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NELUMBO NUCIFERA LEAVES EXTRACT PARTIALLY REDUCES HIGH-FAT DIET INDUCED OBESITY IN EXPERIMENTAL RATS

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Abstract:

Introduction: The global pursuit of reducing body fat among obese individuals has driven extensive research into identifying functional foods that can aid in combating excess body fat. One such focus has been on investigating the potential of *Nelumbo nucifera* (NN) leaf extract to address obesity-related concerns.

Objective: This study aims to assess the anti-obesity properties of *Nelumbo nucifera* leaf extract in rats subjected to a high-fat diet (HFD) that induces obesity.

Materials and Methods: Female albino rats were fed either a standard chow diet (CD) for 40 days to induce obesity. Different doses of NN leaf extract (100, 200, and 400 mg/kg, administered orally) were given daily to distinct treatment groups. Parameters including body weight (measured every 5 days), organ and body fat pad weights, body temperature, locomotor activities, as well as levels of total cholesterol, triglycerides, and glucose were evaluated on the 39th and 40th day of the study.

Results: Rats treated with the high dose (400 mg/kg) of NN leaf extract exhibited significant reductions in body weight, total cholesterol, triglyceride levels, and glucose levels. Additionally, these rats demonstrated increased locomotor activities and elevated body temperatures.

Conclusion: The supplementation of NN leaf extract, particularly at the high dose, effectively mitigated weight gain and positively impacted metabolic parameters in rats. These findings suggest the potential of NN leaf extract as a natural remedy for countering obesity, offering a promising herbal solution for individuals seeking to revert from obesity to a healthier state.

Keyword: Nelumbo Nucifera, Anti-obesity, Herbal, Leaf extract, High-fat diet

Introduction:

The fundamental trigger for obesity lies in the imbalance between energy intake and expenditure, leading to excessive fat accumulation (1). This imbalance arises from a higher anabolic rate compared to the catabolic rate, fostering obesity. The escalating global prevalence of obesity has introduced significant health risks, both independently and in conjunction with various other illnesses. Obesity is a prominent international health concern. In the US, as of 2008, 33.8% of adults with a body mass index exceeding 25 kg/m² and 34.2% with a body mass index over 30 kg/m² were classified as overweight. According to the World Health Organization's 2002 report, over 1.5 billion adults above the age of 20 and more than 300 million children were grappling with obesity. Factors such as insufficient sleep, delayed pregnancy, excessive caloric intake, sedentary lifestyles, and genetic predisposition contribute to obesity (2).

Obesity significantly heightens the risk of chronic conditions, including cardiovascular disease (3,4), respiratory disorders (5), neurological ailments (6), metabolic syndrome (7), osteoporosis, type II diabetes mellitus (8), and various types of cancer (9-13). Furthermore, the consumption of high-calorie diets contributes to type II diabetes (14). Animal models simulating obesity and type II diabetes offer valuable insights for developing novel therapies targeting obesity and related conditions. The study of methods to burn excess body fat and dietary interventions for fat reduction is a global research focus (15).

Nelumbo nucifera (NN), commonly known as lotus or sacred lotus belonging to the Nelumbonaceae family, holds a prominent place in Ayurvedic medicine (16). Many pharmacological studies on lotus have proven its Antidiarrheal, anti-inflammatory, antipyretic, hypoglycemic, immunomodulatory, psychopharmacological, antioxidant, aphrodisiac, lipolytic, antiviral, anticancer and hepatoprotective activities (17). However, no scientific evidence substantiating NN leaf extract's anti-obesity potential using experimental models has been established. Therefore, this study aims to elucidate the impact of NN leaf extract on changes in body weight gain, locomotor activity, total cholesterol, triglycerides, and glucose levels in high-fat diet-induced obesity using female Wistar albino rats as the experimental subjects.

2. Materials and methods

2.1. Drugs and chemicals

All the drugs used were of high grade quality. Glucose, triglycerides and total cholesterol levels were diagnosed at Pathology lab.

2.2. Plant material collection and extract preparation

The NN leaves were meticulously collected from the local region and underwent thorough authentication by a renowned botanist. To ensure the precision and dependability of the plant material utilized in the study, a voucher specimen was meticulously prepared and preserved for future reference.

Subsequently, the collected leaves were subjected to a series of procedures. Initially, they were dried and subsequently ground into coarse powder. This powdered material was then packed into a Soxhlet's column, where it underwent defatting using petroleum ether at a temperature range of 50–60 °C for duration of 24 hours. Following the defatting process, the leaves were extracted with methanol within the same temperature range for another 24 hours. After this extraction process, the obtained extract residue was further processed.

The solvent within the residue extract was entirely eliminated using a rotatory flash evaporator, yielding a concentrated extract. This concentrated extract was subsequently dried utilizing a freeze dryer and was then stored within an air-dried container within a refrigerator. The final yield from this process was approximately 2%, and this yield was used for the subsequent experimental endeavors. The chemical composition of the resulting methanolic extract was confirmed through qualitative analysis.

2.3. Preparation of diet

Cafeteria diet (CD): Cafeteria diet comprises of following partsPart I- Milk (48 gm) + bread slice (48 gm); Part II- Chocolate flakes (18 gm) + biscuits (36 gm) + coconut powder (36 gm); Part IIICheese (40 gm) + boiled potato (60 gm). These three diets were presented to different groups on the 1st, 2nd and 3rd day respectively with repetition in the same succession for 40 days along with normal pellet chow diet.

2.4. Animals

Wistar albino female rats weighing (125-180 gm) were used to induce experimental obesity. The animals were housed in polypropylene cage at constant temperature (25 °C) and 12-h light/dark cycle with free access to food and water at the animal house facility during the entire experimental study. The protocol of this study was approved by the IAEC.

2.5. Acute toxicity study: The acute toxicity studies of mEtoh extract of NN leaves were performed as per OECD guideline no. 420 & 425 followed by fixed dose method and up and down method. The extract did not create any kind of toxicity even at 2000 mg/kg and dose selected for this study starts from 1/5th, 1/10th, 1/20th for further study (18-19).

2.6. Experimental design The animals in each category was randomly divided into six groups each containing six rats (n = 6) and treatment protocol for 40 days of experimental protocol as ordered below-

CD induced obesity: Group I: Vehicle control, receives normal saline; Group II: CD control (CD), receives cafeteria diet as scheduled above; Group III: Treatment LD, receives CD + low dose of NN (100 mg/kg/day p.o); Group IV: Treatment MD, receives CD + medium dose of NN (200 mg/ kg/day p.o); Group V: Treatment HD, receives CD + high dose of NN (400 mg/kg/day p.o); Group VI: Standard control (Std), receives CD + Orlistat (30 mg/kg/day p.o).

The body weight of each animal was measured on every 5th day and % change in body weight was measured upon completion of the protocol. On 39th day, the body temperature and locomotor activities were measured by using rectal thermometer and open field apparatus respectively. On 40th day, animals were anesthetized under light ether inhalation and blood was withdrawn by retro-orbital puncture, collected in eppendorf, and centrifuged to obtain serum for estimation of various biochemical parameters. Ultimately, animals were then euthanized by cervical dislocation and dissected to obtain various body organs and adipose tissue deposits.

2.7. Measurements and parameters: The study was conducted for 40 day and tests/parameters were conducted as detailed-

(i) Body weight-On day 1, the body weight of each rat in every group was recorded on digital weighing balance and was re-scheduled for every 5th day. Ultimately the percentage changes in body weights were calculated at the end of study for individual animal groups.

(ii) Body temperature-The body temperature was recorded using a rectal thermometer on 39th day during half an hour before and after drug administration at regular intervals of 30, 60, 90, 120 and 180 min with 1 min of contact time.

(iii) Locomotor activity- The locomotor activity was recorded on 39th day by open field apparatus with observation time (5 min) after drug administration to individual treatment groups. The test was performed by placing the rats in center of apparatus, were observed and ambulation, the occurrence rate of rearing and grooming were recorded and tabulated (20).

(iv) Biochemical parameters- Blood was collected in eppendorf via retro-orbital puncture on 40th day and centrifuged to obtain serum for measuring the changes in the level of glucose, total cholesterol, and triglyceride using the biochemical kits (21)

(v) Organs and fat pad weights- Firstly, The euthanisation of animals was done using di-ethyl ether as anesthetic and then dissected to obtain different organs such as heart, kidney, liver, spleen and fat pad (including mesenteric, uterine and peri-renal fat pad) and before collecting, the organs were rinsed in cold saline, patted with tissue paper and weighed individually, recorded and the percentage of weight gained by each organ was calculated and tabulated (22)

2.8. Statistical analysis

The results were statistically analyzed as Mean ± SEM. The significance difference between the control, and the treated rats with number of observation 'n' equal to 6 for different parameters was obtained by using one-way analysis of variance (ANOVA) followed by Dunnet's multiple test $p < 0.05$ was considered as statistically significant.

3. Results

Effect on body weight- In CD fed rats, a marked ($p < 0.001$) elevation in body weight was observed when compared to normal control fed rats on normal saline. The administration of NN leaves extract (mEtoh) has significantly (**$p < 0.01$) lower down the body weight in dose dependent-paradigm within individual CD fed rats. The body weight changes of NN extract 400 mg/kg were found to be closer to or list at treated rats.

Effect on body temperature-The body temperature recorded for 180 min was found to be a slight increase with difference of approx. 1.5 °C when compared to the normal rat fed on normal saline. The treatment of mEtoh extract with different doses produces increased body temperature. Among these groups, a dose of 400 mg/kg has markedly (***$p < 0.001$) rose the body temperature upon comparison with HFD control groups towards or list at treated rats.

Effects on locomotor activity- A significant decrease in the ambulation, frequency of rearing and grooming were found when compared to the normal saline fed control rats. Henceforth, NN leaves extract in different doses displayed the significant dose-dependent increase in locomotor activity. Effects on biochemical parameters- The NN leaves extract (mEtoh) at 400 mg/kg significantly (***$p < 0.001$) decreased triglycerides and glucose level but total cholesterol level was not declined significantly as compared with standard drug.

Changes in organs and fat pad weights-The weight of internal organs like heart, liver, kidney, and spleen along with mesenteric, uterine and peri-renal fat pad were significantly increased when compared to the vehicle control. Moreover, the rats fed with HFD for a longtime period develop fatty liver and increase in weight of heart and spleen as well as accumulation of hepatic triglycerides and total cholesterol as compared to the vehicle control group. The NN leaves extract (mEtoh) yields a significant reduction in weight of organs such as heart; kidney and liver while the gain in weight of spleen. The mesenteric, uterine and peri-renal fat pad weights were decreased when compared with HFD control groups.

4. Discussion

The underlying cause of obesity is the disruption between energy intake and expenditure, a phenomenon that manifests as excess body fat accumulation. This global health challenge has far-reaching consequences on well-being and has been linked to decreased life expectancy. High-fat diets (HFDs), such as cafeteria and atherogenic diets, exacerbate fat buildup and are widely utilized as obesity models, providing a closer representation of the obesity scenario in humans (23).

Notably, the consumption of a regular diet (CD) has been observed to boost energy storage rates and contribute to obesity both in humans (Bull, 1988) and animals (24). In animals, the CD has been associated with hyperphagia, leading to increased fat storage and consequent organ and body weight gain (25).

Natural products with anti-obesity properties are categorized into five groups based on distinct mechanisms: reduced lipid absorption, decreased energy intake, increased energy expenditure, inhibited pre-adipocyte differentiation, and heightened lipolysis or diminished lipogenesis (26). Regular consumption of CD triggers obesity in experimental animals by influencing the expression

of genes associated with fatty acid catabolism in the small intestine, ultimately leading to obesity development. Moreover, the combination of a high-fat diet and sedentary behavior results in lipid accumulation in adipose tissue, potentially causing endothelial dysfunction, increased coagulation, insulin resistance, lipid deposition in organs, and alterations in cholesterol components (27).

The CD diet promotes fat storage within muscles rather than oxidation, accompanied by elevated serum or tissue cholesterol levels, contributing to excessive body weight. Cafeteria diets, characterized by energy-rich, palatable, carbohydrate, and fat-rich foods, significantly elevate body weight and fat mass in experimental animals (28).

Phytochemical screening of the NN extract revealed significant components like phenol, flavonoids, and saponins. Saponins have been associated with anti-obesity effects, while phenols and flavonoids exhibit potent antioxidant actions, functioning as free radical scavengers. These compounds could contribute to the reduction of lipids and glucose in the body. Flavonoids are well-known for their various pharmacological activities, including anti-hypertensive, hypoglycemic, spasmolytic, anti-inflammatory, and antioxidant properties (29).

In the animal model of obesity outlined above, excessive body weight due to high fat accumulation in adipose tissues was mitigated by treating the animals with different doses of NN leaves extract. This treatment notably reduced body weight in a dose-dependent manner, possibly by inhibiting pancreatic lipases. Furthermore, treatment with the high dose of NN extract (400 mg/kg) led to a marked decrease in blood glucose levels, demonstrating its potential anti-hyperglycemic effect.

Obesity-associated insulin resistance, characterized by impaired insulin regulation of glucose metabolism in peripheral tissues, is another relevant aspect. In this study, HFD-fed rats displayed elevated blood glucose levels and decreased insulin sensitivity, which were alleviated by the high dose of NN extract. Additionally, obesity often leads to an unfavorable lipid profile, including increased serum triglyceride and cholesterol levels. The rats treated with NN leaves extract exhibited substantial reductions in serum triglycerides and total cholesterol. This decline in triglyceride levels might be associated with increased endothelium-bound lipoprotein lipase (LPL) activity or inhibition of peripheral lipolysis, preventing the conversion of fatty acids into triglycerides (30).

Moreover, obesity is linked to the accumulation of adipose tissue mass and increased organ weights, such as liver, kidneys, heart, and spleen. HFD consumption also contributes to fat deposition in specific regions like mesenteric, uterine, and peri-renal fat pads. The high dose of NN leaves extract significantly reduced fat deposition in these areas. The anti-obesity potential of NN leaves extract aligns with previous research, emphasizing its promising application (31-74).

5. Conclusion Therefore, it is suggested that a comprehensive analysis of the active constituents and rigorous clinical assessments of *Nelumbo nucifera* (NN) leaves could provide valuable insights for effective obesity management strategies. However, it remains crucial to conduct additional extensive studies to substantiate its clinical efficacy, safety profile, and overall value in combating obesity.

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Declaration of competing interest

Authors declare that there are no conflicts of interest.

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Table 1: Effect of methanolic extract of *NN* leaves on body temperature, locomotor activity and biochemical parameters in cafeteria diet induced obesity in rats. (Values are in Mean± SEM from 6 rats in each group)

Treatment Dose (mg/kg)	Percentage change in body weight	Locomotor activity (5 Min)			Glucose (mg/dl)	Cholesterol (mg/dl)	Triglycerides (mg/dl)
		Ambulation	Rearing	Grooming			
Vehicle control (1ml/100g)	13.145±1.15	64.167±3.43	12.733±1.16	6.433±0.543	64.433±0.61	68.569±0.77	68.323±0.49
Cafeteria diet control	55.808±7.94	36.833±3.156	9.01±0.577	5.567±0.715	172.767±1.430	210.333±1.801	229.50±1.92
Cafeteria diet + *NN* 100mg/kg	8.367±0.81	76.167±2.466 ***	19.520±2.045 **	6.967±0.989 ns	172.157±1.537 ns	199.933±3.240 ns	148.83±1.79***
Cafeteria diet + *NN* 200mg/kg	13.462±1.818***	86.698±2.41 ***	23.02±1.52***	18.33±1.45***	144.343±2.246***	176±2.017***	120.16±1.70***
Cafeteria diet + *NN* 400mg/kg	29.172±1.689***	102.5±7.01***	33.40±1.17***	13.987±1.701***	128.166±1.302***	186±2.338***	104.66±2.47***
Reference group: Cafeteria diet + orlistat (30mg/kg)	28.908±2.866***	123.5±12.5493***	34.300±4.09***	17.400±1.87***	109.33±1.87***	119.50±2.60***	103±3.25***

NN- Nelumbo nucifera, *P<0.05, **P<0.01, ***P<0.001, ns= non-significant when compared to the HFD.

Table no.2: Effect of methanolic extract of *NN* leaves extract on body temperature (°C) in rats fed on cafeteria diet. (Values are in Mean± SEM from 6 rats in each group)

Treatment Dose (mg/kg)	Body temperature at different time interval (min.)					
	0 Min	30 Min	60 Min	90 Min	120 Min	180 Min
Vehicle Control (1ml/100g)	32.220 ±0.541	32.667±0.258	37.26 ±0.152	32.30 ±0.15	32.37 ±0.185	32.363 ±0.13
Cafeteria Diet control	31.722 ±0.257	31.812 ±0.314	38.46±0.274	32.43±0.24	32.74 ±0.158	32.643 ±0.19
Cafeteria diet +*NN* 100mg/kg	333.250±0.365 ns	33.680±0.253**	38.73±0.305*	33.83±0.38**	32.58±0.338 ns	31.208±0.26
Cafeteria diet + *NN* 200mg/kg	32.480±0.349 ns	33.680±0.190 **	39.58±0.280 *	31.93±0.22**	32.35±0.338 ns	33.503±0.36 ns
Cafeteria diet + *NN* 400mg/kg	31.817±0.327 ns	31077±0.191***	35.25±0.338 ns	32.46±0.40***	31.35±0.287*	33.350±0.29*
Reference group: Cafeteria diet + orlistat (30mg/kg)	32.838±0.609 ns	35.880±0.54***	36.21±0.41 ns	33.46±0.46***	33.61±0.402***	34.610±0.58***

NN- Nelumbo nucifera, *P<0.05, **P<0.01, ***P<0.001, ns= non-significant when compared to the HFD.

Table no.3: Effect of methanolic extract of *NN* leaves on changes in organ's weight and organ's fat pad weights (g) in rats fed on cafeteria diet (Values are in Mean±SEM from 6 rats in each group)

Treatment Dose (mg/kg)	Change in Organ Weights (g)				Change in organ's fat pad weights (g)			
	Left Kidney	Right kidney	Heart	Spleen	Liver	Mesenteric	Uterine	Peri-renal
Vehicle Control (1ml/100g)	0.543±0.008	0.578±0.09	0.658±0.035	0.707±0.015	5.373±0.051	0.328±0.224	0.742±0.033	1.290±0.063
Cafeteria Diet control	0.682±0.009	0.680±0.010	0.823±0.014	1.085±0.019	5.878±0.121	0.413±0.08	1.420±0.027	2.102±0.025
Cafeteria diet + *NN* 100mg/kg	0.632±0. 006***	0.545±0.008***	0.628±0.012***	0.607±0.022***	5.117±0.056***	0.300±0.020 ns	0.833±0.038***	1.138±0.057***
Cafeteria diet + *NN* 200mg/kg	0.603±0.012***	0.460±0.014***	0.575±0.008***	0.655±0.008***	4.850±0.023***	0.285±0.018 ns	0.732±0.053***	1.101±0.049***
Cafeteria diet + *NN* 400mg/kg	0.543±0.009***	0.333±0.015***	0.555±0.0130***	0.635±0.008***	4.482±0.043**	0.252±0.013*	0.712±0.051***	1.024±0.065***
Reference group: Cafeteria diet + orlistat(30mg/kg)	0.423±0.013***	0.32±0.015***	0.505±0.018***	0.603±0.008***	4.000±0.033***	0.200±0.015**	0.450±0.059***	0.885±0.051***

NN- Nelumbo nucifera, *P<0.05, **P<0.01, ***P<0.001, ns= non-significant when compared to the HFD..