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

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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. Flavonoids 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]
H₂O₂ 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 vehicle 10ml/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 14th 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, kept 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 centrifuged at 10,000×g for 20 min at 4°C used.

1.8 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 30 min and fixation in 10% formalin for 45 min at 37°C, brain slices.

III. Results And Discussion

<table>
<thead>
<tr>
<th>TABLE 1</th>
<th>Percentage yield calculated for Heliotropium indicum using different solvents</th>
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<tbody>
<tr>
<td>Solvents</td>
<td>Wt of HI (gms)</td>
</tr>
<tr>
<td>Pet.ether</td>
<td>30</td>
</tr>
<tr>
<td>Chloroform</td>
<td>30</td>
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<tr>
<td>Ethanol</td>
<td>30</td>
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<tr>
<td>Water</td>
<td>30</td>
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<thead>
<tr>
<th>TABLE 2</th>
<th>Phytochemical screening of Heliotropium Indicum</th>
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<tbody>
<tr>
<td>Chemical constituents</td>
<td>Petroleum ether extract</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>-</td>
</tr>
<tr>
<td>Phenols and Tannins</td>
<td>-</td>
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<tr>
<td>Steroids</td>
<td>+</td>
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<tr>
<td>Alkaloids</td>
<td>-</td>
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<tr>
<td>Glycosides</td>
<td>-</td>
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<tr>
<td>Carbohydrates</td>
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<td>Gums</td>
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<td>Proteins</td>
<td>-</td>
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<tr>
<td>Reducing sugars</td>
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</table>
Neuroprotective effect of ethanolic extract of Heliotropium indicum against transient global ...
It has been shown that natural antioxidants can improve the brain function. 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.

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.

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