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THE ANTIOXIDANT METABOLIC PROTECTIVE EFFECT OF EXERCISE ON D-GALACTOSE INDUCED SPATIAL MEMORY IMPAIRMENT; POSSIBLE ROLE OF HIPPOCAMPAL ASTROCYTES

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ABSTRACT

BACKGROUND: Aging is a complex biological phenomenon distinguished by accumulation of deleterious changes with time including memory impairment. Physical exercise positively affects the brain both during aging and neurodegenerative diseases.

OBJECTIVE: Studying the role of hippocampal astrocytes in the protective effect of exercise in D-galactose induced memory impairment.

MATERIALS AND METHODS: Eighteen rats were divided into 3 equal groups; group I (control) was given saline i.p. for 10 weeks, group II was treated with D-galactose(D-gal 100 mg/kg i.p. for 10 weeks) as it induces memory impairment and group III was co-treated with D-gal and swimming exercise for 10 weeks. Memory and learning performance in Barnes maze test were analyzed. Brain tissue was dissected and processed for histological and immunohistochemical study using glialfibrillary acidic protein (GFAP) and induced nitric oxide synthase (iNOS) markers. Brain homogenate was prepared for estimation of malondialdehyde (MDA), superoxide dismutase (SOD), lactate and pyruvate.

RESULTS: Exercise significantly improved D-gal rats' performance during acquisition and probe phases of Barnes maze test, reduced MDA and increased SOD, lactate and pyruvate levels with reduction of lactate\pyruvate ratio in brain homogenate. Neural cell count increased in hippocampus with decrease of apoptotic cells, GFAP and iNOS expression.

CONCLUSION: Exercise was significantly effective in improving oxidative stress in brain and restoring hippocampal neural and astrocyte metabolic functions with resultant improvement in spatial memory performance.

Key Wards: Swimming, hippocampus, spatial memory, astrocytes.

INTRODUCTION

Aging is characterized by general decline of many physiological functions, with pronounced impact on the cerebral activities and brain executive functions including memory (Terman & Brunk, 2004 and Hayes et al., 2014). Age-associated cognitive decline and neuronal loss are present in old-age neuro-degenerative diseases like dementia of Alzheimer's disease (AD) (Cole et al., 2005). Different theories postulated to explain the diminution of the cerebral activities during aging. The most important one state that the increased formation of reactive oxygen species (ROS) along years resulting in macromolecule damage (Colavitti & Finkel, 2005; Lopez-Torres & Barja, 2008 and Perez et al., 2009).

D-Galactose (D-gal) is a reducing sugar that could induce accelerated aging with neurological impairment and decreased activity of anti-oxidant enzymes (Song et al., 1999). Administration of D-gal in animal model may lead to neuro-degeneration by inhibiting neurogenesis, enhancing apoptosis, gliosis and increasing oxidative damage and mitochondrial dysfunction, with resultant cognitive dysfunction and memory de?cit (Zhang et al., 2005; Cui et al., 2006 and Kumar et al., 2010).

Astrocytes are specialized glial cells constitute the most common cell type in mammalian brain. Glial fibrillary acidic protein (GFAP) is a part of the astrocytes' cytoskeleton intermediate filaments. Reactive gliosis is characterized by hypertrophy of the cell bodies and processes of astrocytes in response to a variety of insults with an increase in the expression of GFAP; the signal that regulates the transition to the reactive state(Sharma et al., 2009). They play a key role in modulation of synaptic neurotransmitter levels, defensive function against oxidative stress, and regulation of synapse formation and remodeling (Bélanger and Magistretti, 2009).They possess a unique morphology and spatial distribution that enable them to provide energy substrates from capillaries to neurons by their perivascular end-feet which form a part of blood brain barrier. Astrocytes mediate the transfer of glucose to the brain and its storage in the form of glycogen to be used by neuron in time of activity.

Physical exercise gained interest as the most effective, low-cost, and low-tech way for successful aging. Therefore, it has the potential to represent a preventive or disease-slowing therapeutic strategy for age-related neurodegenerative diseases (Ahlskog et al., 2011).Physical exercise positively affects the brain both during aging and neurodegenerative processes that are associated with poor cognitive function including dementia of AD (Maesako et al., 2012; Hayes et al., 2014 andYau et al., 2014).However the exact links between physical exercise, increase adult hippocampal neurogenesis and cognition improvement are still unclear (Yau et al., 2014).

Increased lactate production may also account for some of the neuroprotective effects of exercise (Ferris et al., 2007). However, more recent studies verified the occurrence of high CSF lactate levels in patients with AD, which may be ascribed to mitochondria impairment (Liguori et al., 2014).

The role of lactate and the neuro-energetic in brain tissue of age related memory impairment as AD seems to be controversial and highly debated especially with exercise. So, the present work was created to identify the effect of exercise training on spatial memory in relation to biochemical and histological changes in brains of rats injected with D-gal for ten weeks.

MATERIALS AND METHODS

Animals and treatment: Eighteen male albino rats of local strain (age 2 - 3 months, weighting 150-180g) were used in this study. Rats were caged in fully ventilated cages (three rats per cage, 80 x40 x30 cm) and under conditions of controlled illumination (12:12 h light/dark cycle), with food and water provided ad libitum. Animals were acclimatized to these conditions for at least 1 week prior to the experiment. All behavioral testing occurred during the light phase between 8 am and 3 pm, and all experiments were done in accordance with the internationally accepted recommendations in the care and use of animals, and were approved by the Ethical Committee of the Faculty of Medicine, Menoufia University, Egypt. Rats were divided into 3 equal groups:Group 1 (control group) was injected with 0.9% saline intrapretonialy (i.p.) daily. Group II (D-gal treated group) was injected with D-galactose (D-gal) (S.d. fine-CHEM LTd) at a dose of 100 mg/kg dissolved in sterile saline (0.9%) i.p. daily for ten weeks (Cui et al., 2006). Group 3 (exercised D-gal group) was injected with D-gal as group III and was subjected to exercise training program concomitantly with D-gal injection.

Swimming exercise program: Rats were put in a group of 6 in water tank and were urged to swim actively. It started by 5-min/day, 5 days/week and gradually increased over the 10 weeks, until the rats swam continuously for 30 min/ day at the last week of the training (Leosco et al., 2003). Swimming tank dimensions were 100 x 80 x 80 cm. Water was maintained at a thermo neutral temperature of 35 ± 1?C through a thermostat.

Assessment of spatial memory: The acquisition and retention of memory was evaluated by using Barnes maze test which is a visuo-spatial learning and memory task for rats (Barnes, 1979). It was designed at Physiology Department, Menoufia Faculty of Medicine, Egypt according to that described by Sunyer et al. (2007).It consisted of an elevated brown circular surface with eighteen evenly spaced holes around the edge in a well-lit environment. On the Barnes maze, rats used extra-maze visual cues to locate an escape hole that allowed them to escape from open space and bright light into a dark box beneath the maze (Fig. 1).

Figure (1): Rat in Barnes maze that consisted of circular platform (92 cm of diameter) with 18 equally spaced holes (5 cm diameter; 7.5 cm between holes) along the perimeter, and "target box" or (escape box-28 × 22 × 21cm).

Each trial began by placing the animal in a black starting cylinder at the center of the platform that was removed after 10 sec., allowing rats to freely explore the apparatus. Spatial acquisition was evaluated in 4 training sessions (Day 1–4). Each training session consisted of four trials each was 3-min (T 1-4), with inter-trials interval about 20 min. during which rats were placed in their home cage. Rats that failed to find the target box within 3 minutes were gently guided to its location. 180 sec. escape latency was recorded for those rats. All animals stayed in the target box for 1 min after entering.

All trials were recorded by a video-camera for scoring the latency to escape and number of errors. Latency to escape was defined as the time which was taken by animals to completely enter in the target box (all 4 paws out of the platform) and the number of errors was defined as the total number of holes visited during the trial that did not lead to the target box. A hole was considered visited when rats tilted their head over it (nose poke) or introduced their paws into the hole. On Day 5, a 90 sec. probe trial was conducted for evaluation of short-term memory retention during which the target box was removed and the target hole was closed. Rats were allowed to explore the maze and to visit the target hole and the adjacent holes. Latency to reach the target hole for the first time and number of errors before reaching the target hole was recorded.

Brain homogenate Samples for biochemical analysis: Twenty-four hours after the last memory task session, the

animals were anesthetized, the chest was opened and the heart was exposed to perform perfusion technique for fixation of the tissue in-situ by introducing a needle into the left ventricle and making puncture in right atrium. By using triple way cannula, saline was injected in left ventricle to wash out the circulatory blood from puncture of right atrium, then the fixative was injected to fix the tissue in-situ. After fixation, brains were immediately collected and each brain was divided into 2 equal halves, one half was weighed and prepared for tissue homogenization in 10 ml cold buffer (50 mM potassium phosphate at pH 7.5) per gram tissue, Centrifuged at 4000 r.p.m for 15 minutes. The supernatant was collected and stored at -80°C.

Determination of MDA level in brain homogenate*:* Colorimetric method for estimation of malondialdehyde (MDA) was done by using thiobarbituric acid reactive substance for measuring the peroxidation of fatty acids as oxidative stress marker (Biodiagnostic Co., Egypt Ohkawa et al., 1979).

Determination of SOD level in brain homogenate: Colorimetric method for estimation of superoxide dismutase (SOD) depending on the ability of SOD to inhibit the initial rate of photoactivated phenazinemethosulfate mediated reduction of O_2^- to O_2 which then reduce nitroblue-tetrazolium dye (Biodiagnostic Co., Egypt-Nishikimi et al., 1972).

Determination of lactate in brain homogenate: Lactate is oxidized to pyruvate and hydrogen peroxide by lactate oxidase. In presence of peroxidase, hydrogen peroxide reacts with 2, 4, 6-tribromo-3-hydroxybenzoic acid 4-aminoanpyrine to form a red quinoneimine dye (Biodiagnostic Co., Egypt). The color intensity of the formed red quinoneimine dye is directly proportional to the lactate concentration. It is determined by measuring the increase in absorbance at 546 nm (Field et al., 1996).

Determination of pyruvate in brain homogenate: In the presence of an excess NADH, pyruvate is converted to lactate (Biodiagnostic Co., Egypt). The reduction of the absorbance = ΔA, at 340 nm, due to the oxidation of NADH to NAD^+, is a measure of the amount of pyruvate originally present (Wasserman et al., 1985).

Tissue samples for histological and immunohistochemical study: The other halves of brain tissues were fixed in 10% neutral buffered formol saline for 5 days and processed for making paraffin blocks. Sections of 5 microns were cut using microtome and prepared for Hematoxylin and Eosin staining (H&E) with the routine technique for histological and morpho-metric analysisand for immunostaining with anti GFAP &iNOS antibodies (Bancroft et al., 1996).

Immunohistochemistry: Hippocampal sections were immunohistochemically stained for detection of GFAP and iNOS according to a previously published protocol (Cote et al., 1993). Sections were deparaffinized, hydrated and then incubated overnight with polyclonal, rabbit GFAP (Novacastra laboratories Ltd. UK), and with polyclonal rabbit anti iNOS (Transduction laboratories, UK). Sections were rinsed in phosphate buffered saline and few drops of biotinylated mouse anti-rabbit secondary antibody were applied

for 10 minutes (for GFAP) and few drops of biotinylated goat anti-rabbit secondary antibody (Vector laboratory) for iNOS for 30 minutes. Sections were rinsed, then treated with 2 drops of prepared diaminobenzidine tetra hydro-chloride substrate chromogen solutions (DAB) for 15 minutes until the desired brown color was obtained. Sections were counterstained with Mayer's hematoxylin and mounted with aqueous mounting media. Negative control sections were stained after omission of the primary antibody.

Morphometry: For quantitative assessment, two non-overlapping fields (400×) per section were randomly captured by a digital camera (Olympus, Japan) from regions (CA1, CA3 and dentate gyrus) of the hippocampus.The number of pyramidal cells, granular cells and apoptotic cells were counted. Fields taken from at least three anatomically comparable sections /animal were assessed using image J software (Maryland, USA). The numbers calculated for at least five animals/experimental group were considered for comparison and statistical analyses.

Statistical analysis: The SPSS version 20 was used for analysis of data. The results were expressed as mean± S.E.M. The significance of differences between groups were determined by one-way analysis of variance test for non-parametric not normally distributed data (Wallis, 1952), one-way analysis of variance (ANOVA) for parametric normally distributed data and post hoc – Tukey test was done for multiple comparisons between groups. The significance of differences was determined at $p<0.05$.

RESULTS

Barnes maze test results:

Acquisition phase: D-gal rats demonstrated an increase in the mean number of errors infour trials (T1-4) of day 1 and in the mean of total number of errors in day 1 relative to control group ($P < 0.001$). In exercised D-gal rats, they were lower compared to D-gal rats ($P < 0.001$ - Figure 2A and 2C). D-gal rats showed also an increase in the mean of escape latency (s) infour trials (T1-4) of day 1 and in the mean of total escape latency in day 1 relative to control rats ($P < 0.05$), while exercised D- gal rats displayed a reduction in the same parameter relative to D-gal rats ($P < 0.001$ - Figure 2B and 2D). There were no significant changes between groups ($P > 0.05$) in day2, 3 and 4of acquisition phase regarding mean number of errors and mean of escape latency per day (Figure 2C and2D).

Probe phase results: In day 5 of Barnes maze test for assessing recent memory retention, D-gal rats demonstrated an increase in the number of errors relative to control group ($P < 0.05$). In exercised D-gal rats, it was lower compared to D-gal rats ($P < 0.05$ - Figure2E). D-gal rats showed also an increase in escape latency (s) relative to control rats ($P < 0.001$). Exercised D- gal rats displayed a reduction in the same parameter relative to D-gal rats ($P < 0.001$ - Figure 2 F).

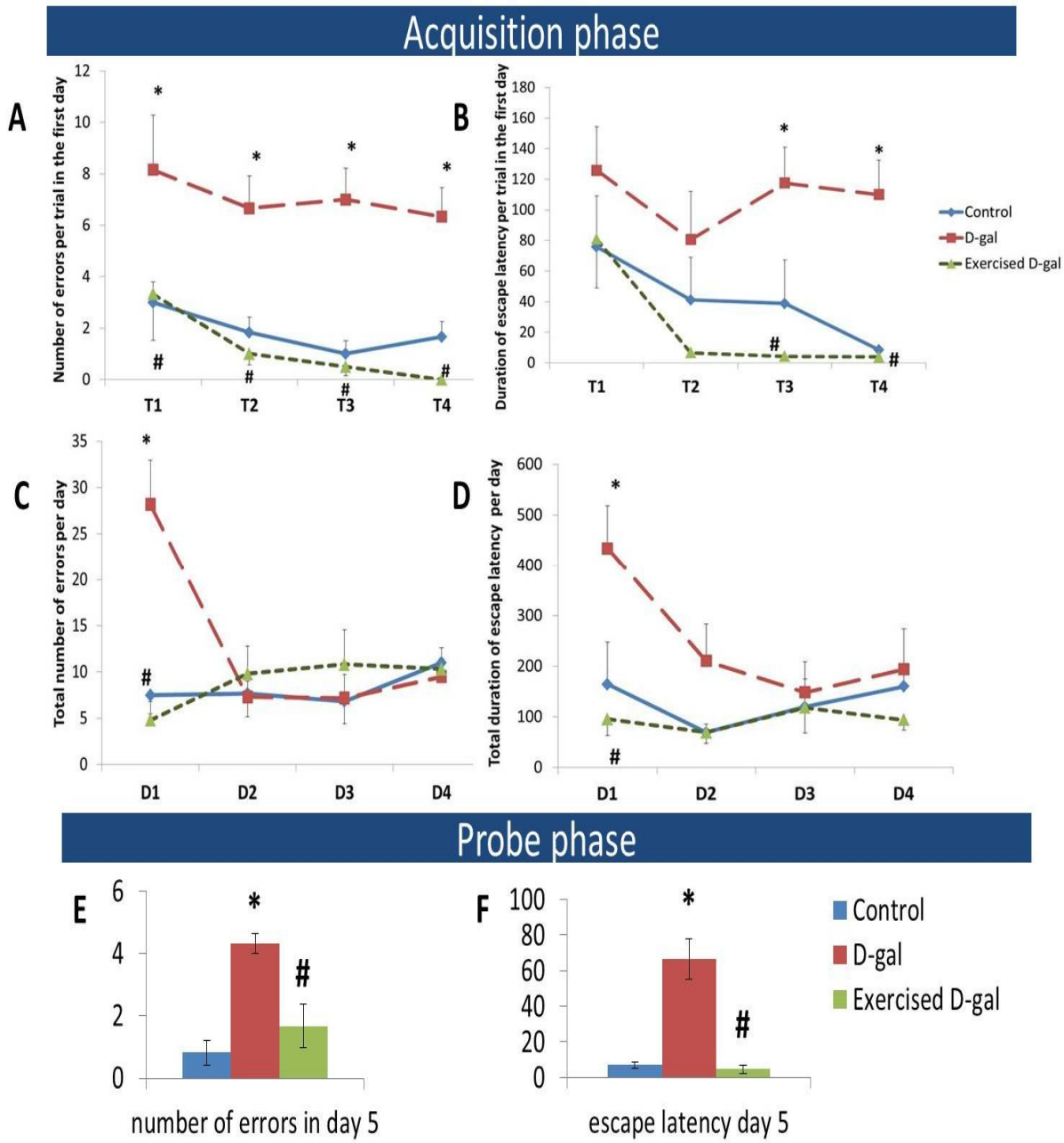

Figure (2): Evidence of improved short-term spatial memoryassociated with exercise training in D-gal rats assessed by Barnes maze test (Mean ± SEM). (A) Number of errors per trial in day 1 of acquisition phase. (B) Duration of escape latency per trial in day 1 of acquisition phase. (C) Total number of errors in day 1-4 of acquisition phase. (D) Total duration of escape latency (seconds) in day 1-4 of acquisition phase. (E) Number of errors in day 5 of probe phase. (F) Duration of escape latency (seconds) in day 5 of probe phase. Data were expressed as mean ±S.E. (n=6). Kruskall-Wallis: *p<0.05, vs control; #p<0.05, vs D-gal treated group.

Biochemical results:

Lactate and pyruvate levels in brain homogenate:

D-gal significantly increased lactate/ pyruvate ratio in rats' brain homogenate compared to control ($p < 0.01$). Exercise training significantly increased lactate ($p < 0.05$), pyruvate ($p<0.01$) and decreased lactate/ pyruvate ratio ($p < 0.01$) compared to corresponding values in D-gal rats. Pyruvate also was higher in exercise training compared to control ($p< 0.05$Table 1).

MDA and SOD level in brain homogenate: D-gal significantly increased MDA level and decreased SOD level in rats' brain homogenate compared to control ($p <0.001$). Exercise training significantly decreased MDA level and increased SOD level in brain homogenate compared to D-gal rats ($p <0.001$). However, SOD level was still significantly lower in exercise training compared to control ($p< 0.05$Table 1).

Table (1): Evidence of metabolic changes and antioxidant effect of exercise training in rat brain homogenate (Mean ± SEM)**.**

Groups / Parameters	Control group (n = 6)	D-gal treated group (n = 6)	Exercised D-gal treated group (n = 6)
Lactate (mmol/l)	3.31±0.48	2.39±0.17	5.22±0.89$^{\#}$
Pyruvate (mmol/l)	1.49±0.11	0.28±0.08	6.27±1.86$^{*\#}$
Lactate/Pyruvate ratio	2.32±0.42	11.73±3.04*	1.17±0.24$^{\#}$
MDA(nm/gm. tissue)	2.68±0.50	24.08± 2.89*	4.32± 1.47$^{\#}$
SOD(U/gm. tissue)	6.55±0.35	4.17±0.24*	5.43±0.16$^{*\#}$

*p<0.05, vs control; #p<0.05, vs D-gal treated group.

Histological and immunohistochemical results:

Histological results: The hippocampus of control group was identified as C-shaped structure in coronal section of brain. It was formed of 3 major areas CA1, CA2 and CA3. The cells of hippocampus was arranged into 3 layers, i.e. the outer polymorphic, the middle pyramidal and the inner molecular layers. The most prominent layer was the pyramidal layer, formed of large pyramidal-shaped neurons with rounded vesicular nuclei and prominent nucleoli. They were densely packed in area CA1 (Figure3A1). The hippocampus of D-gal treated group (area CA1) showed decrease in number and shrinkage of pyramidal cells with disarrangement. Some pyramidal cells were seen in the outer polymorphic layer and many other pyramidal cells showed apoptosis with pyknotic nuclei (Figure

3B1). The hippocampus of exercised D-gal treated group (area CA1) revealed improvement of the histological picture. Most of pyramidal cells appeared normal with euchromatic vesicular nuclei. However, some pyramidal cells were seen in polymorphic layer and other few cells showed pyknosis of their nuclei (Figure3C1).

Sections of area CA3 of hippocampus of control group showed the normal three layers, i.e. polymorphic, pyramidal and molecular layer. The pyramidal cells of this areawere large pyramidal with vesicular nuclei and prominent nucleoli. They were loosely packed compared to area CA1 (Figure3A2).Sections of area CA3 of hippocampus of D-gal treated group showed marked disarrangement of pyramidal cell layer with many apoptotic cells with darkly stained nuclei (Figure3B2).Area CA3 of exercised D-gal treated group showed improvement of their appearance. The pyramidal cells were normally arranged with vesicular nuclei. Some few cells showed darkly stained nuclei (Figure3C2).

Dentate gyrus of control group was formed of three layers of cells forming apex and two blades. The outer molecular layer was formed of small neurons, the middle granular layer formed of outer mature granular cells with large vesicular nuclei, and inner immature granular cells formed of small cells with darkly stained nuclei. The inner most layer was formed of small polymorphic neurons (Figure 3A3). The dentate gyrus of D-gal treated group showed marked reduction of cells' number of all three layers with disarrangement of granular cells and appearance of many apoptotic cells with darkly stained nuclei (Figure3B3).Dentate gyrus of exercised D-gal treated group revealed improvement of the histological results of all layers. The mature granular cells appeared numerous and densely packed with vesicular rounded nuclei compared to D-gal treated group (Figure3C3).

The hilum of dentate gyrus of control group was formed of group of different sized neurons with vesicular rounded nuclei and some neuroglia cells with small densely stained nuclei. Some apoptotic cells were normally seen among hilarcells (Figure3A4).The hilum of dentate gyrus of D-gal treated group showed many apoptotic cells with densely stained nuclei and shrinked cytoplasm among normal hilar cells. Numerous neuroglia cells were also seen denoting active gliosis (Figure3B4).The hilum of dentate gyrus of exercised D-gal treated group revealed normal appearance of most hilar cells which appeared well organized with vesicular rounded nuclei. Very few cells were apoptotic (Figure3C4).

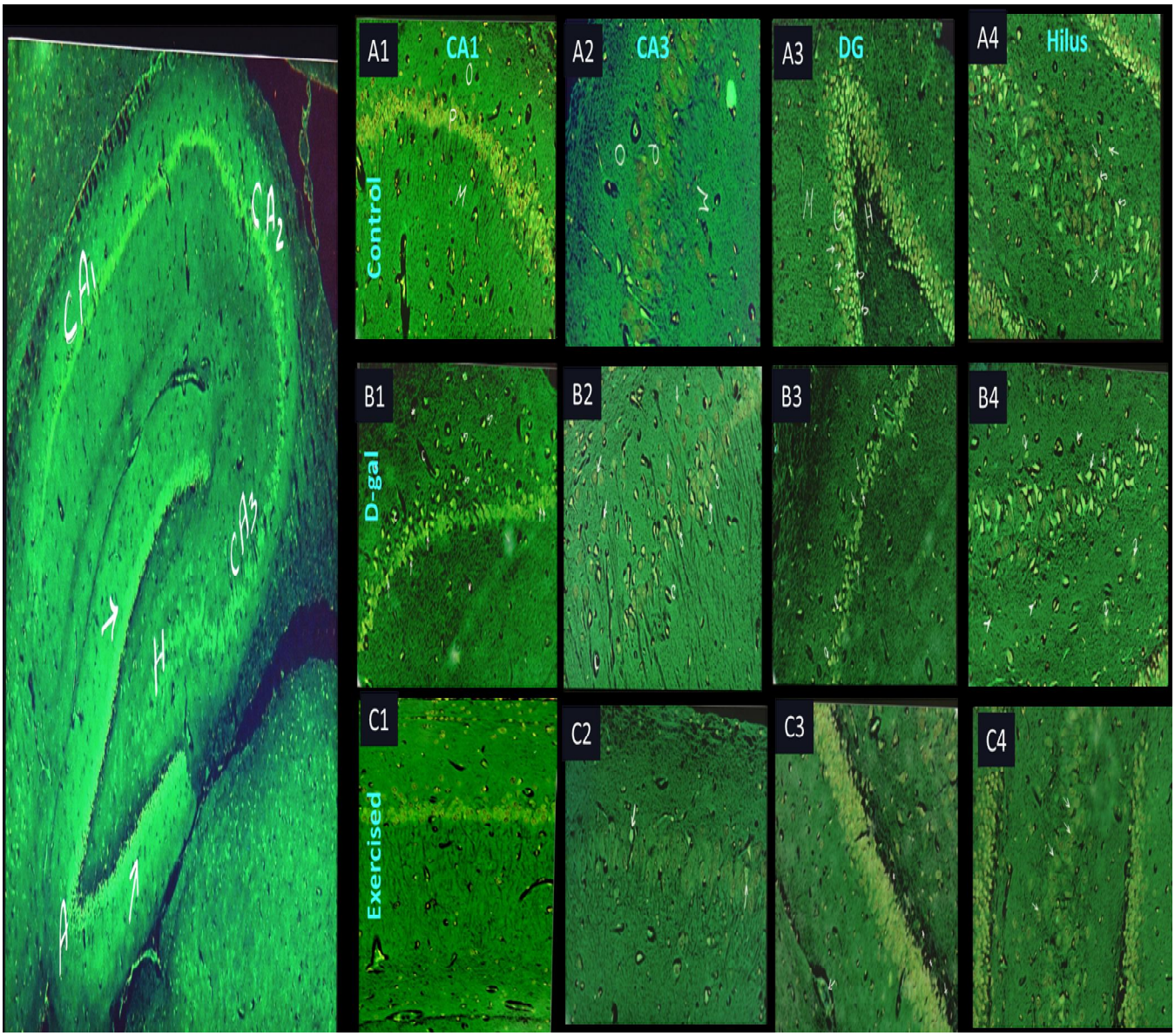

Figure (3): Evidence of improved hippocampal neural cell count and reduction of apoptosis associated with exercise in D-gal rats assessed by hematoxylin-eosin stained sections (H & E X 200) of area CA1, CA3, DG and hilum of DG in control (A1, A2, A3 and A4), D-gal treated group (B1, B2, B3 and B4) and exercised D-gal treated group (C1, C2, C3 and C4).

Morphometry results: The mean ± S.E. of pyramidal cell count for area % in CA1 and CA3 region of D-gal treated groups were 36.67±3.31 and 25.5±3.06 which were significantly lower compared to corresponding values in control group (77.17±2.75 and 54±3.66). In exercised D-gal treated group, they were 63.33±2.11 and 40.83±3.01 which were significantly higher compared to corresponding values in D-gal treated group (Figure4A).

The mean ± S.E. of granular cell count for area % in dentate gyrus of D-gal treated groups was 75±8.56 which was significantly lower compared to corres-

ponding value in control group (148±1.65). In exercised D-gal treated group, it was (109±2.74) which was significantly higher compared to corresponding values in D-gal treated group (Figure 4A).

The mean ± S.E. of apoptotic cell count for area % in CA1, CA3 and dentate gyrus region of D-gal treated groups were 13.33±1.52, 7.83±1.49 and 34.00±7.57 which were significantly higher compared to corresponding values in control group (0.67±0.49, 0.83±0.83 and 1.00±0.52). In exercised D-gal treated group, they were 3.17±1.01, 2.5±1.12 and 2.00±0.37) which were significantly lower compared to corresponding values in D-gal treated group (Figure 4B).

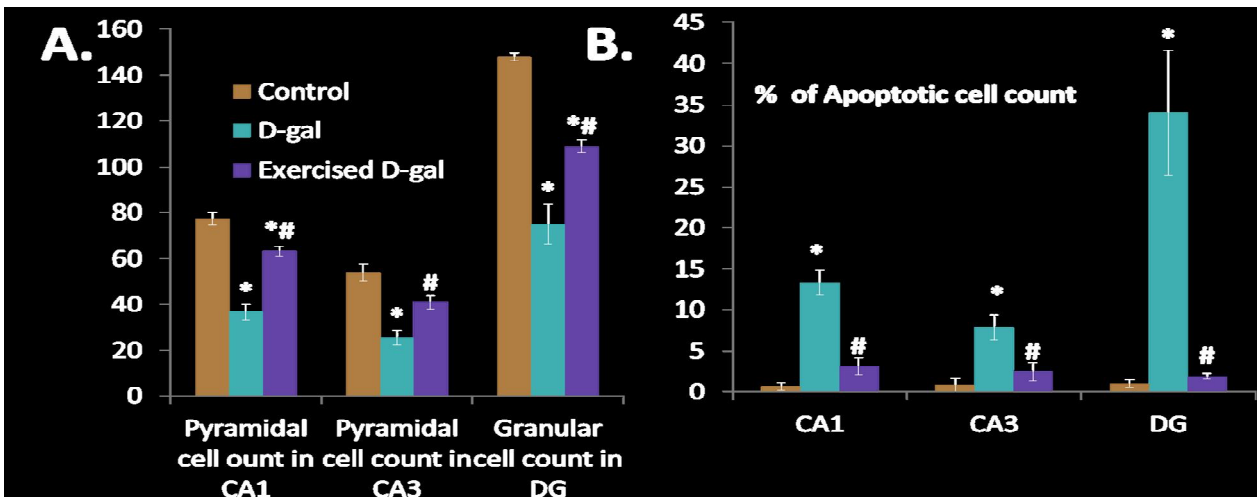

Figure (4): Morphometric analysisusing image J software (Maryland, USA) of (A): pyramidal cell count in CA1 and CA3 and granular cell count in DG(B): percentage of apoptotic cell count in CA1, CA3 and DG.Data were expressed as mean ±S.E. (n=5). One way ANOVA: *p<0.05, vs control; #p<0.05, vs D-gal treated group.

GFAP Immunostain results: GFAP immune reaction of astrocytes of hippocampus showed apparent increase in their number and increase of their processes in D-gal treated group (Figure5B1-5B4) compared to control group (Figure5A1-5A4) indicating active gliosis. In exercised D-gal treated group, GFAP immunoreactivity was apparently decrease in astrocytes and their processes as shown in Figure4 (Figure5C1-5C4) compared to D-gal treated group.

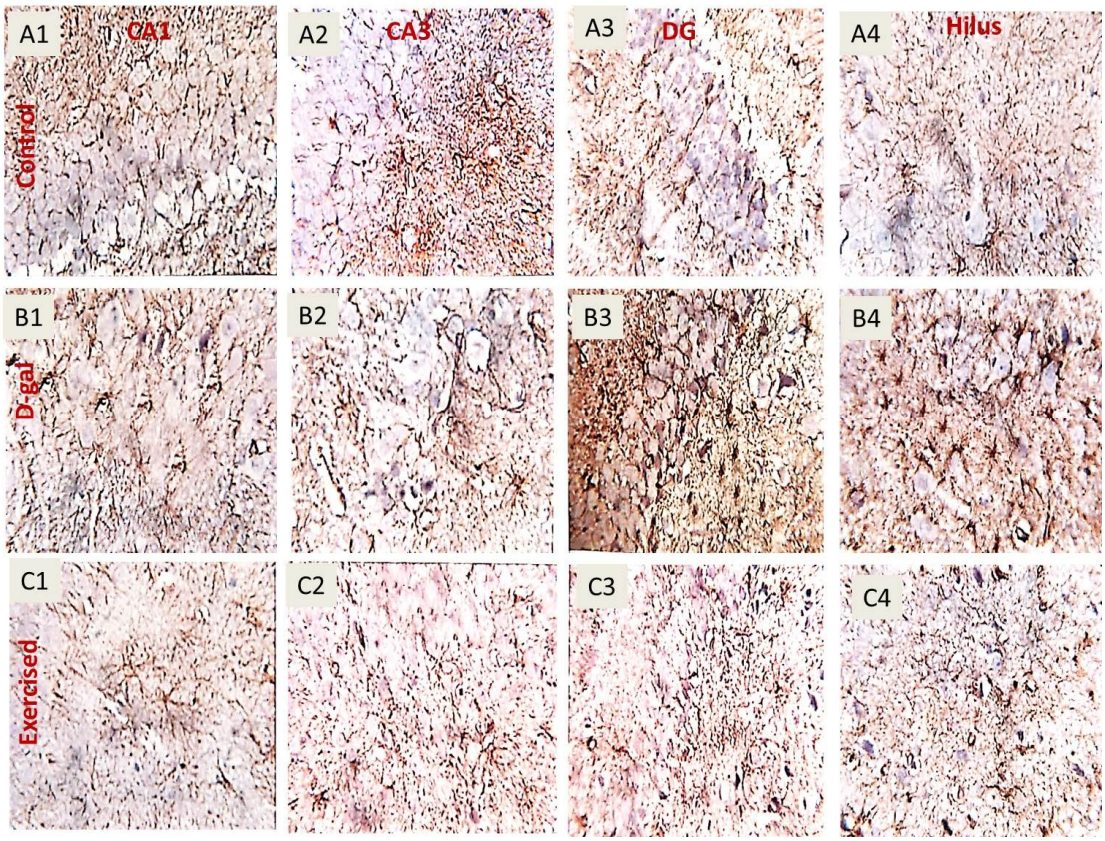

Figure (5): Evidence of apparent reduction of GFAP, which is a marker of reactive gliosis, associated with exercise training in D-gal rats' hippocampi. (A): control rats hippocampi stained with GFAP immunostain in CA1 (A1), CA3 (A2), DG (A4) and hilus (A4). (B): D-gal treated rats' hippocampi showing apparent increase in GFAP compared to Control. (C): Exercised D-gal treated rats hippocampi showing apparent reduction in GFAP compared to D-gal rats.

iNOSimmunostain results: The pyramidal cell layer of area CA1 and CA3 of hippocampus of D-gal treated group revealed overexpression of inducible nitric oxide synthase (iNOS) as indicated by strong immune reaction (Figure 6B1 and 6B2) compared to control group (Figure 6A1 and 6A2). However, in exercised D-gal treated group, the immune reaction for iNOS was decreased and only few pyramidal cells showed +ve reaction (Figure6C1 and6C2) compared to D-gal treated group.

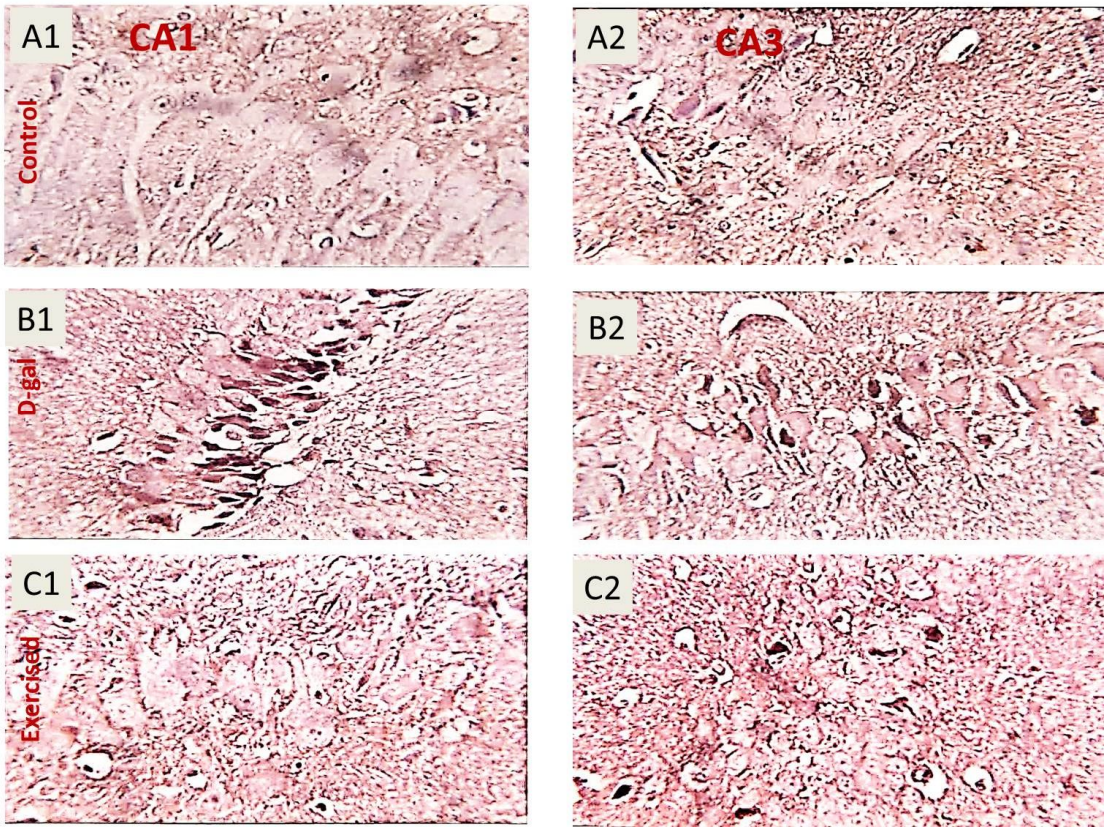

Figure (6): Evidence of reduced iNOS expression, which is a marker of oxidative stress, associated with exercise training in D-gal rats' hippocampi. (A1&2): iNOS immunoreaction in control rats' hippocampi area CA1 and CA3. (B1&2): overexpression of iNOS in hippocampal pyramidal cells of D-gal treated rats**.** (C1&2): decrease in the immune reaction for iNOS in hippocampal pyramidal cells of exercised rats compared to D-gal treated rats**.**

DISCUSSION

Aging is characterized by general decline of physiological function. Brain aging is the key risk factor for the development of cognitive impairment and age relative degenerative pathologies (Miyoshi et al., 2006 and Morcom et al., 2010). Hippocampus including dentate-gyrus is the region of brain that is crucial in cognitive functions such as learning and memory (Tashiro et al., 2007).

In the current study,long term injection of rats with D-gal for 10 weeks was used as a model for studying the effect of exercise on spatial memory impairment and to clarify the mechanisms of oxidative stress-induced brain aging. It was demonstrated that altered brain oxidative and metabolic state in the form of increased lipid peroxidation marker MDA and iNOS expression, reduced antioxidant enzyme SOD, and increased lactate/ pyruvate ratio (LPR) were associated with decreased hippocampal neural cell count, increased apoptosis, gliosis and over expression of GFAP. We have also shown that swimming exercise training was significantly effective in reversing these harmful metabolic and oxidative stress

consequences by reducing gliosis, apoptosis, and in preventing the associated memory impairment of D-gal rats. In this study, we have examined the hippocampus area of the brain, because it is mainly related to the spatial memory, and its neurological function is mainly affected by aging (Ramos, 2000).The spatial learning and memory impairment in D-gal rats that was shown in the present work was in concordance with other studies (Kim et al., 2004; Wei et al., 2005; Hua et al., 2007 and Wang et al., 2009)and was expressed in different ways according to the evaluation method.

We evidenced significant improvement in spatial memoryassociated with swimming exercise training in D-gal rats assessed by Barnes maze test in the form of decrease number of errors and escape latency to locate the escape box. These results were in agreement with previous researches who concluded that exercise (voluntary wheel running) enhances the behavioral performance of animals in spatial memory tasks (Burghardt et al., 2004 and Rodrigues et al., 2010),and also in animal models of brain ischemia (Shih et al., 2013),aging (Praag et al.,2005),and Alzheimer's disease (Adlard et al., 2005).

The possible mechanisms by which exercise was effective in preventing memory impairment and brain aging may be due to restoring oxidant antioxidant balance and preventing the harmful consequences of their disturbance, increasing lactate and pyruvate substrate required for neuronal functions and by improving the role of astrocytes in neural metabolism.

Swimming exercise restored oxidant antioxidant balance: Increased brain MDA and iNOS expression in hippocampus with reduction of brain SOD antioxidant enzyme activity in D-gal rats suggested a compromised oxidant/antioxidant balance in the brain and in particular in the hippocampus; denoting a state of oxidative stress with increased reactive oxygen species (ROS) production, which was in agreement with several other studies (Shen et al., 2002; Ho et al., 2003 and Cui et al., 2006). D-galactose converted into galactitol, which is not metabolized but accumulate in the cell, leading to osmotic stress and production of ROS (Hsieh et. al., 2009). High levels of ROS are linked to pathological processes, such as cellular function impairment, ongoing tissue injury, and cellular apoptosis (Sugamura and Keaney, 2011).Under the oxidative stress, the overproduction of NO by iNOS has been implicated in various pathophysiological processes and contribution to neurotoxicity (Prast and Philippu, 2001).It was also associated with apoptosis in a variety of cells (Yue et al., 2001). Oxidative stress stimulates astrocytes to switch from a resting to a reactive phenotype, thereby causing them to divert some of their energy away from supporting neurons and into defending the brain. This diversion of metabolic support can leave neurons less able to defend themselves from reactive oxygen species which are generated by their own metabolism (Steele and Robinson, 2010). D-galactose induced a significant degree of gliosis marked by increased GFAP and star-shaped astrocyte cell bodies indicating the presence of injured and dysfunctional astrocytes in the hippocampus (Sharma et al., 2009).Also, the significant increase in hippocampal apoptosis with

reduction of neural cell count in D-gal model were in agreement with Ruan et al.(2014). These consequences may be induced by the oxidative stress induced by D-gal.

Swimming exercise was significantly effective in preventing the reactive gliosis which was in agreement with Bernardi and colleagues (2013)who observed a decrease in hippocampal GFAP, induced by treadmill exercise. Thisas well as in preventing the loss of hippocampal neurons associated with D-gal which strongly suggest that these were achieved by its significant effect in restoring the oxidant/antioxidant balance as regular moderate exercise may promote antioxidant defenses (Teixeira-Lemos et al., 2011).That could attenuate central nervous system vulnerability to neuronal degeneration (Panaree et al., 2008). In a systemic review performed by Camiletti-Moir?n et al. (2013),it was reported that aerobic exercise promoted a positive effect (increase or maintain the same level) on SOD brain levels in 100% of the cases. Furthermore, daily moderate exercise has been shown to reduce NO content (measured by nitrite content) in hippocampal homogenate (Bernardi et al., 2013)and reduce the oxidative damage of hippocampal slices from Wistar rats exposed to in vitro ischemia (Scopel et al., 2006 and Cechetti et al., 2007).

Swimming exercise increased lactate and pyruvate substrate required for neuronal functions: Hippocampus mitochondrial dysfunction with impaired electron transfer and increased oxidative damage was observed upon rat aging (Navarro et al., 2008).Ross and his colleagues (2010) found that mitochondrial dysfunction caused by oxidative stress in the brain leads to a metabolic shift from aerobic respiration to glycolytic metabolism, results in robustly increased brain lactate levels. Our findings of increased lactate /pyruvate ratio in D-gal model prove this metabolic shift and the insults that might affected the mitochondrial respiratory chain enzymes. This was in agreement with Marcoux et al. (2008)who suggested that a high LPR may be secondary to mitochondrial dysfunction. Increased LPR indicates disorders of the respiratory chain complex, tricarboxylic acid cycle disorders or suggests a defect in pyruvate metabolism. This glycometabolism block is one of the mechanisms that explain the D-galactose role in the pathogenesis of aging (Shen et al., 2002; Ho et al., 2003 and Cui et al., 2006). However, the brain lactate level did not increase in this study as Ross's results(Ross et al., 2010), but its level was as controls or even lower.Lactate and the LPR need to be combined as screening tools for abnormal metabolism as any attempt to classify the metabolic status as normal or abnormal, i.e., anaerobic using only lactate can lead to an overdiagnosis of anaerobic metabolism when not combined with pyruvate and the LPR (Sahuquillo et al., 2014).

In exercised D-gal rats, the significant lower LPR reflected improvement in mitochondrial chain enzymes in brain neuron and strongly suggested the association between their improvement and the alleviation of the brain oxidative stress state. Lactate and pyruvate showed a significant increase in exercised D-gal rats compared to D-gal and control rats. Both metabolites are considered brain fuel. In the case of exercise, however,

lactate and to some extent pyruvate are the monocarboxylates offered to the brain in increasing arterial concentration (Dalsgaard, 2006).

Intracellular pyruvate is normally produced from glucose in the cytosol of neural cells through the Embden–Meyerhoff pathway, or alternatively, it can be derived from extracellular monocarboxylates after transport to the cytosol (Matsumoto et al., 1994; Desagher et al., 1997; Izumi et al., 1997 and Dienel, 2002). The relative contributions of these two pyruvate sources to the cerebral pyruvate pool and how the corresponding pyruvate precursors mix and interact intracellularly remain, however, less understood. Intracellular redox states are thought to play a dominant role in these processes (Rodrigues et al., 2009).Sharma and colleagues (2009)examined the effect of sodium pyruvate in treating brain injury. They concluded that the neuroprotective effects of pyruvate are mediated through its antioxidant mechanisms which can maintain global redox status, decrease lipid peroxidation and reduce gliosis. So, pyruvate and redox state may have alternating cause-effect relationship, and there is always positive correlation between them.

In this study, the increased brain lactate level in exercised D-gal rats associated with memory improvement may seem to be controversial with previous concepts about the causes and consequences of high brain lactate level. Lactate is considered an important oxidative energy substrate in the cerebrum. The brain is able to take up lactate from the blood during exercise as well as during the initial stage of recovery (Passarella et al., 2008). In support of this, a hypothesis was made proposing an astrocyte-neuron shuttle where glucose is taken up by the astrocytes, converted to lactate and transported through mono-carboxylate transport (MCT) systems for use by the neurons as fuel for mitochondrial respiration (Pellerin & Magistretti., 1994 and Newington et al., 2013).

Researchers noticed a transient decrease in extracellular lactate levels after stimulation both in the human and rodent brain (Hu &Wilson, 1997andMangia et al., 2003) followed by a sustained increase in extracellular lactate (Prichard et al., 1991; Hu and Wilson, 1997 and Lin et al., 2010). These sequences of events closely parallels the temporal changes in extracellular lactate levels observed in vitro by Kasischke and Collaborators (2004). Together, these studies suggest that extracellular lactate is rapidly oxidized by neurons during their activation to meet their energy needs, followed by a sustained activation of the glycolytic pathway in astrocytes, to replenish the extracellular lactate pool (Bélanger et al., 2011).

Swimming exercise improved the role of astrocytes in neural metabolism:The results of this study provided additional evidence for astrocyte-neuron metabolic coupling (Brown, 2004; Brown and Ransom, 2007 and Bélanger et al., 2011).The involvement of astrocytic glycogen-derived lactate in long-term memory formation, and for the in vivo maintenance of long-term potentiation (LTP) of synaptic strength in the mammalian brain, was demonstrated by Suzuki and Colleagues (2011).

Also, the astrocyte seems to be a key player in the redox state of the brain and neurons.It can be expected that episodes of oxidative stress will lower the amounts of antioxidant enzyme and lactate released by astrocytes. These changes will subsequently lower the capacity of neurons to produce energy via the TCA cycle and will also limit their capacity to combat oxidative stress (Steeleand Robinson, 2010).

The morphological and phenotypical characteristics of astrocytes are thus tailored to ideally position them (among other important astrocytic functions) to sense neuronal activity at the synapse and respond with the appropriate metabolic supply via their astrocytic end feet, which enwrap the intracerebral blood vessels. LPR in is taken to indicate insufficient oxygen supply and low cerebral blood flow (Dalsgaard, 2006).In line with this, an increasing body of evidence suggests that astrocytes appear as important players in the neurovascular unit, acting as intermediaries in neuronal signaling to blood vessels and all its function is improved in exercise D-gal rat model.

CONCLUSION

It has become evident that exercise was able to decrease the transformation of astrocytes from a basal to a reactive state in response to oxidative stress, and can trigger a change in their metabolic phenotype. This appears to provide a basis for many of the metabolic changes that occur in exercise induced neural protection and memory improvement.

ABBREVIATIONS

AD: Alzheimer's disease, D-gal: D-galactose, GFAP: Glial fibrillary acidic protein, iNOS: inducible nitric oxide synthase, LPR: lactate/pyruvate ratio, MDA: malondialdehyde, NO: Nitric oxide, ROS: reactive oxygen species, SOD: Superoxide dismutase.

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سوزى فايز عويضة – ماجدة منصور*

قسم الفسيولوجيا الإكلينيكية و الهستولوجيا* - كلية الطب - جامعة المنوفية

خلفية البحث: الشيخوخة هى ظاهرة بيولوجية معقدة تتميز بتراكم تغييرات مؤذية مع الوقت منها إعتلال الذاكرة. الرياضة البدنية تؤثر إيجابا على الدماغ أثناء كلا من الشيخوخة و الأمراض التنكسية العصبية.

الهدف من البحث: دراسة دور خلايا الحصين النجمية فى الوقاية من إعتلال الذاكرة المستحث بسكر ال د-جالاكتوز أثناء ممارسة الرياضة.

طرق و مواد البحث: تم تقسيم ثمانية عشر فأرا إلى ثلاث مجموعات متساوية (كل منها ست فئران): مجموعة ضابطة تحقن بالمحلون الملحى عن طريق الحقن البريتونى لمدة عشر أسابيع، و مجموعة تحقن فى التجويف البريتونى بسكر د-جالاكتوز لانه يسبب إعتلال الذاكرة (100 مجم/ كيلوجرام لمدة 10 أسابيع)، و مجموعة تحقن بسكر د-جالاكتوز مع ممارسة الرياضة لمدة 10 أسابيع. و قد تم تحليل أداء الذاكرة و التعلم للفئران فى متاهة بارنيز و تحليل أنسجة الدماغ و صبغها مناعيا بمضادات البروتينات الليفية الحمضية للخلايا الدبقية و وإنزيم أكسيد النيتريك المستحث. كما تم تجهيز جناسة الدماغ لتقييم مستوى المالونديالدهايد و إنزيم السوبر أكسيد ديسميوتيز واللاكتات و البيروفات.

نتائج البحث: لوحظ تحسن ذو دلالة إحصائية فى المجموعة التى مجموعة تحقن بسكر د-جالاكتوز مع ممارسة الرياضة فى طورى الإكتساب والفحص أثناء إختبار متاهة بارنيز، وإنخفاض المالونديالدهايد وزيادة مستوى إنزيم السوبر أكسيد ديسميوتيز واللاكتات و البيروفات مع إنخفاض نسبة اللاكتات / بيروفات فى جناسة الدماغ. زيادة عدد الخلايا العصبية فى الحصين مع نقص الموت الخلوى المبرمج وإظهار مضادات البروتينات الليفية الحمضية للخلايا الدبقية و وإنزيم أكسيد النيتريك المستحث.

الاستنتاج: هذه الدراسة قدمت دلائل على أن الرياضة فعالة فى تحسين الإجهاد التأكسدى فى الدماغ وإستعادة خلايا الحصين العصبية و الوظائف الأيضية للخلايا النجمية وفى المحصلة تحسن كفاءة الذاكرة المكانية.