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Research Article

ANTIOXIDANT POTENTIAL OF HYDROALCOHOLIC EXTRACT OF *BARRINGTONIA ACUTANGULA* LINN ROOTS ON STREPTOZOTOCIN-INDUCED DIABETIC RATS

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ABSTRACT

Barringtonia acutangula Linn is a plant traditionally used for the cure and treatment of many ailments like diarrhoea, anthelmintic, hemolytic disease (various diseases of blood), abdominal colic, lumbar pain, syphilis, blennorrhoea, febrifuge, malarial and diabetes. Free radical scavenging activity and antioxidant property of the hydroalcoholic extract of plant *Barringtonia acutangula* Linn root (EBA) was evaluated by the method non-enzymatic antioxidant reduced glutathione (GSH), catalase, superoxide dismutase (SOD) and lipid peroxidation (malondialdehyde) activities. All the *in-vitro* models showed dose dependent antioxidant activity. Reactive oxygen species (ROS), such as hydroxyl radicals (-OH), superoxide (O_2, -OOH), hydrogen peroxide (H_2O_2) and reactive nitrogen species (RNS) causing cellular injury at molecular levels. The health benefit of the hydroalcoholic extract of the plant was evaluated by the *in-vitro* models. Natural antioxidants are in main research path in recent terms because of their toxic and mutagenic effects are the primary side effects of the many synthetic antioxidants. The pancreoprotective activity of the hydroalcoholic extracts of the plant *Barringtonia acutangula* Linn was evaluated by the antioxidant property of the plant extract.

Keywords: *Barringtonia Acutangula* Linn, Antioxidant property, Superoxide dismutase, Catalase, Glutathione peroxidase, Lipid peroxidation.

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 the roots is hypoglycemic, aperient and expectorent, the leaf juice is useful in diarrhoea, seeds are carminative and anti-emetic[1]. The diverse activity of *Barringtonia acutangula* Linn inspired us to investigate its antilipidemic activity[2].

Degenerative and pathological process such as alzheimer's disease, rheumatoid arthritis, coronary artery disease, parkinson's disease, cataracts, inflammation, cancer, aging, pancreas damage in diabetes are accompanied by the free radicals and reactive oxygen species oxidative stress[3]. Oxidative stress occurs by the source of stimulated polymorphonuclear leukocytes, macrophages, peroxysomes and aerobic respiration, causing intracellular and extracellular damage of macromolecular like proteins, lipids and nucleic acid[4]. Superoxide dismutase, glutathione peroxidase (GPx) and catalase are the antioxidant enzymes. Hydroxyl radical produced by metal catalyzing process by cleavage of water and hydrogen peroxide, initiates the peroxidation of the cell membrane lipids (lipid peroxides), converts malondialdehyde into carcinogenic and mutagenic compounds[5].

GPx is a selenium containing enzyme, which is active in the reduced form. These enzyme catalyses the oxidations of GSH to GSSG at the expense of hydrogen peroxide[6]. A marked decrease in the hepatic GSH was observed on the Streptozotocin (STZ)-induced diabetes on rats[7]. Such a decrease contributions to the pathogenesis of the complications associated with chronic diabetic state. Treatment with EBA shows a significant protection of this enzymes in diabetic state[8]. Impairment of enzymatic antioxidant system due to reduced levels of insulin in diabetic state increases fatty acyl-coA oxidase and initiates β-oxidation of fatty acids that favours accumulation of free radicals resulting in lipid peroxidation. Increased lipid peroxidation impairs membrane function by decreasing the fluidity and changing the activity of the membrane bound enzymes and receptors. Elevated Thiobarbituric acid reactive substances (TBARS) level in diabetic rats suggests the extent of peroxidative injury, indicating the development of diabetic complications[9]. In the present study STZ-induction caused a significant increase in TBARS of hepatic tissue. But treatment with EBA shows a dose dependant protection against peroxidation and significant reduction in TBARS in hepatic tissues[10].

MATERIALS AND METHODS

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 the plant extracts

The root was chopped to small pieces and dried in shade, powdered and weighed, then 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.

Animals

Inbred adult Wistar albino rats, weighing 180-220 g of either sex were used. They were maintained in a well-ventilated room with 12:12 hour light/dark cycle in polypropylene cages with free access to food and water ad. libitum. The protocol 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.

Reagents

20% Acetic acid, 8.1% sodium dodecyl sulphate (SDS), 0.8 % thiobarbituric acid (TBA), n-butanol-pyridine mixture (15:1v/v), sodium phosphate buffer 0.32 M (pH7), ethylene diamine tetra acetic acid (EDTA)-0.8mM, sodium azide-10mM, reduced glutathione (GSH)-4mM, hydrogen peroxide-2.5 mM, 10%Tricarboxylic acid (TCA), disodium hydrogen phosphate-0.3M, 5,5/-dithiobis (2-nitrobenzonic acid)-DTNB, glutathione standard, phosphate buffer (M/15, p^H7.0), hydrogen peroxide-phosphate buffer-p^H 7-0.1 M, tris-HCl buffer-pH 8.2-5M, Tris-HCl buffer-pH7.4-2 mM pyrogallol solution, absolute alcohol, chloroform.

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).

Estimation of antioxidant enzyme levels-Preparation of liver homogenate

Liver was removed, homogenized with buffer containing (0.25 M sucrose and 0.1M Tris-HCl buffer (pH7.4) to prepare 10% homogenate, prepared by using in Teflon pestle and glass homogenizer and centrifuged at 600 rpm for 10 min. To obtain post mitochondrial supernatant (PMT), this was used to analyse the protein concentration by biuret's method. The post mitochondrial supernatant was again centrifuged at 8000 rpm for 15 min. This supernatant was used to analyze the antioxidant enzyme level in the liver.

Estimation of enzymic antioxidants-Assay of superoxide dismutase[11]

To 1 ml of the sample, 0.25 ml of absolute ethanol and 0.15 ml of chloroform was added. After 15 min of shaking in a mechanical shaker, the suspension was centrifuged and the supernatant obtained constituted the enzyme extract. The reaction mixture for auto-oxidation consisted of 2ml of buffer, 5ml of 2mM pyrogallol and 1.5ml of water. Initially the rate of auto-oxidation of pyrogallol was noted at an interval of 1 min for 3 min. The assay mixture for the enzyme contained 2ml of 0.1M Tris-HCl buffer, 5ml of pyrogallol, aliquots of the enzyme preparation and water to make up 4 ml. The rate of inhibition for pyrogallol auto-oxidation after the addition of the enzyme was noted. The enzyme activity was expressed in terms of units/min/mg protein.

Estimation of catalase[13]

Homogenized the tissue with M/15 phosphate buffer at 1 to 4°C and centrifuged. Stirred the sediment with cold phosphate buffer and allowed to stand in the cold condition with occasional shaking. Repeat the extraction once or twice, supernatants are combined and used for assay. 3ml of H_2O_2 phosphate buffer was taken in one cuvette, added 0.01-0.04 ml sample and read against a control cuvette containing enzyme solution without H_2O_2 phosphate buffer at 240 nm. It was noted for a decrease in the optical density from 0.450 to 0.400. This value was used for the calculations.

Glutathione peroxidase (GPx)[13]

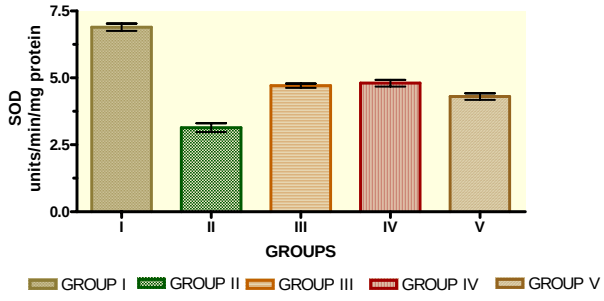
0.2 ml each of EDTA, sodium azide, glutathione (reduced), hydrogen peroxide, 0.4 ml of buffer and 0.1 ml of homogenate was mixed and incubated at 37°C for 10 min. The reaction was arrested by the addition of 5 ml of TCA, the tubes was centrifuged. To 5 ml of supernatant, 4 ml of disodium hydrogen phosphate, 5 ml of DTNB was added and the colour developed was read at 420 nm immediately, standards was also treated similarly. Glutathione peroxidase activity is expressed as n moles of glutathione utilized/minute/mg protein at 37°C.

Estimation of tissue enzyme-estimation of lipid peroxidation[14, 15]

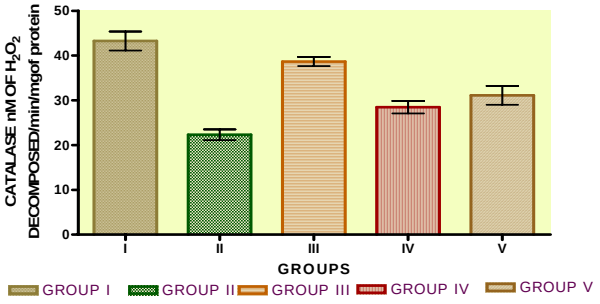
Lipid peroxidation in the liver homogenate was determined by measuring the amounts of malondialdehyde produced. 0.2 ml of tissue homogenate, 1.5 ml of 20% acetic acid, 0.2 ml of sodium dodecyl sulphate and 1.5 ml of thiobarbituric acid was added. The volume of the mixture was made up to 4.0 ml with distilled water and then heated at 95°C in a water bath for 60 min. After incubation the tubes was cooled to room temperature and final volume was made to 5.0 ml in each tube. 5.0 ml n-butanol-pyridine (15:1) mixture was added and the content was vortexes thoroughly for 2 minutes. After centrifugation at 3000 rpm for 10 min, the organic upper layer was taken and its optical density read at 532 nm against an appropriate blank without the sample. The level of lipid peroxides was expressed as n moles of malondialdehyde (MDA)/mg protein in liver homogenate.

RESULTS

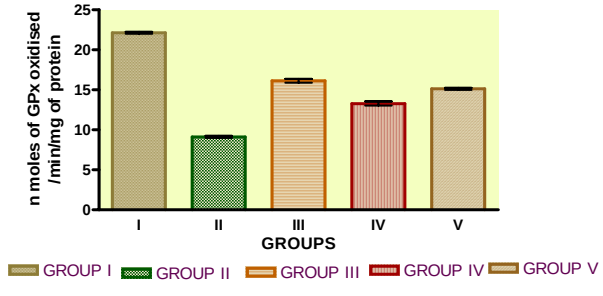
Acute oral toxicity studies reveal that EBA did not produce any mortality or signs of toxicity at the dose of 2000 mg/kg b.w.p.o. in experimental rats. The liver SOD, CAT and GPx levels of diabetic animals treated with EBA (250mg/kg b.w./p.o. and 500mg/kg b.wt/p.o) and glibenclamide (0.5mg/kg b.w./p.o.) showed significant ($p<0.01$) increase when compared to STZ-induced diabetic animals. Whereas, a significant ($p<0.01$) decrease in the liver SOD, CAT, GPx was observed in STZ-induced diabetic animals when compared to control animals. LPO level of diabetic animals treated with EBA (250mg and 500mg/kg b.w./p.o.) and glibenclamide (0.5mg/kg b.w./p.o.) showed significant ($p<0.01$) decrease when compared to STZ-induced diabetic animals. A significant ($p<0.01$) increase in the liver LPO was observed in STZ-induced diabetic animals when compared to control animals (Table 1 and Fig. 1).



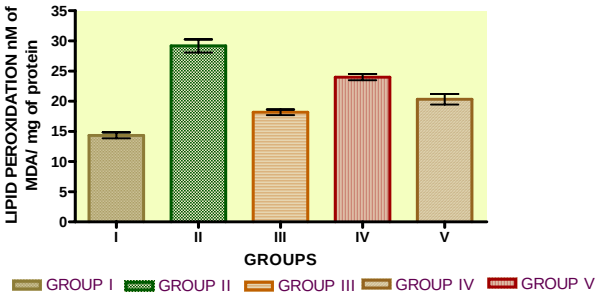
A) Effect of EBA on superoxide dismutase on STZ-induced diabetic rats



A) B) Effect of EBA on catalase on STZ-induced diabetic rats



C) Effect of EBA on glutathione peroxidase on STZ-induced diabetic rats



D) Effect of EBA on lipid peroxidation on STZ-induced diabetic rats

Fig. 1: Effect of EBA on superoxide dismutase, catalase, glutathione peroxidase and lipid peroxidation on STZ-induced diabetic rats

Table 1: Effect of EBA on superoxide dismutase, CAT Liver tissue, GP_X and LPO on STZ-induced diabetic rats

Treatment	Dose (per Kg body weight)	SOD Liver tissue (units/min/mg protein)	CAT Liver tissue (units/min/mg protein)	GP_X Liver tissue	LPO Liver tissue
Control	5ml	6.98 ± 0.13	43.24±2.12	22.14±0.04	14.33±0.494
Disease control (STZ)	45mg	3.14 ± 0.16^{a**}	22.35±1.18^{a**}	9.12±0.01^{a**}	29.17±1.087^{a**}
Standard (Glibenclamide+STZ)	0.5mg	4.71 ± 0.08^{b**}	38.65±1.01^{b**}	16.13±0.10^{b**}	18.17±0.477^{b**}
Test I (EBA+STZ)	250mg	4.80± 0.13^{b**}	28.45±2.12^{b*}	13.29±0.14^{b**}	24.00±0.516^{b**}
Test II (EBA+STZ)	500mg	4.30 ± 0.12^{b**}	31.10±1.39^{b**}	15.13±0.16^{b**}	20.33±0.881^{b**}

The values are expressed as Mean±SEM. Statistical significance test was done by ANOVA followed by Dunnet's test.**p<0.01. ,*p<0.05.

DISCUSSION

Free radicals in the form of reactive oxygen species (ROS) and reactive nitrogen species (RNS) are implicated in numerous pathological conditions. ROS such as hydroxyl radicals (-OH), super oxide (O_2^-, -OOH), and hydrogen peroxide (H_2O_2) are by-products of normal metabolism. Living systems are therefore protected from ROS and RNS by antioxidant enzymes (SOD, CAT, GP_x, LPO etc.) and other endogenous antioxidant sources such as alpha tocopherol, ascorbic acid, beta-carotene and uric acid. The present study results addresses the health benefit of EBA on STZ-induced diabetic rats in the scavenging of free radicals and reactive oxygen species using *in-vivo* model. EBA showed promising dose-dependent antioxidant activity in all the methods tested. Thus, regular consumption of these EBA may improve patient's antioxidant status.

CONCLUSION

The antioxidant study revealed that the hepatic antioxidant enzyme levels (SOD, CAT and GPx) are significantly decreased in STZ-induced diabetic rats with high degree of lipid peroxidation. The enzyme levels increased significantly on treatment with EBA roots at both test doses (250 mg/kg b.w./p.o. and 500mg/kg b.w./p.o.).

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