ACUTE AND CHRONIC TOXICITY STUDIES ON PARTIALLY PURIFIED HYPOGLYCEMIC PREPARATION FROM WATER EXTRACT OF BARK OF *FICUS BENGALENSIS*

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ABSTRACT

Acute and chronic toxicity studies were conducted to assess toxicity of a partially purified preparation from the water extract of the bark of *Ficus bengalensis*, which was demonstrated in our earlier studies to have significant hypoglycemic and hypocholesterolemic effect on alloxan induced, mild and severe diabetes in rabbits. LD₅₀ of this preparation was found to be ~1 gm/kg in rats when given orally. For chronic toxicity studies 3 doses of aqueous preparation were given to 3 groups of rats. First group received 5 times ED₅₀ (50 mg/kg), second group 10 times ED₅₀ (100 mg/kg) and the third group 15 times ED₅₀ (150 mg/kg) for 3 months. Fourth group which served as control was given water. After three months, blood was collected for studying biochemical and hematological parameters. Blood glucose, serum cholesterol, liver and kidney function tests, haemoglobin, total and differential leukocyte count were determined. Animals were sacrificed and histopathological examination of liver, heart and kidneys was carried out. Results of the study showed that partially purified preparation from *Ficus bengalensis* is not toxic by all the above mentioned parameters.

KEY WORDS

Ficus bengalensis, hypoglycemic compound, acute and chronic toxicity.

INTRODUCTION

Diabetes mellitus is a major source of suffering and morbidity and now affects more than 143 million people worldwide (1). Among its major subtypes, type 2 diabetes or non-insulin dependent diabetes mellitus (NIDDM) accounts for 90-95% of all diagnosed cases of diabetes mellitus, whereas type 1 diabetes or insulin dependent diabetes mellitus (IDDM) accounts for 5 to 10% (2). *Ficus bengalensis* is a medicinal plant reputed for its antidiabetic properties (3-7). We have obtained a partially purified preparation from water extract of the bark of this plant (patent applied in India & USA), which was highly effective in controlling blood sugar and cholesterol in mild and severe alloxan induced diabetes in rabbits (8). Water extract of bark of *Ficus bengalensis* was

reported to have hepatotoxic action (9), which was thought to be due to tannic acid content of the extract. However, detanned extract also showed similar toxicity (10). So it was necessary to show whether this hypoglycemic preparation of water extract of the bark is hepatotoxic, in which case it can not be used as treatment for diabetes, or whether the hepatotoxicity was due to some other constituent present in extract prepared by earlier workers. At the same time Ayurvedic system of medicine uses extracts because they contain some useful constituents beside active ingredient. Hence, it is necessary to show that such preparation is not only effective but is also free from undesirable side effects. So the present work was undertaken.

MATERIALS AND METHODS

Animals: Male Wistar rats 4-12 weeks from our animal house, and male albino rabbits (1-1.5 kg) purchased from local breeders were used for studies.

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PREPARATION OF PARTIALLY PURIFIED FRACTION

Since patent is applied for the isolation procedure of the partially purified hypoglycemic compound from water extract of *Ficus bengalensis*, method of preparation of partially purified fraction from water extract of *Ficus bengalensis* is not given here.

Acute and chronic toxicity studies were conducted as described by Ghosh (11) in normal rats.

ACUTE TOXICITY STUDIES

For determination of LD₅₀ different concentrations of partially purified fraction (dry wt. of preparation 0.1, 0.3, 0.5, 0.7, 0.9, 1.1 and 1.3 gm/kg) were given orally to rats, 10 in each group. The animals were closely observed for 3 hours and mortality if any was recorded after 24 hours.

CHRONIC TOXICITY STUDIES

For chronic toxicity studies rats were divided into 4 groups of 10 each. Rats of group 1, 2 and 3 received orally daily a single dose of 5, 10 and 15 times of the ED₅₀ value of purified preparation (i.e. 50 mg, 100 mg and 150 mg/kg) respectively for 3 months. ED₅₀ of partially purified fraction in diabetic rats was determined by using quantal dose effect curve and was found to be 10 mg/kg in previous experiments (8). The 4th group received 0.5 ml of water during the period and served as control. At the end of experiment blood was collected for biochemical and hematological parameters and animals were sacrificed. Liver, heart and kidney were dissected and used for histological examination.

STUDIES WITH DIABETIC RABBITS FOR HYPOGLYCEMIC EFFECT

It was first made sure that partially purified preparation was having hypoglycemic effect as described below and then used for toxicity studies. Rabbits were made diabetic by giving intravenous injection of alloxan (80 mg/kg) in citrate buffer pH 4.0 as described by us earlier (5,8). Fasting blood glucose (FBG) was estimated once every week for one month. When blood sugar got stabilized, animals with FBG 150-250 mg/dl were used as diabetic rabbits. They were given partially purified preparation orally daily once at a dose of 20 mg/kg [effective dose for rabbits was higher (20mg/kg)

than for rats (10 mg/kg) as determined earlier by us (8)] for one month. Food and water was available *ad libitum* during this period. After one month blood was collected from ear veins of over night fasted rabbits for FBG, serum cholesterol, serum triacylglycerol, liver and kidney function tests.

BIOCHEMICAL PARAMETERS

Serum proteins were estimated by method of Reinhold (12), serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), serum alkaline phosphatase (SALP), serum urea, plasma glucose and serum cholesterol were estimated using kits from Orthodiagnostic systems Bombay.

HAEMATOLOGICAL PARAMETERS

Haemoglobin, total leukocyte and differential leukocyte count were determined in control and drug treated groups.

HISTOPATHOLOGICAL STUDIES

Animals were sacrificed, liver, heart and kidneys were removed, washed with cold saline and preserved in 10% formalin in buffered form. Blocks from tissues were routinely processed and embedded in paraffin. 4µm thick sections were cut using rotary microtome (A.O. Spencer) and stained with hematoxylin and eosin for histomorphology evaluation.

RESULTS AND DISCUSSION

Partially purified hypoglycemic preparation from the water extract of *Ficus bengalensis* produced significant fall in fasting blood glucose (FBG) and improvement in glucose tolerance test (GTT) in alloxan induced mild diabetes in rabbits, which is similar to NIDDM in humans. Table 1 shows results of one month treatment of alloxan induced diabetic rabbits, with the partially purified preparation at a dose 20 mg/kg body weight (bw)/day orally. There was significant fall in FBG, cholesterol and triacylglycerol. Moreover there was no adverse effect on kidney and liver function tests (Table 1). It was effective even in alloxan induced severe diabetes in rabbits, which is similar to IDDM in humans (8). These results prompted us to perform acute and chronic toxicity tests with this preparation to assess its usefulness in the treatment of diabetes, without side effects.

In acute toxicity studies, LD₅₀ of this

preparation in rats (oral) was found to be ~1 gm/kg bw (Table 2). This is quite high compared with acute LD₅₀ 760 mg/kg, bw for hypoglycemic drug chlorpropamide (13) and therefore much safer than chlorpropamide.

In chronic toxicity studies, 3 doses were used 5 times ED₅₀ (group 1), 10 times ED₅₀ (group 2) and 15 times ED₅₀ (group 3). ED₅₀ was determined in streptozotocin diabetic rats using quantal dose effect curve and was found to be 10 mg/kg. Rats in group I and II appeared normal in behaviour, while rats in group III showed some dullness in their activity initially, but became active after 2-3 days of initiation of drug treatment. No significant fall in FBG was observed in all the 3 groups even after three months treatment. This means that this partially purified preparation does not produce hypoglycemia in normal rats. Food intake of animals was normal during the entire period of the experiment. Serum protein, blood urea and serum cholesterol values were not affected and were similar to those of control. In enzymes like SGOT, SGPT and S. alkaline phosphatase, there was no significant change in group I and II but in group III there was slight fall in values of SGOT and S. alkaline phosphatase but values were still in normal range (Table 3). Results indicate that this preparation does not affect liver function tests even when given in high doses of 10 and 15 times ED₅₀. Hepatotoxic action of water extract of bark of *Ficus bengalensis* reported by earlier workers (9,10) may be due to the fact that they used crude or only detanned crude extract which possessed some hepatotoxic compound. This also means that the hepatotoxic compound was removed during our

partial purification procedure.

Haematological estimation revealed that there was no change in values of hemoglobin which ranged from 12.9 - 15.2 gm/dl in animals receiving minimum and maximum dose. Total leukocyte count remained between 12.12 to 15.5 x 10⁹/L in rats of group I and II while it dropped to 7.95 x 10⁹/L in group III but values are still in normal range. Differential count also remained similar to control and did not show any change in all the three groups (Table 4).

Weight gain in all the three groups was comparable to that of control (Table 5). It is noteworthy that the weight gain of the animals was not above that of controls even with very high dose (15 times ED₅₀) of this preparation. Weight gain and obesity is a serious drawback with some antidiabetic drugs. This means that there is no risk of overweight after treatment with this preparation. Histopathological examination of heart, liver and kidney showed no change and sections of these tissues in control and treated groups were similar.

Thus the results of the present toxicity studies show that this partially purified hypoglycemic preparation from water extract of bark of *Ficus bengalensis* has high LD₅₀ and is nontoxic even in doses as high as 10 to 15 times ED₅₀. So it has the potential of being used for treatment of diabetes mellitus.

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Table 1

Effect of one month treatment with partially purified hypoglycemic preparation from bark of *Ficus bengalensis* (20 mg/kg body weight/day) on diabetic rabbits.

Parameters	Normal	Diabetic	Diabetic treated	Significance
Fasting blood glucose (mg/dl)	89±6	220±18	80±8	P<0.001
Cholesterol (mg/dl)	35±8.3	80±10.6	51±4.7	P<0.005
Triacylglycerol (mg/dl)	47.8±3.9	147±19	58±2.7	P<0.005
Bilirubin (mg/dl)	0.7±0.2	0.6±0.2	0.7±0.1	NS
Protein (gm/dl)	5.8±0.6	5.7±0.3	5.6±0.1	NS
SGPT (IU/L)	31±2	34.6±3	31±2	NS
SGOT (IU/L)	34±3	70±5	35±4	P<0.005
Alkaline phosphatase (IU/L)	230±10	216±12	222±9	NS
Urea (mg/dl)	35±4	40±5	36±3	NS

Values are mean ± SD of 5 rabbits

NS = Not Significant

P value is between values before and after treatment.

Table 2

LD₅₀ determination in rats (oral) with different doses of partially purified hypoglycemic preparation from water extract of bark of *Ficus bengalensis*.

Dry weight of preparation gm/kg	No. of rats died after 24 hours/total no. of rats	% Mortality
0.1	0/10	0
0.3	0/10	0
0.5	1/10	10
0.7	2/10	20
0.9	4/10	40
1.1	6/10	60
1.3	7/10	70

LD₅₀ from log probit curve ~1 gm/kg.

Table 3

Liver and kidney function tests, plasma glucose and serum cholesterol values in normal rats treated with partially purified hypoglycemic preparation from water extract of bark of *Ficus bengalensis* for three months.

Group	Proteins (gm/dl)	Urea (mg/dl)	SGOT (IU/L)	SGPT (IU/L)	Alkaline phosphatase (IU/L)	Glucose (mg/dl)	Cholesterol (mg/dl)
Group I (5 times ED ₅₀)	7±0.4	45±7	32±6	28±7	218±6	78±7	68±3.2
Group II (10 times ED ₅₀)	7.2±0.6	40±8	34±5	32±5	222±5	70±6	71±2.9
Group III (15 times ED ₅₀)	6.8±0.5	44±4	28±7	38±5	200±8	73±5	69±4
Group IV (0.5 ml water)	7.2±0.8	48±6	35±4	30±6	220±9	80±8	70±4.5

Values are mean ± SD

No. of rats 10 in each group

After treatment values not significantly different from control values

Table 4

Haematological parameters in rats after 3 months treatment with partially purified preparation from water extract of bark of *Ficus bengalensis*.

Group	Hemoglobin gm/dl (mean) (range 12.9-15.2)	Total Leukocyte count x10 ⁹ /L (average value) (range 6.9-15.5)	Differential Leukocyte Count % mean Neutrophils (range 6-9)	Lympho- cytes (range 84-90)	Mono- cytes (range 2-6)	Eosino- phils (range 0-2)	Baso- phils Nil
Group I (5 times ED ₅₀)	13.6±2.7	13.7±2.	7	87	2	1	0
Group II (10 times ED ₅₀)	14.8±1.9	14.6±1.2	7	88	3	1	0
Group III (15 times ED ₅₀)	14.2±0.9	12.8±2.3	6	89	2	1	0
Group IV (0.5 ml water)	13±2.3	13.2±3.3	8	88	3	2	0

No. of rats 10 in each group

After treatment values not significantly different from control values.

Table 5

Effect on weight of normal rats given different doses of partially purified hypoglycemic preparation from water extract of bark of *Ficus bengalensis* for three months.

	Initial weight (gm)	Weight after 3 months (gm)	Change in weight (gm)	Significance between groups
Group I (5 times ED ₅₀)	200 ± 10.3	222.6 ± 8.7	26.6 ± 9	NS
Group II (10 times ED ₅₀)	191.5 ± 15	216.7 ± 13.2	25.5 ± 2	NS
Group III (15 times ED ₅₀)	219.5 ± 12.7	244.2 ± 8.8	24.7 ± 11.5	NS
Group IV Control	200 ± 13.6	227.4 ± 10.4	27.3 ± 4	NS

Values are mean ± SD of 10 rats.

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