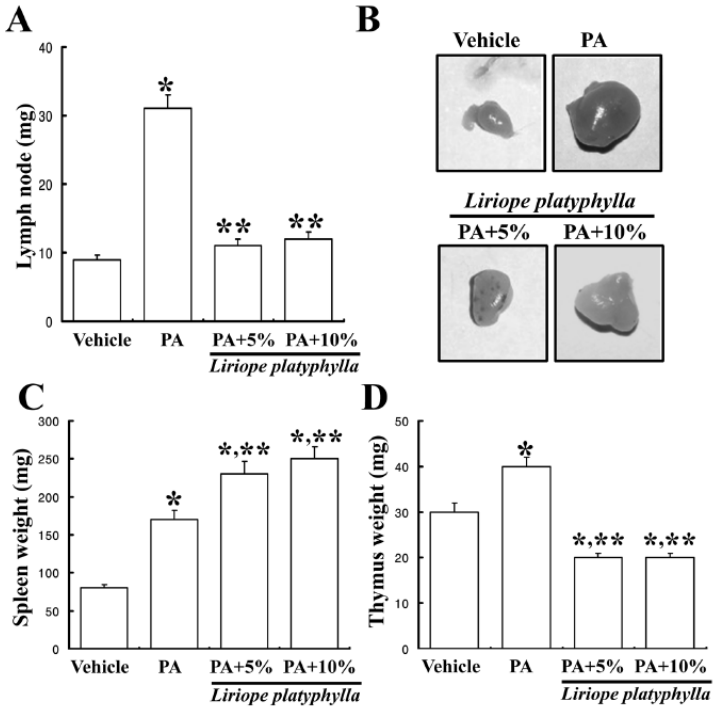

Figure 27. Effects of AELP on weight of three immune organs. After final AELP treatment, all of the animals were immediately sacrificed using CO2 gas in order to prepare the immune organs. The weight of lymph nodes, spleens and thymus were measured using the chemical balances. Data shown are the means±SD (n=5). *P<0.05 is the significance level compared to the vehicle treated group. **P<0.05 is the significance level compared to PA treated group.0

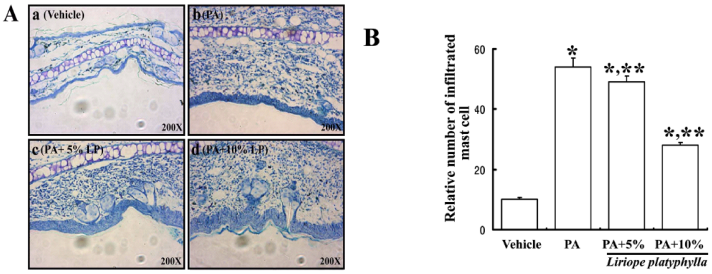

Figure 28. Effects of AELP on the mast cell infiltration. (A) The slide sections of ear tissue were stained with toluidine blue and observed at 400x magnification. Mast cells were stained with purple color in the dermis of ear tissue. (B) In each slide, five fields were randomly chosen and the number of mast cells was counted under a light microscope. Data shown are the means±SD (n=5). *P<0.05 is the significance level compared to the vehicle treated group. **P<0.05 is the significance level compared to the PA treated group.

3. Conclusion

The development and identification of novel therapeutic drugs for human chronic disease was considered as a very important project in the field of pharmacological and clinical research. Among the variety of approaches thus far pursued to develop novel drugs, identification and screening of natural compounds from medicinal herbs has proven a very effective one—not least, because this method saves a great deal of time and cost. Recently, some scientists including our group in Asia countries have reported the therapeutic effects of *L. platyphylla* on the human chronic disease. This chapter introduces some extracts and compound which may prove valuable in efforts to combat chronic diseases such as inflammation, neurodegenerative disorders, diabetes, obesity and atopic dermatitis.

Three extracts prepared with *n*-hexane, ethyl acetate and water was found to significantly induce anti-inflammation and anti-asthmatic effects in model animals. Some extracts of *L. platyphylla* play a role as insulin sensitizer in adipocytes and stimulator in insulinoma cells and the pancreas of mice. Additionally, butanol extracts and spicatoside A markedly induced NGF secretion and expression in some cell lines, while ethanol and methanol extracts induced in mice. Furthermore, recent studies showed that GGEx and water extracts prevented or improved the obesity and atopic dermatitis.

In conclusion, the results of above studies indicated that some extracts and compounds from *L. platyphylla* could contribute the relief and prevent of several chronic diseases including dementia, diabetes, obesity and atopic dermatitis. However, more research was needed to verify the action mechanism and toxicity side effects.

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