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EVALUATION OF ANTIHYPERLIPIDEMIC ACTIVITY OF *NELUMBO NUCIFERA* (LOTUS) FLOWER EXTRACT USING POLOXOMER 407 MODEL

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Abstract: Aim & objective: Evaluation of anti-hyperlipidemic activity of *Nelumbo nucifera* flower extracts (NNFE) (125 mg/kg, 250 mg/kg and 500 mg/kg) using Poloxomer 407 induced hyperlipidemic model. Materials and methods: Hydroethanolic NNFE at different doses (125mg/kg, 250 mg/kg and 500 mg/kg) were evaluated. Hyperlipidemia was induced by Poloxomer 407 at the dose of 1g/kg, i/p and blood was collected at 15 & 24 hrs. Results: From lipid profile, it was found that rats treated with hydroethanolic NNFE at doses 125 mg/kg and 250 mg/kg showed less significant antihyperlipidemic activity whereas rats treated with hydroethanolic NNFE at the dose of 500 mg/kg decreased TC, TG, LDL, VLDL and improved HDL when compared with hyperlipidemic rats. Hydroethanolic NNFE treated rats showed significant results in comparison with atorvastatin (positive control) treated rats and improved Lipid Profile. Conclusion: The results demonstrated that hydroethanolic NNFE has antihyperlipidemic potential. By inhibiting the cholesterol and triglycerides synthesis it, improves total cholesterol and triglycerides level.

Keywords: Antihyperlipidemic activity, Poloxomer 407, *Nelumbo nucifera* flower extracts (NNFE).



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INTRODUCTION

Hyperlipidemia is characterized by elevated serum total cholesterol, low-density, and very low-density lipoprotein and decreased high density lipoprotein levels. It has been ranked as one of the greatest risk factors contributing to the prevalence and severity of coronary heart diseases. Coronary heart disease, stroke, atherosclerosis and Hyperlipidemia are the primary cause of death. Hyperlipidemia associated lipid disorders are considered to cause atherosclerotic cardiovascular disease. Among these, Hypercholesterolemia and hypertriglyceridemia are closely related to ischemic heart disease.¹

The American Heart Association has identified the primary risk factor associated with the development of atherosclerosis. It is the elevated levels of cholesterol and triglyceride in the blood. Therefore the clinician considers the treatment of hyperlipidemia to be one of the major approaches towards decelerating the atherogenic process. Atherosclerosis, referred to as a “silent killer”, is one of the leading causes of death in the developing countries like India. In the general population, the cardiovascular risk from increased LDL cholesterol and is supported by observations that cholesterol-lowering therapy greatly diminishes the clinical manifestations of atherosclerosis, particularly since the advent of inhibitors of 3-HMG Co A reductase (i.e., statins) that profoundly lower LDL cholesterol. In contrast to the situation with LDL cholesterol, the relation between HDL cholesterol and atherosclerosis is an inverse one.^{2, 3, 4}

World health organization (WHO) reports that high blood cholesterol contributes to approximately 56% of cases of cardiovascular diseases worldwide and causes about 4.4 million deaths each year. Hyperlipidemia is a metabolic disorder, specially characterized by alterations occurring in serum lipid and lipoprotein profile due to increased concentrations of Total cholesterol (TC), Low density lipoprotein cholesterol (LDL-C), very low density lipoprotein cholesterol (VLDL-C), and triglycerides (TAG) with a concomitant decrease in the concentrations of high density lipoprotein cholesterol (HDL-C) in the blood circulation. Currently, the use of alternative medicines and especially the consumption of phytochemicals have been rapidly increasing worldwide. As herbal medicines have lesser side effects than synthetic drugs, they have better compatibility and thus improving patient tolerance even on long-term use. Drugs from herbal origin are more promising and exciting than molecular splicing which generates waves of controversy.^{5, 6, 7}

India is sitting on gold mine of well recorded and traditionally well –practiced knowledge of herbal medicines. India is one of the largest producers of herbal medicines and therefore rightly called *Botanical Garden* of the world. India officially recognized over 3000 plants for their medicinal value, estimated over 6000 plants used in traditional, folk and herbal medicine and representing 75% of the medicinal needs of the third world countries.^{8, 9}

India holds the top position in use of herbal drugs. India is one of the most countries exporting plant drugs or their derivatives and excellent in home consumption too. According to the Indian mythology, when the illness and disease get rampant on the earth, the sags learnt the science of healing from lord Indra and recorded them in the scriptures.

Natural products have been derived from higher plants, microbes or animals and these can be of either terrestrial or aquatic or marine origin. The medicinal preparations based on these raw materials were in the form of crude drugs. With the advantage of scientific methods, many of

these ruptured medicinal plants came under chemical investigation leading to isolation of active principles.

Nelumbo nucifera Gaertn. (Family: Nymphaeaceae), is 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 Lord Vishnu – The preserver of the universe by the Hindus and the Lord Buddha by 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*.¹⁰ 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 and 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, skin erysipelas and other external inflammations.¹¹

Flowers are reported to possess aldose reductase inhibitory activity¹², anti-bacterial activity¹³, antioxidant activity¹⁴, antiplatelet activity¹⁵, antipyretic activity¹⁶, aphrodisiac activity¹⁷ and hypoglycaemic activity¹⁸ from the extensive literature survey.

Flowers and petals of *Nelumbo nucifera* yielded quercetin, luteolin, isoquercitrin, glucoluteolin, n-triancontanol, α -amyrin, lupeol, β -sitosterol, amino acids - lysine, proline, hydroxyproline, β -phenylalanine, arginine, kaempferol-3-glycoside anonaine, lotusine, neferine.¹⁹ Flavanoids such as kaempferol along with β -sitosterol-glycopyranoside were also reported in *Nelumbo nucifera*.²⁰ Keeping in view of pathophysiological complications of hyperlipidemia and therapeutic efficacy of herbal medicines, the plant *Nelumbo nucifera* has been evaluated for antihyperlipidemic activity.

MATERIALS AND METHOD:

Collection of Plant Material and Authentication:

Fresh lotus flowers were procured from Flower market, Jamalpur, Ahmedabad.

The flowers were authenticated by Department of Genetics & Plant Breeding, B.A College of Agriculture, **Anand Agriculture University** with authentication number **BACA/GPB/652/14**.

Extraction:

Fresh flowers of lotus were sun dried for 1 day. After drying the flowers, dried flowers were coarsely powdered by mechanical grinder. This coarse powder of *Nelumbo nucifera* (lotus) flowers was packed into Soxhlet Apparatus. The powder packed into Soxhlet Apparatus was successively extracted with mixture of water and ethanol (50:50) as a solvent. The extraction was continued for 12 cycles or until the solvent in the thimble was cleared. With the use of rotary evaporator, extract was concentrated and solvent was recollected. Extract obtained was dried stored under refrigerating condition.²¹

Preliminary Phytochemical Screening:

Hydroethanolic NNFE was screened for presence of various phytoconstituents.^{22, 23}

Experimental Animals:

The complete course of experiment was carried out using 30 Wistar rats of either sex, weighing between 180-200 g, which were procured from Zydus research centre. Animals were housed under standard laboratory condition at room temperature 37 ± 2 °C along with 12/12 dark and light cycle with free access of food and water. After seven days of acclimatization to laboratory conditions, they were allowed to be used for experimental work. Throughout the experiment, animal house was maintained under similar identical conditions as per standard guidelines of CPCSEA.²⁴ The protocol **ASP & BRI/AH/2014/01** was approved by IAEC meeting in January 2014 at our institute.

Acute Oral Toxicity:

LD₅₀ of hydroethanolic NNFE was reported at 2000 mg/kg. So for our current study the dose was selected based on LD₅₀ of the plant. Thus the dose of NNFE was 125 mg/kg, 250mg/kg and 500 mg/kg.²⁵

Hypolipidemic Activity in normal rats:

Animals in the normal control group received normal saline 10 mL/kg orally. Standard group received Atorvastatin at 10 mg/kg orally. The test group of animals were treated with the hydroethanolic NNFE at predetermined therapeutic doses of 125 mg/kg, 250 mg/kg and 500 mg/kg p.o. The blood samples were withdrawn from retro-orbital plexus at 0, 15 and 24 hours. All the lipid profile parameters were determined Total cholesterol, high density lipoprotein (HDL), low density Lipoproteins (LDL), Very low density Lipoproteins (VLDL), Triglycerides (TG) were analysed from serum.

Poloxamer 407 Induced Antihyperlipidemic activity:

Poloxamer 407 is a non ionic surfactant that induces hyperlipidemia. A colloidal solution of Poloxamer 407 was prepared by dissolving Poloxamer 407 in cold normal saline. Rats were injected Poloxamer 407 carefully i.p at dose 1 g/kg. Hydroethanolic NNFE at various doses was administered to screen the antihyperlipidemic activity.^{26, 27}

Biochemical Estimation:

Blood Samples 0.2 mL per sample were collected serially from retro orbital puncture at various time intervals 0, 15 hours, 24 hours. After leaving blood to clot for 30min at room temperature, serum was separated by centrifugation. Lipid parameters like Total cholesterol, HDL, LDL, VLDL and Triglycerides were measured using diagnostic kits. The different test (NNFE) doses, 125 mg/kg, 250 mg/kg and 500 mg/kg were screened for their antihyperlipidemic activity.

Biostatistical Analysis:

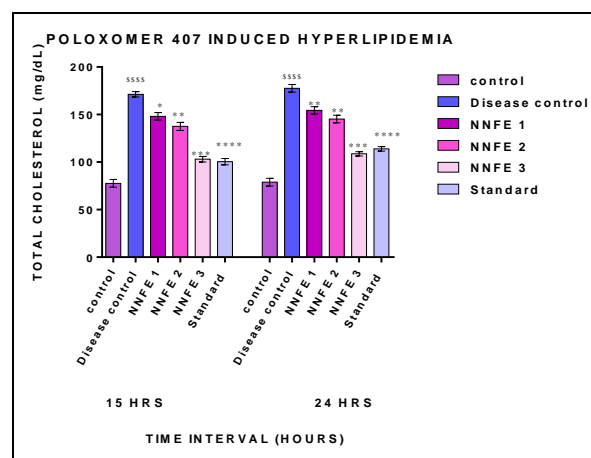
The results were expressed as Mean ± SEM, the statistical analysis were carried out by analysis of variance (ANOVA) followed by Tukey’s test, where **P value < 0.05 is considered as statistically significant**. Data were processed with **Graph Pad Prism** version 6.00 software.

RESULTS:

Phytochemical Screening:

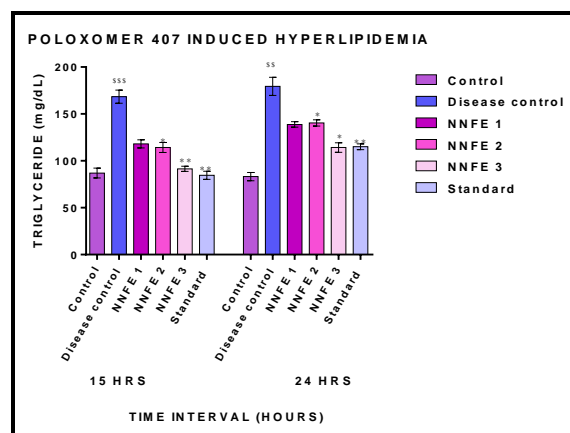
Hydroethanolic NNFE contained flavanoids, alkaloids, amino acids, fats and fixed oils and saponin glycosides.

Effect of Hydroethanolic NNFE on TC:



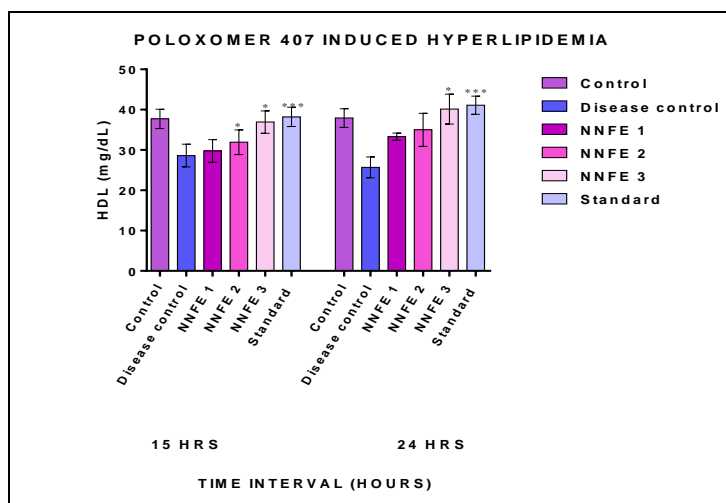
The results indicate that rats treated hydroethanolic NNFE showed highly significant decrease in TC level at the dose of 500mg/kg as compared to P-407 treated rats. Mean ± SEM of NNFE 3 (500 mg/kg) treated animals at 15 and 24 hrs were 100.4 ± 2.81 and 114 ± 2.33, normal saline treated rats has Mean ± SEM at 15 and 24 hrs were 77.6 ±4.01 and 78.8 ± 4.10. At 24 hours, TC level of P-407 treated rats and standard Atorvastatin treated rats were found to be 177.6 ± 3.98 and 94.35±3.26. Thus it indicated that NNFE 3 treated rats (500mg/kg) decreased TC level which was comparable with standard Atorvastatin treated rats. NNFE1 (125 mg/kg) treated rats and NNFE 2 treated rats (250 mg/ kg) showed less significant and significant reduction in TC respectively.

Effect of Hydroethanolic NNFE on TG:



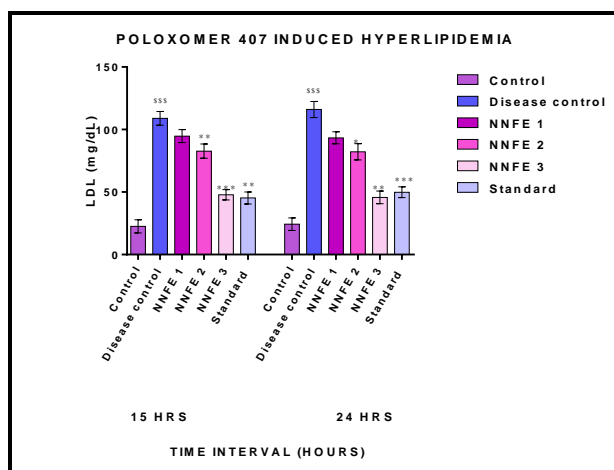
The results indicate that rats treated hydroethanolic NNFE showed decrease in TG level at the dose of 500mg/kg as compared to P-407 treated rats. Mean \pm SEM of NNFE 3 (500 mg/kg) treated animals at 15 and 24 hrs were 91.4 ± 2.76 and 114.2 ± 5.08 respectively, normal saline treated rats has Mean \pm SEM at 15 and 24 hrs were 86.9 ± 5.23 and 83.1 ± 4.47 . At 24 hours, TG level of P-407 treated rats and standard Atorvastatin treated animals were found to be 179.4 ± 9.07 and 115 ± 3.11 respectively. Thus it indicated that NNFE 3 treated rats (500mg/kg) decreases TG level which was comparable with standard Atorvastatin. NNFE1 (125 mg/kg) treated rats showed nonsignificant reduction in TG whereas NNFE 2 (250 mg/ kg) treated rats showed less significant activity.

Effect of Hydroethanolic NNFE on HDL:



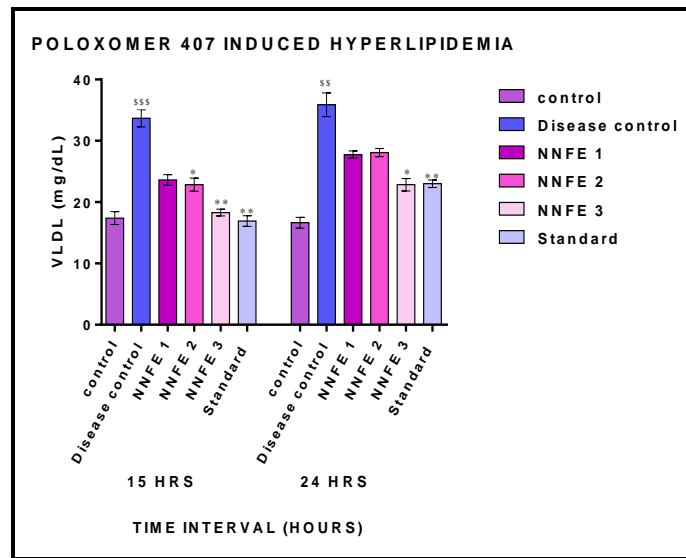
The results indicated that rats treated with hydroethanolic NNFE increases HDL level at the dose of 500mg/kg as compared to rats treated with P-407. Mean \pm SEM of NNFE 3 (500 mg/kg) treated animals at 15 and 24 hrs were 24.9 ± 2.76 and 40.1 ± 3.76 , normal saline treated rats has Mean \pm SEM at 15 and 24 hrs are 37.7 ± 2.39 and 37.9 ± 2.29 respectively. At 24 hours, HDL level of P-407 treated rats and standard Atorvastatin treated rats were found to be 25.7 ± 2.79 and 41.1 ± 2.23 respectively. Thus it indicated that NNFE 3 treated rats (500mg/kg) showed increase in HDL level which was comparable with standard Atorvastatin treated rats. Rats treated with NNFE 125 mg/kg showed nonsignificant enhancement of HDL where as rats treated with NNFE 250 mg/kg showed significant activity.

Effect of Hydroethanolic NNFE on LDL:



The results indicate that hydroethanolic NNFE significant decreases LDL level at the dose of 500mg/kg as compared to P-407 treated rats. Mean ± SEM of NNFE 3 (500 mg/kg) treated rats at 15 and 24 hrs were 47.7 ± 4.13 and 53.9 ± 5.24, normal saline treated rats has Mean ± SEM at 15 and 24 hrs were 22.5 ± 5.23 and 15.3 ± 5.01. At 24 hours, LDL level of P-407 treated rats and standard Atorvastatin treated rats were found to be 116 ± 6.41 and 49.8 ± 4.27. Thus it indicated that NNFE 3 treated rats (500mg/kg) showed decrease in LDL level which was comparable with standard Atorvastatin. NNFE1 (125 mg/kg) treated rats showed insignificant reduction in LDL

Effect of Hydroethanolic NNFE on VLDL:



The results indicate that hydroethanolic NNFE decrease VLDL level at the dose of 500mg/kg as compared to P*707 treated rats. Mean ± SEM of NNFE 3 (500 mg/kg) treated rats at 15 and 24 hrs were 18.3 ± 0.55 and 28.8 ± 1.01 respectively, normal saline treated rats has Mean ± SEM at 15 and 24 hrs are 17.3 ± 1.04 and 16.6 ± 0.89 respectively. At 24 hours, TC level of P-407 treated rats and standard Atorvastatin treated rats were found to be 35.8 ± 1.94 and 23 ± 0.62. Thus it indicated that NNFE 3 treated rats (500mg/kg) showed decrease in VLDL level which was comparable with standard Atorvastatin treated rats. NNFE1 (125 mg/kg) and NNFE 2 (250 mg/kg) treated rats showed less significant reduction in VLDL.

Table 1: Summary of Mean ± SEM at 15 and 24 hours

Time Interval (Hours)	Groups	Estimated Parameters (mg/dl)				
		TC	TG	HDL	LDL	VLDL
15	Control	77.6 ± 4.01	86.9 ± 5.23	37.7 ± 2.39	22.55 ± 5.23	17.39 ± 1.04
	Disease control	171.2 ± 2.81 ^{\$\$\$\$}	168.4 ± 7.01 ^{\$\$\$}	28.6 ± 2.81	108.9 ± 5.48 ^{\$\$\$}	33.68 ± 1.40 ^{\$\$\$}
	NNFE 1 (125 mg/kg)	148.1 ± 3.93*	118.1 ± 4.33	29.7 ± 2.80	94.7 ± 5.20	23.62 ± 0.86
	NNFE 2 (250 mg/kg)	137.5 ± 4.26**	114.2 ± 5.34*	31.9 ± 3.05*	82.7 ± 5.72**	22.84 ± 1.06*
	NNFE 3 (500 mg/kg)	100.4 ± 3.28***	91.48 ± 2.76**	24.92 ± 2.76*	47.78 ± 4.13***	18.30 ± 0.55**
	Standard	91.62 ± 3.29****	84.60 ± 4.30**	38.2 ± 2.40***	45.28 ± 4.82**	16.92 ± 0.86**
	control					

	(10 mg/kg)						
	Control	78.8 ± 4.10	83.18 ± 4.47	37.92 ± 2.29	15.32 ± 5.01	16.64 ± 0.89	
	Disease control	177.6 ± 3.98 ^{\$\$\$\$}	179.4 ± 9.07 ^{\$\$}	25.7 ± 2.59	116 ± 6.41 ^{\$\$\$}	35.88 ± 1.94 ^{\$\$}	
	NNFE 1 (125 mg/kg)	154.4 ± 3.85 ^{**}	138.8 ± 2.90	33.3 ± 0.90	93.34 ± 4.83	27.76 ± 0.58	
	NNFE 2 (250 mg/kg)	145.3 ± 4.04 ^{**}	140.5 ± 3.38	35 ± 4.11	82.2 ± 6.45 [*]	28.08 ± 0.67	
24	NNFE 3 (500 mg/kg)	114 ± 2.33 ^{***}	114.2 ± 5.08 [*]	40.1 ± 3.76 [*]	53.9 ± 5.24 ^{***}	28.84 ± 1.01 [*]	
	Standard control (10 mg/kg)	94.35 ± 3.26 ^{*****}	115 ± 3.11 ^{**}	41.1 ± 2.23 ^{***}	49.8 ± 4.27 ^{***}	23 ± 0.62 ^{**}	

Note:

Values are expressed as Mean ± SEM (n=5) one way ANOVA followed by Tukey's Test

*p<0.05: significant when compared to disease control group

**p<0.01: significant when compared to disease control group

***p<0.001: highly significant when compared to disease control group

\$\$p<0.01: significant when compared to control group

\$\$\$p<0.001: highly significant when compared to control group

\$\$\$\$p<0.0001: highly significant when compared to control group

DISCUSSION:

The present study demonstrates the antihyperlipidemic activity of NNFE against P – 407 surfactant in Wistar rats. The results indicated that rats treated with NNFE shows significant antihyperlipidemic activity at dose of 500 mg/kg.

P-407 induced hyperlipidemia is associated with increases in TC, TG level and decreases HDL level in hyperlipidemic group (positive control) after i.p administration of non ionic surfactant POLOXOMER 407. When the treatment was done with the NNFE at different doses (125 mg/kg, 250 mg/kg and 500 mg/kg), rats treated with 500 mg/kg NNFE showed significant antihyperlipidemic activity. TG (P < 0.05), TC (P < 0.001) were decreased significantly in comparison with rats treated with P-407 (Disease control), where as HDL was significantly improved (p<0.05) in rats treated with 500 mg/kg NNFE. Increase in LDL level is associated with increases in cardiovascular event. LDL (P < 0.01) and VLDL (P < 0.05) were decreased significantly in rats treated with 500mg/kg NNFE when compared with rats treated with P-407 (Disease control).

P-407 is hydrophilic non-ionic surfactant. P-407 induced hyperlipidemia is improved by treatment with NNFE. Hyperlipidemia characterized by abnormally elevated serum TG, TC, LDL-C and VLDL-C, is an established risk factor for the development of coronary artery disease (CAD). In the present study hydroethanolic NNFE was evaluated followed by i/p administration

of P-407. P-407 has been utilized in the hyperlipidemic model due to its convenience and reproducibility. Oral administration of NNFE significantly reduces TC, TG, LDL and VLDL and significantly increases HDL level. P-407 treated rats showed increase in TG by inhibition of Lipoprotein lipase, which is responsible for TG hydrolysis. The results indicate that Hydroethanolic NNFE treated rats showed improvement in lipid profile and thereby reduces the risk of hyperlipidemia probably by increasing Lipoprotein Lipase activity.

The cholesterol lowering effects of NNFE treated rats might be due to the inhibition of hepatic HMG CoA reductase, the rate-limiting enzyme in the biosynthesis of cholesterol since atorvastatin which was used as positive control in this study is a HMG-CoA reductase inhibitor and it was demonstrated that the elevation of serum cholesterol levels following i/p injection of Poloxomer 407 to rats was due to stimulation of 3-hydroxy-3-methylglutaryl-Co-enzyme A (HMG-CoA) reductase activity in the liver by the Poloxomer vehicle.

Phytochemical screening of NNFE shows the presence of saponins, flavanoids, alkaloids, glycosides and phenolic component in the hydroethanolic extract. From the literature review, it can be concluded that flavanoid and phenolic components may be responsible for lowering of TC, TG and LDL levels and markedly responsible for improvement in HDL levels in P- 408 induced hyperlipidemic model.

The results presented in this study indicated that Lipid profiles of the rats treated with hydroethanolic NNFE at the doses of 125 mg/kg, 250mg/kg and 500mg/kg were evaluated after post treatment with Poloxomer 407. Rats treated with 500mg/kg NNFE showed significant improvement in Lipid profile than other two doses (125 mg/kg, 250 mg/kg). It shows higher doses are more protective than lower doses.

CONFLICT OF INTEREST STATEMENT:

We declare that we have no conflict of interest.

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