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Full Length Research Paper

Nelumbo nucifera alkaloid inhibits 3T3-L1 preadipocyte differentiation and improves high-fat diet-induced obesity and body fat accumulation in rats

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The leaf of *Nelumbo nucifera* Gaertn has been used for summer heat syndrome as home remedy in China, and it has been used for the treatment of obesity in traditional medical clinics in China, so we investigated the pharmacological activity of the antiobesity effect of *N. nucifera* leaves extract. *N. nucifera* alkaloid (NNA), which is the main component in the *N. nucifera* leaves. 3T3-L1 preadipocytes and high fat diet (HFD)-induced obese SD rats were treated with NNA, and the effects of NNA on the apoptosis in 3T3-L1 preadipocytes and body weight, as well as plasma TC, TG and LDL-C levels in HFD-induced rats were investigated. The results demonstrated that NNA decreased cell population growth of 3T3-L1 preadipocytes and increased the apoptotic cells in a time- and dose-dependent manner. Furthermore, NNA reduced the body weight, the lee's index, adipose tissue weight, and plasma lipid levels in HFD-induced obese rats. Take together, we demonstrated that NNA can efficiently induces apoptosis in 3T3-L1 preadipocytes, and have further implication in *in vivo* antiobesity effect.

Key words: *Nelumbo nucifera* alkaloid, preadipocyte, antiobesity.

INTRODUCTION

In recent decades, obesity has become a prominent health problem in many countries (Cali and Caprio, 2008), because it is considered to be a risk factor associated with the genesis or development of various diseases, including cardiovascular disease, type 2 diabetes mellitus and metabolic syndrome, which resulting in an increasing morbidity and mortality (Brown et al., 2009). Hence, it is necessary to develop effective prevention and treatment strategies for obesity. Recent reports have proposed mechanisms to reduce obesity, including decreased energy/food intake and increased energy expenditure, decreased preadipocyte differentiation and proliferation, decreased lipogenesis, and increased lipolysis and fat oxidation (Wang and Jones, 2004). The preadipocytes play a key role by differentiating into mature adipocytes and increasing fat mass. Obesity is characterized by the accumulation of adipose tissue, which expands due to an increase in adipocyte size and number (Furuyashiki et al., 2004). Therefore, inhibition of adipogenesis from preadipocytes may regulate the amount of adipose tissue.

Nelumbo nucifera Gaertn, a traditional Chinese herb, is thought to be a useful medicinal plant for the treatment of obesity and other–metabolic diseases (La Cour et al., 1995; Mukherjee et al., 1997). All parts of *N. nucifera* Gaertn, including the leaves, flowers, stamens, embryos, rhizomes, and seed, have been used as traditional medicines and have pharmacologic properties, including hepatoprotection, anti-oxidant activity, antipyretic effects, and prevention of atherosclerosis and fatty liver (Sohn et al., 2003; Jung et al., 2003; Mukherjee et al., 1996; Ho et al., 2010; Lin et al., 2009). The leaf of *N. nucifera* is bitter, sweet and neutral. It is known for cleaning heat, resolving

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summer heat and stop bleeding (Bensky et al., 2004). Alkaloids are the main products in the leaf of *N. nucifera*, and have been used as an effective drug for hematemesis, epistaxis, and hemoptysis (Ou, 1989). However, the literature regarding the effect of alkaloids in the leaf of *N. nucifera* for treatment of obesity still remains unclear. In the present study, we investigated the antiobesity potential of *N. nucifera* alkaloid (NNA) using 3T3-L1 preadipocytes and high fat diet (HFD)-induced obese mice. The objective of this study was focused on the induction of apoptosis and the inhibition of preadipocyte proliferation by NNA in 3T3-L1 preadipocytes. Moreover, we also evaluated the antiobesity effects and lipid lowering activities of NNA in a rat model with high fat diet-induced obesity.

MATERIALS AND METHODS

Preparation of the *N. nucifera* Gaertn extracts

The leaves of *N. nucifera* Gaertn were collected from Qianjiang city (China) in September, 2009, and the voucher specimen has been deposited in our laboratory. The dried leaf of *N. nucifera* (3000 g) was mixed with 60 L hydrochloric acid solution(0.5%, V/V) and incubated for 12 h, then the pH value of hydrochloric acid extracts were adjusted to 9 and incubated at 4°C overnight. The hydrochloric acid extracts was centrifuged to remove insoluble matter. The supernatant was concentrated to dryness on a vacuum rotary evaporator to give *N. nucifera* Gaertn extract. The dried *N. nucifera* Gaertn extract was refluxed twice with 95% aqueous ethanol for 2 h, and extracts were obtained by removal of the solvent in vaccuo. The percentage yield of the ethanolic extracts was found to be 1.5% (w/w).

Isolation of *N. nucifera* alkaloid from *N. nucifera* Gaertn extracts

N. nucifera Gaertn extracts (45 g) were solved in methanol and subjected to column chromatography on silica gel and eluted with increasing gradient of acetone in petroleum ether. After the repeated column chromatography on silica gel, five *N. nucifera* Gaertn extracts were isolated. For proper standardization of *N. nucifera* alkaloids preparation, the presence of each index compounds of the *N. nucifera* Gaertn extracts were confirmed by thin layer chromatography (TLC), the proper *N. nucifera* alkaloid was selected compared with the law herbal material and the amount of *N. nucifera* alkaloid (NNA) was measured by high performance liquid chromatography (HPLC).

Cell culture and differentiation

3T3-L1 preadipocyte cells obtained from department of pharmacology of Tongji Medical College were cultured in DMED containing 10% bovine calf serum (Gibco BRL) and 100 U/ml penicillin-streptomycin at 37°C under a 5% CO_2 atmosphere.

Cell population growth by MTT assay

The MTT assay was performed according to the method of Mosmann (1983). The 3T3-L1preadipocytes were plated into 96-well microtiter plates at a density of 1 x 10^4 cells/well. After 24 h, the culture medium was replaced by 200 µl new medium containing NNA (0-55.25 nM), and the cells were incubated for 12, 24, 48 and 72 h, ten microliters of sterile filtered MTT solution (5 mg/ml) in phosphate buffered saline was added to each well. After 4 h, the unreacted dye was removed, and then the insoluble formazan crystals were dissolved in 200 µl/well dimethyl sulfoxide and measured spectrophotometrically in a microplate reader (BMG Labtechnologies Ltd., Germany) at 490 nm. The inhibition (%) was expressed as the percentage of cell growth compared to control (nontreated cells), and it was calculated by 1-A_{490nm}[NNA]/ A_{490nm}[control] x 100%. The IC_{50} was the concentration that caused 50% inhibition of cell proliferation.

Annexin V-FITC/PI double staining assay

Annexin V FITC/PI double staining of the cells was determined using the Annexin V FITC kit (Serotec Ltd., U.K.). This test employs the property of Annexin V FITC to bind to the membrane phospholipid phosphatidylserine in the presence of Ca^{2+}. To detect early apoptosis, late apoptosis, and necrosis induced by NNA. 3T3-L1 preadipocytes were added to 6-well microtiter plates and treated for 24 h at 37°C in 2 ml of culture medium containing NNA at final concentrations of 5.53, 11.05, 27.63 and 55.25 nM. 3T3-L1 preadipocytes (1 x 10^5 cells) were then stained for 15 min at room temperature with Annexin V FITC and PI in binding buffer and analyzed by flow cytometer (FACsirt BD Biosciences, USA). The percentage of distribution of normal, early apoptotic, late apoptotic, and necrotic cells was calculated using cell quest software (Bio-Rad, USA).

Animals

Male Sprague Drawly (SD) rats of weight 200 ± 20 g were procured from the center of experimental animals of Tongji medical college. The SD rats were housed under standard environmental conditions with a 12 h light/dark cycle at the animal house and had free access to water *ad libitum*. The protocol of this experiment was performed in accordance with the Guidelines for Animal Experimentation of Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China.

Induction of obesity

After 7 days of acclimation, the SD rats were randomly divided into 5 groups (n=10 per group): normal control group, HFD-induced control group and remaining three groups as treatment groups. SD rats in normal control group were fed with normal meals, while the other groups were fed with normal meals containing 20% lard oil, 2% cholesterol, 1% cholate and 10% sucrose *ad libitum*.

Treatment

Treatments were started from 30th day and continued for 30 days. The treatment groups were given 0.7, 1.4 and 2.7 mg/ml NNA (dissolved in 0.9% saline) respectively at 220 mg/kg/day of body weight by oral route. During the course of treatment, the treatment groups were continued to feed with high-fat diet.

Determination of body weight and Lee's index

Throughout the 60 days of study, the body weight and length of each rat was measured once in a week, and the Lee's index (Adejuwon et al., 2010) of each rat was calculated according to

following formula:

$$Lee's \quad Index = \sqrt[3]{\frac{W(g)}{L(mm)}} \times 10^4$$

Where W is the mean values of body weight in each group, and L is the mean values of length in each group.

Determination of lipid profile and measurement of adipose tissue weight

Lipid profiles of all the rats were determined on 30th day (pretreatment) and on 30th day (post-treatment). Blood samples were collected from retinal venous plexus of the overnight fasted rats into micro centrifuge tubes containing heparin. The plasma was separated by centrifugation (10 min, 10,000 rpm) and was analyzed for plasma total cholesterol (TC), triglycerides (TG), low density lipoprotein (LDL-C) and high density lipoprotein (HDL-C) using commercially available diagnostic kits (Sigma Co., USA). After the 30 days treatment, rats were killed by ether anesthesia and the weights of epididymal adipose tissues and retroperitoneal adipose tissue (Okazaki et al., 2010) were measured after quick removal from sacrificed rats.

Rope crawling experiment

Throughout the 60 days of study, rope crawling experiment was performed on all rats to assess their static endurance every 5th day. The experiment was conducted in the following procedure (Feng et al., 2001): rats were placed on the rough surface of a rope of 5 cm in diameter and 150 cm long, with a plate on the top of the rope to prevent them from crawling out; the rope was hung above the deep water with the end about 1 m above the water surface. Then the time of falling was recorded.

Statistical analysis

Statistical analysis was carried out by using SPSS 13.0 software. Values are expressed as mean±SD. For multiple comparisons, one way ANOVA was used followed by Dunnet's test. $P<0.05$ was considered to be significant difference.

RESULTS

Inhibition of population growth in 3T3-L1 preadipocytes

To assess whether NNA inhibited the population growth of 3T3-L1 preadipocytes, cells were treated with 0-55.25 nM NNA and the cell population growth was determined by using MTT assay. As shown in (Table 1 and Figure 1), NNA decreased the cell population growth in a time- and dose-dependent manner with an IC_{50} value of 11.25 nM. NNA caused significant cells growth inhibition as compared with the control.

NNA-induced apoptosis in 3T3-L1 preadipocytes

To quantify the degree of apoptosis, the amount of sub-G_1 DNA was analyzed by flow cytometry. As shown in (Figure 2), flow cytometric analysis of NNA-induced cell apoptosis of 3T3-L1 preadipocytes indicated that the increase of apoptotic cells in a dose-dependent manner. To quantify the modes of cell death (apoptosis or necrosis) induced by NNA, 3T3-L1 preadipocytes were treated with NNA for 48 h, stained with Annexin V-FITC and PI, and analyzed by flow cytometry. As shown in (Figure 3), flow cytometric analysis demonstrated that treatment of cells with NNA decreased the number of normal cells in a dose-dependent manner. The apoptotic cells including early apoptotic and late apoptotic cells were increased in a dose-dependent manner. When the treatment concentrations were increased, the percentage of normal cells decreased from 93.06% (control) to 44.28% (55.25 nM). The percentage of apoptotic cells (including early apoptotic and late apoptotic) increased from 6.69% (control) to 55.26%.

Antiobesity effect of NNA in HFD-induced rats

As shown in (Table 2), after 30 days on the high fat diet, the mean body weight and body weight gain of the HFD-induced rats were more than 125 and 127% higher than those of the normal diet group($P<0.05$), indicating that the high fat diet did induce obesity. NNA administration through oral route (at 0.7, 1.4 or 2.7 g/L) significantly decreased the body weight and body weight gains of HFD-induced rats relative to those of the non- NNA-treated control HFD group (69.0 ± 6.5%,48.7 ± 4.5% or 37.0 ± 7.5% of the control, respectively, $P<0.05$). The weight of adipose tissue was also significantly larger in the HFD group (by 119.8 ± 10.5%) than in the ND group ($P<0.05$). Supplementation of the HFD rats with NNA at 0.7, 1.4 or 2.7 g/L significantly decreased their adipose tissue weight by (90.2 ± 6.1%, 85.7 ± 9.6% or 88.1 ± 7.9%, respectively) relative to that of the non- NNA-treated HFD group ($P<0.05$). The Lee's index is a ratio of the body weight and body length. (Table 2) showed that there was a decrease in the Lee's index in the HFD group in contrast to decreased values for rats from the groups of rats administrated with different concentration of NNA ($P<0.05$).

Effect of NNA on lipid profiles

(Table 3) shows the plasma TG, TC, LDL-C and HDL-C levels of the experimental animals. Significant increase in the levels of plasma TG, TC, LDL-C and decrease in the levels of plasma HDL-C were observed in the HFD fed groups for the initial 30 days compared with normal diet fed group. After administration with high concentration of NNA (at 2.7 g/L), however, the plasma TG, TC and LDL-C levels were significantly decreased while HDL-C level was significantly increased compared with the HFD-induced control group. The treatment with NNA at

Table 1. The inhibition ratio in different time points of every group.

Group	Inhibition ratio (IR, %)			
	12 h	24 h	48 h	72 h
Control	-	-	-	-
0.553 nmol·L^{-1}	0	0	3.4±0.1	15.6±0.3*
2.763 nmol·L^{-1}	0	1.4±0.1	13.1±0.2*	27.1±0.3**
5.525 nmol·L^{-1}	0	12.9±0.4**	24.8±0.3**	40.3±0.2**
11.05 nmol·L^{-1}	1.2±0.1	23.7±0.6**	37.5±0.3**	46.7±0.2**
27.625 nmol·L^{-1}	1.2±0.3	54.3±0.5**	66.3±0.7**	72.6±0.3**
55.25 nmol·L^{-1}	50.2±0.2**	73.8±0.6**	80.6±0.4**	84.7±0.7**

The values were expressed as mean±SD(n=3). *P<0.05 vs control group, ** P<0.01 vs control group.

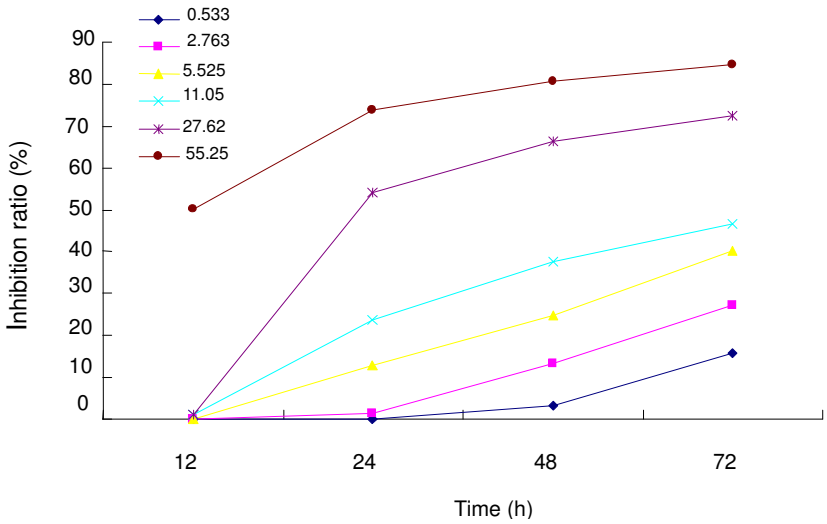

Figure 1. The inhibition ratio of 3T3-L1 preadipocytes in different concentration groups and time points

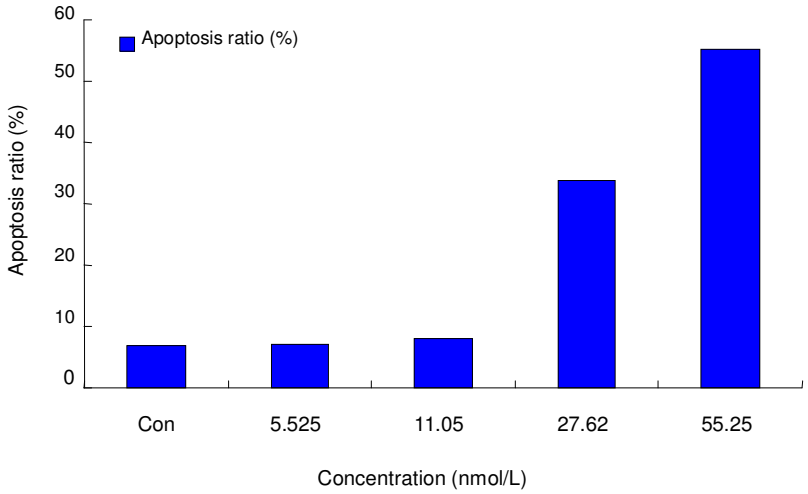

Figure 2. The apoptosis ratio of 3T3-L1 preadipocytes in different concentration groups.

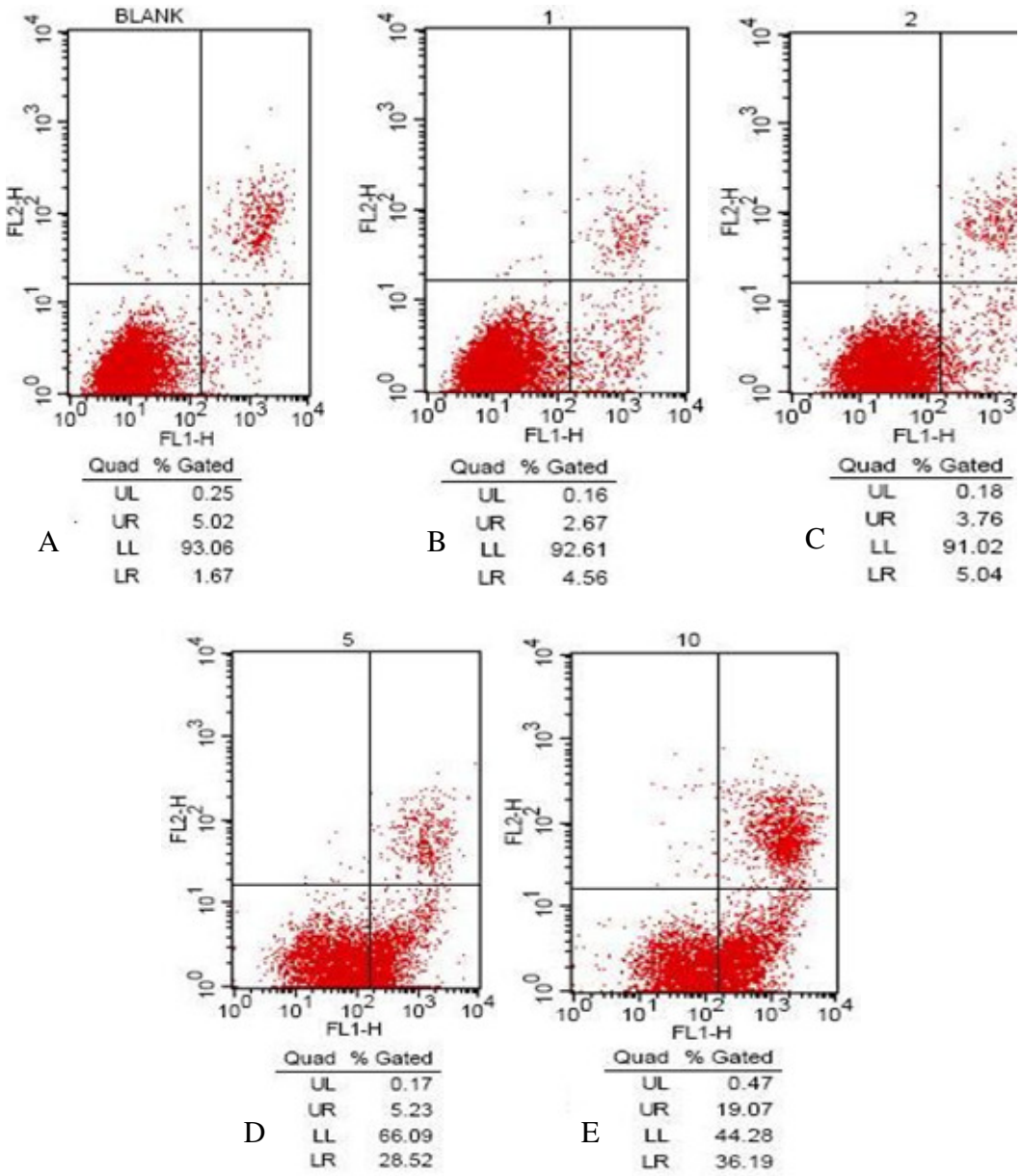

Figure 3. Apoptosis and necrosis induced by NNA in 3T3-L1 preadipocytes. The Figure shows the flow cytometric analysis of annexin V-FITC/PI double stained cells. The cells were untreated or treated with (A) 0 nmol/L, (B) 5.525 nmol/L, (C) 11.05 nmol/L, (D) 27.625 nmol/L, (E) 55.25 nmol/L.

concentration of 2.7 g/L lowered plasma TC levels to 1.74 ± 0.18 mM, plasma TG to 0.93 ± 0.04 mM and plasma LDL-C to 0.52 ± 0.06 mM as compared to TC levels of 2.36 ± 0.28 mM, TG levels of 1.01 ± 0.10 mM and LDL-C levels of 0.72 ± 0.26 mM in HFD control group ($P<0.05$). The plasma HDL-C levels of treatment group with NNA was increased to 0.48 ± 0.09 mM to HDL-C of 0.38 ± 0.09 mM as compared to HFD control group ($P<0.05$). It concludes that NNA have a definite action on lipid metabolism and their antiobesity actions may be correlated with the concentration levels.

Endurance capability study of rats

Results of (Table 4) showed that rope crawling capability of rats in treatment groups administered with different concentration of NNA were similar with that of the normal healthy rats ($P>0.05$), and the behavior of the animals was normal and no appreciable change was observed in the rope crawling experiment. These observations indicated that taking NNA has no implication on rats' endurance and the doses of NNA used for study were safe.

Table 2. Effects of NNA on body weight gain, adipose weight gain and Lee's index in HFD-induced experimental group for 30 days.

Group	NC	HFD	HFD+NNA$_{low}$	HFD+NNA$_{medium}$	HFD+NNA$_{high}$
Initial body weight(g)	221 ± 16	219 ± 14	226 ± 16	221 ± 19	223 ± 12
Final body weight(g)	298 ± 12	328 ± 19	324 ± 12	332 ± 15	328 ± 10
Body weight gain(%)	0.35 ± 0.05	0.4 ± 0.04**	0.44 ± 0.07**	0.51 ± 0.08**	0.47 ± 0.05**
Intake of NNA(g/kg*d)	-	-	1.5	3	6
Body weight gain(g)	27.1 ± 2.2	40.0 ± 1.6**	27.6 ± 2.6$^{\#\#}$	19.5 ± 1.8$^{\#\#}$	14.8 ± 3.0$^{\#\#}$
Adipose weight (g)	1.64 ± 0.31	1.9 ± 0.17**	1.77 ± 0.12$^{\#}$	1.68 ± 0.19$^{\#\#}$	1.73 ± 0.15$^{\#\#}$
Lee's index	303.1 ± 9.5	316. ± 12.9*	302.2 ± 14.8$^{\#}$	301.8 ± 9.2$^{\#}$	300.4 ± 12.7$^{\#}$

The values were expressed as mean±SD(n=10). *P<0.05 vs normal control, ** P<0.01 vs normal control group, $^{\#}$ P<0.05 vs HFD-induced control group, $^{\#\#}$ P<0.01vs HFD-induced control group.

Table 3. Effects of NNA on plasma lipid profiles in HFD-inducd experimental group for 30 days.

Group	Intake of NNA (g/kg)	TC (mmol/L)	TG (mmol/)	HDL-C (mmol/)	LDL-C (mmol/)
Normal control	-	1.54 ± 0.14	0.64 ± 0.12	0.63 ± 0.08	0.27 ± 0.04
HFD-induced control	-	2.37 ± 0.28**	1.01 ± 0.1*	0.38 ± 0.09**	0.72 ± 0.26**
HFD+NNA$_{low}$	1.5	2.51 ± 0.15	0.96 ± 0.06	0.36 ± 0.05	0.66 ± 0.04
HFD+NNA$_{medium}$	3	2.10 ± 0.15$^{\#}$	0.94 ± 0.05	0.36 ± 0.06	0.65 ± 0.04
HFD+NNA$_{high}$	6	1.73 ± 0.18$^{\#\#}$	0.93 ± 0.04$^{\#}$	0.48 ± 0.09$^{\#}$	0.53 ± 0.06$^{\#}$

The values were expressed as mean±SD(n=10). *P<0.05 vs the normal control, ** P<0.01 vs the normal control group, $^{\#}$ P<0.05 vs the HFD-induced control group, $^{\#\#}$ P<0.01vs the HFD-induced control group.

Table 4. Effects of NNA on the rope crawling capability in HFD-induced experimental group.

Group	Intake of NNA (g/kg)	Mean lasting time (s)
Normal control	-	50.4±7.2
HFD-induced control	-	43.0±7.1
HFD+NNA$_{low}$	1.5	48.4±6.2
HFD+NNA$_{medium}$	3	47.9±8.5
HFD+NNA$_{high}$	6	49.0±9.2

The values were expressed as mean±SD(n=10).

DISCUSSION

Obesity can be defined as a disease in which excess body fat has accumulated such that health may be adversely affected. Obesity is characterized at the cellular level by an increase in the number and size of adipocytes differentiated from fibroblasic preadipocytes in adipose tissues. Wang and Jones (2004) proposed that decreased preadipocyte proliferation and adipocyte lipogenesis are mechanisms of antiobesity. The regulation of adipogenesis involves a number of complex, interconnected cell signaling pathways; thus some recent studies have focused on the search for functional herbal extracts that can suppress the accumulation of body fat. *N. nucifera* Gaertn extract has a variety of biological functions, including anti-oxidant activity, antipyretic effects, and prevention of atherosclerosis and fatty liver. In recent years, it has been used as plain extraction or blend tea to treat obesity in China (Gao and Dang, 2000; Liu et al., 2005). NNA is a major component in *N. nucifera* Gaertn extract. In this study, we found that NNA inhibited 3T3-L1 preadipocyte differentiation and suppressed the accumulation of body fat in HFD-fed rats.

The 3T3-L1 preadipocyte line has been well characterized in its ability to undergo complete differentiation into mature adipocytes (Furuyashiki et al., 2004). Adipose tissue consists of adipocytes, which store triacylglycerol as a fuel for the body. The 3T3-L1 preadipocytes can differentiate into mature adipocytes. So, we focused on the effects of NNA on induction of

preadipocytic apoptosis. It is well-known that cell death can be categorized into apoptosis and necrosis. To quantify the modes of cell death (apoptosis or necrosis) induced by NNA, treated preadipocytes were double stained with Annexin V-FITC and PI and analyzed by flow cytometry. The exposure of the membrane phospholipids and phosphatidylserine to the external cellular environment is one of the earliest markers of apoptotic death. Annexin V-FITC binds to phosphatidylserine and can be used to detect the early stages of apoptosis (Vermes et al., 1995).

Our results showed that NNA could lead to a significant increase ($P<0.05$) in the accumulation of the sub-G_1 phase (apoptotic cells) in a time- and dose-dependent manner. Nyska et al. (2003) indicated that natural antioxidants could cause G_1 phase arrest in prostaic carcinoma cells. Other reports indicated that the observed decrease in the number of preadipocytes by NNA could be attributed to its inhibition of cell mitogenesis (Kao et al., 2000; Hung et al., 2005). Thus, our results suggest that NNA has the potential to inhibit cell populaton growth and induce apoptosis in 3T3-L1 preadipocytes.

On the basis of *in vitro* 3T3-L1 preadipocytes inhibitory effects of *N. nucifera* alkaloid, we planned animal studies to further prove the antiobesity effects of *N. nucifera* alkaloid in high fat diet-induced obesity model. Various animal models that imitate obesity in humans have been reported to search of effective antiobesity treatments. Rats fed a high fat diet causes obesity and shows distinctive visceral adiposity, dyslipidemia, hyperglycemia, hyperinsulinemia and hepatic steatosis, which are typically associated with human obesity (Kim et al., 2009). In the present study male SD rats, a widely known model for studying obesity and related metabolic disorders were used.

Our results suggested that NNA significantly suppressed the weight gain of the HFD-induced obesity in SD rats. Feeding a high-fat diet for 30 days produces significant increases in body weight, and some materials, oolong tea (Han et al., 1999), and condroitin sulfate (Han et al., 2000) have antiobesity effects on this model. Adipose tissue is a dynamic organ that plays an important role in energy balance and changes in mass according to the metabolic requirements of the organism. Consistent with our *in vitro* results, the administration of NNA at different concentrations over 30 days to rats fed a HFD decreased the body weight and adipose tissue weight of the rats without changing their endurance capability.

The present result clearly showed that feeding NNA is beneficial for the suppression of diet-induced obesity. Furthermore, as shown in our data, NNA decreases in the levels of serum lipids, such as TC, TG, LDL-C and increases HLD-C in SD rats compared to those for rats fed with HFD only, which could be attributed to the inhibition of lipid absorption in the gastrointestinal tract. Dietary lipids are absorbed into the bloodstream as chylomicron; TG in these chylomicrons are then digested as fatty acids and glycerol by lipoprotein lipase and are eventually transported and stored in the adipose tissues in the form of TG. The remnants of the chylomicrons are taken up mainly by the adipose tissues (such as liver) and are then transformed into lipoproteins, such as VLDL, which transport triglycerides to adipose tissues and LDL, which transports cholesterol to peripheral tissues (Guyton et al., 1996).

In conclusion, our study demonstrates the antiobesity and lipid lowering activities of the *N. nucifera* alkaloid which can be targeted to develop as a nutraceutical for the treatment of obesity and related metabolic disorders. Further studies will focus on identifying the mechanism related to the observed antiobesity effects of *N. nucifera* alkaloid in 3T3-L1 preadipocyte and high fat diet-induced SD rats.

REFERENCES

Adejuwon AA, Olufunmilayo OA, Esther OA (2010). Haematopoietic activity of the seed aqueous extract of Hunteria umbellata (K. schum) hallier f. in experimental anaemia. J. Ethnopharmacol., 130: 307-314.

Bensky D, Clavey S, Stoger E (Eds.), 2004. Materia medica, third ed. Eastland Press Inc., USA, pp. 193-194.

Brown WV, Fujioka K, Wilson PW, Woodworth KA (2009). Obesity: why be concerned? Am. J. Med., 122(4 suppl 1): S4-S11.

Cali AM, Caprio S (2008). Obesity in children and adolescents. J. Clin. Endocrinol. Metab., 93(11 suppl 1): S31-S36.

Feng R, He W, Ochi H (2001). A new murine oxidative stress model associated with senescence. Mech. Ageing. Dev., 122: 547-559.

Furuyashiki T, Nagayasu H, Aoki Y, Bessho H, Hashimoto T, Kanazawa K, Ashida H (2004). Tea catechin suppresses adipocyte differentiation accompanied by down-regulation of PPARgamma2 and C/EBPalpha in 3T3-L1 cells. Biosci. Biotechnol. Biochem., 68(11): 2353-2359.

Gao X, Dang Y (2000). Traditional Chinese Medical Beauty Care. China Science and Technology Press, Peking, pp. 305-306.

Guyton AC, Hall JE (1996). Textbook of medial physiology, 9th ed. Guyton, A.C., Ed; W. B. Saunders; Philadelphia, PA, pp. 869-899.

Han LK, Sumiyoshi M, Takeda T, Chihara H, Nishikiori T, Tsujita T, Kimura Y, Okuda H (2000). Inhibitory effects of chondroitin sulfate prepared from salmon nasal cartilage on fat storage in mice fed a high-fat diet. Int. J. Obes. Relat. Metab. Disord., 24(9): 1131-1138.

Han LK, Takaku T, Li J, Kimura Y, Okuda H (1999). Anti-obesity action of oolong tea. Int. J. Obes. Relat. Metab. Disord., 23(1): 98-105.

Ho HH, Hsu LS, Chan KC, Chen HM, Wu CH, Wang CJ (2010). Extract from the leaf of nucifera reduced the development of atherosclerosis via inhibition of vascular smooth muscle cell proliferation and migration. Food Chem. Toxicol., 48(1): 159-168.

Hung PF, Wu BT, Chen HC, Chen YH, Chen CL, Wu MH, Liu HC, Lee MJ, Kao YH (2005). Antimitogenic effect of green tea(-)-epigallocatechin gallate on 3T3-L1 preadipocytes depends on the ERK and Cdk2 pathways. Am. J. Physiol. Cell. Physiol., 288(5): C1094-C1108.

Jung HA, Kim JE, Chung HY, Choi JS (2003). Antioxidant principles of Nelumbo nucifera stamens. Arch. Pharmacal. Res., 26(4): 279-285.

Kao YH, Hiipakka RA, Liao S (2000). Modulation of obesity by a green tea catechin. Am. J. Clin. Nutr., 72(5): 1232-1234.

Kim M, Kim HK (2009). Perilla leaf extract ameliorates obesity and dyslipidemia induced by high-fat diet. Phytother. Res., 23(12): 1685-1690.

La Cour B, Mølgaard P, Yi Z (1995). Traditional Chinese medicine in treatment of hyperlipidaemia. J. Ethnopharmacol., 46(2): 125-129.

Lin MC, Kao SH, Chung PJ, Chan KC, Yang MY, Wang CJ (2009). Improvement for high fat diet-induced hepatic injuries and oxidative stress by flavonoid-enriched extract from Nelumbo nucifera leaf. J. Agric. Food. Chem., 57(13): 5925-5932.

Liu C, Tseng A (2005). Chinese Herbal Medicine. CRC Press, LLC, pp. 387-388.
Mosmann T (1983). Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J. Immunol. Methods, 65(1-2): 55-63.
Mukherjee PK, Das J, Saha K, Giri SN, Pal M, Saha BP (1996). Antipyretic activity of Nelumbo nucifera rhizome extract. Indian. J. Exp. Biol., 34(3): 275-276.
Mukherjee PK, Saha K, Pal M, Saha BP (1997). Effect of Nelumbo nucifera rhizome extract on blood sugar level in rats. J. Ethnopharmacol., 58(3): 207-213.
Nyska A, Suttie A, Bakshi S, Lomnitski L, Grossman S, Bergman M, Ben SV, Crocket P, Haseman JK, Moser G, Goldsworthy TL, Maronpot RR (2003). Slowing tumorigenic progression in TRAMP mice and prostatic carcinoma cell lines using natural anti-oxidant from spinach, NAO-A comparative study of three anti-oxidants. Toxicol. Pathol., 31(1): 39-51.
Okazaki Y, Kakehi S, Xu Y, Tsujimoto K, Sasaki M, Ogawa H, Kato N (2010). Consumption of sericin reduces serum lipids, ameliorates glucose tolerance and elevates serum adiponectin in rats fed a high-fat diet. Biosci. Biotechnol. Biochem., 74(8): 1534-1538.
Ou M (1989). Chinese-English manual of common-used in traditional Chinese medicine. Joint Publishing (H.K.) Co., Ltd., Hong Kong, pp. 518-519.
Sohn DH, Kim YC, Oh SH, Park EJ, Li X, Lee BH (2003). Hepatoprotective and free radical scavenging effects of Nelumbo nucifera. Phytomed., 10(2-3): 165-169.
Vermes I, Haanen C, Steffens NH, Reutelingsperger C (1995). A novel assay for apoptosis. Flow cytometric detection of phosphatidylserine expression on early apoptotic cells using fluorescein labelled Annexin V. J. Immunol. Methods, 184(1): 39-51.
Wang YW, Jones PJ (2004). Conjugated linoleic acid and obesity control: efficacy and mechanisms. Int. J. Obes. Relat. Metab. Disord., 28(8): 941-955.