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Research article

Evaluation of Antidiabetic activity of *Barringtonia acutangula* (L.Gaertn) leaf extract in Alloxan induced Diabetic rats

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Abstract



The present study was investigated the Antidiabetic effect of oral administration of ethanolic leaf extract of *Barringtonia acutangula* (L.Gaertn) in normal and Alloxan induced diabetic rats. Albino Wistar strain rats (150 to 200 gm) were induced diabetic condition by administration of Alloxan (150mg/kg bw) intraperitoneally. Extract of *Barringtonia acutangula* (L.Gaertn) leaves was orally administrated to normal and Alloxan induced diabetic rats for 21 days to determine the antidiabetic activity. Serum and tissue biochemical analysis including fasting blood sugar level in normal and Alloxan induced diabetic rats were investigated. Administration of Alloxan 150mg/kg (i.p) lead to elevation of fasting blood glucose levels, along with significant decrease in body weight over a period of 21 days and it was partially restored or improved upon administration of *Barringtonia acutangula*. Ethanolic extract (250 and 500 mg/kg bw/day) shows significantly ($P < 0.01$) decreased 40-50% of the elevated blood glucose level, cholesterol, triglycerides, urea, creatinine, bilirubin comparison to untreated diabetic Wister strain albino rats 21 days of daily treatment.

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Key Words

Antidiabetic activity, *Barringtonia acutangula*
(L.Gaertn), Glibenclamide, ethanolic extract,
Alloxan induced diabetic rats

INTRODUCTION

Diabetes mellitus is often termed as diabetes. It is a metabolic disorder characterized by hyperglycaemia and with imbalance in serum lipids and proteins¹. In the year 2000, according to the World Health Organization, at least 170 million people worldwide (2.8% of the population) suffer from diabetes. Its incidence is increasing rapidly, and it is estimated will almost double by the year 2030². Based on its etiology, diabetes mellitus is generally divided into three classifications: type 1, type 2 and gestational. Type 1 is an autoimmune disease characterized by a defect in insulin secretion such that the pancreas produces little or no insulin. It occurs most often in children and young adults and accounts for 5%–10% of cases of diabetes. Type 2 diabetes is a metabolic disorder characterized by a defect in insulin secretion and/or tissues are resistant to its uptake and for 90%–95% of cases of diabetes. The chronic hyperglycemia that characterizes diabetes mellitus results from either defects in insulin secretion or insulin action and/or both. When left unchecked, hyperglycemia precipitates micro and/or macrovascular complications including: cardiovascular disease, nephropathy, neuropathy, and retinopathy. Insulin and oral hypoglycemic agents are used in the management of type 1 and type 2 diabetes mellitus respectively. Medicinal plants continue to provide valuable therapeutic agents, in both modern medicine and traditional systems. Over four hundred traditional plant treatments for diabetes have been reported, although a small number of

these have received scientific and medical evaluation to assess their efficacy and safety.^{3,4,5}

Barringtonia acutangula (L) Gaertn, is an evergreen tree of moderate size, grows on the banks of freshwater rivers, the edges of freshwater swamps and lagoons and on seasonally flooded lowland plains, commonly on heavy soils.

The present study was to investigate Antidiabetic effect of oral administration of ethanolic leaf extract of *Barringtonia acutangula* (EBA) in normal and Alloxan induced diabetic rats and its effect on the vital organ.^{6,7,8,9}

MATERIALS AND METHODS

Drugs and Chemicals

Glibenclamide drug was obtained from Micro lab Hosur, Alloxan received from Himedia laboratory Mumbai. 90% Ethanol were purchased from SD Fine Chem Ltd, Mumbai; all other chemicals used for the study were of analytical grade.

Plant material

Herbal plant *Barringtonia acutangula (L. Gaertn)* collected from Pondicherry Botanical garden. The plant material was authenticated from Dr A.K.Pradeep, Herbarium curator, Dept. of botany, University of Calicut, Kerala.

Preparation of ethanolic extract of *Barringtonia acutangula* (EBA)

All the plant material first washed with water, plant material was dried under

shade until complete removal of moisture content, and such dried leaves were pulverized by using cutter mill and passed through sieve no 80. Dried powder extract with 300 ml of 90% Ethanol solvent at 60-70° C, the extract material made dried and stored in -2° C ¹⁰.

Animals

Albino Wistar strain rats of either sex, weighing about 150–200g each, were used for the study. They were fed with standard chow and water *ad libitum*. They were housed in polypropylene cages maintained under standard conditions (12h light - dark cycle; 25 ± 2 °C; 35–60% humidity). The experimental protocol was subjected to the scrutiny of the Institutional Animal Ethics Committee, CPCSEA / PCP / IAEC / MPHARM / 126 / 2013 and was cleared by same before beginning the experiment.

METHODS

Preliminary phytochemical analysis

Ethanol extract of *Barringtonia acutangula* leaves was subjected to preliminary photochemical analysis to know the nature of various phyto-constituents.

Induction of experimental animals

Albino wistar strain rats either male or female (100-150 g) maintained under standard laboratory conditions, were fasted overnight prior to Alloxan 150mg/kg bw administration. Fasting blood glucose of all animals was

determined. Single dose of Alloxan (150 mg/kg) dissolved in normal saline was administered intra peritoneal (i.p.) to develop diabetes. Control animals were treated with normal saline. Glucose was determined again on fourth day of Alloxan (150 mg/kg) administration and the animals exhibiting fasting blood glucose range >200 mg/dl were used in the experiment. Blood glucose level was again estimated in all the groups at the end of study (21 days) before sacrificing the rats.

Acute Toxicity Studies (OECD)

Animals were fasted for 3-4 h prior to dosing. Following the period of fasting, all extracts at doses of 200, 300, 600, 1000, 1500 2000 and 2500 mg/kg b.w. were administered to six groups with 6 rats each. Animals were observed individually after dosing at least once during the first 30 min. periodically during the first 24 h, with special attention given upto first 4 h. Time of onset and length of recovery period were observed. Additional observations include change in skin and fur, eyes and mucous membranes, and also somatomotor activity and behavior pattern. Attentions were given to observations of tremors, convulsions, salivation, diarrhea, sleep and coma.

Evaluation of hypoglycemic activity

Experimental animals are divided in five groups each group containing six animals (albino -wistar strain rat) Group I- received normal saline 5ml/kg b.w/p.o. per orally for 21 days, Group II- diabetes induced animals received

normal saline 5ml/kg b.w/p.o. for 21 days, Group III-diabetes induced animals received GBC 0.6mg/kg b.w/p.o for 21 days, Group IV&V diabetes induced animals received EBA 250 & 500 mg b.w/ p.o for 21 days respectively.

Biochemical estimation

For experimental methods of estimation like Total cholesterol (CHOD-POD phosphotungestan method), triglycerides (GPO-POD method), serum creatinine, (Modified Jaffe's kinetic method), urea (DAM method) and billirubin (M&E method) was used^{11, 12}.

Pancreatic histopathology

For histopathological study, animals from all groups were anaesthized with ether and dissected. Pancreas are excised and transferred immediately into 10% formalin solution in a stoppered container. These samples were then fixation (using Bouin's solution), dehydration, embedding (in paraffin), sectioning (with standard microtome) and staining (Haematoxylin or eosin). The slides so prepared were then examined.

Statistical analysis

The data were analyzed by one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison tests to determine level of significance. A value of $P < 0.01$ was considered significant results are expressed as mean SEM.

RESULTS AND DISCUSSION

Phytochemical screening

The results of the phytochemical screening of *Barringtonia acutangula* leaves extracts showed the presence of alkaloids, proteins, steroids, tannins, flavanoids, glycosides, sterols, saponins, terpenoids, phenols and carbohydrates. An absence of gums and mucilage were noted.

Acute toxicity

Ethanollic extract of *Barringtonia acutangula* leaves at a dose 2500 mg/kg body wt. showed no toxic effects or mortality upto 14 days. The LD50 cut off value of the extracts was 2500 mg/kg body wt.

Selection of the dose

The LD50 cut off value was found to be 2500 mg/kg body wt. For the assessment of hypoglycemic activity two doses were selected i.e. first dose 1/10th of the LD50 cut off value and second dose twice that of one tenth dose i.e. (250 and 500mg/kg respectively).

Alloxan induced hyperglycemia

Administration of Alloxan 150mg/kg induced Diabetes, by damaging the insulin secreting cells of the pancreas leading to hyperglycemia (i.p) lead to elevation of fasting blood glucose levels, along with significant decrease in body weight over a period of 21 days.

Tab 1. Effect of various extract of *Barringtoniaacutangula* on Glucose, Creatinine, Urea, Bilirubin level against Alloxan induced diabetic rats

| Groups | Blood glucose level (mg/dl) | Creatinine (mg/dl) | Urea (mg/dl) | Bilirubin (mg/dl) |
|----------------------------|-----------------------------|--------------------|--------------|-------------------|
| Normal | 88.16 ± 2.02 | 1.13±0.02 | 32±1.2 | 1.23±0.07 |
| Diabetic control | 285 ± 5.12# | 1.89±0.09 | 69.5±3.4 | 1.9±0.05# |
| Standard | 97.16 ± 3.3 | 1.28±0.03 | 32.5±2.3 | 1.2±0.02 |
| EBA extract (250 mg/kg bw) | 114.16 ± 1.01 * | 1.16±0.02* | 33.5±0.7* | 1.21±0.02* |
| EBA extract (500mg/kg bw) | 111.5 ± 1.05** | 1.07±0.02** | 31.3±0.7** | 1.14±0.02** |

Values are expressed as mean ± SEM.,(n=5) *P<0.05, **P<0.01Creatinine, urea, Billirubin level was compared with diabetic control, P-values are * Indicates significance & ** Indicates Highly significance,# indicates control

Acute hypoglycemic activity

The blood glucose concentration of the group I (88.16mg/dl) is considered normoglycemic, while that of the group II (285.00mg/dl) is considered hyperglycaemic for the experiment. There were significant reductions ($p < 0.01$) in blood glucose levels for the test (IV & V) groups (EBA 250 mg and EBA 500 mg) and the non-diabetic groups that received extracts it was partially restored or improved upon administration ethanol extract of *Barringtonia acutangula* significantly ($P < 0.01$) decreased the elevated blood glucose level, however, hypoglycemic activity between the two different doses of EBA was found to be slightly similar (Tab 1).

Biochemical estimation

The treatment with EBA (250 mg/kg b.w/p.o and 500mg/kg b.w/p.o) showed significant ($p < 0.01$) decrease in serum cholesterol, triglycerides,

creatinine, urea, bilirubin when compared to alloxan induced diabetic rats. As well as animals treated with both the doses of EBA shows significant increase in body weight when compared to standard drug treatment (Tab 2).

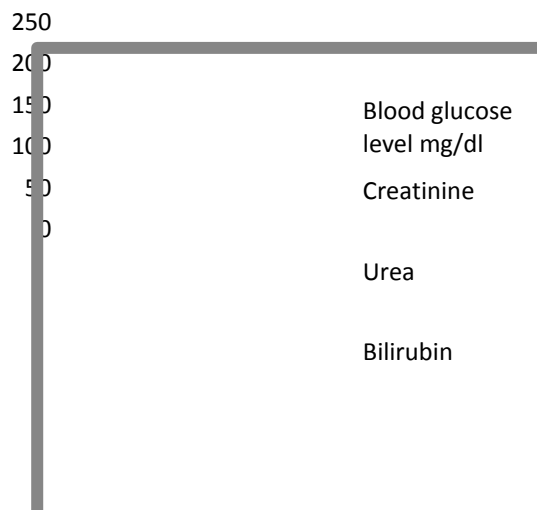


Fig 1. Graphical Representation of biochemical profiles of EBA treated and untreated animals

Tab 2. Effect of various extract of *Barringtoniaacutangula* on Lipid profile, body weight level against Alloxan induced diabetic rats

| Groups | Cholesterol (mg/dl) | Triglycerides (mg/dl) | Bodyweight (gm) |
|----------------------------|------------------------|--------------------------|--------------------|
| Normal | 62.1±2.5 | 96.6±2.6 | 155.83±18.0 |
| Diabetic Control | 102.3±3.1# | 183.8±4.4# | 110±10.5# |
| Standard | 65.33±1.4 | 122.6±5.4 | 133.33±6.05 |
| EBA extract (250mg/kg) | 66.5±0.8* | 106±5.1* | 140±7.07* |
| EBA extract (500 mg/kg) | 59.5±0.5** | 99.3±4.4** | 148.33±7.52** |

Values are expressed as mean ± SEM., (n=5), ***P<0.05**, ****P<0.01** Cholesterol, Triglycerides, body weight level was compared with diabetic control. P-values are * Indicates significance & ** Indicates Highly significance, # indicates control.

Histopathological study of pancreas

| Figure no | Report |
|---|--|
| 3(a) Normal Control (Group I) | H & E stained in the pancreatic section of normal rat showed cells with well preserved cytoplasm, i.e. normal islets and acinar cells. the endocrine components were found as nodules within the substance of exocrine pancreas. |
| 3 (b) Diabetic Control (Group II) | reveals advanced changes of diabetes as destruction of beta cells with pycknosis of nuclei. There was distortion of cells and reticular changes of islets as evidence of fibrosis |
| 3(c) GBC treated (Group III) | Standard drug treated rats showed preserved pancreatic islet of Langerhans cells returned to normal. |
| 3 (d) EBA 250mg/kg body weight (Group-VI) | The pancreatic section of EBA 250mg/kg treated rats showed small islet cells slight vacuolar degeneration and in some cases, there was marked improvement in islets structure. |
| 3(e) EBA 500 mg/kg body Weight (Group V) | The section of EBA 500mg/kg treated rats showed size of islets and there was more or less improvement or restoration of normal cellular population. |

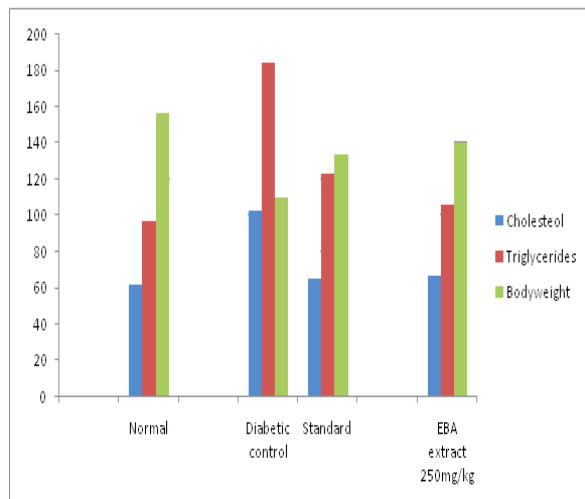


Fig 2. Graphical Representation of Lipid profiles of EBA treated and untreated animals

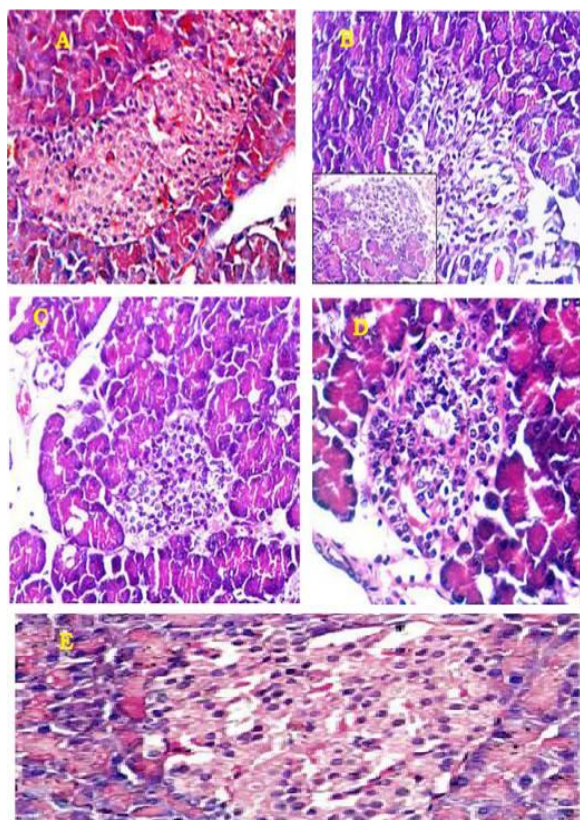


Fig 3 (a) (Normal control), (b) (Diabetic control), (c) GBC treated, (d) EBA treated (250 mg), (e) EBA treated (500 mg)

CONCLUSION

The treatment of EBA leaves showed marked decrease in elevated blood glucose level, as well as marked increase in body weight. The EBA extract treatment remarkably decreased in cholesterol, triglycerides, creatinine, urea levels in serum of alloxan induced diabetic rats was observed. It was confirmed that earlier studies was reported on root part of same plant for hypoglycemic activity¹³. The EBA produced significant beneficial effects on the lipid profile on hyper lipidemic rats at both test doses i.e. 250 mg/kg b.w/p.o and 500mg/kg b.w/p.o. Histopathological studies confirmed the necrosis of β -cells in alloxan-treated group of rats. Whereas, the glibenclamide treated group showed preserved cytology. At 250mg/kg b.w., EBA-treated rats showed small islet cells, whereas 500 mg/kg b.w. EBA-treated rats showed an hyperplastic effect i.e. confirmation of regeneration of β -cells in EBA at 500 mg/kg b.w. group. Further study is needed to find out the exact mechanism and the phytoconstituents responsible for observed effect.

Abbreviations used: BA= *Barringtonia acutangula*; EBA= ethanolic extract of *Barringtonia acutangula*; OECD= Organization of Economic Co-Operation and Development; GBC= Glibenclamide; CHOD-POD= Cholesterol oxidase-peroxidase enzyme; GPO-POD method= Glycerokinase Peroxidase- Peroxidase, DAM=Diacylmonoxime; M&E=Malloy and evelyn method.

References

1. Sheen J A. Drug treatment of non-insulin dependent diabetes mellitus in the 1990s. Achievement and future development. *Drugs* 1997; 54: 355-368.
2. World Health Organization. The Health Situation in the South-East Asia Region: 2001-2007. WHO Regional Publication, South-East Asia Series, 2009: No. 46.
3. Diagnosis and Classification of Diabetes Mellitus. *Diabetes Care*. 2001; 23: S4-19.
4. Jain R C, Vyas C R. Effects of onion and garlic in atherosclerotic heart disease. *Medikon*. 1977; 6:12-4.
5. Nazar S. Hypoglycemic Activity of Nineteen Sudanese Medicinal Plants with Emphasis on: *Allium cepa* L. (Red onion). Thesis for M. Pharm, Faculty of Pharmacy, University of Gezira, Sudan; 2006.
6. Jain S K. Dictionary of Indian Folk Medicine and Ethno botany, National Botanical Research Institute, Lucknow, India, 1991; 33.
7. Ahmad K J. Pharmacognosy of the leaf and root of *Barringtonia acutangula*, Gaertn. *Planta Medica*. 1969; 17 (4) : 338-345.
8. Anantaraman R, and Pillai K S M. Barringtonol and barringtogenic acid, two new triterpenoidsapogenins. *Journal of the Chemical Society*. 1956; 4369-4373.
9. Anjaneyulu A S R, Sastry C S P, Narayan G K A S S, and Row L R. New triterpenes from *Barringtonia acutangula* Gaertn. *Journal of the Indian Chemical Society* LV. 1978; 11: 1169-1174.
10. Mukhargee P K. Quality control of herbal drugs. 1st ed. Calcutta : Business horizons pharmaceutical publishers; 2002.
11. Turner RA. Screening procedures in pharmacology. New York : Acedamic press; 1972; 22-34.
12. Babu V, Gangadevi T, Subramoniam A. Antidiabetic activity of *Cassia Kleinii* leaf on streptozotocin-induced diabetic rats and isolation of active fraction and toxicity evaluation of the extract. *Indian journal of pharmacology* 2003;35:290-296.
13. Nilesh P B, Subal D, Manjunath Y S, Gajanan D, Hariprasath K, Kukkala S. Hypolipidemic effect of hydro-alcoholic extract of *barringtonia acutangula* Linn root extract on streptozotocin-induced diabetic rats. *Journal of Pharmaceutical Science and Technology* 2010; 2 (11):368-371.

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