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Hypolipidemic Effect of Hydro-Alcoholic Extract of *Barringtonia acutangula* Linn Root Extract on Streptozotocin-induced Diabetic Rats

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Abstract: Our present study was to find out the hypolipidemic activity of an hydro-alcoholic extract of *Barringtonia acutangula* Linn root (EBA) on streptozotocin (STZ)-induced rats. Normal as well as diabetic albino rats was divided into groups (n=6) receiving different treatments i.e. vehicle (control), ethanolic extract, EBA (1g and 2g/kg b.w), STZ and glibenclamide. Blood samples was collected by cardiac puncture and was analyzed for lipid profile on days 0,7,14 and 21. STZ-treated diabetic rats has abnormal lipid profile, whereas the *Barringtonia acutangula* Linn root extract (EBA) treated groups showed significant improvement in the lipid profile comparable to glibenclamide treated group. However, improvement in lipid profile was less than that achieved with the standard drug glibenclamide. We conclude that administration of EBA improves lipid profile on euglycemic as well as diabetic rats. The probable mechanism of this action is delaying the intestinal absorption of dietary fat by inhibiting pancreatic lipase activity by saponin which is present in EBA.

Keywards: *STZ-induced diabetic, Barringtonia acutangula Linn root, hypolipidemic effect, serum total cholesterol, serum triglycerides, glycogen in liver.*

Introduction:

Barringtonia acutangula Linn (Family-Lecythidaceae) locally known as Hijjol, is a small to medium-seized evergreen tree native to coastal wetlands in Southern Asia and Northern Australia. In India, it is common in low lying areas and has been used by Indigenous groups for a wide range of medicinal purposes. Extract of roots is hypoglycemic, aperient and expectorent, the leaf juice is useful in diarrhoea, seeds are carminative and anti-emetic (Sahoo T.A et.al., 2008)[1]. The diverse activity of *Barringtonia acutangula* Linn inspired us to investigate its antilipidemic activity **(**Ung-Kyu Choi et.al., 2010)[2].

There has been an increasing demand from patients for the use of natural products with antihyperlipidemic activity. The undesirable side effects and contraindications of synthetic drugs, does not appear without risk and the fact that they are not suitable for use during pregnancy, have made scientists look towards natural products with antihyperlipidemic activity. Lipids play an important role in the pathogenesis of complications involved with diabetes mellitus, where elevated level of serum cholesterol and reduced level of serum HDL cholesterol, possese to be a risk factor for developing microvascular complications leading to atherosclerosis and further leads to cardiovascular diseases like coronary heart disease[3,4].

Insulin is the main regulator of glycogenesis in muscle and liver. The decrease of liver glycogen level observed in this study may be due to lack of insulin in diabetic condition or oxidative stress by diabetes may inactivate the glycogen synthatase[5]. The marked reduction in liver and muscle glycogen level is observed (21 days) on STZ-induced diabetic animals, whereas on treatment with EBA remarkably increased the glycogen level in liver and muscle.

Materials and methods:

Collection and authentication of plant material:

The root of *Barringtonia acutangula* Linn was obtained from Abhirami botanicals, Tuticorin, Tamilnadu. The plant material was identified and authenticated by resident botanist, Dr.S. Jayaraman, Plant Anatomy Research Centre (PARC), Chennai. The voucher specimen was submitted at Dept of Pharmacology, C.L.Baid Metha College of Pharmacy, Chennai,T.N.

***Preparation of plant extracts*[6]:**
The root was chopped to small pieces and dried in shade, powdered and a weighed, subjected to hot solvent extraction in a soxhlet apparatus using aqueous EtOH (50:50), at a temperature range of 60-70˚C. The extract was concentrated to dryness at 40˚C under reduced pressure in a rotary vacuum evaporator. The aqueous EtOH (50:50) extract yielded a brown semi-solid (12.0%) and the extract was preserved in a refrigerator for it's usage.

***Experimental animals*[7]:**
Inbred adult Wistar albino rats, weighing 180-220 g of either sex was taken, maintained in a well-ventilated room with 12:12 hour light/dark cycle in polypropylene cages. The project was approved by IAEC (Institutional Animal Ethical Committee) of CPCSEA (Committee for the Purpose of Control and Supervision of Experimentation on Animals) through it's reference no: IAEC/XII/02/CLBMCP/2008-2009, dated:24/11/2008.

***Toxicological evaluation*[8,9]:**
Acute oral toxicity study (OECD 423): This procedure was followed by using OECD guide lines (Organization of Economic Cooperation and Development) 423 (Acute Toxic Class Method). For experimental methods of estimation like Total cholesterol (CHOD-POD phosphotungestan method), HDL-cholesterol (CHOD-POD phosphotungestan method), HDL-cholesterol, triglycerides (GPO-POD method), LDL-cholesterol, serum creatinine (Modified Jaffe's kinetic method) was used[10,11].
LDL-cholesterol = Total cholesterol− [HDL-C + (Triglycerides/5)],
VLDL- cholesterol = (Triglycerides /5)

Biochemical estimation

***Estimation of glycogen in liver and muscle*[12,13]:**
This estimation indicates the distinction between 'free' and fixed glycogen content in tissue by using anthrone reagent (solution containing 0.05 % anthrone, 1% thiourea and sulphuric acid-72 %, potassium hydroxide-30%, ethanol-95%. Accurately weighed 100 mg of liver and muscle, was digested with 2 ml of 30% boiling KOH and cooled, 3ml of 95% ethanol was added and heated until the bubbles was formed. These mixtures was cooled and centrifuged at 1000 rpm for 5 min and supernatant was discarded. The residue was dissolved in 2ml of distilled water, 10 ml of anthrone reagent was added and immersed in an ice bath to prevent excessive heating. Tubes was incubated at 100^0C for 4 minutes for color development and immersed in an ice bath. Absorbance was measured at λ620 nm using a spectrophotometer. The glycogen content in wet tissue is expressed as mg of glycogen per 100gm.

Results and discussion:
Present study showed that STZ-treated diabetic rats has abnormal lipid profile, whereas the EBA treated group showed significant improvement in the lipid profile comparable to glibenclamide treatment group. Insulin is the main regulator of glycogenesis in muscle and liver. The decrease of liver glycogen level observed in this study may be due to lack of insulin in diabetic condition or oxidative stress by diabetes may inactivate the glycogen synthatase. The marked reduction in liver and muscle glycogen level is observed (21 days) in STZ-induced diabetic animals. Treatment with EBA extract remarkably increased the glycogen level in liver and muscle.

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, LDL-cholesterol, VLDL-cholesterol, creatinine when compared to STZ-induced diabetic rats. As well as the treatment with EBA (250 mg/kg b.w/p.o and 500mg/kg b.w/p.o) showed significant ($p<0.01$) increase in serum HDL-cholesterol and glycogen level in liver when compared to STZ-induced diabetic rats.

However, glibenclamide (0.5mg/kg b.w/p.o) treatment showed significant ($p<0.01$) increase of HDL-cholesterol and glycogen level in liver when compared to STZ-induced diabetic rats. Whereas, glibenclamide (0.5mg/kg b.w/p.o) treatment showed significant ($p<0.01$) decrease of serum triglycerides, LDL-cholesterol, VLDL-cholesterol and creatinene when compared to STZ-induced diabetic rats. The

Table-1: Effect of EBA on serum total cholesterol, HDL Cholesterol in STZ-induced diabetic rats.

G	*Treat.*	*Dose (Kg $^{-1}$ Body W)*	*Total Chol. (mg/dl)*	*HDL -Chol. (mg/dl)*	*TG (mg/dl)*	*LDL (mg/dl)*	*VLDL (mg/dl)*	*Creat. (mg/dl)*	*liver gly. level (mg/g wet tissue)*	*muscle gly. level (mg /g of wet tissue)*
I	Control	5 ml	97 ±0.894	41.50 ±0.428	92.33 ±1.382	36.17 ±0.600	17.83 ±0.30730	0.9617 ±0.980	52 ±2.140	8.33 ±550
II	Disease control (STZ)	45 mg	157.8 ±3.177^{a**}	26.50 ±0.619^{a**}	171.5 ±1.648^{a**}	99.83 ±1.167^{a**}	34.67 ±0.332^{a**}	1.625 ±1.672^{a**}	18.67 ±0.71^{a**}	2.83 ±0.30^{a**}
III	Std. (Gli.+STZ)	0.5 mg	108.5 ±1.668^{b**}	38 ±0.651^{b**}	95.50 ±2.012^{b**}	51.17 ±0.6009^{b**}	18.50 ±0.22^{b**}	1.16 ±0.022^{b**}	43.50 ±0.76^{b**}	7.12 ±0.25^{b**}
IV	Test I (EBA+STZ)	250mg	122.7 ±1.726^{b**}	33.17 ±0.609^{b**}	124.8 ±1.515^{b**}	64.17 ±0.4773^{b**}	23.83 ±0.307^{b**}	1.480 ±0.0161^{b*}	23.50 ±0.99^{b*}	4.33 ±0.30^{b*}
V	Test II (EBA+STZ)	500mg	115.2 ±1.195^{b**}	36.83 ±0.477^{b**}	112.3 ±1.174^{b**}	55.50 ±5672^{b**}	21.58 ±0.42^{b**}	1.243 ±0.0172^{b**}	32.83 ±54^{b**}	6.16 ±0.30^{b**}

The values are expressed as mean ± SEM of 6 animals. Statistical significance test for comparison was done by ANOVA, followed by Dunnet's test. **p<0.01 , *p<0.05, G = group, Treat.= treatment, Chol. = cholesterol, TG = triglycerides, gly. = glycogen, Gli. = glibenclamide, Creat = creatinine, W = weight.

treatment with EBA (250 mg/kg b.w/p.o and 500mg/kg b.w/p.o) showed significant (p<0.01) increase in serum HDL-cholesterol and glycogen level in liver when compared to STZ-induced diabetic rats **(Table 1).**

Conclusions:

The treatment of EBA roots showed marked decrease in glycosylated haemoglobin, as well as marked increase in body weight, protein, HDL-cholesterol levels in serum of STZ-induced diabetic rats. At the same time treatment of EBA roots showed significant decrease in total cholesterol, LDL-cholesterol, VLDL-cholesterol, triglycerides and creatinine levels was observed in serum of diabetic rats. The EBA extract treatement remarkably increased the glycogen level in liver and muscle on STZ-induced diabetic rats. The EBA produced significant beneficial effects on the lipid profile on hyperlipidemic rats at both test doses i.e. 250 mg/kg b.w/p.o and 500mg/kg b.w/p.o.

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