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EVALUATION OF PROTECTIVE EFFECT OF *LUFFA ACUTANGULA EXTRACT* AGAINST BILATERAL CAROTID ARTERY OCCLUSION (BCAO) INDUCED STROKE IN RATS

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ABSTRACT

Luffa acutangula (Family: Cucurbitaceae) is commonly known as Ridge gourd. It is a widely growing vegetative climber. To evaluate the cerebroprotective effect of Petroleum ether extract of whole plant of *Luffa acutangula* (ELA) against the global model of ischemia in rats. In the present study, the animals were pre-treated with ELA for a period of 1week (250 and 500 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 ELA. 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 250mg/kg and 500 mg/kg ELA 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, Petroleum ether extract of whole plant of *Luffa acutangula* produced cerebroprotective effects in global cerebral ischemia as evident from reduction in behavioral score, hyper locomotion and neuronal damage.

Keywords: Whole plant of *Luffa acutangula*, Bilateral Carotid Artery Occlusion, Cerebral ischemia.

INTRODUCTION

A stroke, also known as a cerebrovascular accident (CVA), is the rapid loss of brain function(s) due to disturbance in the blood supply to the brain. This can be due to ischemia (lack of blood flow) caused by blockage (thrombosis, arterial embolism), or a hemorrhage (leakage of blood) [1]. As a result, the affected area of the brain cannot function, which might result in an inability to move one or more limbs on one side of the body, inability to understand or formulate speech, or an inability to see one side of the visual field. A stroke is a medical emergency and can cause permanent neurological damage, complications, and death. It is the leading cause of adult disability in the United States and Europe and the second leading cause of death worldwide. Risk factors for stroke include old age, hypertension (high blood pressure), previous stroke or transient ischemic attack (TIA), diabetes, high cholesterol, cigarette smoking and atrial fibrillation.[2] High blood pressure is the most important modifiable risk factor of stroke [2].

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A silent stroke is a stroke that does not have any outward symptoms, and the patients are typically unaware they have suffered a stroke. Despite not causing identifiable symptoms, a silent stroke still causes damage to the brain, and places the patient at increased risk for both transient ischemic attack and major stroke in the future. Conversely, those who have suffered a major stroke are at risk of having silent strokes [3]. In a broad study in 1998, more than 11 million people were estimated to have experienced a stroke in the United States. Approximately 770,000 of these strokes were symptomatic and 11 million were first-ever silent MRI infarcts or hemorrhages. Silent strokes typically cause lesions which are detected via the use of neuroimaging such as MRI. Silent strokes are estimated to occur at five times the rate of symptomatic strokes [4,5]. The risk of silent stroke increases with age, but may also affect younger adults and children, especially those with acute anemia.

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 [6-8].

Luffa acutangula (Family: Cucurbitaceae) is commonly known as Ridge gourd. It is a widely growing vegetative climber. The fruits are base ball club shaped. Various pharmacological activities include hepatoprotective activity, antidiabetic activity, antioxidant activity, fungistatic property, CNS depressant activity etc. Its chemical constituents were found to be carbohydrates, carotene, fat, protein, phytin, aminoacids, alanine, arginine, cystine, glutamicacid, glycine, hydroxyproline, leucine, lectin, serine, tryptophan, pipecolic acid [9-14]. This present study carried out to assess the validity of the folkloric uses of this plant in brain disorders 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 folklore claim was supported in our study by various behavioral studies.

METERIALS AND METHODS

Plant collection

The whole plant of *Luffa acutangula* has been collected from Sri Venketeswara University near Tirupati, Andhra Pradesh during the month of December 2011 and dried under shade. The plant was authentified 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

Whole plant of *Luffa acutangula* were shade dried, and the dried whole plant were powdered to get coarse granules. The coarse powder was subjected to continuous hot extraction in Soxhlet apparatus using Petroleum ether. The solvent was removed by distillation under reduced pressure, which produced a greenish sticky residue (yield 10%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 Petroleum ether extract of whole plant *of Luffa acutangula* 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, $1/8^{th}$ (250mg/kg) and $1/4^{th}$ (500mg/kg) of this dose was selected for further study [15].

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 250mg/kg of ELA (p.o). Group 3 - Animals with sham operation (without occlusion) and treated with 500mg/kg of ELA (p.o). Group 4 - Animals (Negative Control) with BCAO and treated with Control vehicle only (p.o). Group 5- Animals with BCAO and treated with 250mg/kg of ELA (p.o). Group 6 - Animals with BCAO and treated with 500mg/kg of ELA (p.o).

Induction of cerebral ischemia

In the present study, the animals were pre-treated with ELA for a period of 1week (250 and 500 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

aneurism 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 ELA [16].

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 [17,18].

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 5 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 [17].

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 ELAh animal [18].

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 ELAh session, a random sequence of four starting poles along the perimeter of the pool was generated. All animals followed this sequence for that session. ELAh 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 rELAh 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 ELAh 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. ELAh 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) [19].

Statistical analysis

The statistical analysis was carried out using Graph pad prism 4.0 software. All values were expressed as Mean ± 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 ELA 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 250mg/kg and 500 mg/kg ELA showed significant (P<0.01) improvement in social behaviour when compared with negative control group. The group treated with 250 mg/kg and 500 mg/kg ELA showed the significance of (P<0.01) as shown in Table 1.

Effect of ELA 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 250mg/kg and 500 mg/kg ELA showed significant (P<0.01) improvement in motor activity when compared with negative control group. The group treated with 250 mg/kg and 500 mg/kg ELA showed the significance of (P<0.01) as shown Table 2.

Effect of ELA 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 250mg/kg and 500 mg/kg ELA showed significant (P<0.01) improvement in muscle coordination when compared with negative control group. The group treated with 250 mg/kg and 500 mg/kg ELA showed the significance of (P<0.01) as shown in Table 3.

Effect of ELA 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 250mg/kg and 500 mg/kg ELA 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 250 mg/kg and 500 mg/kg ELA showed the significance of (P<0.01) as shown in Table 4.

Table 1. Effect of ELA on Juvenile Recognition Test

GROUP	1	2	3	4
SCORE	0.0	$5.27\pm0.02^{a**}$	$2.42\pm0.22^{b**}$	$2.14\pm0.36^{b**}$

*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+ELA (250mg/kg), Group 4: Ischemia+ELA (500mg/kg)*

Table 2. Effect of ELA on Motor Activity

GROUP	1	2	3	4
No. of cut off	474.12±2.32	$34.22\pm1.12^{a**}$	$102.24\pm2.10^{b**}$	$112\pm3.02^{b**}$

*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+ELA (250mg/kg), Group 4: Ischemia+ELA (500mg/kg)*

Table 3. Effect of ELA on Rotor Rod Test

GROUP	1	2	3	4
Time In Seconds	147.14±2.15	$18.52\pm1.24a^{**}$	$58.34\pm1.12b^{**}$	$68.51\pm2.24b^{**}$

*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+ELA (250mg/kg), Group 4: Ischemia+ELA (500mg/kg)*

Table 4. Effect of ELA on Morris Water Maze Test

Sessions	**Group**	1	2	3	4
I	**Escape**	74.25±0.14	88.14±1.33	75.42±0.21	77.15±0.14
II	**Latency**	52.14±0.13	68.12±0.27	52.14±1.17	55.14±0.28
III	**(in secs)**	34.12±0.14	47.14±0.32	32.12±0.14	34.12±0.14

*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+ELA (250mg/kg), Group 4: Ischemia+ELA (500mg/kg).*

DISCUSSION AND CONCLUSION

The Present study demonstrates the protective effect of Petroleum ether extract of whole plant of *Luffa acutangula* treatment against to short-term global brain injury in rats. To our knowledge, this is the first report that investigates the effect of ELA 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 [20].

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 [21,22]. Thus based on this analysis, the group treated with 250 mg/kg and 500 mg/kg ELA 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 [23]. Vascular dementia occurs when the blood supply to the brain is reduced by a blocked or diseased vascular system [24] 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 [25,26].

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 250/500mg/kg ELA 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 250mg/kg and 500 mg/kg ELA 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 ELA protects rats from ischemia induced brain injury. This protection was evident from in-vivo behavioral tests. In conclusion, Petroleum ether extracts of Whole plant of *Luffa acutangula* produced cerebroprotective effects in global cerebral ischemia as evident from reduction in behaviour pattern, hyper locomotion and neuronal damage.

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