

Neuroprotective effect of Exercise on Alzheimer's disease in rats: Role of Nuclear Factor Erythroid 2- Related Factor 2 (NRF2)

Hebatallah M. Aboudeya*, Trez N. Michel, Maha M. Attia, Azza Saad

Human Physiology Department, Medical Research Institute, Alexandria University, Egypt

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- Amyloid beta (A β).

Abstract

Alzheimer's disease (AD) is a common neurodegenerative disease that leads to memory and cognitive impairment. Exercise is suggested to prevent it. However, the exact mechanism remains unclear. This study investigated the effect of moderate exercise on cognitive function, oxidative stress and neuro-inflammation in the hippocampal tissue of experimentally-induced AD rats and the possible role of Nuclear Factor Erythroid 2- Related Factor 2 (NRF2) in mediating this effect. Forty adult male albino rats were divided into control and AD groups. Each group is further subdivided into sedentary and exercised ones. AD was induced by intraperitoneal injection with aluminium chloride (70 mg/kg b.w.) for 6 weeks. Exercise protocol was done by swimming 60 minutes, 5 times a week for 4 weeks. The following parameters were evaluated in all groups: hippocampal tissue assessment of NRF2, amyloid beta, malondialdehyde, interleukin 6 and total antioxidant capacity. Assessment of cognitive performance was done using Morris water maze at weeks 3, 4 and 6 after AD induction. Results revealed significantly lower hippocampal NRF2 and TAC levels with significant higher A β , MDA, IL-6 and impaired cognitive dysfunction in sedentary AD rats. These were reversed by swimming exercise. NRF2 was negatively correlated with A β , IL-6 and MDA in both AD groups with positive correlation with TAC. In conclusion, moderate swimming exercise exerts neuroprotective effects in AD through improvement of cognitive function, restoration of the antioxidant and anti-inflammatory capacity. The upregulation of NRF2 could mediate these effects. Therefore, targeting NRF2 could be promising as a therapeutic agent for neurodegenerative diseases.

Corresponding author: Hebatallah Aboudeya drheba_mohammed@yahoo.com, Postal address: 165, Horreya Avenue, Hadara Alexandria, Egypt

INTRODUCTION

Alzheimer's disease (AD), the most common cause of dementia, is a neurodegenerative disease characterized by progressive behavioral and cognitive impairments. Its prevalence increases worldwide, thus becoming a great healthcare problem (1). It affects about 47 million people worldwide and its incidence is expected to increase to 135 million people by 2050. (2)

Although the cause of AD is still not fully understood, amyloid plaques and neurofibrillary tangles (NFT) deposition appear to be important contributing factors. Their production and accumulation could be associated with synaptic dysfunction and neuronal degeneration that lead to progressive and irreversible memory deterioration, affecting language, personality, and cognition (3).

Mitochondrial damage and oxidative stress have been suggested to play a role in the initiation and the progression of several neurodegenerative diseases, including AD (4). Nuclear Factor Erythroid 2- Related Factor 2 (NRF2) is a master transcription factor that regulates oxidative stress-related genes containing the antioxidant response element (ARE) in their promoters. It protects our bodies from dangerous stress by increasing antioxidative defense pathway, decreasing inflammation with maintenance of protein homeostasis. The damaged function and altered localization of NRF2 are found in most neurodegenerative diseases including AD. Recent studies revealed that NRF2 activators have therapeutic effects in AD animal models and in cultured human cells that express AD pathology (5, 6).

Exercise is proposed to play a role not only in preventing the pre-clinical stage of AD but also in slowing its clinical progression. Various human and experimental studies suggested that exercise slows the onset and progression of cognitive decline in AD patients. There is no consensus about the best type of exercise for the amelioration of clinical outcomes in AD (7). A large number of studies have focused on the beneficial effect of aerobic exercise. Some studies suggested that this effect is due to a decrease of deposits in brain parenchyma. Others suggested that exercise could promote neurogenesis, synaptogenesis and angiogenesis leading to increase in blood flow, brain-derived neurotrophic factor (BDNF), insulin-like growth factor 1 (IGF-1), hormones, and second messengers, all of which act in a synergistic manner to induce neuroplasticity and neurogenesis (7,8).

Moreover, Dao et al (9) have reported that cognitive impairments in case of aging and brain insults can also be limited by regular exercise. However, the exact mechanisms by which physical activity improves cognitive performance remain unclear.

Aluminium, a well-established neurotoxicant, is reported to be involved in the etiology of AD due to its easy admittance and accumulation in central nervous system. It promotes the formation of amyloid beta ($A\beta$) protein plaques by aggregating tau proteins in the brain. Administration of aluminum chloride (ALCl₃) predominantly accumulates in the hippocampus and this region is known to be particularly susceptible in AD and has an important role in learning and memory functions (10, 11). For this reason, ALCl₃ model was selected for the present study.

Therefore, the present study was aimed to investigate the effect of moderate swimming exercise on cognitive function, markers of oxidative stress and neuro-inflammation in the hippocampal tissue of experimentally-induced AD rats. The possible role of NRF2 in mediating this effect was also studied.

Materials and Methods

Ethical approval

All animal procedures and experimental protocols were performed in accordance with the Guide for the Care and Use of Laboratory Animals (1985) (12) and were approved by the Research Ethical Committee of the Medical Research Institute of Alexandria University (Approval reference number: 01219123132). All efforts were made to minimize the rats' suffering during the experimental period. The ethical principles of the journal are understood, and the study complies with the checklist of animal ethics.

Animals

Forty adult male wistar (albino) rats (aged 2-3 months) with initial weight (150-160 grams) were obtained from the animal house of Medical Research Institute, Alexandria University, Egypt. Rats were housed under controlled room temperature 25°C with natural light-dark cycle (12/12 h) for one week before the experiment for acclimation with free access of diet and water *ad libitum* throughout the experimental period. Rats were randomly equally divided into 2 main groups: A control group and Alzheimer's disease group. Each group is further subdivided equally into two subgroups; sedentary and exercised.

Induction of Alzheimer's disease

AlCl₃-hydrated (AlCl₃.6H₂O) was purchased from Sigma Chemical Co. (St. Louis, MO, USA). It was freshly dissolved in distilled water.

The rats of AD group were injected intraperitoneally with aluminium chloride (70 mg/kg body weight) for 6 weeks according to Ali et al., 2016 (10).

Moderate exercise protocol

The rats from both exercised control and Alzheimer's groups were subjected to the moderate swimming exercise protocol that included 2 phases: adaptation and training. The rats were adapted for the first three days of training. On the first day, the animals exercised swimming in the pool (area 100 cm², with water depth 35–45 cm at 37 °C. for 15 min). The exercise period was increased by 15 min each day until animals were swimming for one hour, 5 times a week for 4 weeks starting at the beginning of 3rd week of induction (13).

Assessment of cognitive performance

Morris water maze (MWM) test is a hippocampus dependent spatial learning and memory task (14). It consisted of a circular pool (100 cm diameter, 50 cm height). The pool was filled with water at the temperature of 25 ± 2°C. The MWM was divided imaginary into four equal quadrants: North, South, East, and West. A white platform (11 cm diameter and 16 cm height) was centered in the South–East quadrant 1 cm below the water surface.

The water was whitened by addition of liquid milk so that the platform was not seen by rats at water surface. The position of the platform was kept unaltered during the training session. If rats failed to reach the platform within permitted time,

the trial was ended and they were gently guided to the platform. Each animal had four training sessions per day. During each trial session, the time taken to reach the platform was recorded using stopwatch.

In the present work, time was recorded using the stopwatch at the 3rd, 4th and 6th weeks of AD induction, this reflects the start, mid and the end of exercise program.

At the end of experiment, all rats were sacrificed by cervical dislocation under anesthesia using ketamine/xylazine at a dose of 100/10 mg/kg in accordance with the literature (15) and brain tissues were rapidly removed and rinsed with ice-cold phosphate buffered saline (PBS) solution to remove any blood cells and clots. Then, the hippocampus was dissected, cleaned from adhering matters, washed with physiological saline. Then it was minced and homogenized in phosphate buffer (pH 7.4). Homogenates were centrifuged at 5000xg for five minutes and the clear supernatants were collected and stored at -80°C after protein content estimation in hippocampal homogenates by Lowry method (16) for biochemical determination.

Biochemical measurements

Hippocampal A β was determined using a sandwich enzyme-linked immunosorbent assay (ELISA) kit purchased from Elabscience Biotechnology Inc., USA.

Lipid peroxidation was estimated by measurement of MDA levels in hippocampal tissues using thiobarbituric acid reactive substances (TBARS) (17). TAC determination was performed by the reaction of antioxidants in the sample with a defined amount of exogenously provide Hydrogen peroxide (H₂O₂). The remained

H₂O₂ was determined by an enzymatic reaction colorimetrically (18).

IL-6 and NRF2 protein levels were quantified using a commercially available ELISA kit purchased from (R&D Systems, Inc., USA) and (MyBioSource), respectively according to their manufacturer's instructions.

Statistical analysis

The data were analyzed statistically using Statistical Package for Social Sciences (SPSS) program version 20. Data were expressed as mean \pm SD. Analysis of variance (ANOVA) test was used for comparison between the different studied groups and Post Hoc test (Tukey) for pairwise comparison. Pearson correlation was also performed between NRF2 and the other studied parameters. For all statistical tests, level of P equal to or less than 0.05 was considered significant.

Results

Biochemical results:

Biochemical analysis of the brain hippocampal tissue demonstrated significant differences between the 4 studied groups using ANOVA test in all parameters. F values of amyloid beta (A β) (pg/mg protein), interleukin 6 (IL-6) (pg/mg protein), malondialdehyde (MDA) (nmol/mg protein) and total antioxidant capacity (TAC) (U/mg protein were (589.492, 223.365, 208.574, 163.429 respectively, (P<0.001). Findings revealed significant increase in the mean values of A β , IL-6 and MDA in sedentary AD group as compared to other groups (P<0.001). However, a significant decrease in TAC was noticed (P<0.001).

Moderate exercise swimming for 4 weeks induced improvements in the all studied parameters. Significant reduced levels of A β , IL-6 and MDA were noticed in AD exercised group as compared to

sedentary group ($P < 0.001$). On the contrary, TAC levels were significantly increased ($P < 0.001$) in the same group (Table 1).

Effect of exercise on cognitive performance (MWM):

Assessment of cognitive performance using MWM after 3 weeks of AD induction showed no significant changes between the 4 studied groups. However, after the 4th and 6th weeks of AD induction, significant differences among groups were noticed ($F = 16.242$, $F = 44.818$, $P < 0.001$).

Findings revealed a significant increase in time taken by sedentary AD rats to find the platform as compared to control ($p < 0.001$), indicating learning and memory impairments. Meanwhile, following swimming exercise, the time taken by the exercised AD rats was significantly decreased as compared to sedentary ones ($p < 0.001$), indicating cognitive improvement by exercise (Table 2).

Table (1): Effect of exercise on hippocampal levels of amyloid beta ($A\beta$) (pg/mg protein), interleukin 6 (IL-6) (pg/mg protein), malondialdehyde (MDA) (nmol/mg protein), total antioxidant capacity (TAC) (U/mg protein) in the different studied groups.

	Sedentary Control (n=10)	Exercised Control (n=10)	Sedentary AD (n=10)	Exercised AD (n=10)	F	P
Aβ (pg/mg protein), Mean \pm SD. Sig. bet. Grps	2.4 \pm 0.2	2.3 \pm 0.1	20.2 \pm 1.8	12.1 \pm 1.4	589.492*	<0.001*
	p ₁ =1.000, p ₂ <0.001*, p ₃ <0.001*, p ₄ <0.001*, p ₅ <0.001*, p ₆ <0.001*					
IL-6 (pg/mg protein) Mean \pm SD. Sig. bet. Grps	27.6 \pm 5.8	23.1 \pm 3.0	64.8 \pm 3.4	41.1 \pm 3.0	223.365*	<0.001*
	p ₁ =0.068, p ₂ <0.001*, p ₃ <0.001*, p ₄ <0.001*, p ₅ <0.001*, p ₆ <0.001*					
MDA (nmol/mg protein) Mean \pm SD. Sig. bet. Grps	4.2 \pm 0.4	3.7 \pm 0.4	9.3 \pm 0.7	5.5 \pm 0.5	208.574*	<0.001*
	p ₁ =0.335, p ₂ <0.001*, p ₃ <0.001*, p ₄ <0.001*, p ₅ <0.001*, p ₆ <0.001*					
TAC (U/mg protein) Mean \pm SD. Sig. bet. Grps	1.5 \pm 0.1	1.6 \pm 0.1	0.9 \pm 0.1	1.3 \pm 0.1	163.429*	<0.001*
	p ₁ =0.052, p ₂ <0.001*, p ₃ <0.001*, p ₄ <0.001*, p ₅ <0.001*, p ₆ <0.001*					

F: F for ANOVA test, Pairwise comparison bet. each 2 groups was done using Post Hoc Test (Tukey)

p: p value for comparing between the studied groups

p1: p value for comparing between sedentary and exercised control.

p2: p value for comparing between sedentary control and AD

p3: p value for comparing between sedentary control and Exercised AD.

p4: p value for comparing between exercised control and sedentary AD

p5: p value for comparing between exercised control and AD.

p6: p value for comparing between sedentary and exercised AD.

*: Statistically significant at $p \leq 0.05$

Hippocampal NRF2 results:

Measurement of hippocampal Nrf2 showed significant changes between the 4 studied groups by using ANOVA test ($F = 318.613$, $P < 0.001$).

Results showed significant decrease in the NRF2 mean values in sedentary AD group as compared to other groups. However, this was reversed after swimming exercise as significant increase in its mean values was detected in AD-exercised group as compared to sedentary ones (149.3 ± 7.0 pg/mg vs 94.9 ± 8.5 pg/mg < 0.001) (Table 3 and fig 1).

Correlation studies:

The present study revealed significant negative correlations of hippocampal Nrf2 with $A\beta$, IL-6 and MDA in both sedentary AD and exercised groups ($p < 0.001$) (Fig 2-4). However, it showed significant positive correlation with TAC in the same groups ($p < 0.001$) (Fig 5).

Table (2): Effect of exercise on the cognitive performance using Morris water maze (seconds) after 3, 4 & 6 weeks of Alzheimer's disease induction.

	Sedentary Control (n=10)	Exercised Control (n=10)	Sedentary AD (n=10)	Exercised AD (n=10)	F	P
Maze (seconds)						
3week						
Mean ± SD.	15.4 ± 2.8	16.1 ± 2.7	15.2 ± 1.6	14.8 ± 5.4	0.249	0.861
4week						
Mean ± SD.	12.3 ± 3.8	12.9 ± 2.4	20.7 ± 2.4	12.9 ± 3.7	16.242*	<0.001*
Sig. bet. Grps	p ₁ =0.979, p ₂ <0.001*, p ₃ =0.976, p ₄ <0.001*, p ₅ =1.000, p ₆ <0.001*					
6week						
Mean ± SD.	8.8 ± 2.0	6.3 ± 2.4	20.9±1.9	11.5 ± 4.8	44.818*	<0.001*
Sig. bet. Grps	p ₁ =0.267, p ₂ <0.001*, p ₃ =0.190, p ₄ <0.001*, p ₅ =0.002*, p ₆ <0.001*					

F: F for ANOVA test, Pairwise comparison bet. each 2 groups was done using Post Hoc Test (Tukey)

p: p value for comparing between the studied groups

p₁: p value for comparing between sedentary and exercised control.

p₂: p value for comparing between sedentary control and AD

p₃: p value for comparing between sedentary control and Exercised AD.

p₄: p value for comparing between exercised control and sedentary AD

p₅: p value for comparing between exercised control and AD.

p₆: p value for comparing between sedentary and exercised AD.

*: Statistically significant at p ≤ 0.05

Table (3): Effect of exercise on hippocampal levels of Nuclear Factor Erythroid 2- Related Factor 2 (NRF2) (pg/mg protein) in the different studied groups.

	Sedentary Control (n=10)	Exercised Control (n=10)	Sedentary AD (n=10)	Exercised AD (n=10)	F	P
NRF2 (pg/mg protein)						
Mean ± SD.	169.2 ± 8.8	190.8 ± 3.6	94.9 ± 8.5	149.3 ± 7.0	318.613*	<0.001*
Sig. bet. Grps	p ₁ <0.001*, p ₂ <0.001*, p ₃ <0.001*, p ₄ <0.001*, p ₅ <0.001*, p ₆ <0.001*					

F: F for ANOVA test, Pairwise comparison bet. each 2 groups was done using Post Hoc Test (Tukey)

p: p value for comparing between the studied groups

p₁: p value for comparing between sedentary and exercised control.

p₂: p value for comparing between sedentary control and AD

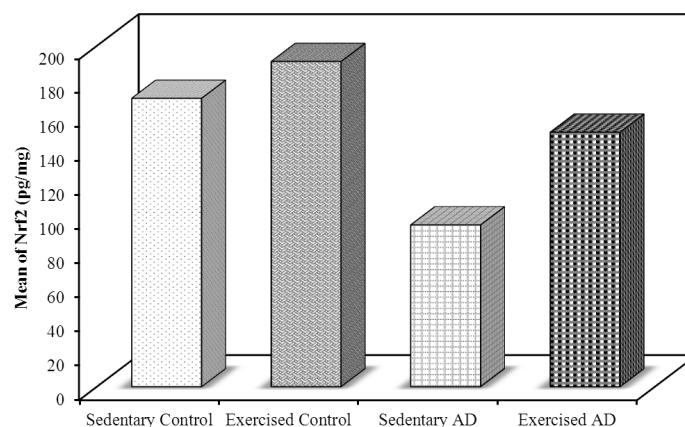
p₃: p value for comparing between sedentary control and Exercised AD.

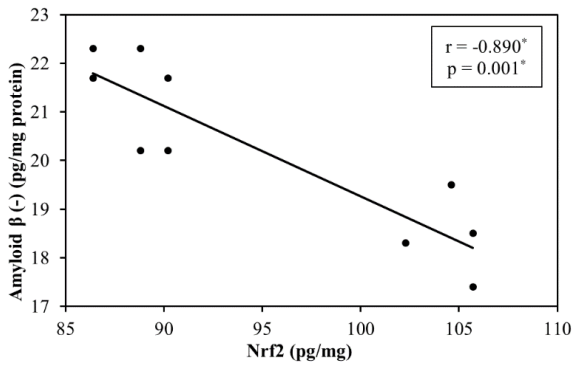
p₄: p value for comparing between exercised control and sedentary AD

p₅: p value for comparing between exercised control and AD.

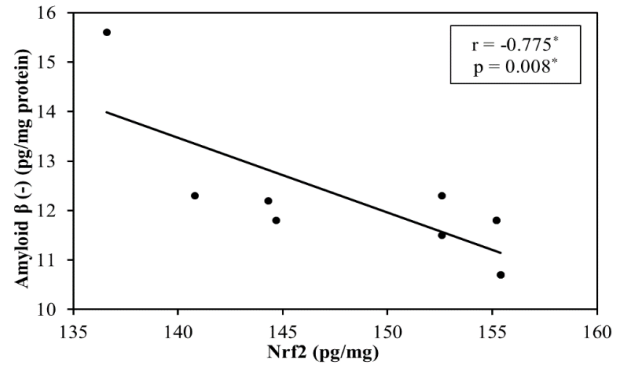
p₆: p value for comparing between sedentary and exercised AD.

*: Statistically significant at p ≤ 0.05

**Figure (1):** Hippocampal levels of Nuclear Factor Erythroid 2- Related Factor 2 (NRF2) (pg/mg protein) in the different studied groups.

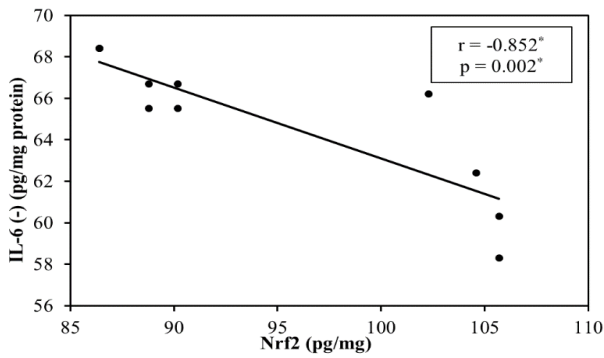


(A)

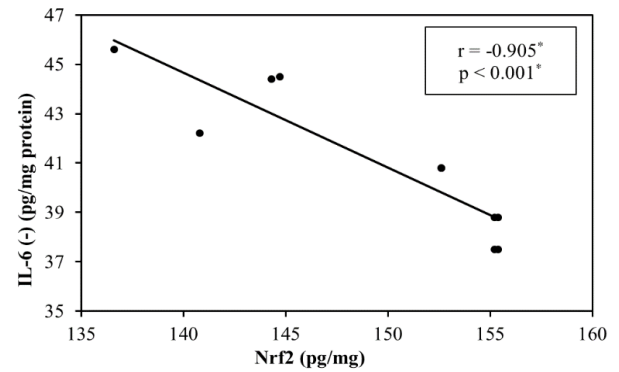


(B)

Figure (2): Correlation between Nrf2 (pg/mg) and Amyloid β in sedentary (A) and exercised (B) AD groups.

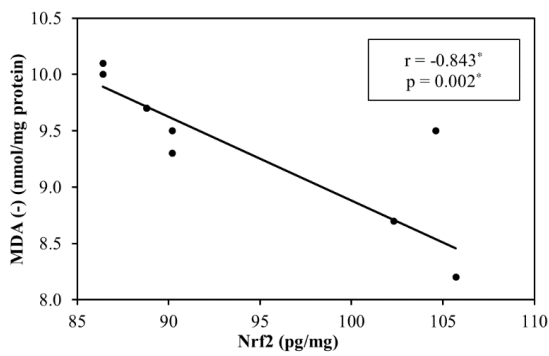


(A)

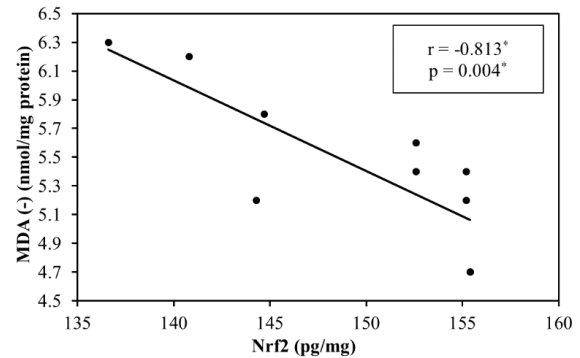


(B)

Figure (3): Correlation between Nrf2 (pg/mg) and IL-6 in sedentary (A) and exercised (B) AD groups.

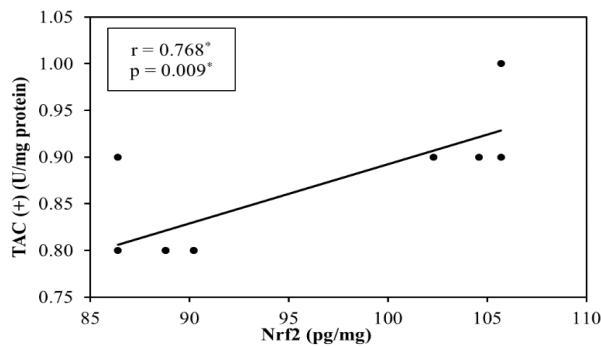


(A)

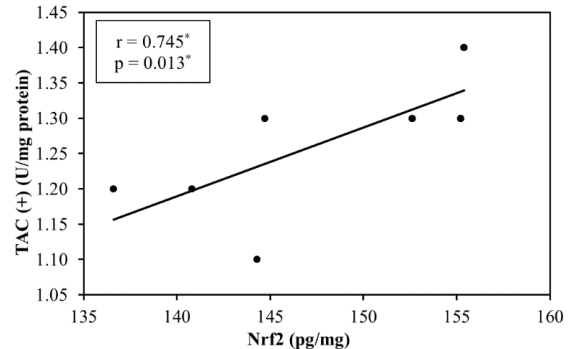


(B)

Figure (4): Correlation between Nrf2 (pg/mg) and MDA in sedentary (A) and exercised (B) AD groups.



(A)



(B)

Figure (5): Correlation between Nrf2 (pg/mg) and TAC in sedentary (A) and exercised (B) AD groups.

Discussion

Alzheimer disease is a progressive common neurodegenerative disorder characterized by early cognitive dysfunction and later behavioural deterioration. The hippocampus is the primary neuronal injury region involved in the disease pathophysiology (19). The accumulation of A β in the brain is one of the major pathological hallmarks of AD (20). Exercise has been suggested as an effective intervention in AD. However, mechanisms underlying its neuroprotective role have not yet been fully elucidated (21). In the present study, we investigated the effect of 4-weeks moderate swimming training on beta amyloidosis, cognitive functions, oxidative stress and inflammation and the possible role of NRF2 in modulating neuronal damage in a rat model of AD induced by aluminum chloride injection.

Findings of the current study revealed significant decrease of A β accumulation in the hippocampus by exercise reflecting its possible role in enhancement of hippocampal A β clearance and contributing to a neuroprotective action. Xia et al (22) have reported that overactivation of the unfolded protein response (UPR) signaling may lead to β -amyloidogenesis. They suggested that treadmill exercise may suppress this overactivation of the UPR signaling as well as it may inhibit the amyloidogenic pathway. Another study suggested that the 12-week treadmill exercise program reduced A β deposition in the hippocampus of amyloid precursor protein /presenilin 1 (APP/PS1) mice, possibly by regulating a disintegrin and metalloproteinase domain-containing protein 10 (ADAM10) and β -site amyloid precursor protein cleaving enzyme 1 (BACE1) levels and by

decreasing cholesterol-mediated lipid raft formation, indicating that exercise represents a therapeutic intervention to treat AD (20).

In addition, the present results showed that exercise induced improvement of cognitive function measured by Morris water maze. Significant decrease of time taken by exercised AD rats after 4 and 6 weeks of induction when compared to sedentary AD rats. This finding illustrates the importance of exercise training in improving learning and memory performance.

Previous studies have shown the effect of exercise on brain adaptations, especially in the hippocampus. Sarlak et al (23) studied the effect of aerobic exercise before and after the induction of AD by intra-hippocampal injection of A β . They found worse performance in AD group than in sham group in the memory and learning tests and the effects of exercise in both A β and sham groups indicated an improvement in the cognitive function as a result of aerobic exercise. Exercise and physical activity lead to neurogenesis and changes in the hippocampal synaptic plasticity that improves cognitive functions, such as learning and memory (24).

Results of the present work indicated the presence of oxidative stress manifested by significant increased MDA and decreased TAC as well as inflammation manifested by significant increased hippocampal IL-6 levels in AD sedentary rats in comparison to exercised ones.

The oxidative stress implicated in the neurodegenerative process of AD may be either due to excessive production of free radicals or loss of antioxidant defenses or both (19). Loss of glutathione has been suggested as an early signaling event in mitochondrial dysfunction and

apoptotic cell death involved in the disease process (19). Reactive oxygen species (ROS)-induced damage to biomolecules, such as lipids, proteins, DNA and RNA has been proposed as a major cause in the advancement of neurodegenerative diseases as AD (25).

Moreover, acute inflammation in the brain was reported as a well-established defense against any brain lesion, but when a disruption of anti-inflammatory and pro-inflammatory signaling occurs, as seen in AD, it results in chronic inflammation. This sustained chronic neuro-inflammation is thought to be related to the neuronal loss occurring in this disorder (26).

However, it has been demonstrated that a persistent immune response in the brain is associated with both neurodegeneration and exacerbation of A β and neurofibrillary tangles (NFT) deposition. It has been suggested that the inflammatory response may provide a link between A β plaques formation and NFTs development (27).

The neuroprotective effect of exercise was investigated in several studies. Belviranli et al (27) suggested that different exercise types could equally attenuated non-cognitive and cognitive disturbances in a rat model of AD. The elevated neurotrophic factors with improved oxidative stress could mediate these improvements. While, a study by Freitas et al (28) showed a positive effect of six weeks of High intensity interval training (HIIT) on hippocampal oxidative stress by reducing lipoperoxidation and inflammation, as well increasing antioxidant defenses and BDNF content. Others reported that overtraining might lead to learning and memory disturbance by

increasing the inflammatory and oxidative stress markers (29).

Zhang et al (30) demonstrated that treadmill exercise could control neuroinflammation through reducing expression of pro-inflammatory factors and increasing the expression of anti-inflammatory mediators in the hippocampus accompanied with shift in activated microglia from the M1 to M2 phenotype. They added that treadmill exercise may lead to marked reduction in MDA level and increase of both Superoxide dismutase (SOD) and Manganese superoxide dismutase (Mn-SOD) activities in the hippocampus thus controlling oxidative stress. These findings could suggest that treadmill exercise can effectively prevent the decrease in hippocampal-dependent cognitive function and A β deposits in early AD progression possibly by modulating microglia-mediated neuro-inflammation and oxidative stress.

NRF2 is a regulator of endogenous inducible defense systems in the body. Normally, NRF2 is mainly located in the cytoplasm. But in response to oxidative stress, it translocates to the nucleus and binds to specific DNA sites known as “anti-oxidant response elements” or “electrophile response elements” to start transcription of cytoprotective genes and activate expression of antioxidant enzymes (25).

In the present study, oxidative stress and neuroinflammation noticed in the hippocampal tissue of AD rats are accompanied with decreased NRF2 indicating its role in AD. This is clearly evident from the negative correlation obtained between NRF2 and both of MDA and IL-6.

It was reported that acute oxidative stress in brain, is increased in animals with deficient NRF2. Insufficient NRF2 activation in humans has been

associated with chronic diseases as Parkinson's disease, AD and lateral sclerosis (31).

The transcription factor NRF2 has been demonstrated as a regulator of inflammatory responses. It was reported that it regulates oxidant metabolism and several cytoprotective responses. It is now known that it exerts immune regulatory functions by inducing the expression of anti-inflammatory genes and repressing the expression of the pro-inflammatory genes. Other mechanisms may involve the control of reactive oxygen species (ROS) levels, which regulate the nuclear factor kappa B (NF- κ B) response, or inhibition of the infiltration of immune cells (32).

The underlying mechanisms behind the poor NRF2 functioning in chronic neurodegenerative diseases are not known. However, one possible link between the NRF2 system and AD could be due to proteinopathies or accumulation of A β plaques (33). This is clearly evident from the negative association between NRF2 and A β in rat models of AD in the present study.

Activation of the NRF2-antioxidant response element signaling pathway is an important mechanism in the cellular defense. Hayes & Dinkova-Kostova et al (34) have reported that induction of the NRF2 pathway exerted anti-oxidative, anti-inflammatory, and anti-amyloid effects on AD cells, suggesting that it could target all these three processes. Besides regulating cellular redox balance and inflammatory reactions, NRF2 has been demonstrated to modulate cellular metabolism and regulate metabolic remodeling during stress. Oksanen et al (35) reported that activation of NRF2 poses several beneficial effects on AD astrocytes. They explained that this activation of NRF2 pathway reduces amyloid

secretion, normalizes cytokine release, and increases Glutathione (GSH) secretion in AD astrocytes. Moreover, NRF2 induction also activates the metabolism of astrocytes and increases the utilization of glycolysis.

Although exercise training was reported to regulate antioxidant defenses system, informations regarding its exact mechanism are limited. It was reported that a single bout of acute exercise in mice has been shown to increase NRF2 gene expression and NRF2 protein abundance in skeletal muscle and mouse myocardium (36, 37). The activation of NRF2 in response to exercise may be an important mechanism that explains how exercise exerts its well-known systemic effects.

Tutakhail et al (38) have demonstrated that only vigorous and longer duration aerobic exercise reduced inflammation by increasing NRF2 protein level in the hippocampus and heme oxygenase 1 (HO-1) protein level in the cortex of normal mice.

Contrary to previous studies, the regular moderate exercise training used in this study was found to be effective in preventing hippocampal neuronal damage in AD rats. This could be explained by the significant upregulation of hippocampal NRF2 in addition to its negative associations with MDA, IL-6 and A β content as well as its positive association with TAC.

In conclusion, the current study indicated that moderate exercise exerts beneficial neuroprotective effects in AD via improving cognitive function, restoring the antioxidant and anti-inflammatory brain capacity. The upregulation of NRF2 could mediate these beneficial effects. Therefore, targeting NRF2 could be promising as a therapeutic agent for neurodegenerative diseases.

Acknowledgments

Not applicable.

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