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NEUROPROTECTIVE ROLE OF *BENINCASA HISPIDA* L. ON HIPPOCAMPAL CELL MORPHOLOGY IN COLCHICINE INDUCED EXPERIMENTAL RAT MODEL OF ALZHEIMER'S DISEASE

[1]*Chandan Roy and [2]Debjani Guha

[1]Dept. of Physiology, Berhampore Girls' College, University of Kalyani, Berhampore, West Bengal, India.

[2]Nagendranath Das Professor (Retd.), S.N. Pradhan Centre for Neurosciences, University of Calcutta, West Bengal, India.

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***Corresponding Author**
Dr. Chandan Roy
Dept. of Physiology,
Berhampore Girls' College,
University of Kalyani,
Berhampore, West Bengal,
India.

ABSTRACT

The present study was designed to undertaken the role of pulp extract of *Benincasa hispida* L. (BH) on hippocampal cell morphology in colchicine induced experimental rat model of Alzheimer's disease. After intracerebroventricular (ICV) infusion of colchicine in colchicine induced experimental rat model of AD produced massive destruction and disintegration of dentate granule cells at CA3 region of hippocampus when compared with control group. But pretreatment with BH pulp extract did not show any destruction or disintegration of CA3 dentate granule cells of the hippocampus. Similar results were obtained in case of saline treated control animal. It is reported that BH has an antioxidant activity on colchicine induced experimental rat model of AD.

KEYWORDS: *Benincasa hispida*, hippocampal cell morphology, colchicine, Alzheimer's disease, CA3 region, hippocampus.

INTRODUCTION

Alzheimer's disease (AD) is a progressive degenerative disorder, which is associated with excessive loss of memory **(Terry and Davies, 1980; Wisniewski and Iqbal, 1980)**. The brain of patients with Alzheimer's disease trends to be smaller than normal and there is generally evidence of atrophy, especially in the cortex and hippocampus **(Tomlinson, 1980)**. The principal neuropathophysiological features of AD are the formation of neurofibrillary

tangles and neuritic plaques or amyloid plaques, neuronal cell loss and synaptic pathology **(Ball, 1976; Tomlinson et al., 1970; Braak and Braak, 1997)**. Colchicine, as a microtubule-disrupting agent (James and Dennis, 1981), binds with tubulin and disrupts its microtubule polymerization. Moreover, blockade of axonal transport **(McClure, 1972)** and induction of neurofibrillary degeneration (**Wisniewski and Terry, 1967)** also have been observed after colchicine treatment. Recently it has been shown that colchicine is a neurotoxic agent and can destroy certain neural cells selectively **(Goldschmidt and Steward, 1982; Goldschmidt and Steward, 1980)**. Intradentate injection of colchicine destroyed granule cells in the dentate gyrus of the hippocampus **(Goldschmidt and Steward, 1982; Lothman et al., 1982; Jarrard et al., 1984; Walsh et al., 1986; Tilson and Peterson, 1987)** and induced learning impairment in various learning tasks **(Walsh et al., 1986; Tilson et al., 1988; Nanry et al., 1989; Tandon et al., 1991)**, all the previous reports show the integrity of granule cell layer plays an important role for memory function.

According to free radical hypothesis, AD may result from acceleration of the normal ageing in specific brain regions exposed to oxygen radicals. Oxidative stress has been implicated in the development of several neurodegenarative diseases including AD **(Harman, 1993; Volicer, 1990)**. Evidence has accumulated that free radical injury may contribute to the pathogenesis of AD **(Halliwell, 1989; Jenner, 1994)**.

Evidences suggest that the hippocampal infusions of colchicine increased the glutamate (GLU)/Gamma amino butyric acid (GABA) ratio in the cortex of the mice brain **(Yu et al., 1997)** and also the nitric oxide (NO) by increase in the NADPHd - positive neurons in the different areas of hypothalamus of guinea pig. This relative increase in GLU activity and NO **(Coyle and Puttfarcken, 1993)** may cause oxidative stress and brain damage.

Antioxidants such as vitamin C and E may have an important role in protecting cells from radical damage. The major constituents of the fruits of *Benincasa hispida* (BH) are triterpenoids, flavonoids, glycosides, saccharides, beta-carotene, vitamin C, E, beta sitosterin and uronic acid **(Roy et al., 2007)**. **Roy et al., (2007)** reported that BH exerts its antioxidative role through the alterations of Superoxide dismutase (SOD), Catalase (CAT), Reduced glutathione (GSH) level and Lipid peroxidation (LPO) level on colchicine induced experimental rat model of AD.

So, the aim of our study is to elucidate the neuroprotective role *Benincasa hispida* on hippocampal cell morphology and senile plaque formation in colchicine induced experimental rat model of AD.

MATERIALS AND METHODS

Animal used and Maintainance

Male Holtzman strain adult albino rats weighing between 200-250gm were used in the following studies. The rats were kept in standard laboratory conditions (room temperature 27 ± 1^0C, humidity 60% and 12h light/dark cycle) in accordance with **'Institutional Ethical Committee'** rules and regulations. They were allowed free access to standard laboratory diet, which supplemented the necessary proteins, carbohydrates and minerals. Drinking water was supplied ad libitum. Body weight of the rats were recorded every day and maintained in the laboratory throughout the experimental period. Also the animal's health was evaluated by checking the breathing for wheezing or rattling, the presence of mucus around the eyes, the presence of blood in the urine, the condition of fur and rapid and large changes in body weight or food intake. Before the experiment, the rats were allowed to get accustomed to laboratory conditions (for seven days) during which their motor behavior, food and drinking habits, micturation and fecal output were noted for future comparison.

Collection and preparation of water extract from the pulp of BH

The fruit of BH was purchased from the local market and the identity of the plant was authenticated by the Botanical Survey of India, Howrah, West Bengal and kept in S. N. Pradhan Centre for Neurosciences, University of Calcutta, Kolkata. Fruit of BH were cut into pieces, sun dried and ground with the help of an electrical grinder to get a free flowing powder. This powder was subjected to extraction with water (1:3) at room temperature for 48 hours. The extract obtained was filtered through Whatman filter paper and vaccum dried at 40^0 - 50^0 C to get a dry powder, which was dissolved in double distilled water for final use.

Treatment

The control animal was treated with normal saline. The BH pulp extract was given orally through orogastric cannula at the standard dose of 400mg/kg b.w. for fourteen consecutive days (between 10:00 and 11:00 hrs). The dose was standardized in the laboratory.

Grouping of Animal

The animals were divided into four groups.

1. Control animal
2. Colchicine treated experimental rat model of AD.
3. BH treated control animal
4. BH treated colchicine treated experimental rat model of AD.

Preparation of experimental Alzheimer's model by colchicine

Prior to surgery, all the animals were subjected to overnight fasting though drinking water was not withdrawn. The rats were anaesthetized with anesthetic ether (Kobra Drugs Ltd, India). The anaesthetized animals were placed on stereotaxic-instrument (INCO, India Ltd.) equipped with a custom-made ear bar**,** which prevents the damage of the tympanic membrane. Head was fixed in such a position that lambda and bregma sutures were in the same horizontal plane by introducing the incisor bar properly attached to the mouth. For aseptic surgery, absolute alcohol or rectified spirit was applied. The scalp was incisioned in the midline and the pericranial muscles and fascia were retracted laterally. After retracting the nuchal musculature the overlying bone was drilled at the specific loci in the lateral ventricle following the coordinanates of the stereotaxic atlas **(Pellegrino and Cushman, 1967)** (Coordinates for the lateral ventricles were: 0.6 mm posterior to bregma, 1.8 mm lateral to the midline and 2.7 mm below the cortical surface). After two-trephine hole was burred in the skull, the subjects were infused through a 10 µl Hamilton syringe with 15 µgm of colchicine in 5 µl of artificial CSF (ACSF; in mM: 147 Nacl, 2.9 Kcl, 1.6 $Mgcl_2$, 2.2 Dextrose and 1.7 $Cacl_2$) in the lateral cerebral ventricles bilaterally. A total volume of 10 µl was delivered to the injection site and the injection cannula was left in place for 2-3 min following infusion. After injecting colchicine the trephine hole was covered with gel foam and sterile bone wax and skin and muscle were sutured back separately. Neosporin powder was sprayed over the wound site as antiseptic measure. Also, Penicillin or PCN (10,000 IU) were injected on the day of the operation and for the next two consecutive days. 2-3 ml of freshly prepare dextrose solution was intraperitoneally (i.p) injected to maintain blood volume. Dilute food was supplied on the day of operation.

Histology

After termination of the experiment, colchicine induced experimental Alzheimer animals were sacrificed with a lethal dose of sodium pentobarbitone. The brain was perfused through the heart with formal- saline mixture. The brain was fixed in 10% formol and was then processed for paraffin section, which was cut at 10 µ thickness. Paraffin sections are stained

with Haematoxylene eosine staining for cellular morphology, Cresyl fast violet for granular cell degeneration and disintegration in CA3 region of dentate gyrus of the hippocampus.

RESULTS

Result of Haematoxylene eosine staining

After intracerebroventricular (ICV) infusion of colchicine in colchicine induced experimental rat model of AD produced massive destruction and disintegration of dentate granule cells at CA3 region of hippocampus when compared with control group. But pretreatment with BH pulp extract did not show any destruction or disintegration of CA3 dentate granule cells of the hippocampus. Similar results were obtained in case of saline treated control animal. The result is shown in Fig-1.

Result of Cresyl fast violet staining

After intracerebroventricular (ICV) infusion of colchicine in colchicine induced experimental rat model of AD produced massive destruction and disintegration of dentate granule cells at CA3 region of hippocampus when compared with control group. The thickness of the CA3 granule cell layers was also decreased in colchicine treated experimental AD model when compared with saline treated control, BH treated control and BH treated colchicine treated experimental AD group. Pretreatment with BH pulp extract did not show any destruction or disintegration of CA3 dentate granule cells of the hippocampus. Similar results were obtained in case of saline treated control animal. The result is shown in Fig-2.

Result of Hagamethanamine silver staining method

In our present study, intracerebroventricular (ICV) infusion of colchicine (bilaterally) in colchicine treated experimental AD model there was an extensive formation of senile plaques in discrete brain regions e.g. temporal lobe and hippocampus compared to that of control group. Pretreatment with BH in BH treated colchicine treated experimental AD rat model, BH prevented this senile plaque formation when this group was compared with colchicine treated experimental rat model of AD. In only BH treated control group, there was no senile plaque. Similar results were obtained in case of saline treated control animal. This result is shown in Fig-3 & 4.

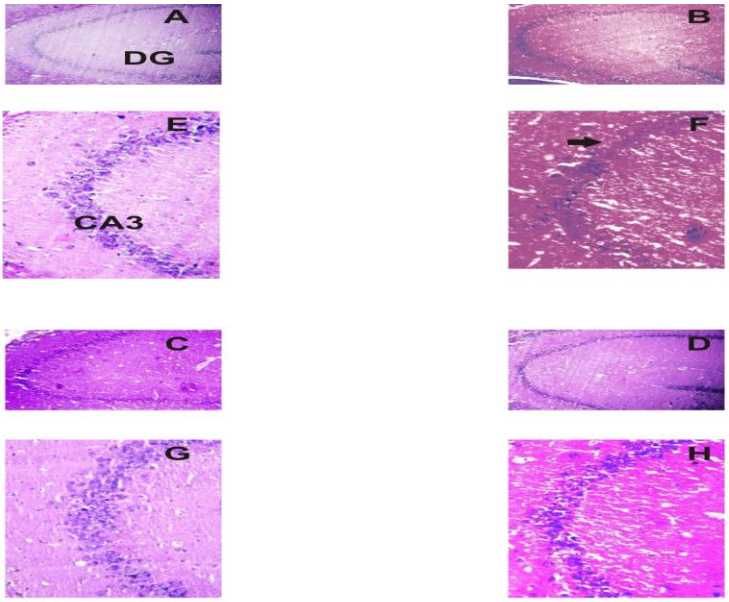

Fig - 1 : Representative photomicrographs of hippocampus. Normal Haematoxylene-eosine staining of the cell morphology of the hippocampal formation. A, B, C & D illustrate the dentate gyrus (DG) of hippocampus. E, F, G & H illustrate the CA3 region of hippocampus. A, E are from a control animal ; B, F are from colchicine treated animal ; C,G are from BH treated control animal; D, H are from BH pretreated colchicine treated animal. Cellular disintegration obtained after colchicine infusion which is indicated by an arrow in F. A, E, C, G, D & H did not show any cellular disintegration or destruction. A, B, C & D indicates low magnification (5 × 10X=50X) and E, F, G & H indicates high magnification (15 × 10X = 150X).

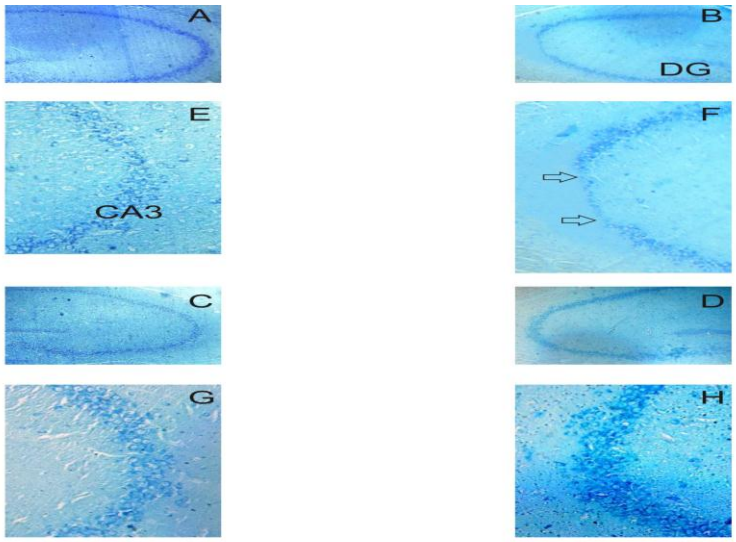

Fig - 2 : Representative photomicrographs of hippocampus. Cresyl fast violet staining of CA3 granular cells in the dentate gyrus of the hippocampal formation from Holtzman strain adult male albino rats. All photos are of coronal sections. A, B, C & D illustrate the dentate gyrus (DG) of hippocampus. E, F, G & H illustrate the CA3 region of hippocampus. A, E are from a control animal; B, F are from colchicine treated animal; C, G are from BH treated control animal; D, H are from BH pretreated colchicine treated animal. Degeneration of granule cells in CA3 region of hippocampus is evident after colchicine administration which is indicated by an open arrow in F. Cellular disintegration obtained after colchicine infusion (indicated by an open arrow in F). A, E, C, G, D & H did not show any cellular destruction or any disintegration of dentate granule cells. A, B, C & D illustrate low magnification (5× 10X=50X) and E, F, G & H illustrate high magnification (15 × 10X = 150X).

DISCUSSION

The bilateral injection of colchicine into the hippocampus produced a variety of histological and behavioral alterations. In Alzheimer's disease, a loss of neurons has been reported in the hippocampus and cortical areas **(Tomlinson, 1980)**. It has been previously reported that intradentate injection of colchicine destroyed granule cells in the dentate gyrus of the hippocampus **(Goldschmidt and Steward, 1982; Lothman et al., 1982; Jarrard et al., 1984; Walsh et al., 1986; Tilson and Peterson, 1987)** and induced learning impairment in various learning tasks **(Walsh et al., 1986; Tilson et al., 1988; Nanry et al., 1989; Tandon et al., 1991)**. All these previous reports show the integrity of granule cell layer plays an important role for memory function. In our present study, intracerebroventricular (ICV) infusion of colchicine (bilaterally) produced a massive destruction of dentate granule cells at CA3 region of the hippocampus - these was obtained from histological study by using Haematoxylene eosine staining and Cresyl fast violet staining method. But *Benincasa hispida*, containing vitamin A, C, E, flavonols and flavonoids, protects dentate gyrus from destruction of dentate granule cells. **Roy et al., (2007)** reported that BH has an antioxidant activity on colchicine induced experimental rat model of AD. It is hypothesized that beta amyloid at normal physiological levels in normal media acts as an antioxidant with the ability to chelate copper and prevent/reduce oxidative damage. However as levels of beta amyloid increase in the case of AD, beta amyloid may transition from antioxidant properties to pro-oxidant behavior**.** Free radicals promote beta amyloid aggregation and plaque deposition **(Friedlich and Butcher, 1994)**. This protein becomes toxic by producing superoxide radicals when interacting with blood vessels **(Thomas et al., 1996)**.

Colchicine, as a microtubule-disrupting agent **(James and Dennis, 1981)** produces marked destruction of hippocampal granule cells, mossy fibers and septohippocampal pathways (SHC, a cholinergic link between medial septum and vertical limb of diagonal band). It induces neurofibrillary degeneration by binding to tubulin, the principal structural protein of microtubule **(McClure, 1972; Wilson and Fried-Kin, 1966; Walsh et al., 1986)**. This event is associated with loss of cholinergic neurons and decrease in acetylcholine transferase, thereby resulting in impairment of learning and memory **(Kevin et al., 1989; Dwaine and Thomas, 1990)**. Evidences suggest that the hippocampal infusions of colchicine increased the glutamate (GLU)/Gamma amino butyric acid (GABA) ratio in the cortex of the mice brain **(Yu et al., 1997)** and also the nitric oxide (NO) by increase in the NADPHd - positive neurons in the different areas of hypothalamus of guinea pig. This relative increase in GLU

activity and NO **(Coyle and Puttfarcken, 1993)** may cause oxidative stress and brain damage. So, from our present study, ICV infusion of colchicine produced an excessive amount of free radicals along with a loss of cholinergic cells. Formation of ROS possibly altered the endogenous antioxidant status leading to vigorious oxidative stress resulting in the massive destruction and disintegration of granule cell layers at CA3 region of the dentate gyrus of hippocampus. But in BH pretreated colchicine treated experimental rat model of AD, BH protected the dentate gyrus from the massive destruction of dentate granule cells possibly by modulating the oxidative stress. So, it prevented the actions of colchicine possibly through its antioxidant scavenging action. Because, BH containing vitamin A, C, E, flavonols and flavonoids has an antioxidative role on colchicine induced experimental rat model of AD **(Roy et al., 2008)**. Both vitamin C and beta carotene were found to protect rat neurons against oxidative stress. Vitamin C and E may have an important role in protecting cells from radical damage. Studies **(Jeandal et al., 1989; Zaman et al., 1992)** have found that low plasma vitamin E levels were present in AD compared to controls, whereas supplementation with vitamin E was intimately related with slower development of the pathology **(Sano et al., 1997)**. A low plasma concentration of vitamin C in Alzheimer patients has also been reported **(Jeandel et al., 1989)**.

So, from our present study it may be concluded that BH protects rat neurons from colchicine induced oxidative stress by preventing the destruction of dentate granule cells at CA3 region of the dentate gyrus of hippocampus in colchicine induced experimental rat model of AD. All these effects exerted by its antioxidant scavenging action.

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