This journal is the official publication of Bangladesh Society of Physiologists (BSP)
Web URL: www.banglajol.info/index.php/JBSP

Abstracted /indexed in Index Copernicus, Director of Open Access Journal, HINARI Index Medicus for South East Asia Region, Google Scholar, 12OR, infobse index, Open J gate, Cite factor, Scientific indexing services

pISSN-1983-1213; e-ISSN-2219-7508

Article

Article information:
Received: Feb 2024
Accepted: April 2024
DOI:https://doi.org/10.3329/jbsp.v19i1.79567

Corresponding author:
Taskina Ali, Department of Physiology, Bangabandhu Sheikh Mujib Medical University, Dhaka. E-mail: dr.taskinaali@gmail.com

Cite this article:
Taskina Ali, Rokhsana Binte Amin, Puspita Basak, Fhamida Akter, Md. Saiful Islam, Kazi Rafiqul Islam. Spatial memory impairment by hippocampal oxidative stress in male Long-Evans rats-Can it be prevented and/or alleviated by climbing exercise? J Bangladesh Soc Physiol 2024;19(1): 9-22.

Spatial memory impairment by hippocampal oxidative stress in male Long-Evans rats - Can it be prevented and/or alleviated by climbing exercise?

Taskina Ali[1], Rokhsana Binte Amin[1], Puspita Basak[1], Fhamida Akter[1], Md. Saiful Islam[1], Kazi Rafiqul Islam[2]

1. Department of Physiology, Bangabandhu Sheikh Mujib Medical University, Dhaka
2. Department of Pharmacology, Faculty of Veterinary Science, Bangladesh Agricultural University, Mymensingh

Abstract

Background: Drug treatment for Spatial memory impairment has a wide range of adverse effects. **Objective:** To assess the effects of climbing exercise (CE)on spatial memory performance and hippocampal oxidative stress in colchicine induced memory impaired rats. **Methods:** Thirty male Long-Evans rats (8±2weeks; 225±75 gm)were grouped (6 rats/group)into normal control, sham control, colchicine control, pre colchicine CE and post colchicine CE. In CE, each rat performed 08 to 12 climbs/hour in a climbing tool for consecutive 28 days. Then, reference memory (RM) [Mean escape latency (EL) in acquisition phase, average EL of 5^{th} and 6^{th}acquisition days; number of target crossing (TC) and time spent in target (TT) in probe trial] and working memory (WM) [Mean EL and savings in training and test phase] were assessed by Morris water maze (MWM) test. Then, after sacrifice, hippocampal malondialdehyde (MDA) and glutathione peroxidase (GPx) were estimated. Data were expressed as mean±SEM and analyzed by one way ANOVA followed by Bonferroni's post hoc test where $p \leq 0.05$ was considered as statistically significant. **Results:** This study indicate that intrahippocampal colchicine administration significantly impaired spatial memory, and elevated MDA, decreased GPx level in hippocampus of colchicine control rats. In contrast, both pre and post treatment of CE significantly improved spatial memory retention and attenuated the oxidative damage almost to normal. **Conclusion:**CE is equally effective in prevention as well as alleviation of colchicine induced spatial memory impairment along with hippocampal oxidative stress in male Long-Evans rats.

Key words: Memory impairment, colchicine, oxidative stress, climbing exercise, malondialdehyde, glutathione peroxidase, hippocampus, Morris water maze, reference memory, working memory.

Introduction

Memory, the processes by which learned knowledge is encoded, stored, and later retrieved.[1] In it, nonspatial memory represents visual shapes and color (i.e., what) of an object whereas spatial learning and memory is important for locating objects, remembering previous locations, and finding one's way around the environment (i.e., where).[2] Memory impairment is unusual forgetfulness which is different from age-related forgetfulness. According to WHO, currently more than 55 million people have dementia worldwide[3] and memory impairment is one of the important components of dementia. Physical inactivity, sedentary lifestyle as well as stressful life style linked with spatial memory impairment. Besides this, many clinical conditions, such as brain trauma, stroke, brain infections, brain tumors, Alzheimer's disease, Parkinson's disease are also associated with memory impairment.[4]

Presently, the long term memory is known as reference memory.[5] Here, the information available for solving reference memory tasks is constant throughout the trials, and reinforced by repeated training.[6] On the other hand, working memory is a form of short term memory that keeps information available, usually for very short periods of time, while an individual plans action based on it.[6] Both of these memories depend on the hippocampus, the most medial portion of the temporal lobe cortex. Neurodegeneration of this hippocampus can cause memory impairment and oxidative stress is one of the postulated mechanisms of this neurodegeneration.[7]

Oxidative stress is caused by reactive oxygen species (ROS), which are part of a group of molecules called free radicals, leads to cellular abnormalities.[8] The final product of lipid peroxidation is malondialdehyde (MDA) which is commonly measured as an indirect index of oxidative stress.[9] On the other hand, antioxidant is the scavenging molecule that limits or prevents the damaging effects of oxidants. Among them, superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) act as first line defense against oxidative injury. Brain is very sensitive to oxidative stress because of its high metabolic activity, high density of oxidizable substances, and relatively low antioxidant defense.[10]

Among various physical exercises, those use oxygen more efficiently, such as swimming, running, cycling, jogging, climbing[11] would be a good alternative for memory impairment and oxidative stress improvement.[12-13] However, among different aerobic exercises, climbing exercise (CE) is much preferable for it's cost effectiveness and easy accessibility in performance in any naturalistic settings. This CE was found to induce cognitive benefits in healthy human[14] as well as in type 1 diabetic rats[15], aged rats[16] and Alzheimer's disease like model of rats[17-18]by decreasing oxidative damage.

Furthermore, in perspective of exercise timing, this CE was found to be effective when treated during[17] as well as before[18] spatial memory impairment by reducing oxidative damage. In them, different time schedules of CE, that is six (6) weeks[17]or eight (8) weeks[18] duration, were applied.

As far as we searched, no published information was found regarding the effect of this exercise after development of any sort of memory impairment. Based on this background, this present study has been designed to evaluate the effects of CE on memory performance as well as hippocampal oxidative stress markers both before and after colchicine induced spatial memory impairment in male Long-Evans rats.

Methods

Study design and setting

This experimental study was conducted in the Department of Physiology, Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka,

Bangladesh from March 2023 to February 2024 after obtaining ethical clearance from Institutional Review Board (Reg No: 1001) of that University.

Drugs and chemicals

Colchicine was obtained from the Incepta Pharmaceuticals Ltd, Bangladesh. Normal saline (Popular Pharmaceuticals Ltd, Bangladesh), gentamicin (Incepta Pharmaceuticals Ltd, Bangladesh),thiopental sodium (Gonosasthaya Pharmaceuticals Ltd) and di-ethyl ether (MERCK, Germany) were purchased from local market.

Procurement and maintenance of animals

Thirty (30) male Long-Evans rats (8±2 weeks; 225±75 gm) were collected from the central animal house of BSMMU. Then under a 12/12 hour light/ dark cycle (Wren-Dail et al. 2016), the rats were kept in specially built plastic cages (6 rats/cage). The ambient room temperature was maintained at 27°C. All rats were provided with standard laboratory food and cooled boiled water ad libitum. All the experiments were conducted according to the þGuidelines for the Animal Experimentation Ethics Committee (AEEC, 15/05/2023)'of the International Centre for Diarrhoeal Disease Research, Bangladesh and advice of an expert veterinarian of Bangladesh Agricultural University, Mymensingh, Bangladesh. All the experiments were performed at daytime between 08:00 and 16:00 hours to avoid circadian influences.

Experimental design and dose schedule

On the basis of treatments, all the rats were randomly divided into five (5) groups (6 rats/ group):

- Normal control (NC): no stereotaxic surgery (SS), no normal saline (NS), no climbing exercise (CE).
- Sham control (SC): SS and hippocampal infusion of 1 µl NS, no CE.
- Colchicine control (ColC): SS and hippocampal infusion of 15 µg of colchicine in 1 µl NS, no CE.
- Pre colchicine climbing exercise (Pre CE Exp):CE for 28 days followed by SS and hippocampal infusion of 15 µg of colchicine in 1 µl NS.
- Post colchicine climbing exercise (Post CE Exp): SS and hippocampal infusion of 15 µg of colchicine in 1 µl NS followed by CE for 28 days.

Climbing exercise (CE) protocol

According to Neto et al. (2016)[20],rats were allowed to 8-12 climbs on the climbing tool (Figure 1) for consecutive sixty (60) minutes/day in morning, for consecutive twenty-eight (28) days. Baits (Jilapi) were given into the top box to attract the rats for climbing.

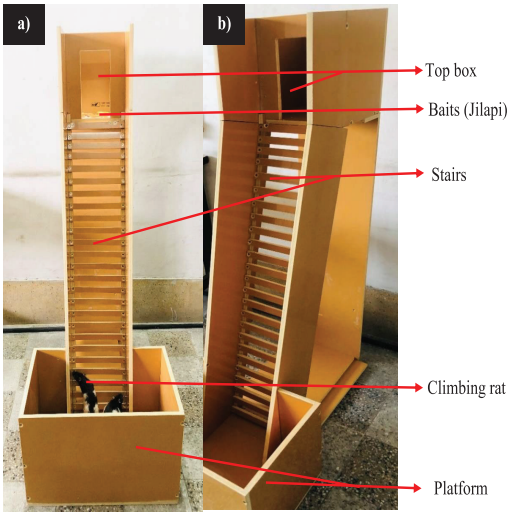

Figure 1: Climbing tool for climbing exercise (a. front view; b. side view)

Hippocampal colchicine application by stereotaxic surgery

According to previous researches[21-22], the colchicine was administered into the rat hippocampus by stereotaxic surgery. After deeply anesthetized with thiopental sodium (45 mg/kg; i.p.), rat was positioned in stereotaxic apparatus. The scalp was incised, retracted and two holes were drilled in the skull according to the coordinates of the hippocampus: -3.6 mm anterior-posterior, ± 2 mm lateral-medial, and -3.4 mm dorso-ventral relative to bregma. Then infused 15 µg of colchicine in 1 µl NS in each hippocampus through a Hamilton micro syringe with very slowly over 1 minute and the micro syringe was kept place for the next minute before being slowly withdrawn. The scalp was then

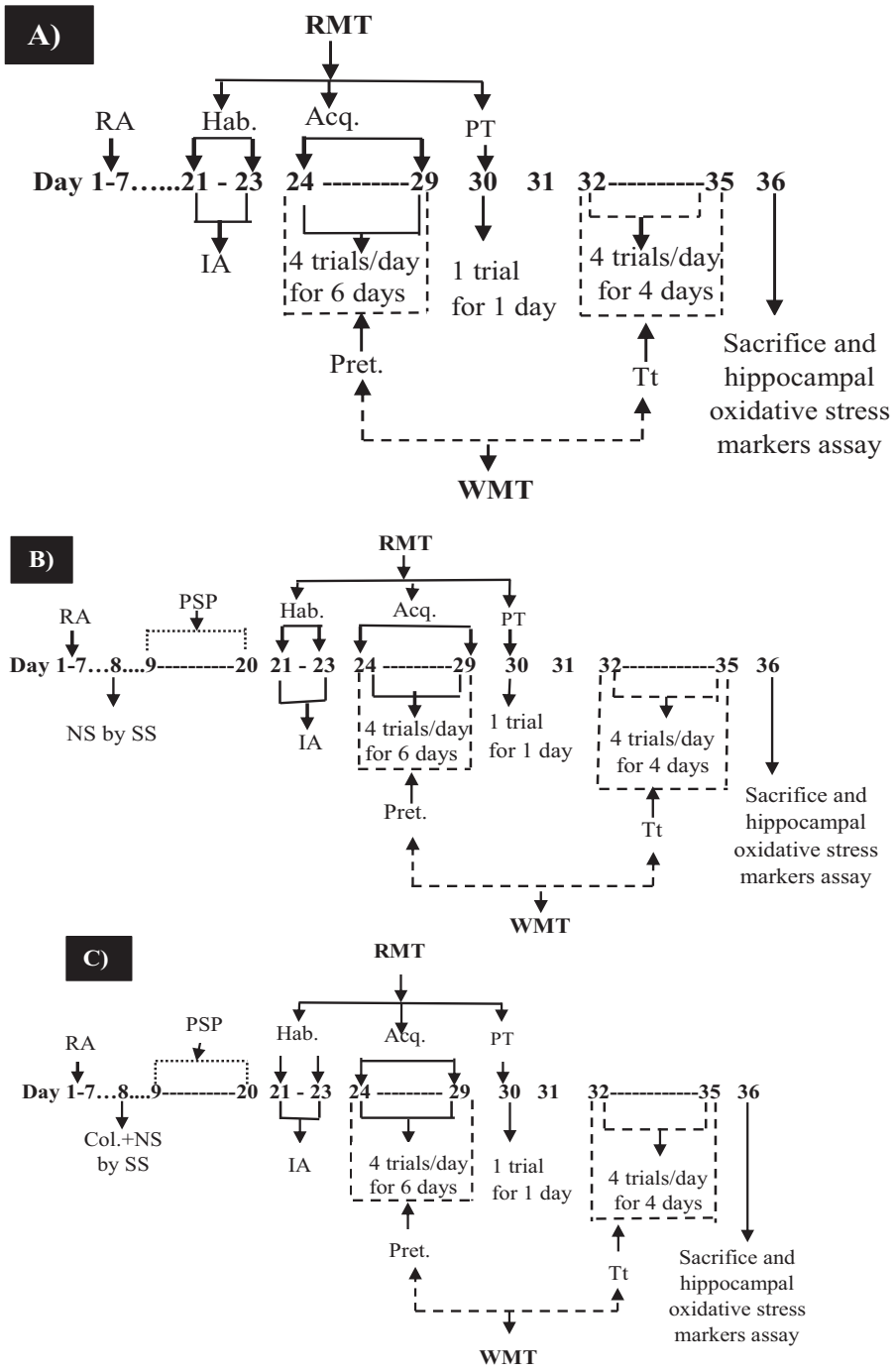

Figure 2: Working plan for rats A) normal control; B) sham control; C) colchicine control RMT: Reference memory test; WMT: Working memory test; RA=Room acclimatization; PSP=Post-surgical period; Hab.=Habituation; Acq.=Acquisition; PT=Probe trial; IA=Instrumental acclimatization; Pret.= Pretraining; Tt=Training and test; NS=Normal saline; SS=Stereotaxic surgery; Col.=Colchicine

closed with sutures and injected gentamycin (5 mg/kg, i.p.) to prevent sepsis.

Spatial memory assessment by Morris Water Maze (MWM) test

Test tools and circumstances

According to previous researchers[23-24], the apparatus consisted of a large circular pool (150 cm diameter X 50 cm high) filled with water. The whole inner wall of the pool and platform was painted with non-toxic black colour to avoid visual clue in the pool. The pool was placed in a room containing extra maze cues, those could be used by rats for their orientation. Eight points, north (N), south (S), east (E), west (W), north-east (NE), north-west (NW), south-east (SE) and south-west (SW) were used as starting positions and divided the pool arbitrarily into four quadrants. A black round platform (15 cm diameter X 28 cm high) was placed in the center of the NE quadrant with its top 2 cm below the water surface so as to be invisible from within the pool.

All the working plan of five different groups of rats were shown in figure 2 and figure 3.

Reference memory test

As shown in figure 2 and figure 3, rats were swimming for 3 minutes without platform for consecutive 3 days for instrumental acclimatization as well as for habituation phase. Then in acquisition phase, rats were given four trials/day over 6 consecutive days with fixed platform position (NE quadrant). In each trial, rats were released from a different starting point in a different sequence in each day and given 60 sec to find the platform. A 50 sec (20 sec on platform+30 sec for self-drying) intertrial time was

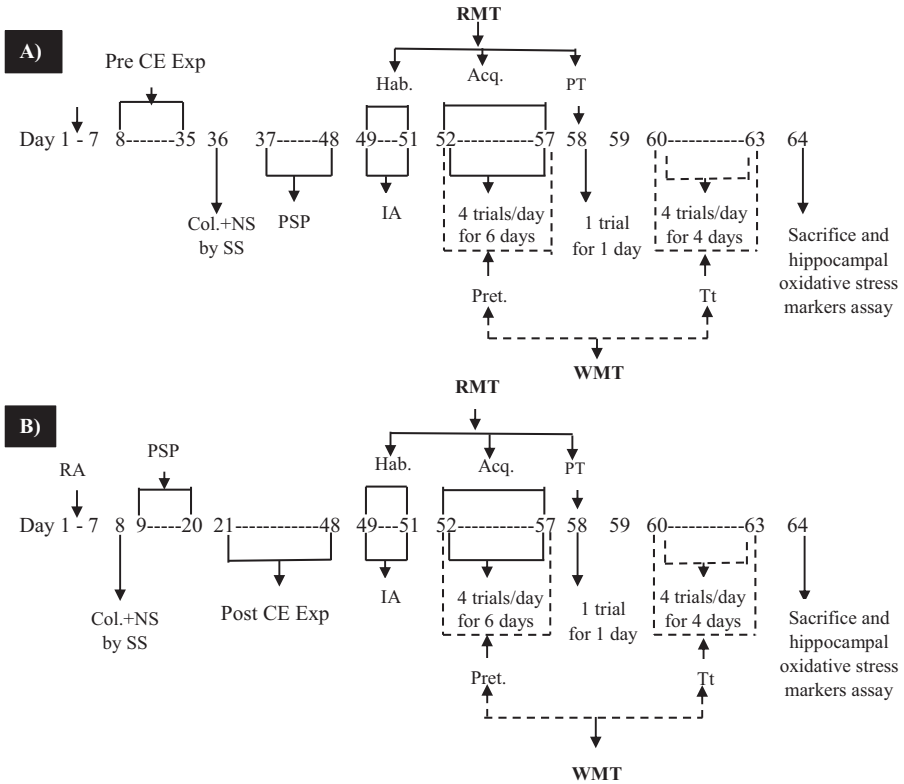

Figure 3: Working plan for rats A) Pre colchicine climbing exercise; B) Post colchicine climbing exercise.RMT: Reference memory test; WMT: Working memory test; RA=Room acclimatization; PSP=Post-surgical period; Hab.=Habituation; Acq.=Acquisition; PT=Probe trial; IA=Instrumental acclimatization; Pret.= Pretraining; Tt=Training and test; NS=Normal saline; SS=Stereotaxic surgery; Col.=Colchicine; Pre CE Exp=Pre colchicine climbing exercise; Post CE Exp=Post colchicine climbing exercise.

permitted. Mean escape latency (EL, time from the moment of entrance into the water up to arrival at the platform] was measured by using a stop watch to assess learning ability. Similar 4 trials were given for consecutive 6 days. On the first trial each day rats reached the platform by chance, that constituted the information stage and the subsequent trials required matching to the novel position for every day, as platform was fixed for 6 days. The average EL of 5th and 6th acquisition days was measured to assess memory consolidation. About 24 hours after last trial of day 6 (day 7), the platform was removed from pool and probe trial was given to assess learning strength/ retrieval. In this trial, rats were allowed to swim ad libitum for 60 sec during which target crossings (TC; number of passing the quadrant by rats, within 60 seconds from where the platform was removed) and time spent in target (TT; time spent in the quadrant, from where the platform was removed within 60 seconds) were measured.

Working memory test

As shown infigure 2 and figure 3, after 48 hours of probe trial, the working memory version of the test was administered using a testing paradigm adapted from Sarihi et al. (2000).[31]Here, t he 6 days pre training phase was considered as acquisition phase of reference memory. Then, training and test phase was conducted over 4 consecutive days with 4 trials/day. Every day, the platform position was changed but kept constant for daily 4 trials. However, every rat was released from 4 different starting points in the 4 daily trials all of which were distant from platform position. The mean EL in training and test phase was recorded as above to assess learning ability and savings (the difference in latency scores between trials 1 and 2, expressed as percentage of trial 1) was measured to assess leaning efficiency.[25-26]

Hippocampal oxidative stress marker assessment

Rats were sacrificed by decapitation under di-ethyl ether (99%) anesthesia. The brains were then quickly removed and hippocampus was separated immediately followed by washing with ice cold phosphate buffer solution [(PBS); 0.1 M, pH:7.40]. Next, using a glass homogenizer, the tissues were weighed and homogenized in PBS using Weight (gm): Volume (ml) = 1:4 ratio. After that, the homogenate was centrifuged at 3500 rpm for 10 minutes in order to extract the supernatant. The supernatant was collected for analysis of MDA and GPx through ELISA. If there was any unintended delay then the supernatant was kept in -20^{0}C in laboratory.

Statistical analysis

Results were expressed as mean±SEM of the study variables shown in table I. Data were statistically analyzed by using ANOVA followed by Bonferroni post hoc test in SPSS (version 25.0), where $p \leq 0.05$ was considered as statistically significant.

Results

Reference memory performance

Here, our ColC rats showed significantly ($p \leq 0.001$) higher mean EL in all acquisition days in comparison to those of SC rats. However, climbing exercise (CE) improved our rats' learning ability performance, as evidenced by statistically significant ($p \leq 0.001$) differences in mean EL of our ColC and experimental (Pre CE Exp and Post CE Exp) rats. Strikingly, the differences of this variable between our experimental (Pre CE Exp and Post CE Exp) and NC rats in last acquisition day as well as between Pre CE Exp vs Post CE Exp rats were found statistically non-significant in all acquisition days (Figure 4A). These data demonstrated that the learning ability of reference memory was impaired by colchicine and this impairment was reversed almost to normal by CE.

As shown in figure 4B, the mean value of average EL in 5th and 6th acquisition days was significantly ($p \leq 0.01$) higher in our ColC rats compared to those of SC rats.Moreover, CE showed significantly ($p \leq 0.05$) lower average EL in both groups of our experimental (Pre CE Exp and Post CE Exp) rats than that of ColC rats. On the contrary, the differences of this variable between our NC vs Pre CE Exp, NC vs Post CE Exp along

Table I: Study variables

	Spatial memory type	Spatial memory perspective	Variables; Units
For memory performance	Reference memory	Learning ability	Mean escape latency (in acquisition phase); seconds
		Consolidation	Average escape latency of 5th and 6th acquisition days (in acquisition phase); seconds
		Learning strength (Retrieval)	Target crossing; frequency/minute Time spent in target; seconds/minute
	Working memory	Learning ability	Mean escape latency (in training and test phase); seconds
		Learning efficiency	Savings; seconds
		Oxidative perspective	Oxidative stress markers; Units
For hippocampal oxidative stress		Oxidation	Malondialdehyde (MDA); ng/mg protein
		Antioxidation	Glutathione peroxidase (GPx); pg/mg protein

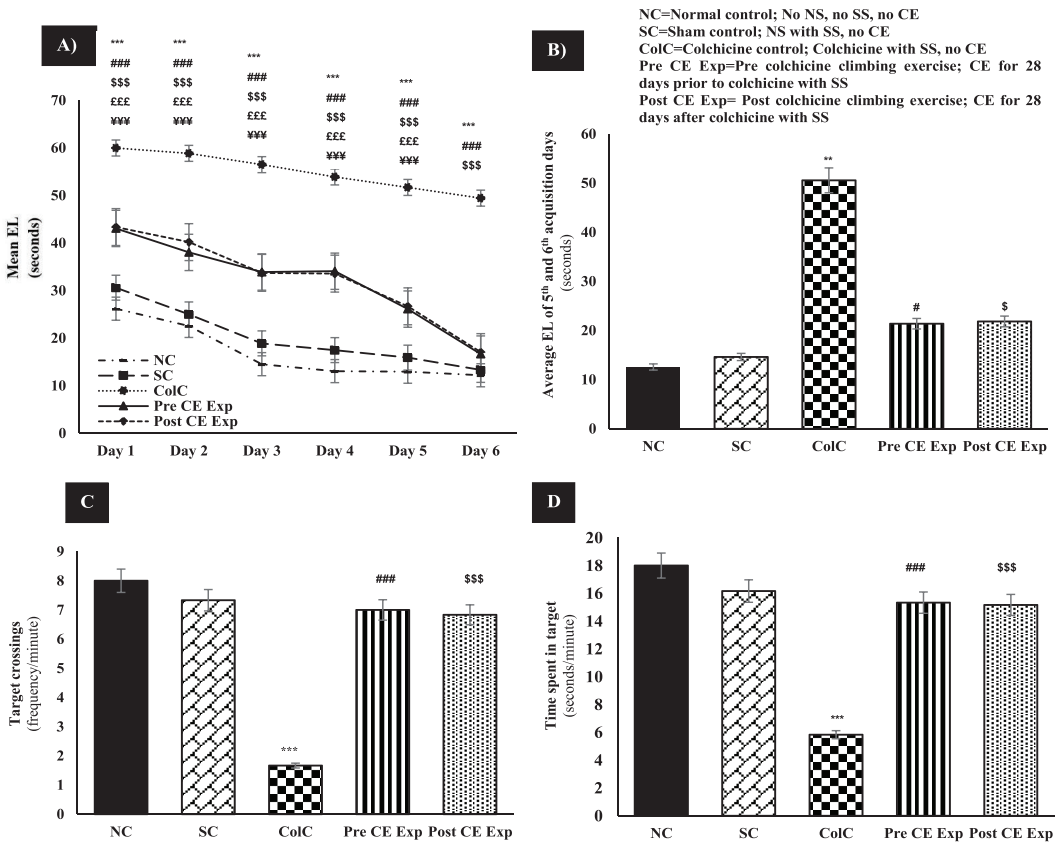

Figure 4: Reference memory performance in the Morris Water Maze (MWM) test. Mean EL of 4 trials (1st/2nd/3rd/4th) in consecutive 6 days (A); average EL of 5th and 6th acquisition days (B); target crossing (C) and time spent in target (D). Data were expressed as mean±SEM.Statistical analysis was done by ANOVA (among groups) followed by Bonferroni's post hoc test (between groups). *=SC vs ColC, # = ColC vs Pre CE Exp, $ = ColC vs Post CE Exp, £ = NC vs Pre CE Exp, ¥ = NC vs Post CE Exp. In the interpretation of results, p≤0.05 was considered as significant. */#/$/£/¥=p≤0.05; **/##/$$/££/¥¥=p≤0.01; ***/###/$$$/£££/¥¥¥=p≤0.001. EL=Escape latency; NS=Normal saline; SS = Stereotaxic surgery; CE = Climbing exercise; n=6.

with Pre CE Exp vs Post CE Exp rats were found statistically non-significant. This result indicated that colchicine induced consolidation disability in our rats and CE improved it similar to normal.

In our study, ColC rats showed retrieval impairment, as evidenced by significantly ($p \leq 0.001$) lower TC and TT than those of SC rats.OurCE caused significantly ($p \leq 0.001$) higher TC and TT in both of our experimental (Pre CE Exp and Post CE Exp) rats than that of ColC rats. And, Statistical analysis revealed that the differences in these variables between our NC vs Pre CE Exp, NC vs Post CE Exp, and Pre CE Exp vs Post CE Exp rats were not significant (Figure 4C, Figure 4D). These data indicated that learning strength (retrieval) was hampered by colchicine and prevented as well as alleviated almost to normal by CE.

Working memory performance

Here, figure 5A showed that ColC rats had significantly ($p \leq 0.001$) higher mean EL in comparison to those of SC rats in all the trials in all the test days. However, CE improved our rats' learning ability performance, as evidenced by statistically significant differences in mean EL of our ColC and experimental [Pre CE Exp ($p \leq 0.05$ in trial 1; $p \leq 0.001$ in trial 2, 3 and 4) and Post CE Exp ($p \leq 0.05$ in trial 1; $p \leq 0.01$ in trial 2; $p \leq 0.001$ in trial 3 and 4)] rats.Strikingly, the differences of this variable were statistically non-significant between NC vs Pre CE Exp, NC vs Post CE Exp in trial 4 and Pre CE Exp vs Post CE Exp in all the trials in all the test days. These data revealed that colchicine impaired the learning ability of working memory and this impairment was reversed near to normal by CE.

In our experiment, ColC rats showedsignificantly ($p \leq 0.01$) lower savings (i.e., learning inefficiency) in comparison to those of SC rats. ButCE could not improve savings in our Pre CE Exp and Post CE Exprats, as evidenced by non-significant difference between our ColC and experimental (Pre CE Exp and Post CE Exp) rats.However, the mean values of savings in Pre CE Exp ($p \leq 0.01$) as well asPost CE Exp ($p \leq 0.001$) were significantly lower in comparison to that of NC rats.On the contrary, differences of this variable between Pre CE Exp vs Post CE Exp rats were statistically non-significant(Figure 5B). Above data indicated that

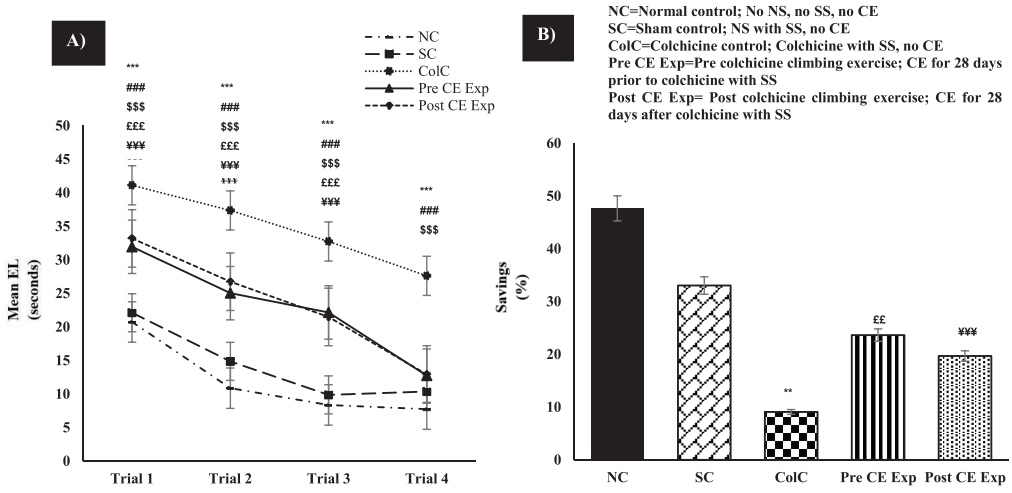

Figure 5: Working memory performance in the Morris Water Maze (MWM) test. Mean EL of 4 trials (1s,2s,3s,4s) of 6 rats in consecutive 4 days (A); savings (B). Data were expressed as mean±SEM. Statistical analysis was done by ANOVA (among groups) followed by Bonferroni's post hoc test (between groups). *=SC vs ColC, #=ColC vs Pre CE Exp, $=ColC vs Post CE Exp, £= NC vs Pre CE Exp, ¥= NC vs Post CE Exp. In the interpretation of results, $p \leq 0.05$ was considered as significant. **/##/$$/££/¥¥=$p \leq 0.01$; ***/###/$$$/£££/¥¥¥=$p \leq 0.001$. EL=Escape latency;NS=Normal saline; SS=Stereotaxic surgery; CE= Climbing exercise; n=6.

our CE schedule could not prevent or alleviate colchicine induced learning inefficiency.

Hippocampal oxidative stress

As shown in figure 6, we observed that our ColC rats showed significantly ($p \leq 0.001$) higher hippocampal MDA and lower GPx level in comparison to those of SC rats. However, CE caused a significantly lower MDA and higher GPx in Pre CE Exp ($p \leq 0.01$; $p \leq 0.001$) as well as in Post CE Exp ($p \leq 0.01$; $p \leq 0.001$) rats in comparison to those of ColC rats. Strikingly, there was non-significant differences between NC vs Pre CE Exp and NC vs Post CE Exp as well as Pre CE Exp vs Post CE Exp rats.These data denoted that, colchicine caused hippocampal oxidative stress and our CE could prevent and alleviate this stress almost near to normal.

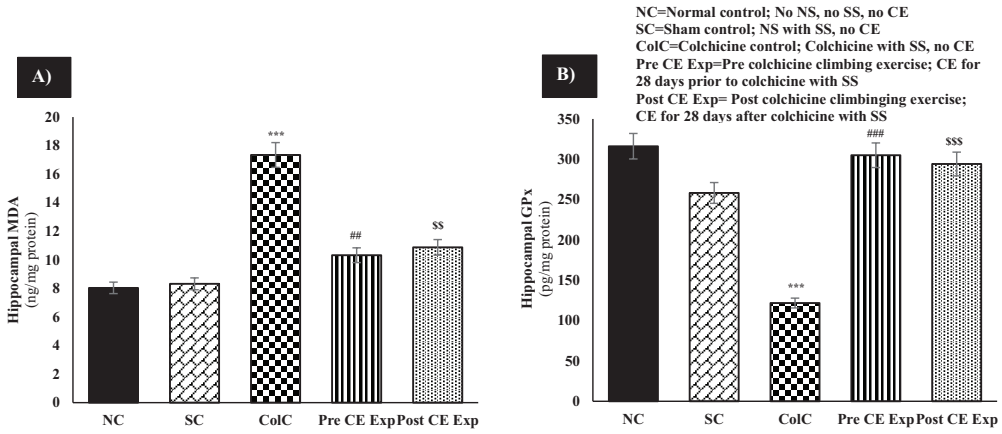

Figure 6: Hippocampal Malondialdehyde (MDA) (A) and hippocampal Glutathione peroxidase (GPx) (B) level in different groups of rats. Data were expressed as mean±SEM. Statistical analysis was done by ANOVA (among groups) followed by Bonferroni's post hoc test (between groups). *=SC vs ColC, #=ColC vs Pre CE Exp, $=ColC vs Post CE Exp. In the interpretation of results, $p \leq 0.05$ was considered as significant. **/##/$$=$p \leq 0.01$; ***/###/$$$=$p \leq 0.001$.NS=Normal saline; SS=Stereotaxic surgery; CE = Climbing exercise; n=6.

Discussion

Memory, specially the spatial memory is one of the brain's most complex processes and is crucial for the proper functioning of any living organism. If memory is impaired, it can significantly hinder one's intellectual ability, interfering with personal behaviors and social communications.

Here, our spatial memory impaired rats showed both reference and working memory deficit along with oxidative stress as evidenced by lowered learning ability, consolidation, learning efficiency, retrieval ability as well as increment of malondialdehyde (MDA) and decrement of glutathione peroxidase (GPx) in comparison to those of normal and sham group rats.Similar findings were reported by Kumar et al.[27] and Pourkhhodadad et al.[28] in colchicine induced memory impaired rats.

Here, the intrahippocampal colchicine might bind with microtubule binding protein (tubulin)[29], depolymerizing microtubule[30] and blocked axoplasmic flow[31], cellular growth as well as cellular differentiation.[21] These processes might lead to hippocampal granule and pyramidal neurons damage.[21]Moreover, colchicine might increase the formation of Beta amyloid protein (BAP)[32] by increasing the action of â and ã

secretase on amyloid precursor protein (APP).[6] This BAP might accumulate extracellularly forming Aâplaques[6-33]resulted in neuronal death.

Moreover, this microtubule disrupting agent also might induce direct nitric oxide (NO) production[34] by activation of inducible nitric oxide synthase (iNOS) enzyme.[35] This NO might react with superoxide anions (a reactive oxygen species, ROS) rapidly, form peroxynitrite ($ONOO^{\cdot}$) (reactive nitrogen species, RNS) resulted in oxidative stress and hippocampal neuronal death. In addition, this neurotoxic agent might elevate glutamate binding site[21] iná-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) and N-methyl-D-aspartate (NMDA) receptors in hippocampal neurons causing excess Ca^{2+} influx. This process might be responsible for mitochondrial stress, ROS production, oxidative stress[36] and neuronal death in hippocampus. Moreover, this markedly increased ROS and RNS might affect double bonds of polyunsaturated fatty acids of neuronal cell membranes (lipid peroxidation)[8] and might elevate hippocampal MDA level in our memory impaired rats.[28]In addition, this increment of oxidants might cause increased consumption of GPx[8] for oxidative balance and decrease GPx concentration[28] in hippocampal neurons

All or any of the above-mentioned mechanisms might responsible forspatialmemory impairment along with oxidative stress in our colchicine treated rats.

However, CE improved both reference and working memory impairment as evidenced by improved learning ability, consolidation and retrieval ability of our experimental (pre and post treated) rats in comparison to those of memory disabled rats. Similar findings were observed in different animal models of memory impairment after various forms of exercises.[12,16]However, our CE schedule could not improve the working memory learning inefficiency (savings) in our experimental rats (Figure 5B).

Here, CE, being an aerobic exercise, might cause increment of brain derived neurotrophic factor (BDNF)[37], an important marker of memory and cognition.[38] As a consequence, BDNF might activate synaptogenesis[39], neurogenesis[40], as well as hippocampal volume increment[37], to replenish the microtubule disrupted neuronal death in our experimental rats. Moreover, CE might cause decrement of Aâ plaques in rat hippocampus[17], resulted in decreased neuronal death. This decrement of neuronal death might be responsible for prevention as well as alleviation of spatial memory impairment in our experimental rats.

Moreover, CE reduced oxidative stress in our experimental (pre and post treated) rats as evidenced by decrement of MDA and increment of GPx when compared to spatial memory impaired rats.Many researchers found similar effects of resistance training and other exercises in different models of memory impaired rats.[12,16,17,41]

In this aspect, our 28 days CE might stimulate the hippocampal ROS induced metabolic adaptation[42], enhancement of the activity of nuclear erythroid 2-related factor 2/antioxidant response element (Nrf2/ARE) signal transduction pathway and formation of GPx[16,41]in our experimental rats. This increased GPx might mitigate the increased MDA level in both of our pre and post exercise treated experimental rats.

As a whole, both of our experimental (pre and post treated) rats showed almost similar reference memory and working memory performances as well as oxidative stress status when compared to those of our normal rats, except savings. From these findings, we propose that our CE schedule could reverse all the aspects of spatial memory disability and oxidative stress almost near to normal. These results also revealed that the improvement of learning disability of our experimental rats were somewhat slower than those of normal rats (Figure 4A; Figure 5A). It indicated that, their speed of acquisition for learning[26] was slow.

Furthermore, when we compared all of these spatial memory and oxidative stress variables between our two experimental groups, they were visualized to be statistically similar preliminarily (Figure 4 – Figure 6). However, to scrutinize the reference memory escape latencies (Figure 4A) more precisely, we analyzed them through a trial by trial basis (Figure 7; Table II).

Here, in the 1st trial of 1st acquisition day, the latencies to find the platform, that is escape latency (EL), was significantly higher in our spatial memory impaired rats in comparison to those of other rats and were not decreased in any of the following acquisition days. It indicated that our colchicine induced memory impaired rats neither acquire nor retain any new information in whole of our reference memory version of MWM.

On the contrary, all the other rats quickly improved their performances (EL) with repeated trials (from trial 1 to 4), though our experimental (both pre and post treated) rats required a relatively longer EL in 1st trial from the 3rd acquisition day onwards in comparison to controls. In addition, the normal rats reached a steady performance (i.e. similar EL in 2nd, 3rd, 4th, trials along with similar EL between last and first trial of two consecutive days)[25] almost on 3nd acquisition day. Whereas, both of our experimental rat groups could avail this steady state on day 5 (Table II). These findings exhibited that, both of our CE application regimens (before or after hippocampal damage) were equally fruitful to regain the learning ability as well as consolidation power, though it was a little bit slower to develop in comparison to those of normal rats.

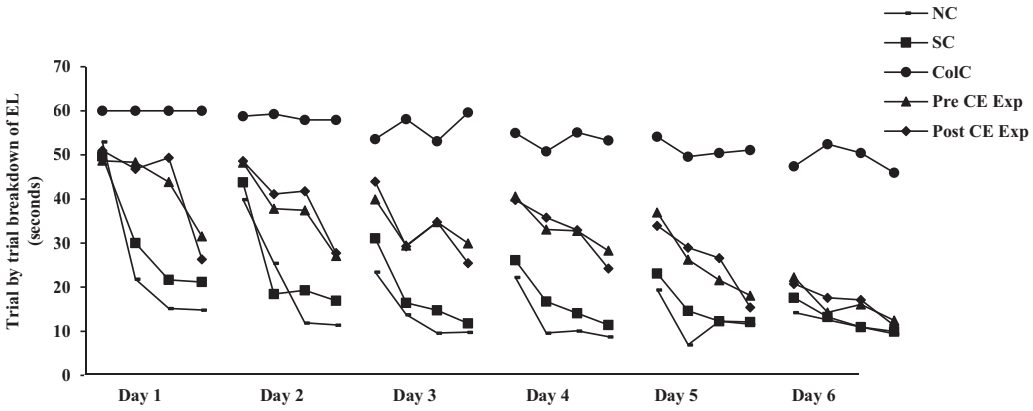

Figure 7: Trial by trial breakdown of reference memory escape latency (EL) in different groups of rats

Table II: Statistical analysis of reference memory escape latency (EL) between last and first trials of any two consecutive days

Breakdown EL in	p values in				
	NC	SC	ColC	Pre CE Exp	Post CE Exp
Day1Trial4 vs Day2Trial1	0.002	0.000	1.000	0.000	0.000
Day2Trial4 vs Day3Trial1	1.000	0.002	1.000	0.004	0.000
Day3Trial4 vs Day4Trial1	1.000	0.002	1.000	0.039	0.000
Day4Trial4 vs Day5Trial1	1.000	0.024	1.000	0.261	0.063
Day5Trial4 vs Day6Trial1	1.000	1.000	1.000	1.000	1.000

In addition, in our study, we used 15 µg of colchicine in each hippocampus to cause spatial memory impairment by extensive hippocampal cells damage along with hippocampal atrophy, as proposed by Nakagawa et al. (1987).[21] As we found both of prevention as well as alleviation of spatial memory impairment with our 28 days CE schedule, so it is functionally verified that both of hippocampal neurogenesis as well as volume increment have been occurred here. This proposal is further supported by Barrett et al. (2019).[6] But it was our limitation that we could not demonstrate it morphologically.

Conclusion

Climbing exercise was equally effective in prevention as well as alleviation of colchicine induced spatial reference and working memory impairment along with hippocampal oxidative stress in male Long-Evans rats. Moreover, this climbing exercise schedule was sufficient enough to reverse these alarming consequences to almost normal, but slowly.

Conflict of interest

Authors declare no conflict of interest.

Acknowledgement

We acknowledge Bangabandhu Sheikh Mujib Medical University (BSMMU) for financing this research partially by a research grant. We also like to thanks Professor Dr. Birendra Nath Mallick (Director of Amity Institute of Neuropsychology and Neuroscience, Uttar Pradesh, India) for his valuable suggestion and helping us in proper conduction of stereotaxic surgery. We also like to thanks Incepta Pharmaceuticals Ltd, Bangladesh for providing us colchicine powder.

References

1. Shohamy D, Schacter DL, Wagner AD. Learning and memory. In: Kandel ER, Koester JD 1, Mack SH, Siegelbaum SA, editors. Principles of neural science. 6thed. USA: McGraw-Hill. 2021. p. 1291-1311
2. Ashwlayan VD, Singh RA. Reversal effect of Phyllanthus emblica (Euphorbiaceae) Rasayana on memory deficits in mice. Int J Appl Pharm 2011;3:10-5.
3. World Health Organization. Dementia [Internet]; 2023. Available from: https://www.who.int/news-room/fact-sheets/detail/dementia.
4. Gironi M, Bianchi A, Russo A, Alberoni M, Ceresa L, Angelini A, Cursano C, Mariani E, Nemni R, Kullmann C, Farina E. Oxidative imbalance in different neurodegenerative diseases with memory impairment. Neurodegener Dis 2011; 8(3):129-137. doi:10.1159/000319452.
5. Cassel JC, de Vasconcelos AP. Importance of the ventral midline thalamus in driving hippocampal functions. Prog Brain Res 2015; 219:145-61. doi:10.1016/bs.pbr.2015.03.005.
6. Barrett KE, Barman SM, Brooks HL, Yuan. Learning, Memory, Language & Speech. In: Ganong's review of medical physiology. 26thed. New York: McGraw-Hill Medical. 2019. p 283-295.
7. Nunomura A, Castellani RJ, Zhu X, Moreira PI, Perry G, Smith MA. Involvement of oxidative stress in Alzheimer disease. J Neuropathol Exp Neurol 2006; 65(7):631-641.doi:10.1097/01.jnen.0000228136.58062.bf.
8. Kumar V, Abbas AK, Aster JC. Robbins basic pathology. 10th ed. Philadelphia, Pennsylvania: Elsevier. 2018; p.31-56.
9. Deavall DG, Martin EA, Horner JM, Roberts R. Drug-induced oxidative stress and toxicity. J Toxicol 2012; 2012. doi:10.1155/2012/645460.
10. Halliwell B, Gutteridge J. Free Radicals in Biology and Medicine. 5th ed. United Kingdom. Oxford: OUP. 2015; p.600-603.
11. Toppo S, Sultana D (2014). Effect of aerobic training on selected physical variables on normal and overweight school boys. Int J Multidiscip Educ Res 3(6):159-166.
12. Belviranlý M, Okudan N. Voluntary, involuntary and forced exercises almost equally reverse behavioral impairment by regulating hippocampal neurotrophic factors and oxidative stress in experimental Alzheimer's disease model. Behav Brain Res 2019; 364: 245-255. doi:10.1016/j.bbr.2019.02.030
13. Molaei A, Hatami H, Dehghan G, Sadeghian R, Khajehnasiri N. Synergistic effects of Quercetin and regular exercise on the recovery of spatial memory and reduction of parameters of oxidative

stress in an animal model of Alzheimer's disease. EXCLI J 2020; 19: 596-612. doi:10.17179/excli2019-2082.

14. Stenling A, Moylan A, Fulton E, Machado L. Effects of a Brief Stair-Climbing Intervention on Cognitive Performance and Mood States in Healthy Young Adults. Front Psychol 2019; 10:2300. doi: 10.3389/fpsyg.2019.02300

15. Zarrinkalam E, Ranjbar K, Salehi I, Kheiripour N, Komaki A. Resistance training and hawthorn extract ameliorate cognitive deficits in streptozotocin-induced diabetic rats. Biomed Pharmacother 2018; 97:503-510. doi: 10.1016/j.biopha.2017.10.138

16. Rahmati M, Keshvari M, Xie W, Yang G, Jin H, Li H, Chehelcheraghi F, Li Y. Resistance training and Urticadioica increase neurotrophin levels and improve cognitive function by increasing age in the hippocampus of rats. Biomed Pharmacother 2022; 153:113306. doi: 10.1016/j.biopha.2022.113306

17. Özbeyli D, Sarý G, Özkan N, Karademir B, Yüksel M, Çilingir Kaya ÖT, KasýmayÇakýr Ö. Protective effects of different exercise modalities in an Alzheimer's disease-like model. Behav Brain Res 2019; 328:159-177. doi: 10.1016/j.bbr.2017.03.044

18. Schimidt HL, Carrazoni GS, Garcia A, Izquierdo I, Mello-Carpes PB, Carpes FP. Strength training or green tea prevent memory deficits in a â-amyloid peptide-mediated Alzheimer's disease model. Exp Gerontol 2021; 143: 111186. doi:10.1016/j.exger.2020.111186.

19. Wren-Dail MA, Dauchy RT, Ooms TG, Baker KC, Blask DE, Hill SM, Dupepe LM, Bohmjr RP. Effects of colored enrichment devices on circadian metabolism and physiology in male Sprague-Dawley rats. Comp Med 2016; 66(5):384-391. PMID: 27780005

20. Neto WK, Silva WA, Ciena AP, Anaruma CA, Gama EF. Vertical Climbing for Rodent Resistance Training: a Discussion about Training Parameters. Int J Sports Sci 2016; 6(1A): 36-49. doi:10.5923/s.sports.201601.07

21. Nakagawa Y, Nakamura S, Kaœe Y, Noguchi T, Ishihara T. Colchicine lesions in the rat hippocampus mimic the alterations of several markers in Alzheimer's disease. Brain Res 1987; 408(1-2): 57-64. doi:10.1016/0006-8993(87)90358-1

22. Paxinos G, Watson C. The Rat Brain in Stereotaxic Coordinates. 6th ed. Paris. Armstredium: Elsevier; 2007; p XII, p XXIX

23. Sarihi A, Motamedi F, Naghdi N, Pour AR. Lidocaine reversible inactivation of the median raphe nucleus has no effect on reference memory but enhances working memory versions of the Morris water maze task. Behav Brain Res 2000; 114(1-2): 1-9.doi:10.1016/S0166-4328(00)00176-5

24. Topuz RD, Gunduz O, Tastekin E, Karadag CH. Effects of hippocampal histone acetylation and HDAC inhibition on spatial learning and memory in the Morris water maze in rats. Fundam Clin Pharmacol 2020; 34(2): 222-228. doi:10.1111/fcp.12512

25. Wisman LA, Sahin G, Maingay M, Leanza G, Kirik D. Functional convergence of dopaminergic and cholinergic input is critical for hippocampus-dependent working memory. Neurosci J 2008; 28(31): 7797-7807. doi:10.1523/JNEUROSCI.1885-08.2008

26. Netto CA, Hodges H, Sinden JD, Le Peillet E, Kershaw T, Sowinski P, Meldrum BS, Gray JA. Effects of fetal hippocampal field grafts on ischaemic-induced deficits in spatial navigation in the water maze. Neurosci 1993; 54(1): 69-92. doi:10.1016/0306-4522(93)90384-R

27. Kumar A, Aggrawal A, Pottabathini R, Singh A. Possible neuroprotective mechanisms of clove oil against icv-colchicine induced cognitive dysfunction. Pharmacol Rep 2016; 68(4): 764-72. doi:10.1016/j.pharep.2016.03.005

28. Pourkhodadad S, Alirezaei M, Moghaddasi M, Ahmadvand H, Karami M, Delfan B, Khanipour Z. Neuroprotective effects of oleuropein against cognitive dysfunction induced by colchicine in hippocampal CA1 area in rats. J Physiol Sci 2016; 66: 397-405. doi:10.1007/s12576-016-0437-4

29. Margolis RL, Rauch CT, Wilson L. Mechanism of colchicine-dimer addition to microtubule ends: implications for the microtubule polymerization mechanism. Biochem 1980; 19: 5550-5557

30. Osborn M, Weber K. Cytoplasmic microtubules in tissue culture cells appear to grow from an organizing structure towards the plasma membrane.

Proc Nat Acad Sci 1976; 73(3): 867-871. doi:10.1073/pnas.73.3.867

31. McClure WO. Effect of drugs upon axoplasmic transport. Adv Pharmacol 1972; 10: 185-220. doi:10.1016/S1054-3589(08)60524-4

32. Joy T, Rao MS, Madhyastha S, Pai K. Effect of N acetyl cysteine on intracerebroventricular colchicine induced cognitive deficits, beta amyloid pathology, and glial cells. Neurosci J 2019; 2019:7547382. doi:10.1155/2019/7547382

33. Sil S, Ghosh T. Role of cox-2 mediated neuroinflammation on the neurodegeneration and cognitive impairments in colchicine induced rat model of Alzheimer's Disease. J Neuroimmunol 2016; 291:115-124. doi:10.1016/j.jneuroim. 2015. 12.003

34. Dufourny L, Leroy D, Warembourg M. Differential effects of colchicine on the induction of nitric oxide synthase in neurons containing progesterone receptors of the guinea pig hypothalamus. Brain Res Bull 2000; 52(5): 435-43. doi:10.1016/S0361-9230(00)00286-0

35. Sil S, Ghosh T, Ghosh R, Gupta P. Nitric oxide synthase inhibitor, aminoguanidine reduces intracerebroventricular colchicine induced neurodegeneration, memory impairments and changes of systemic immune responses in rats. J Neuroimmunol 2017; 303:51-61. doi:10.1016/j.jneuroim.2016.12.007

36. Coyle JT, Puttfarcken P. Oxidative stress, glutamate, and neurodegenerative disorders. Sci 1993; 262(5134): 689-695. doi:10.1126/science. 7901908

37. Erickson KI, Prakash RS, Voss MW, Chaddock L, Heo S, McLaren M, Pence BD, Martin SA, Vieira VJ, Woods JA, McAuley E. Brain-derived neurotrophic factor is associated with age-related decline in hippocampal volume. J Neurosci 2010; 30(15): 5368-5375. doi:10.1523/JNEUROSCI.6251-09.2010

38. Vaynman S, Ying Z, Gomez Pinilla F. Hippocampal BDNF mediates the efficacy of exercise on synaptic plasticity and cognition. Eur J Neurosci 2004; 20(10): 2580-2590. doi:10.1111/j.1460-9568. 2004. 03720.x

39. Alsina B, Vu T, Cohen-Cory S. Visualizing synapse formation in arborizing optic axons in vivo: dynamics and modulation by BDNF. Nat Neurosci 2001; 4(11): 1093-1101.doi: 10.1038/nn735

40. Lee J, Duan W, Mattson MP. Evidence that brain derived neurotrophic factor is required for basal neurogenesis and mediates, in part, the enhancement of neurogenesis by dietary restriction in the hippocampus of adult mice. J Neurochem 2002; 82(6): 1367-1375. doi:10.1046/j.1471-4159.2002.01085.x

41. Alzoubi KH, Halboup AM, Alomari MA, Khabour OF. Swimming exercise protective effect on waterpipe tobacco smoking induced impairment of memory and oxidative stress. Life Sci 2019; 239. doi:10.1016/j.lfs.2019.117076

42. Stone V, Kudo KY, Marcelino TB, August PM, Matté C. Swimming exercise enhances the hippocampal antioxidant status of female Wistar rats. Redox Rep 2015; 20(3): 133-138. doi:10.1179/1351000214Y.0000000116