

## ROLE OF MORINGA OLEIFERA ON HIPPOCAMPAL CELL MORPHOLOGY AND SENILE PLAQUE FORMATION IN COLCHICINE INDUCED EXPERIMENTAL RAT MODEL OF ALZHEIMER'S DISEASE

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Received: 15 March 2014, Revised and Accepted: 29 March 2014

### ABSTRACT

The present study was designed to undertake the role of *Moringa oleifera* (MO) on hippocampal cell morphology and senile plaque formation in colchicine induced experimental rat model of Alzheimer's disease (AD). Paraffin sections are stained with Haematoxyline eosine staining for cellular morphology, Cresyl fast violet for granular cell degeneration and disintegration at CA3 region of dentate gyrus of the hippocampus and Hagemethanamine silver method for presence of senile plaques in the hippocampus. MO leaf extract at a dose of 250 mg/kg body weight, did not show any destruction or disintegration of CA3 dentate granule cells of the hippocampus. MO at a dose of 250 mg/kg body weight prevented the senile plaque formation in discrete brain regions e.g. temporal lobe and hippocampus. MO may provide neuroprotection against colchicine induced oxidative stress possibly by preventing the destruction of dentate granule cells at CA3 region of the dentate gyrus of hippocampus and extracellular deposition of senile plaques and all these effects exerted by its free radical scavenging action.

**Keywords:** *Moringa oleifera*, Colchicine, Alzheimer's disease, Haematoxyline eosine, Cresyl fast violet, Hagemethanamine silver, Hippocampus.

### INTRODUCTION

Alzheimer's disease (AD) is a neurodegenerative disease characterized clinically by both severe memory loss and personality changes, and pathologically by both synaptic loss and neuronal death in the vulnerable areas associated with the formation of neurofibrillary tangles (NFTs) and senile plaques (SPs), both pathological hallmarks of AD. Colchicine has been used in vivo to model several aspects of neuropathology in Alzheimer's disease. Administration of colchicine directly to the dentate gyrus or intraventricularly results in the selective death of neurons in the hippocampal formation [1-2], the loss of cholinergic neurons in the basal forebrain, and cognitive impairment [3].

Different sources of oxidative stress in Alzheimer's disease suggest several pharmacological approaches to influence disease progression. Two major types of therapeutic agent can be described according to their pharmacological point of attack.

(a) Radical scavengers – agents directly interacting with free radicals. These include ginkgo biloba, vitamin A, C and E, and oestrogen.

(b) Antioxidants, which are able to prevent or decrease the production of free radicals. These include selegiline and tenilsetam.

In the latter study, vitamin E and selegiline demonstrated marginal superiority to placebo in slowing functional deterioration in patients with moderately advanced Alzheimer's disease. The beta amyloid protein accumulation is promoted by oxygen radical species and beta amyloid aggregates in the senile plaque have also been related with the production of oxygen free radicals [4]. Both vitamin E and  $\beta$  carotene were found to protect rat neurons against oxidative stress. MO is rich in vitamin A, C, E, essential amino acids, flavonols, flavonoids etc.

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

### MATERIALS AND METHODS

#### Subjects

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  $27\pm 1^\circ\text{C}$ , 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, micturition and fecal output were noted for future comparison.

#### Preparation of water extract from leaves of MO

Fresh, Young, healthy leaves of MO were shaded, dried and grinded with the help of an electrical grinder to get a free flowing powder. This powder was subjected to extraction with water at room temperature for 24 hours. The extract obtained was filtered through whatman filter paper and vacuum dried at  $40^\circ\text{--}50^\circ\text{C}$  to get a dry powder, which was dissolved in double distilled water for final use [5].

#### Treatment

The control animal was treated with normal saline. The MO leaf extract was given orally through orogastric cannula at the standard dose of 250mg/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. MO treated control animal
4. MO 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 incised 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 coordinates of the stereotaxic atlas<sup>6</sup> (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  $\mu$ l Hamilton syringe with 15  $\mu$ g of colchicine in 5  $\mu$ l of artificial CSF (ACSF; in mM: 147 NaCl, 2.9 KCl, 1.6 MgCl<sub>2</sub>, 2.2 Dextrose and 1.7 CaCl<sub>2</sub>) in the lateral cerebral ventricles bilaterally. A total volume of 10  $\mu$ l 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 prepared 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% formalin and was then processed for paraffin section, which was cut at 10  $\mu$  thickness. Paraffin sections are stained with Haematoxyline eosine staining for cellular morphology, Cresyl fast violet for granular cell degeneration and disintegration at CA3 region of dentate gyrus of the hippocampus and Hagemethanamine silver method for presence of senile plaques in the hippocampus.

### RESULTS

#### Result of Haematoxyline 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 MO leaf 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, MO treated control and MO treated colchicine treated experimental AD group. Pretreatment with MO leaf 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.

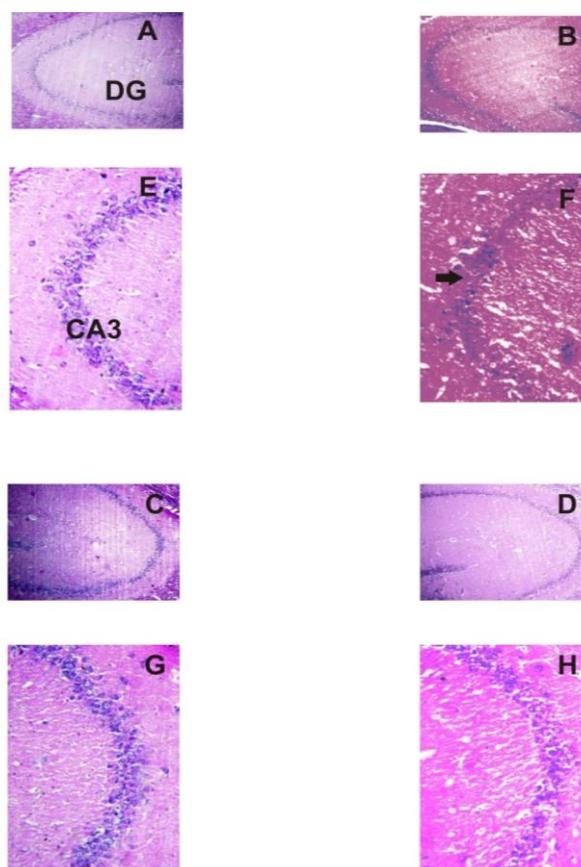
#### Result of Hagemethanamine silver staining method:

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 MO in MO treated colchicine treated experimental AD rat model, MO prevented this senile plaque formation when this group was compared with colchicine treated experimental rat model of AD. In only MO 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.

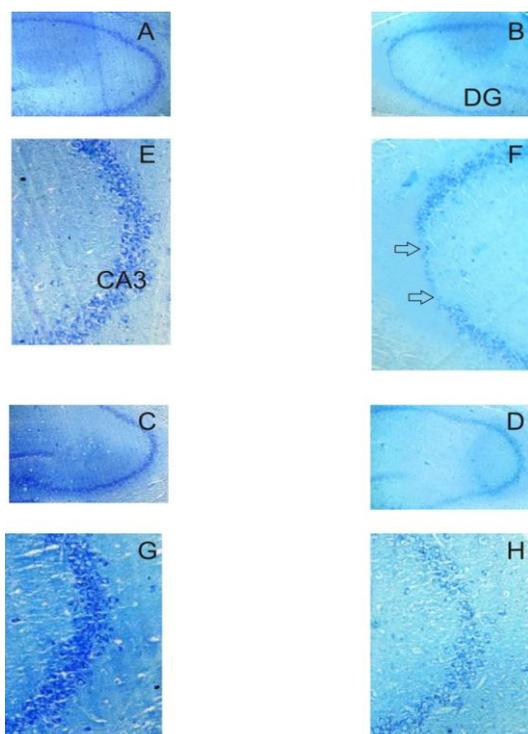
### DISCUSSION

The bilateral injection of colchicine into the hippocampus produced a variety of histological and behavioral alterations. In

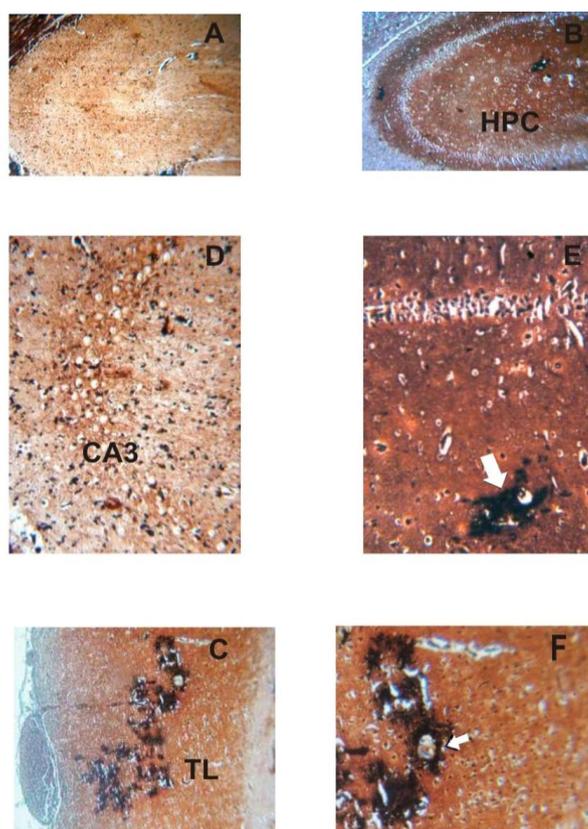
Alzheimer's disease, a loss of neurons has been reported in the hippocampus and cortical areas<sup>7</sup>. It has been previously reported that intradentate injection of colchicine destroyed granule cells in the dentate gyrus of the hippocampus<sup>2,8</sup> [11] and induced learning impairment in various learning tasks [10], [12]-[14]. 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 massive destruction and disintegration of dentate granule cells at CA3 region of the hippocampus and the extracellular deposition of senile plaques- all these were obtained from histological study by using Haematoxyline eosine staining, Cresyl fast violet staining and Hagemethanamine silver staining method. But *Moringa oleifera* (MO) containing vitamin A, C, E, flavonols and flavonoids protects dentate gyrus from both of massive destruction and disintegration of dentate granule cells. Senile plaques are the extracellular deposition of beta amyloid protein. 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 [15].



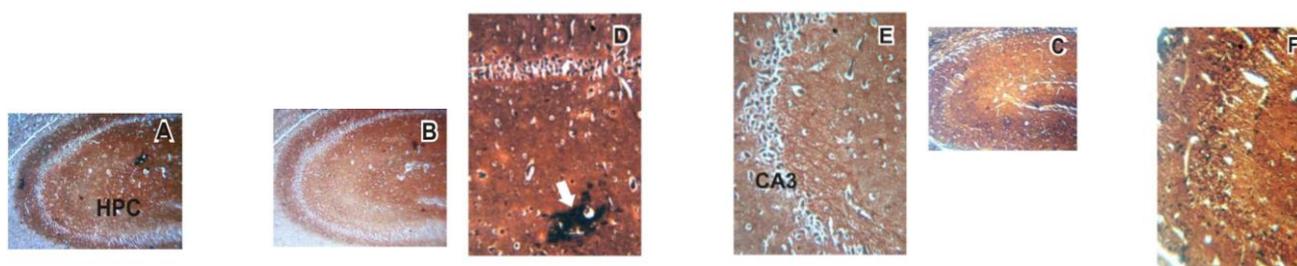
**Fig. 1: Representative photomicrographs of hippocampus.** Normal Haematoxyline-eosine staining of the cell morphology of the hippocampal formation. A, B, C & D illustrate the dentate gyrus (DG) of hippocampus. E, F, G & H illustrate the CA3 region of hippocampus. A, E are from a control animal; B,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



**Fig. 3:** Hagemethanamine silver staining for Senile plaques from Holtzman strain adult male albino rats. All photos are of coronal sections. A, D are from a control animal; B, E, C & F are from colchicine treated animal. B & E illustrate the senile plaque formation in the hippocampal region after colchicine administration (indicated by an open arrow in E). C & F illustrate the senile plaque formation in the temporal lobe after ICV infusion of colchicines (indicated by an open arrow in F). A, B & C indicates low magnification ( $5 \times 10X = 50X$ ) and D, E & F indicates high magnification ( $15 \times 10X = 150X$ ). HPC- hippocampus; TL- temporal lobe.



**Fig. 4:** Hagemethanamine silver staining for Senile plaques from Holtzman strain adult male albino rats. All photos are of coronal sections. A, D are from a colchicine treated animal; B, E, are from a MO treated control animal. C & F are from MO pretreated colchicines treated animal. A & D illustrate the senile plaque formation in the hippocampal region after colchicine administration (indicated by an arrow in D). B, E, C & F did not show any senile plaque formation in the hippocampal region. A, B & C indicates low magnification ( $5 \times 10X = 50X$ ) and D, E & F indicates high magnification ( $15 \times 10X = 150X$ ). HPC- hippocampus.

Colchicine, as a microtubule-disrupting agent [16] 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 [17]-[18]. [10]. This event is associated with loss of cholinergic neurons and decrease in acetylcholine transferase, thereby resulting in impairment of

learning and memory [19]-[20]. The hippocampal infusions of colchicine increased the glutamate (GLU)/Gamma amino butyric acid (GABA) ratio in the cortex of the mice brain [21] 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 [22] may cause oxidative stress and brain damage. From our present study, ICV infusion of colchicines produced the excessive amount of free radicals along with a massive

amount of beta amyloid. The accumulation of beta amyloid protein is promoted by oxygen radical species, and beta amyloid aggregates in the senile plaque have also been associated with the production of oxygen free radicals [4]. This free radical forms reactive oxygen species and beta amyloid aggregates to form the extracellular deposition of senile plaques, which was evident from our present study. Formation of ROS possibly changed the endogenous antioxidant status resulting in the generation of vigorous oxidative stress which in turn possibly produced both of the destruction and disintegration of granule cell layers at CA3 region of the dentate gyrus of hippocampus. But in MO pretreated colchicine treated experimental rat model of AD, MO provides neuroprotection to the dentate gyrus from both of the massive destruction and disintegration of dentate granule cells and thereby prevented the senile plaque formation in the hippocampal region possibly by decreasing the oxidative stress. So, MO containing vitamin A, C, E, flavonols and flavonoids may prevent the actions of colchicine possibly through its antioxidant scavenging action. Vitamin C and E may have an important role in protecting cells from radical damage.

So, from our present study the conclusion reached is that MO may provide neuroprotection against colchicine induced oxidative stress possibly by preventing the destruction of dentate granule cells at CA3 region of the dentate gyrus of hippocampus and extracellular deposition of senile plaques and all these effects exerted by its free radical scavenging action.

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