



In this study, in addition to establishing the hypoglycemic action of the preparation, an attempt is made to find out the possible mode of action.

## METHODS

Rats of original Wistar strain bred in our laboratory for over 45 generations were used in this study. Experiments were carried out in male and female rats 2.5 to 3 months old and weighing between 175-250 gm. They were allowed to get acclimatized to a standard laboratory diet (Hindustan Lever Ltd.) and constant room temperature at 22°C - 24°C with 12 hour day and night cycle. Drinking water was allowed *ad libitum*.

The study was placebo-controlled and the effect of D-400 was evaluated in normal and alloxan-induced diabetic rats. In normal and diabetic rats the study was further subdivided into four parts and two parts respectively. The detailed procedure for each part is follows:

### 1. In normal rats:

- (a) *Effect of D-400 on blood sugar in normal rats:* The effect of D-400 on fasting blood sugar was studied in normal male and female rats following 90 days of treatment. In this experiment, 50 rats (20M+30F) were divided into two groups of 25 each (10M+15F) in a randomize manner. Group I rats received 10 ml/kg body weight of tap water once a day orally for 90 days and served as controls. Group II rats received 2 gm/kg body weight of D-400 fine powder in the form of an aqueous suspension once a day orally for 90 days and served as test animals. Fasting blood was sampled by tail nipping for determination of the glucose levels before, and 30 and 90 days after assigned treatment. Glucose was determined by using dextrostix strips and a Glucometer (Ames Co.).
- (b) *Interaction with Adrenaline in normal rats:* In this experiments, on day 0, fasting blood sugar (FBS) of 8 rats were determined. 100 µg of adrenaline was injected subcutaneously to each rat and blood sugar levels monitored at 30, 60, 90, 120, 180 and 240 min. After basal blood sugar profile following adrenaline, the rats received D-400 treatment once a day

orally for 21 days at a dose of 2 gm/kg. On day 21, following overnight fast, the blood sugar levels were assessed as on day 0.

- (c) *Interaction with Tolbutamide in normal rats:* The hypoglycemic effect of tolbutamide was studied before and after D-400 treatment. Six rats were used for this experiment. On day 0, fasting blood sugar (FBS) was determined and 250 mg/kg body weight of tolbutamide was administered orally to each rat as an aqueous suspension. Blood sugar was determined at every one hour for 4 hours following administration of tolbutamide. After basal blood sugar profile following tolbutamide, the rats were administered 2 gm/kg body weight of D-400 aqueous suspension once a day orally for 21 days. On day 21, blood sugar levels were assessed as on day 0.
- (d) *Interaction with Insulin in normal rats:* The hypoglycemic effect of insulin was studied before and after D-400 treatment in normal rats. On day 0, in 8 rats followed by fasting blood sugar (FBS), 0.25 IU of insulin was injected intravenously and blood sugar was determined at 30, 60, 90, 120, 180 and 240 minutes. The same rats then received 2 gm/kg body weight of D-400 once a day orally for 21 days. On day 21, following overnight fast, the blood sugar levels, were assessed as on day 0.

### 2. In alloxan-induced diabetic rats:

- (e) *Effect of D-400 on blood and urine sugar in alloxan-induced diabetic rats:* The hypoglycemic effect of D-400 was evaluated in alloxan-induced diabetic rats. Sixteen female rats were used in this experiment and fasting blood sugar (FBS) was determined after overnight fast with free access to water. Following this, alloxan monohydrate was given intravenously at a dose of 50 mg/kg body weight and stable hyperglycemia was confirmed on day 8. Out of 16 rats, only 12 rats responded to alloxan monohydrate and were divided into two groups of 6 each. Rats in Group I received tap water at a dose of 10 ml/kg body weight once a day orally for 42 days, while Group II rats received 2 gm/kg

body weight of D-400. In both groups, after overnight fast, blood and urine sugar were monitored on days 20, 30 and 40. On day 42, after an overnight fast, the rats were sacrificed for liver glycogen estimation (21)

- (f) *Interaction with Insulin in alloxan-induced diabetic rats:* The hypoglycemic effect of D-400 along with insulin was studied in alloxan-induced hyperglycemic rats. Six rats were taken for this experiment. 50 mg/kg body weight of alloxan monohydrate was given intravenously. After confirming stable hyperglycemia on day 8, 0.5 IU of insulin was given intravenously and blood sugar determined at 30, 60, 90, 120, 180 and 240 min. The same procedure was repeated after D-400 treatment at a dose of 2 gm/kg body weight once a day orally for 21 days.

**RESULTS**

**1. In normal rats:**

- (a) The aqueous suspension of D-400 exerted a significant hypoglycemic effect on day 30 in

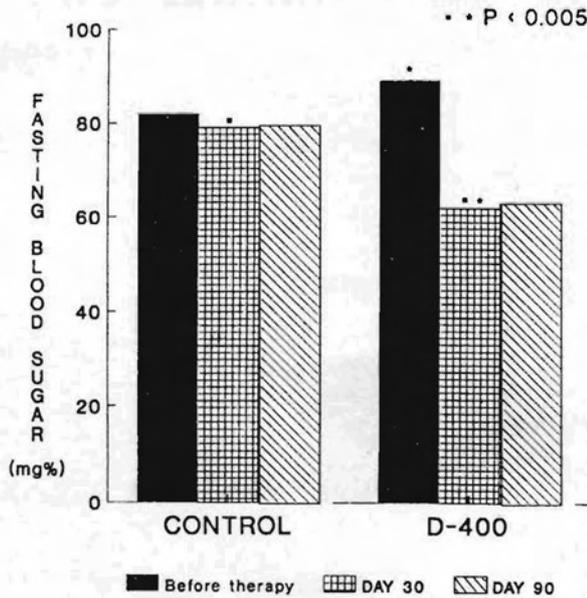


Fig. 1 : Mean fasting blood sugar (mg%) before, 30 and 90 days after D-400 treatment in normal male and female rats (n=25).

both male and female rats. No further lowering of blood sugar was noticed following 90 days of D-400 treatment (Fig. 1)

- (b) Hyperglycemic response to adrenaline at 2 and 3 hours significantly lowered following D-400 treatment. The total area under the time curve concentration (AUC) was reduced significantly as compared to day 0 profile (Fig. 2).

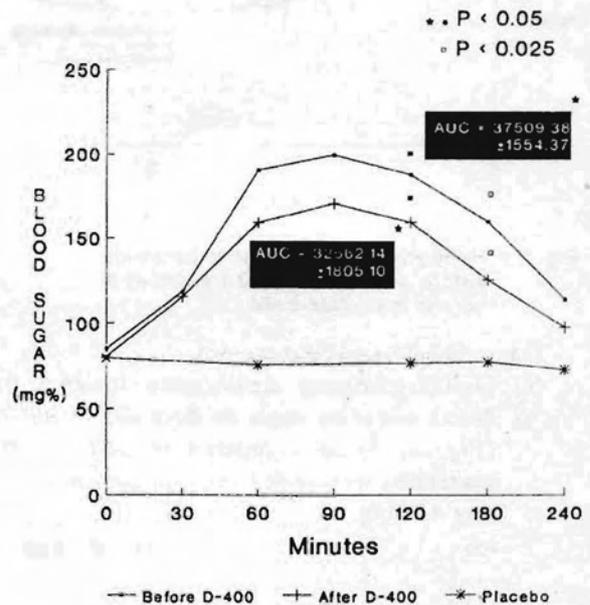


Fig. 2 : Mean blood sugar levels following adrenaline injection before and after D-400 treatment in normal male rats (n=8).

- (c) Tolbutamide 250 mg/kg caused a peak fall in blood sugar levels at 2 hours that normalized at 4 hours. Following D-400 treatment for 21 days, the blood sugar was further decreased at 3 hours and remained so at 4 hrs. The difference was significant at both the time points as compared to tolbutamide alone (Fig. 3).

- (d) In normoglycemic rats, interaction of insulin with D-400 on blood sugar profile elicited comparatively same findings.

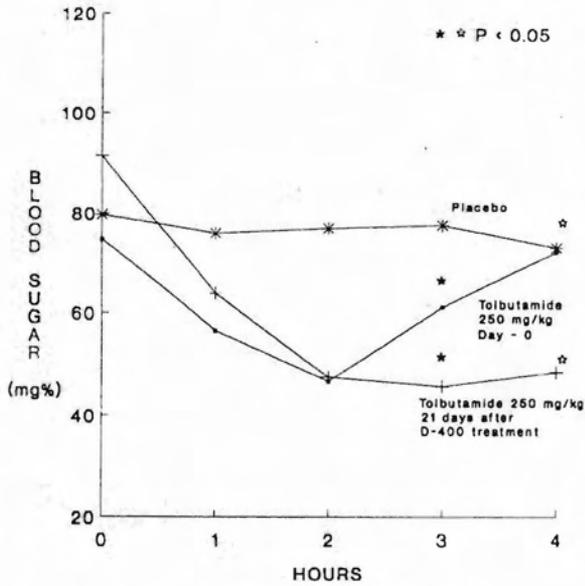


Fig. 3 : Hypoglycemic effect of tolbutamide before and after D-400 treatment in normal male rats (n=6).

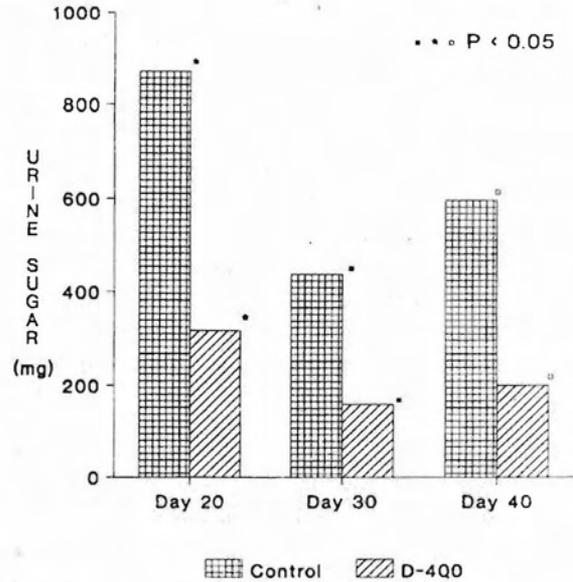


Fig. 5 : Mean urine sugar excretion (mg) and effect of D-400 treatment in alloxan-induced diabetic rats (n=6).

2. In Alloxan-induced diabetic rats:

(e) D-400 treatment significantly lowered the blood and urine sugar on days 20, 30 and 40 (Fig. 4, 5) as compared to placebo and correlated well with liver glycogen levels on day 43 (Fig. 6).

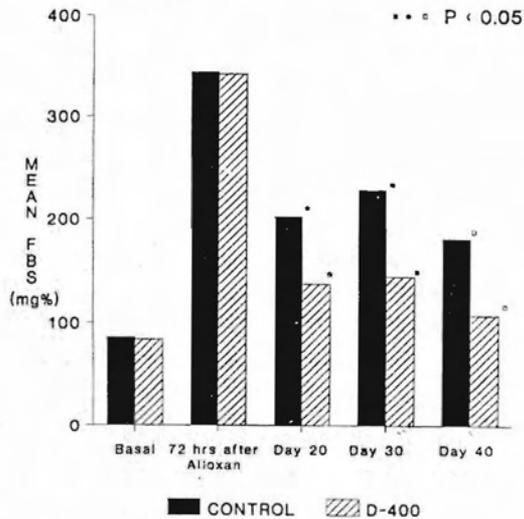


Fig. 4 : Mean FBS (mg%) levels on days 20, 30 and 40 following D-400 treatment in alloxan-induced diabetic rats (n=6).

(f) In hyperglycemic rats, D-400 has significantly potentiated the hypoglycemic effect of insulin at 90, 120, 180 and 240 min (Fig. 7).

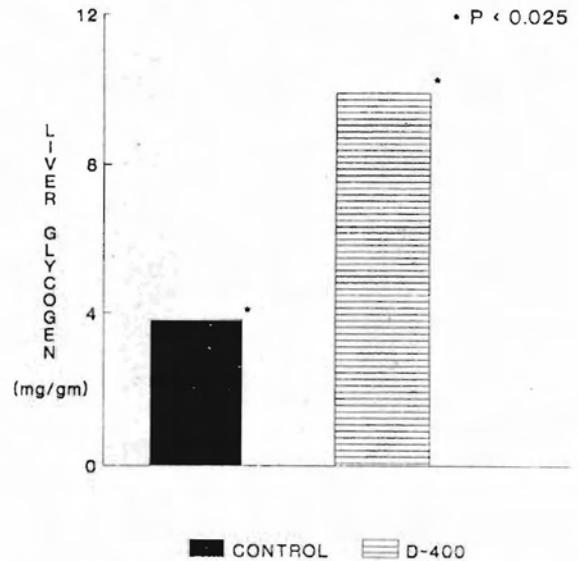


Fig. 6 : Effect of D-400 treatment on liver glycogen in alloxan-induced diabetic rats (n=6).

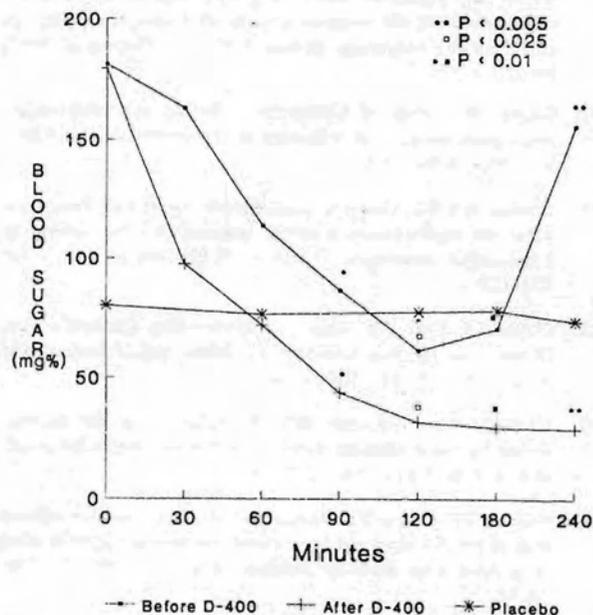


Fig. 7 : Blood sugar levels following insulin injection before and after D-400 treatment in alloxan-induced diabetic rats (n=6).

## DISCUSSION

In the present study, D-400 exhibit a small but significant hypoglycemic effect in normal rats. The hypoglycemic effect of D-400 on fasting blood sugar levels was attained after 20 days of treatment and did not decrease further even upto 90 days. This may be due to the fact that, these being normal animals in whom carbohydrate metabolism was already in

homeostasis, further fall beyond certain limits was not possible. This is a desirable feature.

In another experiment, the hyperglycemic response to adrenaline was reduced following D-400 treatment in rats. Adrenaline is known to cause suppression of insulin release via alpha receptors thereby raising blood sugar levels. It appears that D-400 may possess the ability to stimulate the pancreas, thereby antagonizing the effect of adrenaline and resulting in raising the level of circulating insulin. This in turn decreases blood sugar levels.

D-400 prolonged the hypoglycemic effect of tobutamide in normal rats. This could be due to the action of D-400 on liver glycogen thereby preventing the hepatic glycogenolysis, which is further substantiated by the rise in liver glycogen levels in diabetic rats treated with D-400. This prevention of glycogenolysis by D-400 must have counteracted the rise in blood sugar two hours after tolbutamide administration.

In alloxan-induced diabetic rats, D-400 produced a significant hypoglycemic effect and reduced urinary sugar excretion. The hypoglycemic effect of insulin was enhanced and the liver glycogen store was significantly higher following treatment with D-400. All these findings suggest that D-400 may be acting through some mechanism, viz. improvement in the receptor responsiveness to insulin causing increased glucose uptake by the tissues. Hence, further studies are essential to substantiate the present findings and also to establish the probable mechanism of action of D-400 distinctly.

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