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Oriental Pharmacy and Experimental Medicine 2007 **7(2)**, 205-210

Pharmacodynamic evaluation of hypoglycemic effect of Damtab in healthy adult male volunteers

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SUMMARY

The objectives of the study were (1) To evaluate the safety and tolerability of Damtab. (2) To characterize hypoglycemic effect of Damtab, if any. (3) To evaluate insulin sensitivity effect of Damtab, if any. Hypoglycemic effect of Damtab (700 mg and 1,400 mg) were examined. Gliclazide (80 mg) was used as an active control. Placebo was used as control. Breakfast was given, half an hour before dosing whereas lunch, snacks and dinner were given at 6, 10 and 14 h post dose. An oral glucose tolerance test was conducted to calculate the insulin sensitivity index from the values of glucose and insulin during oral glucose tolerance test. Both giclazide 80 mg and Damtab 1,400 mg significantly lowered plasma glucose level up to 6 h. Insulin sensitivity index of Damtab (1,400 mg) was found to be similar to that of placebo. A significant increase in insulin level at 1 h post dose of Damtab (1,400 mg) was observed. Damtab 700 mg shows placebo like effect whereas Damtab 1,400 mg possesses hypoglycemic effect.

Key words: Hypoglycemic; Insulin sensitivity index; Damtab; Unani medicine; Gliclazide; Oral glucose tolerance test; Healthy volunteers

INTRODUCTION

Recently, there has been increasing interest in the clinical use of indigenous drugs. Traditional and folklore medicines are receiving attention in health care system. In Unani system of medicine, many single and compound formulations have been used for the treatment of diabetes.

Damtab is a polypharmaceutical Unani tablet formulation having constituents from plant and animal sources. It contains leaf, bark and seed of *Azadirachta indica*, seed of *Gossypium herbaceum*, leaves of *Gymnema sylvestre*, petals of *Rosa domascena*, seeds of *Syzigium cumini* as the plant constituent. The animal constituent is kushta baiza murgh i.e ash of egg shells of hen (Anonymous, 1997; Najmi *et al*., 1999). It is used as antidiabetic drug by Unani physicians in Non Insulin Dependent Diabetes Mellitus (NIDDM) (Vohora, 1983).

There are a number of indigenous drugs available throughout the world having very good therapeutic value but their importance is yet to be explored. Many drugs of questionable value and doubtful utility have also come under the indigenous medicine. Systematic investigation is needed not only to exclude these drugs but also to precisely identify their ill effects and to give emphasis on the

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useful drugs.

The present study was a preliminary attempt to have some clinical data on Damtab, which will be helpful for large trials.

MATERIALS AND METHODS

Drugs

Damtab is manufactured by Hamdard (Wakf) Laboratories, Delhi, India. It is available as tablet formulation containing 700 mg of active ingredients. Gliclazide is a sulphonylurea available in the medicine stores. It is used as an antidiabetic drug in the treatment of NIDDM. We have taken Gliclazide (80 mg) tablets (Reclide) manufactured by Dr. Reddy's Laboratories, India. Since this was a double blind study, both the formulations were put in capsules of similar color and size in Department of Pharmaceutics, Faculty of Pharmacy, Jamia Hamdard, New Delhi, India. Placebo capsules containing cellulose were also manufactured in above-mentioned department.

Study design

A randomized, double blind, three treatment, three period cross over pharmacodynamic study was conducted to evaluate the effect of Damtab 700 mg, Gliclazide 80 mg and placebo on plasma glucose level. After getting the results a fourth period was conducted with the same volunteers to evaluate the effect of Damtab 1,400 mg on plasma glucose level. In all these periods laboratory tests on hematology and biochemical parameters were performed and blood pressure, pulse rate and electrocardiogram were checked to evaluate safety & tolerability of Damtab. Breakfast was given in the morning half an hour before dosing whereas lunch, snacks & dinner were served at 6, 10, 14 h post dose respectively.

In another study (study 2) an oral glucose tolerance test was done to find any insulin sensitivity of Damtab (1,400 mg). It was a two-way crossover double blind study. After 10-12 h overnight fasting and subsequent dosing, subjects continued fasting till 2 h post dose on the day of drug administration. Drug washout period was at least 7 days between two periods.

Subject demographics and dropouts

Subjects age ranged from 18 - 31 years with mean (± SD) age of 22.77 (± 3.83), height ranged from 162 - 172 cm with mean (± SD) height of 165.66 (± 2.96) & weight ranged from 49 - 67 kg with mean (± SD) weight of 56.66 (± 6.40). They were neither overweight nor underweight as per height/weight ratio of Life Insurance Corporation of India for non-medical cases. All the subjects were male. They were found to be in normal health and their clinical laboratory test values were within normal range.

Nine subjects were selected for study 1. Subsequently eight of them participated in study 2. There were no dropouts on all the 6 periods.

Study restrictions

The subjects were instructed not to take any medication (prescribed or OTC) for at least two weeks prior to the study and during the study. Subjects were instructed to abstain from any alcoholic products for 48 h prior to dosing in each period and throughout the sampling time in each period. Dinner, breakfast, lunch & snacks served in study 1 were standardized for all the 4 periods. Similarly dinner served on the day before drug administration was standardized for both the periods. Drinking water was not allowed from 1 h pre dose to 2 h post dose except that needed for drug administration and it was freely allowed at rest of time.

Sampling schedule

Study 1: Blood samples (5 ml each) were collected at pre-breakfast and at 24 h post dose for clinical laboratory testing for safety measurements. Blood sample (2 ml each) were collected at pre dose & at 1, 2, 4, 6, 8, 10 & 12 h post dose during all the 4 periods.

Study 2: Blood samples (4 ml each) were collected at pre dose and 1, 2 h post dose during both the periods for measurement of plasma glucose & serum insulin.

Glucose estimation

Glucose was estimated by the GLU method on the Dimension clinical chemistry system (Henry, 1974; Teitz, 1976; Kunst *et al*., 1983). The GLU method is an adaptation of the hexokinase-glucose-6-phosphate dehydrogenase method, presented as a general clinical laboratory method by Kunst *et al*. (1983).

Insulin estimation

Insulin was estimated quantitatively by enzyme linked immunosorbent assay (ELISA). For this purpose DSL-10-1600 ACTIVE™ Insulin ELISA kit was used (Nokagawa *et al*., 1973; Gemmeltoft, 1984; Hwang *et al*., 1985; Rosen, 1987; Rasmussen *et al*., 1990).

Briefly, the DSL-10-1600 ACTIVE™ Insulin ELISA is an enzymatically amplified one-step sandwich type immunoassay. In the assay, standards, controls & unknown serum samples were incubated with anti-Insulin antibody in microtitration wells, which have been coated with another anti-insulin antibody. After incubation and washing, the wells were incubated with the substrate tetramethyl benzidine (TMB). An acidic stopping solution was then added and the degree of enzymatic turnover of the substrate was determined by dual wavelength absorbance measurement at 350 and 620 nm.

The absorbance measured is directly proportional to the concentration of insulin present. A set of insulin standards was used to plot a standard curve of absorbance versus insulin concentrations from which the unknowns can be calculated.

Oral glucose tolerance test (OGTT)

In the morning, after a 10 to 12 h overnight fasting, subjects received a 75 g OGTT. Blood samples were taken at 0, 1 and 2 h for the measurement of plasma glucose and serum insulin concentrations. Orange flavor was added to the glucose load to avoid the unpleasant sensation of drinking glucose solution (Kirsten *et al*., 1991; Matsuda and DeFronzo, 1999).

Insulin sensitivity index (ISI)

Insulin Sensitivity Index was obtained from OGTT applying the formula given by Matsuda and Defronzo, 1999.

$$ISI = 10{,}000/\sqrt{(FPG \times FPI) \times \text{mean glucose conc. in OGTT} \times \text{mean insulin conc. in OGTT}}$$

Where 10,000 simply represents a constant that allows one to obtain numbers ranging from 0 to 12. Square root conversion was used to correct the non-linear distribution of values. FPG = Fasting plasma glucose. FPI = Fasting plasma insulin.

Statistical analysis

The data from the experiments are expressed as mean ± SD. The differences in the evaluation parameters in Study 1 were examined statistically by ANOVA (Kulkarni, 1999). The differences in the evaluation parameters in Study 2 were examined statistically by paired Student's *t*-test. When the probability (*P*) was < 0.05, the difference was considered to be significant.

Ethical considerations

This research work was carried out according to the ethical principles enunciated in Declaration of Helsinki (1996) and the protocol approved by Jamia Hamdard Institutional Review Board.

RESULTS

Plasma glucose level (Study 1)

Single dose hypoglycemic effects of Damtab 700 mg, Damtab 1,400 mg, gliclazide 80 mg, placebo at pre-breakfast, pre dose, 1, 2, 4, 6, 8, 10, 12 and 24 h post dose are shown as mean ± SD in Table 1.

Damtab 700 mg does not show any statistically significant change in plasma glucose as compared to placebo. Gliclazide 80 mg shows statistically

Table 1. Plasma glucose levels at pre breakfast, pre dose and 1, 2, 4, 6, 8, 10, 12 and 24 h after administration of Placebo, gliclazide 80 mg, Damtab 700 mg, Damtab 1,400 mg during study 1

Time	Placebo	Gliclazide (80 mg)	Damtab (700 mg)	Damtab (1,400 mg)	F-value
Pre-Breakfast	93.5 ± 7.81	91.33 ± 6.40	94.11 ± 7.09	88.33 ± 4.38	-
Pre dose	131.44 ± 15.18	132.88 ± 13.29	134.88 ± 29.30	128.68 ± 12.91	0.21
1 h pd	107.53 ± 11.45	104.11 ± 14.26	103.55 ± 11.38	95.66 ± 12.56	1.39
2 h pd	98.00 ± 12.16	87.66 ± 19.18	98.77 ± 10.12	81.22 ± 10.13	3.57*
4 h pd	91.88 ± 4.67	78.33 ± 5.91	90.44 ± 6.04	83.88 ± 4.54	12.37*
6 h pd	88.00 ± 4.21	74.77 ± 5.02	89.77 ± 5.56	80.88 ± 3.82	19.33*
8 h pd	119.22 ± 13.58	106.22 ± 8.67	117.11 ± 20.06	110.11 ± 13.72	1.33
10 h pd	102.77 ± 05.19	91.44 ± 12.66	104.33 ± 12.70	102.55 ± 12.70	2.22
12 h pd	106.77 ± 9.88	98.55 ± 4.00	102.3 ± 11.22	98.11 ± 8.59	1.87
24 h pd	94.66 ± 4.08	93.50 ± 7.55	94.77 ± 5.82	88.22 ± 4.38	-

Each value is the mean ± SD of 9 volunteers. *P < 0.05; pd: post dose. Differences were examined by ANOVA.

Table 2. Plasma Glucose at pre dose, 1 and 2 h after administration of placebo and Damtab 1,400 mg during study 2

	Placebo	Damtab (1,400 mg)
Pre dose	88.13 ± 6.9	88.75 ± 2.12
1 h pd	127.63 ± 25.17	123.63 ± 34.73*
2 h pd	89.75 ± 10.63	95.88 ± 14.07**

Each value is the mean ± SD (mg/dl) of 8 volunteers. *P < 0.01; **P < 0.001; pd: post dose. Differences were examined by the paired student's 't' test.

Table 3. Serum Insulin Level at pre dose, 1 and 2 hours after administration of Placebo and Damtab 1400 mg during study 2

	Placebo	Damtab (1,400 mg)
Pre dose	3.45 ± 2.14	3.44 ± 1.60
1 h pd	75.13 ± 50.76	85.45 ± 65.04*
2 h pd	37.95 ± 20.00	36.33 ± 14.40

Each value is the mean ± SD (mIU/ml) of 8 volunteers. *P < 0.01; pd: post dose. Differences were examined by the paired student's 't' test.

significant decrease (P < 0.001) on plasma glucose at 4 and 6 h post dose as compared to placebo. Damtab 1,400 mg decrease plasma glucose level significantly as compared to placebo at 2 h post dose (P < 0.02), 4 and 6 h post dose (P < 0.01).

Plasma glucose level in OGTT (Study 2)

The glucose level at pre dose, 1 and 2 h post dose of Damtab, 1,400 mg & placebo are shown as mean ± SD in Table 2. The glucose level was decreased at 1 h post dose (P < 0.01) and then increased at 2 h post dose in Damtab (1,400 mg) (P < 0.001) treatment as compared to placebo treatment.

Serum insulin level in OGTT (Study 2)

The serum insulin level at pre dose, 1 and 2 h post dose of Damtab (1,400 mg) and placebo are shown as mean ± SD in Table 3. The serum insulin level was increased at 1 h post dose (P < 0.01) in Damtab (1,400 mg) as compared to placebo. However no statistically significant difference was found at 2 h post dose.

Insulin sensitivity Index

The insulin sensitivity index (Matsuda and Defronzo, 1999) calculated from glucose and insulin levels during OGTT for placebo & Damtab 1,400 mg were found to be 7.327 and 7.001 respectively.

DISCUSSION

Constituents of Damtab from plant source like leaves of *Azadirachta indica*, *Gymnema sylvestre*, seeds of *Syzigium cumini* have some reported anti diabetic effect (Chopra *et al.*, 1996; Najmi *et al.*, 2005). *Azadirachta indica* (neem) has shown moderate hypoglycemic activity in animal models of diabetes (Murty *et al.*, 1978; El-Jamary and Knolief, 1990;

Santhoshkumari and Devi, 1990). Both its oil in a dose of 2.5 ml/kg and its active constituent nimbidin (200 mg/kg) were half as potent as tolbutamide. Nimbola (neem oil in capsule form) has been reported to result in gradual reduction in requirement of anti diabetic drugs in a limited clinical trial (Pillai and Santhakumari, 1981; Bhargava, 1987).

In the 9 volunteers participating in the present study, Damtab was found to be well tolerated up to 1,400 mg as a single dose. Difference in safety parameters at screening time, pre dose & 24 h post dose was not clinically significant for both Damtab (700 mg and 1,400 mg) and Gliclazide (80 mg). However, multiple dose studies are required to have some more information on its safety.

Damtab (700 mg) did not show any statistically significant change in plasma glucose as compared to placebo in the subjects included in this study whereas Damtab 1,400 mg showed significant decrease in plasma glucose at 2, 4 and 6 h post dose. The decrease in plasma glucose up to 2 h post dose by Damtab (1,400 mg) is more than Gliclazide (80 mg) but at 4 and 6 h post dose the effect of Damtab is slightly reduced as compared to Gliclazide.

Glucose lowering effect of Damtab (1,400 mg) is better than Gliclazide (80 mg) up to 2 h but later on its effect was slightly decreased in comparison to Gliclazide (80 mg). Hence a controlled clinical trial on patients may be considered to see whether Damtab is free form hypoglycaemia which is a common side effect of sulphonylureas (Campbell, 1993).

There was a statistically significant increase in serum insulin level at 1hour post dose in Damtab (1,400 mg) treatment as compared to placebo. However, no statistically significant difference was found in serum insulin at 2 h post dose between placebo and Damtab (1,400 mg) in the oral glucose tolerance test (OGTT) conducted on 8 healthy volunteers.

The insulin sensitivity Index calculated from glucose & insulin levels during OGTT for both placebo and Damtab (1,400mg) were found to be nearly same. Hence Damtab might not have any insulin sensitivity effect. However, the first hour significant increase in insulin level shows that Damtab may have insulin-releasing effect like sulphonylureas (White, 1996; Groop and DeFronzo, 1998). To know its mechanism of action at molecular level further studies on large scale are needed.

Damtab did not have any cardiovascular adverse effect in the participated volunteers. No statistically significant difference was found in sitting blood pressure (systolic & diastolic) and pulse rate between placebo, Glicazide (80 mg), Damtab (700 mg) & Damtab (1,400 mg). Again the electrocardiograms (ECGs) at pre dose, 2 and 12 h, post dose in all the treatments in all individuals were found to be normal. The corresponding PR intervals and QRS complexes were also of normal value.

All these data were received from 9 subjects. Further studies on large-scale trials will give valuable information on the safety & efficacy of this drug as well as its mechanism of action.

ACKNOWLEDGEMENTS

The authors thank management of Ranbaxy Clinical Pharmacology Unit for allowing to utilize their excellent research facilities, Hamdard (Wakf) Laboratories for providing Damtab tablets & insulin kit and Jamia Hamdard Institutional Review Board for approving to conduct this study in healthy adult male subjects.

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