Forced exercise activates the NrF2 pathway in the striatum and ameliorates motor and behavioral manifestations of Parkinson's disease in rotenone-treated rats

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Abstract

Background: Parkinson's disease (PD) is a common neurodegenerative disorder characterized by progressive loss of nigrostriatal dopaminergic neurons leading to dopamine depletion and problems of movement, emotions and cognition. While the pathogenesis of PD is not clear, damage of dopaminergic neurons by oxygen-derived free radicals is considered an important contributing mechanism.

This study aimed to evaluate the role of treadmill exercise in male Wister rats as a single treatment and as an aid-therapy with L-dopa for rotenone-induced PD. To study the role of NRF2-ARE pathway as a mechanism involved in exercise associated improvement in rotenone rat model of PD.

Method: Animals were divided into 5 groups, (Control, rotenone, rotenone\exercise, rotenone\L-dopa, and rotenone\exercise\L-dopa (combination) groups). After the PD induction, rats in the rotenone\exercise and combination groups were daily treadmill exercised for 4 weeks.

Results: Treadmill exercise significantly improved behavioral and motor aspects of rotenone model of PD. When treadmill exercise introduced as a single intervention, it amended most behavioral aspects of PD, gait fully corrected, short-term memory, and motor coordination. Where L-dopa corrected locomotor activity and motor co-ordination but failed to improve short-term memory and only partially corrected the gait of rotenone-treated rats. When treadmill exercise was combined with L-dopa, all features of PD were corrected. It was found that exercise upregulated some of its associative genes to NRF2 pathways such as TFAM, NRF2, Noq.1 mRNA expression.

Conclusion: This study suggests that forced exercise improved parkinsonian like features by activating NRF2 pathway.

Background

Parkinson's disease (PD) is a common neurodegenerative disorder characterized by persistent loss of nigrostriatal dopaminergic neurons leading to depletion of dopamine and consequent problems of movement, emotions and cognition. While many factors contribute to the pathogenesis of PD, damage of dopaminergic neurons by oxygen-derived free radicals is considered an important contributing mechanism (1, 2). Rotenone is a potent inhibitor of complex I (NADH: ubiquinone oxidoreductase) of the mitochondrial electron transport chain. This allows for accumulation and overproduction of reactive oxygen species which eventually lead to cell damage.(3, 4) Chronic rotenone exposure in rats causes both neuropathological findings and behavioral symptoms of PD (5). The rotenone model mimics the gradual progression of PD as observed in humans. Systemic mitochondrial inhibition by rotenone leads to selective nigrostriatal degeneration (6).

Treadmill exercise is indicated as a physical therapy to improve motor symptoms in patients with PD. Exercise can improve and alleviate memory loss in elderly, and decrease the risk of developing PD. The neuroprotective potentials of exercise are great, but the underlying mechanisms remain a debatable

issue. Evidence suggests that exercise neuroprotection is due to its neurotrophic effects (7), as exercise increases the availability of several neurotrophic factors. Long-term exercise benefits brain functioning by increasing the blood and oxygen flow to the brain, mobilizing growth factors that promote neurogenesis and synaptic plasticity, releasing of neurotransmitters, such as dopamine (DA), noradrenalin, serotonin and glutamate and consequently improve manifestations of the disease either at the motor or cognitive. levels in Alzheimer's animal model (8).

Exercise increases the antioxidant status in the striatum of animals and protects against neurological oxidative challenges. The nuclear factor erythroid derived 2-like 2 (NRF2)-antioxidant response element (ARE) signaling pathway, a major cellular defense mechanism against oxidative stress. Exercise activates NRF2 in human skeletal muscles and mouse heart. The NRF2-ARE signaling pathway appears to be a strong mechanism for exercise-induced neuroprotection (9). Activation of NRF2 gene activates genes which encode for antioxidant enzymes within the cells like heme oxygenase and NADH quinone oxidoreductase (NQO1). Also NRF2 activates the mitochondrial transcription factor A (TFAM)which regulates for mitochondrial DNA (mtDNA) replication (10)

In this study, we investigated weather treadmill exercise as a single therapeutic intervention, and as add-on therapy with L-dopa improve manifestations of PD in rotenone-treated rats. In addition, we assessed NRF2-ARE pathway as a possible mechanism activated by treadmill exercise.

Materials And Methods

Animals and experimental design

Sixty adult male Wister rats with body weight 275±25 gm, 9 months age; were purchased from Faculty of Science, Sohag University and were housed in Medical Animal Laboratory in Sohag Faculty of Medicine. Animals allowed free access to food and tap water. Rats were housed in standard cages, at normal light/dark cycle and room temperature. The rats were randomly assigned into 5 groups (n=10 in each group). Control group, rotenone-injected group, rotenone\exercise group, rotenone\L-dopa treated-group and combined (rotenone\L-dopa\exercise) group. The study was approved by Research Ethics Committee considering care and use of laboratory animals (permission number: SOH-IACUC-17050301).

Induction of Parkinsonism

Rats were subcutaneously injected with either vehicle, or rotenone (R8875; 95%, Sigma-Aldrich, USA) 2 mg\kg, for 4 weeks, dissolved in 1ml dimethyl sulfoxide (DSMO) & migylol 812 N (Sigma). L-dopa (L-3, 4 dihydroxyphenylalanine methyl ester hydrochloride, Sigma) was administered at a dose 6 mg\kg\day for 4 weeks.

Exercise protocol and treatments

After induction of PD, rats in exercise group were forced to run in 3-channel treadmill (Heath Life V4000M). Exercise regimen continues for 30 min/day, 5 times a week for 4 weeks. The treadmill speed

accelerates beginning with 2 m/min during first 5 minutes, at 3 m/min during second 5 minutes, and then at 5 m/min for the last 20 minutes (11). The efficiency of this exercise protocol was previously assessed by measuring the serum lactate dehydrogenase and creatine phosphokinase in non-published experiment (supplementary figure 1)

Behavioral and motor analysis:

these tests assess motor activity, and behavior of the rats, they were performed at the end of the experiment for all groups, and included.

Open field test (OFT)

OFT was performed according to the previously described method (12). Each animal was then given a score for total locomotor activity; calculated as the sum number of line crosses and rears, a score for exploratory behavior; the sum of the number of central square entries and the duration of time spent in the central square, and the anxiety score is equal to the sum of urination & defecation boli (12).

Object recognition test (ORT)

ORT was performed as described by Walsh and Cummins (13). In order to assess the short-term memory (STM) and long term memory (LTM), four objects used were made of plastic material. The objects and arena were washed with 10% ethanol solution after each trial. Training was conducted by placing each rat for 5 min into the field, where two identical objects (objects A1 and A2) were put in two adjacent sides, 10 cm from the walls. In a STM test a rat given 1.5 h after training, the rats explored the open field for 5 min in the presence of one familiar (A) and one novel (B) object. LTM test given 24 h after training, the same rat explore the field for 5 min in the presence of a familiar object A and a novel object C. Exploration is defined as sniffing or touching the object with the nose and/or forepaws. is calculated by these equations: STM= [B / (A1+B)] 100, and LTM= [C / (A1+C)] 100.

Foot print test

Foot print test was used to measure gait analysis by permitting rats to run in a wood corridor apparatus (65cm× 5 cm×15 cm), which was lined with a pre-cut piece of white paper. Rats were trained to run to the end of the corridor by placing the rats at the far end of the corridor and encouraging them to move towards the end. Training was conducted twice for each rat until the animal could run to the end box without encouragement. For testing, the paws of the animal were painted with four non-toxic water colors as described previously (14). For each animal, the gait was calculated using 4 paw prints; this allowed 5 values-yield/ rat; [front stride length (FSL), front stride width (FSW), hind stride length (HSL), hind stride width (HSW) and overlap (OL)] which were then averaged to provide gait measurements (14, 15).

Rota-rod test

To assess the motor coordination of the animals, we used an accelerating Rota-rod (Harvard Apparatus, UK). The Rota-rod consisted of a suspended rod, accelerating for 60 s, beginning from 5 rounds per minute (RPM) to reach 15 RPM and continuing at that speed for a further 60 s. A trial was stopped when the rat fell off the Rota-rod or after the complete 120 s. The mean latency time of three trials was taken. Animals were trained for five days to perform the test (16).

Tissue Sampling

After behavioral tests, the rats were anaesthetized with Zoletil (1mg\kg i.p (Vibac Laboratories, Carros, France).

Rats were transcardially perfused with 0.05 M phosphate-buffered saline (PBS). The brain was removed, snap freezed in liquid nitrogen, and kept for 1 hour in - 80°C. Striatum was dissected through multiple manual coronal sections with sharp razor blade. Samples stored in Eppendorf tubes at - 80°C till further analyses.

RNA extraction

Total RNA was extracted from 30 mg of tissue samples according to manufacture instructions (RNA Extraction kit (#K0731, Thermo scientific, Lithuania)). They were extracted to measure NRF2, Noq.1, TFAM and using housekeeping GAPDH by real time polymerase chain reaction (real time PCR).

Reverse transcription

Extracted RNA concentration was quantified using Nanodrop spectrophotometry (Quawell 5000, USA); then 110 ng of total RNA transcribed using RNA reverse transcriptase kits ((#K0251) (Thermo scientific, Lithuania)). Thermal cycler was programmed at 25°C for 10 minutes, 37°C for 120 minutes, 85°C for 5 minutes and 4°C for 20 hours.

Real time PCR

Prepared cDNA, was used in the qPCR analyzer (Step One, Applied Biosystems, Singapore) using the MAXIMA SYBR Green qPCR Master Mix with the following program: 1 cycle at 95°C for 10 minutes; 40 cycles of 95°C for 15 seconds, 60°C for 30 seconds and 72°C for 30 seconds; one cycle at 95°C for 15 seconds, 60°C for 1 minute and 95°C for 15 seconds. The specific primers (table 1) of NRF2, TFAM, NADPH dehydrogenase (NQO1) & housekeeping GAPD were purchased from Metabion international AG. Fold expression was measured according to the relative expression to. the relative expression to housekeeping gene as in the fold = $2^{-\Delta\Delta ct}$.

Tyrosine hydroxylase:

Homogenized striatum of the right hemispheres of brain were used to measure the levels of Tyrosine hydroxylase enzyme by ELISA (Tyrosine hydroxylase (TH) rat ELISA kits (#:96791) from Glory Science

Co., (Ltd, China) with detection range 0.625- 20 ng\ ml

Statistical analysis:

Statistical package for social sciences (IBM-SPSS), version 24 IBM- Chicago, USA (May 2016) was used for statistical data analysis. Data expressed as mean ± standard deviation (SD), number and percentage. Student t test was used to compare the means between two groups, and one-way analysis of variance (ANOVA) test was used to compare means of more than two groups. Post hoc test was used for individual P value which is considered significant when $P < 0.05$.

Results

Treadmill exercise, L-dopa and their combination improved exploration, and locomotion in rotenone treated groups (figure 1):

After 4 weeks of continuous administration of rotenone, rats spent less time in locomotion and exploration of open filed environment compared to vehicle-injected group ($p<0.001$, Fig. 1). But the anxiety score in terms of urination and defecation times were not different between both groups. To sum, rotenone injection decreased exploration and locomotion scores in the open field test:

It was noticed that after injection of rotenone, rats practiced 5 times/week a treadmill exercise spent less time in central arena and increased number of line crossings in open-field test compared to non-exercised group *($p<0.001$)*. Similarly, after rotenone-administration, in rats treated with L-dopa and L-dopa co-treatment with exercise practice recovered the locomotor and exploratory activities after rotenone treatment. However, L-dopa\rotenone and the combined exercise\L-dopa\rotenone groups showed significantly higher locomotor scores than the exercise\rotenone group, *($p<0.001$)*, There was an insignificant difference regarding exploration *($p= 0.50$)* in-between the three treatment groups. Also, there was no significant change as regard the anxiety score between the five groups under the study.

Treadmill exercise alone and in combination with L-dopa improved STM in the rotenone-treated groups in ORT(figure 2)**:**

There was a significant decrease in STM of rotenone-treated group compared to control group *($p<0.05$).* No significant difference as regard LTM in either groups.

L-dopa alone didn't improve STM in the rotenone treated rats ($p=0.23$) while, exercise alone or its combination with L-dopa have produced significant improvements in the STM in comparison with the rotenone-injected group ($p<0.05$). Meanwhile LTM was insignificantly changed among all groups *($p =0.38$).*

Treadmill exercise alone and in combination with L-dopa fully corrected the gait, while L-dopa alone caused partial correction of gait analysis

Rotenone injection caused a significant gait impairment in comparison to the control rats in terms of increased overlap (OL) distance *(p<0.001*), shortened stride length, both hind (HSL) and front (FSL) steps, and similarly significant wide base, as detected by increased FSW and HSW *(p<0.05),* Table 2.

Treadmill excercise alone and exercise /L-dopa treatment corrected OL distance induced by rotenone injection (p<0.001). Additionaty, increases of FSL and HSL (p<0.001), and decreases of HSW and FSW (p<0.001). L-dopa treatemnt improved the asymmetrical gait and caused an elongation in the stride length, and decrease in OL in rotenone-injected group (p<0.001), while it did not affect HSW (p=0.08) and FSW (p=0.12). To sum, treadmill exercise alone and in combination with L-dopa treatemnt fully corrected the gait of rotenone-injected rats .

Treadmill exercise, L-dopa and their combination prolonged the latency time to fall in Rota-rod test (figure 3)

There was a significant decrease in latency time to fall after 4 weeks of daily injection of rotenone in rats compared to control group *(p <0.001)*. Rats in all groups exhibited significant increases in the latency time to fall, in comparison to the rotenone-injected group (p<0.001). However, the L-dopa and the exercise/L-dopa-treated group showed longer latency time than the exercise\rotenone group (p<0.05). Therefore, forced exercise, L-dopa treatment and their combination prolonged the latency to fall in Rota-rod test (Fig.3).

Effects of treadmill exercise, L-dopa and their combination on tyrosine hydroxylase (TH) levels in the striatum:

Rotenone administration in rats significantly reduced TH levels (to 40% of the control value) in corpus striatum when compared to vehicle-injected group *(p<0.001)*. Whereas, forced exercise, and their combined applications caused significant increase in striatum TH levels increased up to 62% and 78% of normal) when compared to rotenone-injected group *(p<0.001)*. L-dopa alone didn't significantly increase the TH level in comparison to the rotenone group. In addition, the tyrosine hydroxylase level in the L-dopa\rotenone group was significantly lower than that of the exercise\ rotenone and the combined group (Fig.4).

Effects of treadmill exercise, L-dopa and their combination on NrF2 expression in the striatum

Rotenone caused a significant increase of NrF2 mRNA expression in the striatum of rats in comparison to vehicle-injected group *(p<0.05)*.

Forced exercise alone and in combination upregulated NrF2 mRNA expression in corpus striatum when compared to rotenone-injected group *(p<0.001)*. However, treatment with L-dopa did not affect NrF2 mRNA expression *(p=0.62)*. Moreover, NrF2 mRNA expression was lower in L-dopa\rotenone group compared to exercise\rotenone group *(p=0.001)*. Interestingly, the combination between forced exercised and L-dopa treatment showed a significant increase in NrF2 mRNA expression in comparison to the exercise\rotenone group *(p<0.05)* and L-dopa\rotenone group *(p<0.001)*. This study showed that

administration of rotenone increased NrF2 expression in the corpus striatum, such upregulation was augmented by forced exercise, dramatically increased by the combination of treatment of L-dopa and forced exercise, but was not affected by single treatment of L-dopa (Fig.5.A).

Effects of treadmill exercise, L-dopa and their combination on target genes of NrF2 in the striatum

Rotenone significantly increased expression of NQO1 in rotenone group compared to control *(p<0.001)* as shown in figure 6.B. Exercise produced significant increase in NQO1 mRNA expression level when compared to rotenone group (P<0.001). However, the L-dopa treatment reduced NQO1 mRNA expression in rotenone-injected group *(p=0.001)*. Whereas when exercise performed with L-dopa treatment did not change NQO1 mRNA expression compared to rotenone group *(p=0.49)*, but decreased NQO1 mRNA expression when compared to the exercise\rotenone group *(p=0.003*). Additionally, there was a significant increase in NQO1 expression exercise\L-dopa\rotenone of group compared to L-dopa\rotenone group *(p<0.001)* (Fig.5.B).

Consistently, treadmill exercise caused a significance increase in TFAM mRNA expression after injection of rotenone and when practiced in combination with L-dopa treatment compared to rotenone group (p <0.001) and L-dopa\rotenone (p< 0.001 and <0.05, successively). Similar to NQO1 expression, L-dopa treatment did not affect TFAM mRNA expression in rotenone-injected group *(p=0.73).* There was a significant increase in TFAM mRNA expression in the combination group when compared to exercise\rotenone group *(p<0.05*), (Fig.5.C).

Overall, our results demonstrated that 4 weeks of continuous treatment with rotenone increased NrF2-related genes, NQO1 and TFAM, expression in the corpus striatum. Such expression was enhanced by forced exercise and was not affected by single treatment of L-dopa (Fig.5.B, C).

Discussion

Parkinson's disease (PD) is a common neurodegenerative disease, about 1% above the age of 65 years suffer from. In PD, there is a progressive degeneration of dopaminergic neurons of the substantia nigra of the midbrain (17). This results in the characteristic motor impairment & extra motor manifestation of the disease. Rotenone is known to induce PD in rats by targeting dopaminergic neurons (18, 19). It acts by inhibiting complex I of the mitochondrial electron transport chain and causing accumulation of reactive oxygen species (ROS) and subsequently cell damage. Age related mitochondrial dysfunction and oxidative stress has been strongly involved in the pathophysiology of PD (20, 21).

In this study, Rotenone 2mg\kg\day was injected by the subcutaneous route for 4 weeks to induce rotenone-Parkinson's disease rat model. Rotenone treated group, showed a significant decrease in exploration and locomotion as regards the open field test, gait impairment; (asymmetrical foot pattern, shortened stride length and widened base), a significant decline in motor co-ordination observed by shortened latency time on Rota-rod. Cognitive function impairment of PD also existed; as a significant decrease in novel objects preference in short-term memory test. Tyrosine hydroxylase levels in the

striatum significantly decreased in rotenone treated rats. Similar to our study, Vijayalakshmi et al. (22) reported significant decrease in locomotion in Open field test in rotenone-treated Wister rats. Similarly, Valdez et al. (23) reported 60% decrease in locomotive activity in the open field test in rotenone-treated rats. von Wrangel et al., (24) reported that rotenone-treated rats showed significant impairment in Rota-rod and significant decline in tyrosine hydroxylase in the striatum. Similarly, (25), in their study reported that rotenone treatment in rats caused significant Rota-rod test impairment, and reduction in tyrosine hydroxylase. Cannon et al. (26) reported significant decreased locomotion, gait impairment and 50% loss in tyrosine hydroxylase activity in rotenone-treated rats (27). As regard foot print test, the results of this study were in accordance with Madiha et al. (28) their data showed significantly impaired walking pattern and shortened stride length in rotenone treated rats.

Treadmill exercise significantly increased exploration and locomotion in rotenone-treated rats. Treadmill exercise corrected the gait impairment in rotenone-treated rats; the asymmetrical patterns were corrected by significant reduction in overlap, accompanied by significant increase in stride length and a significant decrease in stride width. Treadmill exercise significantly improved short-term memory novel object recognition in rotenone treated group. Tyrosine hydroxylase levels significantly increased in brain after treadmill exercise. Exercise also improved motor co-ordination as it increased latency time to fall on Rota rod significantly. Recently many authors studied the effects of treadmill and other means of exercise on rat models of PD. Lee et al., and Shin et al. (27, 29), both studied the effects of treadmill exercise on rotenone-treated rats. They reported that rotenone injection shortened the latency time; meanwhile, treadmill running lengthened the latency to fall of the rotenone-treated. Shin et al, reported decrease in depressive-like behaviors in exercised rotenone treated rats (27, 29). In accordance with our study, Chen et al. (30) reported significant improvement of Rota rod test in treadmill exercised 6-OHDA group , and significant gait improvement with significant improvement in overlap, stride length and base width with treadmill exercise.

When L-dopa was administrated in rotenone-treated rats, a significant improvement regarding exploration and locomotion was found. Motor co-ordination significantly improved ever more than exercise alone. The gait showed significant partial improvement, the asymmetrical pattern significantly corrected, and the stride length significantly increased, meanwhile, short-term memory did not significantly improve. Tyrosine hydroxylase levels did not significantly increase in brain but to a Our results was agreed with Alam et al. (31), who found significant increase in locomotor activity on L-dopa treatment of rotenone-treated rats. This is also agreed with Shin et al.(27) who reported significant increase in exploration and locomotive activity of Open field test in rotenone-treated rats. In accordance with our results also, Vijayalakshmi et al. reported that L-dopa significantly increased exploration and locomotion in rotenone-treated rats (22). Sgroi et al. (32), reported a significant increase in locomotion and a significant increase in motor co-ordination with 8 mg\kg L-dopa treatment for ten days in 6-OHDA model of PD.

When L-dopa treatment was accompanied by treadmill exercise, there was a significant increase in exploration and locomotion as animal behavior observed in open field. A significant increase in latency time of Rota rod was found together with improved motor co-ordination as Rota-rod latency time

significantly increased. There was a significant gait improvement with L-dopa and exercise co-treatment, the asymmetrical pattern was fully corrected, with a significant increase in stride length and a significant decrease in stride width. Short-term memory significantly improved. Tyrosine hydroxylase levels in the brain were significantly increased.

In our study, we investigated some genes of the NrF2-ARE pathway as a possible mechanism involved in treadmill exercise impacts in brain. Exercise causes an increase in O2 consumption with increase in ROS production, especially H2O2. Oxidative stress leads to inhibition of KEAP-1, dissociation of NrF2 from KEAP-1IN cytoplasm, migrates into nucleus and activates of NrF2-ARE(33). NrF2 activates anti-oxidant gene expression with increased productions of detoxifying enzymes: Heme oxygenase, and NADPH quinone oxidoreductase (Nqo.1). Activation of NrF2 together with PCG1α, which also is activated by exercise; caused increase in mitochondrial transcription factor A (TFAM) expression in the striatum. TFAM is a nuclear factor which controls replication of mitochondrial DNA (mt DNA). Upregulation of TFAM increases number of copies and packaging of mtDNA . In addition, adequate levels of TFAM are required to prevent mtDNA release into the cytoplasm and initiates inflammatory response (10).

This study measured the rate of expression of NrF2, TFAM, and Nqo.1 in the striatum of rotenone-treated rats. Rotenone downregulated expression of the TFAM in the rotenone group while upregulated NrF2 and Nqo1 in comparison to the control rats. Treadmill exercise showed significant increase in NrF2, TFAM, and Nqo.1 in the striatum in exercise\rotenone group in comparison to control and rotenone groups. L-dopa\rotenone group showed insignificant change in the expression of these genes. However, exercised \L-dopa\rotenone group, showed a significant increase in NrF2, TFAM and Nqo.1 expression, when compared to both control and rotenone group.

Whether rotenone increases or decreases the expression of NrF2 and Nqo1 is a matter of debate. Many studies (25). found that rotenone in in-vivo and in vitro studies downregulated the expression of these genes and enhanced apoptosis of the neurons of the nigrostriatum which was reversed by administration of danshensu herbal extract. Similarly, Cui, et, al. (34) found decreased expression of NrF2 and NQO1 protiens measured by western blotting in rotenone treated rats which was corrected with pretreatment with curcumin. On the contrary Wei et, al., (35) found that rotenone increased NrF2 and NQO1 expression which was further enhanced by ellagic acid. They measured NrF2 in the cytoplasm and in the nucleus of the cell indicating that increased transcription of this transcription factor in the cell and subsequently its associative genes also increased. In addition, Zagoura, et, al., (36) found that rotenone increased activation of NrF2, and NQO1 while downregulated the Keap1 in dose dependent manner in neuronal model derived from human induced pluripotent stem cells.

As regard expression of TFAM, rotenone in this study downregulated its expression which may underlie the instability in the mtDNA and consequent damage of the dopaminergic neuron as detected by decreased level of tyrosin hydroxylase measured in the striatum. Noteworthy, chen et. Al., (37) in their study on post mortem human parkinsonian patients found that expression of TFAM in substantia nigra is lower and their mtDNA is less stable in comparison to age matched elderly control subjects.

As regards our study results, treadmill exercise activated NrF2 pathway in the striatum of rotenone treated rat. This activation can be one of the mechanisms involved in treadmill exercise beneficial effects on rotenone-treated model of PD. These results were in accordance with, Aguir et al., 2016, the author reported significant increased Nrf2 and TFAM in the striatum of exercised 6-OHDA-treated mice, in comparison to sedentary 6-OHDA-treated mice. In line with that, Li et al. (38) reported that exercise activate NfR2-pathway with different exercise duration. Other studies reported TFAM activation with exercise in different pathological conditions, Lashgarie et, al.(10) reported that treadmill exercise increased TFAM expression in the heart of nicotine sensitized rats, which attenuated mitochondrial mediated damage in the myocardium induced by nicotine.

Conclusion

In this study, treadmill exercise activated NrF2 pathway, and some of its associative genes. It caused significant improvement in motor and behavioral aspects of rotenone model of PD. When treadmill exercise introduced as a single measure, it caused improvement of all loccomotor activity and short-term memory. Where L-dopa corrected motor co-ordination but failed to improve short-term memory and only partially corrected the gait of rotenone-treated rats. When treadmill exercise was combined with L-dopa, all behavioral motor and non-motor aspects of PD were corrected.

Abbreviations

ARE**:** antioxidant response elements, DA: Dopamine, FSL: front stride length, FSW: front stride width, HSL: hind stride length, HSW: hind stride width, NQO1: NAD(P) H quinone oxidoreductase 1: NAD(P)H dehydrogenase, Nrf2: nuclear factor erythroid 2 (NFE2)-related factor 2, OFT: Open field test, OL, overlap, ORT: object recognition test, PA:Physical activity, PD: Parkinson's disease, PGC1α : peroxisome-proliferator-activated receptor-γ coactivator-1α, ROS: Reactive oxygen species, TFAM: Mitochondrial transcriptional factor A

Declarations

Ethics approval:

The study was approved by Sohag University research Ethics Committee considering care and use of laboratory animals (permission number: SOH-IACUC-17050301).

Consent for publication:

All authors accept publication in this journal

Availability of data and materials

The datasets used and/or analyzed during the current study are available from

the corresponding author on reasonable request.

Competing interests

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Author contribution:

Amany Abdelrahman selected the point of research, Motamed Mahmoud and Dina Monir equally contributed to the practical part of the study, Ibrahim Rehan collected the data and shared in the analysis of the behavioral tests, All authors shared in the writing of the research with final revision from Omyma Galal and Amany Abdelrahman.

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Tables

Table (1): Forward and reverse primers for Real time PCR

Gene name	Forward primer	Reverse primer	Access number	References
NRF2	CACATCCAGACAGACACCAGT	CTACAAATGGGAATGTCTCTGC	NM_031789	*(1)*
TFAM	AGTTCATACCTTCGATTTTC	TGACTTGGAGTTAGCTGC	NM_031326.1	*(2)*
NADPH dehdrogenase(NQO1)	CAGCGGCTCCATGTACT	GACCTGGAAGCCACAGAAG	NM_017000	*(1, 3)*
GAPDH	CAGGCATATGGTGGTCCATAGAG	TCATGGGATCCACCTGCAGC	NM_017008	*(1))*

Table references

1. Yamashita Y, Ueyama T, Nishi T, Yamamoto Y, Kawakoshi A, Sunami S, et al. Nrf2-inducing anti-oxidation stress response in the rat liver-new beneficial effect of lansoprazole. PLoS One. 2014;9(5).
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Table (2) Effects of exercise, L-dopa and their combination on gait measured by Foot print test (overlap, front stride width, front stride length, hind stride width & hind stride length in cm).

Group	Control G	Rotenone G	Rotenone\ exercise G	Rotenone\L-dopa G	Combined G	P- value by ANOVA
OL	1.50±0.3	2.10±0.45	1.40±0.3	1.35±0.2	1.55±0.3	<0.001
		*	#	#	#	
FSW	4.95±1.23	5.95±0.83	3.40±0.99	5.20±0.91	4.15±0.8	0.006
		*	#		#	
FSL	11.89±1.8	9.70±1.65	13.5±3.2	13.1±1.44	11.1±2.5	0.002
		*	#	#	#	
HSW	6.15±0.78	7.00±0.88	4.55±1.2	6.15±0.78	5.65±0.7	<0.001
		*	#		#	
HSL	12.65±1.6	10.1±1.46	12.8±1.53	13.5±1.24	11.7±2.5	<0.001
		*	#	#	#	

rotenone injection caused a significant gait impairment in comparison to the control rats. There was a significant increase in OL *(p<0.001*), shortened stride length, as indicated by a significant decrease in HSL and FSL *(p<0.05),* significant wide base, as detected by significant increase in FSW and HSW *(p<0.05).* Treadmill exercise alone and in combination with L-dopa fully corrected the gait of rotenone-treated rats. There was a significant decrease in OL between exercise\rotenone and the combined groups in comparison to rotenone group (p<0.001). Also a significant increase in FSL and HSL existed (p<0.001), and a significant decrease regarding HSW and FSW (p<0.001). L-dopa improved the asymmetrical gait and caused significant elongation in the stride length, decrease in OL (p<0.001), while it showed an insignificant decrease regarding HSW (p=0.08) and FSW (P=0.12). P value is considered significant if <0.05.n=10 in each group. * significant when compared with control group.# significant when compared with rotenone group.OL: overlap, FSW: front stride width, FSL: front stride length, HSW: hind stride width, HSL: hind stride length. Data was expressed as mean± SD.

Figures

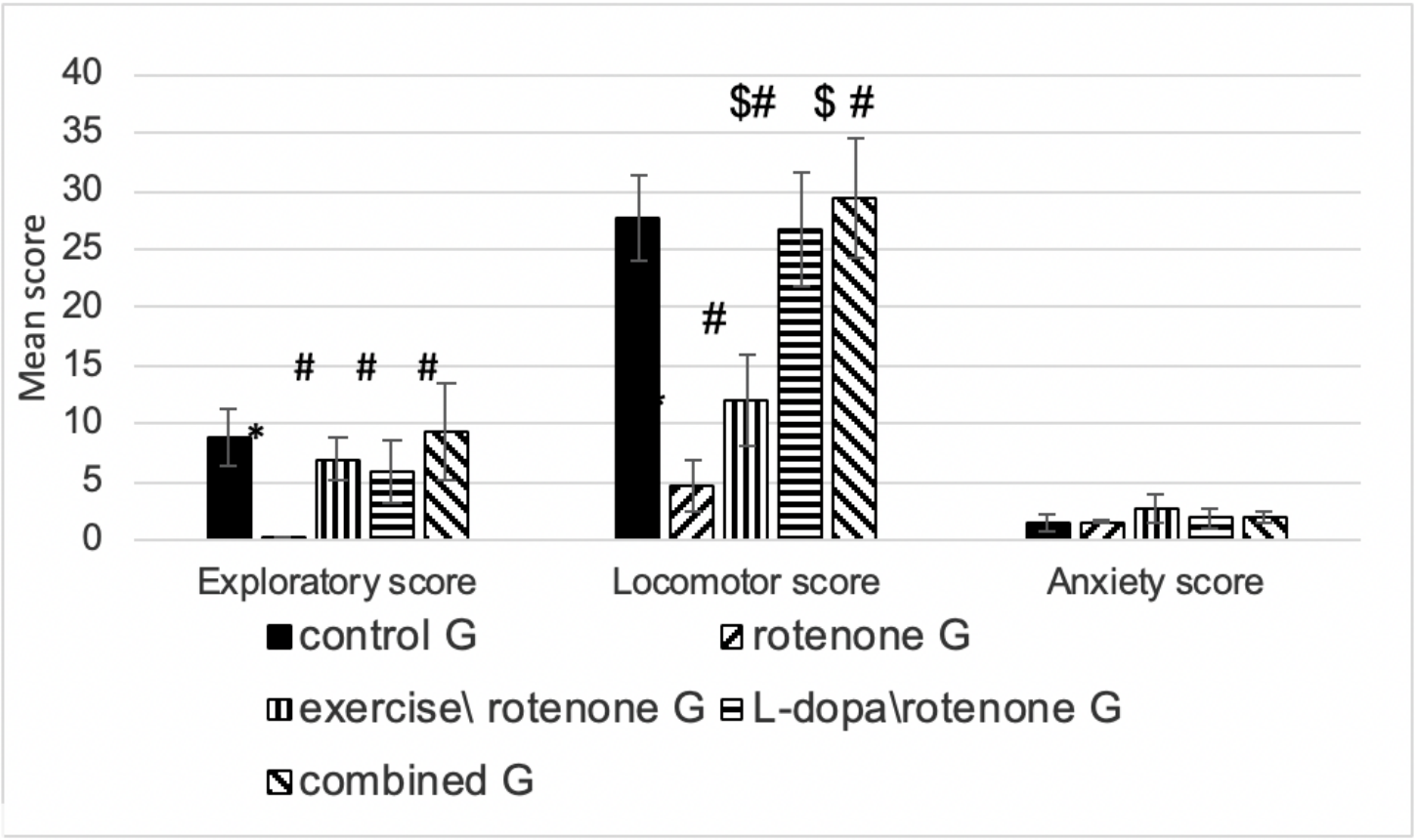
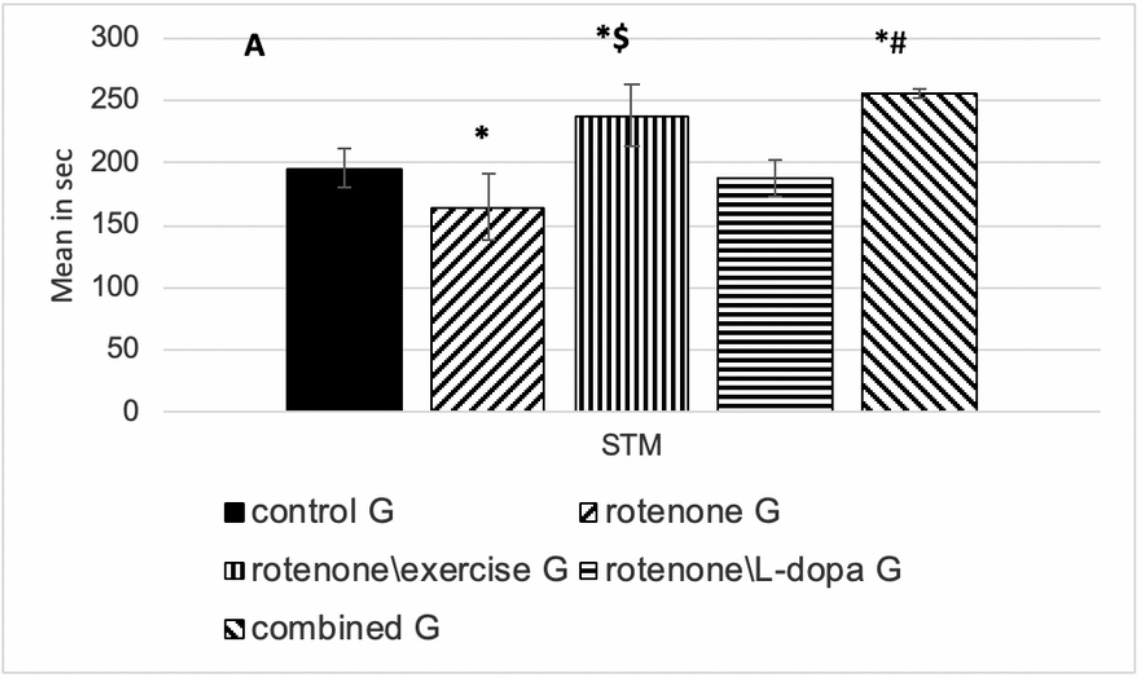


Figure 1

open field test effect of exercise (4 weeks treadmill) , L-dopa (6mg\kg ip), and their combination on rotenone treated rats (2mg\kg sc for 4 weeks). Rotenone induced significant decrease ($P < 0.001$) in the locomotor and exploration scores in comparison to control group. while, exercise, L-dopa and their combination produced significant improvement in the locomotor scores in comparison with the rotenone treated group ($p<0.001$). L-dopa and the combination groups improved locomotor activity more than exercise ($p< 0.01$). no difference as regard the exploration score inbetween the 3 treatment groups ($p=0.5$). no significant difference between either groups as regard the anxiety score. N= 10 rats\group. P-value < 0.05, * compare to control group, # in comparison with the rotenone treated group, $ in comparison with exercise\ rotenone group.



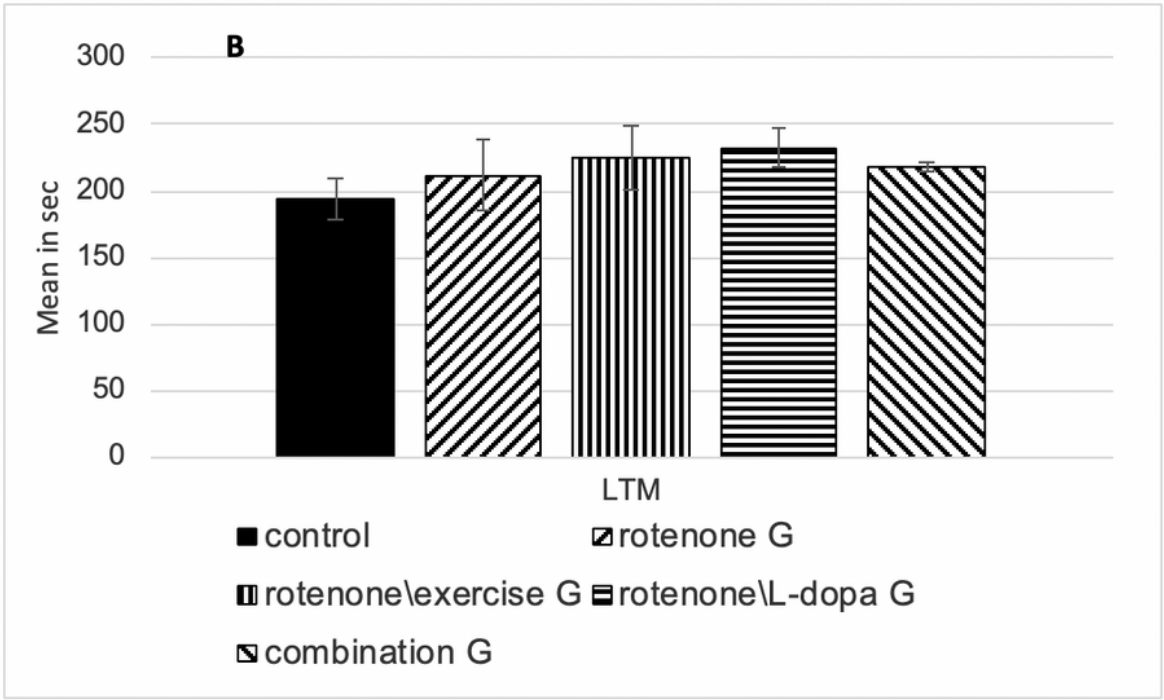


Figure 2

bject recognition test, effect of exercise (4 weeks treadmill), L-dopa (6mg\kg ip), and their combination on rotenone treated rats (2mg\kg sc for 4 weeks). Rotenone induced significant decrease ($P < 0.001$) in the STM (fig A) but has no effect on LTM (figB) in comparison to control group. L-dopa alone didn't improve STM in the rotenone treated rats ($p<0.23$) while, exercise alone or in combination produced significant improvement in the STM (fig A) in comparison with the rotenone treated group ($p<0.05$). No difference

between either group as regard LTM (P = 0.38) as shown in fig B. P-value is considered significant when P < 0.05, n= 10 rat in each group. * compare to control group, # in comparison with the rotenone treated group. STM: short term memory, LTM: long term memory.

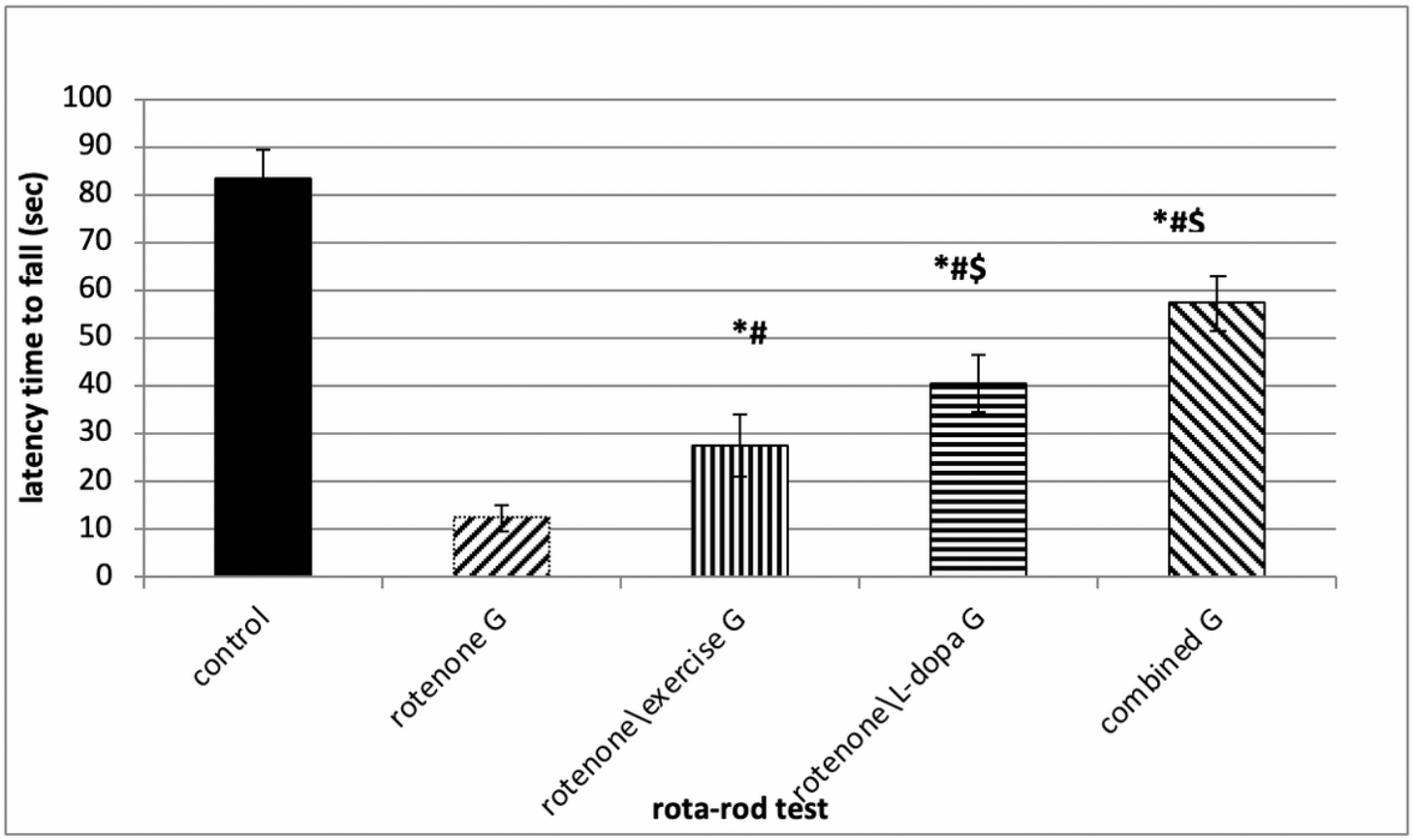


Figure 3

Rota-rod test , effect of exercise (4 weeks treadmill) , L-dopa (6mg\kg ip), and their combination on rotenone treated rats (2mg\kg sc for 4 weeks). rotenone significantly decreased the latency time to fall in rotenone group compared to control group (p <0.001). all treadmill exercise, L-dopa and their combination increased the latency time to fall in comparison to the rotenone group: However, the L-dopa\rotenone and the combined groups significantly increased the latency time to fall more than the exercise\rotenone group (P<0.05). P value is considered significant if <0.05. n=10 in each group. * significant when compared with control group.# significant when compared with rotenone group.$ significant when compared to rotenone\exercise G.

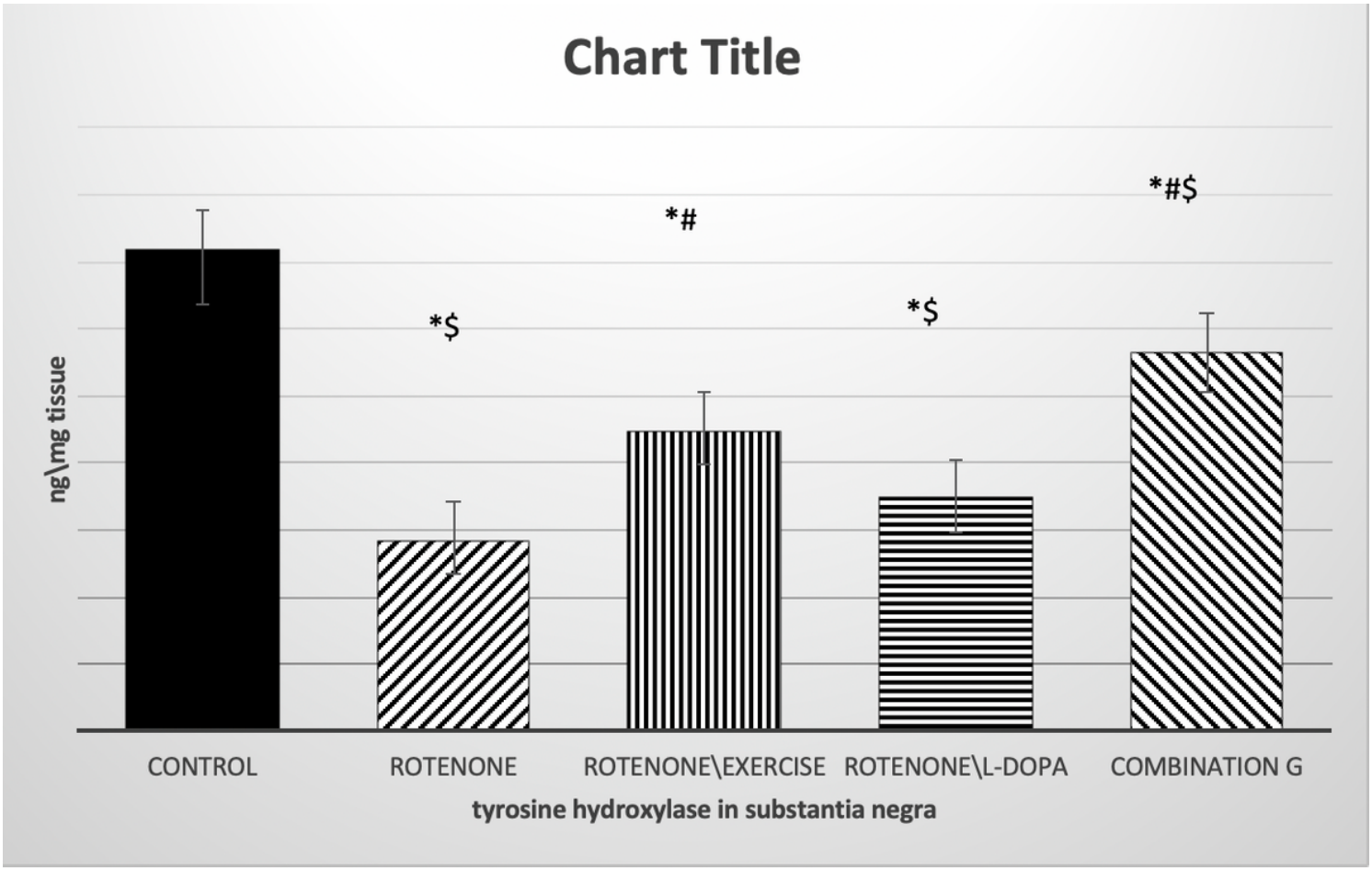


Figure 4

striatal tyrosine hydroxylase measured by ELISA, effect of exercise (4 weeks treadmill), l-dopa (6mg\kg, ip), and their combination on tyrosyne hydroxylase level in rotenone-treated rats (4 weeks, 2mg\kg, sc),rotenone decreased TH level compared to the control G, the exercise rotenone and combination, groups showed significant increase in TH level in comparison to the rotenone group. However the L-dopa group showed significantly lower levels of TH in comparison to the exercise groups (P<0.001) and didn't differ significantly from the rotenone G .P value is considered significant if <0.05. * significant in comparison to control group, # significant when compared to rotenone group, $ significant when compared to rotenone\ exercise group. n=10 in each group.

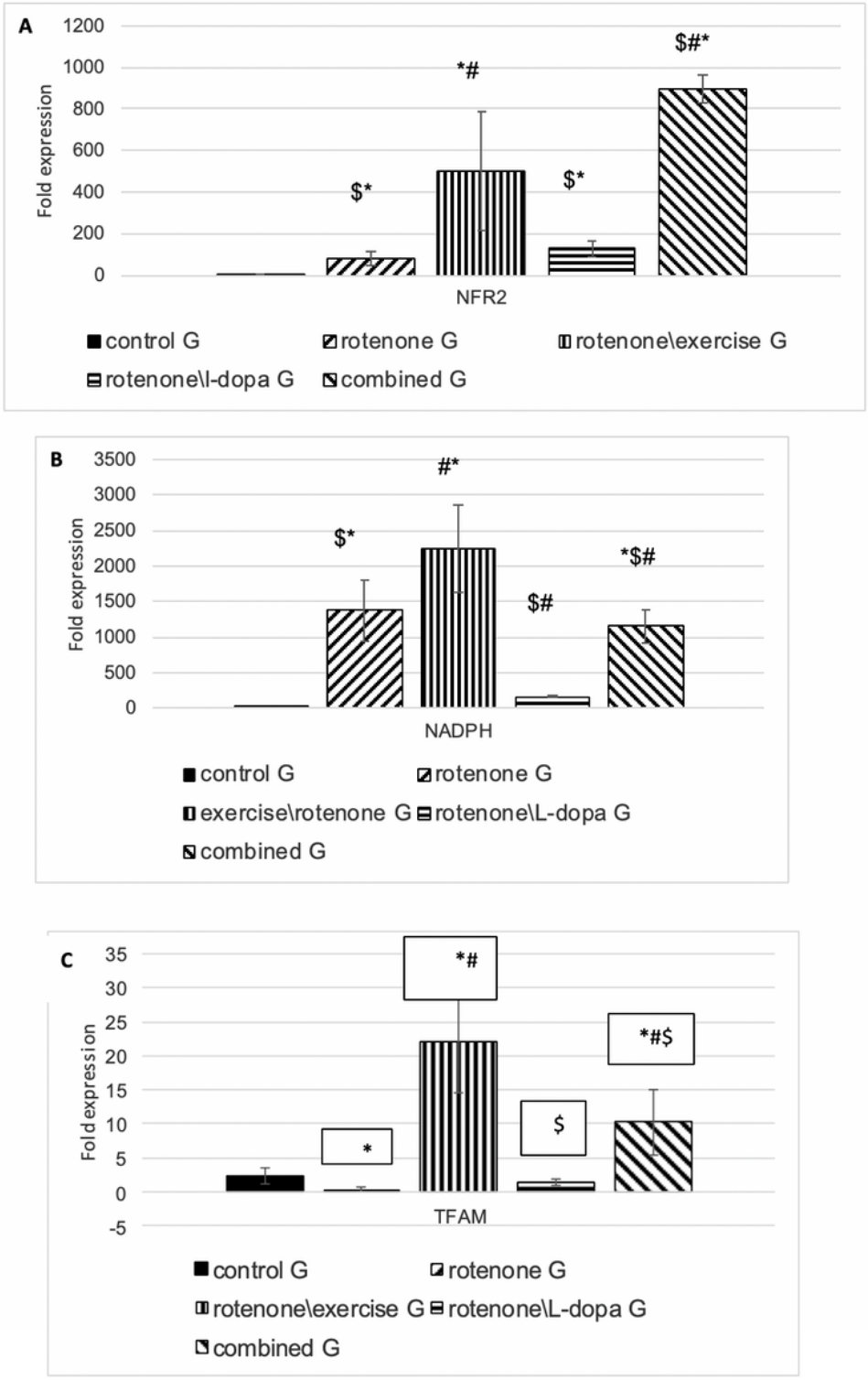


Figure 5

expression levels of genes by PCR in the corpus striatum, A: NrF2 gene, rotenone (2mg\kg, sc, daily for weeks) significantly increased expression of NrF2 gene in comparison to control group, both exercise\rotenone and combination group showed significant increase in expression in comparison to the rotenone group, the L-dopa\rotenone group showed significantly decreased expression when compared to both exercise\rotenone and combination groups. B: expression level of NADPH dehydrogenase (NQO1)

mRNA, rotenone significantly increased expression of NADPH when compared to control group, Exercise produced significant increase in NADPH expression level while the L-dopa group significantly decreased expression in comparison to the rotenone group. The L-dopa and combination groups showed significantly decreased expression of NADPH when compared to the exercise group. C: expression level of TFAM mRNA, rotenone decreased TFAM mRNA expression significantly in comparison to control group Both exercise and combination groups had significantly higher level of TFAM m RNA expression than that of rotenone and L-dopa\rotenone groups. The TFAM expression is lower for the combination group than the exercise\rotenone group. P value is considered significant if <0.05. n= 10 rats in each group. *significant when compared to control G. # significant when compared to rotenone group. $significant when compared to the rotenone\exercise group.

Supplementary Files

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- SuppFig1.docx