Nootropic Effect of Berberine in Colchicines Induced Experimental Alzheimer's Disease Model: Effect on Cholinergic Neurotransmission

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Abstract

Background & Objectives: Colchicine administration by ICV is well known model which shows sporadic dementia of the Alzheimer type in humans, causing cognitive impairment and oxidative damage. Berberine (BBR) is a naturally occurring flavonoid. Literature suggests multiple activities berberine. Hence it may act as a promising agent to combat AD. The present study has been designed to investigate the protective effects of berberine against the colchicine-induced cognitive impairment by modulating cholinergic neurotransmission in mice.

Methods: Colchicine (15 microg/5 microL), administered intracerebroventricularly, resulted in poor memory retention in both the Morris water maze task paradigms. Mice received chronic treatment of BBR at a sub effective and effective dose of (5 and 40 mg/kg per day, PO respectively) along with nicotine and mechamylamine respectively for a period of 25 days beginning 4 days prior to colchicine administration. For cholinergic system modulation study Nicotine and Mecamylamine was given I.C.V as agonist and antagonist respectively.

Results: In present investigation, BBR in sub effective dose do not show any ant amnesic activity but when it is given along with Nicotine it significantly decreases the latency time on as compared to BBR alone in MWM task. Similarly is the case with mecamylamine and BBR at effective.

Conclusions: Our results suggest that BBR provides ant amnesic effects and that may be through modulation of nicotinergic receptors in colchicine's induced memory impairment model and further investigation of the BBR for therapeutic use in treating AD is warranted.

Keywords: AD, Berberine, Nicotine, Colchicine, Morris Water Maze.

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Introduction

AD is characterized by memory impairment and behavioral disturbances. Extracellular senile plaques and intraneuronal neurofibrillary tangles (NFTs) are the two classic hallmark microscopic pathologies of AD[1]. Cholinergic neurons get degenerated in AD both in human and in experimental animal models. Microtubular dysfunction is mainly responsible for sporadic dementia of Alzheimer's type (SDAT) and neurofibrillary tangles and senile plaques are specific characteristics[2]. It is also well reported that central administration of colchicine (a microtubule disrupting agent) produces time and dose-dependent behavioral, anatomical and neurochemical deficits, when injected directly into the areas associated with learning and memory such as dentate gyrus of the hippocampus and nucleus basalis of the neocortex[3].

AD is a progressive neurodegenerative disorder characterized by cholinergic dysfunction and deposition of Aβ in regions of the brain which are associated with learning and memory process[4]. Most common neuropathological feature of AD is the loss of cholinergic neurons in the basal forebrain. The neurotransmitter involved in several cognitive functions such as learning and memory is acetylcholine (ACh). For nicotinic receptors, there is considerable evidence for a reduction in both $\alpha_4\beta_2$ and α_7 receptors in AD, thus implicating these receptors in the pathogenesis of this dementia[5]. Nicotine has been proved improve memory performance in various studies and may be useful for treatment of disease related memory impairments such as AD[6]. It has been reported that expression of $\alpha_4\beta_2$ and α_7 nicotinic acetylcholine receptors is reduced in AD, approximately 50% decrease in the number of α_7-containing neurons in the temporal cortices of patients with AD, without overall loss in neuron number[7].

Alzheimer's disease pathogenesis of is mainly linked to ACh deficiency in the brain. A number of reports proved that berberine exerts AChE inhibitory activities[8,9,10]. In diabetic rats, chronic treatment with berberine during MWM training session improved cognitive performance and AChE activity[11].

In conclusion, the findings of the present literature survey suggest that berberine exerts its beneficial effects in treating cognitive and neural dysfunction. So the present study was designed to investigate the antiamnesic activity of berberine using colchicines induced memory impairment model in mice and its modulation by cholinergic neurotransmitter system.

Materials and Methods

Animals and housing: Swiss Albino Mice (20–25g) were used in present study. The animals were housed under standard laboratory conditions and having free access to food and water. Mice were acclimatized to the laboratory conditions before experimentation.

All protocols were carried out with prior approval of Institutional Animal Ethics Committee, (IAEC Protocol no. IAEC/PC/03/2011-2012), in compliance with the CPCSEA guidelines, Ministry Of Environment And Forests, Government of India, New Delhi.

ICV Administration of Colchicine

Surgery for ICV administration was done as per the previously published protocol[12]. In short animals were anaesthetized with ketamine and xylazine combination and properly placed in a stereotaxic apparatus. A midsagital dorsal incision was made through the scalp and bregma and lamda were identified to place cannula. A guide cannula was sterotaxically implanted in both lateral ventrical with the stereotaxic coordinates from Paxinos and Watson, 1998 (AP -0.82 mm; ML +1.5mm and DV +2.0 mm; respected to bregma). A stainless steel dummy cannula was used to occulude the guide cannula when not in use. The animals were then allowed to recover for week. During this recovery time they were habituated to the experimental protocols to minimize nonspecific stress. For I.C.V administration of colchicines and aCSF, microsyringe was connected to the internal cannula (29 gauges) by PE-10 polyethylene tubing. The internal cannula was inserted into the guide cannula and a volume of 1 µl/ mouse was injected over a period of 1min. The internal cannula was held in position for another 1min in order to allow for the diffusion of the injected volume and prevents pressure-induced damage.

Drugs and treatment schedule

Colchicine, Berberine, Nicotine and Mecamylamine (Sigma Aldrich) solutions were made fresh at the beginning of each experiment. Colchicine, Nicotine and Mecamylamine were prepared in artificial cerebrospinal fluid (aCSF), such that a 15 µg dose was delivered in a volume of 10µl injection ICV administration in each mouse. The aCSF was prepared by dissolving various salts in double distilled water. Berberine was suspended in normal saline and was given orally using oral gavage. The sub effective and effective doses of berberine were selected based on the previous reports in the literature[13]. Nicotine and mecamylamine were given by ICV route.

Treatment groups are as follows

1. Colchicine (15 µg/10 µL) + vehicle for berberine
2. Berberine (5 mg/kg, PO) + colchicine
3. Berberine (40 mg/kg, PO) + colchicine
4. Nicotine (0.25µg/µl) + colchicine
5. Berberine (5 mg/kg, PO) + nicotine + colchicine
6. Mecamylamine (50 ng/µl) + colchicine
7. Berberine (40mg/kg, PO) +Mechamylamine + colchicines

Assessment of cognitive function

Morris water maze test: Memory functions of rats was assessed by using MWM apparatus (VJ Instruments) which consist of a circular pool, painted black (120cm diameter × 51 cm) as described earlier with slight modifications (Morris, 1984). A black painted platform (10cm diameter) was placed in the water dipped below the water so that animals were unable to see it. Animals were trained to swim to reach a platform placed in any one quadrant (N, S, E, and W). The MWM was filled with water (26±2° C) to depth of 30 cm. The mice were required to find the platform throughout the testing for 5 consecutive days and 4 acquisition trials were given per day. Mice were given 4 trials/day by changing the platform in each trial and inter trial interval was 30 sec. The cut-off time was 60 sec to find the platform and mice were allowed to stay on it for 15 sec.

Acquisition test: in Acquisition test mice were given trials to find the hidden platform placed in a quadrant. For 4 consecutive days, mice were given 2 trials per day by changing the platform placed at the quadrant. The mice were 60 sec to search the hidden platform in MWM. Escape latency i.e. time require to find out platform, was calculated in sec.

Retrieval Test (Probe Test): To check the ability of animal to retain and recall the previously learned information this test was performed. After 4 days of acquisition trial, a retrieval trial was performed at time interval of 24 hours during which the platform was removed from the pool and the trained animal was allowed to swim freely for 30 sec. The time spent by the mice to search the platform in the target quadrant where the platform was previously placed during the acquisition test was calculated.

To find out the mechanism of Berberine in AD, mice received chronic treatment of BBR at a sub effective 5 mg/kg and effective dose of 40 mg/kg per day, PO (based on previous studies)[13] along with nicotine and mecamylamine respectively for a period of 25 days beginning 4 days prior to colchicine administration. For cholinergic system modulation study Nicotine and Mecamylamine was given I.C.V as agonist and antagonist respectively.

Animals were subjected to MWM on days 18, 19, 20, and 21 60 min after the berberine treatment. Probe trial was conducted for 30 sec on next day of acquisition test.

Locomoter activity

Locomoter activity was assessed in the actophotometer (Inco, Ambala), for the period of 5 mins. Apparatus which has a lid covered square chamber (30cm × 30cm× 25 cm), and equipped with six pairs of light sources and sensors these are connected to digital counter to record the number of interruption. Locomoter activity was expressed in the terms of total number of counts of light beam irruptions in 5 min.

Statistical analysis

All values are expressed as mean±SEM. The behavioral assessment data were analyzed by a repeated measures one way ANOVA and two-way ANOVA with drug-treated groups as between and sessions as the within-subjects factors. Post-hoc comparisons between groups were made using Post Hoc Bonferroni Multiple Comparision Test and Tukey test. The value $P < 0.05$ was considered as significant.

Results

Effect of Nicotine on Berberine mediated acquisition in MWM

Prior administration of Nicotine with sub effective dose of Berberine (5mg/kg) significantly decreased escape latency as compared to Berberine group. Application of two way ANOVA showed significant interaction between variables namely; treatments and acquisition days [F (9, 80) = 1.89, P < 0.0001]. Post Hoc Bonferroni Multiple comparision Test revealed that prior administration Berberine along with Nicotine significantly reduce latency time on 3rd and 4th days but not on day first and second of the activity. On third day from 47.66±10.43 to 19.83±4.03(P < 0.05), and fourth day from 41.33±9.56 to 9.166±1.96 (P < 0.01).Two Way ANOVA showed a main effect of treatment [F (3,80) = 7.45, P < 0.0001] and acquisition days [F (3, 80) = 9.23, P < 0.0001].(Table 1, Fig. 1a).

Table 1: Effect of Nicotine on Berberine mediated acquisition in MWM

Treatment	Mean escape latency (sec ± S.E.M)			
	Day 1	Day 2	Day 3	Day 4
COL.	58±5.958	56.5±5.210	57.5±2.100	59.83±3.81
BBR. 5 mg	57.16±10.31	53.5±8.057	47.66±10.43	41.33±9.56
NICO.	57.5±9.77	48.16±7.49	39.66±5.840	34.5±5.38*
NICO.+ BBR. 5 mg	56.83±7.3	45±5.754	19.83±4.03@	9.16±1.96 @@
BBR. 40mg	56.50±9.77	39.33±7.49	30±5.840**	18±5.38***
MECA.	57±9.77	56.5±7.49	51.66±5.840	50.5±5.380
MECA. + BBR. 40mg	59.5±2.51	57.83±2.66	53.16±2.22#	40.83±3.451#

Table 1. Effect of effective and subeffective doses of berberine with cholinergic agonist and antagonist. Values are represented as mean±SEM, n=6, ***P<0.001, **P<0.01, *P< 0.05 v/s colchicine, @@P < 0.01, @ P < 0.05 v/s Berberine (5 mg/kg) and #P<0.05 v/s Berberine (40 mg/kg). (Colchicine: COL, Berberine: BBR, Nicotine: NICO, Mecamylamine: MECA).

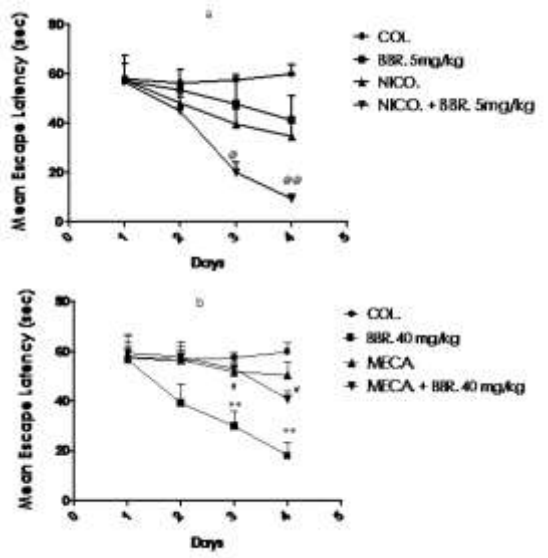

Fig. 1: Effect of Nicotinergic agonist Nicotine (1a) and antagonist Mecamylamine (1b) on Berberine mediated memory performance in MWM. ***P<0.001, **P<0.01, *P< 0.05 v/s colchicine, @@P < 0.01, @ P < 0.05 v/s Berberine (5 mg/kg) and #P<0.05 v/s Berberine (40 mg/kg). Values are represented as mean±SEM and analysed by Two Way ANOVA followed by Post hoc Bonferroni multiple comparison. (Colchicine: COL, Berberine: BBR, Mecamylamine: MECA, Nicotine: NICO)

Effect of Mecamylamine (Nicotinergicantagaonist) on Berberine mediated acquisition in MWM

Prior administration of Mecamylamine with effective dose of Berberine (40 mg/kg) significantly decreased escape latency as compared to Berberine group. Application of Two Way ANOVA showed significant interaction between variables viz; treatments and acquisition days [F 9, 80) = 1.73, P < 0.0001]. Post Hoc Bonferroni Multiple Comparision Test revealed that prior administration Berberine along with Mecamylamine significantly reduce latency time on 3rd and 4th day but not on day first and second of the activity, on third day from 53.166±2.22 to 30±5.840 (P < 0.05), and fourth day from 40.83±3.451 to 18±5.38 (P < 0.05). Two Way ANOVA showed a main effect of treatment [F (3, 80) = 5.10, P < 0.0001] and acquisition days [F (3, 80) = 11.18, P < 0.0001].(Table 1, Fig. 1b)

Effect of Nicotinergic Modulators Berberine mediated retrival in MWM

Prior administration of Nicotine with Berberine significantly showed an increase in time spent in target quadrant as compared to Berberine group while no significant difference was found in case of Mecamylamine. It was found that Nicotine and

Berberine treated mice spent 30 sec in target quadrant as compared to Berberine (5 mg/kg) and for Mecamylamine and Berberine treated mice spent 25 sec in target quadrant as compared to Berberine (40mg/kg) (Fig. 2).

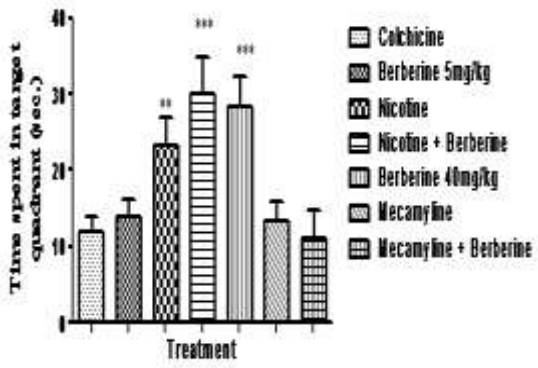

Fig. 2: For probe trial data was analysed by One Way ANOVA followed by Tukey's, test. Values are represented as mean±S.E.M, n=6, *P<0.05 v/s colchicine, @P<0.05, @@P<0.01 v/s Berberine (5 mg/kg), #P<0.05 v/s Berberine (40 mg/kg). (Colchicine: COL, Berberine: BBR, Mecamylamine: MECA, Nicotine: NICO)

Effect of Nicotinergic Modulators with Berberine on locomotor activity

In present experiment mean scores of locomotor activity for each mouse was relatively stable and showed no significant difference. Mice treated with cholinergic modulators did not cause any alteration in locomotor activity compared among the groups (Fig. 3). Repeated measures ANOVA revealed that there was no significant difference in drug treatment, session, and a significant drug treatment- session interaction.

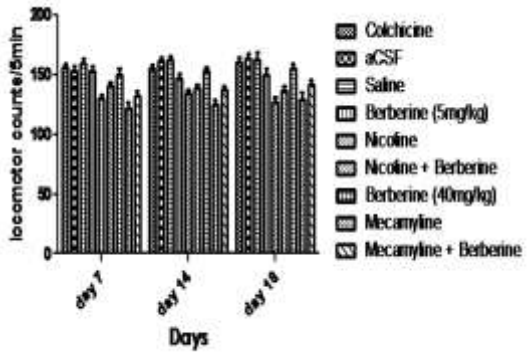

Fig. 3: Effect on locomoter activity, no statistically significant data obtained when data was analyzed by two way ANOVA followed by Post hoc Bonferroni multiple comparison. (Colchicine: COL, Berberine: BBR, Mecamylamine: MECA, Nicotine: NICO)

Discussion

The present study showed that chronic administration of Berberine was found to improve the memory acquisition and retention induced in AD like disease model induced by ICV colchicine administration and there is involvement of cholinergic neurotransmitter modulatory action. Many reports revealed that central administration of colchicine produces a time and dose-dependent anatomical, behavioral and neurochemical changes following colchicine administration[14]. Memory impairment has a slow onset and takes about 14–21 days. It is characterized by a progressive deterioration of cognitive functions, microtubule disruption. Thus, the ICV model can be considered as a relevant model to explain sporadic dementia of Alzheimer's type (SDAT)[15].

The cholinergic system has long been implicated in cognition, and there is a huge data showing the relation between cholinergic deficits and cognitive impairments in animal models and those accompanying neurodegenerative diseases or normal ageing in humans. Acetylcholine (ACh) is central neurotransmitter involved in several cognitive functions including learning and memory. In light of this observation we hypothesized that berberine mediated improvement in learning and memory which may be mediated by cholinergic systems in AD like condition.

To find out nicotinergic modulatory activity on berberine we have used nicotine as agonist and mecamylamine as antagonist. In present investigation, berberine in subeffective dose do not show any antiamnesic activity but when it is given along with Nicotine it significantly decreases the latency time on 3rd and 4th day as compared to Berberine alone. This means nicotinergic transmission may modulate the antiamnesic activity of Berberine through nicotinic receptors.

To further confirm our hypothesis, we investigated the effect of nicotinic receptor antagonist, mecamylamine, on Berberine induced effect in MWM. In line with the above findings, in mecamylamine pretreated mice, berberine at effective dose do not showed any significant increase in latency time as compared to Berberine treated group, indicating attenuation of anticholinergic pathway by Berberine induce antiamnesic activity.

From the above results the amelioration of colchicine induced memory impairment could be due to enhancement of Nicotinergic neurotransmission which indirectly modulate the cholinergic neurotransmission and chronic administration of Berberine may enhance memory by acting through one of this neurotransmission system.

The use of Berberine warrants evaluation for the treatment of neurological disorder, which is associated with free radical generation and cognitive impairment such as AD.

Conclusion

In summary the result of the present study suggest that antiamnesic activity of Berberine might be related to Nicotinergic system by stimulating nAChR which may enhance memory.

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Conflict of interest: Authors do not have any conflict of interest.

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