Hypoglycemic and hypolipidemic effects of *Nelumbo nucifera* flower in Long-Evans rats

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**Implication for health policy/practice/research/medical education:**
Flowers of *N. nucifera* may be utilized for both diabetic and hyperlipidemic patients. Thus, further studies on it may reveal new active compounds having anti-diabetic and lipid lowering properties.


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**Article Type:**
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**Abstract**

**Introduction:** The study was conducted to assess the hypolipidemic and hypoglycemic effects of *Nelumbo nucifera* flowers powder in Long-Evans rats.

**Methods:** Experimental rats were made hyperlipidemic and diabetic (type 2) by feeding high fat diet (Lab diet, Dalda and Coconut oil = 4: 3: 1) and injecting alloxan respectively. *N. nucifera* flowers powder in different percentages was mixed with the regular Lab Diet for 21 days feeding. Serum total cholesterol, triglyceride, low-density lipoprotein (LDL), high-density lipoprotein (HDL) and blood glucose levels were evaluated in various groups.

**Results:** Feeding with *N. nucifera* flowers powder at different percentages to hyperlipidemic groups showed diverse but a significant (*P* < 0.05) decrease in serum total cholesterol, triglyceride and LDL-cholesterol levels when compared to control group while HDL-cholesterol level was increased significantly (*P* < 0.05). Routine feeding with *N. nucifera* flowers powder for 21 days resulted in significant decrease in the blood glucose levels of alloxan-induced diabetic rats. Both 20% and 10% of *N. nucifera* flower powders with Lab Diet significantly (*P* < 0.05) decreased blood glucose level up to 48% and 34%, respectively in comparison to the drug control group treated with glibenclamide which was found with the decreasing capability up to 66% where the higher percentage of *N. nucifera* flower powder was found to exert more prominent effect in lowering blood glucose level. During comparison to the control group, the above mentioned percentages of *N. nucifera* flowers powder was found to reduce the blood glucose level around 32.05% and 47.92% respectively. Although not prominent, but the data revealed that the sample was endowed with the body weight declining capability.

**Conclusion:** Results of the experiments affirm that the flower of *N. nucifera* has potent hypoglycemic and hypolipidemic properties and might be useful in these patients.

**Keywords:**
Alloxan monohydrate
Diabetes
Hypoglycemia
Hypolipidemia
Medicinal plant

**Introduction**

Lipid abnormalities are generally connected with diabetes which ultimately is considered as one of the most contributory factors for cardiovascular morbidity and mortality in patients suffering from diabetes. Type II diabetes is the most common one which accounts for about 90% of the diabetic population affecting minimum 15 million people. It is directly connected with other difficulties including hypertension, atherosclerosis and microcirculatory disorders. Among all the endocrine disorders, diabetes mellitus (type 2) is highly reported and is projected that almost three hundred million will be affected by the disease by 2025 and greater portion of diabetic population will be from India, China and...
Hypoglycemic and hypolipidemic effects of *N. nucifera*

United States (1-6). According to recent epidemiological studies, in Bangladesh more than 11% diabetic patients are suffered from type II diabetes and the presence of atypical fasting glucose, which triggers the initiation of diabetes, goes beyond 6% in Dhaka city (7). Therefore, it is now inevitable to search for novel oral drugs that will be therapeutically effective both as hypoglycemic and hypolipidemic agents at affordable cost particularly both for the developing and under-developed countries. In the experiments of hypoglycemic effects evaluation, animals are made diabetic usually by injecting alloxan or streptozotocin (STZ) intraperitoneally (IP) or intravenously (IV). For the studies of hypolipidemic activity, the subjected animals are generally fed with high fat diet to produce hyperlipidemia in those animals. *Nelumbo nucifera* is an aquatic perennial which belongs to the family of Nelumbonaceae and is familiar by many local names (e.g. Indian lotus, Chinese water lily, and Sacred lotus). Lotus flowers powder is utilized in traditional medicine to treat high blood pressure, diarrhea, fever, weakness, infection, skin inflammation, and body heat imbalance (8). It is also an effective treatment against abnormal bleeding such as hematemesis, epistaxis, hemoptysis, hematuria, and metrorrhagia (9). Lotus flowers extracts are found to possess a powerful antioxidant and radical scavenging ability along with inhibitory activity of diabetic complications (10-13). Lotus flower extract has been reported to treat obesity, too (14-16). Furthermore, lotus flowers extracts were observed to modulate lipolysis-activity and decrease adipogenesis in human pre-adipocytes (17) as well as to increase cholesterol profile in mice and reduce levels of phospholipids and triglycerides (18). It is notable to mention that anti-HIV principles have also been constructed using the ethanolic extract of the lotus flowers (19). This study was conducted to assess the hypolipidemic and hypoglycemic effects of *N. nucifera* flowers powder in Long-Evans rats.

**Materials and Methods**

**Experimental design**

This experiment was conducted according to Figure 1. The study was carried out in Biomedical and Toxicological Research Institute (BTRI), Bangladesh Council for Scientific and Industrial Research (BCSIR), Dhaka, Bangladesh. Flowers of *N. nucifera* were brought from National Botanical Garden, Mirpur, Dhaka, Bangladesh and identified by the Bangladesh National Herbarium, Dhaka. At first, the flowers of *N. nucifera* were washed vigorously using water and then dried at room temperature. The flowers were then air dried and subsequently oven dried at 37°C temperature. Later on, the dried flowers were grinded to powder form which then screened to find out the fine powder. Powder size of <0.5 mm after passing through a 35-mesh sieve was considered for the experiment. Healthy male rats (Long-Evans) of local strain, having around 150-200 g and 200-250 g weights were taken for the hypolipidemic and hypoglycemic effects studies, respectively. The proper environmental condition for the experimental rats was confirmed, kept under firm supervision for a week and maintained at a constant room temperature of 25°C ± 5°C with humidity of 40% to 70%.
with natural day-night cycle. For the hypolipidemic effect studies, the rats were segregated into a total of eight groups consisting of six in each maintaining the congruity of body weight. These included:

- **Group-A** (feeding with lab diet).
- **Group-B1** (feeding with 10% powder of \( N. nucifera \) flower and 90% lab diet).
- **Group-B2** (feeding with 20% powder of \( N. nucifera \) flower and 80% lab diet).
- **Group-B3** (feeding with 30% powder of \( N. nucifera \) flower and 70% lab diet).
- **Group-C** (feeding with high fat diet after inducing hyperlipidemia).
- **Group-D1** (feeding with 10% powder of \( N. nucifera \) flower and 90% high fat diet after inducing hyperlipidemia).
- **Group-D2** (feeding with 20% powder of \( N. nucifera \) flower and 80% high fat diet after inducing hyperlipidemia).
- **Group-D3** (feeding with 30% powder of \( N. nucifera \) flower and 70% high fat diet after inducing hyperlipidemia).

For evaluation of hypoglycemic activity of \( N. nucifera \) flower, the rats were segregated into five groups comprising of six in each with the congruence of body weight. These included:

- 1) **Group-A** (Normal Control, rats fed with 100% lab diet).
- 2) **Group-B** (Alloxan induced diabetic rats fed with 100% lab diet, diabetic control).
- 3) **Group-C** (Alloxan induced diabetic rats fed with Lab Diet plus glibenclamide, given at a dose of 5 mg/10 mL (9.9 mL H\(_2\)O + 0.1 mL Twin 20)/kg body weight, drug control (23)).
- 4) **Group-D** (Alloxan induced diabetic rats fed with 10% powder of \( N. nucifera \) flower plus 90% lab diet).
- 5) **Group-E** (Alloxan induced diabetic rats fed with 20% powder of \( N. nucifera \) flower plus 80% lab diet).

Before starting the experiment, the weights of the rats were taken precisely and the rats were marked on the tail, right front, right back, left front, left back and kept unmark which was subsequently applied as identification purpose for a particular rat, so that the reaction of a specific rat before and after the drug administration could be observed individually.

### Preparation of hyperlipidemic rats and estimation of the lipid profile

The rats were made hyperlipidemic by feeding high fat diet for 10 days containing Lab Diet, Dalda and Coconut oil in 4:3:1 ratio.

For evaluation of hypolipidemic activity, the powder of the \( N. nucifera \) flower was administered orally with high fat diet and lab diet at doses of daily 10%, 20% and 30% of the regular diet for 21 days. Blood samples were collected on the 22nd day by sacrificing the rats after making anesthesia using ketamine hydrochloride. After sacrificing about 2 mL of blood was taken cautiously. The blood samples were then centrifuged after 20 minutes at 4000 rpm for 10 minutes and re-centrifuged at 2000 rpm for 5 minutes. After that the serum samples were separated and taken into Eppendorf tubes. Then the serum triglyceride, total cholesterol, high-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C) were measured. One milliliter of serum was aliquoted and kept frozen at -20°C until analysis of serum for lipid profile.

Serum total cholesterol was measured by enzymatic colorimetric (Cholesterol Oxidase /Peroxidase, CHOD-PAP) method (Randox Laboratories Ltd., UK) using autoanalyzer, AutoLab. Serum HDL-cholesterol was estimated by enzymatic colorimetric (Cholesterol CHOD-PAP) method (Randox Laboratories Ltd., UK) using microplate reader (Bio-Tec, ELISA) and Serum triglyceride (TG) was examined by enzymatic colorimetric (GPO-PAP) method (Randox Laboratories Ltd., UK) using auto analyzer, Auto Lab. Then, serum LDL cholesterol was calculated manually. The calculated formula was:

\[
LDL - C = TC - \left( \frac{TG}{5} + HDL - C \right)
\]

All the groups of rats were remained under similar environmental conditions and provided with the measured food and water throughout the experiment. The body weight of each rat was measured and compared with the controls.

### Preparation of diabetic rats and measurement of blood glucose level

Alloxan monohydrate (\( C_{4}H_{8}N_{2}O_{4}H_{2}O \)) was available in colored bottles containing 25 g powder. The solution was prepared by dissolving 10 g in 100 mL of distilled water (10%).

The rats were made diabetic (diabetes mellitus) by injecting alloxan monohydrate 150-mg/kg-body weight intravenously (20). Three days after injection of the alloxan monohydrate, blood glucose of all the surviving rats was determined by the Diagnostics EliTech method. The rats with blood glucose levels above 6 mmol/L were considered as diabetic and considered for further study. For the assessment of hypoglycemic effect, \( N. nucifera \) flowers powder was fed orally with Lab Diet at doses of daily 10% and 20% of regular diet for 21 days. The drug, glibenclamide was administrated orally as a drug control, at a dose of 5 mg/10 mL (9.9 mL H\(_2\)O + 0.1 mL Twin 20)/kg body weight to the respective grouped rats.

Fasting blood glucose level was measured using glucometer on the 3rd (initial), 12th and 22nd days by collecting blood samples nicking the lateral tail vein using a sterile scalpel blade under ketamine hydrochloride anesthesia. Just before cutting, the tail was immersed into warm water (40°C) for around 25 seconds for vasodilatation.

### Data analysis

The data analysis was performed using SPSS. 11.5
Chronic effects of Nelumbo nucifera flower powder on body weight of rats

**Chronic effect of N. nucifera flower powder on body weight of normal control rats fed with lab diet and rats fed with lab diet plus sample**

The impacts of *N. nucifera* flowers powder on body weight of the rats during 21 days of feeding with Lab Diet plus sample in different percentages are shown in Table 1. Body weight of each rat was measured at every 11-day intervals and compared with that of the normal control group fed with 100% Lab Diet.

**Chronic effect of Nelumbo nucifera flower powder on body weight of hyperlipidemic model rats fed with high fat diet plus sample in comparison to hyperlipidemic model rats fed with only high fat diet**

The impacts of *N. nucifera* flower powder on body weight of hyperlipidemic rats administered with 100% high fat diet and the grouped rats fed with high fat diet plus sample at various percentages during 21 days of feeding are delineated in Table 2. Body weight of each rat was measured at every 11-day intervals.

### Table 1. Chronic effect of *Nelumbo nucifera* flower powder on body weight (BW) of rats fed with lab diet plus sample in comparison to normal control rats fed with lab diet

<table>
<thead>
<tr>
<th>Group</th>
<th>BW, Initial day (g)</th>
<th>BW, 12th day (g)</th>
<th>BW, 22nd day (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A (100% high diet)</td>
<td>174.17±28.04</td>
<td>172.17±27.78</td>
<td>171.67±25.87</td>
</tr>
<tr>
<td>Group B (lab diet + sample)</td>
<td>171.27±04 (100%)</td>
<td>173.83±26.68</td>
<td>177.85±26.11</td>
</tr>
<tr>
<td>Group C (100% high diet)</td>
<td>172.67±26.16</td>
<td>174.50±26.09</td>
<td>174.83±15.67</td>
</tr>
</tbody>
</table>

Data are presented as Mean ± SD and compared using one-way ANOVA (Duncan post hoc test), n = number of rats.

*Statistically significant in comparison to the control group (*P* < 0.05).

### Table 2. Chronic effect of *Nelumbo nucifera* flower powder on body weight (BW) of hyperlipidemic rats fed with high fat diet plus sample in comparison to hyperlipidemic rats fed with only high fat diet

<table>
<thead>
<tr>
<th>Group</th>
<th>BW, Initial day (g)</th>
<th>BW, 12th day (g)</th>
<th>BW, 22nd day (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group C (100% high diet)</td>
<td>172.33±10.46</td>
<td>176.83±15.52</td>
<td>180.33±15.63</td>
</tr>
<tr>
<td>Group D (high fat diet + sample)</td>
<td>171.67±11.00</td>
<td>172.20±11.16</td>
<td>172.42±11.58</td>
</tr>
<tr>
<td>Group E (high fat diet + sample)</td>
<td>171.50±18.17</td>
<td>172.58±17.06</td>
<td>172.95±17.17</td>
</tr>
<tr>
<td>Group F (high fat diet + sample)</td>
<td>174.25±9.3</td>
<td>174.88±15.47</td>
<td>174.79±15.92</td>
</tr>
</tbody>
</table>

Data are presented as Mean ± SD and compared using one-way ANOVA (Duncan post hoc test), n = number of rats.

*Statistically significant in comparison to the control group (*P* < 0.05).
Table 3. Chronic effects of Nelumbo nucifera flower powder on lipidemic status (total cholesterol & triglyceride) of rats fed with lab diet plus sample in comparison to normal control rats fed with lab diet

<table>
<thead>
<tr>
<th>Group</th>
<th>BW, Initial day (g)</th>
<th>BW, 12th day (g)</th>
<th>BW, 22nd day (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group C (100% high fat diet) (n=6)</td>
<td>172.33±10.46 (100%)</td>
<td>176.83±10.52 (102.61%)</td>
<td>180.33±59.63 (104.64%)</td>
</tr>
<tr>
<td>Group D (high fat diet + sample)</td>
<td>D1 (90% high fat diet + 10% sample) (n=6)</td>
<td>171.67±11.00 (100%)</td>
<td>172.20±11.62 (100.31%)</td>
</tr>
<tr>
<td></td>
<td>D2 (80% high fat diet + 20% sample) (n=6)</td>
<td>171.50±8.17 (100%)</td>
<td>172.5±8.07 (100.38%)</td>
</tr>
<tr>
<td></td>
<td>D3 (70% high fat diet + 30% sample) (n=6)</td>
<td>174.25±9.33 (100%)</td>
<td>174.88±5.47 (100.51%)</td>
</tr>
</tbody>
</table>

Data are presented as Mean ± SD and compared using one-way ANOVA (Duncan post hoc test), n = number of rats.

Table 4. Chronic effect of Nelumbo nucifera flowers powder on lipidemic status (total cholesterol & triglyceride) of hyperlipidemic rats fed with high fat diet plus sample in comparison to hyperlipidemic model rats fed with only high fat diet

<table>
<thead>
<tr>
<th>Group</th>
<th>TC, 22nd day (mg/dL)</th>
<th>TG, 22nd day (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group C (100% high fat diet) (n=6)</td>
<td>119.33±11.13</td>
<td>100.83±18.49</td>
</tr>
<tr>
<td></td>
<td>D1 (90% high fat diet + 10% sample) (n=6)</td>
<td>85.17±9.26*</td>
</tr>
<tr>
<td></td>
<td>D2 (80% high fat diet + 20% sample) (n=6)</td>
<td>72.67±8.14*</td>
</tr>
<tr>
<td></td>
<td>D3 (70% high fat diet + 30% sample) (n=6)</td>
<td>60.17±8.18*</td>
</tr>
</tbody>
</table>

Data are presented as mean ± SD and compared using one-way ANOVA (Duncan post hoc test), n = number of rats.

Discussion

It is obvious that there was a tendency to decline the body weight in all sample fed rat groups whereas normal control rats displayed increase in body weights at the end of study period (Table 1). Mathematically around a maximum 0.90% body weight reduction was observed in comparison to the body weights measured at first day considered as 100%

It is noticed from the data presented in Table 2 that there was a raise in body weight in each group. But the sample administered groups displayed slower enhancement in body weight than high fat diet fed groups. Data also suggest that after feeding of only high fat diet for 21 days, body weight augmentation was almost maximum of 10% if the initial body weight was considered as 100%. But, when along with the high fat diet, the sample at different percentages was given, the body weight increase was only a maximum of 0.20% considering the initial body weight as 100%.

According to data presented in Table 3, a significant diminution (P < 0.05) of TC and TG values in group B1 (90% lab diet + 10% sample), group B2 (80% lab diet + 20% sample) and group B3 (70% lab diet + 30% sample) compared to group A (100% Lab Diet) was observed and this diminution was more significant in higher percent of

Table 5. Chronic effect of Nelumbo nucifera flower powder on lipidemic status (HDL-C and LDL-C) of rats fed with lab diet plus sample in comparison to normal control rats fed with lab diet

<table>
<thead>
<tr>
<th>Group</th>
<th>HDL-C, 22nd day</th>
<th>LDL-C, 22nd day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A (100% lab diet) (n=6)</td>
<td>55.50±54.42</td>
<td>16.85±2.44</td>
</tr>
<tr>
<td></td>
<td>B1 (90% lab diet + 10% sample) (n=6)</td>
<td>69.83±7.91*</td>
</tr>
<tr>
<td>Group B (lab diet + sample)</td>
<td>B2 (80% lab diet + 20% sample) (n=6)</td>
<td>76.59±7.81*</td>
</tr>
<tr>
<td></td>
<td>B3 (70% lab diet + 30% sample) (n=6)</td>
<td>80.83±5.70*</td>
</tr>
</tbody>
</table>

Data are presented as mean ± SD and compared using one-way ANOVA (Duncan post hoc test), n = number of rats.

Statistically significant in comparison to the control group (P < 0.05).
Sample treated groups. Data reveal that the TC and TG values of the highest percentage sample treated group, group B3 in comparison to the group A after 21 days of feeding, were found to be lowered by around 43% and 44%, respectively.

Data shown in Table 4, confirm that samples have the characteristics of TC and TG diminishing abilities at a significant level in comparison to that of control one. In Table 5, significant variations were observed in the HDL-C and LDL-C levels among all the test groups after 21 days of sample treatment. In case of group B3 (70% lab diet + 30% sample) HDL-cholesterol was mostly augmented compared to the group A (100% lab diet). In Groups B1 (90% lab diet + 10% sample) and B2 (80% lab diet + 20% sample) the HDL-C levels were also found to be augmented in a significant level although lower than group B3. Furthermore, LDL-C level was observed to be reduced significantly. More specifically, in case of group B3 where the maximum of around 48% of HDL-C level was observed to be increased and the highest of 62% of LDL-C level seen to be reduced in comparison to that of group C.

It is worth mentioning from Table 5 and Table 6 that the increase of HDL cholesterol level was prominently higher in sample diet fed groups compared to that of normal control groups (both group A and group C). Significantly increment tendency of HDL cholesterol level was observed with the higher percentage of sample diet. It is also clear from Table 5 and Table 6 that the reduction of LDL cholesterol level was higher with the higher percentage of sample diet which surely implies to the hypolipidemic activities of the sample, *N. nucifera* flowers powder.

In Table 7, blood glucose levels were depicted and more significant (*P* < 0.05) anti-diabetic activity was observed on 22nd day in alloxan induced type 2 diabetic model rats. In a study it has been suggested that a 25% reduction in blood glucose level is considered a significant hypoglycemic effect. The results of the study were satisfactory and revealed that the 10% & 20% *N. nucifera* flowers powder exhibited significant (*P* < 0.05) hypoglycemic activity. In case of 10% sample induced group, the reduction of blood glucose level was 33%. The reduction of blood glucose level in alloxan induced rat was found highest, 44% with the *N. nucifera* of 20%.

**Conclusion**

According to the experimental results, it can be affirmed that *N. nucifera* flowers powder is endowed with strong hypoglycemic and hypolipidemic properties. Therefore, powder of *N. nucifera* flower may be utilized for the management of diabetes mellitus and other related complications directly linked to the lipid disorders. On the whole, *N. nucifera* is a very promising herbal plant
having enormous medicinal values. The plant should be examined vigorously to find out for its active ingredients for which hypoglycemic and hypolipidemic activities are being exerted and mode of actions behind the observed effects.

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We are expressing our heartfelt gratitude to the Biomedical and Toxicological Research Institute (BTRI), IFST, BCSIR, Dhaka, Bangladesh, for protruding the required hands during the study in all respects including financial and logistic supports.

Authors’ contribution
DI generated the idea. AH, S, LCM, SKD, AS and EPL executed the studies in accordance with the designed procedure working in the laboratories and capturing data. DI, AH and UKP performed data analysis. S and AS prepared the initial draft. AH revised the draft and produced the final manuscript with the consent of DI. All are well concerned about the latest version and agreed for publication.

Conflict of interests
The authors of the manuscript affirm that they have no conflict of interest.

Ethical considerations
The research protocol and all the tests performed in this study were approved by the Animal Care Committee of BCSIR (No. 39.307.099.00.00.166.2010–455). All the associated ethical matters were considered to the best by the authors.

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