

## **Effect of D-400, an Ayurvedic Herbal Formulation on Experimentally-induced Diabetes Mellitus**

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### **ABSTRACT:**

*In the present study, after D-400 treatment a significant reduction in blood sugar levels in alloxan induced diabetes was observed, an oral glucose tolerance test (OGTT) showed a significant lowering of AUC in streptozotocin induced diabetes in rats. There was a rise in hepatic glycogen level closer to normal after D-400 treatment. In the pancreas of diabetic rats, D-400 therapy showed a significant increase in islet number and beta cell count and appeared to bring about blood glucose homeostasis by increasing insulin secretion through repair/regeneration of endocrine pancreas which may be responsible for the prevention of hepatic glycogenolysis.*

*Keywords:* Alloxan, streptozotocin; liver glycogen; antihyperglycaemic action; herbal treatment

### **INTRODUCTION**

In non-insulin dependent diabetes mellitus (NIDDM), insulin resistance is a major pathophysiologic factor influencing glucose homeostasis. NIDDM accounts for over 85% of diabetes worldwide and is associated with morbidity and mortality, resulting from its microvascular, macrovascular and neuropathic complications (Huse *et al.*, 1988). The treatment of hyperglycaemia in patients with NIDDM is directed towards achieving euglycaemia and eliminating or minimizing the chronic complications. Unfortunately, none of the oral hypoglycaemic agents have been successful in maintaining euglycaemia, and in addition have a number of side effects (Holman and Turner, 1991).

In the present study D-400, a crude drug formulation consisting of herbs and minerals derived from the traditional system of medicine in India, Ayurveda, was evaluated for its antihyperglycaemic action.

### **MATERIALS AND METHODS**

**Animals:** Wistar strain albino rats (160-180 g) were used. The rats were housed in colony cages at an ambient temperature of  $25^{\circ} \pm 2^{\circ}\text{C}$  and 45%-55% relative humidity with 12h light-dark cycle. The animals had free access to standard pellet chow (Hindustan Lever Ltd.) and water was given through drinking bottles. All the experiments were conducted between 0900 and 1400 h.

**Test drug:** D-400 is a polyherbal formulation consisting of herbs procured from authentic sources. It consists of medicinal plants, namely *Gymnema sylvestre* R. Br. (Asclepiadaceae, leaves) 30 mg, *Momordica charantia* Linn. (Cucurbitaceae, fruit) 20 mg, *Tinospora cordifolia* (Wild) (Cucurbitaceae, fruit) 20 mg, *Tinospora Cordifolia* (Wild) Miers. (Menispermaceae, stem) 10 mg, *Perocarpus marsupium* Roxb. (Leguminosae, stem) 20 mg, *Casearia esculenta* Roxb. (Samydaceae, stem) 20 mg, *Eugenia jambolana* Lam. (Myrtaceae, bark) 20 mg, *Ocimum sanctum* Linn. (Labiatae, whole plant) 10 mg, *Balsamodendron mukul* Hook. *Ex stocks* (Burseraceae, resin) 30 mg. Shilajit (purified) 30 mg. The drug was suspended in distilled water and was administered orally through an orogastric tube at a dose of 1 g/kg. The volume of the vehicle being kept constant at 5 ml/kg. Control animals received only the vehicle in the same volume and through the same route.

**Alloxan induced diabetes mellitus:** Sixteen female rats were used in this experiment, and fasting blood sugar was determined after an overnight fast with free access to water. On the following day, alloxan monohydrate was administered at a dose of 50 mg/kg and stable hyperglycaemia was confirmed on day 8. Twelve rats showing stable hyperglycaemia were divided into two groups of six rats each. Group 1 received vehicle and group 2 received D-400. In both the groups after an overnight fast, blood was collected for blood sugar estimation, on days 20, 30 and 40. On day 42 rats were killed by decapitation and liver was removed from each rat for glycogen estimation.

**Blood sugar estimation:** Blood was collected by ocular vein puncture under mild ether anesthesia. Blood sugar estimation was done by using GOD/POD enzymatic method (Teitz, 1976).

**Liver glycogen estimation:** Liver glycogen estimation was done by the method as described by Scifter *et al.*, 1949. Immediately after excision from the animal, approximately 1 g of the liver was dropped into a previously weighed test tube containing 3 mL of 30% potassium hydroxide solution. The weight of the liver sample was determined. The tissue was then digested by heating the tube for 20 min in boiling water bath, and following this the digest was cooled, transferred quantitatively to a 50 ml volumetric flask, and diluted to the mark with water. The contents of the flask were then thoroughly mixed and a measured portion was then further diluted with water in a second volumetric flask so as to yield a solution of glycogen of 3-30  $\mu\text{g/ml}$ . Five mL aliquots of the final dilution were then pipetted into Evelyn tubes and the determination with anthrone was carried out. The amount of glycogen in the aliquot used was then calculated using the following equation:

$$\mu\text{g of glycogen in aliquot} = 100 U/1.11S$$

$U$  is the optical density of the unknown solution.  $S$  is the optical density of the 100  $\mu\text{g}$  glucose and 1.11 is the factor determined by Morris standard (Morris, 1948) for the conversion of the glucose to the glycogen.

**Streptozotocin induced diabetes mellitus:** Diabetes was induced in overnight fasted rats by intravenous (via tail vein) injection of 50 mg/kg streptozotocin (Upjohn) using a 5% solution of freshly prepared streptozotocin in 0.1 M citrate buffer (pH 4.5). Control rats received citrate buffer only. Fasting blood glucose was measured and glycosuria was detected in all the animals. 7 days after streptozotocin administration. Blood glucose estimation was done on day 7. Eighteen rats showing blood sugar levels more than 300 mg % were selected for the study. They were divided into two groups of nine rats each. Group I received the vehicle and Group II received the test drug. After 21 days of treatment, an oral glucose tolerance test (OGTT) was carried out according to the method of Cole and Harned (1938) as modified by Wexler and Fischer (1963). After an overnight fast, blood samples were collected and then 200 mg glucose in 2 mL. Solution was administered and blood samples were collected at 0, 30, 60, 90, 120 and 180 min.

**Histologic studies on the pancreas:** The whole pancreas from all 18 animals in the streptozotocin treated group perfused with formalin was collected and removed immediately together with the spleen. The three regions of the pancreas, namely the duodenal (head), gastric (body) and splenic (tail), were dissected (Jeffe, 1951), cut into smaller fragments and fixed separately in Bouin's fluid for 24 h. The segments were dehydrated with ethanol and embedded in paraffin-wax (56°C). Serial sections (5 µm) were taken and stained with chrome-haematoxylin and phloxin (Gomori, 1941). Serial sections were studied for the number of islets and beta cell content in each of the three regions of pancreas from each rat.

Statistical analysis was done by using unpaired Student's *t*-test. The minimum level of significance was set a  $p < 0.05$ .

## RESULTS AND DISCUSSION

No mortality was observed in any experimental group throughout the period of investigation although a steady decline in body weight was observed in the alloxan treated group. D-400 not only attenuated the decrease in body weight but induced a steady rise (Table 1). D-400 treatment appeared to regulate diabetes at the cellular level resulting in declination of body weight to near normal levels. D-400 treatment resulted in a significant reduction in blood sugar level in alloxan treated rats (Table 2). The oral glucose tolerance test following streptozotocin-induced hyperglycaemia showed a significant reduction of AUC from  $71694.80 \pm 987.57$  to  $41489.12 \pm 584.14$  under the influence of D-400. There was a significant reduction in pancreatic weight, number of islets, diameter of each islet and beta cell counts/islet in streptozotocin treated group. Treatment with D-400 resulted in restoration of near normal architecture of pancreatic islet (Table 3, Figs. 1 and 2). This suggests a possible regeneration or repair of the cells of the islets of Langerhans in the streptozotocin treated group, under the influence of D-400. Unlike sulphonylureas D-400 did not have any hypoglycaemic effect in normal rats. It is here that D-400 assumes significance, because of its capacity to partially regenerate the damaged endocrine tissue so that the islet number increases with therapy.

| Table 1: Effect of D-400 on body weight profile in alloxan-induced diabetic rats |                                |                    |                    |                    |
|--|--------------------------------|--------------------|--------------------|--------------------|
| Group  | Body weight (g) mean $\pm$ SEM |                    |                    |                    |
|  | Day 0                          | Day 20             | Day 30             | Day 40             |
| Vehicle  | 157.00 $\pm$ 8.75              | 175.00 $\pm$ 9.70  | 189.00 $\pm$ 9.14  | 198.00 $\pm$ 9.30  |
| Alloxan  | 163.00 $\pm$ 6.00              | 161.00 $\pm$ 11.00 | 153.00 $\pm$ 9.00  | 158.00 $\pm$ 8.00  |
| Alloxan + D-400  | 159.00 $\pm$ 5.00              | 158.00 $\pm$ 11.00 | 164.00 $\pm$ 12.00 | 175.00 $\pm$ 10.00 |

n=6 rats in each experimental group.

| Table 2: Effect of D-400 on fasting blood sugar in alloxan-induced diabetic rats |  |                                 |                                 |                                 |
|--|--|---------------------------------|---------------------------------|---------------------------------|
| Group  | Blood sugar on days (mg%) mean $\pm$ SEM |                                 |                                 |                                 |
|  | Day 0                                    | Day 20                          | Day 30                          | Day 40                          |
| Vehicle  | 79.70 $\pm$ 3.37                         | 75.90 $\pm$ 1.62                | 77.00 $\pm$ 4.97                | 77.70 $\pm$ 4.29                |
| Alloxan  | 343.00 $\pm$ 25.00                       | 202.00 $\pm$ 26.00              | 228.00 $\pm$ 20.00              | 180.00 $\pm$ 20.00              |
| Alloxan + D-400  | 341.00 $\pm$ 23.00                       | 137.00 $\pm$ 11.00 <sup>a</sup> | 144.00 $\pm$ 19.00 <sup>a</sup> | 107.00 $\pm$ 10.00 <sup>a</sup> |

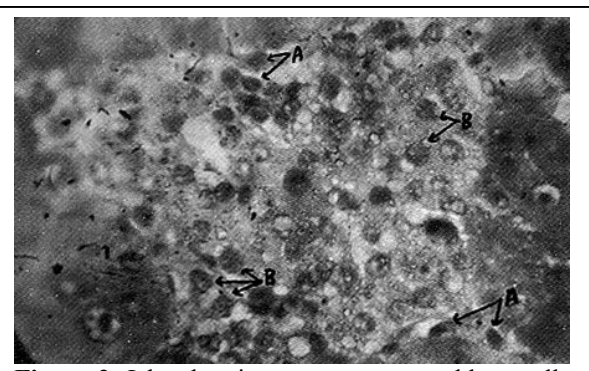
n=6 in each experimental group; statistical significance <sup>a</sup> $p < 0.05$  compared with the alloxan-treated group.

| Table 3: Effect of D-400 on pancreatic weight, number and size of the islets of Langerhans and number of beta-cells/islet in streptozotocin (STZ) induced diabetic rats |                                |                               |                                 |                               |
|---|--------------------------------|-------------------------------|---------------------------------|-------------------------------|
| Group   | Pancreas weight (g)            | No. of islets                 | Diameter ( $\mu$ m)             | Beta-cells/islet              |
| Vehicle   | 1.120 $\pm$ 0.015              | 11.43 $\pm$ 0.57              | 296.47 $\pm$ 0.381              | 51.00 $\pm$ 2.09              |
| STZ   | 1.033 $\pm$ 0.43 <sup>a</sup>  | 07.34 $\pm$ 0.40 <sup>c</sup> | 68.41 $\pm$ 0.071 <sup>b</sup>  | 15.33 $\pm$ 0.56 <sup>d</sup> |
| STZ + D-400   | 1.126 $\pm$ 0.014 <sup>a</sup> | 11.09 $\pm$ 0.50 <sup>c</sup> | 163.43 $\pm$ 0.320 <sup>b</sup> | 26.73 $\pm$ 1.88 <sup>d</sup> |

n=9 rats in each experimental group; statistical significance (<sup>a</sup> $p < 0.05$ , <sup>b,c,d</sup> $p < 0.001$  compared with STZ treated group).



**Figure 1:** Islet component showing imperfections and atrophic changes in streptozotocin treated rats (x1000). A-alpha cells; B-beta cells.



**Figure 2:** Islet showing compactness and beta cells appear dark, well developed and granulated in streptozotocin + D-400 treated rats (x 1000). A-alpha cells; B-beta cells.

In diabetes, glucose-6-phosphatase increases in the liver, facilitating glucose release into the blood. The opposing enzyme, which phosphorylates glucose is glucokinase which decreases in diabetes. As a result, the liver continues to produce glucose even with severe hyperglycaemia. This results in glycogen degradation and inhibition of glucose utilization. The elevation of depressed glycogen stores by D-400 in alloxan treated rats (Fig. 3) may be attributed to either an inhibition of hepatic glucose output by improvement in plasma insulin levels or by stimulating the enzyme glycogen synthetase responsible for the incorporation of glucose moieties into pre-existing glycogen chains. The findings of the present study lend credence to the use of D-400 as an anti-hyperglycaemic agent.

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