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A nootropic effect of *Moringa oleifera* on Ach and ChAT activity in colchicine induced experimental rat model of Alzheimer's disease: Possible involvement of antioxidants

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Abstract: *Context:* The fruit and leaf of *Moringa oleifera* (MO) is an important ingredient of 'Kusmanda lehyam' (Ayurvedic medicine), which is widely used, in nervous disorders. *Objective:* To determine the cognition facilitating effect of MO leaf extract in colchicine induced experimental rat model of AD and to investigate the role of central cholinergic system in the nootropic effect of MO leaf extract with the possible involvement of antioxidant enzymes. *Materials and methods:* The behavior study, Acetylcholine concentration, cholineacetyl transferase activity, antioxidant level 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 frontal cortex (FC) and hippocampus (HPC) in colchicine induced experimental Alzheimer rat model before and after treatment with MO. *Results:* MO (250 mg/kg p.o.) induced statistically significant reversal of colchicine induced cognitive deficits. MO (250 mg/kg p.o.) markedly induced frontal, cortical and hippocampal concentrations of Ach and ChAt activity, the effects being statistically significant on days 7, 14 and 21 respectively. Moreover, MO significantly increased SOD, CAT, GSH activities and significantly decreased LPO level on day 7, 14 and 21 respectively. *Discussion and conclusion:* The aqueous pulp extract of MO (250 mg/kg body weight) containing vit- A, C, E results significant protection in the level of antioxidant status in frontal cortex and hippocampus after a certain period of co administration on colchicine induced oxidative stress without causing any general and metabolic toxicity and possibly thereby induced frontal cortical and hippocampal concentration of Ach and ChAT activity.

Keywords: *Moringa oleifera;* Colchicine; Alzheimer's disease; Acetylcholine; Cholineacetyl transferase; Antioxidant

Introduction

Moringa oleifera (MO) which belongs to the family *Moringaceae,* is prevalent almost all over the Asian and African countries. Its fruits and leaves which show anti-inflammatory and hypotensive effect are consumed as food by the people [1-3]. It has been found recently that MO leaf extract which is not toxic even at higher concentration levels, enhances memory via nootropics activity and provides substantial antioxidants like vitamin C and E to combat oxidative stress in AD [1, 4-6]. Wealth of studies substantiated that monoamines entailed in the memory loss are altered by *Moringa oleifera* leaf extracts [1,7]. Several lines of evidence also suggest that colchicines-induced AD can be ameliorated by ethanolic extract of *Moringa oleifera* by modifying the brain monoamines (norepinephrine, dopamine, and serotonin) and electrical activity in a rat model [1]. Studies on rats demonstrated that the oral administration of Trasina, a herbal formulation, once daily for 21 days can effectively ameliorate colchicine induced effects like reduced frontal, cortical and hippocampal acetylcholine (Ach) concentrations, choline acetyltransferase (ChAT) activity, and muscarinic cholinergic receptor (MCR) binding [8]. Anwala churna (*Emblica officinalis* Gaertn.), an Ayurvedic preparation showed an exemplary improvement in memory and brain cholinesterase activity, thus ameliorating the scopolamine induced amnesia in young and aged mice [9].

Colchicine, as a microtubule-disrupting agent [10] 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 [11-13]. This event is associated with loss of cholinergic neurons and decrease in acetylcholine transferase, thereby resulting in impairment of learning and memory [14-15]. Oxidative stress due to increase in free radical generation of impaired endogenous antioxidant mechanism is an important factor that has been implicated in neuronal damage and in AD and cognitive defects seen in elderly [16]. A number of in vitro studies have shown that antioxidants, both endogenous and dietary, can protect nervous tissue from damage by oxidative stress. Vitamin C has been described to be a major hydrophilic antioxidant in human plasma [17], CSF [18-19] and the central nervous system [20]. The same study showed that vitamin E prevented neuronal damage from reactive nitrogen species [21]. Both vitamin E and β carotene were found to protect rat neurons against oxidative stress from exposure to ethanol [22].

Moringa oleifera (MO) leaves are rich source of vitamins and antioxidants. They contain good amount of proteins, minerals, vitamin A, vitamin B complex, essential amino acids and a high content of vitamin E [23]. Studies reveal that these compounds not only have antioxidant property but also have memory facilitating effect [24].

Objective of study: 1) to determine the cognition facilitating effect of MO leaf extract in colchicine induced experimental rat model of AD and 2) to investigate the role of central cholinergic system in the nootropic effect of MO leaf extract with the possible involvement of antioxidant enzymes.

Material and Methods

Subjects: Male Holtzman strain adult albino rats approximately 120 days old and weighing 250-300gm were used in the following studies. The animals were individually housed and maintained under standard laboratory conditions with natural dark and light cycle (approximately 12-h light/10-h dark cycle) and room temperature (27 ± 1^0C) and constant humidity (60%) in accordance with 'Institutional Ethical Committee' rules and regulations. Food and water were freely available except during testing. Drinking water was supplied ad libitum. Five days prior to behavioral training, animals were reduced to 85% of their free feeding weight by limiting their daily ration of food. Food deprivation was maintained throughout testing except for 3 days immediately prior to, and following surgery. Body weights of the rats were recorded everyday and maintained in the laboratory throughout the experimental period. The behavioral procedure was carried out between 12:00 and 14:00 h.

Collection and Preparation of ethanolic extract from the leaves of Moringa oleifera (MO): Fresh, young, healthy leaves of MO after identification were shade dried and ground with the help of an electrical grinder to get a free flowing powder. This powder was subjected to extraction with dehydrated alcohol at room temperature for 24 hours. The extract obtained was filtered through Whatman filter paper and vacuum dried at 40–50° C to get a free flowing powder. This powder was subjected to extraction with dehydrated alcohol at room temperature for 24 hours. The extract obtained was filtered through Whatman filter paper and vacuum dried at 40–50° C to get a dry powder, which was dissolved in double distilled water for final use [25].

Treatment: The control animal was treated with artificial cerebrospinal fluid or ACSF. The MO leaf extract was given orally through orogastric cannula at the standard dose of 250 mg/kg p.o. for seven, fourteen and twenty one consecutive days respectively (between 10:00 and 11:00 hrs). The dose was standardized in the laboratory. The animals were sacrificed by cervical dislocation and the different parts of the brain like Frontal cortex (FC), Hippocampus (HPC), Cerebral cortex (CC), Cerebellum (CB), Caudate nucleus (CN), Pons & Medulla (PM) and Midbrain (MB), were isolated for biochemical estimation after seven, fourteen and twenty one days respectively.

Grouping of Animal: The animals were divided into four groups:

1. Control (ACSF) rats
2. Colchicine induced Alzheimer's rat model
3. Control rats treated with MO leaf extract
4. Colchicine induced Alzheimer's rat model treated with MO leaf extract.

Behavioural study: The apparatus used consisted of a shuttle box with two identical compartments, separated by a hurdle. During training, each rat was placed in one compartment and after 5 sec a buzzer, situated in the ceiling of the shuttle box, was sounded (2.8 kHz, 70 dB) (conditioned stimulus, CS) for 3 sec, followed by electric shock (1.5 mA, 2 sec) (unconditioned stimulus, UCS) through the grid floor. If the rat crossed to the unelectrified safe compartment during presentation of CS, an avoidance response was recorded, otherwise UCS was applied. Each rat was given 20 trials for 5 days, with an intertrial interval of 30 sec, before lesioning, until it reached the criterion of 100% active avoidance response. Rats not reaching this criterion were discarded from the study [26]. Retention of the acquired active avoidance response, in the different treatment groups, was assessed on days 7, 14 and 21, following lesioning with colchicine or ibotenic acid, by noting the number of trials required to criterion of 100% active avoidance response.

Preparation of experimental Alzheimer's rat model by colchicines: Prior to surgery, all the animals were subjected to overnight fasting though drinking water was not withdrawn. During procedures, the animals were anaesthetized with sodium pentobarbital (50mg/kg b.w.) and re-strained in a stereotaxic apparatus (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 and retracted. An incision was made in the scalp and two holes were drilled in the skull for placement of the injection cannula into the lateral cerebral ventricles. The stereotaxic coordinates for intracerebroventricular injection were: 0.8 mm posterior to bregma, 1.8 mm lateral to the sagittal suture and 3.6 mm below the cortical surface [27].

Subjects were infused through a 10 µl Hamilton syringe with 15 µgm of colchicine (Wako chemicals) in 5 µl of artificial cerebrospinal fluid (ACSF; in Mm: 147 Nacl, 2.9 Kcl, 1.6 $Mgcl_2$, 2.2 Dextrose and 1.7 $Cacl_2$) in lateral cerebral ventricle 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.

Postoperative care: After surgery, all aseptic measures and care were taken for feeding until recovery from surgical stress. Penicillin was given post operatively to all animals for 3 consecutive days by intramuscular 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.

Biochemical Estimation:

Tissue preparation: Rats were sacrificed by cervical dislocation on day 7, 14 and 21 immediately after behavior study. The Frontal cortex (FC) and Hippocampus (HPC) were dissected out. The tissues were weighed and homogenized in ice-cold phosphate buffer and prepared for biochemical estimation.

Estimation of Ach and ChAT activity: Rats of the colchicine group were killed by decapitation at the predetermined time intervals and the frontal cortex and hippocampus were dissected out [28]. The tissues were homogenised in 10 volumes (w/v) of ice-cold Tris-HCl buffer (pH 7.6) and divided into aliquots for estimation of acetylcholine (Ach) levels by a fluorimetric technique [29], choline acetyltransferase (ChAt) activity by a radiometric method [30].

Estimation of SOD, CAT, GSH activity and LPO level: Catalase activity was estimated by the method of Cohen *et al.* [31], Roy *et al.* [32]; Superoxide Dismutase (SOD) was estimated by the method of Mishra & Fridovich [33]; Roy *et al.,* [32], Reduced glutathione (GSH) level was measured according to the method of Ellman [34] and Lipid Peroxidation (LPO) was estimated by the method of Bhattacharya *et al.* [35], Roy *et al.* [32].

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

Results of Behavioural parameter: Colchicine, injected i.c.v., induced marked deficits of the learned active avoidance task, as compared to their ACSF treated counterparts, after 7, 14 and 21 days following administration of the neurotoxins. The retention deficit was evident by day 7 and increased progressively on days 14 and 21. MO (250 mg/kg p.o.) induced dose-related statistically significant reversal of colchicine induced cognitive deficits, when assessed on day 7, remained statistically non-significant (Table-1).

Table-1: Effect of MO on the retention of an active avoidance learning acquisition in cognitive deficits induced by colchicine (15 µg, i.c.v.) in rats (values are Mean ± SEM)

Treatments (mg/kg)	N	Number of trials required to achieve 100% avoidance response		
		Colchicine group		
		Day 7	Day 14	Day 21
ACSF	6	4.9 ± 0.02	3.4±0.02	2.8±0.03
MO	6	2.7±0.02[a]	2.0±0.03[a]	1.4±0.03[a]
COLCHICINE	6	7.9±0.03[b]	8.4±0.03[b]	9.4±0.04[b]
MO+COLCHICINE	6	5.44±0.03[c]	3.54±0.02[c]	2.27±0.04[c]

Values are mean ± SEM, n = 6; [a]p < 0.001, [b]p < 0.001 when compared with ACSF group. [c]p < 0.001 when compared with colchicine treated group. Data were analyzed statistically using one-way ANOVA Test followed by student 't'– test.

Table-2: Effect of MO on acetylcholine concentrations of frontal cortex and hippocampus in colchicines (15 µg i.c.v.) administered rats (values are Mean ± SEM)

Treatments (mg/kg)	N	Acetylcholine concentrations (nmol/g)					
		Frontal cortex			Hippocampus		
		Day 7	Day 14	Day 21	Day 7	Day 14	Day 21
ACSF	6	24.45±0.02	26.22±0.03	23.68±0.02	29.18±0.03	28.34±0.02	30.64±0.03
MO	6	28.26±0.04[a]	34.72±0.02[a]	30.04±0.04[a]	33.82±0.03[a]	35.44±0.04[a]	38.21±0.04[a]
COLCHICINE	6	18.31±0.02[b]	15.41±0.04[b]	11.49±0.03[b]	23.44±0.04[b]	19.15±0.02[b]	14.42±0.03[b]
MO+COLCHICINE	6	23.52±0.02[c]	30.69±0.04[c]	24.88±0.05[c]	28.92±0.04[c]	33.23±0.02[c]	31.44±0.02[c]

Values are mean ± SEM, n = 6; [a] p < 0.001, [b] p < 0.001 when compared with ACSF group. [c] p < 0.001 when compared with colchicine treated group. Data were analyzed statistically using one-way ANOVA Test followed by student 't'– test.

Table-3: Effect of MO on choline acetyltransferase activity of frontal cortex and hippocampus in colchicine (15 µg, i.c.v.) administrated rats (Values are Mean ± SEM)

Treatments (mg/kg)	N	Choline acetyltransferase activity (nmol/mg protein/h)					
		Frontal cortex			Hippocampus		
		Day 7	Day 14	Day 21	Day 7	Day 14	Day 21
ACSF	6	21.72±0.04	20.46±0.02	19.88±0.07	19.34±0.04	18.86±0.06	19.44±0.04
MO	6	21.55±0.02[a]	24.48±0.03[a]	22.48±0.02[a]	22.12±0.05[a]	22.42±0.04[a]	23.32±0.01[a]
COLCHICINE	6	16.78±0.04[b]	14.36±0.04[b]	11.43±0.04[b]	14.78±0.04[b]	12.56±0.02[b]	9.78±0.06[b]
MO+COLCHICINE	6	18.54±0.02[c]	21.54±0.06[c]	23.56±0.02[c]	17.46±0.02[c]	19.68±0.04[c]	16.64±0.02[c]

Values are mean ± SEM, n = 6; [a] p < 0.001, [b] p < 0.001 when compared with ACSF group. [c] p < 0.001 when compared with colchicine treated group. Data were analyzed statistically using one-way ANOVA Test followed by student 't'– test.

Table-4: Effect of MO on muscarinic cholinergic receptors in frontal cortex and hippocampus in colchicine (15 µg, i.c.v.) administered rats (Values are Mean ± SEM)

Treatments (mg/kg)	N	(^{3}H) QNB binding (pmoles/mg protein)					
		Frontal cortex			Hippocampus		
		Day 7	Day 14	Day 21	Day 7	Day 14	Day 21
ACSF	6	1.55±0.01	1.70±0.02	1.52±0.02	1.35±0.03	1.44±0.03	1.41±0.02
MO	6	1.54±0.02[a]	1.74±0.02[a]	1.98±0.01[a]	1.57±0.01[a]	1.68±0.01[a]	1.84±0.01[a]
COLCHICINE	6	0.86±0.02[b]	0.52±0.02[b]	0.44±0.01[b]	0.72±0.02[b]	0.52±0.02[b]	0.46±0.02[b]
MO+COLCHICINE	6	1.24±0.01[c]	1.36±0.01[c]	1.46±0.02[c]	1.06±0.04[c]	1.40±0.02[c]	1.56±0.01[c]

Values are mean ± SEM, n = 6; [a] $p < 0.001$, [b] $p < 0.001$ when compared with ACSF group. [c] $p < 0.001$ when compared with colchicine treated group. Data were analyzed statistically using one-way ANOVA Test followed by student 't' – test.

Results of parameters of cholinergic system: Colchicine, administered i.c.v., markedly reduced frontal cortical and hippocampal concentrations of Ach and ChAt activity, as compared to the ACSF administration control group. The effects were discernible by day 7, and increased progressively, thereafter, on days 14 and 21. MO (250 mg/kg p.o.) tended to reverse the deleterious effects of colchicine on all these biochemical parameters, the effects being statistically significant on days 14 and 21, but not on day 7 (Tables-2, 3 & 4).

Results of parameters of oxidative stress: Colchicine, administered i.c.v., markedly reduced SOD, CAT and GSH activity and markedly increased LPO level in different mentioned brain parts respectively, as compared to the ACSF administration control group. The effects were discernible by day 7, and increased progressively, thereafter, on days 14 and 21. MO (250 mg/kg p.o.) tended to reverse the deleterious effects of colchicine on all these biochemical parameters, the effects being statistically significant on days 7, 14 and 21 respectively (Table-5, 6, 7 & 8).

Table-5: Effect of MO on SOD activity in frontal cortex and hippocampus in colchicine (15 µg, i.c.v.) administered rats (Values are Mean ± SEM)

Treatments (mg/kg)	N	SOD (% inhibition unit)					
		Frontal cortex			Hippocampus		
		Day 7	Day 14	Day 21	Day 7	Day 14	Day 21
ACSF	6	13.34±0.02	11.28±0.02	11.42±0.04	11.34±0.03	11.98±0.07	12.54±0.04
MO	6	10.42±0.03[a]	9.64±0.02[a]	9.86±0.04[a]	9.77±0.05[a]	10.33±0.04[a]	10.76±0.02[a]
COLCHICINE	6	20.96±0.06[b]	19.34±0.05[b]	19.82±0.02[b]	18.68±0.06[b]	21.04±0.03[b]	21.22±0.02[b]
MO+COLCHICINE	6	15.34±0.03[c]	14.54±0.06[c]	14.36±0.03[c]	15.02±0.02[c]	14.69±0.02[c]	15.48±0.02[c]

Values are mean ± SEM, n = 6; [a] $p < 0.001$, [b] $p < 0.001$ when compared with ACSF group. [c] $p < 0.001$ when compared with colchicine treated group. Data were analyzed statistically using one-way ANOVA Test followed by student 't' – test.

Table-6: Effect of MO on CAT activity in frontal cortex and hippocampus in colchicine (15 µg, i.c.v.) administered rats (Values are Mean ± SEM)

Treatments mg/kg)	N	CAT (% inhibition unit)					
		Frontal cortex			Hippocampus		
		Day 7	Day 14	Day 21	Day 7	Day 14	Day 21
ACSF	6	13.44±0.03	12.32±0.09	12.42±0.04	13.21±0.04	13.02±0.04	12.35±0.02
MO	6	12.29± 0.04[a]	10.45±0.04[a]	10.65±0.03[a]	11.88±0.03[a]	10.41±0.03[a]	10.14±0.02[a]
COLCHICINE	6	21.78±0.03[b]	20.42±0.04[b]	20.02±0.05[b]	20.73±0.03[b]	20.35±0.04[b]	22.22±0.04[b]
MO+COLCHICINE	6	15.77±0.04[c]	15.43±0.02[c]	15.43±0.06[c]	14.22±0.03[c]	13.62±0.03[c]	15.89±0.06[c]

Values are mean ± SEM, n = 6 ; [a] $p < 0.001$, [b] $p < 0.001$ when compared with ACSF group. [c] $p < 0.001$ when compared with colchicine treated group. Data were analyzed statistically using one-way ANOVA Test followed by student 't' – test.

Table-7: Effect of MO on LPO level in frontal cortex and hippocampus in colchicine (15 µg, i.c.v.) administered rats (Values are Mean ± SEM)

Treatments (mg/kg)	N	LPO (nmol of TBARS / gm mol of tissue)					
		Frontal cortex			Hippocampus		
		Day 7	Day 14	Day 21	Day 7	Day 14	Day 21
ACSF	6	4.05±0.02	3.87±0.04	3.42±0.04	3.83±0.02	3.69±0.04	3.92±0.02
MO	6	2.94±0.01[a]	2.92±0.03[a]	3.21±0.02[a]	2.86±0.03[a]	3.38±0.02[a]	2.87±0.02[a]
COLCHICINE	6	8.56±0.02[b]	7.44±0.04[b]	7.62±0.03[b]	7.45±0.02[b]	7.22±0.02[b]	8.54±0.02[b]
MO+COLCHICINE	6	5.22±0.03[c]	4.66±0.05[c]	4.24±0.02[c]	4.56±0.02[c]	4.62±0.02[c]	5.06±0.02[c]

Values are mean ± SEM, n = 6 ; [a] $p < 0.001$, [b] $p < 0.001$ when compared with ACSF group. [c] $p < 0.001$ when compared with colchicine treated group. Data were analyzed statistically using one-way ANOVA Test followed by student 't' – test.

Table-8: Effect of MO on GSH level in frontal cortex and hippocampus in colchicine (15 µg, i.c.v.) administered rats (Values are Mean ± SEM)

Treatments (mg/kg)	N	Reduced glutathione (µg/g of tissue)					
		Frontal cortex			Hippocampus		
		Day 7	Day 14	Day 21	Day 7	Day 14	Day 21
ACSF	6	30.42±0.06	29.68±0.06	29.67±0.04	23.92±0.06	26.68±0.04	29.48±0.02
MO	6	32.26±0.04[a]	31.26±0.04 [a]	28.12±0.03[a]	26.77±0.04[a]	27.62±0.02[a]	32.24±0.02[a]
COLCHICINE	6	19.86±0.02[b]	18.66±0.04[b]	15.21±0.04[b]	16.27±0.04[b]	17.32±0.02[b]	15.49±0.02[b]
MO+COLCHICINE	6	26.82±0.04[c]	26.86±0.03[c]	24.85±0.04[c]	25.41±0.05[c]	25.15±0.02[c]	24.45±0.02[c]

Values are mean ± SEM, n = 6 ; [a] $p < 0.001$, [b] $p < 0.001$ when compared with ACSF group. [c] $p < 0.001$ when compared with colchicine treated group. Data were analyzed statistically using one-way ANOVA Test followed by student 't' – test.

Discussion

The present study evaluates the nootropic effect of MO on cognition facilitating effect and central cholinergic system in colchicine induced experimental rat model of AD with the possible involvement of antioxidant enzymes. It is evident from the results of the present investigation that intracerebroventricular (icv) administration of colchicine induced marked deficits of the learned active avoidance task, as compared to their ACSF treated counterparts after 7, 14 and 21 days in colchicine treated experimental AD group. But, treatment with ethanolic leaf extract of MO significantly decreased marked deficits of the learned active avoidance task in MO cotreated colchicine treated experimental AD group compared to only colchicine treated experimental AD group. These findings can be explained by alterations of the parameters of oxidative stress namely lipid peroxidation level (LPO) , SOD, CAT and GSH activity along with alterations of AchE and ChAT activity respectively. Thus, the marked deficit in the retention of the learned active avoidance task, in rats induced by colchicine, noted in this study, is consonant with earlier report [36].

Treatment with ethanolic leaf extract of MO was able to reverse cognitive deficits induced by colchicine, the effects being evident after 2 weeks of treatment. The reversal of cognitive deficits, induced by colchicine, was accompanied by attenuation of its cholinotoxic effects, indicating that the drug was capable of promoting cholinergic recovery. From our present investigation, i.c.v. administration of colchicine markedly reduced frontal cortical and hippocampal concentrations of Ach, ChAt activity, as compared to the ACSF administration control group. The effects were discernible by day 7, and increased progressively, thereafter, on days 14 and 21. MO (250 mg/kg p.o.) tended to reverse the deleterious effects of colchicine on all these biochemical parameters, the effects being statistically significant on days

14 and 21, but not on day 7. It was reported that the i.c.v. injection of colchicine significantly decreased the number of cholinergic neurons in the medial septum /vertical limb of the diagonal band, which project to the hippocampus and synapse on granule cells, pyramidal cells and interneurons [15]. Therefore, it was expected that the intracerebroventricularly administered colchicines would preferentially act on the cholinergic neurons [15]. Nootropic agents, like piracetam, which have been shown to facilitate central cholinergic mechanisms [37-38], are known to improve memory only in the presence of cognitive deficits.

Intracerebroventricular infusion of colchicine causes it to bind with tubulin which is the structural and functional 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 and the intracellular deposition of neurofibrillary tangles [15]. Free radicals play a crucial role in the pathogenesis of AD. 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 [39-40]. In our present study, ICV infusion of colchicine significantly increased the LPO level. The results of significant elevation of LPO level in colchicine treated experimental Alzheimer's group is possibly due to the generation of free radicals via auto-oxdidation or through metal ion or superoxide catalyzed oxidation process. In the present experiment, ethanolic leaf extract of MO significantly decreased LPO level in a dose dependent manner compared to other groups. So, from the result of LPO levels it may be concluded that the protection by MO may be due to vitamin E and beta carotene which is present in MO leaf extract.

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 [41]. Colchicine induced oxidative stress is further supported here by the study of antioxidant scavenger enzyme activities. CAT that protects the tissues from highly reactive hydroxyl radical catalyzes the reduction of hydrogen peroxide. 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 [42]. The destruction of superoxide radicals is catalyzed by SOD, is an important defense system against oxidative damage. From our experimental results of the aforesaid antioxidant enzyme activities in brain tissues colchicine significantly decreased SOD, CAT, GSH activities in colchicine treated experimental Alzheimer's group rather than control, MO treated group and MO cotreated colchicine treated experimental Alzheimer's groups. MO containing vitamin E and beta-carotene significantly increased SOD, CAT, GSH activities and significantly decreased the LPO level in a dose dependent manner rather than other groups.

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 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 [43-44].

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 MO leaf contains high level of vitamin E and beta-carotene [23] which protects rat neurons against oxidative stress possibly through the presence of both vitamin E and beta-carotene. Because vitamin E (alpha tocopherol and other tocopherol) is the most potent antioxidant that can break the propagation of free radical chain reactions in the lipid part of biological membranes. It may be inferred

from the present results that MO protects rat neurons against oxidative stress as is evidenced from our results of LPO, CAT, SOD and GSH activities possibly by vitamin E and beta carotene which is present in MO.

So, keeping in this view, i.c.v. administration of colchicine may generate oxidative stress either by producing reactive oxygen species (ROS) or by hampering the endogenous antioxidant enzymes leading to cholinotoxicity. The aqueous leaf extract of MO containing vit- A, C, E results in significant protection of the level of antioxidant status in frontal cortex and hippocampus after a certain period of co administration on colchicine induced oxidative stress and possibly thereby induced frontal cortical and hippocampal concentration of Ach and ChAT activity. The present investigation, together with earlier reported clinical and experimental data, and the similarly of behavioural and biochemical effects on cholinergic markers and different antioxidant enzyme activity along with LPO level, permit the categorization of MO as a nootropic agent.

However, it may be proposed that further research in this field is essential to find out other active ingredients present in the MO leaf 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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