Study of Anti diabetic Effect of a Compound Medicinal Plant Prescription in Normal and Diabetic Rabbits

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Abstract

Alleged antidiabetic property of a folkioric prescription containing Bergenia ligulata (Pakhan bed), Asteracantha longifolia (Tal Makhana), Cinnamomum cassia (Taj) and Argyria cuneater (Samundar Sokh) has been evaluated in rabbits after its oral administration. The indigenous drug prescription exerted a significant hypoglycaemic effect in both normal and diabetic rabbits. In normal rabbits, oral administration of 1 and 2 g/kg body weight doses of the compound powder caused maximum decrease in blood glucose level at 10 and 24 hour intervals after its administration. In addition the 2 g/kg dose also produced a fall in blood sugar at 4 hours. In the alloxan treated diabetic rabbits, the 2 g/kg dose only coul4 produce a significant decrease in blood glucose levels at 10 and 24 hours. The maximum decrease in blood glucose was observed at 24 hours at all dosage levels in normal as well as diabetic rabbits. It is conceivable that this folk medicine contains some hypoglycaemic principles which can reduce the blood glucose levels of diabetics. The treated rabbits did not show any signs of acute toxicity which encourages the possible use of this compound medicinal plant prescription by diabetic patients (JPMA 34 : 239, 1984).

Introduction

Since ancient times, diabetics have been treated orally with several medicinal plants or their extracts based on folk medicine[1]. Several preliminary screenings of such indigenous medicinal plants have been carried out[2,3] It has been observed that some hypoglycaemic principles are present in the dried fruits/whole plant of Momordica charantia, Fumaria parviflora, Buphorbia prostrata and. Cuminum nigrum.[23]. During these surveys, a substantial number of maturity-onset diabetic patients in the local population have been found to be taking a Unani prescription containing equal parts of roots of Bergenia ligulata (Pakhan bed), seeds of Asteracantha longifolla (Tal makhana) and Argyria cuneater (Samundar sokh) and the bark of Cinnamomum cassia (Taj) for the treatment of their diabetes. An investigation was, therefore, undertaken to test the effect of the above said folkloric medicinal plant prescription on blood glucose levels following oral administration to normal and alloxan-diabetic rabbits.

Material and Methods

Medicinal Plant Prescription:

Dried root of Bergenia ligulata (Pakhan bed), seeds of Asteracantha longifolia (Tal makhana) and Argyria cuneater (Samundar sokh) and the dried bark of Cinnamomum cassia (Taj) were purchased from the local medicinal plant market of Faisalabad. They were taken in equal parts and finely powdered with an electric grinder. The powdered preparation was refrigerated in properly sealed bottles at 4°C.

Chemicals Used:

Alloxan-monohydrate (NH-CO-NH CO-CO-Co H2 0), alpha D-glucose (anhydrous) and methanol

were obtained from B.D.H. Laboratories (Chemicals Division) Poole England. Glacial acetic acid, benzoic acid (Sublimed), 0-toluidine, thiourea and trichloracetic acid, were obtained from E. Merck Darmstadt, West Germany. Gum tragacanth was purchased from local market.
All other chemicals and reagent used were of the analytical grades prepared either by E. Merck or B.D.H. Laboratories.

Animals Used:

Male, adult, healthy albino rabbits of local strain weighing between 1000-1200 g were used in these experiments. The animals were kept in air conditioned animal rooms of Physiology and Pharmacology departments at University of Agriculture, Faisalabad. The animals were offered a balanced rabbit feed prepared by the Nutrition department of Agriculture University and allowed tap water ad libitum. The effects of the compound medicinal plant prescription were studied on blood glucose levels of the normal rabbits. In addition, separate experiments were performed to study its effects on blood glucose level of the diabetic rabbits.

Preparation of Diabetic Rabbits:

A group of rabbits, weighing 1000-1500 g were made diabetic by injecting intravenously 150 mg/kg body weight of alloxanmonohydrate[5]. Eight days after injection, the blood glucose levels of all surviving rabbits were determined by 0-toluidine method[6]. Diabetic rabbits with blood glucose level of 250-500 mg/100 ml were used in other experiments[7].

Grouping of Rabbits:

Rabbits were randomly divided into different groups of 6 animals each. The animals of groups I-IV were normal and healthy (non-diabetic) while the animals of groups V-VIII were made diabetic by the administration of alloxan. Groups I and V served as untreated controls as they received orally 20 ml of water only. The animals of groups II-IV and VI-VIlI were treated orally with 0.5, 1 and 2 g/kg body weight of the antidiabetic powder suspended in 20 ml of water, respectively.

Preparation and Administration of DnigSuspension:

The amount of powdered antidiabetic prescription required for each rabbit was calculated on body weight basis and that amount of drug was weighed, well triturated with water and then administered orally to each animal by using a feeding needle.

Collection of Blood:

Just after drug administration, the animal was held in a wooden rabbit holder and immediately 0.1 ml of blood was collected from saphenous vein. Similarly, samples for blood glucose were collected after 4, 10 and 24 hours of drug administration. After pricking the vein with needle, the blood was collected with 0.1 ml blood sugar pipette. After collecting blood, the pricked site of vein was pressed with cotton swab soaked with 70% ethyl alcohol to protect the rabbit against infection.

Determination of Blood Glucose Levels:

Blood glucose was determined by using the 0-toluidine reagent[6]. This method gives results very close to the glucose oxidase method and is one of the most widely used manual methods.

Acute Toxicity Studies:

In order to study any possible toxic effects of the powdered antidiabetic prescription, adult healthy rabbits of a local strain and weighing between 1000-1500 g were used. Rabbits were randomly divided into 5 groups (I-V) of five animals each. The rabbits of group I served as a control. These animals received orally 20 ml of water only. The animals of groups 2, 3, 4 and 5 were treated orally with 1, 2, 4 and 6 g/kg body weight of the powdered drug suspended in 20 ml of water, respectively.

StatisticalAnalysis:

Mean blood glucose levels were expressed as mg/100 ml ± SEM in all the experiments and Student's 't' test was used to check their significance.

Results

Effect of Alloxan Administration to Rabbits:

These effects have already been described[2]. The rabbits with blood glucose levels above 200 mg percent were considered diabetic and were used in these studies. Such hyperglycaemic rabbits have already been employed in experiments[2,8,9]

1. Effect of Plant Prescription on Blood Glucose in NormalRabbits:

The mean blood glucose concentration of control and drug-treated animals after oral administration of different doses of the powder at various time intervals are shown in table I.

Table I

Mean Blood Glucose Levels of Normal Rabbits Expressed in mg/100ml ± SEM (Standard Error of Means) at Various Time Intervals after Oral Treatment with Water and the Indigenous Plant Antidiabetic Powder (0.5, 1 and 2 g/kg body weight, orally).

Time Interval (Hours)	Group I (Control)	Antidiabetic Powder-Treated Groups		
		Group II (0.5 g/kg)	Group III (1.0 g/kg)	Group IV (2 g/kg)
Zero	90. 33 ± 5.60	82.16 ± 1.19	80.5 ± 1.36	81.16 ± 1.66
4	88.66 ± 7.75	80.00 ± 0.85	77. 66 ± 1.54	69.16 ± 2.71*
10	81.00 ± 2.67	80.83 ± 1.01	74.66 ± 1.38*	63.83 ± 1.07**
24	80.66 ± 0.80	80.16 ± 2.08	72.33 ± 1.20*	59.83 ± 0.79**

* Significant decrease as compared to zero hour level ($P < 0.05$).
** Highly significant decrease as compared to zero hour level ($P < 0.001$).
All other values are non-significant ($P > 0.05$) from zero level.
Number of rabbits in each group = 6.

The administration of water only did not change the blood glucose levels of rabbits. Administration of 0.5 g/kg of powder produced a non-significant ($P > 0.05$) decrease in blood glucose at 0, 4, 10 and 24hr intervals. However, animals treated with 1 g/kg showed a non-significant decrease at 0 and 4 hours but there was a significant ($P < 0.05$) decrease at 10 and 24 hrs. The blood glucose of rabbits treated with 2.0 g/kg body weight of drug was found to be significantly reduced ($P < 0.05$) after 4 hrs. The glucose lowering effect continued to increase even at 10 and 24 hrswhen the lowering of values was highly significant ($P < 0.001$) than at zero level.

In the control experiments, the glucose levels of animals treated with tolbutamide (500 mg/kg) at 4, 8 and 12 hrs were found to be significantly ($P < 0.05$) lower than at zero hr. The level at 24 hrs was non-significantly different from zero hour.

2. Efftct of Plant Prescription on Blood Glucose in Diabetic Rabbits:

Administration of water only to the diabetic rabbits did not alter their blood glucose levels at 0, 4, 8, 12 and 24 hrs. Similarly, blood glucose levels of animals treated with 0.5 and 1.0 g/kg body weight of the powdered drug did not produce any significant change in glucose levels at any of the intervals after drug administration. However, at 2 g/kg body ‘weight, the drug produced a significant decrease ($P < 0.05$) at 10 hour interval and highly significant ($P < 0.001$) at 24 hours in glucose level after drug adminis. tration (Table II).

Table II

Mean Blood Glucose Levels of Alloxan-diabetic Rabbits Expressed in mg/100ml ± SEM (Standard Error of Means) at Various Time Intervals after Oral Treatment with Water and the Medicinal Plant Antidiabetic Powder (0.5, 18 and 2 g/kg body weight, orally).

Time Interval (Hours)	Group V (Control)	Treated with Antidiabetic Powder		
		Group VI (0.5 g/kg)	Group VII (1 g/kg)	Group VIII (2 g/kg)
Zero	418.33 ± 6.91	410.72 ± 4.11	413.66 ± 3.50	412.83 ± 3.74
4	415.00 ± 7.22	412.60 ± 3.63	411.00 ± 2.39	401.16 ± 3.97
10	417.16 ± 3.52	413.52 ± 2.78	409.66 ± 2.83	396.66 ± 3.46*
24	416.5 ± 5.34	408.46 ± 3.96	408.16 ± 2.66	391.5 ± 3.48**

Number of rabbits in each group = 6.

*Significant decrease as compared to zero hour level ($P < 0.05$).

**Highly significant decrease from zero level ($P < 0.001$).

All other values are non-significant ($P > 0.05$).

Moreover, adniinistration of tolbutamide did not produce any significant change ($P > 0.05$) in glucose values at any of the intervals.

3. Acute Toxicity and Behavioural Pattern Studies:

Rabbits treated with high doses of the powdered antidiabetic plant prescription remained alive up to 7 days and did not show any visible signs of acute toxicity e.g. restlessness, respiratory distress, convulsions, coma and death. Moreover, the behavioural pattern studies in rats also revealed no prominent change in the awareness, mood, motor activity, CNS excitation, posture and motor incoordination, autonomic responses, food consumption and body weight.

Discussion

The oral administration of various doses of the indigenous drug has caused a decrease in the blood glucose of normal rabbits (Table I). The crude drug produced a significant and consistent hypoglycaemic response in rabbits. Similar, hypoglycaemic activity has already been reported in several plants. [8,10-12]

For comparison, tolbutamide (250 and 500 mg/kg) was administered orally to normal rabbits. As observed by Augusti[14], these doses of tolbutarnide produced a significant decrease of blood glucose levels at 4, 8 and 12 hrs.[14] However, (Table I), the administration of 1 and 2 g/kg of the drug produced a significant fall in blood sugar even at 24 hrs interval showing that this plant drug possesses longer duration of action than the tolbutamide which produces hypoglycaemia for 6.12 his[15]. Furthermore, calculations show that the blood glucose lowering effect of 2 g/kg of the prescription is about 50 percent'of that produced by the 500 mg/kg of tolbutamide.

Sulfonylureas including tolbutamide have been reported to produce hypoglycaemic effect in normal animals by stimulating pancreatic beta-cells to release more insulin into the blood stream and by increasing the glycogen deposition in the liver. These drugs, however do not decrease blood glucose level in alloxan-diabetic animals.[16] Thus it may be supposed that hypoglycaemic principle(s) in this

plant prescription also exert hypoglycaemic effect in rabbits by initiating the release of insulin from pancreatic beta-cells. Similar mechanism of hypoglycaemic response in normal animals has been proposed to explain the hypoglycaemic effect Momordica charantia, Momordica foetfda, and Tecoma stans. [2,8,10] Thus in an attempt to explore the possible mechanism of hypoglycaemic action of the plant prescription, they were also administered to the alloxan-diabetic rabbits. Data obtained showed that this drug produces a significantly lower blood glucose levels in the diabetic animal too (Table II). These results suggest that the active principle(s) of this plant prescription seem to possess some insulin-like activities. Therefore, further comprehensive chemical and pharmacological investigations are needed to elucidate the exact mechanism of hypoglycaemic effect of antidiabetic prescription containing Bergen la Iigulata, Asteracantha longifolia, Cinnamomum cassia and Argyna cuneater plants.

The acute toxicity studies and behavioural pattern records have also been undertaken. Virtually these plants are quite safe at even high dosages employed as no visible signs of toxicity or adverse effects were observed in the treated animals and no change was observed in the normal behavioural pattern of tested animals.

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