DOI: 10.7860/JCDR/2012/4728.2644

Original Article

Pharmacology Section

Antidiabetic and Hypolipidemic Effect of Salacia Oblonga in Streptozotocin Induced Diabetic Rats

BHAGYAJYOTHI M. BHAT, RAGHUVEER C.V. , VIVIAN D'SOUZA, POORNIMA A. MANJREKAR

ABSTRACT

Objectives: The present study was conducted to evaluate the effect of a standardized hydroalcoholic root extract of Sala¬cia oblonga (SOE) on the Random Blood Glucose (RBG) levels, serum insulin, glycated haemoglobin (HbA1c) and the serum lipid profile in long standing, experimentally induced Diabetes Mellitus (DM) with glibenclamide (Glb) as the standard.

Materials and Methods: Streptozotocin (STZ) induced, diabetic, Wistar rats of either sex were treated with two oral doses of SOE, 100 and 50mg/kg body wt /day, for a period of 16 weeks. The RBG was estimated at day-1 and at the end of the 16 weeks by using a glucometer. The fasting serum insulin was determined by an ELISA technique. The plasma HbA1c was evaluated by a Turbidimetric Inhibition Immunoassay (TINIA) and the lipid profile was estimated enzymatically.

Results and Analysis: A 45% decrease in the RBG was seen after the treatment with the higher dose of SOE, whereas a 44% decrease was observed with the lower dose as com¬pared to the diabetic control. Serum insulin was significantly increased ($P<0.05$) in all the treated groups as compared to the diabetic control. Plasma HbA1c was significantly decreased ($P<0.05$). The serum Triacyl Glycerol (TG) levels were signifi¬cantly decreased ($P<0.05$) in the treated rats as compared to the diabetic control. A significant increase in HDL-cholesterol ($P<0.05$) in the diabetic rats as a result of the 100mg/kg SOE treatment was a remarkable finding.

Conclusion: SOE improves the glycaemic parameters in diabetic rats after a prolonged treatment. The serum TG levels were normalized on treatment. A higher dose of the extract could not alter the parameters significantly, except for HDL-C.

Key Words: Blood glucose, Lipid profile, S. oblonga, STZ- Rats

INTRODUCTION

Diabetes Mellitus (DM) is associated with dyslipidaemia and cardiovascular disease [1]. Several reviews on the medicinal plants which are used in the treatment of DM are available [2,3], but only a few have received scientific scrutiny. Also, a number of plants are known to have a hypolipidaemic activity [4]. However, there are few plants which have both hypoglycaemic and hypolipidaemic effects [5]. The plant, Salacia oblonga (S.oblonga), has been used in treating DM. This plant belongs to the family: Hippocrateaceae, a large woody climber which is distributed in the forests of Sri Lanka and in the southern region of India [6]. Despite its wide use as a folk remedy over a long period of time, the biochemical details of its action on the physiological/ pathophysiological functions have not been systematically investigated. S.oblonga was known to reduce postprandial glycaemia [7,8]. The plants which belong to the Salacia species are known to have the α–glucosidase inhibitory compounds, salalcinol and kotalanol, along with several phenolic compounds, sesquiterpenes and triterpenes [9]. A study on rats revealed that the blood glucose levels in streptozotocin (STZ)-induced diabetic rats were significantly lower after the oral administration of the root extract of S.oblonga [10]. A chronic oral administration of the S.oblonga root extract reduced the cardiac Triacyl Glycerol (TG) and the fatty acid (FA) contents [11]. On the basis of these references, the present study was aimed at evaluating the effects of a standardized hydroalcoholic root extract of S. Oblonga (SOE) on the random blood glucose (RBG), plasma Glycated haemoglobin (HbA1c) and the serum insulin levels and on the serum lipid profile, which included TG, Total Cholesterol (TC) and the High Density Lipoprotein Fraction Of Cholesterol (HDL-C) in rats with STZ induced DM over a period of 16 weeks and at determining the effective dose of the same. The results which obtained with SOE were compared with those of glibenclamide (Glb), a known hypoglycaemic drug.

MATERIALS AND METHODS

Plant extract:

SOE was availed from Natural Remedies Private Limited, Bangalore, taking care to see that all the requirements of the extract came from the same batch of the manufacturing process. The certificate of analysis claimed that this extract had an α-glucosidase inhibitory potential with an IC50 of < 75.0 µg/ml. The powder was dissolved in 0.5% carboxy methyl cellulose to prepare a solution of this extract and it was fed orally.

Animals used:

Albino rats of the Wistar strain, of either sex, which weighed 100 ±10g,were used in the present study. The rats were acclimatized to the laboratory conditions for at least 1 week before any experimental work was undertaken. They were fed ad libitum with a normal laboratory pellet diet and water. The ethical clearance for the study was obtained from the Institutional Animal Ethics Committee, Kasturba Medical College, Manipal University, Mangalore, India.

The induction of experimental DM:

DM was induced by injecting a single dose of STZ (from Sigma –Aldrich Corporation. 3050 Spruce St., St. Louis, Missouri 63103.

United States.), 50mg/Kg body weight, in cold citrate buffer (0.1M) of pH 4.0 intraperitoneally, after they were made to fast for 18-20 hours [12]. The rats were monitored for 72 hours to ensure their survivabilities. On day 1 of the experiment, their RBG levels were checked with ACCU CHEK, an active blood glucose monitor, by using disposable strips. Only those which showed an RBG of 350mg/dl or more were taken up for the study and they were distributed into different groups.

Experimental procedure:

The rats were divided into five groups, with eight rats in each group, as follows:

(i) Group I, normal control rats;

(ii) Group II, STZ-induced diabetic control rats;

(iii) Group III, diabetic rats which were given SOE (100 mg/kg) daily via tube for 16 weeks;

(iv) Group IV, diabetic rats which were given SOE (50 mg/kg) daily via an intragastric tube for 16 weeks and

(v) Group V, diabetic rats which were given Glb (500 µg/kg) daily via an intragastric tube for 16 weeks.

At the end of the 16 weeks, their RBG levels were checked, the 12 hr-fasted rats were sacrificed with a high dose of ether and blood was drawn from them immediately by cardiac puncture. The EDTA blood was used for the HbA1c estimation and the serum was used for the estimation of insulin and the lipid profile.

Analytical methods:

The insulin levels were determined by an ELISA (LINCO Research, 6 Research Park Dr. St.Charles, Missouri 63304 USA) technique by following the instruction manual. The estimation of HbA1c was done by a turbidimetric inhibition immunoassay (TINIA).The concentration of TG was estimated by the Glycerol – 3 - phosphate oxidase – peroxidase method. The concentrations of TC and HDL-C were estimated by the cholesterol oxidase – peroxidase method. The estimation of HbA1c, TC and HDL- C were done in an autoanalyser, HITACHI-917, by using Roche kits.

STATISTICAL ANALYSIS

All the grouped data were evaluated statistically with the SPSS, 17 software (SPSS, Chicago, IL, USA). All the results were expressed as the mean ± SD for eight animals in each group. The comparison of various parameters between the groups was done by using the Kruskal – Wallis test. A P value of < 0.05 was considered to be statistically significant.

RESULTS

[Table/Fig-1] gives the levels of RBG, HbA1c and insulin in the control and the experimental groups of rats. RBG and HbA1c showed a significant increase in the diabetic controls as compared to their levels in the normal control group. The treatment with SOE brought down the values. A 45% decrease in RBG was seen in the treatment with a higher dose, whereas the lower dose showed a decrease of 44% as compared to that in the diabetic control group. The insulin levels were significantly increased in all the treated groups as compared to those in the untreated diabetic control group. The increase in insulin and the decrease in HbA1c with the SOE treatment were dose dependent.

	RBG (mg/dl)	Insulin (ng/ml)	HbA1c (%)
Normal control	97.25± 2.25	0.64 ± 0.13	3.66 ±0.17
Diabetic control	529.38 ±24.78*	0.14 ± 0.03*	7.84 ± 0.32*
Diabetic+ SOE (100mg/kg)	292.13 ±25.32*†	0.54± 0.09*†	6.78 ± 0.81*†
Diabetic+ SOE (50mg/kg)	295.75 ± 69.70* †	0.34 ± 0.07*†	7.14 ±0.32*†
Diabetic+Glb	310.88 ± 76.37*†	0.53 ± 0.05*†	7.09 ± 0.64 * †

[Table/Fig-1]: Levels of RBG, insulin and HbA1c in control and experimental groups of rats
(* - p ≤0.05 compared to normal control; † - p≤ 0.05 compared to diabetic control)

	TG (mg/dl)	TC (mg/dl)	HDL- C (mg/dl)
Normal control	85.38 ± 23.08	60.75 ± 6.82	55.88 ± 8.36
Diabetic control	111.63 ± 20.37*	62.50 ± 10.13	53.25 ± 11.34
Diabetic+ SOE (100mg/kg)	83.38 ± 18.26†	66.75 ± 12.84	63.75 ± 11.17†
Diabetic+ SOE (50mg/kg)	81.88 ± 13.00†	55.38 ± 7.17	50.38 ± 4.90
Diabetic+Glb	82.25 ± 13.30†	60.00 ± 20.88	56.38 ± 7.56

[Table/Fig-2]: TG, TC, HDL- C concentrations in control and experimental groups of rats
* - p ≤0.05 compared to normal control; † - p≤ 0.05 compared to diabetic control

[Table/Fig-2] gives the levels of TG, TC and HDL-C in the control and the experimental groups of rats. Among the lipid profile, the serum TG levels were significantly increased in the diabetic controls as compared to those in the normal control group. The levels reverted to normal on treatment and this decrease was statistically significant as compared to that in the diabetic control group. A significant increase in HDL-C was found with a 100mg/kg SOE treatment.

DISCUSSION

A significant improvement in the glycaemic parameters was shown in the present study, after a period of 16 weeks of treatment with SOE. A significant decrease in serum TG was seen, whereas serum HDL-C has showed a significant increase in the group which received a higher dose of SOE. A study which was done by Krishnakumar, et al., [10] on hypoglycaemic and the anti-peroxidative and the anti-oxidant effects of S.oblonga on STZ-diabetic rats showed that the extract could significantly prevent hyperglycaemia and hypoinsulinaemia. With the dose of STZ which was selected in the present study, a partial destruction of the β-cells might have been attained. The treatment with SOE improved the insulin levels, which was similar to that with Glb [13] thus suggesting that SOE might stimulate insulin secretion from the remnant β-cells or/and cause regeneration of the β-cells of the pancreas. Such an insulinogenic effect of few other plant extracts has been observed by other authors [14,15]. The possible insulinogenic effect of SOE was found to continue for a sustained period of 16 weeks. In this context, a clinical trial which was done by Williams et al., [16] showed that the S.oblonga extract could lower acute glycaemia and insulinaemia in persons with type 2 DM after a high carbohydrate meal. The authors also concluded that this extract may be beneficial in the postprandial glucose control. The present study was in agreement with this conclusion and SOE may also be beneficial for a long standing glycaemic control. Previous workers showed that S.oblonga contains the potent alpha-glucosidase inhibitors, salacinol and kotalanol, and an aldose reductase inhibitor, kotalaganin 16-acetate [9] which have been suggested to be responsible for the hypoglycaemic activity of this plant.

There was no significant decrease in the serum TC levels on treatment with SOE. This might be because a significant hypercholesterolaemia did not exist in the rats. So the hypocholesterolaemic effect cannot be expected. The anti hypertriglyceridaemic effect of SOE, however, has been well established; where the extract could bring back the TG contents to near normal. This effect of SOE is comparable to that of Glb, an insulin secretagogue. Huang, et al.,[11] in 2006, published that they could demonstrate that a chronic oral administration of the S.oblonga root extract reduced the cardiac TG and the FA contents. Another species of Salacia, S.reticulatahas also showed a similar effect, where the adipose TG levels were decreased on treatment [17]. An increase in HDL-C was seen on treatment with a higher dose of SOE (100mg/kg/day), which may be an indication of the progressive metabolic control of S.oblonga. This beneficial effect has not been found in many plants, in spite of them having a hypolipidaemic effect [18]. An increase in HDL-C on treatment with SOE showed that SOE may have an anti atherogenicactivity, however, it could not be proven by the present study. A slight increase in the TC content in the same group might have been due to the increased HDL fraction. This sustained anti hyperglycaemic and anti hypertriglyceridaemic effects and the capacity of SOE to increase the HDL-C levels can be harnessed as a DM treatment option.

CONCLUSION

The sustained anti hyperglycaemic effect of SOE was well established by the present study, which was proven by the decreased RBG and HbA1c and the increased insulin levels. The possible insulinogenic effect was indicated by the increased insulin levels. The sustained anti hypertriglyceridaemic effect was also proven by this study, even though it was not dose dependent. A remarkable finding was an increase in HDL-C by a higher dose, which was not found with many plant extracts, which has to be explored further by doing more controlled studies.

ACKNOWLEDGMENT

The authors are thankful to ICMR, New Delhi, for the financial aid which was received for conducting this study (Ad hoc research project No. 59/18/2005/BMS/TRM).

REFERENCES

[1] Maria P. Solano, Ronald B. Goldberg. Lipid Management in Type 2 Diabetes. *Clinical Diabetes*. 2006;24:27-32.

[2] Akhilesh KTripathi, Pravin K Bhoyar, Jagdish R Baheti, Dinesh M Biyani, M Khalique, Mayuresh.Kothmire, et al. Herbal Antidiabetics: A Review. *Int. J. Res. Pharm. Sci*. 2011;2:30-37.

[3] Raju Patil, Ravindra Patil, Bharati Ahirwar, Dheeraj Ahirwar. Current status of Indian medicinal plants with antidiabetic potential: a review. *Asian Pacific Journal of Tropical Biomedicine*. 2011;1:S291–S298.

[4] Pizziolo VR, Brasileiro BG, Oliveira TT, Nagem TJ. Plants with possible hypolipidemic activity: a review of books published in Brazil between 1998 and 2008. *Rev. Bras. Plantas Med*. [online].2011;13:98-109. http://dx.doi.org/10.1590/S1516-05722011000100015.

[5] Balasubramaniam Dineshkumar, Mitra Analava, Mahadevappa Manjunatha. Antidiabetic and hypolipidaemic effects of few common plants extract in Type 2 diabetic patients at Bengal. *Int J Diabetes and Metab*. 2010;18:59-65.

[6] Hisashi Matsuda, Toshio Morikawa, Masayuki Yoshikawa. Antidiabetogenic constituents from several natural medicines. *Pure Appl. Chem*. 2002; 74: 1301-08.

[7] Collene AL, Hertzler SR, Williams JA, Wolf BW. Effects of a nutritional supplement containing Salacia oblonga extract on postprandial glycemia, insulinemia, and breath hydrogen responses in healthy adults. *Nutrition*. 2005; 21: 848-54.

[8] Heacock PM, Hertzler SR, Williams JA, Wolf BW. Effects of a medical food containing an herbal alpha-glucosidase inhibitor on postprandial glycemia and insulinemia in healthy adults. *J Am Diet Assoc*. 2005; 105: 65-71.

[9] Matsuda H, Yoshikawa M, Morikawa T, Tanabe G, Muraoka O. Antidiabetogenic constituents from Salacia species. *J Tradit Med.* 2005;22(S1):145-153.

[10] Krishnakumar K, Augusti KT, Vijayammal PL. Hypoglycemic and anti-oxidant activity of Salacia oblonga Wall.extract in streptozotocin-induced diabetic rats. *Indian J Physiol Pharmacol*.1999; 43: 510-14.

[11] Huang TH, Yang Q, Harada M, Uberai J, Radford J, Li GQ, et al. Salacia oblonga root improves cardiac lipid metabolism in zucker diabetic fatty rats: modulation of cardia PPAR-alpha-mediated transcription of fatty acid metabolic genes. *Toxicol Appl Pharmacol*. 2006; Jan1;210:78-85.

[12] Claus CR. Drugs producing diabetes through damage of the insulin secreting cells. In: Pharmacological reviews. *Williams and Wilkins Company*. 1970; 22: 508-17.

[13] G Rajiv Gandhi, P Sasikumar. Antidiabetic effect of Merremia emarginata Burm. F. in streptozotocin induced diabetic rats. *Asian Pacific Journal of Tropical Biomedicine.* 2012; 281-286.

[14] Pari L, Latha M. Effect of Cassia auriculata flowers on blood sugar levels, serum and tissue lipids in streptozotocin diabetic rats. *Singapore Med. J*. 2002; 43: 617–21.

[15] Subbiah Rajasekaran, Kasiappan Ravi, Karuran Sivagnanam, Sorimuthu Subramanian. Beneficial effects of Aloe vera leaf gel extract on lipid profile status in rats with Streptozotocin diabetes. *Clinical and Experimental Pharmacology and Physiology*. 2006; 33: 232–37.

[16] Williams JA, Choe YS, Noss MJ, Baumgartner CJ, Mustad VA. Extract of Salaciaoblonga lowers acute glycemia in patients with type 2 diabetes. *Am J Clin Nutr*. 2007; 86: 124-30.

[17] Masayuki Yoshikawa, Hiroshi Shimoda, Norihisa Nishida, Miki Takada, Hisashi Matsuda. Salacia reticulate and Its Polyphenolic Constituents with Lipase Inhibitory and Lipolytic Activities Have Mild Antiobesity Effects in Rats. *J. Nutr*. 2002; 132: 1819–24.

[18] Cleide de Sousa Lino, João Paulo Luz Diógenes, Bruno Azevedo Pereira, Rozilaine Aparecida Pelegrine Gomes Faria, Manoel Andrade Neto, Renata Sousa Alves, et al. Antidiabetic Activity of Bauhinia forficate Extracts in Alloxan-Diabetic Rats. *Biol. Pharm. Bull*. 2004; 27: 125—27.

AUTHOR(S):

1. Dr. Bhagyajyothi M. Bhat
2. Dr. Raghuveer C.V.
3. Dr. Vivian D'Souza
4. Dr. Poornima A. Manjrekar

PARTICULARS OF CONTRIBUTORS:

1. Senior Research Fellow, Department of Biochemistry, Kasturba Medical College, Mangalore, India.
2. Medical Director, Srinivas Institute of Medical Sciences, Mukka, Surathkal, Mangalore, India.
3. Professor, Department of Biochemistry and Assoc. Dean, Kasturba Medical College, Mangalore, India.
4. Professor and Head, Department of Biochemistry, Kasturba Medical College, Mangalore, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:

Dr. Poornima A. Manjrekar,
Professor and Head, Department of Biochemistry,
Centre for basic sciences, Kasturba Medical College,
Bejai, Mangalore, 575004, India.
Phone: 09449033990
E-mail: drpamanjrekar@gmail.com

FINANCIAL OR OTHER COMPETING INTERESTS:
None.

Date of Submission: **Jun 20, 2012**
Date of Peer Review: **Aug 03, 2012**
Date of Acceptance: **Nov 01, 2012**
Date of Publishing: **Dec 15, 2012**