Effect of Ethanolic Extract of *Tinospora cordifolia* on Behavioral Alterations Induced By Long Term Cerebral Hypoperfusion in Rats

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Abstract: Cognitive deficit which represent many neuropsychiatric conditions and/or alone as developmental factor for the demand use of nootropic agent to enhance cognitive functions. In current scenario, there is tremendous demand of medicinal plants to explore cognition enhancing potential with fewer side effects. 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 *Tinospora cordifolia* have been attributed to its anti-stress, antioxidant, neuroprotective, adaptogeneic and nootropic properties. The present study evaluated possible role of ethanolic extract of *Tinospora cordifolia*, in long term cerebral hypoperfusion (a model of cerebrovascular insufficiency and dementia) induced changes in behavioral alterations. *Tinospora cordifolia* (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 *Tinospora cordifolia* in cerebrovascular insufficiency states and dementia.

Keywords: Cerebral hypoperfusion, Learning and memory, dementia, antioxidant, Nootropic.

INTRODUCTION

In Ayurveda system of medicine, *Tinospora cordifolia* is categorized as ‘Rasayana’, which focused on the well being of person from the point of health promoters and to increase longevity by increasing defensive mechanism of body and revitalization of body especially in debilitated conditions [1]. *Tinospora cordifolia* (TC) or Amrita or Giloya in Hindi and Guduchi in Sanskrit in Ayurveda (the classical Indian system of medicine), has been used for centuries, for a variety of diseases [2]. *Tinospora cordifolia* (Guduchi) is a large glabrous, deciduous, climbing shrub of Menispermaceae family found throughout tropical India [3]. It is classed as *Medhya Rasayana* (Learning and memory enhancer) [4]. Neuroprotective and ameliorative properties are due to their antioxidant and trace element contents [5]. *Tinospora cordifolia* is known to be a rich source of trace elements (Zinc and Copper) which act as antioxidants and protects cells from the damaging effects of oxygen radicals generated during immune activation [6]. *Tinospora cordifolia* has been claimed to possess learning and memory enhancing, [7] antioxidant, [8, 9] and anti-stress activity [10]. *Tinospora cordifolia* enhanced the cognition in normal and cognition deficits animals [11]. Mechanism of cognitive enhancement is by immunostimulation and increasing the synthesis of acetylcholine, this supplementation of choline enhances the cognition [12]. Guduchi may also be attributed to its immunomodulatory properties [13].

Chemical constituents’ classes are alkaloids, diterpenoid lactones, glycosides, steroids, sesquiterpenoid, phenolics, aliphatic compounds, lignans and polysaccharides [14]. The polyphenols have the ability to penetrate the blood brain barrier and act as potential neuroprotective agent. Chronic cerebral hypoperfusion induced by permanent occlusion of bilateral common carotid arteries (BCCA) in rats induces a state of chronic low grade ischemia in rat’s brain over an extended period of several months [15, 16]. Extensive investigations report that rats subjected to permanent occlusion of BCCA show impaired spatial learning/ memory capabilities and/or structural
alterations [17]. 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 [18,19]. Chronic reduction in blood flow and brain energy metabolism causes behavioral and cognitive defects [20, 21]. Therefore, present investigation was designed to assess the possible protective activity of extract of *Tinospora cordifolia* 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 raw material of test formulation was procured from Ayurvedic Garden; Banaras Hindu University campus in the month of January. It was authenticated in Department of Dravya Guna, Faculty of Ayurveda, IMS, BHU. The specimen copy has been kept in department for future references. Dried and powdered stem of *T. Cordifolia* was defatted with petroleum ether to remove lipids and fats, and then the residue was extracted with ethanol using soxhlet apparatus. The ethanolic extract was evaporated under reduced pressure at 40°C, using a rotary vapour evaporator. The extract was kept in 4°C for further analysis and experimental studies. The dose selection for the experimental studies was made according to our initial pilot experimental results.

**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, *T. Cordifolia*100 mg/kg/day p.o. was administered during the experimental period in sham operated animals (*T. Cordifolia* per se). Third group animals underwent permanent BCCAO (hypoperfusion group). In the fourth group, *T. Cordifolia* was administered 60 min before BCCAO. *T. Cordifolia* was then continued up to 15th 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.[22] 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 *T. Cordifolia* all animals were subjected to behavioral testing in open field paradigm and Morris’ water maze.

**ii) Behavioral Testing:**

*T. Cordifolia* was then continued up to 15th 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 [23]. 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) [24]. Water was made opaque by adding Indian ink. On day 15 after surgery, spatial learning and memory was tested in water maze. On 14th day the rats received habituation (exposure
Behavioral despair/ Porsolt’s swim test: The rats were placed individually in cylinder (45 x 20 cm) containing 38 cm water (25 ± 2°C), so that the rats could not touch the bottom of cylinder with his hind limbs or tail or climb to the edge of chamber. Two swim sessions were conducted, an initial 15 min pre test followed by 5 min test, 24 h later. Drug was administered after pre-test. The periods of immobility (duration of floating in water without struggling or making only those movements necessary to keep its head above the water level), during 5 min test period was noted [25].

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. T. Cordifolia pretreatment (100 mg/kg. p.o. x 15 days) prevented these alterations. In sham operated animals T. Cordifolia per se did not have any effect on any of the parameters of open field test (Table 1).

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

<table>
<thead>
<tr>
<th>Groups</th>
<th>Ambulation (number)</th>
<th>Immobility (S)</th>
<th>Rearing (number)</th>
<th>Grooming (number)</th>
<th>Fecal pellets (number)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham-operated control</td>
<td>56.50±3.49</td>
<td>33.16±1.76</td>
<td>24.00±0.62</td>
<td>7.69±0.83</td>
<td>4.25±1.05</td>
</tr>
<tr>
<td>Per se</td>
<td>57.16±4.76</td>
<td>28.50±2.67</td>
<td>23.46±2.01</td>
<td>6.76±0.66</td>
<td>4.36±0.46</td>
</tr>
<tr>
<td>Hypoperfusion</td>
<td>25.00±1.40*</td>
<td>46.96±1.82*</td>
<td>15.80±1.79*</td>
<td>1.79±0.60*</td>
<td>1.76±1.22</td>
</tr>
<tr>
<td>T. cordifolia</td>
<td>60.16±4.23*</td>
<td>34.18±2.47*</td>
<td>24.93±1.46*</td>
<td>6.21±1.28*</td>
<td>2.10±0.92</td>
</tr>
</tbody>
</table>

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. T. cordifolia per se is compared with sham-operated control group. Superscript *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 T. cordifolia 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. T. cordifolia (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 T. cordifolia treatment. The result of new platform trial show that hypoperfused animals found the new platform slower than sham-operated rats. T. cordifolia treated animals found the new visible platform quicker than the hypoperfused animals (Table-2).

Table 2: Effect of T. cordifolia (100 Mg / Kg P.O X 15 Days) On Learning and Memory in Long Term Hypoperfused rats in Morris’ water maze.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Escape latency (sessions)</th>
<th>Probe trial</th>
<th>New platform trial</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>I</td>
<td>II</td>
<td>III</td>
</tr>
<tr>
<td>Sham-operated control</td>
<td>68.21 ±4.28</td>
<td>28.74± 1.52</td>
<td>28.41± 1.56</td>
</tr>
<tr>
<td>Per se</td>
<td>69.53 ± 4.52</td>
<td>29.11± 2.42</td>
<td>29.32± 1.42</td>
</tr>
<tr>
<td>Hypoperfusion</td>
<td>75.26 ±2.90</td>
<td>48.74± 1.55*</td>
<td>40.72± 2.34*</td>
</tr>
<tr>
<td>T. cordifolia</td>
<td>67.21 ±2.89</td>
<td>28.00 ± 1.76a</td>
<td>22.55± 2.63a</td>
</tr>
</tbody>
</table>

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. T. cordifoliaper se is compared with sham-operated control group. Superscript * and b indicate p-value less than 0.01 and 0.05 respectively. Statistical analysis was done by one-way ANOVA followed by Tukey test. Behavioural Despair/ Porsolt’s swim test.

Table 3 shows that compared to sham operated animals, hypoperfused animals stayed for significantly for more time without despair. No significant difference was found among sham operated and Tinospora cordifolia per se group. Tinospora cordifolia treatment significantly reduces this time period in comparison to hypoperfused rats.

Table 3: Effect of T. cordifolia (100 mg / kg p.o. x 15 days) on long term hypoperfused rats in Porsolt’s swim test

<table>
<thead>
<tr>
<th>Group</th>
<th>Immobility time in sec</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham control</td>
<td>24.6 ± 1.20*</td>
</tr>
<tr>
<td>Per se</td>
<td>25.3 ± 1.20</td>
</tr>
<tr>
<td>Hypoperfused</td>
<td>39.2 ± 2.39**</td>
</tr>
<tr>
<td>T. cordifolia</td>
<td>27.1 ± 1.54*</td>
</tr>
</tbody>
</table>

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. T. cordifoliaper se is compared with sham-operated control group. Superscript * and b 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 [15, 26, 27]. 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 [18, 19]. Chronic reduction in cerebral blood flow and brain energy metabolism leads to progressive cognitive deficit [21]. Long term hypoperfusion studies have been subjected to critical appraisal, through behavioral analysis [23, 24, 26]. 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 [23]. The reduction in total activity of long term hypoperfused rats in the open field paradigm with significant reduction in ambulation, rearing and grooming with percent to sham operated animals suggest a propensity towards anxiety and listlessness. Neuroprotective and ameliorative properties are due to their antioxidant and trace element contents [28]. Tinospora cordifolia has been claimed to possess

Available online at http://saspublisher.com/sjams/
learning and memory enhancing, antioxidant, and anti-stress activity [7-10].

*T. cordifolia* 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 [27, 29]. Chronic reduction in blood flow secondary to BCCAO has been reported to cause progressive dysfunction in cognitive deficits [18, 19, 21]. 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. *T. cordifolia* alleviated the changes in long term hypoperfusion induced anxiety and listlessness, along with improvement of learning and memory deficits. Alleviation by *T. cordifolia* of these alterations suggests that *T. cordifolia* improve spatial learning and memory in long term hypoperfused rats. It is not out of place to mention that *T. cordifolia* is known to have nootropic activity. These findings are in agreement with cognitive enhancing and memory improving effects of *T. cordifolia* [7]. *Tinospora cordifolia* has been reported to have antidepressant activities. In our study, we also found the antidepressant activity of *Tinospora cordifolia* in Porsolt’s swim test model.

Several studies have identified and isolated the active principle of *T. cordifolia* which lignans, such as 3(a, 4-dihydroxy-3-methoxybenzyl)-4-(4-hydroxy-3-methoxybenzyl) and alkaloids (jatrorrhizine, palmatine, beberine, tembeterine, choline), has gained attention, as it represents the major constituents responsible for modulation in neurotransmitter levels in the brain of rodents [30, 31]. As, *Tinospora cordifolia* contains sterols (such as Giloinsterol, β-Sitosterol, 20a- Hydroxy ecdysone) which are nominated for their potent immunomodulatory property [32-35]. They have been reported to inhibit the release of pro-inflammatory cytokines [36]. *Tinospora cordifolia* has been reported for its immunomodulatory, antioxidant properties. This explains the observed alternations of behavioural disturbances following long term cerebral hypoperfusion via *Tinospora cordifolia*. Medhya Rasayana, a class of ayurvedic rasayasans is mentioned to have potential effect on cognitive functions [37,38]. *Tinospora cordifolia* was coined one of the Medhyarasanaya which claimed its potential therapeutic effect [39]. The cognition enhancing potential of medhyarasanaya is best seen in children with cognitive deficit or in the condition in which memory is compromised due to head injury, aging and prolonged illness. The present study indicates the substantial role of *T. cordifolia* in long term hypoperfusion.

**CONCLUSION**

The present study therefore, implies role of *T. cordifolia* in long term cerebral hypoperfusion. The present study apart from supporting the earlier observed cognition enhancing property of *T. cordifolia* 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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**REFERENCES**


