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Antihyperglycaemic effect of Diamed, a herbal formulation, in experimental diabetes in rats

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Abstract

Diamed is a herbal formulation composed of the aqueous extracts of three medicinal plants (*Azardirachta indica*, *Cassia auriculata* and *Momordica charantia*). We have investigated Diamed for its possible antihyperglycaemic action in rats with alloxan-induced experimental diabetes. Oral administration of Diamed (1.39 (0.25 g), 1.67 (0.30 g) or 1.94 (0.35 g) mL kg^{-1}) for 30 days resulted in a significant reduction in blood glucose, glycosylated haemoglobin, and an increase in plasma insulin and total haemoglobin. The effect was highly significant after administration of the 1.94 mL (0.35 g) g^{-1} body weight dose. Diamed also prevented a decrease in body weight. An oral glucose tolerance test was performed in experimental diabetic rats in which there was a significant improvement in glucose tolerance in the animals treated with Diamed. The effect was compared with 600 $\mu g\,kg^{-1}$ glibenclamide. The results showed that Diamed had antihyperglycaemic action in experimental diabetes in rats.

Introduction

Diabetes mellitus is considered to be a serious endocrine syndrome. In many countries it is traditional to use medicinal plants to control diabetes. The antihyperglycaemic effect of several plant extracts and herbal formulations which are used as antidiabetic remedies has been confirmed (Sharma et al 1992; Stanely Mainzen Prince et al 1998). Synthetic hypoglycaemic agents can produce serious side effects including haematological effects, coma and disturbances of the liver and kidney. In addition, they are not suitable for use during pregnancy (Larner 1985). Compared with synthetic drugs, drugs derived from plants are frequently considered to be less toxic with fewer side effects (Momin 1987). Therefore, the search for more effective and safer antihyperglycaemic agents has become an area of active research.

Diamed is a herbal formulation composed of the aqueous extracts of three medicinal plants (for composition and concentrations see Table 1). These plant extracts are known to possess antidiabetic properties and have been used in indigenous systems of medicine to treat diabetes (Shrotri et al 1963; Dixit et al 1986; Cakici et al 1994). The mechanisms of antidiabetic action of these plants are under study. This investigation has been undertaken to study the action of Diamed on blood glucose, glycosylated haemoglobin and oral glucose tolerance in alloxan-induced experimental diabetes in rats.

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Table 1 Composition and concentration of Diamed.

Serial no.	Botanical name	Family	Part used	Concn (mg mL^{-1})
1	*Azardirachta indica*	Meliaceae	Seed	20
2	*Cassia auriculata*	Caesalpiniceae	Flower	120
3	*Momordica charantia*	Cucurbitaceae	Pericarp	40

Materials and Methods

Plant materials

Azardirachta indica (seed), *Cassia auriculata* (flower) and *Momordica charantia* (pericarp) were collected freshly from Panruti, Cuddalore District, Tamil Nadu, India. Voucher specimens were deposited at the herbarium of the Botany Department, Annamalai University.

Preparation of Diamed

Diamed was prepared on the basis of an ayurvedic antidiabetic formulation proposed by Pandey et al (1995). Five hundred grams of *A. indica* seeds, *C. auriculata* flowers and *M. charantia* pericarp were extracted individually with 1500 mL water by the method of continuous hot extraction (Jain 1968) and evaporated. The yields obtained were 79, 68 and 49 g, respectively. The residual extracts were mixed in the ratio of 1:6:2.

Animals

Male albino Wistar rats (180–200 g) bred in the Central Animal House, Raja Muthiah Medical College, Annamalai University, were used. The animals were fed on a pellet diet (Hindustan Lever, Ltd, India) and water was freely available.

Experimental induction of diabetes

The rats were injected intraperitoneally with alloxan monohydrate dissolved in sterile normal saline at a dose of 150 mg kg^{-1}. Alloxan is capable of producing fatal hypoglycaemia as a result of massive pancreatic insulin release, therefore the rats were treated with a 20% glucose solution (15–20 mL) orally after 6 h. To prevent hypoglycaemia, for the following 24 h the rats had access to a 5% glucose solution bottle in their cages (Gupta et al 1984). After two weeks, the rats which had developed moderate diabetes, as indicated by glycosuria (indicated by Benedict's test for urine) and hyperglycaemia with a blood glucose range of 200–260 mg/100 mL, were used for the experiment. Blood was collected from the eyes (venous pool).

Experimental design

In this experiment a total of 56 rats (40 diabetic surviving rats, 16 normal rats) were used. Diabetes was induced in rats two weeks before the start of the experiment. The rats were divided into six groups (n = 8) after the induction of diabetes. Group 1 was the normal untreated rats. Group 2 was the diabetic rats. Group 3 was the diabetic rats given Diamed (1.39 mL (0.25 g) kg^{-1}) in aqueous solution daily using an intragastric tube for 30 days. Group 4 was the diabetic rats given Diamed (1.67 mL (0.30 g) kg^{-1}) in aqueous solution daily using an intragastric tube for 30 days. Group 5 was the diabetic rats given Diamed (1.94 mL (0.35 g) kg^{-1}) in aqueous solution daily using an intragastric tube for 30 days. Group 6 was the diabetic rats given glibenclamide (600 μg kg^{-1}) in aqueous solution daily using an intragastric tube for 30 days. Group 7 was the normal rats given Diamed (1.94 mL (0.35 g) kg^{-1}) in aqueous solution daily using an intragastric tube for 30 days.

After 30 days the rats were killed by decapitation. Blood was collected in a tube containing potassium oxalate and sodium fluoride solution for the estimation of blood glucose.

Determination of blood glucose, haemoglobin and plasma insulin

Fasting blood glucose was estimated by the *o*-toluidine method (Sasaki et al 1972). Haemoglobin was estimated by the cyanmethaemoglobin method (Drabkin & Austin 1932). Plasma insulin was estimated by radioimmunoassay kit (Boehringer Mannheim).

Determination of glycosylated haemoglobin

Glycosylated haemoglobin was estimated by the method of Sudhakar Nayak & Pattabiraman (1981), modified by Bannon (1982).

Oral glucose tolerance test

After overnight fasting, a 0-min blood sample (0.2 mL) was taken from the rats in the normal, diabetic control, diabetic + Diamed (1.94 mL (0.35 g) kg^{-1}), diabetic + glibenclamide (600 μg kg^{-1}) and normal + Diamed (1.94 mL (0.35 g) kg^{-1}) groups by orbital sinus puncture. Glucose solution (2 g kg^{-1}) was administered orally immediately. Four more samples were taken at 30, 60, 90 and 120 min after glucose administration. All blood

samples were collected with potassium oxalate and sodium fluoride solution for the estimation of blood glucose.

Statistical analysis

Statistical analysis of the results was performed with the Student's *t*-test. Results are presented as the mean ± s.d.

Results and Discussion

Table 2 shows the blood glucose, plasma insulin, total haemoglobin, glycosylated haemoglobin, change in body weight and urine sugar of normal and experimental animals. There was a significant ($P < 0.001$) elevation in blood glucose and glycosylated haemoglobin, while the level of plasma insulin and total haemoglobin were significantly decreased ($P < 0.001$) during diabetes when compared with the corresponding groups. Administration of Diamed at 1.39 (0.25 g), 1.67 (0.30 g) and 1.94 (0.35 g) mL kg^{-1} and glibenclamide (600 μg kg^{-1}) tended to bring the values closer to normal. Diamed at a dose of 1.94 mL (0.35 g) kg^{-1} showed a highly significant effect when compared with glibenclamide. Administration of Diamed to normal rats showed a significant effect on blood glucose and plasma insulin whereas the level of haemoglobin and glycosylated haemoglobin remained unaltered.

Table 2 Blood glucose, plasma insulin, total haemoglobin, glycosylated haemoglobin, change in body weight and urine sugar of normal and experimental group.

Group	Body weight (g)		Fasting blood glucose (mg/100 mL)	Plasma insulin (μU mL^{-1})	Haemoglobin (g/100 mL)	Glycosylated haemoglobin (mg (g Hb)$^{-1}$)	Urine sugar
	Initial	Final					
Normal	194.60 ± 8.48	205.83 ± 9.87	81.04 ± 9.87	23.51 ± 1.66	13.36 ± 0.53	0.20 ± 0.03	Nil
Diabetic control	182.32 ± 8.36	159.15 ± 9.48†††	248.5 ± 9.48†††	11.35 ± 0.92†††	6.03 ± 0.61†††	0.56 ± 0.06†††	+ + +
Diabetic + Diamed (0.25 g kg^{-1})	183.50 ± 7.01	188.32 ± 9.10**	186.7 ± 9.10***	12.84 ± 1.00	7.39 ± 0.48**	0.43 ± 0.04**	+ +
Diabetic + Diamed (0.30 g kg^{-1})	187.93 ± 9.20	195.08 ± 6.92***	119.3 ± 6.92***	15.58 ± 1.33**	9.27 ± 0.63***	0.38 ± 0.02***	+
Diabetic + Diamed (0.35 g kg^{-1})	189.50 ± 7.90	198.60 ± 7.82***	86.74 ± 7.82***	21.32 ± 1.82***	11.92 ± 0.79***	0.21 ± 0.07***	Nil
Diabetic + glibenclamide (600 μg kg^{-1})	185.02 ± 7.34	192.82 ± 4.88***	94.34 ± 4.88***	19.23 ± 1.60***	10.39 ± 0.63***	0.24 ± 0.02***	Trace
Normal + Diamed (0.35 g kg^{-1})	184.70 ± 8.51	193.51 ± 9.10	67.77 ± 3.10***	26.32 ± 1.32**	12.32 ± 0.41	0.19 ± 0.03	Nil

Values are given as mean ± s.d. of six rats in each group. Diabetic control was compared with normal, †††$P < 0.001$. Experimental groups were compared with diabetic control,***$P < 0.001$,**$P < 0.01$. + Indicates 0.25% sugar and + + + indicates more than 1% sugar.

Table 3 Oral glucose (2 g kg^{-1}) tolerance test in normal and experimental groups.

Group	Blood glucose levels (mg/100 mL)				
	0 min	30 min	60 min	90 min	120 min
Normal	83.5 ± 6.5	172.5 ± 5.8	143.4 ± 7.6	104.1 ± 8.4	88.4 ± 5.8
Diabetic control	253.4 ± 7.8***	328.7 ± 6.3***	384.1 ± 9.4***	351.5 ± 6.9***	320.8 ± 8.2***
Diabetic + Diamed (0.35 g kg^{-1})	93.5 ± 4.8***	177.4 ± 7.0***	157.5 ± 9.6***	120.7 ± 3.8***	99.5 ± 8.6***
Diabetic + glibenclamide (600 μg kg^{-1})	98.2 ± 4.9***	193.6 ± 7.2***	171.4 ± 8.4***	133.7 ± 5.9***	115.8 ± 7.4***
Normal + Diamed (0.35 g kg^{-1})	91.2 ± 3.8	176.2 ± 9.2	131.2 ± 7.2†	98.2 ± 6.0†††	77.5 ± 4.2†††

Values are given as mean ± s.d. of six rats in each group. Diabetic control was compared with normal. Experimental groups were compared with the diabetic control group.***$P < 0.001$ compared with diabetic control. †††$P < 0.001$, †$P < 0.05$ compared with normal group.

The significant antihyperglycaemic effect of Diamed may be due to the potentiation of plasma insulin effect by increasing either the pancreatic secretion of insulin from the existing β-cells or its release from the bound form, as evidenced by the significant increase in the level of insulin by Diamed in diabetic rats (Table 2). This was clearly evidenced by the increased level of insulin in normal rats treated with Diamed. Administration of Diamed to normal rats showed a significant decrease in the level of blood glucose and an increase in the level of plasma insulin. This showed that the Diamed had an effect on the secretion of insulin from pancreatic β-cells.

In diabetic rats we observed a decrease in body weight and in total haemoglobin, and an increase in glycosylated haemoglobin. The decrease in total haemoglobin concentration was due to the increased formation of glycosylated haemoglobin. In diabetes, concentration of glycosylated haemoglobin is approximately 16% (Koeing et al 1976). The increase in glycosylated haemoglobin is directly proportional to the fasting blood glucose level (Jackson et al 1979). The Diamed-treated groups showed that there was a significant reduction in blood glucose, and glycosylated haemoglobin, and an increase in total haemoglobin and body weight, when compared with the diabetic control.

Table 3 shows the blood glucose levels of control, diabetic control, Diamed- and glibenclamide-treated animals after oral administration of glucose (2 g kg^{-1}). Diamed- and glibenclamide-treated animals showed a significant decrease in blood glucose concentration after 1 and 2 h. Diamed-treated animals tended to have values nearer to normal.

To our knowledge, this is the first study in which a follow up was made on the changes in glycosylated haemoglobin and oral glucose tolerance. Previous studies showed that *A. indica* (Bopanna et al 1997), *C. auriculata* (Satyavati et al 1976) and *M. charantia* (Uma & Grover 1998) had antihyperglycaemic action in experimental diabetes. An increased glycogenesis was one of the proposed mechanisms of action (Perez et al 1998). The antihyperglycaemic action of Diamed could be due to the decreased absorption of glucose by the intestine, increased glycolysis, decreased glycogenolysis or enhanced glycogenesis (Kumar et al 1993).

It is interesting to note that in glucose-fed rats, the Diamed effectively prevented the increase in blood glucose levels without inducing the hypoglycaemic state.

In conclusion, Diamed was shown to exhibit an antihyperglycaemic activity in alloxan-induced diabetic rats, which was more effective than glibenclamide. Studies are necessary to elucidate in detail the mechanism of action of the drug at the cellular and molecular levels. The compounds responsible for lowering blood glucose require purification and characterization.

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