



## ANTIAMNESIC EFFECT OF BERBERINE IN COLCHICINES INDUCED EXPERIMENTAL ALZHEIMER'S DISEASE MODEL

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### ABSTRACT

Central administration of colchicine is well known to cause cognitive impairment and oxidative damage, which simulates sporadic dementia of the Alzheimer type in humans. Berberine, a natural product alkaloid, has been shown to display a wide array of pharmacological effects. The present study has been designed to investigate the effects of berberine against the colchicine-induced cognitive impairment in mice. Memory performance task was accessed using Morris water maze. Colchicine (15µg/5µL) was administered ICV, resulted in poor memory retention in the Morris water maze task. Mice received chronic treatment of four doses (5, 10, 20, 40mg/kg per day, PO) of berberine for a period of 21 days beginning 4 days prior to colchicine administration. Berberine treatment significantly ameliorated learning deficits, long-term spatial memory retention. Our results suggest that berberine provides anti-amnesic effects in colchicines induced memory impairment model and promising agent for the treatment of AD.

**KEYWORDS:** Alzheimer's disease (AD), Morris Water Maze; Berberine, Colchicine, Memory



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## INTRODUCTION

Alzheimer's disease (AD) is a progressive neurodegenerative disorder and causes significant dementia in elderly. Sporadic dementia of Alzheimer's type (SDAT) has been shown to be associated with microtubular dysfunction and is characterized by the appearance of specific cytoskeletal cellular abnormalities, including neurofibrillary tangles and senile plaques<sup>1</sup>. It is also well reported that central administration of colchicine a microtubule disrupting agent produces marked destruction of hippocampal granule cells, mossy fibers and septohippocampal pathways. It induces neurofibrillary degeneration by binding to tubulin, the principal structural protein of microtubule<sup>2-3</sup>. Biochemical analysis demonstrated that the intracerebroventricular (ICV) administration of colchicine injection significantly induced lipid peroxidation, increased nitrite, and depleted reduced glutathione (GSH) and acetyl cholinesterase (AChE) level in rat brains and consequent oxidative damage leads to cognitive dysfunctions<sup>4-5</sup>. Thus, the ICV colchicine model is relevant to SDAT in humans as both are characterized by a progressive deterioration of cognitive functions, microtubule destruction and decrease in ChAT activity<sup>6-7</sup>. Thus, colchicines-induced cognitive impairment and oxidative stress in animals via ICV administration is widely accepted as a suitable model to study the disease pathogenesis, drug screening and to examine new therapeutic modalities in the treatment of AD. The flavonoids are a group of naturally occurring polyphenolic compounds found in plants and are frequently consumed in human diet. Flavonoids show wide array of activities. In particular, polyphenols have been reported to exert their neuroprotective actions through the potential to protect neurons against injury induced by neurotoxins, an ability to suppress neuroinflammation, and the potential to promote memory, learning, and cognitive function<sup>8</sup>. Flavonoids are highly effective in reversing age-related declines in neuro-

cognitive performance through their ability to interact with the cellular and molecular architecture of the brain responsible for memory and by reducing neuronal loss due to neurodegenerative processes<sup>9</sup>. Berberine has been found in the roots, rhizomes, and bark of a number of plants and its based formulations, are widely used in traditional systems of medicine including, Ayurveda and Traditional Chinese Medicine<sup>10</sup>. Berberine has demonstrated a wide range of pharmacological activities including; antihypertensive, anti-inflammatory, antioxidant, antidepressant, anticancer, anti-diarrhoeal, hepatoprotective and antimicrobial<sup>11</sup>. Many reviews have indicated that berberine has a well-documented neuroprotective effect against cerebral ischemia, mental depression, schizophrenia, anxiety, and AD<sup>12-14</sup>. The multiple activities of berberine, including antioxidant, AChE and butyrylcholinesterase (BChE) inhibitory, monoamine oxidase (MAO) inhibitory, A $\beta$  level-reducing and cholesterol-lowering activities, which suggest that berberine may act as a promising multipotent agent to combat AD<sup>11</sup>. 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 anti-amnesic activity of berberine using colchicines induced memory impairment model in mice which closely mimic the AD type of dementia.

## MATERIALS AND METHODS

### *Animal and housing*

Swiss Albino Mice (20–25 g) were used in present study. The animals were housed under standard laboratory conditions, maintained on natural 12:12h light and dark cycles, relative humidity (50-70%) and temperature controlled (25  $\pm$  1°C) and having free access to food and water. Animals were acclimatized to the laboratory conditions prior to experimentation.

All the experiments were carried out between 09:00 and 15:00 h. All protocols were carried out with prior approval of the Institutional Animal Ethics Committee (IAEC) no. IAEC/PC/03/2011-2012, Rashtrasant Tukdoji Maharaj Nagpur University, Nagpur, in strict compliance with ethical principles and guidelines of the Committee For The Purpose Of Control And Supervision Of Experimental Animals (CPCSEA), Ministry Of Environment And Forests, Government Of India, New Delhi.

#### **(i) Surgery and ICV Administration of Colchicines**

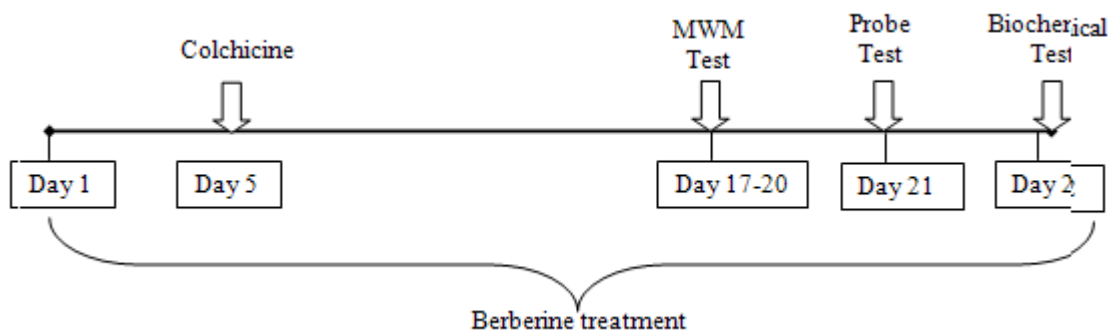
Surgery was performed according to previously described protocol with minor modifications<sup>5</sup>. Animals were anaesthetized with Ketamine (100 mg/kg, I.M) and Xylazine (5mg/ kg, I.M) combination and positioned in a stereotaxic apparatus. A midsagittal dorsal incision was made through the scalp and the skin was retracted. The soft tissue overlying the skull was then removed. Two holes were drilled through the skull for placement of injection cannula into the lateral cerebral ventricle. The scalp was then closed with suture. In sham-operated mice, the surgery was identical except for drilling of holes and placement of the cannula. Coordinates for the injection were 0.5 mm caudal and 1 mm lateral from bregma, depth 2.5 mm. Colchicine (15µg/5 µl), dissolved in freshly prepared artificial cerebrospinal fluid (aCSF), was administered slowly by intracerebroventricular (ICV route). To promote diffusion, the micro syringe was left in place for a period of 2 min following injection. Special care was taken during the postoperative period to provide food and water inside the cage of the mouse<sup>15</sup>.

#### **Drugs and treatment schedule**

Colchicine and berberine (Sigma Chemicals Co., St. Louis, MO, USA) solutions were made fresh at the beginning of each experiment. Colchicine was prepared in artificial cerebrospinal fluid (aCSF) such that a 15 µg dose was delivered in a volume of 5µl injection for intracerebroventricular (ICV) administration in each mouse. aCSF (aCSF; in mmol/l: 147 NaCl, 2.9 KCl, 1.6 MgCl<sub>2</sub>, 1.7 CaCl<sub>2</sub>, and 2.2 dextrose) was prepared by dissolving various salts in double distilled water or water for injection IP. For oral administration, berberine was suspended in normal saline and was given using oral gavage. Animals were divided randomly based on their body weights into seven groups. The groups were set as shown below. Doses of berberine were selected based on the previous reports in the literature.

1. Sham-operated (vehicle for berberine)
2. aCSF (aCSF + vehicle for berberine)
3. Colchicine (15 µg/5µl + Saline)
4. Berberine (5 mg/kg, PO) + colchicine
5. Berberine (10 mg/kg, PO) + colchicine
6. Berberine (20 mg/kg, PO) + colchicine
7. Berberine (40mg/kg, PO) + colchicine

To study the influence of berberine in AD like condition induced in mice, different group were treated with saline (10 ml/kg, p.o) or berberine at various doses (5, 10, 20, 40 mg/kg p.o) for the period of 21 days and colchicines (15 µg/ mouse) in 5µl, I.C.V was injected on 5<sup>th</sup> day after start of saline or berberine treatment. Animals were subjected to Morris water maze (MWM), 60 min after the berberine treatment P.O on days 17-20 after start of berberine treatment. Probe trial was conducted on 21<sup>st</sup> day for 30 sec (Fig 1).



**Figura 1**  
**Schematic representation for experimental procedure**

### **Behavioral Assessment**

#### **(i) Morris water maze (MWM) test**

Cognitive function of rats was assessed by using MWM test as described earlier with minor modifications<sup>16-17</sup>. Animals were trained to swim to reach a platform in a circular pool, painted black (120cm diameter × 51 cm located in test room. The pool was filled with water (26± 2° C) to depth of 30 cm. A round platform of 10cm diameter was placed in the water dipped 1cm below water. The mice were required to find the platform using only distal spatial extra-maze cues available in the testing room. The cues were maintained constant throughout the testing. For 5 consecutive days, 4 acquisition trials were given per day with a fixed platform at the centre of one quadrant and inter trial interval was 10 min. The mice were given a maximum time of 60 sec (cut-off time to find the platform) and were allowed to stay on it for 15 sec.

**Acquisition Test:** Acquisition test involves training of mice to find the hidden platform placed in a quadrant. For 4 consecutive days, mice were given 4 trials per day by changing the platform placed at the quadrant. The mice were allowed to swim for 60 sec. in MWM. Escape Latency i.e. time require to find the platform, was noted in sec.

**Retrieval Test (Probe Trial):** Retrieval test (probe test was performed to check the ability of animal to retain and recall the previously learned information. A retrieval trial was performed 24 hours after the last acquisition

test during which the platform was removed from the pool and the trained animal was allowed to swim freely for 30 sec. In this probe trial the spatial accuracy of the animal was determined represented by the time spent by the mice in searching the platform in the target quadrant where the platform was previously placed during the acquisition test.

#### **(ii) Assessment of Gross Behavioral Activity (Closed field activity)**

Gross behavioral activity was observed on same days on which MWM paradigm was tested. Animal was placed in a square (30 cm×30 cm×25 cm) closed arena equipped with infrared light-sensitive photocells using digital photoactometer. The animals were observed for a period of 5 minutes and the values were expressed as counts/5 minutes.

#### **Tissue collection and preparation of homogenate**

Biochemical tests were carried out 24 h after the last behavioral test on day 17 following colchicine injections i.e. on day 18. Animals were sacrificed by decapitation and the brains were removed and rinsed with ice-cold isotonic saline. Brain tissue samples were then homogenized with 10 times (w/v) ice cold 0.1 M phosphate buffer (pH = 7.4). The homogenate was prepared and then centrifuged at 10,000 × g for 15 min and aliquots of supernatant was separated and used for biochemical estimation.

### **Estimation of biochemical parameters**

#### **(i) Measurement of MDA**

Malondialdehyde (MDA) level was estimated in mice brain as described previously<sup>18</sup>. Brain homogenate (300 µl) was mixed with 30% trichloroacetic acid (TCA), 5 N HCl followed by the addition of 2% thiobarbituric acid (TBA) in 0.5 N NaOH. The mixture was heated at 90°C for 15 min and centrifuged at 12,000 g for 10 min. The pink colour of the supernatant was measured at 532 nm using UV visible spectrophotometer then MDA concentration was calculated using standard curve which was prepared with Tetra ethoxy propane and expressed as nmol/mg protein.

#### **(ii) Measurement of GSH**

Glutathione (GSH) level was estimated by Ellman method<sup>19</sup>. The brain homogenate and 10% TCA (1:1) were mixed and kept on ice for 10 min, then centrifuged at 2,000 g for 10 min at 4°C and supernatant was collected and used for GSH estimation. The supernatant was mixed with phosphate buffer (pH 8.4) and DTNB. The absorbance was read at 412 nm using UV visible spectrophotometer. GSH concentration was calculated by using standard curve prepared with reduced glutathione and expressed as µg/mg protein.

#### **Protein estimation**

The protein content was estimated by biuret method<sup>20</sup> using bovine serum albumin as a standard.

#### **Statistical analysis**

Values are expressed as mean ± SEM. The behavioral assessment data were analyzed by a repeated measures two-way ANOVA with drug-treated groups as between and sessions as the within-subjects factors. The biochemical estimations were separately analyzed by one way ANOVA. Post-hoc comparisons between groups were made using Tukey's test. The value  $P < 0.05$  was considered significant.

## **RESULTS**

#### **(i) Effect of berberine on colchicine induced memory impairment in MWM in acquisition trial**

In the present experiment sham operated, aCSF injected mice showed significant decrease in escape latency from day one onwards of Morris water maze task which indicate spatial learning. In contrast colchicine treated group learn poorly and significantly increased escape latency to reach the platform in acquisition training as compared to aCSF group on successive three days except first day in MWM session. Application of repeated measure two-way ANOVA showed interaction between variables viz, treatment and acquisition days in aCSF and colchicines group (Fig 2). Effect of berberine at various doses on colchicine induced memory impairment was studied using MWM paradigm on day 13,14,15,16 after colchicine injection. Prior administration of berberine with colchicines significantly decreased escape latency in dose dependent manner. Treatment with berberine (5mg/kg) showed significant reduction in escape latency on day three onwards in acquisition test. Similarly, berberine 10 mg/kg, 20 mg/kg and 40 mg/kg showed significant reduction in latency time to reach the platform on day second onward as compared to colchicine group in acquisition test (fig 2). Application of repeated measure two way ANOVA showed a significant interaction between variables viz; treatments and acquisition days [ $F(18, 90) = 5.09, P < 0.0001$ ]. Post Hoc Tukey multiple comparison tests revealed that prior administration of berberine significantly decreased the escape latency. Two way ANOVA revealed a main effect of treatment [ $F(6, 30) = 118, P < 0.0001$ ] and acquisition days [ $F(3, 15) = 389, P < 0.0001$ ].

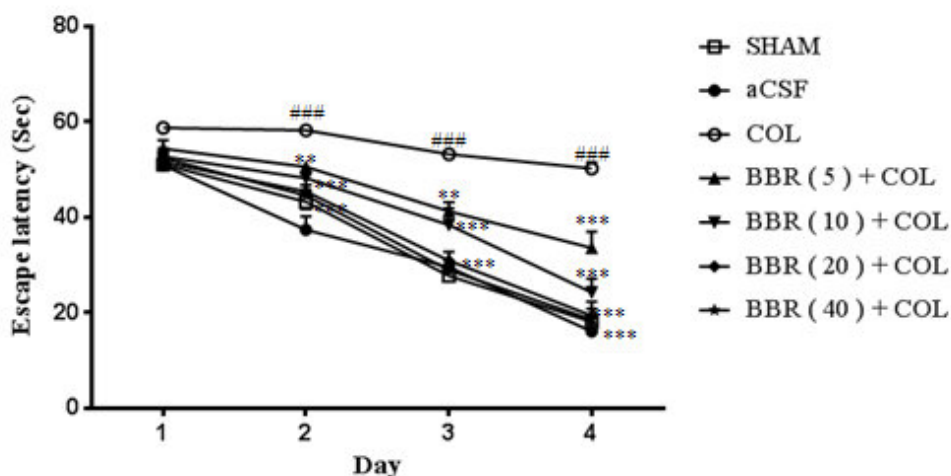
#### **(ii) Effect of berberine on colchicine induced memory impairment in MWM in retention trial**

Colchicine significantly decreased time spent in target quadrant in probe trial session, i.e on 17<sup>th</sup>

day after colchicine treatment, as compared to aCSF group. Further berberine (BBR) at various doses there was increase in time spent in target quadrant during probe trial session i.e. on day 17 of activity after colchicine treatment.

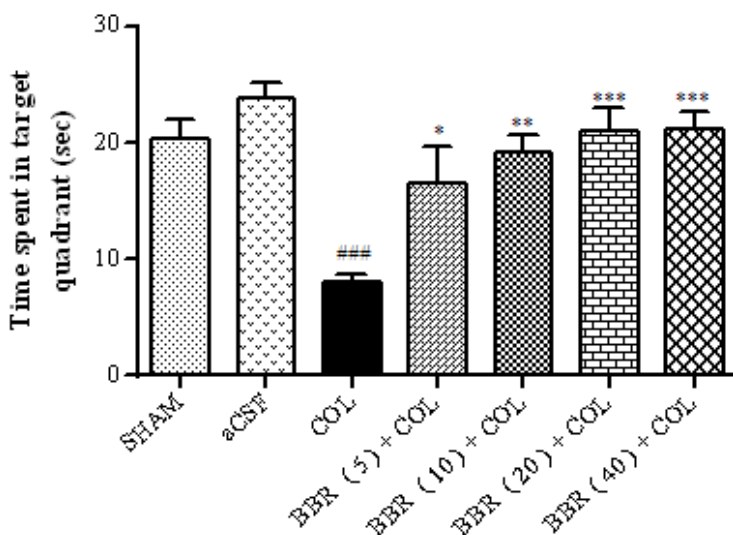
Application of One Way ANOVA showed significant difference in berberine group (5, 10, 20 and 40 mg/kg) when compared with colchicine treated mice (fig 3).

**Figure2**  
**Effect of various doses of Berberine on Acquisition trial in MWM**



Effect of various doses of berberine (BBR) on acquisition trial in MWM task in colchicines (COL) injected mice on day 13,14,15,16 after colchicine treatment. Values are represented as mean  $\pm$  SEM (n=6), ###P < 0.001 as compared with aCSF group and \*\*P < 0.01, \*\*\*P < 0.001 considered as statistically significant as compared with colchicine group. Data was analyzed by One Way ANOVA followed by Tukey test.

**Figure3**  
**Effect of various doses of Berberine on probe trial in MWM task**



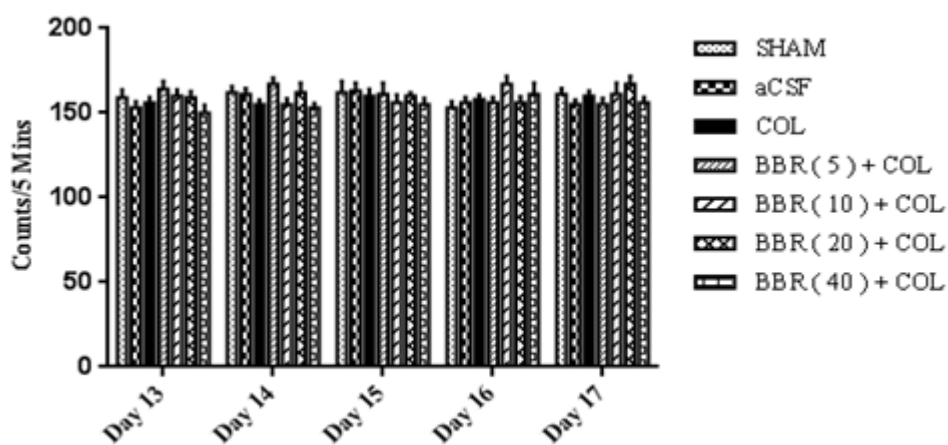
Effect of various doses of Berberine (BBR) on probe trial in MWM task in colchicines (COL) injected mice on day 17 after colchicine treatment. Values are represented as mean  $\pm$  SEM (n=6), ###P < 0.001 as compared with aCSF group and \*P < 0.05, \*\*P < 0.01, \*\*\*P < 0.001 considered as statistically significant as compared with Colchicine group. Data was analyzed by One Way ANOVA followed by Tukey test.

**Effect of Berberine on gross behavioral activity (Closed field activity)**

In the present series of experiments, the mean score of gross behavioral activity on for each mouse was relatively stable and showed no significant variation. The mean scores in sham-operated, aCSF- and colchicine- injected mice remained unchanged from the mean scores of gross behavioral activity observed on all days throughout the entire observation period.

Chronic administration of berberine (5, 10, 20 and 40 mg/kg/day, po) in colchicines injected mice did not cause any alteration in the gross behavioral activity as compared to colchicine-injected mice on day 13,14,15,16 and 17 after colchicine treatment. Repeated measures ANOVA revealed that there was no significant effect of drug treatment, session, and a significant drug treatment × session interaction (Fig 4).

**Figure4**  
**Effect of berberine on locomotor activity**



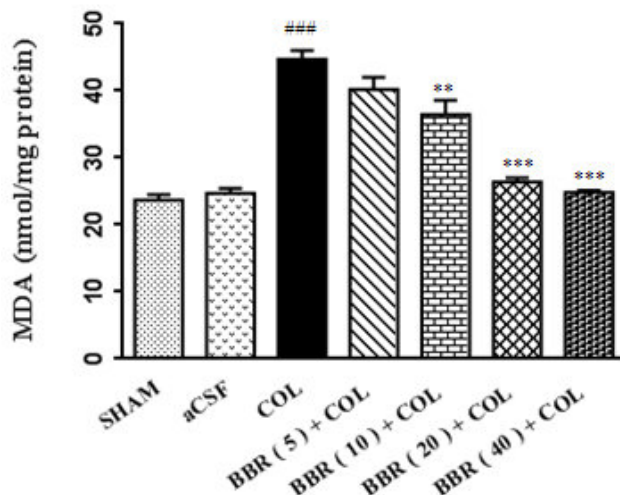
*Effect of berberine (BBR) 5, 10, 20 and 40 mg/kg, PO on locomotor activity in intracerebroventricular colchicines-(COL) injected mice. Values are mean ± SEM. Data was analyzed by two way ANOVA (n = 6 in each group).*

**Effect of berberine on MDA level in colchicine-induced memory deficit mice brain**

ICV administration of colchicine caused a significant increase in MDA level in mice brain as compared with aCSF groups. Preventive treatment with berberine at all doses (10, 20 and 40 mg/kg, PO) except 5mg/kg, significantly decreased MDA level in colchicine-injected mice brain. P < 0.05, was considered as statistically significant (Fig 5).

**Figure 5**

**Effect of berberine on MDA level in colchicine-induced memory deficit mice brain**



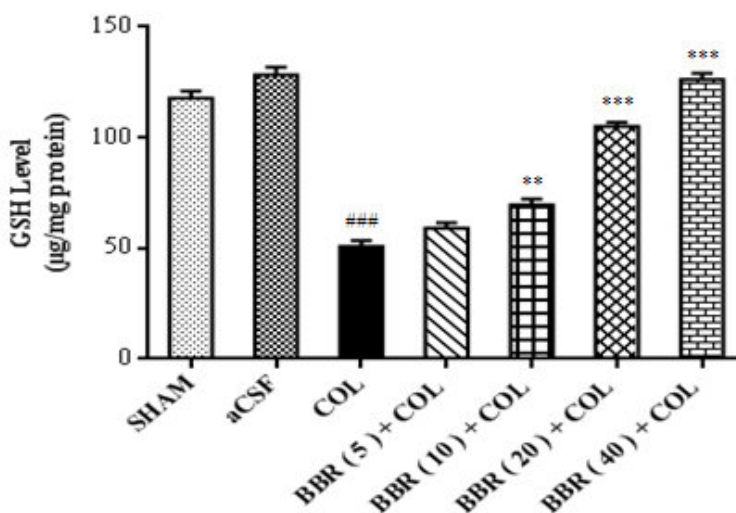
Effect of Berberine (BBR) 5, 10, 20 and 40 mg/kg, PO) on MDA (nmol/mg protein) level in colchicine treated mice brain. Results were expressed as mean  $\pm$  S.E.M. Significant difference ###P<0.001) vs aCSF group and \*\*P < 0.01, \*\*\*P < 0.001 vs colchicine group. Data was analyzed by One-way ANOVA followed by Turkey's test for multiple comparisons.

**Effect of berberine on GSH level in colchicine-induced memory deficit mice brain**

A significant fall in the levels of GSH was observed in the colchicine group as compared to the aCSF treated groups. Treatment with berberine at all doses (10, 20 and 40 mg/kg, PO) except 5mg/kg, significantly prevented the decrease in GSH levels in the brain of colchicine-injected mice. P< 0.05, was considered as statistically significant (Fig 6).

**Figure 6**

**Effect of berberine on GSH level in colchicine-induced memory deficit mice brain**



Effect of Berberine (BBR) 5, 10, 20 and 40 mg/kg, PO) on GSH (µg/mg protein) level in colchicine treated mice brain. Results were expressed as mean  $\pm$  S.E.M. Significant difference ###P<0.001 vs aCSF group and \*\*P < 0.01, \*\*\*P < 0.001 vs colchicine group. Data was analyzed by One-way ANOVA followed by Turkey's test for multiple comparisons.



## DISSCUSSION

The present study investigated the effect of berberine in the prevention of sporadic dementia of Alzheimer's type using intracerebroventricular (ICV) colchicine in mice. The findings of this study indicated that chronic treatment with berberine caused a significant improvement in the memory performance tasks as assessed in Morris water maze. Colchicine is a cytotoxic agent which binds irreversibly to microtubules and causes their depolymerization, thereby inhibiting their assembly. Microtubules are vital components of the neuronal cytoskeleton and play a crucial role in cell growth and differentiation, axonal and dendritic transport. This leads to impaired intracellular trafficking of neurotrophic factors, synaptic loss, and increased axonal excitotoxicity<sup>3, 21</sup>. Cytoskeletal disruption has been linked to neurodegeneration in AD<sup>22</sup>. Central administration of colchicine is characterized by progressive deterioration of learning and memory, oxidative stress, and decrease in acetylcholine turnover<sup>6-7</sup>. Thus, the ICV colchicines model can be considered as a relevant model to explain sporadic dementia of Alzheimer's type (SDAT)<sup>4</sup>. In the present study, intracerebroventricular administration of colchicine at a dose of 15 µg/mice in 5 µl induced spatial memory impairment as indicated by no significant reduction in escape latency time in Morris water maze test. This finding is in agreement with previous studies reporting impairment in memory following colchicine administration<sup>23-24</sup>. In our study chronic administration of berberine was able to improve the cognitive deficit in Morris water maze task. Preventive treatment with berberine dose dependently improved the memory in colchicines treated mice. This may be due to attenuation of oxidative stress, suggesting that berberine improves cognitive task. There was no significant effect on locomotor activity excluding the possibility that alteration in

locomotor activity may have contributed to the observed behavioral effects in Morris water maze after the ICV colchicine administration. Lipid peroxidation plays a major role in oxidative damage of lipids. Free radicals are normal products of cellular aerobic metabolism. However, when the production of free radicals increases or defense mechanism of the body decreases, they cause cellular dysfunction by attacking at the polyunsaturated sites of the biological membranes leading to lipid peroxidation. The key metabolites of lipid oxidation are malondialdehyde (MDA). It has been reported that oxidative stress parameters are generally higher in AD<sup>25-26</sup>. In present study, colchicine caused a significant increase in the MDA levels which are more responsible for the oxidative damage thereby leading to learning and memory deficits. Our results proved that the treatment with berberine able to ameliorate the colchicine induced increase in MDA levels in brain. Glutathione is an endogenous antioxidant present majorly in the reduced form within the cells. It reacts with the free radicals and prevents the generation of hydroxyl radicals. During this defensive process, reduced glutathione is converted to oxidized form with the help of the enzyme glutathione peroxidase<sup>27</sup>. Colchicines treatment decreases the level of reduced glutathione. In our study preventive berberine treatment was able to restore the GSH levels in colchicines treated animals.

## CONCLUSION

The present study showed that treatment with berberine ameliorated colchicine-induced memory impairment in mice. Furthermore, the beneficial effects may be attributed to reduced oxidative stress in the brain. Thus, the use of berberine is promising for the treatment of AD and other neurodegenerative disorders.

## ACKNOWLEDGEMENT: Nil

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