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EVALUATION OF HYPOLIPIDEMIC AND INSULIN RESISTANCE EFFECTS OF HYDRO-ALCOHOLIC EXTRACT OF RHIZOMES OF *NELUMBO NUCIFERA* IN EXPERIMENTAL RATS

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ABSTRACT

Objectives: The present study was designed to evaluate the hypolipidemic and insulin resistance effects of hydro-alcoholic extract of rhizomes of *Nelumbo nucifera* in experimental rats by HFHS diet induce model. **Materials and Methods:** Following Institutional Animal Ethics Committee permission, the study was conducted in Wistar rats (180-200 g). The model was developed using a high fat (Vanaspati ghee: coconut oil, 3:1) oral diet along with 25% fructose (high sugar) added in drinking water over a period of 6 weeks. Atorvastatin (2.1 mg/kg/day), metformin (2.7 mg/kg/day) and NNHE (300 mg/kg/day) were administered 3 weeks after initiation of HFHS diet and continued for another 3 weeks. During the study period food and water intake, body weight, lipid profile, blood glucose level, insulin level was assayed. **Results:** HFHS diet significantly increase the Blood glucose, Insulin & lipid profile. Treatment of standards & test drug decrease the blood glucose level, insulin level & also lipid profile parameters. Therefore, confirming the efficacy of model.

KEYWORDS: Coronary heart disease, high fat, high sugar, hyperlipidaemia, insulin resistance, *Nelumbo nucifera.*

INTRODUCTION

Hyperlipidemia is a term used to describe several conditions in which high concentrations of lipids exist in the bloodstream and it results from abnormalities in lipid metabolism or plasma lipid transport or a disorder in the synthesis and degradation of plasma lipoproteins.[1]

Hyperlipidemia is a family of disorders that are characterized by abnormally high levels of lipids (fats) in the blood, high blood levels of fats increase the risk of coronary heart disease (CHD). Two common lipid abnormalities are characterized either by high blood cholesterol levels (hypercholesterolemia) or high blood levels of triglycerides (hypertriglyceridemia).[2]

Hypercholesterolemia can eventually lead to a heart attack due to CHD or a stroke due to narrowed arteries supplying the brain. Hypertriglyceridemia is characterized by high blood levels of triglycerides, Elevations in blood triglycerides may promote atherosclerosis by altering the size, density, and composition of LDL.[2]

Insulin resistance is the driving factor that leads to type 2 diabetes, gestational diabetes and prediabetes. Insulin resistance is closely associated with obesity; however, it is possible to be insulin resistant without being overweight or obese. Around 15-30 percent of people with prediabetes go on to be diagnosed with type 2 diabetes within 5 years, according to figures from the Centers for Disease Control and Prevention (CDC). Similarly, the American Heart Association (AHA) say that about half of people with high blood sugar go on to develop type 2 diabetes within a decade.[21]

Due to change of lifestyle and food practice several factors implicated in the development of CHD and diabetes. The fundamental aspect in the etiology of these disorders is IR, which is linked to a wide array of other complications including hyperlipidemia. Thus, it is importance to establish an animal model, to have a better understanding of the pathological process involved in IR and hyperlipidemia, which would further aid in developing new therapeutic drugs which would modulate both the conditions.[21]

Herbal medicines are represented as the most potential field of alternative medicines all over the world. *Nelumbo nucifera Gaertn (Nelumbonaceae)* known by numerous common names including Indian lotus, sacred

lotus, bean of India, or simply lotus, is one of two species of aquatic plant in the family Nelumbonaceae.[3] Virtually, all parts of the lotus plant are used: the rhizome is used as food, seed as medicine, thalamus as fruit, leaves as plate (thali), stalks as pickle, petals for color extraction, and tender leaves as food after being blended with vegetables.[4]

Traditionally all parts of *N. nucifera* have various medicinal uses. Rhizomes are prescribed as demulcents for haemarrhoids and are beneficial in dysentery, chronic dyspepsia, and have nutritive, and diuretic activities.[5,6]

The plant has been widely studied for its chemical constituents. Alkaloids sterols and reducing sugars have been detected on preliminary phyto-chemical study form various extract of rhizomes.[7] The medicinal properties are attributed to the presence of flavonoids and quercetin.[8] The methanol extract of the rhizome has been found to possess a steroidal triterpenoid – betulinic acid.

N. nucifera has been screened scientifically for various pharmacological activities like anti-ischaemic activity, antioxidant activity, hepatoprotective activity, anti-inflammatory activity, anti-fertility activity, anti-arrhythmic activity, anti-fibrosis activity, antiviral activity, anti-proliferative activity, antidiarrhoeal activity, psychopharmacological activity, diuretic activity, antioxidant activity, antipyretic activity, immunomodulatory activity, Hypoglycaemic activity, aldose reductase inhibitory activity, antibacterial, aphrodisiac activity, antiplatelet activity, cardiovascular activity, anti-obesity activity, Lipolytic activity, Hypocholesterolaemic activity, hepatoprotective activity, anticancer activity.[9,11]

MATERIALS AND METHODS[21,23]

Docking study[12]

Requirement of software

Chem office version 7.0 supplied by Cambridge software company, USA, was used for ligand Preparation. Docking study was done by PyRx software. It provides binding affinities score and RMSD values for each ligand with nine different poses and docked poses will be analyzed by Discovery studio client 4.5.

Preparation of ligands

Ligand molecules will be drawn by using Chem Draw Ultra module. Transformation of two-dimensional (2D) structures into three dimensional (3D) structures was done by using the Chem3D Ultra module. Energy-minimization of resulting 3D structures was done by using the molecular mechanics (MM2) method and then energy minimized molecules will be re-optimized using Austin model molecular orbital package (MOPAC). These three dimensional molecules will be saved in protein data bank format (.pdb) for final docking study.

Protein preparation

The target protein for docking study HMG-CoA reductase **(PDB Code: 1DQ8)**, can be Downloaded from Protein Data Bank (www.rcsb.org) and crystallographic water molecules will be removed from the protein by using Discovery studio client 4.5.

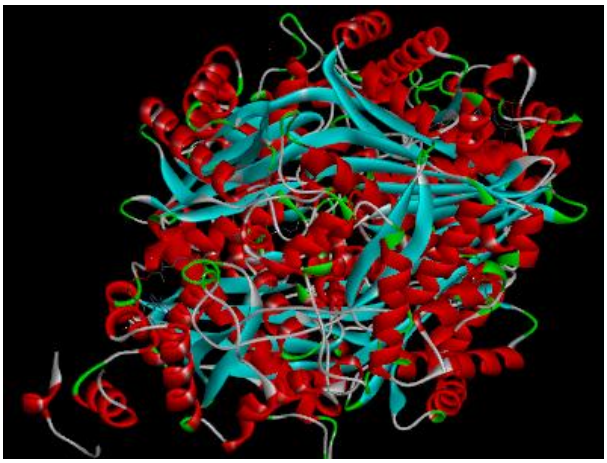


Fig. 1: Targeted protein HMG-CoA reductase (PDB Code: 1DQ8).

Virtual screening

A library of generated ligands was subjected for virtual screening against identified potential drug targets using PyRx. The grid for docking calculations was centered on HMG-CoA reductase (PDB Code: 1DQ8). During virtual screening ligands and proteins will be selected and converted in to PDBQT format for Auto-dock Vina program. The default parameters of Vina will be used for docking simulation and run Vina-Wizard forward for docking study. Finally, the lowest energy binding conformation and root mean square deviation (RMSD) for Ligand binding can be analyzed for each protein Ligand interactions in 9 different poses. Low value of RMSD (<2) indicate good interaction between ligand and protein. Efficiency of all the ligands will be analyzed using binding energy value predicted by software.

Chemicals and reagents: *Nelumbo nucifera* Rhizomes, Atorvastatin calcium[17,18], Metformin[19,20], Ethanol, Chloroform, CMC & all the chemicals & other reagents used in the study were of analytical grade & provided from Sapience Bio Analytical Research lab Bhopal, [M.P.]. All drug solutions were freshly prepared in saline before each experiment.

Animals Used: Adult Wistar rats of 150-200 g were used for the study. The animals were maintained under controlled conditions of temperature (23 ± 2 C), humidity (50 ±5%) and 12 h light-dark cycles. All the animals were acclimatized for seven days before thestudy. The animals were randomized into experimental and control groups and housed individually in sanitized polypropylene cages containing sterile husk as bedding. They had free assessed to standard pellets as basal diet and water *ad libitum*. All the studies conducted

were approved by the Institutional Animal Ethical Committee (IAEC) of Sapience Bioanalytical Research Lab Bhopal **(Proposal no: SBRL/ IAEC/JULY 2017/02)** according to prescribed guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animal, Govt. of India. **(Approval no. 1413/PO/E/S/11/CPCSEA).**[22]

Collection and Authentication of plant: The rhizome was collected in the month of May from the Local Market of Bhopal (M.P.). Herbarium file of plant part was prepared and authenticated by Dr. Zia Ul Hasan (Professor & Head) Department of Botany, Saifia College Bhopal and the specimen voucher no. assigned was 445/Bot/Saifia/17.

Drying and size reduction of plant material: The rhizome was dried under shade. It was pulverized to coarse powder and passed through sieve No. 20 to maintain uniformity and stored in cool and dry place. This material was used for the further study.

Preparation of *Nelumbo nucifera* rhizome extract: 500 gm of fine sieved powder was weighed using aHigh precision balance (WENSAR/PGB 200) and subjected to sequential Soxhlet extraction at room temperature, using hydro-alcoholic solvent (50:50) system. Extract was filtered and dried on room temperature and stored in a refrigerator for the further analysis.

Phytochemical Analysis of Crude Extract[13]
The crude extracts of *Nelumbo nucifera* obtained by solvent extraction was subjected to various qualitative tests to detect the presence of common chemical constituents as: alkaloid, glycoside, carbohydrate, phytosterol, saponins, tannin, flavonoid and protein etc.

Acute toxicity (LD_{50}) studies[14,15,16]
An attempt was made to determine LD_{50} of hydro-alcoholic extracts of *Nelumbo nucifera* rhizomes at a dose of 2000 mg/kg/p.o., in male albino wistar rats. The extracts were found devoid of mortality of the animals. Hence 5000 mg/kg was considered as cut off value. Therefore, the screening doses (Hydro-alcoholic extract 300 mg/kg), selected for the evaluation of hypolipidemic & insulin resistance activity as per OECD guidelines no.425.

Preparation of High Fat High Sugar diet (HFHSD)
Bread (30 g) + Biscuits (30 g) + Vanaspati ghee (3 ml) + coconut oil (1 ml) 25% fructose was added in drinking water bottle.

These diets were fed along with normal diet for a total period of 6 weeks to rats.

Experimental protocols[2,21]
In HFHS Diet model the animals were divided into five groups and each groupcomprised of six animals.
Animals were grouped as follows:
Group I: Normal Control group (Normal pellet diet (ND)
Group II: Positive Control group (ND + HFHSD)
Group III: (Standard Control Group) HFHSD + ND + Atorvastatin Calcium (2.1 mg/kg, p.o. body weight)
Group IV: (Standard Control Group) HFHSD + ND + Metformin (2.7 mg/kg, p.o. body weight)
Group V: (Test Control Group) HFHSD + ND + Hydro-alcoholic extracts of *N. nucifera* (300 mg/kg, p.o. body weight)
All the treatments were carried out for 42 days.

Before and after the treatment, the animals were fasted for 2 h to improve the absorption rate.

Parameters studied for this test were body weights, average feed intake, blood glucose, total cholesterol, HDL, LDL, VLDL, triglycerides, and insulin level.

Statistical Analysis: The values are expressed as Mean ± SEM. The data was analyzed by using one-way ANOVA followed by Dunnett's test using Graph Pad Prism software. Statistical significance was set at P = 0.05.[2]

RESULTS

Docking Result
The results showed that all five phyto-constituents present in plant showed binding free energy ranging between -7.9 kcal/mol to -7.4 kcal/mol while standard atorvastatin showed -7.9 kcal/mol binding free energy. All five compounds have shown significant HMG-CoA reductase inhibitory activity in which steroidal triterpenoid derivative have shown maximum.

Table (1): Binding Affinity of designed ligands with HMG-CoA enzyme estimated by Auto-dock Vina.

S. No.	Phyto-constituent name	Docking score	RMSD (A^o)
1.	Betulinic acid	-7.9	1.822
2.	Quercetin	-7.8	1.555
3.	Roemerine	-7.6	1.875
4.	Pronuciferine	-7.4	1.921
5.	Nuciferine	-7.4	1.920

Percentage yield: The percentage yield of extract rhizome *Nelumbo* nucifera was obtained as 11.94%(w/w) on dry basis.

Phytochemical screening: There is a presence of alkaloids, carbohydrates, flavonoids, glycosides, proteins and saponins in hydro - alcoholic extract of *Nelumbo nucifera* rhizomes.

Table (2): Phytochemical screening of hydro - alcoholic extract of *Nelumbo nucifera* rhizomes.

S. no.	Identification Test	Test name	Present	Absent
1	Alkaloids	Mayer's test		-
		Wagner's test	+	
		Dragendroff's Test	+	
2	Carbohydrates, gums and mucilage's	Molisch's Test		-
		Fehling's Test		-
3	Flavonoids	NaOH	+	
		H_2SO_4	+	
4	Glycoside	Killer-killani test	+	
5	Protein	Xanthoproteic acid test	+	
6	Saponins	Foam test	+	
7	Tannins and Phenolic compound	Gelatin test		-
		Ferric chloride test	+	

(+) = Present, (–) = Absent

Acute Toxicity Studies (LD_{50}): In both phase I and Phase II procedures, none of the animal mortal or any signs of behavioral changes or show any toxicity upon the single administration of HANN (2000 mg/kg p.o.). Thus, 300 mg/kg dose was selected for the present study.

Table (3): Results of Acute oral toxicity study of HANN.

Group name	Animal mark	Dose mg/kg	Body weight			Observation	Mortality
			1 day	7 day	14 day		
Control	H	Normal saline	168	149	169	No sign of toxicity & no lethality occur.	All animals Survived.
	B		105	102	119		
	T		96	98	116		
Test	HT	2000 mg/kg of Extract	209	210	205		
	BT		165	166	191		
	NM		119	105	100		

Evaluation parameters

1. Effect on Feed and water intake

Table (4): Total feed and water intake.

Group	Normal Pellet Diet (gm)	HFHS Diet (gm)	Water intake (ml)
Normal control	61.54 ± 10.34 b**	0	266.67 ± 37.78
Positive control	35.45 ± 8.59 a**	126.07 ± 18.54	300.68 ± 44.44
Standard	42.42 ± 10.68 a**	155.37 ± 10.04b*	329 ± 28.99
Test	46.88 ± 9.62 a**, b*	165.82 ± 8.96 b**	363.33 ± 15.32 a*

All values are Mean ± SEM, n=06. *p <0.05, **p< 0.01 when compared to positive control group as well as normal control group (a = significant difference as compared to normal control group, b = significant difference as compared to positive control group). Following Repeated measures ANOVA (parametric methods, using Dunnett Test.

2. Effect on Body weight

Table (5): Body weight (gm)

Group	0^{th} day	14^{th} day	28^{th} day	42th day
Normal control	168.5 ± 11.53	167.67 ± 12.13	159.33 ± 9.80	153.17 ± 9.42
Positive control	143.5 ±4.69	148.83 ± 4.51	155.83 ± 4.20	159.83 ± 3.96
Standard(A)	165.67 ± 4.67	143.83 ± 4.66	148.5 ± 8.67	144.5 ± 3.74
Standard(M)	148.5 ± 8.84	148 ± 5.09 a*	136.17 ± 6.23	135.83 ± 3.88 b*
Test	164.5 ± 7.86	133.83 ± 5.72 a*	129.17 ± 4.98 a*, b*	121.67 ± 5.17 a**, b**

All values are Mean ± SEM, n=06. *p <0.05, **p< 0.01 when compared to positive control group as well as normal control group (a = significant difference as compared to normal control group, b = significant difference as compared to positive control group). Following Repeated measures ANOVA (parametric methods, using Dunnett Test.

3. Effect on HDL level

Table (6): HDL level (mg/dl).

Group	0th Day	14th Day	28th Day	42th Day
Normal control	86.5 ± 5.078b**	100.83 ± 6.539 b**	92.83 ± 4.549b*	100.18 ± 2.023 b**
Positive control	50.5 ± 4.097 a**	63.5 ± 4.552a**	65 ± 6.424 a*	73.68 ± 4.702 a**
Standard (A)	67.67 ± 6.601	85.83 ± 10.753	102.68 ± 6.965 b**	104 ± 3.615b**
Standard (M)	67.33 ± 6.888	91.83 ± 8.228	96 ± 7.916 b**	106.33 ± 4.137 b**
Test	61.33 ± 5.463 a*	71.68 ± 8.316 a*	85.68 ± 5.914 b*	106.33 ± 4.137b**

All values are Mean ± SEM, n=06. *p <0.05, **p< 0.01 when compared to positive control group as well as normal control group (a = significant difference as compared to normal control group, b = significant difference as compared to positive control group). Following Repeated measures ANOVA (parametric methods, using Dunnett Test.

4. Effect on LDL level

Table (7): LDL level (mg/dl).

Group	0th Day	14th Day	28th Day	42th Day
Normal control	58.83 ± 5.218	66.5 ± 5.644	64.17 ± 4.785	58 ± 5.459 b**
Positive control	48.83 ± 2.428	63.5 ± 2.029	74 ± 3.367	85.67 ± 4.006 a**
Standard (A)	39.83 ± 2.762 a*	53.33 ± 3.343	58.5 ± 4.153	55.33 ± 3.853 b**
Standard (M)	56.33 ± 6.349 a*	54.33 ± 4.652	67.5 ± 5.772	84.67 ± 4.193 a**
Test	45.83 ± 5.850	51.17 ± 4.607 a*	49.17 ± 5.689 b**	58.33 ± 5.200 b**

All values are Mean ± SEM, n=06. *p <0.05, **p< 0.01 when compared to positive control group as well as normal control group (a = significant difference as compared to normal control group, b = significant difference as compared to positive control group). Following Repeated measures ANOVA (parametric methods, using Dunnett Test.

5. Effect on CHL level

Table (8): CHL level (mg/dl).

Group	0th Day	14th Day	28th Day	42th Day
Normal control	139.5 ± 7.753	155.68 ± 3.273	153.67 ± 8.793	159.5 ± 10.704 b**
Positive control	143.68 ± 6.859	189.33 ± 16.386	178.83 ± 6.478	207.18 ± 3.637 a**
Standard (A)	144.33 ± 10.999	161.18 ± 12.448	175 ± 11.696	185.17 ± 5.856 b*
Standard (M)	130.68 ± 3.783	156.68 ± 8.846	166.83 ± 10.716	173.33 ± 8.950b*
Test	153 ± 10.405	169.83 ± 7.120	170.5 ± 5.685	180.17 ± 6.343*b

All values are Mean ± SEM, n=06. *p <0.05, **p< 0.01 when compared to positive control group as well as normal control group (a = significant difference as compared to normal control group, b = significant difference as compared to positive control group). Following Repeated measures ANOVA (parametric methods, using Dunnett Test.

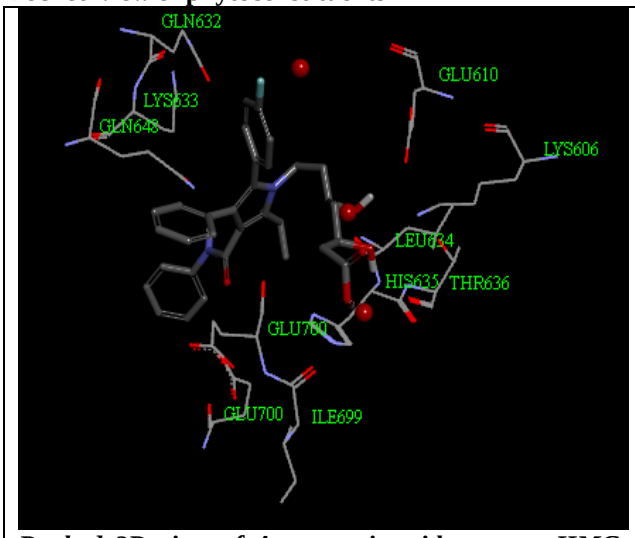
6. Effect on serum insulin level and blood glucose level

Table (9): Serum insulin level & Blood glucose level.

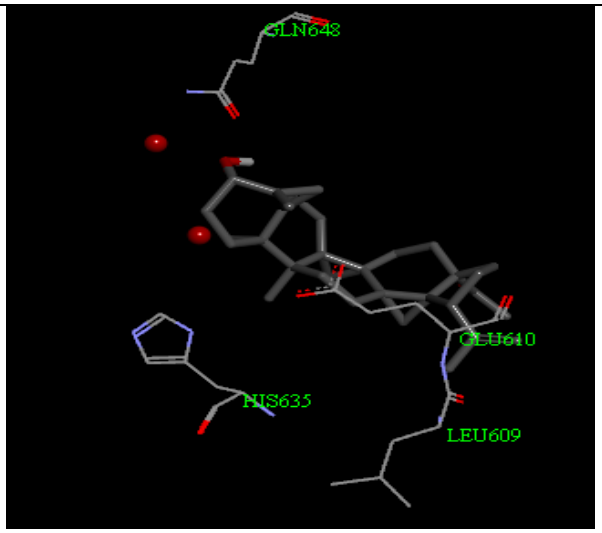
Group	Serum insulin level(ulU/ml)	Blood glucose level(mg/dl)
Normal control	3.133 ± 0.088 b**	129 ± 4.872 b**
Positive control	1.65 ± 0.099 a**	242.5 ± 7.886 a**
Standard (A)	3.017 ± 0.047 b**	125.17 ± 3.240 b**
Standard (M)	2.967 ± 0.072 b**	125.83 ± 3.260 b**
Test	2.867 ± 0.072 b**	129.5 ± 3.566 b**

All values are Mean ± SEM, n=06. *p <0.05, **p< 0.01 when compared to positive control group as well as normal control group (a = significant difference as compared to normal control group, b = significant difference as compared to positive control group). Following Repeated measures ANOVA (parametric methods, using Dunnett Test.

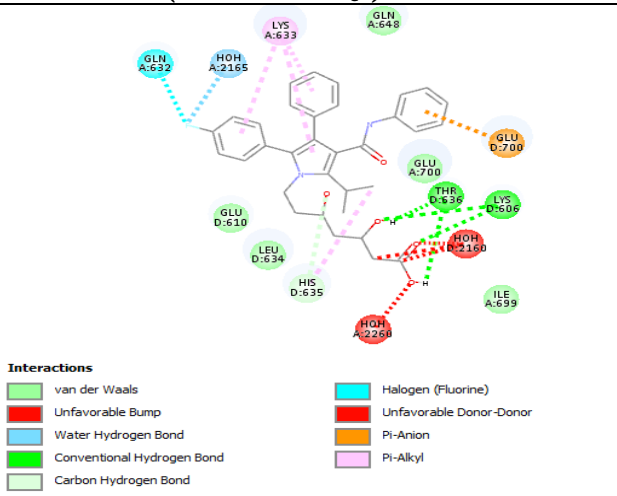
Docked view of phytoconstituents



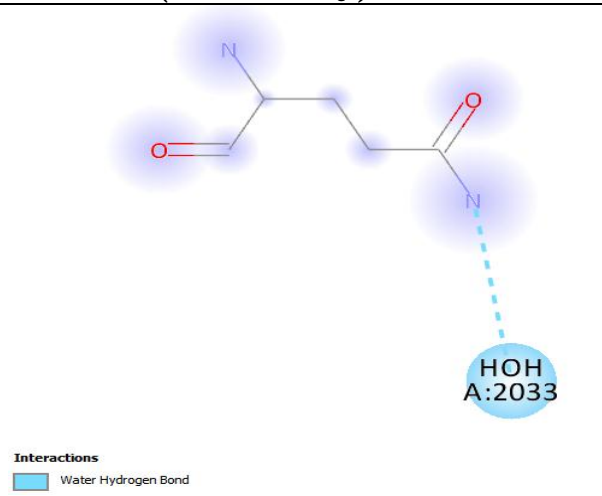
Docked 3D view of Atorvastatin with enzyme HMG-CoA reductase (PDB Code: 1DQ8)



Docked 3D view of Betulinic acid with enzyme HMG-CoA reductase (PDB Code:1DQ8)



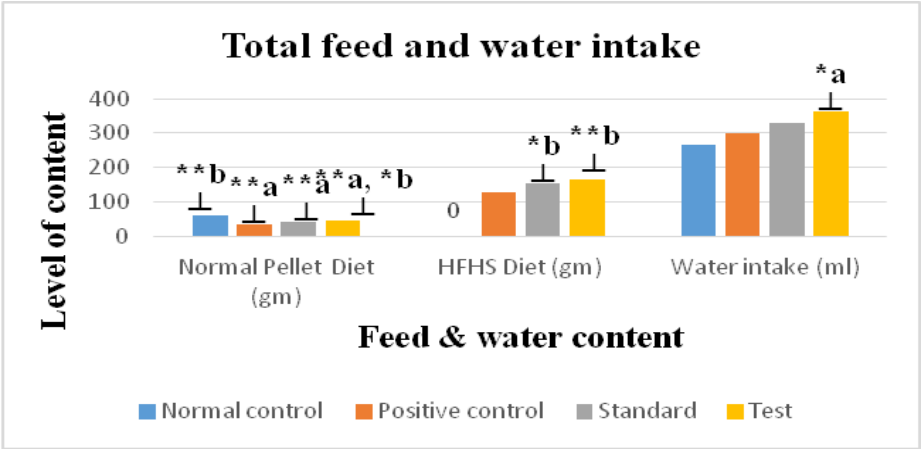
Docked 2D view of Atorvastatin with enzyme HMG-CoA reductase (PDB Code: 1DQ8)



Docked 2D view of Betulinic acid with enzyme HMG-CoA reductase (PDB Code: 1DQ8)

Graphical representation

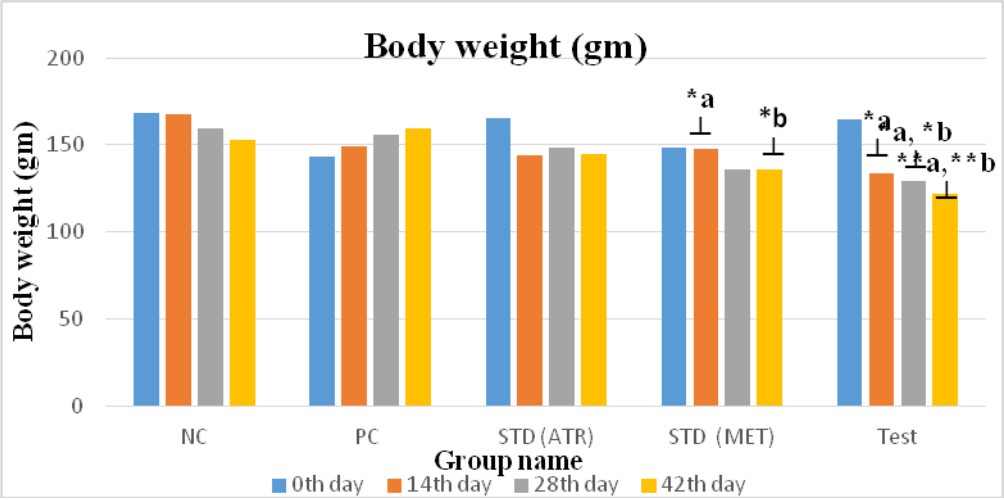
- **Effect on total feed and water intake**



Effect of HFHS diet on total feed and water content. Values are expressed as mean ± standard deviation; (n = 6). All values are Mean ± SEM, n=06. *p <0.05, **p< 0.01 when compared to positive control group as well as normal control group (a = significant difference as compared to normal control group, b = significant

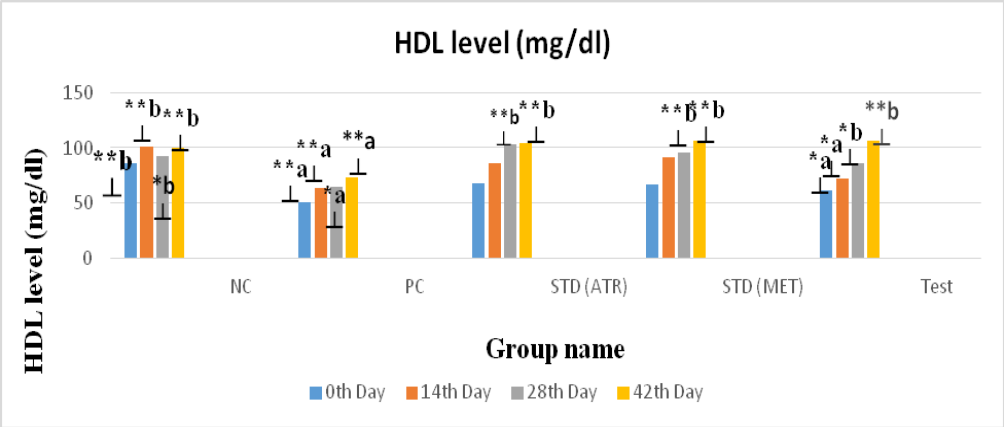
difference as compared to positive control group). Following repeated measures ANOVA parametric methods, using Dunnett Test.

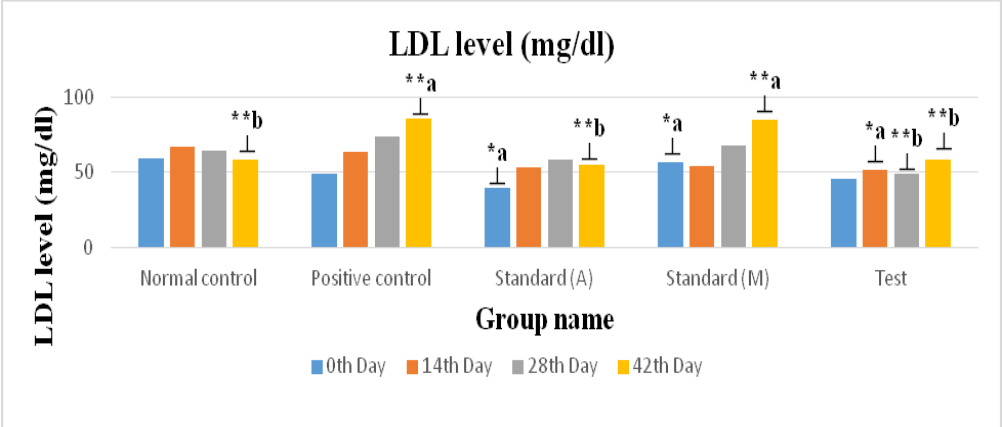
➢ **Effect on body weight**

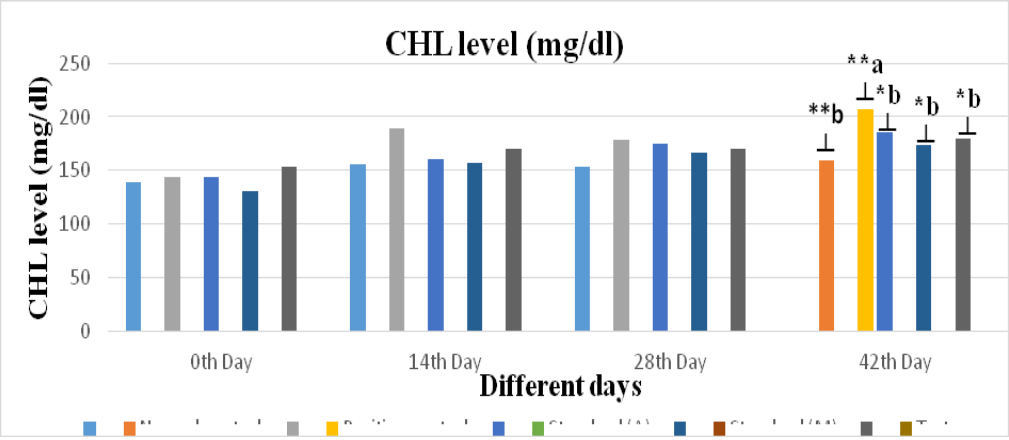


Effect of HFHS diet, atorvastatin, metformin, and Nelumbo nucifera on Body weight. Values are expressed as mean ± standard deviation; (n = 6). All values are Mean± SEM, n=06. *p <0.05, **p< 0.01 when compared to positive control group as well as normal control group (a = significant difference as compared to normal control group, b = significant difference as compared to positive control group). Following repeated measures ANOVA parametric methods, using Dunnett Test. NC = Normal control group, PC = Positive control group or High fat high sugar diet group, Standard (A) = ND + HFHS + atorvastatin (2.1 mg/kg/day), Standard (M) = ND + HFHS + metformin (2.7 mg/kg/day), Test control = ND + HFHS + Nelumbo nucifera (300 mg/kg/day).

➢ **Effect on lipid profiles**

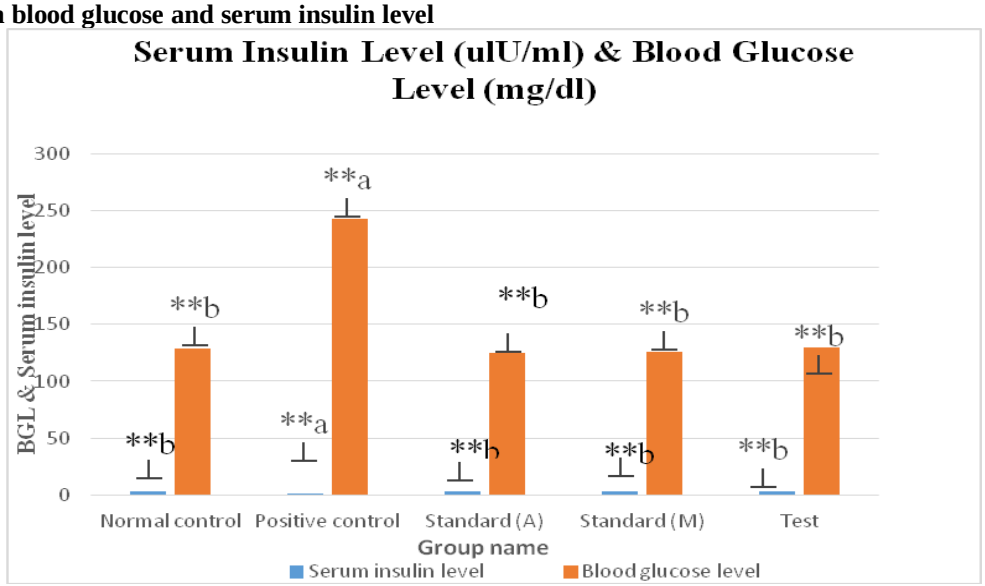






Effect of HFHS diet, atorvastatin, metformin, and *Nelumbo nucifera* on Lipid levels. Values are expressed as mean ± standard deviation; (n = 6). All values are Mean± SEM, n=06. *p <0.05, **p< 0.01 when compared to positive control group as well as normal control group (a = significant difference as compared to normal control group, b = significant difference as compared to positive control group). Following repeated measures ANOVA parametric methods, using Dunnett Test. NC = Normal control group, PC = Positive control group or High fat high sugar diet group, Standard (A) = ND + HFHS + atorvastatin (2.1 mg/kg/day), Standard (M) = ND + HFHS + metformin (2.7 mg/kg/day), Test control = ND + HFHS + *Nelumbo nucifera* (300 mg/kg/day).

- **Effect on blood glucose and serum insulin level**



Effect of HFHS diet, atorvastatin, metformin, and *Nelumbo nucifera* on blood glucose level and serum insulin level. Values are expressed as mean ± standard deviation; (n = 6). All values are Mean± SEM, n=06. *p <0.05, **p< 0.01 when compared to positive control group as well as normal control group (a = significant difference as compared to normal control group, b = significant difference as compared to positive control group). Following repeated measures ANOVA parametric methods, using Dunnett Test. NC = Normal control group, PC = Positive control group or High fat high sugar diet group, Standard (A) = ND + HFHS + atorvastatin (2.1 mg/kg/day), Standard control (M) = ND + HFHS + metformin (2.7 mg/kg/day), Test control = ND + HFHS + *Nelumbo nucifera* (300 mg/kg/day).

DISCUSSION

The study was carried out to evaluate hypolipidemic and insulin resistance activity of *Nelumbo nucifera* rhizomes in HFHS diet induced model of rats.

The plant material wasextracted using Soxhlet apparatus with hydro-alcoholic solvent system (1:1). The obtained practical yield of extract was 10.801gm and percentage yield of extract was 11.94%.

After the extraction, pharmacognostical evaluation and various chemical tests for preliminary identification of various phytoconstituents was done. According to Docking and literature survey Steroidal triterpenoid – betulinic acid, Flavonoids and Quercetin present, which regulate the blood lipid profiles in rats.

In acute oral toxicity study, there were no behavioral changes and no mortality was observed at the maximum tested dose level of 2000mg/kg per oral. It was considered maximum safe dose. Thus, $1/10^{th}$ of these dose was taken as the effective dose.

During the total experiment period total feed intake and water intake show slight decrement. Whereas, during the study body weight were increased with extreme significance and upon treatment with HANN, body weight was decreased. Also Results showed significant decrease blood glucose level and serum lipid profile such as total cholesterol, LDL and increasing serum HDL and serum insulin level at dose of 300 mg/kg (effective dose Ed_{50}).

CONCLUSION

The data of our study suggest that N. nucifera rhizome extract has hypoglycemic effects and also has optimal effects on improvement of lipid profiles levels in diet induced experimental rats.

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REFERENCES

1. Azezli A, Kubat. (2005): Turkey Clinics J, Int Med Sci, 1: 42-48.
2. Arun Babu Vuyyuru, Sarita Kotagiri, Vrushabendra Swamy BM, Archana Swamy P., (2012): Antihyperlipidemic Activity of *Ananas comosus* L. Leaves Extract in Albino Rats; Research Journal of Pharmaceutical, Biological and Chemical Sciences; Volume 3: 1229.
3. Munish G and Singhal T., (2013): Doxorubicin induced cardio myopathy and its herbal solution. Inter J Pharma Sciences Research, 4(9): 3341-3346.
4. Mandal R N, Bar R, (August 2013): The Sacred Lotus - An Incredible Wealth of Wetlands, Resonance.
5. Kirtikar KR and Basu BD, (1975): Indian Medicinal Plants, Edition 2. New Delhi, International Book Distributors: 116–120.
6. Chatterjee A and Pakrashi SC, (1999): The Treatise on Indian Medicinal Plants, Edition 1. New Delhi, Publication and Information Directorate, I: 94–96.
7. Pulok k. Mukherjee, R. Balasubramanian, Kakalisaha, B. P. saha & M. Pal, (4 April 1996) A review on *Nelumbo nucifera* gaertn. Ancient science of life, 15: 268 -276.
8. 5 health benefits of sacred lotus flower (*Nelumbo nucifera*), live a little longer.com accesed 30/7/17.
9. Dhanarasu S, Al-Hazimi A, Phytochemistry, (2013): Pharmacological and Therapeutic Applications of *Nelumbo nucifera*, Asian Journal of Phytomedicine and Clinical Research, 1(2): 123 – 136.
10. Yadav Chhavi, Chaubey Suresh, Singh Tejbeer, Singh D.C, Kumari Sangeeta, (August 2016): A intact review on *nelumbo nucifera* w.s.r to its therapeutic potential, IJAPR, 4(8).
11. Nishkruti R Mehta, Ekta P Patel Pragnesh V Patani; Biren Shah (2013): *Nelumbo Nucifera* (Lotus); A Review on Ethanobotany, Phytochemistry and Pharmacology, Indian J. Pharm. Biol. Res, 1(4): 152-167.
12. Kunal Roy et al. In Understanding the Basics of QSAR for Applications in Pharmaceutical Sciences and Risk Assessment. 2015 (accessed on 25/6/2017).
13. Kokate CK, Khandelwal KR, Pawar AP, Gohale SB. (1995): Practical Pharmacognosy, 3 Ed Pune, Nirali Prakashan, 137-139.
14. Bhardwaj s, Deepikagupta et al., (2012): study of acute, sub-acute and chronic toxicity-gupta, IJAPRB, 2(2): 103-129.
15. Horn H.J, (1956): simplified LD_{50} calculations: biometrics; international biometric society, 12(3): 311-322.
16. OECD 2001-guideline on acute oral toxicity (AOT), Environmental health and safety monograph series on testing and adjustment, No. 425.
17. Atorvastatin - DrugBank.html accesed 31/7/2017.
18. Atorvastatin _ Side Effects, Dosage, Uses & More.html healthlineaccesed 31/7/2017.
19. Metformin _ Side Effects, Dosage, Uses & More.html healthlineaccesed 31/7/2017.
20. Metformin- DrugBank.html accesed 31/7/2017.
21. Renuka P. Munshi, Samidha G. Joshi, and Bhagyeshri N. Rane, (2014): Development of an experimental diet model in rats to study hyperlipidemia and insulin resistance, markers for coronary heart disease. Indian J Pharmacol, 46(3): 270–276.
22. CPCSEA Guideline for Laboratory Animal Facility.
23. Vogel Gerhard, (2008): Drug Discovery & Evaluation: Pharmacological Assays, Springer 3rd edition, volume 2nd: 1661-1717.