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EFFECT OF *NELUMBO NUCIFERA* AQUEOUS LEAVE AND FLOWERS EXTRACT ON LIPID PROFILE OF ALLOXAN INDUCED DIABETIC ALBINO RATS

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ABSTRACT

Introduction:

Nelumbo nucifera Gaertn (Nymphaeaceae), a perennial aquatic plant, has been used as a medicinal herb in China and India. Different part of plant (leave, seeds, flowers, and rhizomes) can be used in traditional system of medicine. In traditional system of medicine, the different parts of plant is reported to possess beneficial effects as in for the treatment of pharyngopathy, pectoralgia, spermatorrhoea, leucoderma, smallpox, dysentery, cough, haematemesis, epistaxis, haemoptysis, haematuria, metrorrhagia, hyperlipidaemia, fever, cholera, hepatopathy and hyperdipsia. Following the traditional claims for the use of *N. nucifera* as cure of numerous diseases considerable efforts have been made by researchers to verify its utility through scientific pharmacological screenings. The purpose of this study was to elucidate the effect of its aqueous leave and flowers extract on serum lipid profile of alloxan induced diabetic rats.

Methods: Therefore, in the design, twenty four rats were divided into four groups of six each and maintained under ideal laboratory conditions. Group 1 & 2 was taken as normal & diabetic control. Group 3 & 4 was treated orally with 200mg/kg body weight aqueous leave and flowers extract of *N. nucifera* respectively for 45 days. The diet and water were given *ad libitum*. Blood was collected by retro-orbital puncture for lipid profile; serum triglyceride, total cholesterol, HDL-cholesterol, LDL-cholesterol and VLDL-Cholesterol estimation.

Results: The results showed that administration dose of *N. nucifera* leave and flowers extract had significant reduction ($P < 0.05$) in serum lipid profile while significant elevation ($P < 0.05$) was found in HDL-cholesterol when compared with diabetic control.

Conclusion: This result clearly indicated that *nucifera* leave and flowers extract can be key contributor in treatment of diabetes and also could play a cardio protective role.

INTRODUCTION

Scientific evaluation of medicinal plant is important to the discovery of novel drugs and also helps to assess risks associated with the use of herbal preparation and other conventional drugs of plant origin. In recent past numerous workers all over the globe have tried to explore the possibilities of using medicinal plants for prevention, treatment and management of various diseases¹. One of such diseases is *Diabetes mellitus* which is a disorder of carbohydrate metabolism in which glucose molecules in the body are not oxidized due to a defect in either the production or function of insulin². It is a major degenerative disorder that characterized by hyperglycemia and hyperlipidemia which has been rated as a major contributing factors underlying the development of several metabolic diseases³.

More than 400 plant species having hypoglycemic activity have been available in literature, however, searching for new antidiabetic drugs from natural plants is still attractive because they contain substances which take alternative and safe effect on diabetes mellitus⁴. *Nelumbo nucifera* is aquatic plant grown in Asian countries for its edible rhizomes, seeds, flowers and leaves. Several pharmacologically active constituents have been used for its medicinal properties that include improving learning and memory⁵, hepatoprotective⁶ anti-obesity, anti-HIV activity, anti-tumor effect⁷, diuretic activity, antipyretic activity, antidepressant, anti-inflammatory, and antihypertensive effect⁸.

With increasing incidence of diabetes mellitus in population throughout the world and due to adverse effects of synthetic medicine, there is a clear need for development of indigenous, inexpensive botanical sources for anti-diabetic crude or purified drugs. The confinements of available oral antidiabetic agents either in terms of efficacy/safety coupled with the emergence of the disease into a global epidemic have encouraged a concerted effort to discover herbal drugs that can manage diabetes and cardiovascular diseases more efficiently.

MATERIALS AND METHODS

Plant Material and Preparation of Aqueous extract: Fresh leaves and flowers of lotus used in the present study were collected from village Ichoi, Milkipur and authenticated by horticulture department of NDUAT, Kumarganj, Faizabad (UP). The leaves and flowers of plant *N. nucifera* were dried and then pounded in a mortar; it was then further ground to powder and stored in an air tight container until required. Two hundred grams of the powder was mixed with 1000ml of distilled water and shaken thoroughly at intervals to ensure adequate extraction. It was then

soaked for 48h and then filtered using muslin cloth after which a filter paper was used to obtain a pure filtrate. The filtrate was collected and then evaporated to dryness in a steam bath to give a brownish black residue which was stored in a small plastic container at 4°C⁹.

Experimental Animals: Adult male *albino* wistar rats weighing around 120- 150 g were purchased from Disease free animal house, CCSHAU, Hissar, India. The animals were kept in polypropylene cages (six in each) at an ambient temperature of 25±20°C and 55-65% relative humidity 12±1 h light and dark schedule was maintained in the animal house till the animals were acclimatized to the laboratory conditions, and were fed with commercially available rat chow (Asshirwaad Industries, 1544, Sector 38-B, Chandigarh). The water was given *ad libitum*. The experiments were designed into four groups; first group received only normal diet (control group), second group was diabetic control group (without treatment), third and fourth group was experimental group which was treated with 200mg/kg body weight aqueous leave and flowers extract of *Nelumbo nucifera* orally for 45 days and conducted according to guidelines approved by the institutional animal ethics committee CPCSEA Registration No. 574/02/ab/CPCSEA.

Induction of diabetes: Diabetes was induced by injection of a single intraperitoneal (i.p.) dose of alloxan monohydrate freshly prepared in sterile normal saline (0.9%)¹⁰. The diabetic state was determined after 7 days of alloxination by moderate blood glucose level and gain of body weight. At every 15 days interval, blood glucose level was estimated by glucometer sticks and at the end of 45 days treatment, serum lipid profile were examined.

Collection of serum: Animals from each group were deprived of food overnight but with free access of water before taking the sample of blood. On every 15 days of the experiment, the blood samples were collected in test tubes containing EDTA from the orbital plexus by pricking a needle and centrifuged at 2000 rpm at 4°C for 10 minutes to separate serum for the estimation of the various biochemical parameters.

Biochemical analysis: Serum total cholesterol (TC) estimated by CHOD/PAP method¹¹, serum Triglyceride (TG) carried out by enzymatic method¹² and serum high density lipoprotein cholesterol (HDL-C)¹³ and serum low density lipoprotein cholesterol (LDL-C) and very low density lipoprotein-cholesterol (VLDL-C)¹⁴ as per equation: Serum VLDL-C= Serum Triglyceride/5 ; Serum LDL-C=Total serum cholesterol-(Serum HDL-C+ Serum VLDL-C).

Statistical analysis: All the values of blood biochemical estimations were expressed as Mean \pm SD of six determinations. Statistical analysis was done by Analysis of Variance (ANOVA) between the groups were considered significance at $p \leq 0.05$ level ¹⁵.

RESULTS

Table 1: Effect of aqueous extract of *N. nucifera* (Gaertn.) parts (Leave and Flowers) on lipid profile on liver tissue of control and experimental Animals

Groups	TG Triglyceride (mg/100ml)	TC Cholesterol (mg/100ml)	HDL-C (mg/100ml)	VLDL-C (mg/100ml)	LDL-C (mg/100ml)
C-1	265.50 \pm 4.94a	173.40 \pm 8.43a	34.13 \pm 2.18a	53.10 \pm 0.99a	85.98 \pm 9.64a
C-2	274.35 \pm 4.65b	213.66 \pm 23.02b	22.55 \pm 2.29b	54.87 \pm 0.93b	136.24 \pm 23.43b
E-1	253.65 \pm 12.24cd (7.54% \downarrow)	165.53 \pm 4.25c (22.53% \downarrow)	37.00 \pm 4.38c (64.07% \uparrow)	50.70 \pm 2.49cd (7.59% \downarrow)	77.83 \pm 3.62c (42.87% \downarrow)
E-2	254.47 \pm 10.97dc (7.24% \downarrow)	169.34 \pm 4.64d (20.74% \downarrow)	38.03 \pm 3.86d (68.68% \uparrow)	50.88 \pm 2.20dc (7.27% \downarrow)	80.36 \pm 7.17d (41.01% \downarrow)

Values are Mean \pm SD (n=6), Column with different superscripts are significantly different at $P \leq 0.05$ level. C-1: Normal (Control), C-2: Diabetic (Control), E-1: Diabetic+200mg/kg *b wt* Leave aqueous extract and E-2: Diabetic+200mg/kg *b wt* Flowers aqueous extract. Data in parenthesis is percent increase (\uparrow) and decrease (\downarrow) when compared to C-2 group.

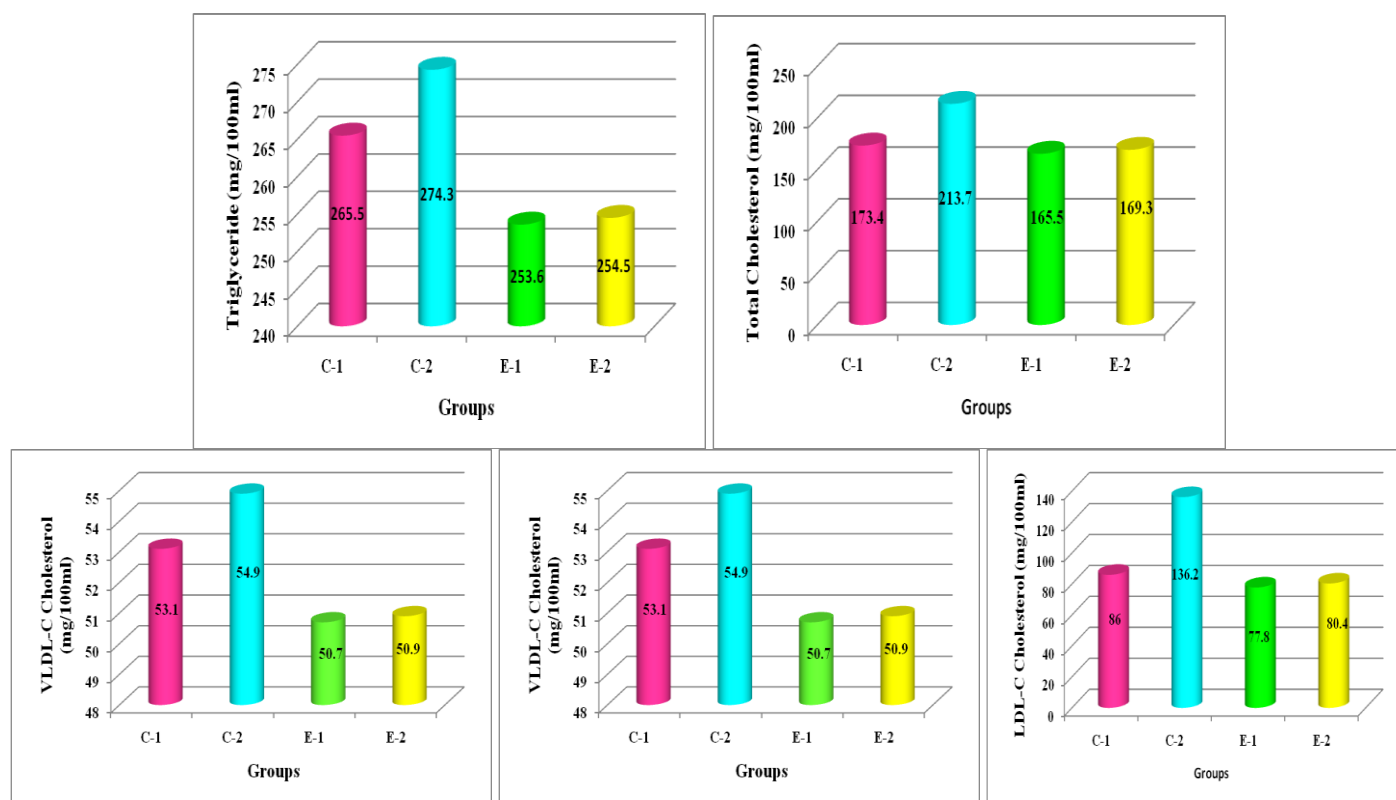


Figure 2: Effect of aqueous extract of *N. nucifera* (Gaertn.) parts (Leave and Flowers) on lipid profile on liver tissue of control and experimental Animals

As shown in table 1 and Figure 2 alloxan produced significant hypercholesterolemic action that a significant increase in level of serum triglyceride (TG), total cholesterol (TC), very low density lipoprotein cholesterol (VLDL-C), low density lipoprotein cholesterol (LDL-C) level while lipoprotein cholesterol (HDL-C) level was significantly decreased when compared to normal group(C-1).

The groups treated with plant part extracts recorded a significant decrease in TG and TC (mg/100ml) level when compared to diabetic control group (C-2). The liver tissue TG and TC of E-1 (253.65 ± 12.24 and 165.53 ± 4.25) and E-2 (254.47 ± 10.97 and 169.34 ± 4.6) showed significant decrease ($p \leq 0.05$) when compared to diabetic control C-2 (274.35 ± 4.65 and 213.66 ± 23.02). The maximum percent reduction was observed in E-1 (7.54% and 22.53%) whereas minimum in E-2 (7.24% and 20.74% respectively). E-1 and E-2 both were insignificant to each other for VLDL-C while significant differences were observed in LDL-C values.

The value of HDL-C (mg/100ml) of C-2 group (22.55 ± 2.2) was significantly reduced at $p \leq 0.05$ level when compared to C-1 group (34.13 ± 2.18). In alloxan treated rats, the HDL-C value of groups E-1 and E-2 was 37.00 ± 4.38 and 38.03 ± 3.86 respectively. From data, maximum increased value was found in E-4 (85.32%), minimum increased was in group E-1 (64.07%).

The value of VLDL-C and LDL-C (mg/100ml) of diabetic control (C-2) group was 54.87 ± 0.93 and 136.24 ± 23.43 which was significantly higher ($p \leq 0.05$) than normal control (C-1) group 53.10 ± 0.99 and 85.98 ± 9.64 respectively. In experimental groups, values of VLDL-C of groups E-1 and E-2 was 50.70 ± 2.49 and 50.88 ± 2.20 while 77.83 ± 3.62 and 80.36 ± 7.17 for LDL-C respectively. The highest percent reduction in was observed in E-1 (7.59% and 42.87%) whereas lowest reduction in E-2 (7.27% and 41.01%) for VLDL-C and LDL-C (mg/100ml) respectively. E-1 and E-2 both were insignificant to each other in VLDL-C whereas significant differences were observed in LDL-C.

DISCUSSION

In the present study, alloxan induced diabetic untreated rats showed significantly increased serum lipid profiles except HDL compared with the control normal rats. Oral administration of the aqueous extracts of *Nelumbo nucifera* plant parts to the diabetic rats significantly reduced the level of TG, TC, and LDL and increased the level of HDL. The results suggest that aqueous extract of the plant parts possesses potential therapeutic value in combating atherosclerosis, which is one of the major complications of diabetes by lowering serum lipids particularly total

cholesterol, triglyceride and low density lipoprotein level. Repeated oral administration of aqueous extracts of *Nelumbo nucifera* plant parts decreased TC, TG and LDL-C but increased HDL-C in treated diabetic rats. These results indicate that *N. Nucifera* plant parts possess hypoglycemic and hypolipidemic activities. In this context numbers of other plants have also been reported to have antihyperglycemic and insulin-release stimulatory effect ^{16, 17}. The oral administration of aqueous extract of rhizomes and leave of *Curcuma longa* and *Passiflora edulis* (*P.edulis*) Sims respectively in diabetic rats for 30 days reduced the total cholesterol, triglycerides (TG) and low density lipoprotein (LDL-C) but increased high density lipoprotein (HDL-C) in the extract treated diabetic rats when compared to diabetic controls by ^{18,19}. Likewise, Sakuljaitrong, *et al.*, ²⁰ also observed that *Nelumbo nucifera* flower extract decreased the levels of total cholesterol (TC), triglycerides (TG) and low density lipoprotein (LDL-C) but increased high density lipoprotein (HDL-C) in the extract treated diabetic rats when compared to diabetic controls. All these studies underscore the importance of diabetes mellitus. The elevated serum TG, TC, VLDL-C, LDL-C level and decreased HDL-C level in alloxan-induced diabetic rats observed in this study is in accord with the previous reports regarding alteration of these parameters under diabetic condition ²¹. Sharma, *et al.*, ²² stated that medicinal plants have hypoglycemic and hypolipidemic effects that may prevent or be helpful in reducing the complications of lipid profile seen in some cases of diabetes in which hyperglycemia and hypercholesterolemia coexist. These effects may be due to low activities of cholesterol biosynthesis enzymes and low level of lipolysis which are under the control of insulin. The overwhelming evidence demonstrated above indicates that hyperglycemia coupled with hyperlipidemia increases the risk for cardiovascular diseases. *Nelumbo nucifera* plant parts have beneficial effect on plasma insulin and these findings strengthen the observation that naturally compounds of plant origin have antidiabetic and antilipidemic effects.

CONCLUSION

The study had shown that the aqueous extract of *N. Nucifera* leave and flowers possesses cholesterol and triglycerides lowering effect in alloxan induced diabetic rats. Therefore, it may be regarded as a useful therapy for hyperlipidemia which can help to prevent cardiovascular diseases and may be used for the control and management of diabetes. Further chemical and pharmacological investigations are required to elucidate the exact mechanism of action of this extract and to isolate the active principles responsible for such effects.

REFERENCES

1. Sarwar M., Attitalla I.H., Abdollahi M. A., "review on the recent advances in pharmacological studies on medicinal plants: Animal studies are done but clinical studies needs completing", Asian Journal of Animal and Veterinary Advances, 2011; Vol. 6: 867-883.
2. Sodipo O. A., Abdulrahman F. I., Sandabe U. K., "Biochemical kidney function with aqueous fruit extract of *Solanum macrocarpum* Linn in albino rats chronically administered triton-X to induce hyperlipidemia", International Journal of Medical Sciences , 2012; Vol. 3:093– 098.
3. Hou Z., Zhang Z., Wu H., "Effect of *Sanguisdraxonis* (a Chinese traditional herb) on the formation of insulin resistance in rats", Diabetes Research and Clinical Practice, 2005; Vol.68: 3-11.
4. Arjun P., MohanaPriya S., Saranya Sivan P.S., Krishnamoorthy M., Balasubramanian K., "Antioxidant and antimicrobial activity of *Nelumbo nucifera* Gaertn. Leaf extracts", Journal of Academia and Industrial Research, 2012; Vol.1 (1):15-18.
5. Gong Y. S., Liu L. G., Xie B. J., Liao Y. C., Yang E. L., Sun Z.D., "Ameliorative effects of lotus seedpod proanthocyanidins on cognitive deficits and oxidative damage in senescence-accelerated mice", Behavioural Brain Research, 2008; Vol. 194: 100-107.
6. Sohn D. H., Kim Y. C., Oh S. H., Park E., LiX., Lee B. H., "Hepatoprotective and free radical scavenging effects of *Nelumbonucifera*", Phytomedicine, 2003; Vol.10:165–169.
7. Duan Y., Zhang H., Xie B., Yan Y., Qin Y., "Whole body radio protective activity of an acetone-water extract from the seedpod of *Nelumbo nucifera*- Gaertn. Seed pod", Food and Chemical Toxicology, 2010; Vol.48: 3374-3384.
8. Sugimoto Y., Furutani S., Nishimura K., Itoh A., Tanahashi T., Nakajima H., Oshiro H., Sun S., Yamada J., "Antidepressantlike effects of neferine in the forced swimming test involve the serotonin1A (5-HT1A) receptor in mice" European Journal of Pharmacology, 2010; Vol.634(1-3):62-67.
9. Nagappan R., "Evaluation of aqueous and ethanol extract of bioactive medicinal plant, *Cassia didymobotrya* (Fresenius) Irwin & Barneby against immature stages of filarial vector, *Culex quinquefasciatus* Say (Diptera: Culicidae)", Asian Pacific Journal of Tropical Biomedicine, 2012; Vol.2 (9):707-711.
10. Esmerino L. A., Ranali R., Rodriguer L. A., "Blood glucose determination of normal and alloxan diabetic rats after administration of local anaesthetics containing vasoconstrictors", Brazilian Dental Journal, 1998; Vol.9 (1):33-37.
11. Stein E. A., "Lipids, lipoproteins and Apolipoproteins. In: N.W. Treitz, (Ed.), Fundamentals of clinical chemistry", 3rd Edn., W.B Saunders, Philadelphia, 1987; 470-479.
12. Tietz N. W., "Clinical guide to lab test", 2ndEdn, W.B. Saunders Company, Philadelphia, U.S.A., 1990; 554- 556.
13. Warnick G. R., "Comparison method for quantification of HDL cholesterol", Clinical Chemistry, 1985; Vol. 31:217.
14. Friedewald W. T., Levy R. I., Fredrickson D. S., "Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultra-centrifuge", Clinical Chemistry, 1972; Vol.18:499-502.
15. Duncan R.C., Knapp R.G., Miller M.C., "Test of Hypothesis in Population Means. In: Introductory Biostatistics for the Health Sciences" John Wiley and Sons Inc., NY. 1997; 71-96.
16. Latha M., Pari L., "Preventive effects of *Cassia auriculata* L. flowers on brain lipid peroxidation in rats treated with streptozotocin", Molecular and cellular biochemistry, 2003; Vol. 243(1-2):23-28.
17. Venkateswaran S., Pari L., "Effect of *Coccinia indica* on blood glucose, insulin and key hepatic enzymes in experimental diabetes", Pharmaceutical Biology, 2002; Vol. 40(3):165-170.
18. Olatunde A., Joel E. B., Tijjani H., Obidola S. M., Luka C. D., "Anti-diabetic Activity of Aqueous Extract of *Curcuma longa* (Linn) Rhizome in Normal and Alloxan-Induced Diabetic Rats", Researcher, 2014; Vol. 6(7):58-65.
19. Kanakasabapathi D., Gopalakrishnan V. K., "Evaluation of Antidiabetic Potential of Aqueous Extract of *Passiflora edulis* Sims on Alloxan Induced Diabetes Mellitus in Wistar Albino Rats", International Journal of Pharmaceutical Sciences Review and Research, 2015; Vol. 34(1):27, 171-177 .
20. Sakuljaitrong S., Chomko S., Talubmook, C., Buddhakala N., "Effect of flower extract from lotus (*Nelumbo nucifera*) on haematological values and blood cell characteristics in streptozotocin-induced diabetic rats", ARPN Journal of Science and Technology, 2012; Vol. 2(11): 1049-1054.
21. Luka C., Tijjani H., "Comparative studies of the aqueous extracts of *Ocimum gratissimum*, *aloe vera*, *brassica oleracea* and *ipomoea batatas* on some biochemical parameters in diabetic rats", IOSR Journal of Pharmacy and Biological Sciences, 2013; Vol. 6, 23-29.
22. Sharma S. B., Nasir A., Prabhu K. M., Murthy P. S., Dev G., "Hypoglycaemic and hypolipidemic effect of ethanolic extract of seeds of *Eugenia jambolana* in alloxan-induced diabetic rabbits", Journal of Ethnopharmacology, 2003; Vol. 85(2):201-206.