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EVALUATION OF ANTIHYPERLIPIDEMIC ACTIVITY OF *NYMPHAEA ALBA*

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ABSTRACT: Hyperlipidemia is the greatest risk factor of coronary heart disease, currently available hypolipidemic drugs have been associated with number of side effects. Literature claims that flavonoids are able to reduce hyperlipidemia. Based on high flavonoids content in herbal plants, *Nymphaea alba* (NA) family Nymphaeaceae were selected and the present study focus on the antihyperlipidemic activity of methanol extract of leaves of NA against triton induced hyperlipididemia in rats. NA was administered at a dose of 100mg &200mg /kg (p.o) to triton induced hyperlipidemic rats. Fenofibrate was used as a reference standard. The statistical analyses were carried out using one way ANOVA followed by dunnets multiple comparisions test. NA shows a significant decrease in the levels of serum cholesterol, phospholipids, triglyceride, LDL, VLDL and significant increase in the level of serum HDL at the dose of 100 & 200mg/kg (p.o) against trition induced hyperlipidemic in rats. Methanol extracts decreased serum level of total cholesterol by 74.25%. On the other hand aqueous extract of NA increased the serum HDL cholesterol level by 23.27%. The reduction of LDL cholesterol level by extract was 32.76%.

INTRODUCTION: Hyperlipidemia has been ranked as one of the greatest risk factors contributing to the prevalence and severity of coronary heart diseases [1]. Coronary heart disease, stroke, atherosclerosis and hyperlipidemia are the primary cause of death [2]. Although many efficacious lipid-lowering synthetic drugs exist, none is effective for all lipoprotein disorders, and all such agents are associated with some adverse effects.

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Therefore it is a need of the day to search other materials from natural sources that are less toxic, less expensive, which can provide better safety and efficacy on a long term usage. Natural products from plants are a rich source used for centuries to cure various ailments.

Plants are well known in traditional herbal medicine for their hyperlipidaemic activities, and available literature indicates that there are more than 800 plant species showing hyperlipidaemic activity *Nymphaea alba* is also known as the European white water lilly, white lotus, is an aquatic flowering plant of the family Nymphaeaceae having erected perennial rhizomes. The leaves may be upto 30cm in diameter and they take up and spread of 150cm per plant. The flowers are white and they have many small stamens inside.

It is rich in tannic acid, gallic acid, alkaloids, sterols, flavonoids, glycosides, hydrolysable tannins and high molecular weight polyphenolic compounds [3].

All the parts of the plant have medicinal use in traditional system of medicine. It is used as an aphrodisiac, anodyne, antiscrophulatic, astringent, cardiotonic, demulcent, sedative and anti-inflammatory. Further it also produces calming and sedative effects upon the nervous system and is useful in the treatment of insomnia, anxiety and similar disorders [4-6]. It anticarcinogenic action and inhibition of renal oxidative stress and hyperproliferative resoponse were reported [7-9]. It also possesses good anxiolytic activity [10]. Gallic acid and ellagic acid are two widely occurring phenolic compounds present in *Nymphaea alba*, to which many biological activities including anticancer and antiviral activity have been attributed [11].

Based on above medicinal properties of *Nymphaea* species, the present study was conducted to investigate the antihyperlipidemic activities of methanol extract of *Nymphaea alba* leaves.

MATERIALS AND METHODS:

Plant material:

Nymphaea alba leaves were freshly collected at Japali tirtham ponds, Tirupati, Chitoor district of Andhra Pradesh, India. The plant material was authenticated by Dr. Madhavachetty, Department of Botany, Sri Venkateswara University.

Animals:

Normal healthy male Wistar albino rats (150-200g) were housed under standard environmental conditions at temperature (25±2ºC). All the experimental protocols used in this study were reviewed by the Institutional Animal Ethics Committee and were in accordance with the guidelines of the CPCSEA.

Experimental Animals:

Wistar albino adult male rats weighing 200-250g and mice weighing 20-25g for acute toxicity studies were obtained from the Mahaveer enterprises, Hyderabad, India.

Acute toxicity studies:

Albino mice weighing 20-25g selected by random sampling technique were used in the study. Acute oral toxicity was performed as per OECD- 423 guidelines (acute class method) [12].

The animals were fasted overnight, provided only water after which extract was administered to the groups orally at the dose level of 5mg/kg body weight by gastric incubation and the groups were observed for 14days. If mortality was observed in 2 or 3 animals among 6 animals then the dose administered was assigned as a toxic dose. If mortality was observed in one animal, then the same dose was repeated again to confirm the toxic dose. If mortality was not observed, the procedure was repeated for further higher doses such as 50, 300 and 2000 mg/kg body weight. The animals were observed for toxic symptoms such as behavioral changes, locomotion, convulsions and mortality for 72 hours.

Antihyperlipidemic studies:

The animals were divided into five groups of five rats each. The first was given standard pellet diet, water and orally administered with 5% CMC. The second group was given a single dose of triton administered at a dose of 400 mg/kg, p.o. after 72 hours of triton injection, this group received a daily dose of 5% CMC (p.o) for 7 days. The third and fourth group was administered a daily dose of NA aqueous extract 100 mg/kg and 200 mg/kg suspended in 5% CMC, p.o., for 7 days, after inducing hyperlipidemia. Fifth group was administered with the standard Fenofibrate 65 mg/kg, p.o for 7 days [13].

Collection of Blood:

On the 8th day, blood was collected by retero orbital sinus puncture, under mild ether anaesthesia. The collected samples were centrifuged for 10 minutes. Then serum samples were collected and used for various biochemical experiments. The animals were then sacrificed and the liver collected [14].

Liver lipid extraction:

The liver was homogenized in cold 0.15 M KCL and extracted with CHCl3 CH3OH (2%V/V) this lipid extract was used for the estimation of lipid parameters [15].

Biochemical Analysis:
The serum and liver extract were assayed for total cholesterol, triglycerides, phospholipids, high-density lipoprotein (HDL), low density lipoprotein (LDL), very low density lipoprotein (VLDL) using standard protocol methods [16].

Statistical analysis:
The results were expressed as mean ± SEM. Statistical analysis was carried out by using ANOVA followed by Dunnet's multiple comparison tests using Graph pad PRISM software version 4.03 (2005). P values < 0.05 were considered as statistically significant.

RESULTS & DISCUSSION: *N. alba* methanol extract was found to be non–toxic up to the dose of 2 g/kg and did not cause any death of the tested animals. Hyperlipidemia is associated with heart disease, which is the leading cause of death in the world. The results are discussed under the lipid profile in serum and the lipid profile in liver. Lipid profile in serum and liver indicates that increased phospholipids (PL), triglyceride (TG) and cholesterol levels were significantly reduced by treatment of 100 and 200 mg/kg of NA. LDL and VLDL levels were significantly increased in triton-injected animals to control rats. The results are shown in **Tables 1, 2**.

The NA markedly lowers the levels of serum cholesterol and VLDL. The decrease in cholesterol may indicate increased oxidation of mobilized fatty acids of inhibition or lipolysis. The present investigation shows that all triton induced rats displayed hyperlipidemia as shown by their elevated levels of serum and liver cholesterol, triglyceride, PL, VLDL, LDL and the reduction in the HDL level. It can be concluded that NA 100 and 200 mg/kg treatment was effective in cholesterol, PL, TG, VLDL, LDL and HDL in a dose dependant manner.

Triton Wr-1339 has been widely used to block clearance of triglyceride-rich lipoproteins to induce acute hyperlipidemia in several animals [17]. This model is widely used for a number of different aims particularly, in rats it has been used for screening natural or chemical hypolipidemic drugs [18].

TABLE 1: EFFECT OF METHANOLIC EXTRACT OF *NYMPHAEA ALBA* ON HDL, LDL, VLDL IN SERUM OF CONTROL AND EXPERIMENTAL RATS

Groups	Parameters		
	HDL	LDL	VLDL
Group-I Control	18.31±1.22	20.25±2.64	11.31±3.16
Group-II Trition treated	19.28±3.20	15.49±7.21	20.01±1.06
Group-III Trition + NA (100mg/kg)	21.81±2.46	38.76±2.70	18.35±2.78
Group-III Trition + NA (200mg/kg)	23.27±4.12	32.76±3.21	14.20±4.10
Group-III Trition + fenofibrate	23.28±4.09	26.35±4.80	13.35±2.35

TABLE 2: EFFECT OF METHANOLIC EXTRACT OF *NYMPHAEA ALBA* ON CHOLESTEROL, TRIGLYCERIDES, PHOSPHOLIPIDS IN SERUM OF CONTROL AND EXPERIMENTAL RATS

Groups	Parameters		
	Hyperlipidemic	Triglycerides	Phospholipids
Group-I Control	60.26±3.21	70.23±4.25	152.70±8.21
Group-II Trition treated	190.01±7.35	110.10±4.20	200.30±9.26
Group-III Trition + NA (100mg/kg)	85.36±4.21	92.06±2.85	189.42±6.25
Group-III Trition + NA (200mg/kg)	74.25±3.28	78.20±4.80	175.20±4.20
Group-III Trition + fenofibrate	68.35±5.22	71.32±5.21	81.35±6.32

CONCLUSION: Interestingly, the results of the present study show that extract of *N. alba* produced a significant reduction in cholesterol level and also it reversed Triton induced hyperlipidemia in rats.

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