Research Article

Effect of *Nelumbo nucifera* Petal Extracts on Lipase, Adipogenesis, Adipolysis, and Central Receptors of Obesity

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Received 22 July 2013; Revised 30 September 2013; Accepted 2 October 2013

1. Introduction

Several herbs have been indicated for weight management [1]. One such plant used for weight management is *Nelumbo nucifera* Gaertn. *N. nucifera*, known by a number of names including Indian lotus, sacred lotus, bean of India, or simply lotus, is one of two species of aquatic plant in the family Nelumbonaceae. Almost all parts of *N. nucifera* are edible, and in many Asian countries it was found in the recipe of food [2]. Extracts of *N. nucifera* flowers, seeds, rhizomes, and leaves have been reported to have varied therapeutic potential including antistress [3], antiobesity [4], antioxidant [5], hepatoprotective [6], antiadipogenic activity [7], anti-inflammatory [8, 9], antipyretic [10], antibacterial [11], and immunomodulatory [12, 13] activities.

Several bioactive phytocompounds derived from these plant parts were belonging to different chemical groups, including alkaloids, flavonoids, glycosides, triterpenoid, and vitamins [14]. Leaves, root, and the embryonic stage of *N. nucifera* have been reported to contain alkaloids such as roemerine, nuciferine, nornuciferine, nelumboside, anonaine, 5-methoxy-6-hydroxyaporphine, liensinine, and asimilobine [15]. Bisbenzyloquinoline alkaloids from *N. nucifera* were shown to be bioavailable after oral administration to rats at a dose of 20 mg/kg [16]. *N. nucifera* alkaloid was shown to inhibit 3T3-L1 preadipocyte differentiation and improve high-fat diet-induced obesity and body fat accumulation in rats [17].

Several flavonoids and nonflavonoids from flowers of *N. nucifera* reported by several authors were consolidated in a review by Mukherjee et al. [14]. Flavonoids include myricetin-3-O-b-D-glucopyranoside, quercetin-3-O-b-D-glucuronide, astragalin, quercetin, 3,4-dihydroxybenzoic, kaempferol, p-hydroxybenzoic acid, and b-sitosterol which were isolated from ethanol extract of the petals of *N. nucifera* [18]. Nonflavonoid compounds, including adenine, myo-inositol, arbutin, and sitosterol glucopyranoside, were identified in flower extract [14]. Wu et al. [19] demonstrated the antiobesity effect of a flavonoid-enriched extract from *N. nucifera* leaf (NLFE) in high-fat diet (HFD) fed C57BL/6 mice and...
concluded its action via lipid-regulated enzymes, thereby attenuating body lipid accumulation and preventing obesity. Antiobesity action of leaves and seeds of *N. nucifera* was extensively studied in *in vitro* and *in vivo* models by many researchers [4, 20–22]. The present study was designed to investigate the effect of *N. nucifera* petal extracts on lipase, adipogenesis, adipolysis, and central receptors *in vitro*.

2. Materials and Methods

2.1. Chemicals. Dexamethasone, isobutylmethylxanthine (IBMX), oil red O, porcine lipase enzyme, thiazolyl blue tetrazolium bromide (MTT), 4-methyl umbelliferyl oleate, GW 803430, melanocyte concentrating hormone (MCH), melanotan II, DL-isoproterenol hydrochloride, and orlistat were procured from Sigma-Aldrich. AM630, CP 55,940, melanocyte concentrating hormone (MCH), tetrazolium bromide (MTT), 4-methyl umbelliferyl oleate, 2.1. Chemicals.

2.2. Plant Material. The petals of *N. nucifera* (100 g each) were procured from a local commercial supplier and were authenticated at National Institute of Science Communication and Information Resources (NISCAIR), New Delhi. A voucher specimen (no. 811) was deposited in our herbarium. Dried petals were extracted with methanol (∼400 mL) by refluxing at 70°C for 1 h. Extract solution was filtered, and the remaining raw material was subjected to methanol extraction by repeating the above steps twice. The liquid filtrates were combined and concentrated by distillation under vacuum to a thick paste, followed by drying under vacuum at temperature 70°C. The dried extract was named as methanol extract and utilized to perform *in vitro* experiments. Phytochemical investigation of methanol extract of *N. nucifera* was carried out by subjecting methanol extract to HPLC analysis to identify the flavonoids as per the method described by Xingfen et al. [23].

Methanol extraction of the raw material was carried out as mentioned above. The leftover raw material after methanol extraction was further boiled with water at 85–90°C (3 times each with 500–600 mL water for 1 h) and filtered each time. The liquid filtrates were combined and concentrated by distillation under vacuum to a thick paste, followed by drying under vacuum at temperature 80°C. The dried extract was named as methanol extract and utilized to perform *in vitro* experiments.

2.3. Cell Lines and Culture Conditions. 3T3-L1 cell line was procured from American Type Culture Collection (ATCC). 3T3-L1 fibroblasts were cultured in DMEM supplemented with 10% BCS and incubated at 37°C; 5% CO2. The U2OS cell line coupled with 5-HT1c or MC4 receptor and CHO-K1 cell line coupled with CNR2 or MCHR receptor were obtained from DiscoveRx. U2OS and CHO-K1 cells were maintained in cell plate reagent in 96-well tissue culture plates for 48 h and 24 h, respectively.

2.4. Cell Viability Determination. Initial experiments using 3T3-L1, CHO-K1, and U2OS cells were conducted to assess the cytotoxic concentrations of both methanol and successive water extracts of *N. nucifera*. Cell viability was determined by a colorimetric MTT assay as described by Mosmann [24]. In brief, cells were cultured in 96-well plates at a seeding density of 5 x 10^3 cells/well. After 24 h of seeding, the cells were treated with and without *N. nucifera* extracts up to a concentration of 100 μg/mL. Thereafter, the cells were rinsed and further incubated with MTT for 1 h. After 1 h, MTT crystals were dissolved in 200 μL DMSO. Optical density was read at 570 nm, and, consequently, the noncytotoxic concentrations were chosen for conducting *in vitro* studies.

2.5. Adipogenesis Assay. Effect of *N. nucifera* petal extracts on adipogenesis was evaluated by examining their ability to inhibit the differentiation of preadipocytes to adipocytes using 3T3-L1 cells as a test system. On day 0, mouse 3T3-L1 fibroblasts were seeded at a density of 3 x 10^4 cells/well in a 48-well plate containing DMEM supplemented with 10% BCS. On day 1, cells were changed to DMEM medium supplemented with FBS (5%), IBMX (0.5 mM), insulin (10 μg/mL) and dexamethasone (1 μM) with and without *N. nucifera* extracts. Guggulsterone was used as a reference control. On day 3, cells were changed to DMEM supplemented with FBS (5%) and insulin (5 μg/mL) with and without *N. nucifera* petal extracts. On days 5 and 7, the cells were changed to DMEM supplemented with 5% FBS. On day 8, 3T3-L1 adipocytes were rinsed with PBS and fixed using 10% formalin for 30 min followed by another rinse with 60% isopropanol solution and allowed to dry. The cells were stained with oil red O solution (0.5% oil red O in isopropanol, diluted in proportion of 3 parts of oil red O stock and two parts of distilled water) for 15 min at room temperature. Dye retained in adipocytes was extracted with isopropanol and quantified by measuring the absorbance at 500 nm.

2.6. Adipolysis Assay. Adipolysis assay was performed to evaluate the possible lipolytic activity of *N. nucifera* petal extracts by examining their ability to release glycerol from differentiated 3T3-L1 cells. On day 0, 3T3-L1 cells were seeded at a density of 3 x 10^4 cells/well in a 48-well plate containing DMEM medium supplemented with 10% BCS. On day 1, cells were changed to DMEM supplemented with 5% FBS, IBMX (0.5 mM), insulin (10 μg/mL), and dexamethasone (1 μM). On day 2, cells were changed to DMEM, supplemented with FBS (5%) and insulin (5 μg/mL) and left undisturbed for 2 days. On day 4, the cells were allowed to grow in FBS (5%) for consecutive three days. On day 7, the cells were starved overnight with DMEM containing 2% BSA. On day 8, cells were treated with noncytotoxic concentrations of *N. nucifera* extracts and DL-isoproterenol hydrochloride (reference control) separately for 4 h in KRB (Krebs-Ringer Bicarbonate) buffer (pH 7.2). The supernatant was collected and estimated for glycerol content by adding 100 μL of
glycerol working reagent and 10 μL of sample/standard per well in a 96-well assay plate. Plate was tapped to mix and incubated at room temperature for 20 min. Read the color intensity at 570 nm in VersaMax plate reader.

2.7. Lipase Assay. The inhibitory effect of \textit{N. nucifera} on porcine pancreatic lipase was evaluated. The assay was based on the principle of conversion of the substrate 4-methyl umbelliferyl olate to 4-methyl umbelliferone by an active porcine lipase enzyme [25]. In brief, the total reaction volume of 50 μL contained 15 μL of Tris buffer/reference control (orlistat)/\textit{N. nucifera} extract, 5 μL of lipase enzyme, 5 μL of demineralized water, and 25 μL of substrate (4-methyl umbelliferyl olate). Mix these reagents and determine the change in fluorescence at 25°C for 20 min at an excitation and emission wavelength of 360 nm and 460 nm, respectively, using FLUOstar Optima.

2.8. Receptor Assays. Both methanol and successive water extracts of petals of \textit{N. nucifera} were screened for possible agonistic and antagonistic activity towards selected receptors at a concentration of 10 μg/mL.

2.8.1. Agonist Assays
5HT\textsubscript{2C} and MC\textsubscript{4}R Receptor Assays. The U2OS cell line coupled with either 5-HT\textsubscript{2C} or MC\textsubscript{4}R receptor was plated at a density of 10\textsuperscript{4} cells/well in 96-well tissue culture plates, containing cell plating reagent. After 48h incubation, \textit{N. nucifera} petal extracts or reference agonist, (AL34662 for 5-HT\textsubscript{2C}; melatonin II for MC\textsubscript{4}R) were added in separate wells on inhibition of forskolin 20 μM. After 30 min, 60 μL of prepared detection reagent solution was added to each well. After 60 min incubation at room temperature, the plate was read using luminescence plate reader (FLUOstar).

2.8.2. Antagonist Assays
Cannabinoid Receptor 2 (CNR\textsubscript{2}). Effect of both methanol and successive water extracts of \textit{N. nucifera} on inhibition of CP 55,940 (CNR\textsubscript{2} agonist) elicits CNR\textsubscript{2} activity in Gi/Go coupled CHO-K1 cell line was studied. 10\textsuperscript{4} cells/well were plated in 96-well tissue culture plates containing cell plating reagent. After 48 h incubation, \textit{N. nucifera} or reference antagonist (AM630) was added to the respective wells and incubated for 30 min at 37°C; 5% CO\textsubscript{2}. 55 μL of prepared detection reagent solution was added to each well and incubated for 60 min at room temperature, and plate was read using luminescence plate reader (FLUOstar).

Melanin Concentrating Hormone Receptor (MCHR\textsubscript{1}) Assay. Antagonistic potential of \textit{N. nucifera} extracts on MCHR\textsubscript{1} receptor was studied using MCHR\textsubscript{1} Gi coupled CHO-K1 cells. Cell density of 3 × 10\textsuperscript{4} cells/well was plated in 96-well tissue culture plates containing cell plating reagent and incubated for 24 h. After incubation, the entire medium was aspirated and 45 μL of cell assay buffer and antibody mixture was added to each well. \textit{N. nucifera} or reference antagonist (GW 803430) was added to the respective wells and incubated for 15 min at 37°C and 5% CO\textsubscript{2}. Agonist compound (MCH—62.5 nM + Forskolin 20 μM) was added to the respective wells and incubated for 30 min. 60 μL of prepared detection reagent solution and CAMP solution D was added to each well and incubated for 60 min at room temperature in the dark. 60 μL of CAMP Solution A was added and incubated for 3 hr at room temperature in the dark. Plate was read using luminescence plate reader (FLUOstar).

2.9. Data Analysis. For adipogenesis and adipolysis assays, statistical analysis was performed by one-way analysis of variance using the Graphpad Prism statistical software. Results are represented as Mean ± SD from three replicates per treatment group. Differences with \( P < 0.05 \) in comparison to control were considered to be statistically significant. For lipase assay, mean of the relative fluorescence unit (RFU) of \textit{N. nucifera}/reference control tested in triplicate was calculated. From the mean values, percentage inhibition (\%I) was calculated using the following equation:

\[
\%I = \frac{(RFU \text{ of Control} - RFU \text{ of Sample})}{RFU \text{ of Control}} \times 100. \tag{1}
\]

IC\textsubscript{50} was calculated by the Finney software. For receptor assays, Student's \( t \)-test was performed using GraphPad Prism 5 statistical software to test for differences among all treatments. Differences with \( P < 0.05 \) were considered to be significant.

3. Results
3.1. Effect of \textit{N. nucifera} on Differentiation of 3T3-L1 Cells. Nontoxic concentrations up to 50 μg/mL and 100 μg/mL were selected for methanol and successive water extract of \textit{N. nucifera}, respectively, for \textit{in vitro} cell-based assays. To test whether \textit{N. nucifera} extracts inhibit adipocyte differentiation, the differentiated adipocytes were stained by oil red O. The staining results showed that incubation of \textit{N. nucifera} during the differentiation period significantly inhibited 3T3-L1 adipogenesis. It was found that treatment of 3T3-L1 cells with \textit{N. nucifera} successive water extract significantly decreased the cell differentiation and lipid accumulation in a dose-dependent manner, compared with control cells. Methanol extract of \textit{N. nucifera} exhibited significant inhibition of adipocyte differentiation by 19% at a concentration of 2 μg/mL. The reference control, guggulsterone, demonstrated a potent inhibitory activity towards lipid accumulation at 20 μM with a percentage inhibition of 48 (Figure 1).

3.2. Lipolytic Effect of \textit{N. nucifera} on Differentiated 3T3-L1 Cells. \textit{N. nucifera} petal extracts displayed lipolytic activity as evident by significant increase of glycerol release from the differentiated 3T3-L1 cells. Methanol extract of \textit{N. nucifera} showed significant dose-dependent release of glycerol at
concentrations ranging from 2 μg/mL to 50 μg/mL. Maximum lipolytic activity of 3.5-fold increase was observed with methanol extract of N. nucifera at a concentration of 50 μg/mL. Successive water extract showed significant increase of glycerol levels at highest concentrations (20 and 100 μg/mL), where a fold increase of 1.9 was observed. Isoproterenol, a known lipolytic agent, elicited a marked glycerol release by fat cells at the tested concentration of 10 μM with a 4-fold increase over control (Figure 2).

3.3. Lipase Inhibitory Effect of N. nucifera. Pancreatic lipase inhibition of both methanol and successive water extracts of N. nucifera was determined at a screening concentration of 50 μg/mL. Percent lipase inhibitory effect of methanol and successive water extract at 50 μg/mL of N. nucifera was found to be 52 and 10, respectively. Further, lipase inhibitory potential of methanol extract was tested to determine the IC50 (the concentration required to inhibit a lipase activity by 50%). Methanol extract of N. nucifera exhibited a dose-dependent lipase inhibitory effect with an IC50 value of 47 μg/mL. However, it was not more effective than orlistat which showed an IC50 value of 26 ng/mL (Table 1).

3.4. Central Target Action of N. nucifera

3.4.1. 5-HT2C and MC4 Receptor Assays. Both methanol and successive water extracts of N. nucifera at 10 μg/mL showed significant equipotential stimulatory activity of about 4-fold increase towards 5-HT2C receptor (Table 2). The reference control AL34662 demonstrated a potent dose-dependent agonist activity towards 5-HT2C receptor with an EC50 value of ~10.8 nM. Both methanol and successive water extracts did not show significant agonist activity towards MC4 receptor at a concentration of 10 μg/mL (Table 2). Melanotan II, a known MC4 R agonist, demonstrated a concentration-dependent potent agonist activity towards MC4R receptor with an EC50 value of ~3.3 nM.

3.4.2. CNR2 and MCHR1 Receptor Assays. At a concentration of 10 μg/mL, methanol extract of N. nucifera petals displayed 26.2% antagonism against CP 55,940 activity towards CNR2 receptor, whereas the successive water extract was found to be inactive at a concentration of 10 μg/mL (Table 2). AM630, a known CNR2 antagonist, demonstrated a potent antagonist activity towards CP 55,940 activated CNR2 receptor with an IC50 value of ~62.3 nM. Both of these two extracts did not show antagonist activity towards MCHR1 (Table 2). GW 803430, a known MCHR, antagonist, demonstrated a potent antagonist activity towards MCH induced MCHR1 receptor with an IC50 value of ~13 nM.

3.5. Phytochemical Analysis of Methanol Extract of N. nucifera. HPLC analysis of crude methanol extract of N. nucifera confirmed the presence of flavonoids as major phytochemicals. HPLC followed by UV spectral analysis confirmed the identity of flavonoid glycosides (quercetin and kaempferol glycosides) which is in compliance with the earlier report by Xingfen et al. [23].

4. Discussion

Dietary fat is not directly absorbed by the intestine unless the fat has been subjected to the action of pancreatic lipase. Therefore, pancreatic lipase is one of the most widely studied mechanisms for determining natural products and potential efficacy as antiobesity agents [26]. In this study, we report the inhibitory effects of Nelumbo nucifera petal extracts on pancreatic lipase. Methanol extract elicited an inhibitory effect on lipase enzyme with an IC50 value of 47 μg/mL. Similar to our study but on lotus leaf, authors have showed antiobesity activity through a concentration-dependent inhibition of lipase enzyme and also upregulated the lipid metabolism [4]. Nelumbo nucifera is known as sacred lotus and found to have various pharmacologically active substances including alkaloids, flavonoids, triterpenoids, polyphenols, steroids, and glycosides [23]. A phytochemical investigation of N. nucifera leaves led to the isolation of eight alkaloids and some of these significantly inhibited pancreatic lipases [20]. Total flavonoids from N. nucifera leaves showed high inhibitory
Table 1: Effect of *N. nucifera* extracts on pancreatic lipase activity.

| Test sample                      | Concentration | % Lipase inhibition | IC<sub>50</sub>  |
|----------------------------------|---------------|---------------------|------------------|
| *Nelumbo nucifera* methanol extract |              |                     |                  |
| 12.5 µg/mL                       | 14.78         |                     |                  |
| 25 µg/mL                         | 29.28         |                     |                  |
| 50 µg/mL                         | 51.81         |                     | 47 µg/mL         |
| 100 µg/mL                        | 71.94         |                     |                  |
| 200 µg/mL                        | 89.87         |                     |                  |
| *Nelumbo nucifera* successive water extract | 50 µg/mL | 10                   | —                |
| 5 ng/mL                          | 28.31         |                     |                  |
| 10 ng/mL                         | 36.89         |                     |                  |
| Orlistat (reference standard)    | 25 ng/mL      | 44.88               | 26 ng/mL         |
| 50 ng/mL                         | 66.15         |                     |                  |
| 100 ng/mL                        | 68.22         |                     |                  |
| 200 ng/mL                        | 71.91         |                     |                  |

Table 2: Effect of petal extracts of *N. nucifera* on central receptors.

| Name of the test item | Solvent       | Concentration | 5HT<sub>2C</sub> (Mean ± SD) | MC<sub>4</sub>R (Mean ± SD) | MCHR<sub>1</sub> (Mean ± SD) | CNR<sub>2</sub> |
|-----------------------|---------------|---------------|-----------------------------|----------------------------|-----------------------------|---------------|
| Negative              | —             | NA            | 35947.0 ± 410.12            | 2322.3 ± 111.11            | NA                          | NA            |
| DMSO                  | —             | 0.1%          | 36188.5 ± 3324.11           | 2405.3 ± 246.41            | NA                          | NA            |
| *N. nucifera* methanol extract | DMSO | 10 µg/mL | 146121.5 ± 3987.37<sup>*</sup> | 2276.5 ± 289.21<sup>(NS)</sup> | 321.5 ± 38.89<sup>(NS)</sup> | 106480.0 ± 2791.66<sup>*</sup> |
| *N. nucifera* successive water extract | DMEM | 10 µg/mL | 150760.5 ± 15262.89<sup>*</sup> | 2071.5 ± 282.14<sup>(NS)</sup> | 320.0 ± 46.67<sup>(NS)</sup> | 122709.0 ± 6588.82<sup>*</sup> |
| Agonist AL-34662 (reference standard) | DMSO | 50 nm<sup>*</sup> | 136099.9 ± 2258.78<sup>*</sup> | NA | NA | NA |
| Agonist melanotan II acetate salt (reference standard) | DMSO | 950 nm<sup>*</sup> | NA | 11798.5 ± 27.58<sup>*</sup> | NA | NA |
| Agonist TP 55,940      | DMSO         | 4.5 nm<sup>i</sup> | NA | NA | NA | 144375.5 ± 7012.38 |
| Agonist MCH            | DMSO         | 62.5 nm<sup>i</sup> | NA | NA | 545.0 ± 1.41 | NA |
| Antagonist AM630 (reference standard) | DMSO | 173.2 nm<sup>*</sup> | NA | NA | NA | 4025.4 ± 994.31<sup>*</sup> |
| Antagonist GW-803430 (reference standard) | DMSO | 18.1 nm<sup>*</sup> | NA | NA | 1294.5 ± 142.13<sup>*</sup> | NA |

RLU: relative luminescence units; NA: not applicable; <sup>*</sup> indicates a concentration at which maximum response of reference controls was observed; <sup>i</sup> indicates EC<sub>80</sub> concentration of agonist against which *N. nucifera* petal extracts/reference controls were evaluated for their antagonistic potential. *P < 0.05.*

Adipocytes primarily store triglycerides and release them in the form of free fatty acid with the change of energy demand in the body. *N. nucifera* petal extracts demonstrated significant dose-dependent inhibitory effects on lipid accumulation in 3T3-L1 adipocytes. Alkaloids isolated from *N. nucifera* showed stronger inhibitory effect on adipocyte differentiation [20] and could probably contribute to the activity of petals of *N. nucifera*. Similar to this study but on lotus seed, epicarp extracts of *N. nucifera* were studied using an *in vitro* 3T3-L1 preadipocyte cell model. Results showed that the lotus seed epicarp extracts inhibited preadipocyte differentiation to adipocyte in a concentration-dependent manner [21]. Antiobesity effect of *N. nucifera* leaves extract (NNE) using high-fat diet-induced obesity in mice was studied. NNE significantly decreased the high-fat diet-induced weight gain, parametrial adipose tissue weight, and liver triacylglycerol levels in mice. Authors concluded that NNE impaired digestion, inhibited absorption of lipids and carbohydrates, accelerated lipid metabolism, and upregulated energy expenditure [4].
In this study, we have demonstrated that the petal extracts of *N. nucifera* clearly exhibit lipolytic activity in a dose-dependent manner in murine 3T3-L1 fibroblasts. Previous studies have reported that a liquid leaf extract of *Nelumbo nucifera* stimulated lipolysis activity in differentiated adipocytes. Also, authors have showed that treatment of adipocyte cultures with 0.5% lotus leaf extract solution significantly increased the content of free glycerol. Likewise, cultivation of cells with 1% lotus leaf extract solution induced a significant release of free glycerol compared to control cells [28]. In another study, 50% ethanol (EtOH) extract prepared from the leaves of *N. nucifera* stimulated lipolysis in the white adipose tissue (WAT) of mice and possible involvement of beta-adrenergic receptor (beta-AR) pathway was attributed to this effect. *N. nucifera* in preventing diet-induced obesity emerged to be due to various flavonoids and that the activation of beta-AR pathway was involved, at least in part [29].

Based on the 5-HT$_{2C}$ receptor study, *N. nucifera* petal extracts showed significant agonist activity towards 5-HT$_{2C}$ receptor and antagonistic activity towards CNR$_2$, indicating its role in central targets of obesity as appetite suppressant. Alkyl 4-hydroxybenzoates were isolated from seeds of *N. nucifera* and shown to enhance and inhibit 5-HT-stimulated inward current (I(5-HT)) mediated by the human 5-HT(3)A receptors expressed in Xenopus oocytes [30]. Similar to this study, Oh et al. [31] demonstrated that chronic treatment with ethanol extract from *Morus alba* leaves exerts an antiobesity effect in diet-induced obese mice via its direct MCH receptor antagonist activity. Various phytochemicals, namely, alkaloids, anthocyanin, and nonanthocyanin flavonoids, have been isolated from petals of *N. nucifera* [32,33] and shown to have other biological activities; however, their role in antiobesity activity needs to be determined.

Flavonoid-enriched *N. nucifera* leaf extract significantly inhibited the high-fat diet-induced abnormal blood lipids and liver damage [27]. Galleano et al. [34] consolidated the proof linking flavonoid intake with metabolic disorders, namely, obesity, hypertriglyceridemia, hypercholesterolemia, hypertension, and insulin resistance. However, a number of molecular mechanisms have been identified; the effects of flavonoids on endpoints of metabolic syndrome are still inconclusive. These convolutions were explained by the complex associations among the risk factors of metabolic syndrome, the multiple biological targets controlling these risk factors, and the high number of flavonoids (including their metabolites) present in the diet and potentially responsible for the *in vivo* effects. As a result, extensive basic and clinical research is warranted to assess the relevance of flavonoids for the treatment of metabolic syndrome [34].

Acute and subchronic oral toxicity studies of *N. nucifera* stamens extract in rats were performed and found to have safety threshold for acute toxicity which is above 5000 mg/kg bodyweight, and no-observed-adverse-effect level (NOAEL) of the extract for both male and female rats is considered to be 200 mg/kg/day [35].

In conclusion, *N. nucifera* petal extract showed antilipase activity, lipolytic and antiadipogenesis effect in adipocytes *in vitro*. *N. nucifera* extract showed agonist and antagonistic effect towards central receptors involved in food intake. Thus, it is worthwhile to further investigate *N. nucifera* petal extract and phytoconstituents for its potential pharmacological effect in metabolic disorders, in particular obesity.

**Conflict of Interests**

All authors declare that they have no conflict of interests.

**Acknowledgment**

This research received support from DBT, India, under Indo-Spanish joint call for collaboration in the field of biotechnology.

**References**

[1] C. V. Chandrasekaran, M. A. Vijayalakshmi, K. Prakash, V. S. Bansal, J. Meenakshi, and A. Amin, “Review: herbal approach for obesity management,” *American Journal of Plant Sciences*, vol. 3, pp. 1003–1014, 2012.

[2] W. La-Ongsri, C. Trisomthi, and H. Balslev, “Management and use of *Nelumbo nucifera* Gaertn. in Thai wetlands,” *Wetlands Ecology and Management*, vol. 17, no. 4, pp. 279–289, 2009.

[3] M. Kulkarni and A. Juvekar, “Attenuation of acute and chronic restraint stress-induced perturbations in experimental animals by *Nelumbo nucifera* Gaertn.,” *Indian Journal of Pharmaceutical Sciences*, vol. 70, no. 3, pp. 327–332, 2008.

[4] Y. Ono, E. Hattori, Y. Fukaya, S. Imai, and Y. Ozihumi, “Anti-obesity effect of *Nelumbo nucifera* leaves extract in mice and rats,” *Journal of Ethnopharmacology*, vol. 106, no. 2, pp. 238–244, 2006.

[5] S. Rai, A. Wahile, K. Mukherjee, B. P. Saha, and P. K. Mukherjee, “Antioxidant activity of *Nelumbo nucifera* (sacred lotus) seeds,” *Journal of Ethnopharmacology*, vol. 104, no. 3, pp. 322–327, 2006.

[6] D.-H. Sohn, Y.-C. Kim, S.-H. Oh, E.-J. Park, X. Li, and B. Lee, “Hepatoprotective and free radical scavenging effects of *Nelumbo nucifera*,” *Phytotherapy Research*, vol. 10, no. 2-3, pp. 165–169, 2003.

[7] P. K. Mukherjee, K. Saha, J. Das, M. Pal, and B. P. Saha, “Effect of *Nelumbo nucifera* rhizome extract on blood sugar level in rats,” *Journal of Ethnopharmacology*, vol. 58, no. 3, pp. 207–213, 1997.

[8] P. K. Mukherjee, K. Saha, J. Das, M. Pal, and B. P. Saha, “Studies on the anti-inflammatory activity of rhizomes of *Nelumbo nucifera*,” *Planta Medica*, vol. 63, no. 4, pp. 367–369, 1997.

[9] P. V. Chakravarthi and N. Gopakumar, “Anti-inflammatory activity of red and white lotus seeds (*Nelumbo nucifera*) in albino rats,” *Veterinary World*, vol. 3, no. 4, pp. 157–159, 2010.

[10] P. K. Mukherjee, J. Das, K. Saha, S. N. Giri, M. Pal, and B. P. Saha, “Antipyretic activity of *Nelumbo nucifera* thizome extract,” *Indian Journal of Experimental Biology*, vol. 34, no. 3, pp. 275–276, 1996.

[11] M. Li and Z. Xu, “Quercetin in a lotus leaves extract may be responsible for antibacterial activity,” *Archives of Pharmacal Research*, vol. 31, no. 5, pp. 640–644, 2008.

[12] D. Mukherjee, A. Biswas, S. Bhadra et al., “Exploring the potential of *Nelumbo nucifera* thizome on membrane stabilization, mast cell protection, nitric oxide synthesis, and expression of costimulatory molecules,” *Immunopharmacology and Immunotoxicology*, vol. 32, no. 3, pp. 466–472, 2010.
[13] D. Mukherjee, T. N. Khatua, P. Venkatesh, B. P. Saha, and P. K. Mukherjee, “Immumomodulatory potential of rhizome and seed extracts of *Nelumbo nucifera* Gaertn,” *Journal of Ethnopharmacology*, vol. 128, no. 2, pp. 490–494, 2010.

[14] P. K. Mukherjee, D. Mukherjee, A. K. Maji, S. Rai, and M. Heinrich, “The sacred lotus (*Nelumbo nucifera*): phytochemical and therapeutic profile,” *Journal of Pharmacy and Pharmacology*, vol. 61, no. 4, pp. 407–422, 2009.

[15] R. P. Rastogi and B. N. Mehrotra, *Compendium of Indian Medicinal Plants*, vol. 1 of (1960–1969), pp. 288-289, Central Drug Research Institute, Lucknow and Publications & Information Directorate, New Delhi, India, 1991.

[16] Y. Huang, L. Zhao, Y. Bai, P. Liu, J. Wang, and J. Xiang, “Simultaneous determination of liensinine, isoliensinine and neferine from seed embryo of *Nelumbo nucifera* Gaertn. in rat plasma by a rapid HPLC method and its application to a pharmacokinetic study,” *Arzneimittelforschung*, vol. 61, no. 6, pp. 347–352, 2011.

[17] B. Xie, J. Wan, W. Wang, C. Shi, X. Hou, and J. Fang, “*Nelumbo nucifera* alkaloid inhibits 3T3-L1 preadipocyte differentiation and improves high-fat diet-induced obesity and body fat accumulation in rats,” *Journal of Medicinal Plant Research*, vol. 5, no. 10, pp. 2021–2028, 2011.

[18] X. Shuangshuang, D. Wenjuan, F. Lei, S. Yu, D. Hongjing, and W. Xiao, “Isolation and characterization of chemical constituents from the petals of *Nelumbo nucifera*,” *Asian Journal of Chemistry*, vol. 24, pp. 4619–4622, 2012.

[19] C. Wu, M. Yang, K. Chan, P. Chung, T. Ou, and C. Wang, “Improvement in high-fat diet-induced obesity and body fat accumulation by a *Nelumbo nucifera* leaf flavonoid-rich extract in mice,” *Journal of Agricultural and Food Chemistry*, vol. 58, no. 11, pp. 7075–7081, 2010.

[20] J. H. Ahn, E. S. Kim, C. Lee et al., “Chemical constituents from *Nelumbo nucifera* leaves and their anti-obesity effects,” *Bioorganic and Medicinal Chemistry Letters*, vol. 23, no. 12, pp. 3604–3608, 2013.

[21] S. Qi and D. Zhou, “Lotus seed epicarp extract as potential antioxidant and anti-obesity additive in Chinese Cantonese Sausage,” *Meat Science*, vol. 93, no. 2, pp. 257–262, 2013.

[22] J. S. You, Y. J. Lee, K. S. Kim, S. H. Kim, and K. I. Chang, “Antiobesity and hypolipidemic effects of *Nelumbo nucifera* seed ethanol extract in human preadipocytes and rats fed a high-fat diet,” *Journal of the Science of Food and Agriculture*, vol. 83, no. 4, pp. 407–412, 2003.

[23] G. Xingfeng, W. Daijie, D. Wenjuan, D. Jinhua, and W. Xiao, “Preparative isolation and purification of four flavonoids from the petals of *Nelumbo nucifera* by high-speed counter-current chromatography,” *Phytochemical Analysis*, vol. 21, no. 3, pp. 268–272, 2010.

[24] T. Mosmann, “Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays,” *Journal of Immunological Methods*, vol. 65, no. 1-2, pp. 55–63, 1983.

[25] US patent publication number: US, 2008/0317821 A1, dated December 2008.

[26] R. B. Birari and K. K. Bhutani, “Pancreatic lipase inhibitors from natural sources: unexplored potential,” *Drug Discovery Today*, vol. 12, no. 19-20, pp. 879–889, 2007.

[27] S. Liu, D. Li, B. Huang, Y. Chen, X. Lu, and Y. Wang, “Inhibition of pancreatic lipase, α-glucosidase, α-amylase, and hypolipidemic effects of the total flavonoids from *Nelumbo nucifera* leaves,” *Bioorganic & Medicinal Chemistry Letters*, vol. 19, no. 21, pp. 6309–6312, 2009.

[28] R. Siegner, S. Heuser, U. Holtzmann et al., “Lotus leaf extract and L-carnitine influence different processes during the adipocyte life cycle,” *Nutrition and Metabolism*, vol. 7, article 66, 2010.

[29] E. Ohkoshi, H. Miyazaki, K. Shindo, H. Watanabe, A. Yoshida, and H. Yajima, “Constituents from the leaves of *Nelumbo nucifera* stimulate lipolysis in the white adipose tissue of mice,” *Planta Medica*, vol. 73, no. 12, pp. 1255–1259, 2007.

[30] U. J. Youn, J. Lee, Y. J. Lee, J. W. Nam, H. Bae, and E. Seo, “Regulation of the 5-HT3A receptor-mediated current by alkyl 4-hydroxybenzoates isolated from the seeds of *Nelumbo nucifera*,” *Chemistry and Biodiversity*, vol. 7, no. 9, pp. 2296–2302, 2010.

[31] K. Oh, S. Y. Ryu, S. Lee et al., “Melanin-concentrating hormone-1 receptor antagonism and anti-obesity effects of ethanolic extract from *Morus alba* leaves in diet-induced obese mice,” *Journal of Ethnopharmacology*, vol. 122, no. 2, pp. 216–220, 2009.

[32] S. Chen, Y. Xiang, J. Deng, Y. Liu, and S. Li, “Simultaneous analysis of anthocyanin and non-anthocyanin flavonoid in various tissues of different lotus (*Nelumbo*) cultivars by HPLC-DAD-ESI-MS(n),” *Public Library of Science One*, vol. 8, no. 4, Article ID e62291, 2013.

[33] S. Nakamura, S. Nakashima, G. Tanabe et al., “Alkaloid constituents from flower buds and leaves of sacred lotus (*Nelumbo nucifera*, Nymphaeaceae) with melanogenesis inhibitory activity in B16 melanoma cells,” *Bioorganic Medicinal Chemistry*, vol. 21, no. 3, pp. 779–787, 2013.

[34] M. Galleano, V. Calabro, P. D. Prince et al., “Flavonoids and metabolic syndrome,” *Annals of the New York Academy of Sciences*, vol. 1259, pp. 87–94, 2012.

[35] P. Kunanusorn, A. Panthong, P. Pittayanurak, S. Wanauppathamkul, N. Nathasaen, and V. Reutrakul, “Acute and subchronic oral toxicity studies of *Nelumbo nucifera* stamens extract in rats,” *Journal of Ethnopharmacology*, vol. 134, no. 3, pp. 789–795, 2011.