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STUDY OF GLYCEMIC PROFILE OF *CAJANUS CAJAN* LEAVES IN EXPERIMENTAL RATS

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ABSTRACT

The present study deals with the evaluation of glycemic profile of aqueous extract of Cajanus cajan leaves *in streptozotocin induced type 2 diabetic rats. Single oral administration of graded doses of aqueous extract of Cajanus cajan* leaves *showed significant increment of 14.3 % in fasting blood glucose levels of normal rats. The sub diabetic and mild diabetic models have also shown hyperglycemic effect from the same variable doses of the extract. The dose of 1000 mg/kg showed the maximum rise of 17.1, 71.2 and 50.7 % in Blood glucose levels of normal, sub and mild diabetic rats respectively during glucose tolerance test. The study of leaves was taken into consideration on the basis of earlier reported hypoglycemic activity of* Cajanus cajan *seeds. However the results observed were found just opposite and therefore it may be useful in controlling hypoglycemia, occasionally caused due to excess of insulin and other hypoglycemic drugs.*

KEY WORDS

Cajanus cajan, Fabiaceae, Pigeon pea, Diabetes mellitus, Hyperglycemic

INTRODUCTION

There is an increasing demand by diabetic patients to use the natural products with antidiabetic activity. Ethnopharmacological surveys indicate that more than 1200 plants are used world wide in traditional medicine for their alleged hypoglycemic activity (1-6). The investigation of anti-diabetic agents of plant origin which are used in traditional medicine is thus of great significance.

Cajanus cajan Millsp. (Fabiaceae) is commonly known as Pigeon pea or red gram in English and Arhar in Hindi. It is one of the most important pulse crops cultivated in India (7). Arhar is consumed in the form of split pulse or dal or when tender, as a vegetable. The green leaves and tops of the plant are used as fodder and as green manure (8).

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The protein fraction isolated from its seeds brought down the contents of Total Cholesterol (TC), Triglycerides (TG) and phospholipids in the tissues of liver aorta and serum of high fat cholesterol diet fed rats (9). Recently the *Cajanus cajan* leaves have been discovered as a new source of antioxidant due to the presence of polyphenols and ascorbic acids (10).

Since, the seeds have already been investigated for their glycemic activity therefore, the present study was carried out to evaluate the glycemic profile of aqueous extract of *Cajanus cajan* leaves, on blood glucose level (BGL) of normal rats and streptozotocin induced sub diabetic and mild diabetic rats during fasting blood glucose (FBG) and glucose tolerance test (GTT) studies.

MATERIAL AND METHODS

Chemicals : Streptozotocin was purchased from Sigma - Aldrich Co., U S A. Glucose level was assayed using kit from Ranbaxy Diagnostics, New Delhi, India.

Preparation of leaves extract : Fresh leaves of *Cajanus cajan* were collected from nearby fields and identified by Taxonomist, Department of Botany, University of Allahabad, Allahabad, India. A voucher specimen has been deposited at herbarium of the university. The leaves were crushed and extracted with

distilled water mostly at 60-70°C for 10 h. The resulting extract was cooled and filtered using Whatman no.1filter paper. The filtrate was evaporated in vacuum to give a residue (yield: 14.7 %w/w).

Animals : Male Albino Wistar rats of body weight 170-220 g were selected for all the experiments. Animals were kept in our animal house at an ambient temperature of 25° C and 45-55 % humidity. Animals were fed pellet diet (Golden Feed, New Delhi) and water ad libitum. The study was approved by the Institutional Ethical Committee (83 a / a / 04 / CPCSEA).

Induction of diabetes in rats : A freshly prepared solution of streptozotocin (45 mg/kg b. w.) in 0.1 M citrate buffer, pH 4.5 was injected intraperitonial to overnight fasted rats (11). FBG levels were estimated at the time of induction of diabetes and Post Prandrial Glucose (PPG) levels were checked regularly upto stable hyperglycemia, usually one week after streptozotocin injection. Depending on their FBG levels the animals were divided arbitrarily into two groups: (A) Sub diabetic animals: with nearly normal FBG (80 – 120 mg /dl) and abnormal glucose tolerance. (B) Mild diabetic animals: with abnormal FBG (120 – 250 mg/dl) and abnormal glucose tolerance.
Blood glucose level was estimated by glucose oxidase method (12).

EXPERIMENTAL DESIGN

Initial screening of variable doses of aqueous extract for its glycemic profile was done in normal healthy rats by conducting FBG and GTT studies. The study was extended for sub and mild diabetic rats too through GTT.

Assessment of glycemic profile of aqueous extract in normoglycemic models : Initial FBG testing was carried out with different doses of aqueous extract of leaves in overnight fasted 24 normal healthy male rats to evaluate its glycemic profile. The animals were divided into four groups of six rats each. Control rats (group I) were given vehicle (distilled water) only. While other groups II – IV received variable doses of extract at 500, 750 and 1000 mg/kg, respectively suspended in distilled water orally. Blood samples were collected from tail vein and BGL was estimated before and after 2, 4, 6 and 8 h of extract administration.

Assessment of glycemic activity of extract on glucose tolerance in normal, sub and mild diabetic rats : The glycemic activity of *Cajanus cajan* leaves aqueous extract in normal, sub and mild diabetic rats was assessed by its effect on glucose tolerance. FBG was checked in overnight fasted rats and each type of (normal, sub and mild diabetic) rats were divided into four groups. Control groups of normal, sub and mild diabetic animals received vehicle (distilled water) only whereas, variable doses of 500, 750 and 1000 mg/kg of extract were administered orally to rest of the three groups of each type. The rats of all the groups were given glucose (3 g/kg) after 90 min of the extract administration. Blood samples were collected just prior to glucose administration (0 h) and after 1, 2 and 3h of glucose loading.

Statistical analysis : Results were expressed as mean ± SD. Statistical analysis of the data was performed with one – way analysis of variance (ANOVA) and by student's 't'- test. Significant differences were indicated by p values lower than 0.05.

RESULTS

Effect of extract on FBG in normoglycemic rats : Results of the effect of graded doses of aqueous extract of *Cajanus cajan* leaves on FBG levels of normoglycemic rats are presented in Table 1. All the three doses (500, 750 and 1000 mg/kg) of leaves extract produced significant hyperglycemic

Table 1: Effect of graded doses of *Cajanus cajan* leaves aqueous extract on FBG levels in normoglycemic rats (mean ± S.D.)

			Blood glucose levels (mg/dl)				
			Pretreatment	Post treatment (hours)			
Expt. Gp.	Treatment	Dose (mg/kg)	FBG	2	4	6	8
I	DW	__	72.3 ± 2.8	71.8 ± 3.2	71.2 ± 4.6	70.5 ± 3.8	70.2 ± 4.2
II	Extract	500	74.3 ± 3.2	77.5 ± 3.9	78.1 ± 3.4*	79.9 ± 4.3*	78.9 ± 4.1
III	Extract	750	78.1 ± 3.8	82.3 ± 4.1	83.5 ± 4.6*	86.1 ± 4.4*	85.1 ± 3.7
IV	Extract	1000	76.3 ± 4.8	80.6 ± 4.4	81.9 ± 4.6*	87.2 ± 3.5*	84.4 ± 5.1

* $P < 0.05$ as compared to pretreatment hour

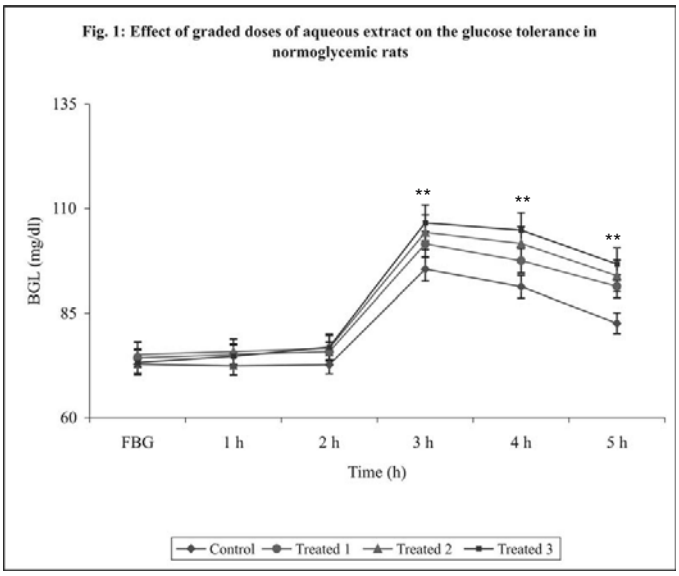
effect after 6 h of dose administration. However, it was more marked in animals receiving dose of 1000 mg/kg. This dose produces a increase of 14.3 % in BGL after 6 h of oral administration. An increase of 7.6 and 10.2 % was observed in BGL at doses 500 and 750 mg/kg, respectively. However slight lowering in BGL was observed after 6 h and exactly at 8^{th} h of extract administration.

Effect of extract on glucose tolerance in normal, sub and mild diabetic rats : Fig – 1, 2 and 3 describes the hyperglycemic effect of graded doses of leaves aqueous extract on glucose tolerance in normal, sub and mild diabetic rats respectively. The dose of 1000 mg/kg produced a maximum increase of 17.1 % in normal rats in BGL after 3 h of extract administration. The same dose also produces a maximum increase of 71.2 % in sub and 50.7 % in mild diabetic rats, respectively, after 2h of glucose administration. The dose of 500 mg/ kg produced an increase in BGL of sub diabetic rats by 19.9 % in 1 h and 41.2 % in 2 h, whereas the same dose produces an increase of 14.3 % in 1 h and 30.9 % in 2h in BGL of mild diabetic rats. The dose of 750 mg/kg produced an increase in BGL of sub diabetic rats by 24.3 % in 1 h and 59.8 % in 2 h and it produces an increase of 15.8 % in 1h and 37.3 % in 2 h in BGL of mild diabetic rats.

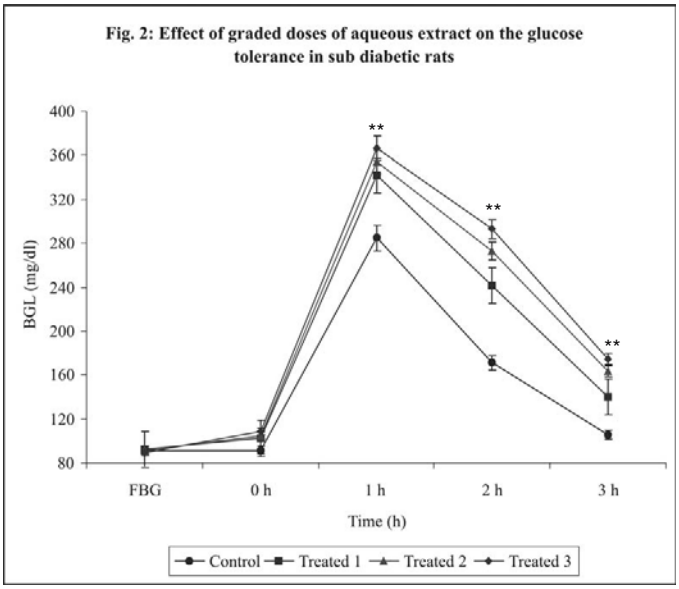
DISCUSSION

Pulse of *Cajanus cajan* seeds given along with animal feed reduced the BGL in normal rats (13). A suspension of powdered un roasted seeds of *Cajanus cajan* (60% and 80%)showed hypoglycemic activity in normal and alloxan diabetic mice (14).

Hence the present study has been carried out with *Cajanus cajan* leaves to get the similar effect. But it was found unexpectedly that the aqueous extract prepared from leaves was devoid of any anti hyperglycemic effect in normal and streptozotocin induced sub and mild diabetic rats. In fact all the graded doses of 500, 750 and 1000 mg/kg of the extract showed a significant rise in BGL after 6 h in case of normal animals instead of fall. However, the maximum rise in BGL was found by the dose of 1000 mg/kg. During GTT in case of sub diabetic rats the dose of 500, 750 and 1000 mg/kg of extract showed an initial increase of 19.9, 24.3 and 28.7 % respectively in BGL after 1 h. This initial rise in BGL was further raised to 41.3, 59.8 and 71.2 % after 2 h of treatment. Similarly in mild diabetic rats the dose of 500, 750 and 1000 mg/kg of extract showed an initial increase of 14.3, 15.8 and 25.7 % respectively after 1 h in BGL. In this case also BGL was further increased to 30.9, 37.3 and 50.7 % after 2 h of treatment. The absence of antihyperglycemic effect in our experimental

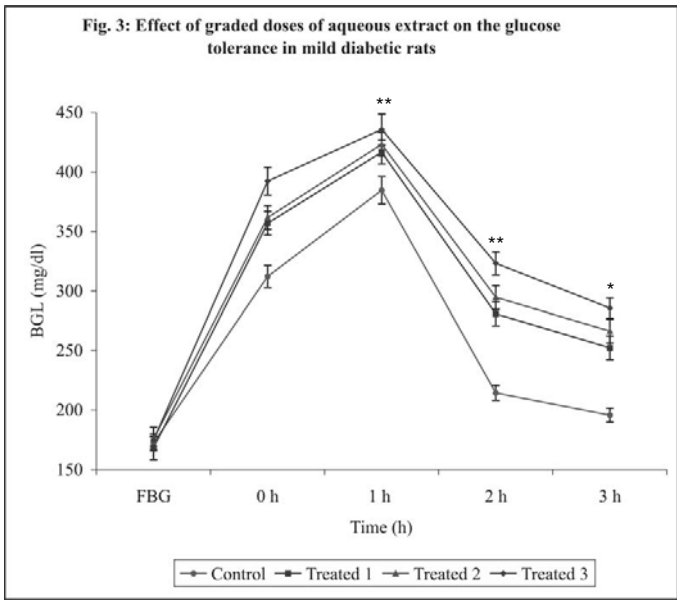


**P<0.01 as compared with control
Control - Distilled water, Treated 1-500 mg/kg, Treated 2 - 750 mg/kg, Treated 3 - 1000 mg/kg



**P<0.01 as compared with control
Control - Distilled water, Treated 1-500 mg/kg, Treated 2 - 750 mg/kg, Treated 3 - 1000 mg/kg

models do not rule out its potential effect in patients with diabetes mellitus, because any mechanism not related to direct hypoglycemic effect may still work in those patients. This is in the case of very effective antidiabetic plant drug Metformin (15). In conclusion, the presence of hyperglycemic effect of the extract of *Cajanus cajan* leaves suggests that it can be useful in controlling hypoglycemia occasionally caused due to excess of insulin and other hypoglycemic drugs. However



*P<0.05; **P<0.01 as compared with control
Control - Distilled water, Treated 1-500 mg/kg, Treated 2 - 750 mg/kg, Treated 3 - 1000 mg/kg

its possibility of being used in diabetes cannot be ruled out completely as its study in severe cases has not been performed due to high risk of mortality.

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