

Evaluation of the Anti-Hyperlipidemic Activity of *Nelumbo nucifera* **Fruit in Rabbits Fed with High Cholesterol Diet**

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How to cite this paper: Rajput, M.A., Khan, R.A. and Assad, T. (2017) Evaluation of the Anti-Hyperlipidemic Activity of *Nelumbo nucifera* Fruit in Rabbits Fed with High Cholesterol Diet. *Pharmacology & Pharmacy*, **8**, 205-213. https://doi.org/10.4236/pp.2017.86014

Received: May 7, 2017 **Accepted:** June 20, 2017 **Published:** June 23, 2017

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Abstract

Recently use of herbal therapies and diet rich in flavonoids and vitamins has augmented significantly to manage minor to modest illnesses. Several anti-hyperlipidemic agents are currently available; however most of them have various unwanted effects. That's why more people are switching towards safer alternatives, specially derived from plants with fewer side effects. Hence current study was focused to determine the anti-hyperlipidemic potential of Nelumbo nucifera fruit in rabbits fed with high cholesterol diet. This study would surely help in the exploration of a novel agent from the plant source and therefore may recommend the application of traditional medicines in humans. 28 white healthy rabbits of either sex weighing 1000 - 1500 gm were divided into four groups. Control group was given gum tragacanth and 3 groups served as test groups and were given N. nucifera fruit extract 50, 100 and 200 mg/kg for 45 days. Initially animals of all four groups received high cholesterol diet (HCD) 0.125 gm/kg cholesterol in 0.5% corn oil for 30 days. All drugs were given orally. Blood samples were taken thrice from the ear vein of animals, 1st after 24 hours of thirty days of HCD then again after 24 hours of thirty and forty five days dosing of N. nucifera fruit extract. The results showed remarkable cholesterol lowering effects of N. nucifera fruit extract at doses 50, 100 and 200 mg/kg but were particularly dominant at 200 mg/kg at which fruit extract highly significantly reduced TC, TG and LDL-C after 30 and 45 days and increased HDL-C significantly after 30 days and highly significantly after 45 days. Flavonoids, saponins, tannins and procyanidin are significant constituents of N. nucifera fruit which may have contributed in lipid lowering effects of N. nucifera fruit which can contribute in lowering the risk of CVDs and stroke but more preclinical and clinical studies are required to confirm these findings.

Keywords

Nelumbo nucifera, High Cholesterol Diet, Flavonoids, Procyanidin

1. Introduction

Herbal Medicine or Herbalism is the practice or art of employing herbs and herbal preparations in order to remain healthy and also for the treatment and improvement in prognosis of diseases [1]. The World Health Organization (WHO) has given its estimation that more than 2/3rd of the global population in recent times depends on alternative sources of treatment to fulfill the basic healthcare requirements and this most importantly embroils the usage of plant products. This means that nearby two-thirds of the people globally trust on plants as a reliable way of their medication [2].

These days vigorous research is ongoing to discover nontoxic and beneficial herbs. Several anti-hyperlipidemic agents are currently available; however most of them have associations with various unwanted effects. That's why more people are switching towards safer alternatives, specially derived from plants with fewer side effects [3].

N. nucifera belongs to family Nymphaeaceae, and is frequently named as bean of India, Chinese water lily, Indian lotus or merely lotus. According to Linnaean classification N. nucifera Gaertn is at present documented name of plant species and is widely cultivated in the tropical regions of Pakistan, India, China, Thailand and Australia [4].

N. nucifera fruit is commonly known as Paban in native language of Sindh province of Pakistan and is cultivated especially in the water gardens, ponds and lakes of interior Sindh (Gotki, Thatta, Larkana and Matiari districts) and in Muzaffergarh regions of southern Punjab, Pakistan. It is also found abundantly in the lakes and streams of Azad Jammu Kashmir (AJK), Pakistan.

Fruit consists of seeds and seed pods. The latter is also known as lotus bulbs, are green in color and provides attachment to the seeds, which are black in color, hard in nature, ovoid, oblongish or roundish in shape, up to 1.5 cm broad and 1.0 cm long and are organized in whorls [5]. The seeds are the edible portion and are studded in the pod or bulb and have to be peeled individually before they are eaten. The seed is perhaps the strongest source of protein in the plant [6].

The N. nucifera seeds are loaded with fat, amino acid, protein, asparagines, unsaturated fatty acids, minerals, starch, saponins and tannins. The seed has three main components; cotyledons, plumule and integuments, which occupies approximately 93.23%, 3.03% & 3.74% in terms of its mass correspondingly. The average burden of hundred seeds is approximately 87.35 g. The seeds also contain substantial percentage of various minerals such as calcium (22.10%), potassium (28.5%), magnesium (9.2%), sodium (1.0%), iron (0.19%), chromium (0.004%), manganese (0.35%), zinc (0.08%) and copper (0.046%). Other significant dietary elements include fat (72.17%), proteins (2.7%), total powdery residue (4.5%), wetness (10.50%) and raw fiber (10.60%). The seed are highly nutritious and possesses 348.45 cal per 100 g [7].

The qualitative phytochemical analysis of the seed pods of N. nucifera fruit exposed the presence of flavonoids, alkaloids, saponins, tannins and terpenoids



[1]. Procyanidin was also identified and sequestered from the pods of *N. nucife-ra* fruit. The chief secondary metabolites present in *N. nucifera* seeds are alkaloids, particularly lotusine, liensinine, isoliensinine, dauricine, nuciferine, pronuciferine, roemerine, procyanidin, neferine and armepavine. The seeds also have carbohydrates, Gallic acid and isoquininolinol [4].

Traditionally fruits of *N. nucifera* are used in enormous conditions such as fever, inflammation, insomnia, palpitation, hypertension, arrhythmia, enteritis, chronic diarrhea, spermatorrhea, leucorrhoea, halitosis, skin diseases, leprosy and menorrhagia. It is also used as an antiemetic, antidote, refrigerant and diuretic [8] [9]. Recent study has revealed the LD50 of *N. nucifera* fruit to be greater than 5000 mg/kg and same study has also demonstrated its anxiolytic and antidepressant activities [1]. However, very limited literature is available regarding its effect on lipid profile, thus current study was focused to determine the anti-hyperlipidemic potential of *N. nucifera* fruit in rabbits fed with high cholesterol diet. This study would surely help in the exploration of a novel agent from the plant source and therefore may recommend the application of complimentary medicines in humans [10].

2. Materials and Methods

Experimental design & methodology

Research work was executed utilizing the laboratory facilities of Pharmacology department and the Research Institute of Pharmaceutical Sciences, University of Karachi, following approval from the Board of Advance Studies & Research (BASR), and departmental research & ethical committee.

2.1. Selection of Animals

Anti-hyperlipidemic effect was evaluated in 28 healthy white rabbits of either sex weighing 1000 - 1500 gm which was divided in to four groups; control and 3 test groups. 7 rabbits were placed in each group. Rabbits were chosen as experimental animals for current study as biochemical changes produced in rabbits are very much similar as observed in humans [11]. In addition to it sufficient amount of blood samples can easily be drawn, very convenient to handle and is also economical [12].

2.2. Animal Housing

Animals were placed in plastic cages with preservation of room temperature at $23^{\circ}C \pm 2^{\circ}C$ and moisture kept at 50% to 60% in a substituting 12-h light/dark succession. Every animal was provided with normal diet prepared in laboratory plus water as desired. The animals were shifted to the laboratory approximately an hour before the commencement of experiments. The experiment was performed during day time. Prior to dose administration, overall fitness of animals were assessed throughout the adaptation phase utilizing the laboratory conditions for a week particularly observing lack of activity, edema, diarrhea and ulceration.

The research committee Department of Pharmacology permitted the use of animals for these experiments in agreement with the guidelines of NIH [13] and NACLAR [14].

2.3. Plant Material and Preparation of Extract

N. nucifera fruits were obtained from the local fruit & vegetable market of Qasimabad, Hyderabad, Sindh, Pakistan. The N. nucifera fruits were identified and authenticated in the Department of Pharmacognosy. The receipt sample no NNF-03 was placed in the section of Pharmacognosy, University of Karachi.

Grounding of crude extract followed cold extraction procedure [15]. 6 kg fruits were washed and the seeds were peeled off from the seed pods of the fruits manually and were then chopped. After that chopped seeds were dried under shade for 6 days. The dried material was ground to coarse powder. The seed pods on the other hand were dried under shade for 3 days and then chopped to fine powder. The seed powder along with the seed pod powder was macerated in 10 liters of 98% ethanol for 30 days with occasional shaking and stirring until the color of the solvent becomes black.

The solvent was filtered with the help of Whatman No. 1 filter paper. Afterwards the extract was evaporated under reduced pressure in a rotary evaporator at 40°C to 45°C followed by freeze drying at -30°C. The solid Lyophilized material so obtained was saved at -20°C until further use in doses of 50, 100 and 200 mg/kg orally [1]. The final quantity of the extract obtained was 400 g of dry mass.

2.4. Preparation of Drugs

0.5% Corn oil was purchased from local medical stores in Karachi, while gum tragacanth and cholesterol powder were supplied by Merck. High cholesterol diet (HCD) *i.e.* 0.125 gm/kg cholesterol in 0.5% corn oil was given to rabbits for 30 days in order to derange lipid profile [16].

The suspending agent (gum tragacanth powder) was consumed to prepare suspensions of the control and 3 doses of test group (N. nucifera fruit extract 50, 100 & 200 mg/kg). It was administered to control group as placebo in the dose of 10 ml/kg per oral, every time fresh suspensions were made for the purpose of dosing [17] [18].

2.5. Effect of N. nucifera Fruit on Lipid Profile

A population of 28 white healthy rabbits of either sex was divided in to four groups. Control group was given gum tragacanth and 3 groups served as test groups and were given N. nucifera fruit extract 50, 100 and 200 mg/kg for 45 days. Initially animals of all four groups received high cholesterol diet (HCD) i.e. 0.125 gm/kg cholesterol in 0.5% corn oil for 30 days. All agents were given PO [16]. Blood samples were taken thrice from the ear vein of animals, 1st after 24 hours of thirty days of HCD then again after 24 hours of thirty and forty five days dosing of N. nucifera fruit extract i.e. on 31st and 46th day of experiment.

Blood samples (5 ml) were collected in gel tube. Serum was instantly separated



by centrifuging blood samples on 14 K Humax centrifuge at 3000 rpm for 15 min. Lipid profile was investigated on Humalyzer 3000 (semi-automatic chemistry analyzer, Model #16700) (Human Germany) using standard kits supplied by Human. TC and LDL-C was assessed by CHOD-PAP method; TG by GPO-PAP method [19] and HDL-C according to the method of Friedwald [20].

2.6. Statistical Analysis

The data was subjected to analysis by taking average and standard error to the average utilizing two sample student T-test and values of P < 0.05 were considered as noteworthy and P < 0.005 as extremely noteworthy. All statistical techniques were executed using SPSS software version 20 [21].

3. Results

Effects of N. Nucifera Fruit on Lipid Profile

Table 1 and **Table 2** gives the comparative effect of total serum cholesterol (TC), triglycerides (TG), high density lipoproteins (HDL-C) and low density lipoproteins (LDL-C) in animals of control and 3 extract groups after 30 and 45 days.

Table 1. Effect of N. nucifera fruit of	on lipid profile after 30 days.
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Groups	Parameters			
	Cholesterol mg/dl	Triglycerides mg/dl	HDL-C mg/dl	LDL-C mg/dl
Control 10 ml/kg	139.0 ± 1.30	249.8 ± 1.80	32.7 ± 0.68	179.8 ± 1.10
<i>N. nucifera</i> 50 mg/kg	133.8 ± 0.40*	223.1 ± 0.40**	32.1 ± 0.74	170.3 ± 0.68**
<i>N. nucifera</i> 100 mg/kg	118.0 ± 0.65**	180.5 ± 1.30**	33.8 ± 0.34	152.0 ± 0.82**
<i>N. nucifera</i> 200 mg/kg	90.8 ± 0.86**	142.0 ± 0.82**	36.5 ± 1.1*	129.3 ± 1.0**

n = 7; Values are Mean \pm S.E.M; *p < 0.05 noteworthy in comparison to control; **p < 0.005 extremely noteworthy in comparison to control.

Table 2. Effect of N. nucifera fruit on lipid profile after 45 days.

Groups	Parameters			
	Cholesterol mg/dl	Triglycerides mg/dl	HDL-C mg/dl	LDL-C mg/dl
Control 10 ml/kg	90.4 ± 1.0	187.8 ± 0.80	32.0 ± 0.72	144.4 ± 1.2
<i>N. nucifera</i> 50 mg/kg	83.8 ± 0.74**	172.0 ± 0.82**	35.7 ± 1.2*	133.1 ± 0.91**
<i>N. nucifera</i> 100 mg/kg	71.3 ± 0.89**	151.4 ± 0.90**	39.8 ± 0.40**	$112.4 \pm 1.10^{**}$
<i>N. nucifera</i> 200 mg/kg	54.3 ± 0.97**	131.0 ± 1.60**	43.0 ± 0.53**	92.4 ± 0.87**

n = 7; Values are Mean \pm S.E.M; *p < 0.05 noteworthy in comparison to control; **p < 0.005 extremely noteworthy in comparison to control.

No deaths of animal were recorded in any group all through the entire period of study. N. nucifera fruit ethanol extract at a dose of 50 mg/kg exhibited considerable lowering effects on TC after 30 days and extremely noteworthy effects after 45 days in comparison to control. The effects of the same dose of extract highly significantly lowered TG and LDL-C after 30 and 45 days whereas HDL-C was raised significantly after 45 days. N. nucifera fruit at a dose of 100 mg/kg revealed highly significant lowering effects on TC after 30 and 45 days as compared to control, whereas the TG and LDL-C lowering effects were also highly significant after 30 and 45 days in comparison to control. The HDL-C was highly significantly increased after 45 days but was not significantly altered after 30 days in comparison to control. N. nucifera fruit ethanol extract 200 mg/kg revealed highly noteworthy TC lowering effects after 30 and 45 days in comparison to control, whereas the TG and LDL-C lowering effects were also highly significant after 30 and 45 days in comparison to control. The HDL-C was significantly increased after 30 days and extremely considerably raised after 45 days as compared to control.

4. Discussion

Hypercholesterolemia or high levels of cholesterol in the blood are primarily a metabolic derangement that can cause many diseases such as CVD and stroke. Hypercholesterolemia or hyperlipidemia may occur due to abnormalities in lipoproteins, the particles that bring cholesterol to the blood stream. Hyperlipidemia has a strong association with atherosclerosis [22]. LDL cholesterol gets deposited in the blood vessel walls and plays major role in the formation of atherosclerotic plaque. These pathological processes can be reversed by lowering the serum LDL level [23]. Moreover increase in serum HDL level has shown to reduce the progression of atherosclerosis [24] [25].

Current study was distinctively planned to assess the hypolipidemic activities of N. nucifera fruit after 30 and 45 days in rabbits kept on high cholesterol diet for 30 days. The results showed remarkable cholesterol lowering effects of N. nucifera fruit extract at doses 50, 100 and 200 mg/kg but were particularly dominant at 200 mg/kg at which fruit extract highly significantly reduced TC, TG and LDL-C after 30 and 45 days and increased HDL-C significantly after 30 days and highly significantly after 45 days.

Previous studies have established an inverse relationship between flavonoids intake and total lipid concentration in plasma. Diet rich in flavonoids protects against coronary artery diseases [26] [27]. Similarly tannins and procyanidin have demonstrated lipid auto-oxidation and free radical scavenging activity in recent studies [28]. In several studies saponins have been shown to inhibit the intestinal absorption of cholesterol and there by reduces plasma lipid levels in variety of animal models [29] [30].

Previous studies on anti-obesity and hypolipidemic activities of N. nucifera seed resulted in inhibition of lipid accumulation and reduced expression of peroxisome proliferator activated receptor gamma (PPAR), leptin in cultured hu-



man adipocytes and glucose transporter (GLUT 4) indicating the inhibitory effect on the differentiation of pre-adipocytes in to adipocytes [31]. Moreover flavonoids, saponins, tannins and procyanidin are significant constituents of *N. nucifera* fruit, hence it can be postulated that lipid lowering effects of *N. nucifera* fruit can be due to the presence of all of these constituents which can contribute in lowering the risk of CVDs and stroke.

5. Conclusion

N. nucifera fruit has exhibited remarkable anti-hyperlipidemic activity which may be due to the presence of secondary metabolites especially flavonoids, saponins, tannins and procyanidin in both segments *i.e.* seeds and pods of this fruit which may be useful in managing conditions such as CVDs and stroke but more preclinical and clinical studies should be encouraged to confirm these findings.

Acknowledgements

The authors are thankful to Dr. Mohtesheem, Associate Professor and Chairman Department of Pharmacognosy for identification and authentication of *N. nuci-fera* fruits. The authors are also thankful to Prof. Dr. Iqbal Chaudary, Director, HEJ, Research Institute of Chemistry for granting permission to use Institute laboratory and workshop facilities.

Conflict of Interest

The authors declare no conflict of interest.

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