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Original Article

Torvoside A extracted from *Solanum torvum* fruits does not directly affect 3T3-L1 –adipogenesis, adiponectin synthesis and glucose uptake

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Abstract

The present study was aimed to determine the effects of Torvoside A from methanolic extract of *Solanum torvum* on adipogenesis, fat accumulation, and adiponectin synthesis as well as glucose uptake activities. For adipogenesis assay, 3T3-L1-preadipocytes were treated with Torvoside A (0, 5, 25, 50 µM) during 7 days of adipocyte differentiation, while 3T3-L1 mature adipocytes were treated with Torvoside A for 2 days in the fat accumulation study. There were no significant differences in the intracellular triglyceride among Torvoside A treated cells and the untreated control ($p>0.05$) in both assays. Torvoside A did not significantly change adiponectin synthesis in both transcriptional and translational levels ($p>0.05$), measured by real-time RT-PCR and western blot, respectively. Finally, Torvoside A did not affect glucose uptake activity and GLUT4 transcript as well. These results can be concluded that Torvoside A could not directly promote adipocyte hyperplasia and hypertrophy as well as the anti-diabetic activities.

Keywords: Torvoside A, adipogenesis, glucose uptake, *Solanum torvum*, fat accumulation

1. Introduction

Adipose tissue found under a layer of the skin, particularly around the waist or belly, consisted of adipocytic cells. In obesity, increasing of numbers and size of adipocytic cells in adipose tissue is due to increasing of adipogenesis and fat accumulation, respectively (Drolet *et al.*, 2008; Jo *et al.*, 2009). The adipogenesis is the process of adipocyte differentiation into mature adipocyte. This process needs the activation of specific adipocytic gene expression via ERK1/2, PI3K/Akt, PPARγ and C/EBPα, respectively (Chuang, Yang, Tsai, Ho, & Liu, 2007). The transcription factors, CCAAT/enhancer-binding protein alpha (C/EBPα) and Peroxisome proliferator-activated receptor gamma (PPARγ) are important and necessary for the cell differentiation into mature adipocytes.

Adiponectin is an adipokine synthesized from mature adipocyte (Scherer, Williams, Fogliano, Baldini, & Lodish, 1995). The reduction of adiponectin in the blood-stream is associated with an increase in insulin resistance (Katsuki *et al.*, 2003; Yamauchi *et al.*, 2001). Previous studies showed that adiponectin levels were decreased in patients with obesity, diabetes type II and cardiovascular disease (Asayama *et al.*, 2003; Elokely, Shoukry, Ghonemy, Atia, & Amr, 2012; Parul, Mazumder, Debnath, & Haque, 2011). In addition, adiponectin also reduces the accumulation of macrophage foam cells by reducing fat oxidized LDL uptake and increasing cholesterol efflux mediated by HDL (Fu, Luo, Klein, & Garvey, 2005; Tian *et al.*, 2009) which in effect reduces the occurrence of atherosclerosis risk factors.

There are several studies on the effects of many kinds of glycosides on adipocyte differentiation or adipogenesis in 3T3-L1 cell, for example, Diosgenin increased adipocyte differentiation by increasing PPARγ mRNA, which resulted in an increases of specific adipocytic gene expressions such as Glucose transporter type 4 (GLUT4), adipocyte protein 2 (aP2), lipoprotein phospholipase (LPL) mRNA level (Uemura *et al.*, 2010). Ginsenoside 20 (S)-Protopanaxatriol found in ginseng activated the PPAR-trans-activation activity, and controlled the PPARγ gene expression (Han, Jung, Sohn,

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& Hwang, 2006). Ginsenoside-Rb1 extracted from the root of ginseng stimulated the PPARγ and C/EPBα expression, while Ginsenoside Rg3 from Red Ginseng effectively inhibited adipogenesis and gene expression of PPAR-γ (Hwang *et al.*, 2009).

Methanol extracts of fruits from *Solanum torvum* Swartz (Solanaceae), a small shrub distributed widely in Thailand, contained steroidal glycoside, named Torvoside H and Torvoside A (Arthan, Kittakoop, Esen, & Svasti, 2006; Arthan *et al.*, 2002). Torvoside H effectively inhibited of the growth of the Herpes simplex type I virus (Arthan *et al.*, 2002), and *Trichomonas vaginalis* infection (Arthan *et al.*, 2008), as well as bacterial infections such as *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* (Chah, Muko, & Oboegbulem, 2000). A methanol extract of *Solanum torvum* from leave and fruits can inhibit ulceration by promoting the production of mucus and reducing gastric-acid secretion (Nguelefack *et al.*, 2008). In addition, the ethanolic extract of *Solanum torvum* can reduce blood pressure and the levels of uric acid, glucose, triglyceride and cholesterol in a blood circulation of hypertensive rats (Mohan, Jaiswal, & Kasture, 2009). However, little is known on the biological activities of Torvoside A. We report herein the effects of Torvoside A isolated from a methanol extract of *S. torvum* fruits on the adipogenesis, fat accumulation, adiponectin synthesis and glucose uptake activity in 3T3-L1 cells (mouse embryonic fibroblast).

2. Materials and Methods

2.1 Materials

Mouse 3T3-L1 pre-adipocytes obtained from American type culture collection (ATTC®CL173; Manessas, VA, USA). Dulbecco modified Eagle medium (DMEM) and fetal bovine serum (FBS) were purchased from Gibco (Gibthai, Thailand). Dexamethasone, 3-isobutyl-1-methylxanthine (IB MX), Oil Red O, sorbitol, insulin, cycloheximide, were purchased from Sigma-Aldrich (St. Louis, Mo, USA). Antibodies used included mouse anti-adiponectin (Chemicon International, Inc., Temecula, CA), mouse anti-actin (Santa Cruz Biotechnology, California, USA), and goat anti-mouse HRP conjugates (Bio-Rad Laboratories, Hercules, CA).

2.2 Torvoside A extraction and isolation

S. torvum Swartz fruits (2.5 kg) were obtained from Pak Klong Talad Market, Bangkok, Thailand. Crushed fruits were macerated in methanol (6 L) for two days at room temperature. The extract was evaporated and dissolved in 70% aqueous methanol, which was sequentially extracted with hexane and ethyl-acetate (equal volume, three times). Aqueous methanol, hexane, and ethyl-acetate layers were evaporated to dryness. A crude extract of the aqueous methanol layer was applied to a Sephadex LH-20 column. Twenty fractions (ca. 100 ml) were collected and evaporated to dryness. Fractions 3–6 containing Torvoside A were combined and further purified by MPLC (C18 reversed phase column) using water: methanol (60:40, v/v) as eluent, to yield Torvoside A (2.1 g).

2.3 Cell cytotoxicity

MTT (Thiazolyl Blue Tetrazolium Bromide) assay (Bagchi, Kuszynski, Balmoori, Bagchi, & Stohs, 1998; Mos mann, 1983) was used to study cell viability after Torvoside A treatment. MTT assay is based on the principle that enzyme in viable cells can metabolize the MTT tetrazolium dye to purple insoluble formazan product. Pre-adipocyte 3T3-L1 cells (approximately $1x10^4$ cells) were cultured in a 96-well plate at 37 ºC in 5% CO_2. After cell attachment, pre-adipocyte 3T3-L1 cells were further incubated with DMSO (control) or Torvoside A (0, 7.8, 15.6, 31.2, 62.2, 125 and 250µM) for 48 hours. After that, fresh MTT solution (20 µL of 5 mg/mL) was added and incubated at 37ºC for 4 hours to allow the MTT to be metabolized. Formazan product was solubilized with 200 µL of dimethyl sulfoxide (DMSO) and the absorbance (A) was measured at 570 nm against background wavelength at 630 nm. Percentage of viable cells was then calculated by comparing to control using the following equation: [A value of sample/A value of control] x 100.

2.4 Cell culture and Torvoside A treatment

Pre-adipocyte 3T3-L1 cells were cultured in adipocyte medium, comprised of DMEM containing 25 mM glucose (DMEM/High glucose), supplemented with 10% FBS, 100 U/mL penicillin and 100 µg/mL streptomycin, at 37 ºC in 5% CO_2. For Torvoside A treatment during adipogenesis, ten thousands per well of 3T3-L1 cells were cultured in 6-well plates in adipocyte medium supplemented with adipogenesis inducer (0.5 mM IBMX, 250 nM dexamethasone) alone as control or with the inducer and additional Torvoside A treatments (5, 25, and 50 µM) for 2 days. Then, adipogenesis inducer was substituted with insulin (5 µg/mL) for two days. After that, cells were further incubated in adipocyte medium without insulin supplement for 3 days. After seven days of differentiation, cells were harvested and kept at -70ºC for RNA isolation or triglyceride and protein determination.

For Torvoside A treatment on mature adipocytes, cells were grown to confluence in 6-well plates and stimulated after two days of post-confluence with the adipogenesis induction medium (adipocyte medium supplemented with 0.5 mM IBMX, 250 nM dexamethasone, and 5 µg/mL insulin) for two days. Then, cells were cultured in 3T3-L1 adipocyte medium supplemented with 5 µg/mL insulin for 4-6 days. The 3T3-L1 adipocyte medium was changed every two days. When 3T3-L1 adipocytes attained maturity, which is about 6-8 days after the induction**,** mature adipocytes were treated with Torvoside A (0, 5, 25, 50 µM) for 2 days. After that, cells were harvested and kept at -70 ºC for RNA isolation or triglyceride and protein determination.

2.5 RNA isolation and amplification using real-time reverse transcriptase-polymerase chain reaction

Total RNA were isolated from 3T3-L1 cells using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's protocol, and were then treated with DNAse (Promega, Madison, WI) to remove residual contaminating DNA. cDNA synthesis was performed by the

iScript™ Reverse Transcription Supermix for RT-qPCR (Bio-Rad, Hercules, CA). First-strand cDNA synthesis was performed in a total reaction volume of 20 µL containing 1 U of Moloney Murine Leukemia virus (MMLV) reverse transcriptase at 42°C for 30 min. PCR amplifications were done by Real-time PCR using the iTaq™ Universal SYBR® Green Supermix (Bio-Rad, Hercules, CA). Specific primers sequences shown in Table 1 were synthesized by Integrated DNA technologies, Singapore. Quantification of gene expression was enabled using Applied Biosystems® 7500 Fast Real-Time PCR Systems (Applied Biosystems, Foster City, CA) and 7500 software v2.3. Briefly, the cDNA was denatured at 95 °C for 5 min followed by 35 cycles of PCR (95°C, 30 sec, 56-57 °C, 30 sec and 72 °C, 1 min). Melting curve analysis for PCR products was performed at the final step with the default settings on the Applied Biosystems® 7500 Fast System. The melting curve analysis revealed a single product at specific melting temperatures (Figure 1A). The PCR products were separated by electrophoresis on a 2% agarose gel, visualized by ethidium bromide staining (Figure 1B). The mRNA levels of all genes were normalized using actin as an internal control. Differences in gene expression between Torvoside A treated cells and control were presented as the relative expression ratios (R) or fold changes calculated using the delta-delta method ($R=2^{-\Delta\Delta Ct}$). All results were obtained from at least three independent experiments.

2.6 Adiponectin synthesis and Western blot

Pre-adipocyte 3T3-L1 cells were cultured and induced to become mature adipocytes, which is about 6-8 days after the induction**.** Mature adipocytes were treated with Torvoside A (0, 5, 25, 50 µM) for 2 days. After that, cells were further incubated with 10 µg/mL cycloheximide (CHX) for 8 hours (Miller *et al.*, 2008) to stop new protein synthesis. Cells were then harvested and prepared for analysis of adiponectin. Cell lysates were incubated with the sample buffer (20 mM Tris-HCl, pH 6.8, 4% (w/v) SDS, 16% (v/v) glycerol, and 0.004% (w/v) bromophenol blue) containing 10% dithiothreitol (DTT), as a reducing agent, for 5 min at 100°C, to change oligomeric to monomeric forms, and resolved in 12.5% (w/v) SDS-polyacrylamide gel electrophoresis. After electrophoresis, proteins were transferred to nitrocellulose membranes. After that, the membranes were incubated overnight with mouse anti-adiponectin antibody (1:5,000) and mouse anti-actin antibody (1:1,000), followed by incubation for 1.5 hours with goat anti-mouse HRP conjugates (1:5,000). Target proteins were then detected by chemiluminescence (Roche Diagnostics). Adiponectin and actin protein band intensities were assessed and analyzed with ImageJ software. Adiponectin band intensity was normalized with that of actin, and the data were presented as fold changes compared with control.

2.7 Glucose uptake assay

Glucose uptake assay was analyzed by a commercial colorimetric assay kit (Biovision Inc., CA, USA). Assays were performed according to the manufacturer's instruction. Briefly, matured 3T3-L1 adipocytes on 96-well plates were starved in serum-free adipocyte medium overnight to increase glucose uptake before being washed with phosphate buffered saline (PBS) and incubated with the Krebs-Ringer-Phosphate-HEPES (KRPH) buffer containing 2% bovine serum albumin (BSA) for 40 minutes. Next, cells were treated with various concentrations of Torvoside A (5, 25, 50 µM) and 50 µM Stevioside as a positive control for 20 minutes to activate glucose transporter. This was coupled with the acute stimulation from 1 µM insulin. Then, 2-deoxy-glucose (2-DG) was added to initiate glucose uptake. 2-DG can be taken up by glucose transporters and metabolized to 2-DG-6-phosphate (2-DG6P), which cannot be further metabolized and accumulates in the cells. After 20 minutes, cells were washed with 1xPBS to remove exogenous 2-DG and lysed with extraction buffer, freeze/thaw and heat at 85°C for 40 minutes. The cell lysate was analyzed by enzyme-based glucose colorimetric assay, NADPH recycling amplification reaction.

2.8 Statistical analyses

The results are expressed as the mean±SEM for triplicate of three independent experiments. Treatments were compared by one-way ANOVA using Tukey's posthoc test to identify statistical differences at $p<0.05$.

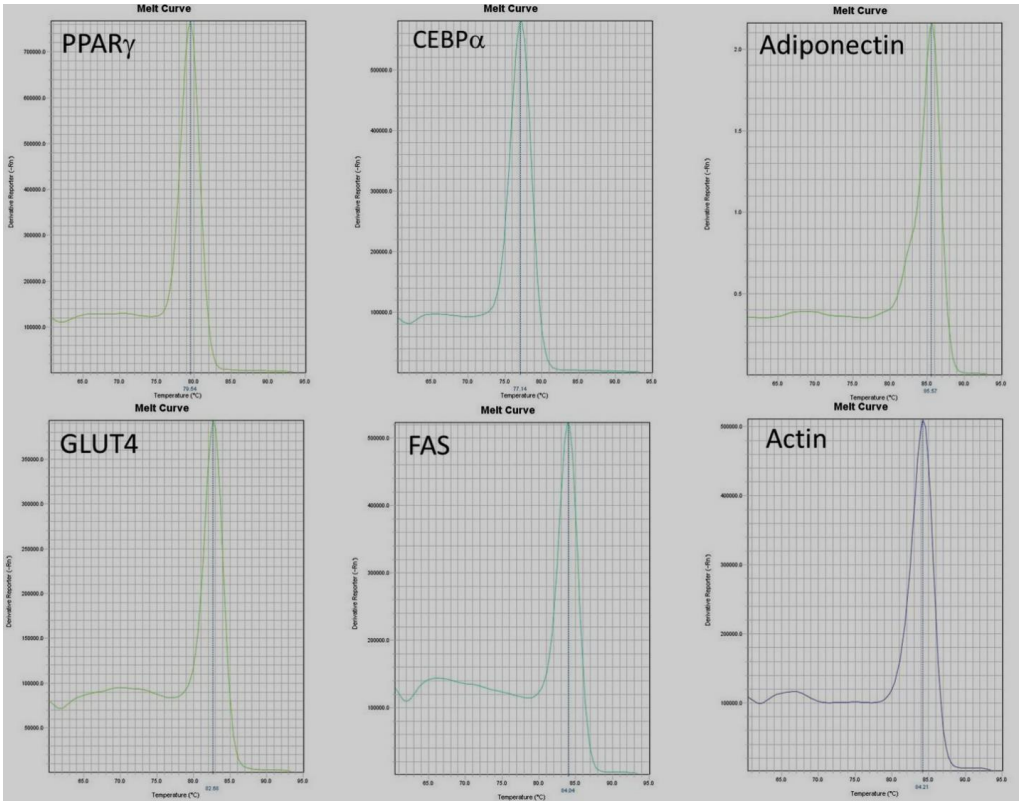
3. Results

3.1 Torvoside A and cell cytotoxicity

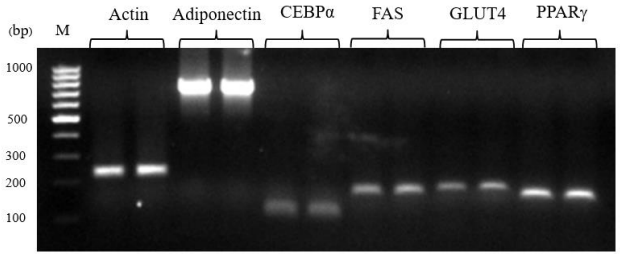
MTT assay was used to detect the effects of Torvoside A on 3T3-L1 cell cytotoxicity. The results showed that after treatments with Torvoside A for 48 hours at the concentrations of 0-250 µM, the percentages of viable cells treated with Torvoside A at the range of 0.05 and 100 µM were

Table 1. Primer sequences and PCR product sizes for Real time-PCR.

Name	Primer sequences	PCR (bp)	References
FAS	F: TGGGTTCTAGCCAGCAGAGT R: TACCACCAGAGACCGTTATGC	159	(Hsu, Lin, Ho, & Yen, 2012)
PPARγ	F: GTGCCAGTTTCGATCCGTAGA R: GGCCAGCATCGTGTAGATGA	142	(Suzuki *et al.*, 2011)
Adiponectin	F: GAAGATGACGTTACTACAAC R: GGTAGTTGCAGTCAGTTGGT	704	(Fujimoto *et al.*, 2005)
actin	F: AAGAGAGGTATCCTGACCCT R: TACATGGCTGGGGTGTTGAA	218	
CEBPα	F: GTGTGCACGTCTATGCTAAACCA R: GCCGTTAGTGAAGAGTCTCAGTTT	97	(Kwak *et al.*, 2013)
GLUT4	F: TTCCTTCTATTTGCCGTCCTC	168	(Gong *et al.*, 2004)



(A)



(B)

Figure 1. Melting curve analysis (A) and Ethidium bromide-stained agarose gel separation of specific PCR products of Actin, adiponectin, CEBPα, FAS, GLUT4 and PPARγ (B). A single band of expected size on agarose gel and a melting curve analysis revealed single PCR product. No primer-dimers were observed during 35 cycles of the real-time PCR amplification protocol.

greater than 80% (Figure 2). Therefore, all Torvoside A concentrations used in this study (5, 25 and 50 µM) caused minimal cytotoxic effects on the cells.

3.2 Torvoside A and 3T3-L1 adipogenesis

3T3-L1 pre-adipocytes were treated with adipocyte medium supplemented with adipogenesis inducer (Materials and Methods). Fat droplets are accumulated during cell differentiation to become mature adipocytes. Therefore, measuring the triglyceride in fat droplets within the cells is to detect 3T3-L1 adipogenesis. It was shown that the fold change of triglyceride/protein of Torvoside A treated cells were not significantly different from that of control (p>0.05) (Figure 3). In addition, the important transcription factors, C/EBPα and

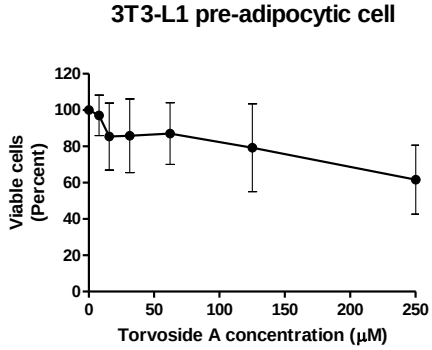
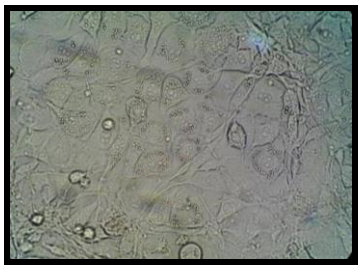
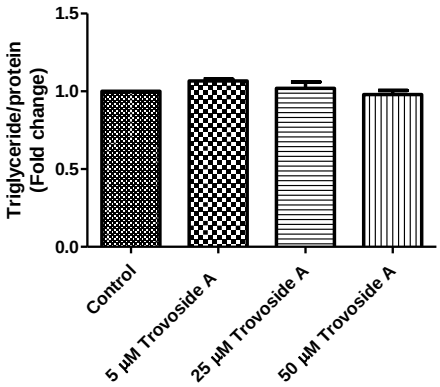


Figure 2. Effects of Torvoside A on viability of 3T3-L1 cells, determined by the MTT assay.



(A)



(B)

Figure 3. Effects of Torvoside A on adipogenesis of 3T3-L1 cells. Mature adipocytes, shown many fat droplets in cytosol, were differentiated from 3T3-L1 pre-adipocytes during adipogenesis (A). 3T3-L1 adipogenesis assay was determined by the amounts of triglyceride in fat droplets synthesized within the cells. Data are presented as fold changes of triglyeceride/protein, compared with the control. These results are shown as mean±SEM from three experiments (B).

PPARγ, for the cell differentiation into mature adipocytes were not significantly different among Torvoside A treated cells and control (p>0.05) (Figure 4).

3.3 Torvoside A and fat accumulation in mature adipocytes

Torvoside A was tested for its effects on fat accumulation in 3T3-L1 mature adipocytes. After 3T3-L1 adipocytes attained maturity, mature adipocytes were treated with Torvoside A (5, 25, 50 µM) for two days. For fat accumulation, cells were harvested for measuring intracellular triglyceride and protein. The fold changes of triglyceride/protein ratios compared with control were 1.083±0.107, 1.107±0.267, 1.160±0.249 for 5, 25, and 50 µM Torvoside A treatments, respectively. It showed that fat accumulation in Torvoside A treated mature adipocytes was not significantly different from that of control (p>0.05) (Figure 5A). For the study of fatty acid synthase (FAS), mRNA expression was analyzed by Real-time PCR and presented as fold changes of FAS/actin mRNA ratios compared with control. Fold changes of FAS/actin mRNA ratios were 1.071±0.274, 0.982±0.240, 0.877±0.226 for 5, 25, and 50 µM Torvoside A treatments, respectively. FAS mRNA levels in Torvoside A treated mature adipocytes were not significantly different from that of control (p>0.05) (Figure 5B).

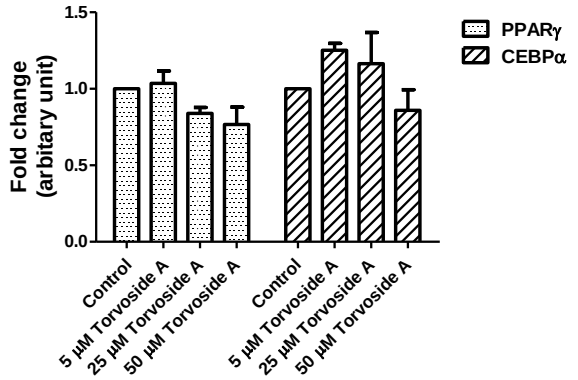
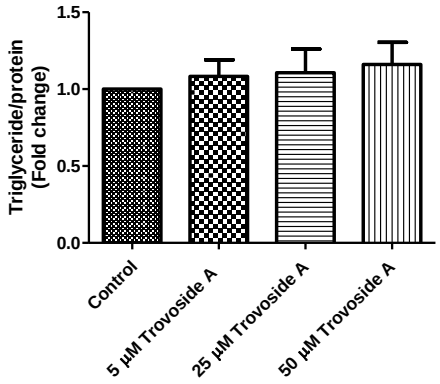


Figure 4. Effects of Torvoside A on PPARγ and CEBPα transcriptiton in 3T3-L1 cell during adipogenesis. Level of PPARγ and CEBPα mRNA measured by relative quantitative RT-PCR kept under control conditions or incubated with various concentration of Torvoside A during adipogenesis. PPARγ and CEBPα mRNA levels were normalized relative to the actin. (mean±SEM, n=3).



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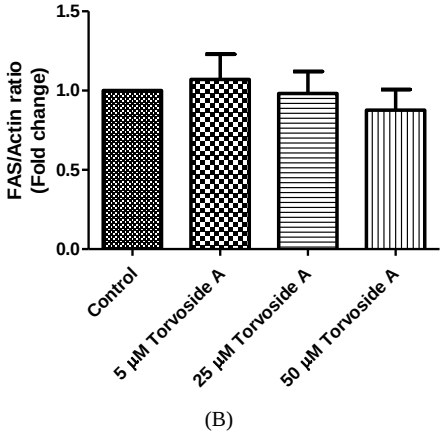
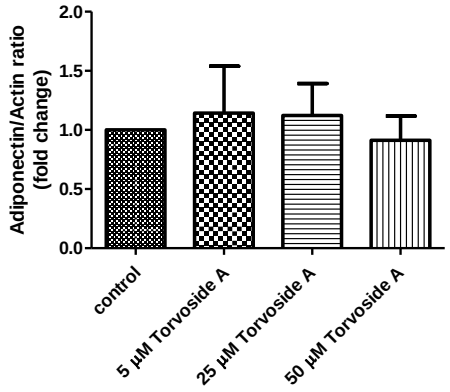


(B)

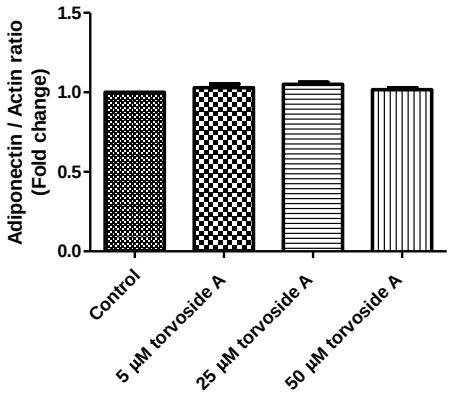
Figure 5. Effects of Torvoside A on fat accumulation within 3T3-L1 mature adipocytes represented as fold changes of trigly ceride/protein ratios of Torvoside A treated cell, compared with control (A). Effects of Torvoside A on FAS transcripts, presented as fold changes of FAS/actin mRNA ratios, compared with control (B). Results are shown as mean±SEM from three experiments.

3.4 Torvoside A and adiponectin gene expression

Torvoside A was tested for its effects on adiponectin gene expression both transcriptional and translational levels. After 3T3-L1 cells were differentiated to mature adipocytes, they were treated with Torvoside A (5, 25, 50 µM) for 48 hours. Cells were collected to determine the amount of adiponectin mRNA by real-time RT-PCR and adiponectin protein synthesis by western blot. The adiponectin/actin ratios by Torvoside A were calculated as fold change compared with control (Figure 6A, and 6B). For adiponectin transcripts, the fold changes of the adiponectin/actin ratios were 1.143±0.397, 1.122±0.270, 0.913±0.204 for 5, 25, and 50 µM Torvoside A treatments, respectively. The results showed that the adiponectin/actin ratios in Torvoside A treated cells were not significantly different from that of control (p>0.05). For adiponectin protein synthesis, the fold changes of the adiponectin/actin ratios were 1.030±0.023, 1.051±0.012, 1.017±0.011 for 5, 25, and 50 µM Torvoside A treatments, respectively indicating that Torvoside A did not significantly change adiponectin synthesis, compared with control (p>0.05).



(A)

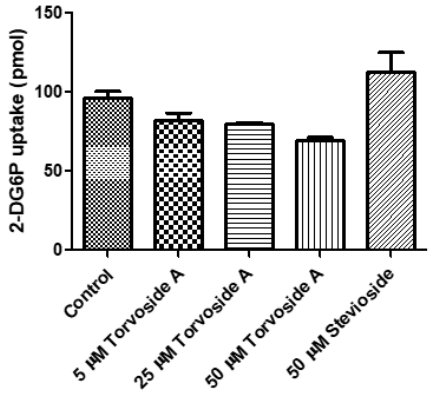


(B)

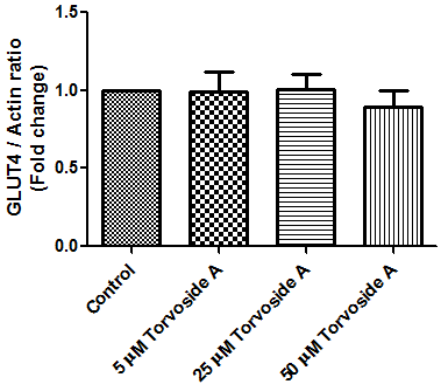
Figure 6. Effects of Torvoside A on adiponectin gene expression in transcriptional level (A), and translational level (B). The data was presented as fold changes of the adiponectin/actin ratios, compared with the control. Results are shown as mean±SEM from three experiments.

3.5 Torvoside A and glucose uptake

To examine the effects of Torvoside A on glucose uptake in 3T3-L1 cells, we evaluated glucose uptake in 3T3-L1 mature adipocytes treated with various concentrations (5, 25, and 50 µM) of Torvoside A. As shown in Figure 7A, glucose uptake was not significantly different when compared with control (p>0.05). Furthermore, Torvoside A did not affect the glucose transporter type-4 (GLUT4) mRNA expression by showing no significant change of GLUT4 mRNA levels among Torvoside A treated cells and control (p>0.05) (Figure 7B).



(A)



(B)

Figure 7. Effects of Torvoside A on glucose uptake (A), and GLUT4 mRNA expression (B) presented as fold changes of the GLUT4/actin ratios, compared with the control. Results are shown as mean±SEM from three experiments.

4. Discussion

Torvoside A structure is similar to various kinds of steroidal glycosides from plant extracts, that have been previously mentioned to their effect on adipogenesis and adiponectin synthesis (Han *et al.*, 2006; Hsu, Lin, Ho, & Yen, 2012; Hwang *et al.*, 2009). In addition, *Solanum torvum* extracts can reduce the levels of glucose, triglyceride, and cholesterol in hypertensive rats (Mohan *et al.*, 2009). However, little is known about the biological activity of Torvoside

A. In this study we have explored its biological activity on adipogenesis, fat accumulation and adiponectin synthesis in the mature adipocyte. Our results showed that Torvoside A did not affect adipogenesis, fat accumulation, and adiponectin gene expression in the 3T3-L1 cell.

Fruits of *S. torvum* are edible and utilized as vegetable and an essential ingredient in the diet. In the present study, it was noted that Torvoside A, known as a furostanol glycoside (Li, Wang, Guo, & Li, 2014), isolated from methanol extract of *S. torvum* fruit did not have the effects on GLUT4 gene expression and the glucose uptake activity on mature adipocytes, 3T3-L1 cells. According to previous studies, methanolic extract of *S. torvum* fruit presenting high levels of phenolic compounds, mainly rutin, caffeic acid, gallic acid, and catechin, has reported lowering blood glucose levels in the streptozotocin-induced diabetic rat (Gandhi, Ignacimuthu, & Paulraj, 2011). In addition, methyl caffeate isolated from the fruit of *S. torvum* was demonstrated to possess an anti-diabetic property by up-regulation of GLUT4 and regeneration of β-cells in the pancreas (Gandhi, Ignaci muthu, Paulraj, & Sasikumar, 2011). Methyl caffeate also moderates inhibitory action against α-glucosidase, resulting in the delay of carbohydrate digestion, increasing the carbohydrate digesting time, and thus reduces the rate of glucose absorption (Takahashi *et al.*, 2010). It is likely to explain that Torvoside A does not have the anti-diabetic activity, whereas the phenolic compounds in the methanol fruit extract of *S. torvum* do. Furthermore, adiponectin gene expression was also not affected by this compound. Therefore, Torvoside A does not show any effect as anti-diabetics.

Obesity is a risk factor associated with metabolic dysfunction leading to type 2 diabetes and cardiovascular diseases. *Solanum torvum* fruit water extract can reverse the level of total cholesterol and triglyceride to their normal level and reduce body weight gain in High Fat Diet (HFD)-induced obese male rats. In addition, lipid accumulation in the liver tissue of the HFD induced obese male rats was slightly reduced after treatment of *S. torvum* (Wannasiri, Chansakaow, & Sireeratawong, 2017). There was no previous study about methanol fruit extract of *S. torvum* on body weight gain and hyperlipidemia. Our results showed that Torvoside A did not increase the CEBPα and PPARγ gene expression during the 3T3-L1 adipogenesis, including mature adipocyte production. In addition, there was no effect of Torvoside A on fat accumulation in 3T3-L1 mature adipocytes, supported by no change of FAS mRNA level, which FAS is an enzyme for fatty acid synthesis. These results likely indicate that Torvoside A probably does not affect the two mechanisms of obesity; hyperplasia (adipogenesis) and hypotrophy (fat accumulation).

5. Conclusions

According to the results obtained in the present investigation, it can be concluded that Torvoside A, a glycolside compound isolated from methanol extract of *S. torvum* fruit did not increase adiponectin synthesis and glucose uptake. It does not only have anti-diabetic activity but also could not promote adipocyte hyperplasia and hypertrophy, subsequently cause obesity. This is the first report demonstrating that Torvoside A has no effect on obesity and diabetes (in vitro). The anti-diabetic activity of Trovoside A in insulin-resistant cells, including the other pharmacological activities like anti-oxidant, anti-inflammatory, anti-bacterial, and anti-ulcerogenic properties should be investigated in future studies.

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