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EFFECT OF *BARRINGTONIA ACUTANGULA* LEAVES EXTRACT ON ANTIOXIDANT ENZYMES LEVELS IN RAT BRAIN AFTER INDUCTION OF CONVULSION

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ABSTRACT

The leaves of *Barringtonia acutangula* is used traditional Indian medicine to treat epilepsy. In present study the effect of aqueous extract of *Barringtonia acutangula* (EBA) on antioxidant enzymes in rat brain after induction of epilepsy by PTZ were observed. In which Superoxide dismutase, glutathione peroxidase, glutathione reductase 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 aqueous extract of *Barringtonia acutangula* treated rats. Similar dose dependent results were obtained in PTZ model also. Whereas EBA significantly decreased lipid peroxidation in PTZ model. The anticonvulsant activity of EBA might be presents of antioxidant properties and it delays the generation of free radical in PTZ induced epilepsy.

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

INTRODUCTION

Serious attention is now paid to the cytotoxicity of active oxygen/free radicals as the cause of various pathological conditions. Antioxidants are believed to play a very important role in the body defense system against reactive oxygen species (ROS), which are the harmful by products generated during normal cell aerobic respiration [1]. It is widely accepted that antioxidants are radical scavengers, which protect the human body against free radicals that may cause pathological conditions such as ischemia, anaemia, asthma, arthritis, inflammation, neurodegenertion, Parkinson's diseases, epilepsy, mongolism, ageing process and perhaps dementias [2].

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 spleenic 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 [3-9].

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On the basis of the traditional use of the plant for treating convulsion, but no previous pharmacological (or) clinical study was carried out to test the antiepileptic activity of this plant. Since the antiepileptic effect of *Barringtonia acutangula* has been experimentally not confirmed. Therefore, the aim of the present investigation was to evaluate the effect of *Barringtonia acutangula* (L.) leaves extract on antioxidant enzymes in rat brain after induction of epilepsy by PTZ in albino wistar rats.

MATERIALS AND METHODS

Plant material

The leaves 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, Tirupathi. The voucher specimen of the plant was deposited at the college for further reference. The leaves were dried under shade, powdered and stored in an air tight container.

Preparation of extract

The collected leaves were 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 10.5%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 [10].

Experimental Animals

Wister albino rats weighing between 150-200gm each maintained in a 12 h light/dark cycle at a constant temperature 25 ºC 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* (EBA) (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 convulsiometer. The duration of various phases of epilepsy were observed.

Pentylenetetrazole (90mg/kg b.w, *s.c*) was administered to other groups to induce clonic convulsions after above respective treatment. Animals were observed for a period of 30mins post– PTZ administration [11].

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 [12], glutathione peroxidise [13], superoxide dismutase [14], glutathione reductase [15] and lipid peroxidation [16].

Statistical analysis

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

Table 1. Effect of EBA on antioxidant enzymes in rat brain after induced seizure by PTZ

Group	Design of Treatment	**Superoxide dismutase** Units/mg protein	**Catalase** Units/mg protein	**Glutathione Reductase** Units/mg protein	**Glutathione Peroxidase** Units/mg protein	**Lipid peroxidation** N mol MDA/mg protein
I	Vehicle Control (SCMC 1ml/100gm)	13.28 ± 0.52	23.14 ± 0.45	34.20 ± 0.45	27.63 ± 0.57	3.19 ± 0.20
II	PTZ (SCMC 1ml/100gm)	8.22±0.24^{a}**	15.68±0.24^{a}**	25.33±0.57^{a}**	20.39±0.27^{a}**	4.46±0.27^{a}**
III	EBA 250 mg/kg,*p.o*	11.29±0.56^{b}*	21.12±0.52^{b}**	27.72±0.36^{b}**	24.19±0.17^{b}**	4.22±0.26^{b}*
IV	EBA 500 mg/kg,*p.o*	13.65±0.75^{b}**	22.45±0.57^{b}*	30.33±0.75^{b}**	26.32±0.70^{b}**	3.42±0.27^{b}*

Values are expressed as mean ± 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

RESULTS

Effect of EBA on antioxidant enzymes in seizure induced rats by PTZ

The levels antioxidant enzymes such as superoxide dismutase, glutathione peroxidase, glutathione reductase and catalase were significantly reduced ($p<0.01$) due to induction of seizure by PTZ in Group II, whereas lipid peroxidation enzymes significantly increased ($p<0.05$) in both models. Administration of EBA 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 EBA 250 and 500 mg/kg. (Table 1).

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 [17,18] and PTZ seizure models[6]. Inactivation of oxygen free radicals can be carried out by antioxidative enzymes, like superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) and catalase [19,20]. Previous study was reported, the intraceribroventricularly administered glutathione (GSH) inhibited pentylenetetrazole (PTZ) induced convulsions in mice [12]. The results of this study showed that EBA at the doses of 250 & 500mg/kg significantly increased the levels of antioxidant enzymes such as superoxide dismutase, glutathione peroxidase, glutathione reductase and catalase on rat brain.

Whereas lipid peroxidation level increases in brain during epileptic seizures [21-24]**.** 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 H_2O_2 detoxification. H_2O_2 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 [25-27]**.** 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 [28-34]**.**

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

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