

**EVALUATION OF ANTI-DIABETIC POTENTIAL OF *ALBIZZIA LEBBEK* BARK
IN NORMAL AND ALLOXAN-INDUCED DIABETIC MICE.**

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Summary

Bark of *Albizzia lebbek* (Family-Mimosaceae), reported to have a large number of medicinal properties was evaluated for its hypoglycemic and anti-hyperglycemic activity. Administration of different doses of aqueous-methanol extract of *A. lebbek* bark lowered blood glucose level of normal and alloxan-induced diabetic mice in a dose- and time-dependent manner. The higher dose of 450 mg/kg body weight proved to be lethal to normal mice. In diabetic mice, however, a prolonged anti-hyperglycemic activity of the extract was observed with no apparent toxicity at the same dose. Glucose tolerance was similarly improved in both normal and diabetic mice on administration of the extract. Metformin, Glibenclamide and Insulin were used as reference drugs for comparison.

Keywords: Hypoglycemic, Anti-hyperglycemic, Alloxan, *Albizzia lebbek*

Introduction

Diabetes mellitus is a chronic metabolic disorder which affects a significant portion of the population worldwide [1, 2]. Both type 1 and type 2 diabetes are known to be multifactorial diseases caused by a combination of genetic (inheritance) and environmental (diet and lifestyle) factors [3, 4]. Its most prevalent form, type 2 diabetes mellitus, in which people are afflicted worldwide, is characterized by peripheral insulin resistance with an insulin-secretory defect resulting in elevated levels of sugar in the plasma [5, 6]. In addition to hyperglycemia, diabetic patients exhibit altered metabolism of lipids, carbohydrates and proteins and an increased risk of developing atherosclerotic arterial disease [7, 8]. In fact, chronic hyperglycemia has been established to be the principal cause of diabetic microvascular and macrovascular complications [9, 10].

Treatment of type 2 diabetes mellitus with insulin and oral hypoglycemic agents, unfortunately, has adverse side-effects associated with them [11, 12, 13, 14, 15]. Therefore, the search for safer and more effective drugs has continued. Herbal remedies have gained importance throughout the world because of their fewer side-effects and relatively low costs [16, 17, 18]. Subsequently, many investigations of oral anti-hyperglycemic agents of plant origin used in traditional medicines have been conducted and many of the plants have been reported to possess activity [19, 20, 21, 22]. Different mechanisms are involved in reducing blood sugar level by plants and their extracts. Some plants are known to exert hypoglycemic effect by involving pancreatic mechanism like the sulphonylurea, glibenclamide [23, 24]. Others exhibit anti-hyperglycemic effect through extrapancreatic mechanism as the biguanide, metformin [25, 26]. The mechanism of action for some of these active principles has also been described [27].

Further, *Momordica charantia* has been reported to have P-insulin (or v-insulin) which is a large polypeptide that is structurally and pharmacologically comparable to insulin [28], besides having a role in the renewal of beta cells in STZ-diabetic rats or alternately may permit the recovery of partially destroyed beta cells [29]. In India, many plants have also been reported to possess anti-diabetic property [30, 31, 32, 33].

Albizzia lebbek B. of the Mimosaceae family, a large tree with dark grey or brownish bark native to deciduous and semi deciduous forests in Asia has been reported to have a large number of medicinal properties. The bark which has acrid taste has been used for bronchitis, leprosy, paralysis and helminth infections [34]. Literature survey conducted has shown that there is no report on the hypoglycemic and anti-hyperglycemic effects of *A. lebbek* on blood glucose level. The present investigation was, therefore, undertaken to study the effects of *A. lebbek* bark extract on fasting blood glucose level and glucose tolerance in normal as well as alloxan-induced diabetic mice. The results were compared with those of insulin, glibenclamide and metformin to provide some insight into its mechanism of action.

Materials and Methods

Chemicals

Alloxan was procured from Sigma Co. USA, Glibenclamide from Hoechts, Insulin from Knoll Pharmaceutical Ltd., Metformin from USV limited, Maharashtra, while other chemicals used were of analytical grade obtained from E. Merck and Hi-media, India.

Test Animals

Healthy, adult female Swiss albino mice, weighing 20-30 gms were used for the study. Mice were housed in a room kept under controlled conditions with temperature maintained at 22 °C on a twelve hour light/dark cycle and were fed with balanced mice feed obtained from Amrut Laboratory, Pune, India. Institutional guidelines were followed for all experiments.

Plant material

Bark of *A. lebbek* was collected from Meghalaya (Voucher No: 4192 NEHU). The specimen was submitted and identified by Dr. P.B.Gurung Curator herbarium, Department of Botany, NEHU, Shillong Meghalaya.

Extraction

The bark was separated, weighed, washed, shredded and dried in the shade. It was then powdered, homogenized and repeatedly extracted with 10 volume of aqueous-methanol solution (1:4) [35]. The mixture was filtered and the filtrate evaporated to dryness at 40 °C in a rotary evaporator (Yamato RE800). The dried mass obtained was used for the investigation. The yield of methanol extract (w/w from dried starting material) was 7 %. Prior to use, weighed powder was dissolved in 2% ethanol and centrifuged at low rpm for 10 minutes. The clear supernatant was used for further study.

Normoglycemic studies

Experimental design

Following the method used in our earlier studies [32], mice were divided into one control and four test groups to study the effects of varying doses of the extracts of *A. lebbek* in normal (Table-I) and diabetic mice (Table-II). Each group comprised 6 mice (n=6). Varying doses of the extract of *A. lebbek* ranging from 150-450 mg/kg body weight (b.w.) were administered to the test groups intraperitoneally (i.p) and glucose level was monitored at different time intervals up to 24 hours following administration of extract. The control group received only 2% ethanol, being the solvent used for preparation. Food, but not water was withheld during the test period not exceeding 24 h. Food, fluid intake and body weights were monitored for 4 weeks after administration of the extract.

Table –I

The animals were divided into five groups. Each group comprised of six mice.

1	Control	Normal mice untreated
2	T-1	Normal mice treated with 150 mg/kg b.w. of plant extract
3	T-2	Normal mice treated with 250 mg/kg b.w. of plant extract
4	T-3	Normal mice treated with 350 mg/kg b.w. of plant extract
5	T-4	Normal mice treated with 450 mg/kg b.w. of plant extract

Anti-hyperglycemic studies

Induction of non insulin dependent diabetes mellitus (NIDDM)

Animals were administered alloxan monohydrate (150 mg/kg b.w. i.p) prepared in acetate buffer (0.15 M, pH-4.5) as described earlier [32]. The control group received only the buffer. Prior to administration, mice were fasted overnight but given water *ad libitum*. Mice with more than 3-4 fold increase in their blood sugar levels were considered diabetic and used for further tests.

Administration of extract to alloxan-induced diabetic mice

Following the same experimental design (Table-II) as with normoglycemic studies, alloxan-induced diabetic mice were administered the test extract (i.p) at varying doses (150-450 mg/kg b.w.) and the blood glucose level was measured at varying time intervals. All animals treated were observed for behavioral changes like polydipsia and polyphagia.

Table –II

The animals were divided into five groups. Each group comprised of six mice

1	Control	Diabetic mice untreated
2	D1	Diabetic mice treated with 150 mg/kg b.w. of plant extract
3	D2	Diabetic mice treated with 250 mg/kg b.w. of plant extract
4	D3	Diabetic mice treated with 350 mg/kg b.w. of plant extract
5	D4	Diabetic mice treated with 450 mg/kg b.w. of plant extract

Oral Glucose Tolerance Test (OGTT)

Experimental design

Mice were divided into one control and four test groups to study the glucose tolerance in normal (Table-III A) and alloxan-induced diabetic mice (Table-III B) following administration of the effective dose of extract of *A. lebbek*. Normal or alloxan-diabetic mice, fasted overnight but provided water *ad libitum*, were administered the test samples intraperitoneally 1.5 h prior to the oral glucose load of 2 gm/kg b.w. according to the method used earlier [32]. Glucose concentration was measured before administration and subsequently at 30, 60, 120, 480 and 1440 minutes after the glucose load. The control group received only the glucose load, while the reference drugs metformin [36], glibenclamide [37], and insulin [38] were administered following the respective cited method. Each group comprised of 6 mice.

Toxicity studies

Normoglycemic mice were administered up to a dose of 450 mg/kg b.w. and kept under observation up to 4 weeks for any signs of distress, convulsion, coma or death [39].

Collection of Blood and Determination of Blood Glucose level

Blood samples from the control and experimental mice were collected by orbital sinus puncture using heparinised capillary glass tubes [24]. The blood samples so collected were analyzed for glucose levels employing glucoStix with the glucometer (Ames).

Statistical Analysis

Student's 't'-tests was used for determining the levels of significance between the control and the test values. Results are expressed as Mean \pm SEM.

Table-III

(A) Glucose Tolerance Test on normal mice

The animals were divided into five groups. Each group comprised of six mice

1	Control	Mice given oral glucose load of 2 gm/kg b.w.
2	OT-1	Mice given oral glucose load of 2 gm/kg b.w. + 250 mg/kg b.w. of plant extract
3	OT-2	Mice given oral glucose load of 2 gm/kg b.w. + 10 mg/kg b.w. of glibenclamide
4	OT-3	Mice given oral glucose load of 2 gm/kg b.w. + 500 mg/kg b.w. of metformin
5	OT-4	Mice given oral glucose load of 2 gm/kg b.w. + 10 U/kg b.w. of insulin

(B) Glucose Tolerance Test on diabetic mice

The animals were divided into five groups. Each group comprised of six mice.

1	Control	Mice given oral glucose load of 2 gm/kg b.w.
2	OT-D1	Mice given oral glucose load of 2 gm/kg b.w. + 250 mg/kg b.w. of plant extract
3	OT-D2	Mice given oral glucose load of 2 gm/kg b.w. + 10 mg/kg b.w. of glibenclamide
4	OT-D3	Mice given oral glucose load of 2 gm/kg b.w. + 500 mg/kg b.w. of metformin
5	OT-D4	Mice given oral glucose load of 2 gm/kg b.w. + 10 U/kg b.w. of insulin

Results**Normal mice**

The effect on blood glucose level of normoglycemic mice after intraperitoneal administration of the methanol extract of *A. lebbek* bark at varying doses showed significant reduction, in a time- and dose-dependent manner (Fig 1). Hypoglycemic effect was observed for all the doses used (150-450 mg/kg b.w.). At the doses of 150 mg/kg b.w. and 250 mg/kg b.w. the blood glucose levels were observed to be minimal at 4 hrs being 39% and 35% respectively from that of control. However, at 350 mg/kg b.w., a prolonged reduction was observed even at 24 hours with glucose level being 28% from that of control. The higher dose of 450 mg/kg b.w. was found to be toxic with severe hypoglycemia at 4 hours with glucose level being 29% from that of control. Mice, however, did not survive beyond 6 hours following administration of the extract at this dose.

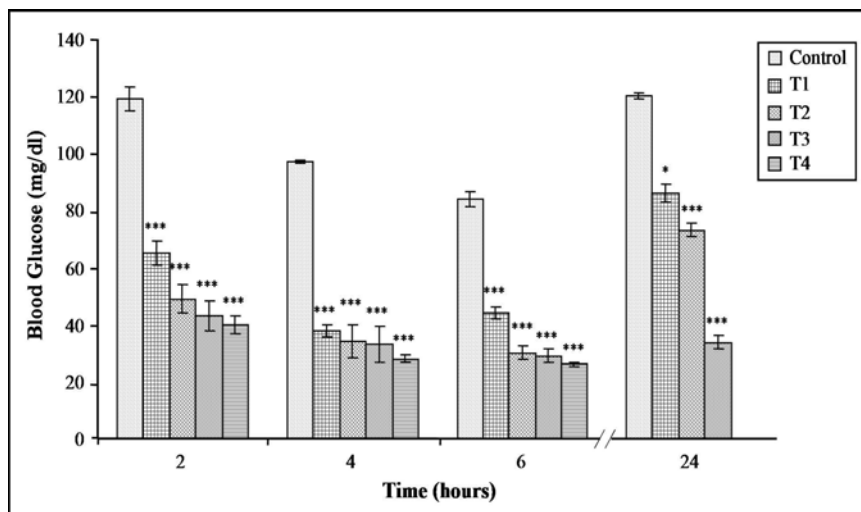


Fig 1: Effect of different doses of methanol extract of *A. lebbek* on fasting blood glucose level of normal mice assayed at different time intervals. Values are expressed as Mean \pm SEM. (*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$). Experimental details are described under materials and methods.

Diabetic mice

Intraperitoneal administration of the methanol extract of *A. lebbek* bark to overnight fasted alloxan-induced diabetic mice also elicited a marked and prolonged anti-hyperglycemic effect in a time- and dose- dependent manner similar to that observed in normal mice (Fig-2). The anti-hyperglycemic effect was observed for all the doses used but was more pronounced at the 24th hour following extract administration. Blood glucose levels were found to be 39%, 32%, 18% and 14% from that of control at 24 hours at the doses of 150 mg/kg b.w., 250 mg/kg b.w., 350 mg/kg b.w. and 450 mg/kg b.w. respectively. Significantly, no fatality was observed for all these doses.

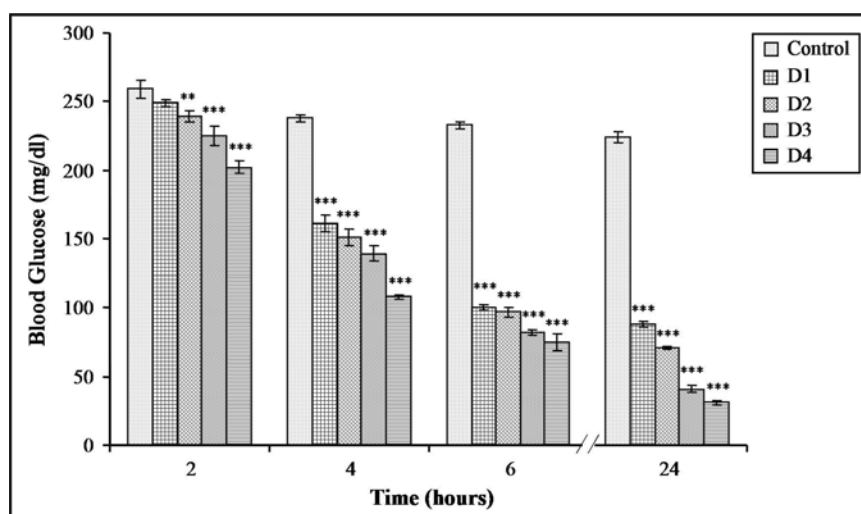


Fig 2: Effect of different doses of methanol extract of *A. lebbek* on fasting blood glucose level of diabetic mice assayed at different time intervals. Values are expressed as Mean \pm SEM. (*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$). Experimental details are described under materials & methods.

Glucose Tolerance Test

Intraperitoneal administration of the extract (250 mg/kg b.w.), one and a half hours prior to the oral glucose load to overnight fasted normoglycemic mice improved glucose tolerance (Fig-3). Blood glucose level was maximally suppressed at 480 minutes following oral glucose load with glucose level being 43% from that of control.

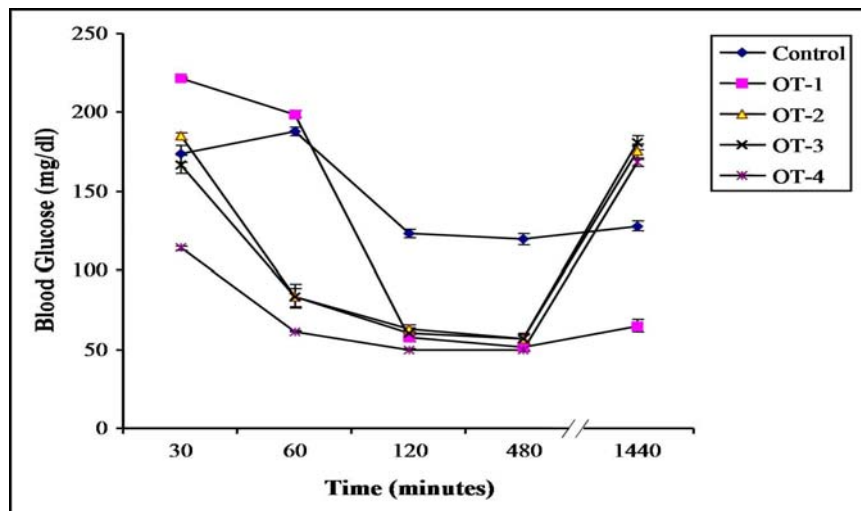


Fig 3: Oral Glucose Tolerance Test (OGTT) of *A. lebbek* extract (250 mg/kg b.w.) and reference drugs on blood glucose level of normal mice assayed at different time intervals. Values are expressed as Mean \pm SEM. Experimental details are described under materials and methods.

Glucose tolerance in alloxan-induced diabetic mice exhibited a similar pattern to that of normal mice wherein maximum suppression of the glucose peak was also observed at 480 minutes following oral glucose load with glucose level being 76% from that of the control.

Toxicity studies carried out on normal mice up to a dose of 350 mg/kg b.w. did not show any adverse effects during the 4-weeks of observation. However, the higher dose of 450 mg/kg b.w. resulted in severe hypoglycemia followed by death within 24 h following administration of extract. In contrast, in diabetic mice, this dose (450 mg/kg b.w.) did not prove to be fatal which indicated that in normal mice hypoglycemia could be the probable cause of death.

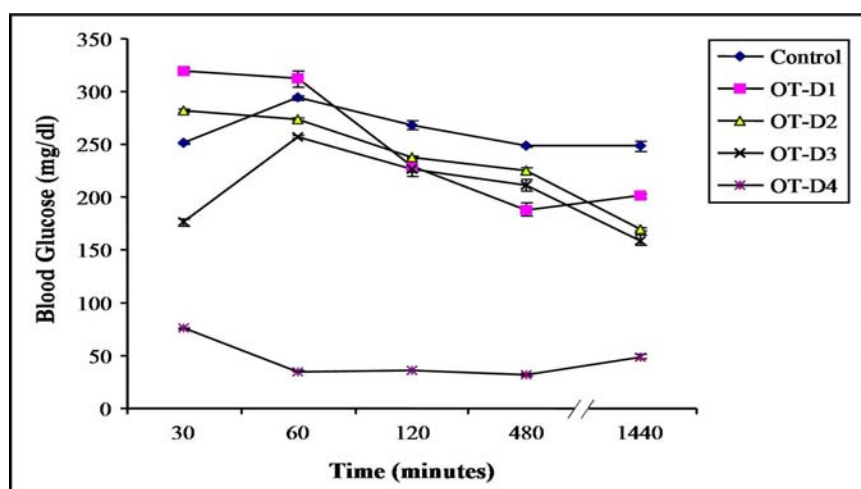


Fig 4: Oral Glucose Tolerance Test (OGTT) in alloxan-diabetic mice treated with extract of *A. lebbek* (250 mg/kg b.w.) and reference drugs, assayed at different time intervals. Values are expressed as Mean \pm SEM. Experimental details are described under materials and methods.

Discussion

The results obtained in this study indicated that the extract of *A. lebbek* bark exerted hypoglycemic as well as anti-hyperglycemic activity in a dose- and time- dependent manner. The hypoglycemic effect may be compared to glibenclamide, a sulphonylurea which is known to cause hypoglycemia by increasing the secretion of insulin from the pancreas [40, 41]. It is active in mild alloxan-induced diabetes but inactive in intense alloxan diabetes [42]. The significant decrease in the level of fasting blood glucose in diabetic mice treated with the extract may be due to stimulation of the residual pancreatic mechanism and enhanced peripheral glucose utilization, similar to glibenclamide [23, 43].

Similarity to metformin, a biguanide, which produces anti-hyperglycemic effect, is ruled out as this drug does not cause hypoglycemia in the normal state. Metformin acts by decreasing hepatic glucose production and intestinal absorption as well as increasing peripheral glucose uptake and insulin sensitivity [44, 26]. The finding that *A. lebbek* bark extract effect hypoglycemia in the normal state suggests that this plant extract influenced blood glucose levels by mechanism comparable to glibenclamide. Death in normal mice at the higher dose of 450 mg/kg b.w. was most probably due to pronounced hypoglycemia, as the fatality was not observed with diabetic mice at the same dose.

A prolonged glucose lowering effect was observed in diabetic mice which persisted even at 24 h. The more prolonged and increased effect of the extract in diabetic mice compared to normal mice may be due to the limited or compromised action of insulin in diabetic condition and therefore, a greater role for the hypoglycemic principle present in the extract.

The oral glucose tolerance test (OGTT) which is the 'gold standard' for the diagnosis of diabetes mellitus [45], reflecting the extent of intestinal glucose absorption and hepatic glucose metabolism was similarly improved in both normal as well as alloxan-induced diabetic mice. In Fig 3 and 4, the suppression of the peak by the test extract suggested that the extract contains principle(s) that are comparable to both the oral anti-diabetic agents, glibenclamide and metformin in pattern and magnitude of effect.

While it is tempting to infer a glibenclamide-type of action as reported for *Curculigo orchoides* [46] and *Trigonella foenum-graecum* [47], however, the magnitude of effect in the reduction of glucose level at 480 minutes (Fig 4) was much higher for the extract (24%) compared to glibenclamide (9%) but not as high as insulin (87%). Other probable mechanisms by which the methanol extract of *A. lebbek* bark lowered blood glucose level in both normal and alloxan-induced diabetic mice could be a more direct insulin-like effect as has been reported for *Momordica charantia* L [48].

In conclusion, the methanol extract of *A. lebbek* bark exhibited both hypoglycemic and anti-hyperglycemic activities in mice. Further comprehensive pharmacological investigations are needed to elucidate the exact mechanism of the plant to access its efficacy in the treatment of diabetes.

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