International Journal of Phytopharmacology

www.onlineijp.com

e- ISSN 0975 – 9328
Print ISSN 2229 – 7472

Research Article

CEREBROPROTECTIVE EFFECT OF METHANOLIC EXTRACT *BOUGAINVILLEA SPECTABILIS* LEAVES AGAINST BILATERAL CAROTID ARTERY OCCLUSION INDUCED STROKE IN RATS

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ABSTRACT

Bougainvillea spectabilis (Nyctaginaceae) is having more traditional values that is used as Antiinflammatory activities, diabetic and also show extensively studies against various Neurological disorders like strees, learning and memory diseases, depression, anxiety. To evaluate the cerebroprotective effect of *methanolic extract* of leaves *Bougainvillea spectabilis* of on cerebral ischemia *(BCAO)* induced in rats. Ischemia induced neuronal injury was assessed by measurement of brain infract area by histopathological studies. In the present study, the animals were pretreatede with *MEBS* for a period of 1 week (250and 500mg/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 on both arteries and later 10 min clamps were removed to allow reperfusion and animals were then returned to their cages. After 24 h of reperfusion the animals behaviors were evaluated by various methods such as behavior 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 *MEBS*. The present studies suggest that, there was decrease in the escape latency in water maze and decrease in the social olfactory memory in Juvenile recognition test in stroke induced groups. The group treated with 250 and 500mg/kg of *MEBS* showed significant ($p<0.01$) improvement in the behaviour pattern and spatial learning, which was confirmed in trial sessions in water maze test and Juvenile recognition test when compared with the negative group. In conclusion, *methanolic extract* of leaves of *Bougainvillea spectabilis* produced cerebroprotective effect in cerebral ischemia as evident from reduction in behavioral score, social olfactory memory, hyper locomotion and neuronal damage.

Key words: Leaves of *Bougainvillea spectabilis*, Bilateral Carotid Artery Occlusion *(BCAO)*, cerebral ischemia.

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INTRODUCTION

A stroke or brain attack occurs when the blood flow to an area of the brain is interrupted by a blocked (ischemic stroke) or brust (hemorrhagic stroke) blood vessel. As a consequence, brain cells begin to die, and the abilities controlled by the parts of the brain are lost. Stroke is the major cause of adult disability and death in the world (Ma *et al.*, 2005). In US stroke is a leading cause of the brain death, killing nearly 130,000 people each year, and a leading cause of serious, long-term adult disability (Miniño *et al.*, 2008). Risk factors for stroke include old age, hypertension, previous stroke or transient ischemic attack (TIA), diabetes, high cholesterol, cigarette smoking and atrial fibrillation. High blood pressure is the most important modifiable risk factor of stroke. A silent stroke is a stroke that does not have any outward symptoms, and the patients are typically unaware they have suffered a stroke (Teng L *et al.*, 2006). Clinical

Access this article online

Home page: http://onlineijp.com/

DOI: http://dx.doi.org/10.21276/ijp.2017.8.3.3

Quick Response code

Received:06.04.17 **Revised**:22.04.17 **Accepted**:12.05.17

diagnosis typically relies on medical imaging methods such as computed tomography (CT) and magnetic resonance imaging (MRI). Although these techniques are effective for identifying the location and type of a stroke, they are not suitable for being used in emergencies because of their high cost and lack of rapidity and portability. Recently, the authors in proposed a method based on microwave tomography (MWT), to supplement these medical imaging techniques, and focused on stroke detection.

Bougainvillea spectabilis (Nyctaginaceae) native to tropical South America, *Bougainvillea* grows around the world as an ornamental climber and shrub. *Bougainvillea spectabilis* was first identified in Brazil at 1798. German botanist Carl Ludwig Willdenow is credited with this identification. It is a popular woody scandent shrub that grows in tropical and subtropical forests in India. The leaves are large and ovate. It is one of the most commonly grown plants in subtropical and tropical gardens and is a Mediterranean favorite. *Bougainvilleas* are easy to grow in frost-free conditions and there are hundreds of cultivars with different colored flowers. Pinitol possesses potent antihyperglycemic properties like insulin, as reported. *Bougainvillea spectabilis* further strong then the ethnomedicical use of this plant, various herbal formulation for the treatment of Diabetes (Adebayo J *et al*., 2005). It is good enough for use as a first aid disinfectant for minor wound dressing. In aspirin plus pyloric ligation induced gastric 4kg *Bougainvillea spectabilis* show significant reduction in gastric volume, free acidity, total acidity and ulcer soon, Used for cough and sore throat, Used as Brain tonic has reported. So this plant is choosen for the cerebroprotective activity, present study carried out to assess the validity of the folkloric uses 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.

METERIALS AND METHODS

Plant Collection and Authentification:

The leaves *Bougainvillea spectabilis* willd (Nyctaginaceae) are collected in local areas near chittoor. It is identified and Authenticated by Dr. K. Madhava chetty, Plant Taxonomist (IAAT:357), Assistant professor, Department of Botany, Sri Venkateswara University, Tirupati, Andhra Pradesh, India. Voucher Number: 2263 is deposited in herbarium of the Department of Botany, Sri Venkateswara University, Tirupati, Andhra Pradesh, India.

Preparation of Plant Extract

The leaves of *Bougainvillea spectabilis* willd (Nyctaginaceae) are collected, washed with fresh water and dried under shade at room temperature. The leaves were powdered and stored. However, the extraction followed here using 90% Methanol by using Soxhlet apparatus for 48hrs. The solvent is removed from extracts by distillation under reduced pressure, which produced a blackish green sticky residue (yield 10%w/w with respect to dried plant material). The concentrated extract was kept in a desicator and was used for further study.

Animals

Inbred adult Wistar albino rats (150-200g) were obtained from the animal house of Sri Venkateswara College of Pharmacy, Chittoor, Andhra Pradesh. 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 animals were maintained in a well-ventilated room with 12:12 hour light/dark cycle in polypropylene cages. 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. Ethical committee clearance was obtained from IAEC of CPCSEA the approval no: SVCP/IAEC/055/2016-17.

Acute Toxicity Study

The acute toxicity of methanol extract of *Bougainvillea spectabilis* leaves was determined as per the OECD guidelines no.423 (Acute toxic class method). It was observed that the test extract was safe to use even at the 2000mg/kg, p.o. From this high dose 1/4th and low dose 1/8th of 2000mg/kg body weight was selected for further pharmacological studies.

Experimental Design

The male or female wistar strain rats will be randomly select 6 different groups (n=6 per each group)

- **Group1**– Animals (positive control) without occlusion & treated with control vehicle only.
- **Group2**–Animals (negative control) with *BCAO*& treated with control vehicle only.
- **Group 3**– Animals with *BCAO*& treated with 250mg/kg of *MEBS*.
- **Group 4**– Animals with *BCAO* & treated with 500mg/kg of *MEBS.*

Induction of Cerebral Ischemia:

In the present study, the animals will be pre-treated with *MEBS* for a period of 1week (250 and 500 mg/kg). The animals will be 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 mins) clamps are removed to allow reperfusion and animals will returned to their cages. After 24 hours of reperfusion, the animal behaviors will

be evaluated by various methods. The treatment was continued for another week after surgery with *MEBS* (OECD, 2002).

INVIVO 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 (Ergun R *et al*., 2002).

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 (Rockwood K *et al.*, 2000).

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 120seconds was assigned. The trial was repeated 3 times, and the latency of the last trial was adopted for each animal (Sekine T *et al*., 1994).

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) (Sharma S *et al.*, 1996).

Statistical analysis

The statistical analysis was carried out using Graph pad prism 4.0 software. All values were expressed as Mean compared with negative control group. The group treated with 250 mg/kg and 500 mg/kg *MEBS* showed the significance of ($P<0.01$).

Histopathology

From the histopathological study it was observed that stroke was induced by *BCAO* followed by reperfusion produced shrinkage, atrophy and necrosis of neurons along with the vacuolization and inflammatory infiltration in the forebrain of *BCAO* control group rats

compared to sham operated rats. The reactive changes were significantly attenuated in methanolic extract of *Bougainvillea spectabilis* (250 and 500mg/kg) pretreated rats as compared to *BCAO* control group rats was shown below figure.

RESULTS AND DISCUSSION

Evaluation of Cerebroprotective Activity by *Bougainvillea Spectabilis* leaves:

Table 1. Effect of *MEBS* on Juvenile Recognition Test

Group	I	II	III	IV
Score	0.0 [a**]	5.46±0.33	2.92±0.19 [b**]	2.09±0.52 [b**]

Significant *P<0.05, **P<0.01. 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 (1% CMC), Group 2: Ischemia (1% CMC), Group 3: Ischemia+MEBS (250mg/kg), Group 4: Ischemia+MEBS (500mg/kg)

Table 2. Effect of *MEBS* on Motor Activity

Group	I	II	III	IV
No. of cut off	384.52±2.64 [a**]	38.42±2.28	142.37±2.42 [b**]	192.19±3.52 [b**]

Significant *P<0.05, **P<0.01. 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 (1% CMC), Group 2: Ischemia (1% CMC), Group 3: Ischemia+MEBS (250mg/kg), Group 4: Ischemia+MEBS (500mg/kg)

Table 3. Effect of *MEBS* on Rotor Rod Test

Group	I	II	III	IV
Time In Seconds	148.82±2.52 [a**]	16.22±1.39	51.45±1.29 [b**]	80.33±2.27 [b**]

Significant *P<0.05, **P<0.01. 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 (1% CMC), Group 2: Ischemia (1% CMC), Group 3: Ischemia+MEBS (250mg/kg), Group 4: Ischemia+MEBS (500mg/kg)

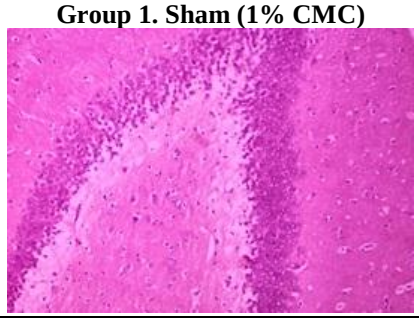
Table 4. Effect of *MEBS* on Morris Water Maze Test

Sessions	Group	I	II	III	IV
1	Escape Latency (in secs)	72.59±1.43 [a*]	85.17±1.46	78.29±1.28 [b*]	74.64±1.17 [b*]
2		56.27±1.14 [a*]	66.29±1.41	58.96±1.22 [b*]	56.19±1.36 [b*]
3		32.64±1.54 [a**]	49.21±1.52	37.45±2.62 [b**]	33.27±1.33 [b**]

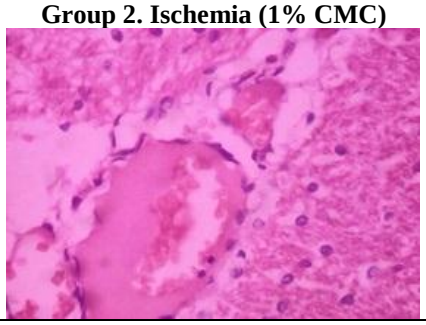
Significant *P<0.05, **P<0.01. Values are expressed as mean ±SEM of 6 animals Comparisons were made between a. Control vs Negative control and b. Negative control vs Treatment group. Group 1: Sham (1% CMC), Group 2: Ischemia (1% CMC), Group 3: Ischemia+MEBS (250mg/kg), Group 4: Ischemia+MEBS (500mg/kg)

Figure 1. Histopathological studies on Brain

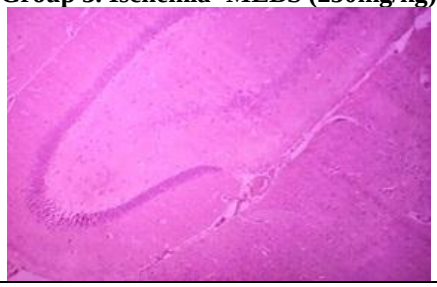
Group 1. Sham (1% CMC)



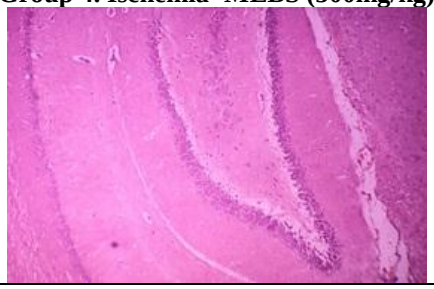
Group 2. Ischemia (1% CMC)



Group 3. Ischemia+MEBS (250mg/kg)



Group 4. Ischemia+MEBS (500mg/kg)



DISCUSSION

The Present study demonstrates the protective effect of methanolic extract of whole plant of *Bougainvillea Spectabilis* treatment against to short-term global brain injury in rats. To our knowledge, this is the first report that investigates the effect of *MEBS* 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 (Morris R. 1984).

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 (Colbourne F *et al.*, 1998). Thus based on this analysis, the group treated with 250 mg/kg and 500 mg/kg *MEBS* showed the significant ($P<0.01$) improvement in locomotor activity (Hirokazu Ohtaki *et al.*, 2005).

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 (Katsuta K *et al.*, 2003). Vascular dementia occurs when the blood supply to the brain is reduced by a blocked or diseased vascular system (Román GC *et al.*, 2002) 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 (Wakita H *et al.*, 1994).

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 *MEBS* 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 *MEBS* 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 present investigation showed the neuroprotective activity of methanolic extract of *Bougainvillea Spectabilis* against Ischemia/reperfusion induced oxidative stress as well as histopathological alterations.

The results of this study confirmed that *MEBS* protects rats from ischemia induced brain injury. This protection was evident from in-vivo behavioral tests. In conclusion, Ethanol extracts of whole plant of methanolic extract of *Bougainvillea Spectabilis* produced cerebroprotective effects in global cerebral ischemia as evident from reduction in behaviour pattern, hyper locomotion and neuronal damage.

CONCLUSION

The methanolic extract *Bougainvillea spectabilis* of has the cerebroprotective effect against Bilateral Artery Occlusion Induced Stroke in rats. In the present study of *BS* leaves it contains flavonoids and other phytochemical like carbohydrates, saponins, terpinoids, tannins, proteins and phenolic compounds. Among this all chemicals, flavonoids have antioxidants activities and during my project work this plants was showing cerebroprotective activity. From the results of toxicity studies, the methanol extract of *Bougainvillea spectabilis* leaves did not possess the toxicity at the higher dose of 2000mg/kg. Hence, it has been concluded that the *MEBS* is safe to use even at the dose of 2000mg/kg, p.o.

Evaluate cerebroprotective activity by methanolic extract of leaves of *Bougainvillea spectabilis* on cerebral ischemia *(BCAO)* induced in rats by following methods like Juvenile recognition Test, Behavioural procedure, Motor Activity, Rotor Rod Test, Morris Water Maze Test. In the present study suggest that, there was decrease in the escape latency in water maze and decrease in the social olfactory memory in Juvenile recognition test in stroke induced groups. The group treated with 250 and 500mg/kg of *MEBS* showed significant ($p<0.01$) improvement in the behaviour pattern, motor activity and spatial learning, which was confirmed in trial sessions in water maze test and Juvenile recognition test when compared with the negative group.

In conclusion, *methanolic extract* of leaves of *Bougainvillea spectabilis* produced cerebroprotective effect in cerebral ischemia as evident from reduction in behavioral score, social olfactory memory, hyper locomotion and neuronal damage. The studies showed that *MEBS* used in cerebroprotective activity against the bilateral carotid artery occlusion in rats. The results showed that this plant could significantly reduce relative infarct size, and rescue neural dysfunction effectively. Furthermore, the formula could prevent neuron cells from death caused by cerebral ischemia or reperfusion to protect from brain damage.

The group treated with 250mg/kg and 500 mg/kg *MEBS* showed significant ($P<0.01$) improvement in the motor activity, muscle coordination, 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 *MEBS* protects rats from ischemia induced brain injury. This protection was evident from *in-vivo* behavioral tests.

In conclusion, methanolic extract of *Bougainvillea spectabilis* leaves produced cerebroprotective effects in global cerebral ischemia as evident from reduction in behaviour pattern, hyper locomotion and neuronal damage. During this preclinical studies we are confirmed that the leaves of *Bougainvillea spectabilis* cerebroprotective avtivity against bilateral artery occlusion to induce stroke. In further cases this plant can be chosen in future to show the cerebroprotective activity against bilateral artery occlusion to induce stroke.

ACKNOWLEDGEMENT

The authors highly acknowledge to Dr.Ravuri Venkataswamy, Chairman and Mr.R.V.Srinivas, Vice Chairman, Srinivasa Educational Academy for providing facilities to pursue this research work.

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Cite this article:
Aruna Kumari T and Saravana kumar A. Cerebroprotective Effect of Methanolic Extract *Bougainvillea spectabilis* Leaves Against Bilateral Carotid Artery Occlusion Induced Stroke In Rats. *International Journal of Phytopharmacology*, 2017;8(3):102-107. DOI: **http://dx.doi.org/10.21276/ijp.2017.8.3.3**