A Thesis for the Degree of Master of Science

Genipin, a metabolite of geniposide from *Gardenia jasminoides*, inhibits adipogenesis and improves lipid metabolism in adipocytes

게니핀의 지방세포에서의 분화 억제 및 지질대사 개선 효능

February, 2014

Department of Agricultural Biotechnology

Seoul National University

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석사학위논문

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이 논문을 석사학위 논문으로 제출함

2014년 2월

서울대학교 대학원

농생명공학부

지 현 경

지현경의 석사학위논문을 인준함

2014년 2월

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학위논문 원문제공 서비스에 대한 동의서

본인의 학위논문에 대하여 서울대학교가 아래와 같이 학위논문 저작물을 제공하는 것에 동의합니다.

1. 동의사항

①본인의 논문을 보존이나 인터넷 등을 통한 온라인 서비스 목적으로 복제할 경우 저작물의 내용을 변경하지 않는 범위 내에서의 복제를 허용합니다.

②본인의 논문을 디지털화하여 인터넷 등 정보통신망을 통한 논문의 일부 또는 전부의 복제.배포 및 전송 시 무료로 제공하는 것에 동의합니다.

2. 개인(저작자)의 의무

본 논문의 저작권을 타인에게 양도하거나 또는 출판을 허락하는 등 동의 내용을 변경하고자 할 때는 소속대학(원)에 공개의 유보 또는 해지를 즉시 통보하겠습니다.

3. 서울대학교의 의무

①서울대학교는 본 논문을 외부에 제공할 경우 저작권 보호장치(DRM)를 사용하여야 합니다.

②서울대학교는 본 논문에 대한 공개의 유보나 해지 신청 시 즉시 처리해야 합니다.

논문제목 : 게니핀의 지방세포에서의 분화 억제 및 지질대사 개선 효능

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Abstract

Genipin is an aglycone metabolite of geniposide which is derived from gardenia (*Gardenia jasminoides*) fruit. Geniposide is deglycosylated to genipin by β-glucosidase in animal intestine and liver. Gardenia fruit is well-known for its food coloring usage and also various health benefits such as anti-inflammatory and hypolipidemic activities as well, which give promising potential to control metabolic disorders and obesity. However, the effects of gardenia fruit and its active components involved in the development obesity have been poorly investigated. Thus, the present study was carried out to identify the effects of genipin, originated from gardenia fruit, on the obesity in the aspects of adipogenesis and lipid metabolism. The data showed that genipin, but not geniposide, inhibits adipogenesis and improves lipid metabolism significantly at 100 μM in 3T3-L1 cells, associated with the down-regulation of adipogenesis-related proteins (peroxisome proliferator-activated receptor gamma; PPARγ, CCAAT/enhancer-binding protein alpha; C/EBPα, and fatty acid synthase; FAS)

expression. However, genipin did not show the suppressive effect on the phosphorylation of protein kinase B (Akt) and extracellular signal-regulated kinase (ERK). Moreover, glycerol release levels which is an indication of lipid metabolism, specifically lipolysis, was also decreased. Taken together, the results elucidate that genipin inhibits adipogenesis in 3T3-L1 preadipocytes and improves lipid metabolism in 3T3-L1 adipocytes. These results suggest that genipin might play a role as a potential chemopreventive compound for the control of body weight/fat.

Keywords: genipin / geniposide / gardenia fruit / obesity / adipogenesis / lipid metabolism / adipocytes / PPARγ / C/EBPα / FAS / glycerol

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1. Introduction

Obesity is the state of having too much body fat which has been a critical global health concern. It is caused by the imbalance between energy intake and expenditure; in that, more energy intake occurs due to much nutrition and little exercise. Obesity and overweight impair one's appearance and also readily lead to insulin resistance and metabolic disorders, such as diabetes, hyperlipidemia, hypertension, cardiovascular disease, and cerebrovascular disease, as well [1]. These are the reasonable causes why it is substantially crucial to optimally manage obesity in many ways.

To the point, the present study was initiated to explore a novel potent anti-obesity compound originated from a food material. Moreover, the study evaluated the anti-obesity activities of selected compounds in the aspects of adipogenesis and lipid metabolism. In the same context, this study was inspired by one of the frequent prescriptions of traditional Korean medicine Bangpungtongseong-san, which has been used to treat obesity for a long time.

Bangpungtongseong-san is composed of 18 plant materials and 3 mineral materials, total 21 materials. So as to select promising candidate compounds that might possess the anti-obesity activities, the indicative components of Bangpungtongseong-san, which are baicalin (from skullcap), glycyrrhizin (from licorice), liquiritin (from licorice), and geniposide (from gardenia), stated by Korea Institute of Oriental Medicine (KIOM) were referred. Meanwhile, all these four compounds are glycosides which generally can be metabolized into aglycone forms in human intestine or liver to increase bioavailability, namely to be easily absorbed and functioned within the body. In this line, aglycones of the above-mentioned four glycoside compounds (baicalin, glycyrrhizin, liquiritin, geniposide) were additionally considered as potential anti-obesity candidates (baicalein, glycyrrhetin, liquiritigenin, genipin), and net eight candidate compounds were prepared. Though, as three among the eight compounds had already been identified their anti-obesity activities, final five candidate compounds (glycyrrhizin, liquiritin, liquiritigenin, geniposide, genipin) were selected in the

end. Then, the anti-obesity effects of these five candidate compounds were evaluated to discover a novel anti-obesity compound. Consequentially, the study found a valid obesity compound, so-called genipin which is an aglycone metabolite of geniposide originated from gardenia fruit.

Gardenia fruit, the fruit of *Gardenia jasminoides*, is popularly used in Asian countries as a natural food colorant; additionally, it is used as a traditional Asian medicine as it possesses an anti-pyretic, anti-inflammatory, hypolipidemic, hepatoprotective, and analgesic effect [2, 3]. Geniposide, an iridoid glycoside and major component within gardenia fruit, is reported to exert an anti-oxidant, anti-inflammatory, hypoglycemic, hepatoprotective, neuroprotective, anti-vascular endothelial cell adhesion, and anti-lipopolysaccharide (LPS) activity [4-10]. Genipin, an aglycone metabolite converted from geniposide, is also reported to have an anti-inflammatory, anti-thrombotic, and bile secretion effect [11-13].

Accordingly, gardenia fruit and its bioactive components

are potentially promising materials for the control of metabolic disorders and obesity; however, their anti-obesity effects and active compounds have been shortly investigated. Thus, it is meaningful to unveil the anti-obesity activity of genipin and its underlying molecular mechanisms involved.

Adipogenesis is a complex process of the differentiation from preadipocytes to adipocytes and accumulate large amount of intracellular lipid droplets in mature cells, which involves various changes including gene expression, hormone sensitivity, and cellular morphology and various other characteristics. The procedure includes mitotic clonal expansion (MCE) as a prerequisite and terminal differentiation [14]. In MCE step, cells synchronously re-enter two-round cell cycle and cell numbers are increased, which is termed as hyperplasia; and, during terminal differentiation, cell size is increased, leading to hypertrophy. Uncontrolled adipogenesis results in the excessive increase in adipocytes number, thereby contributing to the development of

obesity. Thus, suppressing adipogenesis in 3T3-L1 preadipocytes plays a promising action for the prevention or treatment of obesity.

In addition, obesity develops via the imbalance of lipid metabolism in adipose tissue as well, including decreased lipolysis and/or fatty acid oxidation and increased lipogenesis and/or triacylglycerol synthesis. Therefore, the modulation of lipid metabolism in adipocytes may provide pivotal strategies for the control of obesity [19]. Therefore, regulating lipid metabolism within adipose tissue and adipocytes is also important to control obesity.

Here, the study to identify the anti-adipogenic effects of selected compounds commonly uses a cell line model with 3T3-L1 preadipocytes, which differentiate into adipocytes under hormonal stimulation by an adipogenic mixture of isobutylmethylxanthine (IBMX), dexamethasone (DEX), and insulin (INS) (MDI) [15, 16]. During adipogenesis, proteins of peroxisome proliferator-activated

receptor γ (PPARγ) and CCAAT/enhancer-binding protein α (C/EBPα) are expressed as two major transcription factors [17, 18].

In the present study, selected candidate compounds were treated on fully differentiated 3T3-L1 adipocytes in order to evaluate their effects of candidate compounds on lipid metabolism. Furthermore, glycerol releases secreted from adipocytes were estimated to verify whether lipid metabolism is controlled by the lipolytic mechanism.

The study is carried on to elucidate the anti-obesity effects of genipin and geniposide originated from gardenia fruit in relation to the adipogenesis and lipid metabolism and their underlying mechanisms involved. Moreover, this experiment was to evaluate whether the metabolite compound genipin has better anti-obesity activity rather than its parent compound geniposide.

2. Materials and methods

2.1. Materials

3T3-L1 preadipocytes were purchased from American Type Culture Collection (ATCC) (Rockville, MD, USA). Genipin (molecular weight 226.23 g/mol, purity 98%) and geniposide (molecular weight 388.37 g/mol, purity 98%) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Antibodies against PPARγ and horseradish peroxidase (HRP)-conjugated secondary were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Antibodies against C/EBPα and FAS were obtained from Cell Signaling Biotechnology (Beverly, MA, USA). Antibody against β-actin was obtained from Sigma-Aldrich.

Dulbeccos's modified Eagle's medium (DMEM), bovine calf serum (BCS), fetal bovine serum (FBS), antibiotic-antimycotic (AA) were obtained from Gibco (Grand Island, NY, USA). 3-

isobutyl-1-methylxanthine (IBMX), dexamethasone (DEX), insulin (INS), thiazolyl blue tetrazolium bromide (MTT) powder, oil red O (ORO) powder, glycerol standard solution, and free glycerol reagent were obtained from Sigma-Aldrich.

2.2. Cell culture and cell differentiation

3T3-L1 preadipocytes were maintained in DMEM supplemented with 10% BCS and 1% AA and cultured until confluence in a humidified atmosphere at 37℃ and 5% CO_2. Confluent cells were incubated in DMEM supplemented with 10% FBS, 1% AA and standard adipogenic mixture, including 0.5 mM IBMX, 1 μM DEX, and 5 μg/ml INS (MDI), for 2 days to induce differentiation. The medium was replaced with DMEM containing 10 % FBS, 1% AA and 5 μg/ml INS for another 2 days. The medium was changed to DMEM containing 10% FBS and 1% AA for further 2 days until full differentiation. On the other hand, 3T3-L1 adipocytes were maintained in post-differentiation medium,

namely DMEM supplemented with 10% FBS and 1% AA, which was replaced to fresh medium every 2 days.

2.3. Thiazolyl blue tetrazolium bromide (MTT) assay

To analyze the cytotoxicity of genipin and geniposide on 3T3-L1 preadipocytes, 3T3-L1 preadipocytes were seeded in 96-well plates at a density of 0.5×10^4 cells per well, then cultured until confluence. When confluent, the cells were incubated in the presence or absence of genipin and geniposide for 72 h. Subsequent incubation of the cells with MTT solution (0.5 mg/ml) for 30 min at 37℃ allowed formation of a violet precipitate, formazan. It was dissolved in DMSO and the absorbance was measured at 570 nm on a microplate reader.

Otherwise, in order to verify the effect of genipin and geniposide on cell viability in 3T3-L1 adipocytes, 3T3-L1 preadipocytes were seeded and cultured until confluence. Then, the

cells were induced to differentiate into 3T3-L1 adipocytes for 6 days. When fully differentiated, mature adipocytes were incubated in the presence or absence of genipin and geniposide for 96 h. Next, MTT assay was performed according to the procedure stated above.

2.4. Oil red O (ORO) assay

For the investigation of anti-adipogenic effect using genipin and geniposide, 3T3-L1 preadipocytes were seeded in 24-well plates at a density of 2.5×10^4 cells per well, then maintained until confluence. When confluent, the cells were cultured until differentiation in the presence or absence of genipin and geniposide. After the induction of cell differentiation for 6 days, the media were removed and 3T3-L1 adipocytes were fixed in 10 % formaldehyde. The fixed cells were incubated with ORO solution (5 mg/ml) for 30 min at room temperature, which allows staining intracellular lipid droplets. ORO stain was eluted with isopropyl alcohol and the concentration was measured using a spectrometer set at 515 nm.

To detect the effect on lipid metabolism of genipin and geniposide, 3T3-L1 preadipocytes were seeded and cultured until confluence. Then, the cells were induced to differentiate into 3T3-L1 adipocytes for 6 days. When fully differentiated, mature adipocytes were incubated in the presence or absence of genipin and geniposide for 4 days. Next, ORO assay was performed according to the procedure stated above.

2.5. Western blot assay

3T3-L1 preadipocytes were seeded in 6-cm dishes at a density of 1.5×10^5 cells per dish and cultured. The cells were lysed and centrifuged (4℃, 14000 rpm, 10 min) and supernatants were collected. The protein concentration was determined using a protein assay kit (Bio-Rad Laboratories; Hercules, CA, USA) according to the manufacturer's instructions. Proteins were loaded and separated on sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) gels and transferred to membranes.

The membranes were blocked with 5% skim milk and attached with specific primary antibodies followed by HRP-conjugated secondary antibodies. The protein bands were visualized using a chemiluminescence detection kit (Amersham Pharmacia Biotech; Piscataway, NJ, USA).

2.6. Glycerol assay

After differentiation was fully induced in 24-well plates, 3T3-L1 adipocytes were treated with post-differentiation medium in the presence of absence of genipin and geniposide and incubated for 4 days. On day 4, culture supernatants were collected and incubated with free glycerol reagent for 15 min at room temperature. Glycerol release levels were measured using spectrometer at the set of 540 nm.

2.7. Statistical analysis

Data were shown as means ± standard deviations (SD), and the Student's t-test was used for statistical comparisons. A probability value of $P < 0.05$ or $P < 0.01$ or $P < 0.001$ was used as the criterion for a statistical significance.

3. Results

3.1. Genipin, the only one among five candidate compounds, suppresses adipogenesis in 3T3-L1 cells

First, the study assessed the anti-obesity activities using five candidate compounds (genipin, geniposide, liquritin, liquiritigenin, glycyrrhizin; Fig. 1A, B) in order to explore a novel potent anti-obesity compound. Beforehand, this study investigated the concentrations of each compound that do not show cytotoxicity (no cytotoxicity up to 80 μM in case of glycyrrhizin, 100 μM for others) (Fig. 2A, B).

Afterward, the study verified the effects of five candidate compounds on lipid formation. Four compounds (geniposide, liquiritin, liquiritigenin, glycyrrhizin) did not, but only genipin did show anti-adipogenic effect (Fig. 3A, B). Accordingly, this study found a new promising anti-obesity compound, genipin, which is

originated from gardenia fruit.

3.2. Genipin, but not geniposide, inhibits adipogenesis along every each stage, but in a similar extent manner, in 3T3-L1 preadipocytes

The study confirmed the effects of genipin and geniposide on adipocyte differentiation in 3T3-L1 preadipocytes and examined the intracellular lipid accumulation. When post-confluent 3T3-L1 preadipocytes were exposed to genipin and geniposide for 6 days, genipin potently suppressed the lipid accumulation in 3T3-L1 cells in a dose-dependent manner (25, 50 and 100 μM; 93.54, 79.58, and 43.96%; % of the control); otherwise, geniposide did not reduce intracellular lipid droplets at all (Fig. 5A).

Based on the present study's confirming genipin suppresses adipogenesis, subsequent study was performed to assess when genipin exerts its activity during adipogenic process whether on early, intermediate, or late stage. So as to identify on which stage

genipin influences on adipocyte differentiation, 100 μM genipin was treated in 3T3-L1 cells for different time periods (Fig. 5B). Consequently, genipin affected every each phage of adipogenesis, early, intermediate, and late stage, but in a similar extent manner (Fig. 5C).

The viability of 3T3-L1 preadipocytes treated with genipin and geniposide respectively was not significantly affected by concentration up to 120 μM (Fig. 4A, B). These results indicate that the inhibitory effect of genipin on lipid accumulation was not due to cytotoxicity. The suppressive effect of genipin on lipid contents was evident owing to the active function by genipin.

3.3. Genipin inhibits adipogenesis via down-regulation of adipogenesis-related protein expression in 3T3-L1 preadipocytes

To discover the mode of action by which genipin inhibits the lipid accumulation in 3T3-L1 cells, protein expression levels of

representative adipogenic markers PPARγ, C/EBPα, and FAS were measured. Genipin treatment significantly suppressed the expression of these proteins along the induction of adipogenesis for 6 days; whereas, geniposide showed no conspicuous effects on these expression levels as expected (Fig. 6A). These results propose that genipin inhibits adipogenesis through suppressing the expressions of PPARγ, C/EBPα, and FAS.

Subsequently, the study checked how genipin affects the early stage of adipogenesis. When phosphorylation of Akt and ERK was measured, no differences were observed upon genipin treatment for 1 hour and 15 min respectively (Fig. 6B, C). These results indicate that genipin did not inhibit two main pathways, Akt and ERK, during adipocyte differentiation by genipin; on the other word, suppression of adipogenesis was not quite associated with regulation of Akt and ERK phosphorylation.

3.4. Genipin, but not geniposide, improves lipid metabolism in

3T3-L1 adipocytes

Next, the study was performed to elucidate whether and how genipin influences the lipid metabolism during the intermediate and late stages of adipogenesis. For the experiment, 3T3-L1 preadipocytes were firstly fully differentiated into mature 3T3-L1 adipocytes for 6 days. Then, genipin and geniposide were treated within 3T3-L1 cells and incubated for additional 4 days. The results showed that genipin potently suppresses lipid accumulation in 3T3-L1 cells in a dose-dependent manner (25, 50 and 100 μM; 93.78, 90.82, and 87.62%; % of the control); otherwise, geniposide did not reduce intracellular lipid droplets at all (Fig. 8A).

The viability of 3T3-L1 adipocytes treated with genipin and geniposide respectively was not significantly affected by concentration up to 120 μM (Fig. 7A, B). These results indicate that the inhibitory effect of genipin on accumulated lipid contents in adipocytes was not due to cytotoxicity. The suppressive effect of genipin on lipid contents was evident due to the active function by

genipin.

3.5. Genipin decreases glycerol release in 3T3-L1 adipocytes

The contents of the lipid accumulated within mature adipocytes decreased as genipin, but not geniposide, was treated in 3T3-L1 cells. The results may be associated with some possible specific underlying mechanisms; activation of lipolysis/fatty acid oxidation, and/or inhibition of lipogenesis/triacylglycerol synthesis. First of all, this study hypothesized that triacylglycerol mass decreased significantly due to lipolysis activation. It is fully established that when lipolysis functions predominantly along lipid metabolism, triacylglycerol is dissociated into fatty acids and glycerol. In the present study, the contents of glycerol released from adipocytes were measured to assess the lipolytic activity by genipin in 3T3-L1 adipocytes.

3T3-L1 adipocytes were exposed to genipin for 4 days. The study found that genipin weakly but significantly decreases the

glycerol release levels from adipocytes (25, 50 and 100 μM; 89.80, 84.98, and 74.74%; % of the control) (Fig. 8B).

4. Discussion

In the present study, genipin, a natural iridoid originated from gardenia fruit, showed a potential anti-obesity effect, although the precise mode of action has not yet been addressed. This report revealed the mechanism by which genipin exerted its activities of inhibiting adipogenesis in 3T3-L1 preadipocytes and improving lipid metabolism in 3T3-L1 adipocytes. These results suggest the possibility of genipin as a novel modulator of adipocyte differentiation and lipid metabolism for the treatment, prevention, and improvement of metabolic disorders and obesity.

This study using 3T3-L1 cells indicated that genipin inhibits differentiation from preadipocytes to mature adipocytes through the down-regulation of representative adipogenesis markers and lipid metabolism enzymes which are under the transcriptional control by upstream regulators. Whereas, as mentioned previously, geniposide did not show any efficacy, indicating that the bond of glucose within the molecule leads to an almost complete loss of

anti-obesity activity. These observations suggest that an aglycone form is critical for the anti-obesity effect of genipin, probably by maintaining the structure that is essential for the interactions with the target molecule(s).

The major constituent of lipid within adipocytes is triacylglycerol (TAG). When adipogenesis occurs in 3T3-L1 cells, TAG contents within adipocytes are considerably increased according to the differentiation of preadipocytes to adipocytes. However, following the addition of genipin into cells, TAG accumulation is markedly decreased compared with the differentiated control.

At the molecular level, aidpogenesis is regulated by the complex transcriptional cascade which involves PPARγ and C/EBPα, expressed at the early phase of differentiation [20]. In the process of adipogenesis, it is well-reported that PPARγ and C/EBPα are two key transcription factors which regulate the adipocyte differentiation [21]. Furthermore, they induce the expression of

their target genes of lipid metabolizing enzymes such as FAS [22]. Additionally several studies have shown that priorly expressed C/EBPβ and C/EBPδ activate the functions of these master regulators. The expression levels of these adipogenic markers are markedly increased along with the differentiation of 3T3-L1 cells. The current analyses of differentiating protein expression suggest that genipin suppresses the up-regulation of PPARγ, C/EBPα, and FAS. Further investigations are needed to elucidate the molecular mechanisms and targets by which genipin controls adipogenesis.

Previous studies have verified that insulin-stimulated signaling pathways, Akt and ERK, are main cascades during the initial step of adipocyte differentiation [23, 24]. Here, insulin is essential proadipogenic hormone which induces phosphorylation of both Akt and ERK for adipogenic progress [25, 26]. Also, the involvement of Akt and ERK pathways at the early step of adipogenesis as upstreams of PPARγ and C/EBPα has been well reported [27, 28]. However, in this report, genipin did not markedly suppress the phosphorylation of Akt and ERK as depicted earlier.

Obesity is not only caused by adipose tissue hyperplasia, which stimulates the differentiation of preadipocytes into adipocytes, but also by adipose tissue hypertrophy increasing cellular lipid contents. Therefore, this study additionally examined the effects of genipin on differentiated 3T3-L1 adipocytes including densely accumulated lipid droplets inside.

As a result, genipin could modulate the functions of mature adipocytes with which genipin moderately but significantly reduces the amount of accumulated lipids in 3T3-L1 adipocytes, implying the plausible reasons such as decreased triacylglycerol synthesis and increased lipolysis of intracellular lipid. Hence, the subsequent study assessed the glycerol contents secreted from 3T3-L1 cells under the hypothesis that glycerol release might be increased due to the activation of lipolysis. However, the results showed that the glycerol amount is decreased, oppositely from the anticipation. Associated with this result, various further possibilities are currently under the review and discussion.

In summary, the present study illuminates the insight into the promising potential of genipin as an anti-obesity compound via revealing that genipin inhibits adipogenesis and improves lipid metabolism in 3T3-L1 cells. Taken together, newly discovered activities of genipin originated form gardenia fruit on adipogenesis and lipid metabolism suggest the functional/pharmaceutical potentials for the therapies of obesity and other metabolic syndrome.

5. References

1. Kopelman, P.G., *Obesity as a medical problem.* Nature, 2000. **404**(6778): p. 635-43.
2. Lee, I.A., et al., *Antihyperlipidemic effect of crocin isolated from the fructus of Gardenia jasminoides and its metabolite Crocetin.* Biol Pharm Bull, 2005. **28**(11): p. 2106-10.
3. Lee, S.J., P.S. Oh, and K.T. Lim, *Hepatoprotective and hypolipidaemic effects of glycoprotein isolated from Gardenia jasminoides ellis in mice.* Clin Exp Pharmacol Physiol, 2006. **33**(10): p. 925-33.
4. Ma, T., et al., *Hepatoprotective effects of geniposide in a rat model of nonalcoholic steatohepatitis.* J Pharm Pharmacol, 2011. **63**(4): p. 587-93.
5. Yin, F., et al., *Geniposide induces the expression of heme oxygenase-1 via PI3K/Nrf2-signaling to enhance the antioxidant capacity in primary hippocampal neurons.* Biol Pharm Bull, 2010. **33**(11): p. 1841-6.

6. Wu, S.Y., et al., *Effect of geniposide, a hypoglycemic glucoside, on hepatic regulating enzymes in diabetic mice induced by a high-fat diet and streptozotocin.* Acta Pharmacol Sin, 2009. **30**(2): p. 202-8.

7. Wang, G.F., et al., *Geniposide inhibits high glucose-induced cell adhesion through the NF-kappaB signaling pathway in human umbilical vein endothelial cells.* Acta Pharmacol Sin, 2010. **31**(8): p. 953-62.

8. Zheng, X., et al., *Identification of a new anti-LPS agent, geniposide, from Gardenia jasminoides Ellis, and its ability of direct binding and neutralization of lipopolysaccharide in vitro and in vivo.* Int Immunopharmacol, 2010. **10**(10): p. 1209-19.

9. Guo, L.X., J.H. Liu, and Z.N. Xia, *Geniposide inhibits CoCl2-induced PC12 cells death via the mitochondrial pathway.* Chin Med J (Engl), 2009. **122**(23): p. 2886-92.

10. Liu, H.T., et al., *Geniposide inhibits interleukin-6 and interleukin-8 production in lipopolysaccharide-induced*

human umbilical vein endothelial cells by blocking p38 and ERK1/2 signaling pathways. Inflamm Res, 2010. **59**(6): p. 451-61.

11. Nam, K.N., et al., *Genipin inhibits the inflammatory response of rat brain microglial cells.* Int Immunopharmacol, 2010. **10**(4): p. 493-9.
12. Mikami, M. and H. Takikawa, *Effect of genipin on the biliary excretion of cholephilic compounds in rats.* Hepatol Res, 2008. **38**(6): p. 614-21.
13. Suzuki, Y., et al., *Antithrombotic effect of geniposide and genipin in the mouse thrombosis model.* Planta Med, 2001. **67**(9): p. 807-10.
14. Lane, M.D. and Q.Q. Tang, *From multipotent stem cell to adipocyte.* Birth Defects Res A Clin Mol Teratol, 2005. **73**(7): p. 476-7.
15. Poulos, S.P., M.V. Dodson, and G.J. Hausman, *Cell line models for differentiation: preadipocytes and adipocytes.* Exp Biol Med (Maywood), 2010. **235**(10): p. 1185-93.

16. Jessen, B.A. and G.J. Stevens, *Expression profiling during adipocyte differentiation of 3T3-L1 fibroblasts.* Gene, 2002. **299**(1-2): p. 95-100.

17. Clarke, S.L., C.E. Robinson, and J.M. Gimble, *CAAT/enhancer binding proteins directly modulate transcription from the peroxisome proliferator-activated receptor gamma 2 promoter.* Biochem Biophys Res Commun, 1997. **240**(1): p. 99-103.

18. Loftus, T.M. and M.D. Lane, *Modulating the transcriptional control of adipogenesis.* Curr Opin Genet Dev, 1997. **7**(5): p. 603-8.

19. Langin, D., *Adipose tissue lipolysis as a metabolic pathway to define pharmacological strategies against obesity and the metabolic syndrome.* Pharmacol Res, 2006. **53**(6): p. 482-91.

20. Farmer, S.R., *Transcriptional control of adipocyte formation.* Cell Metab, 2006. **4**(4): p. 263-73.

21. Rosen, E.D., et al., *Transcriptional regulation of adipogenesis.* Genes Dev, 2000. **14**(11): p. 1293-307.

22. Rosen, E.D. and O.A. MacDougald, *Adipocyte differentiation from the inside out.* Nat Rev Mol Cell Biol, 2006. **7**(12): p. 885-96.

23. Hu, E., et al., *Inhibition of adipogenesis through MAP kinase-mediated phosphorylation of PPARgamma.* Science, 1996. **274**(5295): p. 2100-3.

24. Sale, E.M., P.G. Atkinson, and G.J. Sale, *Requirement of MAP kinase for differentiation of fibroblasts to adipocytes, for insulin activation of p90 S6 kinase and for insulin or serum stimulation of DNA synthesis.* Embo j, 1995. **14**(4): p. 674-84.

25. Xu, J. and K. Liao, *Protein kinase B/AKT 1 plays a pivotal role in insulin-like growth factor-1 receptor signaling induced 3T3-L1 adipocyte differentiation.* J Biol Chem, 2004. **279**(34): p. 35914-22.

26. Prusty, D., et al., *Activation of MEK/ERK signaling promotes adipogenesis by enhancing peroxisome proliferator-activated receptor gamma (PPARgamma) and*

C/EBPalpha gene expression during the differentiation of 3T3-L1 preadipocytes. J Biol Chem, 2002. **277**(48): p. 46226-32.

27. Tang, Q.Q., T.C. Otto, and M.D. Lane, *Mitotic clonal expansion: a synchronous process required for adipogenesis.* Proc Natl Acad Sci U S A, 2003. **100**(1): p. 44-9.

28. Magun, R., et al., *Expression of a constitutively activated form of protein kinase B (c-Akt) in 3T3-L1 preadipose cells causes spontaneous differentiation.* Endocrinology, 1996. **137**(8): p. 3590-3.

Figure 1

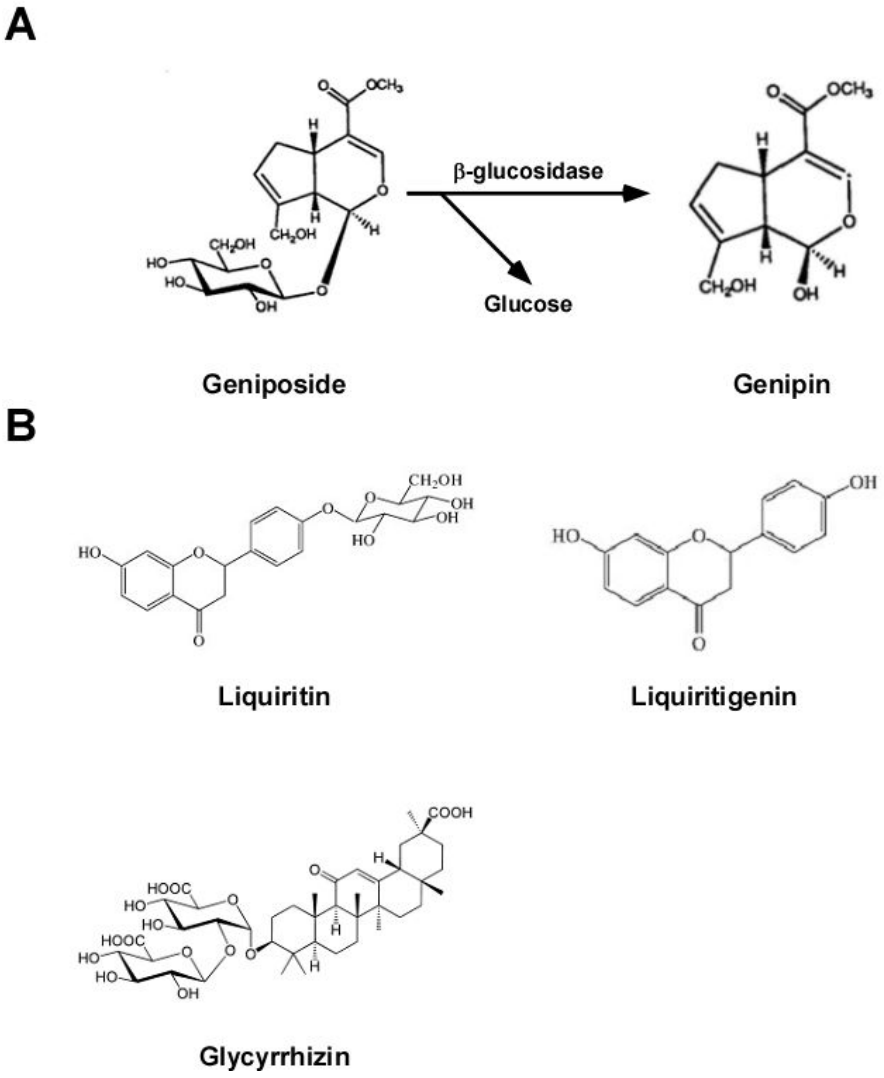

A
β-glucosidase
Glucose
Geniposide
Genipin
B
Liquiritin
Liquiritigenin
Glycyrrhizin

Figure 1. Chemical structures of five candidate compounds

(A) Chemical structures of genipin and geniposide. Deglycosylation of geniposide to genipin by β-glucosidase in animal intestine or liver.

(B) Chemical structures of three other candidate compounds; liquiritin, liquiritigenin, and glycyrrhizin.

Figure 2

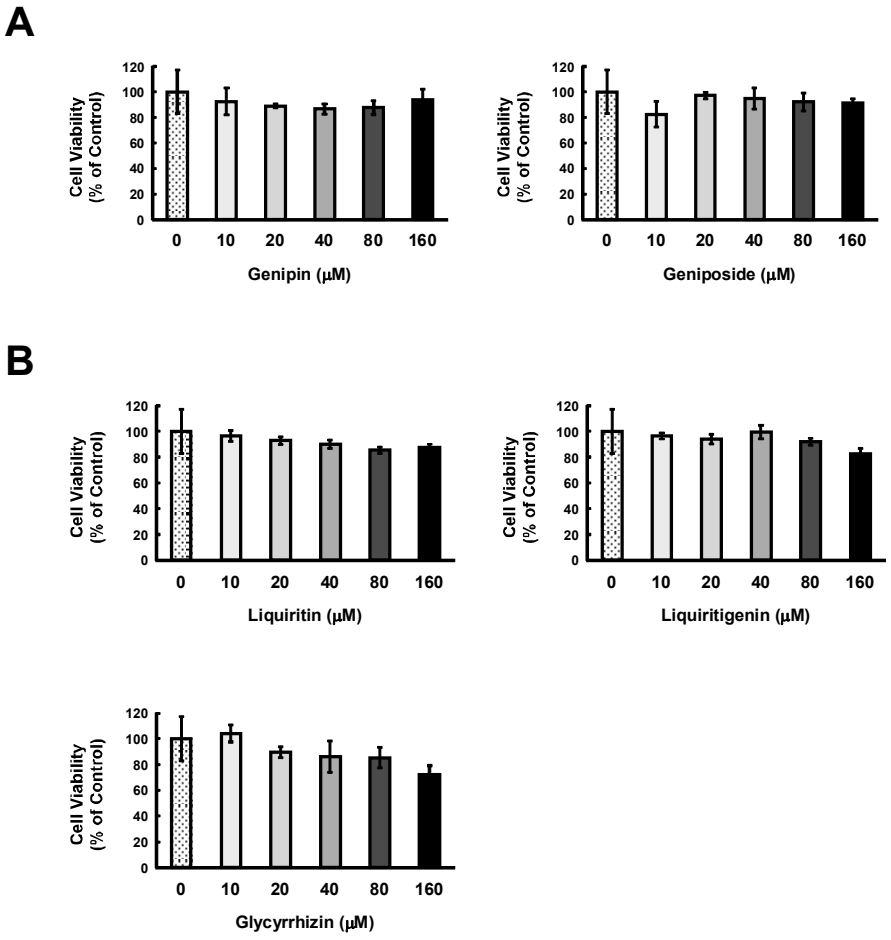

A
Cell Viability (% of Control)
120
100
80
60
40
20
0
0 10 20 40 80 160
Genipin (μM)
Geniposide (μM)
B
Liquiritin (μM)
Liquiritigenin (μM)
Glycyrrhizin (μM)

Figure 2. Effects of five candidate compounds on cell viability in 3T3-L1 preadipocytes

Two-day postconfluent 3T3-L1 preadipocytes (day 0) were treated with indicating concentrations of five candidate compounds for 48 hours.

(A) Cytotoxicity of genipin and geniposide on 3T3-L1 preadipocytes. After 48 hours, the viability of cells was measured by MTT assay. A cell group treated without any substance was used as a control. Data represent means ± SD values from three independent tests and are shown as percentages relative to the control.

(B) Cytotoxicity of three other candidate compounds on 3T3-L1 preadipocytes. After 48 hours, the viability of cells was measured by MTT assay. A cell group treated without any substance was used as a control. Data represent means ± SD values from three independent tests and are shown as percentages relative to the control.

Figure 3

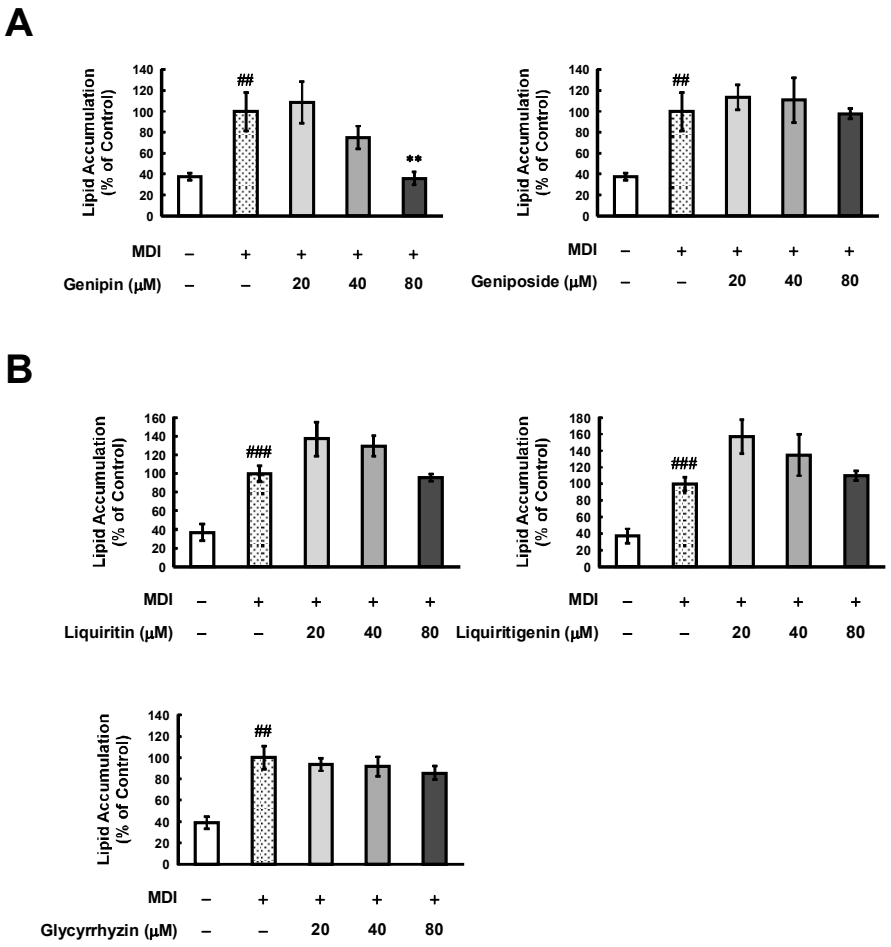

A
Lipid Accumulation (% of Control)
##
**
MDI – + + + +
Genipin (μM) – – 20 40 80
Lipid Accumulation (% of Control)
##
MDI – + + + +
Geniposide (μM) – – 20 40 80
B
Lipid Accumulation (% of Control)
###
MDI – + + + +
Liquiritin (μM) – – 20 40 80
Lipid Accumulation (% of Control)
###
MDI – + + + +
Liquiritigenin (μM) – – 20 40 80
Lipid Accumulation (% of Control)
##
MDI – + + + +
Glycyrrhyzin (μM) – – 20 40 80

Figure 3. Effects of five candidate compounds on lipid accumulation in 3T3-L1 preadipocytes

Two-day postconfluent 3T3-L1 preadipocytes (day 0) were treated with indicating concentrations of five candidate compounds every 2 days for 6 days along the adipogenic process.

(A) Effects of genipin and geniposide on adipogenesis in 3T3-L1 preadipocytes. On day 6, intracellular lipid droplets were stained with oil red O solution and colorimetric intensity was measured. A cell group treated without any substance was used as a control. Data represent means ± SD values from three independent tests and are shown as percentages relative to the control. ##P < 0.01 indicates a significant difference of a differentiated control versus a non-differentiated control. **P < 0.01 indicates a significant difference of experimental groups versus a differentiated control.

(B) Effects of three other compounds on adipogenesis in 3T3-L1 preadipocytes. On day 6, intracellular lipid droplets were stained with oil red O solution and colorimetric intensity was measured. A cell group treated without any substance was used as a control. Data represent means ± SD values from three independent tests and are shown as percentages relative to the control. ##P < 0.01, ###P < 0.001 indicate significant differences of a differentiated control versus a non-differentiated control.

Figure 4

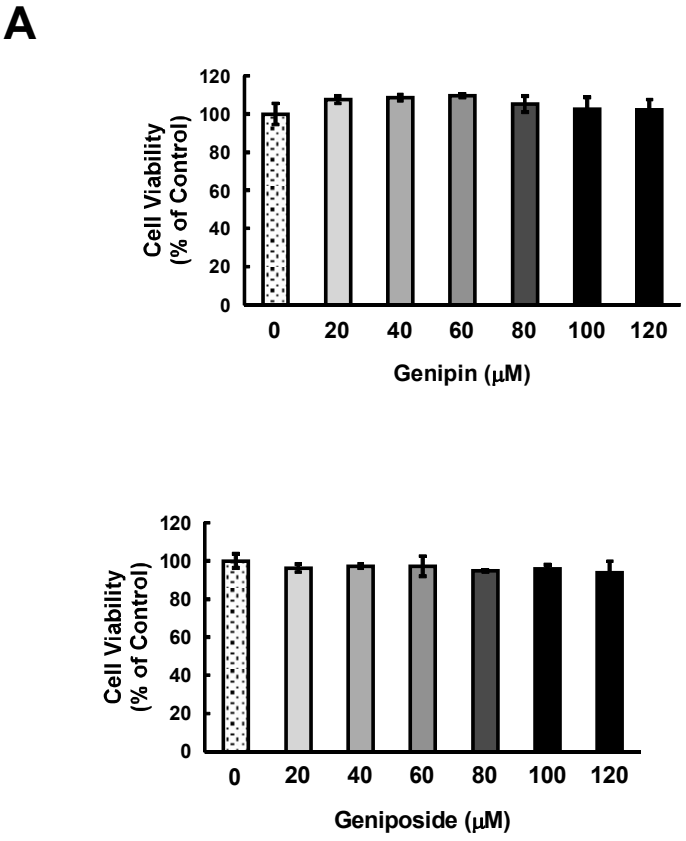

A
Cell Viability (% of Control)
120
100
80
60
40
20
0
0
20
40
60
80
100
120
Genipin (μM)
B
Cell Viability (% of Control)
120
100
80
60
40
20
0
0
20
40
60
80
100
120
Geniposide (μM)

Figure 4. Effects of genipin and geniposide on cell viability in 3T3-L1 preadipocytes

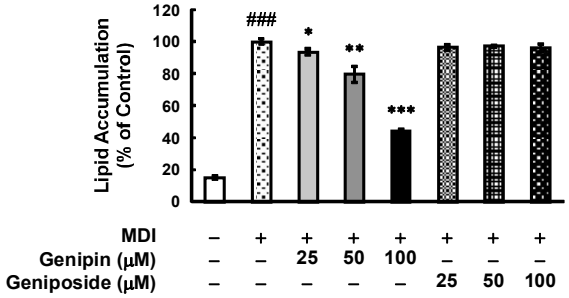
Two-day postconfluent 3T3-L1 preadipocytes (day 0) were treated with indicating concentrations of genipin and geniposide for 72 hours.

(A) Cytotoxicity of genipin on 3T3-L1 preadipocytes. After 72 hours, the viability of cells was measured by MTT assay. A cell group treated without any substance was used as a control. Data represent means ± SD values from three independent tests and are shown as percentages relative to the control.

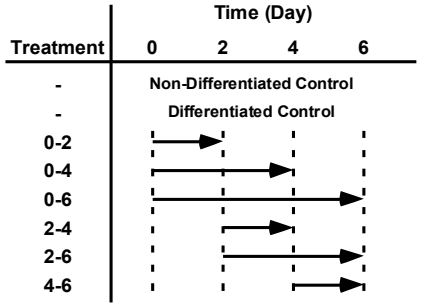
(B) Cytotoxicity of geniposide on 3T3-L1 preadipocytes. After 72 hours, the viability of cells was measured by MTT assay. A cell group treated without any substance was used as a control. Data represent means ± SD values from three independent tests and are shown as percentages relative to the control.

Figure 5

A


Lipid Accumulation (% of Control)
120
100
80
60
40
20
0
###
*
**
***
MDI – + + + + + + +
Genipin (μM) – – 25 50 100 – – –
Geniposide (μM) – – – – – 25 50 100

B


Time (Day)
Treatment
0
2
4
6
-
Non-Differentiated Control
-
Differentiated Control
0-2
0-4
0-6
2-4
2-6
4-6

C

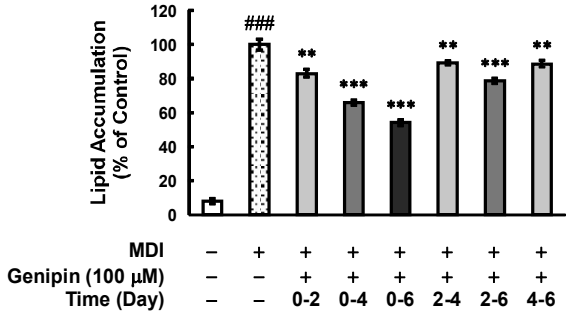

Lipid Accumulation (% of Control)
120
100
80
60
40
20
0
###
**
***
***
**
***
**
MDI – + + + + + + +
Genipin (100 μM) – – + + + + + +
Time (Day) – – 0-2 0-4 0-6 2-4 2-6 4-6

Figure 5. Effect of genipin on adipogenesis in 3T3-L1 preadipocytes

(A) Effects of genipin and geniposide on adipogenesis in 3T3-L1 preadipocytes. Anti-adipogenic effect of genipin, but not geniposide. Two-day postconfluent 3T3-L1 preadipocytes (day 0) were treated with indicating concentrations of genipin and geniposide every 2 days for 6 days along the adipogenic process. On day 6, intracellular lipid droplets were stained with oil red O solution and colorimetric intensity was measured. A cell group treated without any substance was used as control. Data represent means ± SD values from three independent tests and are shown as percentages relative of the control. ###P < 0.001 indicates a significant difference of a differentiated control versus a non-differentiated control. *P < 0.05, **P < 0.01, ***P < 0.001 indicate significant differences of experimental groups versus a differentiated control.

(B) MDI-induced 3T3-L1 cells differentiation for 6 days. Two-day postconfluent 3T3-L1 preadipocytes (day 0) were treated with 100 μM of genipin for indicated time periods during adipogenesis.

(C) Effect of genipin (100 μM) at different stages of adipogenesis in 3T3-L1 preadipocytes. Anti-adipogenic effect of genipin on every each stage of adipogenesis, but in a similar extent manner. On day 6, intracellular lipid droplets were stained with oil red O solution and colorimetric intensity was measured. A cell group treated without any substance was used as a control. Data represent means ± SD values from three independent tests and are shown as percentages relative to the control. ###P < 0.001 indicates a significant difference of a differentiated control versus a non-differentiated control. **P < 0.01, ***P < 0.001 indicate significant differences of experimental groups versus a differentiated control.

Figure 6

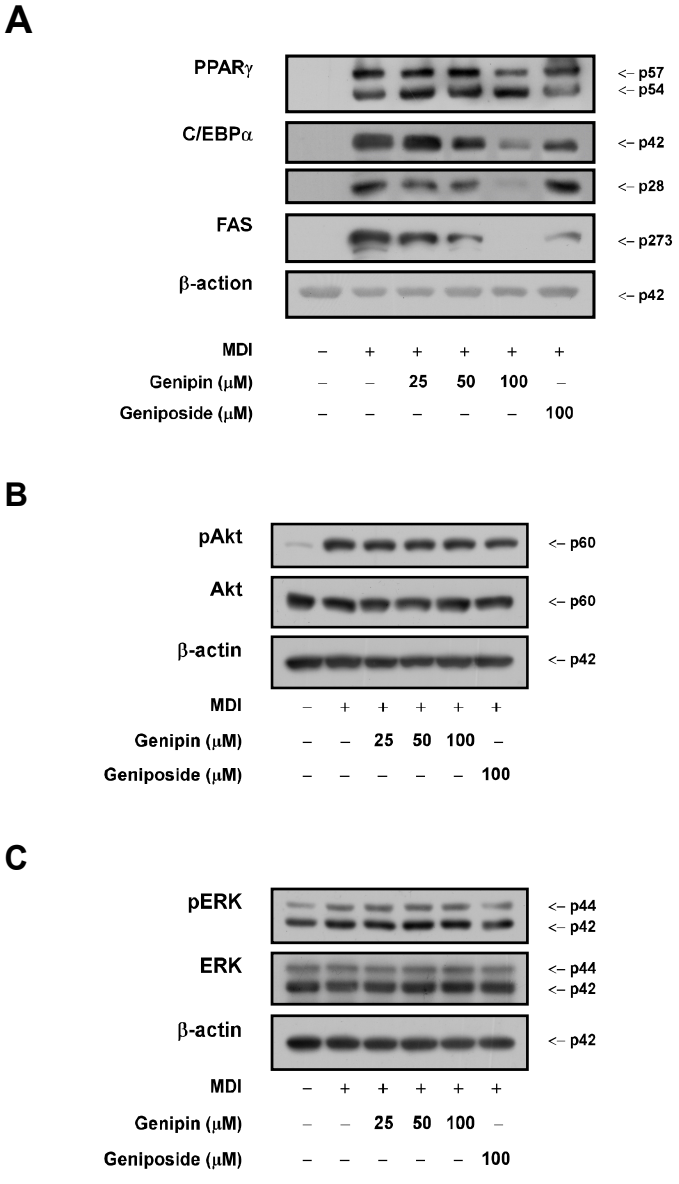

A
PPARγ
C/EBPα
FAS
β-action
<- p57
<- p54
<- p42
<- p28
<- p273
<- p42
MDI – + + + + +
Genipin (μM) – – 25 50 100 –
Geniposide (μM) – – – – – 100
B
pAkt
Akt
β-actin
<- p60
<- p60
<- p42
MDI – + + + + +
Genipin (μM) – – 25 50 100 –
Geniposide (μM) – – – – – 100
C
pERK
ERK
β-actin
<- p44
<- p42
<- p44
<- p42
<- p42
MDI – + + + + +
Genipin (μM) – – 25 50 100 –
Geniposide (μM) – – – – – 100

Figure 6. Effect of genipin on the expression of adipogenesis-related proteins

Two-day postconfluent 3T3-L1 preadipocytes (day 0) were treated with indicating concentrations of genipin and geniposide. Cells were harvested and lysed.

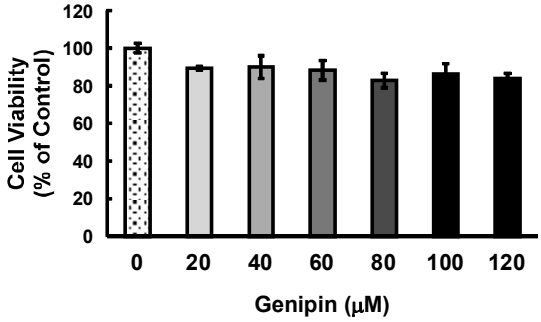
(A) Effect of genipin on adipogenesis-related proteins (PPARγ, C/EBPα, FAS) in 3T3-L1 preadipocytes. On day 6, total cellular proteins were isolated from 3T3-L1 cells treated with genipin or geniposide. The protein levels for the representative adipogenetic markers were quantified by western blot analysis.

(B) Effect of genipin on the phosphorylation of Akt at the early stage of adipogenesis in 3T3-L1 preadipocytes. The protein expression levels of phospho- and total-Akt proteins were determined by western blot analysis.

(C) Effect of genipin on the phosphorylation of ERK at the early stage of adipogenesis in 3T3-L1 preadipocytes. The protein expression levels of phospho- and total-ERK proteins were determined by western blot analysis.

Figure 7

A


Cell Viability
(% of Control)
120
100
80
60
40
20
0
0
20
40
60
80
100
120
Genipin (μM)

B

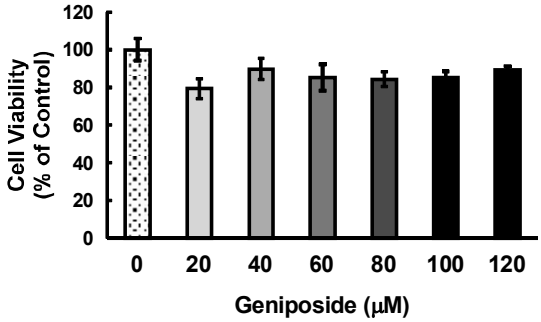

Cell Viability
(% of Control)
120
100
80
60
40
20
0
0
20
40
60
80
100
120
Geniposide (μM)

Figure 7. Effect of genipin and geniposide on cell viability in 3T3-L1 adipocytes

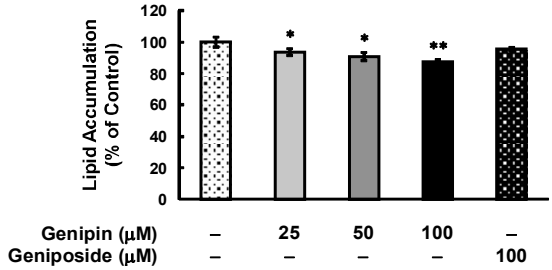
Two-day postconfluent 3T3-L1 preadipocytes (day 0) were differentiated into mature adipocytes for 6 days and treated with indicating concentrations of genipin and geniposide for 96 hours.

(A) Cytotoxicity of genipin on 3T3-L1 adipocytes. After 96 hours, the viability of cells was measured by MTT assay. A cell group treated without any substance was used as a control. Data represent means ± SD values from three independent tests and are shown as percentages relative to the control.

(B) Cytotoxicity of geniposide on 3T3-L1 adipocytes. After 96 hours, the viability of cells was measured by MTT assay. A cell group treated without any substance was used as a control. Data represent means ± SD values from three independent tests and are shown as percentages relative to the control.

Figure 8

A


Lipid Accumulation
(% of Control)
120
100
80
60
40
20
0
*
*
**
Genipin (μM) – 25 50 100 –
Geniposide (μM) – – – – 100

B

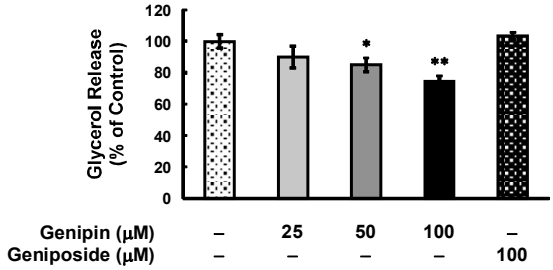

Glycerol Release
(% of Control)
120
100
80
60
40
20
0
*
**
Genipin (μM) – 25 50 100 –
Geniposide (μM) – – – – 100

Figure 8. Effect of genipin on lipid metabolism in 3T3-L1 adipocytes

Two-day postconfluent 3T3-L1 preadipocytes (day 0) were differentiated into mature adipocytes for 6 days and treated with indicating concentrations of genipin and geniposide for 4 days.

(A) Effects of genipin and geniposide on lipid metabolism in 3T3-L1 adipocytes. Improvement of lipid metabolism by genipin, but not geniposide. On day 10, intracellular lipid droplets were stained with oil red O solution and colorimetric intensity was measured. A cell group treated without any substance was used as a control. Data represent means ± SD values from three independent tests and are shown as percentages relative to the control. *P < 0.05, **P < 0.01 indicate significant differences of experimental groups versus a control.

(B) Effects of genipin and geniposide on glycerol release in 3T3-L1 adipocytes. Decrease in glycerol contents by genipin. On day 10, culture supernatants were collected and glycerol release levels were

measured from 3T3-L1 cells treated with genipin or geniposide. A cell group treated without any substance was used as a control. Data represent means ± SD values from three independent tests and are shown as percentages relative to the control. *P < 0.05, **P < 0.01 indicate significant differences of experimental groups versus a control.

국문초록

게니핀은 치자 열매에서 유래한 성분인 게니포사이드의 비배당체이자 대사물이다. 배당체인 게니포사이드는 동물 장이나 간 내에서 β-글루코시데이즈에 의하여 게니핀으로 가수분해된다. 치자 열매는 식용색소의 용도로서 널리 알려져 있고 또한 항염 및 항지혈의 활성과 같은 다양한 건강 도모의 효능도 잘 알려져 있다. 이와 같은 기능성은 치자의 대사성 질환과 비만을 제어할 수 있는 잠재성을 충분히 암시하여 준다. 하지만 치자 열매를 비롯한 이의 활성 성분이 항비만 효능에 관여하는 사실을 뒷받침하는 과학적 근거가 아직 충분하지 않은 실정이다. 따라서 치자 열매에서 유래한 게니핀의 항비만 효능을 규명하고자 지방세포분화 및 지질대사 기전으로 그 활성을 규명하고자 하였다. 결과 자료는 치자의 원성분인 게니포사이드는 그러하지 않았지만 그의 대사체인 게니핀은 100 μM 에서 유의하게 지방세포분화를 억제하고 지질대사를 개선함을

보여주었다. 이와 더불어 지방세포분화와 관련된 대표 단백질인 PPARγ, C/EBPα, FAS, Akt, ERK 의 발현 정도를 측정하였고, 지질대사 기전과 관련하여 글리세롤 양을 관찰하였다. 결과적으로, 연구 결과는 게니핀이 3T3-L1 전구지방세포에서 지방세포분화를 억제하고 3T3-L1 지방세포에서 지질대사를 개선함을 규명하였다. 이에 따라 향후 게니핀을 체중 및 체지방을 조절할 수 있는 유망한 화학적 예방 성분으로서 제안하는 바이다.

주요어: 게니핀 / 게니포사이드 / 치자열매 / 비만 / 지방세포분화 / 지질대사 / 지방세포 / PPARγ / C/EBPα / FAS / 글리세롤

학번: 2012-21186