

Effect of D-400 a Herbomineral Formulation on Liver Glycogen Content and Microscopic Structure of Pancreas and Liver in Streptozotocin-induced Diabetes in Rats

Mitra, S.K., Gopumadhavan, S., Muralidhar, T.S. and Seshadri, S.J.
R&D Centre, The Himalaya Drug Co., Makali, Bangalore, India

SUMMARY

Streptozotocin induces severe and irreversible hyperglycaemia in experimental animals. The effect of oral administration of D-400 (1 gm/kg/day), a herbomineral formulation on streptozotocin induced-diabetes was studied in rats. Liver glycogen content was assayed biochemically on 2, 4 and 8 weeks after D-400 treatment. The microscopic structure of pancreas and liver were examined in both control and treated respectively. Streptozotocin induced a decrease in pancreatic islet cell superoxide dismutase which was reversed by D-400 treatment for a period of 8 weeks. The free radical scavenging activity of D-400 may be attributed to shilajeet, one of its important ingredients. Streptozotocin induced histopathological changes in pancreas and liver was also partially reversed by D-400. The findings indicate that D-400 helps in improving the glycogen stores in the liver and prevents the streptozotocin induced damage through free radicals by increasing the islet cell superoxide dismutase activity.

Non-insulin-dependent diabetes mellitus is among the most common disorders in developed and developing countries¹. Abnormalities of β cell function and secretion exist in patients with non-insulin-dependent diabetes mellitus². Treatment of hyperglycaemia in patients, with NIDDM is directed towards achieving euglycaemia and eliminating or minimizing the chronic complications. The sulphonylureas are reported to regulate blood glucose homeostasis by stimulating pancreatic secretion of insulin³ but have a characteristic profile of side effects⁴.

In diabetes, liver glycogen is degraded and gluconeogenesis are increased while glucose utilization is inhibited. In diabetes, glucose-6-phosphatase increases in the liver, facilitating glucose release into the blood. The opposing enzymes which phosphorylate glucose are hexokinase, which is unaffected by insulin and glucokinase, which decreases in diabetes. As a result, the liver continues to produce glucose even with severe hyperglycaemia. Under these circumstances the normal liver would shut off and deposit glycogen⁵.

D-400 is an Ayurvedic herbomineral formulation which contains herbal extracts and minerals of known antidiabetic action. Its constituents include Shilajeet, *Gymnema sylvestre*, *Momordica charantia*, *Tinospora cordifolia*, *Pterocarpus marsupium*, *Casearia esculanta*, *Eugenia jambolona*, *Ocimum sanctum* and *Balsamodendron mukul* (Guggul).

In previous studies with D-400 in alloxan-induced diabetes, a significant reduction of blood sugar level was reported^{6,7}. In alloxan-induced diabetes, the insulin secreting cells are damaged and to bring about blood glucose and serum insulin homeostasis, either regeneration or repair of beta cells in the islets of langerhans would appear to be necessary. The present study was conducted to evaluate the efficacy of D-400 on β cells and liver glycogen in streptozotocin-induced diabetes.

Streptozotocin was used to induce diabetes, rather than alloxan, since with this agent there is no incidence of spontaneous reversion and greater specificity for islets resulting in >90% of rats becoming diabetic at this dosage used.

MATERIALS AND METHODS

Seventy one inbred female Wistar rats of 2.5-3.0 months age with fasting blood sugar levels of 81 ± 3 mg/dl were used in this study. They weighed 180 to 220 g and were maintained at a room temperature of $22^\circ \pm 2^\circ\text{C}$ with 12 hr light and dark cycle. They were fed with synthetic diet and water *ad libitum*.

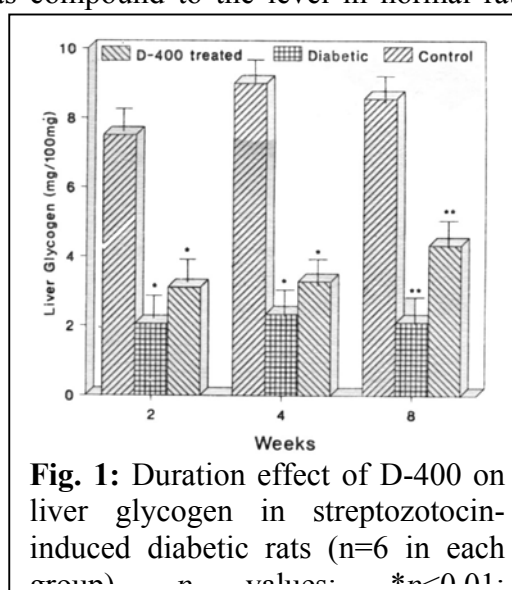
Streptozotocin (Sigma, USA) was dissolved in 0.05M citrate buffer, pH 4.5 and was administered to 52 rats at a dose of 50 mg/kg, iv (via tail vein) after an overnight fast on day '0'. Blood sugar was evaluated on day 7 to confirm stable hyperglycaemia. Twelve rats succumbed to the streptozotocin injection and from among the remaining 40, 36 rats with fasting blood sugar above 250 mg/dl were selected for the study. They were divided into two equally matched groups of 18 each and designated as G2 and G3. G3 received D-400 (1 gm/kg body wt) once daily orally as an aqueous suspension and the other group, viz G2, received water and served as diabetic control. Nineteen normal rats formed G1 in this experiment and received a routine schedule. One of the rats from G1 received 10 gm/kg/body wt. of glucose twice a day orally to serve as a positive control for liver glycogen in PAS reaction.

Six rats from G1, G2 and G3 were sacrificed 2, 4 and 8 weeks after respective assigned treatment to study the duration response of D-400 on liver glycogen content by using the method of Rex Montgomery⁸. At 4th week of sacrifice liver and pancreatic tissues were collected and fixed in 10% buffered neutral formalin routinely. For demonstration of liver glycogen, the liver tissues were fixed in formal alcohol, paraffin sections cut and subjected to PAS reaction. Pancreas were collected from the animals sacrificed on 8th week to study effect of D-400 on superoxide dismutase activity.

RESULTS

Duration response effect of glycogen content (mg/100 mg) normal (G1), streptozotocin-diabetic (G2) and streptozotocin + D-400 treated group (G3) are shown in (Fig. 1).

Liver glycogen content was reduced significantly as compared to the level in normal rats substantiating depletion of liver glycogen and failure of utilisation by hepatocytes in streptozotocin induced diabetic rats. It is further seen that diabetic rats treated with D-400 showed gradual replenishment of glycogen stores in the liver as is seen from the values of 3.110 ± 0.307 , 3.28 ± 0.035 and 4.33 ± 0.0246 as against 2.055 ± 0.261 , 2.33 ± 0.180 and 2.10 ± 0.252 at 2, 4 and 8 weeks respectively. Streptozotocin diabetes resulted in degenerative and lytic changes in the islets of Langerhans of the pancreas (Fig. 2). In some of these sections, the dimensions of the islet was also considerably reduced and shrunken. In the D-400 treated group (G3) it was seen that there was an increase in the size of the islet and evidence of hyperplasia marked by increase in the cellular



components (Fig.3). A section of the liver from rats designated to serve as a positive control for demonstration of glycogen revealed the appearance and deposition of reddish purple material in hepatocytes (Fig.4) by PAS staining. Sections of the liver from the diabetic group (G2) and the diabetic group treated with D-400 (G3) examined with PAS staining showed absence of demonstrability of PAS positiveness in G2 (Fig.5), while in G3, PAS positiveness was evident with the hepatic chords showing a reddish purple material in the hepatocytes, representative of glycogen buildup (Fig.6).

The pancreatic islet cell SOD values (u/mg protein) decreased significantly in the streptozotocin treated group. D-400 (1 gm/kg) induced a discernible and statistically significant increase in SOD concentrations induced by streptozotocin following 8 weeks of treatment. (Fig. 7)

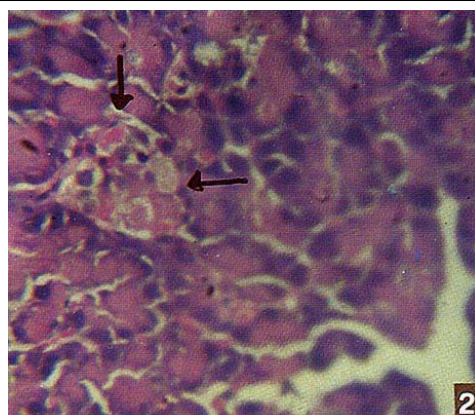


Fig. 2: Section of pancreas from rat with streptozotocin-induced diabetes (G2). Note shrunkenness of islet (→) with lytic and vascular changes of cellular components (H&Ex450).

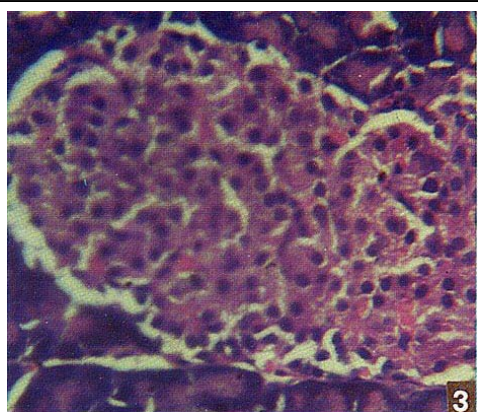


Fig. 3: Section of pancreas from rat with streptozotocin-induced diabetes and treated with D-400 (G3). Note marked hyperplasia and enlargement of islet (H&Ex450).

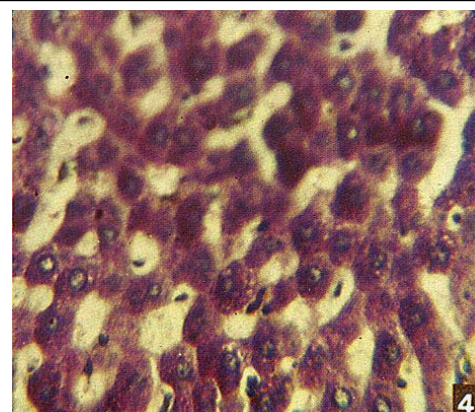


Fig. 4: Section of liver of a normal rat fed with glucose to cause rise in blood sugar level. Note the hepatocytes distended with reddish purple deposition representative of glycogen (PASx450).

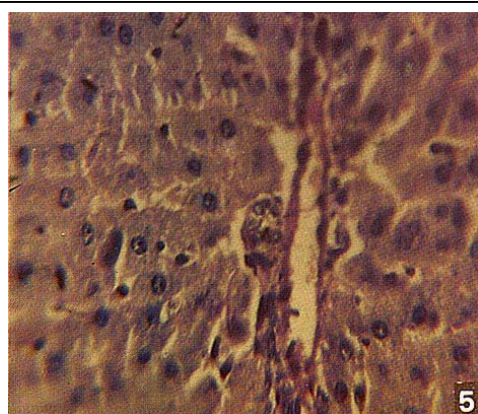


Fig. 5: Section of liver of streptozotocin-induced diabetes rat (G2). Note absence of PAS positiveness in hepatocytes indicating absence of glycogen. PAS positiveness is only observable as a reddish purple colour representing vascular matrix (PASx450).

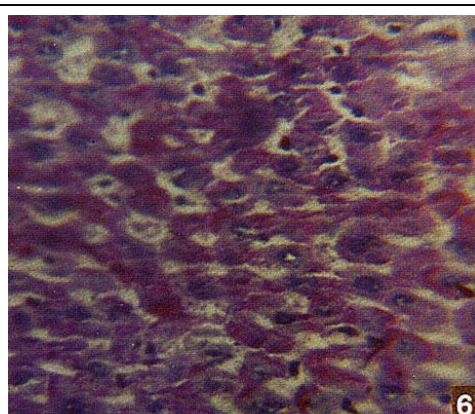


Fig. 6: Section of liver of rat with diabetes-induced by streptozotocin and treated with D-400 (G3). Note the patchy to streaky appearance of reddish purple material build up within the hepatocytes representative of glycogen (PASx450).

DISCUSSION

D-400 is a herbal formulation with known potential of correcting diabetes by acting as an antihyperglycaemic agent rather than by inducing hypoglycaemia, as is verified from clinical observations in earlier studies⁹. The above fact has also been convincingly substantiated in an earlier experiment conducted in streptozotocin induced diabetic rats subjected to D-400 treatment, in that the glucose tolerance test conducted in these two groups provided valuable knowledge on the favourable response to treatment with D-400 as compared to streptozotocin induced diabetic rats¹⁰. In this study the results of the glycogen level in the liver of rats in Groups G2 and G3 when compared with normal rats supported an interpretation that D-400 did enable the liver cells to build up glycogen (G3) that was not available in the streptozotocin induced diabetic group (G2). Though the levels of glycogen build up had not touched those observed in normal rats (G1) within the short duration of this experiment, it has provided a dependable observation that the capacity to rebuild glycogen reserve has been achieved by D-400 treatment.

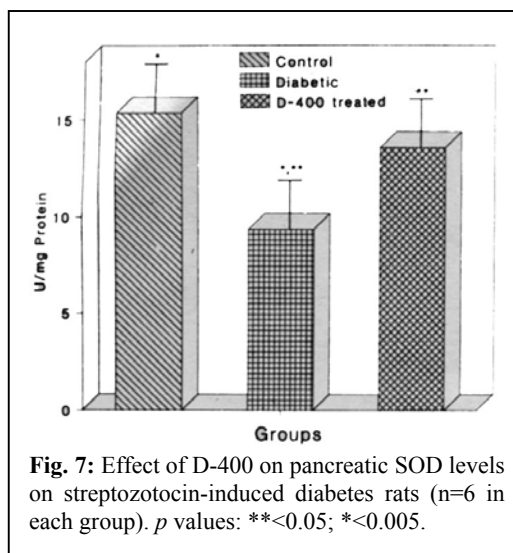


Fig. 7: Effect of D-400 on pancreatic SOD levels on streptozotocin-induced diabetes rats (n=6 in each group). *p* values: **<0.05; *<0.005.

Histopathological examination of pancreas in Group G3 clearly proves that D-400 helps in restoring the activity of islets of Langerhans as compared to Group G2 where the same had been damaged or made atrophic. Sections of liver from Group G2 did not show demonstrability of PAS positiveness to indicate the presence of glycogen while sections of G3 which represented D-400 treated rats showed PAS positiveness proving the presence of glycogen content, a finding that has correlated well with the quantitative estimation of liver glycogen content in Groups G2 and G3.

Streptozotocin a naturally occurring nitrosamide has relative pancreatic islet β cell cytotoxicity in animals. The precise mechanism of actions of streptozotocin is unknown, but there is evidence that it interferes with cellular metabolite oxidative mechanisms. Generation of oxygen free radicals has been proposed to be a major mechanism involved in the cytotoxicity. The alteration of SOD activity by D-400 could be attributed to shilajeet one of its main ingredient which is known for its antioxidant and free radical scavenging activity.

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