Phytochemical Analysis and Anti Hyperlipidemic Activity of *Nelumbo Nucifera* in Male Wistar Rats

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Research Article

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Abstract

Hyperlipidemia is the leading cause for the development of various diseases made pharmaceutical companies to turn towards the herbal products with fewer side effects. In the present research, the Hyperlipidemia activity of *Nelumbo nucifera* Flower (NN) along with their phytochemical evaluation has been done. The Hyperlipidemia effect of hydroalcoholic extract of *Nelumbo nucifera* Flower was evaluated in Poloxamer 407 induced hyperlipidemic in male Wistar rats. Hyperlipidemia was induced by giving Poloxamer 407 intrapertonial route for 15 days. The groups of rats selected for the study were treated with Atorvastatin, ethanol extract of *Nelumbo nucifera* (NN). The analysis of lipids profile such as cholesterol, HDL, LDL, VLDL, Triglycerides and liver markers such as SGOT, SGPT were carried out at the end of the study. Administration of NN significantly decreases the Lipid profile and Liver Markers. Likewise, remarkable increase in the level of HDL-C when compared to standard Atorvastatin drug. The levels of SGOT and SGPT were estimated and found to be significantly less than that of hyperlipidemic control group. The results reveal that NN is a rich source for phytoconstituents and can be used as a potent anti Hyperlipidemic agent in pharmaceutical industry.

Keywords: *Nelumbo nucifera, Hyperlipidemia, Poloxamer 407, Lipid profile, Liver Markers, Phytoconstituents.*

Introduction

Hyperlipidemia is characterized by elevated serum lipid profile in the blood circulation (Ochani et al., 2009). Hyperlipidemia has been ranked as one of the greatest risk factors contributing to prevalence and severity of coronary heart diseases, stroke, atherosclerosis and hyperlipidemia are the primary cause of death (Grundy, 1986; Smith et al., 1993). The World Health Organization (WHO) reported that the high blood cholesterol contributes to approximately 56% cases of cardiovascular diseases worldwide and causes about 4.4 million deaths each year. In India it is presumed that more than 111% of the people's death by cardiovascular disease when compared to the year 1990 (Rajasekhar et al., 2011). This is much higher than that predicted to any other region both in Asia as well as outside Asia. In India, the prevalence of CHD is much higher in south when compared to north India (Mohan et al., 2001; Gupta et al., 2002).

In addition hyperlipidemia is induced by secondary effect of diabetes, therefore, the agent having some antioxidant and anti-diabetic effect also showed favorable effect to hyperlipidemia. HMG-CoA reductase inhibitor has been used in the treatment of hyperlipidemia and Simvastatin is one of the most prevalently used HMG-CoA reductase inhibitors (Sae Kwang Ku et al., 2006).

Currently available drugs have been associated with number of side effects. The consumption of synthetic drugs leads to hyperuricemia, diarrhoea, nausea, myositis, gastric irritation, flushing, dry skin and abnormal liver function. Medicinal plants are used for various research purposes (Brown, 1996).

Medicinal plants are believed to be an important source of new chemical substances with potential therapeutic effects and more than 80% of population of developing countries is dependent on traditional folk medicine therapies for treating their ailments. Lotus (*Nelumbo nucifera*) is a perennial

aquatic plant with yellow flowers. It is utilized as a dietary staple and also for a variety of medicinal purposes in Eastern Asia, particularly in China. This plant is belonging to the Family Nelumbonaceae. All parts of Lotus are used for various medicinal purposes in oriental medicine (Taoying Zhou et al., 2009). This plants are very effective to potential antioxidant activity (Rai et al., 2006), antipyretic (Deepa et al., 2009), Antiplatelet activity (Durairaj et al., 2010) and Hypoglycemic activity (Mani et al., 2010). In the present study, to evaluate hyperlipidemic activity by using hydroalcoholic flower extract of *Nelumbo nucifera*.

Material and Method

Plant Material

This plant was collected from various areas in bulk such as Thovalai, Nagercoil, Tamil nadu, India. This plant was authenticated at Rabinat Herbarium, St. Joseph College, Trichy, Tamil nadu, India.

Extraction

The pulverized plant materials were taken up for extraction using hydroalcohol in the proportion of 30:70. The extraction was carried out by cold percolation method. The extracts were then dried in vacuum and they were stored in desiccator and subsequently to a refrigerator.

Preliminary phytochemical analysis:

Quantitative and Qualitative estimation of Phytoconstituents

The concentration of total phenolic content (Bray et al., 1954), tannin, carbohydrate (Dubois et al., 1956), Vitamin C (Sarojini et al., 1999) and Vitamin E (Jayashree et al., 1985) were estimated in *Nelumbo nucifera* flower extract. The extracts were tested for the presence of various phytoconstituents like flavonoids, tannin, alkaloids by following the method of Trease and Evans, 1958.

Experimental animals

Male albino wistar rats weight about 180-230gm obtained from Swamy Vivekananda College of Pharmacy, animal house were used for the study. They were housed, under standard laboratory conditions at room temperature (21ºC±2ºC) and relative humidity of 55-60%. They were fed with standard pellet diet and water *ad libitum*. The study protocol was approved by the Institutional Animal Ethical Committee (IAEC), Swamy Vivekandha College of pharmacy (Proposal No. SVCP/IAEC/M.Pharm/07/2011) and experiments were conducted in accordance with guidelines set by the CPCSEA (Committee for the purpose of control and supervision of experiments on animals), New Delhi, India.

Acute Toxicity Studies

The acute toxicity of the hydroalcoholic flower extracts of *Nelumbo nucifera* was evaluated in rats using the up and down procedure. Acute oral toxicity was performed as per OECD- 420 guidelines (acute class method) [OECD, 2001].

Rats of either sex (three females and three males, weight: 150-200g, received hydroalcoholic extract of *Nelumbo nucifera* starting at 2g/kg orally by gavage. The animals were observed for toxic symptoms continuously for the first 3 hours dosing, finally, the number of survivors was noted after 24 hours and these animals were then maintained for further 14 days with observations made daily.

Experimental Protocol for Poloxamer 407 (Px) Induced Hyperlipidemia

The 36 male *Wistar rats* were randomly divided into 6 groups each containing six animals (n=6). The experimental hyperlipidemia was induced by 300mg/kg of Poloxamer 407 dissolved in cold water (4^{o}C) injected on 14 days, 30 min after the administration of extract and standard.

Group 1: Administered vehicle 1% CMC p.o., served as normal control.(Yee Hor et al., 2001)

Group 2: Administered 300 mg/kg of Poloaxmer 407 in ip ., served as hyperlipidemic control.
(Nash et al., 1996)

Group 3: Administered 300 mg/kg of of Poloaxmer 407 in ip. + Atorvastatin 20mg/kg b.w.,
suspended in 1% CMC p.o.

Group 4: Administered 300 mg/kg of of Poloaxmer 407 in ip. + NNFE 100mg/kg b.w.,
suspended in 1% CMC p.o.

Group 5: Administered 300 mg/kg of of Poloaxmer 407 in ip. + NNFE 500mg/kg b.w.,
suspended in 1% CMC p.o.

Group6: Administered 300 mg/kg of of Poloaxmer 407 in ip.+NNFE 1000 mg/kg b.w.,
suspended in 1% CMC p.o.

At the end of the experimental period, the animals were fasted overnight, blood was collected by cardiac puncture and plasma was analysed for TGs, TC, HDL-C, LDL, VLDL, SGOT, SGPT and Atherogenic Index (AI).

Plasma total cholesterol and High Density Lipoprotein–Cholesterol (HDL-C) concentrations were determined using enzymatic kits from Accurex Biomedical Pvt.Ltd., Thane. Triglycerides concentration of Plasma was determined by using Triglyceride kit (Accurex Biomedical Pvt.Ltd., Thane). Low Density Lipoprotein-Cholesterol (LDL-C) concentrations were then determined using the Friedewald equation. SGOT levels were determined by using AST reagent kit (Aspen Laboratories, Baddi, H.P). SGPT levels were determined by using ALT Reagent kit (Aspen Laboratories, Baddi, H.P).

Statistical Analysis

The values are expressed in mean ± SEM. One way ANOVA followed by Tukey's multiple comparison Test was used to analyse the effect of different doses of drugs when compared to control, with the help of Graph Pad Instat software, version 3.01. P<0.05 is considered as significant.

Results

In this study the hydroalcoholic extract of *Nelumbo nucifera* showed positive to the following phytoconstituents such as alkaloids, carbohydrates, saponins, tannins, flavonoids, total phenolic substances, glycosides, carotenoids, fatty acids and terpenoids. The results are tabulated in Table 1. Quantitative analysis of NN extract showed the presence of various phytoconstituents in Table 2. The lipid profile and liver markers showed significantly reduced in Table 3.

Table 1. Preliminary Phytochemical Screening of *Nelumbo nucifera* Flower Hydroalcoholic Extract (NNFE)

Name of the Phytoconstituents	**NNFE (µg/100gm)**
Total Phenolic substances	10.20± 0.1
Protein	34.0±1.2
Vitamin C	0.36±0.2
Vitamin E	0.42±0.2
Tannins	4.30±0.3
Carbohydrates	672±2.3

Table 2: Quantitative Phytochemical Screening of *Nelumbo nucifera* Flower Hydroalcoholic Extract

Name of the Phytoconstituents	**NNFE extract**
Carbohydrates	+
Total Phenolic substances	+
Glycosides	-
Alkaloids	+
Proteins and Amino acids	+
Flavonoids	+
Tannins	+
Phytosterols	+
Terpenoids	-
Saponins	-
Fixed oil and Fats	+
Carotenoids	-

Table 3. Effect of *Nelumbo nucifera* Flower Extract on Lipid Profile and Liver Markers in Poloxamer 407 induced Hyperlipidemic Rats

Discussion

Preliminary phytochemical screening of hydroalcoholic extracts of *Nelumbo nucifera* (NN) flower was done. Result showed the presence of the following phytochemical constituents such as total phenolic substances, glycosides, alkaloids, flavanoids, tannins, terpenoids and saponins are the values qualitative in nature (**Table 1**), quantitative phytochemical constituents present in **Table 2**. The hydroalcoholic extract of *Nelumbo nucifera* was found to be non–toxic up to the dose of 2g/kg and did not cause any death of the tested animals. In the present study the results are comparable to the values of Gharak and Asthana, (1995) and Jian Wang (2008). The mechanism of P-407 in hyperlipidemia is enhanced activity of Microsomal 3-Hydroxy-3-methylglutaryl CoA Reductase (Johnston et al., 1997). So that Poloxamer 407 significantly increased total cholesterol and triglyceride level (P<0.001) (Wasan et al., 2003).

The results are discussed under the lipid profile and liver markers. Lipid changes observed in P-407 induced rats are noted to have elevated levels of cholesterol, TGL, LDL, VLDL, SGOT, SGPT and HDL-C was decreased. Hence the flowers were taken up for antihyperlipidemic activity in poloxamer 407 - induced rats. Administration of NN extract in the doses level of 100, 500 and 1000 mg/kg effectively prevented the elevation of lipid parameters and liver markers when compared to poloxamer 407 treated rats. It was also noted that HDL significantly increased in extract group when compared to the standard drugs Atorvastatin 20 mg/kg.

A significant percentage reduction of serum cholesterol, triglyceride, LDL, VLDL, SGOT, SGPT and increase in HDL in test extract was also comparable with the standard drug. A potent hypolipidemic effect of hydroalcoholic extract was evident by a significant reduction in the level of serum cholesterol, LDL, VLDL and triglycerides, SGOT, SGPT in the poloxamer 407 treated animals and also marked increase in the HDL level (**Table 3**). The Atherogenic Index was considerably decreased in the plant extract group which was also comparable with the standard group Atorvastatin against hyperlipidemia. *Nelumbo nucifera* (100, 500 mg/kg) displayed a significant (P<0.001) decrease when compared to hyperlipdemia control group. This indicates that hydroalcholic extract of *Nelumbo nucifera* (NN) significantly reduces the serum

cholesterol level (la Cour et al., 1995). The serum triglyceride levels have been reported to be

Table 3. Effect of *Nelumbo nucifera* Flower Extract on Lipid Profile and Liver Markers in Poloxamer 407 induced Hyperlipidemic Rats

The values are expressed as mean ± SEM; ### P<0.001,##P<0.01, #P<0.05 when the similarity with normal control is looked for the same. *** P<0.001, ** P<0.01, * P<0.05 when compared to hyperlipidemia control. ^P<0.05, ^^P<0.01, ^^^P<0.001 when compared to Atorvastatin 20 mg

Groups	Cholesterol (mg/dl)	TGL (mg/dl	HDL (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)	AI (mmol/dl)	SGOT (IU/L)	SGPT (IU/L)
Normal Control	165.4 ± 6.277	97.5± 6.244	45.8 ± 0.991	82.95 ± 15.04	58.28 ± 17.42	0.338 ± 0.0176	18.8 ± 0.994	31.41 ± 1.828
P – 407 C	414.5 ± 14.863 ###	479.25 ± 74.35 ###	38.31 ± 4.712 ##	236.83 ± 9.673 ###	183.25 ± 47.15 ###	1.0721 ± 0.0784 ###	69.93 ± 6.048 ###	62.18 ± 5.382 ###
Atorvast 20mg/kg	203.16 ± 5.186 **	280.5 ± 15.35 ***	42.76 ± 0.745 ***	86.53 ± 17.23 ***	56.10 ± 3.071 ***	0.812 ± 0.0259 ***	38.61 ± 2.569 **	43.16 ± 2.272 **
NN 100 mg/ kg	221.33 ± 4.208 ## ***	308.25 ± 74.705 ## ***	44.01 ± 0.761 *	138.33 ± 4.849 ## ***	38.613 ± 2.085 ### ***	0.647 ± 0.025 ###**	50.45 ± 2.896 ## # ***	48.1 ± 2.769 ## # ***
NN 500 mg/ kg	195.83 ± 40.746 ***	196.1 ± 9.030 ## #***	45.33 ± 2.009 ***	151.75 ± 8.849 ***	37.75± 3.979 ## ***	0.559 ± 0.036 ***	51.88 ± 3.671 ***	50.9 ± 3.278 ***
NN1000 mg/ kg	270.23 ± 17.781 ***	168.6 ± 9.030 *** ^^^	52.23 ± 3.335 ***^	153.33 ± 11.197***^	60.83± 14.595***^^ ^	0.7571± 0.084***	56.83 ± 3.260 ***	56.6 ± 2.365 ***

an important risk factor as it influences lipid deposition and clotting mechanisms (Rumsey et al., 1994). Like cholesterol, it tends to damage vascular endothelial cells (Harnafi et al., 2009). HDL is considered to be a beneficial lipoprotein as it is involved in reverse cholesterol transport and has an inhibitory effect in the pathogenesis of atherosclerosis. So increased HDL-C has a cardio protective effect (Berliner et al., 1996). Flavonoids are also reported to increase HDL-C concentration and decrease in LDL and VLDL levels in hypercholesteremic rats. The plant extract used in the study (P<0.001) has characteristically reduced the TC, TG, LDL VLDL, as well as increased (p< 0.001) HDL – C level. Significant lowering of TC and increase in HDL are very desirable biochemical state for the prevention of atherosclerosis and ischemic condition (Dobiasova, 2006). Liver damage is always associated with cellular necrosis, increase in lipid peroxidation and depletion in the tissue GSH levels. In addition serum levels of many biochemical markers like SGOT, SGPT, ALP and bilirubin levels are elevated (Felig et al., 1970; Mascolo et al., 1988; Mossa et al., 1991). The plant extract showed significant lowering of SGOT, SGPT levels.

Conclusion

The *Nelumbo nucifera* flower hydroalcoholic extract (NNFE) is found to be a potential anti-hyperlipidemic activity in poloxamer - 407 induced hyperlipidemia in male albino Wistar rats and it is observed to reflect a significantly reduction of cholesterol, triglycerides, LDL-C, VLDL-C, and increases HDL-C. The *Nelumbo nucifera* also reduces SGOT and SGPT levels particularly in liver. The hypolipidemic effect of NNFE may be due to the presence of phenolic substance, flavonoids, tannins, alkaloids and Vitamin C and Vitamin E. It is observed that by estimating various biochemical parameters like lipid profile and qualitative and quantitative phytochemical evaluation an ocean changes is obtained. The extracts of NN exhibit its activity as dose dependent. From our study, the plant extract of NN was proved to be hypolipidemic and also possess antioxidant activity against poloxmer 407 induced rats. The drug does not cause any side effect on experimental rats. *Nelumbo nucifera* extract administered animals showed reduction in serum cholesterol level that was mainly due to decrease in VLDL and LDL levels. It thus, reduced the atherogenic index.

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AUTHORS' CONTRIBUTIONS

Authors contributed equally to all aspects of the study.

PEER REVIEW

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CONFLICTS OF INTEREST

The authors declare that they have no competing interests.