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EFFECT OF *BARRINGTONIA ACUTANGULA* ROOT EXTRACT ON ANTIOXIDANT ENZYMES IN RAT BRAIN AFTER MES INDUCED SEIZURE RATS

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ABSTRACT

The root of *Barringtonia acutangula* is used traditional Indian medicine to treat epilepsy. In present study the effect of ethanol extract of *Barringtonia acutangula* (EEBA) on antioxidant enzymes in rat brain after induction of epilepsy by Maximal Electroshock (MES) were observed. In which Superoxide dismutase and catalase was significantly ($P<0.01$) decreased in rat brain due to epilepsy and it was significantly ($P<0.01$) restored by administration of ethanol extract of *Barringtonia acutangula* treated rats. Similar dose dependent results were obtained in MES model. Whereas EEBA significantly decreased lipid peroxidation in both models. The anticonvulsant activity of EEBA might be presents of antioxidant properties and it delays the generation of free radical in MES induced epilepsy.

Keywords: Antioxidant enzymes, *Barringtonia acutangula* L, Superoxide Dismutase, Glutathione Peroxidase, Glutathione Reductase; Catalase, Lipid peroxidation.

INTRODUCTION

Barringtonia acutangula (L.) Gaertn. (Family: Lecythidaceae) an evergreen tree of moderate size is called as Hijja or Hijjala in Sanskrit. The fruit is spoken of as samudra-phala and various part of this plant used as a folklore medicine for curing various ailments like hemiplegia, pain in joints, eye diseases, stomach disorders, anthelmintic, diarrhoea, cough, dyspnoea, leprosy, intermittent fever, and splenic disorders. An ethanol extract of the bark is found hypoglycemic and is reported to be used in pneumonia, diarrhea, asthma and leaf juice is given for diarrhea. Fruit is bitter, acrid, anthelmintic, emetic, expectorant and vulnerary. It is prescribed in gingivitis, as an astringent and tonic. Whole plant was

reported to possess flavonols, phenolic acids, triterpenoids, tannins and steroidal compounds such as barringtogenic acid, tangulic acid and acutangulic acids. The fruit possessed saponins based on barringtogenol B, C and D. The therapeutic potential of this plant were reported to be antitumor, antibiotic, inhibit growth of *Helicobacter pylori* and antifungal activities [1-7].

Therefore, the aim of the present investigation was to evaluate the effect of *Barringtonia acutangula* (L.) root extract on antioxidant enzymes in rat brain after induction of epilepsy by MES in albino wistar rats.

MATERIALS AND METHODS

Plant material

The root of *Barringtonia acutangula* was collected from Tirumala hills, Tirupati, Andhra Pradesh, India. The plant was identified and authenticated by Dr.K.Madhava Chetty, Department of botany, S.V.University, Tirupati. The voucher specimen of the plant was deposited at the

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college for further reference. The root were dried under shade, powdered and stored in an air tight container.

Preparation of extract

The collected root was dried at room temperature, pulverized by a mechanical grinder, sieved through 40mesh. About 120g of powdered materials were extracted with ethanol (90%) using soxhlet apparatus. The extraction was carried out until the extractive becomes colourless. The extracts is then concentrated and dried under reduced pressure. The solvent free semisolid mass thus obtained is dissolved in normal saline and used for the experiment. The percentage yield of prepared extract was around 12.5%w/w.

Phytochemical analysis

The ethanol extract of *Barringtonia acutangula* was subjected to qualitative analysis for the various phyto-constituents. Standard methods were used for preliminary qualitative phytochemical analysis of extract [8].

Experimental Animals

Wister albino rats weighing between 150-200gm each maintained in a 12 h light/dark cycle at a constant temperature 25 °C with free access to feed (Sai durga feeds and foods, Bangalore) and water. All animals were fasted prior to all assays and were allocated to different experimental groups each of 6 rats. Moreover the animals were kept in specially constructed cages to prevent coprophagia during the experiment. All experiments were carried out according to the guidelines for care and use of experimental animals and approved by Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

Experimental Design

Albino wistar rats were divided into four groups of

six animals each. Group I received vehicle control (1% w/v SCMC, 1ml/100 g) whereas Group-II and III, received ethanol extract of *Barringtonia acutangula* (EEBA) (250 and 500 mg/kg body weight) *p.o* respectively for 20 days. On the 20th day, Seizures are induced to all the groups by using an Electro convulsimeter. The duration of various phases of epilepsy were observed.

Estimation of antioxidant enzymes in rat brain after induction of seizure

On the day of experiment, 100 mg of the brain tissue was weighed and homogenate was prepared in 10 ml tris hydrochloric acid buffer (0.5 M; pH 7.4) at 4°C. The homogenate was centrifuged and the supernatant was used for the assay of antioxidant enzymes namely catalase [9], superoxide dismutase [10], and lipid peroxidation [11].

Statistical analysis

The data were expressed as Mean \pm S.E.M. and statistically analyzed using one way ANOVA followed by Tukey-Kramer's Multiple comparison test, $p < 0.05$ was considered significant.

RESULTS

Effect of EEBA on antioxidant enzymes in seizure induced rats by MES

The levels antioxidant enzymes such as superoxide dismutase and catalase were significantly reduced ($p < 0.01$) due to induction of seizure by MES in Group II, whereas lipid peroxidation enzymes significantly increased ($p < 0.05$) in both models. Administration of EEBA at the doses of 250 and 500mg/kg significantly increased ($p < 0.05$) the levels of the enzymes on the rat brain. Lipid peroxidation was significantly decreased ($p < 0.05$) by the administration of EEBA 250 and 500 mg/kg. (Table 1).

Table 1. Effect of EEBA on antioxidant enzymes in rat brain after induced seizure by MES

Group	Design of Treatment	Superoxide dismutase Units/mg protein	Catalase Units/mg protein	Lipid peroxidation N mol MDA/mg protein
I	Vehicle Control (SCMC 1ml/100gm)	12.28 \pm 0.26	22.17 \pm 0.44	3.64 \pm 0.22
II	MES (SCMC 1ml/100gm)	8.19 \pm 0.14 ^{a**}	14.29 \pm 0.44 ^{a**}	4.94 \pm 0.24 ^{a**}
III	EEBA 250 mg/kg, <i>p.o</i>	11.27 \pm 0.26 ^{b*}	20.19 \pm 0.51 ^{b**}	4.12 \pm 0.16 ^{b*}
IV	EEBA 500 mg/kg, <i>p.o</i>	13.42 \pm 0.72 ^{b**}	23.46 \pm 0.27 ^{b*}	3.67 \pm 0.21 ^{b*}

Values are expressed as mean \pm SEM of six observations. Comparison between: **a-** Group I Vs Group II, **b-** Group II Vs Group III and Group IV. Statistical significant test for comparison was done by ANOVA, followed by Tukey-Kramer's Multiple comparison test * $p < 0.05$, ** $p < 0.01$

DISCUSSION AND CONCLUSION

The study showed that, high level of oxidative damage was detected both in case of electrically generated seizures, viz. electroshock induced seizures [12,13] and MES seizure models. Inactivation of oxygen free radicals can be carried out by antioxidative enzymes, like superoxide dismutase (SOD) and catalase [14,15]. The

results of this study showed that EEBA at the doses of 250 & 500mg/kg significantly increased the levels of antioxidant enzymes such as superoxide dismutase and catalase on rat brain. Whereas lipid peroxidation level increases in brain during epileptic seizures [16-17]. We documented that changes in glutathione peroxidase activity in brain homogenates were inversely correlated with intensity of

lipid peroxidation. It may be supposed that decrease in glutathione peroxidase activity causes failure of H₂O₂ detoxification. H₂O₂ accumulated in brain tissue iron ions present in the brain may undergo Fenton's reaction in which hydroxy radicals are produced. These reactive oxygen species participate in lipid peroxidation processes [18-20]. Increases in lipid peroxidation in brain observed in the present study were dependent on decrease in glutathione peroxidase activity. They suggested that oxidative stress and lipid peroxidation rise might occur during seizure and participate in the pathophysiology of epilepsy. Participation

of oxygen free radicals and oxidative stress in seizure etiology may indirectly be confirmed by anticonvulsant activity of antioxidant enzymes [21-30].

In conclusion, EEBA at the doses of 250 & 500mg/kg significantly increased the levels of antioxidant enzymes such as superoxide dismutase and catalase on rat brain. Inversely lipid peroxidation decreased in EEBA treated rats. Hence the antioxidant properties of EEBA extract delays the generation of free radical in MES induced epilepsy.

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