



**PROTECTIVE ROLE OF ETHANOLIC EXTRACT OF *HYPERICUM PERFORATUM* IN  
LONG TERM CEREBRAL HYPOPERFUSION INDUCED BEHAVIORAL  
ALTERATIONS IN RATS.**

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**ABSTRACT**

Chronic cerebral hypoperfusion induced by permanent occlusion of bilateral common carotid arteries in rats is associated with behavioral alterations. In Ayurveda, the medicinal properties of *Hypericum perforatum* Linn have been attributed to its anxiolytic, antioxidant, antidepressant and nootropic properties. The present study evaluated possible role of ethanolic extract of *Hypericum perforatum* Linn, in long term cerebral hypoperfusion (a model of cerebrovascular insufficiency and dementia) induced changes in behavioral alterations. *Hypericum perforatum* (100 mg/kg, p.o. x 15 days) attenuated the long-term hypoperfusion induced anxiety and listlessness (open field paradigm) along with improvement of learning and memory deficits (Morris water maze testing). The present data report the protective role of *Hypericum perforatum* in cerebrovascular insufficiency states and dementia.

**KEYWORDS:** Cerebral hypoperfusion, Learning and memory, dementia, antioxidant, nootropic.

**INTRODUCTION**

A number of herbal drugs have been evaluated for their possible role in neurodegenerative disorders and cognitive functions. *Hypericum perforatum* (HP) or St. John's Wort known as *Basanti* in Ayurveda (the classical Indian system of medicine), has been used for centuries, for a variety of diseases.<sup>[1]</sup> Ethanolic extract of *H. perforatum* is reported to have antioxidant, anti-inflammatory<sup>[2]</sup> and antidepressant properties.<sup>[3]</sup> Standardized extract of *H. perforatum* is known to possess anxiolytic<sup>[4]</sup> and nootropic activity on the basis of neurotransmitter receptor mechanism.<sup>[4,5]</sup>

Earlier investigations have indicated that *H. perforatum* contains many bioactive constituents; phenyl propanoids, flavonol glycosides, biflavones, oligomeric proanthocyanidins, xanthenes, naphodianthrones and prenylated phloroglucinols.<sup>[6]</sup> The presence of many polyphenolic compounds in this herb suggests that they could have important antioxidant, anti-inflammatory properties.<sup>[2]</sup> The polyphenols have the ability of penetrate the blood brain barrier and act as potential neuroprotective agent. Recently, hyperforin, a prenylated phloroglucinol present in this plant, has been targeted as the major component responsible for the antidepressant activity of *H. perforatum*.<sup>[7]</sup> and inhibition of the uptake of several neurotransmitters *in vitro*.<sup>[8]</sup>

Chronic cerebral hypoperfusion induced by permanent occlusion of bilateral common carotid arteries (BCCAO) in rats induces a state of chronic low grade ischemia in rat's brain over an extended period of several months.<sup>[9]</sup> Extensive investigations report that rats subjected to permanent occlusion of BCCA show impaired spatial learning/ memory capabilities and/or structural alterations.<sup>[11]</sup> Long-term cerebral hypoperfusion induced by bilateral – common carotid arteries occlusion (BCCAO) causes a reduction of blood flow from about 30-45% in cortex to 20% in hippocampus.<sup>[12, 13]</sup> Chronic reduction in blood flow and brain energy metabolism causes behavioral and cognitive defects.<sup>[14, 15]</sup>

This study was designed to assess the possible protective activity of standardized extract of *H. perforatum* on long term cerebral hypoperfusion.

**MATERIAL AND METHODS**

**Animals**

After approval of Institutional Ethics Committee, the present study was conducted on inbred Charles Foster (CF) albino rats of either sex weighing 250-300 g, obtained from the central animal house of the Institute of Medical Sciences, Banaras Hindu University, Varanasi. They were kept in the departmental animal house in colony cages at an ambient temperature of 25±2°C and 45-55% relative humidity with 10:14 hours light: dark

cycles. They had free access to standard rodent pellet diet and drinking water. The food was withdrawn 18-24 h before the surgical procedure, however, water was allowed *ad libitum*. Principles of laboratory animal care (NIH Publication No. 86-23, revised 1985) guidelines were followed throughout.

### Drug Treatment

The plant was collected during August from the company garden, Saharanpur, India. A 50% ethanolic extract (yield 26.75% w/w, standardized for 4.5-5% hyperforin, HPLC) of the dried overground parts (leaves, flowers and stem) of the plant, as administered orally as a 0.3% carboxymethyl cellulose (CMC) suspension, in dose of 100 mg/kg. p.o. once daily. The choice of particular dose was made according to our initial pilot experimental results<sup>[5]</sup>.

### Study Design

For long term hypoperfusion studies, again animals were divided into four groups of six animals each. First group served as sham-operated control. In second group, *H. perforatum* 100 mg/kg/day p.o. was administered during the experimental period in sham operated animals (*H. perforatum* per se). Third group animals underwent permanent BCCAO (hypoperfusion group). In the fourth group, *H. perforatum* was administered 60 min before BCCAO. *H. perforatum* was then continued up to 15<sup>th</sup> post surgical day in sham operated and hypoperfused (treatment group) animals.

### Experimental Methods

#### i) Surgical procedure

Surgical technique for induction of cerebral hypoperfusion by bilateral common carotid artery occlusion (BCCAO) was adapted.<sup>[16]</sup> Rats were anaesthetized by an intra-peritoneal injection of ketamine (100 mg/kg). After a midline skin incision in the neck, both common carotid arteries were identified and isolated carefully from accompanying vagosympathetic nerve.

For long-term hypoperfusion studies, BCCAs were doubly ligated with 3-0 silk sutures and cut in between. The skin was then sutured and animals were returned to their home cage.

On the day 15, 60 min after last dose of *H. perforatum* all animals were subjected to behavioral testing in open field paradigm and Morris' water maze.

#### ii) Behavioral Testing

*H. perforatum* was then continued up to 15<sup>th</sup> post surgical day sham operated and hypoperfused (treatment group) animals. Animals were subjected to behavioral analysis.

**Open Field Test:** Locomotor activity was evaluated in an open field paradigm.<sup>[17]</sup> The open field is made of plywood and consisted of a floor (96 x 96 cm) with high

walls (61 x 61 cm). Entire apparatus is painted black except for 6 mm thick white lines that divide the floor into 16 squares. The entire room except the open field was kept dark during the experimentation. The open field was lighted by a 60 watt bulb focusing on to the field from a height of about 100 cm from the floor. Each animal was placed at one corner of the apparatus and for next 5 min, it was observed for the ambulation (number of squares crossed), total period of immobility (in seconds), number of rearing, grooming and fecal pellets.

**Morris' Water Maze Test:** The maze consisted of a black circular pool (diameter 2.14 m, height 80 cm) filled to a depth of 44 cm with water (25°C).<sup>[18]</sup> Water was made opaque by adding Indian ink. On day 15 after surgery, spatial learning and memory was tested in water maze. On 14<sup>th</sup> day the rats received habituation (exposure in water maze for 1 min) in which there was no platform present. Then, on day 15, 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 (reference memory procedure). 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 s to find the hidden platform. The animal was allowed a 20 s 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 s. The procedure was repeated for all the four start locations.

Two sessions of four trials each were conducted on first day of testing separated by 4h and one session of four trials was conducted on the next day. After that, the platform was removed and a probe trial (without platform) was conducted 4h later. Each rat was placed in the pool at the same randomly selected starting pole and swimming path was observed and time spent in the quadrant of pool which initially contained platform was measured.

On completion of the probe trial, a black platform that extended 1 cm above the surface of water was placed in a quadrant other than that chosen for the submerged platform. Each rat was then given four trials of 90 s to locate it. The latency to reach the platform was recorded (working memory procedure).

### Statistical Analysis

Statistical analysis of data was performed by applying one-way analysis of variance (ANOVA) followed by Tukey Test for biochemical parameters and behavioral observations. A p-value of <0.05 was considered statistically significant.

## RESULTS

### Behavioral observations

#### Open Field Test

Animals with permanent BCCAO (hypoperfusion group) showed marked alterations in locomotor activity in open field paradigm. As demonstrated on day 15, permanent BCCAO was associated with reduced number of

ambulation, rearing and grooming along with increase in period of immobility. *H. perforatum* pretreatment (100 mg/kg, p.o. x 15 days) prevented these alterations. In sham operated animals *H. perforatum* per se did not have any effect on any of the parameters of open field test (Table 1).

**Table 1: Effect of *H. perforatum* (100 mg / kg p.o. x 15 days) on open field parameter in long-term hypoperfused rats.**

Groups	Ambulations (number)	Immobility (S)	Rearing (number)	Grooming (number)	Fecal pellets (number)
Sham-operated control (A)	58.50±4.49	32.16±1.86	23.00 ± 0.52	7.50 ± 0.53	4.63 ± 1.25
<i>Per se</i> (B)	59.16±5.76	29.50±1.67	22.96 ± 1.01	6.56 ± 0.76	4.26 ± 0.76
Hypoperfusion (C)	26.00±4.40 <sup>a</sup>	48.06±1.52 <sup>a</sup>	14.80 ± 1.19 <sup>a</sup>	1.89 ± 0.40 <sup>a</sup>	1.96 ± 1.02
Treatment (D)	61.16±5.23 <sup>a</sup>	32.18±2.47 <sup>a</sup>	22.93 ± 1.36 <sup>a</sup>	6.41 ± 0.98 <sup>a</sup>	2.60 ± 0.82

All data are expressed as mean ± S.E.M. Number of animals in each group = 6. Sham –operated control and treatment groups are compared with hypoperfusion group. *H. perforatum per se* is compared with sham-operated control group. Superscript <sup>a</sup> indicate p-value less than 0.01. Statistical analysis was done by one-way ANOVA followed by Tukey test.

#### Morris' Water Maze Test

In Morris water maze testing, no difference was observed between sham-operated control and *H. perforatum per se* groups. All rats located the hidden platform during the sessions of escape trial, although hypoperfused animals required more time than sham-operated control. A rising trend in time taken for rats to find submerged platform was observed during second session in hypoperfused rats and this increase in escape latency further increased in third session as compared to sham-operated rats but not during the first session. *H. perforatum* (100 mg/kg, p.o. x

15 day prevented this delay in escape latencies in second and third sessions but not during the first session. Analysis of swimming performance during the probe trial revealed that hypoperfused rats spent less time in quadrant of former platform position than did sham operated rats. This change was significantly reversed by *H. perforatum* treatment. The result of new platform trial show that hypoperfused animals found the new platform slower than sham-operated rats. *H. perforatum* treated animals found the new visible platform quicker than the hypoperfused animals (Table-2).

**Table 2: Effect of *H. perforatum* (100 mg / kg p.o. x 15 days) on learning and memory in long term hypoperfused rats in Morris' water maze.**

Groups	Escape latency (sessions)			Probe trial	New platform trial
	I	II	III		
Sham-operated control (A)	68.01 ± 5.28	28.40 ± 1.92	28.20 ± 1.85	30.00 ± 1.21	17.16 ± 1.35
<i>Per se</i> (B)	69.83 ± 4.22	29.73 ± 2.32	29.83 ± 1.62	33.26 ± 1.94	15.43±1.95
Hypoperfusion (C)	76.26 ± 3.90	48.26 ± 1.23 <sup>a</sup>	40.83 ± 2.44 <sup>a</sup>	22.00 ± 2.37 <sup>b</sup>	28.40 ± 1.86 <sup>a</sup>
Treatment (D)	68.21 ± 3.89	27.00 ± 1.46 <sup>a</sup>	22.76 ± 2.53 <sup>a</sup>	32.56 ± 1.55 <sup>a</sup>	16.53 ± 1.06 <sup>a</sup>

All data (time in seconds) are expressed as mean ± S.E.M. Number of animals in each group = 6. Sham –operated control and treatment groups are compared with hypoperfusion group. *H. perforatum per se* is compared with sham-operated control group. Superscript <sup>a</sup> and <sup>b</sup> indicate p-value less than 0.01 and 0.05 respectively. Statistical analysis was done by one-way ANOVA followed by Tukey test.

## DISCUSSION

Permanent BCCA occlusion in rats has been used as one of the animal models for cerebrovascular insufficiency state, white matter lesions, neurodegenerative conditions and dementia.<sup>[9, 19, 20]</sup> Reduced blood flow in the magnitude of 30-45% in cortex to 20% in hippocampus has been observed one week after permanent BCCAO in rats. In addition to reduced glucose utilizations by 20-30% and 15% in cortex and hippocampus respectively.<sup>[12, 13]</sup> Chronic reduction in cerebral blood flow and brain energy metabolism leads to progressive cognitive deficit.<sup>[15]</sup>

Long term hypoperfusion studies have been subjected to critical appraisal, through behavioral analysis.<sup>[17, 18, 21]</sup> In

the present study investigations on open field behavior according to accepted tests, showed that long term hypoperfused animals were more susceptible to develop anxiety when exposed to novel environment.<sup>[17]</sup> The reduction in total activity of long term hypoperfused rats in the open field paradigm with significant reduction in ambulation, rearing and grooming with percept to sham operated animals suggest a propensity towards anxiety and listlessness. *H. perforatum* has significantly prevented long term hypoperfusion induced anxiety. Hypoperfused animals also had deficit of spatial learning and memory as indicated by Morris water maze testing which is in accordance with earlier reports of ischemia induced disturbances of spatial learning and memory.<sup>[20, 21]</sup> Chronic reduction in blood flow secondary to BCCAO

has been reported to cause progressive dysfunction in cognitive deficits.<sup>[12, 13, 15]</sup> Long term hypoperfused animals consistently took longer time to find out the submerged platform (longer escape latencies) reflecting a defective learning process. Moreover, probe trail and new platform trails show deficits of reference working memory in the hypoperfused rats. *H. perforatum* alleviated the changes in long term hypoperfusion induced anxiety and listlessness, along with improvement of learning and memory deficits. Alleviation by *H. perforatum* of these alterations suggests that *H. perforatum* improve spatial learning and memory in long term hypoperfused rats. It is not out of place to mention that *H. perforatum* is known to have nootropic activity.<sup>[22]</sup>

Of the many properties of bioflavonoids, an important action in the present context is their vasodilatory properties.<sup>[23]</sup> Therefore, it is likely that by virtue of bioflavonoids content helps in establishment of collaterals leading to improvement of cerebral perfusion and microcirculation after BCCAO. Over earlier study indicated that *H. perforatum* prevents oxidative stress during cerebral ischemia reperfusion injury.<sup>[24]</sup>

Several studies have indicated that increase in serotonergic neurotransmission can interfere with learning acquisition and memory consolidation.<sup>[25]</sup> The role of 5-hydroxytryptamine (5-HT) in anxiety is now well established that it has been conclusively shown that increase in central serotonergic activity leads to anxiety, whereas decrease in 5-HT activity result in anxiolysis.<sup>[26]</sup> It was reported that neurochemical effects induced by *H. perforatum* and neurotransmitter receptor mechanism contributed to nootropic and anxiolytic actions.<sup>[22]</sup> The present study indicates the substantial role of *H. perforatum* in long term hypoperfusion.

## CONCLUSION

The present study therefore, implies role of *H. perforatum* in long term cerebral hypoperfusion. The present study apart from supporting the earlier observed cognition enhancing property of *H. perforatum* suggests that it may be useful in cerebral hypoperfusion states such as cerebrovascular insufficiency and dementia. Thus, the present observations provide a platform for further detailed investigation in this regard.

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