# Hypoglycemic Properties of Ethanolic Extracts of Salvia Hypoleuca in Rats

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**Summary**

The aim of this study was to elucidate the effects of ethanolic extracts of Salvia hypoleuca on normal and alloxan-induced diabetic rats. Test groups were made diabetic with intra-peritoneal injection of alloxan and treated with 250 mg and 450 mg/kg body weight of Salvia hypoleuca extract. Two non-diabetic groups were also administered with 250 mg and 450 mg/kg body weight extract. The levels of blood glucose were examined in all experimental groups. In diabetic rats, blood glucose levels were significantly reduced on consumption of the extracts, with greater effect exhibited by the 450 mg/kg extract. In non-diabetic rats, blood glucose levels were reduced by on consumption of the seed extract. Histological studies showed a degenerative effect on the pancreatic islet cells of diabetic rats. The results suggested restorative effect of the extract on pancreatic islet cells. Administration of ethanolic extract of Salvia hypoleuca may contribute significantly to the reduction of blood glucose levels and can be useful in the management of diabetes. From the results it can be concluded that Administration of ethanolic extract of Salvia hypoleuca may contribute significantly to the reduction of blood glucose levels and can be useful in the management of diabetes.

**Keywords:** Salvia hypoleuca, Hypoglycemic effects, Rats.

## Introduction

Phytomedicine has been used in healthcare delivery in many parts of world. Today, medicinal plants are increasingly being used in most parts of the world for gaining of better health. Hypoglycemic agents like medicinal plants have been used in the management of diabetes mellitus. Diabetes mellitus is a multifactorial disease which is characterized by hyperglycemia (1, 2), lipoprotein abnormalities (1), raised basal metabolic rate (3, 4), defect in reactive oxygen species scavenging enzymes and altered intermediary metabolism of major food substances (3-5). Diabetes is a major degenerative disease in the world today (6), affecting at least 15 million people and having complications which include hypertension, atherosclerosis and microcirculatory disorders. There has been increasing demand for the use of plant products with antidiabetic activity. Plants which have been shown to have hypoglycemic action, act on blood glucose through different mechanisms. Some of them may inhibit endogenous glucose production (7) or interfere with gastrointestinal glucose absorption (8); some may have insulin - like substances (9, 10); some may inhibit insulinase activity and some may increase secretion of insulin from the β cells of the pancreas i.e. pancreatotrophic action (11-13), while others may increase beta cells in pancreas by activating regeneration of these cells (14, 15). It is necessary to produce effective drugs with lowest side effect for treatment of diabetes and thus it is the aim of this study to investigate the hypoglycemic effect of the ethanolic extract of Salvia hypoleuca used in iranian traditional medicine for diabetes mellitus management and its possible role on pancreatic tissue.

## Materials and Methods

### Preparation of the extract of Salvia hypoleuca

Air dried Powdered plant was divided in to two equal parts (1 kg each); one part was macerated with ethanol (90 % v/v) in glass percolator and allowed to stand at room temperature for about 24 hours. Then the extract obtained was filtered, concentrated by rotary vacuum pump to get the solid mass. The percentage yield was 19.6 %, second part of powdered was cold macerated with distilled water then the same procedure was repeated as mentioned above. The percentage yield was 10.5%.

### Animal treatments

Albino Wistar rats weighing 150-200 g of either sex, 4 months of age were used for this study. The experimental animals were housed in polypropylene cages and maintained under standard conditions (12 h light and dark cycles, at 25±3º C and 35-60% humidity). Standard pelletized feed and tap water were provided ad libitum. The 6 group of rats were as follows: Diabetic group 1 (DG -1), Diabetic group 2 (DG- 2), Diabetic control (DC), Non –diabetic group 1 (NDG- 1), Non – diabetic group 2 (NDG-2) and Normal control (NC). DG-1, DG-2 and DC groups were made hyperglycemic by intra – peritoneal injection of 150 mg/kg body weight of alloxan monohydrate (Sigma) dissolved in sterile distilled water. The NC group was not treated with alloxan. Diabetes was confirmed 3days after alloxan injection by determining the blood glucose concentration using One Touch Basic Glucometer. Then groups DG-1 and NDG-1 were administered with 250 mg/kg body weight of the extract, while groups DG-2 and NDC-2 were administered with 450 mg/kg body weight of the extract, daily for 14 days, by oral gavage. Groups DC and NC, which served as treatment controls, were gavaged with distilled water.

### Collection and Treatment of Samples

After 14 days, the animals were anaesthetized under chloroform vapour. Blood samples were obtained by cardiac puncture. Aliquots of the blood were poured into screw-cap sample bottles containing fluoride /oxalate anticoagulant for blood glucose determination. All analyses were carried out within 24 hrs of blood collection.

### Blood Glucose Analysis

Blood glucose concentration was estimated by glucose oxidase method, using a reagent kit from Randox Laboratory Ltd, UK.

### Histopathological Study

On the last day of experiment, the tail parts of the pancreas were removed and kept in 10% formaldehyde. Tissue processing was carried out by autotechnicon and the prepared 5µ thick sections were mounted on slides and stained with hematoxylin and eosin (H & E). Stained sections were morphologically evaluated.

### Statistical Analysis

All data were expressed as means ± SD. Student's t – test was used to compare the mean values of test groups and control. Differences in mean values were considered significant at $p < 0.01$.

## Results

The effects of ethanolic extract of Salvia hypoleuca on blood glucose concentrations are shown in Table 1. The blood glucose concentrations in NC and DC groups were 3.91 mmol/L and 14.11 mmol/L respectively after the experimental period. In comparison with the DC group, the other experimental groups had significantly lower mean blood sugar 3.37 – 7.39 mmol/L ($p < 0.05$). No significant differences in blood sugar were observed between the diabetic group which received 450 mg/kg body weight extract (5.17mmol/L), and the normal control (NC) group ($p > 0.01$). The non-diabetic groups (NDG) had lower glucose concentrations (3.21 – 3.40mmol/L) when compared with the diabetic groups. The results showed that treatment with 150mg/kg body weight alloxan after 3 days caused significant increases ($p < 0.01$) in blood glucose levels of rats (mmol/L) in groups DC (15.41), DG-1 (14.30) and DG-2 (13.74), when compared with the NC and NDG groups (3.21 – 3.91). Treatment of the hyperglycemic rats with 250 mg/kg and 450 mg/kg body weight of Salvia hypoleuca extract resulted in significant reductions ($p < 0.01$) of blood glucose, when compared with the DC group. The levels of blood glucose in the non - diabetic groups (3.21 – 3.40 mmol/L) were reduced after extract treatment, when compared with the control groups.

**Table 1**: Effect of ethanolic extract of Salvia hypoleuca on plasma glucose concentration in diabetic rats.

| **Plasma Glucose Concentration** | **NC 0 mg/kg body weight** | **DC 0 mg/kg body weight** | **DG-1 250 mg/kg body weight** | **DG-2 450 mg/kg body weight** | **NDG-1 250 mg/kg body weight** | **NDG-2 450 mg/kg body weight** |
|---|---|---|---|---|---|---|
| **Initial (mmol/L)** | $3.89\pm0.42^{a}$ | $15.41\pm0.99^{b}$ | $14.30\pm1.09^{b}$ | $13.74\pm3.72^{b}$ | $3.53\pm0.52^{a}$ | $3.77\pm0.71^{a}$ |
| **Final (mmol/L)** | $3.91\pm0.32^{a}$ | $14.11\pm0.54^{b}$ | $7.39\pm0.68^{c}$ | $5.17\pm0.76^{d}$ | $3.40\pm0.62^{a}$ | $3.21\pm0.43^{a}$ |

Values are means ± standard deviation (n = 5).

### Histopathological studies of Pancreas

Figure 1 indicated the normal histology of pancreas in NC group. Severe necrotic changes of pancreatic islets, especially in the centre of islets were seen in alloxan treated groups. Nuclear changes, karyolysis, disappearance of nucleus and in some places, residue of destroyed cells were visible. Relative reduction of size and number of islets especially around the central vessel and severe reduction of beta cells were clearly seen (Fig. 2). Study of pancreas of treated diabetic groups (DG-1 and DG-2) showed increased size of islets and hyperchromic nucleus in sections stained with H & E. These was also a relative increase of granulated and normal beta cells in the diabetic group which consumed 450 mg/kg body weight extract, when compared with the diabetic group which consumed 250 mg/kg body weight extract (Figs. 3 & 4 respectively). Pancreas of the non- diabetic group which consumed 250 mg/kg body weight extract (Fig. 5), showed close similarity to group NDG-2, which consumed 450mg/kg body weight extract (Fig. 6), NC group (Fig. 1) and DG-2 group (Fig 4).

**Figure 1:** Pancreas section of normal health rat (NC group), H & E Staining (40X)

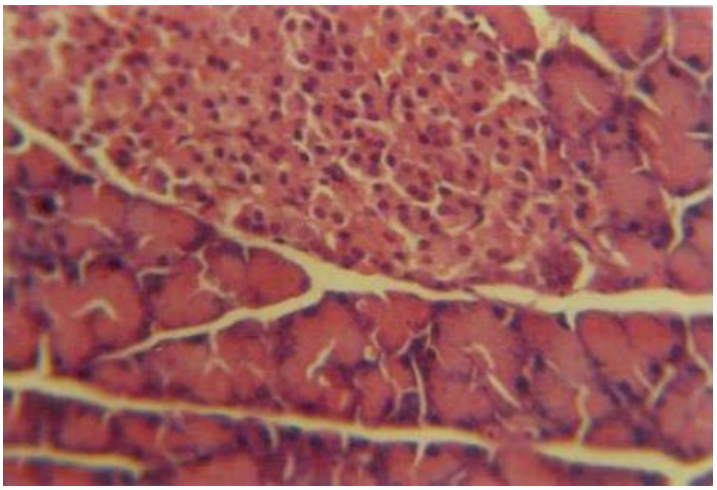


**Figure 2:** Pancreas section of diabetic control Rat (DC group), H & E Staining (40X)

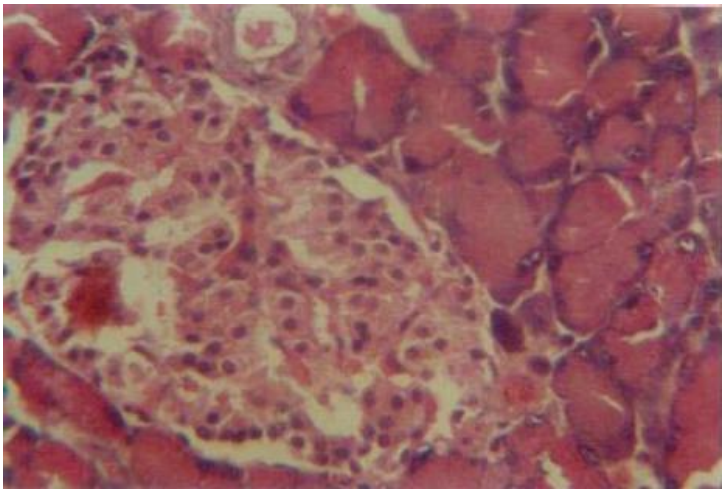


**Figure 3:** Pancreas section of diabetic rat treated with 250 mg/kg body weight Salvia hypoleuca extract (DG-1 group),H & E Staining (40X)

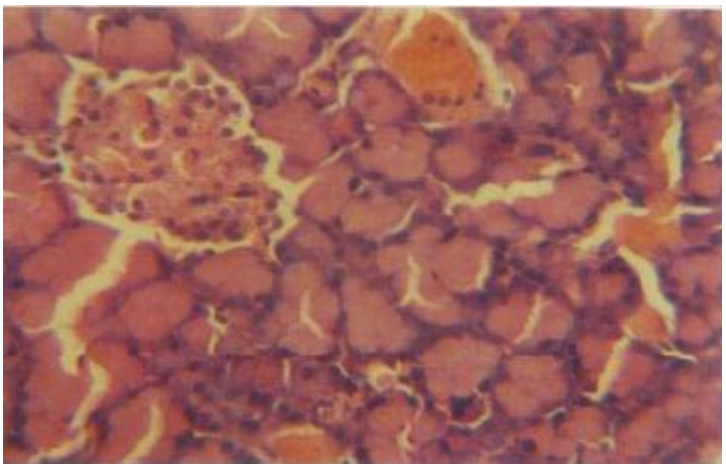


**Figure 4:** Pancreas of diabetic rat treated with 450mg/kg body weight extract of Salvia hypoleuca (DG-2 group), H & E Staining (40X)

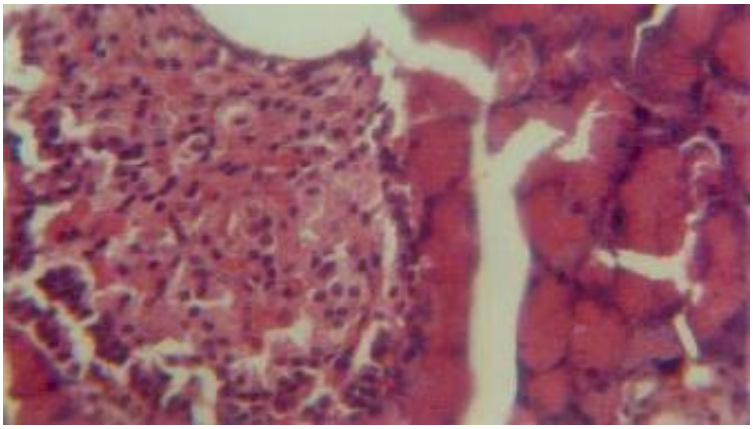


**Figure 5:** Pancreas of normal rat treated with 250mg/kg body weight extract of Salvia hypoleuca (NDG – 1 group), H & E Staining (40X)

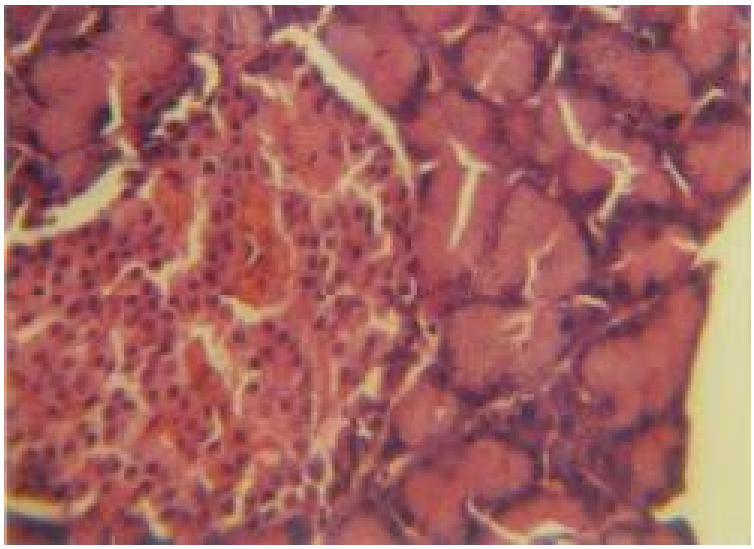
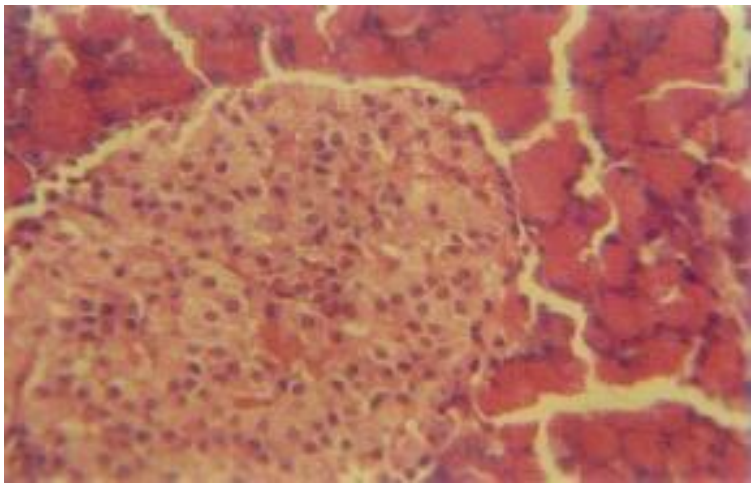


**Figure 6:** Pancreas of normal rat treated with 450mg/kg body weight extract of Salvia hypoleuca (NDG – 2 group), H & E Staining (40X)



## Discussion

The blood glucose concentration of the NC group (3.91mmol/L) is considered normoglycemic, while that of the DC group (15.41mmol/L) is considered hyperglycemic for the experiment (15, 16). There were significant reductions ($p < 0.01$) in blood glucose levels of the test groups (DG-1 and DG-2) and non- significant reductions ($p > 0.01$) for the non-diabetic groups that received extracts. Furthermore, the percentage reduction in glucose levels is high for diabetic groups treated with extract. The results suggest both hypoglycemic and antihyperglycemic effects of the Salvia hypoleuca extracts. The findings may indicate the presence of some hypoglycemic agents in the Salvia hypoleuca, which have been concentrated in the extracts. The hypoglycemic effects of plants may be due to the presence of insulin-like substance in plants (9, 10), stimulation of ß cells to produce more insulin (11), increasing glucose metabolism (17) or regenerative effect of plants on pancreatic tissue (14, 15). In this study, the pancreatic ß cells were destroyed with the help of alloxan. Histopathological study of diabetic untreated (DC) rats showed degeneration of pancreatic islet cells, which was due to alloxan used in this study. This probably gave rise to insulin deficiency. Insulin deficiency (or diabetes mellitus) causes excessive elevation of blood glucose and underutilization leading to hyperglycemia (18). The histopathological study of diabetic treated group (DG-1 and DG-2) indicated increased volume density of islets and increased percentage of beta cells, in the diabetic rats that received the extracts, which may be a sign of regeneration. Signs of regeneration of ß cells, potentiating of insulin secretion from surviving ß cells of the islets of Langerhans and decrease of blood glucose have been reported following consumption of some plant extracts (13-15, 19). Salvia hypoleuca may have some chemical components that exert regenerative effects on ß cells, stimulate these cells to produce more insulin (pancreatotrophic action) or may have some insulin like substances. Induction of regenerative stimulus in diabetic state triggers pancreatic regenerative processes, thereby restoring functional activities of the pancreas (20). A higher dose of the extract has a greater restorative effect on the islet cells of diabetic rats than a lower dose of extract. The hypoglycemic effect was more pronounced in alloxan-diabetic rats than in normal rats.

## Conclusion

The findings of this study indicate that consumption of the ethanolic extract of Salvia hypoleuca exerts significant hypoglycemic effect in diabetic rats. Histopathological studies of the pancreas of diabetic treated rat show evidence of signs of regeneration of ß cells in groups receiving Salvia hypoleuca extracts. These findings support the traditional use of Salvia hypoleuca for controlling hyperglycemia in diabetics, in view of the restorative (protective) effects of the extract on pancreatic islet cells.

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