Hypoglycaemic Effect of the Water Extract of Ficus Bengalensis in Alloxan Recovered, Mildly Diabetic and Severely Diabetic Rabbits.

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SUMMARY

Hot water extract of Ficus bengalensis was given orally to normal rabbits and rabbits with alloxan induced alloxan-recovered, mildly diabetic and severely diabetic states, at a single dose of 50 mg/kg/day for three days. After a gap of five days, the water extract was readministered for three days at the same dose level. There was no significant change in fasting blood glucose (FBG), or glucose tolerance test (GTT) in normal rabbits. There was no fall in FBG but improvement in glucose tolerance in alloxan-recovered rabbits. In mildly diabetic rabbits there was 55.8% fall in FBG values and an improvement in glucose tolerance. The extract produced 68% fall in FBG values in severely diabetic rabbits, an observation not brought out in any of the earlier work in this plant.

INTRODUCTION

The bark of banyan tree (botanical name – Ficus bengalensis, Hindi – Bargad, Telugu – Marrichettu) is used in Ayurvedic system of medicine [1,2,] for the treatment of diabetes mellitus. The hypoglycaemic effect of water extract of the bark of the plant in normal and alloxan diabetic rabbits was demonstrated by Shrotri and Aiman [3] Vohora and Parasar [4] and in humans with mild diabetes by Joglekar et. al [5]. This extract did not bring about any fall in fasting blood glucose (FBG) in normal persons. The same group of workers attributed the hypoglycaemic effect to a water soluble glycoside which can be isolated from the ethanolic extract of the bark [6]. Brahmachari and Augusti found three glycosides in the ethanolic extract of the bark of F. bengalensis and one of them called bengalenoside produced a slight fall in FBG of 15-20% at a high dose of 250 mg/kg in normal and alloxan induced mild diabetes in rats [7]. It was ineffective in severe diabetes. Subramanian and Misra [8] studied the structure of four glycosides isolated from the ethanolic extract of the banyan tree bark. Three of them namely, leucocyanidin (dark green) delphinidin 3-0-∞-L rhamnoside (red), and a substituted leucoanthocyanidin (dark brown) are insoluble in water. The fourth, a dimethylether of pelargonidin -3-0-∞-L rhamnoside (yellow red) is soluble in water. Out of the above four glycosides, leucocyanidin and pelargonidin were found to have antidiabetic effect either by a single dose of 250 mg/kg or on long term treatment with 100 mg/kg/day in alloxan induced mild diabetes in rats by Augusti and his coworkers [9, 10]. Even the pure glycoside did not show any hypoglycaemic effect in severe diabetes. In short, none of the earlier workers could demonstrate any beneficial effect of F. bengalensis in severe diabetes. Further, all of them were less potent than tolbutamide.

Our group initiated work on F. bengalensis in early 1980 with the hope of isolating active principle from the bark of this plant which would be useful not only in mild diabetes but also in severe diabetes. We isolated one acetone soluble highly active hypoglycaemic principle from the ethanolic extract of the bark [11,12,13], which is more potent than tolbutamide. A single dose of 50 mg\kg produced 40% fall in blood glucose during GTT [12] in mildly diabetic rabbits and also increased the release of insulin during GTT [13]. Further it was effective in alloxan induced severe diabetes in rabbits.

Our above mentioned studies were with water insoluble compound. There is no mention so far of any water soluble F. bengalensis compound active in severe diabetes. In this communication we report that the hot water extract prepared under the conditions described by us has favourable hypoglycaemic effect in three types of alloxan induced diabetes of varying severity in rabbits.

MATERIALS AND METHODS

Chemicals: Alloxan monohydrated and glucose oxidase were purchased from Sigma Chemical Co. St. Louis, U.S.A. Peroxidase was from CSIR Center for Biochemicals (now Center for Biotechnology) Delhi. All the other chemicals were of highest purity locally available.

Alloxan Induced Diabetes Mellitus

Male albino rabbits (1-1.5 Kg) were made diabetic by intravenous injection of alloxan (80 mg/kg) in citrate buffer pH 4.0. Fasting blood glucose (FBG) and glucose tolerance test (GTT) were performed every week for one month. Then the animals were arbitrarily divided for convenience into three groups as indicated below based on their FBG and GTT pattern. Five rabbits were used in each group. Animals were given Hindustan Lever rabbit feed and water ad libitum unless otherwise stated.

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1) Alloxan recovered sub-diabetic rabbits (AR). Animals with near normal or slightly elevated FBG (below 120 mg/dl) but having abnormal glucose tolerance are called alloxan recovered subdiabetic animals (AR).
2) Mildly diabetic rabbits (MD). Those animals with FBG between 120-250 mg/dl and abnormal glucose tolerance come under this group.
3) Severely diabetic rabbits (SD) – Rabbits with FBG above 250 mg/dl are referred to as severely diabetic animals.

Rabbits in groups 1 and 2 come under non-insulin dependent diabetes mellitus (NIDDM) but those in group 3 could be either of NIDDM or insulin dependent type depending on the extent of destruction of pancreas.

Preparation of Hot Water Extract

The bark of F. bengalensis was cleaned well by removing the outer green layer and dried in sun. Sixty grams of dried bark was extracted with 500 ml of distilled water by boiling for thirty minutes over a mild flame and filtered through cotton. The filtrate was centrifuged at room temperature at 11,000 r.p.m., for twenty minutes. The clear supernatent free from finely suspended particles was used as the hot water extract.

Assessment of Hypoglycaemic Activity

The effect of hot water extract was tried in normal rabbits and in rabbits with the above mentioned three types of alloxan induced diabetes mellitus. In normal and AR status animals, as the FBG values are normal and near normal respectively, improvement in glucose tolerance pattern was taken as criterion for assessment of hypoglycaemic activity. GTT was performed with overnight fasted rabbits. Hot water extract of the bark of F. bengalensis prepared as above was then given orally at a single dose of 50 mg/kg/day for three days and GTT was performed on the fourth day. Treatment was stopped for five days. Water extract was given again for another three days. GTT was performed as before.

In rabbits with mild diabetes (FBG 120-250 mg/dl) water extract was given at a single dose of 50 mg/kg/day for three days. After a gap of five days, the treatment was repeated at the same dose for another three days. Their FBG was determined before and after treatment. To severely diabetic rabbits with FBG above 250 mg/dl, water extract was given at a single dose of 50 mg/kg/day for three days. Their FBG was determined. After a gap of five days water extract was given to them a second time for fifteen days continuously. FBG was again determined after second treatment.

Estimation of Blood Glucose

Blood glucose was estimated by the method of Hugget and Nixon [14].

Estimation of Serum Insulin

Sub diabetic and severely diabetic animals were divided in two groups. Blood was drawn from overnight fasted animals. One group in each was given water extract single dose (50 mg/kg) another group was given plain water. After one hour glucose was given orally 2 gm/kg., to sub diabetic group and blood sample was drawn at 1 hr. In severely diabetic group GTT was performed next day. Fasting and 1 hr. blood samples were collected. Serum insulin in blood samples was estimated by the RIA method using the kit from Bhabha Atomic Research Center.

RESULTS

Effect of oral administration of hot water extract of F. bengalensis on FBG, and one hour and two hours blood glucose during glucose tolerance test in normal rabbits is shown in Table 1. The results reveal that the water extract did not have any significant hypoglycaemic effect in normal animals. In fact it is an indication that the mechanism of glucose homeostasis in normal animals is not deranged by administration of the water extract. This is in agreement with the studies on normal humans by Joglekar et al [5].

Table 1
Effect of Water Extract of F. Bengalensis on Glucose Tolerance in Normal Animals

	Blood glucose mg/dl		
	Fasting	**1hr**	**2hr**
Before treatment	94 ±3	154.8 ±18	109 ±16
After 1st treatment	90 ±7	152 ±6.7	107 ±9.8
After 2nd treatment	88 ±8.8	155 ±11.2	96 ±7

The values are mean ± SD of 5 rabbits.

The same extract produced good improvement in glucose tolerance in AR rabbits. Even at the end of the first treatment there was improvement (Table 2) in glucose tolerance (28% and 15% fall in 1 hr and 2 hr blood glucose values). By the end of the second treatment, the GTT was almost normal. This favourable effect persisted for ten days after stopping the second treatment.

Table 2

Effect of Water Extract of F. Bengalensis on Glucose Tolerance in Alloxan Recovered Rabbits

	Blood glucose mg/dl		
	Fasting	**1hr**	**2hr**
Before treatment	105 ±17	220.8 ±9.2	117 ±16
After 1st treatment	96 ±7	178.8 ±30	101.6 ±6.6
After 2nd treatment	94 ±8	154 ±4.8	100 ±16
The values are mean	P > 0.30	P < 0.05	P > 0.15

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± SD of 5 rabbits.

In the case of rabbits with FBG between 120-250 mg/dl (MD) the extract produced lowering of (Table 3) FBG from a starting value of 194± 27 mg/dl to 87± 4.5 mg/dl (55% fall at the end of second treatment). There was improvement in glucose tolerance and it was nearly normal to start with (data not shown). FBG remained normal for ten days after stopping the treatment.

Table 3
Effect of Water Extract of F. Bengalensis on the Fasting Blood Glucose Level in Mildly Diabetic Rabbits

Rabbit No.	Fasting blood glucose mg/dl			
	Before treatment	**After 1st treatment**	**After IInd treatment**	**% fall in FBG before and after Treatment**
1	220	120	90	59
2	150	100	80	53
3	200	105	85	57
4	190	112	90	53
5	210	140	90	57
Mean ± S.D.	194 ± 27	115.4 ± 15.6	87 ± 4.5	55.8 ± 2.7
		(P < 0.001)		

In the severely diabetic group with FBG value of 447± 221 mg/dl (Table 4) after the first treatment of only three days the mean FBG value came down to 243± 125 mg/dl and at the end of second treatment for 15 days, the FBG value was only 132± 56 mg/dl (68% fall) which means near normalcy in a very short period of less then one month. In severely diabetic rabbits, GTT cannot be performed because the animals die due to glucose load. Further the FBG values widely from 250-730 mg/dl. In order to appreciate the remarkable improvement brought about by the hot water extract in severely diabetic rabbits, the FBG values of individual rabbits before and after the first and second treatments are given in Table 4. It is of interest to note that out of two rabbits with FBG 700 mg/dl the untreated rabbit died after one week, while the drug treated rabbit had only FBG of 214 mg/dl at the end of the second treatment. In other words, even the rabbit in its terminal stages was saved by the treatment with this extract. All the treated animals survived and were nearly normal. Further the favourable effect persisted for 10-15 days, after stopping the treatment. Thereafter, the FBG values started increasing. Improvement in sever diabetes with F. bengalensis has not been reported so far excepting by our group with one water insoluble principle purified from the acetone extract of the bark [13].

Table 4
Effect of Water Extract of F. Bengalensis on the Fasting Blood Glucose Level in Severely Diabetic Rabbits

Rabbit No.	Fasting blood glucose mg/dl			
	Before treatment	**After 1st treatment**	**After IInd treatment**	**% fall**
1	730	424	214	70
2	517	230	123	72
3	268	150	90	66
4	275	170	100	64
5	700(untreated control died after one week)			
Mean ± S.D.	447 ± 221	243.5 ± 124	132 ± 56	68 ± 3.7
		(P value -0.006)		

In order to understand the possible mechanism of action, the effect of the water extract on serum insulin levels (Table 5) was investigated. Since blood glucose and insulin levels reach peak at about one hour during GTT, serum insulin levels were estimated in fasting sample and in sample of blood collected one hour after giving glucose orally. In untreated sub diabetic rabbits since FBG values are nearly normal because of partial preserved beta cell function. Hence the fasting serum insulin level was 20 µu/ml and it continued to be lower (25 µu/ml) even after one hour. However, in the treated animals of the same group, there was a significant increase in the serum insulin level to 55µu/ml. In the severely diabetic rabbits, the FBG was high 257 mg% and serum insulin level was only 7µu/ml indicating considerable destruction of the pancreas. In such animals treatment with single dose resulted in some increase of serum insulin to 19 µu/ml. These results clearly show that water extract stimulates the release of insulin after oral glucose load.

Table 5
Effect of Water Extract of F. Bengalensis on the Blood Glucose and Serum Insulin Level

	Blood glucose mg%		Serum insulin µu/ml	
	Fasting	1hr	Fasting	1hr
Sub-diabetic				
Before treatment	100	294	20	25
After treatment	100	260	20	55
Severely diabetic				
Before treatment	257	426	7	8
After treatment	200	306	14	19

Thus the results of our present studies show that the hot water extract of F. bengalensis also has a beneficial effect in all the three types of diabetes mellitus induced by alloxan in rabbits. The first type of AR status is similar to the state seen in the early stages of diabetes in humans with near normal or slightly elevated FBG but high post prandial blood glucose i.e. impaired glucose tolerance. The hot water extract improved glucose tolerance, without causing hypoglycaemia. In mild diabetes it improved both FBG and glucose tolerance.

DISCUSSION

Ours is the first study which shows beneficial antidiabetic effect with the hot water extract of Ficus bengalensis in severe diabetes. The failure of earlier workers to observe any favourable hypoglycaemic effect in severe diabetes perhaps lies in the method followed by them. Shrotri and Aiman [3,4] prepared water extract by keeping the bark at 60ºC in water for eight days. It is not known whether the hypoglycaemic principle was incompletely extracted or inactivated. Augusti and his coworkers [9,10] isolated water soluble principle from the ethanolic extract of the bark. But our method is by extracting the bark with boiling water for 30 minutes which is somewhat similar to that of making Kashayams in Ayurveda.

Our study brings out the following important aspects:

1. In order to retain therapeutic efficacy proper method of extraction of the bark is important.
2. The hypoglycaemic principles of the bark have longer lasting effect (10 to 15 days) even after stopping treatment.
3. The hot water extract prepared by us is effective at a dose of 50 mg/kg/day in contrast to 100 mg/kg/day with pelargonidin or 250 mg/kg/day with extracts by other workers.
4. The treatment required is much shorter than that reported by other workers in all the three types of diabetes.
5. It is active in severe diabetes also at the lowest dose of 50 mg/kg/day reported for water extract so far.
6. Our studies also seem to throw some light on the mechanism of action of active principle(s). Its action seems to be probably both pancreatic by releasing insulin because it is active in AR and mildly diabetic rabbits with some functional pancreas. It also has extra pancreatic effect of stimulating utilization of glucose in peripheral tissues because it is active even in severely diabetic rabbits with FBG of 500 or even 700 mg/dl in whom beta cell reserve is likely to be destroyed almost completely.
7. Since even the hot water extract is active at a small dose of 50 mg/kg, the active principle is likely to be active at a much smaller dose reported so far any principle from F. bengalensis.

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