

## Anti-adipogenic Activity of *Acer tegmentosum* and its Constituent, Catechin in 3T3-L1 Cells<sup>†</sup>

Qing Liu<sup>1</sup>, Eunjin Shin<sup>1</sup>, Mi-Jeong Ahn<sup>2</sup>, Bang Yeon Hwang<sup>1</sup>, and Mi Kyeong Lee<sup>1,\*</sup>

<sup>1</sup>College of Pharmacy, Chungbuk National University, Cheongju 361-763, Korea

<sup>2</sup>College of Pharmacy, Gyeongsang National University, Jinju 660-751, Korea

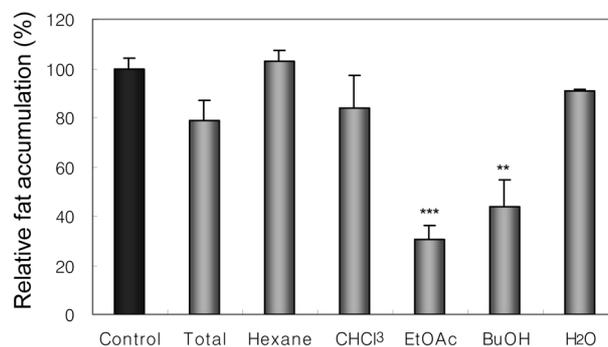
**Abstract** – In the course of screening anti-adipogenic activity of natural products employing the preadipocyte cell line, 3T3-L1 as an *in vitro* assay system, the EtOAc fraction of the stem barks of *Acer tegmentosum* Maxim (Aceraceae) showed significant inhibitory activity on adipocyte differentiation as assessed by measuring fat accumulation using Oil Red O staining. Activity-guided fractionation led to the isolation of active constituent, (+)-catechin. (+)-Catechin showed inhibitory activity on adipocyte differentiation in dose-dependent manner. Further studies with interval treatment demonstrated that (+)-catechin exerted inhibitory activity on adipocyte differentiation via acting on early stage of adipogenesis. Our present study also showed that (+)-catechin significantly inhibited the preadipocyte proliferation. Taken together, these results suggest that (+)-catechin, a constituent of *A. tegmentosum* might contribute the anti-adipogenic activity of *A. tegmentosum*.

**Key words** – *Acer tegmentosum*, adipogenesis, (+)-catechin, adipocyte differentiation, 3T3-L1 preadipocytes

### Introduction

Adipogenesis is a differentiation process by which undifferentiated preadipocytes are converted to fully differentiated adipocytes, fat cells. Adipogenesis is known to be closely related obesity and obesity-related disorders such as diabetes, hypertension and atherosclerosis (Kopelman, 2001). In obesity, adipocytes undergo abnormal growth characterized by increased cell numbers and differentiation (Farmer, 2006; Tontonoz and Spiegelman, 2008). Therefore, inhibition of mitogenesis of preadipocytes and their differentiation to adipocytes would be beneficial for the prevention and progression of obesity (Farmer and Auwerx, 2003; Rayalam *et al.*, 2008).

*Acer tegmentosum* Maxim (Aceraceae) is a deciduous tree that has been used in traditional medicine for the treatment of hepatic disorders (Ahn, 1998). In addition, antioxidative and cytotoxic activities have been reported (Park *et al.*, 2006; Tung *et al.*, 2008). Related to its phytochemical constituents, flavonoids, phenylethyl glycosides and quinones have been reported (Park *et al.*, 2006; Tung *et al.*, 2008). In the course of screening of anti-adipogenic activity of natural products employing a



**Fig. 1.** Effects of each fraction of *A. tegmentosum* on adipocyte differentiation.

Cultures were induced to differentiate for 8 days and treated with 100 µg/ml each fraction for whole differentiation process. On day 8, cultures were subjected to Oil Red O staining and quantitated. Values are expressed as means ± S.D. of triplicate experiments. \*p < 0.01, \*\*p < 0.001 compared with differentiated control.

mouse preadipocyte cell line, 3T3-L1 as an *in vitro* assay system, the EtOAc fraction of *A. tegmentosum* showed significant inhibitory activity (Fig. 1). Therefore, we have attempted to isolate anti-adipogenic compounds and evaluate their anti-adipogenic activity.

### Methods and materials

**Plant material** – The stem barks of *A. tegmentosum* were purchased from the local herbal market, Chungbuk,

<sup>†</sup>Dedicated to Prof. Young Choong Kim of the Seoul National University for her leading works on Pharmacognosy

\* Author for correspondence

Tel: +82-43-261-2818; E-mail: mklee@chungbuk.ac.kr

Korea in May 2009. They were identified by the herbarium of College of Pharmacy at Chungbuk National University, where a voucher specimen was deposited (CBNU200905-AT).

**Extraction and Isolation** – The stem barks of *A. tegmentosum* (1 kg) were extracted twice with 80% MeOH, which yielded the methanolic extract (32.8 g). The methanolic extract was then suspended in H<sub>2</sub>O and partitioned successively with *n*-hexane, CHCl<sub>3</sub>, EtOAc and *n*-BuOH. The EtOAc fraction (2.2 g), which showed significant inhibitory activity, was subjected to silica gel column chromatography with a mixture of *n*-hexane-EtOAc to give 11 fractions (E1-E11). E8 was subjected to column chromatography over Sephadex LH-20 eluted with MeOH to give 5 subfractions (E81-E85). Compound 1 (35 mg) was obtained from E83.

**Culture of 3T3-L1 cells and adipogenesis** – 3T3-L1 mouse embryo fibroblasts were obtained from the American Type Culture Collection (Manassas, VA). Cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS) until confluence. Two days after confluence (day 0), cells were stimulated to differentiate with differentiation medium containing DMEM with 10% FBS, 0.5 mM 3-isobutyl-1-methyl-xanthine, 1  $\mu$ M insulin and 1  $\mu$ M dexamethasone for 2 days (day 2). Cells were then maintained in DMEM supplemented with 10% FBS and 1  $\mu$ M insulin for another 2 days (day 4), followed by culturing with DMEM with 10% FBS for an additional 4 days (day 8). All media contained 100 IU/mL penicillin and 100  $\mu$ g/mL streptomycin. Cells were maintained at 37 °C in a humidified atmosphere of 95% air-5% CO<sub>2</sub>. The cultures were treated with test samples for whole culture period (day 0-8) for general experiment. In some experiment, test samples were treated after differentiation was induced (fully differentiated cells) for three day. For the evaluation of time dependent activity, test samples were treated for indicated time-periods.

**Oil Red O staining** – Lipid droplets in cells were stained with Oil Red O. Eight days after differentiation induction, cells were washed three times with PBS and fixed with 10% formalin at room temperature for 1 hr. After fixation, cells were washed once with PBS and stained with freshly diluted Oil Red O solution (3 parts of 0.6% Oil Red O in isopropyl alcohol and 2 parts of water) for 15 min. Cells were then washed twice with water and visualized. For quantitative analysis, Oil Red O staining was dissolved with isopropyl alcohol and optical density was measured at 520 nm by ELISA plate reader.

**Measurement of cell viability** – 3T3-L1 cells were treated with vehicle or samples to be tested for 72 hr. Cell viability was assessed by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. 3T3-L1 cells were incubated with 0.5 mg/mL of MTT in the last 2 hr of the culture period tested. Reduction of MTT to formazan was assessed in an ELISA plate reader.

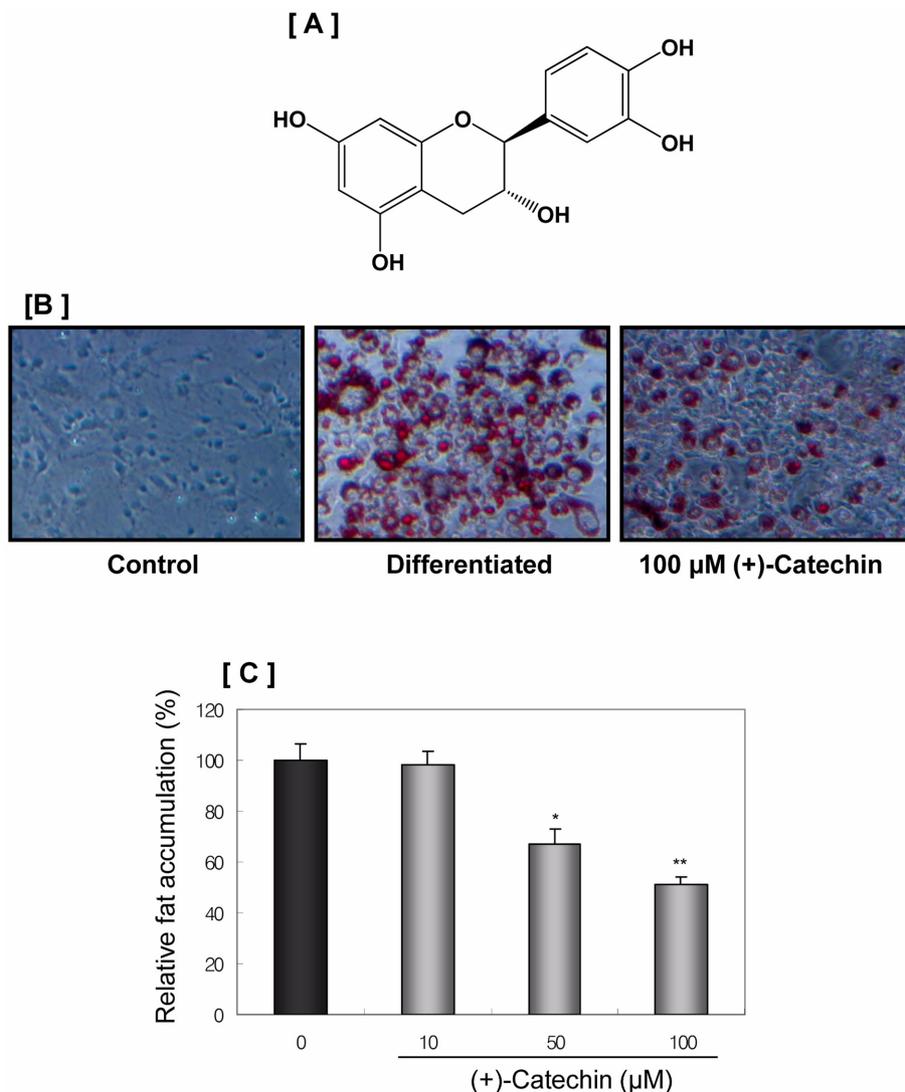
**Statistical analysis** – The evaluation of statistical significance was determined by one-way Anova test with a value of  $p < 0.05$  or less considered to be statistically significant.

## Results and discussion

In the course of screening of anti-adipogenic activity of natural products employing a mouse preadipocyte cell line, 3T3-L1 as an *in vitro* assay system, the EtOAc fraction of *A. tegmentosum* showed significant inhibitory activity (Fig. 1). Activity-guided isolation of EtOAc fraction yielded active constituent, which was identified as (+)-catechin (Fig. 2A) by the direct comparison of those of previously reported (Park *et al.*, 2006).

The inhibitory activity of (+)-catechin on adipocyte differentiation was evaluated in our assay system. Morphological observation of cells showed a decrease in cellular lipid by the treatment of (+)-catechin (Fig. 2B). At the concentration of 100  $\mu$ M, (+)-catechin inhibited fat accumulation up to 50% of fully differentiated adipocytes (Fig. 2C). However, this compound showed weak inhibitory activity when treated to differentiated adipocytes (data not shown). These results suggest that (+)-catechin might be effective in the prevention of adipogenesis.

Adipogenesis, the development of mature fat cells from preadipocytes, is an intensely studied model of cellular differentiation. The process of adipogenesis includes alteration of cell shape, growth arrest, clonal expansion, and a complex sequence of changes in gene expression and storage of lipid, which occurs in several stages (Gregoire *et al.*, 1998; Lefterova and Lazar, 2009). For differentiation, preadipocytes enter growth arrest and continue to subsequent differentiation process by appropriate mitogenic and adipogenic signals. During the initial stage of differentiation, a dramatic decrease of preadipocyte factor-1 (Pref-1) expression accompanies a rapid increase in the expression of PPAR and CCAAT/enhancer-binding proteins (C/EBPs). During the terminal stage of differentiation, enzymes involved in triacylglycerol metabolism such as fatty acid synthase and glycerol-3-phosphate dehydrogenase increase to a great extent (Farmer, 2006; White and Stephens, 2010). Therefore, we



**Fig. 2.** Effect of (+)-catechin on adipocyte differentiation in 3T3-L1 cells.

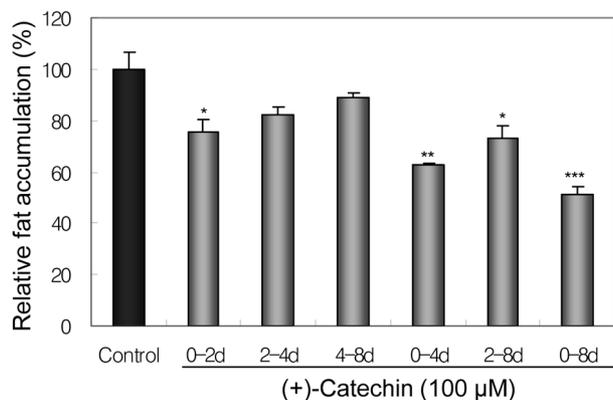
Cultures were induced to differentiate for 8 days and treated with (+)-catechin for whole differentiation process. On day 8, cultures were subjected to Oil Red O staining, photographed (A) and quantitated (B). The pictures are representatives of three independent experiments with similar results. Values are expressed as means  $\pm$  S.D. of triplicate experiments. \* $p < 0.01$ , \*\* $p < 0.001$  compared with differentiated control.

further examined which stage of adipogenic differentiation was effectively inhibited by (+)-catechin. Investigation on interval treatment of (+)-catechin during days 0 - 2, 2 - 4, 4 - 8, 0 - 4, 2 - 8, 0 - 8 during differentiation showed that treatment between days 0-4 significantly inhibited adipocytes differentiation (Fig. 3). From these results, we suppose that (+)-catechin might inhibit early stage of adipogenesis.

Adipocyte mass can be reduced both by inhibiting adipogenesis and reducing adipocyte number (Rayalam *et al.*, 2006). Adipocyte number increases as a result of increased preadipocyte proliferation and clonal expansion during differentiation. Therefore, we investigated the

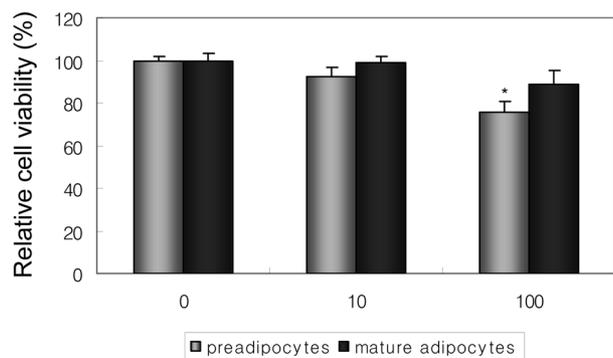
effect of (+)-catechin on adipocyte number. As shown in Fig. 4, (+)-catechin significantly reduced proliferating preadipocytes number. However, (+)-catechin showed weak inhibitory effect on mature adipocyte number.

Taken together, EtOAc fraction of *A. tegmentosum* inhibited fat accumulation in 3T3-L1 cells and (+)-catechin was isolated as an active constituent. (+)-Catechin is a constituent of green tea together with (-)-epigallocatechin gallate (EGCG). Green tea, especially EGCG, is well known for beneficial effect on obesity by inhibiting fat accumulation and stimulating lipolysis (Lee *et al.*, 2009). Consistent with previous report, (+)-catechin inhibited fat accumulation in dose-dependent manner. Our



**Fig. 3.** Effect of (+)-catechin on adipocyte differentiation during differentiation process.

Cultures were induced to differentiate and 100  $\mu$ M (+)-catechin was added at different period of differentiation, namely, days 0 - 2, 2 - 4, 4 - 8, 0 - 4, 2 - 8 and 0 - 8. On day 8, fat accumulation was quantitated using Oil Red O assay. Values are expressed as means  $\pm$  S.D. of triplicate experiments. \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$  compared with differentiated control.



**Fig. 4.** Effect of (+)-catechin on cell viability of proliferating preadipocytes and mature adipocytes.

(+)-Catechin was treated to proliferating preadipocytes and mature adipocytes for 3 days. Cell viability was measured by MTT assay. Values are expressed as means  $\pm$  S.D. of triplicate experiments. \* $p < 0.05$  compared with control.

present study also showed that (+)-catechin is more effective in the prevention of adipogenesis than treatment of obesity by acting early stage of adipogenesis. In addition, (+)-catechin reduced preadipocytes number.

Therefore, *A. tegmentosum* and its constituent (+)-catechin might be useful in the prevention of obesity. More investigation is needed about the anti-adipogenic constituents of *A. tegmentosum*.

### Acknowledgement

This work was supported by the research grant of the Chungbuk National University in 2010. We thank to Ms. Yewon Lee for her technical support.

### References

- Ahn, D.K., *Illustrated Book of Korean Medicinal Herbs*. Kyohak Publishing, Seoul, Korea, 523-524 (1998).
- Farmer, S.R. and Auwerx, J., Adipose tissue: new therapeutic targets from molecular and genetic studies. *Obesity Rev.* **5**, 189-196 (2003).
- Farmer, S.R., Transcriptional control of adipocyte formation. *Cell Metab.* **4**, 263-273 (2006).
- Gregoire, F.M., Smas, C.M., and Sul, H.S., Understanding adipocyte differentiation. *Physiol. Rev.* **78**, 783-809 (1998).
- Kopelman, P.G., Obesity as a medical problem. *Nature* **404**, 635-643 (2001).
- Lee, M.S., Kim, C.T., Kim, I.W., and Kim, Y., Inhibitory effects of green tea catechin on the lipid accumulation in 3T3-L1 adipocytes. *Phytother. Res.* **23**, 1088-1091 (2009).
- Lefterova, M.I. and Lazar, M.A., New developments in adipogenesis. *Trends Endocrinol. Metab.* **20**, 107-114 (2009).
- Park, K.M., Yang, M.C., Lee, K.H., Kim, K.R., Choi, S.U. and Lee, K.R., Cytotoxic phenolic constituents of *Acer tegmentosum* Maxim. *Arch. Pharm. Res.* **12**, 1086-1090 (2006).
- Rayalam, S., Della-Fera, M.A. and Baile, C.A., Phytochemicals and regulation of the adipocyte life cycle. *J. Nutr. Biochem.* **19**, 717-726 (2008).
- Tontonoz, P. and Spiegelman, B.M., Fat and beyond: the diverse biology of PPARgamma. *Annu. Rev. Biochem.* **77**, 289-312 (2008).
- Tung, N.H., Ding, Y., Kim, S.K., Bae, K.H. and Kim, Y.H., Total peroxyl radical-scavenging capacity of the chemical components from the stems of *Acer tegmentosum* Maxim. *J. Agric. Food Chem.* **56**, 10510-10514 (2008).
- White, U.A. and Stephens, J.M., Transcriptional factors that promote formation of white adipose tissue. *Mol. Cell. Endocrinol.* **318**, 10-14 (2010).

Received April 16, 2011

Revised July 15, 2011

Accepted July 19, 2011