EFFECT OF *NELUMBO NUCIFERA* FLOWERS ON PLASMA LIPIDS AND GLUCOSE IN YOUNG, MIDDLE-AGED AND AGED RATS

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Summary

In the present study, the effect of *Nelumbo nucifera* was assessed by determining changes in plasma lipids and glucose of young, middle-aged and aged rats by virtue of its hypolipidemic properties. Male albino rats of Wistar strains were divided into six groups: groups 1, 3 and 5 were control young, middle-aged and aged rats received normal chow diet alone and groups 2, 4 and 6 were young, middle-aged and aged rats administered with *N. nucifera* (1000 mg/kg body weight/bidose/day) mixed with normal chow diet for 4 weeks. The increased levels of total cholesterol, triglycerides, free fatty acids, phospholipids, LDL cholesterol, VLDL cholesterol, glucose and a decreased in HDL cholesterol was observed in middle-aged and aged control rats when compared to young control rats. Administration of *N. nucifera* prevented the age associated changes on total cholesterol, triglycerides, free fatty acids, phospholipids, LDL cholesterol, VLDL cholesterol and glucose. HDL cholesterol level was found to be increased significantly in young, middle-aged and aged rats after treatment with *N. nucifera*. These finding demonstrated that *N. nucifera* normalized the age associated altered levels of lipids and glucose status in middle-aged and aged rats thereby decreasing the risk factors for cardio vascular diseases during aging process.

Keywords: Aging; coronary heart disease; glucose; lipids; *Nelumbo nucifera*

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Introduction

Aging is associated with complex and diversified changes of cardiovascular structure and function. Advanced age may induce a decline in bodily functions and overall cardiovascular performance even in the absence of overt disease [1]. Coronary artery disease (CAD) is the single most important disease entity in terms of both mortality and morbidity in the entire world population. Both men and women between the age group 40 and 60 are susceptible to it. The underlying cause of CAD is atherosclerosis – a disease involving a complex array of circulating blood proteins, lipoproteins and cells, and their interaction with the cells and matrix proteins of the arterial wall. It is well established that high circulatory serum cholesterol, low density lipoprotein and low levels of circulating high density lipoprotein cholesterol are the main causatives of this disease [2]. Aging is a predisposing factor in the process of atherogenesis. It is therefore relevant to compare the effects of aging and those of hyperlipidemia to determine if the early process of atherogenesis is equivalent to accelerated aging.

Hypercholesterolemia refers to levels of cholesterol in the blood that are normally higher than normal individual. Overwhelming evidence indicates that hypercholesterolemia and other lipid abnormalities provide an important modifiable risk factor for coronary heart disease (CHD) [3]. Risk of CHD increases progressively with higher levels of low-density lipoprotein cholesterol (LDL-c) while higher levels of high density lipoprotein cholesterol (HDL-c) reduces the risk significantly. Furthermore, CHD is also found to be associated with coronary endothelial dysfunction and myocardial perfusion abnormalities [3]. Dyslipidemias represent a group of disorders that comprise a variety of lipid abnormalities. These abnormalities are common key factors in the development of chronic allograft dysfunction, and they are independent factors associated with cardiovascular complications [4].

Nelumbo nucifera Gaertn. (Family: Nymphaeaceae), an aquatic herb with stout creeping rhizome found throughout India up to an altitude of 1,800m. *N. nucifera* is commonly found growing in ponds, tanks and jheels; it is often cultivated for its elegant sweet flowers. It is associated with Vishunu - preserver of the universe by the Hindus and the Lord Buddha the Buddhists. All parts of this plant are employed medicinally in the indigenous systems of medicine. The white flower is considered to be nutritive and a good tonic in general. In Siddha System of Medicine, *N. nucifera* is reported to cure cardiac diseases, liver disorders and dysentery. Flower decoction is used to reduce the body heat due to drug toxicity. Aphrodisiac, expectorant, cooling and sedative action was reported for the flowers of *N. nucifera* [5]. A decoction of the flowers is used in the treatment of premature ejaculation. The flowers are recommended as a cardiac tonic. It is used in treating bleeding gastric ulcers, excessive menstruation, post-partum haemorrhage. The flowers and fresh leaves ground with sandalwood or emblic myrobalans are used as a cooling application to the forehead in cephalagia, sking erysipelas and other external inflammations [6].

Flowers and petals of *N. nucifera* yielded quercetin, luteolin, isoquercitrin, glucoluteolin [7], n-triancontanol, α-amyrin, lupeol, β-sitosterol, amino acids - lysine, proline, hydroxyl-proline, β-phenylalanine, arginine, kaempferol-3-glycoside anonaine, lotusine, neferine. Flavanoids such as kaempferol along with β-sitosterol-glycopyranoside were also reported in *N. nucifera* [8]. The flowers of *N. nucifera* was reported for hypoglycemic [9], anti-pyretic [10], anti-inhibitory [11], antihypertensive [12], hepatoprotective [8,13], cardiovascular pharmacological [14], psychopharmacological [15], anti-arrhythmic and inhibits platelet aggregation [16,17].

In the present study, the effect *N. nucifera* was assessed by determining changes in plasma lipids and glucose of young, middle-aged and aged rats by virtue of its hypolipidemic properties.

Methods

Plant materials

Collection and Identification

The flower petals of *Nelumbo neucifera* were collected from the Kuttalam located in Nagapattinum district, Tamilnadu, India, during the months of July-August 2005. The flowers were identified and authenticated by Dr.M.Jegadessan, Professor and Head, Department of Environmental and Herbal Sciences, Faculty of Science, Tamil University, Vakaiyur, Thanjavur, Tamilnadu. The voucher specimens of *N. neucifera* have been deposited in the Tamil University Herbarium (Acc. No. TUH 267).

Preparation of the herbal drugs

The flower petals of *N. neucifera* were air-dried under shade and pulverized and used as drug in the powder form. The flower powders of *N. neucifera* were mixed with normal rat chow-diet at the time of use and given to the experimental rats throughout the study.

Therapeutic dose fixation

The therapeutic dose of *N. nucifera* was determined. Rats were supplemented with different doses (250, 500 and 1000mg/kg b.wt) orally for 2 weeks. Blood samples were collected on the 5th, 10th and 15th days. The activities of marker enzymes and lipid profiles were determined. Results were compared with related groups. Based on the results obtained from the study, the dose of 1000mg/kg body weight of *N. nucifera* exerted the maximum effect on controlling the activities of marker enzymes and lipid profiles [18]. This dose was selected to study the cardioprotective effect of *N. nucifera* flower petals on young, middle-aged and aged rats.

Animals and diet

Male albino rats of Wistar strain approximately 3–4 months old (young), 13–15 months old (middle-aged) and >24 months old (aged) were used in this study. Generally, 4-month-old male Wistar rats are considered as young rats, 16-month-old rats as adult older rats,

and 24-month-old rats as senescent rats [19]. The animals were housed in large spacious cages and were given food and water *ad libitum*. The animal room was well ventilated and had 12-h light/dark cycles throughout the experimental period. The animals were maintained on a commercial rat feed which contained 5% fat, 21% protein, 55% nitrogen-free extract, and 4% fiber (wt/ wt) with adequate mineral and vitamin contents. All animal experiments were conducted as per the instructions of Institutional Animal Ethics Committee.

Experimental design

The animals were divided into following six groups of each containing six animals.

Group 1 : control young rats received normal chow diet alone,
Group 2 : young rats administered *Nelumbo nucifera* (1000 mg/kg bw/bidose/day) mixed with normal chow diet for 4 weeks,
Group 3 : control middle-aged rats received normal chow diet alone,
Group 4 : middle-aged rats administered *Nelumbo nucifera* (1000 mg/kg bw/bidose/day) mixed with normal chow diet for 4 weeks,
Group 5 : control aged rats received normal chow diet alone,
Group 6 : aged rats administered *Nelumbo nucifera* (1000 mg/kg bw/ bidose/day) mixed with normal chow diet for 4 weeks.

Preparation of samples

On completion of the experimental period, animals were anaesthetized with thiopentone sodium (50mg/kg). Blood was collected from jugular vein with EDTA as anticoagulant. Plasma was separated out at 6000rpm for 5 min and used for the biochemical estimations.

Determination of lipid profile and glucose

The total cholesterol was estimated by the method of Allain et al. [20]. Triglycerides was estimated by the method of Werner et al. [21]. Free fatty acids were estimated by the method of Falholt et al. [22]. Phospholipid content was estimated by the method of Zilversmit and Davis [23] and liberated phosphorus was estimated by using Fiske and Subbarrow method [24]. HDL cholesterol was separated by adding phosphotungstic acid and magnesium chloride to the fresh samples to precipitate other lipoproteins and the HDL cholesterol was estimated by the method of Allain et al. [20]. The concentration of LDL cholesterol was calculated by using the Friedwald formula [25] and VLDL cholesterol was calculated by dividing the triglycerides value (in mg/dl) by 5. Serum glucose was estimated by the oxidase method [26].

Statistical analysis

Values were expressed as mean ± standard deviation for six rats in the each group and statistical significant differences between mean values were determined by one way analysis of variance (ANOVA) followed by the Tukey's test for post-hoc multiple comparison tests. Graphpad Instat Software Package was used and $p<0.05$ was considered to be significant.

Results

Table 1 shows the levels of plasma total cholesterol, triglycerides, free fatty acids and phospholipids in control and *N. nucifera* treated young, middle-aged and aged rats. The levels of total cholesterol, triglycerides, free fatty acids and phospholipids were found to be increased in plasma of middle-aged (21.48, 16.04, 24.75 and 17.45%) and aged (36.19, 38.62, 40.06 and 25.90%) control rats as compared with young control rats. As well as between middle-aged and aged control rats, 18.74% for total cholesterol, 20.35% of free fatty acids, 10.24% of phospholipids and 26.90% for triglycerides was increased in aged control rats. Reduced levels of total cholesterol (14.53, 25.71%), triglycerides (14.96, 23.87%), free fatty acids (22.27, 24.31%) and phospholipids (14.19, 13.79%) were observed in *N. nucifera* supplemented middle-aged and aged rats, respectively. *N. nucifera* supplementation did not bring noticeable alteration in young rats as compared with young control rats.

Table 1. Effect of *N. nucifera* on plasma total cholesterol, triglycerides, free fatty acids and phospholipids in young, middle-aged and aged rats.

Treatment	Total Cholesterol (mg/dL)	Triglycerides (mg/dL)	Free fatty acids (mg/dL)	Phospholipids (mg/dL)
Young Control	84.32 ± 3.12	76.34 ± 3.76	15.14 ± 1.04	84.33 ± 4.16
Young Treated	79.84 ± 3.23	72.16 ± 3.88	13.87 ± 1.09	80.62 ± 4.10
Middle-aged Control	107.38 ± 3.36 ^{a*}	90.92 ± 4.11 ^{a*}	20.12 ± 1.11 ^{a*}	102.16 ± 3.86 ^{a*}
Middle-aged Treated	91.78 ± 3.28 ^{b*}	77.32 ± 3.94 ^{b*}	15.64 ± 1.18 ^{b*}	87.66 ± 4.14 ^{b*}
Aged Control	132.14 ± 4.42 ^{a*,b*}	124.38 ± 3.86 ^{a*,b*}	25.26 ± 1.48 ^{a*,b*}	113.81 ± 4.52 ^{a*,b*}
Aged Treated	98.17 ± 4.37 ^{c*}	94.69 ± 3.96 ^{c*}	19.12 ± 1.24 ^{c*}	98.11 ± 4.33 ^{c*}

Values are expressed as mean ± SD for six rats in each group. Values are considered significantly different at $p < 0.05$ with post hoc Tukey-Kramer Multiple Comparisons Test. Statistically significant variations are compared as follows: ^{a}young control vs. young treated, middle-aged control and aged control; ^{b}middle-aged control vs. middle-aged treated and aged control; ^{c}aged control vs. aged treated. Statistical significance: $^{*}p < 0.001$.

Table 2. Effect of *N. nucifera* on plasma lipoproteins in young, middle-aged and aged rats.

Treatment	LDL-C (mg/dL)	VLDL-C (mg/dL)	HDL-C (mg/dL)
Young Control	27.91 ± 1.22	15.27 ± 0.71	41.14 ± 1.42
Young Treated	21.03 ± 1.14 ^{a**}	14.43 ± 0.69	44.38 ± 1.38 ^{a*}
Middle-aged Control	52.26 ± 1.02 ^{a**}	18.18 ± 0.68 ^{a**}	36.94 ± 1.26 ^{a**}
Middle-aged Treated	33.61 ± 1.07 ^{b**}	15.46 ± 0.78 ^{b**}	42.71 ± 1.22 ^{b**}
Aged Control	79.70 ± 2.74 ^{a**,b**}	24.88 ± 0.68 ^{a**,b**}	27.56 ± 1.21 ^{a**,b**}
Aged Treated	32.10 ± 1.42 ^{c**}	18.94 ± 0.77 ^{c**}	47.13 ± 1.23 ^{c**}

Values are expressed as mean ± SD for six rats in each group. Values are considered significantly different at $p < 0.05$ with post hoc Tukey-Kramer Multiple Comparisons Test. Statistically significant variations are compared as follows: ^{a}young control vs. young treated, middle-aged control and aged control; ^{b}middle-aged control vs. middle-aged treated and aged control; ^{c}aged control vs. aged treated. Statistical significance: $^{*}p < 0.01$, $^{**}p < 0.001$.

Table 2 represents the levels of plasma LDL, VLDL and HDL cholesterol in control and *N. nucifera* treated young, middle-aged and aged rats. The levels of LDL and VLDL were found to be increased (46.59, 64.98% for LDL and 16.01, 38.63% for VLDL) and HDL was decreased (10.21, 33.01%) in plasma of middle-aged and aged control rats as compared with young control rats. On comparison with middle-aged and aged control rats, 34.43% of LDL and 26.93% of VLDL were increased and 25.39% of HDL was decreased in aged control rats. *N. nucifera* supplementation decreased the contents of LDL (35.69, 59.72%), VLDL (14.96, 23.87%) and increased the level of HDL (13.51, 41.52%) in plasma of middle-aged and aged rats, respectively. *N. nucifera* supplementation showed significant reduction in plasma LDL (24.65%) and increase in the level of HDL cholesterol (7.30%) in young rats as compared with young control rats.

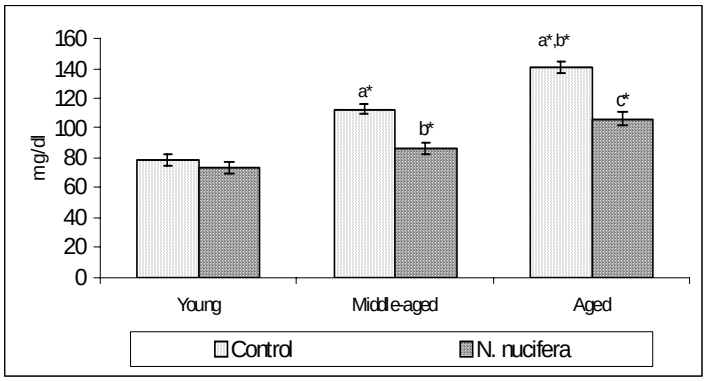

Figure 1. Effect of and *N. nucifera* on plasma glucose in young, middle-aged and aged rats. Values are expressed as mean ± SD for six rats in each group. Values are considered significantly different at $p<0.05$ with post hoc Tukey-Kramer Multiple Comparisons Test. Statistically significant variations are compared as follows: [a]young control vs. young treated, middle-aged control and aged control; [b]middle-aged control vs. middle-aged treated and aged control; [c]aged control vs. aged treated. Statistical significance: $^{*}p < 0.001$.

Figure 1 depicts the level of plasma glucose in control and *N. nucifera* treated young, middle-aged and aged rats. The level of glucose was found significantly ($p<0.001$) increased in middle-aged and aged control rats when compared with young control rats. The increase was 30.57% for middle-aged and 44.33% for aged rats respectively. On comparison with middle-aged and aged control rats, 19.82% of glucose was increased in aged control rats. After administration of *N. nucifera*, a significant decrease in the level of plasma glucose ($p<0.001$) in middle-aged (23.30%) and aged (24.24%) rats was found. *N. nucifera* did not bring any significant changes in plasma glucose level in young rats.

Discussion

Plasma lipid profile is generally considered as a reflection of the tissue metabolism and the permeability of cell membrane to various ions, which in turn depends on lipid composition [27]. Lipids play an important role in the pathogenesis of various diseases. In the present study, the level of plasma total cholesterol was increased in the middle-aged and aged control rats as compared with young control rats. Chylomicrons and VLDL transport the cholesterol in the circulation. HDL is the main substrate for LCAT for cholesterol esterification and incorporation. Fielding et al. [28] suggested that HDL and LCAT might play concerted roles in transporting cholesterol from peripheral tissues. Glomset [29] showed that addition of LCAT to the system led to an increase in HDL unesterified cholesterol and esterified cholesterol levels representing greater total cholesterol uptake

than in the absence of LCAT. Administration of *N. nucifera* depressed the total cholesterol levels in aged and middle-aged rats as compared with control rats. These effects of *N. nucifera* could result in efficient regulation of the key enzymes of lipid metabolism by normalizing the circulatory lipid concentrations. Treatment with *N. nucifera* decoction showed significant decrease in cholesterol levels [30]. In the present study, the reduction of total cholesterol levels elicits hypocholesterolemic effect of *N. nucifera* in different aged rats. Recent evidences show the benefits of reducing cholesterol is associated with a reduced risk of CHD [31].

In the present study, the level of plasma triglycerides was increased in the middle-aged and aged control rats as compared with young control rats. The elevated plasma triglycerides level observed in aging may be due to the lower activity of heart LPL. LPL, which is an enzyme normally expressed in a variety of tissues, most notably skeletal muscles, myocardium and adipose tissue. In the presence of its cofactor, apolipoprotein C-11, LPL hydrolyzes triglycerides contained in the triglyceride rich lipoproteins, namely VLDL and chylomicrons. Chylomicrons and VLDL transport triglycerides and cholesterol in the circulation. This results in the release of FFA that are taken up by myocytes for energy production or by adipocytes for energy storage. The increased synthesis of lipids would lead to a greater production and secretion of hepatic VLDL into plasma. This effect would compound the factors leading to the hypertriglyceridemia [32]. Hepatic LPL selectively hydrolyses the VLDL-TG forming partial glycerides and free fatty acids. Extra-hepatic LPL such as in heart is involved in the uptake of TG-rich lipoproteins from the circulation [33]. Administration of *N. nucifera* decreased the triglycerides levels in aged and middle-aged rats as compared with control rats, may be due to mobilization of fat and normalization the plasma lipids. Lowering of plasma TG concentrations may be attributed to the reduced availability of the precursor FFAs and to enhanced peripheral tissue clearance through increased LPL activity. Flavonoids administered rats showed a reduction in the level of triglycerides by enhanced the activity of lipoprotein lipase in heart of animals [34]. Treatment with *N. nucifera* decoction showed significant decrease in triglycerides levels [30]. In the present study, the reduction of triglycerides levels elicits hypotriglyceridaemic effect of *N. nucifera* in different aged rats.

In the present study, the level of plasma free fatty acids was increased in middle-aged and aged control rats as compared with young control rats. The significant elevation noted in the levels of free fatty acids might be due to enhanced breakdown of membrane phospholipids in plasma by the lipolytic action of phospholipase A2 [35]. The excess free fatty acid may be used for the synthesis of triglycerides [36]. Treatment with *N. nucifera* to middle-aged and aged rats reduced the elevated concentrations of free fatty acids by inhibit the activity of lipogenic enzymes.

Phospholipids are the essential structural components of animal cell membrane and cytoskeleton. The phospholipids are suitable targets in the course of oxidative processes due to the presence of esterified polyunsaturated fatty acids in their molecular structures and therefore study on content of phospholipids provides a measure of extent of susceptibility of membrane lipids towards peroxidation [37]. In the present study, the level of plasma phospholipids was increased in middle-aged and aged control rats as compared

with young control rats may be due to free radicals generated during aging process, which attacks the membrane phospholipids. The age-associated increase in plasma phospholipids has been reported in humans and rats [38,39]. Higher concentrations of phospholipids affect the physicochemical properties of the membrane and blood rheology [40]. Treatment with *N. nucifera* to middle-aged and aged rats, the level of phospholipids was decreased in plasma. The reduction of phospholipids observed may be due to the enhanced activity of phospholipase [39]. Maintenance of ambient levels of phospholipids in plasma of *N. nucifera* treated rats could be due to the scavenging of free radicals levels and decrease the process of lipid peroxidation by their antioxidant property. Previous investigation showed that *N. nucifera* has a cholesterol and phospholipid reducing effect [41].

In the present study, middle-aged and aged control rats revealed a drastic change in the lipoprotein fractions, mainly with the decreased HDL-c and increased LDL-c and VLDL-c concentrations. HDL is considered to be a beneficial lipoprotein [42] and has a negative effect in the development of atherosclerosis. It helps in the scavenging of cholesterol from extrahepatic tissues in the presence of LCAT and brings it to the liver. LDL-c and VLDL-c, both these lipoproteins have a positive role in atherosclerosis [43]. Lipoproteins are chemically modified by oxidation or glycation in the initial stages of atheroma formation. These oxidized or modified lipoproteins do not react with LDL receptors, leading to esterification of cholesterol and conversion of macrophages to foam cells, thereby contributing to the development of atherosclerosis [44]. Thus, LDL receptor plays an important role in LDL metabolism. Under normal conditions, about 80% of the circulating LDL particles are cleared by the liver primarily through an LDL receptor-mediated process [45]. The elevated levels of LDL cholesterol observed in the present finding with aging could be due to LDL receptor deficiency. Further, the lowered HDL concentration can be attributed to the decreased LPL and LCAT activities in plasma. LCAT an enzyme that catalyzes esterification of cholesterol with FFAs along with LPL that is responsible for HDL synthesis. HDL plays an important role in cholesterol and triglycerides transport and their metabolism [46]. The decreased level of HDL can be related to the decreased activity of LCAT in plasma that indicates impairment in HDL synthesis as well as triglycerides metabolism in aging rats.

Treatment with *N. nucifera* to middle-aged and aged rats, the HDL concentration was increased, whereas those of LDL and VLDL were decreased. The rise in the plasma concentrations of HDL might evidence the increased synthesis of HDL constituents. Moreover, stimulation of LPL leads to a rise in HDL production and reduction in VLDL constituents [47]. Recent evidences show the benefits of reducing LDL-c is associated with a reduced risk of CHD [31]. Increased circulating HDL-cholesterol levels, which lends evidence to its protective role in the development of cardiovascular disease [46]. Niacin (nicotinic acid), an essential B-group vitamin, increases HDL-c and decreases LDL-c [48]. It has been reported that niacin has the plasmic lipid-regulating properties and is beneficial for cardiovascular effects [49,50]. The effect of flavonoids and flavonoid rich extracts on reducing lipid levels effectively has been studied [51]. The lipid lowering ability of *N. nucifera* related to the presence of flavonoids which could be due to inhibition of cholesterol biosynthesis and to increased faecal bile acid excretion.

Furthermore, the pre-diabetic state that is characterized by obesity, hyperinsulinemia, and hyperglycemia, may be a sufficient alert for elevated cardiovascular disease risk [52]. Glucose intolerance, a component of type 2 diabetes, has been independently associated with the risk of cardiovascular disease [53,54]. In the present study, the level of plasma glucose was increased in middle-aged and aged control rats as compared with young control rats. It suggests that the increase in glucose levels is due to release of glucose from the liver and also due to the reduced glucose oxidation that leads to hyperlipidemia causing accumulation and increased synthesis of cholesterol, triglycerides, lipoproteins and free fatty acids resulting from excess mobilization of fat from the adipose due to the under utilization of glucose.

Administration of *N. nucifera* had reduced the blood glucose levels in middle-aged and aged rats, evidencing the increased utilization of glucose, further suppressing the mobilization of fat and normalized the plasma lipids. The possible mechanism for the observed anti-hyerglycemic effect of *N. nucifera* may be due to increased ability of insulin to mediate tissue glucose uptake, and thus helpful to maintain glucose homeostasis. Lowering of glucose along with plasma lipids levels seems to be associated with a decrease in the risk of vascular diseases. *N. nucifera* markedly reduced blood sugar level of normal, glucose-fed hyperglycemic and streptozotocin induced diabetic rats [55]. The ameliorative effect of *N. nucifera* in glucose levels can be related to the reduction of hyperinsulinemia which is a common phenomenon that occur in the elderly and may suggest the beneficial effect on glucose regulation in aged rats.

In conclusion, the administration of *N. nucifera* prevented the age associated changes on total cholesterol, triglycerides, free fatty acids, phospholipids, LDL cholesterol, VLDL cholesterol and glucose. HDL cholesterol level was found to be increased significantly in young, middle-aged and aged rats after treatment with *N. nucifera*. From this, the present study suggests the possible role of *N. nucifera* flower petals as therapeutic agent in normalizing the age associated altered levels of lipids and glucose status and decreasing the risk factors for cardio vascular diseases and diabetes mellitus during aging process.

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