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**CEREBROPROTECTIVE EFFECT OF *IPOMOEA ERIOCARPA*
AGAINST CEREBRAL ISCHEMIA IN RATS**

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ABSTRACT

Ischemic hypoxic brain injury often causes irreversible brain damage. Lack of efficient and widely applicable pharmacological treatments for ischemic stroke has necessitated attention towards novel traditional medicines. Traditionally, the leaves of *Ipomoea eriocarpa* is used for various diseases, including blood and neuronal disorders. To evaluate the cerebroprotective effect of Ethanol extract of leaves of *Ipomoea eriocarpa* (EEIE) against the global model of ischemia in rats. In the present study, the animals were pre-treated with EEIE for a period of 1week (200 and 400 mg/kg) p.o. The animals were anaesthetized with thiopentone sodium (45mg/kg) and stroke was induced by Bilateral Carotid Artery Occlusion (BCAO) for defined period with aneurism clamps placed on both arteries and later (10 minutes) clamps were removed to allow reperfusion and animals were then returned to their cages. After 24 hours of reperfusion, the animal behaviors were evaluated by various methods such as behaviour pattern, Juvenile recognition, Motor activity, rotar rod test, Morris water maze test in stroke induced animals. The treatment was continued for another week after surgery with EEIE. The present studies suggest that, there was a decrease in the escape latency in water maze in stroke induced (negative control) group. The group treated with 200mg/kg and 400 mg/kg EEIE showed significant ($P<0.01$) improvement in the behaviour pattern, and spatial learning, which was confirmed in trial sessions in water maze test when compared with the negative control group. In conclusion, Ethanol extract of leaves of *Ipomoea eriocarpa* produced cerebroprotective effects in global cerebral ischemia as evident from reduction in behavioral score, hyper locomotion and neuronal damage.

Keywords: *Ipomoea eriocarpa*, Bilateral Carotid Artery Occlusion, Cerebral ischemia.

INTRODUCTION

Stroke is the world's second leading cause of mortality, resulting around 6,000,000 deaths annually [1]. It is estimated that the lifetime risk for stroke is between 8% and 10% [2]. Pathogenically, stroke involves a heterogeneous group of processes. Vessel occlusions (ischemic stroke) account for 85% of all strokes, while primary intracerebral bleeding (hemorrhagic stroke) accounts for the remainder [3]. Embolisms cause

approximately 75% of all cerebral vessel occlusions, and are the most frequent cause of focally-obstructed blood flow within the brain [4]. Ischemia is defined as a reduction in blood flow sufficient to alter normal cellular function. Brain tissue is exquisitely sensitive to ischemia, such that even brief ischemic periods to neurons can initiate a complex sequence of events that ultimately may culminate in cellular death. Different brain regions have varying thresholds for ischemic cell damage, with white matter being more resilient than gray matter [5]. In addition, certain populations of neurons are selectively more vulnerable to ischemia; for example, in the hippocampus, CA1 pyramidal neurons are highly susceptible to ischemia, whereas dentate granule neurons are more resistant [5]. Early restoration of blood flow remains the treatment of choice for limiting brain injury following stroke.

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Improved educational efforts that emphasize recognition of the early signs and symptoms of stroke, coupled with the widespread application of thrombolytic therapy to patients with acute ischemic stroke, has decreased morbidity and mortality following stroke [6,7]. While reperfusion of the ischemic brain is clearly desirable, tissue damage often results from both the transient ischemic insult and the reperfusion process; the latter process inducing an inflammatory response that causes additional injury to the cerebral microcirculation and adjacent brain tissue.

An ischemic stroke is occasionally treated in a hospital with thrombolysis (also known as a "clot buster"), and some hemorrhagic strokes benefit from neurosurgery. Treatment to recover any lost function is termed stroke rehabilitation, ideally in a stroke unit and involving health professions such as speech and language therapy, physical therapy and occupational therapy. Prevention of recurrence may involve the administration of antiplatelet drugs such as aspirin and dipyridamole, control and reduction of hypertension, and the use of statins. Selected patients may benefit from carotid endarterectomy and the use of anticoagulants [6,7]. Moreover, a growing concern in traditional medicines has raised due to lack of effective and widely applicable pharmacological treatments for ischemic stroke.

Ipomoea eriocarpa R.Br. (Family: Convolvulaceae) often called annual morningglories, are summer annual or perennial broadleaf plants. *Ipomoea eriocarpa* R.Br. are often cultivated as ornamentals, however, under favorable conditions they can become troublesome weeds. They are also a major agricultural weed problem in the San Joaquin Valley of California, where several species of *Ipomoea* are found. Control is critical from crop emergence to harvest. Destroy seedlings while they are small, because once they have twined up stems they are difficult to control without injuring the crop. Seeds remain viable in soil for long periods. Seeds of *Ipomoea* species contain many types of alkaloids, including some that are neurotoxins to humans and animals when consumed. Fortunately, there is typically not enough seed in contaminated grain to cause harm to livestock. Most seedlings emerge following irrigation, but they may also appear when surface soil is too dry to allow germination of other annuals. Cotyledons (seed leaves) are butterfly shaped and more deeply notched and much larger than those of field bindweed. First true leaves are heart shaped with deep lobes at the base. Mature plants have long stems that climb and twine. Leaves are large, heart shaped and/or three lobed, and are alternate to one another along the stem. Both leaf types can occur on the same plant. The funnel-shaped flower varies in color depending on the species, from violet or blue to pink and red. Fruit are pods that release seeds through slits. Seeds germinate down to a depth of 4 inches (10 cm) or more, much deeper than most annuals [7,8].

This present study carried out to assess the validity of the folkloric uses of this plant in anticoagulant property and establish the possible mechanisms of pharmacological action. Scientific evaluation of this claim using experimental model of Bilateral Carotid Artery Occlusion (BCAO) in rats induced cerebral ischemia was ascertained in this study. This was supported in our study by various behavioral studies.

MATERIALS AND METHODS

Plant collection

The leaves of *Ipomoea eriocarpa* has been collected from Sri Venketeswara University near Tirupati, Andhra Pradesh during the month of June 2011 and dried under shade. The plant was authenticated by Mr. K. Madhava chetty, Assistant Professor, Department of Botany of S. V. University, Tirupati. The voucher specimen of the plant was deposited at the college for further reference.

Preparation of extracts

Leaves of *Ipomoea eriocarpa* were shade dried, and the dried leaves were powdered to get coarse granules. The coarse powder was subjected to continuous hot extraction in Soxhlet apparatus using ethanol. The solvent was removed by distillation under reduced pressure, which produced a greenish sticky residue (yield 13.5% w/w with respect to dried plant material). The concentrated crude extract were stored and used for the further study.

Animal Used

Albino Wistar rats, weighing 220–250 g were used. The selected animals were housed in acrylic cages in standard environmental conditions (20–25° C), fed with standard rodent diet and water *ad libitum*. The experiments on animals were conducted in accordance with the internationally accepted principles for laboratory animal use and the experimental protocols duly approved by the Institutional Ethical Committee.

Acute Toxicity Study

The acute toxicity of Ethanol extract of leaves of *Ipomoea eriocarpa* was determined as per the OECD guideline no. 423 (Acute Toxic Class Method). It was observed that the test extract was not lethal to the rats even at the 2500 mg/kg doses. Hence, 1/10th (200mg/kg) and 1/5th (400mg/kg) of this dose were selected for further study [9].

Experimental design

The male Wistar strain rats were randomized into 6 different groups (n=6 per group). Group 1 - Animals (Positive Control) with sham operation (without Occlusion) and treated with control vehicle only (p.o). Group 2 - Animals with sham operation (without occlusion) and treated with 200mg/kg of EEIE (p.o). Group 3 - Animals with sham operation (without occlusion) and treated with 400mg/kg of EEIE (p.o). Group 4 - Animals (Negative

Control) with BCAA and treated with Control vehicle only (p.o). Group 5- Animals with BCAA and treated with 200mg/kg of EEIE (p.o). Group 6 - Animals with BCAA and treated with 400mg/kg of EEIE (p.o).

Induction of cerebral ischemia

In the present study, the animals were pre-treated with EEIE for a period of 1 week (200 and 400 mg/kg) p.o. The animals were anaesthetized with thiopentone sodium (45 mg/kg), and stroke was induced by occlusion of bilateral carotid artery (BCAO) for the defined period with aneurysm clamps placed on both arteries and later (10- 15 minutes) clamps were removed to allow reperfusion and animals were then returned to their cages. After 24 hours of reperfusion, the animal behaviors were evaluated by various methods. The treatment was continued for another week after surgery with EEIE [10].

In Vivo Pharmacological Examination

Social Recognition

Juvenile recognition test

The juvenile recognition test is a suitable model for testing amnesia in animals to assess the social olfactory memory which is impaired in cerebral ischemia. The juvenile recognition test was conducted in three open Perspex arenas (73 * 48 * 30 cm) with a thick bedding of wood shavings. Lighting in the room was bright. There was no visual contact between the arenas [11].

Behavioural Procedure

The test animal was placed in the arena for a habituation period of 10 min. An unfamiliar juvenile female was then introduced into the arena for 10 min (first exposure E1). Both animals were subsequently returned to their home cages. After a variable Inter Exposure Interval (IEI), the male animal was placed in the arena for another habituation period of 10 min, and thereafter the juvenile was reintroduced for 3 min (second exposure E2). E2 was limited to 3 min because only the first 3 min of the observation period were used for behavioral scoring. The rate was blind to the treatment of the animals. Based on the scoring pattern the social recognition of the animals was assessed.

Parameters

Score: 0 - *Body/mouth sniffs*: Sniffing part of the female's body (not genitals) or sniffing or licking the corner of the mouth. Genital Sniff/Follow: Following the female closely and/or sniffing at the ano-genital region Aggression: Side-to-side threatening position, kicking, pursuing, and fighting. Score: 0.5 - *Running*: Running around in the arena
Score: 1 - *Digging*: Digging in the corners of the arena
Score: 2 - *Inactivity*: Sitting inactively
Score: 3 - *Other nonsocial*: Joint category for a variety of nonsocial behaviors, e.g., self-grooming (cleaning fur, etc.),

and exploratory behavior, e.g., walking, sniffing at bedding, walls, etc.

Motor activity

The motor activity was monitored by using actophotometer. Before measuring the cognitive task the animal was placed in Actophotometer record for 10 min. The locomotor activity was expressed in terms of total photo beam interruption counts / min / animal [12].

Rotor Rod Test

Rats were tested on an accelerating rotor-rod (diameter, 5.8 cm) that was turned at a speed of 20-25 rpm, at which all the control animal could maintain position for 120 seconds. If the experimental animal fell within 120 seconds, the latency was recorded. If the animal maintained their position for 120 seconds, a time of 120 seconds was assigned. The trial was repeated 3 times, and the latency of the last trial was adopted for each animal [13].

Morris Water Maze Test

On day 15 after surgery, spatial learning and memory was tested in water maze. The maze consisted of a black circular pool (diameter 2.14 m, height 80 cm) filled to a depth of 44cm with water (25°C). On 14th day the rats received habituation (exposure in water maze for 1 min) in which there was no platform present. Then, on day 15th, a circular platform (9 cm in diameter) was kept hidden 2 cm below water level in the center of one of the quadrants. The platform remained in the same position during training days.

At the beginning of each session, a random sequence of four starting poles along the perimeter of the pool was generated. All animals followed this sequence for that session. Each rat was placed in the water facing the wall at the start location and was allowed 90 sec. to find the hidden platform. The animal was allowed a 20 sec. rest on the platform. The latency to reach the platform was recorded. If the rat was unable to locate the hidden platform, it was lifted out and placed on the platform for 20 sec. The procedure was repeated for all the 4 start locations. Two sessions of four trials each separated by 4 h were conducted on the first day of testing and one session of four trials was conducted on the next day (reference memory procedure). After that, the platform was removed and a probe trial (without platform) was conducted 4 h later. Each rat was placed in the pool at the same randomly selected starting pole and swimming path was observed. The time spent in the quadrant of pool, which initially contained platform, was measured (working memory procedure) [14].

Statistical analysis

The statistical analysis was carried out using Graph pad prism 4.0 software. All values were expressed as Mean \pm S.E.M. Data analysis was done by one-way ANOVA followed by Dunnett's multiple comparison tests.

Difference level at $P < 0.05$ was considered as statistically significant condition.

RESULTS

Effect of EEIE on Juvenile Recognition Test

There was an increase in Score of Social recognition in Stroke induced (negative control) group when compared with the control group and negative control group which showed significance of ($P < 0.01$) when compared with control group. The group treated with 200mg/kg and 400 mg/kg EEIE showed significant ($P < 0.01$) improvement in social behaviour when compared with negative control group. The group treated with 200 mg/kg and 400 mg/kg EEIE showed the significance of ($P < 0.01$) as shown in Table 1.

Effect of EEIE on Motor Activity

There was a decrease in the motor activity in Stroke induced (negative control) group when compared with the control group and negative control group which showed significance of ($P < 0.01$) when compared with control group. The group treated with 200mg/kg and 400 mg/kg EEIE showed significant ($P < 0.01$) improvement in motor activity when compared with negative control group. The group treated with 200 mg/kg and 400 mg/kg EEIE

showed the significance of ($P < 0.01$) as shown Table 2.

Effect of EEIE on Roto Rod Test

There was a decrease in the Muscle coordination in Stroke induced (negative control) group when compared with the control group and negative control group which showed significance of ($P < 0.01$) when compared with control group. The group treated with 200mg/kg and 400 mg/kg EEIE showed significant ($P < 0.01$) improvement in muscle coordination when compared with negative control group. The group treated with 200 mg/kg and 400 mg/kg EEIE showed the significance of ($P < 0.01$) as shown in Table 3.

Effect of EEIE on Morris Water Maze Test

There was an increase in the escape latency in Stroke induced (negative control) group when compared with the control group and negative control group which showed significance of ($P < 0.01$) when compared with control group. The group treated with 200mg/kg and 400 mg/kg EEIE showed significant ($P < 0.01$) improvement in Spatial learning which was confirmed in trial sessions and probe trial when compared with negative control group. The group treated with 200 mg/kg and 400 mg/kg EEIE showed the significance of ($P < 0.01$) as shown in Table 4.

Table 1. Effect of EEIE on Juvenile Recognition Test

GROUP	1	2	3	4
SCORE	0.0	3.48±0.13 ^{a**}	2.51±0.44 ^{b**}	1.34±0.16 ^{b**}

Significant * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. Values are expressed as mean ±SEM of 6 animals Comparisons were made between a. Control vs Negative control and b. Negative control vs Treatment group. Group 1: Sham (saline), Group 2: Ischemia (saline), Group 3: Ischemia+EEIE (200mg/kg), Group 4: Ischemia+EEIE (400mg/kg)

Table 2. Effect of EEIE on Motor Activity

GROUP	1	2	3	4
No. of cut off	449.16±3.41	33.48±1.48 ^{a**}	98.46±2.14 ^{b**}	124±4.19 ^{b**}

Significant * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. Values are expressed as mean ±SEM of 6 animals Comparisons were made between a. Control vs Negative control and b. Negative control vs Treatment group. Group 1: Sham (saline), Group 2: Ischemia (saline), Group 3: Ischemia+EEIE (200mg/kg), Group 4: Ischemia+EEIE (400mg/kg)

Table 3. Effect of EEIE on Rotor Rod Test

GROUP	1	2	3	4
Time In Seconds	142.26±2.44	15.19±1.22a ^{**}	55.46±1.24b ^{**}	69.15±2.39b ^{**}

Significant * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. Values are expressed as mean ±SEM of 6 animals Comparisons were made between a. Control vs Negative control and b. Negative control vs Treatment group. Group 1: Sham (saline), Group 2: Ischemia (saline), Group 3: Ischemia+EEIE (200mg/kg), Group 4: Ischemia+EEIE (400mg/kg)

Table 4. Effect of EEIE on Morris Water Maze Test

Sessions	Group	1	2	3	4
I	Escape Latency (in secs)	64.16±0.14	79±1.15	69.48±0.14	72±0.19
II		47.45±0.14	60.16±0.47	44.25±1.19	49.64±0.42
III		29.33±0.16	37.46±0.19	26.12±0.48	25.18±0.27

Significant * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. Values are expressed as mean ±SEM of 6 animals Comparisons were made between a. Control vs Negative control and b. Negative control vs Treatment group. Group 1: Sham (saline), Group 2: Ischemia (saline), Group 3: Ischemia+EEIE (200mg/kg), Group 4: Ischemia+EEIE (400mg/kg).

DISCUSSION AND CONCLUSION

The Present study demonstrates the protective effect of Ethanolextract of leaves of *Ipomoea eriocarpa* treatment against to short-term global brain injury in rats. To our knowledge, this is the first report that investigates the effect of EEIE treatment against to short-term global brain ischemia/reperfusion injury in rats. Bilateral carotid artery occlusion is the basic experimental inducing model of global cerebral ischemia in animals and common carotid arteries is the main arteries supplying blood to the brain from heart. The occlusion of these arteries for a period of 10 minutes leads to reduction in blood supply to the brain and the pathophysiological events starts and continues followed by reperfusion [15].

BCAO for 10 min in rats resulted in selective loss of pyramidal neurons in the CA1 area of hippocampus within 96 h to become apparent morphologically. There was substantial hippocampal neuronal death (80–85%) in ischemic animals as compared with the sham operated animals. Ischemic animals showed hyper locomotion on initial day of reperfusion. This was found to be consistent with the findings stating that on the first day after reperfusion, ischemia induced increase in locomotor activity is prominent, following two days it starts decreasing [16,17]. Thus based on this analysis, the group treated with 200 mg/kg and 400 mg/kg EEIE showed the significant ($P<0.01$) improvement in locomotor activity.

Global cerebral ischemia causes marked damage to pyramidal neurons in the hippocampal region within days after ischemia in animals and humans. Hippocampal neurons are highly susceptible to ischemia and reperfusion-induced injury. Hippocampus is involved in the regulation of short-term memory. Vascular dementia is the second most common type of dementia following Alzheimer's disease-related dementia [18]. Vascular dementia occurs when the blood supply to the brain is reduced by a blocked or diseased vascular system [19] and leads to a progressive

decline in memory and cognitive function. Cerebral hypoperfusion can be induced by bilateral occlusion of common carotid arteries (BCAO) in rats, resulting in significant white matter lesions, learning and memory impairment, and hippocampal neuronal damage. Thus, BCAO in rats provides a model useful for understanding the pathophysiology of chronic cerebrovascular hypoperfusion and for screening drugs with potential therapeutic value for stroke [20,21].

Therefore, Morris water maze has been employed in present study to evaluate impairment of short-term memory as a result of cerebral ischemia and reperfusion. BCAO induced cerebral ischemia have markedly attenuated ischemia and reperfusion-induced cerebral infarct size in a group III rats and at the doses of 200/400mg/kg EEIE has significantly prevented the ischemia and reperfusion-induced impairment of short-term memory and motor in coordination.

The present studies suggest that In-vivo behavioral studies such as motor activity, rotor rod, and Morris water maze tests were carried out in order to assess the behavior of the animals. There was a decrease in the motor activity and escape latency in the water maze in stroke induced (negative control) group. The group treated with 200mg/kg and 400 mg/kg EEIE showed significant ($P<0.01$) improvement in the motor activity, muscle co-ordination, and spatial learning, which was confirmed in trial sessions in water maze test when compared with the negative control group.

The results of this study confirmed that EEIE protects rats from ischemia induced brain injury. This protection was evident from in-vivo behavioral tests. In conclusion, Ethanolextracts of *Leaves of Ipomoea eriocarpa* produced cerebroprotective effects in global cerebral ischemia as evident from reduction in behaviour pattern, hyper locomotion and neuronal damage.

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