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Studies on the Hypoglycemic and Hypolipidemic Effects of *Nelumbo nucifera* Leaf in Long-Evans Rats

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Abstract

The study was carried out to evaluate the hypolipidemic and hypoglycemic effect of *Nelumbo nucifera* leaf powder. In rats, hyperlipidemia was induced by feeding High Fat Diet (Lab diet: Dalda: Coconut oil = 4:3:1) and type 2 diabetes was built inside by injecting Alloxan. The sample *N. nucifera* leaf powder was added in different percentages with the regular Lab Diet for 21 days feeding. In case of hyperlipidemia developed groups, feeding of *Nelumbo nucifera* leaf powder at, 30% (70% Lab diet + 30% Sample), 20% (80% Lab diet + 20% Sample) and 10% (90% Lab diet + 10% Sample) showed varied but in a nutshell significant ($p < 0.05$) decrease in serum total cholesterol, triglyceride and LDL-Cholesterol levels when compared to control group while HDL-Cholesterol level was augmented significantly ($p < 0.05$). Daily feeding of *Nelumbo nucifera* leaf powder for 21 days resulted significant decrease in the blood glucose levels of alloxan-induced diabetic rats. Both the percentages of *Nelumbo nucifera* leaf powder having 20% with 80% Lab Diet and 10% with 90% Lab Diet significantly ($p < 0.05$) decreased blood glucose level up to 44% and 33% respectively where the higher percentage of *Nelumbo nucifera* powder was found to be exerted to the more prominent effects in lowering the blood glucose level. *Nelumbo nucifera* leaf powder had 75% efficacy rate in lowering the blood glucose level in comparison to the drug control group treated with glibenclamide which was found with the decreasing capability up

to 66%. Although not significant, it, based on the data, can be stated that the sample is endowed with the quality of decreasing capability of body weight. Thus, results of the experimental study reveal that the leaf of *Nelumbo nucifera* has potent hypoglycemic and hypolipidemic properties.

Keywords

Diabetics, Hypoglycemia, Hypolipidemia, Alloxan Monohydrate

1. Introduction

Lipid disorders are usually associated with diabetes which in turn acts as one of the prime causative factors for cardiovascular morbidity and mortality in patients suffering from diabetes [1]. The most common diabetes is type II diabetes which accounts for about 90% of the diabetic population [1] affecting minimum 15 million people which is directly connected with other difficulties including hypertension, atherosclerosis and microcirculatory disorders [2]. Among all the endocrine disorders, diabetes mellitus is the most prevalent one and almost three hundred million will suffer from the disease by 2025 [3] and major portion of diabetic population will be from India, China and United States [4] [5]. According to the current conducted epidemiological study in Bangladesh, more than 11% diabetic population are attacked by type II diabetes and the prevalence of impaired fasting glucose, which triggers the inception of diabetes, exceeds 6% in Dhaka city [6]. Therefore, it is now inevitable to move forward for a sophisticated oral drug that will be therapeutically effective as hypoglycemic side by side hypolipidemic agents at low cost particularly both for the developing and under-developed countries. For biological activities evaluation, as animal models, mainly rats, mice, rabbits or guinea pigs etc. are used. In the studies of hypoglycemic effects, the subjected animals are being made diabetic using alloxan, streptozotocin (STZ) etc introducing inside injecting intraperitoneally (IP) or intravenously (IV). For the hypolipidemic activity investigation, the selected animals are normally fed high fat diet to originate hyperlipidemia inside those animals. It is known that over 400 species around the world have been enlisted to exhibit hypoglycemic hypolipidemic effects, although very limited number has been considered [7] [8] [9] [10]. Ethnopharmacological studies reveal that more 1200 plants are utilized in traditional medicine for their alleged hypoglycemic activity [11].

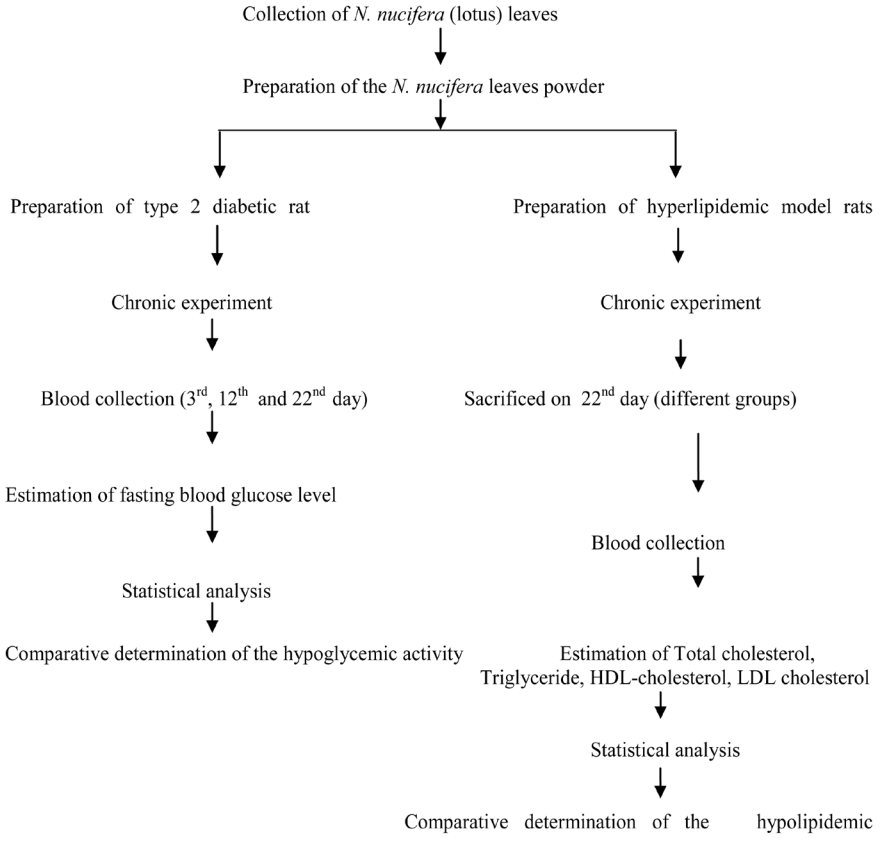
Nelumbo nucifera is an aquatic perennial belonging to the family of Nelumbonaceae, which has several common names (e.g. Indian lotus, Chinese water lily, and sacred lotus). Lotus leaves are used in traditional medicine to treat hypertension, diarrhea, fever, weakness, infection, skin inflammation, and body heat imbalance [12]. They are also an effective treatment against abnormal bleeding such as hematemesis, epistaxis, hemoptysis, hematuria, and metrorrhagia [13]. Extracts from the lotus leaves have shown a strong antioxidant and

radical scavenging ability, as well as inhibitory activity of diabetic complication factors [14] [15] [16] [17]. Again, extracts of the lotus leaf have been used to treat obesity, and have had reported to possess anti-obesity and anti-hyperlipidemia effects on rodents [18] [19] [20]. Additionally, leaf extracts were found to modulate lipolysis-activity and decreased adipogenesis in human pre-adipocytes [21] as well as to lower elevated cholesterol levels in mice and reducing levels of phospho-lipids and triglycerides [19]. Two anti-HIV principles have been isolated from the ethanolic extract of the lotus leaves [22].

2. Methods & Materials

2.1. Experimental Design

This experiment was carried out as per the following experimental design:



2.2. Place of the Study

The study was conducted in Biomedical and Toxicological Research Institute, Institute of Food Science & Technology (IFST), Bangladesh Council for Scientific and Industrial Research (BCSIR), Dhanmondi, Dhaka, Bangladesh.

2.3. Plant Materials

Large 2-foot-wide standing leaves of *N. nucifera* were collected from National Botanical Garden, Mirpur, Dhaka, Bangladesh and Kishorgonj, Bangladesh. This plant was identified by the Bangladesh National Herbarium, Dhaka.

2.4. Preparation of *N. nucifera* Leaves Powder

The leaves of *Nelumbo nucifera* was first washed throughly with water and then dried at room temperature. The leaves were then air dried followed by oven dried at 37˚C temperature. After that the dried leaves were grinded to powder form which subsequently screened to get the fine powder. Powder size of < 0.5 mm after passing through a 35-mesh sieve had been used for the experiment. Around 300 pieces of lotus leaves dried in oven yielded 1000 g of *N. nucifera* leaf powder.

2.5. Chemicals Used

Alloxan monohydrate, Glucose estimation & Lipid profile estimation kits were used.

2.6. Animals Used

Healthy male rats (Long-Evans) of local strain, weighing around 150-200gm and 200 - 250 gm were taken for the hypolipidemic and hypoglycemic experiments respectively. All the experiments conducted in this study were approved by the Animal Care Committee of BCSIR. The proper environmental condition for the experimental rats were 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 12 h day-night cycle.

For the hypolipidemic activity evaluation, rats are divided into a total of eight groups consisting of six in each keeping the congruity of body weight. These include:

Group-A (feeding with 100% Lab Diet).

Group-B1 (feeding with 10% powder of *N. nucifera* leaf and 90% Lab Diet).

Group-B2 (feeding with 20% powder of *N. nucifera* leaf and 80% Lab Diet).

Group-B3 (feeding with 30% powder of *N. nucifera* leaf and 70% Lab Diet).

Group-C (feeding with High Fat Diet after inducing hyperlipidemia).

Group-D1 (feeding with 10% powder of *N. nucifera* leaf and 90% High Fat Diet after inducing hyperlipidemia).

Group-D2 (feeding with 20% powder of *N. nucifera* leaf and 80% High Fat Diet after inducing hyperlipidemia).

Group-D3 (feeding with 30% powder of *N. nucifera* leaf and 70% High Fat Diet after inducing hyperlipidemia).

For the investigation of hypoglycemic activity of *N. nucifera*, the rats were divided into five groups comprising of six in each with the congruence of body weight. These include:

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 rat fed Lab Diet plus Glibenclamide, given at a dose of 5mg/10 ml (9.9 ml H_2O + 0.1 ml Twin 20)/kg body weight, Drug Control [23];

4) Group-D (Alloxan induced diabetic rats fed with 10% powder of *N. nucife-*

ra leaf plus 90% lab diet);

5) Group-E (Alloxan induced diabetic rats fed with 20% powder of *N. nucifera* leaf plus 80% lab diet).

Before commencing an experiment, the rats were weighed accurately and carefully marked on the tail, right front, right back, left front, left back and kept unmark which was later used as identification marks for a particular rat, so that the response of a particular rat before and after the drug administration could be noted separately.

2.7. Estimation of the Lipid Profile

2.7.1. Preparation of Hyperlipidemic Rats

The rats were made hyperlipidemic by feeding High Fat Diet for 10 days which contains Lab Diet, Dalda and Coconut oil in 4:3:1 ratio.

2.7.2. Dose and Route of Administration

For the evaluation of the hypolipidemic activity, the powder of the *N. nucifera* leaf were administered orally with High Fat Diet and Lab Diet at a dose of daily 10%, 20% and 30% of the regular diet for 21 days.

2.7.3. Collection of Blood Sample for Measuring Blood Lipid Profile

Blood samples were collected on the 22nd day by sacrificing the rats making anesthesia using ketamine hydrochloride. After sacrificing about 2 ml of blood was taken cautiously. The blood were then centrifuged after 20 min at 4000 rpm for 10 min and re-centrifuged at 2000 rpm for 5min. After that the serums were separated and taken into eppendorfs. Then the serum triglyceride, total cholesterol, High-Density Lipoprotein Cholesterol (HDL-C) and Low-Density Lipoprotein Cholesterol (LDL-C) were measured. 1ml of serum was aliquoted and kept frozen at -20˚C until analysis of serum for lipid profile.

2.8. Analytical Methods

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 micro-plate 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 by manually. The calculated formula was:

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

2.9. Chronic Effect on Body Weight

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.

2.10. Preparation of the Alloxan Solutions

Alloxan monohydrate ($C_2H_2N_2O_4 \cdot H_2O$) was available in colored bottles containing 25 gm powder. The solution was prepared by dissolving 10 gm in 100 ml of distilled water (10%).

2.11. Preparation of Diabetic Rats

The rats were made diabetic (diabetes mellitus) by injecting alloxan monohydrate 150-mg/Kg-body weight intravenously [24]. Three days after injection of the alloxan monohydrate, blood glucose of all the surviving rats was determined by the Diagnostics Elitech method. Rats with blood glucose levels above 6 mmol/l were considered as diabetic and considered for further study.

2.12. Dose and Route of Administration

For the evaluation of the hypoglycemic activity, the powder of the *N. nucifera* leaf were administered orally with Lab Diet at a dose of daily 10% and 20% of regular diet for 21 days. For all the pharmacological studies, the drug glibenclamide administrated orally as a drug control, at a dose of 5 mg/10 ml (9.9 ml H_2O + 0.1 ml Twin 20)/kg body weight for Type 2 model rats.

On the 22nd day blood samples were collected to measure fasting blood glucose level nicking the lateral tail vein using a sterile scalpel blade.

2.13. Blood Sample Collection and Measurement of Blood Glucose Level

Fasting blood samples were collected on the 3rd (initial), 12th and 22nd days by 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 approximately 22 seconds for vasodilatation. Then the level of blood glucose was measured by glucometer.

2.14. Data Analysis

The data analysis performed by using SPSS. 11.5 windows program. For charts and graphical representation Microsoft word and Microsoft excel were used.

3. Results and Discussion

During the entire study period laboratory protocols described in Methods and Materials section were followed strictly. The findings observed based on experimental data summarized below:

3.1. Chronic Effect of *Nelumbo nucifera* Leaf Powder on Body Weight (BW) of Rats

3.1.1. Chronic Effect of *Nelumbo nucifera* Leaf Powder on Body Weight (BW) of Normal Control Rats Fed with Lab Diet and Rats Fed with Lab Diet Plus Sample

The effects of *Nelumbo nucifera* leaf powder on body weight of model rats dur-

ing 21 days of feeding with Lab Diet plus sample in different percentage are presented in Table 1. Body weight of each rat was taken at every eleven days interval and compared with that of the normal control group fed with 100% Lab Diet. It is evident from the table that there was a tendency to decrease the body weight in all sample fed rat groups where as normal control rats showed rise in body weights at the end of study period. This scenario tells that of having the capabilities of sample to lessen the body weight which is a maximum of around 0.90% reduction in comparison of the body weights taken at first day considered as 100% (Figure 1).

3.1.2. Chronic Effect of *Nelumbo nucifera* Leaf Powder on Body Weight (BW) of Hyperlipidemic Model Rats Fed with High Fat Diet Plus Sample in Comparison to Hyperlipidemic Model Rats Fed with Only High Fat Diet.

The effects of *Nelumbo nucifera* leaf powder on body weight of hyperlipidemic rats fed with 100% High Fat Diet and model rats fed with High Fat Diet plus Sample at different percentage during 21 days of feeding are depicted in Table 2. Body weight of each rat was taken at every eleven days interval. It is found from the table that there was an increase in body weight in all groups. But, Sample provided groups show slower increase in body weight than High Fat Diet in duced group. Data also delineates that after feeding of only high fat diet for 21 days, body weight increment were almost maximum of 10% if the initial body weight was considered as 100%. But, when along with the high fat diet, sample at various percentages were given, the body weight increment was only a maximum of 0.20% considering the initial body weight as 100% (Figure 2).

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

Group		BW_Initial day (gm)	BW_12th day (gm)	BW_22nd day (gm)
Group A (100% Lab Diet) (n = 6)		171 ± 4.43	171.67 ± 4.76	172 ± 5.67
Group B (Lab Diet + Sample)	B1 (90% Lab Diet + 10% Sample) (n = 6)	163.33 ± 5.99	163.31 ± 4.59	163.29 ± 4.27
	B2 (80% Lab Diet + 20% Sample) (n = 6)	167.83 ± 3.43	167.17 ± 5.23	166.33 ± 5.28
	B3 (70% Lab Diet + 30% Sample) (n = 6)	169.17 ± 5.08	168.33 ± 4.63	168.17 ± 3.66

Data are presented as Mean ± SD and compared using one way ANOVA (Bonferroni post hoc test), n = number of rats. $P < 0.05$.

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

Group		BW_Initial day (gm)	BW_12th day (gm)	BW_22nd day (gm)
Group C (100% High Fat Diet) (n = 6)		171.17 ± 3.19	182.17 ± 4.67	187.50 ± 3.21
Group D (High Fat Diet + Sample)	D1 (90% High Fat Diet + 10% Sample) (n = 6)	169.50 ± 3.73	173.50 ± 5.21	175.33 ± 5.20
	D2 (80% High Fat Diet + 20% Sample) (n = 6)	169.17 ± 4.22	170.83 ± 4.22	171.33 ± 5.09
	D3 (70% High Fat Diet + 30% Sample) (n = 6)	168.50 ± 4.37	169.67 ± 4.84	168.83 ± 5.00

Data are presented as Mean ± SD and compared using one way ANOVA (Bonferroni post hoc test), n = number of rats. $P < 0.05$.

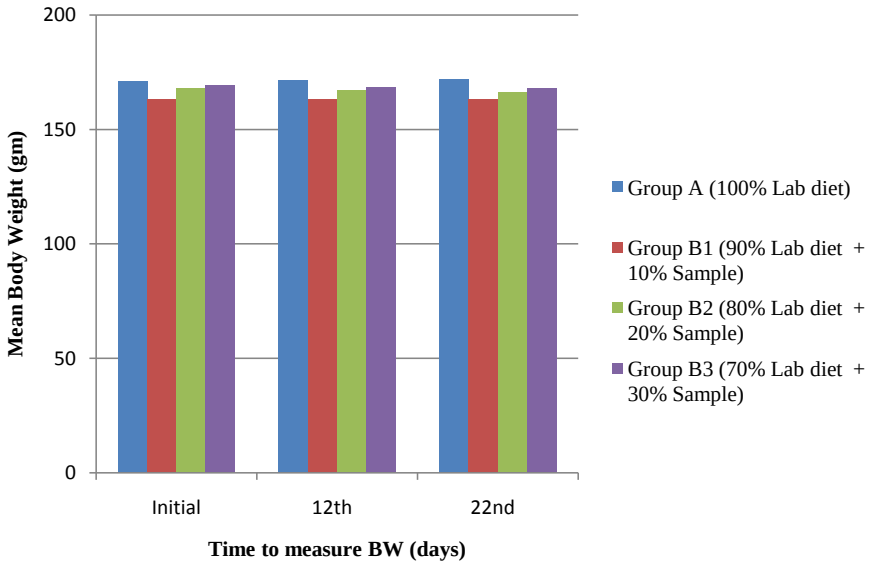


Figure 1. Chronic effect of N*elumbo nucifera* leaf powder on body weight of rats fed with lab diet plus sample in comparison to normal control rats fed with lab diet.

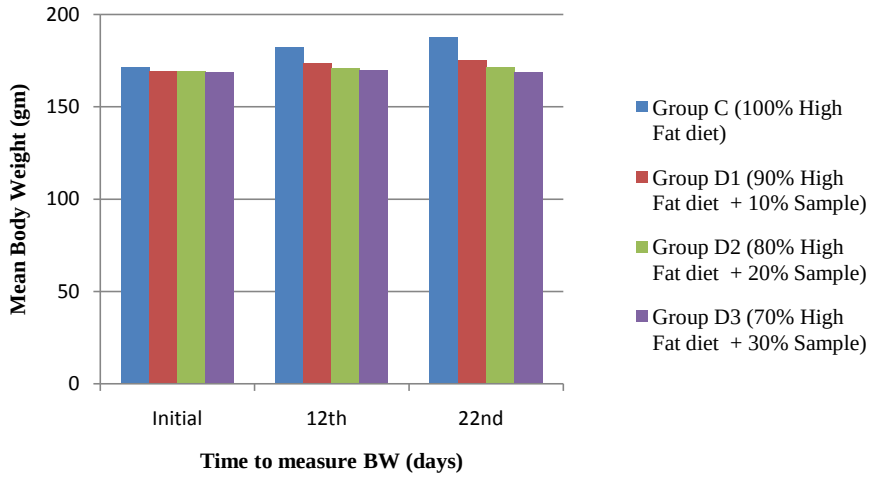


Figure 2. Chronic effect of *Nelumbo nucifera* leaf powder on body weight (bw) of hyperlipidemic model rats fed with high fat diet plus sample in comparison to hyperlipidemic model rats fed with only high fat diet.

3.2. Chronic Effects of *Nelumbo nucifera* Leaf Powder on Lipidemic Status [Total Cholesterol (TC) & Triglyceride (TG)] of Rats

3.2.1. Chronic Effects of *Nelumbo nucifera* Leaf 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

The mean serum total cholesterol and triglyceride levels of normal control rats fed with Lab Diet and model rats fed with Lab Diet plus powder of *Nelumbo nucifera* leaf for 21 days followed by blood collection on 22nd day are shown in Table 3, Figure 3 and Figure 4. To determine whether there was a statistically significant difference in hypolipidemia achieved by the powder sample on the 22nd

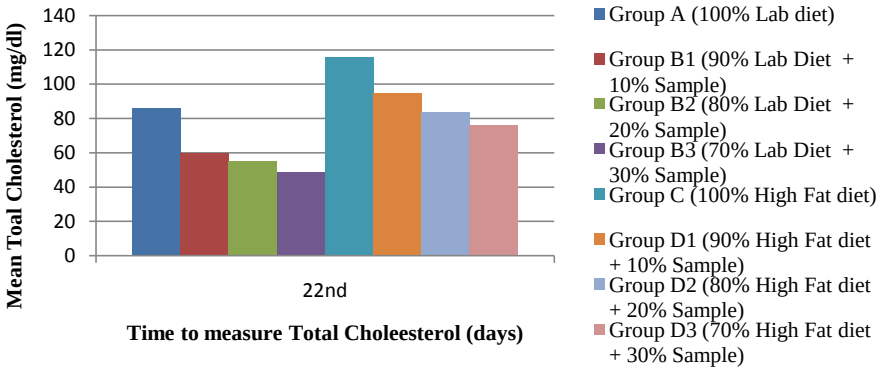


Figure 3. Chronic effects of *Nelumbo nucifera* leaf powder on lipidemic status (total cholesterol) of rats fed with lab diet plus sample in comparison to normal control rats fed with lab diet.

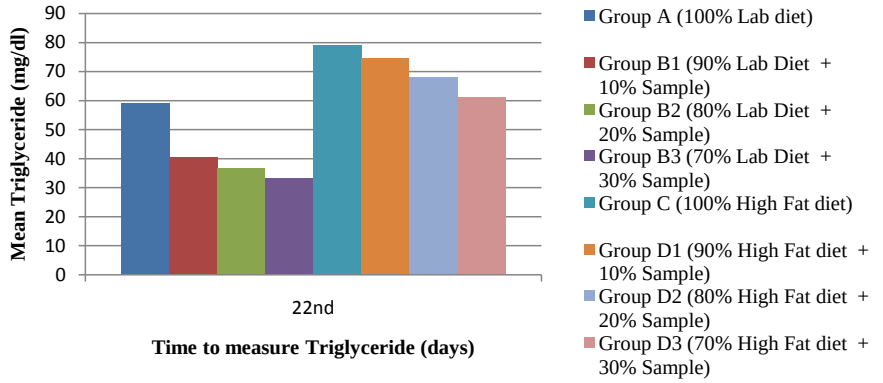


Figure 4. Chronic effect of *Nelumbo nucifera* leaf powder on lipidemic status (triglyceride) of hyperlipidemic model rats fed with high fat diet plus sample in comparison to hyperlipidemic model rats fed with only high fat diet.

Table 3. Chronic effects of *Nelumbo nucifera* leaf 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.

Group		TC_22nd day (mg/dl)	TG_22nd day (mg/dl)
Group A (100% Lab Diet) (n = 6)		86.33 ± 11.24	59.17 ± 6.74
Group B (Lab Diet + Sample)	B1 (90% Lab Diet + 10% Sample) (n = 6)	60 ± 5.87	40.33 ± 3.93
	B2 (80% Lab Diet + 20% Sample) (n = 6)	55.17 ± 6.88	36.83 ± 6.59
	B3 (70% Lab Diet + 30% Sample) (n = 6)	48.83 ± 3.25	33.17 ± 7.47

Data are presented as Mean ± SD and compared using one way ANOVA (Bonferroni post hoc test), n = number of rats. $P < 0.05$.

day, the one-way ANOVA (Bonferroni p test) was applied and compared to the control group. A significant reduction ($P < 0.05$) 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 found and this

reduction was more significant in higher percent of Sample provided groups. Data reveals that the TC and TG values of maximum sample treated group, Group B3 in comparison to the Group A after 21 days of feeding, were found to be decreased by around 43% and 44% respectively.

3.2.2. Chronic Effect of *Nelumbo nucifera* Leaf Powder on Lipidemic Status [Total Cholesterol(TC) & Triglyceride(TG)] of Hyperlipidemic Model Rats Fed with High Fat Diet Plus Sample in Comparison to Hyperlipidemic Model Rats Fed with Only High Fat Diet

The mean serum total cholesterol and triglyceride levels of High Fat Diet induced hyperlipidemic rats fed with only high fat diet (treated as control) and model rats fed with High Fat Diet plus powder of *Nelumbo nucifera* for 21 days followed by blood collection on 22nd day are shown in Table 4, Figure 3 and Figure 4. The data showed of possessing a significant level of TC and TG lowering capabilities of samples in comparison to that of control one.

It can be asserted from the Table 3, Table 4 and Figure 3 that *Nelumbo nucifera* leaf powder possesses the serum total cholesterol lowering efficiency where 30% sample diet is more significant in exerting the effects than that of 20% and 10% diet sample.

From the Table 3, Table 4 and Figure 4, the serum triglyceride lowering efficiency of *Nelumbo nucifera* leaf was found and among all of them 30% sample diet is more significant than 20% and 10% sample diet.

3.3. Chronic Effect of *Nelumbo nucifera* Leaf Powder on Lipidemic Status [HDL-Cholesterol (HDLC) and LDL-Cholesterol (LDLC)] of Rats

3.3.1. Chronic Effect of *Nelumbo nucifera* Leaf Powder on Lipidemic Status (HDL-Cholesterol and LDL-Cholesterol) of Rats Fed with Lab Diet Plus Sample in Comparison to Normal Control Rats Fed with Lab Diet.

The effect of *N. nucifera* leaf powder on atherogenic lipids (HDL-Cholesterol and LDL-Cholesterol) of rats fed with lab diet plus sample and normal control rats fed with lab diet is depicted in Table 5, Figure 5 and Figure 6. There were

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

Group		TC_22nd day (mg/dl)	TG_22nd day (mg/dl)
Group C (100% High Fat Diet) (n = 6)		116 ± 4.34	79 ± 6.78
Group D (High Fat Diet + Sample)	D1 (90% High Fat Diet + 10% Sample) (n = 6)	94.67 ± 8.33	74.67 ± 8.59
	D2 (80% High Fat Diet + 20% Sample) (n = 6)	83.50 ± 8.38	68.17 ± 8.68
	D3 (70% High Fat Diet + 30% Sample) (n = 6)	76 ± 11.35	61.17 ± 9.45

Data are presented as Mean ± SD and compared using one way ANOVA (Bonferroni post hoc test), n = number of rats. $P < 0.05$.

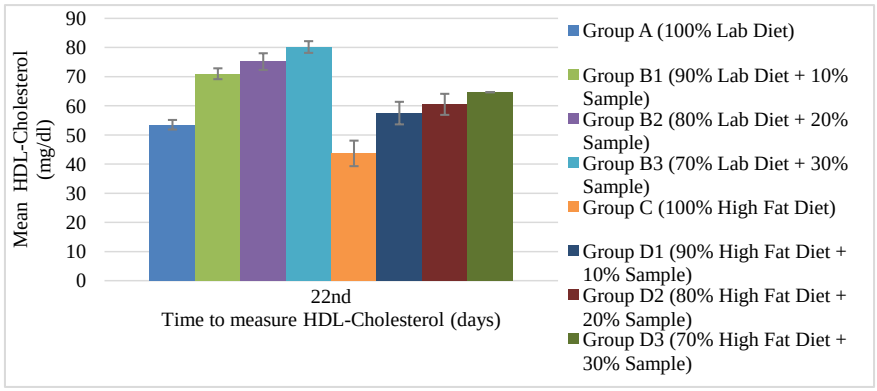


Figure 5. Chronic effect of *Nelumbo nucifera* leaf powder on lipidemic status (HDL-cholesterol) of rats fed with lab diet plus sample in comparison to normal control rats fed with lab diet. Figure embedded with standard error bars where in all cases $P < 0.05$ were found.

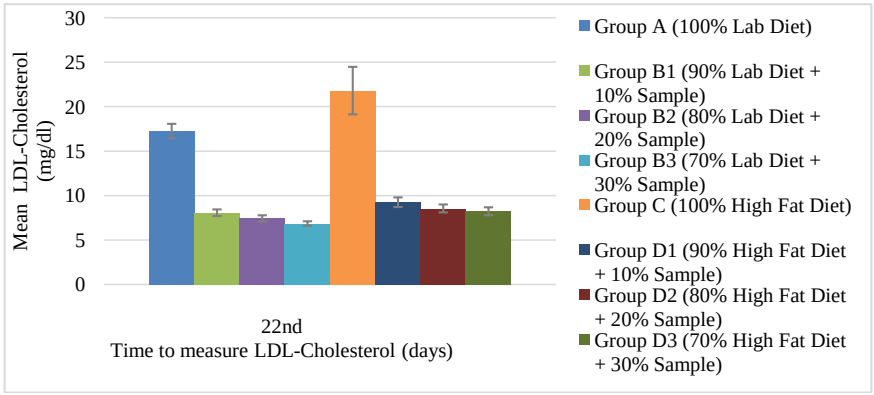


Figure 6. Chronic effect of *Nelumbo nucifera* leaf powder on lipidemic status (LDL-Cholesterol) of high fat diet induced hyperlipidemic model rats fed with high fat diet plus sample in comparison to hyperlipidemic model rats fed with only high fat diet. Figure embedded with standard error bars where in all cases $P < 0.05$ were found.

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

Group		HDLC_22nd day	LDLC_22nd day
Group A (100% Lab Diet) (n = 6)		53.50 ± 4.04	17.25 ± 2.00
Group B (Lab Diet + Sample)	B1 (90% Lab Diet + 10% Sample) (n = 6)	71.00 ± 4.56	8.07 ± 0.91
	B2 (80% Lab Diet + 20% Sample) (n = 6)	75.17 ± 7.00	7.42 ± 0.88
	B3 (70% Lab Diet + 30% Sample) (n = 6)	80.17 ± 4.92	6.85 ± 0.63

Data are presented as Mean ± SD and compared using one way ANOVA (Bonferroni post hoc test), n = number of rats. $P < 0.05$.

significant changes in case of HDL-cholesterol and LDL-cholesterol level among all the test groups after 21 days of feeding. In case of Group B3 (70% Lab Diet + 30% Sample) HDL-cholesterol was mostly increased compared to the Group A

(100% Lab Diet). In Group B1 (90% Lab Diet + 10% Sample) and B2 (80% Lab Diet + 20% Sample) the HDL-cholesterol level were also found to be increased in a significant level although lessen than Group B3. On the other hand, LDL-cholesterol in experimental groups was found to be decreased significantly. Among all the groups, Group B3 (70% Lab Diet + 30% Sample) was identified with the lowest level of LDL-cholesterol. In group B3, the HDLC level was found to be increased by almost 49% and the LDLC level was seen to be reduced to 60% in comparison to that of Group A after 21 days of sample feeding.

3.3.2. Chronic Effect of *Nelumbo nucifera* Leaf Powder on Lipidemic Status (HDL-Cholesterol and LDL-Cholesterol) of High Fat Diet Induced Hyperlipidemic Model Rats Fed with High Fat Diet Plus Sample in Comparison to Hyperlipidemic Model Rats Fed with Only High Fat Diet

The effect of *N. nucifera* Leaf powder on atherogenic lipids (HDL-Cholesterol and LDL-Cholesterol) is depicted in Table 6, Figure 5 and Figure 6. There were significant increases in case of HDL-cholesterol and decreases in the LDL-cholesterol level among all the test groups after 21 days of chronic experiment. The lipidemic status was measured on 22nd day. In case of Group D3 (70% Lab Diet + 30% Sample) HDL-cholesterol was mostly increased compared to the Group C (100% Lab Diet). In Group D1 (90% Lab Diet + 10% Sample) and D2 (80% Lab Diet + 20% Sample) the HDL-cholesterol was increased in a significant level. LDL-cholesterol in experimental groups was found to be decreased significantly. Here, in case of group D3 where the maximum of around 48% of HDLC level was found to be augmented and the highest of 62% of LDLC level found to be reduced in comparison to that of group C.

It is evident from the Table 5, Table 6 and Figure 5, the increment 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 found with the higher percentage of sample diet.

Table 6. Chronic effect of *Nelumbo nucifera* leaf powder on lipidemic status (HDL-cholesterol and LDL-Cholesterol) of high fat diet induced hyperlipidemic model rats fed with high fat diet plus sample in comparison to hyperlipidemic model rats fed with only high fat diet.

Group		HDLC_22nd day	LDLC_22nd day
Group C (100% High Fat Diet) (n = 6)		43.67 ± 10.73	21.80 ± 6.54
Group D (High Fat Diet + Sample)	D1 (90% High Fat Diet + 10% Sample) (n = 6)	57.50 ± 9.48	9.25 ± 1.33
	D2 (80% High Fat Diet + 20% Sample) (n = 6)	60.50 ± 8.85	8.55 ± 1.10
	D3 (70% High Fat Diet + 30% Sample) (n = 6)	64.67 ± 0.09	8.23 ± 1.10

Data are presented as Mean ± SD and compared using one way ANOVA (Bonferroni post hoc test), n = number of rats. $P < 0.05$.

It is also clear from the Table 5, Table 6 and Figure 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, *Nelumbo nucifera* leaf powder.

3.4. Chronic Effect of *Nelumbo nucifera* Leaf Powder on Fasting Blood Glucose (FBG) Concentration of Alloxan Induced Type 2 Diabetic Model Rats

The effects of *Nelumbo nucifera* leaf powder 10% with 90% Lab Diet and 20% with 80% Lab Diet which are expressed as change in blood glucose level are shown in Table 7 and Figure 7. Fasting blood glucose level of each rat was taken at every eleven days interval. More significant ($p < 0.05$) anti-diabetic activity was observed on 22nd day in alloxan induced type 2 diabetic model rats.

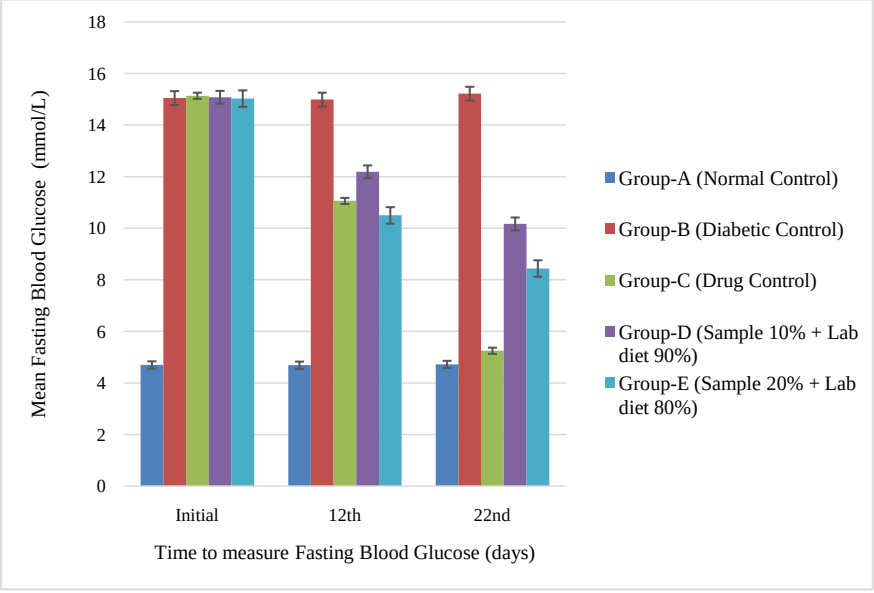


Figure 7. Chronic effect of *Nelumbo nucifera* leaf powder on fasting blood glucose concentation of alloxan induced type 2 diabetic model rats. Figure embedded with standard error bars where in all cases P < 0.05 were found.

Table 7. Chronic effect of *Nelumbo nucifera* leaf powder on Fasting Blood Glucose(FBG) concentration of alloxan induced type 2 diabetic model rats.

Group	FBG_Initial day (mmol/L)	FBG_12th day (mmol/L)	FBG_22nd day (mmol/L)
Group-A(Normal Control)	4.70 ± 0.34	4.69 ± 0.34	4.72 ± 0.36
Group-B(Diabetic Control)	15.05 ± 0.62	14.99 ± 0.69	15.22 ± 0.63
Group-C (Drug Control)	15.14 ± 0.29	11.06 ± 0.40	5.25 ± 0.18
Group-D (Sample 10% + Lab Diet 90%)	15.08 ± 0.41	12.19 ± 0.73	10.17 ± 0.81
Group-E (Sample 20% + Lab Diet 80%)	15.03 ± 0.59	10.50 ± 0.88	8.44 ± 0.88

Data are presented as Mean ± SD and compared using one way ANOVA (Bonferroni post hoc test), n = number of rats. P < 0.05.

Khan and Shechter (1991) have suggested that a 25% reduction in blood glucose levels is considered a significant hypoglycemic effect. The results of the study were satisfactory and revealed that the 10% & 20% *Nelumbo nucifera* leaf powder have 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. nucifer* of 20%.

4. Conclusion

Based on the data obtained from the experiments, it can be asserted that *Nelumbo nucifera* leaf powder possesses remarkable hypoglycemic and hypolipidemic properties. Therefore, powder of *Nelumbo nucifera* leaf may be taken into consideration for the management of diabetes mellitus and other associated complications directly related to lipid abnormalities. Basically, *Nelumbo nucifera* is a very promising herbal plant endowed with huge medicinal values. The plant is needed for further detailed investigation to explore its active principles and mode of actions behind the exerted effects.

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