J Nat Med (2012) 66:394–399
DOI 10.1007/s11418-011-0593-z

NOTE

Hypoglycemic effects of clove (*Syzygium aromaticum* flower buds) on genetically diabetic KK-A^{y} mice and identification of the active ingredients

Minpei Kuroda · Yoshihiro Mimaki · Takayuki Ohtomo · Junji Yamada · Tozo Nishiyama · Tatsumasa Mae · Hideyuki Kishida · Teruo Kawada

Received: 23 February 2011 / Accepted: 16 September 2011 / Published online: 11 October 2011

Abstract Clove (*Syzygium aromaticum* flower buds) EtOH extract significantly suppressed an increase in blood glucose level in type 2 diabetic KK-A^{y} mice. In-vitro evaluation showed the extract had human peroxisome proliferator-activated receptor (PPAR)-γ ligand-binding activity in a GAL4-PPAR-γ chimera assay. Bioassay-guided fractionation of the EtOH extract resulted in the isolation of eight compounds, of which dehydrodieugenol (**2**) and dehydrodieugenol B (**3**) had potent PPAR-γ ligand-binding activities, whereas oleanolic acid (**4**), a major constituent in the EtOH extract, had moderate activity. Furthermore, **2** and **3** were shown to stimulate 3T3-L1 preadipocyte differentiation through PPAR-γ activation. These results indicate that clove has potential as a functional food ingredient for the prevention of type 2 diabetes and that **2**–**4** mainly contribute to its hypoglycemic effects via PPAR-γ activation.

Keywords Clove · *Syzygium aromaticum* · PPAR-γ ligand-binding activity · Neolignan · Triterpenoid

M. Kuroda (✉) · Y. Mimaki · T. Ohtomo · J. Yamada
School of Pharmacy, Tokyo University of Pharmacy and Life Sciences, 1432-1, Horinouchi, Hachioji, Tokyo 192-0392, Japan
e-mail: kurodam@toyaku.ac.jp

T. Nishiyama · T. Mae · H. Kishida
Frontier Biochemical and Medical Research Laboratories, Kaneka Corporation, 1-8 Miyamae-cho, Takasago-cho, Takasago, Hyogo 676-8688, Japan

T. Kawada
Laboratory of Molecular Function of Food, Division of Food Science and Biotechnology, Graduate School of Agriculture, Kyoto University, Uji, Kyoto 611-0011, Japan

Introduction

Metabolic syndrome is a cluster of type 2 diabetes, obesity/abdominal obesity, hypertension, and dyslipidemia [1, 2]. A crucial role in the development of metabolic syndrome is played by adipocytes, which are highly specialized cells involved in energy regulation and homeostasis. Adipocyte differentiation is a tightly controlled process programmed by determinant genes such as those of peroxisome proliferator-activated receptor-γ (PPAR-γ) and CCAAT/enhancer binding protein-α [2, 3]. The nuclear receptor PPAR-γ belongs to the superfamily of ligand-dependent transcription factors [4], and is the predominant molecular target for insulin-sensitizing thiazolidinedione drugs such as troglitazone, pioglitazone, and rosiglitazone, which have been approved for use in the treatment of type 2 diabetes patients [5, 6]. We previously reported that EtOH extracts of licorice (*Glycyrrhiza uralensis* F. roots) [7] and turmeric (*Curcuma longa* L. rhizomes) [8, 9], and a hydrophobic flavonoid-enriched fraction prepared from the EtOH extract of licorice (*G. glabra* L. roots) [10, 11] were effective in preventing and/or ameliorating diabetes, abdominal obesity, and hypertension in animal models of metabolic syndrome. The activities were strongly suggested to be associated with PPAR-γ ligand-binding activities of some flavonoids in licorice [12, 13], and curcuminoids and a sesquiterpenoid in turmeric [8, 9]. As part of our systematic search for functional foods with preventive and ameliorative effects against metabolic syndrome, we found that the EtOH extract of clove (*Syzygium aromaticum* Merr. et Perry, flower buds) suppressed an increase in blood glucose levels in type 2 diabetic KK-A^{y} mice. In order to determine the mechanisms of action, the extract and its components were evaluated for their PPAR-γ ligand-binding activities.

Results and discussion

The effects of the clove EtOH extract in genetically diabetic KK-A^y mice were investigated using pioglitazone as a positive control. Three weeks of feeding the extract at 0.5 g/100 g diet did not significantly affect body weight gain or food intake (Table 1). The intake of the extract, calculated from total food intake and mean body weight gain of the mice, was 657 mg/(kg body weight day) at 0.5 g/100 g diet feeding. Compared with before feeding, the mean blood glucose level in the control group was increased more than 3-fold (Table 1), indicating hyperglycemia after 3 weeks of feeding. The blood glucose level in the pioglitazone group remained the same as before feeding. Compared with the control, the blood glucose level was lower ($P < 0.01$) in mice fed the clove EtOH extract for 3 weeks (Table 1), suggesting that the extract is effective for the prevention and/or amelioration of type 2 diabetes mellitus.

Identification of the ingredients of clove with PPAR-γ ligand-binding activity and their effects on preadipocyte differentiation in 3T3-L1 adipocytes

The cloves (200 g) were percolated with EtOH (2 L) at room temperature for 3 days twice and concentrated under reduced pressure. The clove EtOH extract (13.0 g) exhibited strong PPAR-γ ligand-binding activity and its relative luminescence intensity was around 3.2 at a sample concentration of 30 μg/mL, which was more potent than that of a 0.22 μg/mL troglitazone, a potent synthetic PPAR-γ agonist (Fig. 1). The extract was passed though a porous-polymer polystyrene resin (Diaion HP-20) column eluted with 30% MeOH, 50% MeOH, 80% MeOH, MeOH, EtOH, and EtOAc. The EtOH-eluted fraction (3.35 g) showed significant PPAR-γ ligand-binding activity (Fig. 1) and was subjected to a series of chromatographic separations to obtain **1** (193 mg), **2** (10.0 mg), **3** (15.2 mg), **4** (431 mg), **5** (10.1 mg), **6** (11.2 mg), **7** (3.5 mg), and **8** (413 mg). Compounds **1**–**8** were identified by comparison of their physical and spectral data with those of reported compounds eugenol (**1**) [14], dehydrodieugenol (**2**) [15], dehydrodieugenol B (**3**) [16], oleanolic acid (**4**) [17], arjunolic acid (**5**) [18], corosolic acid (**6**) [19], asiatic acid (**7**) [19], and betulinic acid (**8**) [19], respectively (Fig. 2).

Although eugenol (**1**), a phenylpropanoid derivative, had little PPAR-γ ligand-binding activity, neolignan derivatives dehydrodieugenol (**2**) and dehydrodieugenol B (**3**) exhibited significant activities (Fig. 3). The activity of **2**

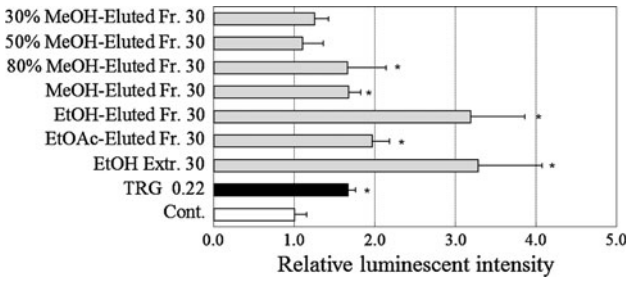

Fig. 1 PPAR-γ ligand-binding activity of the clove extract and fractions. PPAR-γ ligand-binding activity of the clove extract and fractions (30 μg/mL), as well as that of troglitazone (TRG) (0.22 μg/mL) used as a positive control, were measured using a GAL-4-PPAR-γ chimera assay. All samples were dissolved in DMSO, and added to medium to obtain the indicated concentrations. The luminescence intensity ratio (test group/control group) was determined for each sample, and PPAR-γ ligand-binding activity was expressed as the luminescence intensity of the test sample relative to that of the control sample. Values are means ± SD, $n = 4$ experiments. *Statistical significance at $P < 0.05$

Table 1 Effects of 3 weeks of feeding of the clove EtOH extract to KK-A^y mice in experiments on the prevention of diabetes[a]

	Control	Pioglitazone 0.02 g/100 g diet	Clove EtOH extract 0.5 g/100 g diet
Body weight (g)			
Day 0	26.7 ± 1.1	27.4 ± 0.5	27.1 ± 1.1
Day 21	42.7 ± 2.5	45.4 ± 1.4	42.5 ± 2.1
Food intake[b] [g/(mouse day)]	5.37	5.06	5.10
Pioglitazone or clove EtOH extract intake[c] [mg/(kg body weight day)]	0	25	657
Blood glucose level (mg/dL)			
Day 0	163 ± 14	156 ± 16	171 ± 14
Day 21	514 ± 60	164 ± 16**	311 ± 90**

** $P < 0.01$

[a] Body weights and blood glucose levels are expressed as means ± SD of five mice

[b] Calculated as (total food intake)/(number of mice day)

[c] Calculated as (mean food intake × dietary content of pioglitazone or clove EtOH extract)/(mean body weight of mice)

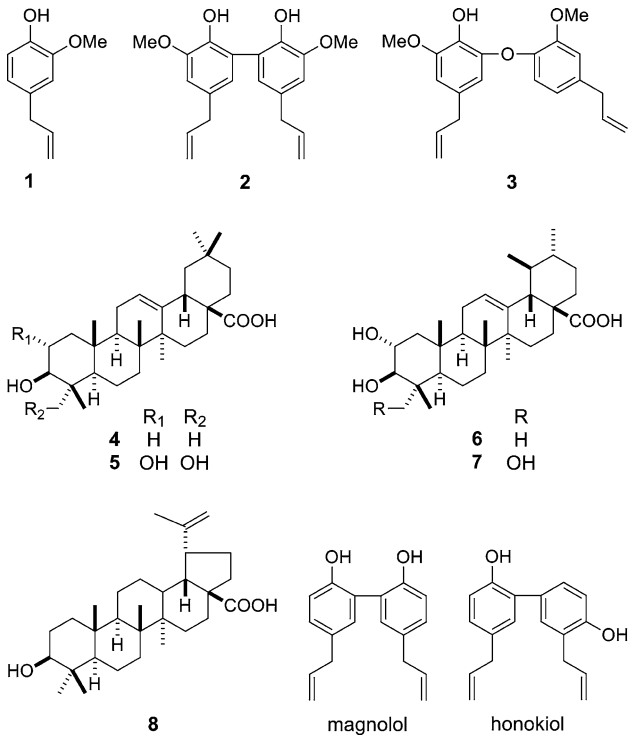

Fig. 2 Structures of compounds **1–8**, magnolol, and honokiol

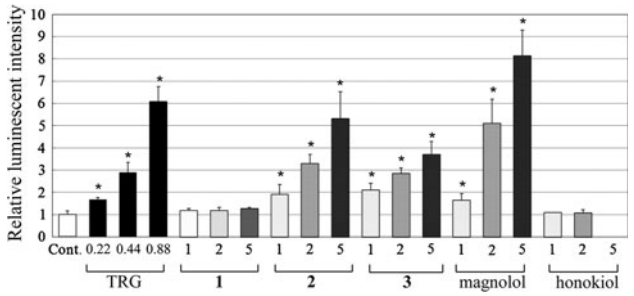

Fig. 3 PPAR-γ ligand-binding activities of **1–3**, magnolol, and honokiol. PPAR-γ ligand-binding activities of **1–3**, magnolol, and honokiol (1, 2, and 5 μg/mL), as well as that of troglitazone (TRG; 0.22, 0.44, and 0.88 μg/mL) used as a positive control, were measured using a GAL-4-PPAR-γ chimera assay. Values are means ± SD, $n = 4$ experiments. *Statistical significance at $P < 0.05$. (*a*) Data could not be obtained due to the cytotoxicity of honokiol at 5 μg/mL

at a sample concentration of 5.0 μg/mL was almost as potent as that of 0.88 μg/mL troglitazone. Since neolignans **2** and **3** are structurally similar to magnolol and honokiol, the main secondary metabolites contained in *Magnolia obovata* barks [20], their PPAR-γ ligand-binding activities were also evaluated. Magnolol at a concentration of 5.0 μg/mL showed more potent activity than troglitazone (0.88 μg/mL). In contrast, honokiol, which is an isomer of magnolol, showed little activity (Fig. 3). Although the PPAR-γ ligand-binding activities of **2**, magnolol, and honokiol have been independently reported by different researchers [21–23], our consecutive evaluation of the biphenyl-type neo-lignans for PPAR-γ ligand-binding activities shed further light on the structure–activity relationship; that is, symmetric structural features of the biphenyl moiety are responsible for the appearance of the potent PPAR-γ ligand-binding activities. Among the isolated triterpenoid derivatives, oleanolic acid (**4**), which was one of the major components in the EtOH extract, showed moderate PPAR-γ ligand-binding activity (Fig. 4).

PPAR-γ agonists are known to promote the maturation of preadipocytes into adipocytes [24]. To further characterize the profile of **2** and **3**, we examined their effects on differentiation of 3T3-L1 preadipocytes. Incubation with dehydrodieugenol as well as troglitazone markedly stimulated preadipocyte differentiation, as indicated by the staining of lipids with Oil Red O (Fig. 5). In addition, we investigated the effect of dehydrodieugenol on PPAR-γ target gene aP2 in differentiated 3T3-L1 cells. As reported previously [25, 26], exposure to troglitazone resulted in a substantial increase in aP2 protein level in 3T3-L1 cells (Fig. 6). Similarly, **2** altered aP2 protein level (Fig. 6). These results suggest that **2** stimulates adipose differentiation via PPAR-γ activation in adipocyte. As for the effect of **2** at 5 μg/mL being weaker than at 10 μg/mL, cytotoxicity might be reflected by the high dose. In contrast, 3T3-L1 cells exposed to both 5 and 10 μg/mL of **3** expressed moderate levels of aP2 protein (Fig. 6). Possibly related to this result, the effect of **3** on preadipocyte differentiation was not significantly stimulated (Fig. 5). However, **3** had the tendency to stimulate adipocyte differentiation, although weakly. It is reported that 3T3-L1 cells were more strongly differentiated after 14 days than 7 days [27]. Further time course studies would be required to demonstrate the effect of **3** on adipocyte differentiation. Accordingly, it was concluded that **2–4** contribute to the potent PPAR-γ ligand-binding activity of the clove EtOH extract, and the hypoglycemic effects of this extract on

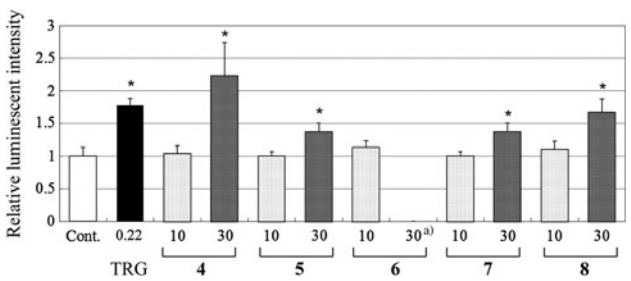

Fig. 4 PPAR-γ ligand-binding activities of **4–8**. PPAR-γ ligand-binding activities of **4–8** (10 and 30 μg/mL), as well as that of troglitazone (TRG; 0.22 μg/mL) used as a positive control, were measured using a GAL-4-PPAR-γ chimera assay. Values are means ± SD, $n = 4$ experiments. *Statistical significance at $P < 0.05$. (*a*) Data could not be obtained due to the cytotoxicity of **6** at 30 μg/mL

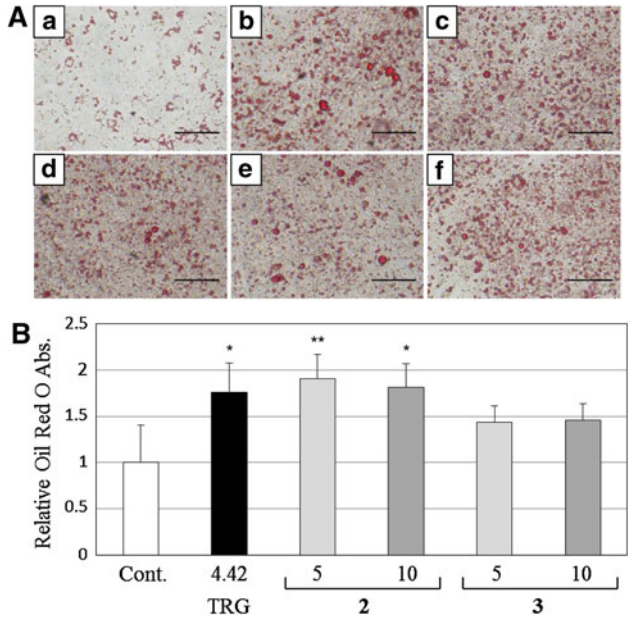

Fig. 5 Effects of **2** and **3** (5 and 10 μg/mL) on 3T3-L1 adipocytes differentiation. Compounds **2**–**3** and troglitazone (TRG; 4.42 μg/mL) were dissolved in DMSO, and added to medium to obtain the indicated concentrations. 3T3-L1 cells grown to subconfluence were induced to differentiate for 7 days. **A** Cells were subjected to Oil Red O staining. *a* DMSO, *b* **2** (5 μg/mL), *c* **2** (10 μg/mL), *d* **3** (5 μg/mL), *e* **3** (10 μg/mL), *f* TRG (4.42 μg/mL) as a positive control. *Scale bars* 100 μm. **B** Lipid accumulation was assessed by quantification of OD_{500} in destained Oil Red O with isopropanol. Values are means ± SD, $n = 4$ experiments. *,**Statistical significance at $P < 0.05$ or $P < 0.01$

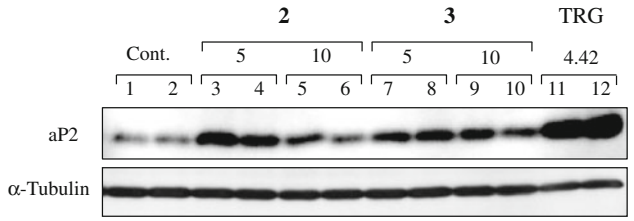

Fig. 6 Effects of **2** and **3** (5 and 10 μg/mL) on the expression levels of aP2 in 3T3L1 cells. Effects **2** and **3** on the expression levels of aP2, as well as that of troglitazone (TRG; 4.42 μg/mL) used as a positive control, were measured using ECL Advance detection reagent. All samples were dissolved in DMSO, and added to medium to obtain the indicated concentrations. $n = 2$ experiments. Alpha-tubulin was used as a loading control

genetically diabetic KK-A^y mice could in part be mediated through this nuclear receptor. Further study needs to clarify whether clove and its ingredients induce PPAR-γ mediated-adipocyte differentiation or not, by using a PPAR-γ antagonist.

Although clove extracts have been reported to show a variety of pharmacological actions, including anti-oxidative [28, 29], antinociceptive and anti-inflammatory [30], anti-allergy [31], and anti-stress activities [32], we discovered a possible new application of clove and its ingredients to the amelioration of type 2 diabetes, a representative insulin resistance syndrome in this work.

Experimental

General

Diaion HP-20 (Mitsubishi-Chemical, Tokyo, Japan), silica gel (Fuji-Silysia Chemical, Aichi, Japan), and ODS silica gel (Nacalai-Tesque, Kyoto, Japan) were used for column chromatography (CC). TLC was carried out on precoated Silica gel 60 F_{254} (0.25 mm, Merck, Darmstadt, Germany) and RP-18 F_{254S} (0.25 mm thick, Merck) plates, and spots were visualized by spraying with 10% H_2SO_4 followed by heating. HPLC was performed using a system comprising a CCPM pump (Tosoh, Tokyo, Japan), a CCP PX-8010 controller (Tosoh), and a Rheodyne injection port. A Capcell Pak C_{18} UG120 column (10 mm i.d. × 250 mm, 5 μm, Shiseido, Tokyo, Japan) was used for preparative HPLC.

Plant material

Clove was purchased from a local market in Lampung, Sumatra, Republic of Indonesia, in May 2001 and identified by Dr. Yutaka Sashida, professor emeritus of Tokyo University of Pharmacy and Life Sciences. A voucher specimen has been deposited in our laboratory (voucher No. 01-8-SA, Laboratory of Medicinal Pharmacognosy).

Preparation of the clove EtOH extract

The cloves (2 kg) were twice percolated with EtOH (10 L) at room temperature for 7 days, and the extract was concentrated under reduced pressure to give 217 g of EtOH extract.

In-vivo evaluation of the clove EtOH extract

Female KK-A^y mice were obtained from Clea Japan (Tokyo, Japan), and housed in an environmentally controlled animal laboratory. Mice at 6 weeks of age were randomly divided into three groups (five mice per group) on the basis of body weight and blood glucose level. The mice were fed a basal diet (Oriental Yeast, Tokyo, Japan) in the control group, whereas the mice were fed the clove EtOH extract at 0.5 g/100 g diet or pioglitazone at 0.02 g/100 g diet in the treated groups. Diet and water were given ad libitum for 3 weeks. Blood samples were taken from the tail veins of the mice and glucose concentrations

were measured using a Glutest Ace Blood Glucose Level Monitor (Sanwa Kagaku, Nagoya, Japan) before and after the 3-week feeding. Experiments were performed according to the Guidelines for the Care and Use of Experimental Animals of the Japanese Association for Laboratory Animal Science.

Extraction and isolation

The cloves (200 g) were twice percolated with EtOH (2 L) at room temperature for 3 days. The EtOH extract (13.0 g) was passed though a Diaion HP-20 column eluted with 30% MeOH, 50% MeOH, 80% MeOH, MeOH, EtOH, and EtOAc. The EtOH-eluted fraction (3.35 g) was chromatographed on silica gel eluted with $CHCl_3$–MeOH gradients (49:1; 19:1; 9:1), and finally with MeOH alone, to give four fractions (I–IV). Fraction I was chromatographed on silica gel eluted with $CHCl_3$–MeOH (49:1) and hexane–acetone (3:1; 2:1) to afford **1** (193 mg), **2** (10.0 mg), and **3** (15.2 mg). Fraction II was separated by a silica gel column eluted with $CHCl_3$–MeOH (49:1) and hexane–acetone (2:1; 1:1) to give **4** (431 mg) and **8** (413 mg). Fraction III was subjected to silica gel CC eluted with $CHCl_3$–MeOH (19:1) and ODS silica gel with MeOH–H_2O (9:1), and finally purified by preparative HPLC using MeOH–H_2O (4:1) to yield **5** (10.1 mg), **6** (11.2 mg), and **7** (3.5 mg), respectively.

PPAR-γ ligand-binding activity

PPAR-γ ligand-binding activity was carried out using a GAL-4-PPAR-γ chimera assay system [33]. CV-1 monkey kidney cells from the American Type Culture Collection (ATCC; Manassas, VA, USA) were inoculated into a 96-well culture plate at 6×10^3 cells/well and incubated in 5% CO_2/air at 37°C for 24 h. For the medium, Dulbecco's modified Eagle medium (DMEM; Gibco, Grand Island, NY, USA) containing 10% fetal bovine serum (FBS), 10 mL/L penicillin–streptomycin (5000 IU/mL and 5000 μg/mL, respectively, Gibco), and 37 mg/L ascorbic acid (Wako Pure Chemical, Tokyo, Japan) was used. Cells were washed with OPTI–minimum essential medium (OPTI-MEM) (Gibco) and transfected with pM-hPPAR-γ and p4 × UASg-tk-luc using LipofectAMINE PLUS (Gibco). In a mock control, pM and p4 × UASg-tk-luc were transfected into CV-1 cells. After 24 h of transfection, the medium was changed to DMEM containing 10% charcoal-treated FBS and each sample, and the cells were further cultured for 24 h. The samples were dissolved in dimethyl sulfoxide (DMSO), to which the medium was added to obtain the final concentration of 0.1% (v/v) of DMSO. DMSO was also added to the control wells. The cells were then washed with Ca^{2+}- and Mg^{2+}-containing phosphate-buffered saline (PBS+), to which LucLite (Perkin-Elmer, Wellesley, MA, USA) was added. The intensity of emitted luminescence was determined using a TopCount microplate scintillation/luminescence counter (Perkin-Elmer). The luminescence intensity ratio (test group/control group) was determined for each sample, and PPAR-γ ligand-binding activity was expressed as the luminescence intensity of the test sample relative to that of the control sample.

Adipocyte differentiation

3T3-L1 preadipocytes from ATCC were incubated into a 12-well culture plate at 4×10^5 cells/well and incubated in 5% CO_2/air at 37°C for 48 h in DMEM containing 10% FBS and 10 mL/L penicillin–streptomycin. For differentiation, the medium was changed to DMEM containing 10% FBS, 10 mL/L penicillin–streptomycin, 1 μg/mL insulin, and each sample, and the cells were further cultured for 7 days. Medium was renewed every 2 days. The samples were dissolved in DMSO, to which the medium was added to obtain the final concentration of 0.1% (v/v) of DMSO. DMSO was added to the control wells.

Oil Red O staining

Fixed cells were washed with PBS+, and placed in 60% isopropanol for 1 min, after which they were stained for 15 min at 37°C in freshly diluted Oil Red O (Wako Pure Chemical, Tokyo, Japan) solution (0.3% stock in isopropanol by water at 6:4), followed by color separation with 60% isopropanol for 1 min. After washing off the excessive dye, bound dye was solubilized with 100% isopropanol and photometrically quantified with at 500 nm.

Western blotting

Proteins were resolved on 15% SDS-polyacrylamide gels and detected using ECL Advance Western Blotting Detection Reagents (GE Healthcare, Buckinghamshire, England) as described previously [34]. Goat anti-aP2 antibody was obtained from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Mouse anti-α-tubulin antibody was obtained from Sigma-Aldrich (St. Louis, MO, USA). For quantitative analysis, the signal intensities of the bands detected on the blots were measured and transformed into relative values using a calibration curve generated with known amounts of protein.

Statistical analysis

Statistical analysis was performed using Dunnett's multiple comparison test with SAS statistical software (SAS

Institute, Cary, NC, USA). Each value in the text is presented as the mean ± SD, and significance was set at levels of $P < 0.05$ and $P < 0.01$.

References

1. Nicholas SB (1999) Lipid disorders in obesity. Curr Hypertens Rep 1:131–136
2. Unger RH (2002) Lipotoxic diseases. Annu Rev Med 53:319–336
3. Burn RP, Kim JB, Hu E, Altiok S, Spiegelman BM (1996) Adipocyte differentiation: a transcriptional regulatory cascade. Curr Opin Cell Biol 8:826–832
4. Mangelsdorf DJ, Thummel C, Beato M, Herrlich P, Schutz G, Umesono K, Blumberg B, Kastner P, Mark M, Chambon P, Evans RM (1995) The nuclear receptor superfamily: the second decade. Cell 83:835–839
5. Kaplan F, Al-Majali K, Betteridge DJ (2001) PPARs, insulin resistance and type 2 diabetes. J Cardiovasc Risk 8:211–217
6. Moller DE (2001) New drug targets for type 2 diabetes and the metabolic syndrome. Nature 414:821–827
7. Mae T, Kishida H, Nishiyama T, Tsukagawa M, Konishi E, Kuroda M, Mimaki Y, Sashida Y, Takahashi K, Kawada T, Nakagawa K, Kitahara M (2003) A licorice ethanolic extract with peroxisome proliferator-activated receptor-γ ligand-binding activity affects diabetes in KK-A^y mice, abdominal obesity in diet-induced obese C57BL mice and hypertension in spontaneously hypertensive rats. J Nutr 133:3369–3377
8. Nishiyama T, Mae T, Kishida H, Tsukagawa M, Mimaki Y, Kuroda M, Sashida Y, Takahashi K, Kawada T, Nakagawa K, Kitahara M (2005) Curcuminoids and sesquiterpenoids in turmeric (*Curcuma longa* L.) suppress an increase in blood glucose level in type 2 diabetic KK-A^y mice. J Agric Food Chem 53:959–963
9. Kuroda M, Mimaki Y, Nishiyama T, Mae T, Kishida H, Tsukagawa M, Takahashi K, Kawada T, Nakagawa K, Kitahara M (2005) Hypoglycemic effects of turmeric (*Curcuma longa* L. rhizomes) on genetically diabetic KK-A^y mice. Biol Pharm Bull 28:937–939
10. Nakagawa K, Kishida H, Arai N, Nishiyama T, Mae T (2004) Licorice flavonoids suppress abdominal fat accumulation and increase in blood glucose level in obese diabetic KK-A^y mice. Biol Pharm Bull 27:1775–1778
11. Aoki F, Honda S, Kishida H, Kitano M, Arai N, Tanaka H, Yokota S, Nakagawa K, Asakura T, Nakai Y, Mae T (2007) Suppression by licorice flavonoids of abdominal fat accumulation and body weight gain in high-fat diet-induced obese C57BL/6J mice. Biosci Biotechnol Biochem 71:206–214
12. Kuroda M, Mimaki Y, Honda S, Tanaka H, Yokota S, Mae T (2010) Phenolics from *Glycyrrhiza glabra* roots and their PPAR-γ ligand-binding activity. Bioorg Med Chem 18:962–970
13. Kuroda M, Mimaki Y, Sashida Y, Mae T, Kishida H, Nishiyama T, Tsukagawa M, Konishi E, Takahashi K, Kawada T, Nakagawa K, Kitahara M (2003) Phenolics with PPAR-γ ligand-binding activity obtained from licorice (*Glycyrrhiza uralensis* Roots) and ameliorative effects of glycyrin on genetically diabetic KK-A^y mice. Bioorg Med Chem Lett 13:4267–4272
14. Shokeen P, Bala M, Singh M, Tandon V (2008) In vitro activity of eugenol, an active component from *Ocimum sanctum*, against multiresistant and susceptible strains of *Neisseria gonorrhoeae*. Int J Antimicrob Agents 32:174–179
15. Suarez M, Bonilla J, De Diaz AMP, Achenbach H (1983) Dehydrodieugenols from *Nectandra polita*. Phytochemistry 22:609–610
16. De Diaz AMP, Gottlieb HE, Gottlieb OR (1980) Dehydrodieugenols from *Ocotea cymbarum*. Phytochemistry 19:681–682
17. Seo S, Tomita Y, Tori K (1975) Carbon-13 NMR spectra of urs-12-enes and application to structural assignments of components of *Isodon japonicus* Hara tissue cultures. Tetrahedron Lett 16:7–10
18. Jayasinghe L, Wannigama GP, Macleod JK (1993) Triterpenoids from *Anamirta cocculus*. Phytochemistry 34:1111–1116
19. Aguirre MC, Delporte C, Backhouse N, Erazo S, Letelier ME, Cassels BK, Silva X, Alegría S, Negrete R (2006) Topical anti-inflammatory activity of 2α-hydroxy pentacyclic triterpene acids from the leaves of *Ugni molinae*. Bioorg Med Chem 14:5673–5677
20. Fujita M, Itokawa H, Sashida Y (1973) Studies on the components of *Magnolia obovata* Thunb. 3. Occurrence of magnolol and hõnokiol in *M. obovata* and other allied plants. Yakugaku Zasshi 93:429–434
21. Choi SS, Cha BY, Lee YS, Yonezawa T, Teruya T, Nagai K, Woo JT (2009) Magnolol enhances adipocyte differentiation and glucose uptake in 3T3-L1 cells. Life Sci 84:908–914
22. Kotani H, Tanabe H, Mizukami H, Makishima M, Inoue M (2010) Identification of a naturally occurring rexinoid, honokiol, that activates the retinoid X receptor. J Nat Prod 73:1332–1336
23. Fakhrudin N, Ladurner A, Atanasov AG, Heiss EH, Baumgartner L, Markt P, Schuster D, Ellmerer EP, Wolber G, Rollinger JM, Stuppner H, Dirsch VM (2010) Computer-aided discovery, validation, and mechanistic characterization of novel neolignan activators of peroxisome proliferator-activated receptor gamma. Mol Pharmacol 77:559–566
24. Spiegelman BM (1998) PPAR-gamma: adipogenic regulator and thiazolidinedione receptor. Diabetes 47:507–514
25. Farmer SR (2006) Transcriptional control of adipocyte formation. Cell Metab 4:263–273
26. Rosen ED, MacDougald OA (2006) Adipocyte differentiation from the inside out. Nat Rev Mol Cell Biol 7:885–896
27. Schmidt W, Pöll-Jordan G, Löffler G (1990) Adipose conversion of 3T3-Ll cells in a serum-free culture system depends on epidermal growth factor, insulin-like growth factor I, corticosterone, and cyclic AMP. J Biol Chem 265:15489–15495
28. Shukri R, Mohamed S, Mustapha NM (2010) Cloves protect the heart, liver and lens of diabetic rats. Food Chem 122:1116–1121
29. Abdel-Wahhab MA, Aly SE (2005) Antioxidant property of *Nigella sativa* (black cumin) and *Syzygium aromaticum* (clove) in rats during aflatoxicosis. J Appl Toxicol 25:218–223
30. Tanko Y, Mohammed A, Okasha MA, Umah AH, Magaji RA (2008) Anti-nociceptive and anti-inflammatory activities of ethanol extract of *Syzygium aromaticum* flower bud in wistar rats and mice. Afr J Trad CAM 5:209–212
31. Kim HM, Lee EH, Hong SH, Song HJ, Shin MK, Kim SH, Shin TY (1998) Effect of *Syzygium aromaticum* extract on immediate hypersensitivity in rats. J Ethnopharmacol 60:125–131
32. Singh AK, Dhamanigi SS, Asad M (2009) Anti-stress activity of hydro-alcoholic extract of *Eugenia caryophyllus* buds (clove). Indian J Pharmacol 41:28–31
33. Takahashi N, Kawada T, Goto T, Yamamoto T, Taimatsu A, Matsui N, Kimura K, Saito M, Hosokawa M, Miyashita K, Fushiki T (2002) Dual action of isoprenols from herbal medicines on both PPARγ and PPARα in 3T3-L1 adiopocytes and HepG2 hepatocytes. FEBS Lett 514:315–322
34. Momose A, Fujita M, Ohtomo T, Umemoto N, Tanonaka K, Toyoda H, Morikawa M, Yamada J (2011) Regulated expression of acyl-CoA thioesterases in the differentiation of cultured rat brown adipocytes. Biochem Biophys Res Commun 404:74–78