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RESEARCH ARTICLE

Antihyperglycemic, Hypoglycemic and Cytotoxic Activity of *Albizia lebbek* and *Trigonella corniculata*

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ABSTRACT:

Antihyperglycemic, hypoglycemic and cytotoxic activities of methanolic extract of *Albizia lebbeck* and *Trigonella corniculata* were studied. Hypoglycemic action of plants was investigated using normoglycemic rabbits (acute study only), whereas antihyperglycemic activity was studied using alloxan induced diabetic rabbits (acute and chronic study). Results revealed both plant extracts and their mixture having significant anti diabetic potential at dose of 200mg/kg comparable to standard drug Glibenclamide (0.5 mg/kg). Brine shrimp assay was used for the determination of cytotoxicity and results showed plants to have low toxic potential (LD50>1000 µg). The study conducted proves both plants and their mixture to have high potential for the treatment of diabetes.

KEYWORDS: Antidiabetic; Alloxan; Rabbit; Methanolic; Ethnopharmacology.

INTRODUCTION:

Diabetes mellitus is a metabolic disorder found worldwide. It is characterized by hyperglycemia, the major sign of the disease, resulting from either defect in insulin secretion, action or both. Type II Diabetes is the most common type found in which there is decreased action of insulin together with low plasma insulin levels. In type I there is complete deficiency of insulin due to defect in the endocrine function of pancreas. Type II or NIDDM (non-insulin dependent diabetes mellitus) has spread like an epidemic in many countries and 90-95% of the diabetic patients suffer from this type. [1,2]

Toxicity studies are routinely performed by scientists on plant extracts and isolated compounds in order to determine their safety. Brine shrimp (*Artemia salina*) are often sensitive to bioactive compounds and small amount of sample can cause death of the animal thus proving the cytotoxic potential of the substance under investigation.[4,5]

Medicinal plants are of great importance for the treatment of diseases and different compounds isolated from them are used in modern medicine. *Albizia lebbeck* belongs to the family Fabaceae has been traditionally used for the treatment of diarrhea, dysentery, constipation and purities.[6]

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It has been studied for its anti-inflammatory, antibacterial, anti-oxidant, diuretic any many other activities thus proving it to be a very useful medicinal plant.[7-10] *Trigonella corniculata* is an annual herb that belongs to family Fabaceae. It is used as food and also as a medicinal herb for the treatment of diabetes and hypercholesterolemia. Studies show the plant having antioxidant and hepatoprotective activites.[11]

In the present study, we evaluated the antidiabetic and cytotoxic potential of *Albizia lebbeck* and *Trigonella corniculata*. Antidiabetic activity was studied in normal and alloxan induced diabetic rabbits with the extracts separately and the mixture of extracts whereas cytotoxic acivity was studied against *Artemia salina*.[3]

MATERIALS AND METHODS:

Plant material used:

The plant material was collected from different parts of Lahore. Plants were identified and authenticated by Dr. Muhammad Ajaib, Dr. Sultan Ahmed Herbarium, Govt. College University, Lahore. The voucher number for *Trigonella corniculata* and *Albizia lebbeck* were GC.Bot.Herb.2208 and GC.Bot.Herb.2209 respectively. The plant material was shade dried at room temperature and ground to coarse powder and stored in air tight polyethylene bags.

Preparation of extract:
3 kg of each powder was extracted twice using methanol of analytical grade. Each extraction continued for 7 days. The extracts were filtered through muslin cloth and then through filter paper and finally dried using rotary evaporator.

Chemicals:
Alloxan monohydrate and Glucose was purchased from Merck, Germany. Glibenclamide was provided by Aventis Pharmaceutical Industry. Methanol was purchased from Panreac, Spain. Etoposide was purchased from Pfizer Pharmaceutical industries. *Artemia* cyst form Ocean Nutrition, USA. Sea salt was purchased from a local aquarium shop.

Animals used:
Adult Rabbits of local breed from either sex were used. Rabbits having weight between 1-1.5 Kg were used in the study. The animals were housed in wooden cages under standard conditions (light period: 12 hours, relative humidity and temperature 25 ± 2^{o}C). The animals were provided free access to food (green fodder, leaves and pulses) and water *ad libitum*. The study protocol was approved by the ethical committee of The department of Pharmacy, The University of Lahore.

Induction of diabetes:
The overnight fasted rabbits were first fed with glucose solution (2 gm/kg in 10ml distilled water) and then after 30 min, injected with Alloxan monohydrate 150 mg/kg dissolved in normal saline.[13,14] Three days (72 hours) after injection blood glucose level was determined using glucose oxidase kit (Accu-check Active, Roche, Switzerland). Any rabbit having blood glucose level ranging from 300-350 mg/dL was considered diabetic and used for further study.

Experimental design:
Both antihyperglycemic and hypoglycemic effects were studied using diabetic and normal rabbits.[12]

Hypoglycemic study (acute):
The animals were randomly divided into 5 groups (n=5). Group I served as normal control and received vehicle only. Group II consisting of normoglycemic rabbits (NR) received Glibenclamide. Group III, IV and V (all NR) received *Trigonella corniculata* 200mg/kg, *Albizia lebbeck* 200mg/kg and a mixture of both plants 200mg/kg (100mg each). The study was performed at 0, 2, 4, 6, 8 hour interval after single dose.

Antihyperglycemic study (acute):
The animals were randomly divided into 6 groups (n=5). Group I served as normal control and received vehicle only. Group II, III and IV which consist of diabetic rabbits were administered *Trigonella corniculata* 200mg/kg, *Albizia lebbeck* 200mg/kg and their mixture 200mg/kg. The diabetic Group V received Glibenclamide. Acute study was performed at 0, 2, 4, 6, 8 hour interval after single dose.

Antihyperglycemic study (chronic):
For long term study in diabetic rabbits the animals were divided randomly into 6 groups (n=5). Group I served as normal control which received vehicle only. Group II, III and IV consisted of diabetic rabbits were administered *Trigonella corniculata* 200mg/kg, *Albizia lebbeck* 200mg/kg and their mixture 200mg/kg. The diabetic Group V received Glibenclamide. The study was performed only on Alloxan induced diabetic rabbits for 28 days and blood glucose level was determined after every 7 day interval.

Brine shrimp lethality assay:
Brine shrimp lethality was determined by the method described by Hussain et al., 2010 with some minor modifications.[15] The eggs were hatched in sterilized petridishes containing artificial sea water (38 g/L in D.W, pH 7.4) at 27^{o}C. After 48 hours the eggs hatched and mature free floating shrimps were used for the study.

20mg of extract was weighed in eppendorf tube and 2ml DMSO was added to make test solution. From this 5,50 and 500 µl was added to separate vials (10, 100 and 1000 µg/ml). 10 larvae were transferred to each vial and the volume was made 5ml with seawater. The vials were incubated at 27^{o}C for 24 hours under illumination and then the number of survived shrimps were counted. Etoposide was used as a standard drug. The experiment was performed in triplicate.

Statistical analysis:
The data was expressed as mean ± standard deviation (SD) and analyzed using analysis of variance (ANOVA). For Brine shrimp lethality test LD50 was calculated by probit analysis using SPSS statistical package.

RESULTS AND DISCUSSION:

Effects in normal rabbits after single dose (acute study):
Hypoglycemic effect of Albizia lebbek (ALME), Trigonella corniculata (TCME) and mixture after single dose in normal rabbits is shown in table 1. Both plants and mixture showed significant reduction in blood glucose level. The mixture was found to be more effective producing 14.6% reduction comparable to the standard group. TCME started lowering blood glucose at 6 h significantly ($P<0.05$) whereas at 8 h the glucose level decreased up to 13.2% ($P<0.001$). ALME produced much rapid effects starting at 2 h ($P<0.05$) and continuing till the end of study i.e. 8 h ($P<0.001$). These effects have also been shown in figure 1. TCME had slow onset of action but effects were similar to ALME and mixture. All three extracts showed high hypoglycemic activity compared to normal control and standard drug (glibenclamide) group.

Effects in Alloxan induced diabetic rabbits after single dose (acute study):
The antihyperglycemic study was carried on alloxan diabetic rabbits. After single dose treatment the blood glucose level decreased significantly in all three test groups when compared to normal control ($P<0.001$). TCME, ALME and Mix showed a maximum reduction of 12.8%,

31.6% and 29.6% respectively at 4 h. The effect of ALME and Mix was much higher than standard group i.e. 24.4% at the same time. ALME started showing significant activity at 1 h, where the other test groups at 2 h. The results are also shown in figure 2.

Table 1: Effect of single dose of TCME, ALME and their mixture on blood glucose level after administration in normal rabbits.

Group	Dose mg/kg	Duration; glucose level in mg/dl (mean ± S.D); percentage reduction					
		0 Hour	1 Hour	2 Hours	4 Hours	6 Hours	8 Hours
NC		134.80±3.701	133.60±3.362 0.9%	132.40±4.278 1.8%	132.80±5.891 1.5%	132.60±4.827 1.63%	132.60±4.722 1.63%
Drug	**0.5**	133.80±5.891	124.20±6.943* 7.2%	116.20±3.564*** 13.2%	113.00±3.391*** 15.5%	105.80±3.834*** 20.9%	98.80±2.864*** 26.2%
TCME	**200**	134.40±4.336	132.60±3.975 1.34%	130.40±3.435 3%	127.00±3.000 5.5%	125.20±2.387* 6.8%	116.60±3.362*** 13.2%
ALME	**200**	132.80±6.870	127.40±7.162 4.1%	124.20±6.301* 6.5%	121.00±5.657** 8.9%	116.80±5.630*** 12%	117.40±6.504*** 11.6%
Mix	**200**	133.00±5.874	131.00±5.148 1.5%	127.60±6.107*** 4.1%	120.40±5.128** 9.5%	118.00±3.873*** 11.3%	113.60±2.702*** 14.6%

* (P<0.05); ** (P<0.01); *** (P<0.001); **NC** normal control; **TCME** *Trigonella corniculata* methanolic extract; **AL** *Albizia lebbek* methanolic extract; **Mix** mixture of TCME and ALME 100+100mg; **Drug** glibenclamide.

Table 2: Effect of single dose of TCME, ALME and their mixture on blood glucose level after administration in alloxan diabetic rabbits.

Group	Dose mg/kg	Duration; glucose level in mg/dl (mean ± S.D); percentage reduction					
		0 Hour	1 Hour	2 Hours	4 Hours	6 Hours	8 Hours
NC		135.80± 3.701	134.20±2.864 1.2%	132.80±4.266 2.2%	131.40±5.128 3.2%	132.80±4.817 2.2%	134.60±3.912 0.9%
DC		314.20± 14.601	309.60±14.328 1.5%	303.60±14.639 3.4%	299.20±14.061 4.8%	304.60±15.274 3.1%	310.20±14.822 1.3%
TCME	**200**	304.20± 19.942	295.60±20.440 2.8%	284.40±19.970* 6.5%	265.40±14.206*** 12.8%	271.80±14.721** * 10.7%	287.20±19.892** 5.6%
ALME	**200**	304.00± 17.593	266.40±13.428*** 12.4%	234.40±10.877*** 22.9%	208.00±12.570*** 31.6%	228.80±10.257** * 24.7%	277.40±8.019*** 8.8%
Mix	**200**	317.40± 18.849	292.80±15.255 7.8%	272.40±11.305** 14.2%	223.60±13.649*** 29.6%	227.80±14.272** * 28.2%	239.80±8.585*** 24.4%
Drug	**0.5**	319.60± 13.612	263.40±11.194*** 17.6%	249.20±11.077*** 22%	241.60±12.361*** 24.4%	256.40±8.355*** 19.8%	269.20±9.682*** 15.8%

***** (P<0.05); ** (P<0.01); *** (P<0.001) ; **NC** normal control; **DC** diabetic control; **TCME** *Trigonella corniculata* methanolic extract; **ALME** *Albizia lebbek* methanolic extract; **Mix** mixture of TCME and ALME 100+100mg; **Drug** glibenclamide.

Table 3: Effect of TCME, ALME and their mixture on blood glucose level during 28 day administration in alloxan diabetic rabbits

Group	Dose mg/kg	Duration; glucose level in mg/dl (mean ± S.D); percentage reduction				
		0 Day	7 Days	14 Days	21 Days	28 Days
NC		136.80±3.0330	136.20±3.633 0.4%	134.40±3.286 1.8%	134.40±4.450 1.8%	135.40±3.20 91%
DC		311.20±10.060	309.20±8.614 0.6%	304.40±8.112 2.2%	311.00±11.85 30.1%	309.40±11.30 50.6%
TCME	**200**	304.20±19.942	275.40±19.769** 9.5%	246.80±20.192*** 18.7%	217.00±27.304*** 28.7%	187.00±23.184*** 38.5%
ALME	**200**	301.60±17.558	271.40±27.619** 10%	240.60±29.484*** 20.2%	205.00±22.136*** 32%	174.60±18.636*** 42.1%
Mix	**200**	317.40±18.849	293.40±22.799 7.6%	266.80±21.347** 15.9%	216.40±21.149*** 31.8%	189.40±13.428*** 40.3%
Drug	**0.5**	321.60±14.741	203.00±5.000*** 36.9%	146.20±5.630*** 54.5%	116.60±6.693*** 63.7%	98.80±3.033*** 69.3%

* (P<0.05); ** (P<0.01); *** (P<0.001) ; **NC** normal control; **DC** diabetic control; **TCME** *Trigonella corniculata* methanolic extract; **ALME** *Albizia lebbek* methanolic extract; **Mix** mixture of TCME and ALME 100+100mg; **Drug** glibenclamide.

Effects in alloxan diabetic rabbits over a 28 day period (chronic study):

Both TCME and ALME started showing significant reduction in sugar level at day 7 ($P<0.01$) when compared to normal control (NC). A 7.6% decrease was observed in mixture at day 7 (317.40 to 293.40 mg/dl). The antidiabetic activity of all three test samples increased each time tested up to a maximum of 38.5%, 42.1%, 40.3% for TCME, ALME and Mix respectively at day 28 ($P<0.001$). The maximum reduction for the standard was found to be 69.3%. Figure 3 suggests that all three samples have almost similar effects and the lines are rising continuously whereas for the standard, the line is curved showing that the effect reached its maximum and then decreased with the passage of time.

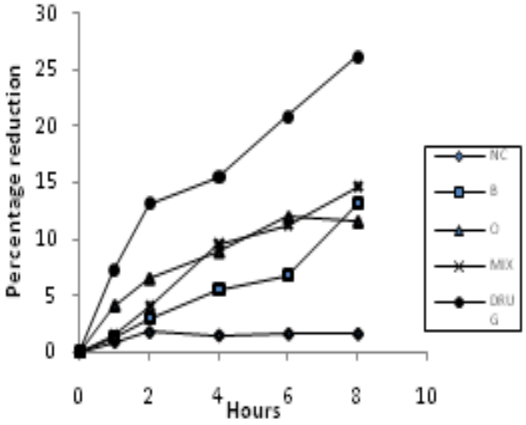

Figure 1: Single dose study in normal rabbits

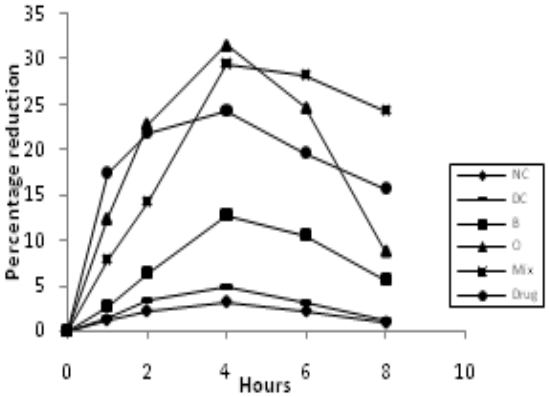

Figure 2: Single dose study in diabetic rabbit

Brine shrimp lethality test:

Brine shrimp assay was carried on TCME and ALME. Study revealed both plants not having significant cytotoxicity and LD50 values calculated, were above 1000 µg. The results reflect plants low toxicity and safety for use in living organisms. The LD50 for etoposide was 7.4625 µg/ml.

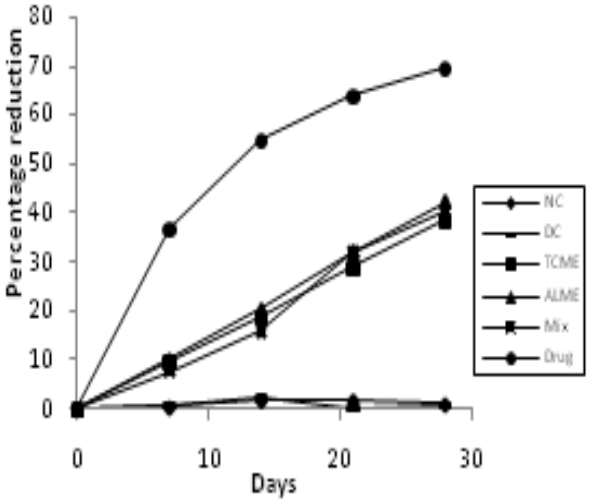

Figure 3: 28 day study in diabetic rabbits

CONCLUSION:

Diabetes and cancer are some of the most widespread diseases seriously affecting its victims. Both diseases especially cancer, are fatal and there is a continuous need of efforts in discovering new remedies more potent and safe then previous ones. Our study proves the high antidiabetic activity of the two plants and their mixture. Cytotoxicity was found to be low and so can be safely used in animal trials. Results from brine shrimp assay make the anticancer potential of plants doubtful. However other anticancer assays may be performed before any final decision. Further studies regarding isolation of bioactive compounds may be carried out.

REFERENCES:

1. Frode, T S and Medeiros Y S. Animal models to test drugs with potential antidiabetic activity. **Journal of Ethnopharmacology**. 115 (2); 2008: 173-183.
2. Jung M et al. Antidiabetic agents from medicinal plants. **Current medicinal chemistry.** 13 (10); 2006: 1203-1218.
3. Ebong P E et al. The Antidiabetic Efficacy of Combined Extracts from Two Continental Plants: Azadirachta indica (A. Juss)(Neem) and Vernonia amygdalina (Del.)(African Bitter Leaf). **American Journal of Biochemistry and Biotechnology.** 4 (3); 2008: 239-244.
4. Hussain F et al. Cytotoxicity and phytotoxicity of some selected medicinal plants of the family Polygonaceae. **African Journal of Biotechnology.** 9 (5); 2010: 770-774.
5. Krishnaraju A V et al. Assessment of bioactivity of Indian medicinal plants using brine shrimp (Artemia salina) lethality assay. **Int. J. Appl. Sci. Eng.** 2 (1); 2005: 125-134.
6. Idu M, and Osemwegie O O. Some medicinal flora of okomu forest reserve in Southern Nigeria. **Res. J. Medicinal Plant.** 1 (1); 2007: 29-31.
7. Babu N P, Pandikumar P and Ignacimuthu S. Anti-inflammatory activity of Albizia lebbeck Benth., an ethnomedicinal plant, in acute and chronic animal models of inflammation. **Journal of ethnopharmacology.** 125 (2); 2009: 356-360.
8. Seyydnejad S M et al. Antibacterial Activity of Hydroalcoholic Extract of Callistemon citrinus and Albizia lebbeck. **American Journal of Applied Sciences.** 7 (1); 2010: 13-18.

9. Pandey S, Devmurari V and Rathanand M. Biological Evaluation of Free Radical Scavenging Activity of Albizia Lebbeck Methanolic Extract in Arthritic Rats. **Annals of Biological Research.** 1 (1); 2010: 116-123.
10. Sivakumar B et al. Diuretic Activity of Methanolic Extract of Albizia lebbeck. **Research and Reviews: Journal Of Pharmacology And Toxicological Studies.** 1 (1); 2013: 1-3.
11. Pribac G, et al. Trigonella Foenum-Graecum And Trigonella Corniculata Seed Extracts Exert A Protective Action On Alcohol Toxicity In Brl3a Rat Liver Cells**. Studia Universitatis Vasile Goldis Arad, Seria Stiintele Vietii.** 19 (1); 2009: 87-93.
12. Satyanarayana T, et al. Antihyperglycemic and hypoglycemic effect of Thespesia populnea fruit in normal and alloxan-induced diabetes in rabbits. **Saudi pharmaceutical journal.** 12 (2); 2005: 107-111.
13. Alam S A, et al. Alloxan induced diabetes in rabbits. **Pakistan Journal of Pharmacology.** 22 (2); 2005: 41-45.
14. Akhtar M S, Khan M A, and Malik M T. Hypoglycaemic activity of Alpinia galangal rhizome and its extracts in rabbits. **Fitoterapia.** 73 (7); 2002: 623-628.

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