Alteration of brain monoamines & EEG wave pattern in rat model of Alzheimer's disease & protection by *Moringa oleifera*

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Background & objectives: The monoaminergic systems which exert a modulatory role in memory processing, are disturbed in Alzheimer's disease (AD) and *Moringa oleifera* (MO) has been shown to exert its effect in CNS by altering the brain monoamines. The present study aims to see whether chronic oral treatment of ethanolic extract of MO leaves can alter the brain monoamines (norepinephrine, dopamine and serotonin) in distinct areas of brain in rat model of AD caused by intracerebroverticle (ICV) infusion of colchicine and hence can provide protection against monoaminergic deficits associated with AD.

Methods: Rats were given ICV infusion of colchicine (15 µg/5µl) and MO leaf alcoholic extract was given in various doses. The effective dose was standardized by radial arm maze (RAM) training. From the selected dose of 250 mg/kg body weight, the biochemical estimations and EEG studies were performed.

Results: Stereotaxic ICV infusion of colchicine significantly impaired the RAM performance together with decrease in norepinephrine (NE) level in cerebral cortex (CC), hippocampus (HC) and caudate nucleus (CN). Dopamine (DA) and serotonin (5-HT) levels were decreased in CC, HC and CN. The EEG studies showed a decrease in beta and alpha waves and increase in biphasic spike wave pattern in experimental Alzheimer rat model. Treatment with MO extract markedly increased the number of correct choices in a RAM task with variable alteration of brain monoamines. The EEG studies showed an increase in beta waves and a decrease in spike wave discharges.

Interpretation & conclusions: Our results showed that brain monoamines were altered discreetly in different brain areas after colchicine infusion in brain. After treatment with MO, leaf extract the monoamine levels of brain regions were restored to near control levels. Our findings indicated that MO might have a role in providing protection against AD in rat model by altering brain menoamine levels and electrical activity.

Neurodegenerative diseases often affect mental performance, in particular memory processing[1]. Alzheimer's disease (AD) is an irreversible, progressive brain disorder that occurs gradually and results in memory loss, unusual behaviour, personality changes and a decline in thinking abilities. In this disease, the capacity to memorize is seriously reduced because of compromised neuronal transmission. It is known that there is a loss of cholinergic neurons in AD both in human and in experimental animal models. Apart

from this, pathological changes have been reported to occur in glutamatergic, noradrenergic and serotonergic transmitter systems in AD patients[2]. These transmitter systems may have different preconditions for serving various processes. Monoaminergic projection systems may be more suited for exerting modulatory functions. Literature showed that noradrenergic (NE) deficits are linked to depression, dementia, diminished alertness and concentration. Dopamine (DA) mediated neurotransmission is related to response selection and habit learning in rats, and 5-hydroxytryptamine (5-HT) neurons are involved in anxiety state in AD[1].

Moringa oleifera (MO), commonly called 'drumstick' belongs to family *Moringaceae*, a multipurpose tree found almost all over the Asian and African countries and its fruit and leaves are consumed as food by the people. The leaf of this plant has been shown to have anti-inflammatory and hypotensive effect[3,4]. Since MO leaves are consumed as food, the chance of toxicity is very less. Majumdar *et al*[5] reported that alcoholic extract of MO leaf is not toxic even when consumed in a higher quantity as is evident from its LD_{50} value ($LD_{50:}$ 2.8 g/kg). Recently it was reported that MO leaf possesses nootropics activity and hence can enhance memory[6]. Earlier we showed that MO leaf can provide protection against oxidative stress generated in AD by providing necessary antioxidants[7]. Recently it was also observed that the leaves of MO provide protection in hypobaric hypoxia by alteration in the brain monoamines, which are associated with memory loss[8].

The present study was undertaken to observe the neuroprotective activity of alcoholic extract of MO leaves given orally on the brain monoaminergic systems in distinct brain regions in experimental model of Alzheimer is disease caused by intracerebroventricle (ICV) infusion of colchicine.

Material & Methods

Animals: Pure colony-bred male Holtzman strain adult albino rats were obtained from Indian Institute of Chemical Biology, Kolkata, weighing between 200-250 *g* were housed individually in a photoperiod cycle of 12 : 12 h (light and dark), at room temperature (around 28°C) and constant humidity (60%) with standard laboratory diet, which supplemented the necessary proteins, carbohydrates and minerals. Drinking water was supplied *ad libitum*. Body weight of the rats was recorded every day and maintained in the laboratory throughout the experimental period. The experiments were performed at S.N. Pradhan Centre for Neurosciences. The study protocol was approved by the Institutional animal ethical committee.

Preparation of ethanolic extract of MO: Fresh, young, healthy leaves of MO (2kg) were bought from local market and kept in the laboratory. The identity of the plant was authenticated by Botanical Survey of India, Howrah. The leaves 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 h. The extract obtained was filtered through Whatman filter paper and vacuum dried at 40-50°C to get a blackish green semisolid mass, which was dissolved in saline (0.9% NaCl) solution for final use. The yield obtained was about 10 per cent. *i.e.* 200 g of extract was obtained from 2 kg MO leaves. Ten gram of the extract was dissolved in 20 ml of distilled water, and was mixed finely to make a solution. From this solution 250 mg/kg dose was calculated[8].

Groups and treatment

Schedule I: In the first set of experiments, the effective dose of MO was standardized on rats by observing their performance in radial arm maze (RAM) task. Seventy two rats were divided into 9 groups- control (group I), colchicine group (group II) and seven MO treated colchicine groups (groups III-IX). Each group consisted of 8 animals. Group I rats were treated with 0.9 per cent saline (5 ml/kg, po). Group II rats were not treated with any drug as these were infused with colchicine only. Groups III-IX rats were treated with MO leaf extract orally using an orogastric cannula in the doses of 50, 100, 150, 200, 250, 300 and 350 mg/kg respectively between 1000 and 1100 h for 14 days[9] and behavioural task by RAM training was given to each rat for seven days (10 trials daily) and on each day, the performance in RAM task was noted. On the 8th day, colchicine (15 μg/5 μl) was infused in lateral ventricle in rats of groups III-IX. Seven days following the colchicine infusion, the RAM task was again performed in all the groups for 7 consecutive days. The group which showed minimum impairment in the RAM performance was selected as effective dose of MO. In our experiments the rats showed minimum impairment after infusion of 250 mg/kg of colchicine, therefore all future experiments were carried out with the same dose.

Schedule II: From the standardized dose of MO (250 mg/kg body weight, bw), the second set of experiments

were performed. The electrical activity and levels of brain monoamines (NE, DA and 5-HT) were estimated. Twenty four rats were taken and divided in four groups; group I- control, group II- MO treated control, group III-colchicine infused group and group IV-MO (250 mg/kg bw) treated colchicine group. Bipolar electrodes were implanted on somatosensory cortex and electrical activity was recorded (8 channel EEG, Medicare and Recorder, Chandigarh) for 5-6 h without interruption.

After EEG recording, rats were sacrificed by cervical dislocation and brain was stored at -20°C for monoamine estimation.

Preparation of experimental Alzheimer 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 sodium pentobarbitone at 40 mg/kg bw dose (Neon Laboratories, India). The anesthetized animals were mounted on stereotaxic instrument (INCO, India Ltd.) equipped with a custom-made ear bar that 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. The surgery was performed under strict aseptic conditions. 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 co-ordinates of the stereotaxic atlas[10]. (According to the co-ordinates: 0. 6 mm posterior to bregma, 1.8 mm lateral to the midline and 2.6 mm below the cortical surface). Colchicine (15 μg / 5 μl of artificial CSF or ACSF) was then slowly infused (0.125 μl/min) into the lateral ventricle.

Post-operative care: After surgery, all aseptic measures were taken for different periods and particular care was taken for feeding for 3 days until they recovered from surgical stress. Antibiotic was given post-operatively to all animals for 3 consecutive days by intramuscular route. After 3 days of surgery, experimental sessions started and continued routinely until sacrificed.

Behavioural testing by radial arm maze training

Habituation session: During RAM training the animals were food deprived to about 80% of their *ad libitum* body weight and trained for 5 days to run on a radial arm maze. (Brown, wooden, 60x10 cm arms) extending from an octagonal central platform. The maze was kept in the centre of a dimly lit room (15 x 10 ft) with many posters and objects hanging on the wall. The animals were placed in the center of the maze with all 8 arms accessible and baited with chocolate chips. The rats were removed from the maze after visiting all the arms. Arms were rebaited only after the animal left the arm and the maze was cleaned with 50 per cent alcohol solution between animals. Only animals reaching this criterion were trained on the memory tasks. Entry into an arm previously visited within any daily trial was scored as an error[11,12].

Following habituation session, the animals were trained for 10 trials per day on RAM task.

Biochemical estimation of serotonin (5-HT), norepinephrine (NE) and dopamine (DA): The animals were sacrificed by cervical dislocation on day 25 (between 1100 and 1200 h). Brain tissues were dissected out, washed in ice cold saline (4°C) and homogenized in 10 ml acidified butanol. Homogenate (4 ml) was mixed with 10 ml 10 per cent heptane and 5ml 0.001 N HCl and then shaken for 5 min and centrifuged at 200 *g* for 10 min. Acid layer (4.5 ml) was eluted and mixed with 200 mg alumina and 1 ml of 2M sodium acetate. The mixture was shaken for 5 min and centrifuged at 200 *g* for 10 min.

Supernatant was taken for estimation of 5-HT and precipitate was used for estimation of DA and NE. Supernatant was mixed with 3 volume of 10 per cent isobutanol, shaken twice with equal volume of salt saturated buffer at *p*H 10. Then 2 volumes of 10 per cent heptane was added and shaken well and then the mixture was made 0.3 N with respect to HCl. This was used for estimation of 5-HT.

Cold distilled water (5 ml) was added to the precipitate, shaken well and then centrifuged at 200 *g* for 3 min. Supernatant was transferred to glass stoppered centrifuged tube. 1.2 ml of freshly prepared ethylenediamine and ethylenediamine dihydrochloride mixture (7:5) was added to it and incubated at 50ºC for 40 min. Mixture was cooled at room temperature and saturated with sodium chloride and then 4 ml 10 per cent isobutanol was added. It was centrifuged at 200 *g* for 3 min. The supernatant was taken for estimation of DA and to the precipitate 4 ml of distilled water was added. This was taken for the estimation of NE. The fluorescence of 5-HT, DA and NE was measured in the Perkin Elmer MPF 44B Fluorescence spectrophotometer, USA with activation and emission wavelength set at 295 and 550 nm (for 5-HT), 320 and 370 nm (for DA) and 385 and 485 nm (for NE)[13].

Surgical procedures for EEG studies: For electrocorticography recordings rats were anaesthetized with pentobarbitone sodium (40 mg/kg, ip). Each rat was placed in a stereotaxic instrument (INCO, India Ltd.) and surgery was done by a midline incision at the back of the head. The skin and the muscles overlying the cranium was retracted on both sides as far as possible, and the exposed bone was cleared of muscle and fascia so that it appeared dry and bone sutures were clearly visible.

At the first stage, bipolar electrodes were implanted on the surface of the somatosensory cortex through trephined holes and fixed with dental cement and acrylic paste. A reference electrode was implanted over the frontal bone and all electrodes were then soldered to a multiple plug, which was fastened to the calvarium with dental cement.

The incised wound of the head was stitched and treated with antibiotics. All possible antiseptic measures were undertaken by injecting antibiotics to prevent any sepsis. The electrical activity from the cerebral cortex was monitored frequently through an 8-channel EEG (Recorder & Medicare India Ltd, Chandigarh). The EEG machine was first calibrated and the signals were amplified such that 50 μV= 5 mm. Recordings were taken approximately every 5 min throughout the session before and after MO treatment. The recording of EEG were analyzed[8, 9,14].

Statistical analysis: The differences between control, colchicine infused animals and MO treated animals were tested by two way ANOVA and appropriate pair-wise comparisons were performed by two-tail test in behavioural changes in RAM task. Changes in brain monoamine activity and EEG studies were analysed using one way analysis of variance (ANOVA) followed by multiple comparison t test. *P*<0.05 was considered statistically significant.

Results

Behavioural analysis by RAM training: Prior to surgery, all rats acquired the RAM task and were making approximately 9 correct choices (>90% accuracy) in their first 4 arms selections (acquisition). Two way ANOVA revealed significant trial effects (*P*<0.05) in the chosen experimental groups but no significant treatment effects. ICV infusion of colchicine (15 μg/5 μl of ACSF) produced significant (*P*<0.001) impairments in the RAM performance (re-acquisition) after 3 days of surgery compared to control group. Two way

ANOVA revealed significant trial effects in the chosen experimental groups (*P*<0.05) as well as significant treatment effects (*P*<0.05). The correct choices out of 10 daily trials, decreased to 2.4 (73.3% decrease) and the latency time was increased to about 1315.4 seconds (186.6% increase) when compared to control. There was a significant impairment after ICV infusion of colchicine (*P*<0.001) in case of making correct choices as well as latency period. Moreover treatment with colchicine exhibited less accurate performance than the control group. Treatment with MO leaves extract (50-350 mg/kg) for 14 days improved RAM performance dose-dependently. At lower doses (50 and 100 mg/kg), the effect was not significant *i.e.*, the RAM performance was not improved. The maximum effect of MO was seen at the dose of 250 mg/kg. The impairment was lowest in this group when compared to control. The impairment in the number of correct choices was by 35.5 per cent and the latency time was by 67.2 per cent. At higher doses, the RAM performance also improved but the improvement was lesser than this dose (Table I).

Changes in norepinephrine (NE) level: Since in behavioural studies 250 mg/kg dose of MO leaf extract was the most effective dose, further studies with monoamines and electrical activity were done with this dosage. After MO treatment in control animals, NE was increased significantly in cerebral cortex (CC) and hippocampus (HC). Following ICV infusion of colchicine, NE was decreased significantly in CC (74.6%), caudate nucleus (CN) (75%), and HC (92.2%) whereas after treatment with MO leaves (250 mg/kg), for 14 days in colchicine infused animals, NE level was increased in CC, HC and CN. When compared to control, the percentage of damaged neurons in these regions was significantly reduced (Table II).

Changes in dopamine (DA) level: In MO treated control rats, differential effect was noticed. In CN and HC the DA level increased whereas no significant alteration was observed in CC. In colchicine infused rats, DA was decreased significantly in all three brain regions *i.e.*, 76.9 per cent in CC, 49.5 per cent in HC and 84.4 per cent in CN but treatment with MO leaf extract (250 mg/kg) for 14 days, caused significantly less impairment in the DA level in HC (9.5%) , CC (34.9%) and CN (63.9%) (Table III).

Changes in serotonin (5-HT) level: In MO treated control animals, there was no alteration in 5-HT level in HC but was increased in CC (65%) and decreased

Table I. Effect of *M. oleifera* (MO) leaf extract on behavioural parameters (RAM test) induced by infusion of colchicine (15 μg of colchicine/5μl of ACSF)

	Acquisition	Acqu Acquisition	Re-acquisition	Re-acquisition
	No. of trials (out of 10)	Latency (in seconds)	No. of trials (out of 10)	Latency (in seconds)
Sham control (Group I)	8.4±0.42	488.2±21.26	9.0±0.27	458.9±24.24
Colchicine (group II)	8.75± 0.25 (4.2)	452.8± 13.34 (7.3)	2.4±0.32 (73.3)	1315.4± 66.97[*] (186.6)
MO+ colchicine (Group III) (50 mg/kg)	9.0± 0.27 (7.14)	515.9± 24.81 (5.7)	2.5± 0.19[*] (72.2)	1218.6± 68.85 (165.5)
MO+colchicine (Group IV) (100 mg/kg)	8.3± 0.41 (1.19)	467.6± 27.75 (4.2)	3.5± 0.19 (61.1)	1157.5± 24.61 (152.2)
MO+ colchicine (Group V) (150 mg/kg)	7.9± 0.44 (5.92)	459.4± 20.51 (5.9)	3.5± 0.19 (61.1)	1060.5± 27.06[†] (131.1)
MO+ colchicine (Group VI) (200 mg/kg)	9.0± 0.33 (7.14)	461.7± 13.25 (5.4)	4.4± 0.18[††] (51.1)	855.7± 16.59[††] (86.5)
MO+colchicine (Group VII) (250 mg/kg)	9.0± 0.27 (7.14)	421.1± 26.88 (13.7)	5.8± 0.25[††] (35.5)	767.3± 25.99[††] (67.2)
MO+colchicine (Group VIII) (300 mg/kg)	8.6± 0.42 (2.38)	433.4± 11.27 (11.2)	5.6± 0.26[††] (37.8)	770.0± 21.41[††] (67.8)
MO+colchicine (Group IX) (350 mg/kg)	7.8± 0.313 (7.14)	427.7± 24.88 (12.5)	5.1± 0.35[††] (43.3)	794.1± 21.92[††] (73)

Values are mean ± SEM, (n=6) *$P<0.001$ compared to sham control; †$P<0.01$, ††$P<0.001$ compared to colchicine infused rats. Values in parentheses are percentage changes observed compared to control data; ACSF, artificial cerebrospinal fluid

Table II. Changes in norepinephrine level in discrete brain regions of different groups

Treatment groups	Norepinephrine level (μg/100g tissue)		
	Cerebral cortex (CC)	Hippocampus (HC)	Caudate nucleus (CN)
Sham control	0.059 ± 0.005	0.1417 ± 0.02	0.100 ±0.002
MO + control	0.085 ± 0.004** (44.1)	0.178 ± 0.02* (25.6)	0.125 ± 0.017
Colchicine	0.015±0.002*** (74.6)	0.011 ± 0.009*** (92.2)	0.025±0.002** (75.0)
MO + colchicine (250 mg/kg)	0.047±0.005## (20.3)	0.111 ± 0.02 ## (21.7)	0.067±0.002# (33.0)

Values are mean ± SEM, (n=6); ***$P<0.001$ compared to sham control; ###$P<0.001$, ##$P<0.001$, #$P<0.05$; compared to colchicine infused rats. Values in parentheses are percentage changes observed compared to control data

Table III. Changes in dopamine level in discrete brain regions of different groups

Treatment groups	Dopamine level (μg/100g tissue)		
	Cerebral cortex (CC)	Hippocampus (HC)	Caudate nucleus (CN)
Sham control	0.169 ±0.002	0.095 ± 0.004	2.364 ± 0.151
MO + control	0.226 ± 0.041	0.113 ± 0.010* (18.9)	2.975 ± 0.21* (25.8)
Colchicine	0.039±0.004*** (76.9)	0.048 ± 0.002*** (49.5)	0.368±0.043*** (84.4)
MO+colchicine (250 mg/kg)	0.110±0.004### (34.9)	0.086 ± 0.005### (9.5)	0.854 ± 0.023# (63.8)

Values are mean ± SEM, (n=6) ***$P<0.001$ compared to sham control; P# <0.05 ##<0.01, ### <0.001 compared to colchicine infused rats. Values in parentheses are percentage changes observed compared to control data

CN (10.7%). In colchicine infused experimental rats, the 5-HT level was decreased in CC (18.3%) and HC (28.6%) but increased in CN (190%). Following treatment with MO leaves (250 mg/kg) for 14 days, 5-HT level was increased in CC (3%) and decreased in CN (16.5%). No significant alteration was observed in other areas (Table IV).

EEG studies: The electrical activity of the brain of the normal mock operated restrained rats was predominantly rapid with varying voltages (50-200 µV) but no high voltage spike could be seen in these animals, either spontaneously or when provoked by flickering light and the frequency of alpha and beta waves were normal. In the control animals, the EEG pattern showed predominance of low voltage fast waves or β waves with a few high voltage slow waves or α waves. Treatment with MO in control animals showed frequent occurrence of α waves but β waves were predominant. Infusion of colchicine caused a frequent occurrence of biphasic spike discharges with a decrease in the number of β and α waves. After treatment with MO leaves extract for 14 days in colchicine infused rats, the EEG showed a significant decrease in spike discharge pattern with a prominent increase in beta waves but alpha waves were not significantly increased (Table V).

Discussion

Colchicine, a microtubule inhibitory drug, when infused in intracerebroventricle (ICV), destroys the microtubule integrity of the neurons thereby causing degeneration of cells. Colchicine, after ICV infusion diffuses through the septal areas to the hippocampus and its surrounding regions including the cerebral cortex[11]. In our study, ICV infusion of colchicine produced impairments in the performance of RAM task in which the correct choices decreased and the latency period increased significantly. The impairment in RAM task can be associated with a significant alteration of various neurotransmitters levels in different regions of the brain. In this study NE level was decreased in CC, HC and CN. NE is released from locus ceruleus in the brain and is responsible for various functions like attention and motor process. Earlier studies have shown that activation of locus ceruleus promotes learning and memory[15]. Treatment with MO for 14 days helped to improve memory (which was evident from the RAM performance) by increasing the correct choices in daily trials and decreasing the latency time together with a significant increase in NE level in CC, HC and CN. In higher cortical structures such as the hippocampus, norepinephrine, via beta-adrenergic receptor (AR) activation, has been shown to reinforce

Table IV. Changes in serotonin level in discrete brain regions of different groups

Treatment groups	Serotonin level (µg/mg tissue)		
	Cerebral cortex (CC)	Hippocampus (HC)	Caudate nucleus (CN)
Sham control (group I)	0.060±0.003	0.049 ± 0.004	0.711 ± 0.009
MO + control	0.099 ± 0.004** (65)	0.049 ± 0.006	0.635 ± 0.018* (10.7)
Colchicine	0.049±0.010** (18.3)	0.035 ± 0.001*** (28.6)	2.062±0.100*** (190)
MO+colchicine (250mg/kg)	0.078±0.008# (3)	0.034 ± 0.001	0.828±0.027### (16.9)

Values are mean ± SEM, (n=6). ***$P<0.001$ compared to sham control; ### $P<0.001$, ##$P<0.001$, #$P<0.05$ compared to colchicine infused rats
Values in parentheses are percentage changes observed compared to control data

Table V. Effect of MO extract on brain electrical activity of colchicine infused rats
(Over duration of 4 h)

Groups	Alpha waves (per epoch or 300 mm)	Beta waves (per epoch or 300 mm)	No. of spikes (per epoch or 300 mm)
Sham control	3.17 ± 0.38	11.00 ± 0.45	0
MO + control	3.92 ± 0.49	12.67 ± 1.02* (15.18)	0
Colchicine	2.51 ± 0.24*** (20.82)	2.75 ± 0.11*** (75)	11.67 ± 0.84*** (116.7)
MO + colchicine	2.49 ± 0.16	6.83 ± 0.70### (37.8)	2.75 ± 0.36### (76.44)

Values are mean ± SEM, (n=6). ***$P<0.001$ compared to sham control; ### $P<0.001$, ##$P<0.001$,#$P<0.05$ compared to colchicine infused rats.
Values in parentheses are percentage changes observed compared to control data

the cognitive processes of attention and memory[16]. MO leaf extract may possibly act by stimulating the adrenergic receptors in different brain areas but further research is required to understand the mechanism. Our study corroborates with earlier reports which revealed that noradrenergic deficits is linked to depression, dementia, diminished alertness and concentration[17]. MO acts in these regions differentially to protect the neurons causing less diminution of neurotransmitters like NE and thereby less impairment in memory.

Dopaminergic neurons arise from corpus striatum and project to mesolimbic and mesocortical areas of the brain, which constitute the behavioural pathway. Dopaminergic activity has been related to motivational behaviour and drive in rats[18]. DA-mediated neurotransmission has also been related to response selection and habit learning in rats[1]. Thus, DA can be involved in plural process supporting learning and memory[1]. In our study, ICV infusion of colchicine decreased DA level in CC, HC and CN significantly. Reduced DA may be due to reduced availability of NE as NE neurons are affected in these regions. Studies reveal that decreased DA level in caudate nucleus is associated with AD[19,20] whereas excess of DA can lead to psychosis, elation and confusion[14]. Stahl[21] has reported that reduced dopamine turnover in pre frontal cortex and medial striatum leads to impairments in acquisition process. MO treatment for 14 days, increased DA level in CC, HC and CN. This region specific effect may be due to differential effect of the MO extract in various brain regions. MO may have a protective action on dopaminergic neurons in cortical and hippocampal region and thus may support memory process.

Most of the brain 5-HT is localized in the thalamus, hypothalamus, midbrain and raphe nuclei of the lower brain stem[22]. There has been growing evidence that the 5-HT system is important in the regulation of memory and thus might be associated with AD while research results on this issue have been inconsistent[23]. Serotonin has been shown to be linked to emotional behaviour in rat[24]. Thus it may be presumed that the anxiety state in Alzheimer's disease may be linked with disturbed serotonergic activity. In our results, colchicine infusion in lateral ventricle produced a significant decrease in 5-HT level in CC but increased in CN whereas following MO treatment 5-HT level was increased significantly in CC only.

Long-term potentiation (LTP) has been advanced as a leading candidate for the neurophysiological substrate in learning and memory[25] and LTP is affected by changes in cholinergic, dopaminergic, noradrenergic and serotonergic systems[26]. It is difficult to assign behavioural effects to alteration in single neurotransmitter due to the complex nature of the brain neuronal circuitry, interaction of neurotransmitters and different effects that these transmitters at different synapses. Our EEG studies showed that α and β wave frequency was significantly suppressed in colchicine infused rats and spike wave pattern was significantly increased. In Alzheimer rats, the co-ordinated function of the brain is lost and also the thinking abilities are diminished which was evident from our EEG results. The spike wave pattern in Alzheimer rats may be either due to decreased DA level in CN or decreased 5-HT level in cerebral cortex or both. The spike discharge may lead to aggression state in AD. It well known that DA has an inhibitory action in CN[27] and decreased DA level in CN may cause excitation leading to spike discharge. On the other hand, 5-HT shows inhibitory action in CNS. So, decreased 5-HT in CC may have led to increased number of spike discharges. Treatment with MO showed an increased in beta wave frequency together with a significant decrease in the number of spike discharge suggesting a role in improving co-ordinated and integrated function of the brain. MO leaf is a potent source of antioxidants like vitamin C, E and other flavonoid compounds, aminoacids, alkaloids and various other compounds, which may be a potent memory enhancer.

In conclusion, based on our results it may be suggested that MO helps to provide some protection against Alzheimer's disease in rat model by causing alteration in brain electrical activity and altering monoamine level in discrete brain regions. Further studies need to be done to understand the mechanism.

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