# Antidiabetic potential of oleanolic acid from *Ligustrum lucidum* Ait.[1]

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**Abstract:** *Ligustrum lucidum* Ait. has been used in traditional Chinese medicine for over 1000 years because of its anti-tumor, antimutagenic, antidiabetic, and hepatoprotective properties. The aim of this study was to determine whether oleanolic acid (OA) is the principal active compound of *L. lucidum* responsible for its antidiabetic properties, and to examine its effect on the expression of thyroid hormones and insulin secretion, thus revealing the mechanism by which *L. lucidum* modulates insulin levels in diabetes. When rats with streptozotocin-induced diabetes were treated with OA (100 and 200 mg/kg body mass per day, for 40 days), the changes in blood glucose levels and in oral glucose tolerance tests showed that hypoglycemia was more pronounced in OA-treated groups than in the diabetic control rats, and that the levels of triglyceride, total cholesterol, and low-density lipoportein cholesterol in OA-treated rats were lower than those in the diabetic control rats, whose high-density lipoprotein cholesterol increased. OA-treated rats also gained weight, and exhibited increased serum insulin levels. In contrast, OA treatment did not effect the levels of thyroid hormone or TSH in rats with streptozotocin-induced diabetes. These results indicate that OA has hypoglycemic and hypolipidemic effects. OA treatment might stimulate insulin release, and consequently, results in the modulation of glucose levels and regulation of lipid metabolism.

*Key words:* hypoglycemic effect, hypolipidemic effect, *Ligustrum lucidum*, oleanolic acid, diabetes.

**Résumé :** Le *Ligustrum lucidum* Ait. est utilisé en médecine chinoise traditionnelle depuis plus de 1000 ans pour ses propriétés antitumorales, antimutagènes, antidiabétiques et hépatoprotectrices. La présente étude a pour but de déterminer si l'acide oléanolique (AO) est le principal composé actif de *L. lucidum* à l'origine de ses propriétés antidiabétiques, et d'examiner son effet sur l'expression des hormones thyroïdiennes et la sécrétion d'insuline afin d'identifier le mécanisme par lequel *L. lucidum* module les taux d'insuline chez les diabétiques. Chez des rats rendus diabétiques par streptozotocine et traités avec de l'AO (100 et 200 mg/kg par jour, pendant 40 jours), les modifications du glucose sanguin et les résultats du test de tolérance au glucose ont montré que les groupes traités à l'OA ont eu une hypoglycémie plus marquée que celle des rats diabétiques témoins, et que les taux de TG, de TC et de LDL-c des rats traités à l'OA ont été plus faibles que ceux des rats diabétiques témoins, alors que la teneur en HDL-c augmenté. L'AO a aussi provoqué un gain de poids corporel des rats. De plus, les rats traités à l'AO ont montré de plus hauts taux d'insuline. À l'opposé, le traitement à l'AO n'a pas eu d'effet sur les taux d'hormone thyroïdienne et de TSH chez les rats rendus diabétiques par STZ. Ces résultats ont indiqué que l'AO présente une activité hypoglycémique et hypolipidémique. Le traitement à l'AO pourrait stimuler la libération d'insuline et ainsi entraîner la modulation des taux de glucose et la régulation du métabolisme des lipides.

*Mots-clés :* effet hypoglycémique, effet hypolipidémique, *Ligustrum lucidum*, acide oléanolique, diabète.

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## Introduction

Diabetes mellitus is a major endocrine disorder that presents a growing health problem in many countries. Despite numerous preventive strategies and medical treatments, 300 million people worldwide are expected to develop diabetes by 2025 (Scheen 2000; Seidell 2000), and in spite of the introduction of many hypoglycemic agents, diabetes and its related complications continue to be major medical problems. Herbal remedies have become a key component of human health care because they may be perceived to have fewer side effects than prescription drugs.

The mechanisms of the active ingredients of herbs used to

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treat diabetes have been proposed, including an antioxidant effect (Ravi et al. 2004; Latha and Pari 2003), the stimulation of insulin secretion, the regulation of enzyme content (Randin et al. 1986; Nijs et al. 1989), and an increase in insulin receptor sensitivity (Lautt 1999). Previous studies have shown that an increase in thyroid hormones resulted in a dramatic increase in the food intake and glycogen degradation and (or) gluconeogenesis and an enhanced breakdown of triglycerides (TGs), which augmented glucose intolerance in diabetic patients (Machackova et al. 2005; Moon et al. 2006). Thyroid hormones were shown to stimulate virtually all aspects of lipid metabolism, including lipid synthesis, mobilization, and degradation (Bhattacharyya and Wiles 1999).

The ripe fruit of *Ligustrum lucidum* Ait. has been a traditional Chinese medicinal plant for several reasons. When mice with hepatocellular carcinoma $H_{22}$ were treated with a 1000 mg/kg dose of *L. lucidum* extract, the size of their tumors decreased by 46.15% (Xiang and Gu 2002). *Ligustrum lucidum* has also been shown to inhibit the proliferation of, and promote apoptosis in, HeLa cells (Zhang et al. 2004). Additionally, *L. lucidum* was shown to have antimutagenic (Wang et al. 1991), antidiabetic (Hao et al. 1992), and hepatoprotective properties (Yin and Yu 1993). Furthermore, *L. lucidum* has also been reported to increase the levels of white blood cells in mice treated with cyclophosphamide (Fan et al. 2004).

To our knowledge, there is no available report on the mechanism of action of *L. lucidum* and oleanolic acid (OA), in particular, and their effect on the treatment of streptozotocin (STZ)-induced diabetes in rats. OA, one of the active components of *L. lucidum* is a pentacyclic triterpene compound (Maia et al. 2006). In this study we investigated and evaluated the hypoglycemic and hypolipidemic effects of *L. lucidum* and determined whether OA was its principal antidiabetic active ingredient. To examine its effect on the expression of hormones and insulin secretion, we attempted to explore its possible mechanism of action and its antidiabetic properties, thus providing further scientific rationale for the use of *L. lucidum* as a potential natural oral hypoglycemic and hypolipidemic agent or functional food.

## Materials and methods

### Reagents and herb samples

A glucose analyzer and strips were purchased from Roche Diagnostics (Indianapolis, Ind.). The OA standard was from Shanghai Yousi Chemical Center (Shanghai, China). STZ was purchased from Sigma-Aldrich Co. (St. Louis, Mo.). Dried fruits from *L. lucidum* were purchased from a local drug market and authenticated by Dr. Xiaohua Liu at the Hospital of Qinhuangdao Chinese Medicine (Qinhuangdao, China).

### Preparation and characterization of *L. lucidum* extracts

#### *Preparation of OA from* L. lucidum

OA extraction was based on the multicrystal method, with some modification, as described previously (Ma et al. 2003). Briefly, dried *L. lucidum* fruits were dipped into $dH_2O$ for 30 min, boiled at a pressure of 1.5 kg/cm$^2$ for 40 min, and then the seed capsule and kernel were separated. The seed capsule was immersed in a solution of 95% ethanol. HCl (2%, pH 1.0) was added to the ethanol solution to obtain the crude crystals. The crude crystals were added to a 5% NaOH (pH 9.0) solution and boiled at 100 °C for 20 min, and the resultant crystals were redissolved in HCl (2%, pH 1.0). The crystals were washed with $dH_2O$, filtered on active carbon, and dried. The yield of OA from *L. lucidum* was approximately 0.5%.

#### *Characterization of OA from* L. lucidum

The OA extract and OA standard solutions were dotted on a TLC plate, *n*-butanol : acetic acid : $H_2O$ (10:9:1) was used as the solvent system, and a phosphomolybdic acid – ethanol solution was sprayed onto the plate. The plate was heated at 105 °C for 10 min.

The mass spectra (MS/MS) were obtained using a 4000 Q-Trap mass spectrometer (Applied Biosystems/MDS Sciex, Concord, Ont.) via a microIonspray interface. Tandem mass spectra were obtained using an enhanced production ion scan mode with a scan rate of 4000 Da/s. Nitrogen was used as curtain (at a value of 12) and collision gas (set to scale high).

### Preparation of experimental animals

Forty male Wistar rats weighing 200–220 g were purchased from the Animal Department of Beijing Institute of Traditional Medical and Pharmaceutical Sciences. All rats were individually housed in stainless steel cages at a controlled temperature (20–22 °C) and 60%–65% relative humidity with a normal 12 h light : 12 h dark cycle. All experimental animal protocols were approved and in accordance with the *Guide to the Care and Use of Experimental Animals* (Canadian Council on Animal Care). Following 1 week of acclimation, 8 rats were randomly assigned as the normal control (NC) group, and the rest, treated with STZ, became the diabetic model (DM) group, according to the standard method (Ravi et al. 2004). Briefly, a 0.5% solution of STZ was prepared using 0.1 mol/L citric acid in sodium citric acid buffer (pH 4.4). The fasted DM rats were injected with STZ solution intraperitoneally at a dose of 60 mg/kg body mass. After the injection (72 h), the blood sample was collected from the tail vein after overnight fasting, and the level of blood glucose was determined according to the glucose oxidase method (Trinder 1969) using a glucose analyzer. The rats with blood glucose levels above 15.0 mmol/L were defined as DM rats. Twenty-four diabetic rats were chosen and randomly divided into 3 groups: DM control group (DM), DM+OA low dose group (DM+OA LD, 100 mg/kg body mass), and DM+OA high dose group (DM+OA HD, 200 mg/kg body mass).

### Determination of the immediate effect of OA on the oral glucose tolerance test

After overnight fasting with free access to water, the rats were administered, by oral gavage, OA dissolved in a solution of 0.5% carboxymethylcellulose (CMC) at the following doses: 100 mg/kg body mass for the DM+OA LD group, 200 mg/kg body mass for the DM+OA HD group, or with the same volume of 0.5% CMC solution alone for the NC and DM groups. Tail blood samples were drawn from

each rat, and a glucose (2 g glucose/kg body mass) solution was orally administered by oral gavage after 30 min following OA administration. Blood samples were taken at 30, 60, 120, and 240 min intervals following glucose administration, and blood plasma glucose levels were measured at various time points using the glucose oxidase method (Trinder 1969).

### Determination of the long-term effects of OA treatment on blood glucose level

The DM+OA treated group of rats were given OA in a solution of 0.5% CMC by oral gavage (100 and 200 mg/kg body mass for the DM+OA LD and DM+OA HD groups, respectively) for 40 days. The control rats (NC and DM groups) were given the same volume of 0.5% CMC without OA. On days 0, 10, 20, 30, and 40, blood samples were collected from tail veins, followed by overnight fasting and glucose measurement.

### Determination of the effect of OA treatment on blood lipid level

On day 38, the rats fasted overnight. Blood samples were collected from a tail vein and placed at room temperature for 2 h for serum preparation, and then centrifuged at 1000*g* for 15 min at 4 °C. The supernatant was immediately separated from the pellet. The serum was used to measure the levels of TG, total cholesterol (TC), low-density lipoprotein cholesterol (LDL-c) and high-density lipoprotein cholesterol (HDL-c) using an automated chemistry analyzer (Olympus, Japan), by following the manufacturer's instructions (Center of Medical Science and Technology of Capital Medical University, Beijing, China).

### Determination of the effect of OA treatment on serum insulin and thyroid hormone level

On day 40, blood was collected in a sterile tube and kept at room temperature for 2 h to prepare the serum, and then centrifuged at 1000*g* for 15 min at 4 °C. The supernatant was immediately separated from the pellet. The serum insulin level was then determined by using an insulin ELISA kit (Animal Diagnostic Laboratory, San Antonio, Tex.) according to the manufacturer's instructions. Triiodothyronine ($T_3$), thyroxine ($T_4$), and TSH serum levels were assayed using a radioimmunoassay kit, following the manufacturer's instructions (Institute of Shanghai Radioimmunoassay Technology, Shanghai, China).

### Determination of effect of OA treatment on rat body and organ mass

The body mass of the rats was recorded every 10 days with overnight fasting during the experimental period. On day 41, the rats were sacrificed under anesthesia with ether. The liver, heart, lung, thymus, spleen, kidney, and pancreas of each animal were dissected and rinsed with cold saline solution. The mass of various organs was recorded.

### Statistical analysis

Statistical analyses were performed using the SPSS statistical software package. Data are expressed as means ± SE. The effects of OA from *L. lucidum* on body mass, oral glucose tolerance test (OGTT), and blood glucose levels were determined using an analysis of variance (ANOVA) for repeated measurements. To analyze the specific differences, the levels of blood lipids, serum insulin, thyroid hormones, and organ masses were analyzed using a one-way ANOVA followed by Scheffe's method. Results were considered significantly different at the level of $p < 0.05$.

## Results

### Characterization of OA from *L. lucidum*

The OA extract was analyzed by TLC, and no significant impurities were detected. The purified OA sample was further confirmed by electrospray mass spectrometry (Figs. 1A and 1B). OA is a pentacyclic triterpene ($C_{30}H_{48}O_3$) and has a molecular mass of 456.71 g/mol (Maia et al. 2006). The mass spectrum of the purified OA has indicated the presence of a major fragment at *m*/*z* 455.6, corresponding to the deprotonated molecule $[M\text{-}H]^-$ of OA. The low level of contaminants, observed in this spectrum (for instance, at *m*/*z* 393 and at *m*/*z* 403) indicated that the sample purification procedure was efficient. To further confirm its structure, a tandem mass spectrometry experiment (MS/MS) was performed on the molecular ion fragment at *m*/*z* 455.6 (Fig. 1B). Fragmentation of this molecular ion led to the loss of a neutral $CO_2$ molecule detected at *m*/*z* 407.3. Other abundant fragment ions, observed at *m*/*z* 391.4, *m*/*z* 389.4, *m*/*z* 375.4, and *m*/*z* 373.3, corresponded to demethylation and (or) dehydration products of OA. The mass spectrometric analysis results were in agreement with the molecular characteristics of OA. These results indicate that OA is the main active ingredient in the *L. lucidum* extract prepared using the procedure described above.

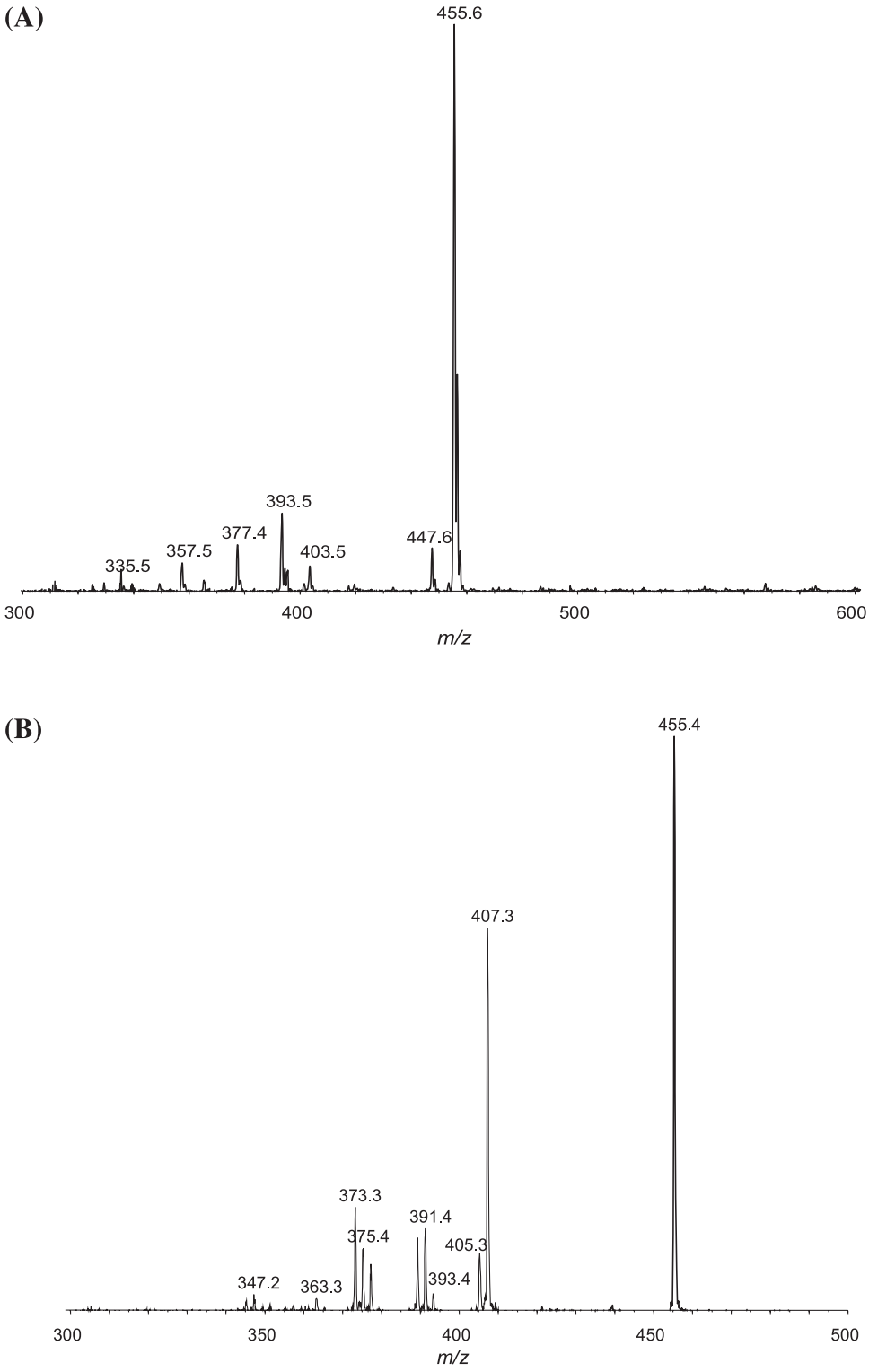
### Effect of OA treatment on oral glucose tolerance

OA supplementation improved glucose tolerance in the rats (Fig. 2). There was no significant difference at 0 min between the DM and OA-treated groups. When glucose was orally administered to the rats, the blood glucose level increased at the same rate in all 3 groups at 30 min, after which, it lowered significantly in the OA-treated groups compared with the DM control group ($p < 0.01$). There was no significant difference between the DM+OA LD and DM+OA HD groups ($p > 0.05$).

### Long-term effects of OA treatment on blood glucose level

Changes in the plasma glucose level as a result of OA treatment are shown in Table 1. Fasting blood glucose levels were measured on days 0, 10, 20, 30, and 40. They consistently remained at similar levels in both the normal control and the diabetic control groups within the time frameof the experiment, i.e., from day 0 to day 40. However, significantly lower blood glucose levels were observed in both of the DM+OA treated groups, compared with the DM control group, during the same time frame ($p < 0.01$). The rates of decrease in plasma glucose levels at day 40 were 58.18% and 60.38% in the DM+OA LD and DM+OA HD groups, respectively. The blood glucose levels in the OA-treated groups indicated that the effect of OA was not dose dependent ($p > 0.05$, DM+OA LD group vs. the DM+OA HD group).

**Fig. 1.** (A) Representative LC–MS chromatogram of oleanolic acid (OA); negative ion mode. (B) Representative LC–MS/MS chromatogram of OA; MS/MS of $m/z$ = 455.6 (negative ion mode).




### Effect of OA treatment on blood lipid levels

The long-term effect of OA treatment on blood lipid levels in the tested groups is presented in Table 2. The results show that the levels of TG, TC, and LDL-c in untreated diabetic control rats were significantly higher ($p < 0.05$ or 0.01), while their level of HDL-c was significantly lower ($p < 0.01$) than those of normal control rats. When diabetic rats were treated with OA for 40 days, the serum levels of TG, TC, and LDL-c significantly decreased ($p < 0.05$ for the DM+OA LD group; $p < 0.05$ or $p < 0.01$ for the

**Table 1.** Effect of oleanolic acid treatments on blood glucose levels in rats with streptozotocin-induced diabetes and control rats.

| | Blood glucose level, mmol/L | | | | |
|---|---|---|---|---|---|
| Group | Day 0 | Day 10 | Day 20 | Day 30 | Day 40 |
| NC | 5.613±0.15 | 5.56±0.11 | 5.59±0.21 | 5.68±0.20 | 5.61±0.16 |
| DM | 18.10±0.33 | 18.04±0.27 | 18.23±0.23 | 18.15±0.34 | 18.24±0.41 |
| DM+OA LD | 18.26±0.44 | 15.60±0.35* | 12.03±0.28* | 8.90±0.24* | 7.64±0.26* |
| DM+OA HD | 18.13±0.27 | 15.33±0.45* | 12.01±0.40* | 8.89±0.28* | 7.10±0.28* |

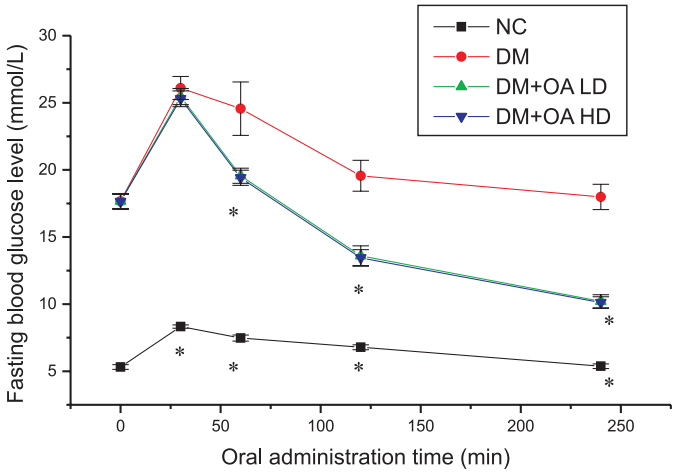
**Note:** Values are means ± SE of 8 rats per group. OA, oleanolic acid; NC, normal control group; DM, diabetic control group; DM+OA LD, diabetic rats treated with a low dose of OA (100 mg/kg body mass); DM+OA HD, diabetic rats treated with a high dose of OA (200 mg/kg body mass). *, $p < 0.01$ compared with the DM group.

**Table 2.** Effect of oleanolic acid treatment on blood lipid levels in rats with streptozotocin-induced diabetes and control rats.

| Group | Triglyceride mmol/L | Total cholesterol mmol/L | Low-density lipoprotein cholesterol, mmol/L | High-density lipoprotein cholesterol, mmol/L |
|---|---|---|---|---|
| NC | 0.93±0.03** | 1.84±0.04* | 0.999±0.04* | 0.93±0.03** |
| DM | 1.22±0.03 | 2.05±0.09 | 1.30±0.07 | 0.74±0.03 |
| DM+OA LD | 1.11±0.03* | 1.81±0.04* | 1.04±0.03* | 0.84±0.02* |
| DM+OA HD | 0.90±0.03** | 1.83±0.05* | 1.03±0.03* | 0.90±0.04** |

**Note:** Values are means ± SE of 8 rats per group. OA, oleanolic acid; NC, normal control group; DM, diabetic control group; DM+OA LD, diabetic rats treated with a low dose of OA (100 mg/kg body mass); DM+OA HD, diabetic rats treated with a high dose of OA (200 mg/kg body mass). *, $p < 0.05$ compared with the DM group; **, $p < 0.01$ compared with the DM group.

**Fig. 2.** Effects of oleanolic acid (OA) treatments on an oral glucose tolerance test administered to control rats and rats with streptozotocin-induced diabetes. Values are means ± SE of 8 rats per group; NC, normal control group; DM, diabetic control group; DM+OA LD, diabetic rats treated with a low dose of OA (100 mg/kg body mass); DM+OA HD, diabetic rats treated with a high dose of OA (200 mg/kg body mass). *, $p < 0.01$ compared with the DM group at the same time point.




DM+OA HD group), but their HDL-c level significantly increased ($p < 0.05$ for the DM+OA LD group, and $p < 0.05$ or 0.01 for the DM+OA HD group), compared with the DM control group. There was no significant difference in blood lipid levels between the NC and DM+OA HD groups ($p > 0.05$), implying that the dose of OA (200 mg/kg) was appropriate for treating diabetes.

### Effect of OA treatment on serum insulin level

Changes in insulin levels are shown in Fig. 3. Within 40 days of OA treatment, serum insulin levels in OA-treated groups was significantly higher than that in the DM group ($p < 0.05$), which implies that OA treatment improved insulin secretion in diabetic rats. In the OA-treated high-dose group, the insulin level was higher than that in the low-dose group, although not significantly so ($p > 0.05$). Additionally, there was no significant difference in insulin level between the NC and the OA-treated groups ($p > 0.05$).

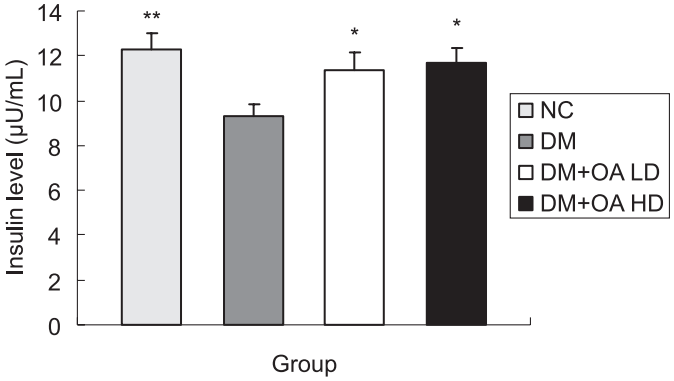
### Effect of OA treatment on serum thyroid hormone levels

The serum thyroid hormone levels in tested groups are shown in Table 3. Serum $T_3$ levels in NC rats were significantly lower, and serum TSH levels in the NC group was significantly higher than those in the DM rats ($p < 0.05$). Serum $T_4$ levels in NC rats were not significantly lower than those in the DM rats ($p > 0.05$). Serum $T_3$ and $T_4$ levels in the OA-treated groups were slightly lower than those in the NC group, but with no statistical significance ($p > 0.05$). There was no significant change in serum TSH levels in the OA-treated groups during the experiment ($p > 0.05$).

### Effect of OA treatment on rat body mass

Body mass changes in the control and experimental groups are shown in Fig. 4. There were no significant differences in the initial body mass (day 0) among the 4 groups ($p > 0.05$). The body mass of the rats in the NC and OA-treated groups increased steadily during the course of the experiment. There were no significant differences either between the NC and OA-treated groups or between the DM+OA LD and DM+OA HD groups. From day 10, the increase in body mass in the DM control group was lower

**Fig. 3.** Effect of oleanolic acid treatment on the insulin levels of control rats and rats with streptozotocin-induced diabetes. Values are means ± SE of 8 rats per group. OA, oleanolic acid; NC, normal control group; DM, diabetic control group; DM+OA LD, diabetic rats treated with a low dose of OA (100 mg/kg body mass); DM+OA HD, diabetic rats treated with a high dose of OA (200 mg/kg body mass). *, $p < 0.05$ compared with the DM group; **, $p < 0.01$ compared with the DM group.




than that in the OA-treated groups ($p < 0.01$). Thus, weight gain improved in the OA-treated groups compared with the DM rats.

### Effect of OA treatment on rat organ mass

The masses of various organs are presented in Table 4. There were no significant differences in the masses of liver, heart, lung, spleen, kidney, and pancreas among the groups ($p > 0.05$). The thymus mass was significantly higher in the OA-treated groups compared with that in the DM and NC groups ($p < 0.05$), while there was no marked difference in high- and low-dose OA-treated groups.

## Discussion

The *L. lucidum* fruit has been used as a traditional medicine for many years in China, owing to its anti-tumor, immunostimulatory, antioxidative, antimutagenic, antidiabetic, and hepatoprotective properties. OA, a pentacyclic triterpene compound, is one of the active ingredients of *L. lucidum*. We optimized multicrystal technology to purify the ethanol extract of OA from *L. lucidum* fruit capsules and confirmed its authenticity by MS and MS/MS analysis.

The results of this study demonstrate that OA significantly improves fasting blood glucose levels and OGTT in the diabetic rat model. The recovered plasma glucose levels approached normal levels following oral administration of OA for 40 days. For example, the blood glucose concentrations that were significantly elevated in diabetic rats during the OGTT were shown to be significantly lower in the OA-treated groups than in the DM control group following 60–240 min of OA administration. The results also suggest that OA could be used in diabetes treatment. Moreover, the changes in blood glucose levels imply that the concentration of OA used (100 mg/kg body mass) was an effective dose.

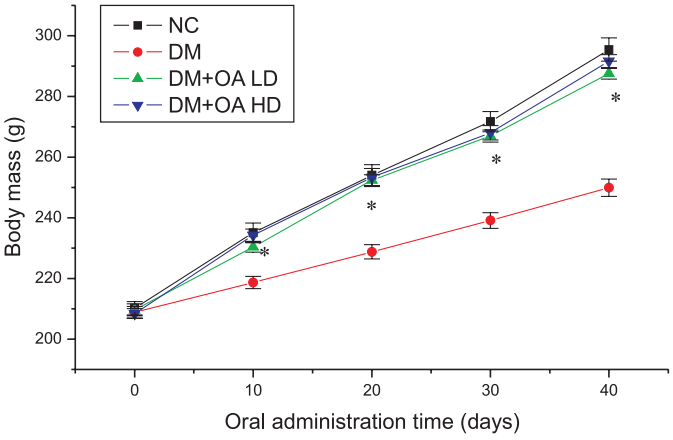
OA also improved the body mass gain of rats with STZ-induced diabetes. Decreased body mass in diabetic rats is due to excessive breakdown of tissue proteins (Ravi et al. 2004). The ability of OA to prevent mass loss seems to be due to its hypoglycaemic and hypolipidemic effects. The results show that OA significantly inhibited the progression of diabetes induced by STZ.

**Table 3.** Effect of oleanolic acid treatments on thyroid hormone levels in rats with streptozotocin-induced diabetes and control rats.

| Group | TSH, μL/mL | $T_3$, ng/mL | $T_4$, ng/mL |
|---|---|---|---|
| NC | 3.109±0.029* | 0.692±0.005* | 72.963±0.409 |
| DM | 2.986±0.035 | 0.710±0.002 | 74.433±0.375 |
| DM+OA LD | 3.073±0.022 | 0.694±0.005 | 73.156±0.414 |
| DM+OA HD | 3.086±0.015 | 0.695±0.004 | 72.866±0.399 |

**Note:** Values are means ± SE of 8 rats per group. OA, oleanolic acid; NC, normal control group; DM, diabetic control group; DM+OA LD, diabetic rats treated with a low dose of OA (100 mg/kg body mass); DM+OA HD, diabetic rats treated with a high dose of OA (200 mg/kg body mass). *, $p < 0.05$ compared with the DM group.

**Fig. 4.** Effect of oleanolic acid treatments on body mass in rats with streptozotocin-induced diabetes and control rats. Values are means ± SE of 8 rats per group. OA, oleanolic acid; NC, normal control group; DM, diabetic control group; DM+OA LD, diabetic rats treated with a low dose of OA (100 mg/kg body mass); DM+OA HD, diabetic rats treated with a high dose of OA (200 mg/kg body mass). *, $p < 0.01$ compared with the DM group.




It was previously shown that an increase in blood glucose level is accompanied by a rise in TC, LDL-c, and TG, and a fall in HDL-c levels (Sharma et al. 2003). An elevation in the levels of plasma lipids usually increases the risk for coronary heart disease (Assmann et al. 2006). It is well known that, in uncontrolled diabetes mellitus, there is an increase in TC, LDL-c, and TG, and a decrease in HDL-c levels, all of which contributes to coronary artery disease (Gotto 2001). Elevated HDL-c levels are associated with a lower risk atherosclerosis development in diabetes mellitus (Taskinen 2002). From this point of view, it is encouraging that the 40 day treatment with OA brought down the elevated levels of TC, LDL-c, and TG, and lowered the level of HDL-c in the diabetic rats. These results indicate that OA may be beneficial to diabetic individuals with atherosclerosis and hypolipidemia. It has been also reported that low HDL-c levels represent one of the most prevalent lipid abnormalities in the subjects affected by coronary heart disease (Genest et

**Table 4.** Effect of oleanolic acid treatments on organ mass in rats with streptozotocin-induced diabetes and control rats.

| Group | Liver, g | Lung, g | Kidney, g | Thymus, g | Pancreas, g |
|---|---|---|---|---|---|
| NC | 7.82±0.15 | 1.59±0.08 | 2.07±0.06 | 0.45±0.04 | 1.36±0.07 |
| DM | 7.92±0.13 | 1.46±0.05 | 2.09±0.07 | 0.43±0.03 | 1.29±0.08 |
| DM+OA LD | 8.06±0.15 | 1.54±0.08 | 2.12±0.08 | 0.53±0.04* | 1.33±0.08 |
| DM+OA HD | 8.09±0.17 | 1.56±0.08 | 2.11±0.07 | 0.54±0.04* | 1.34±0.08 |

**Note:** Values are means ± SE of 8 rats per group. OA, oleanolic acid; NC, normal control group; DM, diabetic control group; DM+OA LD, diabetic rats treated with a low dose of OA (100 mg/kg body mass); DM+OA HD, diabetic rats treated with a high dose of OA (200 mg/kg body mass). *, $p < 0.05$ compared with the DM group.

al. 1992; Rubins et al. 1995), and that high HDL-c levels seem to maintain a significant protective value against in older people (Corti et al. 1995; Whitney et al. 2005).

Treatment with OA did not show any effect on the mass of livers, hearts, pancreases, or kidneys. Whereas the thymus mass significantly increased in diabetic OA-treated rats. The thymus plays an important role in the development of the immune system in the early stages of life, and its cells form a part of the body's normal immune system (Pertsov 2006). It is tempting to speculate that OA might stimulate thymus growth, consequently affecting immune function. Further studies are needed to elucidate the mechanism of thymus weight increase.

Insulin, secreted by the β cells of the pancreas, is directly infused via the portal vein into the liver, where it exerts profound metabolic effects. The action of insulin is important to the ingestion of nutrients, particularly dietary carbohydrates, and stimulates the disposal of ingested glucose into peripheral tissue (Sezik et al. 2005). In this study, we used a STZ-induced diabetic rat model to investigate the hypoglycemic effect of *L. lucidum.* STZ, an *N*-nitroso derivative of glucosamine, is a broad spectrum antibiotic extracted from *Streptomyces acromogenes*. It is a pancreatic β-cell toxin that induces rapid necrosis of β cells and is widely used to induce diabetes in experimental animal models (Junod et al. 1967; Andrade-Cetto and Wiedenfeld 2001; Benwahhoud et al. 2001). In our experiments, insulin was lower in DM rats but insulin was still present, indicating that the level of STZ used did not destroy all β cells. The results showed that the level of insulin increased significantly in OA-treated rats. OA has the ability to amend the pancreatic impairment of rats with STZ-induced diabetes, thus restoring plasma insulin levels results in the stimulation of carbohydrate metabolic enzyme activity to re-establish normal blood glucose levels. There are 2 possible mechanisms by which this may occur. The first is that OA may stimulate the secretory ability of the islet cells of rats with STZ-induced diabetes, and the second is that OA may promote the replenishment of β cell mass. Further detailed studies are necessary to confirm this hypothesis.

It has been reported that an increase in thyroid hormones is accompanied by multiple metabolic abnormalities, which include stimulation of food intake, augmentation of the absorption of glucose by the intestinal system (Beylot et al. 1991), increased energy expenditure, excessive mobilization and utilization of metabolic substrates (Randin et al. 1986; Morrison et al. 1988; Beylot et al. 1991), and enhanced hepatic glucose output due to an increase in glucose metabolism, particularly gluconeogenesis and glycogenolysis (Raboudi et al. 1989; Dimitriadis and Raptis 2001). Additionally, the excess in $T_3$ and $T_4$ levels triggers the breakdown of TGs stored in adipose tissue by enhancing lipid oxidation, which results in the increased concentration of nonesterified fatty acids (Raboudi et al. 1989). In this study, the serum $T_3$ level was significantly lower in the NC group, compared with the DM group, and the level of TSH in the NC rats increased compared with the DM group. The high level of $T_3$ in diabetic rats might result in long-term hyperglycemic and hypolipidemic conditions in rats, which in turn, could result in a hormone imbalance. When the rats were treated with OA, the changes in $T_3$, $T_4$, and TSH levels were insignificant. These results suggest that OA did not modulate the levels of thyroid hormones, and that the modulation of thyroid hormone levels is not mediated by OA's hypoglycemic effects.

In conclusion, this study provides further evidence in support of the use of *L. lucidum* to regulate blood glucose and hypolipidemia. We have shown that OA represents the main active ingredient of *L. lucidum*, and according to our data, OA has potent hypoglycemic and hypolipidemic effects, the former of which could be linked to more than one mechanism. One mechanism might involve the modulation of insulin secretion or action. Thus, OA extract from *L. lucidum* could be used in the future to effectively treat diabetes.

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