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ANTIHYPERGLYCAEMIC, ANTIHYPERLIPIDAEMIC AND ANTIOXIDANT ASSAYS(IN VIVO) OF *NYMPHAEA PUBESCENS* LEAF EXTRACT

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ABSTRACT

The present study was designed to investigate the possible antidiabetic, hypolipidaemic and antioxidant effects of methanol extract of *Nymphaea pubescens* leaves.Diabetes was persuaded in albino rats by injecting of alloxan monohydrate .The methanol extract of *Nymphaea pubescens* leaves at a dose of 250mg/kg and 500mg/kg body weight were administered at single dose per day to diabetes induced rats for a period of 28 days. The effect of methanol extract of *Nymphaea pubescens* leaves extract on blood glucose(48), glycosylated haemoglobin (HbA1C) (3), Alanine aminotransferase (ALT) (38.5) and Aspartate aminotransferase (AST) (56) level. serum lipid profile total cholesterol (TC) (56.5), triglyceride (TG) (44), low density lipoprotein- cholesterol (LDL-C) (10.5), high density lipoprotein- cholesterol (HDL-C) (39) and catalase (CAT) (0.51), lipoprotein peroxidation (LPO) (0.134), blood reduced glutathione (GSH) (0.169) were measured in the diabetic rats. The methanol extracts of *Nymphaea pubescens* leaves elicited significant ($p<0.05$) Declines of blood glucose, lipid parameters except HDL-Cholestrol,serum enzymes and significantly increased HDL-C and antioxidant. In conclusion, the methanol extract of *Nymphaea pubescens* leaves offers promising antidiabetic and hypolipidaemic effects compared with control drug that may be mainly attributed to its potent antioxidant potential. Additional research will be needed in future in order to conclude which one of its active components have the main antidiabetic and hypolidaemic effects.

KEYWORDS: **Alloxan, Diabetes, antioxidant, *Nymphaea pubescens*.**

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INTRODUCTION

Type-2 diabetes (T2D), a chronic metabolic disorder characterized by impaired insulin secretion and insulin sensitivity, has become a worldwide epidemic according to WHO[1]. It is one of the most common endocrine metabolic disorders has caused significant morbidity and mortality due to microvascular (retinopathy, neuropathyand nephropathy) and macrovasculat (heart attack stroke and peripheral vasculat disease) complications[2]. The prevalence of DM for all age-groups worldwide was estimated at 2.8% in 2000 and is projected to increase to approximately 4.4% in 2030. The total number of people with diabetes is projected to rise from 171 million in 2000 to 366 million in 2030[3].The disease is now the world's fourth leading cause of mortality by illness and the global epidemic shows no signs of decline [4]. There is also an increasing demand by patients to use natural products with antidiabetic activity due to the undesirable side effects accompanied with the use of insulin and other hypoglycaemic drugs[5] and also due to the extreme cost of some of these drugs. The available monotherapies and combinatorial treatments are subject to secondary failure which may eventually diminish in efficacy over a period of years. Hence it is a need to constantly attempt to develop safer and more efficacious antidiabetic drugs[6]. Plants are well known in traditional herbal medicine for their hypoglycaemic activities, and available literature indicates that there are more than 800 plant species showing hypoglycaemic. *Nymphaea pubescens* is an aquatic flowering plant of the family Nymphaeaceae having erected perennial rhizomes. It is commonly known as red water lily or hairy water lilly. The whole plant or the parts of the plant used in siddha and folk medicine for treating diabetes, bleeding piles, dyspepsia, jaundice and eye disorders[8]. The flowers are astringent and cardio tonic. The seeds are sweet, cooling, constipating, aphrodisiac, stomachic and restorative. They are useful in vitiated conditions of pita, dipsia, diarrhea and dermatopathy[9]. The plant has to possess antihepatotoxic[9]and hypolipedemic[7] activities. primary and secondary metabolites of the plant was eluted and antimicrobial activity observed[10]. Based on above medicinal properties of *Nymphaea* species, the present study was conducted to investigate the antidiabetic, antihyperlipidaemic and antioxidant activities of methanol extract of *Nymphaea pubescens* leaves in alloxan induced diabetic rats.

MATERIALS AND METHODS

1. Plant material

Nymphaea pubescens 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, The leaves were shade dry and powdered. It was kept in an airtight container in a deep freeze until the time of use.

2. Preparation of plant extract for Antidiabetic and antioxidant

The *Nymphaea pubescens* leaves were shade dried at room temperature and the dried leaves were powdered in a pulverizer. Sixty grams of powdered *Nymphaea pubescens* leaves was packed in a Soxhlet apparatus and extracted with methanol, the extract were subjected to qualitative test for the identification of various phytochemical constituents as per standard procedures[10]. The methanol extracts were concentrated in a rotary evaporator. The concentrated methanol extract were used for antidiabetic & antioxidant studies.

3. Animals

Normal healthy male Wistar albino rats (150-200g) were housed under standard environmental conditions at temperature (25±2º C). Rats were fed with standard pellet diet (Gold mohor Ltd., Mumbai, India) and water by

ad libitum. All the experimental protocols used in this study were reviewed by the Institutional Animal Ethics Committee (1629-PO-A-12-CPCSEA) and were in accordance with the guidelines of the CPCSEA.

4. Acute Toxicity Study

Acute oral toxicity study was performed as per CPCSEA guidelines (acute toxic class method).The group of six animals were kept fasting for overnight and provided only with water, after which the extracts were administered orally at 5mg/kg body weight by gastric intubations and observed for 14 days. If mortality was observed in two out of three animals, then the dose administered was assigned as 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 higher doses such as 250, 500, 1000 and 2000 mg/kg body weight.

5. Induction of Experimental Diabetes

Rats were induced diabetes by the administration of simple intraperitoneal dose of alloxan monohydrate (150 mg/kg)[11].Two days after alloxan injection, rats screened for diabetes having glycosuria and hypoglycemia with blood glucose level of 200 mg/100 ml were taken for the study. All animals were allowed free access to water and pellet diet and maintained at room temperature in plastic cages.

6. Experimental design

In the investigation, a total of 30 rats (20 diabetic surviving rats and 10 normal rats) were taken and divided into five groups of six rats each.

Group I: Normal, untreated rats.

Group II: Diabetic control rats

Group III: Diabetic rats given standard drug glibenclamide (0.5mg/kg of body weight).

Group IV: Diabetic rats given methanol extract of *Nymphaea pubescens* leaves (250 mg/kg of body weight).

Group V: Diabetic rats given methanol extract of *Nymphaea pubescens* leaves (500 mg/kg of body weight).

7. Biochemical analysis

The animals were sacrificed at the end of the experimental period of 28 days by decapitation. Blood was collected, sera separated by centrifugation at 2500rpm/10 minutes. Serum glucose was measured by the GOD-POD method[12]. Triglycerides were measured by GPO-POD method[13].Glycosylated haemoglobin (HbA1c) estimation was carried out by a boronate affinity assay[14, 15]. Cholesterol was estimated by CHOD-POD method[16] (Herbert *et al.,* 1984), Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT) were measured by Modified UV (IFCC), Kinetic Assay, Liver glycogen was estimated by using anthrone reagent[17,18] were analyzed in the normal, diabetic induced and drug treated rats. Catalase (CAT), lipid peroxidation (LPO)[19], reduced glutathione (GSH)[20] were measured by post mitochondrial supernatant (PMS) of pancreas tissue.

8. Statistical analysis

The results were expressed as means ± SEM. Statistical analysis was performed by Two-way analysis of variance (ANOVA) test for multiple comparisons followed by Turkey-Kramer test. Statistical significance set accordingly.

RESULTS AND DISCUSSION

Alloxan is one of the usual substances used for the induction of diabetes mellitus apart from streptozotocin. Alloxan has a destructive effect on the beta cells of the pancreas[21, 22]. Alloxan causes a massive reduction in the insulin release by the destruction of beta cells of the islets of Langerhans, thereby inducing hyperglycaemic[23]. The results of the present study indicated that, *Nymphaea pubescens* leaves extract was found to reduce the glucose level and increase the plasma insulin significantly. The hypoglycaemic effect of

Nymphaea pubescens leaves was found to be inducing insulin release from pancreatic cells of diabetic[24]. It is evident from this study that, there was an increase in insulin levels in diabetic rats treated with plant extract. A significant elevation in serum constituents, Alloxan induced diabetic rats showed significant increased ($p<0.05$) glycosylated haemoglobin (HbA1C) level compared with normal rats. The methanol extract of *Nymphaea pubescens* treated rats showed the decrease in the content of glycosylated haemoglobin. Glycosylated haemoglobin determinations are self-monitoring of blood glucose in (Table-1) therefore play important complementary roles for the management of diabetes mellitus[25].

Table 1

Effect of methanol extract of Nymphaea pubescens on the serum glucose, glycosylated Hb, liver glycogen, Alanine aminotransferase (ALT) and Aspartate aminotransferase (AST) level of normal, diabetic and drug induced adult albino rats on 28th day.

Parameter	Serum glucose levels	Glycosylated hemoglobin	Liver glycogen	ALT levels	AST levels
Group I	66.05±5.135	4.05 ± 0.403	6.24 ± 0.603	34.73± 3.962	55.34±4.983
Group II	261.24±14.44	12.29 ± 1.210	3.93 ± 0.312	150.95 ± 13.526	154.26± 14.52
Group III	63.73±3.379	3.95 ± 0.621	8.88 ± 0.813	39.58 ± 6.615	55.35± 4.467
Group IV	62.71± 5.299	3.50 ± 0.210	2.24 ± 0.812	40.88 ± 5.561	61.15 ± 3.399
Group V	47.54± 2.412	3.05 ± 0.281	3.37 ± 0.712	38.62 ± 6.608	56.32 ± 6.607

Each value is SEM of 6 animals, Comparisons were made between normal control to diabetic control and drug treated groups.

According to above table leaf extract is more potent than control drug with these parameters. The levels of serum total cholesterol (TC), triglycerides (TG), LDL-C and HDL-C in control and diabetic rats were investigated (Table- 2). The glibenclamide and methanol extract of *Nymphaea pubescens* leaves treated rats showed a significant decrease in the content of lipid profile, when compared with diabetic induced rats. HDL helps to scavenge cholesterol from extra hepatic tissues[26]. Decreased HDL can contribute to the increased cholesterol levels. A greater increase of LDL may cause a greater decrease of HDL as there is a reciprocal relation between the concentration of LDL and HDL.

Table 2

Effect of methanol extract of Nymphaea pubescens on the serum Triglycerides, total cholesterol, HDL Cholesterol, LDL-cholesterol levels of normal, diabetic and drug induced adult albino rats on 28th day.

Parameter	Triglyceride	Total cholesterol	HDL-cholesterol	LDL-cholesterol
Group I	36.49 ± 3.320	55.62 ± 4.456	35.40 ± 5.556	12.91 ± 1.671
Group II	140.37 ± 15.58	274.26 ± 13.96	10.26 ± 2.337	235.86 ± 14.10
Group III	48.22 ± 4.489	59.92 ± 4.472	41.385 ± 3.401	8.89 ± 1.500
Group IV	50.58 ± 2.117	60.50 ± 4.437	38.5 ± 5.590	11.88 ± 1.108
Group V	44.23 ± 2.74	56.53 ± 4.774	39.09 ± 3.722	10.590 ± 1.315

Each value is SEM of 6 animals, Comparisons were made between normal control to diabetic control and drug treated groups.

The level of GSH ratio in the blood and the erythrocytes of normal, diabetic induced and drug treated rats were studied. The highly significant reduction of the activity of scavenging mitochondrial enzymes are observed in alloxan induced rats. These adverse changes were reversed to near normal values in the methanol extract of *Nymphaea pubescens* (Table-3). Mitochondria are the energy reservoir of the cells and the damage

inflicted in mitochondria would ultimately result in the reduction of energy production and thereby leading to cell death[27]. Subcellular membrane associated with thiol bearing enzymes, represents sensitive risks for detoxification causing perpetuation of cellular function[28]. Reactive oxygen species can themselves reduce the activities of antioxidant defense mechanism. GSH is a major non-protein thiol in living organisms which plays a central role in coordinating the body's antioxidant defense processes. Perturbation of GSH status of a biological system has been reported to lead to serious consequences[29]. Decline in GSH content in the serum of diabetic induced rats, and its subsequent return towards near normally in plant extracts treated rats reveal the antioxidant effect of *Nymphaea pubescens*. Explanations of the possible mechanism underlying the antioxidant properties of this drug include the prevention of GSH depletion and destruction of free radicals[30]. The increases in the levels of LPO due to the effects of diabetes are shown in (Table 3). The results obtained showed that lipids of the diabetic rats are vulnerable to peroxidation due to the increased oxidative stress during diabetes. LPO plays an important role in aging, atherosclerosis and in a number of diabetic complications[31, 32]. As diabetes and its complications are associated with free radical medicated cellular damage[33], herbal hypoglycaemic agents are administered to diabetic rats to assess their antioxidant potential. In the present study, *Nymphaea pubescens* extract not only have hypoglycemic activity but these compounds also significantly control the LPO levels in diabetic rats. These two factors are believed to attribute to the antioxidant properties of *Nymphaea pubescens.* These results revealed the protective role of plant extracts in decreasing lipid peroxidation and by normalizing antioxidant system.

Table 3

Effect of methanol extract of Nymphaea pubescens on the post mitochondrial supernatant (PMS) tissue of catalase (CAT), reduced glutathione (GSH), lipid peroxidation (LPO) levels of normal, diabetic and drug induced adult albino rats on 28th day.

parameter	CAT (µM/mg tissue)	GSH (µM /mg tissue)	LPO (µM /mg tissue)
Group I	0.576 ± 0.016	0.185 ± 0.033	0.049 ± 0.006
Group II	0.043 ± 0.001	0.040 ± 0.014	0.476 ± 0.052
Group III	0.666 ± 0.018	0.179 ± 0.039	0.058 ± 0.003
Group IV	0.396 ± 0.029	0.013 ± 0.024	0.015 ± 0.011
Group V	0.510 ± 0.045	0.134 ± 0.029	0.016 ± 0.002

Each value is SEM of 6 animals, Comparisons were made between normal control to diabetic control and drug treated groups.

CONCLUSION

In conclusion, the methanol extract of *Nymphaea pubescens* leaves offers a promising therapeutic value in prevention of diabetes. These effects could be mainly attributed to its antioxidant properties as shown by the significant quenching impact on the extract of lipid peroxidation along with, enhancement of antioxidant defense systems in pancreatic tissue. The antioxidative property of *Nymphaea pubescens* extract certainly is due to its chemical constituents. Further studies will be needed in future to determine the main active ingredient having the beneficial antidiabetic, hypolipidaemic and antioxidant effects.

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REFERENCES

1. Nan Shanga, José A. Guerrero-Analcob, Lina Musallama, Ammar Saleem, Asim Muhammad, Brendan Walshe-Roussel, Alain Cuerrier, John T. Arnason, Pierre S. Haddada, Adipogenic constituents from the bark of *Larix laricina du Roi* (K. Koch; Pinaceae),an important medicinal plant used traditionally by the Cree of Eeyou Istchee (Quebec, Canada) for the treatment of type 2 diabetes symptoms. Journal of Ethnopharmacology, 141:1051–1057(2012).
2. Patel DK, Prasad SK, Kumar R, Hemalatha S, An overview on antidiabetic medicinal plants having insulin mimetic property, Asian Pacific Journal of Tropical Biomedicine, 320-330, (2012).
3. S. Semenya, M. Potgieter, L. Erasmus, Ethnobotanical survey of medicinal plants used by Bapedi healers to treat diabetes mellitus in the Limpopo Province, South Africa, Journal of Ethnopharmacology 141: 440– 445 (2012).
4. Diabetes Voice. International Diabetes Federation 5th edition, 52: 1-48 (2007).
5. Kaleem M, Medha P, Ahmed QU, Asif M, Bano B, Beneficial effects of Annona squamosa extract in streptozotocin induced diabetic rats, Singapore Med J, 49:800- 804(2008).
6. Green BD, Irwin N, Duffy NA, Gault VA, Harte FP, Flatt PR, Inhibition of dipeptidyl peptidase-IV activity by metformin enhances the antidiabetic effects of glucagon like peptide-1. European Journal of Pharmacology, 547 :192-199 (2006).
7. Rajagopal K, Sasikala K, Singapore Med, 49(2):137 (2008).
8. Selvakumari S, Shantha A, Antidiabetic activity of Nymphaea pubescens Willd-A plant drug of Aquatic flora interest, Journal of Pharmacy Research, 3(12): 3067-3069 (2010).
9. Muthulingam M, Antihepatotoxic efficacy of Nymphaea pubescens (willd.) on acetaminophen induced liver damage in male Wistar rats, International Journal of Current Research, 3: 012 -016 (2010).
10. Kiran Kumar Angadi, Ravi Kumar Gundampati, Medicherla V. Jagannadham, Ammani Kandru, Phytochemical analysis and Antimicrobial properties of leaf extracts of Nymphaea pubescens, Asian Pacific Journal of Tropical Biomedicine, 1-5 (2012).
11. Nagappa AN, Thakurdesai PA, Venkat Rao N, Sing J, Antidiabetic activity of Terminalia catappa Linn. Fruits, Journal of Ethno pharmacology, 88: 45-50 (2003).
12. Kaplan A, Carbohydrates and Metabolite, In clinical chemistry: Theory, Analysis and co-relation, Kaplan LA and Pesce AJ, Eds. C.V.Mosby, Toronto, pp: 1032-1040 (1984).
13. Kaplan A and Lavernel LS, Disorder of Carbohydrate. In-Clinical Chemistry: Interpretation and Techniques. Lea and Febiger. 2nd Eds. Philadelphia, pp. 301-320 (1983).
14. Gould BJ, Hall PM and Cook JGH, Measurement of glycosylated hemoglobins using an affinity chromatography method., Clinical Chemistry Acta, 125: 41-8 (1982).
15. Yue DK, Mclennan S, Church DB and Turtle JR, The Measurement of glycosylated hemoglobin in man and animals by amino-phenylboronic acid affinity chromatography, Diabetes, 31: 701-705 (1982).
16. Herbert K, Lipids, In Clinical Chemistry: Theory, Analysis and co-relation, Kaplan,

L.A. and Pesce, A.J, Eds, C.V. Mosby, Toronto, pp. 1182-1230 (1984).
17. Seilfter S, Dayton S, Novic B and Muntwyler E, Arch. Biochem, 25:191 (1950).
18. Nicholas V, Carroll, Robert W, Longley and Joseph H, The determination of glycogen in liver, and muscle bye use of anthrone reagent, The Journal of Biological Chemistry, (1955).
19. Niehaus WG and Samuelsson B, Formation of malonaldehyde from phospholipid arachidonate during microsomal lipid peroxidation, Eur J Biochem, 6:126-130 (1968).
20. Jollow D, Mitchell L, Zampaglione N and Gillete J, Bromobenzene induced liver necrosis: Protective role of glutathione and evidence for 3, 4–bromo-benzenoxide as the hepato toxic intermediate, Pharmacol, 11:151-169 (1974).
21. Jelodar G, Mohren M, Shahram S, Effect of walnut leaf, coriander and pomegranate on blood glucose and histopathology of pancreas of alloxan- induced diabetic rats, African J. Traditional Complementary and Alternative medicine, 3:299-305 (2003).
22. Prince S.M, Menon V.P, Hypoglycemic and other related actions of Tinospora cardifolia roots in alloxan induced diabetic rats, J.Ethnopharmacol, 70: 9-15 (2000).
23. Grover J.K, Vats V, Rathi S.S, Antihyperglycaemic effect of Eugenia jambolana and Tinospora cordifolia in experimental diabetes and their effects on key metabolic enzymes involved in carbohydrate metabolism, J. Ethnopharmacol, 73: 461-470 (2000).
24. Sharma N, Garg V, Antidiabetic and antioxidant potential of ethanolic extract of Butea monosperma leaves in alloxan induced diabetic mice, Indian J. Biochem. Biophys, 46: 99-105 (2009).
25. Thai A.C, Yeo PPB, Chan L, Wang KW, Tan BY, Jocobs E, Glycosylated haemoglobin and diabetic control. Singapore Medical Journal, 24: 210-212 (1983).
26. Brewer H.B, Focus on high density lipoproteins in reducing cardiovascular risk, Am. Heart. J, 148: 514-518 (2004).
27. Sohal R, Dubey A, Mitochondria oxidative damage, hydrogen peroxide release and aging, Free Rad. Biol. Med, 16: 621-626 (1994).
28. Kyu B.K, Byung M.L, Oxidative stress to DNA, protein and antioxidant enzymes in rats treated with Benzo (a) Pyrene, Cancer Lett, 113: 205-212 (1997).
29. Uday B, Das D, Banerjee K, Reactive oxygen species: oxidative damage and pathogenesis, Curr. Sci. 77:658-665 (1999).
30. Valenzuela A, Lagos C, Schmidt K, Videla K. Silymarin., Protection against hepatic lipid peroxidation induced by acute ethanol intoxication in the rat, Biochem. Pharmacol, 3: 2209-2212 (1985).
31. Kesavulu M.M, Rao B.k, Giri R, Vijya J.S, Subramanyam A.C.H, Lipid peroxidation and antioxidant enzymes status in type 2 diabetics with coronary heart disease, Diabetes. Res. Clin. Prac, 53: 33-39 (2001).
32. Matkovics B, Varga SI, Szabo L, Witas H, The effect of diabetes on the activities of the peroxide metabolism enzymes, Horm. Metab. Res, 14: 77-79 (1982).
33. Yu B.P, Cellular defense against damage from reactive oxygen species, Physiological Rev, 74: 139-162 (1994).