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Pharmacological activity of Barringtonia Acutangula leaf extract of alloxan induced diabetic animal model

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ABSTRACT

The plant extracts were capable of exerting beneficial effects by reducing the concentrations of blood Sugar level & it possess marked anti hyperglycemic activity by lowering the blood glucose level in Alloxan induced diabetic rats.

Keywords: Barringtonia Acutangula, Alloxan induced diabetic.

INTRODUCTION

In recent years interest in medicinal plants has been increased considerably. Apart from the reliance on the therapeutic values described in ancient texts and current interpretations by specialists in countries have initiated analytical studies to scientifically determine the efficacy of best known medicinal plants in the treatment of diseases. .[1]

Plants provide a large bank of rich, complex and highly varied structures which are unlikely to be synthesized in laboratories. Furthermore, evolution has already carried out a screening process itself whereby plants are more likely to survive if they contain potent compounds which determine animals or insects from eating them. Even today, the number of plants that have been extensively studied is relatively very few and the vast majorities have not been studied at all.

The development of medicinal plants into therapeutic drugs takes several years and millions of dollars are needed, hence making the process very capital-intensive, the risks are also high and the success rate are not very good.

The recent interest in the plant research as a potential source of new drugs, strategies for the fractionation of plant extracts based on biological activity rather than on a particular class of compound, have been developed. The chemical examination follows after the isolation of the Active fraction. [2]

PLANT PROFILE

Barringtonia acutangula

Botanical Classification

Family - lecythidaceae

Botanical – leythidaceae
Ayurvedic – hijjalkul
Kingdom: Plantae Haeckel, 1866 – plants
Subkingdom: Viridaeplantae Cavalier-Smith, 1981 - green plants
Phylum: TracheophytaSinnott, 1935 ex Cavalier-Smith, 1998
Subphylum: Spermatophytina (auct.) Cavalier-Smith, 1998 - seed plants
Infraphylum: Angiospermaeauct.
Class: MagnoliopsidaBrongniart, 1843 - dicotyledons
Superorder: Cornanae
Order: Cornales
Family: Cornaceae
Subfamily: Bombinae
Tribe: Bombini
Genus: Barringtonia Linnaeus, 1753
Species: acutangula
Binomial Name: Barringtoniaacutangula
This is an evergreen tree of moderate size, called by Sanskrit writers Hijja or Hijjala. The fruit is spoken of as Samudra-phala and Dhātriphalaor"nurse's fruit, and is one of the best known domestic remedies. Also called Stream Barringtonia or Itchy Tree (after a catepillar with irritant hairs that sometimes colonises the undersides of the leaves) Barringtoniaacutangula is a tree 5-8m high with rough fissured dark grey bark, red flowers are produced on pendulous racemes about 20cm long. Four sided fruits are produced periodically throughout the year. Partly deciduous in extended dry periods. This species grows on the banks of freshwater rivers, the edges of freshwater swamps and lagoons and on seasonally flooded lowland plains, commonly on heavy soils. Found in Madagascar and tropical Asia, amongst other places. Propagation is by seed. Tolerant of heavy clay soils with poor drainage, it can grow in a range of soils

MATERIALS AND METHODS

Animals

Male albino-Wister strain rats weighing 150-250g were used in the present study. They were housed under standard conditions, maintained on a 12 hours light/dark cycle and had free access to food and water up to the time of experimentation. All the protocol were approved by the IAEC (Institutional Animal Ethical Committee) and conducted according to the guidelines of CPCSEA (Committee for the Purpose of Control and Supervision of Experimentation on Animals) through its reference no: CPCSEA/IAEC/ dated:

Chemicals

Alloxan (Himedia, Mumbai, India) was purchased, preserved at 25ºC and used for the induction of diabetes. Glibenclamide gift sample received from Micro labs, Bangalore. it's an oral antidiabetic preparation with an efficient hypoglycemic action. 10mg of gentamycin CF1 (Himedia, India) was used as positive controls and DMSO used as negative control.

METHODOLOGY

Antibacterial assay

The effects of various alcoholic extracts of Barringtonia acutangula, on the several bacterial strains were assayed by Disc diffusion method. The minimum concentrations of the plant extracts to inhibit the microorganisms were also determined by micro dilution method using plant fractions serially diluted in sterile nutrient broth.

Disc diffusion method [3]

Principle

Paper discs impregnated with specific antibiotics or the test substances are placed on the surface of the Muller Hinton agar medium inoculated with the target organisms, which is recommended for the diffusion of antimicrobial agents as described in NCCLS approved standard. The plates are incubated and the zones of inhibition around each disc are measured.

Protocol

The given samples were extracted using ethanol and the ethanol evaporated using lyophiliser.

- Different concentrations were made using alcohol.
- Overnight bacterial suspension (100µl) adjusted to contain 1x106 CFU/ml of bacteria, spread by a sterile glass rod on Nutrient Agar (NA) medium.
- The inoculated plates were incubated at 27±20C for 24 h, and then the inhibition zones were measured in diameter (mm). Antibiotic discs containing 10mg Gentamicin and was used as p controls The MIC was calculated using the four concentrations of the samples for each extracts separately

Minimum inhibitory concentration (MIC) value was considered as the lowest extract concentration with no visible growth for each plant extract test

pathogens.[100]To measure the MIC values, various concentrations of the stock ie,10,20,40 and 60mg/ml were assayed against the test pathogens. The tubes were then inoculated with standard size of microbial suspension and the tubes were incubated at 37 °C for 24 h for bacteria in a BOD incubator and observed for change in turbidity after 24 h and compared with the growth in controls.[101]

The Agar Disc Diffusion method was used for the antibacterial study. These discs of 6mm diameter were prepared from Whatmann filter paper no.1 and were sterilized in hot air oven at 160°C for 1 hour. The discs were then impregnated with the extract of various concentrations ie, 10, 20,40 and 60.Gentamicin discs were used as standard. Each discs of Gentamycin contained 10mg. The pathogenic strains (Escherichia coli Staphylococcus aureus, Salmonellatyphi, Pseudomonas aeruginosa) were then seeded on the Muller Hinton Agar Media in a petridish by streaking the plate with the help of a sterile swab. Care was taken for the even distribution of culture all over the plate. The seeded plates were allowed to dry and then Gentamicin and extracts discs of various concentrations ie, 10, 20, 40 and 60 were placed on the seeded medium plates and maintained at 4°C for 30mts to allow perfusion of drugs being tested. The plates were then incubated at 37°C for 24hours. After which the zones of growth of inhibition were measured and recorded in millimetre and the control (Gentamicin 10mg) was set up in a similar There is no dependence on traditional medicine for a variety of ailments in a large part of the world population, especially in developing countries; the use of higher plants and preparations made from them to treat infections is a longstanding practice.[4]However many species of plants containing substances of medicinal value have yet to be discovered.(103)The present study reveals the antibacterial potential of extract of leaves of *Barringtoniaacutangula*. Ethanolic extract have shown inhibitory effect against the bacterial test strains. Phytochemical elucidation of antibacterial principles using ethanol fraction should be undertaken with the objective to isolate the active biochemical principles and develop novel antibacterial agents.

Oral toxicity studies(5,6,)

Organization for Economic Co-operation and Development (OECD) guidelines (Guidelines 423; Fixed Dose Procedure) was followed for acute oral toxicity test to plant extract. Before experimentation rats (n=3) were fasted overnight *and was oral administered with fixed extracts dose of 5, 50, 300 and 2000 mg/kg* body weight respectively by gavage using intubation cannula. Administered dose was found tolerable as no death was found. Animals were observed individually after dosing for first 30 min periodically and daily thereafter, till 14 days for any toxicity sign of gross changes in skin, eyes and mucous membranes, circulatory, respiratory, and autonomic and central nervous systems, and behavior pattern if any. Therefore, two dose levels of *Barringtoniaacutangula*ethanolic extract 150 and 300mg/kg b.w were selected for antidiabetic activity.

Induction of Experimental Diabetes

Animals were allowed to fast for 24 hrs and were injected with freshly prepared Alloxan monohydrate 37mg/ml is prepare and administer within five min at a dose of 150mg/kg body weight intra peritonially.[105]After 48 hours of administration the rat with moderate diabetes having glycosuria & hyperglycemia (i.e. blood glucose is more then 300mg/ml) The animals were maintained in the diabetic state over a period of 21 days. Serum glucose was measured by Glucometer Accu check. Rats showing fasting serum glucose levels (>250 mg/dl) were selected for the study.

Experimental design

Experimental animals are divided in Five groups each groups having six animals (albino-wistar strain Rats) Group I. Normal group Received 1 ml of saline and served once a day orally for 21 days by using an intragastric tube. Group II. Positive control group received Alloxon monohydrate 150mg/kg body weight,[7] Administered through intra-peritonially. Group III. Standard group received glibenclamide 600 µgm /kg.[106] body weight once a day orally for 21 days by using an intragastric tube. Group IV. Received Ethanol extraction of *Barringtonia acutangula*(Leaves)] 150 mg/kg body weight once a day orally for 21 days by using an intragastric tube. Group V. Received ethanol extract of *Barringtonia acutangula (Leaves)] 3*00 mg/kg body weight once a day orally for 21 days by using an intragastric tube.

Collection of Pancreas and blood

Blood was collected by retro orbital puncture and serum was separated by centrifugation at 2500rpm. The serum collected was used for biochemical estimations.The rats were sacrificed by cervical dislocation [7] and pancreases were excised immediately and thoroughly washed with ice-cold physiological saline.Pancreas were taken and fixed in 10% buffered formalin. For histopathology study.

STATISTICAL ANALYSIS

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

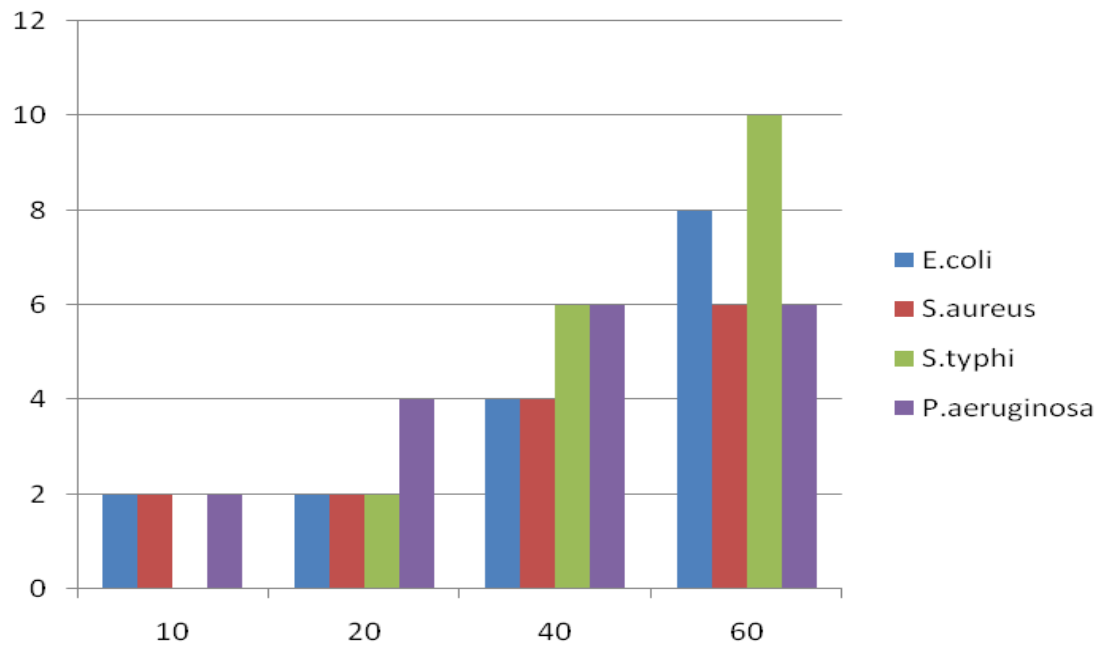
RESULT & DISCUSSION

Antimicrobial evaluation

Anti-microbial study did by Kirby Bauer Disk Diffusion Method. The inoculated plates were incubated at 27±20C for 24 h, and then the inhibition zones were measured in diameter (mm). Antibiotic discs containing 1 µg of Ciproflaxin CF1 and Ampicillin AP1 (Himedia, India) was used as positive controls and DMSO used as negative control. The MIC was calculated using the 4 concentrations of the samples. Against Test organisms: Escherichia coli, *Staphylococcus aureus,* S. typhi, P. Aeruginosa.

Table no 5.3.1. Observation *Barringtonia acutangular*

S.No	Test organisms	Concentration of the sample					Ampicillin
		10 mg	20 mg	40 mg	60 mg	MIC	10 mg
1	Escherichia coli	2 mm	2mm	4mm	8mm	10mg	15mm
2	Staphylococcus aureus	2 mm	2 mm	4mm	6 mm	10 mg	20mm
3	S. typhi	0 mm	2 mm	6mm	10mm	20 mg	25mm
4	P. aurenginosa	2 mm	4mm	6 mm	6 mm	10 mg	20mm



Anti diabetic evaluation

Effect of various extract of *BA,MT,CI*on Glucose level against Alloxan induced diabetic rats

Pharmacological evaluation

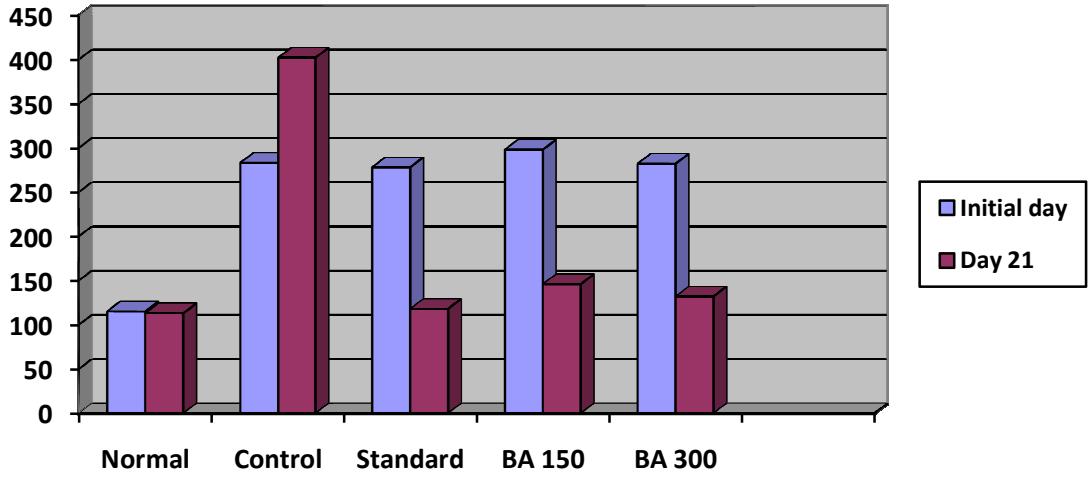
Alloxan induced hyperglycemia

Alloxan induced Diabetes, by damaging the insulin secreting cells of the pancreas leading to hypoglycaemia. 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 *BA* leaves ethanol extract significantly (P<0.01) (P<0.05) decreased the elevated blood glucose level ,when comparison to Alloxan induced diabetic rats[9,10,11]

Effect of BA Leaves extract in Blood Glucose level in diabetic rats on day 21 was compared to initial day between the groups.

S.No	Groups/Treatment	Blood glucose level (mg/dL)	
		Initial day	Day 21
I	Normal	115.27 ± 4.50	113.82 ± 2.40
II	Control	282.81 ± 2.30	402.00 ± 3.40#
III	Standard (6 mg/kg)	278.27 ± 3.20	118.53 ± 3.50
IV	*BA* (150 mg/kg)	298.45 ± 2.37	146.23 ± 0.40*
V	*BA* (300 mg/kg)	282.45 ± 2.37	132.48 ± 1.78**

Values are expressed as mean ± SEM.,(n=5) When groups IV , V compared with diabetic control, *i.e.* group II. (Among the treatment groups IV, V groups shown more significant effect) P –values are * Indicates significance & ** Indicates Highly significance,# indicates control.



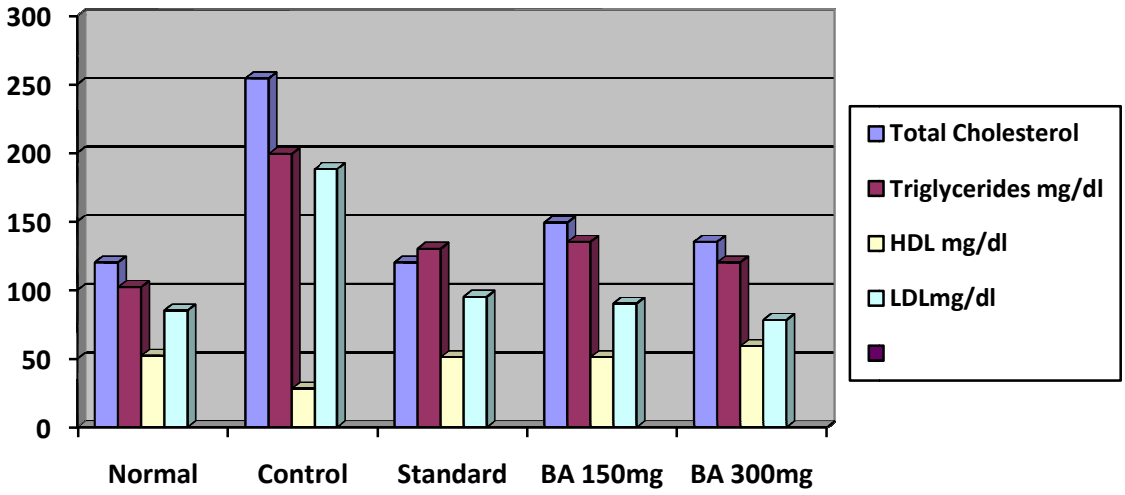
Effect on lipid profile

In the present study the Total Cholesterol, Triglycerides and LDL was reduced in by 21 days treatment with Total cholesterol, Triglycerides, LDL significantly reduced by treatment of ***BA*** leaves Ethanol extractas compared to diabetic control group. HDL cholesterol level was significantly improved by treatment of ***BA*** leaves ethanol extract as compared to diabetic control group. The results of present study indicated that the plant ***BA,*** leavesethanol extract possesses significant hypolipidemic activity.

Effect of *BA* Leaves extract in lipid profile in diabetic rats on the day 21 were compared in between the groups

S.No	Groups/Treatment	Total cholesterol	Triglycerides (mg/dl)	HDL (mg/dL)	LDL(mg/dl)
I	Normal	120.28 ± 3.80	102.42 ± 5.16	52.32 ± 2.9	85.4±3.11
II	Control(ALXN)	254.73 ± 7.60#	199.52 ± 4.71 #	28.23 ± 2.2#	188±11.29#
III	Standard(GLB)	120.54 ± 3.40	130.55 ± 4.62	51.47 ± 5.7	95.10±18
IV	*BA* 150 mg/kg	149.27±3.40*	135.29 ± 1.23*	51.28 ± 5.6*	90.25±2.13*
V	*BA* 300mg/kg	135.27±2.15**	120 ± 1.05**	59.28 ± 4.1**	78.45±3.81**

Values are expressed as mean ± SEM (n=5) *P<0.05, **P<0.01, when groups IV, V compared with diabetic control, i.e. Group II (Among the treatment groups IV, V groups are shown more significant effect) * indicates significance ** indicates more significance # indicates control.



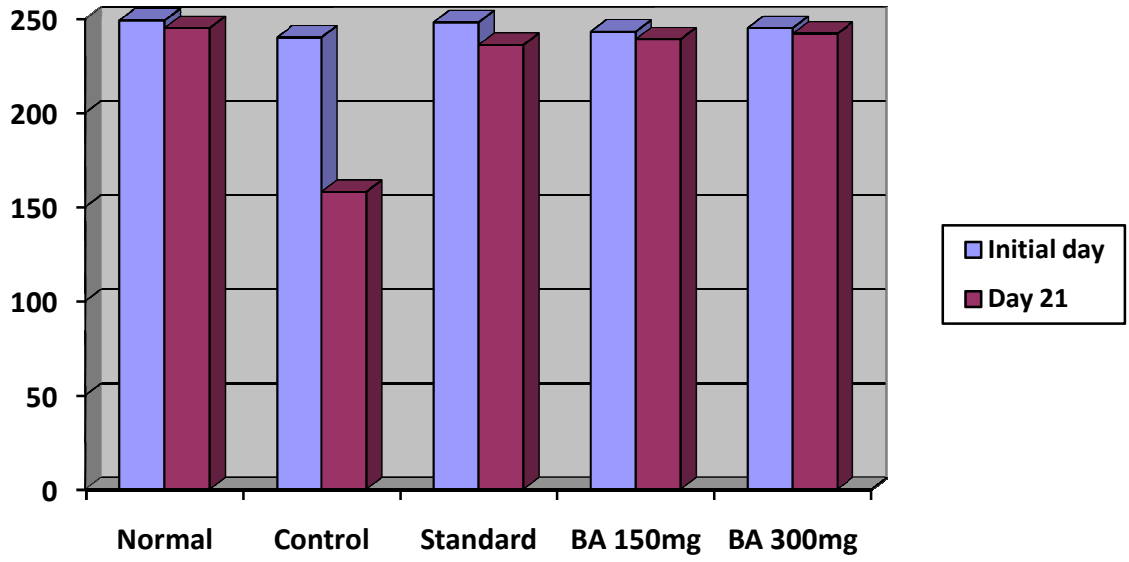
Effect on body weight

The body weight of the diabetic controls (group II) significantly decreased compared with the normal controls (group I). During the observation of the plants *BA* Leaves Extract treated with diabetic rats at doses of 150 mg/kg and 300 mg/kg, respectively there were significant (P<0.01) (P<0.05) weight gains on day 21 relative to day 0 as shown in the Table

Effect of *BA* Leaves extract on body weights in diabetic rats on day 21 were compared to initial day in between the groups

S.No	Groups/Treatment	Body weight	
		Initial day	Day 21
I	Normal	249.20 ± 2.30	245.47 ± 3.20
II	Control	240.23 ± 3.40	158.25 ±4.30#
III	Standard (6 mg/kg)	248.24 ± 2.70	236.14 ± 2.80
IV	*BA* (150 mg/kg)	243.43 ± 3.40	239.73 ± 3.20**
V	*BA* (300 mg/kg)	245.34 ± 2.70	242.25 ± 555555556.231.80**

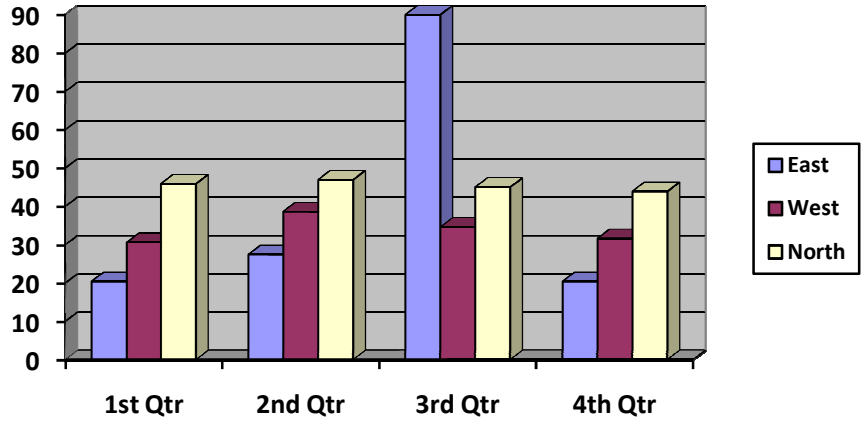
Values are expressed as mean ± SEM (n=5) *P<0.05, **P<0.01, when groups IV, V compared with diabetic control, i.e. Group II (Among the treatment groups IV, V groups are shown more significant effect) * indicates significance ** indicates more significance # indicates

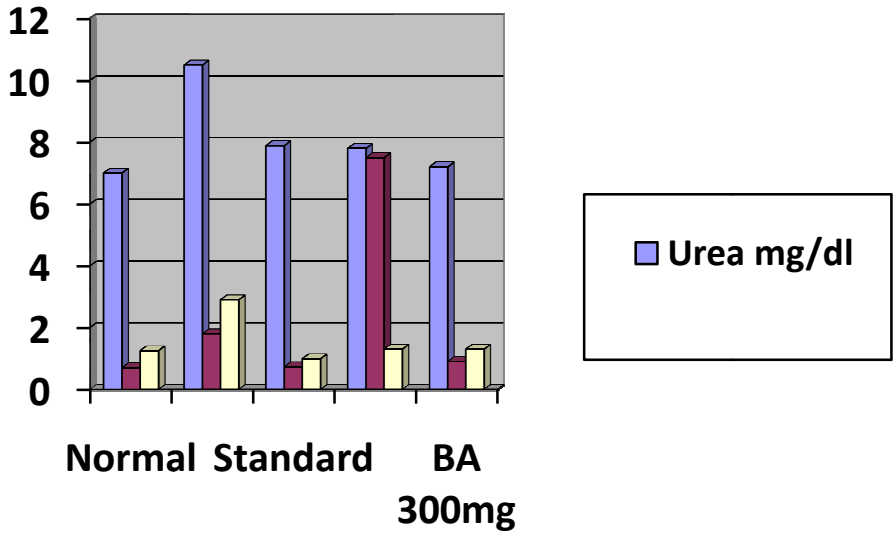


Effect of *BA* Leaves extract in Urea and Serum Creatinine, Bilirubin in diabetic rats on day 21 were compared in between the groups.

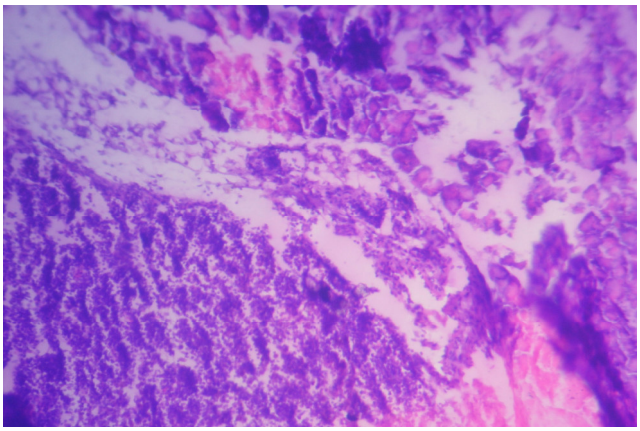
S.No	Groups/ Treatment	Urea mg/dl	Creatinine mg/dl	Bilirubin mg/dl
I	Normal	07.04 ± 0.01	0.7 ± 0.094	1.25 ± 0.08
II	Control(ALXN)	10.58 ± 0.15 #	1.82 ± 0.07#	2.9 ± 0.05#
III	Standard(GLB)	7.89 ± 0.18	0.73 ± 0.06	0.99 ± 0.02
IV	*BA* 150 mg/kg	7.34 ± 7.08**	0.9.34 ± 7.08**	1.01 ±0.108**
V	*BA* 300mg/kg	7.11 ± 7.08**	0.8.34 ± 7.08**	1.34 ± 7.08**

Values are expressed as mean ± SEM (n=5) *P<0.05, **P<0.01, when groups IV, V compared with diabetic control, i.e Group II (Among the treatment groups IV, V groups are shown more significant effect) * indicates significance ** indicates more significance



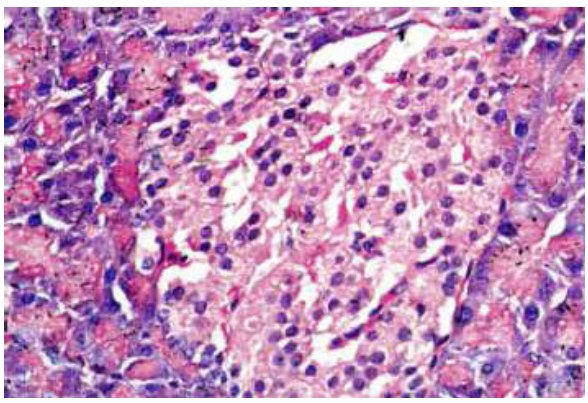


Interpretation of Histopathology



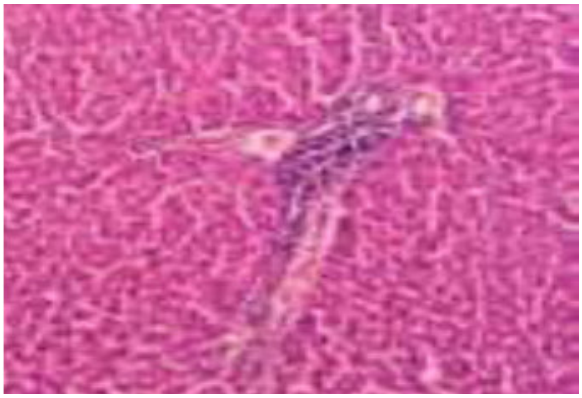
Light microscope section of pancreas of control group

After 21 days , the islets of Langerhans from the pancreas of diabetic control group revealed 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[11,12]



Light microscope section of pancreas of normal control group

Histology of the islets of langerhans of normal control rats which sacrificed on 21 days showed no pathological changes. The exocrine pancreatic tissue composed of acne with draining ductless. The endocrine components were found as nodules with in the substance of exocrine pancreas



Light microscope section of pancreas of standard group
Diabetic standard group

Histopathological study of pancreatic tissues in diabetic rats treated with standard drug glibenclamide was carried after 21 days there was marked improvement and the islets of langerhans was almost returned to normal

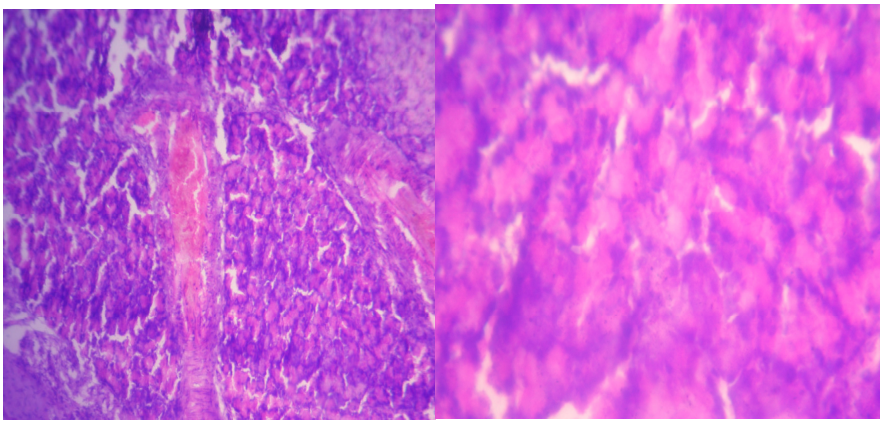


Fig – No 1.Light microscope section of pancreas of BA 150mg/kg
Fig–No.2.Light microscope section of pancreas of BA 300mg/kg

Histopathological study of pancreatic tissues in diabetic rats treated with BA leaf extract was carried after 21 days there was marked improvement and degeneration of the islets of langerhans.

Toxicity study Discussion

Acute toxicity studies on rats showed no mortality at a dose of 2000 mg/kg, during a time period of 14 days. During the study, no noticeable responses were seen in the rats. This helps to predict that it does not contain any type of toxicity and is safe.

Antimicrobial evaluation Discussion

Anti microbial study did by Kirby Bauer Disk Diffusion Method**.** The inoculated plates were incubated at 27±20C for 24 h, and then the inhibition zones were measured in diameter (mm). Antibiotic discs containing 1 µg of Ciproflaxin CF1 and Ampicillin AP1 (Himedia, India) was used as positive controls and DMSO used as negative control. The MIC was calculated using the 4 concentrations of the samples. Against Test organisms: Escherichia coli, *Staphylococcus aureus,* S. Typhi, P. Aurenginosa, We found the MIC (*BA*) 10mg, 10mg,20mg,10mg.

Pharmacological Discussion

- Alloxan induced diabetes showed increased plasma levels of cholesterol, triglycerides, LDL, & HDL is reduced. Insulin deficiency may be responsible for dyslipidaemia. Here repeated administration of *Barringtonia acutangula* (leaf) extract 150mg, 300mg /kg BW extract for a period of 21 days. Renal profile level decreased significantly and Blood glucose level BA (146.23 ± 0.40, 132.48 ± 1.78), Body weight BA (233.73 ± 3.20, 240.25 ± 1.80)
- Insulin has an inhibitory action on HMG-COA reductase, a key rate-limiting enzyme responsible for the metabolism of cholesterol rich LDL particles.
- Among the BA leaf extracts treated rats showed significantly reduced Total cholesterol level (149.27 ± 3.40),(135.27 ± 3.40) and triglyceride(135±1.23),(120±1.05) LDL (90.25±2.13), (81.45±3.81) levels. & increased HDL (51.28 ± 56),(59.28 ± 4.1) at the dose of 150 mg/kg, 300mg/kg respectively.
- This effect may be due to increased insulin secretion and the inhibition of the HMG CoA enzyme.
- Hence, the active constituents of *Baringtoniaacutangula*in the present study have shown significant anti-diabetic and hypolipidemic activity and the activity may be due to increased insulin secretion, probably by the regeneration of the pancreatic beta cells.

Histopathology discussion[13,14]

Histopathology studies in the control group showed reduction in the number of pancreatic islets as well as in the number of beta cells. The islets were irregularly shaped, relatively small and atrophic. Most of the beta cells were destroyed and even if present, they were destroyed partially. Insulin producing beta cells were drastically decreased, whereas glucagon producing alpha cells was predominantly present. Severe vacuolation and de granulation were present in the beta cells of a maximum number of islets. The effect of *BA* leaf extract on beta cells. Showed an increase in the number of pancreatic islets and beta cells in the pancreas.

This indicated that *BA* leaf extract was regenerating beta cells. The regeneration of the beta cells of the Alloxan destructed islets is probably due to the fact that the pancreas contains stable (Quiescent) cells which have the capacity of regeneration.

Therefore, the surviving cells can proliferate to replace the lost cells. Restore the secretion of insulin, and thus correct hyperglycaemia.

BA leaf Extract 300 mg dose treated rats showed more regeneration of the beta cells, increase the number of beta cells may secrete more insulin it may helpful to reduce the elevated hyperglycaemia.

CONCLUSION

- Barringtonia acutangula extracts Barringtonia acutangula leaf extract 300mgshown more significant effect in reduction of blood glucose level. can increase insulin secretion of pancreatic cells and improve the overgrowth of β cells, and also its proved by observation of histopathology investigation.
- More improvement in lipid profile and body weight. Its due to various biological active constituents (Flavonoids, saponins, Triterpenes, tanins) and also its shown significant antimicrobial action
- Barringtonia acutangula (leaf) extract have shown significant Antidiabetic, Hypolipidemic and Nephroprotective effects. Therefore have beneficial effects in diabetes mellitus that holds the hope of new generation of antidiabetic drugs.

REFERENCES

[1]. V.P.S. Varier, A.V. Sala, Kottakkal; Indian medicinal plants, Orient Longman Pvt.Ltd,1994; vol-1: p- ii.
[2]. Trease and Evans Pharmacognosy, Elsevier India private limited, 2005; 15th edition, p-137.
[3]. Trease GE, Evans WC. Introduction and general methods. *Pharmacognosy*(ELBS13th Eds), University Press, Great Britain, Cambridge, 1994: 247 - 264.
[4]. Harborne JB. Methods of plant analysis. Phytochemical methods, Chapman and hall,(2nd Eds), Chaucer press, Great Britain, 1984:1-36.
[5]. Sanjeevaraddi R Sataraddi, Sharanappa T Nandibewoor,Bio synthesis, characterization and activity studies of Ag nanoparticals, by (Costusingneus) insulin plant extract, Der Pharmacia Lettre, 2012, 4 (1):152-158

[6]. Ahmad ZM and Mohammad F. J of Ethnopharmacology, 1998;62: 183-193.
[7]. Nilesh P Babre1*, Subal Debnath1, S.Y. Manjunath1,V.Malla Reddy1, P. Murlidharan 2, G.Manoj2. Antidiabetic Effect of Hydroalcoholic Extract of *Barringtoniaacutangula*Linn. Root on Streptozotocin-induced Diabetic Rats
[8]. International Journal of Pharmaceutical Sciences and Nanotechnology Volume 3 • Issue 3 • October - December 2010.
[9]. S. Pascual ME, Carretero ME, Slowing KV, Villar A. 2002.Simplified screening by TLC of plant drugs.Pharmaceutical Biology, 40(2):139-143.
[10]. P.K. Mukherjee; Quality control of harbal drugs, an approach to evaluation of Botanicals, Business Horizons, 2002; vol-1; p-3.
[11]. H.A. El-Shemy, A.M. Aboul-Enein, K.M. Aboul-Enein, K. Fujita; Willow Leaves'Extracts Contain Anti-Tumor Agents Effective against Three Cell Types, plosOne.2007; 2: e178.
[12]. Grotewold E. The Science of Flavonoids. 6th ed. NY: Springer Science Business MediaInc; 2007. P. 1-2.
[13]. Sarkar SD, Latif Z, Gray AI. Natural product isolation. 2nd ed. New Delhi: Springer;2005. P.323-351.
[14]. Mukherjee PK. Quantity Control of Herbal drug: An approach to evaluation Of Botanicals. 3rd ed. New Delhi: Business Horizones; 2010. P.379-422.
[15]. Heinrich, M.; Barnes, J., Gibbons, S. & Williamson, E.M. (2004). Fundamentals of Pharmacognosy and Phytotherapy. Churchill Livingstone, Edinbrugh, pp. 245–252.