

# Impact of *Cyperus Rotundus* Extract on Brain Ischemia and Learning Dysfunction in a Rat Model

Rahil Khan<sup>1</sup> and Dr. Atul Baravkar<sup>2</sup>

<sup>1</sup>Research Scholar, Department of Pharmacy

<sup>2</sup>Professor, Department of Pharmacy

Sunrise University, Alwar, Rajasthan

**Abstract:** *Cyperus rotundus*, a traditional medicinal plant, has been investigated for its neuroprotective potential in ischemia-induced brain damage and memory dysfunction in rats. This study aimed to evaluate its therapeutic efficacy in mitigating cognitive deficits and neuronal damage following cerebral ischemia. Male Wistar rats were subjected to bilateral common carotid artery occlusion (BCCAO) to induce global cerebral ischemia, followed by oral administration of *Cyperus rotundus* extract. Behavioral assessments, including the Morris water maze and novel object recognition tests, were conducted to evaluate memory function. Biochemical analyses of oxidative stress markers and inflammatory cytokines were performed, along with histopathological examinations of the hippocampus and cortex. The results demonstrated that *Cyperus rotundus* significantly improved cognitive performance, reduced oxidative stress, and inhibited neuroinflammation in ischemic rats. Histological analysis revealed decreased neuronal degeneration and enhanced neuronal survival in the treated group compared to ischemic controls. These findings suggest that *Cyperus rotundus* exerts neuroprotective effects through antioxidant, anti-inflammatory, and neurorestorative mechanisms, highlighting its potential as a natural therapeutic agent for ischemia-induced cognitive impairment. Further research is needed to explore its molecular mechanisms and clinical applicability in neurodegenerative disorders associated with cerebral ischemia.

**Keywords:** *Cyperus rotundus*, ischemia-induced brain damage, memory dysfunction, neuroprotection, oxidative stress, neuroinflammation, cognitive impairment, cerebral ischemia, hippocampal damage, antioxidant therapy

## I. INTRODUCTION

One of the leading causes of mortality in affluent nations is global cerebral ischemia-reperfusion damage (GCIRI) (1,2). Since the hippocampus, particularly the CA1 region, is known to be susceptible to global cerebral ischemia, many of these patients may experience learning and memory impairments as a result of hippocampal damage (3–7). Despite numerous attempts, there is currently no effective method to prevent or treat this cognitive and memory impairment.

Inflammation after cerebral ischemia and reperfusion after brain ischemia have been shown in several studies to augment these inflammatory processes, which may worsen neuronal damage (8–10). Pro-inflammatory cytokine production may greatly raise the likelihood and severity of brain damage (14), as does the release of pro-inflammatory cytokines including necrosis factor $\alpha$  (TNF- $\alpha$ ), interleukin (IL)-1, and IL-6 during ischemia/reperfusion injury after both localized and global cerebral ischemia (11–13).

Delays in neuronal death are caused by a series of cellular and molecular processes that occur during brain ischemia. The excessive activation of glutamatergic receptors and the large release of glutamate are two well-established pathways in the pathologic processes of neuronal death after ischemia (15). For the treatment of stroke, it is thus essential to investigate new therapeutic approaches, such as medications based on herbs. Ayurvedic medicine uses *Cyperus rotundus* (family: Cyperaceae), a classic Indian medicinal plant, as a nootropic and nervine tonic (16). The

goal of the current research was to examine the neuroprotective potential of *Cyperus rotundus* ethanol extract (EECR) in a rat model of global cerebral ischemia/reperfusion. In first pharmacological screening, EECR had a significant central nervous system (CNS) depressive effect in contrast to its other extracts (17). On the other hand, no research on this plant's CNS activity has been published. Through an assessment of the pathophysiology of the hippocampus tissue and spatial memory, the current work has examined the potential neuroprotective effects of EECR on a model of global transient ischemia.

## **II. MATERIALS AND METHODS**

### **Plant material and preparation of crude extract**

After being recognized by Dr. Gholamreza Amin (School of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran), the rhizomes of *C. rotundus* (herbarium No: PMP215) were bought from a neighborhood herbal shop in Tehran, Iran, and the voucher specimen (8005) was placed at the Pharmacy School. The hydro-alcoholic extract was made by macerating 1000 g of pulverized *C. rotundus* rhizomes in 70% ethanol three times, each lasting 24 hours. A 12% yield was obtained after the extract was filtered and concentrated using a vacuum evaporator.

### **Phytochemical screening**

Standard procedures and assays were used to conduct phytochemical analyses of the hydroalcoholic extract of *C. rotundus* rhizomes (18, 19). Using established protocols, chemical tests were performed on the hydroalcoholic extract of *C. rotundus* rhizomes to determine their chemical contents. In order to test for tannins, 1 gram of extract was dissolved in 2 milliliters of distilled water. The filtrate was then treated with filtered water and ferric chloride reagents. The extract was put through Fehling's test for glycosides and the foaming test for saponins. Spots were found after applying Dragendorff's reagent to the alkaloid fraction that was prepared using a traditional acid-base extraction method for alkaloids. The alkaloid fraction was then subjected to TLC analysis in a 25% 8:2:0.5 chloroform: methanol: ammonia solution as the solvent system. By adding 1% aluminum chloride solution to the extract and observing the yellow tint, the presence of flavonoids was identified. Another test for flavonoids was conducted by mixing the extract with diluted ammonia (5 ml) and then concentrated sulfuric acid (1 ml). By mixing 1 milliliter of acetic anhydride with 0.25 grams of methanolic extract of each sample and 1 milliliter of H<sub>2</sub>SO<sub>4</sub>, steroids were found. When steroids were present, the hue shifted from violet to blue or green. Anthraquinones were tested using 0.5 g of extract that had been heated with 10 ml sulfuric acid and then filtered. After shaking the filtrate with five milliliters of CHCl<sub>3</sub>, the CHCl<sub>3</sub> layer was transferred to a different tube, and a color shift was seen when one milliliter of ammonia was added. A sheet of filter paper was dampened with NaOH and placed over a test tube containing a boiling plant extract solution in order to detect coumarins. A positive coumarin test result was determined if the filter paper subsequently displayed any yellow fluorescence when exposed to UV light. Terpenoids (triterpenoids) were detected by mixing 0.5 g of extract with 2 ml of CHCl<sub>3</sub>, followed by 3 ml of finely concentrated H<sub>2</sub>SO<sub>4</sub> to create a layer and a reddish-brown hue at the interface.

### **Induction of brain ischemia/reperfusion and drug administration**

24 A male adult In accordance with the guidelines and protocols of Islamic Azad University's National Institutes of Health Guidelines for Animal Experiments, wistar rats weighing 250–300 g were randomly assigned to three groups (control, ischemia, and treatment) and housed individually in a cage with a 12-hour light/dark cycle and unrestricted access to food and water. In order to create reversible ischemia for 20 minutes, the rats in the control and treatment groups were operated on using the modified global cerebral ischemia model, which may be created by blocking the two common carotid arteries or by two-vessel occlusion (2VO). In short, pentobarbital sodium (40 mg/kg) was used to anesthetize the bilateral carotid arteries before they were clamped using a microsurgery clamp. Twenty minutes after the ischemia started, these clamps were removed. A homoeothermic blanket was used to keep the body temperature at 37°C (20). Only pentobarbital sodium was used to put the control animals to sleep.

All surgical operations were performed on animals in the ischemia group. The ethanol extract of *C. rotundus* (EECR) was gavaged for the treatment group four days before to the induction of ischemia and three days after the ischemia.

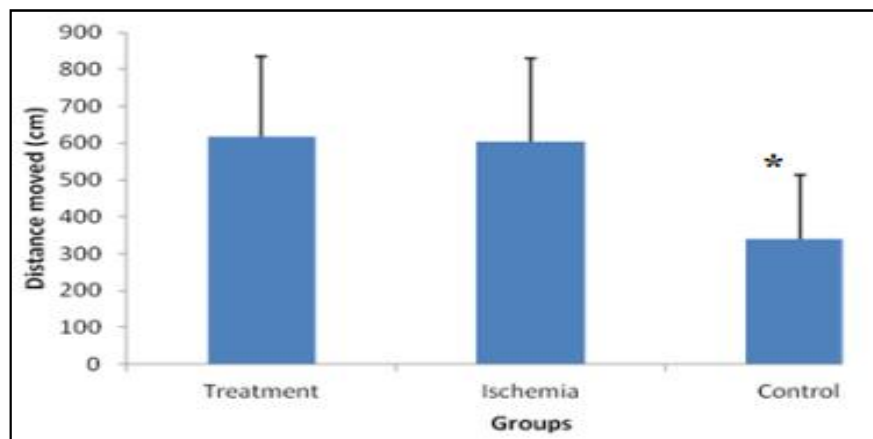
Following ischemia, the animals were housed in an animal home for a week before completing the Morris water maze activity. One day after the conclusion of the Morris water maze training, rats were killed. The Nissl technique was used to extract the brains for histological evaluation.

### Behavioral testing

One week after cerebral ischemia, training in the Morris water maze was conducted. A circular galvanized steel tank of 170 cm in diameter and 45 cm in height, filled to a depth of 13 cm with water at  $22\pm 1^\circ\text{C}$ , and encircled by a number of extra-maze cues, made up the apparatus. The escape platform (diameter: 18 cm) was situated in the southeast (SE) quadrant of the tank, which was separated into four sections. It was fixedly positioned and buried 2 cm below the water's surface. The rats used extra-maze signals to locate a submerged platform during the spatial acquisition phase. For four days in a row, each rat took part in 16 trials, which were arranged into daily blocks of four trials. For no more than sixty seconds, or until the platform was found, the rats were free to swim. The rat was led to the platform and given 20 seconds to stay there if the platform could not be found during this period. Following a day of testing, the animals were killed and their hippocampus damage was assessed.

### Histological procedures

Animals were sacrificed 1 day after water maze testing. For Nissl staining, we used paraffinembedded brain sections that received transcardiac



**Figure 1. Effect of *Cyperus rotundus* on distance moved in ischemia-induced memory deficit model \*P-value  $\leq 0.05$ , statistically different from ischemia group. Data are mean $\pm$ SD**

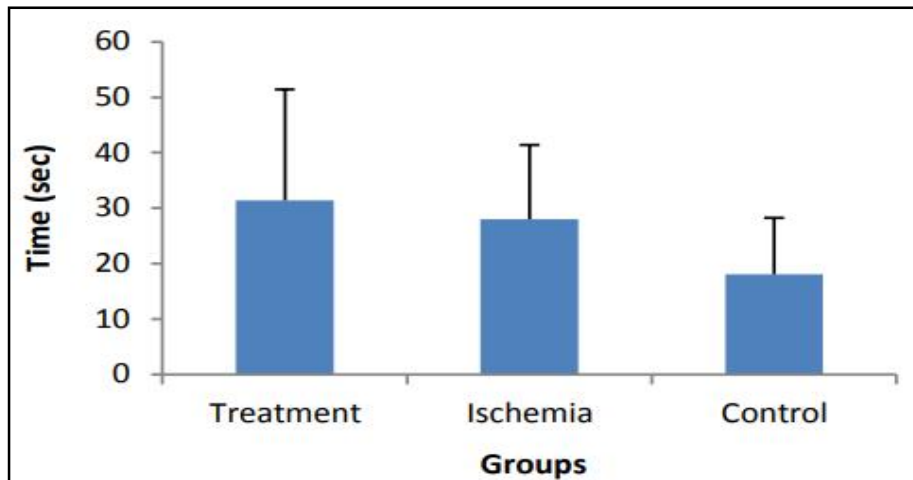
perfusion with 4% formaldehyde in phosphatebuffered saline, followed by immersion fixation for more than 3 days. Paraffin-embedded coronal sections were cut from Bregma 2/3 mm to 5 mm into 10  $\mu\text{m}$  thick sections posterior to Bregma fortune.

### Nissl staining

Rat brain coronal slices (10  $\mu\text{m}$  thick) were produced continuously. These sections were dehydrated, cover-slipped with Entellan, then treated with 1.0% cresyl violet. Under an optical microscope, histological alterations in brains were discovered. Using a light microscope, the diameters and numbers of the CA1 pyramidal cells in the hippocampus were quantified in three stained slices of each rat's hippocampus. A microscope (Olympus AX-70) was used to produce photomicrographs at a magnification of 400 x, and image tool 2 software was used for analysis.

### Statistical analysis

All histological data were analyzed with a oneway ANOVA. Post hoc comparisons between independent groups were made with the Tukey test.



**Figure 2. Effect of Cyperus rotundus on latency time in ischemia-induced memory deficit model. \*P-value  $\leq 0.05$ , statistically different from ischemia group. Data are mean  $\pm$  SD**

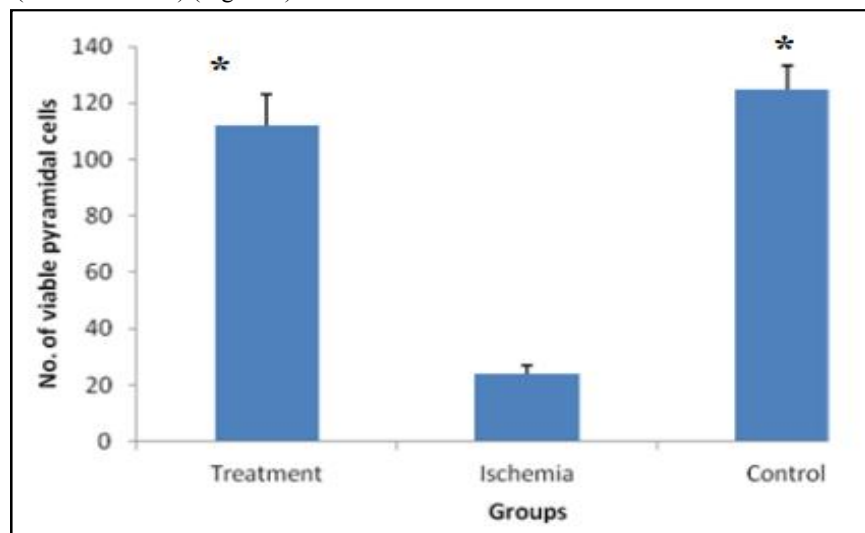
In all cases, the acceptable level for statistical significance was P-value  $\leq 0.05$ .

### III. RESULTS

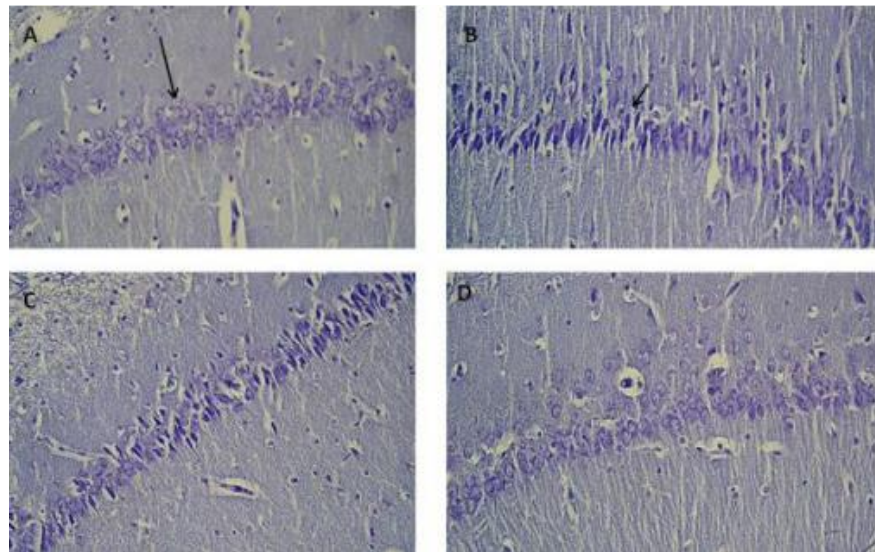
1- Data analyses from Morris water maze test showed that there was statistically significant difference between control and ischemia groups but no statistical difference was seen between ischemia and treatment groups in distance and time (Figure 1, 2).

2- Data of Nissl staining showed that 20 min of bilateral common carotid occlusion caused marked CA1 cell loss. But no statistically significant difference was seen between control and treatment groups (P-value= 0.105) it means that EECR can prevent CA1 cell loss due to ischemia/ reperfusion injury (Figure 3, 4).

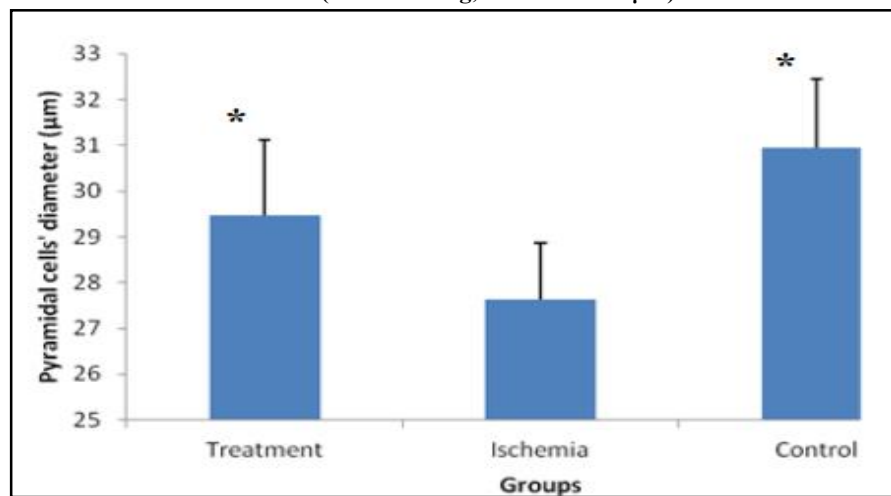
3- Data which were collected from cell diameters showed that 20 min of bilateral common carotid occlusion caused marked reduced cell diameters in CA1 region of hippocampus. There was statistically significant difference between control group versus ischemia group (P-value=0.010). But there was no statistical significant between control and treatment groups (P-value= 0.299) (Figure 5).



**Figure 3. Effect of Cyperus rotundus on number of CA1 pyramidal cells in ischemia-induced memory deficit model \*P-value  $\leq 0.05$ , statistically different from ischemia group. Data are mean  $\pm$  SD**



**Figure 4. Photomicrographs of coronal sections of CA1 region in hippocampus. A) control, B) ischemia, C) vehicle and D) experimental groups. Long arrow shows an intact neuron and short arrow shows a degenerated neuron (Nissl staining, scale bar= 30  $\mu$ m)**



**Figure 5. The effect of Cyperus rotundus on pyramidal CA1 cells diameters ( $\mu$ m) after transient global ischemia \*P-value  $\leq$  0.05, statistically different from ischemia group. Data are mean $\pm$ SD**

#### IV. DISCUSSION

A clinical consequence of cardiac arrest and other events that cause a brief drop in brain oxygen levels, transient global cerebral ischemia may result in the death of CA1 neurons in the hippocampus (21–23). Severe hippocampal dysfunction, including difficulties in memory and spatial learning, is linked to degeneration of the CA1 pyramidal neurons (24).

The neurotrophic impact of EECR is shown in this work using a rat model of complete ischemia-reperfusion. Ischemic neuronal death is caused by a number of pathologic processes, including excitotoxicity, inflammation, and apoptosis (25). We used reperfusion after common carotid blockage to cause the neurological impairment.

Our results showed that rats exposed to bilateral common carotid ligation for 20 minutes had damaged pyramidal cells in the CA1 area and an impairment in spatial memory.

Although the exact mechanism of ischemia/reperfusion (IR) is still unknown, reactive oxygen species (ROS) seem to be one of the key elements that cause neuronal death during IR injury (26).

Destructive free radicals as peroxynitrite, superoxide, and nitrogen oxide (NO) rise after IR damage (27). In the experimental mouse model of cerebral IR damage, nitrogen oxide synthase (NOS) expression and activity are elevated. The pathophysiology of brain ischemia damage involves oxidative products and nitrogen oxide (28).

Due to the oxidative mechanism of ischemia-induced cell death and injury, there is growing interest in neuroprotective drugs that may lessen the harm caused by ROS and NO (29).

Recently, the food industry has begun using *C. rotundus*, a traditional herbal remedy. Through its antioxidant and free radical scavenging properties, which are crucial in preventing neurodegenerative diseases, the neuroprotective benefits of *C. rotundus* rhizome extract (CRE) have been investigated in this work (30).

According to Seo et al., the methanol extract of *C. rotundus* rhizomes could decrease the expression of iNOS mRNA and inducible nitric oxide synthase (iNOS) in proteins, which in turn might influence the generation of O<sub>2</sub> and NO (31).

According to Lee et al., the alcoholic extract of *C. rotundus* possesses antioxidant properties (32). Additionally, in two animal models, investigations have shown that this herb has extremely substantial anti-inflammatory action against the exudative and proliferative stages of inflammation (33, 34).

The evidence that a medication may improve the behavioral impairment caused by IR, backed by histology data, is a crucial discovery in therapeutic settings.

The Stroke Therapy Academic Industry Roundtable (STAIR, 1999) highlights the importance of behavioral measures in the preclinical assessment of medications' neuroprotective benefits prior to the start of clinical trials. EESR was unable to lessen the impact of ischemia on learning and spatial memory in the current investigation.

Lastly, no relationship was seen between the degree of pyramidal cell loss in the CA1 region of the hippocampus in the EECR-treated group and any of the behavioral characteristics examined. Contradictory findings have emerged from attempts to quantify the relationship between ischemia-induced behavioral impairments and the loss of hippocampus pyramidal cells:

Cognitive disruption by ischemia may be determined by various intra- and/or extra-hippocampal consequences, and the number of preserved, undamaged seeming pyramidal cells alone may not be a reliable indicator of behavioral alterations, according to the strong association.

The degree of pyramidal cell loss in the CA1 region of the hippocampus did not correlate with the behavioral impairment assessed in the water maze in our investigation. Bachevalier and Meunier (35), reviewing the relationship between ischemia-induced cognitive deficits and hippocampal cell loss, came to the conclusion that at least three factors may be at play: the type of task used, the specific memory process taxed, and the role of intra- and extra-hippocampal damage. Therefore, we cannot rule out the potential that ischemia may have caused damage to brain areas other than the hippocampus. Given the connection between structure and function, it's also critical to stress that the degree to which methodological constraints like histological evaluation and/or random behavioral variables may affect a correlation analysis's findings is unclear (36).

## V. CONCLUSION

According to the Morris water maze, the current research demonstrated that EECR is unable to lessen the cognitive deficits brought on by temporary, worldwide cerebral ischemia. As far as we are aware, this is the first study to examine how EECR affects the cognitive outcome of temporary, worldwide ischemia in rats. To fully describe the role of EECR in improving function after ischemia-induced brain injury, further research is required.

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