Research Article

Investigative studies on pharmacological potential of HDAC inhibitor on heavy metal induced Neurodegeneration

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Abstract

Objective: To study the neuroprotective effect of Sodium butyrate against $CdCl_2$ induced dementia in rats. **Materials and methods:** The neuroprotective activity of HDAC inhibitor, sodium butyratewas evaluated against $CdCl_2$ induced dementia in adult male Albino Wistar rats. A number of behavioral (Elevated plus maze and Morris water maze; Assessment of learning and memory) and biochemical (brain/ serum levels of nitrite/nitrate, Aortic Superoxide Anion, calcium, GSH, TBARS, CAT, AChE) parameters were assessed. **Results and discussion:** In comparison to the disease control, administration of sodium butyrate (HDACi) significantly attenuates the $CdCl_2$induced behavioral alternations like learning and memory impairment; andincreased the brain/serum levels of acetylcholinesterase (AChE), catalase (CAT), reduced glutathione (GSH), thiobarbituric acid reactive species (TBARS), brain calcium levels, aortic superoxide anionand nitrite/nitrate levels. **Conclusion:** The results clearly indicate that oxidative stress and lipid peroxidation are the primary mechanisms involved in the pathogenesis of $CdCl_2$ induced dementia.The research aims to identify and implement the therapeutic potential of HDACi in the treatment of dementia. It is concluded that a target based drug research against pathogenic mechanisms like oxidative stress and lipidperoxidation may help to alleviate ailments associated with neurodegeneration.

Keywords: Neuroprotective, Dementia, Sodium butyrate, catalase, vascular dementia

Introduction

Neurodegeneration is defined as the gradual atrophy and functional deficit in neurons, which is prevalent in Alzheimer's disease and Parkinson's disease (Serrano-Pozoet al., 2011). Dementia is one of the most prominent features of neuronal dysfunction or synaptic dysfunction; due to neurodegeneration (Lepeta et al., 2016; Srivastava et al., 2019). Neuronal disconnections, damage to hippocampal, basal forebrain and cortical regions may leads to dementia (Arendt et al., 2015). Dementia is a mental disorder is characterized by impaired memory and intellect, which interfere with one's occupational or social activities. Dementia increases exponentially with age. Vascular dementia (VaD) is the second most prominent type of dementia (Sharma and Singh, 2012a) after Alzheimer's dementia. Dementia is commonly referred as the "silent epidemic of the twenty-first century" (Battistin et al., 2010). The prevalence of VaD linearly increases with age and that is varies greatly from country to country, this range from 1.2 to 4.2% of people are over 65 old years, after adjustment of age and sex. The incidences of the VaD are generally homogeneous and it is estimated that about 6-12 cases per 1,000 are concerned with people over 70 years of age (Hébert et al., 1995). Few of the underlying pathogenic mechanisms in the slow progression of neurodegeneration involves: accumulation of misfolded proteins, metal accumulation, protofibril formation, impaired ubiquitin-proteasome system (UPS), excitotoxicity, oxidative and nitrosative stress, mitochondrial deficit, synaptic malfunction, distorted metal homeostasis and interruption of axonal and dendritic transport (Bossy-Wetzel et al., 2011). Metal accumulation is one of the most major pathogenic mechanisms involved in progressive neurodegeneration (Cannon et al., 2011). Metals are required in the human body as they are required to maintain cell structure, regulate: gene expression, neurotransmission, and antioxidant response (Marchetti et

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al., 2014). However, their unnecessary accumulation in the nervous system may leads to toxicity, oxidative stress, mitochondrial dysfunction, and impaired enzymatic activity (Jomova et al., 2011). Metal accumulation may result in severe neurological disorders. Cadmium (Cd) is a highly toxic heavy metal which accumulates in the nervous system and is presently one of the major occupational and environmental pollutants (Choong et al., 2014). Cadmium (Cd) is generally absorbed by the human body through consumption of tobacco or contaminated food. Its biological half life is about 20–30 years in humans and is low rate of excretion causes nephrotoxicity, hepatotoxicity, endocrine and reproductive toxicities (Branca et al., 2018). Furthermore, Cd-induced neurotoxicity is associated with neurodegenerative diseases like Alzheimer's and Parkinson's diseases, amyotrophic lateral sclerosis, and multiple scleroses (Chin-Chan et al., 2015). Cadmium (Cd) is reported to affect a number of activities like cell differentiation, proliferation and apoptosis (Siewit et al., 2010). Disruption in DNA repair mechanisms and production of reactive oxygen species are few of the most prominent pathogenic mechanisms involved in Cd- induced neurotoxicity (Nair et al., 2013). Histone deacetylase inhibitors (HDACi) are reported to alleviate the ailments associated with a number of CNS disorders (Kazantsev et al., 2008; Sleimanet al., 2009). HDACi also improve learning and memory (Fischer et al., 2007; Guan et al., 2009) and support axonal regeneration (Rivieccio et al., 2009; Gaub et al., 2010). HDACi modify gene expression profiles by facilitating chromatin remodeling through histone acetylation (Sleiman et al., 2011). HDACi may facilitate the acetylation of a number of nonhistone proteins (Choudhary et al., 2009), like transcription factors (Brochier et al., 2013) and cytoskeletal proteins (Kim et al., 2012).The objective of the present research is to evaluate the neuroprotective activity of Sodium butyrate against $CdCl_2$ induced VaD in rats.

Materials and methods

Experimental Animals

Healthy adult male Albino Wistar rats (200-250 g) were procured from the Animal house division of Hygia Institute of Pharmaceutical Sciences and Research (HIPER), Lucknow. The animals were kept in polyacrylic cage; under standard housing condition (room temperature 25±1 °C and humidity 60–65%) with 12: 12 light/dark cycle.The animals were exposed to12hrs light and 12hrs dark cycle. The animals were acclimatized to thelaboratory condition five days prior to behavioral study.Animals had free access to standard feed and water ad libitum. The protocol of the study was duly approved by Institutional Animal Ethics Committee (IAEC) and care of the animalswas taken as per the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Environment and Forests and Government of India (Approval No. HIPER/IAEC/13/18/04).

Drugs and Chemicals

Donepezil tablets were provided by Cipla Limited (India). Thiobarbituric acid, sodium butyrate, and 1,1,3,3 tetra methoxypropane were purchased from Sigma-Aldrich (USA).5,5′-Dithiobis (2-nitro benzoic acid), bovine serum albumin, L-Glutathione reduced, and nitrobluetetrazolium were purchased from Sisco Research Laboratories Private Limited (India).

Experimental Protocol

Animals were divided into five groups containing six animals each.

Control Group (Group I): Animals were exposed to EPM for acquisition transfer latency on 1st day and retention transfer latency on 2nd day as well as MWM for acquisition trial from 2nd to 5th day and retrieval trial on 6th day.

Cadmium chloride treatment group (Group II): Cadmium chloride (2.5 mg/kg/day, p.o.) was administered to the rats for 30 days. Animals were exposed to EPM for acquisition transfer latency on 24th day and retention transfer latency on 25th day as well as MWM for acquisition trial from 25th to 29th and retrieval trial on 30th day.

Cadmium chloride and sodium butyrate low dose (Group III): Sodium butyrate (100 mg/kg i.p., daily) was administered to the rats, starting from 7th day of cadmium chloride (2.5 mg/kg/day, p.o.) treatment. Animals were exposed to EPM for acquisition transfer latency on 24th day and retention transfer latency on 25th day as well as MWM for acquisition trial, from 25th to 29th and retrieval trial on 30th day.

Cadmium chloride and sodium butyrate high dose (Group IV): Sodium butyrate (200 mg/kg i.p., daily) was administered to the rats, starting from 7th day of cadmium chloride (2.5 mg/kg/day p.o.) treatment. Animals were exposed to EPM for acquisition transfer latency on 24th day and retention transfer latency on 25th day as well as MWM for acquisition trial, from 25th to 29th and retrieval trial on 30th day.

Cadmium chloride and donepezil (Group V): Donepezil (0.5 mg/kg i.p., daily) was administered to the rats, starting from 7th day of cadmium chloride (2.5 mg/kg/day, p.o.) treatment. Animals were exposed to EPM for acquisition transfer latency on 24th day and retention transfer latency on 25th day as well as MWM for acquisition trial, from 25th to 29th and retrieval trial on 30th day.

Cadmium induced Dementia

Rats were administered cadmium chloride (5mg/kg/day, p.o.) in drinking water for 30 days to produce oxidative stress induced vascular dementia (Gupta et al., 2017; Kukongviriyapan et al., 2014). Body weight of rats was monitored weekly. Animals were exposed to Elevated plus maze for acquisition transfer latency on 24th day and retention transfer latency on 25th day as well as Morris Water Maze for acquisition trial from 26th to 29thdays and retrieval trial on 30th day. The Cadmium chloride treatment was continued throughout acquisition trials on Morris water maze.

Behavioral analysis

Assessment of learning and memory by Elevated Plus Maze (EPM)

Elevated plus maze is a widely used model of anxiety but in previous studies it has also been used as a model to assess learning and memory. Acquisition and retention memory processes were assessed using the EPM. The EPM is made for wood and consisted of two open arms (50×10 cm) and two closed arms (50×10×40 cm) forming a cross, with a quadrangular centre (10×10 cm). The maze was placed about 50 cm above the floor. On the 1st day, the acquisition transfer latency (TL_1) was carried out as follows: the rats were placed individually at the end of one open arm oppositeto the central platform and the time taken to move from the open arm to either of the enclosed arm was noted. The TL was the time when a rat was placed on the open arm and when all its four legs cross to the enclosed arm. In this experiment, when the rat did not enter the enclosed arm in 90 sec, it was gently pushed on the back into the enclosed arm and the transfer latency was assigned 90 sec. The rodent was permitted to move freely in the plus maze for 20 s; after the measurement of transfer latency. The rat is gently taken out of the plus maze and was returned its home cage. Twenty-four hours later the retention transfer latency (TL_2) test was performed in the same manner as in the acquisition trial. The rats are put into open arm and transfer latency was recorded again. If the rat did not enter the enclosed arm within 90 s on the 2nd trial, the transfer latency was assigned 90s (Tamaddonfard et al., 2013).

Assessment of learning and memory by Morris Water Maze (MWM)

In previous studies Morris water maze has been used as exteroreceptive model to assess learning and memory. Escape latency time (ELT) is used as an index of leaning and time spent in target quadrant (TSTQ) is used as an index of memory. The MWM procedure was based on a principle, where the animals were placed in a large pool of water, as animals dislike swimming, their tendency to escape from the water being accomplished by finding an escape platform. MWM consisted of large circular pool (150 cm in diameter, 45 cm in height, filled to a depth of 30 cm with water at 28±1°C). The water was added with nontoxic white colored dye to make it opaque. The tank was divided into four equal quadrants with a help of two threads, fixed at right angle to each other on the rim of the pool. A submerged platform (10 cm^2), painted in white was placed 1 cm below surface of water inside the target quadrant. Position of the platform was kept unaltered throughout of the training session. Each animal was subjected to four consecutive trials on each day with the gap of 5 min. The rat are gently placed in water pool between quadrants and facing wall of pool with the drop location changing on each trial, and allowed 120 sec to locate submerged platform and it was allowed to stay on the platform another 20 sec. If it failed to find the platform within 120 sec, it was guided gently onto the platform and allowed to remain there for 20 sec. The Escape latency time (ELT) is located on the hidden platform in water maze, which was noted as index of acquisition or learning. The animals are subjected to four acquisition trials for daily four consecutive days. On 5th day, the platform was removed and each rat was allowed to explore in the pool for 120 sec. The Mean time spent in the all four quadrants was noted. The mean time spent by the animal in target quadrant searching for the hidden platform was noted as an index of retrieval (memory) (Dutta et al., 2017).

Biochemical analysis

Blood samples for biochemical estimation were collected by retro orbital bleeding. The blood was kept at room temperature for 30 min and then centrifuged at 4000 rpm for 15 min to separate serum which was then used for biochemical estimation. After retro-orbital bleeding, animals were sacrificed by cervical dislocation; thoracic aorta and brain tissue was carefully removed. Thoracic aorta was used for endothelium dependent and independent relaxation as well as for the estimation of superoxide anion, whereas brains were subjected to various biochemical estimations (brain TBARS, GSH and proteins). The removed brains were homogenized in phosphate buffer (pH 7.4, 10% w/v) using Teflon homogenizer and centrifuged at 3000 rpm for 15 min to obtain the clear supernatant. This clear supernatant (TBARS, GSH and proteins) containing was removed carefully from the centrifugation tube and it was then used for different biochemical estimations.

Estimation of serum nitrite/nitrate concentration

Serum nitrite concentration was measured spectophotometrically at 545 nm (UV-1800 ENG.240V; Shimadzu Corp. Japan). Briefly, 400µl of carbonate buffer

(pH 9.0) was added to 100µl of serum or standards sample followed by addition of small amount (0.15 g) of copper-cadmium alloy. The sample tube was incubated at the room temperature for 1 hour and to reduce nitrate to nitrite. Add 100µl of 0.35M sodium hydroxide for the reaction was stopped and 400µl of zinc sulfate solution (120mM) is added to deproteinate the serum samples. The samples was allowed to stand for 10min and then centrifuged at 4000 g for 10 min. Greiss reagent (250µl of 1.0% sulfanilamide prepared in 3N HCl and 250µl of 0.1% N-naphthylethylenediamine (prepared in water) was added to aliquots (500µl) of clear supernatant and serum nitrite was measured spectophotometrically at 545 nm. The standard curve of sodium nitrite (5 to 50µM) was plotted to calculate concentration of serum nitrite (Sharma and Singh, 2012a).

Estimation of Reduced Glutathione (GSH)

The homogenate sample was mixed with the trichloroacetic acid (10% w/v) in the ratio 1:1. The tubes were centrifuged at 1000 g for 10 min at 4 ^{0}C. The homogenate sample is obtained (0.5 ml) and these samples were mixed with 2 ml of 0.3 M disodium hydrogen phosphate. Then 0.25 ml of 0.001 M freshly prepared DTNB [5, 5`-dithiobis (2-nitrobenzoic acid) dissolved in 1% w/v sodium citrate] was added and absorbance was noted spectophotometrically (UV-1800 ENG.240V; Shimadzu Corp. Japan) at 412 nm. In the result, the standard curve is plotted by using 10-100 µM reduced form of glutathione and results was expressed in micromoles of reduced glutathione per mg of protein.

Estimation of Thiobarbituric Acid Reactive Substances (TBARS)

Pipette out 0.2 ml of homogenate sample in a test tube, add 0.2 ml of 8.1% sodium dodecyl sulphate, add 30% of acetic acid 1.5 ml (pH 3.5), add 0.8%ofthiobarbituric acid 1.5 ml and make volume up to 4 ml with distilled water. Sample test tubes were incubated at 95 ^{0}C for 1 h, then cooled the test tube sample at the room temperature and add 1 ml of distilled water. Add 5 ml of n-butanol & pyridine mixture (ratio 15:1 v/v). The sample tubes are centrifuged at 2000 RPM for 10 min. The absorbance of developed pink color was measured spectophotometrically (UV-1800 ENG.240V; Shimadzu Corp. Japan) at 532 nm. The standard curve was prepared by using 1-10 nM of 1, 1,3,3-tetra methoxy propane. The value of TBARS was expressed as nano moles per mg of protein.

Estimation of brain/serum catalase enzyme

Catalase activity was assayed in which the breakdown of hydrogen peroxide (H_2O_2) is measured at 240 nm. Briefly the assay mixture consisted of 12.5 mM H_2O_2 in phosphate buffer (50 mM of pH 7.0) and 0.05 mL of supernatant from the tissue homogenate (10%) brain/serum, and the change in absorbance was recorded at 240 nm using spectrophotometer (UV-1800 ENG.240V; Shimadzu Corp. Japan). The results are expressed as mM of H_2O_2 decomposed mg^{-1} protein min^{-1} (Kumar et al., 2011).

Estimation of Aortic Production of Super Oxide Anion

The superoxide anion was determined spectrophotometrically at 540 nm (UV-1800 ENG.240V; Shimadzu Corp. Japan). Briefly, the aorta was cut into transverse rings l0 mm in length and placed in 5mL buffer at 37^{0}C containing 100 mmolL^{-1} of nitroblutetrazolium (NBT) for 1.5 h. NBT reduction was stopped by addition 5mL of 0.5 molL^{-1} HCl. The rings was minced and homogenized in a mixture of 0.1molL^{-1} NaOH and 0.1% SDS in water containing 40 mgL^{-1} diethylentriamine penta acetic acid. The mixture was centrifuged at 20,000g for 20 mins, the resultant pellet was re-suspended in 1.5 mL of pyridine and kept at 80^{0}C for 1.5 h to extract formazan. The mixture was centrifuged at l0, 000 g for 10 min and the absorbance of the formazan was determined spectrophotometrically at 540 nm (Sharma and Singh, 2012a).

Estimation of Brain Acetyl Cholinesterase (AChE) Activity

This was measured on basis of the formation of yellow color due to the reaction of thiocholine with dithiobisnitrobenzoate ions (Pohanka et al., 2011). The rate of formation of thiocholine from acetylthiocholine iodide in the presence of brain cholinesterase was measured using a spectrophotometer. 0.5 ml of clear supernatant liquid of the brain homogenate was pipette out into 25 ml volumetric flask and dilution was made with a freshly prepared DTNB (5, 5'– Dithiobis (2-nitrobenzoic acid) solution (10 mg DTNB dissolved in 100 ml Sorenson phosphate buffer, pH 8.0). From the volumetric flask, two 4 ml portions were pipette out into two test tubes. Two drops of eserine solution was added in one of the test tubes. 1 ml of substrate solution (75 mg of acetylthiocholine iodide per 50 ml of distilled water) was pipetted out into both of the test tubes. The test tube containing eserine was taken as blank and the change in absorbance per min. of the test sample was read spectrophotometerically (UV-1800 ENG.240V; Shimadzu Corp. Japan) at 420 nm. AChE activity was calculated using the following formula:

R = (δ O.D. X V) / (E X P)

Where R = rate of enzyme activity in 'n' mole of acetylthiocholine iodide hydrolyzed/min/mg protein.

δ O.D. = change in absorbance / minute

V = Volume of assay

E = Extinction coefficient = (13600 / M / cm)

P = Protein content (mg)

Results

Effect on acquisition transfer latency and retention transfer latency using Elevated Plus Maze (EPM)

Administration of cadmium chloride (2.5mg/kg, day, p.o., 30 days) did not show any significant effect on acquisition transfer latency (TL_1) and retention transfer latency (TL_2). In addition, administration of Sodium butyrate: dose 1(100 mg/kg, i.p., daily), Sodium butyrate: dose 2 (200 mg/kg i.p., daily), and donepezil (0.5 mg/kg i.p, daily) did not show any significant effect on TL_1 and TL_2. However cadmium chloride treated rats showed a significant increase in TL_1 and TL_2 time duration, indicating impairment of learning and memory. Administration ofSodium butyrate (dose1 and dose2) and donepezil significantly prevented cadmium chloride induced rise in increase in TL_1 and TL_2time duration, thus indicating reversal of cadmium chloride induced impairment of learning and memory.

Effect on escape latency time (ELT) and time spent in target quadrant (TSTQ), using Morris water maze (MWM)

In comparison to day 1, control group showed significant fall inELT on day 4th, thus reflecting normal learning ability. In respect to time spent in other quadrants, a significant rise in TSTQ was observed on the day 5th; reflecting normal retrieval. In comparison to the control animals, cadmium chloride treated rats showed a significant increase in ELT on the day 4th.In comparison to normal control, cadmium chloride treated ratsshowed significant decrease in TSTQ on day 5th, indicating impairment of memory as well. Sodium butyrate (dose1 and dose2) and donepezil significantly prevented cadmium chloride induced rise in ELT on day 4th, indicating reversal of cadmium chloride induced impairment of acquisition. Sodium butyrate (dose1 and dose2) and donepezil treatment attenuated cadmium chloride induced decrease in TSTQ on day 5th in a significant manner; thus, indicating reversal of cadmium chloride induced impairment of memory.

Effect on Serum Nitrite/Nitrate Level

In comparison to the normal control, cadmium chloride showed significant decrease in the serum levels of serum nitrite. Treatment with Sodium butyrate (dose1 and dose2) and donepezil prevented cadmium chloride induced decrease in serum nitrite level in a significant manner.

Effect on Levels of serum/brain Reduced GSH

Cadmium chloride produced a significant decrease in the brain/serum levels of reduced glutathione (GSH). Treatment with Sodium butyrate (dose 1 and dose2) and donepezil significantly prevented cadmium chloride induced oxidative stress.

Effect on levels of serum/brain TBARS

Cadmium chloride produced a significant increase in brain/serum thiobarbituric acid reactive species (TBARS).

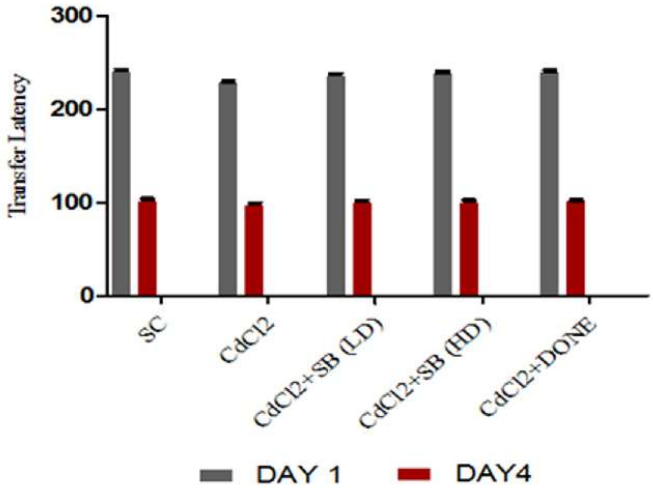

Figure 1. Effect of Sodium butyrate (LD): dose 1, Sodium butyrate (HD): dose 2, and Donepezil on acquisition transfer latency and retention transfer latency of cadmium chloride (CdC) induced VaD in rats using Elevated Plus Maze (EPM).Values are expressed as mean±SEM. (n=6). SEM was statistically analyzed using one–way ANOVA followed by dunnett's multiple comparison tests. P < 0.05 was considered to be statistically significant.*P < 0.05 versus day 1 transfer latency time in respective groups, **P <0.05 versus day 4 transfer latency time in control group, ***P <0.05 versus day 4 transfer latency time in cadmium chloride treated group.

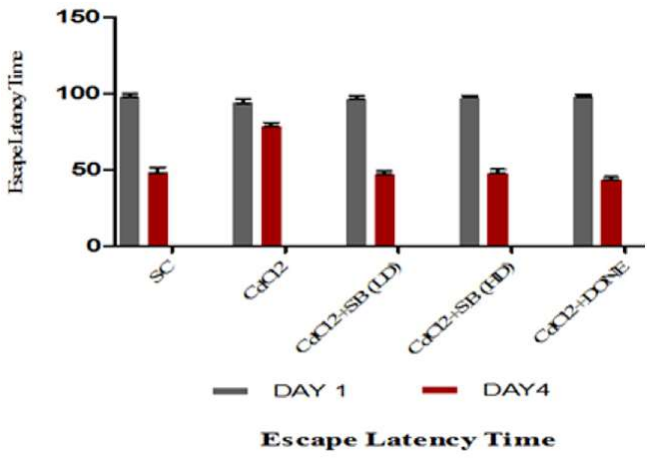

Figure 2. Effect of Sodium butyrate (LD): dose 1, Sodium butyrate (HD): dose 2, and Donepezil on escape latency time (ELT) of cadmium chloride (CdC) induced VaD in rats using Morris water maze (MWM).Values are expressed as mean±SEM. (n=6). SEM was statistically analyzed using one–way ANOVA followed by dunnett's multiple comparison tests. P < 0.05 was considered to be statistically significant *P < 0.05 versus day 1 escape latency time in respective groups, **P <0.05 versus day 4 escape latency time in control group, ***P <0.05 versus day 4 escape latency time (ELT) in cadmium chloride treated group.

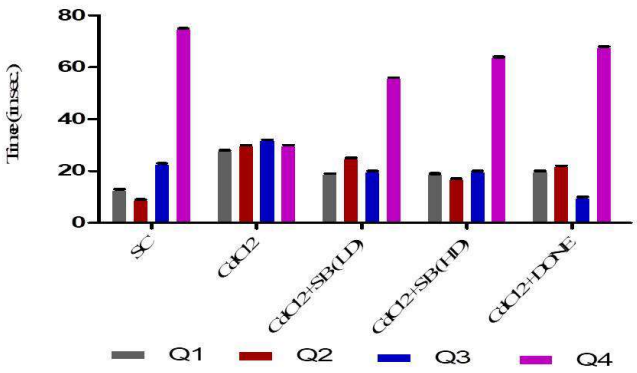

Figure 3. Effect of Sodium butyrate (LD): dose 1, Sodium butyrate (HD): dose 2, and Donepezil on time spent in target quadrant (TSTQ) of cadmium chloride (CdC) induced VaD in rats using Morris water maze (MWM).Values are expressed as mean±SEM. (n=6). SEM was statistically analyzed using one–way ANOVA followed by dunnett's multiple comparison tests. P < 0.05 was considered to be statistically significant.

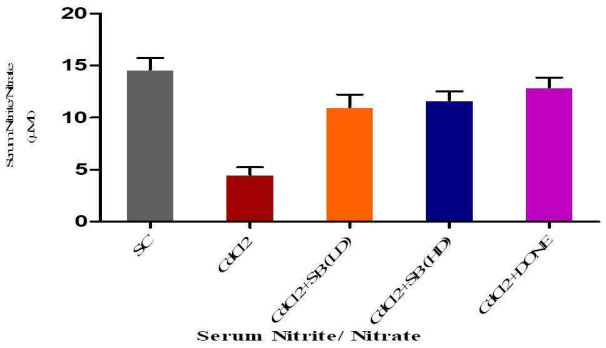

Figure 4. Administration of Sodium butyrate (SB): (dose 1 and dose 2) significantly reversed the $CdCl_2$ induced reduction in serum nitrite/nitrate.*P < 0.05 versus control group, ** P < 0.05 versus $CdCl_2$ treated group; n=6.

Hence it is reflecting induction of oxidative stress. Treatment with Sodium butyrate (Dose1 and Dose2) and donepezil significantly prevented cadmium chloride induced endothelium dysfunction. Further, Sodium butyrate (Dose1 and Dose2) and donepezil did not show any significant per se effect on oxidative stress level.

Effect on levels of Aortic Superoxide Anion Level

Cadmium chloride produced a significant increase in serum levels of aortic superoxide anion. Treatment with Sodium butyrate (Dose1 and Dose2) and donepezil significantly prevented Cadmium chloride induced oxidative stress. Further, Sodium butyrate (dose1 and dose 2) and donepezil did not show any significant per se effect on oxidative stress level.

Effect on serum/brain catalase activity

Cadmium chloride produced a significant decrease in catalase activity. Treatment with Sodium butyrate (dose1 and dose2) and

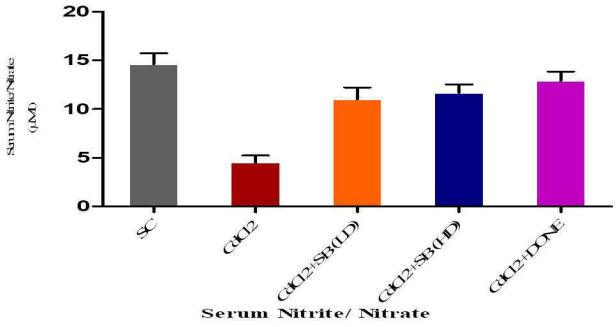

Figure 5. Administration of Sodium butyrate (dose 1 and dose 2) and donepezil significantly increased the serum levels of reduced glutathione (GSH).*P < 0.05 versus control group, ** P < 0.05 versus $CdCl_2$ treated group; n=6.

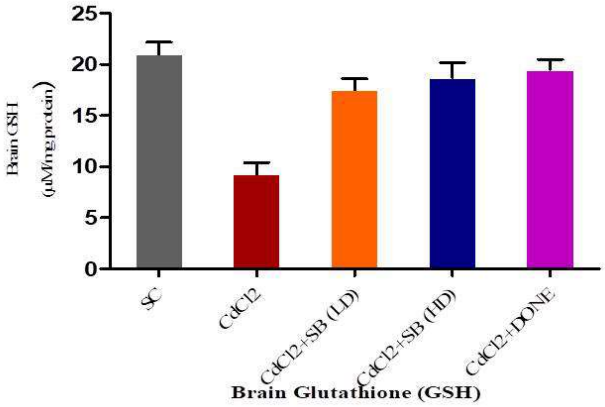

Figure 6. Administration of Sodium butyrate (dose 1 and dose 2) and donepezil significantly increased the Brain levels of reduced glutathione (GSH).*P < 0.05 versus control group, ** P < 0.05 versus $CdCl_2$ treated group; n=6.

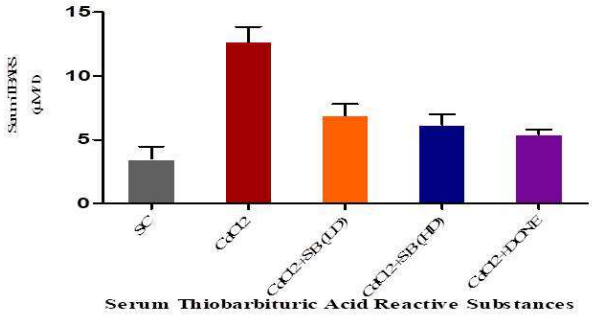

Figure 7. Administration of Sodium butyrate (dose 1 and dose 2) and donepezil significantly reduced the serum levels of thiobarbituric acid reactive species (TBARS).*P < 0.05 versus control group, ** P< 0.05 versus $CdCl_2$ treated group; n=6.

donepezil significantly prevented cadmium chloride induced oxidative stress. Further, Sodium butyrate (Dose1 and Dose2) and donepezil did not show any significant per se effect on oxidative stress level.

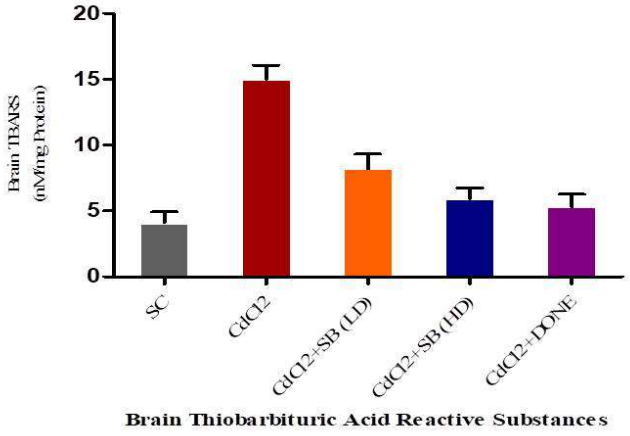

Figure 8. Administration of Sodium butyrate (dose 1 and dose 2) and donepezil significantly reduced the Brain levels of thiobarbituric acid reactive species (TBARS).*P < 0.05 versus control group, ** P < 0.05 versus $CdCl_2$ treated group; n=6.

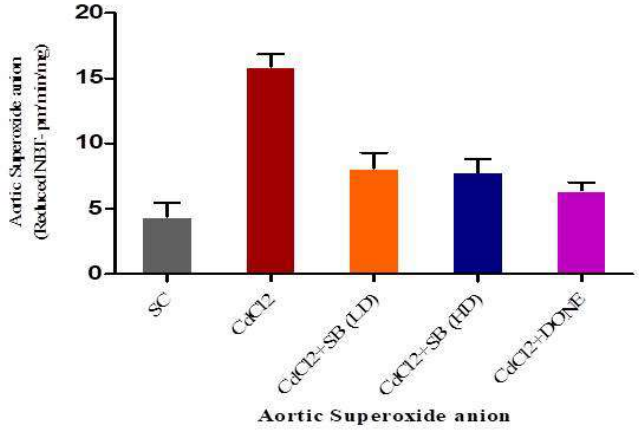

Figure 9. Administration of Sodium butyrate (dose 1 and dose 2) and donepezil significantly reverse the $CdCl_2$ induced increased aortic superoxide generation.*P < 0.05 versus control group, ** P < 0.05 versus $CdCl_2$ treated group; n=6.

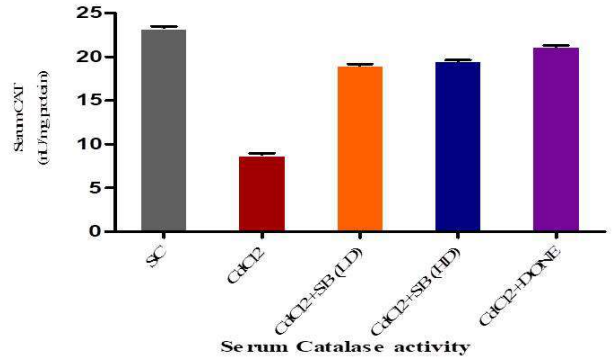

Figure 10. Administration of Sodium butyrate (dose 1 and dose 2) and donepezil significantly reversed the $CdCl_2$ induced reduction in serum levels of catalase (CAT).*P < 0.05 versus control group, ** P < 0.05 versus $CdCl_2$ treated group; n=6.

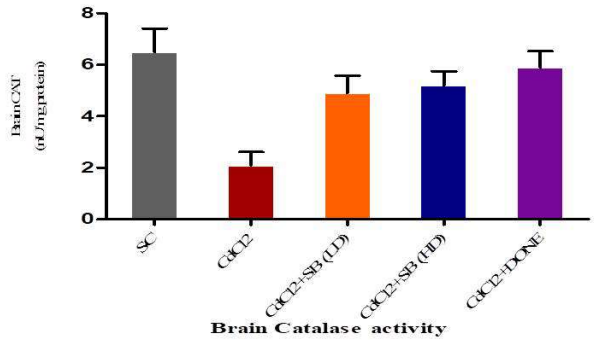

Figure 11. Administration of Sodium butyrate (dose 1 and dose 2) and donepezil significantly reversed the $CdCl_2$ induced reduction in Brain levels of catalase (CAT). *P < 0.05 versus control group, ** P < 0.05 versus $CdCl_2$ treated group; n=6.

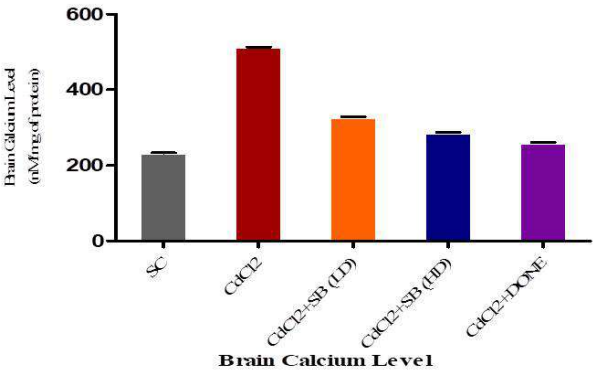

Figure 12. Administration of Sodium butyrate (dose 1 and dose 2) and donepezil significantly reversed the $CdCl_2$ induced reduction in Brain calcium levels. *P < 0.05 versus control group, **P < 0.05 versus $CdCl_2$ treated group; n=6.

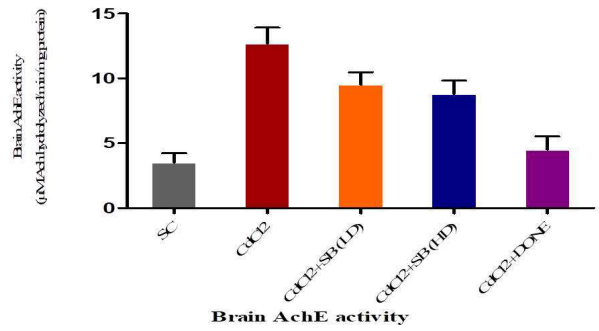

Figure 13. Administration of Sodium butyrate (dose 1 and dose 2) and donepezil significantly reversed the $CdCl_2$ induced reduction in brain acetylcholinesterase (AChE) activity.*P < 0.05 versus control group, ** P < 0.05 versus $CdCl_2$ treated group; n=6.

Effect on brain calcium level activity

Cadmium chloride produced a significant increase in brain calcium levels. Treatment with Sodium butyrate (dose 1 and dose 2) and donepezil significantly prevented cadmium chloride induced increase in brain calcium levels.

Effect on brain acetylcholinesterase (AChE) activity

Cadmium chloride produced a significant increase in AChE activity. Treatment with Sodium butyrate (dose 1 and dose 2), and donepezil significantly prevented cadmium chloride induced increase in AChE activity. Further, Sodium butyrate (dose 1 and dose 2), and donepezil did not show any significant per se effect on increase in AChE activity.

Discussion

The present study observed the neuroprotective effect of Sodium butyrate against $CdCl_2$ induced VaDin rats. HDAC inhibitor, sodium butyrate (Ryu et al., 2003), defend against oxidative stress induced neuronal death; which, suggests that HDACs may still be relevant targets for alleviating ailments associated with neuronal death. A number of behavioral [Elevated plus maze (EPM) and Morris water maze (MWM): Assessment of learning and memory] and biochemical (brain/ serum levels of nitrite/nitrate, Aortic Superoxide Anion, calcium, GSH, TBARS, CAT, AChE) parameters were assessed. Assessment of learning and memory was performed using Elevated plus maze (EPM) and Morris water maze (MWM). Elevated plus-maze (EPM) is employed to evaluate learning skills and memory in rodents. EPM comprises two open and two enclosed arms, which are used to evaluate memory in rodents. Rodents in the plus-maze tend to escape from the open arm to the enclosed arm since they dislike open and high spaces. The time taken by the rodents to move from the open arm to the enclosed arm (transfer latency) was recorded (Itoh et al., 1990). The Morris water maze (MWM) test is performed evaluate spatial learning in rodents which relies on distal cues to navigate from initial positions around the perimeter of an open swimming arena to locate a submerged escape platform. Repeated trials are performed to assess the spatial learning and reference memory is determined by preference for the platform area when the platform is absent. Reversal and shift trials help in revealing spatial impairments (Vorhees et al., 2006). Alzheimer's dementia is generally characterized by the degeneration of the cholinergic system. Therefore the treatment regimens either include muscarinic agonists or acetylcholinesterase (AChE) inhibitors; the latter tends to augment the concentration of acetylcholine by inhibiting AChE activity (Holzgrabe et al., 2007). Oxidative stress is one of the etiopathogenesis associated with dementia. Catalase (CAT) is an important enzyme which protect the cell from oxidative damage and prevent ROS generation by catalysing the breakdown of H_2O_2 into O_2 and H_2O, but in the presence of low concentrations of H_2O_2,itcatalyse the oxidation of electron donors such as ethanol or phenols (Kirkman et al., 1984). Glutathione (GSH) is one of the most important endogenous antioxidants in our bodies, which plays an important role in combating against oxidative stress. Studies with noninvasive magnetic resonance spectroscopy showed that the parietal cortical regions of healthy young male and female subjects have higher amount of GSH in comparison to cortical region in AD patients. Serum levels of catalase CAT, glutathione peroxidase GPX, and superoxide dismutase SOD are reported to be significantly low in patients with AD (Mao et al., 2012). Lipid peroxidation is reported to induce distortion in membrane organization and cause functional deficit in proteins and DNA. Lipid peroxidation involves a series of reactions, which results in the generation of free radicals (Ceaser et al., 2004; Zmijewski et al., 2005). This phenomenon usually occurs in the lipid components of cellular membranes. Polyunsaturated fatty acids frequently endure lipid peroxidation and produce highly reactive electrophilic aldehydes. Amongst them, 4-hydroxy-2-nonenal (HNE) and malondialdehyde are the most common, while acrolein is the most reactive. Thiobarbituric acid reactive substances (TBARS) are produced as a result of lipid peroxidation (Sultana et al., 2012). Lipid peroxidation concerned alterations in cerebral cortex of AD patients, showed a significant decrease in nitrite/nitrate levels in frontal cortex.Nitric oxide (NO) is a free radical produced by nitric oxide synthase (NOS), during the alteration of L-arginine to citrulline. Nitric oxide is a neuronal messenger with a short half life.It converts immediately into nitrite and nitrate (Chen et al., 2000). The present research determines $CdCl_2$-induced alterations in NO production by measuring nitrite and nitrate in the rat brain. Calcium (Ca^{2+}) plays a major role in regulating the fundamental functions of neurons like synaptogenesis, synaptic transmission, plasticity and cell survival. However, Ca^{2+}-regulating systems are compromised in neurodegenerative disorders which results in synaptic dysfunction, impaired plasticity and neuronal death. Most of the pathogenic mechanisms involved in exacerbation of neurodegeneration like oxidative stress, disturbed energy metabolism and aggregation of disease-related proteinsnegatively affect Ca^{2+} homeostasis (Mattson et al., 20007).

Conclusion

The data obtained from the present research clearly indicates that the therapy involving HDACi like Sodium butyrate can be used as a potential therapeutic approach to block further progression of $CdCl_2$ induceddementia and improve disease pathology. HDACi are reported to protect against oxidative stress by their HDAC inhibitory action and also regulate transcription.It is evident that treatment of sodium butyrate(HDAC modulator) significantly attenuates the $CdCl_2$induced behavioral alternations like learning and memory impairment; andsignificantly increased the brain/serum levels of acetylcholinesterase (AChE), catalase (CAT), reduced glutathione (GSH),and nitrite/nitrate levels. In comparison to the disease control,

sodium butyrate administration significantly reduced the brain/serum levels of thiobarbituric acid reactive species (TBARS), brain calcium levels and aortic superoxide anion. The results clearly indicate that oxidative stress and lipid peroxidation are the primary mechanisms involved in the pathogenesis of $CdCl_2$ induced dementia.The aim of this research is to identify and implement the therapeutic potential of HDACi in the treatment of dementia. It is concluded that a target based drug research against pathogenic mechanisms like oxidative stress and lipidperoxidation may help to alleviate ailments associated with neurodegeneration.

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Conflict of Interest

Nil

References

Arendt T, Brückner MK, Morawski M, Jäger C, Gertz HJ. 2015. Early neurone loss in Alzheimer's disease: cortical or subcortical? Acta Neuropathologica Communications , 3:10.

Battistin L, Cagnin A. 2010. Vascular cognitive disorder. A biological and clinical overview. Neurochemical Research, 35(12):1933-8.

Bossy-Wetzel E, Schwarzenbacher R, Lipton SA. 2004. Molecular pathways to neurodegeneration.Nature Medicine, 10 Suppl:S2-9.

Branca JJV, Morucci G, Pacini A. 2018. Cadmium-induced neurotoxicity: still much ado. Neural Regeneration Research,13(11):1879-1882.

Brochier C, Dennis G, Rivieccio MA, McLaughlin K, Coppola G, Ratan RR, Langley B. 2013. Specific acetylation of p53 by HDAC inhibition prevents DNA damage-induced apoptosis in neurons. The Journal of Neuroscience, 33(20): 8621–8632.

Cannon JR, Greenamyre JT.2011. The role of environmental exposures in neurodegeneration and neurodegenerative diseases. Toxicological Sciences, 124(2):225-50.

Ceaser EK, Moellering DR, Shiva S, Ramachandran A, Landar A, Venkartraman A, Crawford J, Patel R, Dickinson DA, Ulasova E, et al. 2004. Mechanisms of signal transduction mediated by oxidized lipids: the role of the electrophile-responsive proteome. Biochemical Society Transactions, 32:151–155.

Chen SM, Swilley S, Bell R, Rajanna S, Reddy SL, Rajanna B. 2000. Lead induced alterations in nitrite and nitrate levels in different regions of the rat brain.Comparative Biochemistry & Physiology, 125(3):315-23.

Chin-Chan M, Navarro-Yepes J, Quintanilla-Vega B. 2015. Environmental pollutants as risk factors for neurodegenerative disorders: Alzheimer and Parkinson diseases. Frontiers in Cellular Neuroscience, 10 (9):124.

Choong G, Liu Y, Templeton DM. 2014. Interplay of calcium and cadmium in mediating cadmium toxicity. Chemico-Biological Interactions, 211:54–65.

Choudhary C, Kumar C, Gnad F, Nielsen ML, Rehman M, Walther TC, Olsen JV, Mann M. 2009. Lysine acetylation targets protein complexes and co-regulates major cellular functions. Science,325 (5942): 834–840.

Dutta D, Hari Kumar SL, Grewal A K. 2017.Neuroprotective Effect of Pirfenidone on Scopolamine Induced Cognitive Impairment and Oxidative Stress. Indian Journal of Physiology and Pharmacology, 61(4): 416–429.

Gaub P, Tedeschi A, Puttagunta R, Nguyen T, Schmandke A, Di Giovanni S. 2010. HDAC inhibition promotes neuronal outgrowth and counteracts growth cone collapse through CBP/p300 and P/CAF-dependent p53 acetylation. Cell Death & Differentiation, 17 (9):1392–1408.

Gupta R, Shukla RK, Chandravanshi LP, Srivastava P, Dhuriya YK, Shanker J, Singh MP, Pant AB, Khanna VK. 2017. Protective Role of Quercetin in Cadmium-Induced Cholinergic Dysfunctions in Rat Brain by Modulating Mitochondrial Integrity and MAP Kinase Signaling.Molecular Neurobiology, 54(6):4560-4583.

Hébert R, Brayne C. 1995. Epidemiology of vascular dementia.Neuroepidemiology, 14(5):240-57.

Holzgrabe U, Kapková P, Alptüzün V, Scheiber J, Kugelmann E. 2007. Targeting acetylcholinesterase to treat neurodegeneration. Expert Opinion on Therapeutic Targets, 11(2):161-79.

Itoh J, Nabeshima T, Kameyama T. 1990.Utility of an elevated plus-maze for the evaluation of memory in mice: effects of nootropics, scopolamine and electroconvulsive shock.**Psychopharmacology (Berl),** 101(1):27-33.

Jomova K, Valko M. 2011. Advances in metal-induced oxidative stress and human disease. Toxicology, 283 (2-3):65–87.

Kazantsev AG, Thompson LM. 2008. Therapeutic application of histone deacetylase inhibitors for central nervous system disorders. Nature Reviews Drug Discovery,7 (10):854–868.

Kim C, Choi H, Jung ES, Lee W, Oh S, Jeon NL, Mook-Jung I. 2012. HDAC6 inhibitor blocks amyloid beta-induced impairment of mitochondrial transport in hippocampal neurons. PLoS One, 7 (8):e42983.

Kirkman HN, Gaetani GF. 1984. Catalase: a tetrameric enzyme with four tightly bound molecules of NADPH. Proceedings of the National Academy of Sciences of the United States of America, 81(14):4343-7.

Kukongviriyapan U, Pannangpetch P, Kukongviriyapan V, Donpunha W, Sompamit K, Surawattanawan P. 2014.Curcumin protects against cadmium-induced vascular dysfunction, hypertension and tissue cadmium accumulation in mice. Nutrients, 6(3):1194-208.

Lepeta K, Lourenco MV, Schweitzer BC, et al. 2016.Synaptopathies: synaptic dysfunction in neurological disorders - A review from students to students. Journal of Neurochemistry, 138(6):785-805.

Mao P. 2012. Oxidative Stress and Its Clinical Applications in Dementia. Journal of Neurodegenerative Diseases, 2013:319898.

Marchetti C. 2014. Interaction of metal ions with neurotransmitter receptors and potential role in neurodiseases. Biometals, 27 (6):1097–1113.

Mattson MP. 2007.Calcium and neurodegeneration.Aging Cell,6(3):337-50. Epub 2007 Feb 28.

Nair AR, Degheselle O, Smeets K, Van Kerkhove E, Cuypers A. 2013. Cadmium-Induced Pathologies: Where Is the Oxidative Balance Lost (or Not)? The International Journal of Molecular Sciences, 14(3):6116-43.

Pohanka M, Hrabinova M, Kuca K, Simonato JP. 2011. Assessment of acetylcholinesterase activity using indoxylacetate and comparison with the standard Ellman's method. The International Journal of Molecular Sciences, 12(4):2631-40.

Rivieccio MA, Brochier C, Willis DE, Walker BA, D'Annibale MA, McLaughlin K, Siddiq A, Kozikowski AP, Jaffrey SR, Twiss JL, Ratan RR, Langley B. 2009. HDAC6 is a target for protection and regeneration following injury in the nervous system. Proceedings of the National Academy of Sciences of the United States of America, 106 (46):19599–19604.

Ryu H, Lee J, Olofsson BA, Mwidau A, Dedeoglu A, Escudero M, Flemington E, Azizkhan-Clifford J, Ferrante RJ, Ratan RR. 2003. Histone deacetylase inhibitors prevent oxidative neuronal death independent of expanded polyglutamine repeats via an Sp1-dependent pathway. Proceedings of the National Academy of Sciences of the United States of America. 100 (7):4281–4286.

Serrano-Pozo A, Frosch MP, Masliah E, Hyman BT. 2011.Neuropathological alterations in Alzheimer disease. Cold Spring Harbor Perspectives in Medicine 1(1):a006189.

Sharma B, Singh N. 2012a. Defensive effect of natriumdiethyldithiocarbamatetrihydrate (NDDCT) and lisinopril in DOCA-salt hypertension-induced vascular dementia in rats. Psychopharmacology, 223(3): 307-17.

Siewit CL, Gengler B, Vegas E, Puckett R, Louie MC. 2010. Cadmium promotes breast cancer cell proliferation by potentiating the interaction between ERalpha and c-Jun. Molecular Endocrinology, 24(5):981-92.

Sleiman SF, Basso M, Mahishi L, Kozikowski AP, Donohoe ME, Langley B, Ratan RR. 2009. Putting the 'HAT' back on survival signalling: the promises and challenges of HDAC inhibition in the treatment of neurological conditions. Expert Opinion on Investigational Drugs.18 (5):573–584.

Sleiman SF, Berlin J, Basso M, Karuppagounder SS, Rohr J, Ratan RR. 2011. Histone deacetylase inhibitors and mithramycin A impact a similar neuroprotective pathway at a crossroad between cancer and neurodegeneration. Pharmaceuticals, 2011;4 (8):1183–1195.

Srivastava A, Singh R. 2019. Pharmacological evaluation of combined neuroprotective effect of Melatonin and Simvastatin against LPS and STZ induced memory impairment in rodents. Asian Journal of Pharmacy and Pharmacology, 5(2): 01-10.

Sultana R, Perluigi M, Butterfield DA. 2012. Lipid peroxidation triggers neurodegeneration: a redox proteomics view into the Alzheimer disease brain. Free Radical Biology and Medicine, 62:157-169.

Tamaddonfard E, Farshid AA, Asri-Rezaee S, Javadi S, Khosravi V,Rahman B, Mirfakhraee Z. 2013. Crocin Improved Learning and Memory Impairments in Streptozotocin-Induced Diabetic Rats. The Iranian Journal of Basic Medical Sciences, 16 (1):91-100.

Vorhees CV, Williams MT. 2006. Morris water maze: procedures for assessing spatial and related forms of learning and memory. Nature Protocols, 1(2):848-58.

Zmijewski JW, Landar A, Watanabe N, Dickinson DA, Noguchi N, Darley-Usmar VM. 2005. Cell signalling by oxidized lipids and the role of reactive oxygen species in the endothelium. Biochemical Society Transactions, 33:1385–1389.