**Scholars Journal of Applied Medical Sciences (SJAMS)** **ISSN 2320-6691 (Online)**
**Sch. J. App. Med. Sci., 2017; 5(7B):2630-2636** **ISSN 2347-954X (Print)**

(An International Publisher for Academic and Scientific Resources)
www.saspublishers.com DOI: 10.36347/sjams.2017.v05i07.027

Original Research Article

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

**AnshumanTrigunayat*[1], Shardendu Kumar Mishra[1]**
[1]Neuropsychopharmacology Laboratory, Department of Pharmacology, Institute of Medical Sciences, Banaras Hindu University, Varanasi-221005, Uttar Pradesh, India

***Corresponding author**
Anshuman Trigunayat
Email: anshuman_trigunayat@rediffmail.com

**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 of penetrate the blood brain barrier and act as potential neuroprotective agent. 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 [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$^0$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$^o$C, using a rotary vapour evaporator. The extract was kept in 4$^o$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 15$^{th}$ 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 15$^{th}$ 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$^o$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 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).

**Behavioral despair/ Porsolt's swim test:** The rats were placed individually in cylinder (45 x 20 cm) containing 38 cm water (25 ± 2ºC), so that the rats could not touched 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 locomotors 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.**

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

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. cordifoliaper se* is compared with sham-operated control group. Superscript [a] 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.**

| Groups | Escape latency (sessions) | | | Probe trial | New platform trial |
|---|---|---|---|---|---|
| | I | II | III | | |
| Sham-operated control | 68.21 ±4.28 | 28.74± 1.52 | 28.41± 1.56 | 31.00 ± 1.31 | 17.74± 1.85 |
| *Per se* | 69.53 ± 4.52 | 29.11± 2.42 | 29.32± 1.42 | 32.26 ± 1.84 | 16.43±1.25 |
| Hypoperfusion | 75.26 ±2.90 | 48.74± 1.55[a] | 40.72± 2.34[a] | 23.00 ± 2.27[b] | 27.40 ± 1.56[a] |
| *T. cordifolia* | 67.21 ±2.89 | 28.00 ± 1.76[a] | 22.55± 2.63[a] | 33.56 ± 1.65[a] | 16.83 ± 1.26[a] |

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 [a] 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**

| Group | Immobility time in sec |
|---|---|
| Sham control | 24.6 ± 1.20[a] |
| Per se | 25.3 ± 1.20 |
| Hypoperfused | 39.2 ± 2.39[ab] |
| *T. cordifolia* | 27.1 ± 1.54[b] |

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 [a] 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 mater 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 tents, 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 percept 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 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*in 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, ß-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 rasayanas 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.

## ACKNOWLEDGEMENT

The authors are thankful to Neuropsychopharmacology Laboratory, Department of Pharmacology, Institute of Medical Sciences, Banaras Hindu University for providing the necessary facility for the experimental studies.

## REFERENCES

1. Weiner MA, Weiner J, In: Quantum Books Ed Herb that heal, Mill Valley Publishers: CA; 1994.
2. Czygan, FC, Kulturgeschichte and Mystik des Johanniskrutes. Zeitschrift fur Phytotherapie. 1993; 14:276-82.
3. Sharma PC, Yelne MB, Dennis TJ, Database on Medicinal plants used in Ayurveda and Sidha. Vol 3. New Delhi: CCRAS, Dept. of AYUSH, Ministry of Health and Family Welfare, Govt. Of India. New Delhi; 2005.
4. Acharya YT, Agnivesha. Caraka Samhita with Chakrapani's Ayurveda DeepikaTeeka. Varanasi: ChoukhambaSanskritaSansthana; 1994.
5. Avinash KR, Manohar GM, Saibal KB. *Rubiacordifolia*, *Fagoniacretica* Linn. and*Tinosporacordifolia* exert neuroprotection by modulating the antioxidant system in rat hippocampal slices subjected to oxygen glucose deprivation. BMC Complementary Alternative Medicine. 2004; 4:11.
6. Chulet R, Pradhan P. A review on Rasayana. Pharmacognosy Reviews. 2009; 3:229-234.

7. Agarwal A, Malini S, Bairy KL, Rao MS. Effect of *Tinospora cordifolia* on Learning and Memory in normal and memory deficit rats. Indian Journal of Pharmacology. 2002; 34:339-49.
8. Singh RP, Banergee S, Kumar PV, Raveesha KA, Rao AR.*Tinospora cordifolia* induces enzymes of carcinogen/ drug metabolism and antioxidant System, and inhibits lipid peroxidation in mice. Phytomedicine. 2006; 13:74-84.
9. Stanely M, Prince P, Menon VP. Antioxidant action of *Tinospora cordifolia* root extract in alloxan diabetic rats. Phytotherapy Research. 2001; 15:213-218.
10. Patil M, Patki P, Kamath HV,Patwardhan B. Anti-stress activity of *Tinosporacordifolia* (Wild) Meirs. Indian drugs. 1997; 34:211-215.
11. Reddy YY, Mohana LS, Saravana KA, Review on Effect of Natural Memory Enhancing Drugs On Dementia. International Journal of Phytopharmacology. 2010; 1:1-7.
12. Asuthosh A, Malini S, Bairy KL, Muddanna SR. Effect of *Tinospora cordifolia* on learning and memory in normal and memory deficits rats. Indian Journal of Pharmacology. 2000; 34:339-349.
13. Manjrekar PN, Jolly CI, Narayanan S. Comparative studies of the immunomodulatory activity of *Tinosporacordifolia* and *Tinosporasinensis*. Fitoterapia. 2000; 71:254-257.
14. Singh SS, Pandey SC, Srivastava S, Gupta VS, Patro B, Ghosh AC. Chemistry and Medicinal properties of *Tinosporacordifolia* (*Guduchi*). Indian Journal of Pharmacology. 2003;35:83-91.
15. Ni JM, Ohta H, Matsumato K, Watanabe H. Progressive cognitive impairment following chronic cerebral hypoperfusion induced by permanent occlusion of bilateral carotid arteries in rats. Brain Research.1994; 653:231-236.
16. Davidson CM, Pappas BA, Stevens WD, Fortin T, Bennett SA. Chronic cerebral hypoperfusion: loss of papillary, visual impairment and retinal neurodegenration. Brain Research. 2000; 859:96-103.
17. Sarti C, Pantoni L, Bartolini L, Inzitari D. Cognitive impairment and chronic cerebral hypoperfusion: what can be learned from experimental models. Journal of Neurological Sciences. 2002; 203/204:263 – 266.
18. Tsuchiya M, Sako K, Uura S, Yonemasu Y. Cerebral Blood flow and histopathological changes following permanent bilateral carotid artery ligation in wistar rats. Experimental Brain Research. 1991; 89:87-92.
19. Tsuchiya MK, Yura S, Yonemasu Y. Local cerebral glucose utilization following acute and chronic bilateral carotid artery ligation in Wistar rats: relation to changes in local cerebral blood flow. Experimental Brain Research. 1993; 95: 1-7.
20. Martinez G, Di Giacomo C, Carnazza ML. MAP2, Synaptophysin immunostaining in rat brain and behavioral modifications after cerebral post-ischemic reperfusion. Developmental Neurosciences. 1997; 19:457-464.
21. Beal MF, Hyman BT, Koroshetz W. Do defects in mitochondrial metabolism underlie the pathology of neurodegenerative diseases? Trends Neurosciences. 1993; 16:125-31.
22. Pappas BA, DelaTorree JC, Davidson CM, Keyes MT, Fortin T. Chronic reduction of cerebral blood flow in adult rat: late – emerging CA1 cell loss and memory dysfunction. Brain Research.1996; 70:50-58.
23. Lister RG. Ethologically based animal models of anxiety disorders. Pharmacology Therapeutics. 1990; 46:321-40.
24. Morris R. Development of a water maze procedure for studying spatial learning in rat. Journal of Neurosciences Methods. 1984; 11:47-60.
25. Willer P. The validity of animal model of depression. Psychopharmacology 1984; 83:1-16.
26. Plaschke K, Yunn S, Martin E, Hoyer S, Bardenheuer HJ. Interaction between cerebral energy metabolism and behavior in a rat model of permanent brain vessel occlusion. Brain Research. 1999; 830: 320-329.
27. Tanaka K, Ogawa, A, Asanuma, M, Kondo, Y, Nomura, M. Relation between cholinergic dysfunction and discrimination learning disabilities in wistar rats following chronic cerebral hypoperfusion. Brain Research. 1996;729: 55-65.
28. Avinash KR, Manohar GM.Saibal KB.*Rubiacordifolia*, *Fagoniacretica* Linn. and *Tinosporacordifolia* exert neuroprotection by modulating the antioxidant system in rat

hippocampal slices subjected to oxygen glucose deprivation. BMC Complementary Alternative Medicine. 2004; 4:11.

29. Jaspers RMA, Block F, Heim C, Sontag KH. Spatial learning is affected by transient occlusion of common carotid arteries (2VO): Comparison of behavioral and histopathological changes after '2VO' and 'four-vessel occlusion in rats. Neuroscience Letters. 1990; 117: 149-153.
30. Sarma DNK, Khosa RL, Chaurasia JP, Ray AK. Effect of *Tinospora cordifolia* on brain neurotransmitters in stressed rat.Fitoterapia.1995; 66(5):421.
31. Pathak AK, Agarwal AK, Jain DC, Sharma RP, Howarth OW. NMR studies of 20 hydroxyecdysone, a steroid isolated from *Tinospora cordifolia,* Indian Journal of Chemistry.1995; 34:674.
32. Kidwai AR, Salooja KC, Sharma VN, Siddiqui S. Chemical Examination of *Tinospora cordifolia*, Journal of Scienceand Industrial Research. 1949; 8:115.
33. Khaleque A, Maith MAW, Huq MS, Basar KA.*Tinospora cordifolia* IV. Isolation of heptacosanol, ß sitosterol and three other compoundstinosporine, cordifol and cordifolone, Pakistan Journal of Sciences&Industrial Research. 1970;7:61.
34. Dixit SN, Khosla RL. Chemical Investigations on *Tinospora cordifolia* (Wild.) Miers, Indian Journal of Applied Chemistry. 1971; 34:46 andChemical Abstracts. 1974; 80:248.
35. Pathak AK, Jain DC, Sharma RP. Chemistry and Biological Activities of the Genus *Tinospora*; International Journal ofPharmacognosy.1995;33 (4):277.
36. Bouic JDP, Plant sterols and sterolins: Review of their immune modulating properties. Alternative Medicine Reviews. 1999; 4:170-177.
37. Singh RH.In:Udupa KN, Tripathi SN (Eds.) Amrit, IMS Publisher: BHU, Varanasi;1988.
38. Singh RH, Kohli KR, Pathak SR. Proceeding of First National Seminar on Development of Safer Therapeutics. Biochemistry Cell, IIT New Delhi.1984:58-65.
39. Acharya YT. Caraka Samhita with Chakrapani's Ayurveda Deepika Teeka. Varanasi: Choukhamba Samskrita Samsthana. 1994:385.