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# Clinical Trial in Patients with Diabetes Mellitus of an Insulin-like Compound Obtained from Plant Source

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## ABSTRACT

**Clinical study of an insulin-like compound obtained from vegetable source (vegetable insulin) was carried out on nine patients with diabetes mellitus. The active hypoglycaemic principle, purified protein extract, was obtained from fruits as well as from tissue cultures of the plant *Momordica charantia* L. This extract was homologous to insulin obtained from animal pancreas. It showed a consistent hypoglycaemic effect in patients with diabetes mellitus. The average fall in blood sugar level at the peak effect of vegetable insulin was found to be statistically significant. The onset of action was within 30–60 min with the peak effect six hours after the administration of the dose of plant insulin. No hypersensitivity reaction to this extract was observed in the group of patients studied.**

## INTRODUCTION

A crude extract obtained from the fruit of a plant known as *Momordica charantia* L. (bitter gourd), has been shown to possess hypoglycaemic activity when tested in rabbits (1, 2, 3). However, this extract was also found to have many side effects, including uterine haemorrhage in gravid female rabbits. An intraperitoneal injection of this extract invariably caused the death of the experimental animal. Khanna & Mohan (4) were able to extract an abortifacient factor present in this crude extract and isolated diosgenin from the fruit as well as from the in vitro tissue cultures of this plant. Subsequently they were able to extract out the active principle in a pure protein from (vegetable insulin, v-insulin) which could be used as a hypoglycaemic agent in human beings after biological standardization (5). Vegetable insulin (v-insulin) is structurally and pharmacologically comparable in many respects to bovine insulin (5). The method of extraction of v-insulin is similar to that of extraction of pure insulin from the pancreas of animals (6). During its extraction, traces of zinc are added, resulting in the formation of colourless, needle-like crystals. The crystals of v-insulin are purified by thin-layer chromatography. The electrophoretic pattern also resembles that of bovine insulin. The infrared spectrum of p-insulin is superimposable on that of standard zinc crystalline insulin. Qualitative amino acid analysis by paper chromatography and quantitative analysis by an amino acid analyser showed that p-insulin consisted of 17 amino acids. The three-dimensional structure was found to consist of two chains of amino acids, bound together with sulphide bonds. The biological assay of the hypoglycaemic activity of v-insulin has been determined in animal experiments (5).

Vegetable insulin is available as a suspension which is stable at 4°C and denatured by heat. The compound is suspended in sterile double-distilled water and ultra-violet light and potassium permanganate fumigation is used for sterilization. The dose is so standardized that the final concentration is 40 units per ml (1.8 mg per 40 units). It can be administered by the subcutaneous or the intramuscular route.

## MATERIAL AND METHODS

Nine in-patients, eight males and one female, with an age range of 16 to 52 years, from S.M.S. Medical College Hospital, Jaipur, India, were studied after informed consent was obtained. All had diabetes mellitus and the duration of their disease ranged between 3 months to 10 years. Diagnosis of diabetes mellitus was confirmed by clinical examination and laboratory investigations. Patients with primary or idiopathic diabetes mellitus were studied.

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Table I. *Clinical data of patients with diabetes mellitus*

| Case no. | Name | Sex | Age (y.) | Body height (cm) | Body weight (kg) | Duration of disease (y.) | Type of diabetes |
|---|---|---|---|---|---|---|---|
| 1 | JC | M | 22 | 168 | 48.5 | 6 | Juvenile |
| 2 | GR | M | 20 | 168 | 45.0 | 6 | Juvenile |
| 3 | GS | M | 16 | 148 | 43.0 | 7 | Juvenile |
| 4 | PL | F | 20 | 152 | 39.5 | 8 | Juvenile |
| 5 | SK | M | 22 | 170 | 58.0 | 10 | Juvenile |
| 6 | US | M | 18 | 165 | 48.0 | 8 | Juvenile |
| 7 | HR | M | 50 | 165 | 55.0 | 0.5 | Chemical |
| 8 | GC | M | 52 | 172 | 57.0 | 0.3 | Chemical |
| 9 | MR | M | 50 | 162 | 60.5 | 1 | Maturity onset |

These patients were placed in two groups, depending on their stage of carbohydrate decompensation, as follows:

(i) Overt, clinical diabetes, i.e. patients with elevated fasting and random blood sugar levels (more than 110 mg per 100 ml). They were further subdivided into juvenile and maturity onset types, depending on the age at onset of diabetic symptoms.

(ii) Asymptomatic diabetes, i.e. patients with normal blood sugar levels (less than 110 mg per 100 ml) but with an abnormal intravenous glucose tolerance test.

There were 6 patients with juvenile diabetes, one with maturity onset and 2 with asymtomatic diabetes mellitus (Table I). All antidiabetic medication in the form of insulin or oral hypoglycaemic agents was stopped 24 hours prior to the study. A fasting blood sugar sample was taken at 7 a.m. before giving v-insulin. The dose of v-insulin was varied according to the severity of the disease and was decided upon arbitrarily according to the fasting sugar level (Table II). Vegetable insulin was administered subcutaneously and samples for blood sugar determination were taken at regular intervals. The first three samples were drawn at half-hourly intervals (7.30 a.m., 8.00 a.m. and 8.30 a.m.) to establish the onset of action and the subsequent samples were collected at 11.00 a.m., 1.00 p.m., 3.00 p.m. and 7.00 p.m. to determine the peak effect and duration of action of v-insulin. All samples were collected intravenously. Blood sugar estimation was done on whole blood by the method described by King & Wooton (7). All the subjects were kept fasting during the interval for collection of samples and only plain lemon water was given if desired by the patients. A provision was kept for the administration of glucose in the event of the development of hypoglycaemic symptoms.

Table II. *Dose of vegetable insulin, according to blood sugar levels*

| Severity of diabetes | Fasting blood sugar level (mg%) | Dose of vegetable insulin |
|---|---|---|
| Mild | Less than 180 | 10 units |
| Moderate | 180–250 | 20 units |
| Severe | 250 and above | 30 units |

Five healthy volunteers (control group I) and 5 patients with overt diabetes mellitus (control groups II) served as controls. A placebo injection was used. The control subjects were kept fasting and blood samples were collected and analysed in a similar way. The administration of v-insulin to control subjects was avoided due to its inherent hypoglycaemic properties, as evaluated in animal experiments.

Table III. *Effect of vegetable insulin and placebo on blood sugar levels*

Results given in percentage of fall in blood sugar levels. Statistical values are shown

| Clinical group | No. of subjects | Fasting values (mean) mg% | % of fall in blood sugar levels | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | 7.30 a.m. | 8.00 a.m. | 8.30 a.m. | 11.00 a.m. | 1.00 p.m. | 3.00 p.m. | 7.00 p.m. |
| Healthy controls I | 5 | 75 ± 7.4 | 5.0 ±1.7 | 5.0 ±1.7 | 5.6 ±1.4 | 5.4 ±1.8 | 5.6 ±1.6 | 5.5 ±1.4 | 5.4 ±1.5 |
| Diabetic controls II | 5 | 210 ±11.8 | 4.6 ±2.0 | 4.5 ±2.6 | 5.0 ±2.1 | 5.8 ±1.8 | 5.4 ±1.6 | 5.8 ±1.8 | 5.7 ±2.0 |
| Diabetes mellitus | 9 | 295 ±15.7 | 21.5 ± 8.9 | 24.8 ±11.0 | 30.2 ±12.1 | 49.2 ±13.7 | 40.3 ±13.4 | 35.9 ±10.3 | 28.8 ±11.4 |

## RESULTS

The placebo injection in the control groups did not produce any appreciable reduction in blood sugar levels at different intervals. A definite hypoglycaemic effect of v-insulin was observed in the patient group in this study. The onset of vegetable insulin effect was observed within ½–1 hours, with the peak effect after 4 hours in 6 juvenile diabetics, after 6 hours in 2 patients with chemical diabetes mellitus, and after 12 hours in one patient with maturity onset of diabetes mellitus.

All values in this study were analysed statistically by applying the paired *t*-test and the calculated *t* was 3.3 and the tabulated *t* was 2.3 at d.f. 8°, which was highly significant for the diabetic patient group compared with the healthy and diabetic controls during peak hours.

The hypersensitivity reactions were conspicuously absent after administration of vegetable insulin and there was no local reaction at the site of injection.

## DISCUSSION

The present investigation revealed that vegetable insulin has a consistent hypoglycaemic effect in patients with diabetes mellitus. The onset of action is similar to that of standard zinc crystalline insulin (30–60 min). However, the peak effect of vegetable insulin was seen after 4–12 hours as compared with, for 2–3 hours regular insulin. The greatest fall in blood sugar levels observed in the patient group was found to be statistically significant. There were no anaphylactic reactions to vegetable insulin however, as regards its long-term use, further studies are required in order to evaluate its antigenic properties.

The availability of vegetable insulin should open new horizons in the treatment of diabetes mellitus, especially where it is taboo to use animal products. Since the active principles are derived from a vegetable source, it can be obtained in abundance. Further clinical trials are needed in order to establish its duration of action, assay, antigenicity and various effects on intermediary metabolism in human beings.

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