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BIOCHEMICAL CHARACTERIZATION AND HYPOLIPIDEMIC ACTIVITY OF *NELUMBO NUCIFERA* (GAERTN,) FLOWERS

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ABSTRACT

Globally, numerous hypolipidemic and cardioprotective herbs are commonly used by traditional practitioners. *Nelumbo nucifera* (Gaertn) (NN) flowers are commonly used s as cardio-tonic agent. In this study, chemical characterization and the hypolipidemic activity of hydroalcoholic extract of NN was carried out. The hypolipidemic effect was evaluated in Triton WR-1339 induced hyperlipidemic animals. The chemical characterization of NN extract was carried out with GC-MS analysis. The GC-MS analysis of methanolic fraction separated from 70 % ethanolic extract of NN showed the presence of fatty acids, nornuciferine and apomorphine. In Triton WR 1339 induced hyperlipidemic rats, animals pre-treated with NN (1000 $mg^{-1}kg$ $b.wt,^{-1}day$ (p.o.)) for 7 days were seen to significantly ($p<0.05$) decrease the level of cholesterol and LDL. The activity was similar to that of atorvastatin. The results of the present study reveals that NN is a rich source of chemical constituents and hypolipidemic agent in nature.

KEYWORDS: lipid profile, atorvastatin, GC-MS, Triton WR-1339.

INTRODUCTION

The genus *Nelumbo* is represented by only two species, *Nelumbo nucifera* and *Nelumbo lutea*. *Nelumbo nucifera* is widely distributed in South-East Asia. It is distributed widely in all parts of India. *Nelumbo nucifera* Gaertn. (Nymphaeaceae) flowers (NN) is used by traditional practitioners as heart tonic.[1] It has also been used to cure abdominal cramps,

cancer, weakness, body heat imbalance, consolidation of kidney function, male sexual disorders, syphilis, stopping bleeding, bloody discharges etc.,[2] Lotus produces a number of important secondary metabolites, like alkaloids, flavonoids, steroids, triterpenoids, glycosides and polyphenols.[3] Floral extracts possess antimicrobial activity[4], vasodilating effect, antihypertensive and antiarrhythmic ability[5], aphrodisiac activity[6], antioxidant[7] and free radical scavenging capacity.[8]

In the present study, we examined the efficacy of *Nelumbo nucifera* flowers on the lipid profile in Triton WR 1339 induced hyperlipidemic rats. The GC-MS analysis of methanolic fraction separated from 70 % ethanolic extract of *Nelumbo nucifera* flowers is carried out.

METHODS

Drugs and chemicals: Triton WR 1339 was obtained from Sigma Chemicals, MO, USA. All other chemicals used were of analytical grade.

Plant material: NN flowers were obtained from Thovalai, Tamilnadu, India. They were identified, authentified and the specimen of the same was maintained in Centre for Advanced Research in Indian System of Medicine, (CARISM) SASTRA University, Thanjavur, Tamilnadu, India (Voucher number: 0092). They were shade dried and coarsely powdered. Extraction was made by soaking the raw material in 70 % ethanol for one week. The solvent was removed by distillation and final traces of solvent was removed under reduced pressure *In vaccuo* (Yield = 8.49 %).

Experimental Animals: Male Wistar albino rats weighing 180 – 220 g were allowed to have a standard pelleted diet M/s Hindustan Lever Foods, Bangalore, India and water *ad libitum*. They were housed under standard environmental conditions. All the animal experiments were performed after getting clearance from Animal ethical clearance (Clearence No. 7/SASTRA/IAEC/RPP).

Hypolipidemic activity: Animals were divided into five groups of six rats each. Group 1 animals received Triton WR 1339. Group 2 animals were pre-treated with Atorvastatin 10 mg^{-1} kg^{-1} day (p.o), for 7 days. Group 3 and 4 animals were pre-treated with NN (70% ethanolic extract) at the doses of 500 and 1000 mg^{-1} kg $^{-1}$day (p.o.), respectively for 7 days. Group 5 animals considered as normal were fed with standard diet. During treatment, on 5^{th} day 1 hour after the administration of NN, all animals except Group 5 was injected with Triton WR 1339, intraperitoneally (i.p.) (dissolved in saline) at the single dose of 400 mg^{-1}kg

b.wt. The animals were fasted for 3 hours before administration of Triton WR 1339 and the fasting was continued up to 48 hours after administration of Triton WR – 1339. All animals were fed with water after the injection of Triton WR 1339.[9] The blood was collected from all animals before and after 48 hr the administration of Triton WR 1339 by retro-orbitol puncture under anesthesia. Plasma total cholesterol,[10], HDL-C[11], TGL[12], LDL[13] were analysed.

GC-MS analysis: 10 mg of NN extract was dissolved in methanol. The methanolic fraction separated from 70 % ethanolic extract was injected for GC-MS analysis. Sample was analyzed by GC–MS on GC Clarus 500 Perkin Elmer using the following experimental conditions: Column type - Elite -5 (5 % diphenyl 95 % dimethyl polysiloxane), Column dimension 30 m X 0.32 mm), carrier gas – Helium 1 ml/min, column temperature from 50 °C up to 285°C at the rate of 10 °C/min and 5 min hold, at 285 °C, injector and detector temperature - 290°C, injection mode split, volume injected: 0.5 µl of a solution prepared from 2 mg/100 ml in methanol. Total run time was 30 minutes. Mass spectrum was taken using Mass detector – Turbo Mass gold – Perkin Elmer. Transfer line temperature – 230 °C, Source temperature – 230 °C, scan range is from 40 – 450 amu, ionisation technique – Electron ionization technique. The component identification was confirmed by comparing mass spectra of compounds with available NIST and Willey mass spectral library. The quantitative composition was obtained by peak area normalization.

Statistical analysis: Results were expressed as mean ± S.D. and One Way ANOVA with DMRT (Duncan Multiple Range Test) was used to assess statistical significance. $p<0.05$ was considered as significant difference. Statistical analysis was performed using SPSS software (Version 12.0).

RESULTS

In the present study the lipid profile has been observed to be increased significantly ($p<0.05$) in diseased (Group 1) against normal (Group 5) animals (Table 1). Atorvastatin treatment for 7 days is seen to decrease the level of cholesterol and LDL level pointedly against (Group 1) Diseased animals ($p<0.05$, Table 1). Likewise treating animals with NN at the dose of 1000 mg/kg b.wt, has also observed to decrease the level of cholesterol and LDL significantly against Group 1 animals ($p<0.05$, Table 1). Large difference has not been observed at the dose of 500 mg/kg b.wt. Moreover noted difference has not been observed in HDL and TGL level in extract or standard drug treated animals.

In order to evaluate the major compounds responsible for the hypolipidemic activity, GC-MS analysis has been performed. Methanolic fraction of 70% hydroalcoholic extract of NN shows the presence of numerous compounds lik hexa decanoicacid (37.8 %), octadcanoic acid (21.6%) and dimthyl derivative of apomorphine (17.5 %) (Fig 1, Tabl 2).

Table 1 Effect of 70 % ethanolic extract of *Nelumbo nucifera* flowers (NN) on plasma lipid profile in Triton WR 1339 induced hyperlipidemic rats

Parameters	Group 1	Group 2	Group 3	Group 4	Group 5
Cholesterol	850.2 ± 8.3****	473.5 ± 7.5**	795.2 ± 3.5***	750.2 ± 3.5***	60.5 ± 8.3*
HDL	60.3 ± 1.5**	65.2 ± 2.0**	60.2 ± 2.3**	63.2 ± 3.0**	12.3 ± 2.2*
LDL	669.8 ± 10.3****	312.3 ± 3.6**	622.9 ± 2.6****	581.8 ± 5.6***	25.9 ± 5.2*
TGL	600.3 ± 10.2**	525.1 ± 12.4**	560.2 ± 5.3**	525.3 ± 1.2**	65.3 ± 8.2*

Values are mean ± SD; n=6; Values not sharing common number of * are differ significantly at P<0.05; Unit is mg/dl.

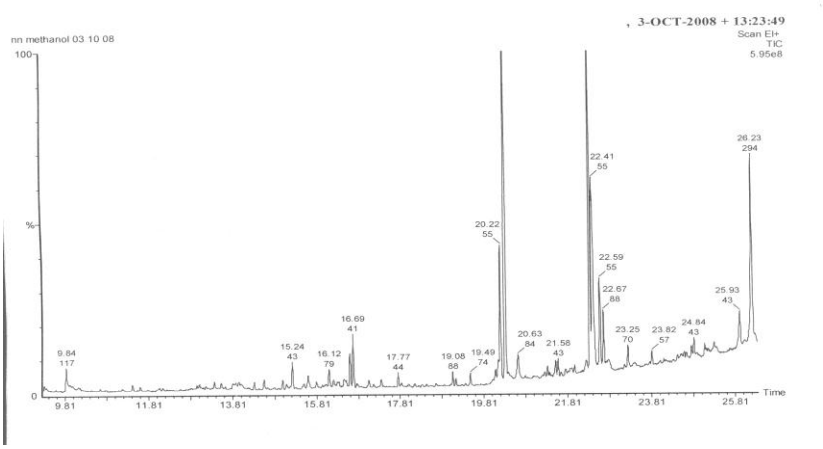

Fig 1 GC chromatogram of methanolic fraction of *Nelumbo nucifera*'s 70 % ethanolic extract

Table 2 Percent composition of various phyto-constituents in methanolic fraction of *Nelumbo nucifera* flowers analyzed by GC-MS

S. No	Peak Name	RT	% Area
1	Propanedioic acid, propyl	9.84	0.3250
2	α-Cardinol	15.24	1.5037
3	Valeranone	16.12	1.6542
4	Patchouli alcohol	16.69	2.7619
5	Myristic acid-ethyl ester	17.77	0.6749
7	Hexadecanoic acid-ethyl ester	20.22	37.8341
8	9,12-Octadecanoic acid, ethyl ester	22.41	21.6367
9	N-nornuciferine	25.93	3.3991
10	Dimethyl derivative of apomorphine	26.23	17.5908

DISCUSSION

The systemic administration of the surfactant Triton WR 1339 to rodents, results in a biphasic elevation of plasma cholesterol and TGL.[14] It has been used for screening natural or chemical hypolipidemic drugs.[15]

The marked decrement of cholesterol and LDL (Table 1) observed in the present study reveals that extract at the dose of 1000 mg/kg b.wt, for 7 days is equal in their activity to that of standard drug (10 mg/kg b.wt,).

The hypolipidemic effect of NN might be due to the presence of octadecadienoic acid (Fig 1, Table 2). The nuclear hormone receptor is called PPAR up regulates the β-oxidation of fatty acids by induction of the acyl-CoA oxidase. Poly unsaturated fatty acid (PUFA) present in the NN extract (Fig 1) may activate the PPARs and thereby exhibit the hypolipidemic activity (Hansiorg et al., 1993). It is well established that polyunsaturated fatty acids (PUFA) lower plasma total low density lipoprotein (LDL) cholesterol concentrations in humans (Goodnight et al., 1982). PUFA also affect the concentrations of apolipoproteins in the rat.[16] N-6 PUFA administration also stimulates the respiration rate in brown adipose tissue thereby, increases the hydrolysis of triglycerides in adipose tissue and exhibits hypolipidemic activity.[17] The hypolipidemic effect of PUFA might be due to the action on various lipid metabolizing enzymes includes decreases liver stearoyl CoA desaturase[18], decreases liver and adipose tissue's fatty acid synthase[19] and Acyl CoA carboxylase, decrease the response of insulin responsive glucose transporter-4 in the adipose tissue[20] and also decreases fatty acid synthase in the adipose tissue.[21] The hypolipidemic activity may also be due to the presence of various compounds like N-nornuciferine, dimethyl apomorphine orvaleranone and patchouli alcohol (Table 2).

The presence of Nornuciferine (Taqble 2) in NN is reported earlier.[22] The presence of other compounds like propanedioic acid, α-Cadinol, Valeranone, Patchouli alcohol, Myristic acid, Tridecanoic acid methyl ester, Hexadecanoic acid ethyl ester, octadecanoic acid ethyl ester, 9,12-octadecadienoic acid ethyl ester, dimethyl derivative of apomorphine have not been reported earlier.

CONCLUSION

Short time treatment with higher dose (1000 mg/kg/b.wt) of 70 % ethanolic NN flowers extract betrays hypocholesterolaemic activity. The hypocholesterolaemic activity of NN

extract is similar to that of atorvastatin. The major compounds analysed from GC-MS spectra of NN extract is PUFA especially hexadecanoic acid acid. The separation, quantitative estimation and activity of PUFA in crude extract are under progress.

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