EFFECTS OF AQUEOUS METHANOL EXTRACT OF *ALBIZZIA LEBBEK BENTH* SEEDS ON VARIOUS BIOCHEMICAL PARAMETERS IN ALLOXAN-INDUCED DIABETIC RABBITS

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Summary

In this study we observed that methanol extract of *Albizzia lebbek Benth* significantly decreased in fasting blood glucose levels, total cholesterol, Triglycerides, serum Glutamate Oxaloacetate and Pyruvate Transaminase (sGOT and sGPT) while it causes increase in the High density lipids (HDLs), however it could not produce any significant change in serum Creatinine clearance in alloxan-induced diabetic rabbits. It also increased the weights of diabetic treated rabbits. In normal rabbits, both methanol extract of *A.lebbek B* seeds and gliclizide produced a significant decrease ($P<0.05$; $P<0.001$) in blood glucose level. The hypoglycemic effect of methanol extract of *A.lebbek B* seeds in normal rabbits was comparable with gliclizide. In Alloxan induced diabetic rabbits, methanol extract of *A.lebbek B* seeds produced a significant decrease ($P<0.001$) in blood glucose level. While, gliclizide could not produce any significant hypoglycemic effect in alloxan-induced diabetic rabbits.

A significant increase ($P<0.05$) in the weights of treated diabetic rabbits receiving 200 mg/kg and 250 mg/kg body weight methanol extract of *A.lebbek B* seeds was noted. These doses also significantly ($P<0.05$) lowered the plasma total Cholesterol, Triglyceride and Low-density lipids (LDLs) in treated rabbits as compared to diabetic rabbits. Furthermore, same dose also significantly ($P<0.05$) increase the plasma HDL levels of treated group when compared with diabetic group. Feeding methanol extract of *A.lebbek B* seeds (200 mg/kg and 250 mg/Kg) improved the liver function in diabetic rabbits as shown by reduction in serum sGOT and sGPT. Acute toxicity studies showed that 2500 mg/kg body weight methanol extract of *A.lebbek B* seeds could not produce any lethal effect in rabbits. This is the first pilot study to provide biochemical evidence of potential of *A.lebbek B* in diabetes.

Kyewords: *Albizzia lebbek Benth*, methanol extract, gliclizide, hypoglycemic effect,

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Introduction

A.lebbek B (*Family: Leguminoseae, Sub Family: Mimosaceae*) commonly called the *siris* tree is native to tropical Africa, Asia and northern Australia, widely planted and naturalized through out the tropics. It is reported to posses anxiolytic (1), Anticonvulsant (2), Antifertility (3), nootropic (4), Antidiarrhoeal (5) and antiasthmatic activity (6).
Traditional herbs and plants have been used for the treatment of various diseases (7, 8). However, still a large number of herbs claimed to be useful in the treatment of diabetes have not been screened (9). A.lebbek B is a well known medicinal plant that has been used in the traditional medicines for treating various diseases including diabetes mellitus (9,10). Therefore, in this study, we have evaluated the antidiabetic activity of aqueous methanol extract of A.lebbek B to establish scientific evidence in support of the folkfore claim. Furthermore, A.lebbek B is having Antihyperlipidemic effect in asthmatics (11). Whereas, experimental diabetes in rabbits use to alter the lipid profile (12,13). So, we also studied its effect on lipid profile, liver (sGOT and sGPT) and kidney function (Creatinine clearance) in Alloxan induced diabetic rabbits.

Materials and Methods

Plant material
A.lebbek B seeds were collected from the *A.lebbek B trees*, situated on the campus of Bahauddin Zakariya University, Multan, Pakistan. They were identified and authenticated by Dr. Bukhari department of Botany (Ex Director) Bahauddin Zakariya University, Multan, Pakistan. The seeds were shade dried and powdered finely with a Chinese herbal grinder.

Preparation of Extract
Seeds were Soxhlet-extracted with aqueous methanol mixture (70 methanol: 30 water). Extract was dried with the help of Rotary evaporator at 40 ^{0}C. The yield of dried aqueous methanol extract was 21%.

Chemicals and Drugs
Alloxan monohydrate and Methanol were of Sigma Chemical Co. The gliclizide was obtained from Servier Research and Pharmaceuticals, Lahore, Pakistan. Glucose oxidized kit (Accu Check advantage II) was that of Roche Chemicals, Switzerland.

Biochemical analysis
The blood glucose levels were measure using Glucose oxidized kits. The serum total Cholesterol,
Triglyceride, HDLs and LDLs , sGOT and sGPT levels were evaluated by enzymatic test kits (Sigma chemical co).
LDLs level was calculated by using following formula
LDL= total cholesterol – HDL – (triglyceride/5) (14).
Creatinine clearance was determined with the help of Chemistry analyzer Microlab 200 (Merck).

Animals
Male albino rabbits belonging to the local strain (Oryctolagus cuniculus) with average weight 1.26 Kg and a range of 1.0-1.5 Kg were used.

Treatment of Rabbits
All animals were housed at the animal house of Department of Pharmacy Bahauddin Zakariya University Multan .Animals were housed in stainless cages under standard laboratory condition (light period 8.00 a.m to 8.00 p.m 21±2 ^{o}C , relative humidity 55% , green fodder and water available ad labitum . Animals received human care. The study protocol was approved by the local ethical committee.

Induction of diabetes and experimental design
Rabbits were divided into four experimental groups (control, diabetic and diabetic with A.lebbek B extract treatment). At the start of the experiment the animals in the latter three groups were injected intravenously with 100 mg/kg of 10%, alloxan (Sigma Chemical Co., St Louis, MO, USA) dissolved in isotonic saline to induce diabetes (12). The control group was injected only with the same volume of isotonic saline as the diabetic groups received. Three days after alloxan injection DM was confirmed by the demonstration of hyperglycemia (Blood glucose >250 mg/dl).

Hypoglycemic activity in normal rabbits
Rabbits were divided into three groups, normal control, normal treated and gliclizide receiving group. Normal treated group was further subdivided into 3 different doses groups (1) receiving 100 mg/kg body weight *Albizzia lebbek B* seed extract (2) receiving 200 mg/Kg body weight A.lebbek B seed extract (3) receiving 250 mg/Kg body weight A.lebbek B seed extract. Gliclizide receiving group was treated orally with 25mg/kg Gliclizide, used as a standard (15). Blood samples of rabbits were taken at 0, 2 hour, 4 hour and 6 hour of treatment and blood glucose level was determined.

Hypoglycemic activity in diabetic rabbits
Rabbits were divided into 4 different groups (1) normal control (2) Diabetic control (3) Diabetic treated (4) Gliclizide treated diabetic group
Diabetic treated group was further subdivided into three groups based on dose of A.lebbek B seeds extract, receiving 100 mg/kg , 200 mg/Kg , 250 mg/Kg body weight A.lebbek B extract every day for one month after induction of DM was confirmed. At the end of experimental period (one month), the animals in all three groups were fasted for 12 hours (over night) and blood samples were taken for biochemical analysis.

Toxicity study
Acute toxicity was determined according to the method of Litch and Wilcoxon (16) by observing the deaths of rabbits (n=10) treated with the extract in the dose range of 200-2500 mg/Kg , which revealed that extract is safe to use even at the doses of 2.5 gm/Kg of body weight orally.

Statistical analysis

The data expressed was expressed as mean ± standard deviation (SD) and analyzed using analysis of variance (ANOVA) .Tukey's test was used to test for differences among means for which ANOVA indicated a significant (P<0.05) F ratio.

Results

Hypoglycemic activity in normal rabbits

Treatment with methanol extract of *A.lebbek B* significantly (P<0.05 or P<0.001) decreased the blood glucose levels of normal treated groups in different doses groups (100 mg/kg, 200 mg/kg, 250 mg/kg body weight A.lebbek B extract respectively). Gliclizide also caused significant decrease (P<0.001) in the blood glucose levels in normal treated rabbits. Hypoglycemic effect shown by the methanol extract of A.lebbek B seeds (200 mg/kg, 250 mg/kg body weight A.lebbek B extract respectively) was comparable with gliclizide.Blood levels of different groups are s hown in Table 1.

Table1. Effect of Albizzia lebbek Benth seeds extract on blood glucose level in normal rabbits at 0, 2, 4 and 6 hours.

Group	Blood glucose level (mg /dl)			
	0 hour	2 hour	4 hour	6 hour
Normal	103.4±6.5	103.5±3.4	102.7±4.5	102.6±5.5
Normal treated				
100 mg/Kg	107±3.5	99.10±8.3	91.57±10.24	86.73±9.3*
250 mg/Kg	106.68±8.4	94.42±10.21	86.31±6.5 *	77.39±7.1* *
Gliclizide treated diabetic Group (20mg/kg)	101.20±6.9	89.4±5.8*	76.64±7.9**	70.85±12.6* *

Number of rabbits in each group=5 Values are expressed as Mean±SD number of estaric shows level of significance (* P<0.05; ****** P < 0.01) compared to normal control group at various time intervals

Hypoglycemic activity in diabetic rabbits

Blood glucose concentration of diabetic rabbits 270.6± 6.05 (Mean ±SD) was increased significantly (P<0.001) as compared to the control group. In control, group level of blood glucose was 102.2±5.51. Treatment with methanol extract of *A.lebbek B* significantly (P<0.05; P<0.01;P<0.001) decreased the blood glucose levels of diabetic treated groups with

mean values 160.28±11.61,139.70±8.02 and 122±4.34 in different doses groups (100 mg/kg , 200 mg/kg , 250 mg/kg body weight A.lebbek B extract respectively) . Blood glucose levels of treated diabetic groups receiving 200 mg/kg and 250 mg/kg body weight *A.lebbek B* extract were with in normal range (75-150 mg/dl). While, gliclizide could not produce any significant hypoglycemic effect in alloxan-induced diabetic rabbits. Blood levels of glucose of control, diabetic and treated groups are shown in Table 2.

Table 2. Effect of *Albizzia lebbek Benth* seeds on blood glucose level in alloxan induced diabetic rabbits for 1 month.

Group	Blood glucose level (mg /dl)
Normal control	102.2±15.5
Diabetic control	270.20±6.05
Diabetic treated	
100 mg/Kg	160.28±18.61*
200 mg/Kg	137.70±8.12* *
250 mg/Kg	120.34±14.21* **
Gliclizide treated diabetic Group (20 mg /kg)	273.54±18.32

Number of rabbits in each group=5 Values are expressed as Mean±SD number of estaric shows level of significance (* P<0.05; ****** P < 0.01**. ***** P < 0.001) compared to diabeteic control group.

Effect on weight of rabbits

Weight of rabbits was measured at o day and 30 day in different experimental groups. A significant decrease (P<0.05) in weights of diabetic rabbits was observed when the weights measured at 0 day and 30th day were compared. While a significant increase (P<0.05; P<0.01) in weights of treated diabetic rabbits receiving 200 mg/Kg and 250 mg/Kg body weight of A.lebbek B aqueous methanol extract was noted, when the weights of treated diabetic rabbits measured at 0 day and 30 day were compared.
Weights of control, diabetic and treated groups are shown in Table 3.

Table 3. Effect of Albizzia lebbek Benth seeds on weight in alloxan induced diabetic rabbits for 1 month.

Group	Weight at 0 day (Kg)	Weight at 30th day (Kg)
Normal control	1.30±0.2	1.28±0.7
Diabetic control	1.40±0.5	0.9±0.6
Diabetic treated		
100 mg/Kg	1.48±0.4	1.18±0.3
200 mg/Kg	1.38 ±0.8	1.42±0.5*
250 mg/Kg	1.23±0.6	1.52±0.2 **

Number of rabbits in each group=5 Values are expressed as Mean±SD number of estaric shows level of significance (* P<0.05; ****** P < 0.01) compared to O day treatment.

Hypolipidemic effects

Cholesterol, Triglyceride and LDL values increased significantly (P<0.05; P<0.01; P<0.001) in diabetic rabbits. Where as HDL values decreased significantly (P<0.05) in diabetic rabbits. A.lebbek seeds treatment decreased the cholesterol, Triglyceride and LDL values and increased HDL values significantly (P<0.05) in diabetic treated rabbits. Lipid profile of normal, diabetic and treated diabetic rabbits is shown in Table 4.

Table 4. Effect of Albizzia lebbek Benth seeds extract on plasma cholesterol, triglyceride, LDLs and HDLs in alloxan-induced diabetic rabbits for 1 month.

Group	Total cholesterol (mg/dl)	Triglyceride (mg/dl)	LDLs (mg/dl)	HDLs (mg/dl)
Normal	40.4±2.17	50.6±10.80	2.68±0.12	27.6±1.14
Diabetic control	60.20±2.42	134.2±30.7	11.76±7.61	21.6±2.07
Diabetic treated				
100 mg/kg	55.8 ±5.98	84.6±14.16******	10.8 ±3.30	29.4 ±1.51
200 mg/kg	50.8±2.60	76±18.2 5******	4.6±2.93	31.20±2.28
250 mg/kg	40.21±2.88*****	69.3 ±5.37*******	2.75 ±1.59*****	32.6±2.3*****

Number of rabbits in each group=5 Values are expressed as Mean±SD number of estaric shows level of significance (* P<0.05; ****** P < 0.01; ******* P < 0.001) compared to diabetic control

Effect on sGOT and sGPT values

sGOT and sGPT values increased significantly ($P < 0.01$; $P < 0.001$) in diabetic rabbits. A.lebbek B extract treatment decreased ($P<0.05$) sGOPT and sGPT values in diabetic rabbits. The effects on sGOPT and sGPT values in normal, diabetic and treated diabetic rabbits are shown in table 5.

Table 5. Effect of the Albizzia lebbek Benth extract on plasma sGOT and sGPT levels in alloxan- induced diabetic rabbits for 1 month.

Group	sGOT (U/dl)	sGPT (U/dl)
Normal	28 ±5.78	26±3.58
Diabetic control	160.6±23.6	173±25.8
Diabetic treated		
100 mg/kg	107.25±11.61**	113.25±18.05**
200 mg/kg	41.3± 29.31***	38.3±12.35***
250 mg/kg	30.3±13.3***	32.0±30.1 ***

Number of rabbits in each group=5 Values are expressed as Mean±SD number of estaric shows level of significance (** $P < 0.01$; ***$P < 0.001$) compared to diabetic control.

Creatinine clearance

Creatinine clearance values were not significantly ($P>0.05$) changed in diabetic rabbits. Table 6 shows values of creatinine clearance in control, diabetic and treated diabetic rabbits.

Table 6. Effect of the *Albizzia lebbek Benth* extract on creatinine clearance values in alloxan-induced diabetic rabbits for 1 month.

Group	Creatinine clearance
Normal	1.2±0.2
Diabetic	1.4±0.5
Diabetic treated	
100mg/kg	1.4±0.5
200mg/kg	1.31±0.3
250 mg/kg	1.3±0.1

Number of rabbits in each group=5 Values are expressed as Mean±SD

Discussion

The results obtained in the present study indicated administration of aqueous methanol extract of A.lebbek B to the normal rabbits, produced significant decrease in blood glucose level. Gliclizide also produced a significant decrease in blood glucose level in normal rabbits
Furthermore, present study indicated that administration of aqueous methanol extract of A.lebbek B to the diabetic rabbits produced significant decrease in blood glucose level. Whereas the blood glucose level of diabetic rabbits receiving 200 mg/Kg and 250 mg/Kg body weight of A.lebbek B aqueous methanol extract were with in normal range (75-150 mg/dl). In diabetic rabbits gliclizide could not produce any significant effect on blood glucose level. The results shown by gliclizide in our study are in line with the previous findings (17). When the weights of treated diabetic rabbits measured at 0 day and 30^{th} day were compared, A significant increase ($P<0.05$) in weights of treated diabetic rabbits receiving 200 mg/Kg and 250 mg/Kg body weight of A.lebbek B aqueous methanol extract was noted. The antidiabetic effect of different plants and miniral compounds such as Shilagit and Ficus bengalensis (18,19) have been found to be associated with their weight promoting effect. The antidiabetic effect shown by A.lebbek B seeds could be through its weight promoting action as noted in treated diabetic rabbits.
Hypercholesterolemia and hypertriglyceridemia have been reported to occur in alloxan induced diabetic rabbits (13,14) and a significant increase observed in our experiments was in accordance to these studies. Oral administration of A.lebbek B extract significantly reduced the total cholesterol and triglyceride in plasma as compared to diabetic group. Diabetic patients are more prone to atheromatous complications such as ischemic heart disease (20). It was found that oral administration of A.lebbek B seeds extract causes an increase in HDL and decrease in LDL levels that probably prevent the diabetic patients from atheromatous disease. Repeated administration of A.lebbek B extract thus had a beneficial effect on the hyperlipidemia associated with hyperglycemia. The strong antihyperlipidemic activity of A.lebbek B extract could be through its control of diabetes, as this is a major determinant of triglyceride, total cholesterol and LDL levels (13).
DM is the commonest cause of liver failure and hepatomegaly (21), which itself represents a huge and rapidly increasing problem. Liver failure is indicated by the increased level of sGOT and sGPT in blood. This study showed that A.lebbek B treatment decreased the elevated levels of sGOT and sGPT in A.lebbek B seeds treated diabetic group. This result shows that A.lebbek B may also decrease the risk of liver failure associated with DM. A.lebbek B treatment showed no effect on kidney function and the Creatinine Clearance values were not significantly different in three experimental groups ($p> 0.05$). Diabetes mellitus was unable to effect on kidney function test. Chronic diabetes mellitus causes renal failure (22). Taken together, these findings show that

1- Aqueous methanolic extract of A.lebbek seeds causes a decrease in blood glucose levels in alloxan induced diabetic rabbits.
2- A.lebbek seeds treatment tends to decrease diabetes induced rise in lipid levels and decreases the risk atheromatous disease.
3- A.lebbek seeds treatment also improves the liver function by decreasing sGOT and sGPT levels in diabetic rabbits. Clinical investigation of *A.lebbek seeds* may enhance the chances of commercial exploitation of this extract as antidiabetic as well as hypolipidemic because these two metabolic disorders follow each other .

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