Prevention of dexamethasone- and fructose-induced insulin resistance in rats by SH-01D, a herbal preparation

Md. Shalam, M.S. Harish**, S.A. Farhana*

ABSTRACT

Objective: To investigate the preventive effect of SH-01D, a herbomineral preparation, on the development of insulin resistance induced by dexamethasone and fructose, in rats.

Materials and Methods: Two models of insulin resistance were used (dexamethasone 10 mg/kg, s.c. once daily and fructose 10% w/v, p.o., *ad libitum*) in rats for a period of 10 and 20 days, respectively. Two doses of SH-01D (30 mg and 60 mg/kg, p.o.) were used. At the end of the experimental period, serum biochemical parameters like insulin, glucose, triglycerides, LDL, HDL and cholesterol were studied. Liver and muscle glycogen were estimated in the fructose model after sacrificing the animals.

Results: In both the models, SH-01D at 60 mg/kg showed significant effect. Fructose feeding increased serum biochemical parameters and decreased liver and skeletal muscle glycogen levels. Dexamethasone caused an increase in serum glucose, triglyceride levels and a decrease in body weight. In fructose-fed rats, SH-01D at 60 mg/kg significantly prevented (a) the increase in serum biochemical parameters and (b) the decrease in glycogen levels. In the dexamethasone model, SH-01D prevented the rise in serum glucose and triglycerides and improved the body weight.

Conclusion: The present study indicates that SH-01D may be useful in the management of insulin resistance.

KEY WORDS: Diabetes mellitus, dexamethasone, fructose, herbal antidiabetic.

Introduction

Diabetes is the world's largest endocrine disease with deranged carbohydrate, fat and protein metabolism. As per a WHO report, approximately 150 million people have diabetes mellitus worldwide, and this number may well double by the year 2025. Statistical projection suggests that the number of diabetics will rise from 15 million in the year 1995 to 57 million in 2025, making India the country with the highest number of diabetics in the world. Although many drugs and interventions are available to manage diabetes, these are expensive for a developing country like India apart from their inherent adverse effects. Therefore, it is necessary to look for new avenues to manage this major health problem.

As part of the pathogenesis of noninsulin-dependent diabetes mellitus (NIDDM), the skeletal muscle, liver and adipose tissues become resistant to the hormonal effects of insulin, which in turn leads to decreased insulin-mediated glucose disposal, hepatic glucose overproduction and a marked increase in lipolysis.^[11] In addition to the above, hyper-

insulinemia is a central pathophysiological feature of NIDDM and has been shown to play a key role in the disease evolution and macrovascular complications. Other than these, insulin resistance has been linked to obesity, hypertension and atherosclerosis, which are responsible for substantial morbidity and premature mortality. Hence, in addition to glycemic control, management of hyperinsulinemia is also essential for limiting these after-effects of NIDDM.^[1]

Numerous herbal drugs like *Momordica charantia*, *Tinospora cordifolia, Terminalia cattapa, Melia azadirachta* and *Syzygium cumini* have been used by people of various cultures to treat diabetes.^[2] Similarly, ayurveda, an ancient Indian system of medicine, mentions several plants that are useful in the correction of metabolic disorders such as diabetes mellitus. One herbomineral preparation that has been formulated with plants mentioned in ayurvedic texts and manufactured by Shrushti Herbal Pharma, Bangalore, India, is SH-01D.

Each tablet of SH-01D contains aqueous extracts equivalent to Guduchi (*Tinospora cardifolia*) 80 mg, Saptachakra (*Salacia reticulata*) 80 mg, Bilva (*Aegle marmelos*) 20 mg, Neem

Departments of Pharmacology and Pharmaceutics*, V.L. College of Pharmacy, Raichur-584103. **Department of Pharmacology, Govt. College of Pharmacy, Bangalore-560 027. India

> Received: 3.12.2005 Revised: 25.4.2006 Accepted: 29.5.2006

Correspondence to: Md. Shalam E-mail: shalam26@yahoo.co.in (*Melia azadirachta*) 50 mg, Musta (*Cyprus rotundus*) 40 mg, Jambu (*Syzygium cumini*) 40 mg, Amalaki (*Phyllanthus emblica*) 120 mg, Haridra (*Curcuma longa*) 40 mg, Vanga Bhasma 15 mg, Shilajit (Mineral pitch) 15 mg, The tablets were provided as a gift sample by Shrushti Herbal Pharma, Bangalore. Lead (lead oxide) was present at 5.5 ppm per tablet as a trace element. Though SH-01D has been claimed to possess antidiabetic activity due to the presence of multiple ingredients, experimental study in laboratory animals with insulin resistance has not been done. Hence the present study was conducted to find out the effect of SH-01D on insulin resistance models in animals.

Materials and Methods

Animals

Wistar albino rats of both sex (175-200 g) and female albino mice (20-25 g) were procured from Drug Testing Laboratory, Bangalore and maintained at $25\pm1^{\circ}$ C temperature and relative humidity of 45-55% under 12:12 h light:dark cycle. The animals of the control and the dexamethasone groups had free access to tap water while the other groups were fed with 10% fructose in drinking water. All the groups were fed with rat-food pellet *ad libitum* (Lipton, India). The experimental protocols were approved by the Institutional Animal Ethics Committee of Government College of Pharmacy, Bangalore, India, and were in accordance with the guidelines of the Committee for the Purpose of Control and Supervision on Experiments on Animals. Water was used as vehicle for preparing drug solutions.

Acute toxicity

Acute toxicity of SH-01D was determined using female albino mice. The animals were fasted 3 h prior to the experiment according to the procedure (OECD guideline no. 425) and were observed for 48 h following oral administration of different doses of SH-01D, as per the guidelines.^[3]

Experimental design

Dexamethasone induced insulin resistance model [4-6]

Animals were divided into 4 groups, each consisting of 6 rats.

Group 1 : Normal control (NC) - oral saline

Group 2 : Dexamethasone control (DC) – dexamethasone sodium phosphate 10 mg/kg, once daily, s.c.

Group 3 : Dexamethasone + SH-01D (30 mg/kg, b.w., p.o.) Group 4 : Dexamethasone + SH-01D (60 mg/kg, b.w., p.o.)

In groups 2-4, dexamethasone was administered to the overnight-fasted rats and continued till the end of the experiment along with SH-01D 30 or 60 mg/kg, b.w., p.o., in two divided doses in water. At the end of the experimental period, i.e. on day 11, the animals were anesthetized with ether; blood was collected by retroorbital puncture and serum separated for the estimation of glucose (glucose oxidase method) and triglyceride (GPO). The animals were weighed at the beginning and at the end of the experimental period.

Fructose-induced insulin resistance model [7, 8]

The animals were divided into three groups of six rats each.

Group1 : Normal control (NC) – Fed with tap water and saline p.o.

Group 2 : Fructose control (FC) – Fed with 10% w/v

fructose solution *ad libitum* in feeding bottle + vehicle for 20 days.

- Group 3 : Fructose + SH-01D Fed with 10% fructose solution *ad libitum* along with SH-01D (30 mg/ kg b.w., p.o., once daily) for 20 days.
- Group 4 : Fructose + SH-01D Fed with 10% fructose solution *ad libitum* along with SH-01D (60 mg/ kg, b.w., p.o., once daily) for 20 days.

All the animals were fasted for half an hour prior to drug administrations. On day 21, the animals were anaesthetized with ether and blood was collected by retroorbital puncture and the serum separated for estimation of glucose, cholesterol (cholesterol oxidase method), triglyceride and insulin (radioimmunoassay method). Then the animals were sacrificed, a piece of liver and thigh muscle tissues were dissected out, weighed and immediately digested with 2 ml of 30 % KOH solution separately, for determination of glycogen as per the method described by Montgomery.^[9] The Fasting Insulin Resistance Index (FIRI) was calculated using the formula, FIRI= (Fasting insulin X Fasting glucose)/25

Chemicals and biochemical estimations

For estimation of glucose, triglyceride and cholesterol, kits (Span diagnostics, India) were used. Serum insulin was estimated by radioimmunoassay (RIA) using RIA kit , INSIK-5 (Diasorin, Italy), with a gamma counter. All the estimations were carried out as per the instructions provided by the kit manufacturers. Glycogen was determined as per the method mentioned earlier.

Statistical analysis

The data were expressed as mean±SEM. The statistical significance between means was analyzed using one-way analysis of variance (ANOVA) followed by Tukey multiple comparison test. A P<0.05 was considered as statistically significant.

Results

Acute toxicity of SH-01D in mice

The animals were observed for mortality for 48 h, and the preparation was found to be safe upto a dose of 5000 mg/kg, b.w.

Effect of SH-01D in dexamethasone-induced insulin resistance model in rats

Dexamethasone significantly increased serum glucose and triglyceride levels compared to control group. [Table 2] The SH-01D (60 mg/kg, p.o.) along with dexamethasone for 10 days had significantly reduced the serum biochemical parameters like glucose and triglycerides (P<0.001) as compared to dexamethasone alone treated group. There was a significant (P<0.001) reduction in body weight in dexamethasone-treated rats when compared to normal control. [Table 2] Treatment with SH-01D at 60 mg/kg significantly prevented the loss in body weight. However SH-01D at 30 mg/kg did not produce any significant effect on the above parameters.

Effect of SH-01D in fructose-induced insulin resistance model in rats

Fructose feeding significantly increased serum glucose, insulin, FIRI index, cholesterol, triglyceride and LDL when compared to normal rats. [Table1, Figure 1] However, there

Table 1

Effect of SH-01D administration (20 days) on biochemical parameters in fructose-induced insulin resistance in rats

Parameters		Serum glucose	Serum insulin	FIRI	Glycogen levels (µg/mg of tissue)	
		(mg/dl)	(µU/mI)		Liver	Muscle
Normal control		71.80±2.31	26.76±1.57	76.85±3.97	22.77 ±1.96	6.34 ± 0.33
10% fructose feeding alone		103.3±1.45***	44.57±2.29***	184.16±6.78***	19.19 ±1.68	5.290 ± 0.44
SH-01D (30 mg/kg) + fructose feeding		98.83±1.621	38.90±1.27	153.77±2.75	20.59 ±1.2	3.501 ±1.51
SH-01D (60 mg/kg) + fructose feeding		63.83±1.621###	28.10±1.27###	69.56±2.75 ^{###}	24.55 ±0.71	4.501 ±0.51
One-way	F	131.02	27.02	211.44	2.728	2.067
ANOVA	Р	<0.0001	<0.0001	<0.0001	>0.05	>0.05

Values are mean±SEM. n=6 in each group. df=3,20. ***P<0.001 Vs. normal control (Tukey test), ###P<0.001 Vs. fructose-fed rats (Tukey test).

Table 2

Effect of SH-01D administration (10 days) on serum glucose, triglyceride levels and body weight change on dexamethasone-induced insulin resistance in rats

Group		Serum glucose (mg/dl)	Serum triglyceride (mg/dl)	Body weight change (g)
Normal control		84.82±5.28	15.95±2.17	4.83±2.07
Dexamethasone control		143.6±4.61***	50.24±4.76 ***	-17.75±4.76 ***
SH-01D (30 mg/kg) + dexamethasone		140.6±3.62	40.85±2.42	-14.55±2.42
SH-01D (60 mg/kg) + dexa	methasone	77.18±5.35 ***	25.85±2.4 ###	-4.95±2.01 ###
One-way	F	51.94	28.88	19.089
ANOVA	Р	<0.0001	<0.0001	<0.0001

Values are mean<u>+</u>SEM. n=6 in each group. df=3,20. ****P*<0.001 *Vs.* normal control (Tukey test), ****P*<0.001 *Vs.* dexamethasone-administered rats (Tukey test). '-' and '+'signs indicate decrease and increase in body weights respectively.



Figure 1. Effect of SH-01D administration (20 days) on lipid profile in fructose-induced insulin resistance in rats

P*<0.05 *Vs* normal control (one-way ANOVA followed by Tukey test), *P*<0.01 *Vs* normal control (one-way ANOVA followed by Tukey test), #*P*<0.05 *Vs* fructose-fed rats (one-way ANOVA followed by Tukey test), #*P*<0.01 *Vs* fructose-fed rats (one-way ANOVA followed by Tukey test). n=6 in each group.

was no significant effect on HDL levels. Giving SH-01D (60 mg/kg, p.o.) along with fructose feeding for 20 days significantly (P<0.001) reduced the said biochemical parameters like glucose, insulin and FIRI values when compared to fructose alone fed group. [Table 1]

Discussion

Insulin resistance in humans has been shown to be present in conditions like NIDDM, obesity and dyslipidemia. Thus interventions to decrease insulin resistance may postpone the development of NIDDM and its complications. Treatment with natural herbs is likely to be fraught with lesser side effects compared to the presently used synthetic oral antidiabetic agents. Among the various constituents of SH-01D, the aqueous leaf extract of Melia azadirachta has been reported to possess significant antidiabetic effect in both type 1 and type 2 diabetes models as well as improved glucose tolerance.^[10, 11] Vats et al,^[12] have shown that the extract of Tinospora cardifolia had significant antihyperglycemic effect. Das et al,^[13] have found the aqueous extract of the leaves of Aegle marmelos to have significant hypoglycemic activity in both normal and streptozotocin-diabetic rats, through stimulation of the surviving cells to release more insulin. Shilajit showed antidiabetic activity in alloxan-induced diabetes.^[14]

Dexamethasone increases triglyceride levels, causing an imbalance in lipid metabolism leading to hyperlipidemia^[5] and an increase in glucose levels leading to hyperglycemia.^[6] Pharmacological doses of glucocorticoids induce ob gene expression in rat adipocyte tissue within 24 h. This is followed by complex metabolic changes resulting in decrease in food consumption, reduction in body weight, profound obesity often accompanied by diabetes and development of insulin resistance with enhanced blood glucose and triglyceride levels. In the present study, dexamethasone for 10 days in rats resulted in increased triglyceride and glucose levels similar to a previous study.^[7] A higher dose of SH-01D prevented the rise in triglyceride and glucose caused by dexamethasone. Further, SH-01D also prevented the progressive decrease in body weight caused by dexamethasone.

The rats fed with high fructose diet induced a nonobese model of hyperlipidemia, insulin resistance, hyperinsulinemia and mild hypertension, which are features associated with obesity-related hypertension. This experimental model of hyperinsulinemia in rodents represents metabolic abnormalities seen in patients with insulin resistance that increase their risk of developing NIDDM and coronary heart disease. The use of 10% fructose in drinking water for a period of 1 week or longer is equivalent to a diet containing 48–57% by calories, and has been found to be most suitable for the production of insulin resistance in rats.^[8] In our study, administration of fructose for 20 days significantly increased the glucose, insulin and triglyceride levels similar to an earlier study.^[15] Administering SH-01D (60 mg/kg) prevented the development of hyperinsulinemia, hyperglycemia and hypertriglyceridemia.

The SH-01D might have improved insulin resistance through enhanced insulin sensitivity in peripheral tissues, as was evident from the decreased glucose and insulin and increased liver and skeletal muscle glycogen stores. The drugs ameliorating hyperinsulinemia are likely to have greater therapeutic potential as they may also exert beneficial effects on the clinical course of NIDDM, hypertension and coronary artery disease conditions.

In conclusion, oral administration of SH-01D at a dose of 60 mg/kg lowers serum glucose, insulin, triglyceride and LDL concentrations in fructose-fed rats and similarly reduces serum triglyceride and glucose levels in dexamethasone-administered rats. If these results are extrapolated to humans then SH-01D might prove useful in the treatment and/or prevention of insulin resistance in nondiabetic states such as obesity and impaired glucose tolerance. However, detailed toxicity profile is needed to determine the suitability of SH-01D for clinical usage.

Acknowledgments

We are grateful to Dr Shubha Hegde, Managing Director, Shrushti Herbal Pharma Ltd, Bangalore, for providing the gift sample of the investigational drug and financial assistance. We also thank Prof. M. Lakshmana, ex-Principal, Government. College of Pharmacy, Bangalore, for providing the necessary facilities for the study.

References

- Grover JK, Vats V, Rathi SS, Dawar R. Traditional Indian antidiabetic plants attenuate the progression of renal damage in streptozotocin induced diabetic Mice. J Ethnopharmacol 2001;76:233–8.
- Marles R, Farnsworth NR. Antidiabetic plants and their active constituents. Phytomedicine 1995;2:137–89.
- OECD 2001-guideline on oral acute toxicity. Environmental health and safety monograph series on testing and adjustment no.425.
- Kaur N, Sharma N, Gupta AK. Effects of dexamethasone on lipid metabolism in rats organ. Indian J Biochem Biophy 1989;26:371–6.
- Wiesenberg I, Chiesi M, Missbach M, Spanka C, Pignat W. Specific activation of the Nuclear Receptor PPARy and RORA by the Antidiabetic thiazolidinedione BRL 49653 and the Antiarthritic Thiazolidinedione Derivative CGP 52608. Mol Pharmacol 1998;53:1131–6.
- Mahendran P, Shyamala Devi CS. Effect of *Garcinia cambogia* extract on lipids and lipoprotein composition in dexamethasone administered rats. Indian J Physiol Pharmacol 2001;45:345–50.
- Dai S, Todd, McNeil JH. Fructose induced hypertension in rats is concentration dependent and duration dependent. J Pharmacol Toxicol Methods 1995;33: 101–3.
- Vikrant V, Grover JK, Tandon SS, Rathi SS, Gupta N. Treatment with extracts of *Momordica charantia* and *Eugenia jambolana* prevents hyperglycemia and hyperinsulinemia in fructose fed rats. J Ethnopharmacol 2001;76:139–43.
- Montgomery R. Determination of Glycogen. Arch Biochem Biophys 1957; 67: 378–86.
- Bajaj S, Srinivasan BP. Investigation into the antidiabetic activity of Azadirachta indica. Indian J Pharmacol 1999;31:138–41.
- Mandal SC, Pal S, Ashok Kumar CK, Mohana Lakshmi S. A study on hypoglycemic activity of methanolic extract of *Azadirachta indica*. Indian J Nat Prod 2000; 16:8–10.
- Vikrant V, Grover JK, Rathi SS. Antihyperglycemic effect of *Eugenia jambolana* and *Tinospora cordifolia* in experimental diabetes and their effects on key metabolic enzymes involved in carbohydrate metabolism. J Ethnopharmacol 2000;73:461–70.
- Das AV, Padyatti PS, Paulose CS. Effect of leaf extract of *Aegle marmelos* (L.) Correa ex Roxb. on histological and ultra structural changes in tissues of streptozotocin induced diabetic rats. Indian J Exp Biol 1996;31:341–5.
- 14. Bhattacharya SK. Activity of shilajit on alloxan-induced hyperglycemia in rats. Fitoterapia 1995;66:328-32.