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HYPOGLYCEMIC AND HYPOLIPIDEMIC EFFECTS OF ALCOHOLIC EXTRACT OF *MIMOSA CATECHU* BARK IN ALLOXAN INDUCED DIABETIC RATS

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ABSTRACT

Herbal medicine has been used for many years by different cultures around the world for the treatment of diabetes and hence the alcoholic bark extract of *Mimosa catechu* (AEMC) Wild plant was investigated for its possible antihyperglycemic effect in alloxan induced diabetic rats. The animals were made diabetic by intraperitoneal injection of alloxan monohydrate at a dose of 150mg/kg body weight. The alcoholic extract of *Mimosa catechu*, AEMC (250 and 500mg kg⁻¹) and the standard drug (Glibenclamide 0.5mg/kg) were administered orally. Control group of rats were administered 0.2ml of 10% acacia mucilage (vehicle). The effect of oral administration of AEMC for 7days on the levels of Serum glucose, total cholesterol, and triglycerides, HDL-cholesterol, LDL-cholesterol in normal and diabetic rats were evaluated and compared with that of standard antidiabetic drug, Glibenclamide. Oral administration of 250 and 500mg kg⁻¹ body wt of AEMC for 7 days exhibited a significant reduction in serum glucose, total cholesterol, triglycerides, LDL-cholesterol and increase in HDL-cholesterol, Plasma insulin in alloxan induced diabetic rats. The antidiabetic effect of AEMC was similar to Glibenclamide. Hence our study reveals the antidiabetic and Hypolipidemic potential of *Mimosa catechu* bark and the study could be helpful to develop medicinal preparations for diabetes and related symptoms.

Key words: *Mimosa catechu*; Hypoglycemic Activity; Hypolipidemic activity; Alloxan; Glibenclamide.

INTRODUCTION

Diabetes mellitus is a global disease that is a major cause of morbidity in the world. This disorder is basically characterized by high levels of blood glucose caused by defective insulin production and action that are often responsible for severe health problems and death¹. The commonly encountered acute and late diabetic complications are already responsible for major causes of morbidity, disability and premature death in Asian countries². Hyperglycemic condition causes increased glycosylation leading to biochemical and morphological abnormalities due to altered protein structure which over a period of time develops diabetic complications such as nephropathy, retinopathy, neuropathy and cardiomyopathy³. Diabetic patients, particularly those with type II diabetes are at considerable risk of excessive morbidity and mortality from cardiovascular, cerebrovascular and peripheral vascular diseases leading to myocardial infarction, strokes and amputations⁴.

Recent decades have shown a resurgent interest in traditional plant treatments for diabetes, which has pervaded nutrition. The pharmaceutical industry and academic research fueled by a growing public interest

and awareness of so called complementary and natural types of medicine⁵. Many traditional plant treatments for diabetes exist, wherein lies a hidden wealth of potentially useful natural products for diabetes control⁶. *Mimosa catechu* Wild (Family: Fabaceae and Subfamily: Mimosidaea) known as Black cutch, is a deciduous thorn like tree mainly found in India and also found in deciduous forests around the world. The leaves and heartwood has many nutritional and medicinal uses. The extract of *Mimosa catechu* has been reported to have various pharmacological effects like immunomodulatory⁷, antipyretic, hypoglycemic⁸, antidiarrhoeal⁹, and hepatoprotective activities¹⁰. However, there are no scientific studies available on the antidiabetic effect of *Mimosa catechu* bark extract. Therefore, the anti-diabetic effect of alcoholic extract of *Mimosa catechu* (AEMC) bark was investigated in diabetic rats.

MATERIALS AND METHODS

Procurement and identification of plant material

The fresh bark of plant was collected from a village named Punyakshethram, which is about 20km away from Rajahmundry, during October. The plant was

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identified and authenticated as *Mimosa catechu* by the Taxonomist, Dr. T.U.Raghuram.

Preparation of the extract

Bark of *Mimosa catechu* was dried under shade for two weeks. Dried bark was coarsely powdered and stored in air tight container at room temperature. Dried powder was then extracted repeatedly with alcohol by maceration followed by hot percolation process. The extract was then concentrated by drying and the yield was found to be 17.78%.

Drugs and chemicals

Alloxan monohydrate was procured from Loba Chemie Laboratory Reagents and Fine Chemicals, Mumbai. Glibenclamide (Batch no: G080851) was a gifted sample from Tablets India Ltd, Chennai. Standard Glucose estimation kits were procured from Robonik (India) Pvt.Ltd, Mumbai. Enzymatic kits for the estimation of lipid profile were obtained from Chema Diagnostica (India).

Animals

Albino rats (150–200 g) of both sexes were selected and acclimatized to the experimental room at temperature $23 \pm 2^{\circ}\text{C}$, controlled humidity conditions (50–55%) and 12 h light/dark cycle. They were caged with a maximum of three animals in polypropylene cage and were fed with standard food pellets and water *ad libitum*.

Experimental design

Induction of experimental diabetes

Albino rats (n=24) were fasted for 16 to 18hrs. Diabetes was induced by administering freshly prepared alloxan monohydrate in normal saline intraperitoneally at a dose of 150mg/kg body weight as single dose¹¹. After 72hrs of alloxan induction, fasting blood was collected and blood sugar was estimated by glucose oxidase method. Only those animals which showed blood glucose levels >250mg/dl were separated and used for the study.

Treatment protocol

All the rats were randomized into four groups comprising of six animals in each group as given below.

- Group I : diabetic control
- Group II : diabetic rats received AEMC - 250mg/kg
- Group III : diabetic rats received AEMC -500mg/kg
- Group IV : diabetic rats received Glibenclamide - 0.5mg/kg

Vehicle or AEMC (250 and 500 mg/kg) and Glibenclamide (0.5mg/kg) were administered orally using an intra-gastric tube once daily for 7days and blood glucose levels were monitored at 0, 2, 4 and 24 hrs after the administration of single dose of AEMC or Glibenclamide (for acute study) as well as on the 1st, 3rd, 5th, and 7th day respectively (for prolonged effect). Percentage reduction in serum glucose was calculated with respect to the initial level.

Collection of blood samples and estimation of Biochemical parameters

For the purpose of biochemical estimation, blood samples were collected by snipping the tail vein on day 1 and 7 before the feeding of morning dose of the drugs. Blood samples were allowed to clot for 30 min and serum was separated by centrifugation at 3000rpm¹².

Determination of blood glucose levels

Serum glucose levels were estimated by glucose–oxidase–peroxidase (GOP –POD) method in Prietest touch auto biochemistry analyzer (version 2.6228) using the standard kits obtained from Robonik (India) Pvt.Ltd, Mumbai.

Determination of serum lipid profile

Serum cholesterol¹³ and triglycerides¹⁴ were estimated on initial and final days of experiment of each model by CHOD – POD method and enzymatic colorimetric method (GPO which is highly influenced by level of fasting). HDL¹⁵ cholesterol was determined by using standard enzymatic kits obtained from ROBONIK (INDIA) PVT.LTD, Mumbai. While the LDLC¹⁶ was derived from cholesterol and triglyceride values, VLDL cholesterol value was derived from cholesterol and HDL values.

Determination of serum insulin levels

For the estimation of serum insulin, blood samples were collected by snipping the tail vein on day 7 before the feeding of morning dose of the drugs. Blood samples were allowed to clot for 30 min and serum was separated after centrifugation at 3000rpm for 10min. Serum insulin levels were determined by solid phase radioimmunoassay method (INSU – R.I.A) (lab code: 100401889/AND15) and (barcode: 14474773/AND15).

Histopathological study

After collecting blood sample on 7th day, immediately the rats were sacrificed by mild ether anesthesia and pancreas was collected, excised and rinsed in ice-cold 0.9% saline solution. For Histopathological studies the pancreas was blotted, dried and fixed in 10% formalin for 48 h. Thereafter, the tissues were dehydrated in acetone for 1 h and embedded in paraffin wax. Section of pancreatic tissues were then taken through microtome and stained with haematoxylin-eosin for photo microscopic observation¹⁷.

Statistical analysis

All the values of body weight, fasting blood sugar, and biochemical estimations were expressed as mean \pm standard error of mean (S.E.M.) and analyzed for ONE WAY ANOVA and post hoc Dunnett's t-test using computerized Graph Pad Prism In Stat version 5.0, Graph Pad software. Differences between groups were considered significant at $P < 0.001$ and very significant at $P < 0.0001$ levels.

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RESULTS

There was a significant elevation in serum glucose, total cholesterol, triglycerides while the serum insulin and HDL-C levels significantly decreased in the diabetic control rats after the single i.p injection of alloxan monohydrate at a dose of 150mg/kg.

Changes in the body weight of diabetic and treated rats

As shown in the Table 1, diabetic control rats showed a slight but significant reduction in body weight during 7 days which is reversed by the alcoholic extract of *Mimosa catechu* bark after 7 days of treatment.

Table 1: Effect of AEMC bark on body weight (gm) in alloxan induced diabetic rats

GROUP	TREATMENT	AVG BODY WT (gm) ± SEM			
		Day 1	Day 3	Day 5	Day 7
I	Diabetic control (DC)	110±12.65	109.67±14.7	109.33±14.9	107.3±13.76
II	DC + AEMC (250mg/kg)	140±5.4	142.67±7.14	144.3±7.57	151.4±11.8
III	DC + AEMC (500mg/kg)	163.3±15.32	162.96±14.7	166.67±13.09	174.03±9.6
IV	DC + GLB (0.5mg/kg)	123.3±9.42	124.33±9.4	124.33±9.4	126.9±11.99

Values are expressed as Mean ± SEM

Changes in serum glucose levels

Tables 2 and 3 represent the hypoglycemic effect of the extract on the fasting blood sugar levels of alloxan induced diabetic rats. Administration of alloxan (150mg/kg, i.p) led to 2–3 fold elevation of fasting blood glucose levels, which was maintained over a period of one week and the daily treatment with extract led to a dose-dependent fall in blood sugar levels by 60 – 77%.

Table 2: Effect of AEMC bark on blood glucose level (mg/dl) in alloxan induced diabetic rats on day 1 of the treatment (ACUTE STUDY)

GROUP	TREATMENT	FASTING BLOOD GLUCOSE LEVEL (mg/dl)			
		0 th hr	2 nd hr	4 th hr	24 th hr
I	Diabetic control (DC)	299.7±4.85	273±6.95*	260.33±9.07**	296±3.92
II	DC + AEMC (250mg/kg)	365.5±10.54	293.8±8.36***	251.3±6.36***	360±0.45
III	DC + AEMC (500mg/kg)	393±0.5	323.8±8.57***	251.8±6.65***	365±0.5
IV	DC + GLB (0.5mg/kg)	370.67±5.24	291.33±2.25***	219.5±0.28***	363±1.03

Values are expressed as Mean ± SEM; *P<0.001; **P<0.001; ***P<0.0001

Table 3: Effect of AEMC bark on blood glucose level (mg/dl) in alloxan induced diabetic rats

GROUP	TREATMENT	FASTING BLOOD GLUCOSE LEVELS (mg/dl)				
		0 th day	1 st day	3 rd day	5 th day	7 th day
I	Diabetic control (DC)	299.7 ± 4.85	293.6 ± 9.07	272.33 ± 6.34	266.6 ± 10.97	251.8 ± 10.29*
II	DC + AEMC (250mg/kg)	365.5 ± 10.54	261.3 ± 7.35***	245.5 ± 6.59***	188.8 ± 3.6***	144.5 ± .50***
III	DC + AEMC (500mg/kg)	393 ± 0.5	261.8 ± 7.65***	191.5 ± 6.13***	148.16 ± 1.12***	97.38 ± 2.82***
IV	DC + GLB (0.5mg/kg)	370.67 ± 5.24	219.5 ± 9.35***	151.5 ± 6.33***	111.33 ± 4.4***	87 ± 8.15***

Values are expressed as Mean ± SEM; **P<0.001; ***P<0.0001

Changes in lipid profile

Alcoholic extract of *Mimosa catechu* bark lowered the triglyceride levels significantly (p<0.0001) from day 1 value of 264mg/dl to 142.5mg/dl and 35mg/dl on day 7

at a dose of 250mg kg⁻¹ and 500mg kg⁻¹ respectively. A slight but significant change was observed in serum cholesterol levels in extract treated group and it was 83mg/dl on day 1 while it was 74.7mg/dl and 68mg/dl on day 7 at a dose of 250mg kg⁻¹ and 500mg kg⁻¹ respectively. A very significant reduction in the VLDL-C levels was observed with the extract treated group of animals. The results were shown in Table 4.

Table 4: Effect of AEMC bark on serum lipid profile in alloxan induced diabetic rats after 7 days treatment

GROUP	TREATMENT	TC	TG	HDL	LDL	VLDL	TC/HDL	LDL/HDL
I	Diabetic control (DC)	264±12	242±12	27.5±0.57	87±2.5	208±20.8	9.6	3.14
II	DC + AEMC (250mg/kg)	83±14.2	142.5±7.4	27.5±1.2	24.3±3.9	30.7±10.4	3.0	1.9
III	DC + AEMC (500mg/kg)	68±15.5	35±5.5	26.2±2.5	30.1±6.5	12.6±2.0	2.6	1.8
IV	DC + GLB (0.5mg/kg)	264±12	242±12	27.5±0.57	87±2.5	208±20.8	9.6	3.14

TC= total cholesterol; TG= triglycerides; HDL= high density lipoproteins; LDL= low density lipoproteins; and VLDL= very low density lipoproteins. All the values are expressed as Mean ± SEM and are very significant at ***P<0.0001

Changes in serum insulin levels of diabetic and treated group of animals

After 7 days treatment period, it was observed that the animals treated with alcoholic extract of *Mimosa catechu* bark (AEMC) showed a significant increase in the serum insulin levels from 2.43µU/ml to 3.21µU/ml (32.09%) at a dose of 250mg/kg and 4.12µU/ml (69.54%) at a dose of 500mg/kg. results were shown in Table 5.

Table 5: Effect of AEMC bark on serum insulin levels in alloxan induced diabetic rats after 7 days treatment

GROUP	TREATMENT	INSULIN LEVELS ± SEM	% INCREASE IN INSULIN LEVELS
I	Diabetic control (DC)	2.43±0.03	-
II	DC + AEMC (250mg/kg)	3.21±0.038***	32.09%
III	DC + AEMC (500mg/kg)	4.12±0.0007***	69.54%
IV	DC + GLB (0.5mg/kg)	4.36±0.016***	80.6%

Values are expressed as Mean ± SEM; and all the values are very significant at ***P<0.0001. DC= Diabetic control; AEMC= Alcoholic extract of *Mimosa catechu*; GLB= Glibenclamide.

Histopathological changes of pancreas

Photomicrographs (Fig.1) showed normal acini and normal cellular population in the islets of langerhans in pancreas of vehicle-treated rats (A). Extensive damage to the islets of langerhans and reduced dimensions of islets (B), restoration of normal cellular population size of islets with hyperplasia by Glibenclamide (C) was also shown. The partial restoration of normal cellular population and enlarged size of β-cells with hyperplasia was shown by the alcoholic extract of *Mimosa catechu* bark (Fig. 1C & 1D).

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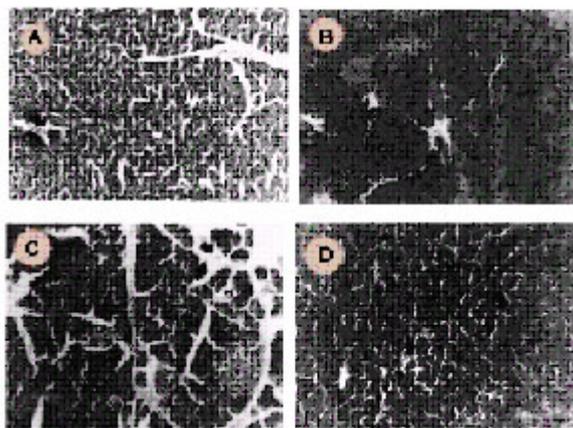


Fig. 1: Photomicrographs rat pancreas stained by haematoxylin and eosin of alloxan-induced diabetic group rats (A); effect of glibenclamide (B); effect of AEMC bark at a dose of 250mg/kg (C) and effect of AEMC bark at a dose of 500mg/kg (D). Microscope magnification (400x).

DISCUSSION

The use of ethno botanicals has a long folkloric history for the treatment of blood glucose lowering abnormalities¹⁸. Therefore the search for more effective and safer antidiabetic or hypoglycemic agents has continued to be an important area of active research. In the present study, *Mimosa catechu* Wild was selected for antidiabetic evaluation owing to its ethno medicinal use in curing diabetes. Therefore the study was undertaken to justify its claimed use. As a result, alcoholic extract of *Mimosa catechu* bark was prepared and stored. Albino rats were selected as experimental animals for the antidiabetic activity.

The present results showed that the alcoholic extract of *Mimosa catechu* bark (AEMC) significantly decreased serum glucose, triglycerides, cholesterol where as it increased HDL-cholesterol and serum insulin levels in treated diabetic rats as compared with the diabetic control rats.

Models of experimental diabetes that utilizes diabetogenic agents (alloxan and STZ) and induced blood glucose levels higher than 300mg/dl¹⁹ or 400mg/dl²⁰ have been considered as severe diabetes. In our study, as observed from the values of parameters known to suffer changes in this illness, the alloxan induced diabetic rats presented clear symptoms of diabetes in the diabetic control group. The induction of diabetes by an intraperitoneal injection of alloxan (150mg/kg) was confirmed, as reflected by the hyperglycemia (serum glucose > 300mg/dl), polyphagia, and polydipsia and body weight loss as compared to the normal rats. Experimentally induced diabetes is generally characterized by loss in body weight which may be due to degradation of structural proteins since structural proteins are known to contribute to the body weight. In our study weight loss was observed and treatment with AEMC bark reversed

the weight loss, which may be due to the increased secretion of insulin by AEMC bark. Treatment with AEMC bark and Glibenclamide showed the reversal of serum glucose near to normal level which is supported by the elevated level of plasma insulin. The elevated insulin in AEMC bark treatment could be due to increased secretion by regenerated β -cells.

Dyslipidemia is a frequent complication noted in chemical-induced diabetes²¹⁻²³ and presents serious risk of vascular disease. The total cholesterol and triglycerides of the diabetic animals treated with the extract were substantially improved, as compared to the diabetic control group. This suggests that the strong hypoglycemic effect of the extract could indirectly be related to beneficial action against the abnormal high concentration of serum lipids observed in diabetic animals.

CONCLUSION

This study demonstrated that the oral administration of alcoholic extract of *Mimosa catechu* bark (AEMC) has beneficial effects in reducing the elevated blood glucose levels and lipid profile of alloxan induced diabetic rats. The extract showed improvement in parameters like body weight, lipid profile as well as regeneration of β -cells of pancreas and resulted in elevation of insulin levels and this might be the possible mechanism contributing to the antidiabetic effect of AEMC bark.

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