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ROLE OF *BENINCASA HISPIDA LINN.* ON BRAIN ELECTRICAL ACTIVITY IN COLCHICINE INDUCED EXPERIMENTAL RAT MODEL OF ALZHEIMER'S DISEASE: POSSIBLE INVOLVEMENTS OF ANTIOXIDANTS.

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ABSTRACT

The present study was designed to undertaken the role of *Benincasa hispida* (BH) on brain electrical activity in colchicine induced experimental rat model of Alzheimer's disease (AD) with possible involvement of antioxidants. The antioxidant enzyme activities such as, superoxide dismutase (SOD), catalase (CAT), reduced glutathione (GSH) and lipid peroxidation (LPO) level were studied in different parts of the brain such as Cerebral cortex (CC), Cerebellum (CB), Caudate nucleus (CN), Pons and Medulla (PM) and Midbrain (MB) in BH treated colchicine induced experimental Alzheimer's rat model. Electroencephalographic recordings were also examined throughout this study. BH significantly increased the superoxide dismutase (SOD), catalase (CAT) and reduced glutathione (GSH) level in CC, CB, CN, MB and PM and significantly decreased the lipid peroxidation (LPO) level. After intracerebroventricular (ICV) infusion of colchicine, it significantly decreased the occurrence of alpha wave activity and significantly increased the occurrence of spike wave discharges in colchicine induced experimental rat model of AD as compared to that of control and BH treated colchicine treated experimental rat model of AD. BH protects rat neurons against oxidative stress as is evidenced from our results of LPO, CAT, SOD, GSH and EEG activities possibly by vitamin E, C and beta carotene which are present in BH pulp extract.

KEYWORDS: *Benincasa hispida*, colchicine, Alzheimer's disease, superoxide dismutase, catalase, lipid peroxidation.

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)**. AD is clinically characterized by a progressive decline in cognitive function and neuropathologically by the presence of neuritic plaques and neurofibrillary tangles. According to the free radical hypothesis, AD may result from acceleration of the normal ageing process 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).** In AD there is increased sensitivity to free radicals in the cerebral cortex **(Richardson, 1993)**, perhaps related to lower activity of antioxidant enzymes such as superoxide dismutase **(Thome et al., 1997)**. Antioxidants such as vitamin C and E may have an important role in protecting cells from radical damage. Studies **(Jeandel 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 associated with slower development of the pathology **(Sano et al., 1997)**.

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). In 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)**.

In AD patients, delta and theta relative power values were increased and alpha relative power was decreased compared with normal controls. The AD patients' relatives had normal resting EEG parameters. Under hyperventilation in the AD relatives, synchronous high-voltage delta and theta activity and sharp waves were revealed, theta and delta relative powers were increased and alpha relative power was decreased compared with the resting EEG in the same persons. It has been suggested that the excitotoxic processes and oxidative stress underlie the EEG alterations in the AD patients' relatives and that these alterations may be related to the

further AD development. A maximum number of spike wave discharges are seen in an AD patient. It has been observed that resting delta and alpha electroencephalographic (EEG) rhythms are abnormal in patients with AD.

The fruit of *Benincasa hispida* (Thunb.) Cogn., commonly called as ash guard, belonging to cucurbitaceous is employed as a main ingredient in kusmanda lehyam, in Ayurvedic system of medicine. The lehyam is used as rejuvenate agent and also numerous nervous disorders. The major constituents of these fruits are triterpenoids, flavonoids, glycosides, saccharides, β carotene, vitamin C, E, β sitosterin and uronic acid. The fruits and seeds of BH possess a number of pharmacological properties and uses: Anthelmintic **(Ansari, 1981)**, laxative, tonic, diuretic, aphrodisiac, antiperiodic, inhaemoptysis, other internal haemorrhages, in insanity, epilepsy and other nervous disorders **(Chopra, 1956)**. It has been observed that BH has significant antiulcer activity, nootropic **(Kumar and Nirmala, 2003)** and antidepressant activity **(Rukumani et al., 2003)**. 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 pathological hallmarks of AD are extracellular neuritic plaques (NPs), intracellular neurofibrillary tangles (NFTs), neuronal cell loss and synaptic pathology **(Braak and Braak, 1991)**.

A major approach to the treatment of AD has involved attempts to augment the cholinergic function of the brain **(Johnston, 1992)**. Four inhibitors of acetyl cholinesterase (AChE), *tacrine* (1,2,3,4-tetrahydro-9-aminoacridine; COGNEX), *donepezil* (ARICEPT) **(Mayeux and Sano, 1999)**, *rivastigmine* (EXCELON) and *galantamine* (REMINYL) were used in the management of AD. The side effects of *tacrine* often are significant and dose limiting; abdominal cramping, anorexia, nausea, vomiting and diarrhea are observed in up to one-third of patients receiving therapeutic doses and elevations of serum transaminases are observed in up to 50% of those treated. The side effects of *donepezil*, *rivastigmine* and *galantamine* are almost similar to that of tacrine such as nausea, diarrhea, vomiting and insomnia. These side effects have prompted the scientific world for the search of alternative herbal remedies of AD. The BH plant in our country is easily available and cheap. This herbal plant has no side effects. The wide use in tribal practice makes this to be potential medicinal plants to improve mental health.

Based on this report, the present study was designed to undertaken the role of BH pulp extract on the brain electrical activity in colchicine induced experimental rat model of AD with the possible involvements of antioxidants.

MATERIALS AND METHODS

Animal used and Maintainance

Twenty-four male Holtzman strain adult albino rats weighing between 200-250gm were selected throughout the experiment. The rats were kept in standard laboratory conditions (room temperature 27 ± 1^{0}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, micturation and fecal output were noted for future comparison. The behavioral procedure was carried out between 12:00 and 14:00 h.

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 and West Bengal and kept in S. N. Pradhan Centre for Neurosciences, University of Calcutta and 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 $40^{O} - 50^{O}$ C to get a dry powder, which was dissolved in double distilled water for final use **(Roy et al., 2007)**.

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.

After fourteen days, the animals were sacrificed by cervical dislocation and the different parts of the brain like Cerebral cortex (CC), Cerebellum (CB), Caudate nucleus (CN), Pons and Medulla (PM) and Midbrain (MB) were isolated for antioxidant estimation.

Grouping of Animal

The animals were divided into four groups.

1. Control rats
2. Colchicine induced Alzheimer's rat model
3. Control rats treated with BH Pulp extract
4. Colchicine induced Alzheimer's rat model treated with BH Pulp extract.

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.

Postoperative care

After surgery, all aseptic measures and care were taken for feeding until recovery from surgical stress. Penicillin or PCN (10,000 IU) was given post operatively to all animals for 3 consecutive days by intramuscular (i.m) route. After 3 days of surgery, experiment was started and continued routinely until sacrificed. Similar procedure was repeated thrice, each at an interval of two days.

Electroencephalographic (EEG) study

For Electroencephalography or EEG recordings, rats were anesthetized with Pentobarbitone sodium (40 mg/kg i.p). Each rat was placed in a stereotaxic apparatus. Bipolar electrodes were implanted on the surface of the sensorimotor cortex through trephine hole. A reference electrode was also implanted over the frontal bone. EEG recordings from conscious rats were recorded from day 3-5 after ICV injection of colchicine using an 8 channel Electroencephalogram. Recording session lasted for 5 hours without interruption. Animal's behavior was critically checked for movement artifacts in the recordings (**Ray et al., 2004; Roy and Das, 2013**).

Biochemical Estimation

Tissue preparation

Rats were sacrificed by cervical dislocation on day fourteen immediately after behavior study. The Cerebral cortex (CC), Cerebellum (CB), Caudate nucleus (CN), Pons and Medulla (PM) and Midbrain (MB) were dissected out. The tissues were weighed and homogenized in ice-cold phosphate buffer and prepared for biochemical estimation.

Estimation of SOD, CAT, GSH and LPO level

Superoxide dismutase (SOD) was estimated by the method of **Mishra and Fridovich (1972); Roy et al., (2008),** Catalase (CAT) activity was estimated by the method of **Cohen et al. (1970); Das and Roy (2012),** Reduced glutathione (GSH) level was measured according to the method of **Ellman (1959); Das and Roy (2011)** and Lipid peroxidation (LPO) was estimated by the method of **Bhattacharya et al. (2001); Roy and Mazumdar (2014).** Detailed procedures of the estimation of SOD, CAT, GSH and LPO level described in methodology section.

Statistical analysis

The data were expressed as MEAN ± S.E.M. and were analyzed statistically using one way analysis of variance (one way ANOVA) followed by multiple comparison *'t'* test. In addition to this, two-tailed Student *'t'* test was performed to determine the level of significance between the means. Difference below the probability level 0.05 was considered statistically significant.

RESULTS

Fourteen days after Intracerebroventricular (ICV) infusion of colchicine, the SOD, CAT, reduced glutathione levels and lipid peroxidation were estimated and EEG activities were done. The normal EEG wave pattern showed predominance of low voltage fast waves or β waves in normal saline treated control rats. Pretreatment with BH pulp extract at a dose of 400 mg/kg body weight, it significantly increased the occurance of α wave activity which persisted for more than five hours. After intracerebroventricular (ICV) infusion of colchicine, there was an increase in the occurance of the spike wave discharges. In BH pretreated colchicine treated experimental rat model of AD, there was a dramatically decline in occurance of the spike wave discharges along with an increase in the occurance of α wave activity in comparison with colchicine treated experimental rat model of AD. The result is shown in Plate - 1.

There was a sharp decline ($p<0.001$) in SOD activity in CC, CB, CN, MB and PM in the colchicine treated experimental AD group as compared to the control group. The SOD activity was significantly ($p<0.001$) increased in BH treated control group rather than control group in CC, CB, CN, MB and PM. BH significantly ($p<0.001$) increased SOD activity in BH treated colchicine treated group in comparison with colchicine treated experimental AD group in all mentioned parts of the brain. The result is shown in Table-1.

There was a significant rise ($p<0.001$) in lipid peroxidation levels in the colchicine treated experimental AD group as compared to the control group in CC, CB, CN, MB and PM. The LPO level was significantly ($p<0.001$) decreased in CC, CB, CN, MB and PM in BH treated control group rather than control group. BH significantly ($p<0.001$) decreased LPO levels in BH treated colchicine treated group rather than colchicine treated experimental AD group in all mentioned parts of the brain. The result is shown in Table-2.

There was a sharp decrease ($p<0.001$) in CAT activity in CC, CB, CN, MB and PM in the colchicine treated experimental AD group as compared to the control group. The CAT activity was significantly ($p<0.001$) increased in BH treated control group rather than control group in CC, CB, CN, MB and PM. BH significantly ($p<0.001$) increased CAT activity in BH treated colchicine treated group rather than colchicine treated experimental AD group in CC, CB, CN, MB and PM. The result is shown in Table-3.

There was a sharp decrease ($p<0.001$) in GSH level in the colchicine treated experimental AD group as compared to the control group in CC, CB, CN, MB and PM. The GSH level was significantly ($p<0.001$) increased in BH treated control group rather than control group in CC, CB, CN, MB and PM. BH significantly ($p<0.001$) increased GSH level in BH treated colchicine treated group rather than colchicine treated experimental AD group in different brain regions. The result is shown in Table-4.

Table – 1: Changes in SOD activity in different brain areas (CC, CB, CN, MB and PM) of BH treated colchicine (15µg/ 5 µl of ACSF) induced Alzheimer rat model.

	SOD (% inhibition unit)				
	CC	**CB**	**CN**	**MB**	**PM**
Control	13.61±0.08	11.42±0.06	11.42±0.02	10.69±0.14	12.39±0.02
Colchicine	21.12±0.03***	20.48±0.08***	19.84±0.03***	19.50±0.02***	20.35±0.03***
BH	10.18±0.06***	9.79±0.06***	9.42±0.02***	8.80±0.02***	9.89±0.02***
BH+Colchicine	16.66±0.03$^{\#}$	15.33±0.03$^{\#}$	15.28±0.07$^{\#}$	14.51±0.03$^{\#}$	15.61±0.03$^{\#}$

Values are mean ± SEM, n = 6; $^{***}p < 0.001$ when compared with control group. $^{\#}p < 0.001$ when compared with colchicine treated group. Data were analyzed statistically using one-way ANOVA Test followed by multiple comparison t – test.

Table – 2: Changes in LPO level in different brain areas (CC, CB, CN, MB and PM) of BH treated colchicine (15µg/ 5 µl of ACSF) induced Alzheimer rat model.

	LPO (nmol of TBARS / gm mol of tissue)				
	CC	**CB**	**CN**	**MB**	**PM**
Control	5.01±0.02	5.09±0.01	4.18±0.04	4.23±0.02	4.18±0.04
Colchicine	9.11±0.02***	9.04±0.03***	8.32±0.03***	9.15±0.03***	9.15±0.04***
BH	2.09±0.02***	3.16±0.03***	2.37±0.05***	2.09±0.02***	2.19±0.03***
BH+Colchicine	6.11±0.03$^{\#}$	6.65±0.02$^{\#}$	5.56±0.02$^{\#}$	6.68±0.02$^{\#}$	5.61±0.02$^{\#}$

Values are mean ± SEM, n = 6; $^{***}p < 0.001$ when compared with control group. $^{\#}p < 0.001$ when compared with colchicine treated group. Data were analyzed statistically using one-way ANOVA Test followed by multiple comparison t – test.

Table – 3: Changes in CAT activity in different brain areas (CC, CB, CN, MB and PM) of BH treated colchicine (15µg/ 5 µl of ACSF) induced Alzheimer rat model.

	CAT (% inhibition unit)				
	CC	**CB**	**CN**	**MB**	**PM**
Control	14.12±0.03	12.37±0.01	12.67±0.02	13.37±0.03	12.31±0.03
Colchicine	20.27±0.02***	20.37±0.02***	20.35±0.04***	20.26±0.02***	20.42±0.04***
BH	10.34±0.02***	9.86±0.02***	9.90±0.02***	9.98±0.02***	9.07±0.04***
BH+Colchicine	15.75±0.03#	15.35±0.03#	15.50±0.02#	16.12±0.03#	16.75±0.03#

Values are mean ± SEM, n = 6; *** $p < 0.001$ when compared with control group. # $p < 0.001$ when compared with colchicine treated group. Data were analyzed statistically using one-way ANOVA Test followed by multiple comparison t – test.

Table – 4: Changes in GSH level in different brain areas (CC, CB, CN, MB and PM) of BH treated colchicine (15µg/ 5 µl of ACSF) induced Alzheimer rat model.

	Reduced glutathione (µg/g of tissue)				
	CC	**CB**	**CN**	**MB**	**PM**
Control	31.44±0.07	30.22±0.03	28.97±0.04	23.36±0.03	26.66±0.02
Colchicine	2.57±0.41***	4.45±0.01***	3.21±0.05***	2.44±0.12***	2.24±0.37***
BH	35.58±0.02***	36.57±0.04***	35.56±0.04***	30.29±0.02***	32.41±0.02***
BH+Colchicine	25.55±0.01#	26.28±0.02#	22.30±0.02#	20.21±0.01#	22.30±0.02#

Values are mean ± SEM, n = 6; *** $p < 0.001$ when compared with control group. # $p < 0.001$ when compared with colchicine treated group. Data were analyzed statistically using one-way ANOVA Test followed by multiple comparison t – test.

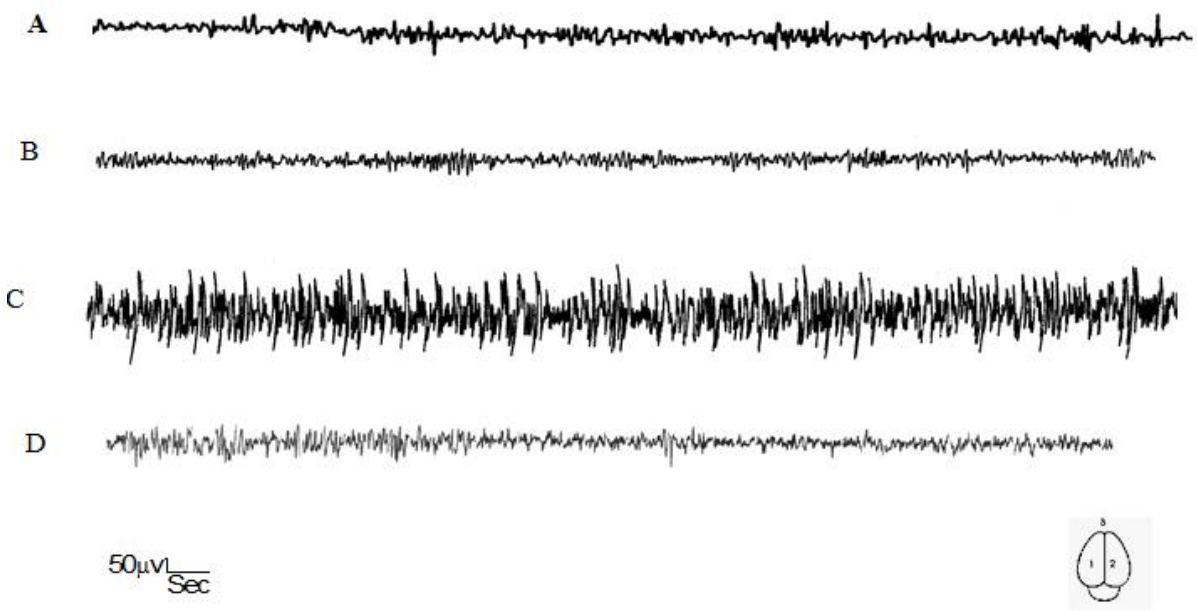

Plate - 1: Electroencephalographic (EEG) recordings.

A: Control model: predominance of low voltage first waves or waves.

B: BH treated model: occurance of wave activity was increased.

C: Colchicine treated model: occurance of spike wave discharges was increased and occurance of wave activity was decreased.

D: BH pretreated colchicine treated model: occurance of spike wave discharges was decreased and occurance of wave activity was increased.

DISCUSSION

In our present study, we have tried to find out the role of BH on the frequency of α wave and spike wave pattern in colchicine induced experimental rat model of AD with the possible involvement of antioxidants. The normal EEG wave pattern showed predominance of low voltage fast waves or β waves in normal saline treated control rats. Pretreatment with BH pulp extract at a dose of 400 mg/kg body weight, it significantly increased the occurance of α wave activity which persisted for more than five hours. After intracerebroventricular (ICV) infusion of colchicine, there was an increase in the occurrence of spike wave discharges. In BH pretreated colchicine treated experimental rat model of AD, there was a markedly decline in the occurence of spike wave discharges along with an increase in the occurance of α wave activity in comparison with colchicine treated experimental rat model of AD. These findings can be explained by alterations of the lipid peroxidation (LPO) level and the antioxidant enzyme activities such as SOD, CAT and also the GSH level in different brain regions.

ICV infusion of colchicine causes it to bind with tubulin which is the structural protein of microtubule and thereby generates more and more reactive oxygen species (ROS) leading to neurodegeneration and ultimately produces a condition akin to AD or produces experimental AD model which is histopathologically characterized by the extracellular deposition of senile plaques. Free radicals play a crucial role in the pathogenesis of AD. The LPO is determined by the balance between the production of oxidants and the removal and scavenging of those oxidants by antioxidants **(Filho, 1996; Halliwell, 1989; Lopez Torres et al., 1993)** Lipid peroxidation can be used as an index for measuring the damage that occurs in membranes of tissue as a result of free radical generation **(Dianzani, 1985; Husain and Somani, 1997)**. In our present study, ICV infusion of colchicine, it significantly increased the LPO level. The results of significant elevation of LPO level in colchicine treated experimental Alzheimer's group indicates increased free radical generation in the colchicine treated rat group which was supported by **Veerandrakumar and Gupta, 2002**. The LPO level was significantly

decreased in BH treated control group rather than control group. So, from the results of LPO level, it may be concluded that BH, the storehouse of vitamin E, C and β-carotene, provides antioxidant protection against colchicines induced oxidative stress, through the changes of LPO level.

Endogenous antioxidant status in colchicine induced experimental Alzheimer's rat model was evaluated here by noting the activities of CAT, SOD and GSH as these are the important biomarkers for scavenging free radicals **(Venkateswaran and Pari, 2003)**. Generally SOD catalyzed to scavenge excess superoxide anions and convert them to H_2O_2 **(Husain and Somani, 1997)**. Biphasis fluxes of SOD activities are common and an increase or decrease may relate to the presence of excess superoxides **(Bondy, 1992)**. Richardson reports an overall increase in peroxidation in AD and a 25-35% decrease in SOD activity in frontal cortex and hippocampus in AD versus control **(Richardson, 1993).** The SOD activity was found to be decreased in the colchicine treated group rather than control, BH treated control and BH treated colchicine treated experimental groups. Inhibition of SOD activity in colchicine treated group may be consequences of decreased de novo synthesis of SOD protein or irreversible inactivation of enzyme protein from increased free radical generation resulting from ICV infusion of colchicine. **Santiard et al., 1995**, supported this line of reasoning. The SOD activity was significantly increased in BH treated control group rather than control group. This increase in the activity of SOD in control, BH treated control and BH treated colchicine treated experimental AD groups is possibly due to the increased de novo synthesis of SOD protein or irreversible activation of enzyme protein from decreased free radical generation.

The primary role of CAT is to scavenge H_2O_2 that has been generated by free radicals or by SOD in removal of superoxide anions and to convert it to water **(Ribiere et al., 1992)**. The activity of CAT was found to be significantly decreased in the colchicine treated experimental AD group rather than control, BH treated control and BH treated colchicine treated groups. CAT activity was significantly increased in BH treated control group rather than control group. The increase in the CAT activity in BH treated colchicine treated experimental AD group is possibly due to excess H_2O_2 production resulting from SOD inhibition.

Glutathione is an endogenous antioxidant, which is present majorly in the reduced form within the cells. It prevents the hydroxyl radical generation by interacting with free radicals.

During this defensive process, reduced glutathione is converted to oxidized form under the influence of the enzyme glutathione peroxidase (GPX). The GSH level was significantly decreased in colchicine treated experimental group rather than control and BH treated colchicine treated experimental group. The decreased level of reduced glutathione in colchicine treated experimental group seen in our study indicates that there was an increased generation of free radicals and the reduced glutathione was depleted during the process of combating oxidative stress **(Reiter, 2000; Schulz et al, 2000)**. The GSH level was significantly increased in BH treated control group rather than control group. The increase in the level of GSH in BH treated colchicine treated experimental AD group rather than colchicine treated experimental AD group is possibly due to the activity of vitamin C, E and β-carotene that is present in BH pulp.

From the results of EEG activities in our study, this has probably been possible either from the low level of ROS production or through a rapid dissolution of ROS that has further been strengthened from the elevated activities of important antioxidant defense enzymes CAT and SOD, studied in this experiment. Literature study has shown that the BH contains high level of vitamin E, C, beta-carotene, flavonols and flavonoids that protects rat neurons against oxidative stress possibly through the presence of both vitamin E and beta-carotene. Because both beta carotene and vitamin E (α tocopherol and other tocopherol) are the most potent antioxidant that can break the propagation of free radical chain reactions in the lipid part of biological membranes.

The conclusion reached is that there is an increased sensitivity to oxygen free radicals, which could be due to either a decrease in free radical defenses (as in a decrease in SOD activity) or an increase in free radical formation in colchicine treated experimental rat model of AD compared to that of BH pretreated colchicine treated experimental rat model of AD. Thus this study clearly shows a deficit in the antioxidant enzyme in colchicine induced experimental rat model of AD and suggests that the mechanism of neuronal cell death may be from a failure to protect against free radical damage which in turn possibly increased the occurance of spike wave discharges and simultaneously decreased the occurance of α wave activity. An increase in free radical activity could lead to increased neuronal cell death, the hallmark of AD **(Terry et al., 1991; Terry and Davies, 1980; McKee et al., 1991).** Thus the overall differences among the control, BH treated control, colchicine treated experimental rat model of AD group and BH pretreated colchicine treated experimental rat model of AD group assays offer

a possible cause for AD. In our present study it appears that increased oxidative stress is unable to induce brain antioxidant activity, resulting in increased lipid peroxidation. Pretreatment with BH pulp extract for fourteen consecutive days decreased oxidative stress possibly by increasing the antioxidant enzyme activity and decreasing the LPO level and thereby significantly decreased the occurrence of spike wave discharges and simultaneously increased the occurrence of α wave activity.

It may be inferred from the present results BH protected rat neurons against oxidative stress as is evidenced from our results of LPO, CAT, SOD, GSH and EEG activities possibly by vitamin E, C and β carotene which are present in BH pulp extract.

Thus the salient findings are that the aqueous pulp extract of this plant results significant neuroprotection in the level of antioxidant status in CC, CB, CN, MB and PM and EEG activities of the brain after a certain period of ICV infusion of colchicine induced oxidative stress without causing any general and metabolic toxicity. From this point of view, it may be proposed that further research on this field is essential to find out other active ingredients present in the BH pulp extract and their specific role by which the therapeutic importance may be evaluated and the outcome of which can be utilized in the protection of AD.

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