IOSR Journal of Pharmacy and Biological Sciences (IOSR-JPBS)
e-ISSN:2278-3008, p-ISSN:2319-7676. Volume 11, Issue 5 Ver. III (Sep. - Oct.2016), PP 06-11
www.iosrjournals.org

Neuroprotective effect of ethanolic extract of *Heliotropium indicum* against transient global ischemia induced brain damage in rats

Dasarapu Santhosha*[1],Alluri Ramesh[1], Emandi Hemalatha[2], Malothu Nagulu[3]
[1.]*Vishnu institute of Pharmaceutical Education and Research,Narsapur,Medak,Telangana-502 313*
[2.]*Department of Pharmacognosy, Asia metropolitanUniversity,Cheras,43200,Selangor,Malyasia*
[3.]*Department of Pharmacology, Swami Ramananthathirtha Institute of Pharmaceutical Sciences,Nalgonda,Telangana-502 313*

Abstract : *Heliotropium indicum is a whole herb used as traditional medicine mainly in India for the treatment of mental disorders and as brain tonic since many years. The present study is to investigate the protective effects of ethanolic extract of whole herb of Heliotropium indicum on cerebral ischemia/reperfusion induced brain damage in rats. Cerebral ischemia was induced by occluding bilateral common carotid arteries for 60 min followed by 3h of reperfusion. Ischemia/reperfusion-induced neuronal injury was assessed by measurement of brain infarct area, biochemical estimations and histopathological studies. Pretreatment of Heliotropium indicum extract (200, 280 and 400 mg/kg, p.o.) significantly reduced the lipid peroxidation, increased the total thiol content, Catalase and glutathione-S-transferase activity in brain homogenates. The decreased cerebral infarction area in Extract-treated groups and histopathological sections confirmed the above findings. These observations reveal that Heliotropium indicum is a neuroprotective agent and may prove to be useful adjunct in the treatment of stroke and neurological disorders.*

Keywords: *Heliotropium indicum ,Ischemia, biochemical estimations ,stroke*

I. Introduction

Neurological disorders ranks third in cause of death and second in neurologic disability. The present scenario in the treatment of ischemic stroke is not sufficient and the conventional drugs for treatment of strokes are not sufficient and not much useful. So, natural products (medicinal plants based products) probably represent an ideal source to develop safe and effective agents for the management of stroke [1] .The scientifically proven flavonoids [2] and drugs like *Gingko biloba[3]*, *Ginseng[4]* have reported successful recovery from ischemia/reperfusion injuries in the Brain. The present study is to know the safe neuroprotective effect of polyherbal formulation against global cerebral ischemia in pre-clinical models. Stroke is one of the leading causes of death and disability worldwide. Despite decades of research, however, treatment options remain limited. Numerous neuroprotective treatments have been identified that show prominent results in animal models of stroke. Unfortunately, nearly all have failed to provide protection in clinical trials. Flavanoids are naturally occurring compounds that readily cross the blood-brain barrier and are well known for their protective effects[5].The polyphenolics including flavanoids which are found in many herbal extracts have been shown to be strong reactive oxygen species scavengers ,antioxidants and protectors of neurons from lethal damage *in invitro.* The plant is chiefly used as a traditional medicine. The extracted juice from the pounded leaves of the plants is used on wounds, skin ulcers and furuncles. The juice is also used as an eye drop for conjunctivitis. The pounded leaves are used as poultice.[6]

High incidences of neurotoxicity may be due to our lifestyle, increased usage of mobile phones whose radiations kill the neurons. It may also take place as side effect of many drugs used for chronic diseases. For example Vinca which is used as anticancer drug, is neurotoxic which is the side effect of Vinca[7].Similarly there are many drugs which are used for Alzheimer's, Parkinson's which have neurotoxicity as common side effect.

There are many mechanisms by which neurons are injured.one of the mechanism is ischemic stroke i.e., obstruction of blood flow to the brain. when blood flow is interfered by fatty material or low levels of oxygen, it blocks the flow of blood which in turn damages neurons. When obstructed blood flow is restored it causes more damage because of increased oxidative stress. So, in both the ways neurons can be damaged.In this study we are going to use plant extract which could possibly show protective effect against this type of injuries.

II. Materials And Methods

2. Materials

2.1.1 Plant:

Heliotropium indicum was collected from Thirupathi hills.Whole herb was collected, dried and made into course powder. It was authenticated by Dr.Madhava chetty, SV University, Thirupati.(Voucher number 939)

2.1.2.Animals

Male albino Wister rats were selected .All animal procedures involving animals and their care were conducted in accordance with the guidelines of OECD. All the experimental protocols are approved by CPCSEA(Reg No 1358/AC/10/CPCSEA).Male albino wister rats were selected weighing about 200-250g for the study. Thirty animals were selected and divided into five groups of six animals each. These were Maintained In 12:12 Day And Night Cycle And Supplied With standard Pellet Diet And Water *ad libitum.* Animals were kept in the animal house one week prior to the experimentation to adapt animals to the environment.[8]

2.1.3 Chemicals

All the solvents were purchased from sd.fine chemicals. 1,3,5-Trichlorotetrazolium chloride was purchased from sd fine chemicals

2.2 Acute Toxicity Studies:

The toxicity studies were conducted as per OECD Guidelines [9]

2.3 Extraction of *Heliotropium Indicum*

Hot continuous soxhlet method was followed to extract *Heliotropium indicum* .The herb was dried, powdered and extracted by successive solvent extraction technique by using soxhlet apparatus..In this method we have used four solvents pet.ether, chloroform, ethanol and water. These four extracts were subjected to phytochemical screening in which ethanolic extract has shown maximum number of phytoconstituents, so it was selected for further study. The ethanolic extract obtained was dried and stored.[10]

2.4 Phytochemical screening [11, 12, 13]

Phyto chemical examinations were carried out for all the extracts as per the standard methods.

1.Detection of Alkaloids 2. Detection of Carbohydrates
3.Detection of Glycosides 4.Detection of Steroids:
5.Detection of Phenols 6.Detection of Flavonoids
7.Detection of Proteins

2.5 Evaluation of *in-vitro* antioxidant activity

The anti-oxidant activity was evaluated by using two methods [14]
H_2O_2 Method and DPPH Method

2.6 Experimental protocol

Animals were divided into five groups of six animals each. First group was treated with only vehicle 10ml/kg b.w. Second group was treated as ischemic control which was treated with vehicle10ml/kg b.w.Third group was treated with 200mg/kg bw p.o of ethanolic extract. Fourth group was treated with 280mg/Kg b.w p.o and fifth group was treated with 400mg/kg b.w p.o.

All the groups were treated with above said protocol for 14days and after 14^{th} day except first group i.e., the control group ,all others groups were subjected to cerebral ischemia. One day before experimentation animals were withdrawn food but supplied with water. Cerebral ischemia was induced by using nylon thread. Bilateral common carotid artery was occluded for one hour followed by three hours of reperfusion. After reperfusion immediately brains were separated and washed in normal saline solution. From each group two animals were used for biochemical estimations, two animals for estimation of brain infarct area and two animals were used for histopathological studies.

Induction of global ischemia by BCAO, followed by reperfusion [15, 16, 17 , 18 ,19, 20]

Rats will be anesthetized by giving thiopentone sodium (40 mg/kg) i.p. Surgical technique for the induction of cerebral ischemia .Under anesthesia midline incision will be given. Common carotid arteries will be identified and isolated carefully from vago-sympathetic nerve. Rats will be ischemic by occluding of bilateral carotid artery with nylon thread for 60 min and reperfusion allowed for 3 h by removing the thread. Body temperature will be maintained around 37 ± 0.5º C throughout the surgical procedure.

Wister albino rats (200–250 g) were divided into five groups of six rats each and fed with drug/ vehicle for 11 days prior the experiment and treated as follows:

HI=*Heliotropium indicum*, BCAO=Bilateral carotid artery occlusion

- Group I: Normal saline (10 ml/kg b.w, orally)
- Group II: Normal saline (10 ml/kg b.w, orally)Ischemic control
- Group III: HI(200 mg/kg b.w, single dose/day, orally), BCAO for 60 min and followed by 3 h reperfusion individually
- Group IV: HI(280 mg/kg b.w, single dose/day, orally), BCAO for 60 min and followed by 3 h reperfusion individually.
- Group V: HI (400 mg/kg b.w, single dose/day, orally), BCAO for 60 min and followed by 3 h reperfusion individually.

1.7 Preparation of brain homogenate

Following decapitation, the brain was removed and washed in cooled 0.9% saline, keep on ice and subsequently blot on filter paper, then weighed and homogenized in cold phosphate buffer (0.1 M, pH 7.4) using a homogenizer. Homogenization procedure was performed as quickly as possible under completely standardized conditions. The homogenates were centrifuge at 10,000×*g* for 20 min at 4°C used.

Biochemical estimations .enzymatic analysis [21]

- Glutathione
- Lipid peroxidation
- Catalase (CAT)
- Superoxide dismutase (SOD)

1.8 Histopathology

1.9 Assessment of brain infarct size by TTC staining:

For TTC staining, two pieces of 2.0 mm thick coronal sections per brain were sliced along the hypophysis. After incubation in 2% TTC for 30min and fixation in 10% formalin for 45min at 37 ◦C, brain slices.

III. Results And Discussion

TABLE 1 Percentage yield calculated for *Heliotropium indicum* using different solvents

Solvents	Wt of HI (gms)	B.P(^{0}C)	Volume (ml)	Time(hrs)	%Yield (gms)
Pet.ether	30	40-60	350	24	0.85
Chloroform	30	61.2	350	24	0.92
Ethanol	30	78.3	350	24	1.40
Water	30	100	350	24	1.29

TABLE 2 Phytochemical screening of *Heliotropium Indicum*

Chemical constituents	Petroleum ether extract	Chloroform extract	Ethanolic extract	Water extract
Flavonoids	-	+	+	+
Phenols and Tannins	-	+	+	+
Steroids	+	+	+	-
Alkaloids	-	+	+	+
Glycosides	-	-	-	-
Carbohydrates	-	-	-	-
Gums	-	-	-	-
Proteins	-	-	+	+
Reducing sugars	-	-	-	-

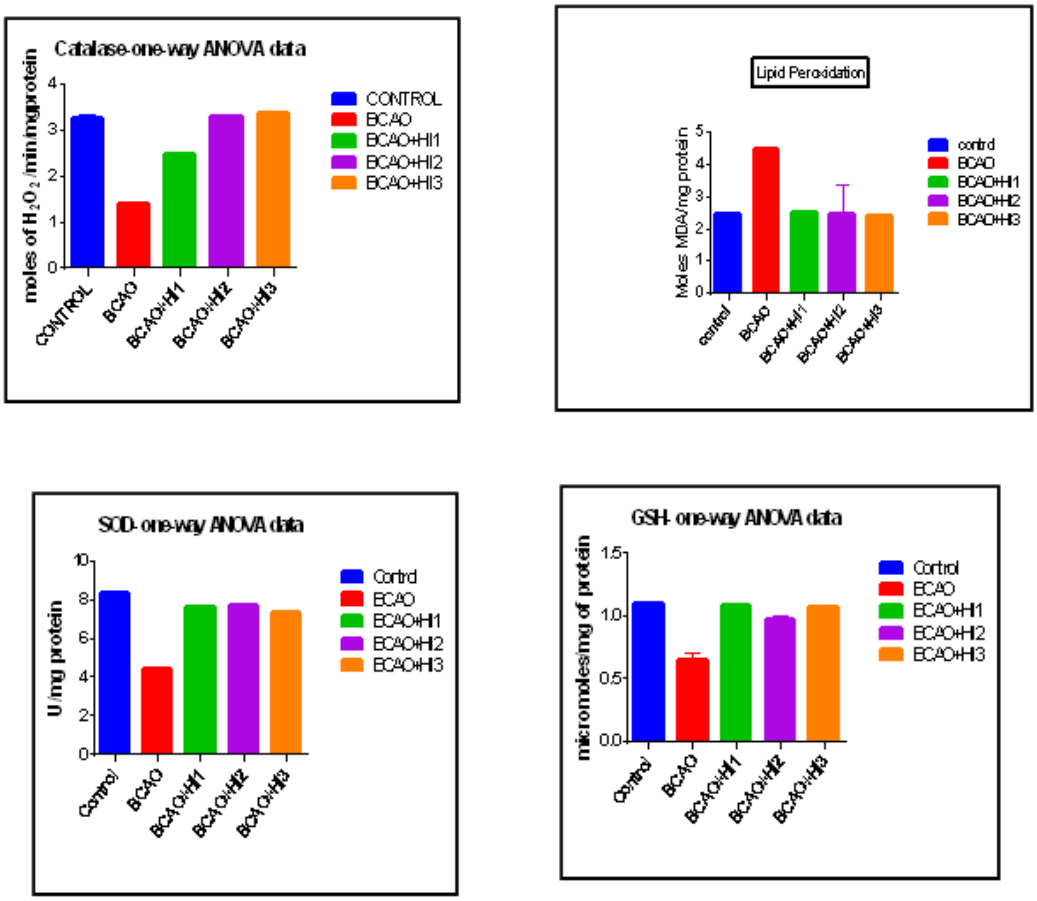

Fig 1 Biochemical Parameters Measured In Brain Homogenate

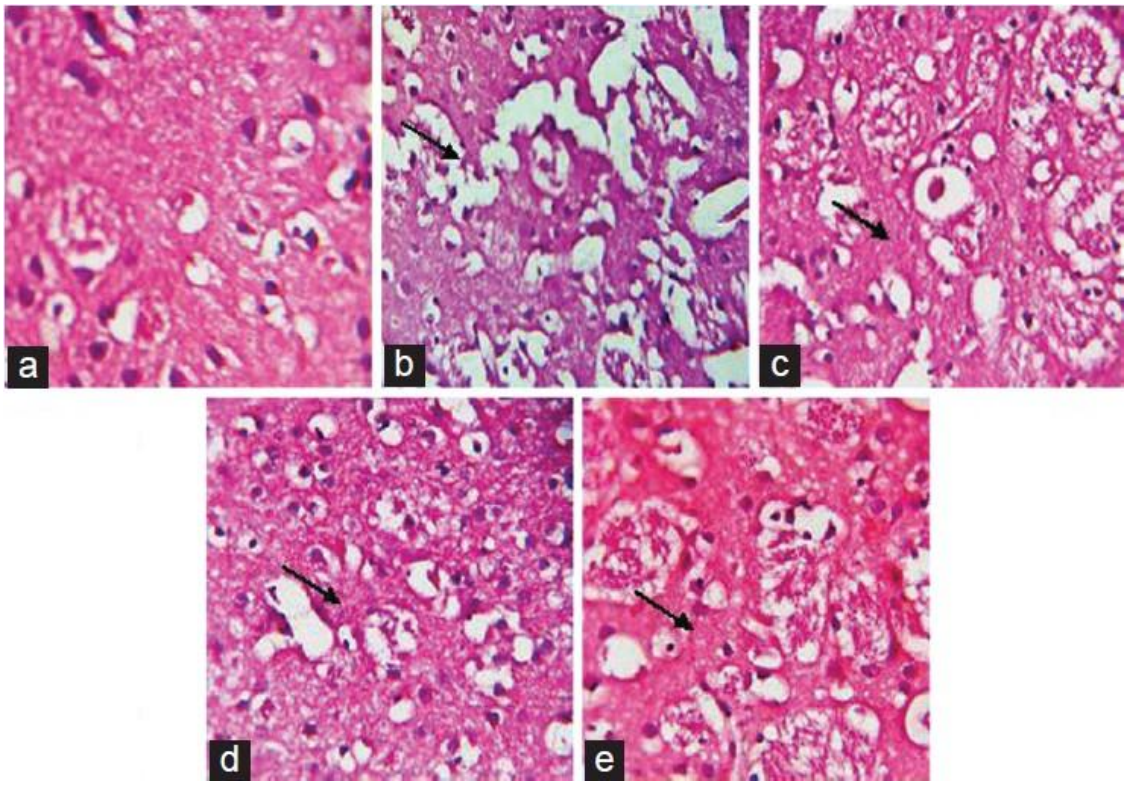

Fig 2 Histopathological Studies Carried Out On Isolated Brain Sections

a=normal rat brain
b= BCAO
c=200mg/kg b.w of HI
d= 280mg/kg b.w of HI
E=400mg/kg b.w of HI

IV. Discussion

The present investigation showed the neuroprotective activity of ethanolic extract of *Heliotropium indicum* against Ischemia/reperfusion induced oxidative stress as well as histopathological alterations.It has shown prominent antioxidant activity *in-vitro*.

The activities of the extract prepared to work by restoring the altered antioxidant enzymes. The severe neuronal loss observed as shrinkage of neurons and atrophy was observed in histological sections of I/R. The percentage of living cells was increased in plant extract treated groups using TTC Staining method.

V. Conclusion

In the present study, the model of focal cerebral ischemia reperfusion was performed in rats by BCAO method. The brain infarct area size,Biochemical parameters and Histopathology of normal rats and formula-treated rats with cerebral ischemia or reperfusion injury were investigated to find out how the plant worked to protect and improve the brain function. 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.

Acknowledgements

We are thankful to DST, Department of science and technology, New Delhi, India for giving financial support to carry out this work. We are also thankful to our Chairman Dr.Vishnu Raju for providing us necessary facilities to carry out this work in college.

References

[1]. AKK Marry, YY Lloyd, KA Brian, LC Robin, AK, Wayne Applied therapeutics the clinical use of drug. WoltersKlawer / Lippincott Williams and Wilkins(9,2009;9:53-1)
[2]. F Dajas, Rivera, FMegret, F Blasina. Neuroprotective by Flavonoids. Brazil J Med Biol Res, 36,2003,1613-1620.
[3]. EJ Lee, HY Chen, IA Ayoub, KI Maynard, Acute administration of Ginkgo biloba extract (Egb 761) affords neuroprotection against permanent and transient focal cerebral ischemia in Sprague-Dawley rat. J Neurosci,68,2002,636-645.
[4]. ZA Shah, RA Gilani, P Sharma, SB Vohora. Cerebroprotective effect of Korean ginseng tea against global and focal models of ischemia in rat. J Ethnopharmacol,101,2005,299-307.
[5]. Nehlig,The neuroprotective effects of cocoa flavanol and its influence on cognitive performance, British Journal of Clinical Pharmacology, 75(3), 2013,716–727.
[6]. Onaylos, Irma Noel. Plants That You Know But Really Don't: Home Remedies from 110 Philippine Medicinal Plants. Cebu City: Our Press, Inc, 40.
[7]. AA GELDOF , O AMINNEBO, JJ HEIMANS ., VINCA-ALKALOID NEUROTOXICITY MEASURED USING AN IN VITRO MODEL, J NEUROONCOL. ,37(2), 1998,109-13.
[8]. Guide for the Care and Use of Laboratory Animals..National Research Council (US) Committee for the Update of the Guide for the Care and Use of Laboratory Animals. 8,2011.
[9]. In series: OECD Guidelines for the Testing of Chemicals, Section 4: Health Effects.
[10]. Anees Ahmad, F Abbas.M.Alkarki, Sufia Hena, Lim han Khim, Extraction, separation and identification of chemical ingredients of Elephantopus Scaber using factorial; design of experiment. International journal of chemistry ,1(1) , 2009.
[11]. J. B. Harborne ,Phytochemical Methods,A Guide to Modern Techniques of Plant Analysis, 1973,
[12]. K khandelwal , Practical Pharmacognosy,Techniques and experiments, Nirali Publication, 2nd edn, 2000.
[13]. O Shoge Mansurat ,G Ndukwe,J Amupitan . Phytochemical and antimicrobial studies on the aerial parts of Heliotropium indicum. Annuals of Biological Research,2(2), 2011,129-136.
[14]. Yasmin Begum, Antibacterial, Antioxidant and Cytotoxic activities of Heliotropium Indicum , The Experiment,23(1),2014,1564-1569.
[15]. RR Pujari [1], NS Vyawahare , VG Kagathara . Evaluation of anti-oxidant and Neuroprotective activity of date palm against against Bilateral common carotid artery occlusion in rats.ijeb.49, 2011, 623-633.
[16]. Bhakta Prasad Gaire, Young Ock Kim,Zhen Hua Jin, Juyeon Park, Hoyoung Choi, Youngmin Bu and Hocheol Kim .Neuroprotective Effect Of Scutellaria Baicalensis Flavones Against Global Ischemic Model In Rats,Journal of NPA, 27 (1), 2014.
[17]. Yaghoob Farbood, Alireza Sarkaki, Sheida Hashemi, Mohammad Taghi Mansouri, Mahin Dianat.The effects of gallic acid on pain and memory following transient global ischemia/reperfusion in Wistar rats. Avicenna Journal of Phytomedicine,3(4), 2013, 329-340.
[18]. R Ionara. Siqueiraa, Helena Cimarostic, Cı´ntia Fochesattoc, Domingos S. Nunesd,Christianne Salbegoc, Elaine Elisabetskya,e, Carlos A. Neuroprotective effects of Ptychopetalum olacoides Bentham(Olacaceae) on oxygen and glucose deprivation induced damage in rat hippocampal slices. Nettoa Life Sciences ,75,2004, 1897–1906.
[19]. Arunachalam, S. Selvakumar, S. Jeganath, A.Arunachalam. Protective Effect of Vernonia Cinerea Against Bilateral Carotid Artery Occlusion Induced Stroke In Rats International Journal of Pharmacy, 2(1), 2012, 28-33.

[20]. Mohammad Bayat, Abolfazl Azami Tameh, Mohammad Hossein Ghahremani, Mohammad Akbari,Shahram Ejtemaei Mehr, Mahnaz Khanavi and Gholamreza Hassanzadeh.Neuroprotective properties of Melissa officinalis after hypoxic-ischemic injury both in vitro and in vivo,. DARU Journal of Pharmaceutical Sciences,20, 2012, 42

[21]. Dupadahalli Kotresha, Rama Rao Nedendla, Tigari Prakash· Neuroprotective activity of Wedelia calendulacea on cerebral ischemia/reperfusion induced oxidative stress in rats.Indian Journal of Pharmacology, 43(6), 2011, 676-682.