International Journal of A J Institute of Medical Sciences 1 (2012) 86-92

Original Research Article

Hepatic anti-oxidants in streptozotocin induced diabetic rats on long term treatment with Gymnema sylvestre

Bhagyajyothi M. Bhat,[1] C.V. Raghuveer,[2] Vivian D'Souza[3], K. K. Srinivasan,[4] Poornima A. Manjrekar[3]

[1] Department of Biochemistry, A.J. Institute of Medical Sciences, Mangalore, India
[2]Yenepoya University, Mangalore, India
[3]Department of Biochemistry, Kasturba Medical College, Mangalore, India
[4]Department of Pharmaceutical Chemistry, Manipal College of Pharmaceutical Sciences, Manipal University, Manipal, India

Abstract

Ability of some of plants to control hyperglycaemia has captured scientific interest worldwide towards better understanding of the underlying mechanism**.** The present study was conducted to evaluate the effect of a standardized hydro alcoholic leaf extract of Gymnema sylvestre on random blood glucose (RBG), serum insulin, plasma glycated haemoglobin (HbA1c) and liver vitamin C, reduced glutathione (GSH), protein thiols (PT), thiobarbituric acid reactive substances (TBARS) and carbonyl proteins (CP) in long standing experimentally induced diabetes mellitus with glibenclamide (Glb) as standard. Streptozotocin (STZ) induced diabetic wistar rats of either sex were treated with two oral doses of Gymnema sylvestre extract, 2.5 and 1g/kg body wt /day for a period of 16 weeks. Increase in the levels of vitamin C, GSH, PT and decreased TBARS and CP in liver was observed on treatment with Gymnema sylvestre ($p \leq 0.05$). A decrease in RBG (45% and 33%) and plasma HbA1c ($p \leq 0.05$) with increased serum insulin ($p \leq 0.05$) was also observed in diabetic rats on treatment. The effect of Gymnema sylvestre on tissue antioxidants is possibly due to reduction in blood glucose level in diabetic rats, which is an additional benefit for this plant towards becoming a drug of choice in controlling chronic diabetes mellitus.

Keywords: Anti-oxidant; Blood glucose; Diabetes mellitus; Gymnema sylvestre

Received 11st July, 2012; Revised 8th August, 2012; Accepted 12th September, 2012.

1. Introduction

Oxidative stress is currently suggested to play a major role in the development of diabetes mellitus.[1] On the contrary, there is growing evidence that excess generation of highly reactive free radicals, largely due to hyperglycaemia, causes oxidative stress, which further exacerbates the development and progression of diabetes and its complications.[2,3] Increased generation of reactive oxygen species and/or decrease in antioxidant defense potential might be the causes for oxidative stress in cells and tissues.[4] Nonenzymatic glycation and lipid peroxidation have been estimated to play an important role in the progression of diabetic complications such as atherosclerosis, neuropathy, nephropathy, retinopathy and

cataractogenesis.[5] Since numerous studies demonstrated that oxidative stress, mediated mainly by hyperglycaemia-induced generation of free radicals, contributes to the development and progression of diabetes and related contributions, it became clear that ameliorating oxidative stress through treatment with antioxidants might be an effective strategy for reducing diabetic complications.[2]

Free radicals are generated as by-products of normal cellular metabolism; however, several conditions are known to disturb the balance between reactive oxygen species (ROS) production and cellular defense mechanisms. This imbalance can result in cell dysfunction and destruction resulting in tissue injury. The increase in the level of ROS in diabetes could be due to their increased production and/ or decreased destruction by nonenzymic and enzymic antioxidants.[6] The ROS generated in turn leads to tissue damage, with lipid peroxidation, inactivation of proteins, and protein glycation as intermediate mechanisms for its complications such as retinopathy, nephropathy, and coronary heart disease. Therefore, recently, interest has focused on plant-based natural antioxidants such as tannins, polyphenols and flavonoids to reduce the negative effect of oxidative stress and free radicals in diabetic patients and to prevent the destruction of β-cells and thus diabetic complications.[7,8]

Gymnema sylvestre also known as Madhunashini is a woody climber, belonging to the family Asclepiadaceae, common in central and southern India. The first scientific confirmation of usage of Gymnema sylvestre in human diabetics came almost a century back when it was demonstrated that the leaves of this plant reduce urine glucose in diabetics.[9] The leaf extract of Gymnema sylvestre was known to contain anti-diabetic and anti-atherogenic principle.[10,11] Gymnemic acid present in the leaves was found to be the active principle responsible for anti-hyperglycaemic activity.[12,13] Anti-oxidant effect of hydro alcoholic leaf extract of Gymnema sylvestre has been shown by previous workers in-vitro.[14,15] It was also suggested that the anti-oxidant property might be due to the presence of acidic compounds, flavonoids, phenols, saponins, tannis (phenolic compounds) and triterpenoids found in the preliminary phytochemical screening.[15] In the present study, the anti-oxidant effect of a standardized hydroalcoholic leaf extract of Gymnema sylvestre was evaluated in hepatocytes of streptozotocin (STZ) induced diabetic rats on treatment for a period of 16 weeks. The results obtained with Gymnema sylvestre were compared with glibenclamide (Glb), a known anti-hyperglycaemic drug.

2. Materials and methods

Ethical clearance for the study was obtained from the Institutional Animal Ethics Committee (IAEC), Kasturba Medical College, Manipal University, Mangalore. Required quantity of Gymnema sylvestre extract was availed from a leading herbal research driven company. Gravimetric phytochemical analysis report of Gymnema sylvestre certified that the active principle, gymnemic acids content was > 10.0 %w/w. The powder was dissolved in 0.5% carboxy methyl cellulose to prepare a solution of this extract and fed orally. Present study was conducted using Albino rats of Wistar strain of either sex, weighing 100 ± 10g. The normal laboratory pellet diet and water was given ad libitum (at one's pleasure). Rats were acclimatized to the laboratory conditions for at least 1 week prior to the experiment. Single dose of STZ, 50mg/Kg body weight, in cold citrate buffer (0.1M) of pH 4.0 was injected intraperitoneally, after 18-20 hours fasting for inducing Diabetes mellitus.[16] The rats were monitored for 72 hours to ensure survivability. Only those rats which showed Random Blood Glucose (RBG) of 350mg/dl or more at day 1were taken for the study. RBG was checked with ACCU-CHEK active blood glucose

monitor, using disposable strips.

Acute toxicity studies were conducted for the plant extracts.[17] Wistar rats of either sex, weighing 100 ± 10g were selected for the study. Thirty animals, fasted overnight, were divided in to 5 groups, each consisting of six animals. Plant extract was administered in 0.5% carboxymethyl cellulose in different doses consisting of 5, 10, 20, 25 and 30g/kg body weight for Gymnema sylvestre extract. The animals were observed continuously for 2 hours, then intermittently once in every 2 hours and then at the end of 24 hours to note the number of deaths. In general, $1/10^{th}$ of the maximum tolerance dose is chosen for the plant extracts. 25g/kg was found to be safe for Gymnema sylvestre extract, so $1/10^{th}$ of these doses, i.e., 2.5g/kg were selected. Since it is always better to use the smallest possible dose with maximum benefits, 1g/kg was selected as the second dose.

The duration of the study was 16 weeks. Rats were divided into five groups of eight rats each.

Group 1- Normal control.

Group 2- STZ-induced diabetic control.

Group 3- Diabetic rats fed Gymnema sylvestre extract at a dose of 2.5g/kg body weight.

Group 4- Diabetic rats fed Gymnema sylvestre extract at a dose of 1.0g/kg body weight.

Group 5- Diabetic rats fed Glb (500 µg/kg) in aqueous solution.

At the end of 16 weeks, RBG was checked. After overnight fast, the rats were sacrificed with high dose of ether. Blood was drawn immediately by cardiac puncture. Serum was used for estimation of insulin, while EDTA blood was used for estimation of HbA1c. The liver was dissected out, weighed, homogenized in 10 volumes of 0.9% normal saline and used for estimation of vitamin C, reduced glutathione (GSH), protein thiols (PT), thiobarbituric acid reactive substances (TBARS) and carbonyl proteins (CP).

Insulin levels were determined by ELISA using anti-rat insulin antibodies (LINCO Research, 6 Research Park Dr. St. Charles, Missouri 63304 USA). Estimation of HbA1c was done by turbidimetric inhibition immunoassay (TINIA). Vitamin C[18] was estimated by reaction with 2,4-dinitrophenyl hydrazine (DNPH), which was measured spectrophotometrically at 520nm. GSH was estimated by the reaction with 5,5′-dithio nitrobenzoic acid (DTNB), by the method of Beutler et al., the yellow colour was measured at 412 nm.[19] TBARS were measured by the method of Stocks and Dormandy, the pink chromogen was read at 532nm. CP was estimated by the reaction with DNPH and was read at 365 nm.[20,21] PT was estimated by the reaction with DTNB and the yellow colour developed was read at 412 nm.[22] Systronics UV-Visible Spectrophotometer 117 was used for manual estimations. All the chemicals used were reagent grade.

All grouped data were evaluated statistically with SPSS 17 software (SPSS, Chicago, IL, USA). All results were expressed as the mean ± SD for eight animals in each group. Comparison of various parameters was done using Kruskal-Wallis test, p-value ≤ 0.05 was considered statistically significant.

3. Results

Treatment with Gymnema sylvestre extract brought down the RBG. A comparison of RBG at the end of 16^{th} week with that of day-1 is given in table-1. Diabetic rats which received higher dose of GSE showed 45% decrease, where as lower dose showed a decrease of 33% in RBG as compared with that of day-1. The decrease in RBG by Glb was 29%. A significant decrease in HbA1c and increase in serum insulin were observed in rats which received the plant extract when compared to diabetic control (Table 1). Treatment with Glb also showed similar results to that of Gymnema sylvestre.

Table 1: Levels of RBG, insulin and HbA1c in control and experimental groups of rats

(n=8)	RBG (mg/dl)		Insulin (ng/ml) at the end of 16 weeks	HbA1c (%)at the end of 16 weeks
	Day-1	End of 16th week		
Group-1	103.88±4.76	97.25± 2.25	0.64 ± 0.13	3.66 ± 0.17
Group-2	531.25±32.44	529.38±24.78	0.14 ± 0.03*	7.84±0.32*
Group-3	483.13 ±19.94	265.75 ±58.47	0.60±0.08 †	6.78±0.73†
Group-4	464.50 ±31.38	309.25±58.48	0.35±0.09 †	6.93±0.36†
Group-5	436.25±23.06	310.88±76.37	0.53 ± 0.05†	7.09±0.64†

*p ≤ 0.05 compared to normal control, †p ≤ 0.05 compared to diabetic control.

(Group-1: Normal control; Group-2: Diabetic control; Group-3: Diabetic rats treated with Gymnmema sylvestre extract at a dose of 2.5g/kg/day; Group-4: Diabetic rats treated with Gymnmema sylvestre extract at a dose of 1g/kg/day; Group-5: Diabetic rats treated with glibenclamide at a dose of 500µg/kg/day)

A significantly decreased vitamin C, GSH and PT with increased TBARS and CP were observed in diabetic controls compared to normal control group. Upon treatment with Gymnema sylvestre, a dose dependent increase in vitamin C, GSH and PT and a decrease in TBARS and CP were observed, which was significant compared to diabetic control. Treatment with Glb also improved these parameters, where statistical significance was achieved only by GSH, PT and TBARS (Table 2).

Table 2: Levels of vitamin C, GSH, PT, MDA and CP in liver of control and experimental groups at the end of 16 weeks

(n=8)	Vitamin C (µg/g tissue)	GSH (µg/g tissue)	PT(µmoles/mg protein)	MDA(µmoles/ g tissue)	CP(mU/mg protein)
Group-1	188.13±35.14	627.0±179.77	79.49±11.07	13.03±1.31	1.33±0.34
Group-2	86.75±13.77*	279.63±32.96*	35.76±2.02*	24.2±2.21*	1.96±0.19*
Group-3	123.88±24.06†	424.25±44.41†	61.46±18.71†	16.00±1.76†	1.83±0.31
Group-4	111.13±17.4†	307.38±43.15	59.64±7.02†	22.2±3.67	2.36±0.47
Group-5	101.75±14.65	431.25±70.93†	68.06±17.22†	18.81±4.05†	2.14±0.52

*p ≤ 0.05 compared to normal control, † p ≤ 0.05 compared to diabetic control.

(Group-1: Normal control; Group-2: Diabetic control; Group-3: Diabetic rats treated with *Gymnmema sylvestre* extract at a dose of 2.5g/kg/day; Group-4: Diabetic rats treated with *Gymnmema sylvestre* extract at a dose of 1g/kg/day; Group-5: Diabetic rats treated with glibenclamide at a dose of 500µg/kg/day)

4. Discussion

An improvement in the glycaemic status of diabetic rats was appreciated on treatment with Gymnema sylvestre extract which was indicated by decreased RBG and plasma HbA1c with increased serum insulin levels. The anti-hyperglycaemic effect of Gymnema sylvestre has been established earlier by several workers.[23,24] Gymnemic acid present in Gymnema sylvestre was found to be responsible for anti hyperglycaemic activity.[25,26] On this basis, it may be said that the gymnemic acid present in Gymnema sylvestre extract used in the present study produced anti hyperglycemic effect. Gymnema sylvestre was also found to cause regeneration of endocrine pancreas[23]. Increased insulin level in rats which received the plant extract is possibly due to this effect. Decrease in HbA1c confirms the anti hyperglycaemic effect of Gymnema sylvestre and the effect lasted for a sustained period of 16 weeks. Numerous studies have revealed lowered anti-oxidant and enhanced peroxidative status in diabetes mellitus.[27] The present study also is in agreement with this finding, where the levels of anti oxidants vitamin C, GSH and PT were decreased and those of oxidants TBARS and CP were increased in diabetic control. Treatment with Gymnema sylvetre extract partially normalised this imbalance which was indicated by an improved anti-oxidants and decreased oxidants in the treated groups. In-vitro anti-oxidant activity of Gymnema sylvestre was demonstrated by previous workers.[14,15,28] Earlier studies showed anti-oxidant effect of Gymnema sylvetre in STZ diabetic rats, indicated by increased TBARS and superoxide dismutase.[29,30] Anti-oxidant activity of a polyherbal formula containing Gymnema sylvestre was observed by Patel et al.[31] Srividya et al. claimed Gymnema sylvestre to have a hepatoprotective role, and it is possible that the capacity of this plant to increase anti- oxidants in hepatocytes might have protected the hepatic peroxidative damage.[32] The present study thus adds to the existing knowledge, where anti-oxidant activity was retained for a sustained period of 16 weeks.

It was also suggested that the anti-oxidant effect of the plant extracts is due to the presence of acidic compounds, flavonoids, phenols, saponins, tannis (phenolic compounds) and triterpenoids present in them.[15] As Gymnema sylvestre contains gymnemic acid, it is possible that this principle has acted in protecting the tissue from oxidative damage. In conclusion, the effect of Gymnema sylvestre on tissue antioxidants is possibly due to reduction in blood glucose level in diabetic rats, which prevented excessive formation of free radicals through various biochemical pathways or on the other hand, the anti oxidant effect of Gymnema sylvestre might have controlled chronic hyperglycaemia.[33] This mechanism has to be elicited further for better understanding of the mechanism of action of Gymnema sylvestre. On either way, Gymnema sylvestre has a dual benefit for treating chronic hyperglycaemia.

5. Limitations

While the estimation of parameters like HbA1c and insulin were performed by latest and sensitive methods, those used for estimation of anti-oxidants were older but established methods. However, the results of the study can be ascertained by sensitive assays by further research.

Acknowledgements

The authors are indebted to ICMR, New Delhi for the financial support.

References

1. Tripathi UN, Chandra D. The plant extracts of momordica charantia and trigonella foenum

graecum have antioxidant and anti-hyperglycemic properties for cardiac tissue during diabetes mellitus. Oxid Med Cell Longev 2009;2:290-296.

2. Johansen JS, Harris AK, Rychly DJ, Ergul A. Oxidative stress and the use of antioxidants in diabetes: Linking basic science to clinical practice. Cardiovasc Diabetol 2005;4:5.
3. Mahjoub S, Roudsari JM. Role of oxidative stress in pathogenesis of metabolic syndrome. Caspian J Intern Med 2012;3:386-396.
4. Gumieniczek A, Hopkala H, Wojtowich Z, Nikolajuk J. Changes in antioxidant status of heart muscle tissue in experimental diabetes in rabbits. Acta Biochim Pol 2002;49:529-535.
5. Shibata S, Natori Y, NishiharaT, Tomisaka K, Matsumoto K, Sansawa H, Nguyen VC. Antioxidant and anti cataract effects of chlorella on rats with STZ induced diabetes. J Nutr Sci Vitaminol 2003;49:334-339.
6. Moussa SA. Oxidative stress in diabetes mellitus. Romanian J Biophys 2008;18:225-236.
7. Kaleem M, Asif M, Ahmed Q U, Bano B. Antidiabetic and antioxidant activity of annona squamosa extract in streptozotocin induced diabetic rats. Singapore Med J 2006;47:670-675.
8. Yin P, Zhao S, Chen S, Liu J, Shi L, Wang X, et al. Hypoglycemic and hypolipidemic effects of polyphenols from burs of castanea mollissima blume. Molecules 2011;16:9764-9774.
9. Saneja A, Sharma C, Aneja KR, Pahwa R. Gymnema sylvestre (gurmar): A review. Der Pharmacia Lettre 2010;2:275-284.
10. Shanmugasundaram ER, Gopinath KL, Shanmugasundaram KR. Possible regeneration of the islets of langerhans in streptozotocin-diabetic rats given gymnema sylvestre leaf extracts. J Ethnopharmacol 1990;30:265-279.
11. Bishayee A, Chatterjee M. Hypolipidaemic and antiatherosclerotic effects of oral gymnema sylvestre R.Br. leaf extract in albino rats fed on a high fat diet. Phytother Res 1994;8:118-120.
12. Yoshikawa M, Murakami T, Matsuda H. Medicinal Foodstuffs. X. Structure of new triterpene glycosides, gymnemosides-c,-d, -e, and f, from the leaves of gymnema sylvestre R. Br.: Influence of gymnema glycosides on glucose uptake in rat small intestinal fragments. Chem Pharm Bull 1997;45:2034-2038.
13. Chowdhary F, Rasool MH. Isolation and characterization of gymnemic acid from indigenous gymnema sylvestre. J App Pharm 2010;3:60-65.
14. Sarkar R, Hazra B, Biswas S, Mandal N. Evaluation of the in vitro antioxidant and iron chelating activity of gymnema sylvestre. Pharmacologyonline 2009;3: 851-865.
15. Rachh PR, Patel SR, Hirpara HV, Rupareliya MT, Rachh MR, Bhargava AS, et al. In vitro evaluation of antioxidant activity of gymnema sylvestre r. br. leaf extract. Rom J Biol Plant Biol 2009;54:141-148.
16. Claus CR. Drugs producing diabetes through damage of the insulin secreting cells. In: Pharmacological reviews. Baltimore:Williams and Wilkins Company 1970;22:508-517.
17. Bidhe RM, Ghosh S. Acute and subchronic (28-day) oral toxicity study in rats fed with novel surfactants. AAPS PharmSci 2004;6:e14.
18. Tietz NW. Methods of determination of ascorbic acid. In: Text book of Clinical biochemistry. Tietz NW (editor). Philedelphia: Saunders company;1986.p.960-962.
19. Beutler E, Duron O, Kelly BM. Estimation of Glutathione content of RBC and whole blood. J Lab And Clin Med 1963;61:882-888.
20. Stocks J, Dormandy TL. The autooxidation of human red cell lipids. Br J Haematol 1971;20:95-111.
21. Levine RL, Garland D, Oliver CN, Amiei A, Climent I, Lenz AG, et al. Determination of carbonyl content in oxidatively modified proteins. Meth Enzymol 1990;186:464-478.
22. Ellman GL. Measurement of protein thiols in plasma. Arch Biochem Biophy 1959;82:70.
23. Shanmugasundaram ER, Venkatasubrahmanyam M, Vijendran N, Shanmugasundaram KR. Effect of an isolate from gymnema sylvestre, R. Br. In the control of diabetes mellitus and the associated pathological changes. Anc Sci Life 1988;7:183-194.
24. Mall GK, Mishra PK, Prakash V. Antidiabetic and hypolipidemic activity of gymnema sylvestre in alloxan induced diabetic rats. GJBBR 2009;4:37-42.
25. Shimizu K, Iino A, Nakajima J. Suppression of glucose absorption by some fractions extracted by gymnema sylvestre leaves. J Vet Med Sci

1997; 59: 245-251.
26. Gholap S, Kar A. Gymnemic acids from gymnema sylvestre. Potentially regulates dexamethasone-induced hyperglycaemia in mice. Pharm Biol 2005;43:192-195.
27. Kaleem M, Asif M, Ahmed QU, Bano B. Antidiabetic and antioxidant activity of Annona squamosa extract in streptozotocin-induced diabetic rats. Singapore Med J 2006;47: 670-675.
28. Fazal H, Ahmad N, Khan MA. Physico-chemical, phytochemical evaluation and dpph-scavenging antioxidant potential in medicinal plants used for herbal formulation in Pakistan. Pak J Bot 2011;43:63-67.
29. Vasil S, Austin A. Effect of herbal hypoglycemics on oxidative stress and antioxidant status in diabetic rats. Open Diabetes Journal 2009;2:48-52.
30. Kang MH, Lee MS, Choi MK, Min KS, Shibamoto T. Hypoglycemic activity of gymnema sylvestre extracts on oxidative stress and antioxidant status in diabetic rats. J Agric Food Chem 2012;60:2517-2524.
31. Patel SS, Shah RS, Goyal RK. Antihyperglycemic, antihyperlipidemic and antioxidant effects of Dihar, a polyherbal ayurvedic formulation in streptozotocin induced diabetic rats. Indian J Exp Biol 2009;47:564-570.
32. Srividya R, Varma SK, Dhanapal SP, Vadivelan R, Vijayan P. In Vitro and In Vivo Evaluation of Hepatoprotective Activity of Gymnema Sylvestre. International Journal of Pharmaceutical Sciences and Nanotechnology 2010;2:768-773.
33. Michael Brownlee. Biochemistry and molecular cell biology of diabetic complications. Nature 2001;414:813-820.

Corresponding author

Dr. Poornima A. Manjrekar
Professor and Head
Department of Biochemistry
Centre for Basic Sciences
Kasturba Medical College, Manipal University
Mangalore, India
Mobile: +91 9449033990
Email: poornima.manjrekar@manipal.edu