International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 9, Issue 9, 2017

Original Article

CANDESARTAN REVERSES MEMORY DEFICIT CAUSED BY COLCHICINE INDUCED CHOLINERGIC DYSFUNCTION AND OXIDATIVE STRESS

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Received: 31 May 2017 Revised and Accepted: 22 Jul 2017

ABSTRACT

Objective: We sought to investigate the protective activity of candesartan against memory impairment, oxidative stress and cholinergic dysfunction induced by activation of the central renin-angiotensin system.

Methods: Male Swiss albino mice were divided into eight groups. Group 1 received vehicle (1.0% w/v gum acacia), orally for 14 d. Group 2 received intracerebrally (*i. c.*) artificial cerebrospinal fluid (aCSF, the vehicle of colchicine) and treated with vehicle for 14 d. Group 3, 4 and 5 injected with *i. c.* colchicine in the doses of 1µg, 2µg, 3µg respectively and treated with vehicle for 14 d. Group 6 and 7 received *i. c* colchicine (3 µg) and treated with candesartan (0.05 and 0.1 mg/kg respectively) orally for 14 d. Group 8 received *i. c* colchicine (3 µg) and treated with standard drug donepezil 5 mg/kg (PO) for 14 d.

Learning and memory behavior was assessed by using morris water maze. Biochemical parameters of oxidative stress and cholinergic function were estimated in the brain on day 18. Parameters of oxidative stress and cholinergic function were estimated after the completion of behavioral studies

Results: Treatment with a higher dose of colchicines (3µg/mice) caused memory deficit as shown by no significant decrease in escape latency time throughout all the sessions. Results of biochemical estimation showed a marked increase in malondialdehyde (MDA), nitrite level, reduced glutathione (GSH) level, cholinotoxic effect of colchicines has been correlated by marked decrease in acetyl cholinesterase (AChE) activity. Colchicine in a dose of 3 µg/mice has been validated. Pretreatment with candesartan in doses 0.05 and 0.1 mg/kg reverses oxidative stress which can be measured by decreased MDA, nitrite level and increased GSH level. Increased AChE activity may imply protection of cholinergic neurons hence improvement in learning and memory behavior.

Conclusion: Preventive treatment with angiotensins receptor blocker, candesartan showed that memory impairment induced by colchicines may be mediated by alteration of central rennin angiotensins system and loss of cholinergic neurons. This study highlighted a number of clinical findings which support marked neuroprotection by blocked of the central AT1 receptor.

Keywords: Colchicine, Antioxidant activity, Neuroprotection

DOI: http://dx.doi.org/10.22159/ijpps.2017v9i9.20391

INTRODUCTION

The primary function of the renin-angiotensin system (RAS) is to maintain fluid homeostasis and regulate blood pressure [1]. Beyond the actions of peripheral RAS, an independent RAS exist in the brain [2]. The brain RAS has been suggested to contribute to the neurodegenerative disorders such as Alzheimer's disease or vascular dementia [3, 4]. Several studies suggested the role of brain RAS in memory consolidation. Though, the participation of angiotensin II (AII) in this process is controversial. Some studies have demonstrated that acute administration of AII in the central nervous system improves the learning ability in rats. [5-7]. Delorenzi and Maldonado have reported, enhancing the effect of both exogenous AII and angiotensin IV (AIV) on memory consolidation on fear contextual memory in the crab, which can be reverted by nonspecific AII receptor antagonist saralasin [8, 9].

In our previous study AT1 receptor antagonist, candesartan improve learning and memory in streptozotocin model of dementia [10]. Another study report, that in step-down inhibitory avoidance task, AII blocked the formation of the long-term memory for a one-trial, when given into the CA1 region of the rat dorsal hippocampus [11]. AII type 1 receptor antagonist E4177 slightly improved the memory dysfunction observed in the aged Dahl salt-sensitive rat [12].

Colchicine induces neurofibrillary degeneration by binding to tubulin, the principal structural protein of microtubules and it inhibiting axoplasmic transport, and mitosis [13]. Its central administration can result in cell death associated with cognitive impairment [14]. In addition, it causes oxidative damage and generation of excessive free radical that can be positively correlated with the extent of cognitive impairment [15]. Colchicine causes loss of cholinergic neurons, destruction of cholinergic pathways, and a decrease in cholinergic turnover, induces hippocampal lesions resulting in learning and memory impairment, reduction in choline acetyltransferase [16], suggesting that it could be used as a suitable model for studying Alzheimer's disease.

Therefore ATI receptor blocker of RAS pathway may have an influence on learning and memory paradigm. The present study investigated the role of central ATI receptor in memory impairment, induced by intracerebral colchicines. The antihypertensive drug, ATI receptor blocker candesartan was used as an experimental tool. Further cholinergic dysfunction was estimated by acetyl cholinesterase assay and oxidative stress was measured by estimating the level of MDA, GSH, and nitrite.

MATERIALS AND METHODS

Animals

Adult male Swiss albino mice (25–30 g) were used. The animals were obtained from the Laboratory Animal Services Division of Central Drug Research Institute, Lucknow. The animals were kept in the polyacrylic cage (22.5×37.5 cm) and maintained under standard housing conditions (room temperature 24-27 °C and humidity 60-65%) with a 12 h light and dark cycle. Food and water were available *ad libitum* but the food was not allowed from 1 h prior to the behavioural study. The experimental protocol was approved by the Institutional Animal Ethical Committee (No. 1213/ac/June 2008/ CPCSEA), and

experiments were conducted in accordance with the CPSCEA guidelines on the use and care of experimental animals.

Materials

The biochemicals i. e. colchicine, chloral hydrate, sodium chloride (NaCl), sodium nitrate ($NaNO_2$), sulphanilamide, naphthylamine diamine dihydrochloric, bovine serum albumin (BSA), acetyl-thiocholine iodide, 5, 5'-dithiobis (2-nitro-benzoic acid) (DTNB), 1, 1, 3, 3-tetraethoxypropane (TEP), glutathione (GSH) and 2-thiobabituric acid (TBA) were purchased from Sigma-Aldrich, USA. Candesartan cilexetil was purchased from local market.

Drug administration

Colchicines: intracerebral (*i. c.*) administration

The animals were anesthetized with chloral hydrate (300 mg/kg, IP). A midline sagittal incision was made in the scalp. A 27 gauge hypodermic needle attached to a 100 µl Hamilton syringe was inserted (2.5 mm depth) perpendicularly through the skull into the brain. Colchicine (1, 2 and 3 µg/10 µl), dissolved in freshly prepared artificial CSF (aCSF), was administered slowly in a volume of 10 µl by intracerebral (*i. c.*) route. The site of injection was 2 mm from either side of the midline on a line drawn through the anterior base of the ears. The syringe was left in the place for a further 2 min for proper diffusion of colchicine [17].

Administration of candesartan

For oral administration, candesartan was suspended in 1.0% w/v gum acacia immediately before administration in a constant volume of 10 ml/kg body weight. To study the effect of candesartan in morris water maze, it was administered at doses of 0.05 and 0.1 mg/kg starting from the first dose of colchicine for 14 d. Candesartan was administered orally one hr before colchicine administration on the 1st day [10].

Administration of acetylcholinesterase inhibitor in mice

Donepezil was administered at a dose of 5 mg/kg for 14 d. It was administered as 1 % aqueous suspension with gum acacia [18], 1 hr before colchicine administration on day 1.

Experimental protocol

Animals were randomly divided into eight groups of 5 animals each.

Group 1: Control mice treated with a vehicle of candesartan (1.0% w/v gum acacia) for 14 d.

Group 2: Mice injected with intracerebrally (*i. c*) artificial cerebrospinal fluid (aCSF, the vehicle of colchicine) and treated with vehicle for 14 d.

Group 3: Mice injected with *i. c.* colchicine (1µg) and treated with vehicle for 14 d

Group 4: Mice injected with *i. c.* colchicine (2µg) and treated with vehicle for 14 d.

Group 5: Mice injected with *i. c.* colchicine (3µg) and treated with vehicle for 14 d

Group 6: Mice injected with *i. c.* colchicine (3µg) and treated with candesartan 0.05 mg/kg (PO) for 14 d

Group 7: Mice injected with *i. c.* colchicine (3µg) and treated with candesartan 0.1 mg/kg (PO) for 14 d

Group 8: Mice injected with *i. c.* colchicine (3µg) and treated with donepezil 5 mg/kg (PO) for 14 d

Animals from Group 1, 2 and 5-8 were sacrificed on day 18 and tissues were taken for biochemical studies

Evaluation of spatial memory by morris water maze test

The Morris water maze consisted of a large circular black pool of 120 cm diameter, 50 cm height, filled to a depth of 30 cm with water at 26±2 °C. A black colored round platform of 8 cm diameter was placed 1 cm below the surface of the water in a constant position. The water was colored with non-toxic black dye to hide the location of the submerged platform. The pool was divided into four hypothetical quadrants.

On the 14th day from colchicine injection, spatial learning and memory of animals were tested in Morris water maze. Trials were given for 5 consecutive days in order to train mice in the Morris water maze. The mice were given a maximum time of 60s (cut-off time) to find the hidden platform and were allowed to stay on it for 30s. The experimenter put the mice on platform himself that failed to locate the platform. The animals were given a daily session of 3 trials per day. Latency time to reach the platform was recorded in each trial. Mean latency time of all three trials is shown in the results. A significant decrease in latency time from that of 1st session was considered as a successful learning [18].

Spontaneous locomotor activity

Locomotor activity was tested one hr before water maze trial on 14th day after colchicine administration. After a period of 15 min for acclimatization in Optovarimex activity meter, each animal was observed for 10 min taking readings every 2 min. Results were expressed as mean counts/2 min.

Estimation of biochemical parameters

AChE and biochemical parameters of oxidative stress, MDA and GSH, and Nitrite were measured in the brain on the 18th day after colchicine Injection.

Brain tissue preparation

The mice were decapitated under ether anesthesia. The skull was cut open and the brain was exposed from its dorsal side. The whole brain was quickly removed and cleaned with chilled normal saline on the ice. A 10% (w/v) homogenate of brain samples in 0.03M sodium phosphate buffer (pH 7.4) was prepared.

Estimation of oxidative stress markers

Brain tissue homogenate (10% w/v in 0.03M sodium phosphate buffer, pH 7.4) was prepared by using homogenizer at a speed of 9,500 rpm.

Measurement of MDA

MDA, a marker of lipid peroxidation, was estimated in the brain tissues, according to the method of Colado *et al.* [19]. After homogenization, tissue homogenate was mixed with 30% trichloroacetic acid (TCA), 5N HCl followed by the addition of 2% thiobarbituric acid (TBA) in 0.5N NaOH. The mixture was heated for 15 min at 90 °C and centrifuged at 12,000 g for 10 min. The pink color of the supernatant was measured at 532 nm on UV-visible spectrophotometer. MDA concentration was calculated by using standard curve prepared with Tetra Ethoxy propane and expressed as nmol/mg protein.

Measurement of GSH

GSH was determined by its reaction with 5, 5'-dithiobis (2-nitrobenzoic acid) (DTNB) to yield a yellow chromophore which was measured spectrophotometrically. The brain homogenate was mixed with an equal amount of 10% TCA and centrifuged at 2,000 g for 10 min at 4 °C. The supernatant was used for GSH estimation. The processed tissue sample was mixed with phosphate buffer of pH 8.4 and DTNB and the mixture was shaken vigorously on vortex. The absorbance was read at 412 nm using UV-visible spectrophotometer. Standard curve was prepared with reduced glutathione and GSH concentration was expressed as µg/mg protein [20].

Nitrite estimation

Nitrite was estimated in the mice brain using the Greiss reagent and served as an indicator of nitric oxide (NO) production [21]. An equal volume of Greiss reagent (1:1 solution of 1% sulphanilamide in 5% Ortho-phosphoric acid and 0.1% naphthylamine diamine dihydrochloric acid in water) and processed tissue sample was mixed and absorbance was measured at 542 nm using a UV-visible spectrophotometer. Nitrite concentration was calculated using a standard curve for sodium nitrite and expressed in µg/mg protein.

Sample preparation and assay of AChE activity

The brain homogenate in the volume of 500 µl was mixed with 1% Triton X-100 (1% w/v in 0.03M sodium phosphate buffer, pH-7) and centrifuged at 100,000 g at 4°C in a Ultracentrifuge for 60 min. The supernatant was collected and stored at 4°C for acetylcholinesterase estimation by Ellman's method [22].

The kinetic profile of enzyme activity was measured by UV-visible spectrophotometer at 412 nm with an interval of 15 s. One unit of acetylcholinesterase activity was defined as the number of micromoles of acetylthiocholine iodide hydrolyzed per minute per milligram of protein. The specific activity of acetyl cholinesterase is expressed in µmoles/min/mg protein.

Protein estimation

Protein was measured in all brain samples for nitrite, GSH and MDA by the method of Lowry *et al.* (1951) and protein for acetylcholine esterase activity by the method of Wang and Smith (1975) [23, 24]. Bovine serum albumin (BSA) (1 mg/ml) was used as standard and measured in the range of 0.01–0.1 mg/ml.

Statistical analysis

The results are expressed as mean±SEM Statistical analysis of Morris water maze data was done by one-way analysis of variance (ANOVA). Biochemical values were analyzed by ANOVA followed by Tukey's test.

RESULTS

Effect of candesartan on colchicine induced memory impairment in mice

As shown in fig. 1 control [F (4, 20) = 66.48, P<0.01] and aCSF [F (4, 25) = 38.3, P<0.01] injected mice showed significant decrease in escape latency time (ELT) from session three onward indicating spatial learning. To induce memory impairment, colchicine was administered intracerebrally at 1, 2 and 3 µg/mice dose and memory function was evaluated 14 d after colchicine administration. Colchicine at 1 µg dose [F (4, 20) = 10.63, P<0.05] showed a significant decrease in ELT at session 4 and 5. Colchicine at 2 µg dose [F (4, 20) = 3.33, P<0.05] showed significant decrease in ELT at session 5 while the dose of 3 µg [F (4, 20) = 0.93, P>0.05] caused memory deficit as shown by no significant decrease in ELT throughout all the sessions. Colchicine in dose of 3 µg/mice has been validated.

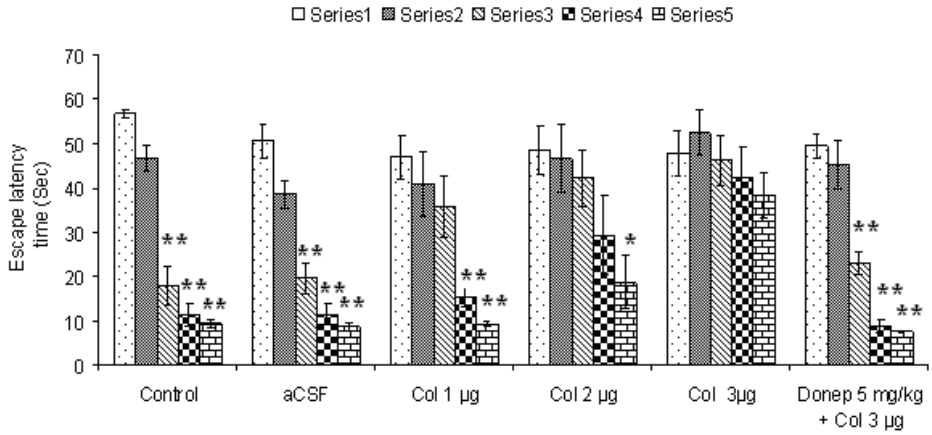

Fig. 1: Effect of various doses of colchicines in morris water maze trial, results are expressed as mean latency time (sec)±Standard error. * Significant difference (P<0.01 and *P<0.05) in latency time in comparison to session 1 (n=5)**

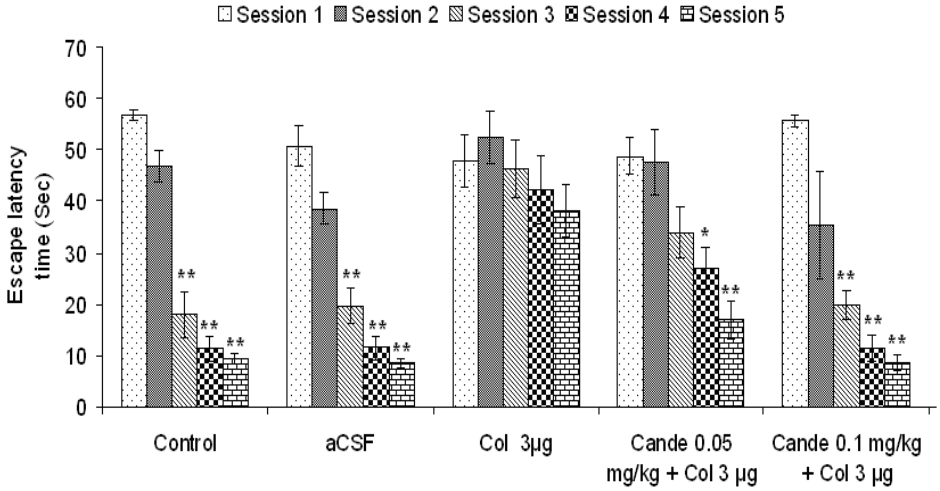

Fig. 2: Effect of candesartan on colchicine induced memory impairment in mice, results are expressed as mean latency time (sec)±Standard error. * Significant difference (P<0.01 and *P<0.05) in latency time in comparison to session 1. (n=5)**

AT1 receptor blocker candesartan was used to study the role of AII in colchicine induced memory impairment. As shown in fig. 2, colchicines induced memory impairment was reversed by candesartan in dose dependent manner.

Candesartan 0.05 mg/kg [F (4, 20) = 8.5, P<0.01] treated mice showed significant decrease in ELT from session 4 onward whereas higher dose [F (4, 20) = 15.09, P<0.01] decreased ELT from session 3 onward.

Biochemical estimations

Effect of candesartan on MDA level in colchicine induced memory deficit mice brain

Colchicine caused a significant increase in MDA level as compared with control and aCSF groups [F (2, 12) = 33.30, P<0.01]. This increase in MDA was attenuated by candesartan treatment [F (2, 12) = 46.24, P<0.01]. However, administration of aCSF (*i. c.*) had no significant (P>0.05) effect on MDA level as compared to control (fig. 3).

Effect of candesartan 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 control and aCSF treated groups [F (2, 12) = 24.86, P<0.01]. Treatment with candesartan dose dependently prevented the decrease in GSH levels in the brain of colchicine-injected mice [F (2, 12) = 15.49, P<0.01] (fig. 4).

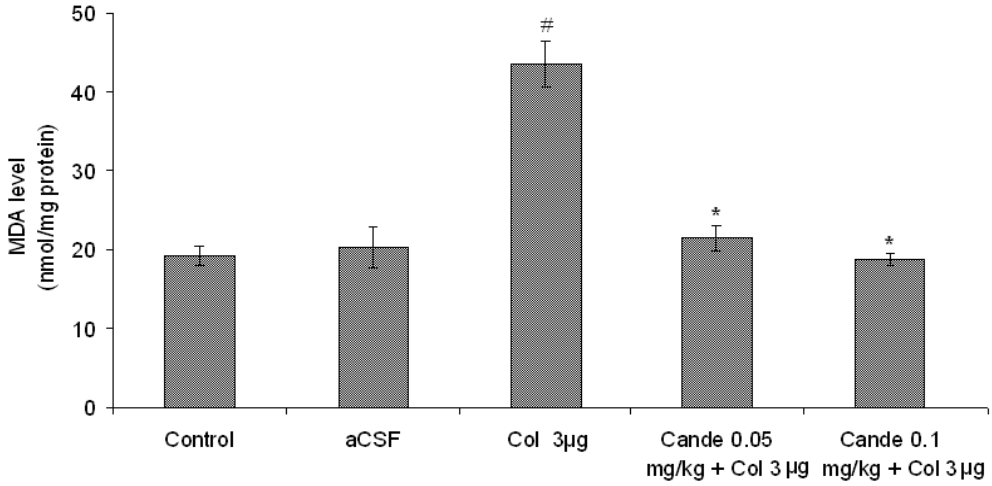

Fig. 3: Effect of candesartan on Malondialdehyde (nmol/mg protein) level in mice brain, results were expressed as a mean±Standard error and analyzed by one way ANOVA followed by Tukey's test. #Significant difference (#P<0.01) vs control and aCSF group; *Significant difference (*P<0.01) vs colchicine group (n=5)

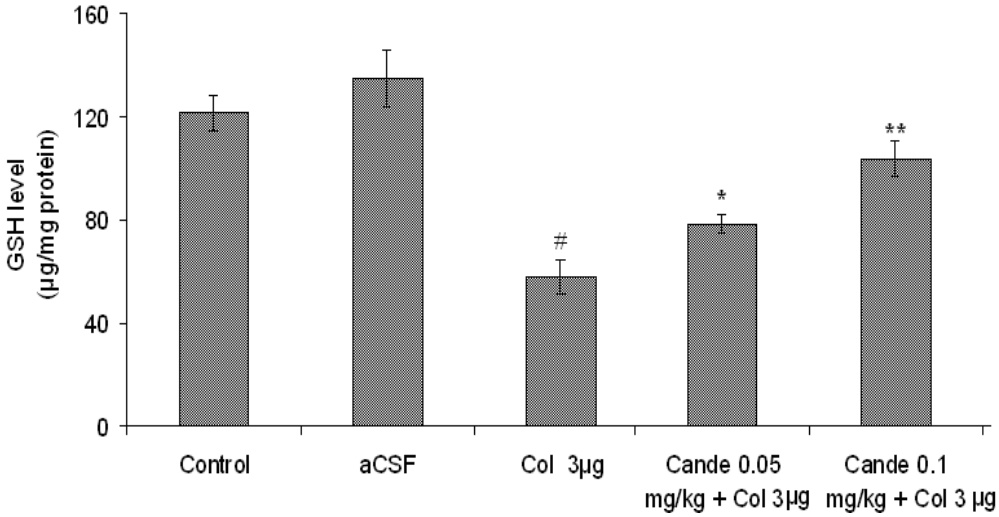

Fig. 4: Effect of candesartan on GSH (µg/mg protein) level in mice brain, results were expressed as a mean±Standard error and analyzed by one way ANOVA followed by Tukey's test. #Significant difference (#P<0.01) vs control and aCSF group; *Significant difference (*P<0.05 and **P<0.01) vs colchicine group. (n=5)

Effect of candesartan on nitrate level in colchicine induced memory deficit mice brain

A significant rise in nitrite level was observed in the brain of colchicine treated mice in comparison to control and aCSF groups [F (2, 12) = 13.57, P<0.01]. Candesartan treatment significantly inhibited this increase in nitrite levels in colchicine treated mice [F (2, 12) = 17.65, P<0.01] (fig. 5).

Effect of candesartan on AChE activity in colchicine induced memory deficit mice brain

AChE activity was significantly decreased in the colchicine treated mice brain when compared with control and aCSF groups [F (2, 12) = 11.11, P<0.01]. Chronic candesartan treatment significantly [F (2, 15) = 6.38, P<0.05] prevented this decrease in AChE activity (fig. 6).

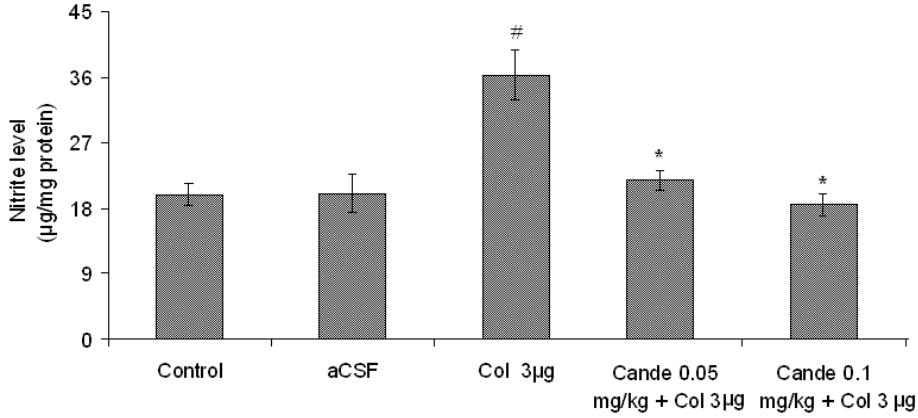

Fig. 5: Effect of candesartan on nitrite (µg/mg protein) level in mice brain, results were expressed as mean±Standard error and analyzed by one way ANOVA followed by Tukey's test. #Significant difference (#P<0.01) vs control and aCSF group; *Significant difference (*P<0.01) vs colchicine group (n=5)

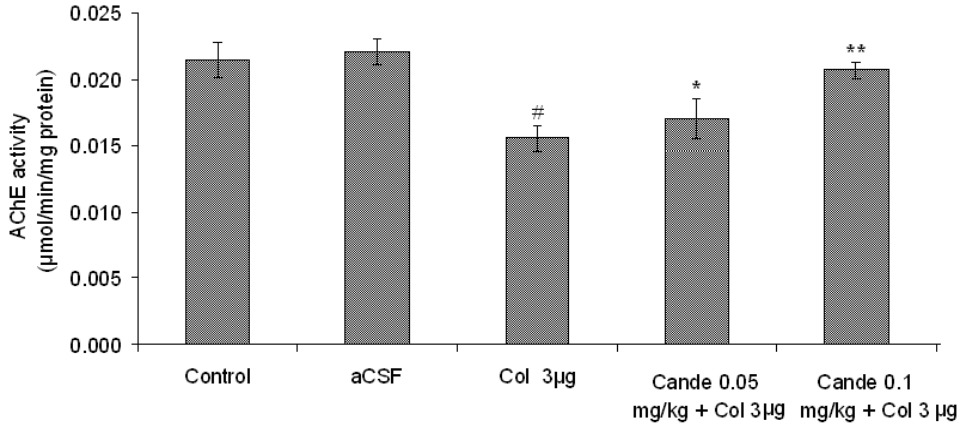

Fig. 6: Effect of candesartan on AChE activity (µg/min/mg protein) in mice brain. Results were expressed as mean±Standard error #Significant difference (#P<0.01) vs control and aCSF group; *Significant difference (*P<0.01) vs colchicine group (n=5)

DISCUSSION

This study investigated the role of central RAS in memory function and its relation with changes in biochemical markers of cholinergic function and oxidative stress in intra-cerebral (*i. c.*) colchicine induced a model of memory deficit in mice. The present study showed that AT1 receptor has the crucial role in preventing memory deficit induced by *i. c.* colchicines. It has been reported that central administration of colchicine induces memory impairment in rodents by causing cholinergic neurodegeneration and oxidative stress [25]. In the present study *i. c.* administration of colchicine at a dose of 3 µg/mice induced spatial memory impairment as indicated by no significant reduction in escape latency time in Morris water maze test. However, a lower dose of colchicine failed to induce memory deficit. Therefore, further studies were carried out by using colchicine at 3 µg/mice dose.

This finding is in agreement with previous studies reporting impairment in memory following colchicine administration [26]. Further, the colchicine induced memory impairment model was validated by clinically used antidementic-anticholinesterase drug donepezil. Preventive treatment with donepezil for 14 d ameliorated colchicine induced memory impairment in mice. Involvement of central RAS in colchicine induced memory impairment was studied by using AT1 receptor blocker candesartan. Candesartan was administered chronically for 14 d in colchicine injected mice and memory function was tested by Morris water maze. Candesartan prevented colchicine induced dementia in mice implicating the role of the central AT1 receptor in memory function. Locomotor activity of experimental animals was not altered. We found elevated nitrosative (increased nitrite level), oxidative stress (decreased GSH and increased MDA) and decreased AChE level in colchicine treated mice brain which was reversed by preventive treatment of ATI receptor blocker candesartan.

CONCLUSION

The treatment with candesartan alleviates colchicine induced memory impairment in mice. Intra-cerebral injection of colchicines leads to memory impairment by micro tubular dysfunction which may lead to loss of cholinergic neuron further oxidation of neuronal cell is also another implication of colchicines which again leads to memory deficit.

Candesartan, an ATI receptor blocker reverses memory deficit caused by colchicines. Which support the hypothesis of reversal of cholinergic dysfunction and oxidative stress by treatment of candesartan.

This study supported a number of clinical findings that central AT1 receptor blockade could be neuroprotective.

CONTRIBUTION OF AUTHORS

Awasthi H researched and Siddiqui HH provided guidance, critical review, revision and approved the final version of this study.

CONFLICT OF INTERESTS

All authors have none to declare

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How to cite this article

- Himani Awasthi, HH Siddiqui. Candesartan reverses memory deficit caused by colchicine induced cholinergic dysfunction and oxidative stress. Int J Pharm Pharm Sci 2017;9(9):184-189.