Hypoglycaemic effects of alcoholic root extract of *Borassus flabellifer* (Linn.) in normal and diabetic rats

Titas Debnath[1], Rajesh Radhakrishnan[2], Murugananthan Gopal[3] , Sahil Talwar[4] and Nandakumar K[4]*

[1]Department of Pharmacology, PES College of Pharmacy, 50 feet Road, Hanumanthanagar, Bangalore, Karnataka, India
[2]Department of Pharmacy Practice, Manipal College of Pharmaceutical Sciences, Manipal, Karnataka, India
[3]Department of Pharmacognosy, PES College of Pharmacy, 50 feet Road, Hanumanthanagar, Bangalore, Karnataka, India
[4]Department of Pharmacology, Manipal College of Pharmaceutical Sciences, Manipal, Karnataka, India

Abstract: The objective of the study was to investigate the alcoholic (ALEBF) extract of *B. flabellifer* for their hypoglycaemic effects in normal and diabetic rats. Diabetes was induced in rats by single dose administration of alloxan (120 mg/kg, i.p.) or by injecting dexamethasone (10 mg/kg, i.p.) for 10 days. In normal rats, ALEBF (100, 200 and 400 mg/kg) had significantly decreased the blood glucose level in a dose dependent manner after repeated administration for 7 days. In alloxan induced diabetic rats, extract (ALEBF) had decreased blood sugar level and improved glucose tolerance in alloxan induced diabetic rats at the end of 1st, 2nd , 3rd and 4th week after test extract treatment. However, the insulin levels of extract treated group did not significantly change after 28 days treatment with the extract. It did not alter the insulin levels. In alloxan model, repeated dose administration of ALEBF had showed significant increase in body weight, prevention of elimination of sugar in urine and reduced the mortality rate induced by alloxan. In dexamethasone induced insulin resistance diabetic rats, repeated administration of ALEBF inhibited the increase in blood glucose level, improved glucose tolerance and reduced the insulin levels as compared dexamethasone induced diabetic rats.

Keywords: *Borassus flabellifer;* Hypoglycaemic activity**,** Anti-diabetic activity, alloxan, dexamethasone.

INTRODUCTION

Diabetes mellitus is a chronic metabolic disorder characterized by a high blood glucose concentration-hyperglycemia (fasting plasma glucose > 7.0 mmol/l or plasma glucose > 11.1 mmol/l 2 hours after a meal) – caused by insulin deficiency, often combined with insulin resistance. Hyperglycemia occurs because of uncontrolled hepatic glucose output and reduced uptake of glucose spills over into the urine (glycosuria) and causes an osmotic diuresis (polyuria), which in turn results in dehydration, thirst and increased drinking (polydipsia) (Rang *et al*., 2003).

India leads the world with largest number of diabetic subjects earning the dubious distinction of being termed the "diabetes capital of the world". International Diabetes Federation, (2006) published the number of people with diabetes in India currently is around 40.9 million and is expected to rise to 69.9 million by 2025 unless urgent preventive steps are taken (Mohan *et al*., 2007).

Pharmacological treatment with of diabetes is based on oral hypoglycaemic agents and insulin (Committee Report, 1997). However, long term treatment with these drugs is expensive and poses life threatening adverse effects. Management of diabetes without any side effects is still a challenge to the medical community. There is continuous search for alternative drugs. Therefore, it is prudent to look for options in herbal medicine for diabetes as well (Noor *et al*., 2008). Further, the World Health Organization (WHO) has recommended that this area warrants further evaluation (WHO, 1980).

Borassus flabellifer Linn is widely distributed plant in coastal and forest area in India and are very likely to be cultivated for both medicinal as well as commercial reason. There are innumerable medicinal uses for all parts of the *B. flabellifer*. Briefly, the young plant is said to relieve biliousness, dysentery, and gonorrhea. Young roots are diuretic and anthelmintic, and a decoction is given in certain respiratory diseases. The cabbage, leaf petioles, and dried male flower spikes all have diuretic activity. The pulp of the mature fruit relieves dermatitis (Morton, 1988). The methanolic extract from the male flowers of *B. flabellifer* was found to inhibit the increase of serum glucose levels in sucrose-loaded rats (Masayuki *et al*., 2007). Decoction of the root is used in gastritis, hiccup and it is taken by labour class people for the treatment of diabetes (Nadkarni & Nadkarni, 2000). However, no systematic study was carried out on the root extracts of *B. flabellifer* Linn, for anti diabetic activity. Hence present study was planned to investigate the hypoglycaemic activity of ethanolic root extract of *B. flabellifer* (ALEBF) in normal and diabetic rats.

MATERIALS AND METHODS

Plant material

Roots of *B. flabellifer* Linn. were collected from the surrounding areas of Bellary, Karnataka, in the month of

**Corresponding author:* e-mail: nandakumar77@rediffmail.com

June, 2006. The plant was identified by Dr. K.P. Sreenath, HOD, Department of Botany, Bangalore University. The roots were dried and powdered with a blender.

Drugs and chemicals

Glibenclamide Tab. (Aventis Pharma Ltd., Mumbai, India); Alloxan (Spectrochem Pvt. Ltd., Mumbai, India); Dexamethasone Sodium Phosphate (Tridoss Laboratories Ltd. Mumbai, India); Glucose estimation kit. (Span diagnostic Ltd., Surat, India); and insulin estimation kit (Diagnostic System Laboratories, Inc., USA) were used in this study. All the other solvents and chemicals used for extraction and phytochemical investigation were as of analytical grade purchased from S.D fine chemicals Pvt.Ltd. Mumbai, India.

Preparation of plant extract

Dried powdered material of the roots were successively extracted in Soxhlet apparatus using petroleum ether (60-80^{0}C) followed by ethanol (95%). Marc obtained from the first extract was air dried and then subjected for alcoholic extract.

Residue obtained from the alcoholic extract then macerated with water for 72 hours. All the extracts were then concentrated in a rotary evaporator under reduced pressure at temperature of 50^0 C and then lyophilized to get a powder. The percentage yield of these extracts was found to be petroleum ether (0.2% w/w), ethanolic (21% w/w) and aqueous extract (2.3% w/w). Pilot study of these extracts was done and it was found that alcoholic extract possessed hypoglycaemic activity (data unpublished). Hence main study was performed with alcoholic extract.

Preliminary phytochemical investigation

The ethanolic extract of *B. flabellifer* was subjected to preliminary qualitative investigations to detect various secondary metabolites present in the extract (Khandelwal, 2000). Ethanolic extract showed presence of alkaloids, saponins, phenolics compounds, tannins, proteins and aminoacids.

Animals

Wistar rats (170-220g) of either sex were purchased from Bioneeds, Nelamangala, Tumkur. They were maintained in the animal house of PES College of Pharmacy, Bangalore for experimental purpose. All the animals were acclimatized for seven days under standard husbandry conditions, i.e.; room temperature of 25 ± 1^{0}C; relative humidity 45-55% and a 12:12h light/ dark cycle. The animals had free access to standard rat pellet diet (Pranav Agro Industries Ltd, Bangalore, India), with water supplied *ad libitum* under strict hygienic conditions. Each experimental group had separate set of animals and care was taken to ensure that animals used for one response were not employed elsewhere. Animals were habituated to laboratory conditions for 48 hours prior to experimental protocol to minimize if any of non-specific stress. The approval of the Institutional Animal Ethical Committee (IAEC) of P.E.S College of Pharmacy Bangalore (Karnataka) was taken prior to the experiments. All the protocols and the experiments were conducted in strict compliance according to ethical principles and guidelines provided by Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

Experimental procedure

Assessment of hypoglycaemic activity in normal rats

Fasted rats were divided in to five groups consisting of 8 animals in each group. First group received vehicle (3% v/v tween 80 in distilled water, orally in a volume of 10 ml/kg, which served as control). Group II received Glibenclamide (5 mg/kg, p.o.) as standard drug suspended in vehicle. ALEBF suspended in vehicle was administered at the doses of 100, 200 and 400 m/kg, p.o. in a volume of 10 ml/kg to the rats of group III, IV and V respectively. Blood samples were collected from the tail vein or by retro-orbital puncture method just prior to and at 1, 2, 4 h after dosing for acute studies and glucose was estimated. For sub-acute studies blood samples were collected on 4th and 7th day after 16 h of overnight fasting for glucose level estimation.

Oral glucose tolerance test (OGTT) was carried by administering glucose (2 g/kg, p.o.), 30 minutes after the extract or standard drug administration after 7 days of pretreatment period. Blood samples were collected from tail vein for glucose analysis prior to glucose administration (0 h) and at 1, 2 and 3 h after glucose loading.

Induction of diabetes

Alloxan induced diabetes

Diabetes was induced by single injection of alloxan monohydrate (120 mg/kg, i.v.) in ice cold normal saline. Five days after the injection of alloxan, the animals were fasted for 16 h and blood sugar was determined of the surviving animals. Rats with blood glucose level >200 mg/dl were considered diabetic and were used in the experiment.

Acute and sub-acute effect of ALEBF in alloxan diabetic rats

Diabetic rats were divided into 5 groups consisting of 8 animals in each group. Group I received vehicle (3 %v/v tween 80, p.o.) which served as control. Group II received glibenclamide as reference drug (5 mg/kg, p.o.) suspended in vehicle. ALEBF at the doses 100, 200 and 400 mg/kg were administered orally to the rats of group III, IV and V respectively. Blood samples were collected from the tail vein just prior to and at 1, 2 and 4 hour after single dosing. In sub-acute studies weekly blood samples were collected by retro-orbital puncture method after 1 h

of extract administration at the end of 1st, 2nd, 3rd and 4th week. OGTT was performed at the end of 28 days study period.

Blood glucose and insulin levels were measured. Weekly body weight and mortality were also recorded at the end of each week.

Dexamethasone induced insulin resistance in rats
Wistar rats of either sex weighing 170-220 g were divided in to 6 groups consisting of 8 animals in group. Group I and II received only vehicle (3% v/v tween 80 in water). Group III received glibenclamide (5 mg/kg, p.o.) which served as reference standard group. ALEBF at the doses 100, 200 and 400 mg/kg were administered orally to the rats of group IV, V and VI respectively. All the treatments were made for a period of 10 days. One hour after test drug administration all the rats received dexamethasone (10 mg/kg, p.o.) daily for 10 days except group I which served as normal control group. Blood samples were withdrawn from tail vein, to determine fasting blood glucose(FBG) and insulin levels, prior to and on 4th, 7th and 10th day 1 hour after dexamethasone treatment. OGTT were also performed with a glucose loading in these rats on 10th day.

Analytical method
Determination of blood glucose concentration
Blood glucose concentration was determined in plasma by commercially available glucose kit based on glucose oxidase method. Instruction manual provided by manufacturer was followed to obtain the glucose level present in the sample and was expressed as mg/dl.

Determination of insulin concentration
Plasma insulin levels were determined by radio-immunoassay using a commercially available kit as per the manufacturer instruction manual provided along with the kit. Insulin levels were expressed as µIU/ml.

STATISTICAL ANALYSIS

The results are presented as mean ± SEM from 8 animals. Statistical difference between the treatments and the controls were analyzed using One-Way Analysis of Variance (ANOVA) followed by Dunnett post-hoc test. $P<0.05$ was considered to be statistically significant.

RESULTS

Hypoglycaemic effect of ALEBF in normal rats
FBG levels were within the range of 95-105 mg/dl in all the groups at 0 h. Single dose administration of ALEBF (100, 200 and 400 mg/kg) did not significantly decrease the blood glucose level (BGL) at various time intervals viz. 1, 2 and 4 h after treatment, indicating that the extracts could not produce significant hypoglycaemic activity after acute treatment (table 1). Repeated administration of ALEBF had significantly reduced the FBG on 4th day with 400 mg/kg and on 7th day with 200 and 400 mg/kg, indicating the alcoholic extract can produce hypoglycaemia on repeated administration (table 2). Glibenclamide (5 mg/kg) significantly reduced BGL after single dose and repeated dose administration as compare to vehicle groups.

Administration of glucose (2g/ kg) to 7 days pretreated

Table 1: Effect of ALEBF on blood glucose concentration in normal rats

Treatment	Dose (mg/kg)	Blood glucose level (mg/dl)			
		0 h	1 h	2 h	4 h
Control (3 % v/v tween 80)	10 ml/kg	98.81 ± 2.02	98.41 ± 1.33	100.00 ± 2.38	98.02 ± 1.89
Glibenclamide	5	99.60 ± 1.29	93.65* ± 1.00	84.13** ± 1.18	61.9** ± 1.18
ALEBF	100	98.41 ± 2.1	97.22 ± 1.43	98.02 ± 2.64	97.62 ± 1.94
ALEBF	200	98.02 ± 1.56	96.83 ± 1.33	97.22 ± 1.29	98.4 ± 1.00
ALEBF	400	100.37 ± 2.57	99.21 ± 1.59	99.21 ± 1.70	98.02 ± 2.08

Values are expressed as mean ± S.E.M from 8 rats. *P<0.05; **P<0.01 significant from the control animals.

Table 2: Hypoglycaemic activity of ALEBF in normal rats after repeated dose administration for 7 days

Treatment	Dose (mg/kg)	Blood glucose level (mg/dl)		
		0 day	4th day	7th Day
Control (3 % v/v tween 80)	10 ml/kg	98.81 ± 2.02	98.81 ± 1.02	98.40 ± 2.66
Glibenclamide	5	99.60 ± 1.29	60.71** ± 1.48	48.41** ± 2.51
ALEBF	100	100.79 ± 1.91	97.62 ± 1.23	96.03 ± 1.46
ALEBF	200	98.02 ± 1.56	94.05 ± 1.60	90.88* ± 1.68
ALEBF	400	100.37 ± 2.57	92.86** ± 1.84	77.78** ± 1.70

Values are expressed as mean ± S.E.M from 8 rats. *P<0.05; **P<0.01 significant from the control animals.

Table 3: Effect of ALEBF of blood glucose level of glucose loaded hyperglycaemic rats (OGTT) after 7 days treatment

Treatment	Dose (mg/kg)	Blood glucose level (mg/dl)			
		0 h	1 h	2 h	3h
Control (3 % v/v tween 80)	10 ml/kg	97.22 ± 1.89	148.02 ± 2.08	115.08 ± 1.18	99.30 ± 1.66
Glibenclamide	5	49.21 ± 0.79	62.7** ± 1.33	52.78** ± 0.73	50.00 ± 1.06
ALBEF	100	95.24 ± 1.51	141.67 ± 2.19	111.84 ± 1.26	97.22 ± 2.25
ALEBF	200	88.09 ± 1.07	123.49* ± 1.60	100.89 ± 1.64	89.68 ± 1.18
ALEBF	400	73.41 ± 1.67	99.30** ± 1.67	79.60* ± 0.78	74.45 ± 1.81

Values are expressed as mean ± S.E.M from 8 rats. **P*<0.05; ***P*<0.01 significant from the control animals.

Table 4: Effect of single dose treatment of ALEBF of blood glucose level on alloxan induced diabetic rats.

Treatment	Dose (mg/kg)	Blood glucose level (mg/dl)			
		0 h	1 h	2 h	4 h
Diabetic Control	10ml/kg of vehicle	262.7±7.70	266.07 ± 6.15	267.14 ± 6.42	257.86 ± 5.84
Glibenclamide	10	266.4 ± 5.90	240.00* ± 5.95	203.57** ± 6.88	172.14** ± 3.99
ALEBF	100	263.2 ± 4.99	262.50 ± 4.79	255.36 ± 3.95	257.14 ± 4.35
ALEBF	200	257.50 ± 5.12	257.86 ± 5.41	259.29 ± 4.93	253.93 ± 5.95
ALEBF	400	263.21 ± 5.74	259.29 ± 5.11	259.57 ± 6.78	257.86 ± 5.84

Values are expressed as mean ± S.E.M from 8 rats. **P*<0.05; ***P*<0.01 significant from the control animals.

Table 5: Effect of repeated dose treatment of ALEBF of blood glucose level and plasma insulin levels in alloxan induced diabetic rats.

Treatment	Dose (mg/kg)	Blood glucose level (mg/dl)				Plasma insulin level (µIU/ml)			
		7^{th} d	14^{th} d	21^{st} d	28^{th} d	7^{th} d	14^{th} d	21^{st} d	28^{th} d
Normal Control	-	95.58 ±1.21	96.62 ±1.56	96.54 ±1.03	97.14 ±0.65	10.38 ±1.08	10.57 ±0.89	10.44 ±1.21	10.12 ±0.86
Diabetic Control	-	282.92 ±6.22	280.00 ±4.70	288.50 ±3.70	291.25 ±3.66	5.66 ±0.92	4.98 ±1.12	5.02 ±1.01	5.15 ±1.23
Glibenclamide	10	151.57** *±2.96	104.64** *±2.91	97.14*** ±1.27	89.64*** ±3.01	6.78 ±0.97	8.43* ±1.12	9.27** ±1.19	9.94** ±1.09
ALEBF	100	280.83 ±3.09	280.00 ±4.48	272.00* ±5.15	256.00** * ± 4.66	5.93 ±0.78	4.77 ±1.39	5.28 ±0.96	5.56 ±0.43
ALEBF	200	275.42 ±2.57	263.75* ±2.45	238.50** *±3.76	198.50** * ±2.72	5.89 ±0.76	4.64 ±0.65	5.99 ±1.02	6.12 ±1.17
ALEBF	400	245.42** * ±4.07	220.00** * ±2.99	169.58** * ±2.35	112.01** * ±3.61	6.21 ±0.40	5.43 ±0.19	7.02 ±1.58	6.43 ±1.23

Values are expressed as mean ± S.E.M. n = 8. * *P* <0.05, ** *P* <0.01 and *** *P* <0.01 Diabetic Control Vs treated groups.

rats significantly suppress the rise in BGL with ALEBF at 1 and 2 h with 400 mg/kg and at 1 h with 200 mg/kg as compare with vehicle control. Glibenclamide (5 mg/kg) showed significant suppress in BGL rise at 1 and 2 h (table 3).

Effect of ALEBF in alloxan induced diabetic rats

FBG levels in normal rats were in the range of 95-100 mg/dl. Treatment with alloxan (120 mg/kg, i.v.) had increased the BGL to a range of 250-270 mg/dl after 5 days. Single dose administration of ALEBF (100, 200 and 400 mg/kg) did not significantly reduced the BGL in alloxan induced diabetic rats, while glibenclamide (5 mg/kg) significantly reduced the BGL at 1^{st}, 2^{nd} and 4^{th} hour after single dose administration in alloxan induced diabetic rats (table 4).

Repeated dose administration with alcoholic extract (100, 200 and 400 mg/kg) had progressively reduced the BGL in a dose dependent manner over a period of 4 weeks (table 5). These results indicate the ALEBF possessed hypoglycaemic activity on repeated administration in alloxan induced diabetic rats. However there is no marked change in insulin levels after repeated dose administration of ALEBF (table 5).

Repeated dose treatment with ALEBF (100, 200 and 400 mg/kg) for 4 weeks had significantly improved the glucose tolerance as compared to diabetic control rats (table 6).

Treatment with alloxan (120 mg/kg, i.v.) had significantly decreased the body weight at the end of 7^{th}, 14^{th} 21^{st} and

Table 6: Effect of repeated dose administration of ALEBF on oral glucose tolerance test in alloxan induced diabetic rats on 28th day.

Treatment	Dose (mg/kg)	Blood glucose level (mg/dl)		
		1 h	2 h	3 h
Diabetic Control	-	52.87 ± 2.08	28.08 ± 1.28	2.81 ± 0.61
Glibenclamide	10	23.8*** ± 3.78	9.04*** ± 1.41	0.46* ± 0.46
ALEBF	100	37.26* ± 1.89	18.64* ± 1.18	1.74 ± 0.22
ALEBF	200	26.65** ± 2.12	11.73*** ± 0.71	0.41* ± 0.38
ALEBF	400	30.14*** ± 2.19	13.03*** ± 1.75	1.02 ± 0.44

Values are expressed as mean ± S.E.M. * *P* <0.05, ** *P* <0.01, *** *P* <0.001, Diabetes Control Vs all treated groups.

Table 7: Effect of repeated dose treatment of ALEBF of body weight in alloxan induced diabetic rats.

Treatment	Dose (mg/kg)	Body weight (percentage change from the initial weight)			
		7 d	14 d	21 d	28 d
Normal Control	-	1.46 ± 0.55	2.59 ± 0.87	4.53 ± 1.13	5.88 ± 0.93
Diabetic Control	10	-5.48 ± 0.92	-8.66 ± 1.23	-10.15 ± 0.80	-10.75 ± 1.19
Glibenclamide	10	-1.61** ± 0.33	-2.6*** ± 0.45	-2.49*** ± 0.45	-2.32*** ± 0.57
ALEBF	100	-5.03 ± 0.54	-7.88 ± 1.01	-9.38 ± 0.85	-9.72 ± 1.03
ALEBF	200	-4.23 ± 0.78	-6.00 ± 0.90	-6.20 ± 1.07	-6.28* ± 0.81
ALEBF	400	-3.55 ± 0.71	-4.93 ± 0.63	-5.21** ± 0.74	-5.40** ± 0.67

Values are expressed as mean ± S.E.M. * *P* <0.05, ** *P* <0.01, *** *P* <0.001, Diabetes Control Vs all treated groups.
"-ve" sign indicates reduction in body weight compared to before alloxan administration.

Table 8: Effect of ALEBF of blood glucose level and plasma insulin levels in dexamethasone induced diabetic rats.

Treatment	Dose (mg/kg)	Blood glucose level (mg/dl)		Plasma insulin level (µIU/ml)	
		5^{th} d	10^{th} d	5^{th} d	10^{th} d
Normal Control	-	100.01±2.32	101.79±1.95	10.03±1.34	10.49±0.67
Diabetic Control	-	159.38±3.26	192.50±4.43	15.67±0.87	19.23±0.98
Glibenclamide	10	103.93**±3.49	85.00***±2.78	10.23***± 0.98	11.24***±1.19
ALEBF	100	146.78±2.46	168.34±3.42	13.98 ± 1.12	17.48*±0.81
ALEBF	200	134.64*±3.43	145.00***±3.74	12.49 **± 0.54	11.48***±0.23
ALEBF	400	120.00**±3.78	115.00***±4.30	11.40**±0.45	10.87***±1.46

Values are expressed as mean ± S.E.M. * *P* <0.05, ** *P* <0.01, *** *P* <0.001, Diabetes Control Vs all treated groups.

28^{th} day as compare to normal animals. Repeated administration of glibenclamide (5 mg/kg) had prevented the reduction in body weight on 7^{th}, 14^{th} 21^{st} and 28^{th} day in diabetic rats. ALEBF 400 mg/kg was able to significantly inhibit the decrease in body weight only after 21^{st} and 28^{th} day and ALEBF 200 mg/kg could do it on 28^{th} day (table 7).

Single administration of alloxan (120 mg/kg, i.v.) had produced mortality of 43% over a period of 4 weeks. Repeated administration of glibenclamide (5 mg/kg) had prevented the mortality in alloxan induced diabetic rats throughout the study. Repeated dose administration of ALEBF (100 and 200 mg/kg) showed mortality of 29% while at 400 mg/kg dose level mortality rate was 14% at the end of 4 weeks. These results indicated that glibenclamide and ALEBF could protect the animals against alloxan induced mortality.

Effect of ALEBF in dexamethasone induced diabetic rats

FBG levels were within the range of 95-105 mg/dl in all the groups on day 0. Dexamethasone treatment produced a elevation of glucose levels and insulin levels over a period of 10 days. Treatment with ALEBF significantly prevented dexamethasone induced hyperglycaemia, hyperinsulinemia (table 8) and improved glucose tolerance (table 9) as compared to diabetic control rats.

DISCUSSION

Results of the above mentioned studies have revealed that ALEBF does not possess any effect on normal rats on acute treatment, while sub acute treatment for 7 days showed hypoglycaemic effect in normal animals. Hence we planned the study of alloxan for 4 weeks period as the extract effect may be stabilized with in that period. In

Table 9: Effect of repeated dose administration of ALEBF on oral glucose tolerance test in dexamethasone induced diabetic rats on 10th day.

Treatment	Dose (mg/kg)	Blood glucose level (mg/dl)		
		1 h	2 h	3 h
Diabetic Control	-	60.15±2.30	40.01±2.11	29.94±2.05
Glibenclamide	10	32.19***±1.95	12.05***±1.86	2.25***±0.80
ALEBF	100	49.60±4.17	39.33±2.94	17.2*±0.46
ALEBF	200	46.86*±3.45	21.90***±3.39	11.05***±2.11
ALEBF	400	39.56***±1.72	17.42***±1.32	6.00***±1.06

Values are expressed as mean ± S.E.M. * P <0.05, ** P <0.01, *** P <0.001, Diabetes Control Vs all treated groups.

alloxan induced diabetic rats ALEBF showed progressive decrease in blood sugar level over a period of 28 days. As far as the effective dose is concern it has been found that 400 mg/kg of ALEBF. This dose brought the blood sugar level nearer to the normal.

Alloxan, a beta-cytotoxin destroys the pancreatic beta cells in a wide variety of animal species, resulting in decrease in endogenous insulin release, which subsequently leads to decreased utilization of glucose by the tissue (Saravanan and Pari, 2005). It has been postulated that glucose transporter and glucokinase are the target molecule for alloxan, leading to decreased insulin levels and uncontrolled BGL (Anonyms, 2006). The hyperglycemia seen in alloxan induced diabetic rats in our study may be due to the above mentioned mechanism. It is well established that sulphonylureas produce hypoglycemia by increasing the secretion of insulin from pancreas and improving the glucose transporter system. These compounds are active in mild to moderate alloxan induced diabetes, but they are ineffective in severe alloxan diabetes (Nammi *et a*l., 2003). Results from the present study suggest that the type of diabetes produced by alloxan in this study may be of moderate in nature. Sub acute treatment with ALEBF for 28 days could show anti-hyperglycemia with improved glucose tolerance with no change in insulin levels suggesting that ALEBF may preferably alter the glucose transporter or glucokinase system with negligible insulin-tropic effects. Hence it was thought worthwhile to find the effect of ALEBF on insulin resistance models.

Diabetes is described as wasting diseases, since in diabetic condition leads to loss of muscle mass. Insulin is said to be anabolic hormone responsible for conversion of glucose to glycogen and stored in skeletal muscle and liver. Apart from this insulin also inhibits lipolysis and helps in deposition of adipose tissue (Guyton, 2006). In alloxan induced model due to lack of insulin production these anabolic effect of insulin will be hampered leading to wastage of muscle mass, resulting in progressive decreased in body weight of the animals and death. Treatment with our ALEBF had substantially reduced the decrease in body weight and mortality produced by alloxan.

Dexamethasone increased triglyceride levels causing an imbalance in lipid metabolism leading to hyperlipidemia and increase in glucose level leading to hyperglycemia. Dexamethasone can cause metabolic changes resulting in decrease in food consumption and decrease in body weight. Profound obesity due to alteration of ob gene expression often associated with development of insulin resistance with enhanced BGL due to impairment of GLUT4 translocation (Hideyuki *et al*., 2000). Occurrence of hyperglycemia and hyperinsulinemia in dexamethasone administered rats may be due to the above mentioned mechanisms. Treatment with ALEBF could control the dexamethasone induced hyperglycemia and hyper-insulinemia suggesting ALEBF could increase the peripheral utilization of glucose by acting on transporter system.

In conclusion, the above observations have clearly demonstrated that ALEBF, as a folk remedy, exerts effectual hypoglycaemic activity in normal and diabetic rats, which confirms the folkloric utilization. Further studies should be conducted to isolate the active ingredients and to know the mechanism of the isolated compound.

ACKNOWLEDGEMENTS

The authors like to thanks the management members of Manipal University and PES College of Pharmacy for financially supporting this research work. We would like to thank Dr. K. P. Sreenath, HOD, Department of Botany, Bangalore University for identifying the plant specimen.

REFERENCES

Anonyms (2006). www.sciencedirect.com/science/ mechanism of action of alloxan and strepzotocin\.mht [cited 2006 Dec 11th].

Committee Report (1997). Report on expert committee on the diagnosis and classification of diabetes mellitus. *Diabetes Care*, **20**: 1183-1197.

Guyton AC and Hall JE (2006). A Text Book of Medical Physiology. 6th Edition, Elsevier Saunders, Philadelphia, pp.971-973.

Hideyuki S, Takehide O, Motonobu A, Makoto F, Kouichi I and Hideki K (2000). Dexamethasone-induced insulin resistance in 3T3-L1 adipocytes is due to inhibition of glucose transport rather than insulin signal transduction. *Diabetes*, **49**: 1700-1708.

Khandelwal KR (2000). Practical Pharmacognosy Techniques and Experiments. Nirali Prakashan, Pune, India, pp.149-150.

Yoshikawa M, Xu F, Morikawa T, Pongpiriyadacha Y, Nakamura S, Asao Y, Kumhara A, Mastuda H **(**2007). Medicinal Flowers XII (1) New Spirostane-Type Steroid Saponins with antidiabetogenic activity from *Borassus flabellifer*. *Chem. Pharm. Bull.*, 55: 308-316.

Mohan V, Sandeep S, Deepa R, Shah B and Varghese C (2007). Epidemiology of type 2 diabetes: Indian scenario. *Indian J. Med. Res.*, **125**: 217-230.

Morton JF (1988). Notes on distribution, propagation, and products of *Borassus palms* (Arecaceae). Economic Botany, pp.420-441.

Nadkarni KM and Nadkarni AK (2000). Indian Materia Medica, Popular Prakashan. Bombay, India, pp.209-210.

Nammi S, Boini MK, Lodagala SD and Behara RBS (2003). The juice of fresh leaves of *Catharanthus roseus* Linn. reduces blood glucose in normal and alloxan diabetic rabbits. *BMC Complementary and Alternative Medicine*, **3**: 4.

Noor A, Gunasekaran S, Manickam S and Vijayalakshmi MA (2008). Antidiabetic activity of *Aloe vera* and histology of organs in streptozotocin induced diabetic rats. *Curr. Sci.*, **94**: 1070-1076.

Rang HP, Dale MM, Ritter JM, Moore PK (2003). *Pharmacology*. 5th Edition, New Delhi: Churchill Livingstone, pp.397-409.

Saravanan R and Pari L (2005). Antihyperlipidemic and antiperoxidative effect of Diasulin, a polyherbal formulation in alloxan induced hyperglycemic rats. *BMC Complementary and Alternative Medicine*, 5: 14.

WHO Expert Committee on diabetes mellitus (1980). Technical Report Series 646, Second Report. World Health Organization, Geneva.