

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

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## ABSTRACT

**Objectives:** The present study was conducted to evaluate the effect of a standardized hydroalcoholic root extract of *Salacia 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 compared 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 significantly 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  $\alpha$ -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  $\alpha$ -glucosidase inhibitory potential with an IC<sub>50</sub> of  $< 75.0 \mu\text{g/ml}$ . 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  $100 \pm 10\text{g}$ , 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 con-

	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

trol 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.

[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.reticulata has 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 atherogenic activity, 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.

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